

A Platform Strategy Utilizing the Unique Solvent Properties of Ionic Liquids to Dissolve and Process Cellulose with Controlled Architecture, Functionality, and Rheology for Reducing or Replacing Synthetic Polymers in Advanced New Materials

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Affirmation Statement: The nominated technology has been discovered, researched, and demonstrated within the past five years. Patents¹ and publications² detail the dissolution of cellulose in a variety of forms using ionic liquid solvents, reconstitution of the cellulose in several architectures, encapsulation of active ingredients to control functionality, and preparation of composites with other polymers to control rheology. Additional patent applications for new cellulose-based materials, as well as, business models which will guide the commercial development and implementation of the technology are in progress.

1. Related patents include: Swatloski, R. P.; Rogers, R. D.; Holbrey, J. D. "Dissolution and processing of cellulose using ionic liquids," 11/30/04, U.S. Patent No. 6,824,599 B2; International Application PCT/US02/31404; International Publication Number WO 03/029329 A2, April 10, 2003. Holbrey, J. D.; Spear, S. K.; Turner, M. B.; Swatloski, R. P.; Rogers, R. D. "Cellulose Matrix Encapsulation and Method," 10/26/04, U.S. Patent No. 6,808,557 B2. Holbrey, J. D.; Swatloski, R. P.; Chen, J.; Daly, D. T.; Rogers, R. D. "Polymer Dissolution and Blend Formation in Ionic Liquids." Provisional U.S. Patent filed March 27, 2004. Mays, J. W.; Bu, L.; Rogers, R. D.; Hong, K.; Zhang, H. "Polymer Formation in Room Temperature Ionic Liquids," U.S. Patent Application U.S. 60/280,201 (Allowed 2004); International Application PCT/US02/10091; International Publication Number WO 02/079269 A1, October 10, 2002.
2. Related publications include: Swatloski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D. "Dissolution of Cellulose with Ionic Liquids," *J. Am. Chem. Soc.* **2002**, *124*, 4974-4975. (Highlighted in: "Ionic Liquids Can Dissolve Cellulose," *Chemical & Engineering News*, April 29, 2002, p 24.) Swatloski, R. P.; Holbrey, J. D.; Spear, S. K.; Rogers, R. D. "Ionic Liquids for the Dissolution and Regeneration of Cellulose," In *Molten Salts XIII: Proceedings of the International Symposium*, Trulove, P. C.; De long, H. C.; Mantz, R. A.; Stafford, G. R.; Matsunaga, M., Eds.; The Electrochemical Society: Pennington, NJ, 2002; Vol. 2002-19, pp 155-164. Turner, M. B.; Holbrey, J. D.; Spear, S. K.; Rogers, R. D. "Production of bioactive cellulose films reconstituted from ionic liquids," *Biomacromolecules* **2004**, *5*, 1379-1384. Swatloski, R. P.; Holbrey, J. D.; Weston, J. L.; Rogers, R. D. "Preparation of magnetic cellulosic composites using ionic liquids," In *Molten Salts XIV: Proceedings of the International Symposium*, Trulove, P. C.; De long, H. C.; Mantz, R. A., Eds.; The Electrochemical Society: Pennington, NJ, 2005; In Press. Swatloski, R. P.; Holbrey, J. D.; Memon, S. B.; Caldwell, G. A.; Caldwell, K. A.; Rogers, R. D. "Using *Caenorhabditis elegans* to probe toxicity of 1-alkyl-3-methylimidazolium chloride based ionic liquids," *Chem. Commun.* **2004**, 668-669. Hong, K.; Zhang, H.; Mays, J. W.; Visser, A. E.; Brazel, C. S.; Holbrey, J. D.; Reichert, W. M.; Rogers, R. D. "Conventional free radical polymerization in room temperature ionic liquids: A green approach to Commodity Polymers with Practical Advantages," *Chem. Commun.* **2002**, 1368-1369.

Eligibility Statement: This project is eligible for the academic award.

Focus Area Statement: The primary focus area for this nomination is the use of alternative synthetic pathways for green chemistry; specifically, alternative feedstocks that are more innocuous and renewable. The utilization of Nature's abundant renewable polymer, cellulose, rather than synthetic polymers, in the formation of advanced materials, will dramatically lessen the dependence on unsustainable petroleum feedstocks--resulting in significant energy and chemical savings.

Technical Abstract: This nomination illustrates the enabling nature of ionic liquids (ILs) as non-volatile solvents for the direct co-dissolution and/or suspension of cellulose and active ingredients which can be easily reconstituted in a variety of forms--yielding advanced composites integrating particulates, complexants, colorimetric or biological receptors, etc. The ability to easily modify the properties of cellulose into new biorenewable or biocompatible materials will lessen the current dependence on diminishing petroleum feedstocks currently used to make polymers and composites. By using ILs to directly dissolve and process cellulose one can effectively utilize the full complexity of Nature's polymer. While this will lead to the commensurate reduction/elimination of environmentally undesirable solvents, feedstocks, and processes, it will also allow us to reexamine and improve current processing strategies.

This research has achieved: (1) efficient, direct dissolution of cellulose, (2) introduction of functional additives, (3) simple product generation, and (4) recovery and recycling of the IL. By combining fundamental knowledge of ILs as solvents with a novel technology for processing cellulose, and using Green Chemistry principles to also guide process development and commercialization, it is envisioned that this platform strategy can lead to a variety of commercially-viable advanced materials which can reduce the dependence of synthetic polymers.

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Executive Summary

Tremendous strides are currently being made towards the utilization of renewable resources by major chemical companies in the form of biorefineries. Interestingly, in a biorefinery, the complexity afforded by Mother Nature in natural polymers such as cellulose, is broken down into simple building blocks (e.g., ethanol, lactic acid) in order to build back up more complex polymers for use as plastics. However, if one could directly utilize the biocomplexity of cellulose in the formation of new materials, we could eliminate many destructive and constructive synthetic steps. Indeed, the full potential of cellulose and cellulose products has not been fully exploited, partially due to the historical shift towards petroleum-based polymers from the 1940's onwards, the inability to easily modify the cellulose polymer properties, and also by the limited number of common solvents in which cellulose is readily soluble. One must ask, however, will synthetic polymers sustain their cost effectiveness as oil becomes less abundant?

The nominated technology combines two major principles of Green Chemistry: the development of alternative solvents which are more environmentally-benign and the utilization of nature's virgin biorenewable feedstocks in the formation of advanced materials. Cellulose from virtually any source (fibrous, amorphous, pulp, cotton, bacterial, filter paper, etc.), can be readily dissolved without derivatization in the low melting IL [C₄mim]Cl by gentle heating (especially using microwaves) to permit rapid dissolution. IL-dissolved cellulose can easily be reconstituted in water in controlled architectures (fibers, membranes, beads, flocs, etc.) using conventional extrusion spinning or forming techniques. If, at the cellulose-in-IL solution stage, functional additives are incorporated into the solution before regeneration, then blended or composite materials can be prepared. The incorporated functional additives can be either dissolved (e.g., dyes, complexants, other polymers) or dispersed (e.g., nanoparticles, clays, enzymes) in the IL medium before or after dissolution of the cellulose. This simple, non-covalent approach lends itself readily to the preparation of encapsulated cellulose composites of tunable architecture, functionality, and rheology. IL recycling can be accomplished by a novel salting-out step or by common cation exchange techniques, both of which save energy compared to a totally evaporative procedure. Current work is aimed at improved, more efficient and economical syntheses of [C₄mim]Cl, studies of the IL toxicology, engineering process development, and commercialization.

We have successfully demonstrated a platform strategy to efficiently exploit the biocomplexity afforded us by Nature's renewable polymer - cellulose; perhaps reducing Society's dependence on non-renewable petroleum-based feedstocks for synthetic polymers. We have developed an apparently nontoxic solvent system for the efficient processing of cellulose which eliminates volatile emissions and negative processing (needed for derivatization or degradation of the cellulose) effects common with traditional techniques. We have shown that by carefully controlling the addition of functional additives (enabled by the same IL solvent system that dissolves the cellulose), the physical and chemical properties of the final materials can be easily tuned for specific applications, leading to a variety of smart advanced materials of controlled architecture, functionality, and rheology.

We are currently in the process of market research and business planning leading to the development of the technology to the point of commercialization of targeted materials, either through joint development agreements with existing chemical companies or through the creation of small businesses. Green Chemistry principles will guide the development work and product selection. For example, targeting thermoplastic materials for the automotive industry could result in materials with lower cost, flexibility, lower weight, lower abrasion, lower toxicity, and improved biodegradability, as well as leading to a significant reduction in the utilization of polypropylene and polyethylene. By combining a fundamental knowledge of ILs as solvents with a novel technology for the dissolution and reconstitution of cellulose and similar polymers, and using Green Chemistry principles to also guide process development and commercialization, it is envisioned that this platform strategy can lead to a variety of commercially-viable advanced materials which can obviate or reduce the use of synthetic polymers.

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Due to increasing environmental awareness and diminishing petroleum-based resources,^{1,2} there is a growing desire for the development of new materials and plastics derived from renewable resources via green processes³ to obviate or reduce the need for synthetic polymers. Cellulose, as one of the most abundant natural resources, offers a wide range of desirable properties including biological compatibility, resistance to chemical attack, and benign decomposition products,⁴ thus, economically- and environmentally-sustainable cellulose-based materials are in demand. However, there are tremendous challenges with regard to developing chemical processing for cheap, easy to manufacture, advanced cellulosic materials, and cellulose is, at the moment, losing market dominance to cheaper synthetics, even though the unique properties of cellulose have yet to be synthetically duplicated.

In our search for new advanced materials, we have sought to combine two Green Chemistry principles, the need for easy to use and readily recoverable greener solvents, with the use of biorenewable feedstocks. We realized that the full potential of cellulose and cellulose products has not been fully exploited, partially due to the historical shift towards petroleum-based polymers from the 1940's onwards, but also due to the inability to easily modify the properties of cellulose and by the limited number of common solvents in which cellulose is readily soluble.

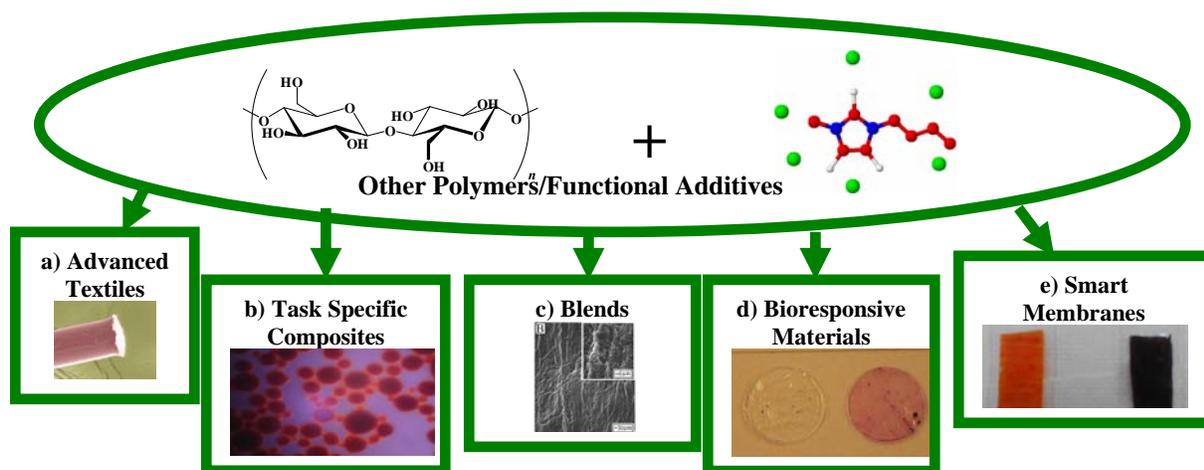
Traditionally there have been four main cellulose systems: a) cellulose nitrate; b) cuprammonium hydroxide; c) xanthate or viscose; and d) amine oxide or lyocell. The viscose process making use of cellulose xanthate, developed by Cross, Bevan, and Beadle in 1891,⁵ is the most widely used processing technique to date, with minimal changes being made to the process in the past 100+ years. In this process, cellulose is dissolved as cellulose xanthate following treatment with alkali and CS₂. The cellulose is then recovered by treatment with sulfuric acid, liberating CS₂ and H₂S, neither environmentally-friendly or sustainable. Yet, more than 3,000,000 tons of 'industrial grade cellulose' are processed using this method per year,⁶ which accounts for approximately 5% of the world's 55 million ton fiber market (natural fibers ~20, cellulose ~3, synthetics 25, and others 7 million tons as reported in 2000).⁵

To put the cellulose resource in perspective, 100 billion tons of vegetation grow and decay annually on land, representing approximately 12% of the planet's total production of vegetation (the other 88% is produced in the oceans).⁷ If one considers the largest cellulose processing process, viscose, it only utilizes approximately one ten thousandth of the earth's annual production of land-based cellulose, or put another way man's annual cellulose requirements are produced by nature every 50 minutes.

Tremendous strides are currently being made towards the utilization of renewable resources by major chemical companies (e.g., Cargill-Dow) in the form of biorefineries. Interestingly, in a biorefinery, the complexity afforded by Mother Nature in natural polymers such as cellulose, is broken down into simple building blocks (e.g., ethanol, lactic acid) in order to build back up more complex polymers such as plastics. *However, if we can directly utilize the biocomplexity Mother Nature has provided with cellulose in the formation of new materials, we could eliminate many destructive and constructive synthetic steps.* A major road block in this latter approach is the lack of an inexpensive, recyclable solvent to directly dissolve cellulose and allow modification of its properties and solution routes to composite materials.

We have thus, concentrated on developing an ionic liquid (IL) solvent which could be used to readily dissolve cellulose without pretreatment or derivatization and at modest temperatures under mild conditions. ILs are a class of novel compounds composed of only ionized species - no neutral molecules - which have melting points (by definition) of 100 °C or less.⁸ One of the most important and unique properties of ILs, is their flexibility. ILs can be fine-tuned to design in certain physical or chemical properties. For example, the choice of dialkylimidazolium cations allows controlled modification of the solvent properties through alkyl substitution, while maintaining a number of desirable features, namely non-volatility, relatively high thermal stability, and wide liquidus range. While, ILs may or may not be 'green' unto themselves, it is hoped that some of the unique solvent properties of this class of materials may be utilized in 'greener' processing technologies.

The Nominated Technology: We have demonstrated that chloride ILs, in particular, 1-butyl-3-methylimidazolium chloride ($[\text{C}_4\text{mim}]\text{Cl}$) can be used as non-derivatizing solvents for the dissolution and (using water or other non-solvent) regeneration of cellulose into a variety of physical forms.⁹ We have also demonstrated the ready modification of cellulose's chemical and physical properties by the encapsulation of dissolved or suspended functional additives in the cellulosic solutions prior to regeneration.¹⁰⁻¹² This year, we have demonstrated the co-dissolution of other natural and synthetic polymers leading to advanced composites of controlled rheology.¹⁰ We have begun demonstrating methodologies to recycle the IL (which dissolves in water during the regeneration step) by a novel salting-out step or by common cation exchange techniques, both of which save energy compared to a totally evaporative procedure.¹³ Current work in our laboratories is aimed at improved, more efficient and economical syntheses of $[\text{C}_4\text{mim}]\text{Cl}$,¹⁴ studies of the IL toxicology,¹⁵ engineering process development, and commercialization. Scheme 1 provides the conceptual basis for the platform strategy.



Scheme 1. Dissolution of cellulose with a functional additive allows the cost efficient formation of advanced, tunable, biorenewable materials: a) SEM of fiber;¹⁶ b) beads impregnated with the Fe_2O_3 ;¹¹ c) 20/80 wt% cellulose/polyacrylonitrile blend;¹⁰ d) cellulose films containing entrapped laccase before (*left*) and after (*right*) contacting and incubation in aqueous syringaldazine solution;¹² e) cellulose sensors for mercury ions, orange before and deep-red after, a 1 min contact with a 10 ppm solution of HgCl_2 .¹⁷

Cellulose from virtually any source (fibrous, amorphous, pulp, cotton, bacterial, filter paper, etc.), can be readily dissolved without derivatization in the low melting IL $[\text{C}_4\text{mim}]\text{Cl}$ by gentle heating (especially using microwaves) to permit rapid dissolution.⁹ IL-dissolved cellulose can easily be reconstituted in water in controlled architectures (fibers, membranes, beads, flocs, etc.) using conventional extrusion spinning or forming techniques (conventional processing using the same approach taken by researchers investigating other advanced cellulose solvents such as NMMO for the Lyocell processes⁶).

If, at the cellulose-in-IL solution stage, functional additives are added to the solution before regeneration, then blended or composite materials can be prepared.¹⁰ The incorporated functional additives (ideally, themselves not readily soluble in the regenerating medium) can be either dissolved or dispersed in the IL medium before or after dissolution of the cellulose. The modified cellulosic materials can then be reconstituted in an identical manner to that described above, simple addition to a non-solvent diluent such as water. The resulting physical and chemical properties of the materials (and indeed their functionality and rheology) are thus, tunable based upon the identity and amount of additive.

This simple, non-covalent approach lends itself readily to the preparation of encapsulated cellulose composites. Entrapped materials have a wide number of uses, from controlled release systems to structural modifiers and sensor or reactive materials. For example, enzyme entrapment on solid supports is a well-established technique for improving stability and separations aspects in enzymatic transformations. Entrapment of enzymes on solid supports can result in improved stability to pH and temperature and aid in separation of the enzyme from the reaction mixture, and also for formation of enzyme electrodes for sensor applications. In another example, nanoparticles and other macroscopic

particles dispersed within the viscous IL-cellulose solution can result in nano-dispersed composite materials with increased strength and conductivity.

There are two primary compositing techniques available for use; (i) dissolution of two (or more) components allowing intimate blending and (ii) dispersion of additives (e.g., nanoparticles) as a way of introducing insoluble particles throughout the matrix. In the latter case, ILs appear to be very effective for maintaining dispersions of particulates, most likely because the polar, electrolytic nature of the IL reduces the tendency of nanoparticles to self aggregate.

Example of the Dissolution Route: Colorimetric indicators can be easily introduced into the cellulose-IL solution providing a rich and flexible route to forming, e.g., disposable metal-sensing composites. We have shown that PAN, an azo-dye, can be readily incorporated into cellulose films, and turns from orange to a deep-red in the presence of mercury ions in solution (Scheme 1, e).¹⁷

A key to full utilization of this approach, is that the ILs that dissolve cellulose are capable of dissolving many organic moieties that are water insoluble, thus, it is possible to introduce high loadings of hydrophobic additives into the cellulose. The hydrophilic nature of cellulose itself may also provide process advantages over hydrophobic, poorly wetting supports (e.g., polystyrene-divinylbenzene).

The flexibility of a dissolved cellulose media also allows the generation of many designed forms of the reconstituted product. Cellulose membranes (made from underivatized cellulose rather than cellulose acetate) for example, may be advantageous over other synthetic polymer membranes in applications which require high porosity for water, and low (in the dry state) gas porosities. This hydrophilicity, or wettability, can be very useful, for example, in providing fast transport of water-soluble ions (metals) to the active sensing sites.

Examples of the Dispersion Route: Introducing macroscopic or nano-scale particles as dispersions in the IL/cellulose systems results in homogeneous, dispersed particle composites. (Such composites are currently of major interest as ‘smart’ materials.) Utilizing a dissolution route rather than a melt processing route offers certain processing advantages, not the least of which is low temperature operation.

We have successfully encapsulated magnetite within a cellulosic matrix to produce homogenous magnetic composites (Scheme 1, b) by directly adding magnetite to cellulose containing IL solutions.¹¹ There appears to be a linear increase in magnetization with respect to magnetite loading, allowing one to make materials with specific magnetic properties. All materials produced exhibited uniform loading of magnetite, and there was little evidence of expanded aggregation from the SEM and AGM results. The use of ILs here offers some unique advantages: due to their inherent ionic nature, there is no need to utilize additives to combat charging/aggregation.

Cellulose is also an ideal material to support many enzyme systems, being both biosourced and biocompatible. Numerous examples of cellulose supported enzymes are known and utilized, most using cellulose-derivatives and covalent bonding via functional linkers to hold enzymes in place. Entrapment or encapsulation using other procedures, especially physically trapping the enzyme, without recourse to chemical attachment is certainly desirable.

We have successfully encapsulated laccase from *Rhus vernificera* (E.C. 1.10.3.2), within cellulose films (Scheme 1, d) and shown retained enzyme activity.¹² Laccase was chosen as a target enzyme because of its ready availability, penchant toward aromatic oxidation in the presence of celluloses, and possibility of measuring activity through colorimetric assays. The oxidation of reduced syringaldazine (colorless) to the oxidized purple form confirmed the encapsulated laccase activity.

The successful introduction of an active enzyme into a cellulose film using the IL processing route, also demonstrated other unique advantages of the IL methodology. The IL used could be supercooled without crystallizing, allowing low temperature processing which helped maintain enzyme activity. Activity could be further improved by pre-treatment of the enzyme with a hydrophobic IL coating providing a less denaturing microenvironment.

Polymer-Polymer Blends: We are now preparing IL solutions of other ‘difficult’ polymers using a correlation of the solubility parameters (Hansen and Hildebrand) for cellulose. These solubility parameters are useful in ensuring the suitability of polymers for dissolution in IL, but also in the formulation of polymer-polymer blends. Many common synthetic polymers such as poly(vinyl alcohol), poly(2-hydroxyethyl methacrylate), polyacrylonitrile, polyaniline (emeraldine base), polypropylene glycol, polyethylene glycol, and biopolymers such as bovine serum albumin, and deoxyribonucleic acid (DNA) salts were found to be soluble in [C₄mim]Cl.¹⁰

For demonstration, a series of cellulose/polyacrylonitrile blends was prepared by mixing solutions of cellulose and polyacrylonitrile in [C₄mim]Cl, and then forming composite films by casting, and regenerating with water. Homogeneous, miscible structures were formed across a range of blend compositions, and suppression of the characteristic porous structure of pure polyacrylonitrile, led to denser, tougher composites even when as little as 20% cellulose was added (Scheme 1, c).

Recycle and Cost of the IL: The recovery and reuse of the hydrophilic ILs could be a significant barrier to implementation, similar to the economic cost of dehydrating *N*-methylmorpholine-*N*-oxide prior to reuse in the Lyocell process.⁵ Dehydration could be accomplished directly by evaporation; however, this is an energy intensive step.

In one attempt to solve this problem, we have demonstrated that aqueous solutions of ILs, including [C₄mim]Cl can be ‘salted-out’ forming an aqueous biphasic system with aqueous solutions of common ‘water-structuring’ inorganic salts (e.g., K₃PO₄).¹³ This is a well known technology used commercially for concentration, and solidification of soaps and detergents from solution. Using readily available, and cheap K₃PO₄, [C₄mim]Cl can be dehydrated to produce relatively concentrated (80-90 wt%) solutions. The energy requirements for the final dehydration would thus be much lower when compared to methods used today. Tightly engineering such a process to recycle all salts is very important and other processing options, including simple cation exchange are being considered.

The cost and availability of the solvent system itself is also important. The technology to prepare the salt is simple alkylation chemistry performed routinely within the surfactant sector. We are currently developing lower cost (and greener!) synthetic routes to ILs,¹⁴ however, we note that BASF has recently announced it is registering and producing [C₄mim]Cl in ton-quantities!

Commercialization. The road from innovation to commercialization requires development beyond the innovative ideas that lead to patents. We have developed two strategies to ensure commercialization of the nominated technology. Both strategies involve initially conducting market research (which leads to viable materials needs) and the construction of business plans (needed to assess viability). Armed with this information we are in discussions with several major chemical companies to discuss jointly developing specific cellulose-based materials. Our second approach is to utilize a small business entity to seek SBIR/STTR funding for development of specific products. Thus, market research and business planning will be used to guide the development efforts where they will likely prove most successful.

It is worth noting that Green Chemistry principles can also guide the development work and product selection. While considerations of market size, market share, and economics of processing will need to be considered in selecting the most viable business model, it is also possible to direct efforts toward replacing those materials which are environmentally damaging. For example, thermoplastics account for 13% of the polymers used in the automotive industry; composites 36%. Advantages of incorporation of cellulosic fibers for these plastics applications include its low cost, flexibility, low weight, low abrasion, low toxicity, and biodegradability. Long fiber (e.g., cellulose) thermoplastic composites demonstrate fiber dominant property improvements across the board. Tensile, flexural, and impact properties are significantly elevated over analogous short fiber composites in a broad range of base resins.

In order to utilize new materials based on the nominated technology, there is a need to develop methods and data showing that long fiber cellulose composites from IL dissolution are better than wood flour compounding based composites. A key issue would be to develop methods and processes for the blending of cellulose with polypropylene and polyethylene. If successful, such an approach could lead to

a significant reduction in the utilization of these synthetic polymers and lead to composites which were more readily biodegradable. Utilization of the nominated technology in pursuit of such materials may thus lead to commercial technologies which reduce or eliminate the use or impact of synthetic polymers; and this is but one example.

Summary: We have successfully demonstrated a platform strategy to efficiently exploit the biocomplexity afforded us by Nature's renewable polymer - cellulose; thus perhaps reducing Society's dependence on non-renewable petroleum-based feedstocks for synthetic polymers. We have developed an apparently nontoxic solvent system for the efficient processing of cellulose which eliminates volatile emissions and negative processing (needed for derivatization or degradation of the cellulose) effects common with traditional techniques. We have shown that by carefully controlling the addition of functional additives (enabled by the same IL solvent system that dissolves the cellulose), the physical and chemical properties of the final materials can be easily tuned for specific applications, leading to a variety of smart advanced materials of controlled architecture, functionality, and rheology. We are currently in the process of market research and business planning leading to the development of the technology to the point of commercialization, either through joint development agreements with existing chemical companies or through the creation of small businesses. By combining a fundamental knowledge of ILs as solvents with a novel technology for the dissolution and reconstitution of cellulose and similar polymers, and using Green Chemistry principles to also guide process development and commercialization, it is envisioned that this platform strategy can lead to a variety of commercially-viable advanced materials which can obviate or reduce the use of synthetic polymers.

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Direct Synthesis of Hydrogen Peroxide by Selective Nanocatalyst Technology

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Project Title: Direct Synthesis of Hydrogen Peroxide by Selective Nanocatalyst Technology

Project Focus Areas: This technology fits in all three specified green chemistry focus areas.

Project Eligibility: This project is not eligible for the “academic” or “small business” categories.

Project Research Location: This project has been researched and the technology developed over the last six years by investigators at Headwaters Technology Innovation, in Lawrenceville, N.J., U.S.A.

Project Milestones:

- Sept. 28, 2004: Joint venture formed with chemical industry leader to pilot test the catalyst technology in industrial conditions.
- Mar. 9, 2005: Direct-synthesis of hydrogen peroxide (DSHP) technology successfully integrated into propylene oxide pilot plant, makes PO for the first time.
- Oct. 10, 2005: Joint venture extended to build full-scale demonstration plant.
- Oct. 9, 2006: Successful start-up of commercial demonstration plant.

Milestone Patents Granted for this Direct Synthesis Technology, 2000-2006:

US Patents 7,144,565 B2 (2006), 7,045,481 B1 (2006), 7,045,479 B2 (2006), 7,011,807 B2 (2006), 6,919,065 B2 (2005), 6,908,873 B2 (2005), 6,746,597 (2004), 6,740,615 (2004), 6,576,214 (2003), 6,534,661 (2003), 6,500,969 (2002), 6,500,968 (2002), 6,168,775 (2001)

Technical Abstract

Researchers in industry and academia have sought an inexpensive, commercially viable and environmentally benign method for synthesizing hydrogen peroxide (H₂O₂) directly from hydrogen and oxygen. A clean, versatile, environment-friendly oxidant, H₂O₂ can be substituted for environmentally deleterious chlorinated oxidants in manufacturing many products—but its relatively high market price has prevented it from being used as widely as it could be. The current H₂O₂ manufacturing process is complex, expensive and energy-intensive. It also uses of large amounts of hazardous and toxic chemicals and creates undesirable byproducts that must be kept out of the environment.

Several technical challenges have kept researchers from developing a safe direct-synthesis technology that can produce H₂O₂ in industrial settings over last 3 decades. Headwaters Technology Innovation developed a highly selective, robust nanocatalyst technology that has overcome these challenges through the adroit use of a palladium-platinum catalyst with proprietary molecular templates. Through the precise control of the crystal structure, size, composition, dispersion and stability of the catalytic nanoparticles, the technology achieves up to 100 percent reaction selectivity for H₂O₂. It requires no hazardous materials and produces no byproduct except water. This technology was successfully demonstrated at commercial scale in partnership with Degussa AG, a major H₂O₂ manufacturer. A full scale commercial plant is currently being designed. Construction of the plant is scheduled to begin in 2008. Degussa AG plans to have the first plant completed and online in 2009. This cutting-edge new direct synthesis of hydrogen peroxide process will disrupt the current 60-year old hydrogen peroxide manufacturing technology, due to the significant lower capital and operation cost, and environmental friendly advantage.

Direct Synthesis of Hydrogen Peroxide by Selective Nanocatalyst Technology

Executive Summary

Headwaters Technology Innovation (HTI) has produced a robust nanocatalyst technology that enables the synthesis of hydrogen peroxide (H_2O_2) directly from hydrogen and oxygen. This breakthrough technology, called NxCat, eliminates all hazardous reaction conditions and chemicals, along with the undesirable byproducts in the existing, costly and complex, H_2O_2 manufacturing process. It produces far greater amounts of H_2O_2 while cutting production energy consumption and costs. Innocuous, renewable feedstocks are used, and no toxic waste is generated.

By contrast, the current manufacturing process requires the use of an anthraquinone working solution containing several toxic chemicals. The working solution is reduced in the presence of a hydrogenation catalyst, forming anthrahydroquinone, which is then oxidized to release hydrogen peroxide in concentrations ranging from only 0.8 to 1.9 percent. The H_2O_2 is removed from the organic mixture with energy-intensive equipment and then concentrated by vacuum distillation. Although the bulk of the working solution is recycled, a fraction of it must be continually replaced.

Unlike other attempts to directly synthesize H_2O_2 , the NxCat catalyst technology enables a simple, commercially viable H_2O_2 manufacturing process that in 2005 was pilot tested in partnership with Degussa AG, a leading H_2O_2 manufacturer, and in 2006 was successfully demonstrated at industrial scale. The technology has the added benefit of producing an effective and environmentally harmless oxidant that, but for its historically higher price, would have been an excellent substitute for the more frequently used—and far more deleterious—chlorinated oxidants. By cutting the cost of H_2O_2 production through the reduction of investment by as much as 50 percent, this technology is expected to generate a more competitively priced supply of H_2O_2 , thereby hastening its acceptance as an environmentally preferred product in the private sector

NxCat works through the precise construction and control of the nanoparticles of a palladium-platinum catalyst. HTI has engineered a set of molecular templates that maintain control of the crystal structure, size, composition, dispersion and stability of catalyst nanoparticles. Each of these controls enhances the production rate and selectivity for H_2O_2 .

NxCat nanocatalyst technology, which enables a long-sought, safe and low-cost method for direct-synthesis H_2O_2 , is an excellent example of “green chemistry” that is expected to serve as a foundational technology supporting environmentally responsible chemical industry growth.

Introduction

Headwaters Technology Innovation (HTI) has produced a robust nanocatalyst technology that synthesizes hydrogen peroxide (H_2O_2) directly from hydrogen and oxygen. Unlike previous attempts to develop a direct route for synthesizing hydrogen peroxide, the new technology, called NxCat catalyst technology, enables a commercially viable H_2O_2 manufacturing process that is being demonstrated at commercial scale. By using a highly selective catalyst made of precisely controlled palladium and platinum nanoparticles HTI has solved longstanding technical problems in a process that has been the subject of decades of scientific investigation. Not only does the new technology eliminate all of the toxic and hazardous feedstocks and byproducts of the standard industrial process,

but it can significantly cut the production costs of the environment-friendly oxidant, making it a competitive alternative to chlorinated oxidants. Another important feature is its adaptability as a new low-cost process technology that has never before been commercially viable. Its only byproduct is water.

The discussion that follows explains why NxCat is a vital addition to a growing palette of green chemistry technologies, embodying the “12 Principles of Green Chemistry” established by the EPA Office of Pollution Prevention and Toxics.¹ As an adroitly managed catalyst technology, NxCat has major performance and economic advantages over current technology and demonstrates that environmentally sound manufacturing practices can be commercially competitive. It can also be applied in other chemical manufacturing processes to lessen their environmental impacts, as will be described.

The Environmental and Economic Significance of Direct Synthesis of Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a clean and versatile oxidant with many industrial uses and the potential for many more. SRI Consulting (Menlo Park, CA) projects that global demand for the oxidant in 2006 will total 1.77 million metric tons, of which 60 percent will be used as a bleaching agent in the pulp and paper industry, 22 percent in manufacturing chemicals and laundry products and the remaining 18 percent divided among environmental, textile, mining and other applications.² The demand for H_2O_2 has grown in recent years but its high production cost makes it more expensive than chlorinated oxidants, which, though cheaper, have many more deleterious environmental impacts. The standard H_2O_2 production process also requires the continual use and recycling of an anthraquinone working solution made of hazardous organic compounds, and generates a waste stream of undesirable quinone-derived byproducts that manufacturers must keep out of the environment.

For both economic and environmental reasons, researchers in industry and academia have for decades sought a safer, cheaper, more environmentally sound method for generating hydrogen peroxide directly from hydrogen and oxygen. Not only could a direct synthesis process lower the H_2O_2 market price to a level competitive with environmentally deleterious chlorinated oxidants, it could eliminate the environmental hazards and byproducts associated with current H_2O_2 production. But until now researchers have been unable to produce a commercially feasible direct synthesis process. A brief patent search in 1998 in support of a research grant proposal found more than 70 patent awards for direct-synthesis technologies over the previous 20 years. Several were granted to major companies, including Atochem, Air Products, Dow Chemical, DuPont, Mitsubishi and Solvay, indicating how intensively the industry has pursued a viable direct synthesis production method. Those methods failed to commercialize due to their low selectivity, low production rates and their need to work with hydrogen gas phase concentrations within the hazardous, explosive range.

In 2003 The Rand Corporation also performed a study of industrial acceptance of green chemistry technologies that compared three direct-synthesis H_2O_2 manufacturing processes, with a description of the issues that prevented them from becoming commercially viable. The authors noted: “There is a fundamental trade-off in direct synthesis between catalyst reactivity and mass transfer concerns. Formulations involving gas phase contact of H_2 and O_2 must address safety concerns, particularly in an industrial environment.” As an example, the report described a direct synthesis route using CO_2 as a solvent (a previous Presidential Green Chemistry Challenge Award winner), which failed to gain

commercial acceptance for lack of a strong catalyst to make the process work at industrial scale.³

Unsuccessful attempts such as these mean that H₂O₂ worldwide is still produced using the environmentally wasteful method described below. Most would agree it should and could be improved.

Chemistry of the Anthraquinone Process

The standard industrial manufacturing method uses a hydrogen carrier of anthraquinone, which is dissolved in an organic solvent, becoming what is termed “the working solution.” It is reduced to anthrahydroquinone in the presence of a hydrogenation catalyst. The anthrahydroquinone in the working solution is oxidized, releasing hydrogen peroxide and regenerating anthraquinone. The hydrogen peroxide, which is stripped from the organic mixture, is mixed into water and purified, and then concentrated by vacuum distillation. In theory the process recycles the working solution in a closed loop, but in practice the hydrogenation cannot be controlled precisely enough to prevent the over-hydrogenation of a fraction of the organic working solution, which must be regularly replaced.

Disadvantages of the Anthraquinone Process

The anthraquinone working solution contains several chemicals that EPA has identified as toxic, hazardous or even carcinogenic.⁴ The four components of the working solution include: (1) anthraquinone; (2) a nonpolar solvent; (3) a polar solvent; and (4) unwanted but unavoidable quinone byproducts. The nonpolar solvent generally consists of benzene, mixed alkyl naphthalenes and mixed or single alkyl benzenes. The polar solvent contains some combination of trioctylphosphate, disobutyl carbinol, and methylcyclohexyl acetate or tetra-alkyl urea.

Hydrogen peroxide manufacturers must use extremely large amounts of these chemicals to achieve a workable rate of production because hydrogen peroxide forms in the working solution at a concentration range of only 0.8 to 1.9 percent. The working solution must also be distilled each time it is recycled, and fresh working solution must be continually added to replace lost and degraded working solution. HP production reached 514,000 tons in US in 2005, and global production reached 3,586,000 tons². Even at the high end 1.9% concentration of HP in the working solution and assuming the residence time is one hour, the amount of hazardous and carcinogenic working solution needed is estimated at 3000 and 21,500 tons for US and global HP production, respectively. The anthraquinone process also consumes a great deal of energy by using reduction and oxidation reactors, a stripping column and a distillation train. The overall process of reduction, oxidation, extraction and purification also makes for high equipment and operating costs.

Today’s H₂O₂ manufacturers have effectively managed the anthraquinone process, but at least three plants have been destroyed and other significant incidents have occurred in the past. That is because under many conditions H₂O₂ decomposes to oxygen and water, releasing heat and pure oxygen while building up pressure. In combination with the great quantities of flammable solvent in the working solution, “these characteristics...must be recognized as potentially hazardous at all stages of design and operation of a [hydrogen peroxide manufacturing] process.”⁵

NxCat: A Commercially Viable Green Chemistry Solution

Advantages of NxCat Technology

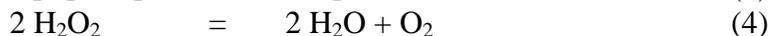
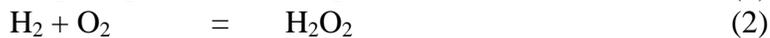
The HTI nanocatalyst technology lays out a safe alternative, a “green” pathway for producing hydrogen peroxide. By replacing an industrial process requiring the continual use of large amounts of hazardous chemical reagents with a catalyzed, direct synthesis route that uses only innocuous, renewable feedstocks of hydrogen and oxygen. By providing alternative reaction conditions it reduces the risk of flammability and explosion of conventional production methods while using an aqueous or organic solvent with minimal human health and environmental impacts. Through precise nanoparticle control the catalytic method allows for a far greater range of reaction selectivity without using energy-intensive equipment. NxCat also completely eliminates the toxic quinone-derived byproducts of the conventional process. The only possible byproduct is water.

This technology is unique in comparison with other methods of direct H₂O₂ synthesis because of the robust action of the nanocatalyst technology, which allows the use of hydrogen gas in concentrations below the 4 percent range, preventing the development of hazardous explosive reaction conditions and making the process safe for large commercial scale production.

Finally, NxCat technology delivers the bonus of producing, safely and at lower cost, large amounts of hydrogen peroxide, a clean, safe oxidant that is expected to replace chlorinated oxidants in many industrial processes. By making available a much simpler manufacturing process, this technology can produce a competitively priced supply of hydrogen peroxide that could speed its inclusion in industrial processes that now use more environmentally deleterious chemicals.

Chemistry of the Catalytic Process

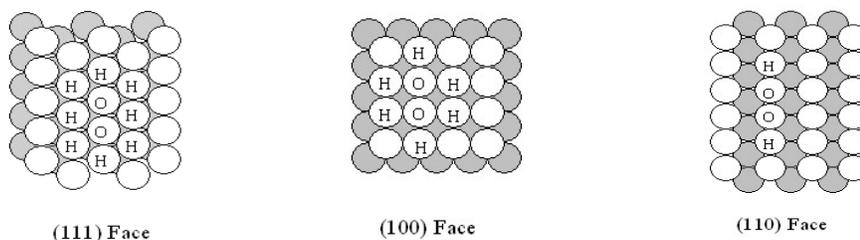
When hydrogen encounters oxygen, the thermodynamically favored reaction product is water (equation 1). Hydrogen peroxide (H₂O₂) is only an intermediate (equations 2 and 3), because hydrogen peroxide is not stable and releases water and oxygen when it decomposes (equation 4).



HTI addressed this challenge by developing a set of proprietary molecular templates that achieve an unprecedented level of control of the catalytic nanoparticles, thereby eliminating unwanted reactions and increasing selectivity for H₂O₂ to up to 100 percent. The molecular templates are usually made of organic molecules or polymers with many functional groups that have specific effects on the catalyst, which is a precisely tailored combination of palladium (Pd) and platinum (Pt). The technology offers control of activity and selectivity by managing nanoparticle crystal structure, size, composition, dispersion and stability (anchoring), as outlined below.

Crystal Structure Control: In catalysis Pd/Pt surface crystal structure plays a pivotal role in control of selectivity for reactions between H₂ and O₂. The schematic diagrams below represent three faces of Pd/Pt crystal. When O₂ adsorbs on face-110, hydrogen atoms can only adsorb on two adjacent Pd/Pt atoms. The only product from reaction on such crystal structure is H₂O₂. However, O₂ adsorbed on face-100 or face-111 can be attacked by 6 or 8 hydrogen atoms adsorbed on the

adjacent Pd/Pt atoms, respectively, increasing tremendously the chance of water formation. Thus the reaction selectivity for H_2O_2 is greatly enhanced if the Pd/Pt in the catalyst retains the face-110 crystal structure.



Under the auspices of a 1998 National Science Foundation Small Business Innovation Research grant (#DM 97760555), HTI, which was then called Hydrocarbon Technologies, Inc., conducted pioneering research that uncovered a relationship between the growth rate of Pd/Pt crystal face and the number of molecular template polyacrylates surrounding each Pd/Pt ion. That allowed the researchers to establish the (proprietary) ideal ratio of the template polymer to Pd/Pt. The resulting face-controlled catalyst selectivity for H_2O_2 improved to about 100 percent, significantly greater than the best results (74 percent) patented by an industry leader using catalysts without controlled Pd/Pt exposition under similar reaction conditions.⁶

Particle Size Control: Uniform, small nanoparticle size is also critical in augmenting the production rate of H_2O_2 . Catalysts developed by other companies contain a majority of Pd/Pt nanoparticles at sizes larger than 20 nm—but HTI's NxCat technology keeps the Pd/Pt particle size at about 4 nm. This enables each gram of HTI catalyst to be an order of magnitude more active, producing 2,400 grams/hour of H_2O_2 as compared with the average of 250-300 grams/hour H_2O_2 in similar conditions with other catalysts. In addition, HTI researchers observed that a 4-nm particle catalyst produced hydrogen peroxide—but a catalyst containing 2-nm particles created water. In a proprietary process, HTI engineered molecular templates of specific size and concentration that allow the template molecules to wrap around the metal atoms of the catalyst, thus keeping the nanoparticles at the desired size.

Particle Composition Control: A careful selection of the functional group of the molecular template enables HTI to control the nanoparticle composition, thus minimizing unwanted reactions. For example, to fabricate a catalyst with specific proportions of Pd and Pt HTI selects a template containing two functional groups, one with an affinity to Pd and the other with Pt. By precisely adjusting the ratio of the two selected groups the research team achieved the exact formulation of the catalyst nanoparticle composition.

Particle Dispersion Control: An even dispersion of catalyst particles is one of the most difficult conditions to achieve because metal nanoparticles have a strong tendency to clump together, minimizing the exposure of particle surface area, thereby weakening the catalyzing effect. HTI overcame this challenge by engineering molecular templates with functional groups that bond closely with the metal atoms, counteracting the tendency of the particles to agglomerate. Thus stabilized, nanoparticles remain evenly dispersed while exposing a large surface area, retaining their powerful reactivity.

Particle Stability Control: Catalytic processes usually involve the confinement of the active

component of the catalyst to a substrate by physical force, such as by Van de Waals force. But the NxCat approach is to use a molecular template to bind the Pd nanoparticles to a substrate of carbon black, silica or other catalyst supports. The chemical bond is much stronger than physical force and more firmly fixes the nanoparticles to the substrate surface, stabilizing the catalyst so it can withstand severe reaction conditions and endure for years in commercial applications. A test of more than 3,000 hours in 2005 confirmed that the catalyst remained stable even in extremely industrial harsh conditions, such as in the presence of sulfuric acid and hydrogen peroxide. The resulting catalyst has an expected industrial lifespan of more than three years.

Demonstrating Commercial Viability

HTI has also taken a step unprecedented in the development of catalytic direct-synthesis processes by partnering with an industry leader to test the process at industrial scale. In 2005, with German manufacturer Degussa AG, HTI completed the milestone of pilot testing the technology in industrial conditions. In 2006 HTI and Degussa AG jointly performed a full-scale technology demonstration—and in 2007 HTI and Degussa AG will start the design of commercial production plant. The new environmentally friendly process has been marketed worldwide. Construction of first commercial plant with 200,000 ton is scheduled for 2008. Several other major manufacturers have expressed interest. It was forecasted that from 2010, every other year a new 200,000 ton plant will be built. The DSHP process is the preferred cutting-edge technology due to its environmental friendly advantage and low cost.

Work is already underway to adapt the flexible catalytic technology to other industrial manufacturing processes. HTI partner Degussa AG has recruited another industry partner to adapt the NxCat technology to the manufacture of propylene oxide. Worldwide, about 55 percent of propylene oxide is produced using a chlorohydrin process that generates large amounts of aqueous waste.⁷ The direct synthesis of H₂O₂ has the potential to eliminate that waste stream, and also reduce plant capital cost by 50 percent and product cost by 20 percent.

NxCat can also be used in the production of various other large-volume chemical products to potentially reduce manufacturing cost and achieve fewer deleterious environmental impacts, including: caprolactam, epichlorohydrin, adipic acid and phenol. HTI long-term plans include applying it in the fabrication of chemicals, energy, environment, pharmaceuticals and other products. The potential range of environmental and economic benefits of this breakthrough technology can as yet scarcely be described.

1. EPA Office of Pollution Prevention and Toxics, "12 Principles of Green Chemistry," <http://www.epa.gov/greenchemistry/principles.html>.

2. Cited by *Chemical Week*, Aug 17, 2005, "Product Focus: Hydrogen Peroxide", p. 36.

3. Robert Lempert, Parry Norling, Christopher Pemin, Susan A. Resetar, Sergej Mahnovski, *Next Generation Environmental Technologies: Benefits and Barriers*: "Production of Hydrogen Peroxide (H₂O₂) Directly from Hydrogen and Oxygen" (Rand Corp., 2003 monograph) pp. A-57-62. Available at: www.rand.org/pubs/monograph_reports/MR1682/ (as of Dec. 29, 2005).

4. Benzene, naphthalene, hydroquinone and quinone all appear on the Environmental Protection Agency EMCI Chemical References Web Pages, a list of substances monitored by EPA's Major Program Systems (Air, Water, Hazardous Waste, Superfund and Toxics Release Inventory). Available at: www.epa.gov/enviro/html/emci/chemref/ (as of Dec. 29, 2005).

5. J.R. Kirchner, *Encyclopedia of Chemical Technology*, 1981, "Hydrogen Peroxide," p. 41-42.

6. L. W. Grosser and J.-A. T. Schwartz. U.S. 4832938 (1989) DuPont de Nemours.

7. Lempert et al., "Environmental Technologies."

Environmentally Benign Medical Sterilization Using Supercritical CO₂

*A Presidential Green Chemistry Award Nomination
December 19th, 2006*

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Small Business Grants: National Institutes of Health
National Science Foundation
Other Investors: KensaGroup LLC
David Burns

Project Title: **Environmentally Benign Medical Sterilization Using Supercritical CO₂**

Recent Milestones:

2000: NovaSterilis (NS) was founded. The Company licensed novel supercritical CO₂ (SCD) sterilization technology for biodegradable polymers developed at MIT [USP 6,149,864; see also Dillow, A., Dehghani, F., Hrkach, J., Foster, N., Langer, R. *Proc. Nat. Acad. Sci.* **1999**, *96*, 10344-48]. In a validation study, NS established that the MIT technology successfully killed vegetative bacterial cells.

2001: NS obtained Phase I SBIR Grant (\$100K) to improve the technology and extend it to human musculoskeletal and allograft tissue. A collaboration with Univ. of Utah scientists established compatibility of SCD sterilization with synthetic skin (hyaluronic acid matrix containing EGF). David Burns was hired as CEO, and the first tissue bank collaboration was formalized.

2002-3: An improved SCD sterilization process (proprietary additives, cycle and pressure optimization) led to the successful sterilization (6 log reduction in bioburden) of bacterial endospores. A scaled-up 20 L sterilization vessel was manufactured. A key collaboration with DuPont established that SCDS worked on existing, commercially available medical packaging (Tyvek), thus making possible “terminal” sterilization of the final packaged product. NS awarded “Best Research Presentation in Show” at the 2003 Annual American Association of Tissue Banks National Meeting.

