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'Smart' polymers in biotechnology and medicine

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Abstract. 'Smart' water-soluble polymers and hydrogels are capable of responding reversibly to slight changes in the properties of the medium (pH, temperature, ionic strength, the presence of certain substances, illumination, electric field), the response of the system being readily seen with the naked eye (the formation of a new phase in a hitherto homogeneous solution, sudden swelling or contraction of the hydrogel). The properties of such polymers and hydrogels are examined. The use of 'smart' polymers and hydrogels for the concentration of protein solutions and the dehydration of suspensions, for the creation of membranes with a controllable permeability, for the isolation and purification of biomolecules, for the immobilisation of biocatalysts, and for the creation of sensor systems and systems for the controlled release of medicinal drugs is discussed. The bibliography includes 261 references.

I. Introduction

In recent years, investigators have devoted much attention to the so called 'smart or intelligent materials'. Materials capable of responding to slight changes in the external medium in a preprogrammed manner are combined under this name.[1] A specialist journal has actually appeared, namely the *Journal of Intelligent Material Systems and Structures* (Technomic Publishing), in which data on this topic are published.

Among the water-soluble polymers and hydrogels, the term 'smart' may be applied to systems which respond reversibly to slight changes in the properties of the medium (pH, temperature, ionic strength, the presence of certain substances, illumination, electric field), the response of the system being readily visible with the naked eye (the formation of a new phase in a hitherto homogeneous solution, sudden swelling or contraction of the hydrogel).

Such systems are evidently attractive for biotechnology and medicine. They place in the hands of investigators a simple method for manipulating the system. Heating/cooling by several degrees or slight changes in the pH can be achieved rapidly and easily and this permits a reversible and repeated transition from a homogeneous to a heterogeneous system and an alteration by a factor of several tens of the degree of swelling of the hydrogels. Such systems have already found practical applications in the isolation and purification of biologically active substances and in the immobilisation of biocatalysts. They can be used for the controlled release of medicinal drugs, in biosensors, and for the modelling of regulation processes in the living cell associated with phase transitions in membranes.

All living organisms move by virtue of the isothermal conversion of chemical energy into mechanical work, expressed, for example, by the contraction of muscles and by the movement of the flagella or cilia of microorganisms. Hydrogels based on 'smart' polymers, capable of markedly swelling (expanding) or, conversely, contracting in response to external stimuli in the form of thermal, chemical, or electrical energy, may be used for the modelling of such systems. The history of the creation of the first systems of this kind in the 1950s is associated with names familiar in polymer chemistry such as those of Kuhn and Katchalsky.[2,3]

A micromotor or a flapping wing, a gel 'beetle' climbing an inclined slope, and a gel 'finger' bending and straightening out in air and not in solution as in most other cases, the motion of all of which was initiated by a gel, were demonstrated in the Section of Polymer Gels of the Congress of the Japanese Polymer Society held in December 1991 in Tsukuba.[4,5]

The aim of the present review was to draw the attention of chemists and biochemists to certain interesting and useful properties of 'smart' polymers and to stimulate, on the one hand, the use of such systems in biochemical and biotechnological research and, on the other, to stimulate the search for and synthesis of new homopolymers and copolymers with such properties.

II. 'Smart' polymers

For a polymer system to become 'smart', i.e. capable of responding strongly to slight changes in the external medium, a first-order phase transition, accompanied by a sharp decrease in the specific volume of the macromolecule, must occur in it. The theoretical foundations of such processes were laid by Flory.[6] One of the main conditions for the manifestation of critical phenomena in swollen polymer networks or linear macromolecules is the presence of a 'poor' solvent. In such a solvent, the forces of attraction between the segments of the polymer chain may overcome the repulsion forces associated with the excluded volume, which leads to the collapse of the polymer chain.

The three-dimensional structure of macromolecules is determined by four fundamental interactions: van der Waals forces, hydrophobic interactions, electrostatic interactions, and

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hydrogen bonds. The electrostatic interactions between like charges of the polymer chain constitute the most effective repulsion forces. The osmotic pressure within a hydrogel, arising due to low-molecular-mass counterions, also promotes the swelling of hydrogels. Van der Waals forces, hydrophobic interactions, hydrogen bonds, and the interaction of ions of opposite sign constitute attraction forces and give rise to phase transitions in polymer solutions or to the collapse of hydrogels. The differences in the nature of the interactions inducing the phase transition are manifested to the greatest extent in the temperature dependence of the swelling or collapse of hydrogels.[7] If van der Waals forces (for example, the phase transition in a partly hydrolysed polyacrylamide gel in water–acetone mixtures)[8] or hydrogen bonds (for example, the phase transition in a gel consisting of interpenetrating networks of polyacrylamide and polyacrylic acid)[9] are responsible for the collapse of the hydrogel, then the hydrogel swells following an increase in temperature and contracts when it is reduced. On the other hand, if the collapse of the gel is induced by hydrophobic interactions (for example, the phase transition in the gel of the *N*-isopropylacrylamide–sodium acrylate copolymer)[10] or the interaction of ions of opposite sign [for example, the phase transition in the acrylamide–acrylate–(*N*-acryloyl-3-aminopropyl)trimethylammonium copolymer],[11] then the opposite temperature dependence is observed — the gel swells at a low temperature and contracts when the temperature is raised. In certain hydrophilic gels, for example in the acrylamide–sodium vinylsulfonate copolymer, there is a possibility of two phase transitions. In 65% acetone at low temperatures, the gel is in a state of collapse but begins to swell rapidly at temperatures above −10 °C. Up to 55 °C, the gel remains in its swollen state and then its collapse begins again.[12]

For highly hydrophilic ionogenic polymers, the solvent can become sufficiently poor as a result of the addition of an organic solvent to water. Critical phenomena (precipitation of the polymer from the solution or the collapse of the polymer gel) in the presence of 30%–70% acetone, 1-propanol, or ethanol have been observed for both synthetic polymers (copolymers comprising acrylamide and ionogenic comonomers)[11,13–16] and natural polymers (hyaluronic acid,[17] DNA,[18–20] gelatin,[20] agarose[20]).

For nonionogenic polymers, the solubility of which in water is determined by the formation of hydrogen bonds, water becomes a poorer solvent with increase in temperature. For example, when aqueous solutions of the homopolymers with ethylene oxide and its copolymers with propylene oxide are heated above the lower consolute temperature (LCT), the solution divides into two phases — the phase enriched in the polymer and the aqueous phase which contains hardly any polymers. On neutralisation of ionogenic groups, aqueous solutions of polyelectrolytes also divide into two layers on heating (for example, polymethacrylic acid at pH ~ 3 and temperatures > 50 °C).[21] As a rule, the LCT for such polymers is 60 °C and above (see, for example, Pandya et al.[22] and Louai et al.[23]), which is relatively unsuitable for biomolecules. However, such polymers can be used successfully to create reversibly soluble catalysts for homogeneous chemical reactions. Thus a homogeneous hydrogenation catalyst, which precipitates on heating to 50–60 °C, has been created on the basis of a block-copolymer of ethylene and propylene oxides (Pluronic) and a rhodium complex. After precipitation, the catalyst can be readily separated from the reaction mixture, dissolved at room temperature, and reused.[24]

For the appearance of critical phenomena in the systems described above, extreme conditions are necessary (a high concentration of the organic solvent, a high temperature, and low pH values), leading to the rapid denaturation of the biomolecules. For this reason, they have not so far found applications in biotechnology. However, the development of enzymology in nonaqueous media and of new methods for the immobilisation and stabilisation of biocatalysts may render such systems promising from the standpoint of biotechnology.

This review deals with polymers and hydrogels in which critical phenomena are observed in aqueous solutions at temperatures not exceeding 40–45 °C and at weakly acid or neutral pH values.

1. Polymers sensitive to changes in pH

Owing to the neutralisation of the charged macromolecule as a result of a change in the pH of the medium, water becomes a poor solvent for the neutral polymer molecule; a possibility of critical phenomena arises. As already mentioned above, for highly charged polyelectrolytes, such as polymethacrylic acid, critical phenomena occur at extreme pH values and temperatures. A decrease in the charge of the macromolecule and an increase in its hydrophobicity as a result of copolymerisation with hydrophobic comonomers make it possible to achieve critical phenomena within an acceptable pH range. For the copolymers of dimethylaminoethyl methacrylate with n-alkyl methacrylates, the collapse of the gels occurs at pH 6.0–6.5 at room temperature. With increase in the content of n-alkyl methacrylate in the gel, the difference between the volumes of the collapsed and swollen gels diminishes and the transition is observed at lower pH values. An increase in the length of the alkyl substituent in n-alkyl methacrylate also diminishes the difference between the volumes of the collapsed and swollen gels but does not exert a systematic influence on the pH of the transition.[25]

Aqueous solutions of methacrylic acid–methyl methacrylate copolymers and derivatives of cellulose which have been rendered hydrophobic [containing carboxy-groups (carboxymethylethylcellulose, cellulose acetate-phthalate, hydroxypropylmethylcellulose phthalate or acetate-succinate)], which are homogeneous at pH > 4 – 5.5, do not separate into aqueous and polymer phases on acidification[26] Such polymers are manufactured on an industrial scale and are widely used in the manufacture of medicinal drugs (Table 1). The shell surrounding a tablet comprising such polymers does not dissolve in the stomach at pH 1.4 and protects the drug against the harmful influence of the contents of the stomach. Having entered the intestine (pH 6.7–7.4), the shell of the tablet dissolves, which enables the drug to be rapidly assimilated into the blood. On the other hand, the copolymer of diethylaminoethyl methacrylate with methyl methacrylate and butyl methacrylate is insoluble at neutral pH values, but dissolves in an acid medium. The shell comprising this polymer protects the tablet against dissolution in the saliva and under these conditions the mouth is not affected by the unpleasant taste of bitter medicines.[27]

Table 2 presents the monomers used in pH-sensitive synthetic polymers and hydrogels. Monomers containing both weak acid or basic groups and strong acids or bases are used to create such systems. The pH-sensitive hydrogels are obtained by radical polymerisation in the presence of a cross-linking agent or by the chemical cross-linking of a water-soluble polymer with the aid of a bifunctional agent. By varying the composition of the monomers and the content of the cross-linking agent, it is possible to obtain hydrogels with various pH-sensitivities and degrees of swelling. The degree of swelling of a hydrogel, i.e. the response of the system to a change in the external conditions, depends mainly on the charge of the monomer, the pK of the ionogenic group, the degree of ionisation, the concentration of the ionic monomer in the polymer network, the degree of cross-linking of the hydrogel, and the pH and the ionic strength of the surrounding medium.[30]

Hydrogels containing acid groups swell in a weakly alkaline medium but collapse in an acid medium. On the other hand, hydrogels with basic groups swell in an acid medium but collapse on raising the pH. Polyampholytic hydrogels, obtained from comonomers one of which is soluble in an alkaline medium but insoluble in an acid medium (for example, methacrylic acid), while the other is actually soluble in an acid medium but insoluble in an alkaline medium [for example, 2-(*N*,*N*-dimethylamino)ethyl methacrylate], swell to the maximum extent at neutral pH values. When such gels are acidified or rendered alkaline, they undergo rapid dehydration.[34]

Table 1. Industrially manufactured polymers used in medicinal preparations.

