Sustainable Molecular Design through Biorefinery: Biomass as an Enabling Platform for Oil Thickening Safe Agents

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- **Title**: *Sustainable Molecular Design through Biorefinery: Biomass as an Enabling Platform for Oil Thickening Safe Agents*

- **Milestones:** Among many, **two** newly designed and engineered biobased sugar-derived amphiphiles that are far less costly, environmentally benign and superior to several polymer derived thickening agents for hydrophobic liquids including crude oil (for oil-spill cleanup) and vegetable oils (applications in food industry and personal care products). Thus far, several food and personal care companies expressed their interest to license the technology; currently we are having negotiations with companies for potential licensing opportunity.
- The technology is eligible for an academic award.
- The primary focus area is best described by "**Use of Renewable Feedstocks"** and **"Designing Safer Chemicals and Products"** through **"Non-Covalent Synthesis and Self-Assembly Protocol."**
- All of the development work on biobased amphiphiles from sugar and fatty acids using enzyme catalysis has been, and continue be done, at the City College of the City University of New York (*a Minority & Hispanic serving institution*).

Abstract

Thus far, a wide range of industrial materials such as solvents, fuels, synthetic fibers, vegetable oil-structuring agents and chemical products are being manufactured from petroleum resources. However, rapid depletion of fossil/petroleum resources is encouraging current and future chemists to orient their research towards designing safer chemicals, products and processes from renewable feedstock using green-chemistry principles with an increased awareness of environmental and industrial impact. We have successfully demonstrated remarkable examples of amphiphiles generated from biobased precursors, which upon *self-assembly* produced soft-materials in aqueous and organic solvents. The present technology focused on developing next-generation biobased amphiphiles to structure the hydrophobic liquids (vegetable oils and related.) for food & cosmetic applications. Structuring of vegetable oil is a process, which involves alteration of physical property of oil with or without altering the chemical properties. Current structuring agents/methods tend to increase content of the saturated fatty acid and trans fatty acids in oils, which has potential risk of increasing vascular and heart diseases that could eventually lead to stroke. Thus, we have developed potent amphiphiles from biobased resources (open-chain sugars) using enzymatic green synthesis, and these amphiphiles exhibited superior structuring ability of vegetable oils without increasing saturated fatty acid content. In addition, these self-assembled materials have shown a remarkable ability in control-release of pheromones. Therefore, this technology offers potential opportunity to replace current oil structuring agents with biobased and safe amphiphiles at lower-cost with enhanced performance.

US EPA Green Chemistry Challenge: *Award focus areas fit to the technology*: "Design and synthesis of biobased amphiphiles using green synthetic route - enzyme catalysis; and utilization of biobased amphiphiles in food and cosmetic industry."

Introduction

With paradigm shift from non-renewable to renewable resources being observed in chemical industry for economic and environmental concerns, integration of sustainable technology in scientific research has become of great significance. The major oil crisis of 1970s and fast depletion of petroleum resources has emphasized the need to increase reliance on biomass feedstock. In addition to utilizing biomass, the processes that transform them into valuable products are required to be selective, energetically efficient, high yielding, and environmentally benign. In this context, methodologies employing principles of biotechnology and green chemistry have been developed to yield pure products and consume less energy. Utilization of nature's catalyst, micro-organisms and enzymes, has dominated in new methodologies. Such a concept of integrating biomass feedstocks with green processes to sustainably produce commodities analogous to petroleum refinery is termed as '***biorefinery***'. Such concept has been recently integrated by industries to efficiently manufacture valuable products such as resins, chemicals, solvents, and fuels from feedstocks such as starch, sucrose and cellulose. We also focus on implementing the biorefinery concept in developing sugar-based amphiphiles that are capable of developing multifunctional soft materials, primarily **molecular gels**.

Molecular gels (MGs) are viscoelastic materials consisting of 3-D network formed by non-polymeric amphiphiles in which large pool of organic or aqueous solvent is immobilized by surface tension and capillary action (Figure 1). The propensity of amphiphiles to form a network is underpinned solely by supramolecular chemistry principles, i.e., self-assembly through non-covalent interactions. The most interesting aspect of MGs is that a subtle change in amphiphilic structure alters the nature of self-assembled micro-structures, thereby changing the macroscopic properties of the resulting gels. Hence, applications of well-designed MGs have been successfully exemplified in various areas such as biomedicine, art restoration, crystal engineering, gel-electrophoresis, catalysis, electronics and photonics. Unfortunately, wide variety of amphiphiles (including sugar/amino acid based gelators) contains structural motifs that are derived from non-renewable resources. In addition, their synthesis is frequently multi-step, involve energy intensive purification steps and require complex and expensive catalysts. These drawbacks impede commercial application of MGs (John, G. et al. *Acc. Chem. Res.* **2008**, *41*, 769). Hence, challenges still exist to identify biobased starting materials and develop efficient synthetic route for ensuring sustainable and environmentally benign advancement of MGs (John, G. et al. *Langmuir* **2010**, *26*, 14783).