2004: NS partnered with (a) six tissue banks to replace gamma radiation and chemical disinfectant washes with SCD for sterilizing musculoskeletal tissue, (b) three major pharma companies to replace ethylene oxide with SCD for sterilizing biodegradable polymer and small molecule drugs, and (c) one biotech company to sterilize biologics. NS obtained approval opinion from FDA for SCD use in tissue sterilization.

2005-6: NS achieved successful sterilization of tendon and bone allograft tissue as well as whole-cell vaccines. NIH SBIR Phase 2 grant awarded (\$933K). Key NS patent issued [USP 7,108,832]. NS launched the commercial Nova 2200™ System and sold four units into the US tissue bank market.

Eligibility: The nominated technology is eligible for the **Small Business Award**.

Focus Areas: The nominated technology falls within Focus Areas 2 (replacing hazardous gamma radiation and ethylene oxide) and 3 (less toxic than the current sterilization process).

Abstract: NovaSterilis has developed SCD sterilization to replace traditional ethylene oxide or gamma radiation sterilants for the terminal sterilization of musculoskeletal allograft tissue, biological materials, medical devices, and small molecule drugs. SCD treatment can also replace the toxic preservatives used in preparing sterile vaccines. Both ethylene oxide and gamma radiation raise toxicity or environmental safety concerns. Ethylene oxide is a mutagenic, carcinogenic, volatile, flammable, readily polymerized gas, residues of which remain in the sterilized material, thus increasing the risk of hemolysis and other toxic side effects. Gamma radiation is highly penetrating and is lethal to all cells, thus causing radiation injuries. Ethylene oxide and gamma radiation sterilization methods account for the majority of the sterilization market, but neither technique can provide full terminal sterilization without eroding the physical integrity of the tissue or material being sterilized.

*EXECUTIVE SUMMARY***Environmentally Benign Medical Sterilization Using Supercritical CO₂**

The need for an effective, reliable, terminal sterilization process for the tissue bank industry became a national issue in 2002 when an otherwise healthy 23-year-old student died from a contaminated knee ligament transplant [Kainer, M. A. et al. "Clostridium Infections Associated with Musculoskeletal-Tissue Allografts," *N. Eng. J. Med.* **2004**, 350, 2564]. Subsequently, several other serious infections and illnesses were traced back to tissue banks that distributed contaminated donor tissue, including a November, 2006 nationwide incident at the world's largest tissue bank, Musculoskeletal Transplant Foundation of Edison, N.J [November 22, 2006 Associated Press Article: "Patient Got Tainted Cadaver Tissue"].

NovaSterilis (NS), a privately held biotechnology company in Ithaca NY, has successfully developed and commercialized a highly effective and environmentally benign technique for sterilizing musculoskeletal allograft tissue using supercritical CO₂ (SCD). Licensing a patent [USP 6,149,864 (2000)] issued to Professor Robert S. Langer and his team at M.I.T. for bacterial inactivation in biodegradable polymers, NS enhanced and optimized the technology to kill bacterial endospores in a commercially viable process. NS's patented technology [USP 7,108,832 (2006)] addresses the unmet market need in tissue banks as well as other needs in the biomedical, biologics, medical device, pharmaceutical and vaccine industries. In mid-2006 NS introduced the Company's first product, the Nova 2200™ sterilizer. NS has now sold four units into the tissue bank market.

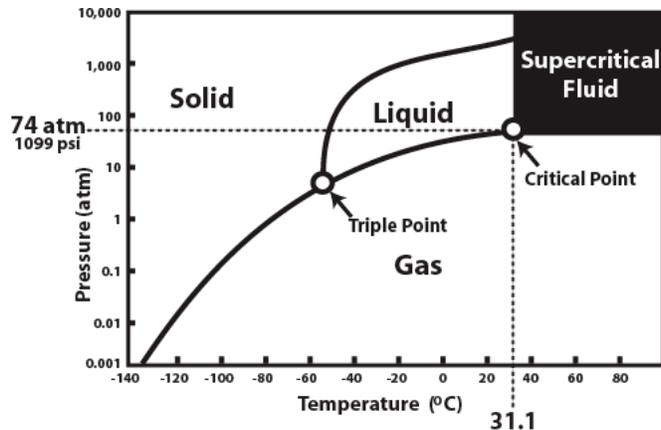
The SCD technology developed by NS uses low temperatures and moderate pressure, and is highly cost-effective. Unlike other sterilants such as gamma radiation and ethylene oxide, SCD sterilization is environmentally benign. Both ethylene oxide and gamma radiation raise toxicity or environmental safety concerns. Ethylene oxide is a mutagenic, carcinogenic, volatile, flammable, readily polymerized gas. Ethylene oxide residues remain in the sterilized material, thus increasing the risk of hemolysis and other toxic side effects. Gamma radiation is highly penetrating and is lethal to all cells, thus causing radiation damage. Ethylene oxide and gamma radiation sterilization methods account for the majority of the sterilization market, but neither technique can provide full terminal sterilization without eroding the physical integrity of musculoskeletal tissue or other sensitive materials requiring sterilization.

Besides being "green," SCD sterilization achieves "terminal" sterilization, i.e. sterilization of the final packaged product, which provides greater assurance of sterility than traditional methods of aseptic processing. The method is also perfectly compatible with sensitive biological materials. NS's technology consistently achieves rapid (less than one hour) and total inactivation of a wide range of microbes, including bacterial endospores. The new technology is effective for a wide range of important biomedical materials, including (a) musculoskeletal tissue (e.g. human bone, tendons, dermis, heart valves) for transplantation, (b) biodegradable polymers and related materials used in medical devices, instruments and drugs, (c) drug delivery systems, and (d) whole-cell vaccines that retain high antigenicity.

Introduction: The Challenge of Supercritical CO₂ (SCD) Medical Sterilization

Carbon dioxide has unique properties that make it a useful and practical reagent for sterilization. At relatively low temperatures and pressures (31.1°C and 1099 psi) carbon dioxide achieves the supercritical state of a fluid (Fig. 1). By combining the permeability of a gas with the flow density of a liquid, SCD fluid can penetrate and permeate deeply into materials, with applications ranging from bioremediation to natural products extraction. Moreover, SCD is non-toxic and can easily be removed by simple depressurization and outgassing.

Figure 1: Phase diagram of carbon dioxide.



For those reasons, SCD has long been considered a potentially attractive sterilization agent.¹ However, the development of such methodology has been hampered by the requirement for a sterility assurance level (SAL) of 10^{-6} . That level is defined as the probability (one in 10^6) that a product containing an initial bioburden of $\geq 10^6$ colony forming units of a bioindicator will be contaminated following the sterilization protocol.² The customary sterilization bioindicators are the sporular forms of various bacteria, which are high resistant to killing.

None of the SCD technologies reported to date is capable of inactivating bacterial spores; at best they achieve some level of disinfection.¹ Most spore-inactivating methods require high temperatures and aqueous environments, which are incompatible with delicate biological materials such as musculoskeletal tissue.

The issue of product packaging has also hampered the development of a successful sterilization technology. Terminal sterilization (i.e. sterilization of the final packaged product) provides greater assurance of sterility than aseptic processing, and can reduce various manufacturing and sterilization costs.

Thus, for SCD sterilization to become a commercially viable, environmentally benign sterilization method, it must consistently and rapidly inactivate bacterial endospores under terminal sterilization conditions without eroding the integrity of the sterilized material. NS has met that challenge by developing the necessary reagents and process chemistry to achieve validated SAL 10^{-6} levels of terminal sterilization under mild and gentle conditions that are compatible with a wide range of delicate biological materials.

The Chemistry and Mechanism of Supercritical CO₂ Sterilization

NovaSterilis has developed an effective and environmentally benign method for replacing ethylene oxide and gamma irradiation with a SCD-based process that relies on pressure cycling and a peroxide-based additive to achieve SAL 10^{-6} levels of terminal sterilization. This

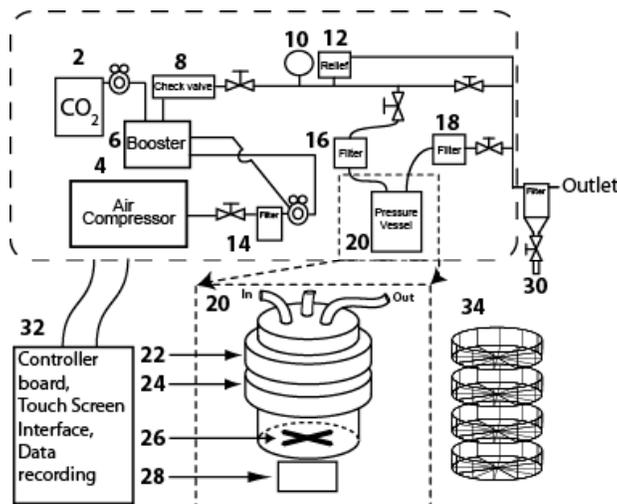
¹ Spilimbergo, S.; Bertucco, A. *Biotechnol. Bioeng.* **2003**, *84*, 627-38.

² Guidance on Premarket Notification [510 (k)] Submissions for Sterilizers Intended for Use in Health Care Facilities. Infect Control Devices Branch, Division of General and Restorative Devices. Food and Drug Administration. 1993.

breakthrough discovery relied on several key chemical and mechanistic findings which merit recognition in the form of a Small Business Green Chemistry Award.

Initial research using a 600 mL version of the current 20 L vessel standard in the Nova 2200 commercial system (Fig. 2) demonstrated that vegetative cells of various bacterial species, including *E. coli* and *S. typhimurium* were readily inactivated by SCD. However, commercially available spore preparations of *B. subtilis* or *B. stearothermophilus* endospores were refractory to SCD, even after exposure for 72 h. Endospores were also resistant to pressure cycling (>30 cycles, 3000–1500 psi), which dramatically accelerated inactivation of vegetative cells. Addition of water, which has been shown to facilitate inactivation of microbes with SCD,³ failed to inactivate endospores.

Figure 2: Schematic diagram of supercritical CO₂ sterilization apparatus.



Several medically approved disinfectants and oxidants were screened to identify additives that could successfully inactivate *B. stearothermophilus* endospores. The most promising of these additives was peracetic acid (PAA), which is non-toxic and has a long history of usefulness and safety in disinfection. When used in conjunction with pressure cycling, the combination of SCD with PAA in the presence of low concentrations of water caused significant log reductions of the standard panel of endospore bioindicators. Under aqueous conditions, PAA is in equilibrium with acetic acid and hydrogen peroxide (Eqn 1).

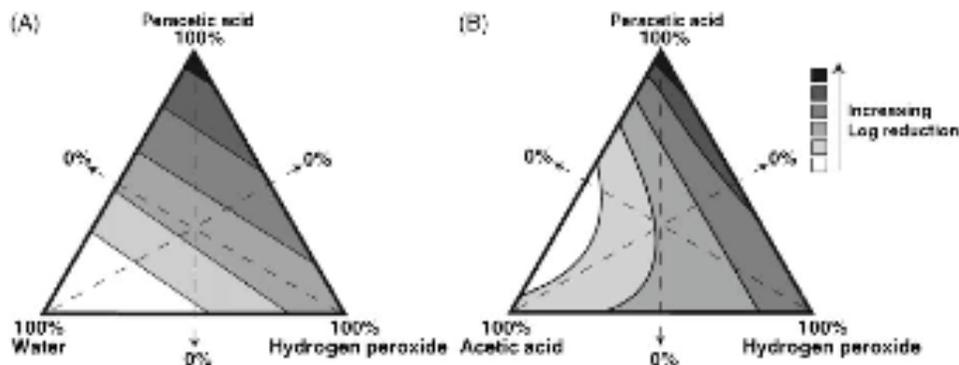


Control experiments using acetic acid and H₂O₂ alone established that the active form of the additive in the SCD process was most likely PAA, and that H₂O played a different role in sterilization (*vide infra*). Mixture analysis led to contour plots of inactivation of *B. stearothermophilus* endospores (Fig. 3), indicating that PAA (not water or acetic acid) promoted endospore inactivation. Hydrogen peroxide did exhibit some sporicidal activity; however, the greatest log reductions in spore counts were associated with higher PAA concentrations.

Additional control experiments in which SCD was replaced with pressurized air confirmed that SCD was essential to the sporicidal activity of PAA, and not simply causing an inadvertent pressure effect. Sterilization runs using PAA in combination with SCD revealed an

³ Dillow, A. K. et al., *Proc. Nat. Acad. Sci.* **1999**, 96, 10344-48.

Figure 3: Mixture analysis of PAA-based additives plotting relative log reductions of colony-forming units for *B. stearothermophilus* spore suspensions. (A) Contour plot of PAA vs H₂O₂ vs water; (B) Contour plot of PAA vs H₂O₂ vs acetic acid.



unexpectedly high (and nonobvious) level of synergy, with SCD-PAA being 10^4 -fold more effective than pressurized air-PAA, and 5-fold more effective than H₂O₂ in SCD.

It was also important to establish that the observed reduction in endospore activity was caused by spore inactivation and not merely inhibition of growth (i.e. bacteriostasis) caused by inadvertent introduction of an inhibitor during culturing.⁴ If bacteriostasis were caused by an inhibitor, then inoculating a treated sample of bioindicator with a low titer of bacteria should show no observable growth, whereas sterilized samples would show renewed bacterial growth after reinoculation. In fact, when samples culturing negative for growth after SCD sterilization were inoculated with 10–100 colony forming units of *B. subtilis* spores, bacterial growth was observed within 48 h, thus confirming spore inactivation, not bacteriostasis.

To determine whether bacterial endospores could be inactivated in a terminal sterilization protocol, test strips of appropriate bioindicator spores were sealed inside double (pouch within a pouch) Tyvek bags and subjected to the optimized SCD-PAA protocol. The double bagging procedure was designed so that tissue banks could ship terminally sterilized musculoskeletal tissue in packages that could be opened in operating rooms by surgical teams immediately prior to use. In fact, terminal SAL 10^{-6} sterilization was reproducibly achieved in double Tyvek bags using the 20 L Nova 2200TM sterilizer. Subsequent runs using bone, tendon and other musculoskeletal tissue confirmed that SCD sterilization was compatible with all existing industry-standard packaging protocols for terminal sterilization.

The mechanism of bacterial inactivation by SCD is still not well-understood, although several possible explanations have been suggested.¹ Plausible mechanisms include (i) cell rupture or lysis, (ii) modification of the bacterial membrane, (iii) extraction or inactivation of essential enzymes and/or intracellular substances, and (iv) acidification by carbonic acid formed *in situ*.

Earlier studies on vegetative cells ruled out the lysis hypothesis: microscopic analysis established that the bacteria remain intact.^{3,5} Since the NovaSterilis process involved substantial additional turbulence caused by vigorous stirring and pressure cycling in the presence of additives, the stability of bacterial cells was reinvestigated. Wet pellets of *S. typhimurium* harvested during log

⁴ Berube, R.; Oxborrow, G. S. **1991**, Methods of Testing Sanitizers and Bacteriostatic Substances. Disinfection, Sterilization and Preservation, 4th ed. Lea & Febiger, Philadelphia, PA, pp. 1058–1068.

⁵ Hong, S.I.; Pyun, Y.R. *J. Food Sci.* **1999**, *64*, 728-33.

phase ($>10^{12}$ colony forming units) were subjected to SCD-PAA. Scanning electron microscopy (Fig. 4) showed that inactivated *Salmonella* remained intact. Thin section transmission electron microscopy (Fig. 5) was used to visualize the interior ultrastructure of the inactivated *S. typhimurium*. Comparisons with untreated cells revealed few differences, except that lipid bilayers in the inactivated group appear to be ‘roughened’ compared to the control. Furthermore, the internal structures of the inactivated cells appeared less distinct than in the untreated group. Similar observations have been reported for SCD-inactivated *L. plantarum*.⁵

Figure 4: Scanning electron micrographs of *S. typhimurium*, untreated (A-C) and inactivated (D-F).

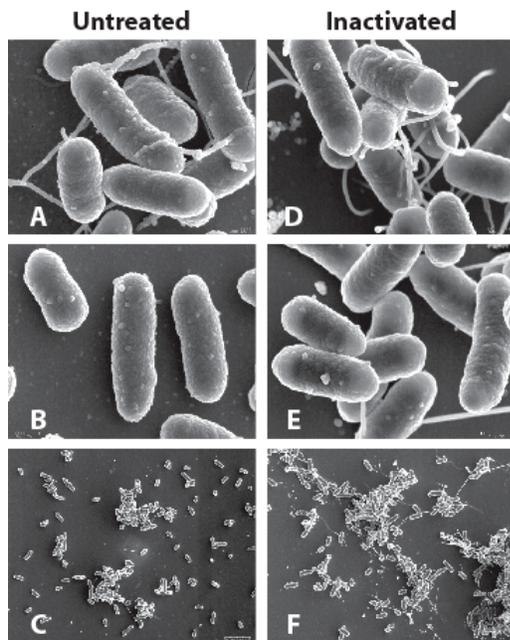
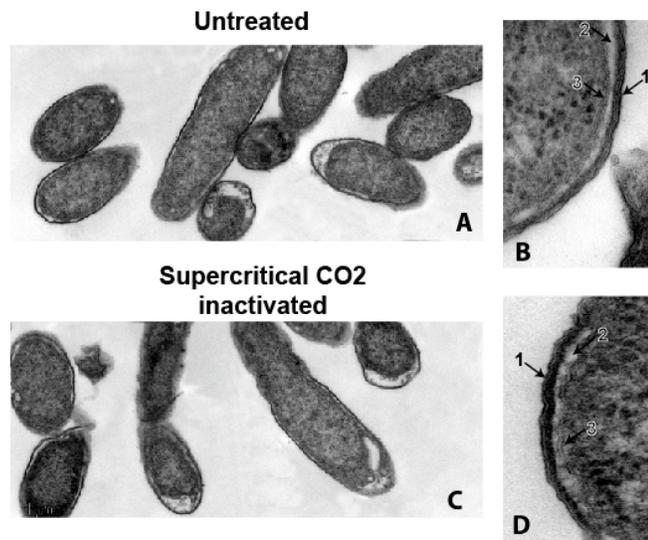


Figure 5: Transmission electron micrographs of *S. typhimurium* treated as in Figure 4. Untreated (A,B) and inactivated (C,D). Higher magnifications (B,D; arrows) reveal few differences in cell wall (1), periplasmic space (2) and lipid bilayers (3)



SCD may inactivate bacteria by disrupting the lipid bilayer in what is referred to as an ‘anaesthesia’ effect.⁶ According to this hypothesis, mass transfer of CO₂ irreversibly increases membrane fluidity and permeability, perhaps by extracting or denaturing essential membrane proteins.

To investigate whether key enzymes or cellular constituents were inactivated or extracted during SCD sterilization, pellets of SCD-inactivated *S. typhimurium* were lysed and analyzed by one-dimensional SDS-PAGE. Both untreated and inactivated cells displayed similar band patterns, suggesting that no wholesale degradation of proteins had occurred. A higher resolution examination using two-dimensional electrophoresis also revealed no appreciable degradation of proteins.

The possible involvement of carbonic acid in SCD sterilization is suggested by several findings. We and others¹ have observed that small amounts of water dramatically improve SCD sterilization, perhaps by enhancing cell wall permeability to SCD. Pressure cycling and

⁶ Isenschmid, A.; Marison, I.W. et al, *J. Biotechnol.* **1995**, *39*, 229–237.

agitation, which are key to effective sterilization, would also be expected to enhance mass transfer of CO₂ into bacteria where it can form carbonic acid by the reaction of CO₂ with water (Eqn 2). The resulting inactivation may be caused by transient acidification of the interior of the cell or by inhibition/denaturation of essential enzymes.



The Problem Addressed by Supercritical CO₂ Sterilization

Of the five widely used methods for medical sterilization (ethylene oxide, gamma radiation, electron beam, steam, and hydrogen peroxide plasma) none is well-suited for sterilizing delicate biological materials. Moreover, the most widely used sterilants (ethylene oxide and gamma radiation, which together account for over 90% of the market) have serious toxic and/or environmental shortcomings.⁷ Ethylene oxide is a mutagenic, carcinogenic, volatile, flammable, readily polymerized gas, residues of which remain in the sterilized material, thus increasing the risk of hemolysis and other toxic side effects. Gamma radiation is highly penetrating and is lethal to all cells, thus causing radiation damage. Both ethylene oxide and gamma radiation alter polymer structures, causing changes in molecular weight, shear and tensile strength, elastic modulus and transparency.^{8,9,10,11} By contrast, carbon dioxide is an environmentally unreactive, nontoxic substance that is compatible with delicate biomaterials.

An independent report recently published on the Web analyzed and compared the cost, performance and overall feasibility of various sterilization methods, including SCD, for medical devices.¹² Table 1 below (reproduced from ref. 12) presents a cost comparison on a ‘per-cubic-foot’ basis. The data were acquired from a major hospital facility, and costs were calculated using various assumptions and data presented in the report. The increased costs for ethylene oxide sterilization include the long cycle time involving outgassing, risk-management-related costs, insurance, safety monitoring equipment, and legal liability. The report concludes that SCD sterilization is a viable option to established sterilization protocols, both economically and logistically, for medical device manufacturers.

Table 1: Cost analysis and comparison of four sterilization methods

Cost (USD)	Steam	Ethylene Oxide	H ₂ O ₂	SCD
Machine	70,000	40,000	123,000	170,000
Maintenance	1,100	2,500	8,300	8,300
Cost per load	25	77	17	13
Cost per cubic ft	1	19	6	6

Data reproduced from Ref. 12.

⁷ Osterman-Golkar, S.; Bergmark, E. *Scand. J. Work Environ. Health* **1988**, *14*, 372-77.

⁸ Goldman, M.; Lee, M. et al. *J. Biomed. Mater. Res.* **1997**, *37*, 43-50.

⁹ Klapperich, C.; Niedzwiecki, S. et al. *J. Biomed. Mater. Res.* **2000**, *53*, 73-5.

¹⁰ Ferreira, S. D.; Dernell, W.S. et al. *Clin. Orthop.* **2001**, *388*, 233-39.

¹¹ Digas, G.; Thanner, J. et al. *Acta Orthop. Scand.* **2003**, *74*, 531-41.

¹² <http://www.devicelink.com/mddi/archive/01/05/005.html>

Microbial Production of Renewable Petroleum™ Fuels and Chemicals

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Abstract

Renewable and scalable fuels and chemicals that are cost competitive with petroleum and compatible with the existing distribution and consumer infrastructure have the greatest potential for rapid and widespread consumer adoption. LS9 has developed a platform technology for the cost effective production of a diversity of advanced biofuels and renewable chemicals by an efficient and simple one step fermentation process (Fig. 1). LS9 has engineered established industrial microorganisms

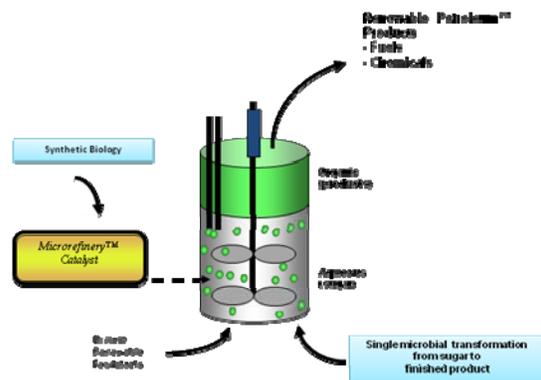


Figure 1. LS9 Renewable Petroleum™ Technology

to convert fermentable sugar selectively to alkanes, olefins, fatty alcohols, and fatty esters each in a single unit operation. The process enables precise genetic control of the molecular composition and performance characteristics of the resulting fuel or chemical product. The technology has been successfully scaled to pilot level for the production of UltraClean™ diesel, which is on schedule for commercial demonstration in 2010. UltraClean™ diesel exceeds all of the specifications ASTM 6751 for on road vehicle use, lacks the environmental pollutants benzene, sulfur, and the heavy metals found in petroleum diesel, and based on the GREET model for life cycle analysis will result in a $\geq 85\%$ decrease in GHG emissions. This same technology platform is being applied through a strategic partnership with Procter and Gamble for the production of surfactants for their consumer chemical products. These and other LS9 drop-in renewable products are on target to facilitate broad environmental benefit through rapid product adoption.

Recent Milestones

- Completion of \$25 million Series C with Chevron as a strategic investor.
- Partnership with Procter and Gamble for the production of surfactants for consumer chemical products.
- UltraClean™ diesel externally validated to be on road vehicle ready in the US and Brazil.
- Successful completion of Pilot Plant and scale up of UltraClean™ Diesel process to 1000L.
- Intellectual property portfolio to >20 patent families for core and product technologies.

Award Eligibility

We submit this proposal for consideration in the Greener Synthetic Pathways as the primary focus area, and the Design of Greener Chemicals and Use of Greener Reaction Conditions as the secondary focus areas. LS9 is also eligible for consideration in the Small Business category.

Scope of the Technology

LS9 has developed a technology platform for the simple and selective conversion of diverse renewable feedstocks to a portfolio of advanced drop-in compatible fuel and chemical products (Fig. 1). The technology leverages the natural efficiency of microbial fatty acid metabolism for the biosynthesis of long hydrocarbon chains and, using synthetic biology, combines this with

new biochemical pathways engineered into a whole cell biocatalyst to convert these intermediates to specific finished fuel and chemical products that are secreted from the cell. The technology enables unique ambient one-step conversion processes from renewable carbohydrate to finished products that are simple, selective, and efficient. The products are immiscible and form a light organic phase that is non-toxic to the whole cell catalyst and easily recoverable by centrifugation. LS9 is actively developing the technology for the production of alkanes (diesel, jet, gas), alcohols (surfactants), esters (Biodiesel, chemical intermediates), olefins (lubricants, polymers), aldehydes (insulation, resins), and fatty acids (soaps, chemical intermediates). Specific product performance is enabled through the genetic control of structure (chain length, saturation, and branching). To date LS9 has advanced its flagship UltraClean™ Diesel process through pilot scale, and it has demonstrated ASTM compliance of the resulting fuel and is in the advanced stages of EPA product certification. In the chemicals area, LS9 is in partnership with Procter and Gamble to develop and commercialize a variety of consumer product chemicals based on the same technology platform. LS9 is advancing the technology to commercial scale in its Renewable Petroleum™ facility, which will come on line in Q2 2010 initially for the production of UltraClean™ diesel followed by other products.

Technology platform

LS9 technology is based on microbial fatty acid metabolism. In nature, hydrocarbon biosynthesis is ubiquitous with the most efficient metabolic pathway proceeding through fatty acid biosynthesis (1). Fatty acid biosynthesis initiates from the terminal product of glycolysis, acetyl coenzyme A (CoA). In bacteria, such as *Escherichia coli*, acetyl CoA is converted to malonyl CoA, which is then recursively condensed, reduced, dehydrated, and further reduced through a series of selective enzymatic conversions to produce a growing hydrocarbon chain that is covalently attached as a thioester to the pantethionyl moiety of the acyl carrier protein (ACP) (Fig. 2)(3). During biosynthesis, a double bond can be incorporated into the growing acyl chain after the addition of the fifth malonyl unit, resulting in a ω -seven cis double bond. Depending on the precursor for biosynthesis or post biosynthetic modification, fatty acids can be straight or branched. Acyl ACP is the terminal product of fatty acid biosynthesis and serves as the precursor for phospholipid biosynthesis. Acyl ACPs from 2-19 carbons in length exist as a dynamic in the cell and can be selectively diverted to a diversity of natural and engineered products (2,4). Fatty acids are primary metabolites used both structurally (membranes) and for energy storage (fat), and accordingly are produced efficiently and at high rates. Fatty acid biosynthesis occurs with a theoretical maximum mass yield of ~35%, which represents 90% energetic efficiency (1). *Escherichia coli*, which is 9.7% structural lipid, naturally produces fatty acids at commercial productivities just to support growth (0.2 g/L.hr per gram of dry cell). By uncoupling production from growth, LS9 has engineered the *E. coli* pathway for commercial production of a diversity of fatty acid derivatives. This is achieved by engineering into *E. coli* new biochemical pathways that divert fatty acyl ACP specifically to new products, such as alkanes, alcohols, esters, and olefins (Fig. 2)(4,5,6).

Technology platform enables multiple products

LS9 technology is enabled by proprietary Microrefinery™ Catalysts. These are microbial cells engineered such that all chemical conversions from feedstock to product occur in each cell. This obviates additional chemical steps and improves process yield and capital costs. Figure 2 is a

scheme of some of the biochemical pathways that LS9 has developed for the production of renewable fuels and chemicals. Each has been enabled and is under development for commercial production.

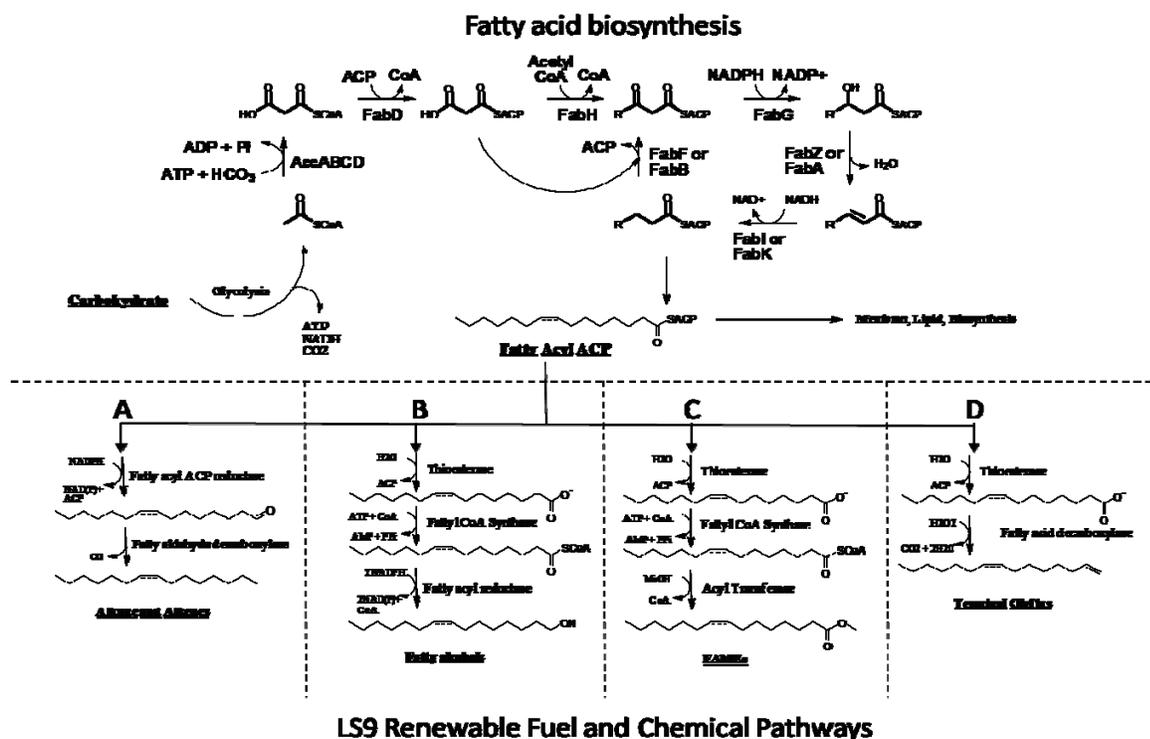


Figure 2. LS9 Technology Platform. The biochemical reactions of fatty acid biosynthesis are shown in the upper half of the figure, in which fatty acyl ACP is the primary product. LS9 introduces new biochemical pathways into the cell that efficiently and selectively divert fatty acyl ACP to a diversity of other products. Shown in the lower half of the figures are the biochemical pathways that LS9 have developed for the production of (A) Alkanes, (B) Fatty Alcohols, (C) Fatty acid methyl esters (FAMES), and (D) Terminal Olefins. All of these products are produced in the cell and then secreted to the medium where they are easily recoverable. The chain length, presence and absence of the ω -7-cis double bond, and branching are all controllable for each product.(1,2,3,4,5,6)

Alkanes. Alkanes are the primary components of liquid transportation fuels and are derived from petroleum. While biological alkane biosynthesis have been researched, the responsible genes and enzymes have remained elusive. LS9 has identified the genes responsible for natural alkane biosynthesis, and has demonstrated their engineered expression and efficient alkane production by fermentation. This two gene pathway encodes an acyl ACP reductase, which catalyzes the NADPH dependent reduction of fatty acyl ACP to a fatty aldehyde, and a fatty aldehyde decarbonylase, which catalyzes the conversion of fatty aldehydes to alkanes. *E. coli* engineered to express this pathway effectively produces and secrete C11-c17 alkanes and alkenes (derived from unsaturated fatty aldehydes) (5). This is the only reported process for the direct production of fuel grade hydrocarbons from renewable carbohydrate by fermentation to our knowledge. All others require at minimum catalytic hydrogenation after recovery. Alkane samples recovered from LS9 fermentations have been positively evaluated by end users, and the process for their production has been taken through lab scale and is moving to pilot scale in Q1, 2010.

Fatty Alcohols. Fatty alcohols are commodity chemicals used for the preparation of surfactants and are derived from the hydrogenation of palm and coconut derived fatty acid methyl esters or ethylene (about 50% from each raw material). The LS9 pathway for renewable alcohol production consists of three biochemical steps. The first is thioesterase (TE) catalyzed hydrolysis of fatty acyl ACP to produce fatty acids and deregulate fatty acid biosynthesis (10), the second is acyl CoA synthase catalyzed ATP and Coenzyme A dependent synthesis of acyl CoA, and the third is fatty acyl CoA reductase catalyzed NADPH dependent reduction of the fatty acyl CoA to fatty aldehyde and fatty alcohol. The chain length of the fatty alcohols are controlled by the selectivity of the TE, enabling the chain length composition to be engineered genetically (2,4). The fatty alcohol process is presently being scaled to pilot level production.

Terminal Olefins. Terminal olefins are used in a diversity of industrial applications and are derived from petroleum through polyethylene chemistry. The LS9 biochemical pathway consists of a TE to produce fatty acids, and a novel enzyme discovered by LS9 that catalyzes the peroxide dependent decarboxylation of fatty acids (6). As with the other LS9 products, the terminal olefins composition is controlled by the chain length selectivity of the TE in the pathway, and scales with similar performance in lab scale processes. LS9 is in active commercial discussions for this product.

Fatty Acid Methyl Esters. Biodiesel is composed of fatty acid methyl esters (FAME), which are produced from the transesterification of plant and animal oils. Biodiesel today is the main renewable replacement for diesel with over 2 billion gallons consumed annually. The LS9 FAME biochemical pathway is the same as that for fatty alcohols except the fatty acyl reductase reaction is replaced by an acyl transferase catalyzed acylation of methanol, which is provided at low level in the fermentation. Unlike traditional production of biodiesel, which is produced from triacylglycerol (TAG)(11), LS9 FAME is produced biochemically directly from the precursor of TAG biosynthesis, fatty acyl ACP. The resulting FAME is secreted from the cell, and its chain length composition is controlled by the selectivity of the TE used in the pathway (Fig. 3)(2,4). The LS9 FAME process is simple and reflects how each of the LS9 renewable fuels and chemicals are produced. The UltraClean™ diesel catalyst is grown in a minimal salts media with a carbohydrate and nitrogen feed. Production occurs throughout and after a short growth phase and can be monitored by the accumulation of oil outside of the cells (Fig. 3). The broth is pumped through a disc stack centrifuge where the light oil phase is separated from cells and aqueous medium. The cells can be recycled, used as fertilizer (assuming approval), burned for energy, or converted to methane through anaerobic digestion. The oil is washed with water and Magnesol to extract any salts and is ready for use as fuel. The resulting UltraClean™ diesel exceeds all ASTM 6751 specifications for use in the US for on road vehicles (external lab validated) and outperforms soy, rapeseed, and palm derived biodiesel for cold flow, volatility, cetane, and oxidative stability. The product is in the advanced stages of product certification by the EPA. The LS9 FAME is our flagship product and the primary component of our UltraClean™ diesel. The UltraClean™ diesel process has been scaled through pilot, and tech transfer to commercial demonstration phase is on schedule for Q2 2010; this will be final scale up step before commercial production scheduled for 2012.

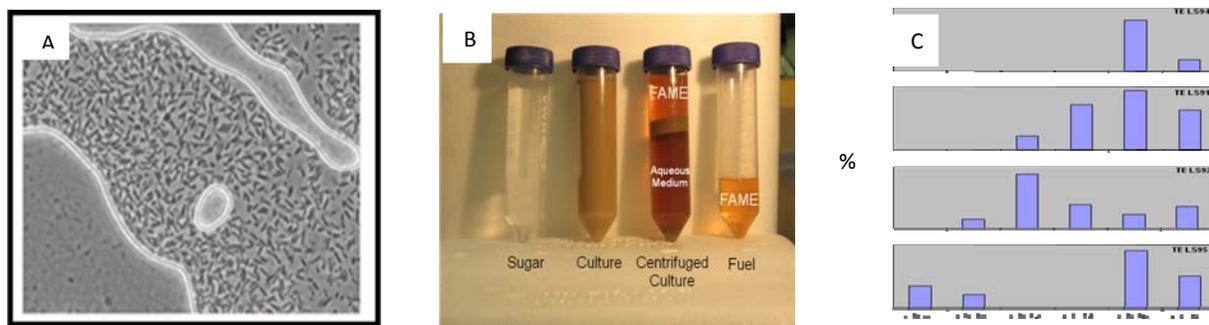


Figure 3. Production, Secretion, recovery, and control of FAME composition. (A) Micrograph of LS9 UltraClean™ diesel Microrefinery™ catalyst producing and secreting FAME. The rods are bacterial cells and the FAME is seen as a second organic phase. The sample was taken directly from the LS9 pilot plant. (B) The oil is directly recoverable by centrifugation and forms a light organic phase easily recoverable from the cells and aqueous phase. (C) Control of chain length distribution of the FAME by substitution of the thioesterase in the engineered pathway (Fig. 2) with thioesterases having different chain length selectivity.

Microrefinery™ catalyst: Host organism

The LS9 platform technology functions in many industrial organisms, however, LS9 has focused on the development of *E. coli*. *E. coli* naturally produces fatty acid metabolites at commercial productivities. It is an established industrial microorganism that has the most precedent for the commercialization of large scale metabolically engineered products, such as 1,3 propane diol, polyhydroxyalkanoates, lysine, threonine, and tryptophan. *E. coli* is tolerant to >30% LS9 products and it is genetically stable for over a week of continuous fermentation. The Microrefinery™ *E. coli* catalyst is a derivative of MJ 1655, which is a “K” strain and the best characterized of the *E. coli* isolates, which naturally utilizes C5 and C6 sugars as feedstocks.

Feedstocks

LS9 has demonstrated production without issue of its products from a diversity of feedstocks including glucose, xylose, arabinose, mannose, sucrose, glycerol, mixed C5 and C6 sugars, and hydrolysates from stover, woodships, and bagasse. Indeed the biphasic nature of the fermentation provides increased tolerance to organic toxins, such as those found in biomass hydrolysates. LS9 commercial production will initially be from Brazilian sugarcane, since it is most cost effective and does not compete with food. As lignocellulose becomes cost effective, LS9 is ready to transition and is working with industrial leaders to integrate their technologies. Recently, LS9 with the Keasling lab (UC Berkeley, JBEI) developed a one step process for the conversion of biomass to LS9 products (2).

Human health and environmental benefits

Renewable Petroleum™ fuels and chemicals result in a significant decrease in the production of green house gases and organic and inorganic toxins relative to their petroleum alternatives. UltraClean™ diesel is representative of these products and contains very low nitrogen, sulfur, and no benzene, lead, manganese, mercury, or arsenic which are found in petroleum-based diesel. Indeed, the LS9 technology is unique amongst its technology peers, as LS9 does not require any heavy metal catalysts for the production of its products, since all the chemistry is performed inside the cell. All other routes to hydrocarbons, such as to “renewable diesel” (UOP, NESTE oil), farnesane (Amyris), Biogasoline (Virent), and butanol derived hydrocarbons (Gevo,

Dupont) require metals (Ni, Pd, Pt, Rh, Ru, etc) which ultimately end up in product or waste streams.

Using the GREET model, developed by Dr. Michael Wang and colleagues at Argonne National Laboratory, the farm-to-wheels greenhouse gas (GHG) emissions lifecycle was evaluated for sugarcane-derived UltraClean™ diesel that is produced in Brazil, shipped by tanker, and consumed in the United States. Our analysis shows that UltraClean™ Diesel achieves more than 85% net reductions in overall GHG emissions relative to petroleum diesel. The large reduction can be attributed to the displacement of fossil energy use, low energy and fertilizer inputs for sugarcane cultivation, and the generation of surplus electrical power during the fuel production process. A major benefit relative to plant derived biodiesel is the significant improvement in land use, with UltraClean™ diesel yielding 400gal/acre at existing sugar cane yields and increasing to ~2000gal/acre with the expected improvements in energy crop yields. Results from these analyses indicate that UltraClean™ diesel is well positioned to exceed the stringent fuel emissions reductions criteria set forth in the Energy Independence and Security Act of 2007. As LS9 transitions to lignocellulosic feedstocks, the environmental benefits of this and other LS9 products shall improve.

Economics

A model of a 100MM gal/year plant for the production of UltraClean™ diesel has been completed. As for all fermentation based biofuels, feedstock costs represent ~ 80% of the final COGs. Today, the most cost effective raw material that does not compete with existing food supplies is newly planted Brazilian sugar cane. Assuming a greenfield plant that sources cane from third party growers, fermentable sugar is produced at \$0.0495/lb (CONSECANA prices for the last 8 years were R\$35/tonne which yields ~331lbs fermentable sugar). At an overall process efficiency of 85% of maximum (85% of 37% (max) = 31.5 % mass yield on sugar), LS9 UltraClean™ diesel will be produced at prices competitive with diesel from \$45-50 barrel oil without subsidy.

Comparison to Other Technologies A diversity of biotechnologies is emerging for the production of fuels and chemicals from renewable resources. A comparison of the unit operations required for the manufacture of diesel by these technologies is shown in figure 4 (4,5,6,7,8,9). The LS9 diesel process stands alone in its high yield (37%) and simple process (1 step). All other technologies start with a lower theoretical yield (based on the metabolic pathways used) and require production of an intermediate prior to chemical conversion to diesel. The additional chemical operations require elevated temperatures, high pressure, hydrogen, and heavy metal catalysts. Recently, algae has re-emerged as a possible catalyst for the photosynthetic production of renewable oil, however, this crude oil (TAG) must be extracted from the cells prior to heavy metal catalyzed and hydrogen dependent catalytic deoxygenation to fuel grade hydrocarbons, similar to the other technologies in figure 4. Not shown are chemical technologies, such as the Virent BioForming™

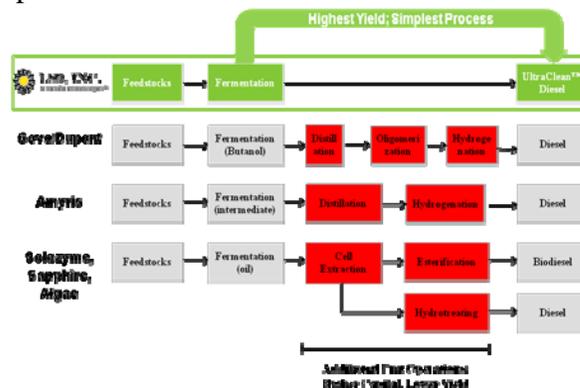


Figure 4. A comparison of the LS9 UltraClean™ Diesel technology to competing technologies. The LS9 process is a single step conversion of carbohydrate to finished product. Other processes require additional operations such as hydrogenation.

technology, which results in the non-selective production of a diversity of light hydrocarbons and oxygenates. The Virent technology is powerful but appears to require the use of significant heavy metal catalysts, such as Nickel, Palladium, Platinum, and Ruthenium. These are known environmental toxins, in some cases running in short supply, and require clean sugar streams that will not foul these sensitive catalysts. Further, the technology appears from the patent applications to produce diverse product mixtures of CO₂(20%), paraffin (10%), monooxygenates (30%), ketones (7%), tetrahydrofurans (8%), cyclic monooxygenates (1%), and unknown aqueous species (28%) (12) making the production of desired compounds require additional chemistry and complex separations. We are unaware of another technology platform that can effectively and selectively produce fuel and chemical grade FAMES, alkanes, fatty alcohols, or olefins that is as efficient in yield, process simplicity, environmental benefit, or economics as the LS9 technology.

Summary

Fossil feedstocks are the major source of environmental pollutants ranging from CO₂ to toxic organics and heavy metals. A rapid and widespread adoption of renewable alternatives is urgently needed to curb the release of these destructive agents. Described here is a simple yet efficient platform technology that supports the cost effective production of renewable fuels and chemicals currently derived from fossil feedstocks. These products contain no toxic organics or inorganics, result in >85% decrease in GHG emissions relative to fossil derived products, are superior in performance, process efficiency, land yield, and economics to existing alternatives. These products can be produced from a diversity of non-food based biological feedstocks, leverage existing infrastructure, and are being actively deployed by LS9 and their partners. The efficiency, affordability, and product performance bodes well for the LS9 technology to become one of the many solutions required to wean our dependence on fossil fuels, and to arrest the ongoing environmental destruction this dependence effects.

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BioAmber Succinic Acid: First Commercial Production and
Substitution of an Existing Industrial Chemical Through Metabolic
Engineering, Novel Integrated Process Improvements and
Breakthrough Downstream Applications for Green Products

December 31, 2010

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PROJECT TITLE

BioAmber Succinic Acid: First Commercial Production and Substitution of an Existing Industrial Chemical Through Metabolic Engineering, Novel Integrated Process Improvements and Breakthrough Downstream Applications for Green Products

RECENT MILESTONES

BioAmber optimized and scaled up succinic acid fermentation technology to become the first company to supply commercial quantities of biobased succinic acid to the market as an alternative to petroleum-derived succinic acid. BioAmber's production facility was commissioned in January 2010 and operates at the 350,000-liter fermentor scale. Strain development was originally sponsored by the U.S. Department of Energy (DOE), and BioAmber then optimized the fermentation and integrated important downstream process innovations. BioAmber has also been actively developing novel applications for succinic acid that will rapidly grow the market for the biobased chemical. BioAmber is the first to market biobased succinic acid that has a lower cost than fossil fuel-derived succinic acid. We believe this to be the first example of a direct substitution of a petroleum-derived chemical by a fermentation-derived chemical.

ELIGIBILITY OF SMALL BUSINESS AWARD

BioAmber, Inc. is a registered Delaware Corporation and a US small business with less than \$40 Million in annual sales. As a renewable chemistry company with innovative technology it is eligible for the EPA Small Business for Green Chemistry Award.

FOCUS AREA

Fits all focus areas, with emphasis on Focus Area 3: design of greener chemicals.

U.S. COMPONENT

BioAmber advanced a novel, integrated production technology with extensive US-based intellectual property that leverages an organism licensed from the DOE. The company has validated commercial scale production as well as downstream applications of biobased succinic acid covering the organism, fermentation, isolation and purification, and transformation into safer, less toxic products.

ABSTRACT

BioAmber introduced high quality, biobased succinic acid to the market in 2010 and is expanding the green chemical market with novel downstream applications. BioAmber has scaled up an *E. coli* biocatalyst technology under license from the DOE and integrated a novel downstream purification process. This development has resulted in a technology that produces succinic acid at a lower cost than fossil fuel derived succinic acid. BioAmber's process also dramatically decreases the environmental footprint when compared to the petroleum-based process. Thanks to BioAmber's achievements, succinic acid joins polylactic acid and 1,3-propanediol as successfully commercialized biobased chemicals, with one important distinction: succinic acid is a platform molecule that can be used to produce a wide range of renewable chemicals. Succinic acid can also directly substitute other chemicals, including highly polluting adipic acid in polyurethane applications and highly corrosive acetate salts in deicing applications. BioAmber's biobased succinic acid technology is cost advantaged over fossil fuel-derived succinic even if oil drops to below \$40 per barrel.

BioAmber's economic advantage has given a number of chemical markets the

confidence to develop new applications for succinic acid. It has also made it economically viable to (i) transform biobased succinic acid to renewable 1,4-butanediol and tetrahydrofuran; (ii) produce succinate esters for use as non-toxic solvents; and (iii) produce biodegradable performance plastics that are also renewable. BioAmber is leading the development of modified polybutylene succinate (mPBS), a 50%-70% biobased and biodegradable polyester with unique performance features including high temperature resistance.

