

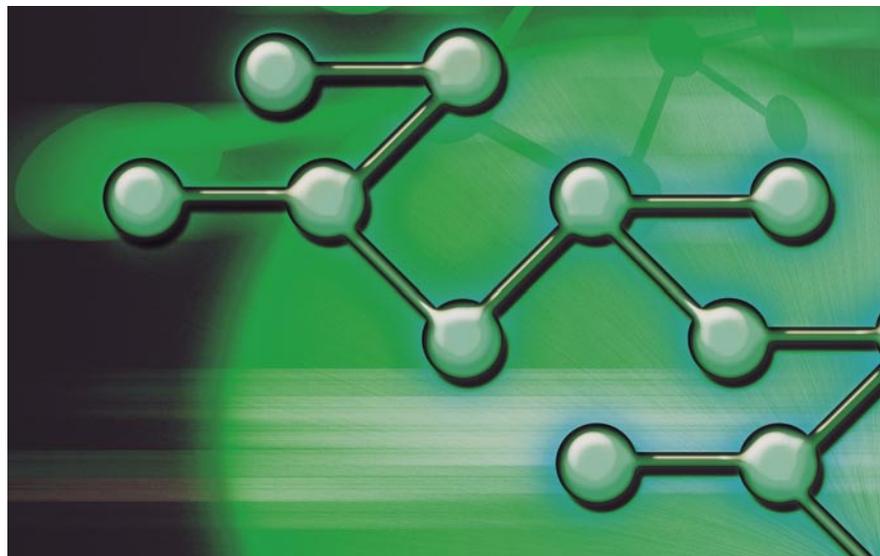
Smart Polymers Are in the Biotech Future

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Life is polymeric in its essence. In every part of life, we are associated with polymers. The most important components of living cells — proteins, carbohydrates, and nucleic acids — are polymeric molecules. Nature uses polymers both as constructive elements and as a part of the complicated cell machinery of living things.

The phrase *smart polymers* is appearing with increasing frequency in scientific and engineering publications as well as in the popular press. Stimulus-responsive or “smart” polymers undergo strong conformational changes with only small changes in their environment such as in pH, temperature, ionic strength, and electric or magnetic fields. Structures of these polymers are called *smart materials* and include those used in sensors and actuators. Smart materials for devices have been developed and studied either embedded in or attached to structural materials.

Why Are They Called “Smart Polymers?” The salient feature of



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functional biopolymers is their highly nonlinear response to external stimuli. Small changes occur in response to varying parameters until a critical point is reached. A significant transition occurs when parameters are varied within a certain narrow range. After the transition is complete, the system shows no significant further response.

The nonlinear response of biopolymers is effective in highly cooperative interactions. Despite the weakness of each particular interaction taking place in each separate monomer unit, when summed through hundreds and thousands of monomer units those interactions represent a significant driving force for the biological processes occurring in such systems. Our growing understanding of the mechanism of such cooperative interactions has generated a large amount of research. Recent decades have brought synthetic functional polymers, each of which

responds in some desired way to a change in temperature, pH, or other stimuli. They were thus termed *stimuli responsive*.

“Smart” Inventions: Why Do We Need Them? Several so-called smart systems have been commercialized and are widely used. Many more are in various stages of development (1):

- Aerospace engineers are interested in smart airfoils to control drag and turbulence.
- Diabetics need medical systems that will sense sugar levels and deliver insulin appropriately.
- Architects are designing smart buildings with self-adjusting windows to control the flow of heat and sunlight.
- Tennis players want smart racquets with which they can make both overhead smashes and delicate drop shots.
- For security, smart motion detectors could monitor authorized

PRODUCT FOCUS: ALL PRODUCTS

PROCESS FOCUS: FORMULATION, DELIVERY, AND DOWNSTREAM PROCESSING

WHO SHOULD READ: PROCESS DEVELOPMENT, PRODUCT DEVELOPMENT

KEYWORDS: HYDROGELS, CONTROLLED RELEASE, ENZYMATICS, SEPARATIONS MEDIA

LEVEL: BASIC

and unauthorized entries into buildings.

- At home, smart toilets could analyze urine to identify health problems.
- In agriculture, smart irrigation systems will be needed to optimize the world's food supply.

CHEMISTRY

Graft-and-block copolymers are of interest because such systems can usually retain the unique properties of their individual components; random copolymers exhibit average properties of their combination. Graft-and-block copolymers also represent a way to combine two unique properties into one polymer structure. Two different polymers grafted together could offer a combination of properties desirable for certain applications.