Fig. 1. A representative gel and its 3-D network

Nature offers a myriad of hydrophilic (sugars, proteins, nucleotides) and hydrophobic molecules (e.g. lipids, phytosterols, waxes, polyphenols) that can be exploited to tailor the structure of amphiphiles for desired application. Hence from MGs perspective, natural building blocks can be utilized to exquisitely control the gelator's structure and consequently develop MGs for unique applications. Adoption of biorefinery concept in designing of biobased amphiphiles will have a significant impact on the qualitative evolution of the field of MGs. Hence, we have focused on demonstrating the feasibility of converting biomass-derived sugars and sugar-like compounds into amphiphiles, thereby highlighting them as an ideal platform for developing functional MGs. Biobased building blocks, namely amygdalin (John, G. et al. *J. Am. Chem. Soc.* **2006**, *128*, 8932), trehalose (John, G. & Dordick, J. S. et al. *Angew. Chem. Int. Ed.* **2006**, *45*, 4772), ascorbic acid (John, G. et al. *Chem. Mater.* **2007**, *19*, 138) and mannitol (John, G. et al. *Angew. Chem. Int. Ed.* **2010**, *49*, 7695) were transformed into amphiphiles by appending a biobased hydrophobic group, fatty acids. Highly regiospecific lipase mediated route was adopted for synthesis, which was optimized to produce amphiphiles in quantitative yield (Figure 2). The resulting amphiphiles exhibited exceptional gelation abilities in a wide range of solvents (aqueous or organic solvent). The utility of these MGs was successfully demonstrated for developing drug-delivery systems (John, G & Karp, J. et al. *Biomaterials,* **2009,** 30, 383) and templates for hybrid-nanomaterials and nanoparticles (John et al. *Chem. Mater.****2007,*** **19**, 138).

Herein, briefly summarize our efforts to generate wide-range of amphiphilic systems from various renewable resources using green enzymatic synthesis, and our strategy to utilize biobased amphiphile derived self-assembled nanomaterials for wide range of applications, namely drug-delivery, oil thickeners for food and cosmetic materials applications.

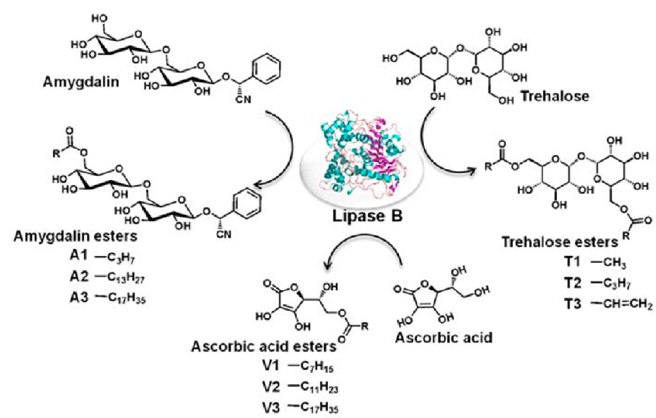

Fig. 2. Enzyme-catalyzed synthesis of sugar amphiphiles.

Amygdalin, a glycoside, is a byproduct of fruit processing industry, chiefly almonds and apricots. The amphiphiles adjoining the non-toxic blocks, sugar and fatty acids *via* an ester linkage, are expected to be both biocompatible and enzyme-labile. Furthermore, the amygdalin-based hydrogels exhibited very high solubilization ability towards hydrophobic drugs (e.g. curcumin, its solubilization in water was increased by 33000 folds. The drug could be released by hydrolase-triggered breaking of the gel under physiological condition (~ 37 °C). Importantly, the drug release kinetics could be modulated by altering the enzyme concentration and/or the temperature. Such hydrogels have the potential to increase the bioavailability of water-insoluble drugs while exhibiting excellent stability in the absence of a hydrolase enzyme without ―leaking the encapsulated drug (John, G. et al. *J. Am. Chem. Soc.* **2006**, *128*, 8932).