Copolymer	Molecular mass	Ratio of carboxy- and ether groups	Trade name	Manufacturer	Country
Methacrylic acid–methyl methacrylate	135000	1:1	Eudragit L	Röhm Pharma GMBH	Germany
	135000	1:2	Eudragit S	Röhm Pharma GMBH	Germany
	–	–	MPM-05, MPM-06	Tanabe Seiyaku Co. Ltd	Japan
Carboxymethylethylcellulose	–	–	CMEC	Freund Sangyo Co. Ltd	Japan
Cellulose acetate-phthalate	–	–	CAP	Wako Pure Chemicals Ltd	Japan
Hydroxypropylmethylcellulose phthalate[28]	–	–	HP-50, HP-55	Shin-Etsu Chemical Co. Ltd	Japan
Hydroxypropylmethylcellulose acetate-succinate[28]	–	–	AS-M, AS-H	Shin-Etsu Chemical Co. Ltd	Japan
Diethylaminoethyl methacrylate–methyl methacrylate (or butyl methacrylate)	150000	–	Eudragit E	Röhm Pharma GMBH	Germany

Table 2. The monomers used in pH-sensitive 'smart' polymers and hydrogels.

Type of hydrogel	Monomer	pH-sensitive group	Ref.
Acid	Methacrylic acid	$-COOH$	27
	Acrylic acid		29
	Maleic acid		30
	N-Acryloyl-*p*-amino-benzoic acid		31
	Styrenesulfonate	$-SO_3Na$	32
	Sulfonyloxyethyl methacrylate	$-SO_3H$	32
Alkaline	Aminoethyl methacrylate	$-NH_2$	32
	N,*N*-Dimethylamino-ethyl methacrylate	$-NMe_2$	25
	N,*N*-Diethylamino-ethyl methacrylate	$-NEt_2$	33
	Vinylpyridine	$-NC_5H_4$	32
	Vinylbenzyltrimethyl-ammonium chloride	$-NMe_3^+Cl^-$	32

Physicochemical models of the swelling of hydrogels usually take into account the change in the free energy as a result of three factors: (1) the osmotic pressure of the mobile counterions within the gel (the Donnan theory); (2) the mixing of the polymer with the solvent (the Flory–Huggins theory); (3) the stretching of the macromolecules, increasing the free energy, which ultimately actually determines the limiting degree of swelling. Thus the increase in entropy as a result of the mixing of the polymer with an effective solvent increases the swelling of the gel. If the solvent is poor, then the entropy gain is inadequate and the gel does not swell. The extension of the macromolecules during the swelling of the polymer network is described by the elasticity theory, which postulates that the polymer network consists of cross-linked polymer chains in a free Gaussian conformation.[33]

The physicochemical models of the swelling describe satisfactorily only the swelling of weakly charged hydrogels with univalent counterions at high degrees of swelling.[16] For low degrees of swelling or for gels with a high charge density, the theory agrees poorly with experiment. Thus, for a high charge density in the gel, the activities of the ions are no longer equal to their concentrations. In a weakly swollen gel, the effective relative permittivity is lower than in the surrounding solution. This situation is energetically unfavourable for any ion and the latter tends to leave the gel. This tendency intensifies with increase in the hydration radius of the ion. For a low degree of swelling, an appreciable proportion of the water in the gel is in a state where it is bound to the polymer. The ability of such molecules to solvate counterions is much lower. In a highly charged gel at a high degree of swelling, it is no longer permissible to neglect the electrostatic interactions between the fixed charged groups and the mobile counterions, as is done in the Donnan theory. The electric potential in the gel ceases to be uniform, since the counterions tend to be localised in the vicinity of the charged groups. Furthermore, the Donnan theory does not take into account the specific ionic effects, for example, the cross-linking of the polymer chains by polyvalent counterions and complex formation.

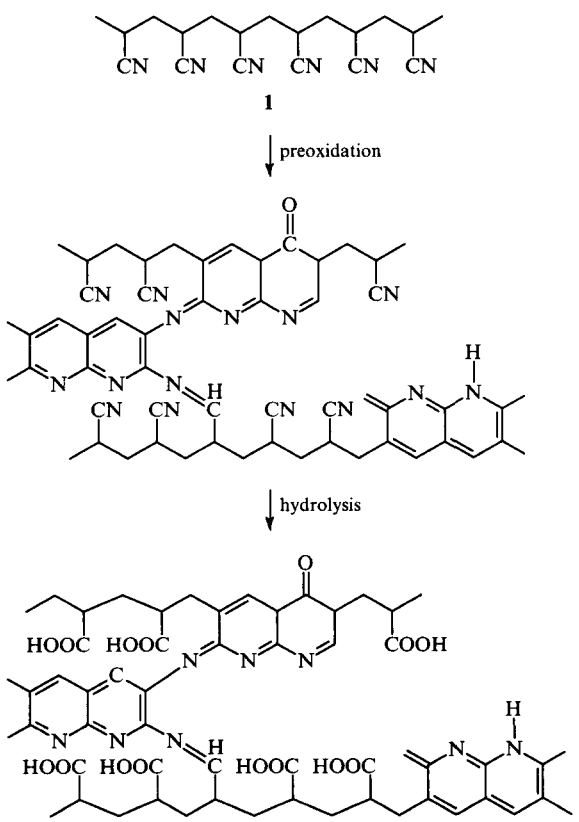
The factors listed above explain the absence of a satisfactory general theoretical model of the swelling of hydrogels.[33]

The change in pH near the electrode is used to create polymer systems responding to the electric potential.[35] Thus a column of a partially hydrolysed (20% of amide groups) polyacrylamide gel 3 cm long in 50% acetone shortens by a factor of 200 under the influence of a potential (> 1.15 V). In the electric field, the protons move to the cathode, while the negatively charged acrylic group of the polymer chain tends to move to the anode. A mechanical stress arises in the bulk of the gel, reaching a maximum in the vicinity of the anode and a minimum near the cathode, which under the conditions of a poor solvent leads ultimately to the collapse of the gel (in a purely aqueous solution, only a gradual decrease in the size of the gel without collapse takes place). The collapse of the gel is reversible and in the absence of an electric field the gel swells again. The response time in such a system is measured in hours. The response can be accelerated by decreasing the size of the gel and by increasing the

electric field strength. The 150–300 μm polyacrylate microparticles undergo a decrease in volume by 90% after 50 s in a 46 V electric field.[36] When the potential of a polypyrrole film is altered from −0.6 to 0.4 V, the pH in its vicinity changes from 4 to 10. The Gaussian polyacrylate gel microparticles on the surface of the film then dissolve in only 15 s. When the initial potential (−0.6 V) is restored, the microgel particles are reformed (in 10 s).[37]

A biodegradable gel, swelling reversibly when the pH is altered, has been obtained from the copolymer of *N,N*-dimethylacrylamide, *N*-*tert*-butylacrylamide, the *p*-nitrophenyl ester of *N*-methacryoylglycylglycine and acrylic acid cross-linked covalently with the aid of *N,N'*-(ω-aminocaproyl)-4,4'-diaminobenzene.[38]

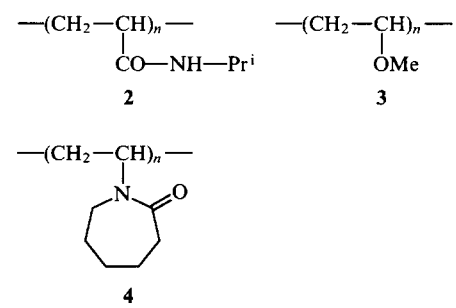
The critical phenomena in highly charged polyelectrolytes, occurring at extreme pH values, are of interest from the standpoint of the possibility of converting chemical energy into mechanical energy and of modelling biomechanical systems. Such systems are nowadays already capable of developing forces sufficient to lift by 0.5 cm a motor-car weighing 800 kg,[39] whilst a composite film consisting of the vinyl alcohol–acrylic acid and vinyl alcohol–acrylic acid–allylamine copolymers is capable of undergoing a change in size in response to a change in the solvent (the replacement of ethanol by 0.01 M NaOH) in only 40 s.[40] The artificial muscle model, created at the Tokyo Technological Institute, is even more striking.[41] Modification by partial oxidation and hydrolysis of the industrially manufactured polyacrylonitrile fibres **1** imparts to the latter the ability to swell strongly in an alkaline medium (1 M NaOH) and to contract in an acid medium (1 M HCl). The elongation reaches 80% in 2 s and the force developed under these conditions is 12 kgf cm^{-2}. For comparison, we may note that skeletal muscles are elongated by 30% in 10^{-2}–10^{-3} s, developing a force of 4–10 kgf cm^{-2}. Thus the effectiveness of artificial muscles almost approaches that of biological muscles.



The mechanical systems capable of converting various types of chemical energy into mechanical work have been examined in an extensive review by Osada,[39] whose coworkers carried out much successful work in this field.

2. Polymers sensitive to changes in temperature

Thermoresponsive polymers and hydrogels with a LCT below 40–45 °C are considered in this section. In their classical work,[42] Taylor and Ceranowski derived a rule according to which there exists a region where the polymer solution has a LCT. One of the most complete lists of polymers having a LCT in aqueous solutions is presented in the review by Galaev and Mattiasson.[43] Polymers with a wide variety of LCT have been synthesised.[44] The possibility of using some of them in biotechnology has been vigorously investigated at the present time. These are mainly poly-*N*-isopropylacrylamide **2** (poly-NIPAA) and other poly-*N*-alkylacrylamides as well as poly-(methyl vinyl ether) **3** (PMVE). Recently poly-*N*-vinylcaprolactam **4** (PVCL) and ethylene oxide–propylene oxide copolymers have attracted the attention of investigators.