Over the past decade, significant interest has developed in mucoadhesive polymers. Mucus membranes represent a large and readily accessible surface area of the body for drug delivery, especially in the eyes (ophthalmic delivery), nasal cavity and sinuses, mouth (buccal delivery), vaginal surfaces, and the gastrointestinal tract. Mucoadhesive polymers can prolong the residence time of a delivery system by adhering to mucosal surfaces at the site where a therapeutic is to be delivered.

Polyacrylic acid (PAAc) is well known for bioadhesive drug delivery systems. It is often applied in lightly cross-linked forms that are commercially available. However, PAAc is also a pH-sensitive polymer in which carboxyl groups ($-\text{COOH}$) are ionized at a certain physiological condition (pH 7.4), which can lead to swelling and rapid disintegration of the PAAc. That causes the associated drug to be released upon contact with the mucosal surface, bursting from its polymer matrix. That usually results in low bioavailability, as well as a lower formulation efficiency. It can also sometimes lead to undesirable side effects. Modification of the mucoadhesive polymer, of the formulation, or of the delivery conditions is therefore necessary.

A graft-and-block copolymer could solve that problem. It is often necessary for a polymeric drug carrier to provide more than one important property. In the above case of delivering a drug to a mucosal surface, both prolonged residence time on the mucosal surface and prolonged release kinetics of the active agent are necessary properties for a successful system. But they can often conflict. Another polymer in use is poly(*N*-isopropylacrylamide), polyacrylamide (PAAm) and temperature sensitive triblock copolymer of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) or (PEO-PPO-PEO, known as pluronic polyol surfactants).

CLASSIFICATION

In general, all smart polymers and hydrogel systems can be divided into three groups: pH-sensitive polymers, heat-sensitive polymers, and reversibly cross-linked polymer networks. These refer to linear polymers only. Matrix polymers are discussed in the next section.

With pH-sensitive smart polymers, poor solvent conditions are created by decreasing their net charge. It can be decreased by changing pH to neutralize the charges on each macromolecule — and hence to reduce repulsion between the polymer segments. For instance, copolymers of methylmethacrylate and methacrylic acid precipitate from aqueous solutions on acidification to about pH 5, whereas copolymers of methylmethacrylate with dimethyl aminethyl methacrylate are soluble at acidic conditions but precipitate at slightly alkaline conditions.

Pills coated with such a polymer film are insoluble in the acidic environment of the stomach, so their active components would be protected against the harmful action of the stomach environment. When arriving in the intestine, with its neutral pH, the polymer coating will dissolve to release the active ingredient.

Thermosensitive smart polymers are uncharged and soluble in water because of hydrogen bonding with water molecules. A change in hydrophobic-hydrophilic balance is

induced by increasing temperature or ionic strength of the solution. The efficiency of hydrogen bonding is reduced on raising temperature. At a critical temperature for some polymers, the efficiency of hydrogen bonding becomes insufficient for macromolecular solubility, so phase separation takes place. When the temperature of a smart-polymer aqueous solution is elevated to a point higher than that critical temperature (the *lower critical solution temperature*, LCST, or *cloud point*), separation into two phases takes place: a polymer-enriched phase and an aqueous phase containing practically no polymer at all. Both can be easily separated. The phase change is completely reversible because the polymer dissolves in water on cooling.

Two thermosensitive smart polymers are most widely studied and used: poly(*N*-isopropylacrylamide) or poly(*N*-IPAM), with an LCST at 32–34 °C; and poly(*N*-vinyl caprolactum) or poly(VCL), with an LCST of 32–40 °C. In contrast to pH sensitive smart polymers, which contain carboxy or amino groups that can be used for covalent coupling of ligands, thermosensitive polymers have no inherently reactive molecular groups.

Reversibly cross-linked polymer networks combine reversible noncovalent cross-linking of separate polymer molecules into an insoluble polymer network. These polymers have found limited application as carriers in affinity precipitation, but they are more promising for the development of smart drug delivery systems capable of releasing drugs in response to a signal; for example, a release of insulin when glucose concentration increases in the blood.

APPLICATIONS

Smart polymeric materials respond to small changes in their environment with a considerable change in their own properties. Such environmental stimuli include altered temperature, pH, chemicals, and light. “Smart” stimuli can involve either synthetic or natural materials. Here I focus on the application of smart materials as tools

to solve biological problems such as bioseparations and drug delivery, as well as enzyme immobilization in smart hydrogels. The goal for such endeavors is to mimic the “smartness” of biological systems and ultimately moderate complex systems (e.g., the immune response) at desired levels. The versatility and untapped potentials of smart polymeric materials makes them an exciting interface of chemistry and biology. Because their stimulus-responsive behavior occurs in aqueous solutions, such polymers and hydrogels are becoming increasingly attractive in biotechnology and medicine.