BioAmber's process also reduces energy consumption by 60% compared to its petrochemical counterpart and consumes CO₂, rather than generating it.¹ In 2011, BioAmber is planning to begin North American construction of a 20,000 ton facility that will offset CO₂ emissions by sequestering 8,000 tons of CO₂ per year; equivalent to 8,000 fewer cross country airline flights, or 2,300 compact cars taken off the road, based on EPA average emissions assumptions.²

The scale up of biobased succinic acid has incorporated green chemistry principles to expand markets, generate source pollutant reductions and increase health benefits along numerous points in the lifecycle of a variety of chemicals.

SCOPE AND SELECTION CRITERIA

BioAmber's technology meets both the scope and selection criteria of the EPA Green Chemistry program through optimization, process integration, and scale up of core US-based succinic acid technology, coupled with novel breakthroughs in downstream applications. BioAmber's technology incorporates "the principles of green chemistry at the earliest design stages of a new product or process which have yielded benefits to human health and the environment along many points in the technology's lifecycle", from feedstock conversion to synthesis, use, and ultimate end of life fate of greener products. Accordingly, the technology fits all three Focus Areas, especially Area 3. The following is a discussion of the structure of succinic acid and the biochemical rationale behind the construction of *E. coli* AFP 184, the foundation of BioAmber's core technology as prelude to improvements, scale up, integration of backend efficiencies, and breakthrough downstream applications including mPBS, a biodegradable polymer with 50%-70% renewable content.

INNOVATIVE CHEMISTRY OF THE NEW TECHNOLOGY

a. Structure and Biochemical Rationale behind the construction of Succinic Acid-Producing Strain *E. coli* AFP 184.

Succinic acid is an organic di-acid with four carbon atoms (C₄H₆O₄) present in a large number of metabolic processes of living organisms. Succinic acid and its derivatives are used in foods, pharmaceuticals, and cosmetics. It is most commonly produced by hydrogenation of maleic acid or recovered as an ester from a by-product stream in the production of adipic acid, both petroleum-derived. It is naturally produced by most living organisms including microbial systems as an intermediate in the Krebs cycle (tricarboxylic acid cycle).

BioAmber is the first and only company to develop and scale an efficient integrated fermentation process with novel purification and separation technology to produce

¹ *Producing Bio-Based Bulk Chemicals Using Industrial Biotechnology Saves Energy and Combats Climate Change*, Herman, Blok, and Patel, Science, Science, Technology & Society, Utrecht University, Heidelberglaan 2, 3584 CS Utrecht, The Netherlands, 41, 7915-7921, 2007.

² See: http://www.epa.gov/climatechange/emissions/ind_assumptions.html

succinic acid for commercial manufacture. *E. coli* K12 was selected as the host organism due to its profusion of genetic tools allowing specific genetic and metabolic reconstruction, and because of its long history of safe industrial production of various products. *E. coli* K12 produces succinate, although as a minor product of fermentation for wild-type strains. *E. coli* principally converts phosphoenolpyruvate and pyruvate formed through the Embden-Meyerhof-Parnas pathway under anaerobic conditions to formate, lactate, and ethanol, and genetic manipulations are necessary to increase succinate production and reduce by-product formation, e.g. lactic or acetic acid.

Diversion of carbon to succinate alone is insufficient to prevent the accumulation of other undesired products; mutations in lactate- and formate-forming steps are used to further improve succinate production. Mutants of *E. coli* deficient only in fermentative lactate dehydrogenase (*ldh*) did not exhibit decreased anaerobic growth, while *E. coli* NZN111, which lacks both the pyruvate-formate lyase (*pfl*) and *ldh* genes, exhibited marginal anaerobic growth on glucose. When transformed with the *mdh* gene encoding malate dehydrogenase, *E. coli* NZN111 grew anaerobically. Donnelly *et al.* (1998) reported an unknown spontaneous chromosomal mutation in NZN111 which permitted anaerobic growth on glucose, and this strain was named AFP111. AFP111 grown anaerobically under 5% H₂-95% CO₂ resulted in a succinate yield of 0.70 and a succinate-to-acetate molar ratio of 1.97.

Moreover, this strain grown aerobically for biomass generation and then subjected to anaerobic conditions and supplemented with CO₂ (dual-phase fermentation) resulted in a succinate yield and a productivity as high as 0.99 and 0.87 g/liter/h, respectively. The causative mutation in AFP111 was mapped to the *ptsG* gene, which encodes a glucose-specific transporter of the phosphotransferase system. *E. coli* AFP111 was derived from strain K12 W1485. Surprisingly and unexpectedly, the metabolism rate and titer for *E. coli* K12 C600 proved to be superior to the W1485 derivatives (including AFP111). Thus, the same mutations *ldh*, *pfl* and *ptsG* were introduced into the C600 host to derive the strain *E. coli* K12 AFP184, which shows performance much higher than AFP111.

b. Process development and cost improvements made by BioAmber

When BioAmber licensed *E. coli* AFP184 from the US DOE, it had only been validated at lab scale, and there was no viable downstream processing available. BioAmber developed a cost-effective production medium and fermentation process in a series of successive sizes, all the way up to the 350,000-liter fermentor scale, with a proprietary aqueous-based downstream process. Unlike other fermentation processes using *E. coli*, BioAmber's process does not produce significant by-product. Today, BioAmber operates the world's only large-scale, dedicated biobased succinic acid plant in Pomacle, France. The plant was commissioned in early 2010.

In BioAmber's production process, the *E. coli* is first propagated aerobically from shake flask to the production fermentor under strict axenic conditions to avoid contamination and to prevent the release of the strain in the environment. Once the cells have reached an optimum physiological state, those same cells



are transferred into a bioconversion fermentor that is operated anaerobically. Then the cells are fed with a proprietary production medium and carbon dioxide (CO₂) under neutral pH to produce succinate salts. Once completed, the succinate salt is recovered and concentrated by novel downstream procedures that produce high purity biobased succinic acid crystals.

Since early 2010, the BioAmber plant has been producing succinic acid from wheat-derived glucose with high purity and increasing yields. Historically, the key challenges associated with scaling industrial fermentations have included lowering production costs, increasing fermentation yields and titers, and reducing by-products. *BioAmber's scale-up has addressed these challenges by improving biomass conversion, developing robust organisms, improving fermentation and purification and achieving other process efficiencies.* As a result, the current cash cost for BioAmber biobased succinic acid is 40% less than fossil fuel-derived succinic acid production, and a cost advantage persists even if oil drops to \$40 per barrel. Further improvements in downstream process efficiency, and the development of a yeast that is able to consume lignocellulose hydrolyzate will further increase the cost advantage. BioAmber's ongoing R&D efforts are implementing these improvements, providing a clear pathway to a cost point that will generate several million tons of demand for biobased succinic acid and will likely lead to "shut-down economics" for the petrochemicals that biobased succinic acid replaces.

Since 2008, BioAmber's IP portfolio has doubled to 47 families of patents, covering the organism, fermentation process, isolation and purification of biobased succinic acid and its transformation into value added derivatives.

ENVIRONMENTAL AND HUMAN HEALTH RISKS THE TECHNOLOGY ADDRESSES

The USDA, DOE and leading academic and industry analysts (BREW project 2006, SRI 2007, and Patel 2007)³ have identified opportunities to substitute (both directly and functionally) existing petrochemical-derived products with biobased succinic acid including maleic anhydride as a feedstock for butanediol and THF, and the partial replacement of adipic acid (2.7 million tons annual production) in polyurethanes and other applications. BioAmber has achieved the program scope of "innovation in chemical design, manufacture, and use" consistent with a study commissioned by the DOE and USDA in 2004, which identified succinic acid as among the twelve strategic platform chemicals that could be produced from renewable resources and substitute directly existing petrochemicals to drive US competitiveness in a low carbon, sustainable economy. *BioAmber's demonstration of cost competitive commercial economics for biobased succinic acid has helped the green chemicals industry to take root in the US and address the expanded market for the chemical derivatives.*⁴

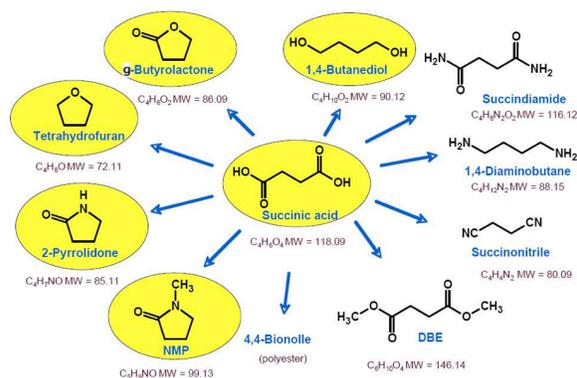
First, environmental benefit is evident in the development of an improved production process. By substituting biomass for petrochemical feedstock, BioAmber's technology addresses environmental risk in chemical production by reducing reliance on fossil fuels, increasing the use of natural resources for the production of safer green chemicals, sequestering CO₂ and achieving significant GHG emission reductions. Succinic

³ Department of Energy, *Top Value Added Chemicals from Biomass Volume 1 – Results of Screening for Potential Candidates from Sugars and Synthesis Gas*, 2004 European Commission's GROWTH Program, BREW report, 2006. SRI, "Chemicals from Biomass," 2007. PATEL, Science, 2007.

⁴ See *Chemical derivative chart above*: <http://www.chemistryinnovation.co.uk/roadmap/sustainable/files/gfx/succinic%20acid%20DOE>.

acid is a chemical currently produced by the reduction of maleic acid, which is produced from either n-butane or benzene feedstocks. The BREW and SRI reports confirm that biobased succinic acid has the potential to significantly reduce GHG emissions, relative to petrochemicals. BioAmber's succinic acid fermentation process consumes significant amounts of CO₂, directly reversing greenhouse gas emissions. BioAmber estimates that a 20,000MT succinic acid biorefinery will sequester 8,000 tons of CO₂ annually, which would be equivalent to the elimination of 8,000 annual cross country airline flights, or heating 1,400 homes per year, or 2,300 compact cars being taken off the road, based on EPA average emissions assumptions.

The production cost of fossil fuel-derived succinic acid restricts significant expansion of the current 50,000 MT market. Yet succinic acid has the potential to be a non-toxic environmentally beneficial substitute in numerous high-volume markets, if the price is competitive. The 2007 study by Patel⁵ indicates a total savings potential from the green chemicals industry of 510 million tons CO_{2,eq} if corn starch is the feedstock, 820 million tons CO_{2,eq} if lignocellulosic sugars are used, and 1,030 million tons CO_{2,eq} if sugar cane is used. The study states that the potential GHG savings for current technologies, using corn starch as a feedstock, have already reached 45%. The report highlights that, "using industrial biotechnology to produce biobased chemicals can contribute significantly to the reduction of climate change and the depletion of fossil energy. It is therefore a key strategy for sustainable development of the chemical industry." BioAmber's advancements in robust fermentation systems and high-yielding strains meet the needs of the chemical and manufacturing sectors, because biobased succinic acid is commercially produced at a lower cost than the fossil fuel chemicals it is seeking to substitute.



Second, in addition to these significant environmental benefits accrued from the process, there are vital health and environmental benefits derived from the chemicals and products themselves. The first benefit centers on end of life biodegradability and cradle-to-cradle options, which directly address point source reductions of any hazardous substance, pollutant, or contaminant including fugitive emissions prior to recycling, treatment, or disposal associated with any chemical or product for which biobased succinic acid is a substitute. The second benefit is safer, less toxic alternative products to replace the stream of insidious yet ubiquitous petro-derived compounds pervasive in our work and homes. For instance, phthalates are under increasing scrutiny from international regulatory bodies, scientists, and consumers with mounting evidence that suggests these chemicals are endocrine system disruptors that affect mammal fetal development. A growing chorus of industry watchdogs are calling for less toxic alternatives, with articles populating main stream media outlets such as Time Magazine's special feature this April, *The Peril of Plastics*, citing international toxicology experts. While the EU has implemented

⁵ Patel, 2007. *Supra* footnote 1

new chemical safety laws known as REACH, the political landscape in the US has relied on industry innovators such as BioAmber to bring alternatives to market. As a concrete example, BioAmber has worked with a number of plasticizer companies to show that certain esters of succinic acid are viable alternatives to phthalates in plasticizer applications.

Through its scale-up advancements and downstream breakthrough applications, BioAmber is leading green chemical innovation for a portfolio of renewable, safer products to replace these petro-derived chemicals and materials. Succinic acid will initially substitute petroleum-derived succinic, and in parallel progressively expand into new markets as technology improvements further reduce cost. BioAmber is working closely with customers to develop environmentally-friendly plasticizers, solvents and other applications, including a proprietary, environmentally friendly deicer that is FAA compliant for use on airport runways (AMS 1435 standard). Biobased succinic acid is also the key to the development of the polybutylene succinate (PBS) market, a biodegradable polymer with strong export potential to emerging Asian PBS markets.

To exploit the emerging PBS market opportunity, BioAmber has developed novel polymers made from succinic acid, through its subsidiary Sinoven Biopolymers. Sinoven has a proprietary technology for modifying PBS, producing a biodegradable polyester that meets US, European and Chinese (ASTM D6400, EN13432, and GB/T 20197) biodegradability standards and has over 50% renewable content if made with biobased succinic acid (see image below). With its unique performance and excellent safety profile, modified PBS can be used in food service, automotive and electronic applications that require heat resistant, biodegradable polymers.

BioAmber's modified PBS offers the following breakthrough performance features; (i) high heat distortion up to 110°C; (ii) strength and stiffness that provide the "feel" of polypropylene or PVC; (iii) drop-in process-ability for extrusion and injection molding; (iv) FDA compliance for food service applications; (v) biodegradability within 90 days when composted; and (vi) over 50% renewable content when made with biobased succinic acid.



SINOVEN mPBS meets US, European and Chinese biodegradation standards (ASTM D6400, EN 13432 and GB/T 20197).

As BioAmber's ongoing improvements further reduce costs and prices, they will accelerate biobased succinic acid's substitution of monomers for which biobased sources are not yet available, such as adipic acid. The final stage in the use of succinic acid will be its substitution for maleic as a precursor for making butanediol, THF and other monomers.

COMPETITIVE ADVANTAGE

The technology advancements resulting from its successful scale up have put BioAmber well ahead of its competition and in a position to address the increasing environmental and health concerns of the market. Technology advantages encompass: (1) a proprietary purification process that reduces utility consumption, is less capital intensive, and has greater scalability and operability than conventional bio-processing, without the production of by-products typically seen in neutral pH fermentation processes; (2) broad intellectual property protection for the production process and the use of succinic acid in selected applications; and (3) an R&D pipeline that includes a new generation yeast organism that is capable of consuming lignocellulosic sugars.

Competing biobased succinic acid companies are currently operating at the 20,000L fermentation scale, with non-continuous purification processes that have yet to be proven at a large scale. In contrast, BioAmber has been operating a \$30 million production plant at almost 20 times greater fermentation volume (350,000 liter) and an integrated, continuous downstream process. By operating at this large scale for the past year, BioAmber has validated the material and energy balance, confirmed its process economics and assessed the impact of a continuous, high volume process on product quality. When a process has large recycle streams operating, large fermentors requiring many more growth cycles for the organism, large volumes of liquid being processed and industrial scale cleaning-in-place (CIP) being used, there is the inevitable emergence of impurities that were not apparent at the smaller pilot scale. This requires process changes and innovation in order to ensure a high quality product that is also cost effective. This continuous process innovation and development feedback loop at industrial scale is a key competitive advantage that BioAmber has achieved over the past year, and that eludes its biobased succinic competitors, who are still 2 to 3 years away from this milestone. BioAmber has incorporated these learnings and innovations into its engineering package, which will in turn dramatically reduce the risk for BioAmber's first North American plant, scheduled to begin construction in 2011.

As a result of BioAmber's disruptive innovation (both in the upstream and downstream processes), it possesses the most advanced, lowest risk technology for making biobased succinic acid. A report published in December 2010, entitled *Lux Research Report: Today's Top Technologies in Bioplastics and Biofuels*, classified BioAmber as a "Dominant" company in the field of fermentation derived chemicals, based on the strength of its technology, execution and stage of development ("dominant" companies are considered the strongest performers in the biobased chemicals and materials sector). The report also identified BioAmber as the clear leader in the field of biobased succinic acid (and the only succinic acid company with a "dominant" positioning).

Thanks to these competitive advantages, BioAmber has secured strategic partnerships with a number of leading companies including Cargill, DuPont, MATRIC (former Union Carbide R&D Center) and Mitsui & Co. BioAmber is proud to be the leader in both the commercialization and further R&D innovation of biobased succinic acid, with the goal of opening up multiple large markets for renewable alternatives to petrochemicals. Thanks to its commercial stage succinic acid technology, BioAmber is positioned as a leader in green chemicals, with both viable economics and broad reaching health and environmental benefits.



2011 EPA Presidential Green Chemistry Challenge Award, submission

**Cost-advantaged Production
of Intermediate and Basic Chemicals
from Renewable Feedstocks**

December 26, 2010

Sponsor: Genomatica

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Project Title:

Cost-advantaged production of intermediate and basic chemicals from renewable feedstocks

Milestones:

- a) Successful *production, at pilot-scale*, of Genomatica's first product, bio-BDO. BDO (1,4-butanediol) is an intermediate chemical with a \$4 billion existing market worldwide, used to make spandex, automotive plastics, running shoes and more. Achieved in first half of 2010, with 3,000-liter fermentations; announced in June 2010 (<http://www.genomatica.com/news/press-releases/genomatica-delivers-pilot-scale-validation/>). We have also done successful conversion testing with a color-free product that exceeds industry purification specs.
- b) Initiated *collaboration for demo-scale production* with strategic partner, to be announced Q1 2011. Partner has substantial experience in manufacturing scale-up of renewable chemicals.
- c) Achieved productivity, yield and titer at lab scale that would allow *cost competitiveness with best-cost-position in North America*, fully-depreciated petro-based chemical plants, if delivered at commercial scale. On track for metrics for *cost-advantaged* production in 2011.
- d) *Confirmed* ability of Genomatica technology platform to facilitate *rapid design and development* of organisms and processes.
- e) Signed *joint development and investment agreement* with strategic partner to create processes for making *chemicals from syngas derived from municipal solid waste*. Provided confirmation of potential of Genomatica's technology platform. To be announced Q1 2011.
- f) *Received patents* related to sustainable nylon (7,799,545) and low-cost chemicals (7,803,589). 25 patent applications published, including recent ones on production of BDO (WO 2010/141920) and downstream separations (WO 2010/141780). Over 100 patents filed.
- g) *Secured additional financing*, Q4 2010, including strategic partners. TBA Q1 2011.

Technology eligibility: Eligible for the small business award.

Focus area: Primary is *focus area 1*, greener synthetic pathways, due to Genomatica's use of only renewable feedstocks. Secondary is *focus area 2*, greener reaction conditions, as processes typically use 60% less energy, produce 70% less greenhouse gases, and proceed at lower temperature and pressure. Elimination of fossil fuels also reduces toxic and environmentally-disadvantageous substances.

U.S. component of technology development: All research and development has been in the U.S. at Genomatica's facilities in San Diego, California.

Abstract:

Genomatica is developing and commercializing sustainable intermediate and basic chemicals made from renewable feedstocks, including readily-available sugars, followed by biomass and syngas. The company aims to transform the chemical industry through cost-advantaged, smaller-footprint production of major industrial chemicals as direct replacements in a trillion-dollar global market. By greening intermediate and basic chemicals 'at source', thousands of downstream chemicals, derivatives and materials are made greener – with no change in their manufacturing processes. Initial lifecycle analyses from our first product show that Genomatica

chemicals will use about 60% less energy to produce, and have 70% lower CO₂ emissions. Genomatica was recognized as one of '10 Big Green Ideas' by Newsweek, featured in a 'most-read' Forbes.com article on transforming the chemical industry, and highlighted in Chemical Week's November 2010 cover story.

Genomatica is now moving to demo-scale production in the U.S. of its first commercial product, bio-BDO (1-4 butanediol), with a strategic partner to be announced in Q1 2011. The company expects early commercial production in 2012, followed by world-scale manufacturing plants starting in 2013. BDO, an intermediate chemical with a \$4 billion market worldwide, is used to make spandex, automotive plastics, running shoes and more. Genomatica has been successfully producing at pilot scale in 3,000-liter fermentations since the first half of 2010. Multiple large chemical companies have successfully used Genomatica's bio-BDO samples for downstream testing and processing.

Genomatica projects capital costs to be 50% lower, and operating costs to be 15-30% lower than petroleum-based processes, at commercial scale. Such economics allow for rapid adoption. During 2010, we achieved productivity, yield and titer at lab scale that would allow cost-competitiveness of our bio-BDO with the competing best-cost-position petro-based chemical plant in North America, if delivered at commercial scale.

Genomatica's unique integrated bio-process engineering platform and extensive intellectual property allow it to rapidly develop organisms and processes for dozens of intermediate and basic chemicals. This provides the potential to multiply economic and environmental benefits. As the chemical industry uses approximately 8% of the world's fossil fuels, Genomatica technology has the potential to reduce carbon emissions by hundreds of millions of tons per year.

1.0 Introduction: adherence to principles of green chemistry

Genomatica's technology directly supports many of the twelve principles of green chemistry:

- #7, *renewable feedstocks*: All Genomatica products use renewable feedstocks.
- #6, *energy efficiency*: Initial lifecycle analyses show that Genomatica chemicals, such as bio-BDO, will use approximately 60% less energy to produce, and have 70% lower CO₂ emissions.
- #2, *atom economy*, and #8, *reduce derivatives*: Genomatica's bio-processes are *direct, one-step* processes that result in the desired, major-existing-market intermediate or basic chemical rather than platform chemicals (e.g. succinic acid or 3-hydroxypropionic acid) that need added chemical processing steps. The processes are efficient, and without derivative waste.
- #12, *safer*: Genomatica's bio-processes are generally at lower temperatures and pressures than the petroleum-based processes they replace.

2.0 Science and innovation

Genomatica's first product, bio-BDO, provides the first-ever direct route to bio-catalytic production of BDO from renewable carbohydrate feedstocks.

Producing BDO biologically is challenging, in that it is highly reduced, and not produced naturally in any known organism. Genomatica's BDO is therefore one of the most complex metabolic engineering endeavors to date, and represents a breakthrough in renewable chemicals production. Once commercialized, this BDO process will be one of the first examples of a

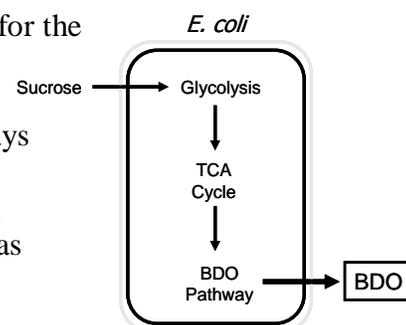
marketed industrial chemical produced in multi-billion pound quantities from petroleum-derived feedstocks being replaced by a complete biological process.

Approximately 2.8 billion pounds of BDO are manufactured globally for use in the production of solvents, fine chemicals and high-performance polymers such as spandex fibers for textiles and polybutylene terephthalate (PBT) for engineered plastics used in the automotive and electronics industries. More than 90% of spandex fibers are made from BDO, making BDO a key ingredient in most performance apparel. BDO is an important ingredient in the plastics that make up car dashboards, bumper fascia and under the hood components. The casings for many small home appliances require BDO derived plastics for durability. BDO is also a precursor to TPU, which is used for shoe soles and other rubber components. All current BDO production involves the use of fossil-derived raw materials such as acetylene, butane and propylene, and requires three to four additional energy-consuming transformations to convert these materials into BDO.

Genomatica's process takes advantage of the selectivity and efficiency of biological systems. Sugar is fed to an engineered strain of *E. coli*, where it is converted through glycolysis and the tricarboxylic acid cycle (TCA cycle) to metabolites that feed into a pathway of enzymes constructed from different organisms, leading to production of BDO. This process demonstrates that complex multi-step transformations can occur inside of cells to produce unnatural chemicals such as BDO in a single-unit operation. In this case, the single-unit operation is fermentation.

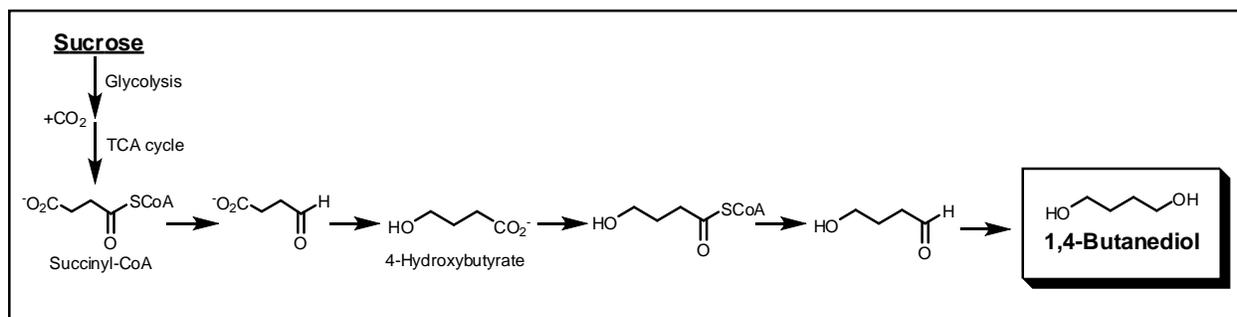
A systems-based metabolic engineering strategy was implemented to construct a strain of *Escherichia coli* capable of producing economically-viable titer, productivity and yield of this highly-reduced, non-natural chemical – and to do so directly, in a single step.

Computational methods were used to elucidate multiple novel pathways for the bio-synthesis of BDO from common metabolic intermediates, and two such heterologous pathways were introduced into *E. coli* to synthesize BDO from succinyl-CoA and α -ketoglutarate, respectively. The pathways were optimized through gene choice, operon arrangement, promoter and ribosome binding site variation, and codon usage. Guided by a patented, proprietary genome-scale metabolic model, an engineered *E. coli* host was developed to enhance anaerobic operation of the oxidative TCA cycle to generate reducing power to drive the BDO pathway and couple BDO production to growth.



BDO production has been demonstrated from glucose, xylose, and sucrose, as well as biomass-derived mixed sugar streams, demonstrating the versatility of this metabolic design. This work also demonstrates an integrated genome-scale computational approach to strain design and development that can enable novel bio-processes to produce many other important commodity chemicals that are not naturally produced by living cells.

Genomatica's process is distinct from bio-based chemical processes that produce succinic acid, which is then converted with traditional methods to other intermediate chemicals, including BDO. By producing the target chemical directly, Genomatica's process reduces unwanted byproducts. By involving fewer steps, the direct production method also reduces manufacturing complexity, capital and energy use. Most importantly, the simplified manufacturing process substantially reduces costs versus the production of BDO from succinic acid, leading to a more competitive final product and process.

Figure 1. A BDO pathway constructed and engineered into *E. coli*

Prior to Genomatica's breakthroughs, no organism had been discovered in nature or engineered to produce BDO from sugars. Three main challenges in developing a BDO production process were: 1) finding the appropriate set of enzymes for a functional BDO pathway; 2) engineering *E. coli* so that it has sufficient energy and reducing power to grow and produce the highly reduced BDO product; and 3) evolving *E. coli* to tolerate commercial levels of BDO. As shown in Figure 1, BDO pathways require up to four equivalents of NADH or NADPH. The pathway enzymes and strain designs, which were discovered through the use of Genomatica's computational modeling and simulation technologies, were engineered into *E. coli* for high-level production of BDO.

Natural metabolism of sugars such as glucose or sucrose via glycolysis and the TCA cycle provides central metabolites such as succinyl-CoA. The non-native pathway shown in Figure 1 permits conversion of succinyl-CoA to BDO through the function of five enzymes. In order to construct this pathway, Genomatica scientists had to screen many candidate enzymes on criteria such as yield, redox and ATP balance to identify those displaying the desired activity on the pathway intermediates. Since many of the pathway intermediates are not natural metabolites, up to 100 different enzymes had to be screened for certain steps. Once active enzymes were found, pathways were constructed on plasmids and assayed for BDO production. Different combinations of plasmids, genes, gene order, promoters and ribosome binding sites were screened to find functional pathways that provided the highest BDO yield.

After four months of intensive screening, several functional BDO pathways were identified. However, in order to increase yield and productivity, the strain had to be engineered to optimize the growth rate, the availability of reducing equivalents, and the carbon flux through the BDO pathway. OptKnock, a computational algorithm that is proprietary to Genomatica, was employed together with genome-scale metabolic models to identify strain designs that provide the most favorable intracellular conditions. OptKnock designs ensure redox and energy balance by coupling the growth of the organism to the production of the chemical of interest. Hence, by introducing specific gene knockouts, *E. coli* becomes dependent upon the BDO pathway to balance $NAD^+/NADH$ levels and to generate ATP required for survival. Introduction of these gene knockouts also greatly reduces or eliminates production of unwanted by-products, thus minimizing waste and increasing yield. Throughout, the close working relationship between the lab and the computational modeling operation is key to refining the engineered microorganism and accompanying process effectively and efficiently.

Genomatica also leveraged a unique adaptive evolution approach using proprietary machines that helped increase the tolerance of *E. coli* to commercially-viable BDO concentrations above 10%.

Through these engineering efforts, BDO production has been increased over 20,000 fold, and Genomatica is well on the way to hitting all commercialization targets. The BDO process has been validated in 3,000 liter fermentations. Smaller fermentations deliver over 70g/L titer. The fermentation broth is processed through cell separation, salt separation, water removal, and BDO purification. Samples are produced with greater than 99.5% purity, and there is no color exhibited in either the BDO or the downstream polymers produced from the BDO. Our fermentation process now is being scaled up to 12,000 liters and a fully-integrated demonstration plant for production of two tons per week of polymer-grade BDO. These demo studies will lead to the complete engineering package used for construction of commercial-scale bio-BDO plants, with the first plant expected to be built in the U.S. for operation by the end of 2013. In addition, BDO analytical testing has commenced with potential customers and sugar-derived BDO is being converted into downstream products such as PBT, polyurethanes, and spandex.

Genomatica's technology has been described in many peer-reviewed publications, issued patents and published patent applications, including the following examples:

Selected publications

Nature Chemical Biology: A detailed technical paper on Genomatica's technology and results has been submitted, and is currently in the review process.

Burk, M. J. (2010) Sustainable production of industrial chemicals from sugars. International Sugar Journal, January 10 issue.

Feist, A. M.; Palsson, B.O. (2008) The growing scope of applications of genome-scale metabolic reconstructions using *Escherichia coli*. Nat. Biotechnol. 26:659-667.

Burgard, A. P., Pharkya, P.; Maranas, C.D. (2003) OptKnock: A bilevel programming framework for identifying gene knockout strategies for microbial strain optimization. Biotechnology and Bioengineering 84:647-657.

Patents

Patent number 7,799,545, Microorganisms for the production of adipic acid and other compounds, issued 9/21/2010

Patent number 7,803,589, Methods and organisms for utilizing synthesis gas or other gaseous carbon sources and methanol, issued 9/28/2010

PCT patent number WO 2010/141920, Microorganisms for the production of 1,4-butanediol and related methods, published 12/9/2010

US patent number 7,127,379, Method for the evolutionary design of metabolism, issued 10/24/06

PCT patent number WO 2008/115840A2, Compositions and methods for the biosynthesis of 1,4-butanediol and its precursors, published 09/25/08

US patent appl. 2009/0075351, Compositions and methods for the biosynthesis of 1,4-butanediol and its precursors, published 03/19/09

PCT patent number WO 2009/023493A1, Methods and organism for growth-coupled production of 1,4-butanediol, published 02/19/09

Details on [25 published applications at WIPO](#) (click link).

3.0 Human health and environmental benefits

Genomatica's bioprocesses for production of intermediate and basic chemicals provide important benefits to health and the environment, when compared to traditional, petrochemical processes.

From an environmental perspective, petrochemical processes contribute to depleting global fossil fuel reserves, require large amounts of energy to convert oil and natural gas to chemical products, use large quantities of hazardous and volatile organic solvents, produce toxic chemical waste by-products, and emit large quantities of greenhouse gases such as methane and carbon dioxide. Genomatica processes, such as for bio-BDO, use 100 percent renewable feedstocks. For each plant that produces 100 million lb per year of BDO, the Genomatica process will eliminate the need for over 100 million pounds of petroleum-based feedstock like acetylene, butane or propylene. Detailed process design and preliminary life cycle analysis (LCA) indicates that BDO production from sucrose requires 59 percent less fossil energy relative to the commonly-used Reppe process, which uses acetylene for BDO production.

As shown in Figure 1, the bio-based BDO pathway actually consumes carbon dioxide through the conversion of phosphoenolpyruvate to the TCA cycle metabolite oxaloacetate and one carbon of bio-based BDO derives from this carbon dioxide. Preliminary LCA indicates that bio-based BDO produced from sucrose will reduce CO₂ emissions by at least 68 percent relative to petrochemical BDO production. The most abundant material used in the bio-based BDO process is natural water, which is recycled – no organic solvents are employed for bio-based BDO. By contrast, large quantities of synthetic organic solvents and flammable raw materials are transported and used to produce petroleum-based BDO.

The bio-based BDO fermentation process requires simple equipment and operates at near-ambient pressure and temperatures, providing a safer environment for workers relative to petro-BDO facilities that require higher pressures and temperatures.

4.0 Applicability and impact

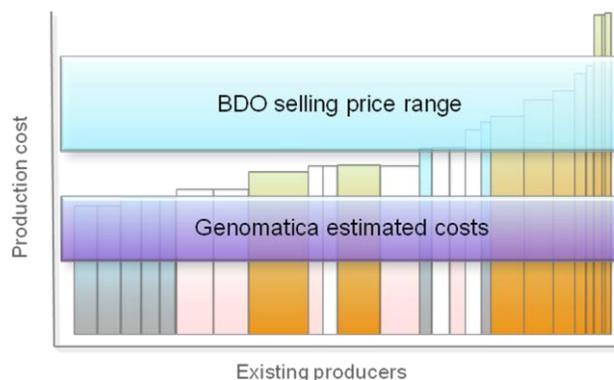
Genomatica's technology provides a practical, highly cost-effective approach to green chemistry. The improved economics, green/environmental benefits, and applicability to dozens of basic and intermediate chemicals at the core of the chemical industry, suggest broad applicability.

- a) *Lower capital:* Plants built using Genomatica's technology are estimated to cost significantly less than the most widely available competing petro processes. Below, estimates for BDO:

Capex, 100M lb plant	
Genomatica	\$110M
Huntsman-Davy	\$218M
Reppe-BASF	\$347M

- b) *Lower operating costs:* We estimate operating costs will be 15-30% lower than petro plants for BDO. Genomatica has achieved productivity, yield and titer at lab scale that would allow cost competitiveness with best-cost-position in North America, fully-depreciated petro-based chemical plants, if current results are delivered at commercial scale plants. Genomatica is on track for demonstrating metrics consistent with cost-advantaged production during 2011.

Genomatica: cost-advantaged production of intermediate and basic chemicals from renewable feedstocks



- c) *Enables smaller plants:* Because Genomatica plants are less capital-intensive, plants can be ‘right-sized’. That means they can be located closer to feedstock sources and/or to downstream plants, reducing fuel used for and emissions from transportation.
- d) *Feedstock flexibility:* Genomatica has already shown production from glucose, xylose, sucrose and biomass-derived sugar streams. This means plants can use whatever feedstock makes most sense in a geography, further reducing costs and environmental footprint.
- e) *Direct production reduces costs:* Genomatica technology carries an important advantage over bio-processes that produce so-called ‘platform’ chemicals. For example, some bio processes are being developed to produce succinic acid. That succinic acid then requires additional chemical processing steps to convert it to larger market chemicals such as BDO, with increases in operating cost, capital equipment cost and environmental footprint. Eliminating intermediates and chemical solvents also reduces unwanted byproducts.

In summary, we expect that Genomatica’s bio-BDO will be competitive at oil prices of \$45 per barrel or natural gas prices as low as \$3.50 per million BTU.

Genomatica’s unique integrated bio-process engineering platform and extensive intellectual property allow it to rapidly develop organisms and processes for intermediate and basic chemicals. This provides the potential to multiply the economic and environmental benefits. As the chemical industry uses approximately 8% of the world’s fossil fuels, Genomatica technology has the potential to reduce carbon emissions by hundreds of millions of tons per year.

5.0 Additional materials



Genomatica’s fermentation broth for BDO, proven at pilot scale.



The first-ever renewable BDO, which major chemical companies have converted to samples of downstream polymers.



Drums of Genomatica’s bio-BDO.



Project Title: NEXAR™ Polymer Membrane Technology

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Contributors:

Kraton Polymers LLC is the sole contributor in the development of the NEXAR™ polymer technology and maintains ownership and responsibility for all technical data reported and all associated financial sponsorship.



Project Title: NEXAR™ Polymer Membrane Technology

Milestones for the Nexar™ Technology

- The initial development and demonstration of the first sulfonated pentablock copolymer was completed in 2007.
- Patent applications were filed between 2006 and 2010 globally, including application specific patents in ERV and performance fabrics. Patents have been issued in South Africa and US.
- First pilot production occurred in 2009, and first successful large scale production (ca 10 metric tons) was completed in third quarter of 2010.
- The first commercial sale occurred in 2009.
- Application specific validation process started in 2010 to demonstrate Nexar™ polymers' superior performance and environmental benefits. Examples include successful lamination of Nexar™ polymers onto substrates, ERV core construction and testing, and others.

Nomination Categories

Focus Area - *Design of Greener Chemicals*.

Focus Area – *Greener Synthetic Pathways*.

Research and Development

Kraton Polymers LLC has conducted the majority of research and development related to this project within the USA at their research facility located at 16400 Park Row, Houston, Texas.

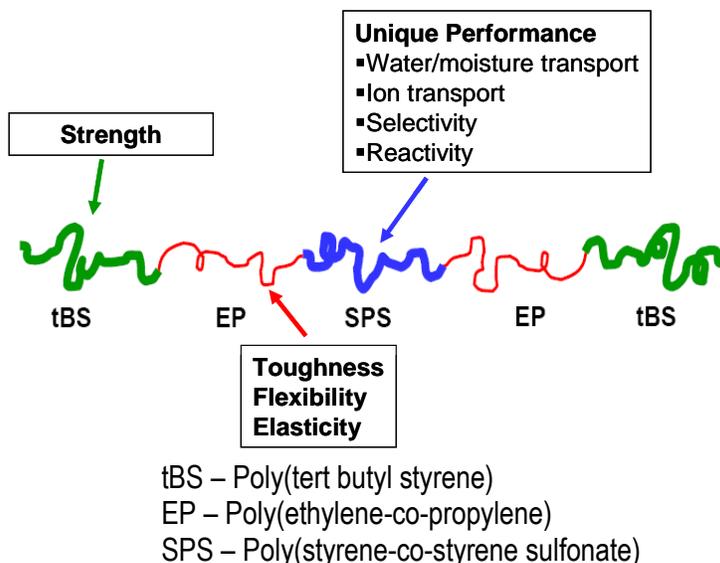
Abstract

The production process of Nexar™ polymers uses less hydrocarbon solvents (by up to 50%) and completely eliminates the need for halogenated co-solvent. Moreover, Nexar™ technology offers enhanced performance and great environmental benefits for a wide variety of different applications. Some are listed below:

- **Reverse Osmosis.** Nexar™ polymers have exceptionally high water flux, up to 400 times higher than current reverse osmosis membranes. This could translate into significant reduction in energy consumption and materials usage.
- **Electrodialysis.** Higher mechanical strength of Nexar™ polymers makes it possible to use thinner membranes than what's currently used in electrodialysis, translating into significant reduction in material usage. More importantly, Nexar™ polymers eliminate the use of poly(vinyl chloride) or PVC in current electrodialysis membranes.
- **Humidity Regulation.** For energy recovery ventilation (ERV) systems, ERV core made of Nexar™ membranes has significantly higher energy recovery efficiency. For other humidity regulation applications, where halogenated products like Nafion® and polytetrafluoroethylene are often used, Nexar™ polymers offer environmental benefits by completely eliminating halogenated products.

Technical Description of NEXAR™ Polymers

Kraton Polymers LLC (Kraton) was the original inventor of styrenic block copolymer (SBC) chemistry in the 1960s.¹ Kraton continues its commitment to engineering precision polymer structures in partnership with innovators worldwide. Nexar™ polymer is a new family of pentablock copolymer. The innovation with the Nexar™ polymer family lies with the unique design in that only the middle block is selectively sulfonated.² The following schematic shows the structure and key characteristics of Nexar™ polymers.



The precursor polymer prior to sulfonation is a poly (t-butyl styrene-b-(ethylene-r-propylene)-b-styrene-b-(ethylene-rpropylene)-b-(t-butyl styrene) copolymer)(tBS-EP-S-EP-tBS). The styrene block is selectively sulfonated via acyl sulfate chemistry. The amount of sulfonation of the styrene repeat units may be adjusted from 10% to 100% based on the requirements of the end use application. To date, sulfonation levels (ion exchange capacity or IEC) from 0.4 to 2.0 meq/g polymer were made. Two grades are currently commercially available both as a solution and membrane: MD9200 (2.0 IEC) and MD9150 (1.5 IEC).

Kraton Nexar™ family of polymers qualifies under EPA's TSCA polymer exemption requirements (40 C.F.R. § 723.250) as it is considered a polymer of low concern from a human health and environmental standpoint. The chemistry has a high molecular weight with no more than 0.5% in the molecular weight range defined as oligomers. Kraton has not requested a CAS # designation to date. Kraton has also initiated registration for the sulfonated polymers under requirements for polymers of low concern in China, Japan and Korea and will be completed in 2011.

¹ Kenzie Nozika, Block copolymers, *US Patent 3189664*.

² Willis; C.L., Handlin, D.L., Trenor; S. R., Mather; B. D.; Sulfonated block copolymers, method for making same, and various uses for such block copolymers, *US Patent 7737224*.



The Nexar™ sulfonated polymers are considered to exhibit similar toxicity profiles as the parent polymer precursors (SBS), which is essentially non-toxic. The SBS family of materials is considered to be non-irritating. The acute oral toxicity reports an LD-50 of >5000 mg/kg and acute dermal also with low toxicity of >2000 mg/kg dermally. The United States Pharmacopoeia (USP) biological reactivity tests provide an assessment of the potential biological effects and systemic toxicity of polymers used in containers (USP Reference 661). A polymer that meets the requirements of the most severe set of criteria (Class VI) may be considered for use in food and drug containers that have direct contact with its contents. Nexar™ polymer precursors have passed the *most severe* USP test requirements at the highest extraction temperature of 121°F (50°C). This testing is considered the ‘worst case’ scenario for potential extractables and their toxicological effect on animals. Kraton has also obtained FDA acceptability for the polymer precursor as articles or as components of articles intended for use in contact with food.

Production of NEXAR™ Polymers

The overall production chemistry and fabrication of the end product membrane is performed in an environmentally friendly manner. The solvents produced are captured and recycled and spent membrane can be recycled as the base polymer structure is essentially a polyalkyl styrene, a class of polymers that is widely used and recycled from consumer products. Kraton Polymers has improved upon the synthesis of sulfonated block copolymers through the total elimination of halogenated hydrocarbons from the manufacturing process which has significant environmental and human health benefits. In addition, the unique structure allows the process to use far less (by up to 50%) aliphatic hydrocarbon in the total synthesis

Performance and Environmental Benefits of Nexar™ for Various Applications

Reverse Osmosis

Almost 40% of the world’s population (roughly 2.3 billion people) lives in water-stressed areas and projected to increase to roughly 3.5 billion by 2025.^{3, 4} The need for clean water has been driving the rapid growth of desalination markets, currently at 12% annual growth rate. Unfortunately, today’s dominant desalination technology, reverse osmosis (RO), has many negative impacts on our environment. Current RO membranes are made of cross-linked aromatic polyamides, which have very low water flux. The low water flux of current RO membranes requires high pressure and lots of membranes to produce enough clean water, which translates into high energy consumption and high materials usage. Energy cost for a RO plant can be as high as 40% of the total cost.⁵

Nexar™ polymers have exceptionally high water flux, up to 400 times higher than current RO membranes. Replacing current RO membranes with Nexar™ membranes could result in

³ Service, R. F., *Science*, **313**, 1088, (2006).

⁴ Fritzmann, C., et. al., *Desalination*, **216**, 1, (2007).

⁵ Moulin, P., et. al., *Water Research*, **43**, 2317, (2009)

significant reduction in energy consumption as well as materials usage. We did some modeling work to compare a RO plant with Nexar™ system to a current RO plant. ⁶ For a medium size RO plant to produce 20 million gallons of clean water per day, 2500 standard membrane cartridges (400 square feet each standard cartridge) are needed. With Nexar™ system, only 653 standard cartridges are needed, resulting in more than 70% savings in membrane cost, as well as more than 50% energy savings. The model has very conservative assumptions. For examples, it only assumes that water flux for Nexar™ polymers is 200 times higher than current RO membranes, while we have lab data showing as much as 400 times higher water flux. The model also makes conservative assumptions that active layer thickness of Nexar™ system is twice the thickness of current RO system. We may be able to achieve thinner active layer thickness, thus more energy savings and materials usage reduction. The table below summarizes the key benefits arising from our modeling of Nexar™ system.

A medium size desalination plant operated at 150 psi to produce 20 million gallons water per day		
	Nexar system	Current RO system
Feed NaCl (ppm)	2000	2000
System salt rejection	98%	98%
Water flux (GFD.µm)	400	2
Active layer thickness (µm)	0.2	0.1
# of standard cartridges needed	653	2500
Total cartridge cost	\$261,200	\$1,000,000
Cartridge savings	74%	
Energy savings	59%	

Electrodialysis

Another potential application of Nexar™ polymers is electrodialysis, which has been used for a variety of different applications, such as brackish water desalination, salt recovery, waste acid recovery, and ultrapure water. Key requirements for electrodialysis are conductivity, permselectivity and mechanical strength. Due to its unique chemical structure, Nexar™ offers much better mechanical strength than traditional ionomers, while maintaining more or less the same conductivity and permselectivity. Higher mechanical strength of Nexar™ polymers make it possible to use thinner membranes, resulting in reduction of material usage, as well as better performance. Energy loss due to membrane resistance is determined by area resistance. The lower the area resistance, the less energy loss. As shown in the following table, ⁷ Nexar™ membranes offer better performance (lower area resistance) with less material. We are in the early stage of research for electrodialysis, and further improvement is expected.

⁶ Nexar system is different from traditional RO plant in design. A traditional RO plant has cartridges in parallel, while cartridges in Nexar system will be both in parallel and series.

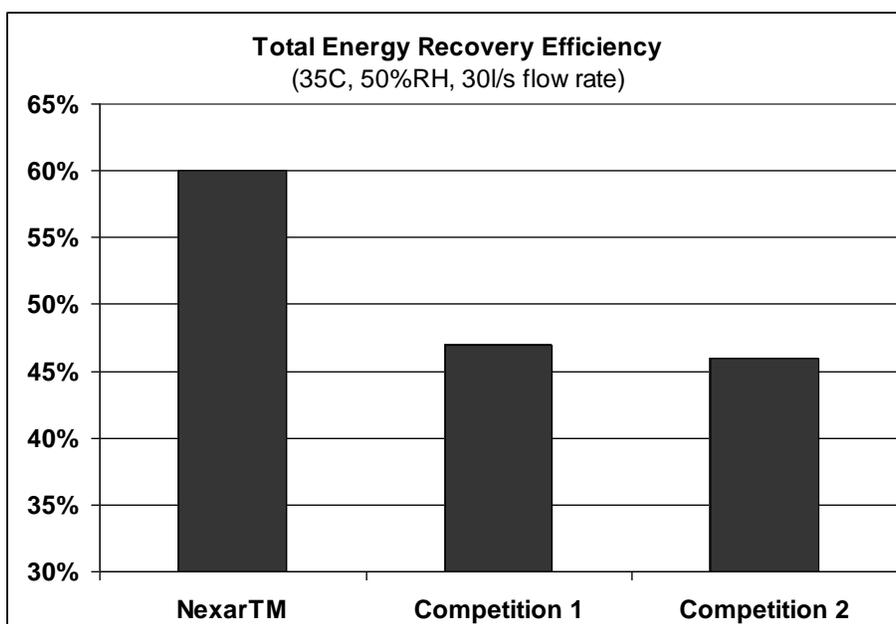
⁷ Permselectivity is measured in 0.1M and 0.5M KCl, while area resistance is measured in 0.5M NaCl solution.