The greatest number of publications have been devoted to NIPAA. The NIPAA homo- and copolymers are readily obtained by radical polymerisation in aqueous[45] or organic[46,47] solvents. Poly-NIPAA is soluble in acetone, chloroform, dioxane, tetrahydrofuran, lower alcohols, and cold water; it forms a separate phase at a temperature of about 32 °C, the LCT being almost independent of the molecular mass (*M*) of the polymer.[47] Poly-NIPAA cannot be fractionated into narrow molecular-mass fractions when an aqueous solution of the polymer separates into layers comprising different phases on heating. A carefully dried mixture of acetone and cyclohexane is used for the fractionation.[48]

Narrow molecular mass fractions of poly-*N,N*-diethylacrylamide with a very narrow distribution of molecular masses (polydispersity less than 1.2) have been obtained by polymerising the corresponding monomer with transfer of the group (live polymerisation) in dry tetrahydrofuran, the process being initiated by the methyl(trimethylsilyl)acetal of dimethylketene and catalysed by tetrabutylammonium acetate.[49] However, the molecular mass of the polymer synthesised is low and amounts to only 4700. The lower consolute temperature is 30 °C for the poly-*N,N*-diethylacrylamide synthesised by radical or live polymerisation and 40 °C for that synthesised by anionic polymerisation.[50,51]

The synthesis of copolymers of NIPAA with acryloyloxysuccinimide (used for the covalent immobilisation of proteins),[52] the modification of poly-NIPAA designed to obtain a porous glass[53] and polymer membranes,[44,54,55] and the preparation of block-copolymers of NIPAA with ethylene oxide,[56] the aqueous solutions of which do not separate into two phases on heating, in contrast to the poly-NIPAA homopolymer, but form a gel[57,58] have been described.

The properties of poly-NIPAA in aqueous solutions and the phase transition on heating have been investigated in detail by various methods: turbidimetric,[47,48] viscometric,[48,49] calorimetric,[48,60,61] light scattering,[60,61,63] fluorescence,[63–66] and EPR.[67] The influence of surfactants and of the inclusion of

hydrophobic or ionogenic comonomers on the LCT of poly-NIPAA has been investigated,[59,68-72] while the influence of the phase transition on the sorption of poly-NIPAA on aerosils with various hydrophobic properties has also been studied.[73]

The phase separation of aqueous solutions of poly-NIPAA on raising the temperature is caused by the conformational transition of the macromolecule from a loose globule to a compact coil. Such a transition is accompanied by a sharp decrease in the size of the macromolecule,[62] a decrease in the mobility of the fluorescent dansyl label bound covalently to the polymer,[64] and a fivefold increase in the nonradiative energy transfer between the pyrene and naphthalene labels bound covalently to the macromolecule.[65] The enthalpy of the transition is consistant with the loss of approximately one hydrogen bond per monomer unit.[45] The heat capacity of poly-NIPAA decreases with increase in temperature. The decrease in the heat capacity of poly-NIPAA reflects the collapse of the polymer chain on heating accompanied by a decrease in the number of contacts between the polymer molecule and water molecules similar to that occurring when a protein is renatured after being denatured by cooling.[61]

The formation of multimolecular micelles, consisting of a hydrophobic core surrounded by solvated poly-NIPAA chains, has been observed for the hydrophobically modified NIPAA copolymers at temperatures below the LCT.[63] The exchange of polymer molecules between the micelles is very slow.[66] At the LCT, the collapsed micelles aggregate and effective mixing of the copolymer molecules becomes possible.[63,66] The endothermic enthalpy of the phase separation of the ternary NIPAA – butyl methacrylate – X copolymers, where X is a hydrophilic, hydrophobic, cationic, or ionic comonomer, decreases linearly with increase in the LCT. Thus the hydrophobic interactions between the polymer side groups intensify with increase in temperature owing to the decrease in the structure formation in the water around these groups. It was concluded that the change in the LCT induced by the incorporation of other comonomers is determined by the overall change in the hydrophilicity of the copolymer and not by the direct influence of the hydrophilicity or charge of the comonomer on the water molecules in a structured arrangement around the hydrophobic groups.[71]

Since the hydrophobic interactions are the main driving force inducing the phase separation, the LCT of poly-NIPAA or of its copolymers may be regulated by influencing the effectiveness of these interactions. The introduction of hydrophobic and hydrophilic comonomers respectively decreases and increases the LCT of the polymer.[74] The LCT of poly-NIPAA diminishes with increase in the ionic strength of the solution and increases in the presence of ionic detergents forming stable complexes with the polymer.[57,68-70,75] Organic solvents miscible with water weaken the hydrophobic interactions of the side groups of poly-NIPAA and increase the LCT. However, in the presence of 40 – 60 vol% of certain solvents (methanol, tetrahydrofuran) the LCT of poly-NIPAA decreases to 0 °C and below.[76]

The gels of poly-NIPAA and other *N*-alkyl-substituted polyacrylamides are obtained by adding cross-linking agents (usually *N,N'*-methylenebisacrylamide) to the initial reaction mixture, by radiation-induced polymerisation,[77] or by cross-linking the copolymer with the aid of a bifunctional agent.[38] The introduction of ionogenic comonomers makes it possible to obtain hydrogels sensitive to changes in both temperature and pH.[78-80] Macroporous gels with high rates of swelling and dehydration have been synthesised at temperatures below the LCT of poly-NIPAA.[81] The poly-NIPAA gel in a granulated form can be obtained by emulsion or suspension polymerisation.[82-84] By employing different *N*-alkyl-substituted polyacrylamides, it is possible to synthesise thermoresponsive hydrogels (TH) with a LCT ranging from 5.5 °C for the poly-*N*-acryloylpiperidine gel to 72 °C for the poly-*N*-ethylacrylamide gel.[85]

The study of the phase transitions in hydrogels of poly-NIPAA[86-97] and other *N*-alkyl-substituted polyacrylamides[93,98-101] was initiated by Ilavsky et al.[98] and Tanaka and coworkers.[10] These investigations are continuing to develop vigorously also at the present time. The dependence of the time required for the phase transition on the size of the gel specimen makes it possible to describe the phase transition approximately as a collective diffusion process.[94] The formation of separate collapsed sections or of a thin film of the collapsed gel on the surface of the specimen plays an important role in this.

The influence of surfactants,[75,102,103] organic solvents,[76,101,104] salts,[105] and poly(ethylene glycols)[106] and of the inclusion of ionogenic comonomers[87,89,98,100,107] on the collapse and swelling of gels has been investigated. The nonradiative energy transfer[108] and mechanical stresses[109] arising on collapse of poly-NIPAA gels have been studied. It is of interest to note that sodium chloride at a concentration of ~1 M induces the collapse of the poly-NIPAA hydrogel at room temperature, whereas other salts exert a typical salting-out effect — the gel is gradually dehydrated with increase in the salt concentration.[105] The strong hydrophobic interaction of the isopropyl groups of poly-NIPAA with the alkyl group of sodium dodecyl sulfate results in the poly-NIPAA gel behaving in the presence of the detergent as a gel of the copolymer of NIPAA with the anionic monomer.[102,103]

A detailed theoretical discussion of the models of the phase transitions in poly-NIPAA gels may be found in recent reviews.[110,111]

Poly(methyl vinyl ether) **3** is obtained by the cationic polymerisation of methyl vinyl ether, this polymer being produced industrially.[85] The LCT for aqueous solutions of PMVE is 34 – 38 °C.[85,112] The PMVE gel is obtained by the γ-irradiation of polymer solutions.[85] Irradiation at temperatures above the LCT[113] or careful selection of the conditions (irradiation dose, polymer concentration)[114] make it possible to obtain gels with high rates of swelling and dehydration when the temperature is altered. The phase transitions in solutions[45,112,115] and in PMVE gels[85,86,113,116-118] have been studied much less than in poly-NIPAA.

As for poly-NIPAA, hydrophobic interactions apparently play the main role in the phase transition in PMVE.[112] The molar enthalpy of the phase transition is lower for PMVE than for poly-NIPAA,[86] but the phase transition in PMVE is also accompanied by the loss of one hydrogen bond per monomer unit,[118] as in poly-NIPAA.

Polyvinylcaprolactam **4** (PVCL) is obtained by the radical polymerisation of vinylcaprolactam in an organic solvent[120] or in bulk.[121,122] The thermodynamics of the phase transition in aqueous PVCL solutions have been investigated by Tager and coworkers.[123-126] The LCT for aqueous PVCL solutions depends on the molecular mass of the polymer and varies from 31 °C for the polymer with $M = 1\,250\,000$ to 40 °C for the polymer with $M = 15\,500$.[43] The Gibbs free energy, enthalpy, and entropy of mixing of PVCL with water are negative over the entire range of compositions. With increase in temperature, the mixing becomes less exothermic. It has been suggested that hydrophilic and hydrophobic hydration of the polymer takes place in aqueous PVCL solutions. In the ranges of temperatures and compositions close to the binodal curve, the hydrophobic hydration predominates, as a consequence of which the miscibility of the polymer with water diminishes and phase separation takes place.[123]

Cellulose derivatives, in particular hydroxypropylmethylcellulose and ethoxyethylcellulose, are also promising for use in biotechnology. They are distinguished by a high chemical stability and a satisfactory biological compatibility. The LCT for aqueous solutions of these polymers is 50 – 60 °C, but in the presence of salts and surfactants it diminishes to almost 0 °C.[127-129]

Among epoxyethane derivatives, nonionic detergents, especially Triton X-114 (*p-tert*-octylphenyl ether of polyoxyethylene with a degree of polymerisation of 7 – 8) have found extensive

application in biotechnology. The copolymers of ethylene and propylene oxides have also attracted the attention of investigators recently. Aqueous solutions of Triton X-114 have a LCT in the region of 18 °C. They are used to extract hydrophobic proteins into the polymer phase (the so called cloud point extraction). However, strictly speaking Triton X-114 ($M = 450 - 500$) and similar compounds are oligomers and not polymers, so that their study is outside the scope of the present review. The theoretical principles and practical aspects of the use of cloud point extraction of detergents have been examined in detail in a brilliant review by Hinze and Pramauro.[130]

