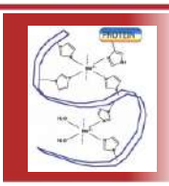


"The name 'smart polymers' was coined due to the similarity of the stimuli-responsive polymers to the biopolymers. There is a strong belief that nature has always been striving for smart solutions in creating life. The goal for the scientists is not only to mimic biological processes, and therefore understand them better, but also to create novel species and invent new processes."

Smart polymeric Biomaterials: *where Chemistry & Biology can merge*

By Ashok Kumar

Smart polymeric materials respond by large changes due to small changes in environment. The polymers that form these smart materials are referred as "smart" polymers or "stimuli-responsive" polymers or "environmentally" sensitive polymers. The smart polymers undergo fast and reversible changes in the microstructure from a hydrophilic to a hydrophobic state that are triggered by small stimuli in the environment. The changes are apparent at the macroscopic level as precipitate formation from a solution accompanied by phase separation from aqueous solution or order of magnitude changes in the hydrogel size. This whole phenomenon is reversible, the system returning to its initial state when the trigger is removed. The driving force behind these transitions varies, with common stimuli including neutralization of charged groups by either a pH shift or the addition of an oppositely charged polymer, changes in the efficiency of the hydrogen bonding with an increase in temperature or ionic strength, and collapse of hydrogels and interpenetrating polymer networks. Even the latest among these have been the electric, magnetic, light or radiation induced reversible phase transitions. Such property change of smart polymers has shown various applications in biological systems.

Life is polymeric

The most important components of living cells; proteins, carbohydrates, nucleic acids are polymers. The functions of living cells are regulated by these biopolymers that form the basis around which all major natural processes are controlled. Nature use polymers both as constructive elements and parts of complicated cell machinery. The salient feature of functional biopolymers is their all-or-none or at least highly nonlinear response to external stimuli. Small changes happens in response to varying parameter until the critical point is reached, then the transition occurs in the narrow range of the parameter varied and after the transition is completed, there is no significant further response of the system. Such nonlinear response of biopolymers is warranted by highly cooperative interactions. Despite the weakness of each particular interaction taking place in a separate monomer unit, these interactions when summed through hundreds and thousands of monomer units could provide significant driving forces for the processes occurring in such systems. Understanding of the mechanism of cooperative interactions in biopolymers has opened floodgates for attempts to mimic cooperative behavior of the biopolymers in synthetic systems. Last two decades witnessed the appearance of synthetic functional polymers, which respond in some desired way to a change in temperature, pH, electric or magnetic fields or some other parameters. These polymers were nicknamed stimuli-responsive. The name 'smart polymers' was coined due to the similarity of the stimuli-responsive polymers to the biopolymers. There is a strong belief that nature has always been striving for smart solutions in creating life. The goal for the scientists is not only to mimic biological processes, and therefore understand them better, but also to create novel species and invent new processes. Recent developments

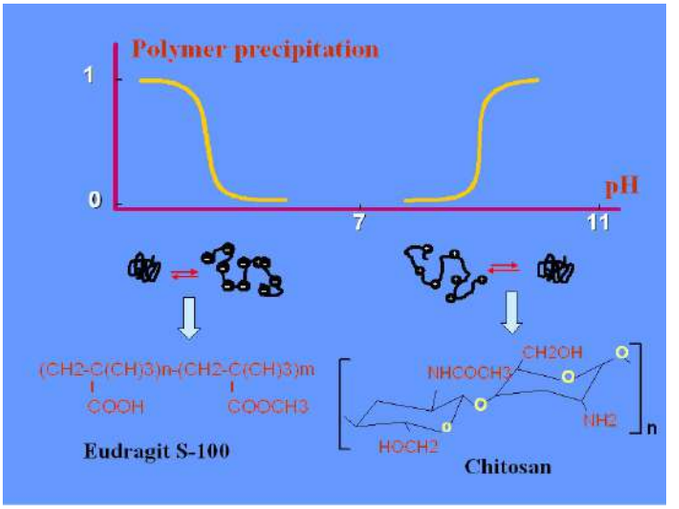


Fig. 1: pH-induced soluble-insoluble behaviour of synthetic (Eudragit S-100) and natural (chitosan) polymers