Trehalose is a sugar, which is commercially manufactured by enzymatic treatment of starch. Trehalose-based amphiphiles exhibited gelation capability in organic solvents such as ethylacetate, isopropanol, acetone, and xylene at a concentration as low as 0.04% wt/v, which is the lowest value reported for sugar ester gelators then. Polymerizable trehalose-based amphiphiles, specifically trehalose acrylate, were used to produce free-standing scaffolds, which when immersed in water produced a self-supporting transparent hydrogel. Such trehalose systems could be used to generate excellent scaffolds for tissue engineering applications (John, G., & Dordick, J. S. et al. *Angew. Chem. Int. Ed.* **2006**, *45*, 4772).

Ascorbic acid (or vitamin C) is abundant in citrus fruits and plants. Commercially, it is produced by fermentation of glucose. Ascorbic acid-based amphiphiles enabled gel formation, metal reduction and metal nanoparticle stabilization steps to occur simultaneously leading to formation of organic-inorganic hybrid materials. This approach of *in situ* synthesis of organic-inorganic hybrid materials was found to be a versatile method because the matrix itself acts as both a reducing agent and a stabilizing agent (John, G. et al. *Chem. Mater.* **2007**, *19*, 138).

The above three studies exemplify the bright prospect of incorporating biorefinery concept in the field of MGs. We believe that swift integration of ideas from biorefinery and materials chemistry is a welcome approach that paves the way for new advances in the development of materials, chemicals, and new energy sources.

Nomination

The technology nominated for the award is inspired from our previous work. It embodies the same biorefinery approach to efficiently design gel-forming multifunctional amphiphiles from sugar alcohols. Sugar alcohols are chemically defined as carbohydrate derivatives in which a ketone or an aldehyde group has been replaced by a hydroxyl group. There are an integral part of present biorefinery plants and are generally produced by microbial fermentation (lactic acid bacteria) of different sugars. The physico-chemical properties of sugar alcohols are markedly different than the typical sugars; for example, sugar alcohols are non-reducing molecules, exhibit greater stability towards external stimuli (alkali, acids and temperature) and do not undergo Maillard reaction. Due to such differences, the amphiphiles developed from them are expected to be functionally different compared to those derived from typical sugars. The utility of this technology was successfully demonstrated in developing three commercially competitive products: *(i) oil spill recovery materials; (ii) controlled release devices for pheromones; and (iii) healthy vegetable oil structuring agents.*

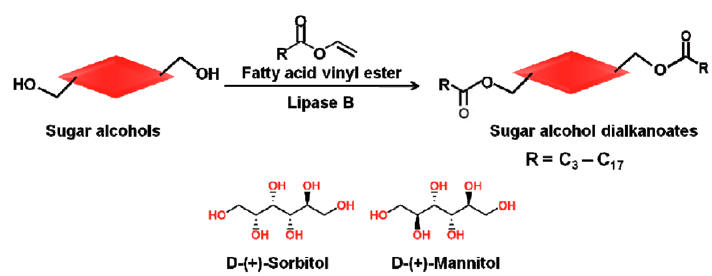

Fig. 3. Schematic representation of enzyme catalysis of sugar alcohols.

***Synthesis*:** The amphiphiles were synthesized by enzyme mediated trans-esterification of sugars with fatty acid donors. Novozyme 435 (*Candida Antartica Lipase B*) was used for trans-esterification. The reaction is highly regioselective, i.e., only the two primary hydroxyl groups of sugars are acylated. The developed biocatalysis protocol is a one-pot synthetic approach with purification involving simple hexane washing process, which ensures minimal solvent handling and hazardous waste generation. The typical synthesis scheme is shown in Figure 3. The ease of synthesis and cheap raw materials (sugar alcohols and fatty acid esters) translate into low cost production of efficient sugar-based gelators. Most importantly, these amphiphiles have been found to be nontoxic and biodegradable (John, G. et al. *Angew. Chem. Int. Ed.* **2010**, *49*, 7695).

***Gelation*:** Among the developed sugar amphiphiles, mannitol dioctanoate (M8) and Sorbitol dioctanoate (S8) were found to be efficient gelling agents. They were able to immobilize wide range of organic solvents at low concentrations. Minimum gelation concentrations (MGC) for the gelators ranged from 1.5 to 5 wt% depending on the gelator and solvent. All the above gels were stable for months. Various characterization techniques (spectroscopic, X-ray diffraction and microscopy) were collectively applied to decipher the gelation mechanism. It was learned that the hydrogen bonding between sugar group and van der Waals interaction between alkyl chains were the primary physical interaction responsible for self-assembly process (Figure 4).