The 50:50 ethylene oxide–propylene oxide copolymer with $M = 4000$ (manufactured by the Union Carbide Company under the trade name UCON 50-HB-5100) has an LCT of 55 °C. However, in the presence of a 0.2 M sodium sulfate solution the LCT diminishes to 40 °C.[131] A more hydrophobic copolymer containing 80 mol.% of propylene oxide ($M = 2000$) has a lower LCT (18 °C).[132]

The methacrylic acid–methacrylate copolymer described above (Eudragit S 100),[27] aqueous solutions of which separate into layers on acidification, forms a separate polymer phase in a neutral medium at 40 °C in the presence of 50 mM Ca^{2+}. The copolymer redissolves when the temperature is reduced.[133,134]

The first communication about reversible gel formation in an inorganic system has appeared recently. The aluminium polyphosphate hydrogel dissolves when the temperature is reduced and reforms at room temperature.[135]

The reversibly flocculating latexes are interesting systems. The minimum NaCl concentration inducing flocculation diminishes with increase in temperature and reaches a constant value at temperatures above the LCT. At a certain NaCl concentration, the latex begins to flocculate after the attainment of a certain temperature, but it can be redispersed on cooling. The formation of such latexes based on poly-NIPAA[136–140] and its copolymers,[77,84,141,142] *N*-acryloylpyrrolidine–*N*-acryloylpiperidine copolymers,[143,144] methacrylic acid esters, and ethylene oxide oligomers with degrees of polymerisation of 1,2,4–5, and 7–8 has been described.[145]

3. Polymeric complexes

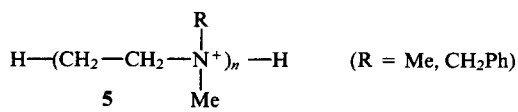
Polymeric complexes, i.e. complexes formed by two different polymers or a polymer and a low-molecular-mass compound, have recently attracted increasing attention by investigators.[146]

The formation of a polyelectrolyte complex is described in a general form by the following scheme:[147]

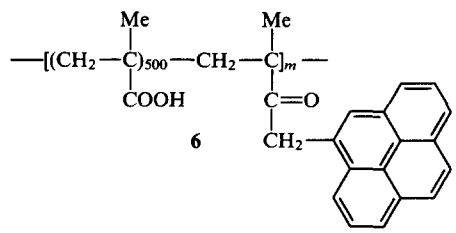
$$(A^-k^+)_n + (K^+a^-)_m \rightleftharpoons \rightleftharpoons [(A^-K^+)_x(A^-k^+)_{n-x}(K^+a^-)_{m-x}] + x\,a^- + x\,K^+ ,$$

where A^- and K^+ are the anionic and cationic groups in the polymer and a^- and k^+ are their low-molecular-mass counterions. The degree of conversion Θ is defined as the ratio of the number of interionic bonds formed to its maximum possible value ($\Theta = x/m$ for $n \geqslant m$ or $\Theta = x/n$ for $m \geqslant n$). The solubility of polymeric complexes in water depends on the fraction of the charged ionic group z and on the relative length of the oppositely charged polyions and also on the pH and the ionic strength of the solution. The polymeric complexes formed by polymers with $z = 1$ have minimum solubility at $\Theta \to 1$. Nonstoichiometric polyelectrolyte complexes in which one of the polyions predominates can be represented as an unusual block copolymer consisting of a charged hydrophilic block containing one polymer chain and a neutral hydrophobic block containing two polymer chains.

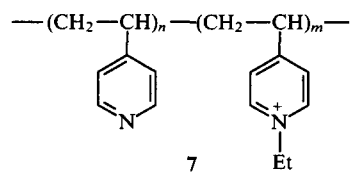
The cooperative nature of the interaction between the polyions makes such polymeric complexes exceptionally stable even for fairly low degrees of polymerisation of the polyions. For example, the dissociation constant of the polymeric complexes of polymethacrylic acid with quaternised oligoethyleneimines **5** becomes negligibly small even for the octamers.[147]



5



6



7

Despite the high stability of the polymeric complexes, they are capable of exchanging the polyions. The nature and length of the polyion are of fundamental importance in exchange reactions.[148] The polymethacrylate complex **6** (PMA) (degree of polymerisation 4100), containing a fluorescent label (1 label for each 500 monomer units), with partially quaternised poly-4-vinylpyridine **7** (P-4-VP) (degre of polymerisation 170) is capable of exchange with polyphosphates (PP) with different degrees of polymerisation (from 20 to 230):

$$\text{P-4-VP.PMA} + \text{PP} \rightleftharpoons \text{P-4-VP.PP} + \text{PMA} .$$

The equilibrium in this reaction in 0.3 M NaCl is fully displaced to the right for the long polyphosphates and to the left for the shortest polyphosphate. For a fixed degree of polymerisation of the polyphosphates ($n = 70$), the reaction equilibrium is fully displaced to the right in 0.3 M LiCl and to the left in 0.3 M KCl, the equilibrium remaining unchanged in 0.3 M NaCl. Such phenomena have no analogues in nonpolymeric systems and can serve as useful models for the regulation of processes in the living cell with the aid of biopolymers.[148]

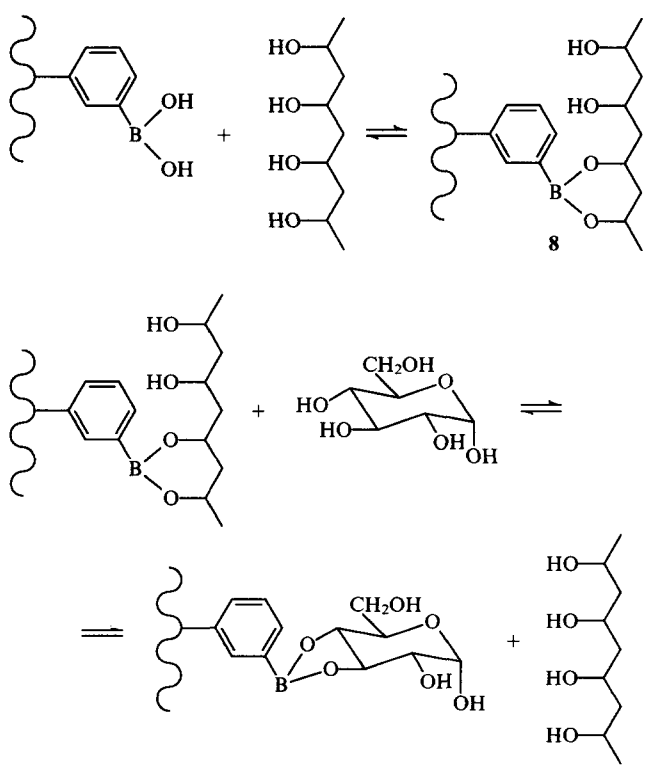
Systems capable of undergoing marked but reversible changes in their properties and of responding to slight changes in the medium may be included among 'smart' polymeric complexes. Nonstoichiometric polyelectrolyte complexes (NPC), formed in the interaction of a polycation (for example, poly-*N*-ethyl-4-vinylpyridinium bromide) and an excess of a polyanion (for example that of polymethacrylic acid) are systems promising for biotechnology.[149–151] The ability of such complexes to be precipitated quantitatively from solution when the pH of the latter is altered by only 0.3 of a unit is an attractive feature.[149]

Another interesting group comprises complexes between uncharged polymers, which are obtained as a result of numerous weak interactions, for example via hydrogen bonding between polyacrylic acid and polyacrylamide.[9] The strength of the hydrogen bonds increases with increase in temperature. Thus the gel formed by interpenetrating polyacrylic acid and polyacrylamide polymer networks collapses on cooling to ~20 °C and swells again on heating.[9]

Kabanov and coworkers[152,153] observed complex formation between polymethacrylic acid and polyoxyethylene (POE). Complexes between high-molecular-mass polymethacrylate (or polyacrylate) and POE are formed in this case when the molecular mass of the latter exceeds a certain critical value, which is 2000 for the complex with poly(methyl methacrylate) and 6000 for that with polyacrylate. The complex between POE and polymethacrylate is stabilised by hydrophobic interactions, its stability increasing with increase in temperature, while that of the complex between POE and polyacrylate depends little on temperature.

The important role of hydrophobic interactions (in which the α-methyl group of the polymethacrylate participates) in the stabilisation of the complex between POE and polymethacrylate has been observed also in the study of the influence of the nature of the solvent. Thus the critical molecular mass of POE, necessary for the formation of its complex with polymethacrylate, increases sharply on passing to more hydrophobic water–methanol mixtures, whereas for the polyacrylate complex it remains virtually constant.[154] The marked reversible temperature dependence of the complex formation reaction between polymethacrylate and POE in the range 25–45 °C renders such systems interesting for biotechnological use.[39,155,156]

Boric acid affords stable complexes with polyol compounds. The complex between poly(vinyl alcohol) and the *N*-vinylpyrrolidinone–*N*-acryloylamidophenylboronic acid copolymer is formed as a result of the interaction of boric acid with the hydroxy-groups of the polyol, but in the presence of an excess of another polyol (for example, glucose) the complex decomposes. The removal of glucose (for example, by dialysis) again leads to complex **8**,[157,158] so that the complex formation reaction depends reversibly on the presence of glucose, i.e. the given system can be used as a detector for the presence or absence of glucose.