have shown an explosive growth in the subject where smart polymeric materials are being tailor made for application in biotechnology and medicine. Of special interest are two most common smart polymer systems with sharp response to external stimuli and thus promising enormous potential in biotechnology, bioengineering and medicine. These systems are developed from pH- and temperature-sensitive smart polymers. For applications, these polymers are utilized in many forms, as can be dissolved in aqueous solution, adsorbed or grafted on aqueous-solid interfaces, or cross-linked in the form of hydrogels.

pH-sensitive smart polymers

This group of smart polymers consists of the polymers for which transition between soluble and insoluble state is created by decreasing net charge of the polymer molecule. The net charge can be decreased by changing pH to neutralize the charges on the macromolecule and hence to reduce the hydrophilicity (increase the hydrophobicity) of the macromolecule. Copolymers of methylmethacrylate (hydrophobic part) and methacrylic acid (hydrophilic at high pH when carboxy groups are deprotonated but more hydrophobic when carboxy groups are protonated) precipitate from aqueous solutions on acidification to pH around 5 while copolymers of methyl methacrylate (hydrophobic part) with dimethylaminethyl methacrylate (hydrophilic at low pH when amino groups are protonated but more hydrophobic when amino groups are deprotonated) are soluble at low pH but precipitate at slightly alkaline conditions. Hydrophobically modified cellulose derivatives with pending carboxy groups, e.g. hydroxypropyl methyl cellulose acetate succinate are also soluble at basic conditions but precipitate in slightly acidic media. The pH-induced precipitation of smart polymers is very sharp and requires usually the change in pH not more than 0.5 units (Fig. 1).

The copolymerization of some specific monomers results in the synthesis of a pH-sensitive polymer with reversible transition in the physiological range of pH 7.0-7.5, thus making them more suitable for biological systems. The charges on the macromolecule can also be neutralized by addition of an efficient counterion, e.g., low molecular weight counter ion or a polymer molecule with the

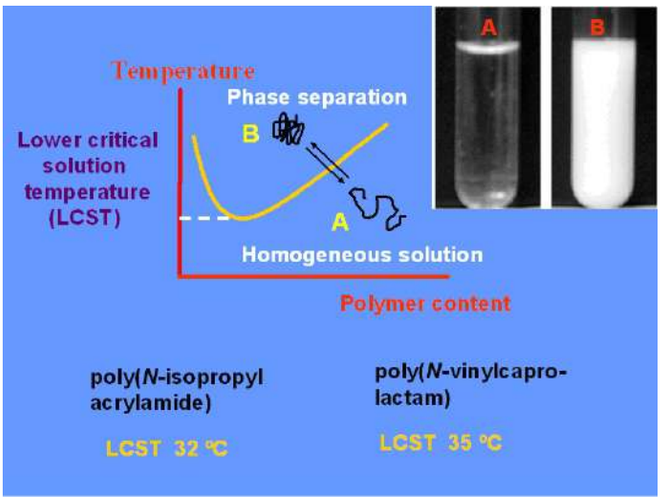


Fig. 2: Temperature response for thermo-sensitive polymers. (A) soluble phase(below LCST); (B) insoluble phase (above LCST)

opposite charges. The latter systems are combined under the name of polycomplexes. The cooperative nature of interaction between two polymers with the opposite charges makes polycomplexes very sensitive to the changes in pH or ionic strength. Many polymer systems have thus been designed that can show the reversible solubility property in any desired pH range where one needs to utilize them.