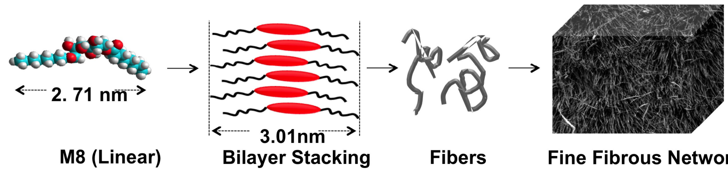

Fig. 4. Schematic representation of molecular assembly of M8. M8 self-assemble via multilayered stacking to form fibers, which further entangle to form a 3-D fibrous network, thereby entrapping an organic liquid and producing a gel.

Application:

a. *Environmentally benign oil spill recovery material:* The M8 and S8 exhibited phase-selective gelation of organic solvents. When these amphiphiles are added to an oil-water mixture, they selectively partition into the oil phase and convert it into a gel (Figure 5). Since oil spills scenarios consist of oil-water mixture, we investigated the ability of developed amphiphiles in selectively gelling crude oil and petroleum refinery products. Interestingly, it was able to selectively gel petroleum products such as diesel, mineral oil etc. at very low concentration. For example, M8 gels diesel at 2.5%wt/v; in other words, it immobilizes diesel to roughly 32 times its own dry weight. The reported amphiphiles are more efficient compared with commercial polymeric oil spill recovering materials (gelation efficiency ~28 times its dry weight). The gelled oil could be conveniently mopped out from the water surface, thereby effecting complete removal of oil from water bodies. Advantageously, the oil could be quantitatively recovered from the gel through simple vacuum distillation (John, G. et al. *Angew. Chem. Int. Ed.* **2010**, *49*, 7695). Such a feat is not possible with polymeric materials. The amphiphiles are easily synthesized and environmentally benign, and can be recovered and reused multiple times. Thus, it not only demonstrates the oil removal ability of the sugar-based molecular gelators but also illustrates, for the first time, recovery of the oil from gel and the recyclability of sugar-based gelators, which are essential for projecting a cost-effective and benign remediation material.

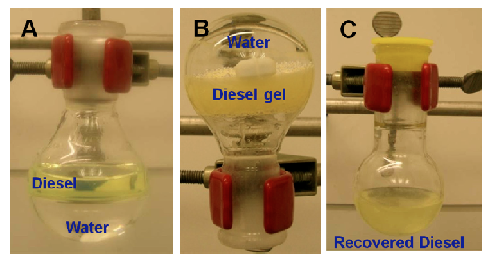

Fig. 5. Phase-selective gelation and diesel recovery from a two phase system. A) Diesel-water biphasic mixture. B) Phase-selective gelation of diesel in the presence of water. C) Recovery of diesel from gel via vacuum distillation.

b. *Efficient controlled-release material for pheromones*: Pheromones are naturally obtained volatile semiochemicals and are considered as effective biopesticides under the integrated pest management concept. Numerous reservoir-type controlled release devices (CRDs) have been developed to overcome the high volatility of pheromones and achieve a sustained release over a period of several weeks, essential for effective pest control. However, most of the devices exhibit low pheromone-holding capacity (~50% wt/wt), low biodegradable and are prone to leak when subjected to compressive force, which is common during shipping and handling. Herein, we propose a biobased molecular gelator as an efficient alternative material for developing reservoir-type release devices.

M8 efficiently gelled pheromones (e.g. lauryl acetate and 2-heptanone) at the concentration of ~7% wt/wt, indicating that approximately 92% of the remaining gel is pheromone liquid (John, G. et al. *Soft Matter* **2011**, *7*, 864). Thus, the loading capacity was found to increase by two fold in comparison to commercial devices. In addition, the pheromone-M8 gel did not exhibit any syneresis on application of high compressive stress, thereby ensuring leak-free performance. Due to such advantages of pheromones-M8 gels, its application in the agricultural industry was demonstrated by utilizing them to develop efficient CRDs. The CRD typically consisted of pheromone-M8 gel (7 %wt/v) wrapped in a fruit film, a carbohydrate material extracted from fruits. The developed CRDs were able to deliver the pheromones uniformly at high concentration for a prolonged time. In addition, owing to minimal solid content and high biodegradability of gelators, the CRDs once spent, will degrade rapidly; thereby, precluding the need and associated cost to recover the spent devices.