Among the complexes between polymers and low-molecular-mass compounds investigated, mention should be made of the complex formed by the poly-2-acrylamido-2-methylpropanesulfonic acid with n-dodecylpyridinium chloride. This system responds to changes in the electric potential by a change in the form of the gel.[159]

III. Applications of 'smart' polymers

1. Concentration of protein solutions and dehydration of suspensions

When hydrogels collapse, their volume contracts by a factor of 1000.[86] Their ability to absorb up to several grams of water per gram of the gel and then to give up the absorbed water after a slight change in conditions is used in the dehydration of suspensions and in the concentration of protein solutions. Such processes are based on the cycle presented in Fig. 1.[160]

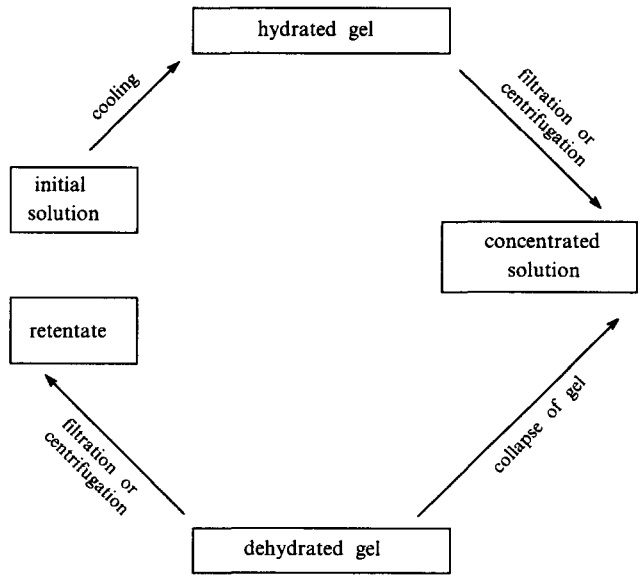


Figure 1. The removal of water from suspensions and concentration of protein solutions.[160]

The gel dehydrated beforehand swells in the initial solution, 'taking up' water. Low-molecular-mass substances, capable of permeating into the gel pores, diffuse within the gel. On the other hand, high-molecular-mass substances, which are incapable of permeating into the pores, remain in solution in a concentrated form. The swollen gel is separated from the solution and regenerated by inducing its collapse (it is heated above the critical temperature or the pH and the ionic strength of the solution are altered). The gel collapses and gives up the previously absorbed water together with the low-molecular-mass substances dissolved in it. After separation by filtration or centrifugation, the dehydrated gel is again ready to concentrate a fresh portion of the initial solution. By regulating the composition and degree of cross-linking of the gel, it is possible to alter its selectivity with respect to the molecular mass and charge of the sorbed solutes.[161–164] When collapsed gels are used for concentration and dehydration (swellex process or gel dewatering process), it is possible to combine the high productivity of liquid extraction with the selectivity of membrane methods.[162]

The idea of concentrating protein solutions by adsorbing water on solid particles had already appeared in the 1960s.[165] However, attention was concentrated only on the stage involving adsorption without the regeneration of the gel, i.e. on the left-hand part of the cycle presented in Fig. 1. Only after the appearance of 'smart' hydrogels has it become possible to carry out economically the entire cycle.

Solutions of casein and bovine serum albumin have been concentrated by a factor of 4.6–4.8 with the aid of a sulfonated polystyrene gel cross-linked by divinylbenzene (the gel was regenerated by means of a concentrated salt solution). The yield of the protein was then 95%–99%.[162]

The appearance of thermoresponsive hydrogels (TH), which collapse on heating, made this type of process even more attractive. The thermoresponsive NIPAA hydrogel has been used to concentrate cellulases from the *Trichoderma reesei* culture liquid.[162]

Cussler and coworkers[166] developed an industrial process for the preparation of soya protein by dehydrating the mother liquor with the aid of poly-NIPAA-TH (Fig. 2).

After the extraction of soya oil, the soya beans are ground up and the proteins are extracted with water at pH 8.5. The mother liquor is separated by centrifugation. In the traditional technology, the proteins are precipitated with acid. The most

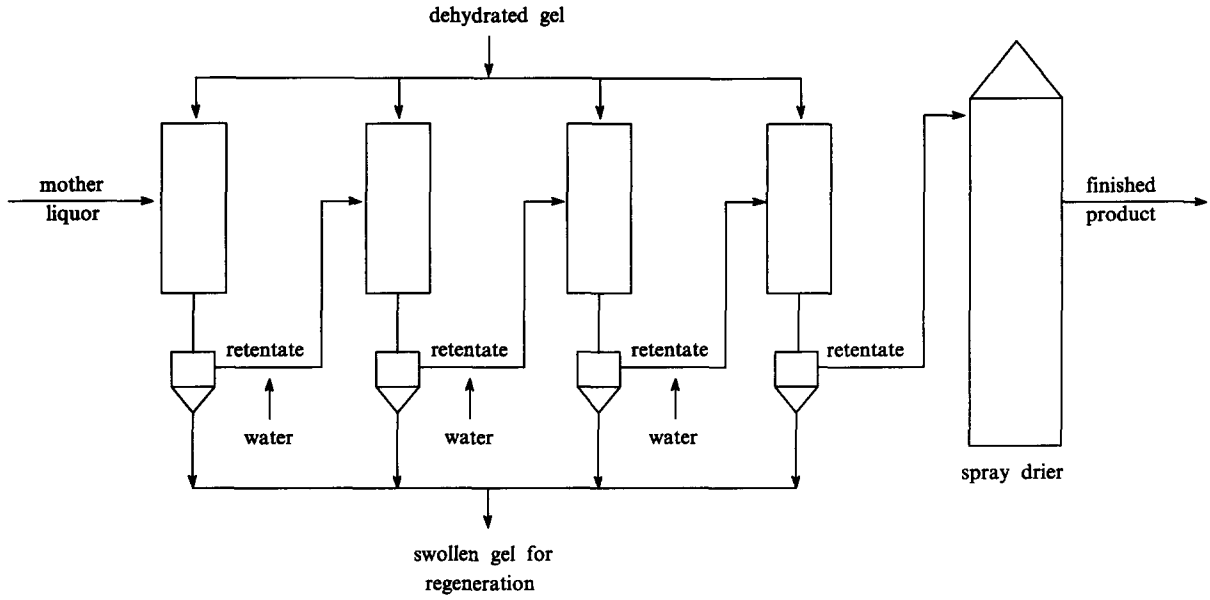


Figure 2. Industrial preparation of soya protein.[160]

valuable among them (the albumin-like proteins) are not precipitated under these conditions and are lost, while the toxic phytins are precipitated. The protein precipitate is dissolved at pH ~7 and dried on a spray dryer. From 100 kg of the initial raw material, 33 kg of the product is obtained.

In the proposed technology, the mother liquor is treated at 5 °C with the collapsed poly-NIPAA-TH, after which the gel swells, adsorbing 40% of water. The swollen gel is separated by centrifugation and the retentate is diluted with water and again treated with the collapsed gel (this procedure is repeated 2–4 times), which makes it possible to separate the protein from low-molecular-mass impurities. After this, the retentate, containing 15% of dry substances, is transferred to the spray dryer. From 100 kg of the initial raw material, one obtains 45 kg of the product. The yield with respect to the protein exceeds 80%.[166]

The new process is not free of disadvantages, one of which is the adsorption of the protein on the surface of the gel particles. However, it does have significant advantages: a high yield of the product as a result of the retention of albumin-like proteins, the absence of the precipitation stage with acidification, which makes it possible to retain a greater amount of the proteins in the native form, and better consumption qualities of the product because of the absence of toxic phytins and a higher protein content. Economic analysis, carried out on industrial apparatus, showed that the capital expenditure on the manufacture of the protein is recovered in 3.2 years.[160]

The PMVE-based thermoresponsive hydrogel is used to remove water from suspensions, in particular the culture liquids of microorganisms.[167,168] This type of process makes it possible to overcome a number of the disadvantages of the traditional filtration. Thus the filtration area may be readily increased by increasing the number of TH particles; the 'pie' comprising the microorganisms, formed on the surface of the particles, is continually disrupted as a result of the increase in the geometrical size of the TH particles on swelling; the path traversed by water from the bulk of the suspension to the filtering surface is greatly shortened.

Engineering calculations have shown that it is necessary to have 7–8 kg of dry PMVE-TH for the preparation of 1 kg of dry substances. In order to compete with the most productive strip filters, the TH particles must withstand at least 2000 cycles, so that an increase in the mechanical strength of the PMVE-TH particles is the primary condition for the successful introduction of the TH into practice.[168]

2. Membranes with a controllable permeability

The ability of 'smart' polymers to undergo a distinct transformation from an uncoiled globule into a compact coil has been used to create membranes with a controllable permeability. The principle of the operation of such a membrane is illustrated in Fig. 3. In the uncoiled conformation, the polymer molecules immobilised on the surface do not prevent the permeation of the solutes into the pores. In the compact conformation, the polymer molecules block or strongly hinder the entry of the solutes into the pores.

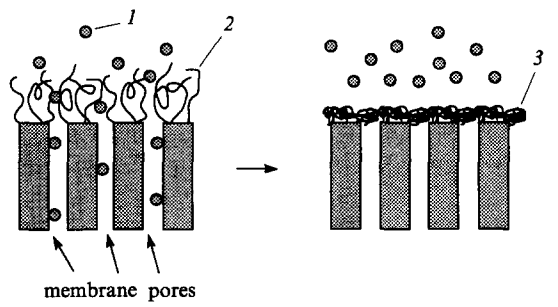


Figure 3. Controllable permeability of a membrane as a function of the conformation of the polymer. (*1*) Solute; (*2*) polymer in the uncoiled conformation; (*3*) polymer in the compact conformation.