Thermo-sensitive smart polymers

The reversible solubility of thermosensitive smart polymers is caused by changes in hydrophobic-hydrophilic balance of uncharged polymer induced by increasing temperature or ionic strength. The uncharged polymers are soluble in water due to the hydrogen bonding with water molecules. The efficiency of hydrogen bonding reduces with increase in temperature. The phase separation of polymer takes place when the efficiency of hydrogen bonding becomes insufficient for solubility of macromolecule. On raising the temperature of aqueous solutions of smart polymers above a certain critical temperature(which is often referred as transition temperature, lower critical solution temperature, LCST, or 'cloud point'), phase separation takes place. An aqueous phase containing practically no polymer and a polymer enriched phase are formed. Both phases can be easily separated by decanting, centrifugation or filtration. The temperature of phase transitions depends on polymer concentration and molecular weight (MW) (Fig. 2). The phase separation is completely reversible and the smart polymer dissolves in water when the temperature is reduced below the transition temperature.

Two groups of thermo-sensitive smart polymers are most widely studied and used.

- Poly(N-alkyl substituted acrylamides) and the most well-known of them, poly(N-isopropyl acrylamide) with transition temperature of 32 °C.

- Poly(N-vinylalkylamides) like poly(N-vinyliso-butyramide) with transition temperature of 39°C or poly(N-vinyl caprolactam) with transition temperature 34-36°C (depending on polymer molecular weight).

Other polymers with different transition temperatures from 4-5°C for poly (N-vinyl piperidine) to 100°C for poly(ethylene glycol) are available at present. Increase in the hydrophilicity of the polymer by incorporation of hydrophilic co-monomers or coupling to

hydrophilic ligands, increases the transition temperature while hydrophobic co-monomers and ligands have the opposite effect.

Block copolymers with a thermosensitive "smart" part consisting of poly (NIPAAM) form reversible gels on increase in temperature while random copolymers separate from aqueous solutions by forming concentrated polymer phase. Thus, the properties of smart polymers important for biotechnological and medical applications could be controlled not only by the composition of co-monomers but also by the polymer architecture. The phase transition at increased temperature of thermosensitive polymers is the result of hydrophobic interactions between polymer molecules. As hydrophobic interactions are promoted by high salt concentrations, the addition of salts shifts cloud point to lower temperatures. On the other hand, addition of organic solvents, detergents and chaotropic agents increases transition temperature as these compounds deteriorate hydrophobic interactions.

Applications in Biotechnology and Medicine

Smart polymers may be physically mixed with or chemically conjugated to biomolecules to yield a large family of polymer-biomolecule systems that can respond to biological as well as to physical and chemical stimuli. Biomolecules that can be polymer conjugated include proteins and oligopeptides, sugars and polysaccharides, single and double-stranded oligonucleotides, DNA plasmids, simple lipids and phospholipids and wide range of other ligands and synthetic drug molecules. These polymer-biomolecule complexes, are referred as affinity smart biomaterials or intelligent bioconjugates. Also such polymers have been used in developing smart hydrogels and smart surfaces that can respond to external stimuli. Such polymeric biomaterials have shown a range of different applications in the fields of biotechnology and medicine (Fig. 3).The researchers have used these polymers for biomedical applications to downstream

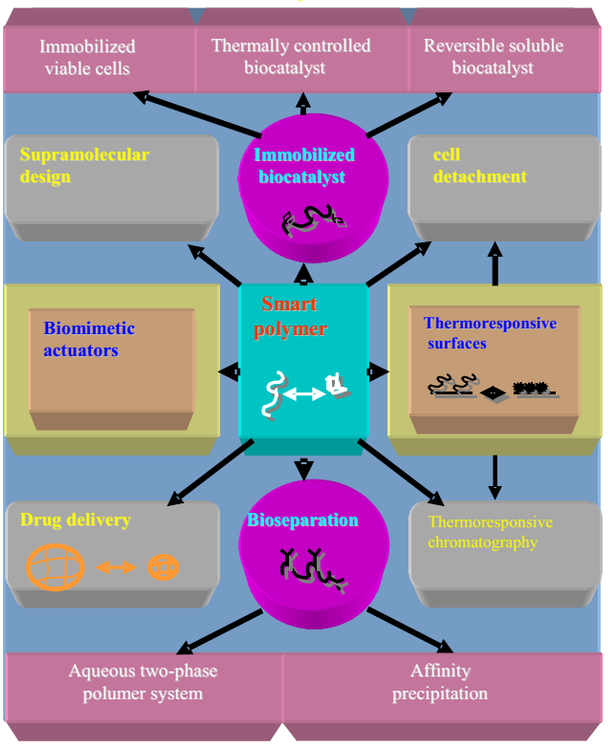


Fig. 3: Application of smart polymeric materials in biotechnology and bioengineering