	Nexar™ Membrane	Traditional Electrodialysis Membrane
Permselectivity	94.4%	96%
Conductivity (mS/cm)	2.6	3.5
Membrane thickness (µm)	40	100
Area resistance (Ω.cm ²)	1.5	2.9

More importantly, Nexar™ polymers eliminate the use of poly(vinyl chloride) or PVC in current electrodialysis membranes. Most of current electrodialysis membranes are made by mixing ionomers with PVC powder and then molding into a sheet.⁸ Without PVC, most ionomers are too weak to be usable. Nexar™ membranes completely eliminate the need for PVC.

Humidity Regulation.

Humidity regulation plays a very important role in our daily life, and one of the important humidity regulation applications is energy recovery ventilation (ERV). ERV is the energy recovery process of exchanging the energy contained in exhaust air and using it to treat the incoming air in HVAC systems. ERV not only reduces the energy consumption for cooling and heating, but also improves indoor air quality. In general, higher moisture transport rate is believed to correlate to higher latent heat transfer. Since Nexar™ membranes have excellent moisture transport rate, they should perform very well in an ERV system. In fact, when we tested an ERV core made of Nexar™ membranes, it outperformed its competition. The chart below demonstrates Nexar™ membranes superior energy recovery efficiency,⁹ which could translate into more efficient HVAC systems.

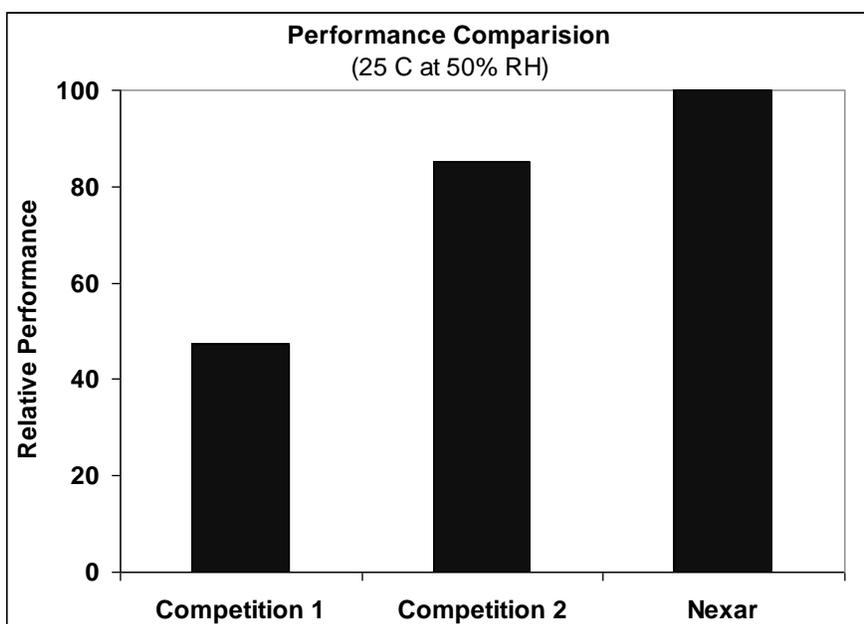


⁸ Baker, R.W., Membrane Technology and Applications, 2nd Et., 2004, John Wiley & Sons,

⁹ The data were generated at an independent testing lab, Exova. The data shown here are for the cooling mode.

There are many other humidity regulation applications. Unfortunately, halogenated materials are often used. For example, Nafion[®] tubes¹⁰ are used for air drying applications, and microporous polytetrafluoroethylene (PTFE)¹¹ are used for performance fabrics. Nexar[™] polymers could potentially replace those halogenated products.

The graph below¹² shows the relative performance between Nexar[™] membranes and halogenated microporous membranes currently used in high performance fabrics. The high moisture transport rate of Nexar[™] membranes allows large amounts of moisture to be moved away from the skin very quickly to maintain a comfortable microclimate for the human body. As the human body's temperature rises, the polymers breathability performance allows the membranes to work better and become more efficient. Other benefits of Nexar[™] membranes in performance fabrics include flexible processing freedom using current commercial equipment, high strength in both wet and dry environments.



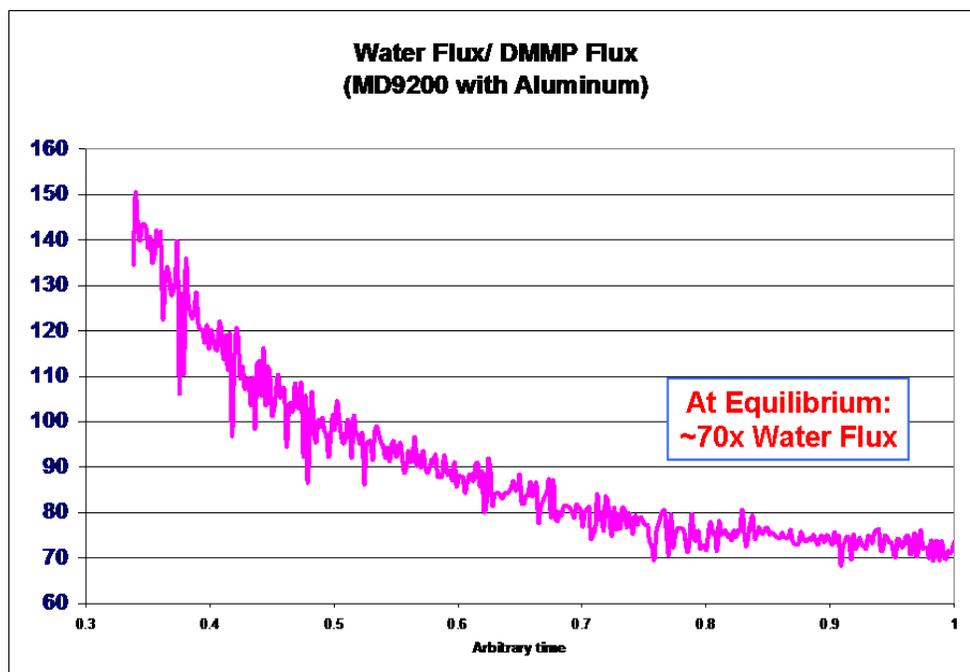
It is worthy to note that unlike other microporous membranes, Nexar[™] membranes are nonporous. The dense nature of Nexar[™] membranes eliminates the potential for pore clogging and loss of performance as is the case for microporous membranes. More importantly, the dense nature of Nexar[™] membranes ensures the capability to block many toxins and pathogens, which is critical for applications in military and medical clothing. For example, when Nexar[™] MD9200 (neutralized with aluminum) was tested against dimethyl methylphosphate (DMMP), a sarin gas stimulant, Nexar[™] performed very well. The data in the following graph show very

¹⁰ To see an example, visit website <http://www.permapure.com/company/our-technology/>

¹¹ To see an example, visit website http://www.gore.com/en_xx/technology/timeline/applications_eptfe.html

¹² The comparison is expressed in relative unit (the best performance = 100). The raw data are from inverted cup test: upstream is liquid water, downstream is air with 50% RH, air flow is regulated by a fan operated at 12V.

good selectivity between water vapor and DMMP,¹³ indicating that NexarTM membranes might be able to provide protection against toxins like sarin gas while maintaining breathability.



Summary

The production process of NexarTM polymers uses up to 50% less hydrocarbon solvents and completely eliminates the need for halogenated co-solvent. NexarTM technology not only offers enhanced performance for a wide variety of different applications, such as reverse osmosis, electrodialysis, humidity regulation, and others, but also provides environment benefits, such as saving energy, reducing materials usage, and eliminating halogenated products. It is these attributes that make NexarTM polymers an environmentally conscious choice.

¹³ Zukas, W.X., Schneider, N.S., "Permeation and Diffusion in Ion Containing Polymers", Presented at the 2009 Chemical and Defense Science and Technology Conference, Dallas, TX, 19-Nov-2009

Water based Alkyd Technology (LAAD)

December 29th, 2010

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The United Soybean Board- Sponsor

Water based Alkyd Technology

Novel Water based Alkyd Technology was developed by The Sherwin-Williams Company based on the concept of green chemistry and engineering. This technology was successfully scaled-up and commercialized in key Sherwin-Williams coatings in 2010. These products are sold through the Sherwin-Williams stores located across United States of America.

The following products based on the Water Based Alkyd Technology abbreviated as LAAD (Low VOC Alkyd-Acrylic Dispersion) were launched nationally in USA in 2010.

- a) "Proclassic" Smooth enamel finish comparable to traditional oil based coatings for trim doors cabinets, custom woodwork, high end residential and commercial interior coatings at < 50 gms/l VOC formula compliant nationwide. Water based alkyd semi-gloss system.
- b) "Promar 200" Interior water based semi-gloss paint for production focused commercial contractors- spray and roll less than 100 gms/l compliant nationwide. Non-yellowing technology.
- c) "Industrial Maintenance water based alkyd High gloss enamel" for Direct to Metal coating Primer and Topcoat with excellent humidity resistance and corrosion resistance.
- d) Several new Products with LAAD technology are being formulated using it as a booster to improve adhesion, flexibility and gloss in Low VOC Water based coating

About 500,000 gallons of paint based on this technology was made and commercially sold in the market. The volume of paint based on this technology is expected to increase to about 5 MM gallons annually with replacement of non compliant solvent containing alkyds with this nationally compliant water based alkyd technology which utilizes renewable resources and recycled material.

- The US patent # 7,129,278 covering the The Sherwin-Williams Water based Alkyd technology (LAAD) was issued in 2006.
- This nomination is neither eligible for award in either the academic category or the small business category.
- The nominated technology covers all of the three focus areas of green chemistry a) Use of greener synthetic pathways. Utilizing a combination of renewable green raw materials based on Soy and recycled plastic (Polyethylene Terephthalate) to create a bio-based Water borne Polymer technology b) Using Greener reaction conditions- Replacing petroleum based solvents with Soybean oil used as a reactive diluent/solvent during process and manufacturing c) Design, development and implementation of the Green Water based Alkyd (LAAD) Polymer technology in key Architectural and Industrial Maintenance Products. LAAD is a novel and hydrolytically stable water based alkyd technology with the best performance and value added benefits of both alkyd and latex technologies for nationally compliant paints and coatings. The LAAD technology offers the best oil based performance and application in a water based system. This Green Water borne Polymer technology is replacing Solvent based alkyds which are becoming non compliant because of higher VOCs. More than 60 % of the components of LAAD Technology are coming from renewable and recycled materials with less solvent , easier to handle and nationally compliant.

- All of the activities for research and development occurred within The Sherwin-Williams Company's United States locations. Coatings based on this Technology are also manufactured within USA

Abstract: The recent record high cost of petroleum and oil based raw materials, as well as uncertain availability due to increased demand overseas, has made complete dependency on synthetic raw materials questionable. There is a strong need to find different materials, preferably based on renewable resources. Furthermore the changing and tightening VOC regulations set forth by OTC (Ozone Transport commission) and SCAQMD (South Coast Air Quality Management District) will necessitate changes in formulation of architectural and industrial coatings. These regulations have necessitated a search for alternate VOC compliant technologies, which perform like the non complying solvent-borne technologies. Today, latex emulsions dominate water borne coatings; whereas as the solvent borne coatings are dominated by alkyds. Each of these technologies has challenges in meeting the performance and application properties of Solvent borne coatings. To address this challenge, The Sherwin-Williams Company developed a novel a low VOC Water based Alkyd Technology by utilizing the concepts of sustainability and green (naturally occurring resource materials). The Water based Alkyd technology was designed to meet key performance attributes of Solvent based Alkyds but at lower VOCs and with excellent hydrolytic stability similar to latex paints for architectural and industrial maintenance coatings applications.

Coatings formulated from The Sherwin-Williams Water-based Alkyd Technology (LAAD) perform like the conventional solvent-based alkyd paints with high gloss, excellent adhesion, excellent moisture resistance and hydrolytic (shelf) stability. This "no surfactant" technology enables alkyd like properties with water clean up and less odor than currently available conventional and high solids alkyd and latex technologies. In addition, because of the gloss enhancing and excellent adhesion characteristics, the LAAD technology could be used as a secondary binder or as a booster emulsion to improve film formation and improve performance properties of conventional and upcoming latex coatings.

Sherwin-Williams Water based Alkyd Technology (LAAD CHEMISTRY)

Recycled PET (polyester) and soybean oil were used along with commonly available raw materials to create a Waterborne Alkyd technology without any surfactants and with low levels of solvents for formulating into value added water based VOC compliant Architectural and Industrial Maintenance Paints and Coatings.

The Polymer reaction steps are:

- a) Synthesize a solvent less Soy PET Alkyd,
- b) Acrylic grafting of the Soy PET Alkyd in Soy Oil.
- c) Dispersion of Soy-PET Acrylate Prepolymer in water

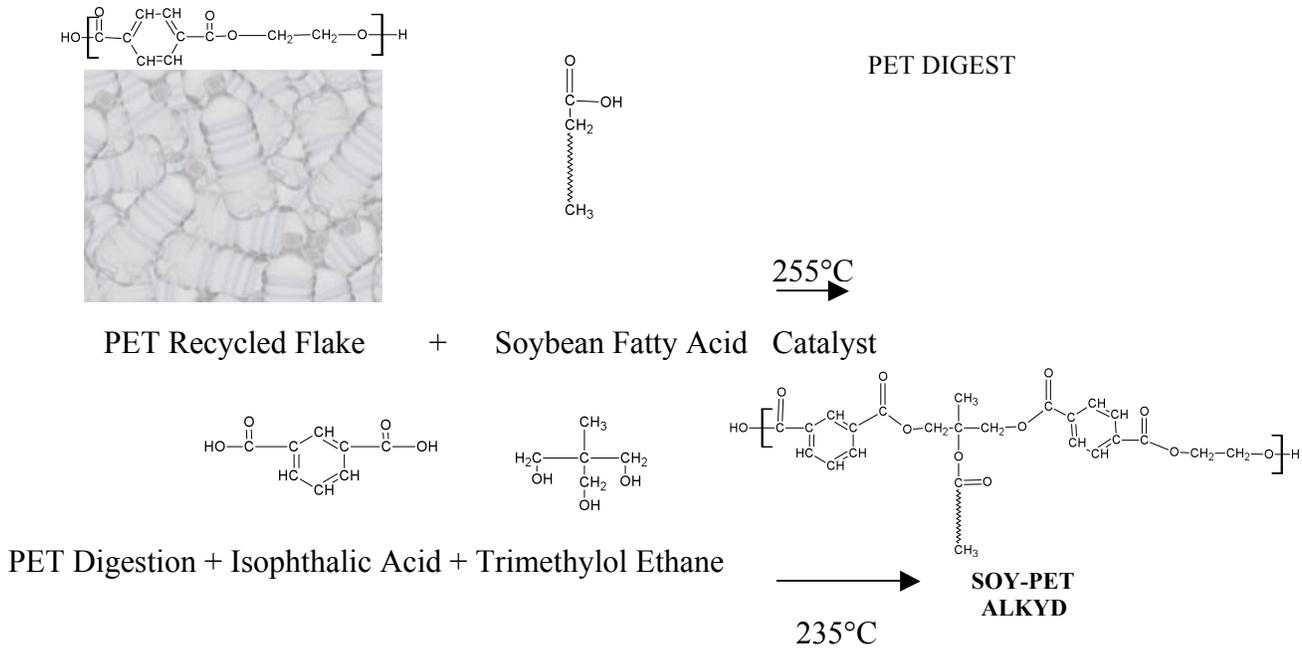
The following steps were carried out to make the LAAD Polymer:

- 1) A controlled digestion of PET with Soya fatty acid with the resulting fatty acid terminated PET units converted into liquid soy functional PET Alkyd by reacting with polyols.(25% PET and 50 % Soy)
- 2) The Soya PET Alkyd is grafted with hydrophobic and hydrophilic acrylic monomers by graft polymerization in presence of Soybean oil which functions as a reactive diluent.

- 3) The anionic pre-polymer was dispersed in water using an amine under high shear conditions. Under these conditions, the polymer inverts from water-in-oil to oil-in-water emulsion.

The resulting novel polymer dispersion has hard PET segments, which bring rigidity, hardness and Hydrolytic resistance. The acrylic functionality for improving dry times and durability and Soya functionality to help in film formation, gloss, flexibility and cure.

I. Soy PET Alkyd



II. ACRYLIC GRAFTING OF SOY PET ALKYD



III. DISPERSION OF ALKYD ACRYLIC



Waterborne Low VOC Alkyd Acrylic Dispersion (LAAD)

Non Volatile Material: 42 % (Water)
 Viscosity: < 2000 cps
 pH: 7.6-8.0
 Dry Tack free: < 30 minutes
 Hydrolytic Stability: > 3 months at 120 °C

Targeted Properties	Specifications	Pigmented Prototype
Viscosity (KU)	90-100	98
Weight per gallon	9.60	9.60
SAG	12 min.	12
pH	8.5 – 9.5	9.0
Gloss @ 60	>80 min.	85
Dry Time	< 4 hrs	2 hrs
Adhesion (Tape)	Tape 5B	5B

Benefits of LAAD Technology

This robust waterborne technology has all the value added performance properties of alkyds but with water clean up and outstanding hydrolytic shelf stability – a feature not seen in Polyester functional technologies like alkyds. Because the soybean oil is the solvent, the technology has low odor and low VOC- a desired feature for consumers. This Technology will eliminate pollution and solvent clean up problems experienced from typical oil based solvent borne alkyd products.

In 2007, 500 MM gallons of Solvent borne Coatings were used. It is expected that replacing conventional alkyd resin technology with The Water based Alkyd Technology will enable

- Up-cycling of about 250 Million lbs of Recycled PET per year.
- Utilize about 320 MM lbs of Soybean oil /year– Helping The United States agro economy and Soybean farmers.
- Replace over 1.0 million barrels per year of crude oil from the solvents and oil based alkyd polymers it can replace.
- Reduce 800 Mm lbs/year of VOCs going into environment and potentially contributing to Ozone depletion.
- About 500,000 gallons of paint based on this technology was commercially manufactured and sold in the market in 2010. The products incorporating this technology sold in the market are Proclassic, Promar 200 and Industrial Maintenance Waterborne Alkyd Enamel. These products have been very well received and accepted in the market by Professional contractors and DIY. It is expected that LAAD Technology will grow by replacing non compliant Alkyd Technologies.

The Waterborne dispersion technology (LAAD) was compared to the other currently available and prior presidential Green Chemistry award winning technologies. The comparison is shown in the tables and the spider diagram which elucidates the value added features compared to Latex, Alkyd, Sucrose ester (Procter & Gamble) and the Reactive Coalescent RC-Sun (ADM).

PERFORMANCE COMPARISON OF TECHNOLOGIES (Table I)

Performance Property	ALKYD	LATEX	HS Sucrose Ester (Procter and Gamble)	RC-Sun Reactive Coalescent (ADM)	Water based Alkyd (LAAD) (SW)
VOC	1	3	2	5	4
Exterior Durability	1	5	2	3	4
Yellowing	1	5	2	3	4
Application	5	2	4	3	4
Gloss	5	1	3	2	4
Adhesion	5	2	3	1	5
Total	18	18	16	19	25

Rating Scale: 5= Best, 1= Worst

The Waterborne Alkyd technology (LAAD) delivers the key performance attributes of conventional solvent borne Alkyd technologies, while delivering on lower VOCs, Exterior Durability and Yellowing. LAAD Technology outperforms latex technology in Gloss, Application and Adhesion properties. LAAD technology was compared to some noteworthy VOC compliant technologies introduced recently in the market. The LAAD technology outperforms solvent borne high solids Sucrose Ester technology (Procter and Gamble) in all of the performance properties. In addition, it has water clean-up attributes. The RC-Sun Coalescent Technology from ADM is a latex booster technology and fared similar to conventional latex technology in performance. LAAD outperformed RC-Sun technology in commonly tested systems. The water based Alkyd technology (LAAD) offers a balance of desired performance properties either as a sole binder or as a booster binder to enhance gloss, adhesion without compromising VOC of coating.

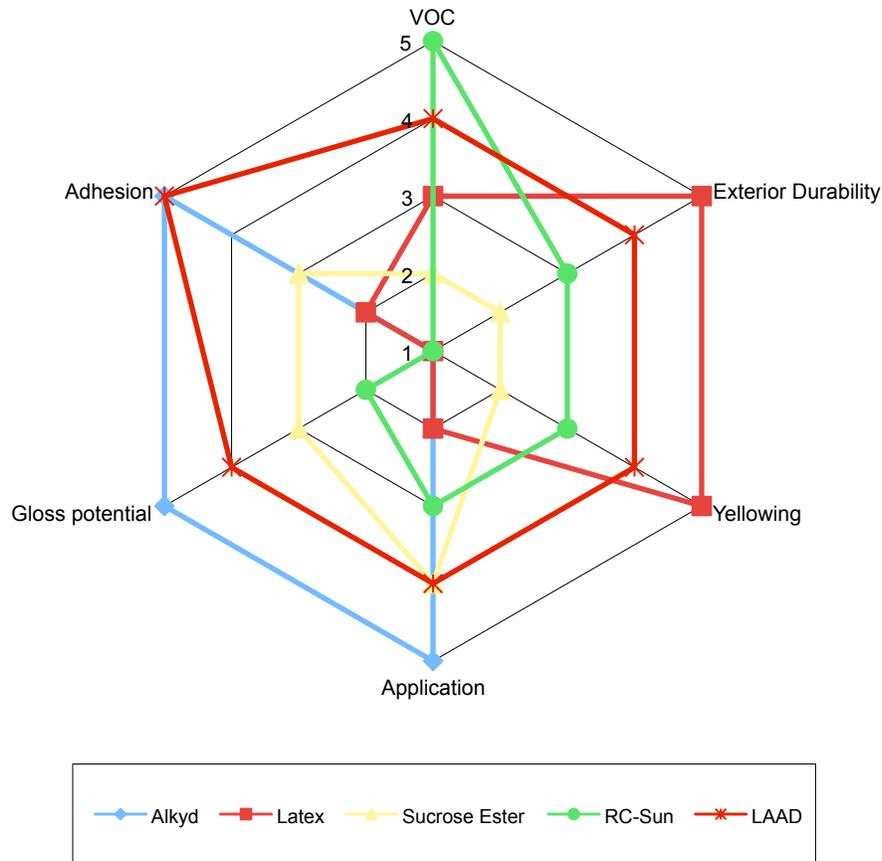
COST COMPARISON OF TECHNOLOGIES (\$/dry lb) Table II

Technology	ALKYD	LATEX	HS Sucrose Ester	RC-Sun-Coalescent	LAAD Technology
VOC Compliance	NO Exceedance Fee to be paid	YES	YES	YES	YES
COST	0.80	1.0	1.30	1.20	1.15

The costs mentioned in Table II are comparative and normalized for disclosure. The lower costing Alkyd and latex technologies lack performance properties as shown in Table I. The VOC Compliant and Performance effective LAAD Technology brings value to replace non compliant Solvent borne alkyd coatings.

SPIDER DIAGRAM COMPARISONS OF TECHNOLOGIES

TECHNOLOGY COMPARISONS



The LAAD Technology offers the best balance of all performance properties. The Technology shows outstanding adhesion, hydrolytic resistance and exterior durability as an Alkyd Binder. It applies like an oil based Alkyd and with less yellowing than any of the current alkyd technologies including the Sucrose ester from Procter and Gamble. It outperforms latex technologies and RC-Sun because of its unique Solvent like performance in a water based paint.

The LAAD technology is surfactant free and environmentally friendly .It is based on utilization of renewable resource based on soy and recycled plastic- Polyethylene Terephthalate (PET). In addition Soybean oil was used as a reactive diluent in place of solvents thereby further reducing ecological impact. This Waterborne alkyd technology performs and applies like conventional Alkyds but has water clean up and VOC compliance nation wide. The low odor and non yellowing feature makes it appealing for Interior coatings.

Conclusion: A novel green water based Alkyd technology (LAAD) with the best performance attributes of both alkyd and latex technologies was developed, manufactured and commercially launched in several key Sherwin-Williams Architectural, Industrial Maintenance product lines in 2010. More products based on this technology will be launched in the near 2011/2012. This national VOC compliant performance delivering water based technology has started replacing conventional non VOC compliant Solvent borne alkyds. This water based technology has delivered customer needs of performance and application, regulatory needs of national VOC compliance while applying the principles of green chemistry and engineering and delivering a series of robust commercial coatings products to benefit the environment. More products are expected to be launched in the next few years based on the LAAD technology. This technology will be leveraged for other coatings applications and can potentially eliminate about 200 mm lbs of VOC or 25 MM gallons of Solvent emissions/ year. This will also up cycle 10-12 Mm lbs of recycled PET/ year.

The Water based Alkyd technology (LAAD) developed based on the principles of green chemistry is proving to be a technical and commercial winner for Sherwin-Williams Company. This Technology is providing key desirable performance and application features for a whole range of nationally compliant products for the future.

An Efficient Biocatalytic Process for Simvastatin Manufacture

Nominated for the 2012 Presidential Green Chemistry Challenge Award

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An Efficient Biocatalytic Process for Simvastatin Manufacture

Recent milestones

- Proof of concept demonstrated and process optimized 2007
- Optimized process using further evolved enzyme (Codexis) 2009
- Simvastatin-ammonium produced biocatalytically at 400 kg scale 2010
- Enzyme manufacturing scaled up to 150-200 kg batch scale 2010
- Process and Composition of Matter patent applications published 2011
- Process has been scaled up and >10 mT simvastatin produced 2011

Eligibility

The nominated technology was co-developed by Prof. Tang and Codexis. Prof. Tang is eligible for the academic award, and Codexis is eligible for the award in Focus Area 1, “An industry sponsor for a technology that uses greener synthetic pathways”.

Focus Areas

This technology can be classified into all three focus areas: the use of greener synthetic pathways, the use of greener reaction conditions, and the design of greener chemicals.

Location

The new simvastatin manufacturing process was conceived and enabled in the United States and then scaled-up in Austria (enzyme manufacture) and India (simvastatin manufacture).

Abstract:

Simvastatin is an important drug for treating cardiovascular diseases. Simvastatin is a semi-synthetic derivative of lovastatin, a fungal natural product, and contains an additional methyl group at the C2' position of the side chain. Until the discovery of the novel, green synthetic pathway nominated here, introduction of this methyl group in lovastatin required multistep chemical synthesis due to the need to protect/deprotect other functionalities in the lovastatin molecule.

Generally, two different routes have been described for the conversion of lovastatin into simvastatin. In the first route lovastatin is hydrolyzed to the triol monacolin J, followed by protection via selective silylation, esterification with dimethylbutyryl chloride and deprotection. The other route involves protection of the carboxylic acid and alcohol functionalities followed by methylation of C2' with methyl iodide followed by deprotection. Both processes are inefficient despite considerable optimization (<70% overall yield), mass intensive as a result of the need for protection/deprotection, and require the use of copious amounts of toxic and hazardous reagents. Therefore, a shortened reaction sequence in which the need for protection/deprotection was circumvented was expected to i. be more efficient resulting in a higher atom economy, ii. reduce waste generation in the manufacture of this product, and iii. provide overall less hazardous process conditions.

Dr. Yi Tang's group at UCLA conceived a new simvastatin manufacturing process and enabled it by first identifying both a biocatalyst for regioselective acylation and a practical, low-cost acyl donor. Codexis licensed the intellectual property for this process from UCLA and subsequently optimized the enzyme and the chemical process for the commercial manufacture of simvastatin.

An Efficient Biocatalytic Process for Simvastatin Manufacture

Introduction:

Statins are important drugs for treating cardiovascular disease. Six statins have been approved by the FDA, three of which (lovastatin, simvastatin and pravastatin) are natural product derived. Lovastatin was the first FDA approved statin and is a secondary metabolite produced by fermentation of *Aspergillus terreus*. Simvastatin is a semi-synthetic derivative of lovastatin, containing a dimethyl group at C2' of the side chain (Fig 1). This subtle structural modification renders simvastatin more potent in the reduction of total and low-density lipoprotein cholesterol (LDL-C) with decreased hepatotoxicity and reduced side effects. In 2005 simvastatin (brand name Zocor®) was Merck's best selling drug and second largest selling cholesterol lowering drug in the world with ~\$5 billion in sales. Since Zocor went off patent in 2006, simvastatin rapidly became the second highest selling generic drug with sales ~ \$2 billion in 2008. Annually, it is estimated that over 300 mTs of simvastatin are produced worldwide.

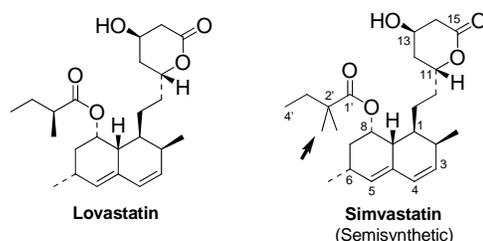


Fig. 1: Chemical structure of lovastatin (left) and its semi-synthetic derivative simvastatin (right) with additional methyl substituent (arrow).

The Challenge:

Manufacturing of simvastatin involves multiple steps to preserve other key chemical functionalities of the molecule. Simvastatin manufacturing processes are generally classified as “hydrolysis/esterification” routes (Fig. 2) or “direct methylation” routes (Fig. 3), both of which require protection/deprotection steps.

According to information obtained from US4,444,784, for the “hydrolysis/deprotection” route, and US4,820,850, for the “direct methylation” route, both routes utilize excess hazardous and

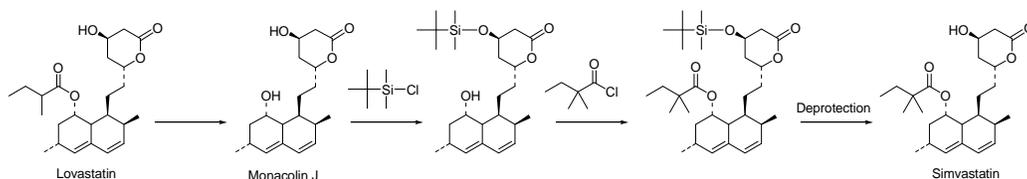


Fig. 2: Simvastatin synthesis via the “hydrolysis/esterification” route involves hydrolysis of the ester bond to generate monacolin J with a free hydroxyl at the 8 position, followed by selective TBDMS protection, esterification with the desired dimethylbutyryl side chain and deprotection.

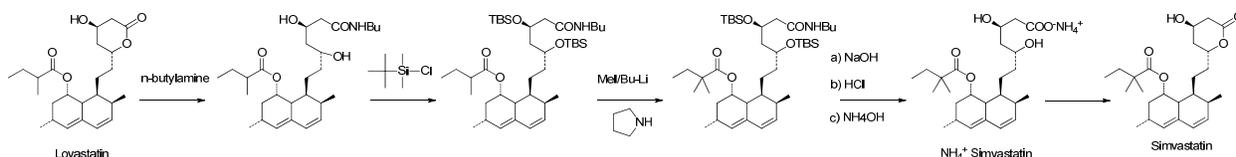


Fig. 3: Simvastatin synthesis via the “direct methylation” route involves protection of the carboxylic and alcohol functions, alkylation using methyl iodide and deprotection to form the simvastatin-ammonium salt. This is subsequently converted to the simvastatin lactone.

toxic reagents (see below) and copious amounts of solvents. Based on this and the low overall yields of the two processes (estimated at <70%) a higher yielding, less waste-generating manufacturing process for simvastatin was deemed to be still highly desirable.

The Approach:

To completely redesign the simvastatin manufacturing process using more efficient and greener chemistry, we decided to follow nature's example and use enzyme technology. Towards this end, we embarked on a protein engineering approach to first identify a biocatalyst for the conversion of lovastatin to simvastatin, and then to improve it towards commercial use. The technology we envisioned is shown in Fig. 4: following the quantitative hydrolysis of lovastatin to monacolin J acid, an enzyme is used to regioselectively acylate the C8 position of monacolin J with a dimethylbutyryl group to yield simvastatin. A novel, non-natural acyl donor was identified to enable this approach. This process circumvents the (de)protection steps required for chemical synthesis and is significantly more efficient, cost effective and environmentally friendly.

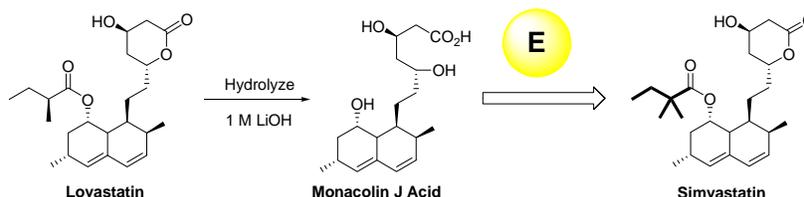


Fig. 4: Enzymatic synthesis of simvastatin from monacolin J.

The Solution:

We first identified LovD as an acyltransferase that selectively transfers the 2-methylbutyryl side chain from a fungal polyketide synthase (LovF) to the C8 alcohol of monacolin J sodium or ammonium salt (Fig. 5) and cloned the corresponding gene (the Tang group at UCLA - *Chem. Biol.* **2006**, 13: 1161; *J. Am. Chem. Soc.* **2009**, 131:8388). This reaction proved to be completely regioselective as only the C8 position was acylated, proving that protective chemistry was not required. The natural side chain donor in *Aspergillus terreus* is 2-methylbutyryl-LovF, an acyl substrate bound to a large enzyme (270 kDa) via a thioester. Since this acyl donor is neither readily available nor scalable for commercial manufacturing, we evaluated the substrate specificity of LovD towards different acyl groups and thioester acyl carriers. We demonstrated that LovF is not required for substrate recognition by LovD and that simple thioesters, e.g. with *N*-acetylcysteamine (SNAC) can be used for the conversion of monacolin J to lovastatin and simvastatin, albeit with low catalytic turnover (k_{cat} for simvastatin conversion was 0.02 min^{-1}). This represented the first demonstration of enzymatic synthesis of simvastatin. LovD can therefore be considered as a simvastatin synthase and a starting point for developing the desired green manufacturing process.

With LovD in hand we developed an *E. coli*-based biocatalytic approach to synthesize simvastatin. We synthesized a series of commercially accessible dimethylbutyryl thioesters and tested their ability to support the transacylation reaction. Dimethylbutyryl-*S*-methylmercapto-propionate (DMB-SMMP) was identified as a catalytically superior acyl donor to DMB-SNAC, thereby establishing a novel route to simvastatin (Fig. 5). DMB-SMMP is very efficient as an acyl donor in the LovD catalyzed reaction with a $k_{\text{cat}} = 0.75 \text{ min}^{-1}$, more than 30-fold faster than that of DMB-SNAC (*Appl. Environ. Microbiol.* **2007**, 73:2054). DMB-SMMP binds readily to LovD and its binding is not competitively inhibited by high concentrations (>30 mM) of mona-

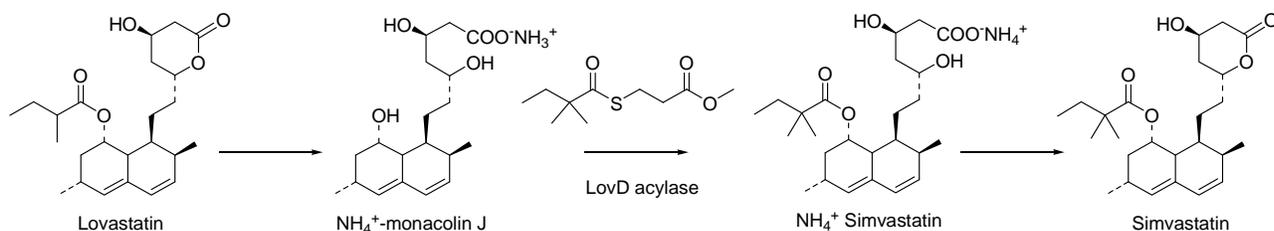


Fig. 5: LovD enabled synthesis of simvastatin. Lovastatin is hydrolyzed and converted to the ammonium salt of monacolin J which is subsequently acylated using DMB-SMMP and LovD as biocatalyst.

colin J. Furthermore, DMB-SMMP can be prepared in a single step from readily available, inexpensive precursors to provide a safer alternative to the typically used acyl donors and the reactions conditions they are used in. In addition, the methyl 3-mercaptopropionate by-product is hydrolyzed under reaction conditions and the acid has been shown to be recyclable.

Having a practical acyl substrate available, we next explored whether an *E. coli* strain overexpressing LovD can be used as a whole-cell biocatalyst for the synthesis of simvastatin. The *E. coli* host was first optimized by eliminating a competing esterase (encoded by *bioH*), which rapidly hydrolyzed the methyl ester bond of DMB-SMMP (*Metab. Eng.* **2007**, 9:379). In a typical laboratory run (1-2 L), the optimized *E. coli* strain harboring the LovD expression plasmid was grown to high cell density in minimal medium. DMB-SMMP and monacolin J were then added to the fermentor and progress of the reaction followed by HPLC. Complete (>99%) conversion of monacolin J to simvastatin (10 g/L) was observed in less than 24 hours. The synthesized simvastatin was readily isolated from the fermentation broth by ethyl acetate extraction at acidic pH. A two step extraction process followed by precipitation with ammonium hydroxide gave nearly pure (>98.5%) simvastatin ammonium salt. An additional recrystallization step afforded >99.5% pure simvastatin in 91% yield from monacolin J acid. Using directed evolution technology we developed LovD variants that were better expressed (*Biotechnol. Bioeng.* **2009**, 102:20), and more active for the *in vivo* process (*Chem. Biol.* **2009**, 16:1064). The structure of LovD was also determined and the basis of catalysis was elucidated (*Chem. Biol.* **2009**, 16:1064).

Based on these proof-of-concept studies this technology was made available for license and Codexis continued to optimize the enzyme for commercial scale use. Using enzyme optimization technology, we developed LovD variants that enabled efficient simvastatin manufacture (Table 1). Over the course of nine iterative rounds of *in vitro* evolution 216 libraries were constructed and 61,779 variants were screened to result in a new LovD variant with improved activity, in process stability, and tolerance to product inhibition. The amino acid sequence of the new variant has 30 mutations and as such is 7% different from the natural enzyme. The Codexis team further developed a one-pot approach in which monacolin J is first converted to the water-soluble ammonium salt that is converted biocatalytically to the water-insoluble simvastatin ammonium salt (SAS), which is readily collected by filtration and used directly to make simvastatin lactone API. The ~1000-fold improved enzyme and the new process not only pushed the reaction to completion at high substrate loading, but also minimized the use of acyl donor as well as the use of solvents for extraction and separation of the product. As a result, SAS is synthesized in >97% yield at a loading of 75 g/L monacolin J. The technology was scaled-up in early 2010 to 400 kg batch size by our first commercial manu-

Parameter	Performance at initiation of evolution program	Final performance
[Monacolin J]	3 g/L	75 g/L
[Thioester]	>3 eq.	1.1 eq.
LovD loading	10 g/L (natural LovD)	0.75 g/L (evolved LovD)
Reaction time	18 hrs	36 hrs
Conversion	50%	97%

Table 1: Comparison of biocatalytic simvastatin manufacturing processes using natural and evolved LovD. The overall volumetric productivity on an enzyme basis was improved over 300-fold from 0.2 to 67 g/L.day.g_{LovD}. At the same time the amount of thioester required for complete conversion was greatly reduced.

facturing partner (who prefers to remain undisclosed). A second API manufacturer (Arch Pharmalabs) was brought on-line in late 2010. Enzyme manufacture has been established at 30,000L scale and over 10 mT simvastatin has now been produced using this biocatalytic process. Customers have evaluated biocatalytically-produced simvastatin and confirmed that it met their stringent specifications.

The Significance:

The economic attractiveness of our biocatalytic acylation process stems from its greenness compared to the previous processes (Table 2). As a result of the simple nature of the biocatalytic process, the number of steps is reduced by avoiding the protection/deprotection strategy. The

Route	“hydrolysis/deprotection”	“direct methylation”	Biocatalytic process
Number of steps	4	5	3
Reagents:			
➤ Protection:			None
- TBDMS-Cl	2.5 equivalents	2.25 equivalents	
- Butylamine		16.6 equivalents	
➤ Deprotection:			None
- Bu ₄ N ⁺ F ⁻	2.3 equivalents	15 equivalents	
- HF (50% aq)			
➤ Reagents			None
- imidazole	2 equivalents	2.5 equivalents	
- n-BuLi/hexane		2.3 equivalents	
- pyrrolidine		2.3 equivalents	
Side chain	4 eq. DMB-chloride	4 eq. methyl iodide	1.1 eq. DMB-SMMP
Solvents	Multiple and significant volumes	Multiple and significant volumes	Small volumes of a single solvent
Non-solvent waste: kg/kg Product	>25	>25	<1

Table 2: Reagent use for the manufacture of simvastatin from lovastatin using the different chemical routes and the biocatalytic route. The information for the chemical processes is derived from gram-scale syntheses (18-50 g starting material; US\$4,444,784 and US\$4,820,850) which to our knowledge is the largest scale information publicly available. Comparison of E-factors for the different processes is problematic as we do not have good visibility on large scale process characteristics for the two chemical processes. The E-factor for the biocatalytic step including water and solvents (MTBE) is 25 (0.6 if all water and solvent is recycled).

use of hazardous and toxic materials is greatly reduced and the formation of 1000s of mTs of non-solvent waste will be prevented with the pending expanded implementation of this process.

The merits of the nominated technology vis-à-vis the selection criteria specified in the Nomination Package for 2011 Awards

Focus areas: The nominated technology addresses all three focus areas of the Presidential Green Chemistry Challenge program:

1. It provides a **greener synthetic pathway** for the manufacture of simvastatin by using a novel enzyme enabling a biocatalytic, green-by-design, regioselective acylation that obviates the need to use hazardous and toxic reagents that generate substantial waste, while at the same time improving the overall yield.
2. The process is run under **greener reaction conditions** compared to the traditional processes in that it is run at ambient temperature and atmospheric pressure. No solvents other than water are used during the biocatalytic process.
3. A new and **green chemical was designed** for this new process. The acylating thioester DMB-SMMP is a safer acylating reagent and the thiopropionate by-product can be recycled at scale.

Science and Innovation:

- The nominated technology is original, never employed before:
 - A new biocatalytic process was conceived and enabled using molecular biology methodology and chemical process development including screening of various side-chain donors.
- The nominated technology is scientifically valid.
 - The technology has undergone repeated peer review and has been published in high impact journals (referenced in the text).
 - It is also proven as a result of its successful adoption for commercial scale manufacturing.

Human health and environmental benefits:

The nominated technology offers several human health and/or environmental benefits:

- Reduced use of toxic and hazardous substances:
 - Avoidance of *tert*-butyl dimethyl silane chloride, a moisture sensitive, flammable solid.
 - Avoidance of methyl iodide, a possible carcinogen and compound that can cause lung, liver, kidney and central nervous system damage.
 - Avoidance of *n*-butyl lithium, a pyrophoric reagent that requires handling under cryogenic conditions (-30°C),
 - Reduction in solvent use due to the aqueous nature of the reaction conditions.
 - Several co-products are avoided and the only new co-product is methyl 3-mercaptopropionic acid, which will be recycled at commercial scale.
- Improved energy efficiency as the reaction is run at ambient temperature.

The merits of the nominated technology vis-à-vis the Twelve Principles of Green Chemistry

1. Waste prevention:
 - The highly selective biocatalytic acylation provides product in substantially higher yield and circumvents the need for protection/deprotection reactions that result in significant waste generation.
 - The main waste streams are aqueous and directly biodegradable.

- 2. Atom Economy:
 - Use of extraction solvents is greatly reduced.
 - The high yield of the reaction, the high purity of the product, the avoidance of protection/deprotection reactions and the low solvent usage result in more of the total atom and mass that is put into the process is recovered in the product.
- 3. Less Hazardous Chemical Syntheses:
 - In this process, the use of hazardous chemicals such as TBDMS, MeI, and *n*-butyl lithium is avoided.
- 4. Design Safer Chemicals:
 - The acyl donor used was designed for use in this process and provides safety advantages over the traditional chemistries.
- 5. Safer Solvents and Auxiliaries:
 - Water is used as a safe and recyclable solvent.
- 6. Design for Energy Efficiency:
 - The reaction runs at or near ambient temperature and at atmospheric pressure.
 - The high activity of the evolved biocatalysts minimizes the fermentation capacity requirement, thereby reducing the energy and water needs, as well as the CO₂ output of the catalyst manufacturing process.
- 7. Use of Renewable Feedstocks
 - The catalyst is produced efficiently from renewable resources.
- 8. Reduce Derivatives
 - No protection/deprotection needed.
- 9. Catalysis
 - The new process is enabled by a regioselective catalyst preventing the need for protection/deprotection reactions.
- 10. Design for Degradation
 - The major waste streams generated by this process are directly biodegraded in biotreatment facilities.
- 11. RTA for pollution prevention
 - The process is monitored in real-time via the pH-stat controlled addition of NH₄OH.
- 12. Inherently Safer Chemistry for Accident Prevention
 - The chemicals used in this process are readily used safely. Hazardous reagents are avoided. The reaction is run at ambient temperature.

Broad applicability:

- The nominated technology is a practical, cost-effective approach to green chemistry:
 - It is a practical operation to manufacture simvastatin.
- The nominated technology embodies features that can be transferred readily to other processes and facilities:
 - Regioselective catalytic acylation reactions are highly desirable as they circumvent the need for extensive protection/deprotection methodology. The nominated technology demonstrates a new biocatalytic acylation process for the manufacture of simvastatin and enabled the development of an economic, green-by-design biocatalytic process.

References to Patent Applications:

WO2007/139871 entitled “Methods and Materials for Making Simvastatin and Related Compounds”, published Dec. 6, 2007; both WO2011/41231 entitled “Variant LovD Polypeptides and their uses” and WO2011/41233 and entitled “Improved LovD acyltransferase mediated acylation” published April 7, 2011. Corresponding national phase applications have been filed.



**Commercial Production and Substitution of Petrochemicals through
Innovative Biorefineries producing Cost Advantaged and Higher Performing
Green Specialty Chemicals based on Nobel Prize-winning Metathesis Catalysis
for Markets of \$176 Billion**

December 31, 2011

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PROJECT TITLE: Commercial Production and Substitution of Petrochemicals through Innovative Biorefineries producing Cost Advantaged and Higher Performing Green Specialty Chemicals based on Nobel Prize-winning Metathesis Catalysis for Markets of \$176 Billion.

RECENT MILESTONES: Since inception in 2007, building upon a collaboration between Cargill and Materia initiated under a Department of Energy (DOE) grant, Elevance has optimized, engineered, scaled, and commercialized Nobel-prize winning innovations in energy efficient, cost advantaged metathesis catalysis using multiple renewable oil feedstocks. In 2009, won a \$2.5M DOE grant to understand the impact of feedstocks and recycle streams, as well as produce platform chemicals and fuels for market development and performance testing from its integrated biorefinery. In 2010, partnered with Wilmar International to build a world-scale biorefinery in Indonesia, and closed \$100M investment in Series C funding. In 2011, expanded technology with XiMo AG licensing agreement to use proprietary molybdenum and tungsten metathesis catalysts. In May 2011, validated technology at commercial scale tolling facilities, converting 1mlbs (450 metric tonnes) of feedstock into green chemicals for market development. Secured sales in performance waxes and candle markets, and in personal care markets. From 2007 – 2011, established partnerships with industry leaders in feedstock, technology and commercialization, including Cargill, Clariant, Dow Corning, Materia, Stepan, Wilmar, DSM, and Hutchinson Worldwide. June 2011, purchased an 80 mgpy biodiesel plant in Mississippi, to repurpose as biorefinery with annual production capacity of 280,000 metric tonnes (610 mlbs). Filed S1 to begin IPO process.

ELIGIBILITY OF BUSINESS AWARD: Elevance was incorporated in the State of Delaware on October 17, 2007, to pursue work started in 2004 in a collaboration between Cargill and Materia, Inc., a leading-edge catalyst technology company founded by Dr. Robert H. Grubbs. To date, Elevance has less than 100 employees, and has generated less than \$40 million in annual revenues.

FOCUS AREA: Fits all focus areas, with emphasis on Focus Area 3: design of greener chemicals.

US COMPONENT: Elevance has grown from 2 employees in 2007 to just under 100 employees at two US facilities at the end of 2011, and has optimized, engineered, scaled and commercialized US-based Nobel Prize-winning technology supported twice by the DOE. As of June 2011, conducted R&D in Bolingbrook, Illinois, then expanded and relocated in September to Woodridge, Illinois. In June 2011, acquired a former 80 million gallon per year biodiesel plant in a USDA designated priority development area in Mississippi, to repurpose into Elevance's second commercial biorefinery. This involves remediating a Brownfield site, helping to save regional rail line from being scrapped, and providing overall revitalization of regional economy, including approximately 165 direct jobs and over 300 construction jobs with an estimated \$275 million investment.