Permeability controlled by varying the pH has been achieved when poly-4-vinylpyridine or polyacrylic or polymethacrylic acid were immobilised on the surfaces of the membranes of nylon capsules. In the first case, the permeability increases with decrease in the pH, while in the second it increases with increase in the pH.[169] When the pH is reduced from 6 to 3, the permeability of the polymethacrylate membrane to fluorescence-labelled dextran (M = 10 000) changes by a factor of 85.[170]

The regulation of permeability by varying the temperature has been achieved in the immobilisation of poly-NIPAA or other poly-*N*-alkylacrylamides on the pore surface.[44,54,55,171] The change in the effective pore size due to the transition of the immobilised NIPAA molecules from the uncoiled conformation to the compact conformation makes it possible to regulate gel-permeation chromatography by varying the elution temperature.[53]

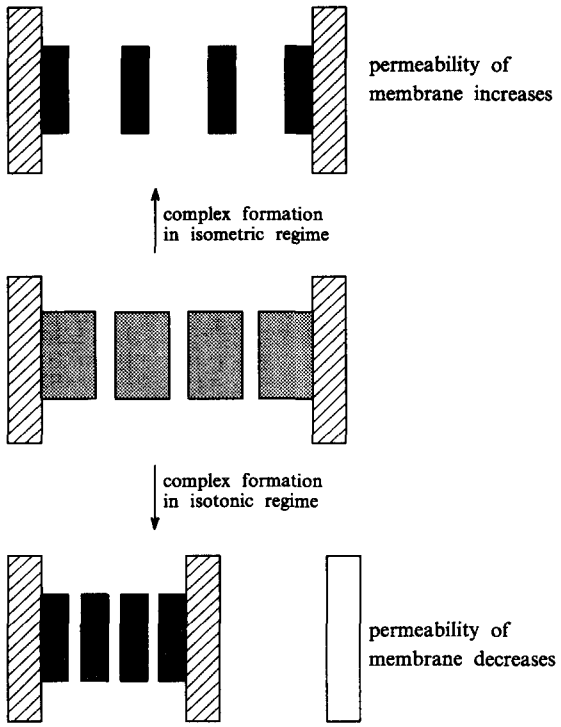


Figure 4. Behaviour of a poly(methyl methacrylate) membrane during the formation of a complex with polyoxyethylene in isometric and isotonic regimes.[39]

If the membrane consists wholly of a polymeric hydrogel, its permeability changes greatly as the degree of swelling of the gel is altered. The permeability of a membrane made from a copolymer comprising 4-acryloylpiperidine (LCT = 5.5 °C), 2-hydroxyethyl methacrylate, and styrene to insulin varies sharply with temperature. Depending on the composition of the copolymer, the permeability either increases or decreases with increase in temperature.[172] A change in the pH or in the electric field alters reversibly the permeability of a poly(methyl methacrylate) membrane by a factor of 50.[173] The complex formation reaction between polyoxyethylene and a poly(methyl methacrylate) membrane can be used both to increase the permeability of the membrane, which has fixed geometrical dimensions (isometric regime), and to decrease the permeability of a freely mounted membrane (isotonic regime) (Fig. 4). In both cases washing of the membrane with a weakly alkaline solution leads to the dissociation of the complex and the restoration of the initial permeability.[39,156]

The acrylamide–acrylic acid copolymer hydrogel, immobilised on porous glass, exists under isometric conditions. Such a membrane allows the passage of aqueous solutions of polyoxyethylene only with $M < 4000$. The collapse of the hydrogel in 50% acetone leads to a sharp increase in the permeability of the membrane: it is now capable of allowing the passage of polyoxyethylene with $M = 80\,000$.[111,174,175]

Thus membranes based on 'smart' polymers can be used to create various systems with feedback.

3. Isolation and purification of biomolecules

The ability of 'smart' polymers in aqueous solutions to form a separate phase after a slight change in conditions has been used to isolate and purify proteins. A general scheme for this process, which has been called affinity precipitation, is illustrated in Fig. 5 (see over).

The conjugate of a 'smart' polymer with a ligand which has an affinity for the given target protein and forms a complex with the latter is added to the initial mixture containing the protein. When the conditions are altered (change in the pH, temperature, and ionic strength, addition of specific complex-forming agents), the polymeric conjugate together with the target protein is segregated as a separate polymeric phase, while the impurities remain in the solution. The polymeric phase is separated from the supernatant. Next the target protein is either eluted from the polymeric phase or the latter is redissolved, altering the conditions in such a way that the complex dissociates, while the polymeric conjugate is reprecipitated but this time without the target protein, which remains in the solution.

This type of approach was first used by Schneider et al.[31] to isolate trypsin. The extract from the pancreas was treated with an acrylamide–*N*-acryloyl-*p*-aminobenzoic acid copolymer containing *m*-aminobenzamidine ligands with a high affinity for trypsin. The trypsin–copolymer complex was precipitated by acidifying the solution to pH 4. The precipitate was separated, washed, and redissolved at pH 8. The polymer was then precipitated but this time at pH 2 (the trypsin–polymer complex is known not to form in an acid medium), whereupon trypsin remained in the solution. After lyophilic drying, the solution contained 90% of trypsin. The copolymer was regenerated (to the extent of 93%) and reused.[31]

Apart from precipitation, reversible flocculation may be used for the purification of biomolecules. The particles of the latex of a copolymer comprising styrene, NIPAA, glycidyl methacrylate, and methacrylic acid are used for this purpose. Thus the antibodies to bovine serum albumin have been isolated from the serum with the aid of the latex containing immobilised bovine serum albumin on its surface.[142]

Examples of the successful employment of the affinity precipitation methods for the isolation and purification of various biomolecules are compiled in Table 3. Mattiasson and coworkers have made a contribution to the development of these methods.[176,179,185–190]

The traditional methods for the purification of proteins by precipitation with ammonium sulfate, polyoxyethylene, or organic solvents are distinguished by simplicity and a high productivity. Their disadvantage is a low specificity. Affinity precipitation makes it possible to combine the simplicity and high productivity of the protein precipitation processes with the high specificity of the affinity chromatographic method. In principle, the same ligands can be used for affinity precipitation as for affinity chromatography. The difference is that affinity precipitation is carried out in a batch regime, which rules out the possibility of the repeated interaction of the protein with the ligand, as in chromatography. Therefore ligands with a high affinity for the protein isolated are preferable for affinity precipitation, which restricts somewhat the choice of the systems employed.[189] Both synthetic and natural polymers are used for affinity precipitation.

The affinity of the target protein for the polymer (for example, the affinity of endo-polygalacturonase for alginate,[187] of agglutinin for chitosan,[176] and of D-lactate dehydrogenase from *Lactobacillus bulgaricus*[186] or xylanase[185] for the methacrylic acid–methyl methacrylate copolymer), is an interesting case, which makes it possible to avoid the complex procedure involving the preparation of the polymer containing the specific ligand.

It is noteworthy that the choice of the polymer is very important for the successful employment of affinity precipitation. The polymeric phase formed should not be too hydrophobic in order not to create an undesirable environment for the protein molecules being isolated.[190] On the other hand, an unduly hydrophilic polymeric phase is difficult to separate from the supernatant, which leads to an appreciable nonspecific trapping of extraneous proteins, i.e. to a decrease in the degree of purification and in the yield of the target protein.[179] The most successful results have been achieved by employing the methacrylic acid–methyl methacrylate copolymer

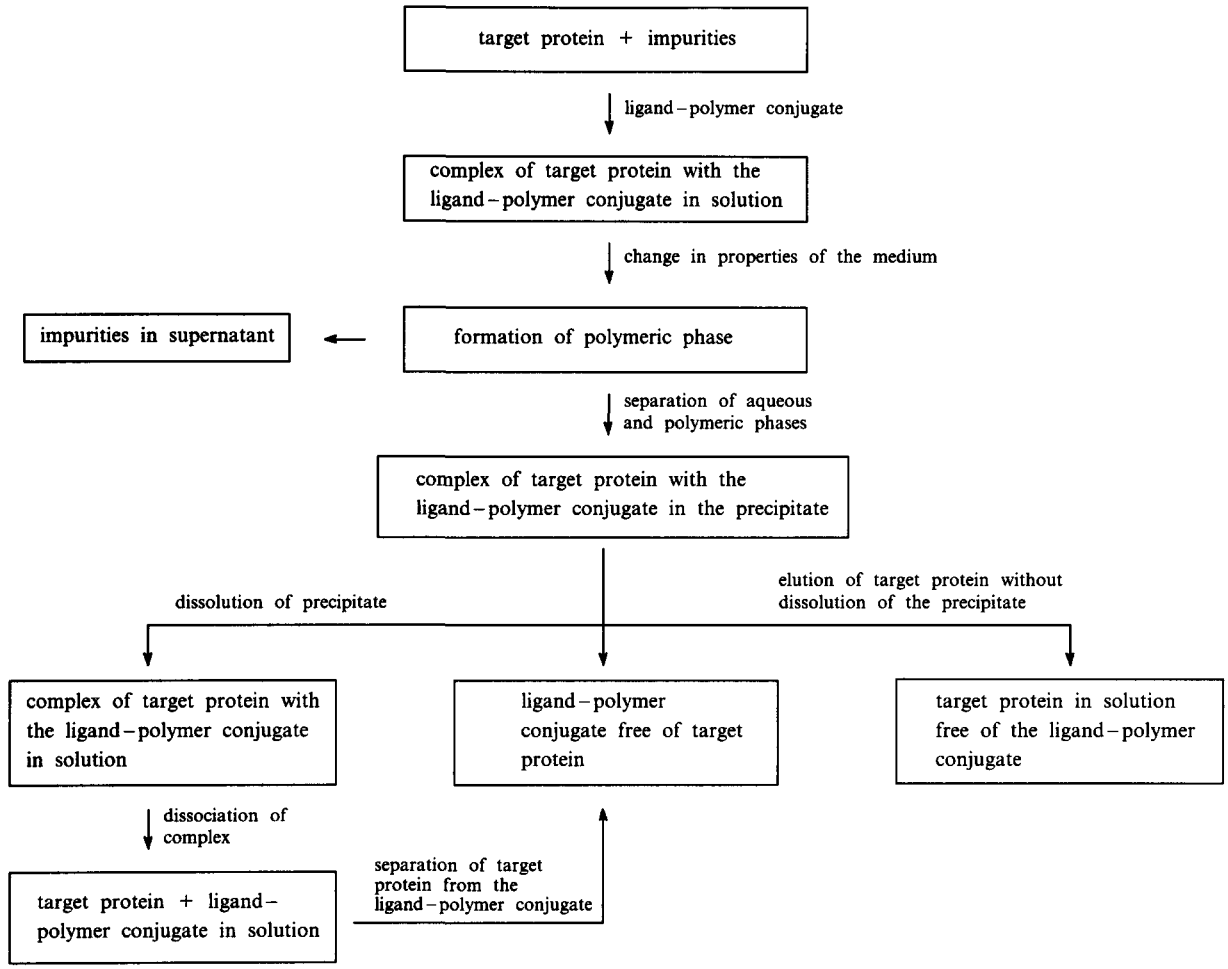


Figure 5. General scheme employed in affinity precipitation methods.