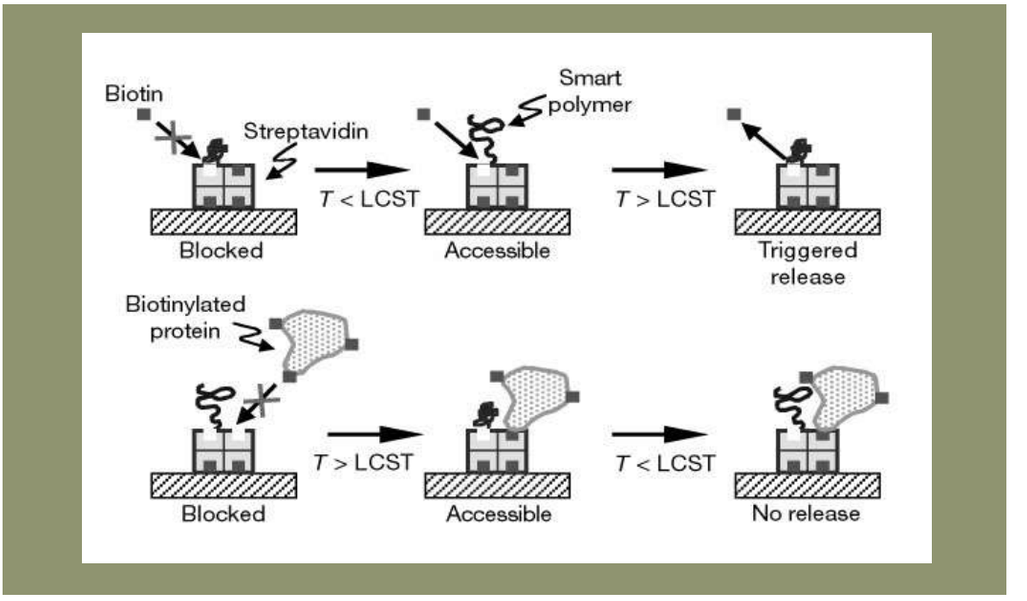


Fig.4: Shielding effects of a conjugated smart polymer on the binding of a small ligand (biotin) and a large macromolecular ligand (a biotinylated protein) to streptavidin. (Source: Nature (2001); 411, 59-62).

processing and biocatalysis and several breakthrough findings have emerged. In the streak of these stimulatory research findings is the latest thrilling breakthrough achieved by the group of Stayton and Hoffman, at the University of Washington, USA. The researchers developed a clever way to use smart polymers that provide size selective switches to turn proteins on and off. If smart polymer chain is attached to the protein molecule farther from the active site, the extended polymer chain would shield the site, blocking larger molecule from attaching. However, when coiled, the chain was far enough out of way, so the big molecule could bind to the site. Such polymers act as a kind of shield or molecular gatekeeper that regulates, based on size, the kind of molecules that can bind to a protein (Fig. 4). This could help bioengineers precisely control the function of proteins and could enable a new class of powerful diagnostic and sensing devices.

Biomimetic actuators

There have been attempts to mimic the efficient conversion of chemical energy into mechanical energy in living organisms. A cross linked gel of poly(vinyl alcohol)chains entangled with polyacrylic acid chains has good mechanical properties and shows rapid electric field associated bending deformation: a gel rod of 1mm diameter bends semi circularly within 1 sec on the application of an electric field. An artificial fish with a gel tail swam forward at a velocity of 2 cm/ sec as the gel oscillated under sinusoidally varied electric fields; a mechanical hand composed of four gel fingers could pick up a fragile quil egg (9g) from a sodium carbonate solution and hold it without breaking, controlled by an electric signal. Polymer gels capable of mechanical response to electric field have also been developed using the cooperative binding of the positively charged surfactant molecules to the polyanionic polymer poly (2 acrylamido- 2 methyl- 1- propane sulfonic acid). Copolymers gels consisting of N-isopropylacrylamide and acrylic acid would be useful for constructing biochemomechanical systems. A pH induced change in the -COOH ionization of acrylic acid alters the repulsive force; the attractive