ABSTRACT:

Elevance produces high performance and cost advantaged green chemicals from renewable oils, to address markets of \$176 billion. Its processes use Nobel Prize-winning innovations in metathesis catalysis, which consume significantly less energy, and reduce GHG emissions by 50% compared to petrochemical technologies. Elevance products enable novel surfactants, lubricants, additives, polymers, and engineered thermoplastics. Elevance has secured strategic partnerships with value chain global leaders to accelerate rapid deployment and commercialization for these high performance products and their applications. Elevance's technology addresses the increasing

demand for everyday products made from non-toxic, environmentally friendly and renewable sources. For instance, Elevance is producing specialty chemicals to enable cold water detergents with more concentrated formulations and improved solvency for better cleaning, to improve sustainability metrics and reduce energy costs for customers and consumers. Other examples include biobased anti-frizz and shine additives for leave-in hair care products to replace petroleum-based petrolatum, alternatives to paraffin for high performance waxes, novel plastic additives for PVC and unique monomers for bio-based polymers and engineered plastics.

Supported in 2004 and 2009 by the Department of Energy, Elevance's process results in lower source pollution, production costs and capital expenditures than petrochemical refineries. The process uses a highly efficient and selective catalyst to break down natural oils and recombine fragments to produce high value chemicals with superior functional attributes previously unavailable commercially. Elevance has completed validation in toll manufacturing and plans are underway to build three world-scale facilities with combined annual production capacity of one million metric tonnes (2.2 billion pounds). The company is currently building one of the world's largest integrated biorefineries in Indonesia with Wilmar International that will start up during 2012, and repurposing a biodiesel plant in Mississippi to operate as a biorefinery in 2013. By 4Q2014, expect third facility in South America. The company's ability to manufacture biochemicals for multiple products—and use a diversity of renewable feedstocks—reduces reliance on petrochemicals, provides more effective and sustainable products to consumers, and helps green chemistry take route in the US for jobs and competitiveness in low carbon, global sustainable economy.

SCOPE AND SELECTION CRITERIA: Elevance's technology fits all three Focus Areas, especially Area 3. It meets the scope and selection criteria of the program through optimization, and scale up of US-based metathesis catalysis technology, coupled with novel downstream green product applications. It incorporates “the principles of green chemistry at the earliest design stages of a new product or process which have yielded benefits to human health and the environment along many points in the technology's lifecycle”, from renewable feedstock conversion to synthesis, use, and end of life of greener products. Elevance's technology is sustainable, with a small environmental footprint. Its cleantech catalytic processes are low-pressure and low-temperature, yielding low-toxicity products and byproducts. The natural oil compounds are synthesized with high efficiency, offering superior performance at a lower cost with less energy than fossil-based oils. In 2009, DOE acknowledged Elevance's innovation with a \$2.5 million grant to advance its metathesis and biorefinery technologies for greener synthetic pathways. The core technology is based on the work of Nobel Laureate Dr. Robert H. Grubbs. Elevance further expanded its proprietary technology in 2011 with a licensing agreement with XiMo AG to use proprietary molybdenum and tungsten metathesis catalysts based on the work of Nobel Laureate Dr. Richard Schrock. Collectively, Elevance's disruptive technology delivers a broad portfolio of high performance products targeted at markets estimated at over \$176 billion.

1. Science and Innovation: Original and Scientifically Valid

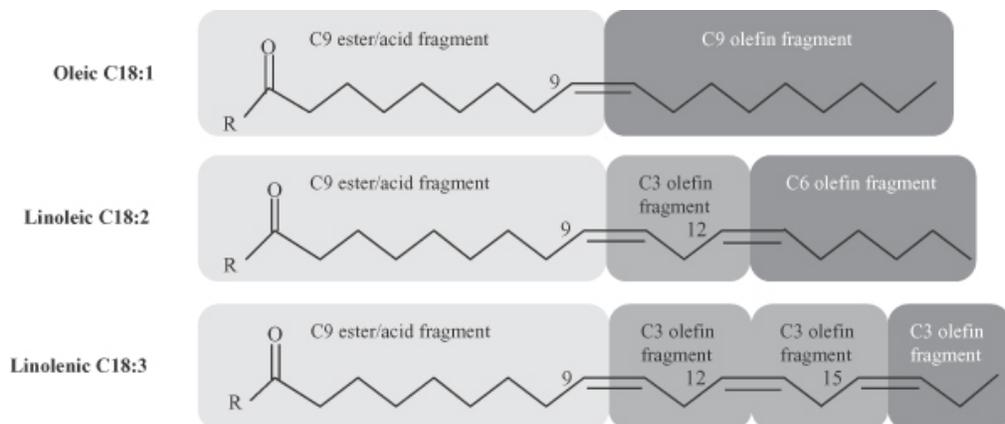
Elevance's proprietary process is based on Nobel Prize-winning innovations in metathesis catalysis, a chemical reaction that uses a highly efficient and selective catalyst to break down and recombine molecules into new chemicals. The company uses metathesis to make novel, di-functional molecules, as building blocks of a specialty chemical business. As shown below, these molecules combine the functional attributes of an olefin, typical of petrochemicals, and a mono-functional ester or acid, typical of oleochemicals, in a single molecule. Elevance is the only company to date that can economically produce these di-functional chemicals, which provide access to a large

functional molecules and polymers, and made metathesis of functionalized molecules, such as natural oils, infeasible.

The 2005 Nobel Prize in Chemistry was shared by three researchers for their breakthroughs that made the metathesis of functional molecules possible. Among these laureates, Dr. Robert Grubbs of the California Institute of Technology (“Caltech”) developed metathesis catalysts that are highly active, stable in air and tolerant of reactants with functional attributes. Dr. Grubbs shared the Prize with Dr. Yves Chauvin and Dr. Richard Shrock. Dr. Chauvin discovered the mechanism by which metathesis operates, and Dr. Shrock invented metathesis catalysts that are highly active. Elevance has an exclusive license for the patents, for use with natural oils, owned and licensed to Materia, which includes all of the innovations created by Dr. Grubbs. Elevance also has a license with XiMo for innovations either created by Dr. Shrock and his team or developed with Elevance.

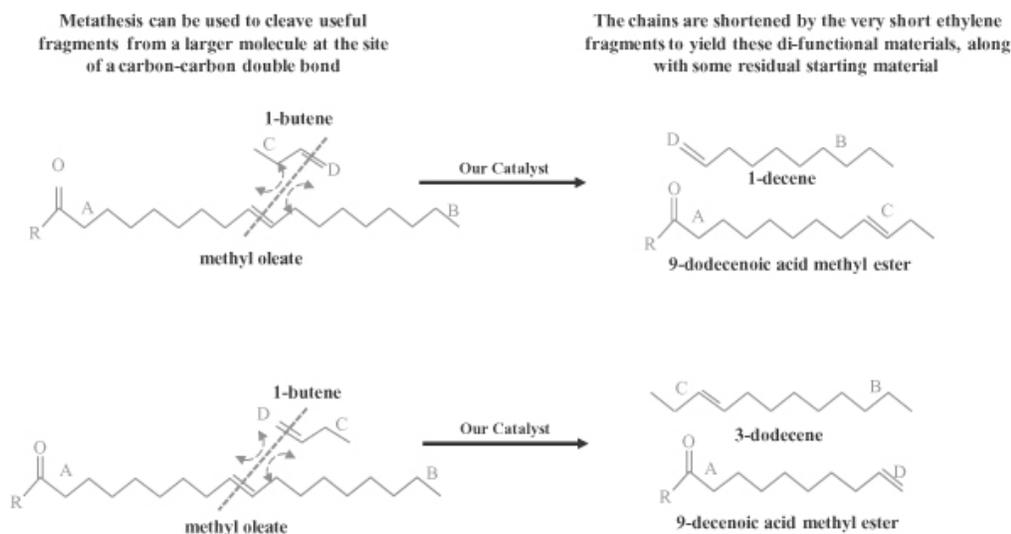
Because natural oils are functionalized molecules, without Dr. Grubbs’ innovations, the metathesis of natural oils would be technically impractical and commercially uneconomic. Elevance’s proprietary biorefinery combines metathesis of natural oils with established industrial processes, such as transesterification and distillation, to economically produce specialty chemicals and direct replacement intermediate chemicals through a single process. Natural oils provide a wide array of useful molecule fragments that can be accessed through metathesis. The fragments below are among the most common that can be created from natural oils.

In Elevance’s proprietary biorefinery design, a stream of natural oils, which are chemically made of triglycerides, is reacted with short-chain olefins, such as butene. This reaction cleaves

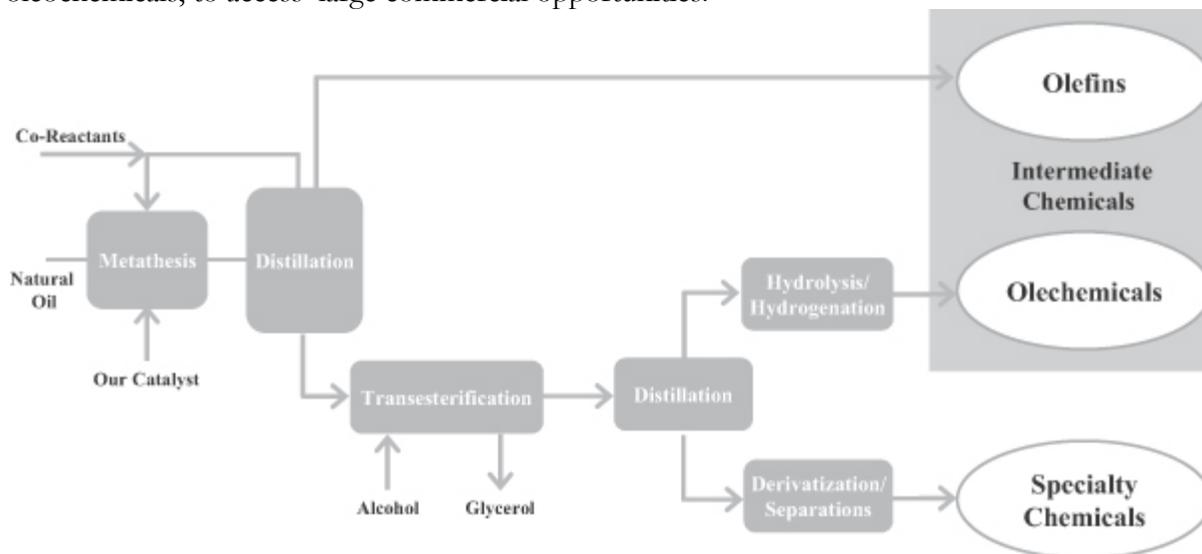


olefin molecules from the triglyceride, producing higher value olefins, including decene. Moreover, the metathesis reaction of the natural oil cleaves its triglycerides into ones rich in valuable, medium

chain-length unsaturated fragments (C10-C15), as well as unreacted saturated long chain fragments (C16-C22). An example of this reacting 1-butene with methyl oleate, a common natural oil component, is



demonstrated to left. When these unsaturated and saturated fragments are freed from the triglyceride's glycerol backbone, the resulting unsaturated fragments produce a distribution of di-functional molecules, including fatty acids and esters, as further described below. The saturated fragments, which are inert to metathesis because they lack a carbon-carbon double bond, produce saturated acids, esters or oleochemicals. The figure below illustrates how Elevance produces products from its novel biorefinery process. The reactor used in toll manufacturing to date operates at a similar scale as will the reactor installed at the first biorefinery. Note three main product streams: one stream of specialty chemicals and two streams of intermediate chemicals, namely olefins and oleochemicals, to access large commercial opportunities.



2. Remedy for Environmental and Health Problems: Innovations for Large Industry Sectors

Environmental Benefits: Elevance's biorefinery design reduces source pollutants and requires less capital than conventional technologies because of the following characteristics: fewer major process steps; lower operating temperatures and pressures providing energy efficiency; and limited production of hazardous and toxic by-products. As a result, environmental benefit is evident in the development of an improved, energy efficient process that achieves a greater than 50% reduction in GHG emissions by substituting biomass for petrochemical feedstock, and using a more energy efficient conversion process. A key criterion of the DOE grant for an integrated biodiesel-renewable chemical biorefinery was to demonstrate a 50% lifecycle GHG emissions reduction compared to petrochemical technologies. Elevance used The GREET 1 model, Version 1.8c.0., with the "energy-based allocation" method to account for co-products for a more conservative value for emission reduction compared to the displacement method. Elevance contracted with Earthshift Inc. to validate calculations. In addition to significant energy and GHG reductions, the process can use a variety of natural oils, including emerging oils, such as those derived from algae. Primary feedstocks include palm, soy and rapeseed oils, though can use many other natural oils. These are available in liquid form in industrial quantities from a variety of geographic regions. This allows for low-cost transportation and storage compared to other renewable feedstocks such as industrial sugars, biomass and waste. The ability to use a range of oils in various geographies optimizes feedstocks dynamically based on the cost to use the oils and the value created from the resulting product mix. This de-risks production and maximizes profitability. Elevance does not expect large price increases of primary feedstocks, as will consume a de minimis percentage of global natural oil supply.

Health Benefits: Beyond the significant environmental benefits accrued from the process described above, there are vital health and environmental benefits derived from Elevance’s broad portfolio of high performance products meeting customer needs for non-toxic, environmentally friendly and renewable sources in three large market platforms: Consumer Ingredients and Intermediates; Engineered Polymers and Coatings; and Lubricants, Additives, Specialty Fuels. Summary tables follow, with markets based on management estimates.

Ingredients & Intermediates

Market (Addressable Market Size)	Platform/Segment	Elevance Value Proposition
Consumer Ingredients & Intermediates (\$31 billion)		
Detergents, cleaners (\$20 billion)		
<ul style="list-style-type: none"> Specialty surfactants 		<ul style="list-style-type: none"> Various formulation benefits including improved cold water performance, concentration, compaction, hard water tolerance and solvency Alternative alcohol feedstock to palm kernel oil, coconut oil and fossil fuels to address price and supply concerns
Personal care products (\$6 billion)		
<ul style="list-style-type: none"> High performance soy wax Emulsifiers 		<ul style="list-style-type: none"> Naturally derived wax, eliminates brittleness, adds body Improved emulsification, improved film forming and emolliency, naturally derived
<ul style="list-style-type: none"> Soy petrolatum to replace petroleum derived petroleum jelly 		<ul style="list-style-type: none"> Naturally derived and elegant aesthetics Anti-frizz and shine for leave-in hair care Moisturizing benefits and smoother feel for skin care
Performance waxes (\$5 billion)		
<ul style="list-style-type: none"> Plastic processing additives 		<ul style="list-style-type: none"> Thermal stability with low volatility and good release/anti-stick effects
<ul style="list-style-type: none"> Soy and palm formulated wax blends for candles 		<ul style="list-style-type: none"> Reliability of supply, increased fragrance loading, alternatives to paraffin
<ul style="list-style-type: none"> Palm wax blends for corrugated coatings 		<ul style="list-style-type: none"> Improved recyclability, reliability of supply, sustainability

Lubricants & Additives

Market (Addressable Market Size)	Platform/Segment	Elevance Value Proposition
Lubricants & Additives (\$29 billion)		
Lubricant base oils (\$17 billion)		
		<ul style="list-style-type: none"> Reduction in formulation costs Improved fuel economy, wear, sludge resistance, stability Less frequent oil changes Alternative and renewable source of olefins for PAO
Lubricant and fuel additives (\$12 billion)		
<ul style="list-style-type: none"> Viscosity improvers, extreme pressure and anti-wear additives, dispersants 		<ul style="list-style-type: none"> Improved lubricity, detergency to control impurities, corrosion prevention Renewable-novel additives improve cold flow, lubricity
<ul style="list-style-type: none"> Cold flow modifiers, lubricity 		<ul style="list-style-type: none"> Supply chain improvements

enhancers, deposit control

Engineered Polymer & Coatings

Market (Addressable Market Size)	Platform/Segment	Elevance Value Proposition
Engineered Polymers & Coatings (\$116 billion)		
Monomers and building block chemicals used in:		
<ul style="list-style-type: none"> Specialty polyamides, polyesters and polyols (\$25 billion) Epoxies and polyurethanes (\$58 billion) Coatings and cross linking agents for coatings (\$33 billion) 	<ul style="list-style-type: none"> Enhanced corrosion, chemical and heat resistance and improved electrical insulation over existing alternatives Light-weight replacement for metal alternatives Produce existing products via lower cost routes New source of C10 molecules Renewable products & polymers 	

3. Applicability and Impact: Practical and Cost Effective Approach

Overall, Elevance has operated innovative source reduction programs by transforming a diversity of natural plant-based oils into cost advantaged and high performance green chemicals as substitutes to petrochemicals. The breakthrough process is practical and cost effective, with lower construction and production costs than alternative routes to comparable products, such as olefins produced 56-79% cheaper than incumbent products. Execution ongoing to build three world-scale facilities by 4Q2014, with combined annual production capacity of one million metric tonnes (2.2 billion pounds). Concurrently building one of the world’s largest integrated biorefineries in Indonesia with Wilmar International Limited, and repurposing a biodiesel plant in Mississippi. Construction of these facilities is estimated to cost \$215 to \$535 per metric tonne (\$0.10 to \$0.25 per pound) of annual production capacity compared to \$920 to \$2,300 per metric tonne (\$0.42 to \$1.04 per pound) for conventional facilities. By 4Q2014, expect third facility in South America. As evidence of widespread market applicability, Elevance has secured significant global partner agreements related to: sales, marketing and application development; manufacturing; technology; and feedstock supply. Accordingly, Elevance benefits from a number of competitive strengths:

- Proprietary technology produces high-value specialty chemicals and direct replacement intermediate chemicals that are cost-advantaged, higher performing compared to incumbents.
- Biorefinery design requires less capital than conventional technologies because: (1) fewer major process steps; (2) lower operating temperatures and pressures; (3) limited production of hazardous and toxic by-products; and (4) ability to integrate process into existing industrial sites.
- Lower production costs because: (1) more direct process, resulting in fewer conversion steps; (2) highly efficient and selective catalyst; (3) feedstock flexibility; and (4) lower operating temperatures and pressures, resulting in greater energy efficiency.
- Strategic partnerships with industry leaders provide sales and marketing expertise, established distribution channels, technical know-how, product and application expertise and infrastructure.
- Feedstock flexibility: technology has the flexibility to use diverse natural oils. Ability to adjust inputs in real time to take advantage of changes in feedstock prices and product demand.
- Targeting large and well-established end markets, currently estimate addressable specialty chemical markets represent \$176 billion in annual commercial opportunity.
- Rapid deployment because: (1) ability to repurpose or integrate into existing sites; (2) low capital requirement; (3) existing large markets; and (4) relatively short engineering, construction cycle.

Silicone Synthesis with Earth Abundant Metal Catalysts

December 31, 2015

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Technology Title: Silicone Synthesis with Earth Abundant Metal Catalysts

Eligibility: Academic award

Name of EPA Area: Greener synthetic pathways

Description of Most Recent Milestone and Year: The discovery air-stable, easily handled iron and cobalt catalysts for commercial silicone synthesis. (Year 2012)

Patent: Delis, J. G. P.; Chirik, P. J.; Tondreau, A. M.; Weller, K. J.; Lewis, K. M.; Nye, S. A. Hydrosilylation Catalysts. U. S. Patent 8,415,443, Issued: April 9, 2013.

-Technology has produced 2 additional issued patents and 13 more applications.

Publication: Tondreau, A. M.; Atienza, C. C. H.; Weller, K. J.; Nye, S. A.; Lewis, K. M.; Delis, J. G. P.; Chirik, P. J. "Iron catalysts for selective anti-Markovnikov alkene hydrosilylation using tertiary silanes." *Science* **2012**, 335, 567-570.

Activities within the United States: All research was conducted at Princeton University in Princeton, New Jersey in conjunction with collaborators at Momentive Performance Materials in Waterford and in Tarrytown, New York.

Abstract (300 word or fewer): Metal-catalyzed chemical reactions have enabled many of the technological innovations of modern society with applications ranging from the synthesis of advanced materials to new medicines. For decades, catalyst technology has principally relied on some of the least abundant elements in the Earth's lithosphere – palladium, platinum, rhodium and iridium. In addition to their high cost, price volatility and toxicity, extraction of these elements from the Earth's crust has significant environmental consequences. Obtaining one ounce of a precious metal often requires mining approximately 10 tons of ore, one mile deep and as a consequence creates a CO₂ footprint that estimated to be 6000 times that of iron.

Silicones are enabling materials found in a range of consumer products including household utensils, adhesives, medical devices, health care products and low rolling resistance tires. The majority of these products are prepared by alkene hydrosilylation, an industrial process uses platinum catalysts to connect alkenes and silanes. Often times the platinum is not recovered and results in a significant environmental footprint for this commercially important process. Professor Chirik and his research group, in collaboration with Momentive Performance Materials, have discovered a new class of hydrosilylation catalysts based on earth abundant transition metals such as iron and cobalt that have superior performance to existing platinum catalysts. The base metal catalysts are also more selective and enable new chemistry, obviating distillation, waste generation and multistep syntheses associated with current industrial practice. This technology is based upon "metal-ligand cooperativity", a broad catalysis concept pioneered by the Chirik group, where electron changes occur concomitantly between the metal and the supporting ligand.

Detailed Description of Technology: Alkene hydrosilylation, the addition of a silicon-hydride (Si-H) across a C=C bond, is one of the largest scale industrial applications of homogeneous catalysis. The reaction is critical for the production of a wide range of consumer goods including hair conditioners, release liners, low rolling resistance tires, surfactants, agricultural adjuvants, paper-coatings, fiberglass composites and dental impressions (Figure 1).¹ Alkene and alkyne hydrosilylations are employed in the manufacture of over 90% of the organosilanes and siloxanes used globally in the \$5billion/year silicones industry.² **Almost all readers of this document will have used a product of hydrosilylation in the past 24 hours and as a consequence have relied on platinum catalyst technology and its associated environmental footprint.**

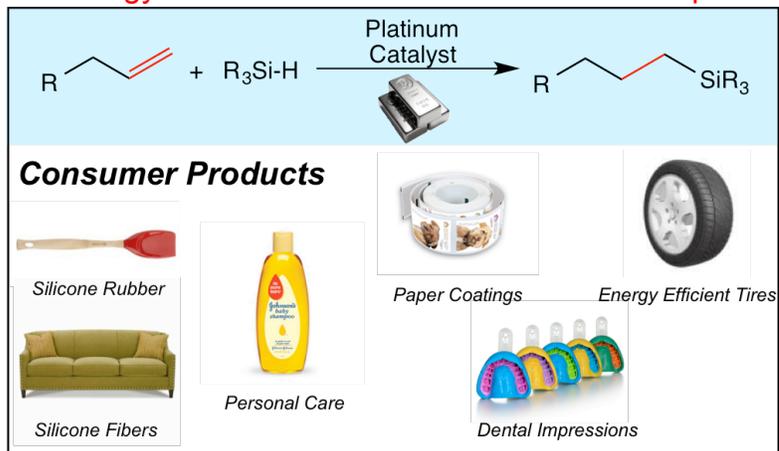


Figure 1. Platinum-catalyzed hydrosilylation used in the commercial production of silicones and application in various consumer products.

According to Johnson Matthey,³ the worldwide silicones industry uses approximately 180,000 troy ounces (5.6 metric tons) of platinum annually and most is not recovered. This corresponds to annual losses of \$230 million and requires fresh supplies of metal to support production and keep pace with the estimated 5% annual growth of the silicones industry. The global supply of platinum is currently threatened as the annual output from principal suppliers (South Africa, Russia, Zimbabwe) has remained constant,⁴ while demand from the jewelry, automotive and fuel cell industries continues to undergo rapid growth. In addition to cost concerns, mining platinum has severe environmental consequences. Due to its low terrestrial abundance, obtaining one ounce of metal requires extraction of 10 tons of ore, generating enormous quantities of waste and dangerous mining conditions. These constraints require large fossil fuel inputs and create a considerable CO₂ footprint. Mining of platinum is estimated to liberate 6000-8000 more equivalents of carbon dioxide than the same molar quantity of iron.⁵

Despite being a mature industrial technology, platinum-catalyzed hydrosilylation also has several disadvantages that violate the principles of green chemistry (Figure 2). Current catalyst technology often isomerizes terminal olefin substrates to internal alkenes (up to 30% depending on the process) and requires distillation to remove the unwanted side products. In other applications, excess of alkene is required for optimal yield of hydrosilylated product. Most platinum-catalyzed hydrosilylations require high temperatures for useful reaction rates, creating additional CO₂ and fossil fuel footprints. Another disadvantage of current platinum catalyst technology is limited substrate scope. In addition to being ineffective for internal alkenes, platinum catalysts are readily poisoned by amine

and phosphorus compounds. The intolerance to amines is significant as γ -amino propyl trialkoxysilanes are one of the largest classes of silanes produced and require a multistep synthesis that generates a variety of waste products including acidic alcohol and alkyl halides that require landfills or incineration for disposal.

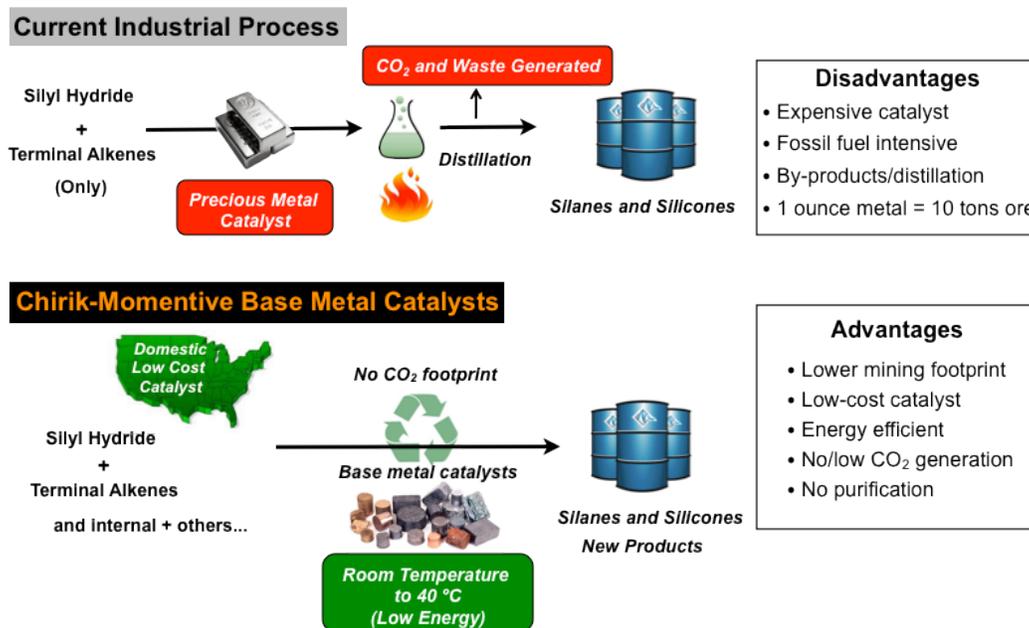


Figure 2. Comparison of existing industrial hydrosilylation processes reliant on precious metal catalyst technology versus Princeton-Momentive base metal catalysts that deliver transformative energy, economic and environmental benefits.

Catalysts based on earth abundant transition metals such as iron and cobalt offer many advantages for more sustainable and green hydrosilylation technology. The terrestrial abundance of these elements provides a vast domestic supply with significantly reduced mining footprints. The lower cost and reduced toxicity of base metals are also advantageous. From a green chemistry perspective, new base metal catalyst technology offers the opportunity to enable new chemistry – processes that provide the desired product exclusively, eliminating distillation steps and avoiding generation of byproducts and unnecessary waste. New catalyst technology also offers the opportunity to open new substrate space, potentially enabling new and atom economical routes to current products that require multistep synthesis. Here we describe the results of a collaboration between the Chirik research group at Princeton University and Momentive Performance Materials that has produced a transformative class of base metal hydrosilylation catalysts. Iron and cobalt examples have been discovered with activity and selectivity superior to platinum that are currently being produced on scale for commercialization. Momentive engineers have estimated that Chirik base metal catalysts offer the potential to significantly reduce separation process steps, cutting energy usage by 85 billion BTUs/year, waste generation by 8.5 million kg/year and carbon dioxide generation by 21.7 million kg/year. For organofunctional silane processes, energy reduction has been estimated on the order of 80%, due to the high-energy costs associated with distillation of the mixtures of products

generated from existing platinum-catalyzed methods.

A. Iron Catalysts for Selective Hydrosilylation. One of the principal challenges for realizing effective base metal hydrosilylation catalysts is overcoming the redox differences between earth abundant and precious metals.⁶ Second and third row transition metals, like platinum tend to operate via two-electron redox chemistry while first row transition metals such as iron and cobalt tend to promote one-electron, radical processes that are often deleterious. To overcome this challenge, the Chirik group introduced the concept of redox-active ligands to base metal catalysis. This approach takes advantage of ligands that can undergo reversible one electron transfer with a transition metal.⁷ By having the ligand and metal work cooperatively in one electron redox chemistry, incoming substrates undergo net two-electron reactivity of the type required for catalytic hydrosilylation.

Bis(imino)pyridines are a readily synthesized, highly modular and relatively inexpensive class of redox active ligands.^{8,9} Bis(imino)pyridine iron dinitrogen complexes are effective pre-catalysts for alkene hydrogenation,¹⁰ hydroboration¹¹ and [2+2] cycloaddition.¹² Combined spectroscopic, magnetic and computational studies have established that fundamental two-electron transformations such as oxidative addition operate via cooperative one electron chemistry occurring concomitantly at the ligand and the metal.¹³ This family of iron compounds also exhibits unprecedented activity and selectivity for the hydrosilylation of alkenes with commercially relevant tertiary silanes (Figure 3).

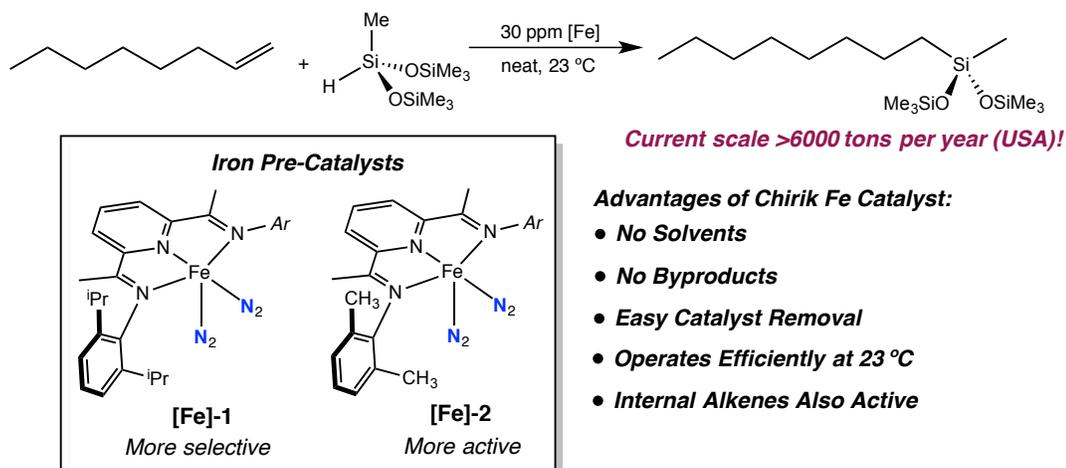


Figure 3. Bis(imino)pyridine iron catalysts for the hydrosilylation of 1-octene with superior activity and selectivity than current industrial platinum technology.

As described in a 2012 publication in *Science*,¹⁴ both **[Fe]-1** and **[Fe]-2** promote the hydrosilylation of alkenes with commercially relevant silanes. The iron catalysts are more active than platinum, operate efficiently at ambient temperature without the need for solvents and produce no byproducts. Significantly, the iron catalysts are also effective for the hydrosilylation of internal alkenes as well as amino alkenes used in the manufacture of fiberglass. In both cases, only the terminally functionalized products were observed, demonstrating that iron catalysts open new chemical space for industrial hydrosilylation.

Iron catalysts **[Fe]-1** and **[Fe]-2** have proven effective for the synthesis of other commercial silicone products. Crosslinking of silicone fluids (Figure 4) finds widespread

application as release coatings, providing the backing for labels and stamps. Because the product is gelatinous, catalyst recovery is impossible and is one of the largest sources of lost platinum in the silicone industry. Iron catalysts effectively promote this reaction at ambient temperature and provide materials indistinguishable from those prepared with platinum. One limitation of the iron catalyst is that residual ligand at the ppm level slightly discolors the product. This limitation has since been overcome by second-generation cobalt catalysts described in Section B.

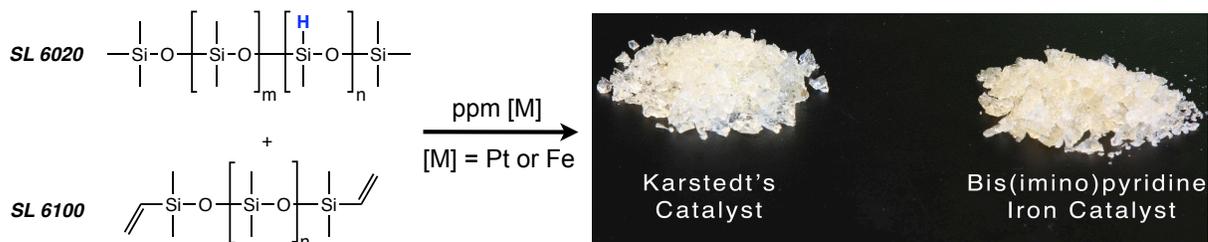


Figure 4. Comparison of Karstedt's Catalyst (platinum) to Chirik-Momentive iron catalysts for the synthesis of silicone release coatings. See U. S. Patent 8,927,674.

Silicones are also important components of agricultural adjuvants and low-rolling resistance, environmentally friendly tires. These applications clearly demonstrate the advantages of Chirik-Momentive base metal catalysts over current platinum technology (Figure 5). The former application is for the manufacture of so-called "super spreaders" – trisiloxanes that are exemplary of the principles of green chemistry. Addition of less than 1 weight percent of these compounds enhances the spreadability and uptake of contact fungicides, herbicides and insecticides, significantly decreasing the amount of these chemicals deployed by farmers that ultimately contaminate ground water,¹⁵ transforming environmental footprint of agriculture, particularly in the developing world. Current platinum catalyst technology used in the synthesis of the trisiloxanes necessitates high temperatures and produces up to 30% of isomerized product which requires rhodium-catalyzed hydrogenation and distillation for removal from the desired product (Figure 5). Chirik-Momentive iron catalysts, **[Fe]-1** and **[Fe]-2**, promote this reaction at ambient temperature and generate only the desired product, obviating the need for separation. These results once again highlight the ability of base metal catalysts to enable new and selective chemistry previously unobserved with precious metals.

Similar advantages of the iron catalysts are apparent in the chemistry used to prepare silicones for application in low rolling resistance tires that offer improved gas mileage and wear.¹⁶ The alkene substrate, 1,2,4-trivinylcyclohexane requires the selective addition of a single [Si-H] unit to a single alkene. Two vinyl groups must remain in tact, ideally those that are adjacent, for subsequent functionalization and incorporation into the rubber matrix. It is ideal if the hydrosilylation occurs exclusively at the 4-position. Commercial platinum catalysts yield a statistical mixture of products with poor regioselectivity even with a slow addition of a substoichiometric amount of silane. This lack of selectivity requires purification by distillation and generates considerable waste. The more sterically protected iron catalyst, **[Fe]-1** exhibits unprecedented selectivity for the monohydrosilylation of the desired 4-alkene and opens a new pathway for the selective synthesis of the desired compound.¹⁷

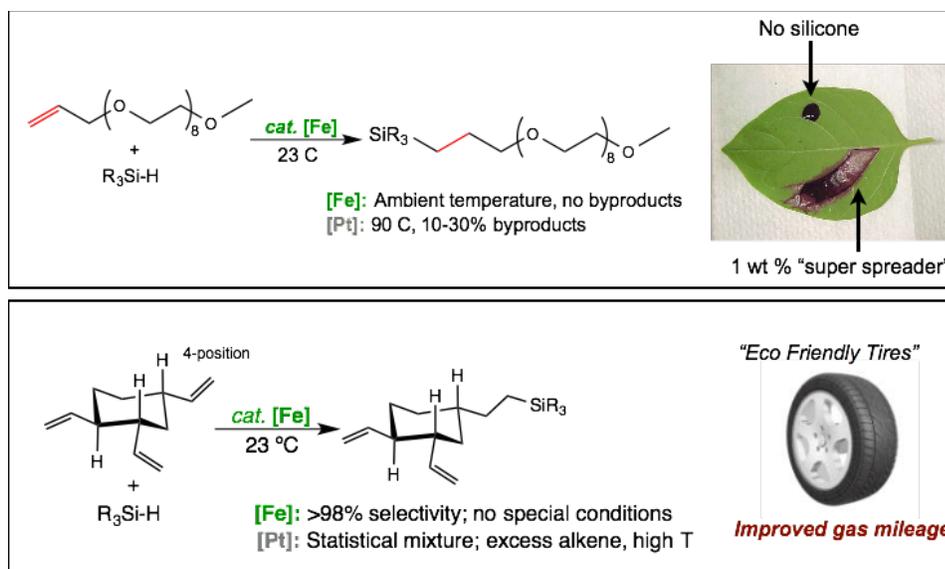
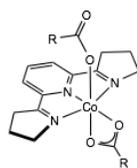
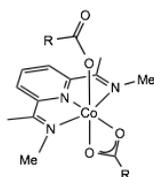


Figure 5. Application of Chirik bis(imino)pyridine iron catalysts for the selective hydrosilylation of allyl polyethers for the synthesis of agricultural adjuvants (top) and 1,2,4-trivinylcyclohexane used in the manufacture of low rolling resistance tires (bottom) (see US 2012/013010).

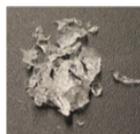
B. Air Stable Cobalt Catalysts for Alkene Hydrosilylation. Second generation catalyst development has centered on cobalt. Primary motivations for this work are to improve the air-stability and overall handling of the pre-catalysts as well as to remove color contamination from crosslinked silicones. It is also of fundamental interest to determine what other base metals are effective for this transformation. Thorough mechanistic studies have determined the necessary ligand effects required to favor hydrosilylation over the more commonly observed and unwanted dehydrogenative silylation processes.¹⁸ Cobalt, like iron, is a terrestrially abundant element and offers significant cost and environmental advantages over platinum. Notably, the supporting ligands that enable cobalt-catalyzed hydrosilylation are colorless and overcome one of the significant limitations of the iron chemistry. More significantly, cobalt precursors have been discovered that allow handling in air, demonstrating that base metals can offer the same ease of handling of well-established precious metal catalysts. Finally, the cobalt catalysts enable the hydrosilylation of new substrates, particularly allyl glycidyl ether – a product used as a finishing material in the glass reinforced plastics (fiberglass) industry and manufactured domestically on a >2000 ton scale per year. Current commercial platinum catalysts suffer from competing alkene isomerization and in some cases opening of the sensitive epoxide ring. Second generation Chirik cobalt catalyst technology promotes the selective hydrosilylation of this alkene with no evidence for byproducts. The cobalt catalyst operates effectively at ambient temperature at part per million levels and is tolerant of air and moisture.

Second Generation Cobalt Catalysts

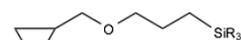


- < \$1.40 gram
- Air stable
- Improved FG tolerance
- Colorless ligands

Silicone Products Enabled by New Catalysts



Colorless
Release Coating



Allyl glycidyl ether



Amino silanes

Applications in Fiberglass



Figure 6. Second generation Chirik cobalt catalysts that are air stable and open direct routes to silicone products. See: Provisional Application Filed July 24, 2015.

C. Current and Planned Commercialization. The discovery of air-stable, readily synthesized iron and cobalt catalysts with unprecedented activity and selectivity have transformed the industrial approach to commercial silicone products. Cost estimates for second-generation cobalt catalysts are \$1.34 per gram (\$717.16 per mol), approximately 3% of the cost of commercially used platinum sources (\$60.00 per gram; \$22,9000 per mol). Both the iron and cobalt catalysts have been prepared on large (>100 g) scale and have undergone pilot plant evaluation. Hydrosilylations of various commercial products described above have been conducted on kilogram scales and the base metal catalysts will likely impact at least five product lines ranging from agricultural adjuvants to tires to health care to fiberglass. Evaluation of important industrial parameters such as recyclability, reproducibility, and process safety are on going and once complete further steps toward commercialization will be conducted.

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**An Application for the 2016 Presidential Green Chemistry Challenge Awards
Program in the Category of Specific Environmental Benefit**

**Instinct® Technology - Making Nitrogen Fertilizers Work More
Effectively for Farmers and the Planet**

December 29th, 2015

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Instinct® Technology - Making Nitrogen Fertilizers Work More Effectively for Farmers and the Planet

This entry is primarily intended for the Specific Environmental Benefit category with a secondary fit for Focus Area 3, the Design of Greener Chemicals.

This entry is not eligible for small business or academic award.

Significant Milestones:

- **US EPA registration approvals:** registration numbers 62719-583 and its alternates, 2009-2015, 62719-657 and its alternates, 2013-2015, 62719-684 and its alternates, 2015, 62719-692 and its alternates, 2015.
- **Commercial launch:** initially in US corn market in 2010, expanding into wheat in 2014.
- **Key patents:** US8377849B2 and US8741805B2, granted in 2014.
- **Commercializing in other countries:** between 2012 and 2015, including Australia, China, Canada, and EU (>12 member states).

The US component of the nominated technology: The active ingredient nitrapyrin was discovered, developed, and commercialized in the US by the Dow Chemical Company (Dow), Midland, MI. Instinct®, an aqueous microcapsule suspension of nitrapyrin, was designed, developed and commercialized by Dow AgroSciences, Indianapolis, IN, USA. Instinct® is currently manufactured at the Dow plant in Pittsburg, CA, and is currently sold in many countries around the world including the US under the Instinct®, eNtrench™, Entrench™ and N-Lock™ brand names.

Abstract

Human activities related to farming account for a significant percentage of nitrate in ground and surface waters and nitrous oxide (N₂O) emissions. Estimates published on the US EPA website stated that about 75% of all N₂O emissions come from agricultural activities such as applied nitrogen fertilizers and manures. Crop genetics and precision application methods have improved the efficiency of applied nitrogen fertilizers, but losses to the environment are still significant after soil bacteria quickly convert N, applied as urea or ammoniacal form, to nitrate (NO₃⁻). Nitrogen fertilizer in nitrate form is susceptible to losses via leaching and N₂O emissions. Furthermore, nitrate fertilizer that leaches out of the plant root zone is no longer available to provide nutrients to the crop. Instinct® nitrogen stabilization technology protects N fertilizer in the ammoniacal form, reducing nitrate leaching to ground and surface waters and atmospheric nitrous oxide emissions, the latter due to a soil denitrification process, thus retaining applied N longer in the plants' root zone for optimal crop utilization and yield.

The active ingredient in Instinct® is nitrapyrin, a powerful nitrification inhibitor and a proven tool for nitrogen stabilization. However, N-Serve®, the first nitrapyrin commercial product introduced by Dow in 1974, is only suitable for use with anhydrous ammonia applications due to the limitations of its physical and chemical properties. In 2010, Dow AgroSciences launched a novel aqueous microcapsule suspension product,

Instinct®. This patented technology can be conveniently used with commonly used N sources and has enabled wide adoption of nitrification inhibitor use in the US. In less than five years, acres treated with stabilized nitrogen have grown more than five-fold. In 2014 alone, based on calculated adoption in the US, it is estimated that Instinct® use added ~50 million bushels of additional corn and reduced carbon dioxide equivalent emissions by ~664,000 MT.

Description of the Nominated Technology

Problem addressed and solutions

One of the greatest challenges facing today's world is to feed and support the growing population in a sustainable and environmentally responsible way. The demand for higher crop yields and agricultural productivity is ever increasing and so are concerns around the negative impacts on the environment caused by agricultural activities. For example, estimates published on the official US EPA website stated that about 75% of all nitrous oxide (N₂O) emissions come from agricultural activities such as use of nitrogen fertilizers and livestock manures. Similarly, these nitrogen sources also result in most of the nitrate (NO₃⁻) leaching into ground and surface water sources. The majority of the nitrogen fertilizer products used today, such as urea, UAN (Urea Ammonium Nitrate liquid fertilizer), manure, and anhydrous ammonia, are ammonium based. Ammonium ions, because of their positive charge, are retained in the soil, which is negatively charged, and are less susceptible to leaching out of the plants' root zone. However, once ammonia based fertilizers are applied to the soil, the ammoniacal form of nitrogen is rapidly converted by the ubiquitous *Nitrosomonas sp* soil bacteria into nitrite (NO₂⁻), and then by *Nitrobacter sp* to nitrate (NO₃⁻), a N form which is very mobile and prone to loss from the root zone via leaching. Furthermore, nitrate ion (NO₃⁻) in soil can undergo a denitrification process to release the nitrogen into the atmosphere in the form of nitrous oxide (N₂O). The impact of one pound of N₂O, a potent greenhouse gas, on atmospheric warming is over 300 times that of one pound of CO₂. Studies show that a significant portion of soil nitrogen can be lost through leaching and denitrification before plant roots can take it up and utilize it.

More than a half century ago, scientists at The Dow Chemical Company (Dow) discovered a powerful nitrification inhibitor, nitrapyrin or 2-chloro-6-(trichloromethyl) pyridine, that can inhibit soil bacteria from rapidly converting NH₄⁺ to NO₃⁻, retaining more nitrogen in the stable ammoniacal form. By keeping N in the root zone for a longer period during the season, nitrapyrin improves NUE (Nitrogen Use Efficiency) and reduces nitrogen loss through leaching and denitrification. Figure 1 illustrates schematically the soil nitrogen cycle and the role of nitrapyrin in stabilizing nitrogen fertilizer applications. Effectiveness of nitrapyrin is due to its suppression of an enzyme in the soil bacteria *Nitrosomonas* that quickly initiates the conversion of NH₄⁺ to NO₃⁻, a N form that is susceptible to leaching loss and denitrification.

The first nitrapyrin commercial product, N-Serve® 24, was introduced by Dow in 1974, for use with anhydrous ammonia applications, primarily on maize crops. However, due to limitations of the formulation's physical and chemical properties, N-Serve® 24 is not suitable for the majority of nitrogen fertilizer sources, including urea, UAN, and manure, which are typically applied to the soil surface or injected into the soil. In 2010, after extensive research and development, Dow AgroSciences launched an aqueous based microencapsulated nitrapyrin suspension, with the brand name **Instinct®**. This robust and innovative technology can be applied conveniently to both dry and liquid nitrogen fertilizers such as urea, UAN and manure and enabled widespread adoption of nitrification inhibitor use in the US. In less than five years, the acres treated with stabilized nitrogen have grown more than five-fold. Figure 2, taken from a published meta-analysis of available research studies found in the scientific literature up to 2004¹, summarizes benefits of using nitrapyrin as a nitrification inhibitor. The meta-analysis showed that the use of nitrapyrin products provided significant benefits for both the environment and the crops including, on average, 1) improving corn yield by about 7%, 2) increasing soil N retention by approximately 28%, 3) reducing N₂O emissions by about 51% and 4) reducing NO₃⁻ leaching by approximately 16%. In 2014 alone, based on calculated adoption in the United States, the estimated impact from the use of Instinct® was adding about 50 million bushels of corn to the US production while reducing carbon dioxide equivalent emissions by about 664,000 MT, through the reduction of nitrous oxide emissions from denitrification.

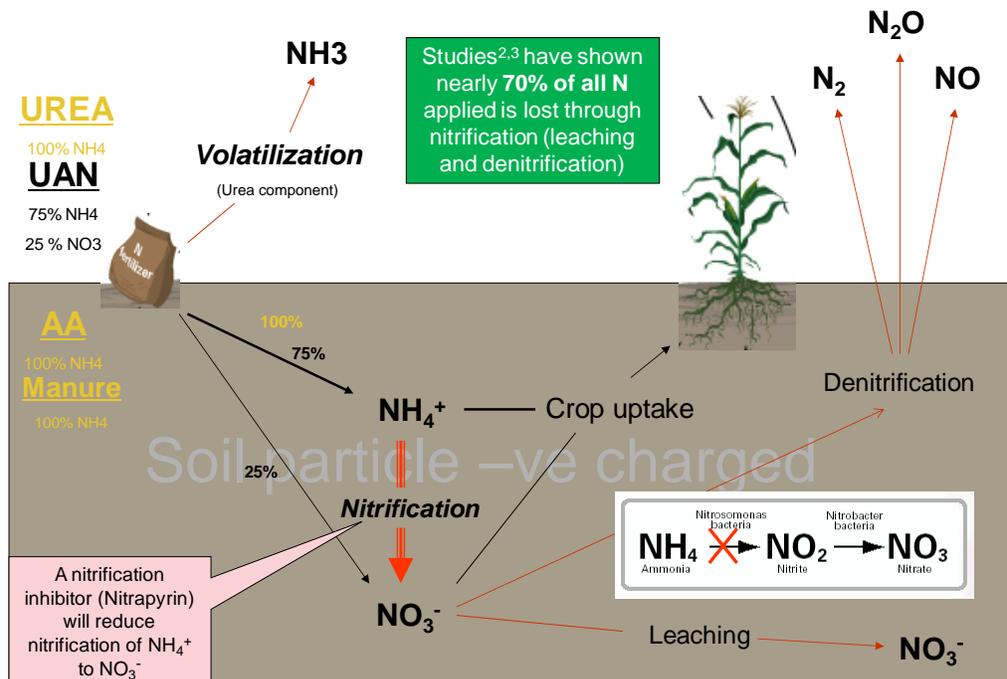


Figure 1. Nitrogen movement and the role of Nitrapyrin in Nitrogen fertilizer applications.