(Eudragit S 100, Table 3). The optimum selection of the conditions makes it possible to attain a tenfold degree of purification of the protein combined with 70%–80% yields.[133,134]

Aqueous solutions of polyoxyethylene and dextran form two immiscible polymeric phases — hydrophilic and hydrophobic — containing more than 80%–95% of water. The hydrophilic and hydrophobic phases consist of dextran and polyoxyethylene respectively. The different distributions of the substances between these two phases are widely used to isolate and purify many biomolecules.[191] One of the problems in this approach is the need to separate the target product from the phase-forming polymer after the mechanical separation of the phases. A convenient way of solving this problem has been proposed by Tjerneld and coworkers.[131,132,192,193]

The principle of the proposed method is illustrated in Fig. 6. A solution of dextran or hydroxyethyl-substituted starch was used as the hydrophilic phase, while the hydrophobic phase consisted of ethylene oxide–propylene oxide copolymers, the aqueous solutions of which separate into aqueous and polymeric phases on heating. When clarified baker's yeast homogenate is added to the two-phase polymer system, enzymes with affinity for the hydrophobic phase (glucose 6-phosphate dehydrogenase, 3-phosphoglycerate kinase, and hexokinase) are extracted into this phase. The extraction of the target product into the hydrophobic phase is facilitated by the addition to the system of the conjugate of the hydrophobic monomer with a ligand having affinity for the target protein (for glucose 6-phosphate dehydrogenase, such a ligand is the dye Procion Yellow).[193] After the mechanical phase separation in the centrifuge, the hydrophobic phase is heated to 24 °C, whereupon the copolymer is segregated in a separate concentrated polymeric phase, whilst the purified enzymes remain in the aqueous phase. The degree of purification and the yield were 5.3% and 50% for glucose 6-phosphate dehydrogenase, 15.3% and 67% for hexokinase, and 15.4% and 72% for 3-phosphoglycerate kinase respectively.[132] The results are entirely comparable with the results of the chromatographic purification of the proteins by traditional methods.

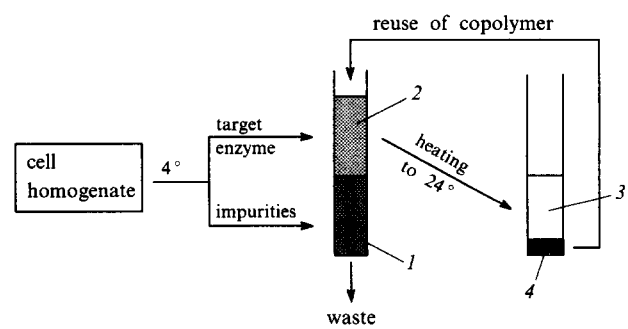


Figure 6. Purification of enzymes in a two-phase system with separation from the phase-forming polymer at a temperature above the LCT.[132] (*1*) Hydrophilic phase — dextran or hydroxypropylstarch; (*2*) hydrophobic phase — copolymer at a temperature below the LCT; (*3*) aqueous phase containing the purified enzyme; (*4*) concentrated polymeric phase — copolymer at a temperature above the LCT.

Table 3. Conditions necessary for the formation of the polymeric phase.

Target product	Ligand	Polymer	Method of formation of polymeric phase	Ref.
Wheatgerm agglutinin	[a]	chitosan	change in pH	176
Trypsin	trypsin inhibitor from soya beans	"	"	177
	p-aminobenzamidine	NIPAA – *N*-acryloyloxy-succinimide copolymer	change in temperature	178
	trypsin inhibitor from soya beans	alginate	addition of Ca^{2+}	179
	"	PVCL	change in temperature	180
Protein A	IgG	hydroxypropylmethylcellulose acetate-succinate	change in pH	181
Human IgG	protein A	galactomannan	addition of tetraborate	182
Alkaline protease	*p*-aminobenzamidine	NIPAA	change in temperature	183
β-Glucosidase	[a]	chitosan	change in pH	184
Hind III restriction endonuclease	λ-phage DNA	NIPAA	change in temperature	183
Xylanase from *Trichoderma viridie*	[a]	Eudragit S 100	change in pH	185
D-Lactate dehydrogenase from *Lactobacillus bulgaricus*	[a]	"	"	186
Endopolygalacturonase from *Aspergillus niger*	[a]	"	"	187
D-lactate dehydrogenase from *Leuconostock mesenteroides ssp. cremoris*	Cibacron Blue	"	"	188
Lactate dehydrogenase, pyruvate kinase from pig muscle	"	"	change in temperature	133
Alcohol dehydrogenase from baker's yeast	"	"	"	134

[a] The target protein exhibits an affinity for the polymer itself.

Another way of separating the target protein from the phase-forming polymer involves the combination of extraction in a two-phase polymer system with affinity precipitation.[194,195] A conjugate of the Eudragit S 100 copolymer with an affine ligand [human immunoglobulin (IgG) (in the purification of recombinant protein A)[194] or Cibacron Blue (in the purification of lactate dehydrogenase)[195]] is added to the polyoxyethylene – hydroxypropylstarch two-phase polymer system. By virtue of the hydrophobic properties of the polymer, the complex of the target protein with the conjugate is extracted into the hydrophobic phase, which is then separated by centrifugation and the target protein – conjugate complex precipitated by acidification. As a result, the recombinant protein A is purified by a factor of 26 (yield 80%),[194] whilst lactate dehydrogenase is purified by a factor of 12 (yield 50%).[195]

An interesting example of the use of the PVCL-based 'smart' polymer has been demonstrated by Galaev et al.[196] Polyvinylcaprolactam forms stable complexes with dyes, including Cibacron Blue, and is sorbed effectively in a chromatographic carrier containing similar ligands (for example Sepharose Blue). At temperatures above the LCT, the polymer exists in the compact conformation, where each polymer molecule can

interact only with a few ligand molecules, leaving the rest accessible for interaction with enzyme molecules. When the temperature is reduced, the PVCL passes to the uncoiled more flexible conformation. Segments of the polymer molecules acquire the ability to interact with a larger number of dye molecules and begin to compete with the sorbed enzyme for the ligand, ultimately displacing it.

Such a system is the first example of the successful purification of an enzyme by elution solely as a result of changing the temperature without any alteration of the composition of the buffer. The column with Sepharose Blue was treated with a solution of PVCL at room temperature and was washed until traces of the polymer were absent from the eluate. At 45 °C, the unpurified pig muscle extract was deposited on this column and was washed in a buffer until the complete absence of the protein from the eluate, after which the column was cooled to room temperature and the homogeneous lactate dehydrogenase was eluted with the same buffer. A 17-fold purification with a yield of 90% was achieved.[196]

The examples described demonstrate the extensive possibilities of the use of 'smart' polymers for increasing the efficiency of traditional methods for the purification of proteins and for the creation of new ones.

4. Immobilisation of biocatalysts

The ability of polymers to form a separate phase in aqueous solutions following a slight change in external conditions can be used to create reversibly soluble biocatalysts, provided that the enzyme molecule is bound covalently to the polymer. The reversibly soluble biocatalyst acquires the advantages of immobilised enzymes (ease of separation from reaction mixture and the possibility of multiple employment) but at the same time it makes it possible to overcome the disadvantages of immobilised and solid matrix enzymes such as diffusional difficulties in the transport of substrates and products (particularly high-molecular mass ones) and the impossibility of using the enzymes in reactions with insoluble substrates or products.

Catalysts which are reversibly soluble as a function of pH have been obtained by the covalent binding of lysozyme to alginate,[197] of trypsin to the acrolein–acrylic acid copolymer,[29] and of cellulase,[198] amylase,[26] α-chymotrypsin, and papain[119] to methacrylic acid–methyl methacrylate copolymers, carboxymethylcellulose, hydroxypropylmethylcellulose phthalate, and cellulose acetate-phthalate. A reversibly soluble cofactor has been obtained by the covalent binding of NAD to alginate with the aid of diepoxyoctane.[199]

Margolin and coworkers[149,151] studied in detail the behaviour of α-chymotrypsin, penicillinamidase, alcohol dehydrogenase, and urease bound covalently to the polyanion or polycation in the nonstoichiometric polymeric complex of polymethacrylic acid and modified poly(*N*-ethyl-4-vinylpyridinium) bromide. The enzymes in the polymeric complexes are readily precipitated from solution on acidification from pH 6.1 to pH 5.7 and are separated by centrifugation. The polymeric precipitate is redissolved in a buffer solution at pH 6.1 and the immobilised enzyme can be used repeatedly. In the precipitate, the α-chymotrypsin bound covalently to the polyanion is entirely accessible to a high-molecular-mass inhibitor, in contrast to the chymotrypsin bound to the polycation, which is accessible to the extent of only 20%. The enzyme bound to the polycation is present within the hydrophobic core of the complex; it is effectively protected by this environment, which increases its thermal stability.[149]

Enzymes which are reversibly soluble as a function of temperature have been obtained by the covalent binding of α-chymotrypsin and penicillinamidase to PVCL,[200] of trypsin,[74] alkaline phosphatase,[178] and thermolysin[201] to poly-NIPAA, and of lipase and wheat germ to the graft-copolymer comprising NIPAA and the acrylamide–acrylic acid copolymer.[202] The reversible flocculation of latexes has been used to obtain reversibly soluble (more precisely reversibly dispersable) biocatalysts also in affinity precipitation. Trypsin[203] and papain[141] preparations were obtained in this way.

Enzymes which are reversibly soluble as a function of Ca^{2+} concentration are formed on covalent binding of phosphoglyceromutase, enolase, peroxidase, and pyruvate kinase to α_{s1}-casein. The enzyme–casein conjugate is soluble at a Ca^{2+} concentration below 20 mM, but is fully precipitated at a Ca^{2+} concentration in excess of 50 mM. When the Ca^{2+}-binding EDTA is added, the precipitate redissolves.[204]

The use of reversibly soluble biocatalysts in reactions which are difficult or impossible to carry out with the aid of enzymes immobilised on insoluble matrices, for example in the hydrolysis of high-molecular-mass substrates (casein,[141,201] dextrin, and starch[26]), in the hydrolysis of insoluble substrates [cellulose[198] and unpurified starch (corn flour)[26,205–208]], and in the preparation of the insoluble benzyl ester of benzyloxycarbonyl-L-tyrosyl-N^{ω}-nitro-L-arginine[119] and of insoluble phenylglycine[209] is of practical interest.