force is produced by hydrophobic interactions arising from the dehydration of the N-isopropylacrylamide moieties. Glucose dehydrogenase catalyses the conversion of neutral glucose into gluconic acid, accompanied by a decrease in the pH. When the reaction occurs inside the gel, the repulsive forces are eliminated owing to the protonation of the COO- groups. As a result, the attractive forces dominate, followed by the collapse of the gel. Changes in the gel's size (about 1.5 times) using immobilized glucose dehydrogenase are initiated by pulses of a 40 mm glucose solution.

The biomimetic actuators could be used in future 'soft' machines that are designed using more biological than mechanical principles. The gel hand consisting of smart polymer can be used as a very useful tool in picking up very tiny little objects (so tiny we can't even see it) in the aqueous solution. All we have to do is to insert the gel hand and raise the temperature a little bit. As the polymer layer shrinks, the "hands" will contract and grab on to the target object. This is totally reversible. When the target compound within the gel hand is destined for release, just decrease the surrounding temperature. In contrast to biological systems, biomimetic actuators can withstand very hostile environments.

Reversible biocatalysts

The ability of the smart polymers to form a separate phase in the aqueous solutions following a slight change in the external conditions can be used to create reversibly soluble biocatalysts, when the enzyme molecule is bound covalently to the polymer. These biocatalyst catalyze an enzyme reaction in their soluble state and thus can be used in reactions with insoluble or poorly soluble substrates or products. As the reaction is complete, the conditions are changed to cause the catalyst to precipitate so that it can be separated from the products and used in the next cycle, after redissolution. Wide range of smart polymers have been used for the development of reversibly soluble biocatalysts whose solubility is controlled by pH or temperature. For example, trypsin immobilized on a pH-responsive copolymer of methylmethacrylate and methacrylic acid (Eudragit S100) is used for repeated hydrolysis of casein. Water-insoluble compound phlorizidin is hydrolyzed by a reversible biocatalyst of β-glucosidase immobilized on a pH responsive, reversibly soluble polymer (hydroypropylmethylcellulose acetate succinate) and was reused for five cycles. Similarly, when *Arthrobacter simplex* cells are immobilized inside beads of a thermosensitive polymer gel as a biocatalyst, thermal cycling cause cyclical changes between the collapsed and the swollen states of the hydrogel beads. The system thus work as a 'hydraulic pump', driving the mass transfer of the substrate (hydrocortisone) into gel and the product (prednisolone) out. A biocatalyst sensitive to magnetic field is produced by immobilizing invertase and γ–Fe_2O_3 in a poly (N-isopropylacrylamide-co-acrylamide) gel. The heat generated by exposure of γ–Fe_2O_3 to a magnetic field causes the gel to collapse, which is followed by a sharp decrease in the rate of sucrose hydrolysis. Polymer bound smart catalysts are useful in waste minimization, catalyst recovery , and catalyst reuse. Polymeric smart coatings have been developed that are capable of both detecting and removing hazardous nuclear contaminants. Such applications of smart materials involving catalysis chemistry, sensor chemistry, and chemistry relevant to decontamination methodology are especially applicable to environmental problems.

Thus the biocatalysts obtained by immobilizing enzymes and cells with the aid of "smart" polymers acquire potentially useful properties. Such biocatalyst can combine the advantages of homogeneous and heterogeneous catalyst, can serve as convenient 'chemical switches' sensitive to slight changes in the external conditions, and are distinguished by a higher productivity as a result of the operation of the 'thermal pump'.