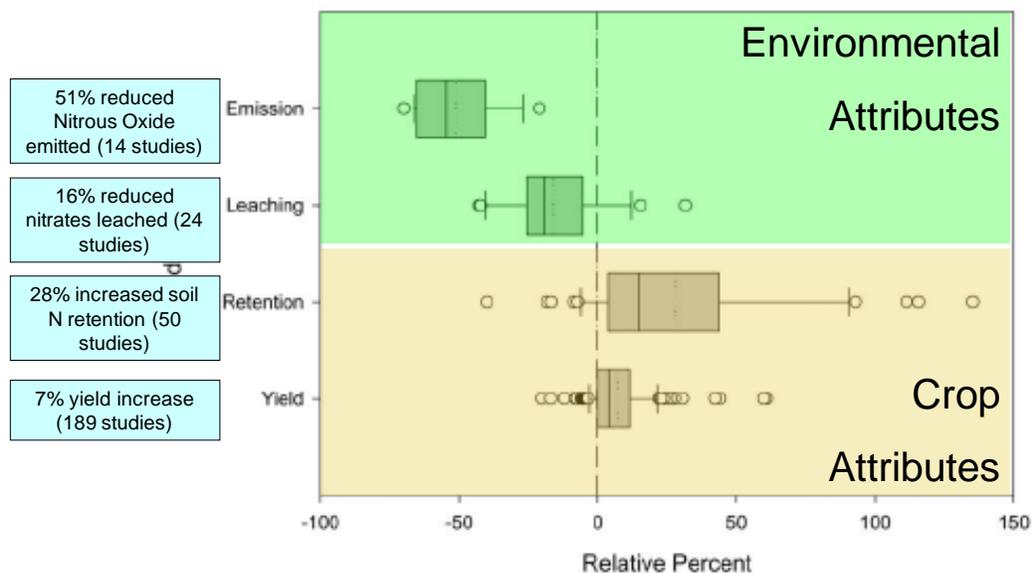


Figure 2. Summary of literature studies¹ on the benefits of using nitrapyrin in nitrogen fertilizer applications.

A more recent meta-analysis⁴ published by Qiao et al. validated the above data and the positive benefits of nitrification inhibitors, such as nitrapyrin, in maximizing crop production and minimizing environmental nitrogen losses.

Details of the Nominated Chemistry

The main limitation that makes nitrapyrin less effective for soil surface applied fertilizers such as UAN, urea and manure is the molecule's intrinsic high vapor pressure (43 Pa @ 25°C), which can result in high losses of nitrapyrin before rainfall, irrigation or mechanical incorporation into the soil occurs to inhibit nitrification. Microencapsulation technology was considered a potential solution to reduce the volatility and improve nitrapyrin stability on the soil surface, thus greatly increasing the application window to allow rainfall, irrigation or mechanical incorporation to get the nitrapyrin into the soil to inhibit the nitrification process. Figure 3 shows the patent protected microencapsulation technology developed at Dow AgroSciences. An oil phase containing nitrapyrin dissolved in organic solvents (e.g. aromatic 200, etc.) and oil soluble polyisocyanate was first mixed under homogenization or mechanical shear with an aqueous phase containing surfactants to form an oil-in-water emulsion with desired droplet size and distribution, after which the addition of a water soluble organic amine (e.g., ethylene diamine) results in a rapid polycondensation reaction to form the microcapsule wall (shell) containing the nitrapyrin in the core. Since one part of the reactant (polyisocyanate) is only oil soluble and the other reactant (amine) is only water soluble, the reaction can only occur at the oil-water interface where a shell or wall of polymer network is formed as illustrated in Figure 3. This process is highly effective to encapsulate an oil soluble active ingredient such as nitrapyrin. The shell then serves as a barrier to nitrapyrin volatility loss. Figure 4 provides a visual image of the microcapsules taken by cryogenic SEM (Scanning Electron Microscope).

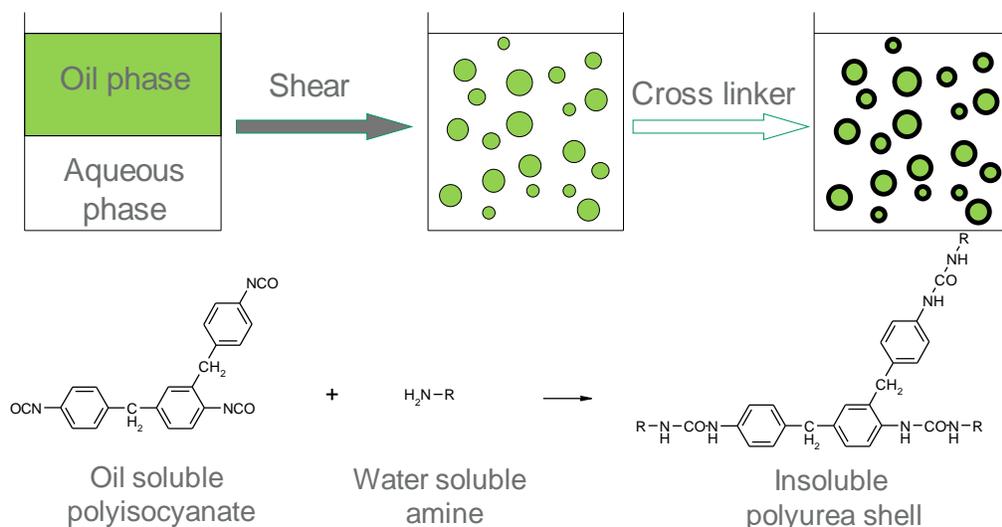


Figure 3. Chemistry and process schematics of interfacial polycondensation to microencapsulate nitrapyrin in aqueous media.

Although microencapsulation using interfacial polymerization is a known technology, the ultimate commercial success of this technology in the novel Instinct® formulation was the result of innovative design and careful optimization by scientists, biologists and engineers at Dow AgroSciences to achieve both superior biological performance and customer convenience across a wide range of applications. First, the capsule size and shell thickness were designed to provide adequate stability on the soil surface while readily moving the microcapsules into the soil with limited rainfall or watering. Secondly, the formulation composition was optimized to allow convenient applications in a wide variety of N fertilizers, including manure, liquid UANs, dry fertilizers, such as urea, and spray mixtures with crop protection chemicals. Instinct was also formulated to optimize good storage and handling properties for a wide range of climate conditions, including cold temperatures (e.g., late fall or early spring), to broaden the opportunities for use with fertilizer applications.

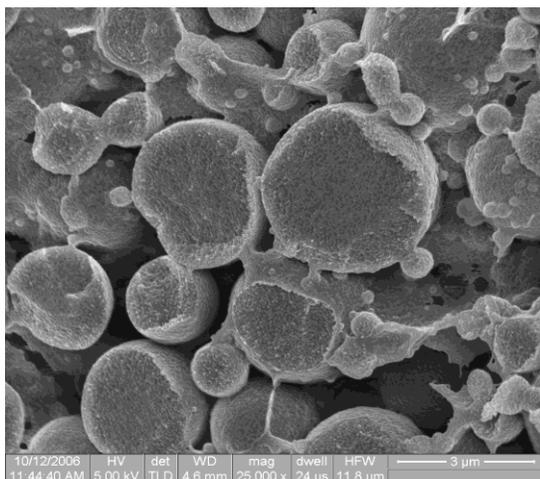


Figure 4. Image of nitrapyrin microcapsules by cryogenic SEM.

Microencapsulation of nitrapyrin as an aqueous suspension also provides additional environmental benefit by significantly reducing the amount of petroleum-based solvent usage input per treated acre. The amount of aromatic solvents used has been reduced by three-fold from approximately 3 kg/kg nitrapyrin in N-Serve[®] 24 to about 1kg/kg nitrapyrin in Instinct[®].

How technology compares

Nitrogen fertilizer efficiency enhancement technologies have been researched and explored for many years. Given the known scale of nitrogen loss with current practices and the potential benefits to the global agricultural industry and the environment, effective technologies in this area are highly valued. The three main technologies developed and commercialized in this area which have been independently and scientifically proven to add value are: nitrification inhibitors such as nitrapyrin, urease inhibitors, and coated or slow-release fertilizers. All three technologies can offer NUE benefits, but the use of coated fertilizers is limited due to high cost and the difficulty designing the technology for optimal release under a wide range of soil moisture and temperature conditions. Urease inhibitors offer value only when urea fertilizer is applied to the soil surface and not quickly incorporated. Urease inhibitors do not affect the conversion of urea or ammonium forms of applied N to nitrate. Effective nitrification inhibitors, such as nitrapyrin, are cost effective for farmers to adopt, flexible in their use to allow wide utilization, and directly impact the main nitrogen loss pathways, nitrate leaching and nitrate denitrification to nitrous oxides.

Nitrapyrin is scientifically proven to be very effective in reducing nitrous oxide emissions and was recognized and published⁵ by the US EPA as the most impactful technology for reducing nitrous oxide emissions in Agriculture. Nitrapyrin was shown to be more effective than other nitrogen use strategies such as split nitrogen applications, reduced nitrogen rates (as low as 70%) and no-till farming practices.

The development of the Instinct[®] formulation has allowed the use of nitrapyrin to be adopted by many more farmers using multiple sources of nitrogen fertilizers, including manures, at a competitive cost, multiplying the positive environmental and agricultural benefits explained above.

Current and planned commercialization

The development of the Instinct[®] micro-encapsulated formulation was important to allow US corn growers to use nitrapyrin with multiple forms of nitrogen, namely UAN, urea and manure. In the US, Instinct[®] is registered for use in corn and wheat crops with the expectation to expand its use to fruit and vegetable crops in the coming years. Current research in the fields of biology and formulation chemistry continues to seek means to broaden the application of the product and improve ease of use.

Since anhydrous ammonia is not widely utilized as a nitrogen source in most of the world, the flexibility of Instinct[®] also enabled Dow AgroSciences to commercialize the product in multiple crops and in additional countries around the world. The product is currently used in the United States, Canada, UK, Germany, Netherlands, Denmark,

Hungary, Poland, Australia and China. In the coming years, use will expand to include most countries in Europe with ongoing exploration in South America. Outside the US, the product is being effectively used in maize, cereals, canola, sugarcane, cotton and rice, further expanding the benefits of improved crop yields, reduced nitrate leaching losses and reduced greenhouse gas emissions.

Impact to Agricultural Productivity

As stated above, Instinct® offers both productivity and environmental benefits to agriculture. Through the protection of the nitrogen fertilizer in the soil, yield potential is maximized and extensive research illustrates the productivity gains that farmers can achieve. To illustrate the contribution of Instinct® to agricultural productivity based on average yield increases, it is estimated that in 2014 alone, Instinct® applications stabilized nitrogen that added about 50 million bushels of additional corn production. This equates to \$205,500,000 additional production revenue for US corn growers in 2014 (with an average bushel price of \$4.11). As the adoption of Instinct® in the US market increases and Dow AgroSciences launches into new markets and countries, we expect these environmental and productivity benefits to be multiplied around the globe.

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Renewable Nylon through Commercialization of BIOLON™ DDDA

December 31, 2015

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Renewable Nylon through Commercialization of BIOLON™ DDDA

This nomination is eligible for the small business award.

This nomination is submitted for the focus area *greener synthetic pathways*.

The integrated process for the production of renewable BIOLON™ DDDA described in this nomination was demonstrated at our 400-liter scale pilot facility throughout 2012 and 2013. Subsequent scale-up at external partner facilities in 2014 and 2015 enabled:

- 1) The production and sale of 70,000 lbs of renewable BIOLON™ DDDA,
- 2) Certification of our product with the USDA Certified Biobased product label,
- 3) The collection of data necessary for the engineering of our first commercial facility to be located in Malaysia, and
- 4) The signing of our first product distribution agreement with European specialty chemical distributor Will & Co., effectively securing off-take for over 25% of our plant's planned capacity.

The technology for this nomination was developed at Verdezyne's R&D headquarters and validated in our pilot plant, both located in Carlsbad, California. Further demonstration of the technology at larger scales was performed at partner facilities located in Michigan and Missouri.

Abstract: Verdezyne has developed a fermentation-based technology platform to provide manufacturers and consumers with renewable alternatives to existing petroleum-based chemical intermediates. The developed technology has focused on the production of the dicarboxylic acid chemical intermediates adipic acid, sebacic acid and dodecanedioic acid (DDDA). The first of these to be commercialized will be DDDA, which is used in many applications including coatings, corrosion inhibitors, adhesives, lubricants, and fragrances. However its main application is in the manufacture of nylon 6,12 for engineered plastics requiring special properties such as high chemical, moisture, or abrasion resistance.

The global market for DDDA is approximately 100 million pounds and is currently produced from fossil-based sources, with the largest volume technology proceeding via trimerization of butadiene followed by hydrogenation and oxidation with nitric acid. The Verdezyne process for production of DDDA uses fatty acid feedstocks sourced from the co-products of vegetable oil refining. In addition to providing a renewable alternative, our process offers improved safe operations without the need for high temperature and pressure or concentrated nitric acid, and offers reduced greenhouse gas emissions.

The technology for renewable DDDA has been developed at Verdezyne's R&D headquarters and with the 2011 commissioning of our pilot plant, the integrated process has been validated at pilot scale (400 L). Throughout 2014 and 2015, Verdezyne has demonstrated the process at larger scales (4,000 and 25,000 L), leading to the production and sale of 70,000 lbs of renewable BIOLON™ DDDA. This product met all industry quality specifications and has earned the USDA Certified Biobased product label, verifying that the product's amount of renewable biobased

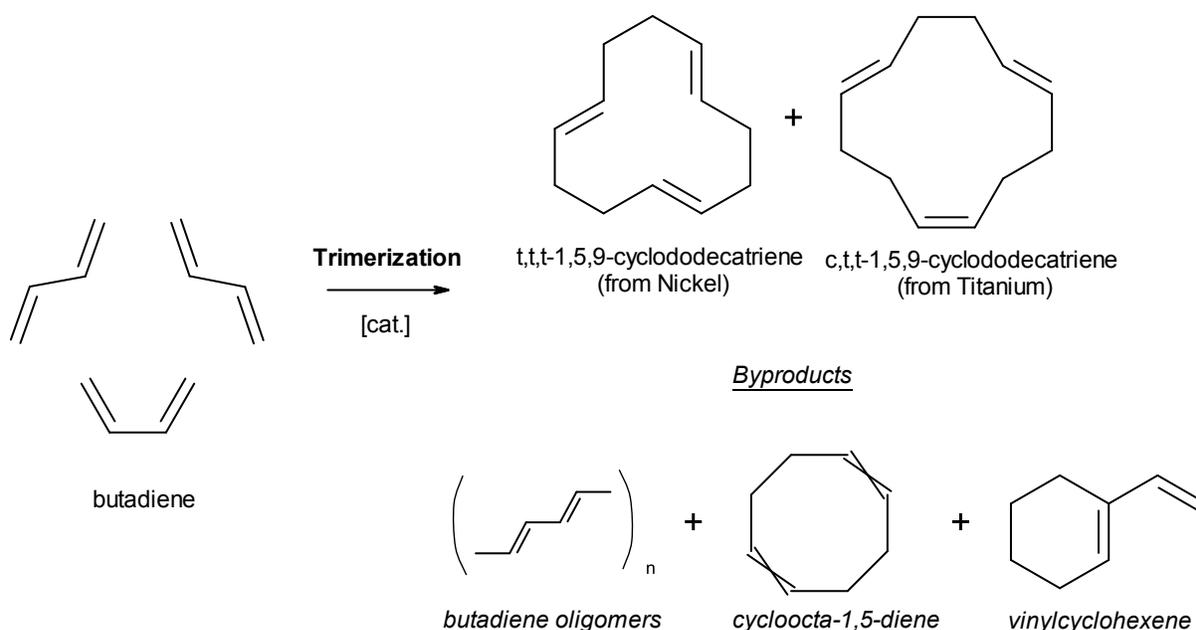
ingredients meets or exceeds levels set by the USDA. Our product is currently the only DDDA in the Biopreferred® catalog. We are currently preparing to break ground on a commercial-scale facility to be located in Nusajaya, Johor, Malaysia for the production of renewable BIOLON™ DDDA.

Renewable BIOLON™ DDDA as a Petrochemical Drop-in Replacement

Verdezyne has developed a robust industrial yeast strain and fermentation process capable of producing bio-based DDDA, a chemical that is currently produced from fossil-based sources. Our technology development has focused on producing renewable alternatives to existing petrochemicals where the fermentation product is the exact chemical currently used in the existing value chain, therefore making them drop-in replacements for petrochemicals. Producing these chemicals at or exceeding industry specifications allows manufacturers to switch to a renewable alternative without having to change equipment or procedures. This affords us the opportunity to take advantage of the existing markets for these chemicals for rapid commercialization.

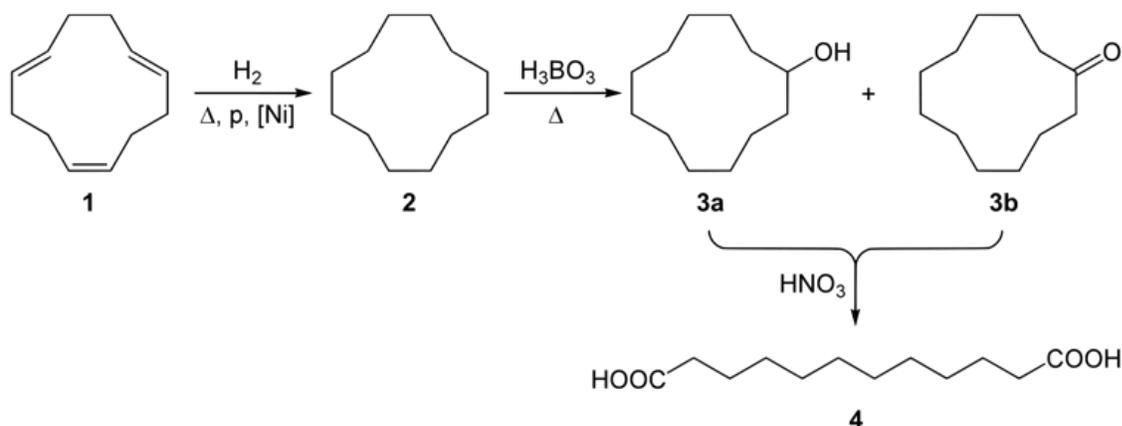
Two major producers, Invista and Cathay, supply the current world market for DDDA, which is approximately 100 million pounds. Specialty chemicals producers Evonik and UBE produce lower quantities, primarily for captive use. The Cathay process for the production of DDDA is fermentation-based, using dodecane purified by distillation from kerosene as the feedstock. The Invista process uses the petrochemical building-block molecule butadiene in a trimerization reaction (Equation 1) with a Ziegler-Natta titanium catalyst producing 1,5,9 cyclododecatriene

Equation 1: Synthesis of 1,5,9 cyclododecatriene from butadiene



(CDT) with a net selectivity of 90% for the desired *cis,trans,trans* isomer.¹ The CDT intermediate is then converted to DDDA in a multi-step process: catalytic hydrogenation at 170-180°C and 26-28 bar pressure absolute, mild oxidation with boric acid at 160-180°C resulting in a mixture of cyclododecanone and cyclododecanol, and ring-opening oxidation at 70-90°C with nitric acid and copper/vanadium catalyst (Equation 2).²

Equation 2: Synthesis of DDDA from 1,5,9 cyclododecatriene

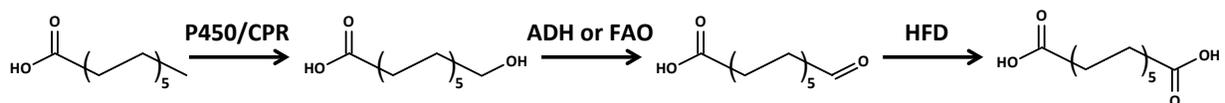


The last nitric acid oxidation step and the process for the production of nitric acid both release N₂O, a gas that has a global warming potential 298 times that of CO₂. The synthesis of adipic acid follows the same final reaction steps with six carbon molecules and releases ~0.3 tons of N₂O per ton of adipic acid produced in the nitric acid oxidation step.³ Using the same stoichiometry, the final DDDA step would release ~0.2 tons of N₂O per ton of DDDA produced and consume ~0.5 tons of nitric acid per ton of DDDA produced. Abatement technologies for the destruction of both NO_x and N₂O in process emissions exist, however their effectiveness depends on the technology employed and the usage factor for the abatement at an individual facility. The amount of N₂O released to the atmosphere from current DDDA production is unknown. Nitric acid production is itself a significant source of greenhouse gas emissions with an estimated release of 36 kt of N₂O (10.7 million metric tons CO₂ equivalent) in 2013 from U.S. production facilities alone.⁴

The technology developed by Verdezyne is an improvement over the incumbent DDDA processes in that it: 1) reduces our dependence on petroleum by using a renewable feedstock, 2) operates under safer conditions with temperatures and pressures closer to ambient, and 3) eliminates the production of N₂O and the use of nitric acid.

Verdezyne's production of BIOLON™ DDDA is an aerobic fermentation process integrated with downstream product isolation and crystallization. The fermentation converts the twelve-carbon fatty acid, lauric acid, to DDDA through the activity of a genetically engineered version of *Candida* yeast. The natural lauric oils coconut oil and palm kernel oil (PKO), both have fatty acid compositions of approximately 50% lauric acid. PKO is the larger volume of the two with

Equation 4: Enzymes involved in the ω -oxidation pathway converting lauric acid to DDDA



(CPR) that regenerates the P450 for another round of oxidation. In our production yeast, a 10-gene family encodes P450 enzymes, with each gene encoding a P450 with different substrate specificity. Identification of the P450 enzyme specific for lauric acid and its subsequent amplification (along with other supporting gene amplifications) have resulted in our commercial production strain with yields in excess of 95% and rapid production of titers in excess of 140 g/L DDDA. The genetic engineering of our production host and the fermentation methods for diacid production are the subject of 4 issued patents and 33 pending patent applications. The technology for production of renewable DDDA from lauric acid feedstocks is the subject of patent application WO 2013/006730 A2.⁵

With the ultimate goal of building and operating a commercial facility for the production of renewable DDDA, we worked with partners to demonstrate the fermentation performance at larger scales. This demonstration was performed at the Michigan Biotechnology Institute, at the 4,000-liter scale and at ICM at the 25,000-liter scale. In both cases the fermentation

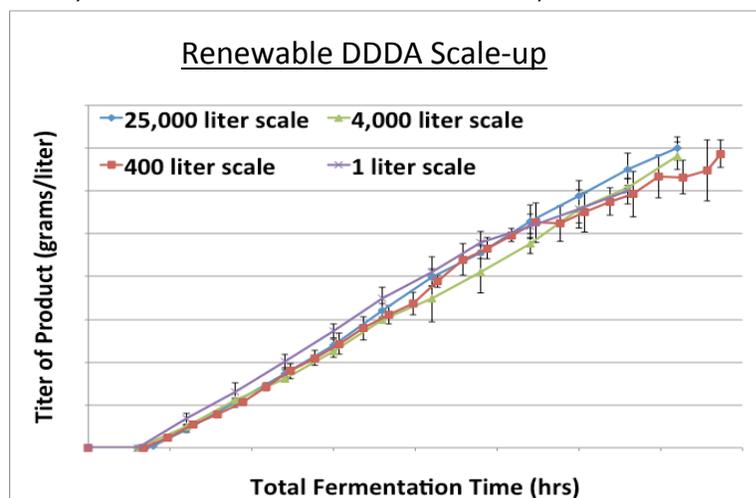


Figure 1: Equivalent fermentation performance at multiple scales

performance was as good as or better in yield, productivity, and final titer than the performance seen at Verdezyne R&D and pilot facilities (Figure 1). DDDA was isolated during each campaign, resulting in the production of 70,000 lbs of finished product that met or exceeded industry specifications. All of this product was immediately sold and is currently being tested by potential future customers in their existing commercial applications. Reports received so far by Verdezyne are that BIOLON™ DDDA performs

equal to or better than the petroleum based product in tested applications. In one application the report stated, "In side-by-side comparisons, Verdezyne's biobased DDDA yielded the highest-quality polymer that we have ever synthesized. Our end application is extremely sensitive to contaminants, which ultimately show up as undesirable colorants in the final product. By contrast, using Verdezyne's raw material we were able to produce the clearest, most functional material to date."⁶ Our product has also earned the USDA Certified Biobased product label and is the only DDDA in the USDA Biopreferred® catalog.

The data collected from these campaigns has also been used in the engineering of our commercial-scale facility for the production of BIOLON™ DDDA. The site selected for the 20



Figure 2: 3D rendering of planned commercial BIOLON™ DDDA facility

million pound facility (Figure 2) is the Bio-XCell bioindustrial park located in Nusajaya, Johor in southern peninsular Malaysia, just across the border from Singapore, strategically located close to the source of our lauric acid feedstock and to nearby ports for product distribution. The park provides existing infrastructure for security, park management, and a central utilities facility to provide electricity, steam, and wastewater treatment. We are currently in the detailed engineering phase for

the project with groundbreaking planned for March 2016 and plant commissioning in late 2017. In compliance with local regulations stipulated by the Malaysian Ministry of Natural Resources and Environment, Verdezyne has officially registered our internal Institutional Biosafety Committee with the Malaysian Department of Biosafety and we have received approval from the Department of Biosafety for the contained use of our genetically modified yeast for production of DDDA.

As we near commercial production, we are putting in place the necessary supply chain, sales, and product distribution infrastructure and agreements. We recently reached a product distribution agreement with European specialty chemical distributor Will & Co, securing off-take for over 25% of the planned plant capacity.⁷ Additional distribution agreements currently under negotiation could bring the total secured off-take to 50% of plant capacity. These agreements, likely to be completed well in advance of the completion of our commercial facility, reflect the surging global demand for biobased alternatives to petrochemicals. Additionally, the 100 million pound market for DDDA is growing at an estimated 5% annually with multiple applications, some of which are detailed below.

Nylon: Nylon 6,12 is based on DDDA and hexamethylenediamine. It is widely used in the manufacture of engineering resins and filaments. These engineering resins have lower moisture absorption and better dimensional stability compared with nylon 6,6 resins and are used in a variety of automotive applications. Flexibility imparted by the presence of DDDA makes nylon 6,12 filaments particularly useful in paintbrushes, toothbrushes, and cosmetic brushes. Nylon 12,12 containing DDDA and dodecanediamine has lower moisture absorption and greater flexibility than nylon 6,12, and is useful in high performance engineering resin applications, as well as in monofilament fishing line.

Powder Coatings: DDDA is widely used as a curing agent or cross-linker for glycidyl methacrylate (GMA) acrylic powder coatings. The use of DDDA in GMA acrylics results in coatings with better toughness, gloss, durability, chemical resistance, and flexibility. DDDA also

imparts good storage stability to powder coating compositions, in part because of its low solid/solid solubility in the bulk acrylic resin. DDDA linear polyanhydride and carboxy-terminated polyesters containing DDDA have also been used as curing agents for GMA acrylic powder coatings.

Lubricants and Greases: Diester lubricants based on DDDA have outstanding friction, wear and viscosity-temperature properties. Their performance is better than that of other shorter chain-length esters or hydrocarbon base oils, and have found applications in both automotive and aviation engine oils. Greases made with DDDA diesters also show bearing torque characteristics and oxidative stability superior to that of comparable greases made from a solvent neutral oil of similar viscosity.

Fragrances: C12 products are used in the manufacture of synthetic macrocyclic musks and also find applications in the synthesis of woody and floral notes. DDDA is used to produce a variety of macrocyclic ketones and lactones for the synthetic musk market.

The Verdezyne technology for production of BIOLON™ DDDA described above provides a renewable drop-in replacement for an existing petrochemical. In addition to reducing our dependence on finite petroleum resources contributing to global warming, the fermentation-based process can be carried out under safer operational conditions. We have successfully scaled-up our production process, verifying key metrics for competitive economics, and have demonstrated the highest product quality at each stage. Plans for our first commercial facility are moving steadily forward, and we are eagerly anticipating scaling up production to meet the world's surging demand for renewable DDDA and other chemicals.

¹ Zakharkin L and Guseva V (1978) The chemistry of 1,5,9-cyclododecatriene and syntheses based on it. Russian Chemical Reviews 47: 955-974.

² Rajendran G (2012) Process for the preparation of dodecanedioic acid. Subject of patent application EP2407444 A2.

³ Reimer R, Slaten C, Seapan M, Koch T, and Triner V (2000) Adipic Acid Industry – N₂O Abatement. In "Non-CO₂ Greenhouse Gases: Scientific Understanding, Control and Implementation", J. van Ham, et al. (eds.). Springer-Science+Business Media, B.V. p 347.

⁴ Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2013. EPA 430-R-15-004. <http://www3.epa.gov/climatechange/ghgemissions/usinventoryreport.html>.

⁵ Beardslee T, Picataggio S, Eirich ED, and Laplaza JM (2013) Biological methods for preparing a fatty dicarboxylic acid. Subject of patent application WO2013/006730 A2.

⁶ Customer testimonial by Pete LeBaron, Ph.D., VP of Technology at ICM Products, April 21, 2015.

⁷ <http://verdezyne.com/2015/06/09/verdezyne-signs-agreement-with-major-european-chemicals-distributor-will-co/>

AlkyClean® Technology

An Inherently Safer Alkylation Technology for the Production of Motor Gasoline Alkylate

December 15, 2015

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AlkyClean® Technology

An Inherently Safer Alkylation Technology for the Production of Motor Gasoline Alkylate

RECENT MILESTONES

- ACS Affordable Green Chemistry Award Winner in 2010 for the development of the AlkyClean process.
- Two U.S. Patents recently issued for AlkyClean technology: 7,875,754 (01/25/2011) for increasing alkylate yield, and 8,163,969 (04/24/2012) for improved catalyst.
- World's first safe and successful start-up of a commercial scale, 2,700 barrels per day, solid catalyst alkylation unit (AlkyClean) on August 18, 2015, at Zibo Haiyi Fine Chemical Co., LTD (Haiyi), Shandong Province, Peoples Republic of China.

SIGNIFICANT U.S. COMPONENT

- Engineering design of the AlkyClean Demonstration Plant at Porvoo (operating between 2002-2004) was executed by ABB Lummus Global, Bloomfield, N.J. (now CB&I). Albemarle's Houston, Texas, plant produced the main component of the catalyst, the zeolite, for the demonstration plant.
- The basic engineering design package for, and commissioning of, the commercial plant at Zibo Haiyi was executed by personnel from the CB&I offices in Houston, Texas.

FOCUS AREAS

- Area 2: Replacement of hazardous liquid acid catalyzed alkylation processes (i.e., HF and H₂SO₄) by a true solid acid zeolite catalyst process that has significantly less impact on human health and the environment.
- Area 1: Greener synthetic pathway to produce motor gasoline alkylate by a novel harmless catalyst.

NOT ELIGIBLE FOR THE ACADEMIC OR SMALL BUSINESS CATEGORIES

ABSTRACT

Refinery alkylate is an ideal clean gasoline blend component that is produced from light olefins and isobutane. It consists of clean combusting isoparaffins, having both low vapor pressures and very high octane numbers. Furthermore, it contains no environmentally unfriendly or toxic components, such as aromatics, olefins, or sulfur compounds. For compliance with ever stricter environmental regulations, alkylate is the preferred gasoline blending component.

The problem for refineries today is that alkylate production, currently about 30 billion gallons/yr worldwide (60% of which is located in North America), requires the use of liquid acid catalyzed processes (HF or H₂SO₄). HF is extremely toxic and, upon release,

forms clouds that can be lethal up to five miles. Sulfuric acid technologies generate huge quantities of corrosive spent acid, on the order of 10-20 billion lbs/yr, that have to be transported and regenerated, thereby, introducing risk into surrounding communities and adding to the generation of environmentally unfriendly emissions.

For more than 40 years, academic and industrial scientists have been trying to replace these liquid acid technologies by a much greener solid acid catalyst technology. AlkyClean technology is now available and has been commercialized. It is inherently safer to operate and has a lower environmental impact than alternative technology because it employs a true solid catalyst.

This breakthrough has been demonstrated by the successful start-up of the commercial scale (2,700 BPD) plant at Zibo which has met or exceeded all performance expectations. The design basis for Zibo was developed at the Neste Porvoo plant which operated for two years. During this demonstration, the technology was optimized and fully proven for commercial application. Product quality is on par with existing technologies. Moreover, production of waste products like acid soluble oils or spent acids is eliminated, and there is no need for product post-treatment of any kind.

EXECUTIVE SUMMARY

Alkylate, the product of the reaction of isobutane with light olefins (C₃-C₅), is highly valued as an ideal “clean fuels” blending component for the gasoline pool because it has no olefins, carcinogenic benzene or other aromatic compounds, a low sulfur content, a limited heavy end, a low vapor pressure and both high research and motor octane numbers. Currently about 30 billion gallons/yr of alkylate are produced worldwide, 60% of which is located in North America.

Refiners now have available a cleaner and inherently safer alkylation technology, the AlkyClean alkylation process. Based on more than two (2) years of successful operation of the prototype, CB&I successfully scaled it up to commercial scale at Zibo. Other solid acid technologies have only been investigated on much smaller pilot scale and/or still use leachable corrosive components, such as halogens. AlkyClean is the only solid acid technology that is in commercial operation.

The new solid acid catalyst (SAC) process produces high quality alkylate without the drawbacks of the existing hydrofluoric (HF) and sulfuric (H₂SO₄) acid based technologies. Neither acid soluble oils, nor spent acids, are produced, and there is no need for product post-treatment of any kind. It is estimated that about 10-20 billion lb/yr of spent sulfuric acid is being transported and regenerated just for alkylation. Besides these processing advantages, eliminating the use of these toxic and corrosive liquid acids greatly reduces maintenance and monitoring requirements while reducing environmental and personnel safety concerns. Benchmarking efforts have confirmed the overall competitiveness of the new technology.

Central to the new technology is the utilization of a novel, “true” SAC with a patented pore distribution. In this situation, “true” SAC means that the catalytic acid function is intrinsic to the solid itself rather than being a separate component, such as an immobilized liquid deposited on a solid substrate. The formulation is zeolite based. It contains no halogens, has acid sites with optimum strength for alkylation, yields high quality alkylate and exhibits the required activity, stability and regeneration capability necessary for a successful and viable process.

The AlkyClean process has proven to be robust, requiring minimal maintenance, while producing high quality product. The zeolite based solid acid catalyst has exhibited tolerance to both upsets and exposure to contaminants. The catalyst’s ability to be easily and repeatedly regenerated, under mild non-oxidative conditions, with full activity recovery, has been demonstrated over long periods of operation. With all of its very positive benefits, this breakthrough technology provides a profitable addition to the processing portfolio of refiners, as they strive to meet regulatory-driven demand for both cleaner fuels and greener/ safer refining processes.

I DESCRIPTION OF PROBLEM

Currently worldwide about 30 billion gallons per year of alkylate is produced by the reaction of isobutane with C₃ and C₄ olefins, mainly from FCC (Fluid Catalytic Cracking) units. The mixture of multi-branched, gasoline-range hydrocarbons that is formed is an excellent clean gasoline blending stock that can be used to replace toxic and carcinogenic aromatic compounds in the refinery gasoline pool.

However, the currently available liquid acid catalyzed HF and H₂SO₄ processes have many drawbacks. Both acids can inflict serious injury via skin contact and/or inhalation. This increases safety risks for operating personnel and nearby communities. Over the years many incidents have occurred, causing injuries as well as fatalities.

In the case of HF units, leaks may lead to the formation of aerosol clouds that can spread lethal doses of HF upwards of five miles. Based on refinery risk management plans, in the U.S. alone, more than 17 million people currently live within such danger zones (USPIRG: Needless Risk Study, 2005). While mitigation options have been developed, they can be very costly, and have not been universally adopted. Significant risks still exist, especially during unit repair and maintenance or process accidents at adjacent units.

In the case of sulfuric acid, worldwide consumption by alkylation units is in the order of 10-20 billion pounds per year. Regeneration of spent sulfuric acid is carried out by incineration. This consumes energy, produces greenhouse gasses, and also increases the risks of SO_x emissions. In addition to the hazards described above, both conventional liquid acid processes require removal of residual acid from the products by scrubbing, washing and neutralization with caustic and/or lime. This leads to the production of waste water and sludge.

Altogether, the incentive to develop and commercialize an environmentally friendly, solid acid catalyst (SAC) alkylation process is very high. Although there have been many attempts during the past 40 years, nobody had of yet succeeded in demonstrating such a technology. Prior approaches have in most cases failed because of poor product selectivity and/or excessively rapid catalyst deactivation, coupled with the lack of an acceptable catalyst regeneration procedure. Also in many cases the catalyst utilized leachable corrosive components such as halogens, trifluorosulfonic (triflic) acid, BF_3 and H_2SO_4 , which could migrate into product streams.

Albemarle Catalysts and ABB Lummus Global (now CB&I) started a co-operation in 1996 to develop a catalyst/ process combination that addresses the aforementioned problems. Commercial success was ultimately proven and demonstrated with the start-up of the Zibo plant in August 2015.

II CHEMISTRY OF ISOBUTANE ALKYLATION

In literature, many papers address the chemistry of alkylation (e.g. Catal. Rev.-Sci. Eng., 35(4), p.483-570, 1993). The preferred alkylation products are C_8 trimethylpentanes (TMP), which are formed by the primary reaction of isobutane with butenes. The research octane number (RON) of the various TMP isomers is about 100-110. However, depending on the process and operating conditions, part of the C_8 s formed will be dimethylhexanes (DMH), which have a RON of only about 50-60. Also C_5 - C_7 (lights) and C_9 + ("heavies") secondary products are formed, which have a lower RON and/or a higher vapor pressure than the favored TMP products.

It is desirable that a catalyst/process combination leads to high TMP/DMH ratios and low by-product formation. Therefore, an understanding of the reaction mechanism is essential for the development of a good SAC process technology. A generally accepted proposal for the mechanism of butene alkylation is presented in Figure 1.

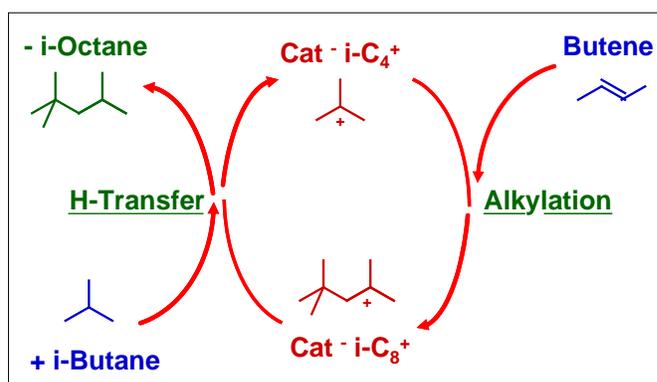


Figure 1. Chemistry

The initial reactions lead to the formation of C_4^+ ions on the acid sites of the catalyst. After reaction with C_4 olefins, C_8^+ ions are formed, which can escape from the catalytic sites after hydrogen transfer reaction with isobutane. In this way, the preferred species,

(isooctanes) are produced and C_4^+ ions are formed again. This catalytic cycle can be repeated many times. However, C_8^+ ions can also react with a second olefin, leading to C_{12}^+ ions. The C_{12}^+ ions can “escape” by hydrogen transfer or react further to form higher molecular weight products. Some larger carbonium ions will split into smaller fragments, leading to the formation of the undesired C_5 - C_7 and C_9+ compounds. It is clear that high isobutane-to-olefin ratios (I/O) in the reactor feed and high hydrogen transfer reaction rates favor the formation of C_8 s over C_5 - C_7 and C_9+ compounds.

However, the rate constant of alkylation reactions leading to undesired long chain molecules is about three orders of magnitude higher than the rate constant of hydrogen transfer reactions. Thus, to achieve our product quality goals, the catalyst, reactor and process design should be directed to maximize hydrogen transfer reactions and to attain high I/O at the acid sites.

III PROCESS AND CATALYST DEVELOPMENT AND COMMERCIALIZATION

Attempts to apply SAC in the past failed, because the catalyst and process conditions used resulted in very rapid deactivation of the catalyst. So, in addition to a novel catalyst and a novel reactor and process design, novel regeneration procedures had to be developed.

Catalyst

After pre-screening in a micro reactor, a true SAC was selected. The zeolite used is of a type well proven in industry. However, we had to optimize its strength and the number of acid sites to enhance hydrogen transfer reactions over multiple alkylation reactions. The catalyst particle size and porosity also needed to be optimized using a pilot plant that allowed the investigation of regeneration procedures as well. Three breakthrough innovations are illustrated by the following U.S. Patents: 5,986,158 (novel process and regeneration procedures), 6,855,856 (catalyst with optimized porosity and particle size), and 8,163,969 (alkylation process with an improved catalyst), all granted to Albemarle (inventors - E.H. van Broekhoven et al.). Other solid acid technologies use a less robust catalyst that still contains leachable components.

Reactor and Process design

The AlkyClean solid acid alkylation process operates in the liquid phase at moderate operating conditions, so no refrigeration, as used in the case of sulfuric acid alkylation, is required. The general process scheme is similar to that employed by both HF and H_2SO_4 alkylation processes with respect to the recycling of isobutane back to the reactors. However, in the case of the AlkyClean process, the reactors contain fixed bed catalyst and are designed to keep olefin concentration low and I/O at the active sites of the catalyst high. In this way hydrogen transfer reactions leading to the desired products are favored as described in the Chemistry Section.

Regeneration procedures

Regeneration of the catalyst is carried out continuously by cyclic switching from olefin addition (alkylation), to addition of dissolved hydrogen (mild regeneration), without changing reactor conditions. Nevertheless, depending on the severity, overall olefin conversion may drop, hence a higher temperature regeneration with hydrogen is carried out every week or so. This restores catalyst activity and selectivity and also distinguishes AlkyClean technology from other developments.

Demonstration Plant Operation

The final Basis of Design was developed and optimized at Neste's Porvoo facility during the time period 2002-2004. All elements of the technology (catalyst, process, regeneration, hardware, etc.) were fully demonstrated and proven.



Figure 2. Photograph of the Zibo Haiyi Plant

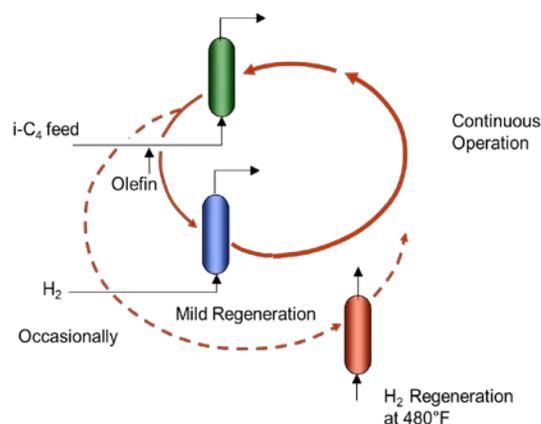


Figure 3. AlkyClean Flow Scheme.

Commercial Scale AlkyClean Unit Start-up

The first commercial-scale, solid catalyst alkylation unit was started up on August 18, 2015, at Zibo Haiyi Fine Chemical Co., LTD (Haiyi). The unit, which employs CB&I's AlkyClean technology, jointly developed by CB&I, Albemarle Catalysts and Neste Oil, has a capacity of 2,700 BPSD of alkylate production (100,000 kta). The unit is located in Zibo, Shandong Province, Peoples Republic of China and has achieved all performance expectations. In addition, the octane value (RON) of the alkylate product has been consistently measured between 96 and 98, quite high when compared with typical alkylate products. The value of alkylate, as a gasoline blend component, increases with higher RON value.

AlkyClean technology uses Albemarle's AlkyStar catalyst, a robust zeolite catalyst, and together with CB&I's novel reactor scheme, high-quality alkylate product can be produced without the use of liquid acid catalysts in the manufacturing process.

Cross-media effects

Since no corrosive media are present, cost of maintenance and repair of the AlkyClean process will be much lower and reliability much higher. This will reduce emissions of hydrocarbons during such periods of time. Catalyst needs replacement only once every five years. The spent catalyst contains zeolite, alumina matrix and a very low concentration of Pt metal; consequently it is relatively harmless and can easily be transported off site for the reclamation of Pt. The noble metal is reused, while solids can be utilized in the construction industry.

Capital investment and operating costs

Capital investment requirements for the AlkyClean process were optimized by CB&I as process licensor. The results indicate that the total installed cost is somewhat higher than an equivalent H₂SO₄ unit, not unexpected when the value proposition for the refiner is the elimination of liquid acid catalyst by a true solid catalyst. The total cost of catalyst and utilities for the AlkyClean process is also somewhat higher than that of H₂SO₄ technology, but we expect to achieve lower costs in the future as we further optimize the plant design.

Existing liquid acid units may be revamped to AlkyClean technology by reusing the existing feedstock pretreatment and distillation facilities and replacing the reaction section. Since AlkyClean technology creates no waste water or acid sludge, no acid neutralization facilities are required which eliminates related costs.

Product properties and yield

The alkylate product from the AlkyClean process meets all specifications (e.g. RON, vapor pressure and end boiling point) for reformulated gasoline blend stock. In addition, the new “green” technology does not produce heavy polymeric side products, while HF and H₂SO₄ technologies form acid-soluble oils waste with this material, which must be incinerated. So the alkylate yield from the AlkyClean process is at least as high as found in conventional liquid acid processes.

IV CONCLUSIONS

In conclusion, the AlkyClean technology has been proven at a commercial scale since August 2015 and is available to refinery operators that want or need an inherently safer technology when compared with conventional liquid acid catalyzed alkylation technologies.

Additional environmental benefits have also been demonstrated with the elimination of waste materials that are typically generated with conventional liquid acid alkylation technologies. Furthermore, alkylate quality and yields are comparable.

Technology title: Green Process for Commercial Manufacture of Etecalcetide Enabled by Improved Peptide Manufacturing Technology

Date: December 31, 2016

Primary Sponsors: Amgen Inc. and Bachem

Contact person:

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Drug Substance Technologies

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Technology title: Green Process Commercial Manufacture of Etelcalcetide Enabled by Improved Peptide Manufacturing Platform Technology

The nominated technology is neither eligible for the small business award nor for the academic award. The focus area of the technology is “Greener Reaction Conditions”.

Parsabiv™ is a novel calcimimetic agent indicated for the treatment of secondary hyperparathyroidism (sHPT) in adult patients with chronic kidney disease (CKD) on hemodialysis therapy. In 2016, the drug was approved in many regions and countries including the European Union (EU), Russia and Japan. In the U.S. the user fee goal date is Feb. 9, 2017. Etelcalcetide, a synthetic peptide, is the active ingredient in Parsabiv™. An improved manufacturing process of Etelcalcetide was validated and implemented for the commercial production at Bachem, holding a leading position in the process development and the manufacturing of peptides with +40 years of experience, in 2015. The patent application of this process was issued in 2015 (Method For Preparing AMG 416 PCT Int. Appl. (2015), WO2015154031).

The process design and development activities were mainly performed in the US by a joint effort between Amgen Inc. and Bachem. The commercial manufacturing is currently performed in Bachem’s facility in Bubendorf, Switzerland.

Abstract:

Parsabiv™ is a novel calcimimetic agent indicated for the treatment of secondary hyperparathyroidism (sHPT) in adult patients with chronic kidney disease (CKD) on hemodialysis therapy. In 2016, the drug was approved in many regions and countries including the European Union (EU), Russia and Japan. In the U.S., the user fee goal date is Feb. 9, 2017. Etelcalcetide, a synthetic peptide, is the active ingredient in Parsabiv™.