c. *Healthy alternative vegetable oil structuring agent:* Structuring of vegetable oil is a process which involves alteration of physical property of vegetable oil with or without altering the chemical properties. In other words, it is a process which converts liquid like vegetable oil into butter like fat. Vegetable oil is structured to improve the consistency, mouth-feel, and stability and also to prevent oil exudation or migration. Structured oil is primarily used in confectionary and baking industries. Margarines and shortenings are the well known examples of structured oil. The existing oil structuring methods, such as hydrogenation, interesterification and addition of fatty acids, tend to increase the saturated fatty acid and trans fatty acid content of oils. Such fatty acids elevate the risk of heart diseases. Therefore, conscious effort are been taken to reduce the level of saturated fatty acid content in the food for the development of more healthy and nutritional food products.

In this context, the medium chain dialkanoates of low calorie sugars (sugar alcohol dioctanoates), M8 and S8, were investigated as healthy oil structuring agents. These amphiphiles, when consumed will be metabolized to its precursors, viz. sugars (mannitol or sorbitol) and caprylic acid (octanoic acid). Both the products do not exhibit any harmful effects on the body. On contrary, they are known to be therapeutic. Mannitol and Sorbitol are non-reducing, low calorie sugars which inhibit growth of oral-bacteria. The caprylic acid is also known to be anti-bacterial in nature.

M8 and S8 were found to gel wide range of edible oils, ranging from saturated coconut oil to highly unsaturated grape-seed oil, in the range of 1-3.5 %wt/v. Advantageously, the physical property of the oil structured by using sugar amphiphiles was on par with the oil structured using stearic acid, which is commercial oil structurant. In a nutshell, the developed amphiphiles are potential food additives and can be used for numerous food applications.

References to Patent Applications:

We obtained a provisional patent based on this invention # WO 2011/014653A2 entitled "Methods for thickening hydrophobic liquids with amphiphilic esters". In a month time, we will convert into utility patent in US, Europe, Brazil, Japan, India and China.

In addition, EPA strongly encourages you to compare the cost, performance and environmental profile of your technology with any competing technologies. This may help you demonstrate the broad applicability of your technology.

Cost. At present, we are working with a Contract Research Organization in India, to produce bio-based amphiphiles in Ton-scale level with the production cost less than the current production of cost of existing thickeners.

Performance. The designed amphiphiles are remarkably effective gelation agents for a variety of organic liquids from crude oil-pheromone-vegetable oil. Such wider gelation ability enables application of these amphiphiles in diverse industries such as petroleum, agriculture and food/personal care/pharmaceutics. Future generation of gelation agents are anticipated that may lead to even better performance, as we begin to accumulate information on a library of sugar amphiphiles, developed through biorefinery approach.

Environmental profile. Given the GRAS- status of building blocks – sugar alcohols and fatty acids – the amphiphiles are expected to have no health or safety issue. The sugar alcohol-based amphiphiles were analyzed for biodegradability tendency and cytotoxicity. Preliminary results demonstrate that the rate of degradation of these amphiphiles is on par with starch, most readily degradable natural material. In addition, the amphiphiles exhibited no toxicity towards HepG2 cells even at high dose of 100 μg/mL.

Applicability. Prudent utilization of natural resources as starting materials for developing functional materials is gaining importance to minimize the carbon footprint as well as to decrease reliance on depleting petroleum resources. The nominated technology embodies similar approach for developing value-added chemicals with a practical and cost-effective technique. It uses renewable feed-stocks (sugar alcohols) and single step enzyme catalysis in GRAS conditions for designing multifunctional sugar-based amphiphiles. These amphiphiles showed remarkable gelation efficiency with a variety of organic liquid at small (< 5%) percentage of amphiphiles. The technology enabled development of new and improved products relevant to personal care, food and crude oil applications. Such value-added chemicals developed through **biorefinery** concept may have an impact on industrial applications and new products.

Summary of key features associated with this technology:

- Adoption of biorefinery approach to produce value-added chemicals.
- Enzyme catalysis optimized to avoid solvent-intensive chromatographic purification and scaled-up to Ton quantities.
- Eliminate energy intensive heating step in inducing gelation of an organic liquid.
- Applicable to oil spill recovery, controlled release technologies (drug & agriculture) and healthy structuring of edible oils.
- A potential eco-friendly substitute for polymeric thickeners of hydrophobic liquids.
- Should stimulate further advances in the use of biobased raw-materials for developing end products capable of substituting petroleum-based ones.

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