Smart drug delivery systems

The application of the smart polymers for drug delivery shows great promise due to modulated or pulsatile drug release pattern to mimic biological demand. Stimuli occurring externally or internally include temperature, photoirradiation, electric current, pH, and metabolic chemicals. When an enzyme is immobilized in smart hydrogels, the products of enzymatic reaction could themselves trigger the gel's phase transition. It would then be possible to translate the chemical signal (e.g., presence of substrate) into the environmental signal (e.g. pH change) and then into the mechanical signal, namely shrinking or swelling of smart gel. The swelling or shrinking of smart hydrogel beads in response to small change in pH or temperature can be used successfully to control drug release, because diffusion of the drug out of the beads depends on the gel state. When a smart polymer integrated into microcapsule wall or a liposomal lipid bilayer, the conformational transition of the polymer affects the integrity of the microcapsule or liposome and allows the regulated release of the drug loaded into the microcapsule or liposome. In a temperature sensitive polymer, a dilute solution (1-3%) of the polymer is watery liquid, while on warming to body temperature the solution gels, becoming viscous and clinging to surface in a 'bioadhesive' form. The hydrogel therefore provides an effective way to administer drugs, either topically or mucosae, over longer timescales than otherwise possible, by dissolving them in a solution of the hydrogel, which also contains hydrophobic regions. By using such drug formulations incorporated into hydrogels pharmaceutical companies will be able to increase the efficiency, cost-effectiveness and range of applications for existing therapeutics.

As an intelligent drug delivery scheme, the development of glucose-sensitive insuln-releasing system for diabetes therapy has become a popular model for the systems using smart polymers. There have been several schemes which researchers developed over the years for the intelligent release of insulin (Fig. 5 & 6).

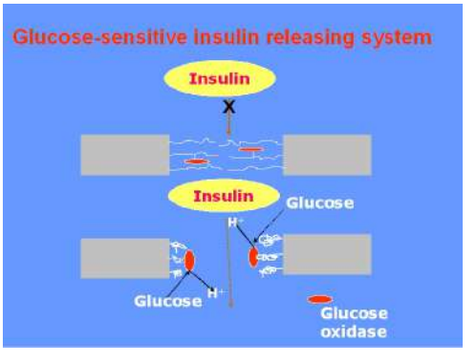


Fig. 5: Insulin delivery system
(Source: TIBTECH (1999), 17(8):335-40)

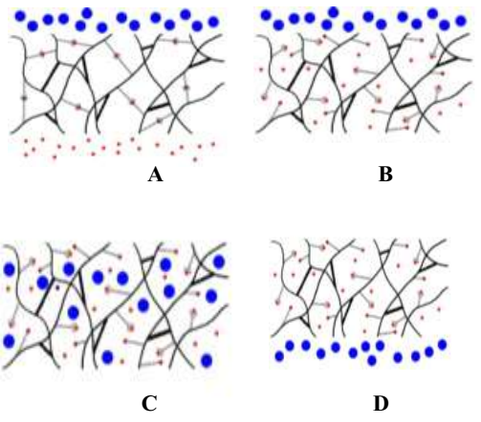


Fig. 6: Schematic of composite hydrogel

- **A- Gel porosity is reduced by affinity crosslinks that exclude large molecules, e.g. insulin (large blue dot).**
- **B- Glucose (small red dot) diffuses in and competitively displaces affinity crosslinks.**
- **C- Insulin is able to diffuse into the more highly porous gel.**
- **D- Insulin diffuses through the gel providing the concentration gradient and glucose concentration is maintained.**