The standard peptide manufacturing platform was used to manufacture drug substance at Bachem for clinical supply up to Phase 3. However, the environmental and manufacturing costs, along with high drug demand exceeding the capacity of all major CMOs, compelled Amgen to redesign, and implement an improved peptide manufacturing process in close collaboration with Bachem. The new process resulted in the following improved attributes:

1. 5-fold increase in manufacturing capacity
2. 76% manufacturing cost reduction
3. 56% decrease in manufacturing operating time
4. Reduction in solvent consumption by 71%
5. Elimination of an ion-exchange column process requiring over 3 L of water for every gram of drug substance
6. Lowered the energy intense lyophilization cycles from 13 per drug substance batch to 1.

The overall impact of these technology innovations from Amgen and Bachem to the Etelcalcetide process will be realized by the annual elimination of 1440 cubic meters or more waste, including over 750 cubic meters of aqueous waste.

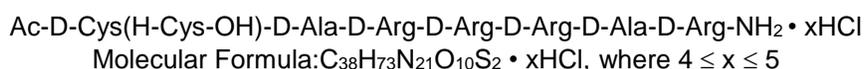
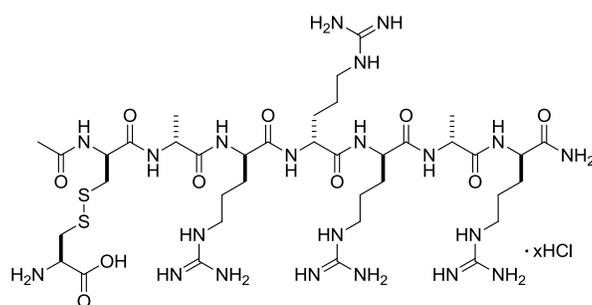
The new, green commercial manufacturing process was implemented and validated prior to the drug approval. As a result, Amgen and Bachem will realize the benefits of this new process for virtually the entire lifetime of this product, which minimizes the environmental impact to the largest extent. In addition, the broad and general applicability of this improved peptide-manufacturing platform has been demonstrated for other peptide drug candidates. The technology developed and implemented by Amgen and Bachem for the manufacture of Etelcalcetide is an excellent example of scientific innovation resulting in manufacturing technology evolution and benefits to the environment.

Introduction

Peptides have gained increased interest as therapeutics over last three decades, largely due to their advantageous properties including high specificity, affinity, as well as superior safety and tolerance. These properties make peptide drugs more desirable than small molecule drugs in certain diseases such as cancer, enzyme deficiency disorders, protein-dysfunction disorders, genetic and degenerative diseases and infectious diseases. Currently, there are over 60 FDA approved peptide drugs on the market. About 140 peptide drugs are in clinical trials and over 500 are in pre-clinical development (<http://www.peptideguide.com>). Thus, peptide-based pharmaceuticals are rapidly becoming an important class of therapeutic agents with the potential to replace many existing small molecule-based pharmaceuticals in the near future.

The solid phase synthesis method invented by Merrifield in the 1960s for the preparation of peptides has matured sufficiently to become the method of choice not only for the drug discovery but also as a key platform technology for commercial production. In particular, large-scale production has been fueled over the last decade by technical improvements in chromatographic equipment used for peptide purification. Accordingly, consistent product quality can be achieved with relatively less need for in-depth process development. However, compared to the manufacturing process of small molecule drugs, the environmental impact of this technology has not garnered much public attention, in part, because the high potency of peptide drugs has rendered supply needs significantly lower (typically less than 10 kg annually) than traditional small molecule drugs. However, closer recent investigation has revealed that, on average, producing 1 kg of peptide requires over 5 metric tons of solvent (typically including DMF, DCM, IPE, Acetonitrile, and IPA), which is *significantly* higher than most other types of synthetic small molecules. As more peptides enter the clinical and commercial spaces, and production volume increases (> 1000 kg peptide produced by a major CMO in 2016), the total environmental impact of manufacturing peptide drugs can be at least as large as for other small molecule drugs and should not be overlooked by pharmaceutical companies and contract manufacturing organizations (CMO) responsible for the manufacture.

Figure 1. Etelcalcetide (AMG 416) Structure



The Etelcalcetide drug substance was manufactured at Bachem using a standard solid phase peptide synthesis (SPPS) manufacturing platform up to the phase 3 stage. The drug substance supply post launch was deemed at high risk due to two major issues 1) the high cost and 2) the exceptionally high demand, which cannot be fulfilled by any of CMOs with existing capacities. One possible solution to address both issues could be simply leveraging 'economy of scale' since the existing phase 3 process was shown to be robust and scalable. However, expanding the manufacturing footprint would require > \$30 M investment and cause the potential delay of the commercialization of the program. More importantly, this approach would not reduce or eliminate the use of large amount of solvents, water, energy or generation of hazardous wastes and therefore doesn't meet the fundamental principles of green chemistry. The environmental and manufacturing cost, along with the high drug demand, compelled Amgen to redesign, and implement an improved peptide manufacturing process in close collaboration with Bachem. The joint team successfully eliminated one of five manufacturing stages and significantly optimized the remaining four stages by developing and implementing innovative technologies. Table 1 summarizes the evolution of the improved manufacturing process, major technologies implemented at each stage and the benefits (on the annual basis) realized during manufacturing. In summary, the improved new process resulted in a 5-fold increase in manufacturing capacity (without equipment upgrades or expansion) and a 56% decrease in manufacturing operating time which mitigated risks to drug supply. This new process will create a reduction of solvent consumption by 71%, completely eliminate an ion-exchange column process requiring over 3 L of water for every gram of drug substance, and reduce the energy intense lyophilization cycles from 13 per a drug substance batch to one. Every year Amgen and Bachem expects to eliminate the formation of 1440 cubic meters or more waste, including over 750 cubic meters of aqueous waste.

Table 1. Manufacturing Process Flow, Evolution, Process Improvements and Benefits

	Standard Peptide Manufacturing Process (Phase 3)	Improved Peptide Manufacturing Process (Commercial)	Technology Implemented	Benefit Realized (per annum)
Stage 1	Solid Phase Peptide Synthesis (Tricyclic amide linker resin, Fmoc-D-Arg(Pbf)-OH, Fmoc-D-Ala-OH, Fmoc-D-Cys(Trt)-OH) AMG 416-Resin	Solid Phase Peptide Synthesis (Rink amide AM resin, Fmoc-D-Arg(Pbf)-OH, Fmoc-D-Ala-OH, Fmoc-D-Cys(Trt)-OH) AMG 416-Resin	High substitution capacity resins In process testing to monitor the reaction and washing end points	2 times capacity increase 50% solvent reduction 420 m ³ DMF reduction 40% reduction on washing cycles
Stage 2	Cleavage (TFA, water, TIPS, DPDS) Precipitation and centrifuge (IPE, ACN) AMG 416-SPy	Cleavage (TFA, water, TIPS, DPDS) Precipitation and filtration (IPE, ACN) AMG 416-SPy	Particle engineering to improve the isolation process	13 times capacity increase 68% solvent reduction 57 m ³ solvent reduction 83% process time reduction
Stage 3	Conjugation (disulfide formation) (TFA, Cys) Precipitation, washing, drying (IPE, ACN) Crude AMG 416	Conjugation (disulfide formation) Conjugation (Aqueous TFA, Cys) Crude AMG 416	Conjugation reaction in aqueous solution	18 m ³ solvent reduction Telescope to the purification step (elimination of stage 3 isolation)
Stage 4	Purification (C18 silica gel, water, TFA, ACN) AMG 416-TFA	Purification (C18 silica gel, water, TFA, ACN) AMG 416-TFA	Purification optimization platform	3 times capacity increase 57% elute reduction (195 m ³ water and acetonitrile) 30% process time reduction
Stage 5	Salt exchange TFA to acetate (Anion exchange resin) Salt exchange acetate to HCl (HCl, lyophilization) Lyophilization AMG 416	Salt exchange TFA to HCl by precipitation:, washing, drying (IPA, HCl, water) Lyophilization AMG 416	Precipitation for salt conversation Lyophilization	10 times capacity increase Elimination of 72 lyophilizations 750 m ³ water saving N/A

The new, greener manufacturing process was subsequently validated and approved for manufacture at Bachem's plant in Switzerland. As a result, Amgen and Bachem will realize the benefits of this new process for virtually the entire lifetime of this product which minimizes the environmental impact to the largest extent. In addition, the broad and general applicability of this improved peptide manufacturing platform has been demonstrated for other peptide drug candidates. The technology developed and implemented by Amgen and Bachem for the manufacture of Etecalcetide is an excellent example of scientific innovation resulting in manufacturing technology evolution and benefits to the environment.

Process improvement and realized benefit

The detailed process improvements in each stage of the manufacturing process and their impacts on the environment, cost, and manufacturing efficiency are provided in the following sections.

Process Improvement of Stage 1 (SPPS)

The purpose of Stage 1 is to assemble the fully protected peptide backbone on a resin by solid phase peptide synthesis. The peptide backbone is built up from the C-terminus to the N-terminus by 1 amino acid per synthetic cycle until the resin carries the complete amino acid sequence using Fmoc (9-fluorenylmethoxycarbonyl) protection strategy. The final Stage 1 reaction is acetylation of the peptide N-terminus. The protected peptide backbone on resin (AMG 416-Resin) is isolated by filtration and dried.

To ensure the complete removal of the byproducts and excess reagents and minimize the impurity generation in the following cycles, sufficient washing is required after both coupling and Fmoc removal steps. A standard SPPS procedure comprises a total of 20 washes of DMF (the primary solvent for both reactions and washings) steps per cycle; (10 after the coupling step and 10 after the Fmoc removal step) and the ratio of solvent to the resin is 10/1 (L/kg), which led to use of 4 L of DMF per 1 g of product and long process time.

The process improvements mainly focused on 1) defining appropriate IPC criteria for washing end points by understanding the impact of residual byproducts and reagents on the product quality; 2) developing/implementing rapid testing methods to monitor the end points of washing (by pH), coupling (by Kaiser color testing), and Fmoc removal (by UV testing) steps and perform additional washing or extend reaction time only when needed, rather than follow a fixed procedure; 3) using a higher substitution solid phase resin (50% capacity increase).

Implementation of those improvements reduced the number of washings to 6 per cycle on average; the amount of DMF to less than 1 L per gram of product (only 25% of a standard SPPS protocol), which is equal to approximately 70 cubic meters of DMF per SPPS batch. In addition, the higher volumetric productivity and operational efficiency increase the plant capacity by > 2-fold.

Process Improvement of Stage 2 (Cleavage)

The purpose of Stage 2 is to cleave the peptide backbone from the resin, remove all side-chain protecting groups and activate the sulfhydryl group of cysteine with 2,2' dipyridyl disulfide (DPDS) in TFA. This procedure yields the AMG 416-SPy intermediate containing a disulfide bond between pyridyl sulfide and the drug substance backbone. In the standard protocol, AMG 416-SPy is precipitated by transferring the cleavage solution in TFA to IPE/ACN. Small particle size and poor filtration characteristics of the material necessitate the use of an inverting centrifuge to isolate the materials and drying has to be performed in a rotavap portion wise. Due to the general size limitation of inverting centrifuges, approximately 8 cleavage batches would be required to make one batch of drug substance and each cleavage batch would require 7-day process time, which represents the major capacity bottleneck.

Therefore the process improvements mainly focused on improving physical properties of the intermediate by redesigning the isolation using small molecule crystallization and particle engineering principles to promote the particle growth and optimize the solvent system. It was found that the addition mode has the most prominent effect and an inverse precipitation procedure (ie, precipitation by addition of the antisolvent mixture ACN/IPE to the cleavage solution instead of the inverse operation) resulted in significantly increased particle size and improved the filtration behavior. This improvement allowed the product to be isolated and dried on a regular filter dryer, which is more amenable to the large scale production and increases the manufacturing capacity by 13 times removing the major capacity bottleneck. In addition, the volume of antisolvent (ACN/IPE) was significantly reduced from 7 times the volume of cleavage solution to 3 times the volume of cleavage solution, which translates to 68% reduction of solvent usage. The purity of isolated AMG 416-SPy was also increased due to more efficient washing to remove byproducts (eg, PySH) and excess reagents (eg, DPDS) in the filter dryer.

Process Improvement of Stage 3 (Conjugation)

The existing platform process included a "Conjugation" operation (disulfide bond formation) in organic solvent (TFA), followed by precipitation in large amounts of organic solvent (IPE) and isolation of the solid intermediate by centrifugation and drying. The isolated crude drug substance is then dissolved in water and advanced through Stage 4 purification.

During the disulfide formation reaction optimization, kinetic studies using NMR indicated that the conjugation reaction is actually very sluggish in neat TFA, but clean and fast in aqueous TFA solution. Further studies proved that the actual conjugation reaction happened in aqueous TFA solution during sample preparation in Stage 4 and in general the presence of water can accelerate the disulfide bond formation. Those findings resulted in eliminating the original conjugation step, telescoping it into the purification step and saving over 3000L organic solvents per drug substance batch, and yielded significant cycle time and cost reduction. This finding also led to a broadly application as a new platform technology in the process of peptides containing disulfide bonds.

Process Improvement of Stage 4 (Purification)

The crude AMG 416 solution is purified by preparative reversed-phase chromatography. The purification process consists of 2 steps: the purification step to remove impurities and the concentration step to increase the fraction concentration. Using the standard purification platform, 2.2 L of eluent was required to produce 1 g of drug substance.

To increase the productivity and reduce the eluent volume while maintaining the produce quality, we developed a platform tool including the mathematical modelling and scoring system and applied this to improve the purification process improvement. This allows systematically investigate the process parameters (e.g. stationary phase, modifier, gradient slope and form, and linear velocity) and build a robust small scale model for the scale up. The optimized operating conditions resulted in a total eluent reduction of 57%, including 59% reduction in the purification step and 50% in the concentration step. This platform tool has become a standard practice to improve the purification efficiency and reduce the elute volume for other peptides developed at Bachem.

Process Improvement of Stage 5 (Salt conversion)

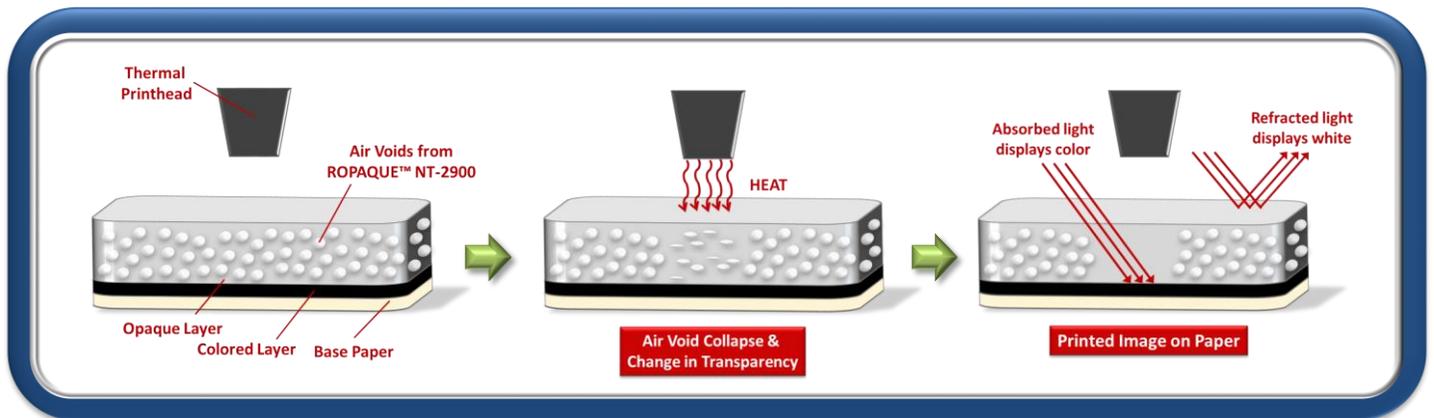
The standard procedure to convert a peptide TFA salt (derived from the TFA-buffered HPLC purification process) to the final salt form, hydrochloride (HCl) salt involved 2 steps: 1) conversion of the TFA salt to the acetate salt using an acetate ion exchange resin; 2) conversion of the acetate salt to the final HCl form by lyophilization of acetate solution in the presence of dilute hydrochloric acid. While this two-step exchange has been proved more practical for the large scale production than direct exchange using the chloride resin due to the low selectivity coefficients of chloride ion and need for large excess of chloride resin, it's still an inherently volume intense and low throughput process. Each batch of AMG 416 drug substance required 3 full-scale ion-exchange columns and 3 lyophilizers, 13 lyophilization cycles in total, 750 m³ of water (not including the resin regeneration process) and > 30 days process time, which represent another major bottleneck of the process and beyond the capacity of all major peptide manufacturers.

During the physical properties characterization of the drug substance and intermediates, the solubility of AMG 416-TFA salt was found to be > 100 times higher than AMG 416 hydrochloride in many organic solvents. Leveraging solubility difference and crystallization process design principles, a precipitation process was carefully designed for direct salt conversion, where the solution of AMG 416-TFA salt in aqueous IPA was added to a solution of HCl in IPA and the AMG 416 HCl salt was isolated by filtration. Process parameters, e.g. equiv of HCl, addition modes, mixing were systemically optimized to ensure effectively removal of 5 equiv of TFA (>33 wt%) and avoidance of the common gel out issue for peptides in solutions. This novel and practical direct salt conversion process can be run at large scale using a standard reactor and filter dryer, and significantly reduced the cycle time and waste while eliminating the use of large ion-exchange columns and 12 lyophilization cycles per drug substance batch. Since its implementation in AMG 416 process, this alternative salt conversion technology has found more general application to several peptide products.

Nomination for the 2017
Presidential Green Chemistry Challenge Award

Voided Structure Technology for Thermal Papers

December 31, 2016



Sponsors:

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Title: Voided Structure Technology for Thermal Papers

This entry is **Not Eligible** for awards in either the academic or small business categories.

Focus Area: 3 – The Design of Greener Chemicals

Most Recent Significant Milestones:

Dow developed the enabling component for the technology (ROPAQUE™ NT-2900 Opaque Polymer) in **2012**.

The formulations for the novel imaging system were developed and optimized by Dow and Koehler to prove that the technology worked while meeting BfR XXXVI and customer performance targets in **2013**.

Koehler conducted field testing throughout **2014** to validate the performance in real world conditions.

Dow optimized the process for scale up and manufactured ROPAQUE™ NT-2900 Opaque Polymer at a commercial scale in **April 2014** and Koehler optimized formulations for large scale paper production.

A marketing campaign was launched in **June 2015** in which product boxes with sample material and sample rolls were delivered to >200 customers. The material was completely sold out in a few months and further production was scheduled.

The final product was re-optimized in **2016** by Koehler and commercial deployment is underway.

United States Component:

The polymeric imaging technology was developed in the U.S. (Dow research centers in Collegeville, PA and Midland, MI). Production and initial commercialization of the thermal paper with customers is occurring in Europe, and will be expanded globally including the U.S.

Abstract:

The Dow Chemical Company and Koehler jointly developed a novel thermal printing paper which needs no chemical developers to create an image. The technology uses an entirely new mode of action based on changing the refractive index of a polymeric coating, a physical, not chemical, transformation. This new technology relies solely on air voids in the paper coating to develop an image. It avoids the use of free, reactive monomers and materials of concern. The resulting thermal paper is compatible with commercial thermal printers on the market today.

Thermal paper is used broadly throughout the world for cash register receipts, tickets, tags, and labels. Current thermal papers use chemical developers such as bisphenol A (BPA) and bisphenol S in the coating. When used in thermal paper, BPA is present as free, monomeric BPA, which is likely to be more available for exposure than BPA polymerized into a resin. Low-dose studies on the health effects of BPA raise questions and concerns, leading consumers and regulators to suggest limiting exposure. Environmental agencies and thermal paper producers are actively looking for replacements. No clearly safer thermal paper developers are available, with most alternatives having Moderate or High hazard designations for human health or aquatic toxicity endpoints.² A new approach is clearly needed. Voided polymer imaging technology allows thermal imaging with chemical developers, avoided the challenges of a reactive system and with no BPA.

The benefits of this new thermal paper imaging technology include:

- Complete elimination of chemical developer (e.g. BPA and BPS) which are under scrutiny for health and safety concerns
- Source reduction – Imaging chemicals are replaced with benign polymer and air
- Printed images are permanent/non-reversible unlike conventional thermal paper
- Compatibility with existing standard thermal printers currently in commercial use across the globe.

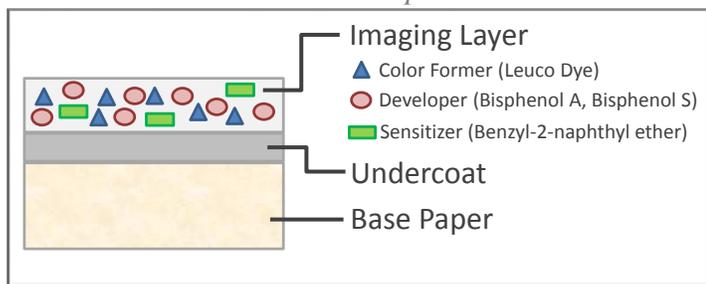
Description of the Nominated Technology

Problem Addressed and Solutions: A phase-out of bisphenols in thermal paper has been the target of environmental agencies and an objective of thermal paper manufacturers. BPA is a high production volume chemical used in manufacturing polycarbonate plastics, epoxy resins, and other uses. Testing shows that human exposure to BPA is widespread and that thermal paper is a source of exposure.¹ In March 2010, the EPA released a chemical action plan for EPA. One component of the plan tasked the EPA Design for the Environment (DfE) branch to conduct an alternatives assessment for BPA in thermal paper. Thermal paper was selected for evaluation based on concerns for potential exposure to consumers and workers, release to the environment, and stakeholder interest. The study attempted to identify safer chemical developers and no clearly safer alternatives to BPA were found. Most alternatives have Moderate or High hazard designations for human health or aquatic toxicity endpoints.² Rather than being limited by thermal developer technology, researchers at Dow and Koehler found a new approach.

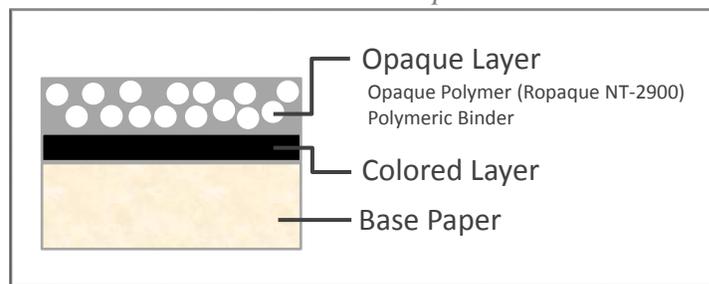
Scientists at Dow and Koehler developed a patented thermal paper technology that completely eliminates the use of chemical developers (BPA or BPS) and other reactive chemistries.^{3,4} This new thermal printing technology relies solely on the collapse of air voids in the paper coating during printing. Polymer properties are tailored so that a physical change, rather than a chemical reaction, creates the image. This is the first commercially viable and technically suitable drop-in alternative for conventional thermal papers introduced to the market. This technology avoids exposure to imaging chemicals for retailers and consumers. Additionally, this technology overcomes a major problem of conventional thermal papers; the new technology does not fade even under severe sunlight. Using this technology, documents such as medical records would not have to be photocopied for long term storage.

Details of the Nominated Chemistry: Direct thermal paper is used for many applications, including point of sale point-of-sale receipts, tickets, tags and labels.⁵ Direct thermal printers are widely used because they are highly reliable, require very little maintenance, and offer high image resolution. The printed image is formed by heating the paper, which contains a reactive combination of Leuco dye and acidic developer, typically BPA. In the presence of heat, the dye is protonated by the developer to facilitate a color change from white to black.

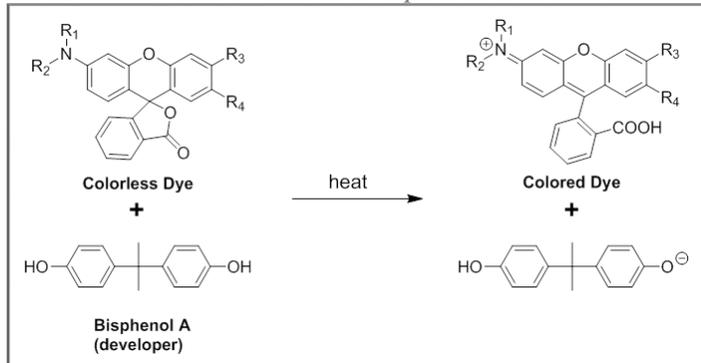
Traditional Thermal Paper Structure



New Voided Thermal Paper Structure



Traditional Thermal Paper Mechanism



New Voided Thermal Paper Mechanism

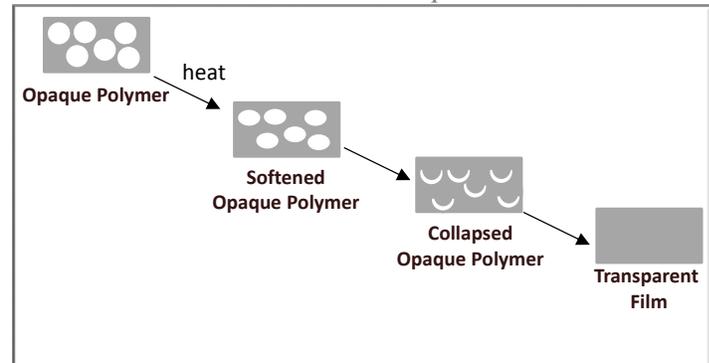




Figure 1. Structure of Thermal Papers (top), Mechanisms for Printing (middle), and Examples of Thermal Paper (bottom)

The new voided thermal paper is composed of three layers, including the top opaque layer, a colored layer, and a base paper (top of Figure 1 on the previous page). The colored layer contains only polymeric binders and permanent pigments such as carbon black. The opaque layer contains hollow particles that create air voids in the coating, resulting in an opaque layer which hides the underlying dark colored layer. When heat is applied to the paper from the thermal printhead, it causes localized collapse of the hollow particles. The opaque coating then becomes transparent only where the collapse has occurred and the underlying colored layer is visible and creates an image. In Figure 2, cross-sections of the opaque layer before and after printing are shown. In this case, the paper was printed at half energy to show the collapsed and retained air voids in the same image. At higher print energies, the air voids completely collapse to a transparent film. The details for the new thermal paper have been discussed in detail in our patents.^{3,4}

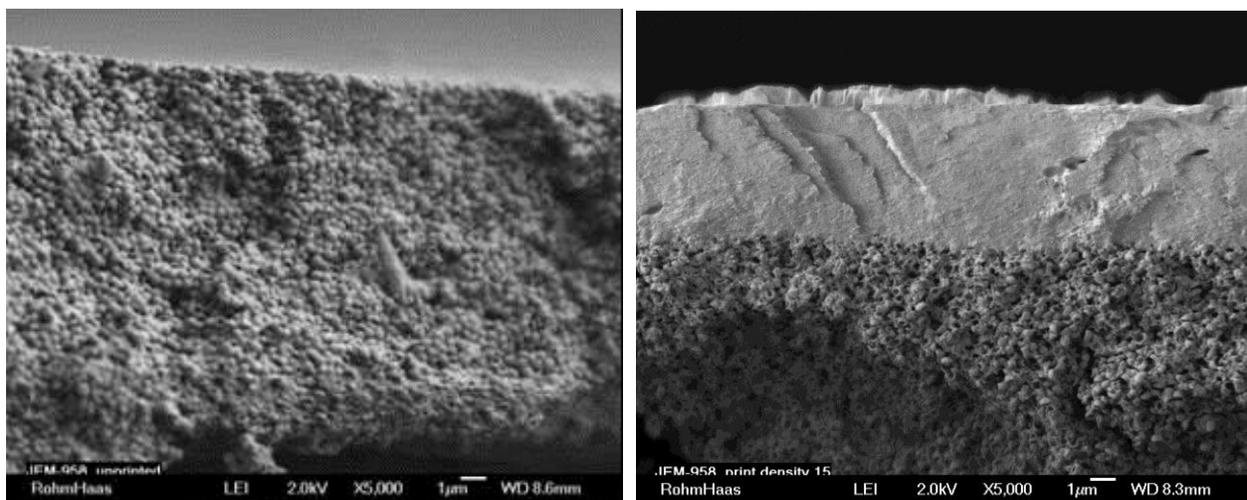


Figure 2. Cross-Section SEM of Opaque Layer Before Printing (left) and After Printing at Half Energy (right)

Hollow opaque polymers were originally invented by The Dow Chemical Company over 40 years ago to replace titanium dioxide in architectural paints. They are now used in a far reaching range of end uses including paints,

sunscreens, coated paper for hiding and gloss generation, white inks, debulking in leather finishes and more. Opaque polymers are synthesized by encapsulating a carboxylic acid functionalized polymeric core with a polymer shell using emulsion polymerization. When neutralized, the carboxylic acid core expands and absorbs water. Then upon drying the water-filled particles, the opaque polymers are left with an air void in the core.

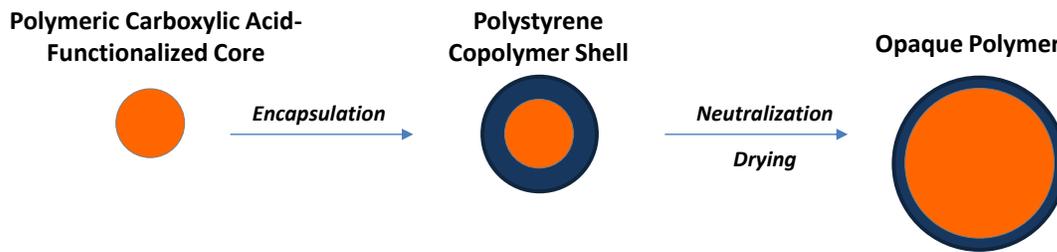


Figure 3. Opaque Polymer Synthesis

One of the biggest technical challenges in developing an opaque polymer which provided the new thermal paper with the same exceptional print contrast as in conventional thermal paper was the stringent requirements for print darkness and readability (such as in barcodes). Ideally, the opaque polymer layer delivers a high level of opacity while also completely collapsing the air void when locally heated. In order for the air voids to collapse completely to a uniform transparent film, the thermal printhead must cause the opaque polymer to soften and collapse. The dot pulse duration of a thermal printer is fractions of a millisecond, limiting the temperature that the thermal paper surface can reach. To further complicate the matter, the thermal conductivity of the opaque polymer is very low due to the insulating capacity of the air voids. These effects drive to thinner layers to improve thermal print performance. Conversely, paper brightness improves as the thickness of the opaque layer increases. Theoretical predictions guided the development of hollow polymer spheres. The opaque layer needs to be at least 20 μm thick to reach the typical brightness of a receipt paper. In reality the opaque layer includes additional binders, reducing light scattering efficiency due to the smaller voids between the particles. Theoretical predictions over-predict the brightness. The calculations show that achieving high brightness in the paper along with good printability is quite challenging. The commercially available opaque polymers at the time could not meet the scattering efficiency and print targets, and therefore it was necessary to develop a breakthrough in opaque polymer technology for this new thermal paper.

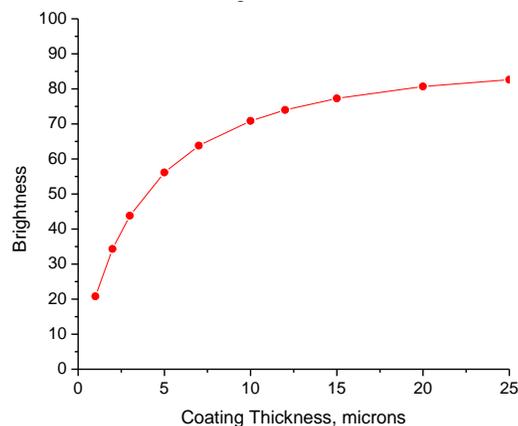


Figure 4. Theoretical Prediction of Paper Brightness vs. Opaque Layer Thickness

In addition to the thickness of the opaque layer, the brightness of the paper is also directly related to the particle morphology and chemistry. The refractive index differences between the internal air void and the polymer shell must be maximized to achieve optimal opacity. As a result, the new opaque polymer was synthesized with high levels of polystyrene to take advantage of its large refractive index difference with air (1.6 for polystyrene vs 1.0

for air). The scattering efficiency is also improved by optimizing the air void diameter to 300 nm by controlling the particle size and acid content of the core.

The second part of the key challenge was to ensure that the air voids would collapse readily during printing. Traditional opaque polymers were designed to be very stable in paints, so the air voids did not collapse significantly during printing.⁶ An improvement occurred when the glass transition temperature (T_g) of the opaque polymer was decreased to facilitate collapse during printing. However, many of the air voids also collapsed during paper manufacturing when utilizing low T_g polymers. A breakthrough in the technology was achieved by changing the morphology of the opaque polymer so that the T_g could be tailored to offer improved performance. The composition of the new opaque polymer is patent pending.³

To illustrate the advantages of the optimized morphology, two opaque polymers were synthesized with identical overall calculated T_g s. The first (OP1) had the traditional composition. The second (OP2) was synthesized through the optimized morphology to an overall T_g that was the same as OP1. OP2 had a higher initial brightness and significantly improved printability (Table 1, higher optical density signifies a darker image). Optimization of the morphology resulted in further improvements in the performance, culminating in development of the unique opaque polymer, Ropaque NT-2900 Opaque Polymer.

Table 1. Improved Brightness and Printing from a New Opaque Polymer Morphology

Opaque Polymer	OP1	OP2
Initial Brightness	67.0	75.0
Printed Optical Density (au)*	0.91	1.06

*Zebra Printer Model XVIII, Print Darkness = 15, Optical Density measured by densitometer

Through the optimization of opaque polymer and the opaque layer, the new thermal paper has a unique bluish-gray color that differentiates it from conventional thermal papers (Figure 1, bottom). There is no mistaking this new thermal paper from conventional thermal papers, which makes it easy for consumers to know when they are getting this differentiated product. To ensure excellent readability, the print contrast is similar to commercial receipt papers since the printed image of the new voided thermal paper is darker than conventional thermal paper.

The composition and performance of the two technologies are summarized below:

Table 2. Comparison of Traditional Thermal Paper to the New Voided Thermal Paper

Type	Traditional Thermal paper	New Thermal paper
Image creation	Uses chemical reaction under heat to create image	Uses only physical changes to create image
Key coating ingredients	Chemical Developers (BPA/BPS), Leuco Dyes, Binder	Opaque Polymer (Polystyrene copolymer), Binder, Wax, Carbon Black
Direct food contact	No	Yes

Human Health Effects: When used in thermal paper, BPA is present as a free monomer, which is likely to be more available for exposure than BPA polymerized into a resin or plastic. European data indicate that the use of BPA in paper may also contribute to the presence of BPA in the stream of recycled paper and in landfills. Global developer volumes for thermal paper are estimated at 19,500 MT.⁷ Although there are currently no estimates for the amount of BPA used in thermal paper in the United States, in Western Europe, the volume of BPA reported to be used in thermal paper in 2005/2006 was 1,890 tons per year (JRC-IHCP 2010). At 10% market penetration, the new thermal paper will reduce the production and release of 189 tons of BPA in Europe only or 1950 MT of common chemical developers globally.^{8,9}

The new voided thermal paper eliminates the use of reactive chemicals for imaging with a benign polymer containing air. Moreover, this new thermal paper will be the first commercially viable thermal paper applicable in direct food contact. Based on the European ISEGA-certificate, it can be used in direct food contact (dry and fatty food goods) and can be recycled without any concerns.

In the table below the Health and hazard information for BPA is compared with that of the ROPAQUE™ NT-2900 Opaque Polymer for the new thermal paper following some of the criteria used in the EPA report:

Table 3. Comparison of GHS Hazards of Thermal Papers

Type	Traditional Thermal Paper	New Voided Thermal Paper
Material	Bisphenol A (BPA)	ROPAQUE™ NT-2900 Opaque Polymer
Generic chemistry	Bisphenol	Polystyrene Copolymer
GHS Signal Word*	Danger	None
Pictograms*		None
GHS Classification Hazard Statements*	H317 Skin sensitization H318 Serious eye damage H335 Respiratory irritation H361 Reproductive toxicity H401 Acute aquatic toxicity H411 Chronic aquatic toxicity	Not a hazardous substance or mixture

*Hazards were obtained from Sigma Aldrich SDS documents

From a source reduction point of view, conventional thermal papers fade due to the reversible chemistry of the dye and developer. This requires important documents such as medical records to be photocopied regularly – a manual and very resource-intensive process. The new thermal paper is irreversible, which overcomes this major deficiency of traditional thermal paper. Trials show that severe sunlight testing resulted in easily readable documents that do not decay and can be archived for an unlimited period of time. The permanent images produced through this new imaging technology offer additional protection against falsification of documents.

Thermal printing is the most efficient printing mechanism from the perspective of material consumption, printer size, electrical consumption and speed. Due to the irreversible imaging, this new thermal paper could substitute inefficient and less environmentally friendly printing solutions such as (heavy metal containing) laser printing and thermal film printing.

Status of Commercialization

The opaque polymer used in the opaque layer, ROPAQUE™ NT-2900 Opaque Polymer, is produced in full scale commercial reactors.

In order to confirm market interest, a series of market tests was carried out at a small food chain in Germany in May 2014. This company was selected due to its very close contact with customers, trained sales personnel, and the reputation of being a high-end food manufacturer. The high level of acceptance of the new thermal paper in four of the company's stores supported further market introduction. Further testing required a larger scale production in May 2014 in Germany.



Figure 9: Production on a 500 mm coating machine in Oberkirch, Germany



Based on this successful production test, a larger test series of about 2,000 cash register rolls was performed at a home center in London, United Kingdom in September 2014.

Material of a large-scale pilot trial was sold to a supplier of checkout equipment in Germany. A first marketing campaign in May 2015 resulted in a very high response rate of about 20% of non-target product communication.

A second campaign in June 2015 was launched and product boxes with product literature and sample rolls were delivered to over 200 customers. A sample box is shown on the left. The material was completely sold out as of October 2015 and further production was scheduled. There is also firm interest by larger chain stores. Encouraged by this positive market feedback, production is continuing with a goal of large-scale commercial roll-out in 2017.

As can be seen from the above interest and production plans going forward, this differentiated thermal paper provides customers with a chemical developer-free solution that can be used on existing printers at an acceptable cost.

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- ⁷Laves Chemie Consulting, *Thermal Paper 2011-2016 – 9th Edition – Worldwide Market Study*
- ⁸Joint Research Centre-Institute for Health and Consumer Protection JRC-IHCP 2010.
- ⁹European Union Risk Assessment Report, 4,4'-Isopropylidenediphenol (Bisphenol-A).

Letemovir: A Case Study in State-of-the-Art Approaches to Sustainable Commercial Manufacturing Processes in the Pharmaceutical Industry

December 21st 2016

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Letermovir: A Case Study in State-of-the-Art Approaches to Sustainable Commercial Manufacturing Processes in the Pharmaceutical Industry

- The technology described herein is not eligible for the academic or small business award.
- **Focus Area 1: Greener Synthetic Pathways**
- The technology described in this package has been demonstrated at validation scale within the last year (2016) and represents the commercial process intended for use for Letermovir.
- The technology design and development described in this award package was carried out at the Rahway, NJ, USA research facility.

Abstract

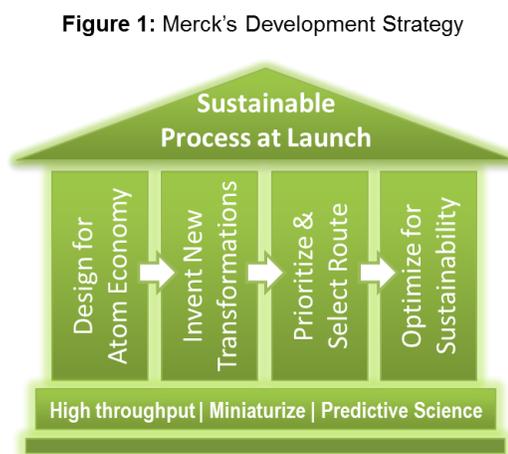
Merck aims to develop the best chemistry at time of regulatory filing, with the ultimate goal of the 'zero waste' API manufacturing process. Achieving this ambitious goal is enabled by an innovative, green by design, development strategy, to progress from initial route design through to a fully optimized sustainable commercial manufacturing process. The strategy commences with the application of green chemistry principles incorporated into route exploration in order to identify the shortest possible routes with maximized atom economy. This is followed by establishing proof of concept demonstration for the different routes with extensive use of high-throughput experimentation, allowing evaluation of vast reaction landscapes with minimal material and solvents. Next, viable routes are carefully evaluated for efficiency and sustainability potential for final route selection supported by computational and predictive science. Lastly, the selected route is fully optimized, with the goal to develop a sustainable process with minimal environmental footprint.

The strategy described above was successfully applied to Letermovir, an antiviral drug candidate, which is currently in phase III clinical trials. A novel asymmetric aza-Michael cyclization, employing a chemically stable and fully recyclable organocatalyst, has been discovered. The remainder of the synthesis has been fully optimized to reduce catalyst loading, minimize the number and amount of solvents, employ telescoped processing where possible, and to maximize atom-economy. Implementation, demonstration and validation of the new process were successfully completed in 2016. Compared to the benchmark process used for the generation of the initial phase III clinical supplies, this revolutionary new synthesis reduces PMI by 73%, increases the overall yield by more than 60%, and reduces raw material costs by 93%. Life-cycle assessment reveals that the new process reduces carbon footprint and water usage by 89% and 90%, respectively, demonstrating the significant impact of our strategy.

Introduction

In the past two decades, Green Chemistry has challenged chemists to consider environmental impacts in developing synthetic methodologies or designing chemical processes.¹ Despite these efforts, the pharmaceutical industry is among the largest waste producing sectors in all chemical industries, partly due to its typical requirement of multi-step syntheses. The sustainability potential of a process is inherent in the original route design, so attempts to improve the sustainability of a poorly designed route will lead to only incremental improvements at best. Often, the invention of novel synthetic methodologies is required to enable transformational changes in sustainability.²

Merck has set out to pursue the most efficient and sustainable process at product launch, with the ultimate goal of the 'zero waste' API manufacturing process. In order to guide the progress from initial route design through to fully developed and validated process, an innovative strategy with sustainability as a main focus has been developed (Figure 1). Merck's integrated strategy involves several stages, and aims to provide a revolutionary solution rather than incremental improvements to historical practices:



1. Design for Atom Economy: Our strategy commences through the application of green chemistry principles to the initial route design. To this end, potential manufacturing routes are proposed and prioritized based on their sustainability potential. The routes should be the shortest and most direct, avoid protecting groups, maximize atom-economy, and use safe, environmentally benign substances whenever possible. Ideally, our goal is to design a process in which all of the atoms in the starting materials are incorporated in the final API.³

2. Invent New Transformations: New synthetic transformations are often required in order to fully realize our goals. At Merck, the invention of new synthetic methodologies is accelerated by application of high-throughput experimentation (HTE) reaction discovery tools, which allow running hundreds of milligram-scale experiments simultaneously. This way, promising routes are rapidly identified for further investigation in the next stage. Additionally, the use of computational and predictive science aids in reaction development, mechanistic understanding and new catalyst/reagent design.⁴

3. Prioritize and Select Route: After exploration of the key synthetic routes, the successful approaches are evaluated based upon their sustainability potential. A systematic assessment is made not only for the key transformations, but also for the entire synthesis starting from raw materials to the final API. Ideally, the selected approach should allow the use of benign solvents, recyclable reagents, and raw materials derived from renewable resources. The routes should

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³ Trost, B. M. *Science* **1991**, *254*, 1471-1477.

⁴ Harper, K. C., et al. *Nature Chem.* **2012**, *4*, 366-374.

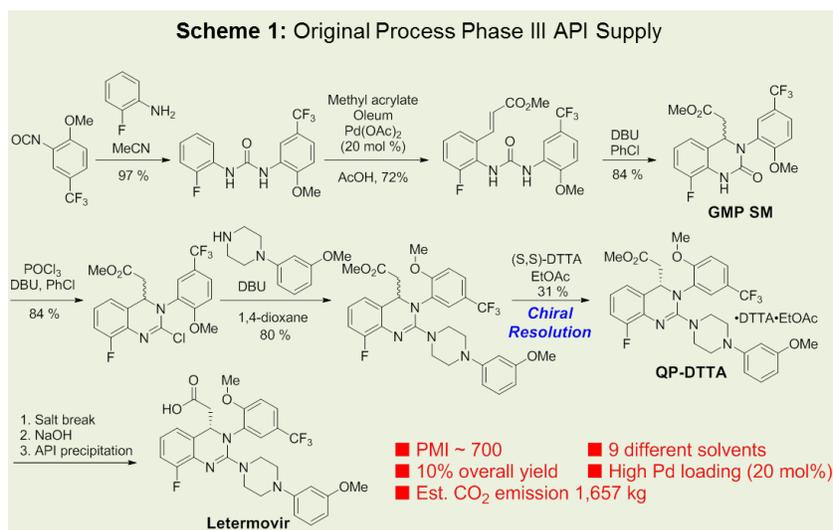
avoid waste production at source (intrinsically low PMI) and promote the use of asymmetric synthesis, biocatalysis, catalysis, and minimize the use of costly and unsustainable reagents where possible.

4. Optimize for Sustainability: Finally the selected route is further optimized with the goal to develop a sustainable process for commercial production. Further solvent consolidation and reduction is evaluated, direct isolations and telescoped steps, avoiding work-ups and solvent switches are implemented where possible. The starting material and intermediate physical properties such as solubility are measured and used to aid further process optimization to further reduce the environmental footprint. Opportunities to maximize solvent, reagent and catalyst recovery and re-use are assessed and implemented. Metric evaluation using PMI and life-cycle assessment (LCA) tools, to compare the new process to prior or alternative synthetic processes, provides a measure of the overall effectiveness of this approach.

The application of this strategy to the development of the commercial manufacturing process for Letemovir is highlighted below.

Original Manufacturing Process for Letemovir

Letemovir is an antiviral drug, currently at the end of phase III clinical trials for the treatment of cytomegalovirus (CMV) infections.⁵ CMV is widely spread in the human population and can cause severe, life-threatening infections especially in immunocompromised patients. Letemovir has been granted Fast Track Status by the FDA and Orphan Product Designation by the European



Medicines Agency for the prevention of CMV viremia in at-risk populations. The chemical process employed to supply most of the phase III clinical trials (Scheme 1) was based on a late-stage chiral resolution to obtain the desired stereoisomer in the penultimate intermediate (QP-DTTA). QP-DTTA was subsequently hydrolyzed, and the amorphous API was obtained using a controlled, patented precipitation process.⁶ An evaluation of this process reveals several elements that are unacceptable for a commercial manufacturing process at Merck. These include (1) a low overall yield of 10% due in part to the late stage resolution to access the stereogenic center, (2) the use of nine different solvents including hazardous dioxane and chlorobenzene, and (3) high palladium loading in the C-H activated Heck reaction. There was also little, if any, opportunity to recover or recycle any of the solvents or reagents. Substantial

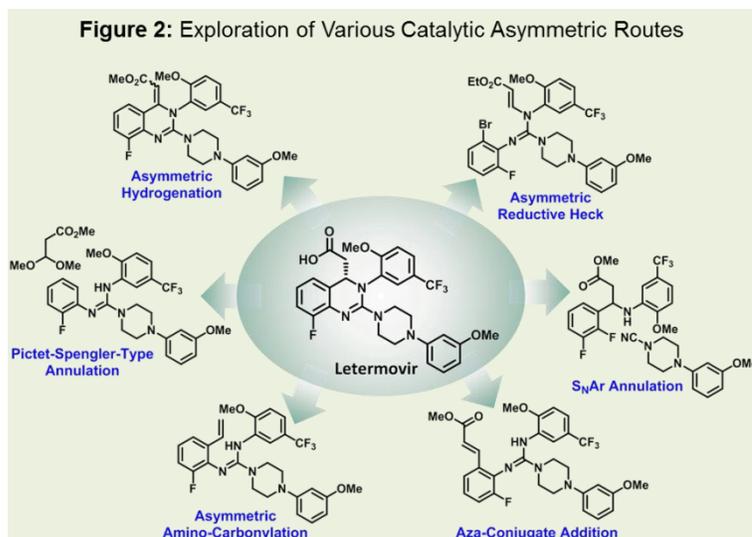
⁵ Kischka, P. et al, *Antimicrob. Agents Chemother.* **2010**, *54*, 1290-1297.