Enzymes Immobilized in Smart Hydrogels: Hydrogels are three-dimensional polymer networks that swell, but do not dissolve in water. The physicochemical principle of their stimulus response is similar to that described above for linear polymers. However, whereas linear and branched polymers undergo a coil-globule phase transition upon a change in their environment, hydrogels swell or collapse. Their advantages for immobilizing enzymes include ease of recovery for reuse, continuous operations, different shapes for specific purposes, and improved physicochemical properties (2).

The technology for immobilizing enzymes is relatively mature. The key now is to come up with new ideas and novel systems of immobilized enzymes that can fulfill specific needs. Recent research is seeking the possibility of constructing immobilized enzymes for which the enzymatic process can be controlled by externally applied stimuli such as light, electric fields, pH, temperature, or mechanical forces.

Since the first use of a cross-linked acrylamide gel for enzyme immobilization by physical entrapment, polymer hydrogels have frequently been used in preparation of immobilized biocatalysts. With the conventional gel-entrapped biocatalysts, the gel material itself served only as a support for maintaining or holding the biocatalyst — no functional properties are attributive to the gels themselves. Recent dramatic development in the field has led to successful synthesis of

many smart hydrogels that undergo continuous or discontinuous volume changes in response to an external stimulus (3).

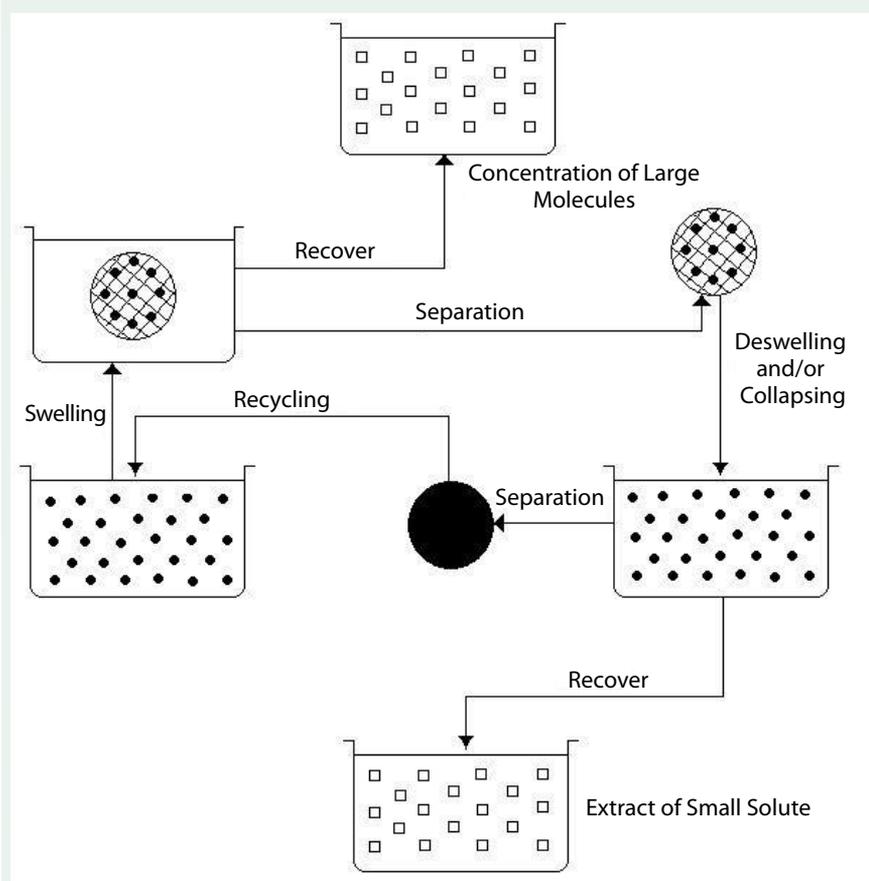
Drug Delivery: When an enzyme is immobilized in a smart hydrogel, the product of the enzymatic reaction could itself trigger the gel’s phase transition. It would then be possible to translate a chemical signal (e.g., presence of a particular substrate) into an environmental signal (e.g., pH change) and then on into a mechanical response, namely shrinking or swelling of the smart gel. This idea lies behind the development of drug-delivery systems in which active ingredients are liberated in response to a chemical signal (e.g., insulin release in response to rising glucose concentration).