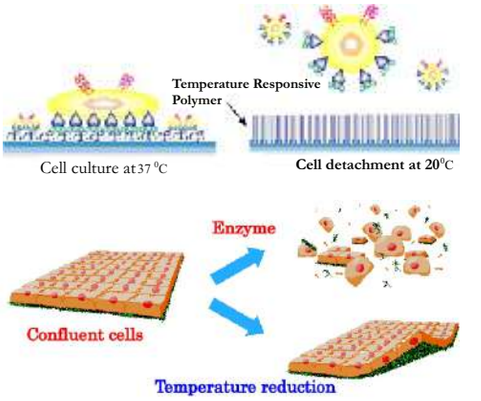
One latest model explains how specific release of insulin can be achieved in response to glucose in the form of 'chemical valve'. Glucose oxidase can be immobilized on a pH responsive poly(acrylic acid) layer grafted onto a porous polycarbonate membrane. Under neutral conditions, polymer chains are densely charged and have an extended conformation,

preventing insulin transport through the membrane by blocking the pores. Upon exposure to glucose the pH drops and the polymer chains become protonated and adopt a more compact conformation. The blocking of pores is reduced and insulin is transported through the membranes. Membrane permeability can be regulated via the conformational changes of the poly(methacrylic acid), poly(2-ethylacrylic acid) or poly(N-isopropylacrylamide) grafted inside the pores.

Thermosensitive polymers as carriers for DNA delivery are recently looked at as an efficient mode of gene transfection and also these Polymer are engineered as carriers of therapeutic proteins. Drug release from thermoresponsive self assembled polymeric micelles composed of cholic acid and poly(N-isopropylacryalmide) were studied as model system to monitor the rate of drug release based on temperature changes.

Stimuli-responsive surfaces

The change in surface properties of the thermoresponsive polymers from hydrophobic above the critical temperature to hydrophilic below it has been used in tissue culture applications. Mammalian cells are cultivated on a hydrophobic solid culture dishes and are usually detached from it by protease treatment, which also cause damage to the cells. This is rather an inefficient way in that only some detached cells are able to adhere onto new dishes because the rest are damaged. At temperature of 37^0C, a substrate surface coated with grafted poly (N-isopropylacryalmide) is hydrophobic because this temperature is above the critical temperature of the polymer and the cells grow well. However, when the temperature is decreased to 20^0C, resulting the surface to become hydrophilic, the cells can be easily detached without any damage. The cells can be used for further culturing. The cells are detached maintaining the cell-cell junction.



source http://www.cellseed.com/

Smart polymer grafted surfaces for cell detachment

This enables the collection of cultured cells as a single "sheet". Cell-sheet is highly effective when transplanted to patients due to tight communication between cells and cells. This technology has recently now been commercialized.

Similarly surfaces with thermoresponsive hydrophobic-hydrophilic properties have been used in modifying chromatographic matrices. Temperature responsive size-exclusion chromatography using poly(N-isopropylacrylamide) is used for high protein resolution with low non-specific interactions. Affinity interactions are also controlled by grafting responsive polymers on affinity matrices (Fig. 7). More interestingly, combinations of pH and temperature-responsive polymers have been used in aqueous chromatography to separate ionic bioactive compounds. These provide the mutual influences of both electrostatic and hydrophobic interactions between the separating compounds and the modified chromatographic matrix.

Bioseparations

The use of smart polymers in bioseparation has developed into a broad area of application since it was begun in the late 1980 s. In affinity

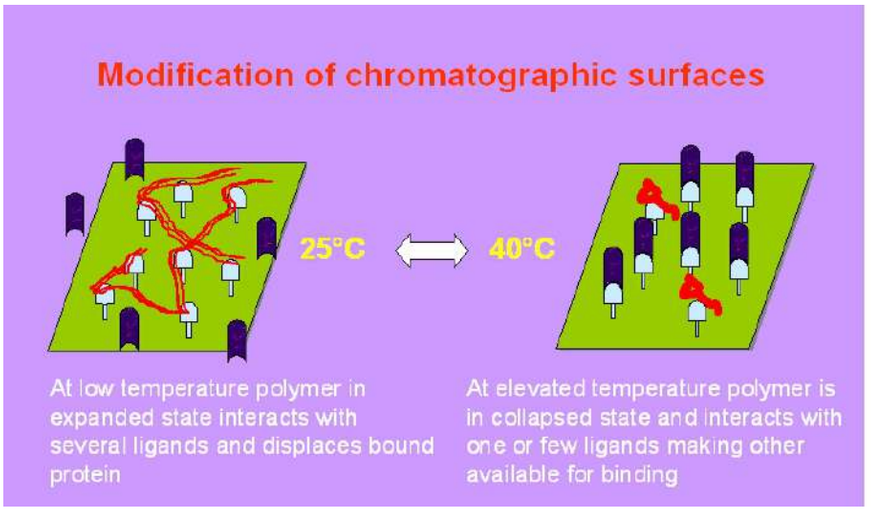


Fig. 7: Modification of affinity chromato-graphic surfaces with temperature responsive polymers to influence the binding and recovery of biomolecules.