⁶ Goosen, K.; Kuhn, O.; Berwe, M.; Kruger, J.; Milizer, H.-C., US 8,372,972

improvements in all of these areas were required in order to meet our aforementioned sustainability goals. Implicit in any redesign of the API synthesis was that the work would be executed without compromising process robustness, product quality or patient safety. Since the API is an amorphous substance, purity upgrade in the conversion of penultimate QP-DTTA salt to the final API is not feasible. The final drug substance attributes are set by a patented, tightly controlled precipitation protocol. For these reasons the redesign work focused primarily on the process to synthesize the penultimate QP-DTTA salt in high yield and purity.

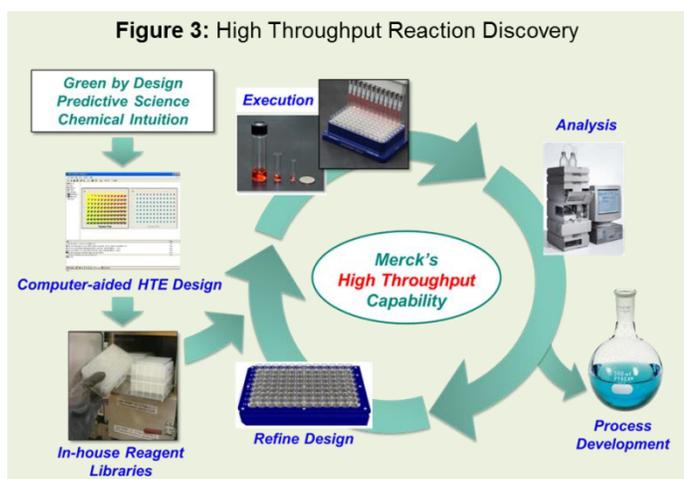
Design for Atom Economy

From the onset, and in spite of a lack of prior art, we recognized the strategic importance of efficiently installing the single asymmetric quinazoline center to our ultimate goal. Following the green chemistry principles, bond disconnections and key transformations were proposed to maximize the incorporation of all potential building blocks used in the process to access Letermovir. Six novel asymmetric reactions were proposed as the key transformations to introduce the stereogenic center in Letermovir (Figure 2). These approaches involved minimal use of protecting groups, preventing waste on the molecular level.



Invent New Transformations

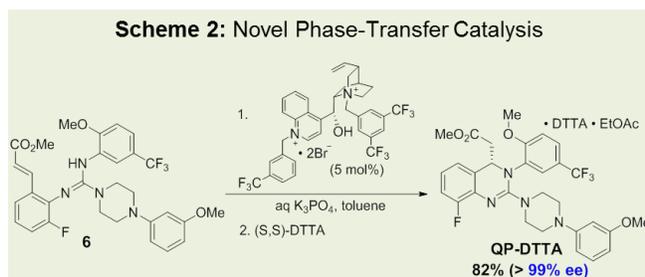
High-throughput reaction discovery tools allowed rapid investigation of these six novel asymmetric transformations with hundreds of potential catalysts and reaction conditions (Figure 3). The HTE workflow involved rapid screening utilizing either 96 or 1,536 well-plates and allowed for thousands of different reaction conditions to be screened and analyzed in a fraction of the time normally needed in a conventional laboratory experimental setting (several days vs. months). Also, since the experiments could be carried out at the sub-milligram scale, this workflow reduced the solvent typically required for this type of investigation by at least a factor of 10. By applying this approach, proof of



concept was achieved on four out of six of the proposed catalytic approaches: asymmetric hydrogenation, reductive Heck coupling, amino-carbonylation, and aza-Michael cyclization (Figure 2).

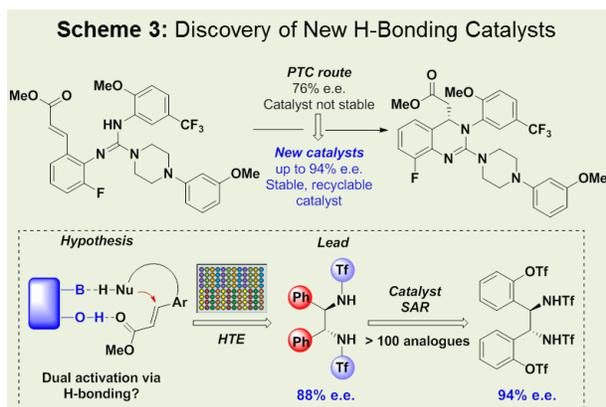
Prioritize and Select Route

Three out of the four successful routes required the use of non-sustainable and costly transition metal catalysts (e.g. Pd, Ru, Rh) as well as expensive chiral ligands. As such, we chose to focus our efforts on the aza-Michael approach with an aspirational goal to develop an economical, stable and fully recyclable organocatalyst to achieve this transformation in an asymmetric fashion. Initial success was achieved using alkaloid-based bis-quaternary ammonium salt catalysts (Scheme 2). Subsequent application of molecular modeling and quantitative structure activity relationship (QSAR) led to the ability to predictively evaluate virtual catalysts *in silico*, ultimately leading to the identification of an improved catalyst that achieved 84% ee for the cyclization of guanidine **6**.



Optimize for Sustainability

The novel PTC reaction provided QP-DTTA salt with a gratifying 82% overall yield and excellent enantiopurity (>99% ee) (Scheme 2). This methodology could be readily scaled up to prepare more than 1MT QP-DTTA salt to resupply phase III clinical studies. While representing a major improvement over the original resolution approach, the new chemistry did not meet all the requirements of our aspirational goal. Specifically, recovery and reuse of the PTC catalyst was not viable due to its decomposition over the course of reaction. Furthermore, this decomposition also added impurities that needed to be purged with multiple extractions, resulting in multiple unit operations and high waste volume. In keeping with our intent to develop the most sustainable chemistry at launch, a second round of reaction discovery was carried out. Based on observations that rapid, non-selective cyclization of **6** (Scheme 2) occurs in polar protic solvents (e.g. MeOH), we hypothesized that an asymmetric hydrogen bonding catalyst might promote the desired reaction in an apolar reaction medium. HTE was again

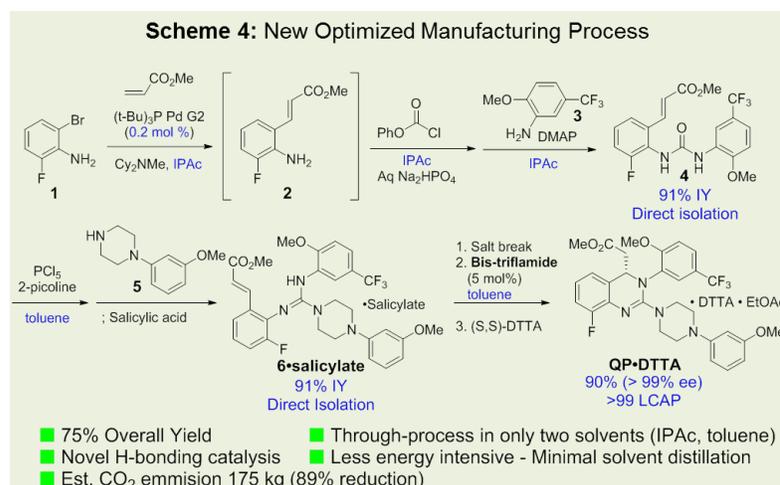


engaged to screen hundreds of potential catalysts. This included acidic catalysts such as chiral phosphoric acids, amino-alcohols, diamines, thioureas, and squaramides. During this effort, chiral bis-triflamide catalysts were found to promote the intramolecular, asymmetric aza-Michael reaction in quantitative yield and high enantiomeric purity (Scheme 3). This represented a major advantage in terms of simplifying the reaction and work-up, and reducing waste production.

The new hydrogen bonding catalysts are stable crystalline solids showing no degradation under the reaction conditions. Application of molecular modeling, QSAR and predictive sciences led to a mechanistic hypothesis that enabled further catalyst optimization to provide up to 94% ee under mild conditions in the absence of any other reagents, bases or additives. Furthermore, the bis-triflamide catalyst is easily recovered and re-used, which was successfully demonstrated at commercial scale.

With the key asymmetric transformation in place, we required a sustainable synthesis of guanidine **6**. Bulk, commercially available inexpensive building blocks (less than or equal to \$100/mole) were chosen as raw materials to maximize economic efficiency, and process optimization was carried out specifically to minimize the number and amounts of solvents used, telescope reactions where possible, apply direct isolations and avoid solvent switches, reaction work-ups and purifications where feasible. This work was executed without compromising process robustness, product quality or patient safety.

First, new conditions were identified for the Heck coupling to introduce the acrylate moiety with a 100 fold reduction of palladium catalyst charge (Step 1, Scheme 4). A 3-step, single solvent (isopropyl acetate), telescoped process was optimized to provide the highly crystalline urea intermediate **4**, by direct isolation, in 92% overall yield. The process was designed to specifically allow for facile



recovery of the solvent used as well as recovery of the trialkylamine and palladium metal utilized in step 1. Next, a one-pot urea activation and subsequent quench with piperazine **5** followed by addition of salicylic acid provides the guanidine salicylate, by direct isolation, in high efficiency (91% overall yield). The optimized asymmetric cyclization process involves simply adding a small amount of inexpensive bis-triflamide catalyst to the guanidine **6** in toluene and warming at 50 °C. A quantitative assay yield of cyclized product is obtained, and the catalyst can easily be removed from the reaction stream prior to direct QP-DTTA salt formation. Optimization using a full design of experiments (DoE) approach provided high purity QP-DTTA salt in 90-92% overall isolated yield (>99.4% ee). Since the solvent is the same as the previous step (toluene), solvent recovery is facile. Moreover, we have demonstrated that (*S,S*)-DTTA can be recovered and re-used at commercial scale.

Conclusion



An innovative green chemistry strategy, to progress from initial route design through to fully validated process, has been developed incorporating HTE and predictive science. This strategy was successfully applied to the commercial route development for Letemovir, which resulted in a novel, highly atom-economical synthesis that enabled simple recovery and re-use of >90% of the solvents and reagents, including catalysts, employed. It is of note that towards our goal of a zero waste process, nearly every atom of every molecule used in the process is either incorporated in the API or is recycled. In order to achieve this goal, a novel organocatalyst was discovered for the key asymmetric transformation in the process, leveraging high throughput experimentation to minimize the environmental footprint in research and development. Implementation, demonstration and validation of the new process was successfully completed in 2016. This revolutionary new synthesis reduces the PMI by 73%, decreases raw material costs by 93% and increases the overall yield by more than 60% (Figure 4). Merck estimates that this optimized process will result in the elimination of more than 15,000 MT of waste over the lifetime of Letemovir. Life-cycle assessment shows that the new process is expected to significantly reduce the environmental impact, decreasing the carbon footprint and water usage by 89% and 90%, respectively, which clearly demonstrates the substantial impact of our new development paradigm (Figure 5).⁷

Figure 4: Process Mass Intensity and Cost Reductions

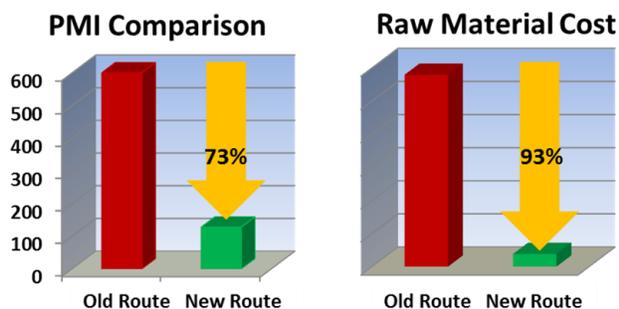
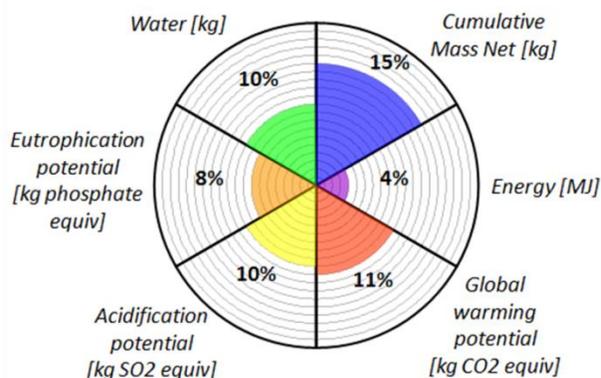


Figure 5: Life-cycle Impact for kilogram of API



⁷ The numbers shown in figures are the impact of the new process in percentage of original process.

Technology Title: “Simple and Efficient Recycling of Rare Earth Elements from Consumer Materials Using Tailored Metal Complexes”

Date of Nomination: 12/21/2016

Primary sponsor: Prof. Eric J. Schelter

Contact Person: Prof. Eric J. Schelter

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Technology Title: “Simple and Efficient Recycling of Rare Earth Elements from Consumer Materials Using Tailored Metal Complexes”

Category: Academic Award

Focus Area: Focus Area 2: Greener Reaction Conditions. The technology described here includes source reduction because it “eliminates energy- or material-intensive separation and purification steps” in the primary mining, separations and recycling of rare earth elements.

Recent Milestones: Results on the separations/recycling approach described here were published in 2016 (*PNAS*, **2016**, *113*, 14887–14892) and 2015 (*Angew. Chem., Int. Ed.* **2015**, *54*, 8222–8225) and a patent was filed in 2015 (PCT/US2015/042703).

U.S. Component: The research and development of the technology occurred in the Chemistry Department at the University of Pennsylvania and was supported by a grant from the U.S. Department of Energy.

Abstract: The rare earths (La–Lu, Sc and Y) are a group of seventeen elements whose intrinsic properties make them extraordinarily useful and irreplaceable in modern technologies. About 17,000 metric tons of rare earth oxides are used in the U.S. annually in diverse materials. Their end use applications include wind turbines, catalysts, lighting phosphors, electric motors, batteries, cell phones, displays and many others. However, the primary mining, refining and purification of rare earths are extraordinarily energy and waste intensive and carry a significant environmental burden. An interesting alternative to mining rare earth elements is recycling them from consumer materials. But they are currently recycled at a rate of only ~1%. The chief barrier to recycling rare earth elements is the cost requirement to purify their mixtures. The technology described here has been developed to purify targeted mixtures obtained from consumer materials, using an operationally simple, fast and low-cost chemical method. Organic compounds that target and separate rare earth elements using solubility differences have been developed. Recycling the rare earth element neodymium at a rate of 10% annually is expected to eliminate waste encompassing ~12 metric tons of fluoride, 19 tons flue dust, 1.4×10^7 – 1.7×10^7 m³ gas containing: flue dust concentrate, hydrofluoric acid, sulfur dioxide, and sulfuric acid; 10^5 m³ acidic waste water and 1500 tons of radioactive waste residue. The technology described here has been patented and development/scaling to an industrial recycling process is underway.

I. The Problem. Rare Earths (REs) are a class of elements that are used widely in technology, are irreplaceable in many cases and are therefore critical to modern life. The REs are not actually rare, but occur as mixtures geologically that must undergo extensive refining through separations chemistry prior to use in technology. Their mining and refining of REs, especially their separations by solvent extraction, carries a large, negative environmental burden. The hard rock mining and refining requires large quantities of water, acid and organic solvents and similarly produces tons of hydrofluoric acid, organics, and radionuclide wastes including uranium and thorium and their decay products. The production of such wastes creates both environmental and human health hazards. The challenges of REs processing have contributed to a virtual monopoly of the supply in the People's Republic of China, though one producer still exists in North America. And there is significant interest in developing new domestic sources. Because of their ubiquitous use in technology including green energy- and defense related technologies, the U.S. Department of Energy has classified several REs as 'critical' materials.ⁱ With the severe environmental and human health hazards around primary source mining, there is significant interest in recycling REs from consumer materials. However, for consumer materials, purified REs are typically blended into mixtures for their application. The challenging separations chemistry of REs is the chief barrier to widespread recycling; REs are currently recycled at a rate of only ~1%. We have developed a new, targeted chemical separations approach that simplifies and reduces the cost of separations of mixtures of REs obtained from consumer materials. Our method is expected to contribute to reducing waste, energy use, CO₂ production and source reduction from primary REs mining by adding recycled REs to the domestic supply chain.

A. Background/Introduction. The seventeen rare earth elements (RE) include the lanthanides (La–Lu), scandium (Sc) and yttrium (Y). The lanthanide series is the first instance in the periodic table of filling the 4f valence shell of electrons. The 4f electron shell creates particular and extraordinary properties for rare earths found in no other elements. These properties make rare earth elements highly useful in varied, especially technological, applications. However, the 4f electrons do not contribute to large chemical differences between rare earth elements because of the small radial extent of the 4f orbitals.ⁱⁱ As such, REs have similar chemical properties between them and co-occur geologically as mixtures of 5–7 elements in ores. And the processes used to purify individual REs, especially the separations chemistry using countercurrent liquid-liquid solvent extraction, are energy and solvent intensive. Such processes used to obtain pure REs are ultimately environmentally harmful due to the large energy investment, water use and generation of toxic- and radioactive wastes. With 90+% of global production in China, the RE marketplace has shown major instability in recent years. Life-cycle studies have indicated that RE recycling is a promising alternative. But less than 1% of RE materials were recycled in 2011. To incentivize the 'urban mining' of REs there is a clear need for transformative chemistry that enables simple separations of the mixtures of REs used in consumer materials.

B. Industrial and Commercial Uses of Rare Earth Elements. Because of the unique properties of the elements, rare earth element containing materials are used in many diverse technologies, including green energy technologies, electronics, lighting and defense applications. More specifically, they are used as components in essential materials for (but not limited to):

- Fluid catalytic cracking catalysts for petroleum refining
- Laptops
- Cell phones
- Wind turbines
- Lighting phosphors
- Automotive systems
- Electric motors
- Generators
- Permanent magnets
- Precision guided munitions
- Aircraft
- Batteries
- High temperature superconductors
- Ceramics
- Glass polishing

C. Forms of Rare Earth Elements in Applied Materials and Potential for Recycling.

An interesting alternative to obtaining rare earth elements from primary sources through mining, beneficiation and separation by solvent extraction is the reclamation of them from spent consumer materials. However, REs are currently recycled at a rate of < 1%. In most cases, the application of rare earth materials requires their variable blending or doping with multiple members of the series. For example, melt-spun or sintered ‘neo’ permanent magnets (Nd₂Fe₁₄B) contain primarily neodymium. But variable amounts of the late metals dysprosium or terbium are added to improve the thermal performance characteristics of the resulting permanent magnets. The amount of dysprosium (or terbium) added to the material varies depending on the application of the magnets. Similarly, phosphor materials include mixtures of yttrium and europium. In all cases it is essential that the individual rare earth elements are purified as a step in the recycling process before they can effectively reenter the supply chain. Because of the large initial investment to establish a separations plant to re-purify the rare earth elements and on-going costs required to run such a plant, *the chief economic barrier to recycling rare earth elements is the requirement to purify the associated mixtures of rare earths from consumer materials.*

II. Description of Invention: Targeted Rare Earth Element Separations/Recycling

Chemistry. A central hypothesis of our work is that tailored organic compounds can provide simple and effective separations for mixtures of RE metals, based on solubility differences of the RE complexes. Phosphors (32% of total market) and magnets (38%), comprising mixtures of Nd/Dy and Eu/Y respectively, are the optimal targets for recycling. The *key problem* is developing new methods to separate Nd/Dy and Eu/Y. Our group has synthesized a new organic compound (a ‘ligand’): tris(2-*tert*-butylhydroxylamino)benzylamine (H₃TriNO_x, Figure 1) for separations.ⁱⁱⁱ Isostructural metal complexes RE(TriNO_x)THF, RE = La–Lu and Y, were synthesized. Analysis of the solution structure of RE(TriNO_x)THF, RE = Nd, revealed two compounds in chemical equilibrium: Nd(TriNO_x)THF and a dimeric compound: [Nd(TriNO_x)]₂ (Figure 1). In the cases of late RE = Eu–Yb (including Dy and Tb), dimeric forms were not observed.

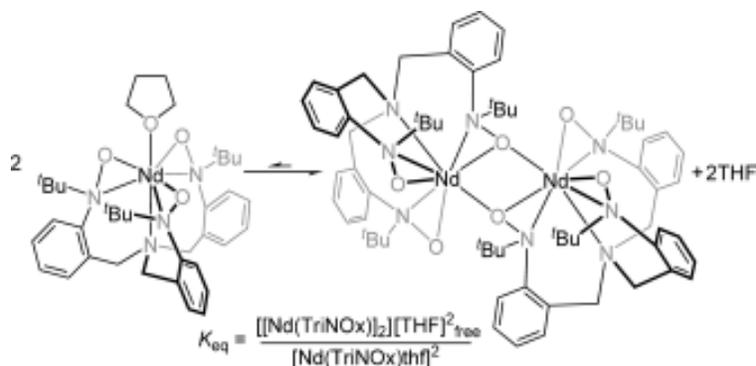


Figure 1. Solution chemical equilibrium process for Nd(TriNOx)THF and [Nd(TriNOx)]₂ that enables the new separations process for mixtures of rare earth elements.

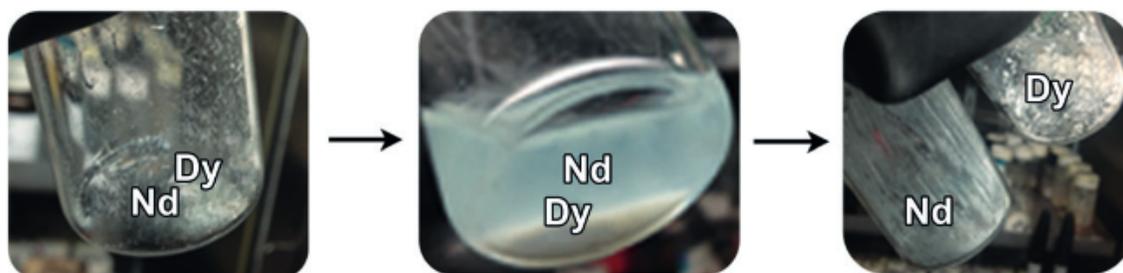


Figure 2. Images of Nd/Dy(TriNOx) separation.

A preferential formation of dimeric structures from the larger Nd with the TriNOx³⁻ ligand, and monomeric ones with the smaller Dy (see the equilibrium between the two forms, Figure 1), indicated targeted separations were possible. Critically, *significantly different solubilities* were observed for the Nd(TriNOx)THF and Dy(TriNOx)THF complexes in organic solvents. Using 50:50 (Nd:Dy) mixtures, Nd(TriNOx)THF was selectively leached into solution leaving a solid residue enriched with Dy(TriNOx)THF. The data showed filtrate enrichment to 95.37% Nd and solid enrichment to 94.56% Dy, providing a separations factor, $S_{Nd/Dy} = 359$. A separations factor is a ratio of the purity of each material following separation. Larger separations factors indicate more efficient separations. The $S_{Nd/Dy} = 359$ indicated an ~10-fold improvement over the commercial separations of Nd/Dy. It should be stressed that the separations method by filtration shown here is highly simplified and immediate (Figure 2), compared to the solvent extraction method used industrially. Treatment of the purified fractions with oxalic acid successfully reclaimed the H₃TriNOx ligand in up to 87% recovery.

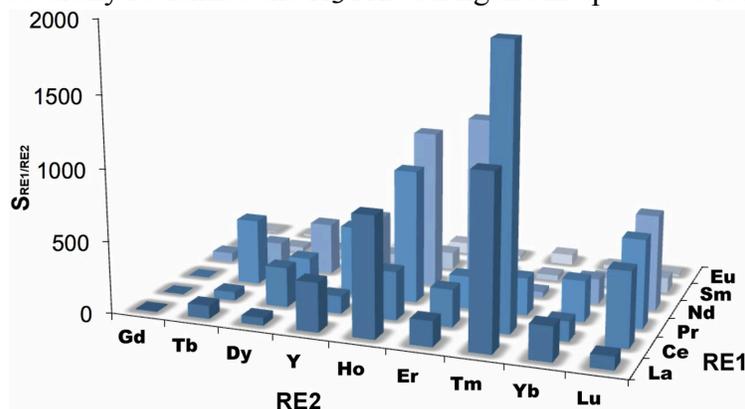


Figure 3. Summary of separations factors for early/late RE metal combinations.

Beyond the initial Nd/Dy separations, we have demonstrated the viability of our method for 54 pairwise combinations of REs (Figure 3), with particular focus on Eu and Y, critical components of phosphor materials in fluorescent light bulbs.^{iv} In general, the separations factors increased as the difference in ionic radii of the pair of RE ions increased. Whereas a moderate separations factor, $S_{Eu/Y} = 28.4$, was observed in the Eu/Y case, a more than sixfold improvement of the separations factor to 189 was obtained through adjusting the solvent to diethyl ether.

III. Potential benefits and drawbacks. According to the USGS,^v in 2015, the estimated consumption of rare earth oxide equivalents by U.S. industry was 17,000 metric tons. The U.S. was a net importer of rare earth elements and the primary supplier was the PRC. The Table 1 below indicates the production of rare earths, in tons of rare earth oxide equivalents, globally:

	Mine production		Reserves	
	2014	2015		
United States	5,400	4,100	1,800,000	
Australia	8,000	10,000	63,200,000	
Brazil	—	—	22,000,000	
China	105,000	105,000	55,000,000	
India	NA	NA	3,100,000	
Malaysia	240	200	30,000	
Russia	2,500	2,500	(with others)	
Thailand	2,100	2,000	NA	
<u>Other countries</u>	<u>NA</u>	<u>NA</u>	<u>41,000,000</u>	
World total (rounded)	123,000	124,000	130,000,000	

The impact of rare earth element mining and refining on the environment and human health was reviewed (for region 8) in an EPA report by Paul and Campbell in 2011.^{vi} The primary minerals targeted in modern, hard rock rare earth element mining are monazite/xenotime ($RE(PO_4)$) and bastnaesite ($RE(CO_3)F$); bastnaesite is the mineral processed at the Mountain Pass mine, CA and at the Baotou mine in China. In all cases, the minerals occur with calcite or barite gangue materials and naturally contain uranium and thorium and their radioactive decay products. As such, the chemical characteristics of the ores and requirements for their refining have a large energy and waste burden. Table 2 indicates particular waste volumes for the production of 1 ton of rare earth elements, as reported by the Chinese Society of Rare Earths.^{vii}

Byproduct (waste)	Amount
Fluoride	8.5 kg
Flue dust	13.0 kg
Gas containing: flue dust concentrate, hydrofluoric acid, sulfur dioxide, and sulfuric acid	9,600-12,000 m ³
Acidic waste water	75 m ³
Radioactive waste residue (²³² Th, natural U and their decay products)	1 ton

Table 2. Estimated Byproducts Produced from Mining and Refining of 1 Metric Ton of Rare Earth Elements using Sulfuric Acid Refining in China

The details of the production of byproducts in the domestic production of rare earth elements at Mountain Pass are proprietary. However, we can estimate upper bound quantities of waste reduction from recycling of rare earths using the Chinese data on byproducts in Table 2. In this context we can roughly estimate that if only 10% of the annual 17,000 metric tons rare earth oxides consumed by U.S. industry in 2015 were obtained from recycled neodymium sources instead of primary mined ores, the annual amount of waste *eliminated* would be:

Summary of Waste Eliminated from Recycling Neodymium Annually at a Rate of 10%:

- 12 metric tons of fluoride
- 19 tons flue dust
- $1.4 \times 10^7 - 1.7 \times 10^7$ m³ Gas containing: flue dust concentrate, hydrofluoric acid, sulfur dioxide, and sulfuric acid
- 10^5 m³ acidic wastewater
- 1500 tons of radioactive waste residue (containing water)

The results of the waste elimination analysis are encouraging for development of this technology. There are some practical limitations that should be addressed to make the new separations chemistry more commercially viable. Chiefly, the RE-TriNOx complexes used are vulnerable to oxidation/degradation in air such that the separations must be performed under nitrogen, which is a drawback of this technology. However, our work demonstrates the concept of molecular design for recycling of REs is viable and worthwhile.

IV. Comparison of Breakthroughs with Existing Technology. The results from the separations based on the RE-TriNOx system were compared with the RE-HCl-HDEHP- and RE-HNO₃-Cyanex 302-based liquid-liquid extraction separations used industrially.^{viii} The most convenient metric for comparison are separations factors, which are ratios of the purity of each material following separation, where larger numbers indicate more efficient separations. On average, the separations factors for the 54 early/late metal combinations RE1 = La-Eu, RE2 = Gd-Lu were approximately five times larger than those for the commercial HDEHP system. In contrast, the separations factors for the TriNOx system were comparable to those of the Cyanex 302 system for RE1 = Pr, Nd, Sm, Eu, and RE2 = Tb, Dy, Ho. The separations factors for the TriNOx system, however, deviated from those of the Cyanex 302-system for RE1 = La, Ce and/or RE2 = Y, Er-Lu. As such, the data showed separations factors for the TriNOx system that were comparable or ~0.4 times smaller than those for the Cyanex 302-system on average.

For the combinations of particular interest to recycling, Nd/Dy, the $S_{Nd/Dy}=359$ achieved for the RE-TriNOx system compared favorably to that of 41.5 determined for the RE^{III}-HCl-HDEHP extraction method used industrially, indicating an approximately 10-fold improvement in the separation of Nd and Dy. It was also an approximately 1.5-fold improvement compared to the separation based on the RE^{III}-HNO₃-Cyanex 302 system. Optimization of solvent conditions using diethyl ether for Eu/Y resulted in a separation factor of 189. This value is higher than the corresponding values for Cyanex-302 ($S_{Eu/Y} = 116$) and HDEHP ($S_{Eu/Dy} = 3.83$, $S_{Eu/Ho} = 4.74$). In general, the TriNOx system performed comparably or better than industry standards for early/late pairwise combinations across the series of REs, and significantly better than industry standards for the Nd/Dy and Y/Eu combinations.

V. Planned Commercialization. A patent was filed in 2015 (PCT/US2015/042703) and the published results have garnered significant interest from VC investors, technology companies and mining concerns. Work is currently underway toward developing the academic separations concepts disclosed here into practical and industrially viable recycling processes. A recent DOE grant award will support the further development of the technology.

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The Uni.System: An Advanced Vanadium Redox Flow Battery for Grid-Scale Energy Storage

December 30, 2016

Primary Sponsor:

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Additional contributors to this project include the US Department of Energy's (DOE) Pacific Northwest National Laboratory (PNNL), with the support of the US DOE Office of Electricity Delivery and Energy Reliability's Grid Storage Program.

The Uni.System: An Advanced Vanadium Redox Flow Battery for Grid-Scale Energy Storage

Eligibility for Small Business Award

The advanced vanadium redox flow battery (VFB) chemistry technology developed at PNNL has been improved up and commercialized at scale by a US-based company and is eligible for consideration for the small business award. UniEnergy Technologies, LLC (UET) in Mukilteo, Washington, licensed the technology developed at PNNL and has further advanced and deployed its product, named the Uni.System™, at a commercial scale to utilities and renewable power sites in the United States, Germany and Italy.

Focus Area 2: Greener Reaction Conditions

Recent Milestone

At the end of 2016 UET deployed the largest capacity containerized flow battery in world in Everett, Washington for Snohomish County Public Utilities District. The 2MW/8MWh VFB will be commissioned and operational in early 2017 and will be used by the PUD for reliability, load balancing and renewables integration.

U.S. Component

UET has licensed the technology developed at the Pacific Northwest National Laboratory and has further advanced and deployed it at a commercial scale to utilities in the United States. Current project sites include Avista Utilities in Pullman, Washington and Snohomish Public Utility District in Everett, Washington. In addition, UET has been awarded projects to deploy additional grid-scale batteries using the advanced vanadium chemistry for energy storage in Alaska, California, Hawaii, Tennessee and New York.

Abstract

There is an increasing demand of electrical energy storage in the electrical power market, from generation, transmission/distribution to the end users of electricity. Customers expect the electrical power chain to be cleaner, more efficient, reliable, flexible and affordable. As a result, energy storage is considered indispensable to modernizing the electrical infrastructure and integrating “green” renewable energy, such as solar and wind power, into the nation’s electrical grid. In order to meet these needs cost-effectively, industry needs energy storage solutions that do not degrade, allow unlimited cycles and use of stored energy, and are operational in any environment and temperature. This improved advanced vanadium redox flow battery, originally developed at the Pacific Northwest National Laboratory (PNNL), and improved and commercialized by UniEnergy Technologies (UET), is a critical contributor to the reduction and eventual elimination of the need for carbon-emitting electricity generation.

This third-generation vanadium redox flow battery, called the UniSystem™, utilizes a breakthrough chemistry: a vanadium electrolyte with double the energy density of prior chemistries, and a much broader operating temperature (-40° to 120°F) that allows the energy

storage system to be deployed in nearly any ambient temperature or environment on earth. These improvements have resulted in fully containerized and deployed megawatt-scale vanadium redox flow battery systems that are approximately one-fifth the footprint than previous flow battery technologies and have significantly reduced the amount of chemicals needed for these energy storage systems. The new vanadium electrolytes, with a chloride based complex chemistry, are proved to have a much improved stability over the traditional sulfate based chemistry. UET's improvements in system integration and controls have made the deployment, operation, and maintenance of this chemical battery much simpler, cost effective and safe. Because the vanadium electrolyte is water-based and does not degrade, the batteries are non-flammable and recyclable.

The problem:

The evolving demands in the energy market call for alternative energy storage technologies capable of both long and short duration benefits and as long-lasting assets to deliver a maximum value to customers. Current battery chemistries based on Li-ion have proved to be suitable, competitive technologies for power and relative short duration (2 hour or less) applications such as frequency regulation, given their excellent power capability and battery cell energy efficiency. However, this is not necessarily a competitive solution for customers that need long duration benefits as an essential part of their overall value propositions. Li-ion batteries degrade over time and have limited cycle life. In addition, they cannot use their full state of state-of-charge typically operated between 20-80% and have noted challenges with thermal runaway and flammability.

As a result, the vanadium redox flow battery (VFB) has emerged recently as the most competitive alternative that is capable of delivering competitive value to utility, commercial and industrial, and microgrid customers that require long duration benefits, such as load leveling, peak shaving, islanding and renewable energy integration, as an essential part of their overall value propositions for energy storage. Until recently however, the VFB's drawbacks included a large footprint to operate, and limited stability in broad temperature ranges.

The chemistry:

The Redox Flow Battery (RFB): The RFB is a unique electrochemical device that stores electrical energy in liquid electrolytes, instead of in electrodes as many other batteries do. The RFB then releases the stored energy according to the demands of the customer, at levels up to multi-megawatt (MW) and mega-watt hours (MWh). As shown in Figure 1, a RFB cell consists of two electrodes or "stacks" (made from carbon felt and other items) and typically two circulating electrolyte solutions (a positive/cathode-side electrolyte or catholyte, and a negative/anode-side electrolyte or anolyte) that are separated by an ion exchange membrane or a separator. The energy conversion from electricity energy to chemical potential energy (charge) and vice versa (discharge) occurs instantly within the electrodes as the liquid electrolytes flowing through the cell.

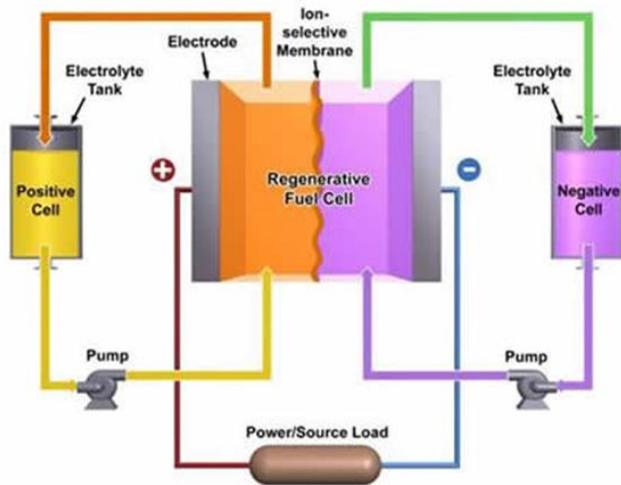


Figure 1: Schematic illustration of the structure of a redox flow battery

Because of this structure, RFB's allow for separate design of energy (kilowatt hours) and power (kilowatts) and the power/energy ratio can be tuned according to the applications wanted by the customer. During operation, the flowing electrolytes carry away the heat generated from the electrode reactions and ohmic resistance such that the electrolyte tanks act as a large heat sink, preventing overheating of the battery stacks and the individual cells within them. The physical separation of cells/stacks and electrolytes also helps avoid thermal run-away as may happen in other batteries. Also, the elimination of repeated ion insertion and de-insertion in electrodes as occurs in other batteries, preserves the structural and mechanical integrity of the cells/stacks, enabling a long-cycle life of the battery. In a true RFB that utilizes liquid electrolytes on both positive and negative sides, its cycle life is generally independent of the battery's stage of charge (SOC) and depth of discharge (DOD). This is not the case with non-RFB batteries that store energy in their solid electrodes.¹

The evolution of the Vanadium Redox Flow Battery (VFB): The RFB may be traced back to Zinc-halide batteries that use “consumable” Zn/Zn²⁺ anodes. True RFBs were first used in the early 1970's by Dr. Larry Thaller's research group at NASA with Iron-chrome (Fe-Cr) chemistries that utilized “inert” electrodes and had both reactants and products dissolved in liquid electrolytes. To avoid the cross-contamination and improve electrochemical activity over the early Fe-Cr RFB, the all-vanadium RFB or VFB was first demonstrated by Dr. Maria Skyllas-Kazacos's research group in 1980's at the University of New South Wales. As shown in Figure 2, the VFB utilizes a V²⁺/V³⁺ aqueous sulfate solution on the anode side and a V⁴⁺/V⁵⁺ aqueous sulfate

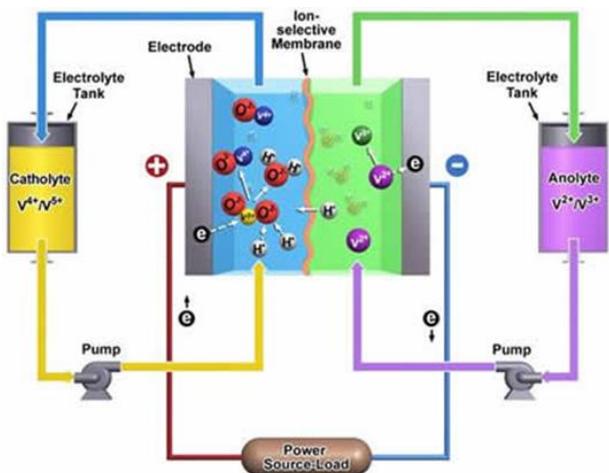
¹ See the following journal articles for history of the flow battery, including mixed acid systems:

Yang Z, Zhang J, Kintner-Meyer MC, Lu X, Choi D, Lemmon JP, et al. Electrochemical energy storage for green grid. *Chem Rev* 2011;111:3577–613.

Wang W, Luo Q, Li B, Wei X, Li L, Yang Z. Recent progress in redox flow battery research and development. *Adv Funct Mater* 2013;23:970–86.

Skyllas-Kazacos M, Chakrabarti MH, Hajimolana SA, Mjalli FS, Saleem M, Progress in Flow Battery Research and Development. *J Electrochem Soc* 2011; 158 (8) R55-R79

solution on the cathode side. A standard voltage of 1.25 V is generated by the VFB through the following reactions:



Cathode:	$VO^{2+} + 2H^+ - e^- \leftrightarrow VO_2^+ + H_2O$
Anode:	$V^{3+} + e^- \leftrightarrow V^{2+}$
Cell:	$VO^{2+} + V^{3+} + 2H^+ \leftrightarrow VO_2^+ + H_2O + V^{2+}$

Figure 2: Schematic of all-vanadium redox flow battery, in a charge process.

The VFB demonstrates an excellent electrochemical reversibility and virtually an unlimited cycle life. Over 10,000 full cycles (from 100% SOC) has been demonstrated in the field. The use of aqueous-base catholyte and anolyte further strengthens the safety record of VFB's. The favorable general features of RFBs and in particular the advantages with the VFB have attracted wide interests in using the VFB for utility and other large scale stationary applications.

However, the traditional VFB is limited in the stability of its basic electrolyte chemistries. Vanadium oxides, e.g. V_2O_5 , tend to precipitate out from the electrolytes via irreversible reactions, resulting in capacity loss and degradation in the battery's durability and reliability. As such, the operation of the traditional VFB is limited in a temperature range to 10 ~ 40 °C (practically, 35°C/ 95° F), imposing the burden of heat management and energy loss due to heat management. Additionally, the limited stability of the traditional VFB electrolytes at elevated temperatures limits vanadium solubility in the electrolytes and as thus energy capacity or density of VFB's, a disadvantage compared with many other batteries.

The Third Generation VFB for the Uni.System: To improve performance, reliability and economics of VFB's, UET has licensed and utilizes a new generation electrolyte chemistry initially developed at the US Department of Energy's (DOE) Pacific Northwest National Laboratory (PNNL), with the support of the US DOE Office of Electricity Delivery and Energy Reliability's Grid Storage Program.² The electrolyte chemistry was developed by a team of

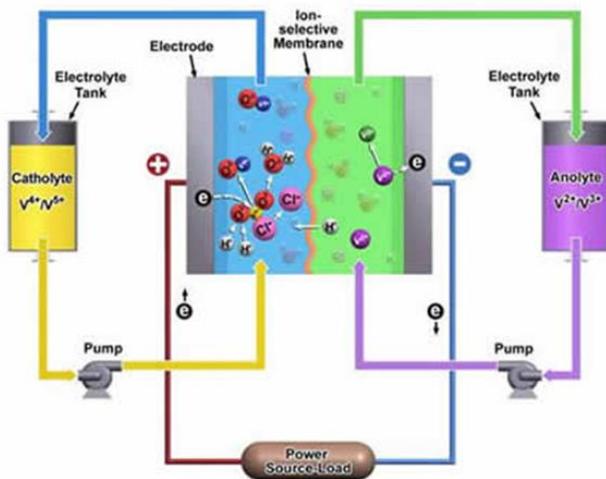
² "A Stable Vanadium Redox-Flow Battery with High Energy Density for Large-Scale Energy Storage," Li, Kim, Wang, Vijayakumar, Nie, Chen, Zhang, Xia, Hu, Graff, Liu, Yang, *Adv Energy Mat.*, Volume 1, pages 394–400, May, 2011, <http://onlinelibrary.wiley.com/doi/10.1002/aenm.201100008/abstract>.

"Chloride supporting electrolytes for all-vanadium redox flow batteries," Kim S, Vijayakumar M, Wang W, Zhang JL, Chen BW, Nie ZM, Chen F, Hu JZ, Li LY, Yang Z, *Phys Chem Chem Phys* 2011; 13: 18186-18193 <http://pubs.rsc.org/is/content/articlelanding/2011/cp/c1cp22638j#ldivAbstract>

researchers at PNNL, two of whom left their lab positions in 2012 to co-found UET (Dr. Gary Yang and Dr. Liyu Li) to further develop and commercialize the VFB.

This new electrolyte is a breakthrough in energy storage technology and received the Federal Laboratory Consortium (FLC) award in 2012. The new vanadium electrolytes with a chloride based complex chemistry were proved to have a much improved stability over the traditional sulfate based chemistry. Negligible Cl_2 and other gas evolution during operation up to 100% state of charge has also been proven.

Meanwhile the UET RFB (see Figure 3) with the new generation electrolytes demonstrates a voltage and reversibility that is at least as excellent as that of the traditional VFBs with the sulfate electrolytes, via the following reactions:



Cathode:	$\text{VO}_2\text{Cl} + 2\text{H}^+ + \text{e}^- \leftrightarrow \text{VO}^{2+} + \text{Cl}^- + \text{H}_2\text{O}$
Anode:	$\text{V}^{2+} - \text{e}^- \leftrightarrow \text{V}^{3+}$
Cell:	$\text{VO}_2\text{Cl} + \text{V}^{2+} + 2\text{H}^+ \leftrightarrow \text{VO}^{2+} + \text{V}^{3+} + \text{Cl}^- + \text{H}_2\text{O}$

Figure 3: Schematic of UET VFB, during a charge process.

Realized potential benefits and drawbacks

With the much improved stability of the new electrolytes and optimized cell/stack design and electronic controls, UET VFB realizes the following benefits:

- A practically doubled energy density due to a higher solubility and a higher utilization of the active vanadium species;
- An operating temperatures range from -40°C to $>50^\circ\text{C}$ easing heat management, mitigating solid vanadium oxide precipitation and capacity fading, and improving energy efficiency;
- No oxygen evolution on the graphite felt cathode electrodes during any overcharging (even locally), avoiding oxidation of the graphite electrode and degradation in the electrode reactivity, and thus raising the voltage up-boundary and electrolyte utilization;
- Advanced balancing management between cathode- and anode-side electrolytes due to a much higher vanadium concentration allowed ($>3\text{M}$) at the cathode side;
- Improved tolerance to impurities in the electrolytes due to the chemistry changes over the traditional sulfate systems.

These features have allowed the VFB to be fully containerized in standard 20-foot shipping containers and cost-effectively deployed to utility, microgrid, and commercial sites where long-duration, grid-scale electrical energy storage is needed.

Comparison of cost, performance and environmental profile with competing technologies

Compared to the traditional sulfate vanadium redox flow battery system, the Uni.System largely improved the stack’s power density and the electrolyte’s energy density. This improvement reduces the materials consumption of balance of plant (BOP) and stacks by more than 50%. UET’s new system also improves the utilization of vanadium for energy storage because of the higher vanadium concentration and “over-charge safe” feature of the chloride-containing electrolyte.

The UET Uni.System demonstrates a significant reduction in the footprint of the battery system, and the amount of materials and chemicals needed to operate a VFB. Figure 4 (below) shows the footprint of the Uni.System is approximately one-fifth of that of the tradition sulfate system, UET’s major competing technology.

Figure 4 – Highlighted yellow area illustrates the footprint of the Uni.System compared to a traditional flow battery



The table below illustrates the reduction in chemicals and electrolyte tank materials based on a 1 MW-4MWh project:

	CONVENTIONAL SULFATE-BASED SYSTEM	UET UNI.SYSTEM’S CHLORIDE-BASED SYSTEM	OVERALL CHEMICAL REDUCTION
VANADIUM, KG	22440	16320	27%
H ⁺ FROM ACID, K MOL	2133	1200	44%
PLASTIC FOR TANKS, KG	8000	4000	50%

Technologies that are commercialized at scale and capable of long duration benefits include sodium-sulfur (Na-S) batteries. Operated around 300°C with molten sulfur positive electrode and molten sodium negative electrode contained in a ceramic tube, the Na-S battery was a major battery technology adopted early for utility applications and only surpassed by Li-ion batteries a few years ago. However, fire accidents led to recall and halting operations around the world. The fire hazardous inherent to the use of molten sodium and sulfur, limits in its flexibility, and costs associated with manufacturing and installation, have drastically slowed down its adoption in the storage market. Meantime, the Uni.System has received a zero flammability rating from Hazardous Material Identification System and has a letter of non-objection from the New York City Fire Department for deployment at an urban hospital. Finally, the ability to containerize and easily deploy the Uni.System VFB in standard 20-foot shipping containers has led to significant cost reductions allowing the systems to be manufactured and deployed at a price point competitive with the incumbent Li-ion energy storage systems. When comparing the total cost of ownership, including the levelized costs of the system and its multiple use applications over their 20-year life period, the Uni.System provides greater value per kilowatt hour in many use scenarios than the incumbent grid-scale storage systems.

UET's new chemistry and unique product, when combined with renewable energy generation such as solar and wind power, will lead to a significant reduction in greenhouse gas emissions as it is further deployed and put into use. According to the 2013 report on Grid Energy Storage prepared by the U.S. Department of Energy, "Storage technology can help contribute to overall system reliability as large quantities of wind, solar, and other renewable energy sources continue to be added to the nation's generation assets, furthering the goals of reducing greenhouse gas emissions and increasing energy security."³

Current and planned commercialization

To date, UET has nearly 100 megawatt hours of VFB energy storage systems deployed, ordered or awarded in three countries and six US states. Representative projects include a 1 MW/4 MWh containerized Uni.System for **Avista Utilities** in Pullman, Washington; a 2MW/8MWh Uni.System for **Snohomish County PUD** in Everett, Washington, which will be the largest capacity containerized flow battery in the world when commissioned in January 2017;⁴ and a 500kW/2MWh DC energy storage system for **Terna** in Sicily, Italy.

³ http://www.sandia.gov/ess/docs/other/Grid_Energy_Storage_Dec_2013.pdf, accessed December 29, 2016

⁴ <http://www.heraldnet.com/news/pud-invests-in-11-2-million-in-energy-storing-units/>, accessed December 29, 2016