The swelling or shrinking of smart hydrogel beads in response to small changes in pH or temperature could be used to control drug release because diffusion of the drug out of those beads would depend on the gel state

(4). When a smart polymer is integrated into a microcapsule wall or liposomal lipid bilayer, the polymer’s conformational transition affects the integrity of that carrier and allows for regulated release of drugs.

In one study, a cross-linked hydrogel component of ethyl acryl amide was prepared with the amine-containing dimethyl amino ethyl methacrylate monomer (5). The result was a polymeric “device” that swelled in response to pH changes (neutral to acidic). Entrapment of glucose oxidase within that material made it glucose-responsive through the formation of gluconic acid. When insulin was coloaded with glucose oxidase into such “biosmart” devices, a twofold increase in insulin release rate was observed when they were immersed in glucose solutions. Insulin release ceased within 10 minutes of glucose removal and could then be restimulated by glucose addition (5).

Figure 1: Process — first step is the preferential partitioning of target substance and impurities between two phases (liquid-liquid or liquid-solid); second is the mechanical separation of the phases (e.g., separation of the stationary and mobile phase in a chromatographic column); third is the recovery of the target substance from the enriched phase.



Specific release of insulin in response to glucose also could be designed in the form of a “chemical valve” (3). Glucose oxidase can be immobilized on a pH-responsive PAAc layer grafted onto a porous polycarbonate membrane. Under neutral conditions, polymer chains are densely charged and have an extended conformation, which prevents insulin transport through the membrane by blocking its pores. Upon exposure to glucose, the pH level drops, and those polymer chains become protonated, adopting a more compact conformation. Pore blockage is reduced, and insulin is transported through the membrane.

Those research results are encouraging, but associated difficulties cannot be ignored: possible toxicity or nonbiocompatibility of the material(s) used, potentially undesirable byproducts of degradation, and the higher cost of controlled-release systems compared with traditional pharmaceutical formulations.

Bioseparations: The cost of the purification is a critical factor in determining the overall production cost of a protein product. Use of smart polymers has led to some simple and economical strategies in bioseparations. Smart materials can “sense” changes in their environment and respond to those changes in a preprogrammed and pronounced way. Water-soluble polymers and hydrogels undergo fast and reversible changes in microstructure triggered by small changes of medium property (e.g., pH, temperature, ionic strength, presence of specific chemicals, light, and electric or magnetic fields). Such microscopic changes of polymer microstructure manifest themselves at the macroscopic level as a precipitate formation in solution and as manifold decreases or increases of a hydrogel’s size (and water content). Figure 1 illustrates a hydrogel-based bioseparation.

RESEARCH

At present, researchers mainly concentrate on applications with the greatest potential: the design of new synthetic materials (not found in nature) based on an understanding of

the relationships between molecular structure and biorecognition. The intelligent response of a smart polymer gel can be used in diverse novel applications such as sensors, actuators, chemical and biological separation techniques, membranes, controlled drug delivery methods, and even some consumer products.

Research at our National Chemical Laboratory (NCL; Pune, India) has been directed toward developing new temperature-sensitive hydrogels and novel gel-based products and applications. Development of new thermoreversible gels is motivated by the fact that currently there is only one such gel — poly(*N*-isopropylacrylamide) or PNIPAAm — applied in general use. Developing new gels requires a thorough understanding of why volume transitions occur. Researchers have demonstrated for the first time that a fine balance between hydrogen bonding and hydrophobic interactions is responsible for such transitions.

One application developed at our lab deals with separating the aqueous phase from oil–water dispersions and emulsions. Another interesting gel-based product we’ve developed is the patented “Gelofuel,” which retains large quantities of a clean fuel such as methanol or ethanol to release slowly for burning.

THE FUTURE

Smart materials are the focus of a great deal of attention in the popular and technical press. Articles have detailed how buildings made from smart materials could withstand earthquakes, how airplanes could fly without conventional hydraulic systems or electrical motors to move their control surfaces, and how a materials revolution could occur in the electronics industry. Some visionaries have even gone so far as to predict materials that “learn,” meaning that their behavior would change and self correct over time.

In the future, new smart polymers with transition temperatures and pH levels in the range at which certain biomolecules are most stable (4–5 °C and pH 5–8) will be developed and introduced commercially. The fields of

smart and genetically produced biomaterials and drug delivery systems have made great progress in recent years. And there is enormous interest in their commercialization in a variety of industries, with a number of applications having already shown commercial success.

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