precipitation of biomolecules, the bioconjugate is synthesized by coupling a ligand to a water soluble "smart" polymer. The ligand-polymer conjugate selectively binds the target protein from the crude extract and the protein-polymer complex is precipitated from the solution by the changes in the environment like pH, temperature, ionic strength or addition of some reagents. Finally, the desired protein is dissociated from the polymer and the later can be recovered and reused for another cycle. Various ligands like protease inhibitors, antibodies, nucleotides, metal chelates, carbohydrates, and trizine dyes have been used in affinity precipitation. New approach is recently developed for purifying His-tag proteins using thermoresponsive polymers by metal chelate affinity precipitation. The incorporation of relatively hydrophilic imidazole moieties into the poly(NIPAM) backbone hinder the hydrophobic interactions and increase the precipitation temperature of the copolymer. However, when Cu(II) ions are be loaded on the copolymer, the precipitation efficiency is promoted by adding NaCl and increasing temperature. The flexible backbone of the water soluble poly(NIPAM) allowed proper co-ordination between the imidazole groups and metal ions and also leaving some co-ordination sites available for proteins (Fig. 8).

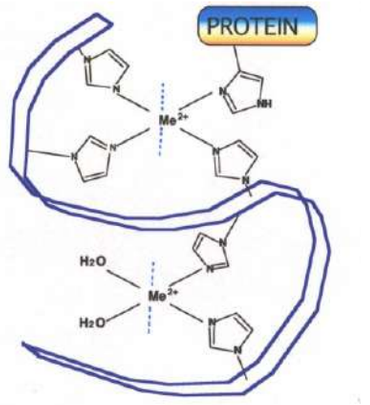


Fig. 8: Smart polymer-metal conjugate for protein purifications

Other emerging strategy involves selective portioning of proteins between two phases in aqueous two-phase systems (ATPS). This is an efficient tool for purifying proteins, cells and some low molecular weight substances. However, the main problem is how to separate the target biomolecules from the phase forming polymer. Smart polymers provide a simple solution to this problem as simple precipitation of the phase forming polymer leaves the protein in supernatant. There have

been several examples where thermoresponsive polymers such as poly(ethylene oxide-co-propylene oxide) or poly(N-vinyl caprolactam-co-vinyl imidazole) from two phase systems with dextran and have been used to purify proteins. Another interesting approach is a combination of affinity precipitation with the extraction in ATPS. Type specific separation of animal cells using pH-sensitive polymer Eudragit S-100 and temperature sensitive polymer poly(NIPAM) as ligand carrier in ATPS has been established.

This seems to be only beginning of the myriad uses and applications of smart polymers in biotechnology. The application of these polymers is only limited by our imagination. Even though the class of these "smart" materials is still very much in the research and development stage, it would not be long before we can really utilize their applications in our daily lives. On the theoretical side, we expect to gain a better understanding of the mechanism of cooperative interactions in these polymer and to increase our knowledge of structure-property relationships to enable the rational synthesis of smart polymers with predefined properties.

About the author: Dr. Ashok kumar is an Associate professor in the Department of Biological Sciences & Bioengineering at IIT Kanpur. He obtained his Ph.D. jointly from IIT Roorkee and Institute of Genomics & Integrative Biology, Delhi. He has postdoctoral research experience from Sweden & Japan. He was a faculty at Lund University, Sweden. His areas of research interest include smart polymeric biomaterials for biotechnological applications, bioprocess technology and bioseparations, affinity interactions, stem cell separations, biosensors and ligand-receptor interaction analysis.