In the hydrolytic cleavage of corn flour to glucose on treatment with amylase, the reaction product (glucose) inhibits the hydrolysis. The use of a reversibly soluble amylase conjugate increases the efficiency of the hydrolysis. The hydrolysis was carried out at pH 5, at which the amylase conjugate is soluble. After each 24 h, the pH was reduced to 3.5, the unhydrolysed solid residue and the precipitated conjugate were separated by centrifugation, the conjugate was redissolved in a fresh portion of the substrate at pH 5, and the hydrolysis was continued. After five repetitions of the cycle, 101 g of glucose was obtained from 150 g (30 × 5) of the unpurified starch. The activity of the amylase after the fifth cycle ws 96% of the initial value.[205]

The cyclic conversion of the unpurified starch to sugar has been used for the preparation of ethanol by fermentation.[205,207,208] The fermentation by yeast in the presence of a biocatalyst was carried out at pH 5 in a reactor with a conical bottom. The fermentation liquid was periodically acidified to pH 4, whereupon the yeast cells flocculated and the biocatalyst precipitated. The precipitate with the unhydrolysed substrate was separated by allowing it to stand for 10 min, after which it was treated with a fresh portion of the substrate. Ethanol was obtained from the supernatant (80% of the initial volume). After five cycles, 61 g of ethanol was obtained from 150 g of the unpurified starch; the productivity was 0.85 g litre^{-1} h^{-1}.[205,207]

Reversibly soluble amylase has been used also to convert (under continuous fermentation conditions) unpurified starch in ethanol[208] or into lactic acid.[206] In the preparation of ethanol, the fermentation, the separation of the cells and the biocatalyst, and their resuspension were carried out in three separate reactors. The continuous fermentation regime made it possible to increase the productivity to 2 g litre^{-1} h^{-1}.[208] A bioreactor with cells of *Lactobacillus casei* suspended in a κ-carrageenan gel was used in the preparation of lactic acid. The amylase conjugate was separated and resuspended in two other reactors. A productivity of 3.1 g litre^{-1} h^{-1} was attained, which is three times higher than for cyclic batch fermentations.[206]

The formation of a water-insoluble polymeric phase by polymers constitutes a convenient method for the immobilisation of enzymes and cells. This process is achieved in the following way: at a temperature below the LCT, the enzyme is dissolved or the cells suspended in a concentrated aqueous solution of the polymer, after which the initial solution is added dropwise to the aqueous solution at a temperature above the LCT. The insoluble polymeric phase formed in the drop 'traps' the enzyme or the cells. After the addition of stabilisers (ovalbumin, egg powder, α-carboxymethylcellulose, tannin) to the initial solution, the polymer particles acquire sufficient stability and do not coagulate on stirring. Trypsin and carboxypeptidase B (stabilised beforehand by covalent binding to the vinylpyrrolidinone–acrolein copolymer),[210] fungal and bacterial cells,[211] and hybridoma cells[212] have been immobilised in a PVCL gel in this way.

The 13:1 NIPAA–vinylferrocene copolymer has been used simultaneously both as a mediator of charge transfer in the enzyme electrode and for the immobilisation of glucose oxidase on the electrode surface.[213-215] At a temperature below 22 °C, the polymer gel film on the surface of the electrode is in a swollen state. The pores in the gel are sufficiently large to allow the permeation of glucose oxidase molecules into their interior and the gel may be 'charged' with the enzyme. At a temperature above 35 °C, the gel collapses, the pores become markedly narrower, and the enzyme is no longer capable of diffusing from the gel, but the gel remains permeable to the low-molecular-mass substrate (glucose). Under these conditions, the glucose concentration is measured with the aid of the electrode. An advantage of such an electrode is the possibility of replacing rapidly and conveniently the inactivated enzyme by fresh enzyme. For this purpose, it is sufficient to wash the electrode below 22 °C in order to remove the inactivated enzyme, charge it with the fresh enzyme, and induce the collapse of the gel by heating it to a temperature above 35 °C.[213]

Thermoresponsive hydrogels were first used for the immobilisation of biocatalysts by Hoffman and coworkers.[216-218] Thus, β-D-galactosidase was immobilised in poly-NIPAA-TH grains (grain size 200–400 μm in the swollen state; critical temperature 37–40 °C). The overall productivity of the bioreactor with the immobilised enzyme at 35 °C differs slightly from its productivity at 30 °C (both temperatures are below the critical temperature). However, cyclic heating and cooling of the reactor between these two temperatures at a rate of 1 °C per minute increases the productivity of the reactor by a large factor. The swelling on cooling and contraction on heating of the TH grains intensify the mass transfer of the substrate and the products into the interior of the grains, the TH operating under these conditions as a pump actuated by a change in temperature and ensuring a more effective supply of the substrate and removal of the products.[217] A similar increase in productivity has been observed also for *Arthrobacter simplex* cells immobilised in poly-NIPAA-TH. The cells catalyse the conversion of hydrocortisone into prednisolone.[218]

Whereas the cyclic heating–cooling between two temperatures below the critical temperature increases the productivity of the bioreactor, heating to a temperature above the critical temperature leads to the complete arrest of the enzymic reaction. The activity of β-D-galactosidase immobilised in poly-NIPAA-TH falls virtually to zero at 50 °C despite the fact that this is the optimum temperature for the soluble enzyme.[219]

exo-1,4-α-Glucosidase has been immobilised in PMVE-TH with a critical temperature of 38 °C. It is noteworthy that the permeability of the TH to the substrate diminishes by five orders of magnitude on heating from 32 to 42 °C, whereupon the reaction stops almost completely. On cooling the TH to 32 °C, the reaction is renewed.[220] A similar effect has been observed also for α-chymotrypsin incorporated in PMVE-TH.[221]

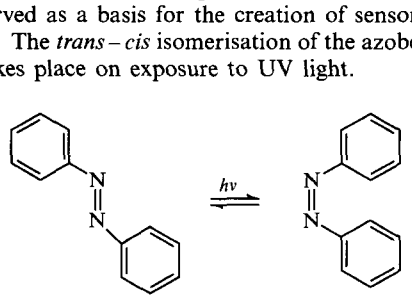
On collapse of the TH, the polymer chains approach one another and the medium within the TH becomes more hydrophobic. Both these factors limit the mobility of the macromolecules incorporated in the gel and hence increase the thermal stability of the immobilised enzyme at temperatures above the critical value.[222] A formal kinetic description of the behaviour of α-chymotrypsin incorporated in a cross-linked poly-NIPAA-TH has been published.[223-225] The temperature dependence of the activity of the immobilised α-chymotrypsin ceases to obey the Arrhenius law at temperatures close to the temperature of the collapse of the NIPAA-TH. This is expressed by the appearance on the Arrhenius plot of a section with an apparent negative activation energy. An increase in the concentration of the cross-linking agent leads to a shift of the break point on the Arrhenius plot towards higher temperatures and to a less marked decrease in the activity of the enzyme.[224]

Thus the biocatalysts obtained by immobilising enzymes and cells with the aid of 'smart' polymers acquire new and unusual properties. They can combine the advantages of homogeneous and heterogeneous catalysts, can serve as convenient 'chemical switches' sensitive to slight changes in external conditions, and are distinguished by a higher productivity as a result of the operation of the 'thermal pump'.

5. Sensor systems

Solutions and hydrogels of 'smart' polymers are sensitive to slight changes in the properties of the external medium, i.e. they function as complete sensors for these properties. For example, a set of solutions of polymers with different LCT can serve as a thermometer. The response of the polymers to changes in the properties of the medium is due to a change in the balance between the hydrophobic and hydrophilic interactions. Covalent binding to the polymer of acceptor molecules capable of undergoing a change in their hydrophobic properties in the presence of the agent to be detected (a definite substance or light) leads to the appearance of two states of the polymer — in the absence of the substance to be detected (characterised by a critical temperature T_1) and in its presence (characterised by a critical temperature T_2). Usually $T_2 > T_1$. If a temperature in the range $T_1 < T < T_2$ is fixed, then the transition from one state of the polymer to the other can be achieved under isothermal conditions with the aid of the agent to be detected. The latter induces a visible change in the solution or hydrogel, which has served as a basis for the creation of sensor systems.[226]

The *trans–cis* isomerisation of the azobenzene chromophore takes place on exposure to UV light.



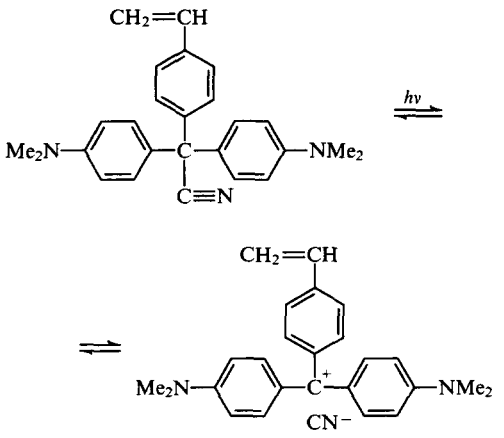
This transition is accompanied by an increase in the dipole moment from 0.5 D (for the *trans*-form) to 3.1 D (for the *cis*-isomer), which indicates an increase in the hydrophilicity of the polymer. The incorporation of a hydrophobic comonomer [*N*-(4-phenylazophenyl)acrylamide] (2.7 mol%) in a copolymer with NIPAA lowers the LCT to 19.4 °C, whilst UV irradiation increases it to 26.0 °C (as a result of the transition of the azobenzene chromophore to the more hydrophilic *cis*-form).[226]

We shall now consider the dimethylacrylamide–4-phenylazophenyl acrylate (7.5 mol%) system.[227] At a temperature slightly above the LCT in the absence of UV irradiation (visible light), the solution is turbid owing to the formation of an insoluble polymeric phase. UV irradiation increases the LCT (from 14 to 21 °C) and the copolymer, which is at a temperature below the LCT, gradually dissolves (the system becomes transparent). When UV irradiation ceases, the LCT is reduced, the copolymer again forms an insoluble polymeric phase, and the system once more becomes turbid. From the foregoing, it is clear that aqueous solutions of most copolymers constitute a sensor responding to UV irradiation.[226]

It is noteworthy that the phase transition in the copolymers becomes sensitive to UV irradiation for a strictly defined content of the acceptor comonomer. This emphasises yet again that the maintenance of a very fine balance between the hydrophobic and hydrophilic interactions is needed for the manifestation of critical phenomena in polymer solutions.

Another molecular acceptor of light quanta is triarylmethyl cyanide (the leuco-derivative of triphenylmethane), which dissociates reversibly on UV irradiation. The NIPAA copolymer containing only 1 mol% of the leuco-nitrile ligands has a LCT of 29.2 °C. After UV irradiation, the copolymer becomes more hydrophilic to such an extent that it loses the capacity for the clearly expressed formation of a polymeric phase following an

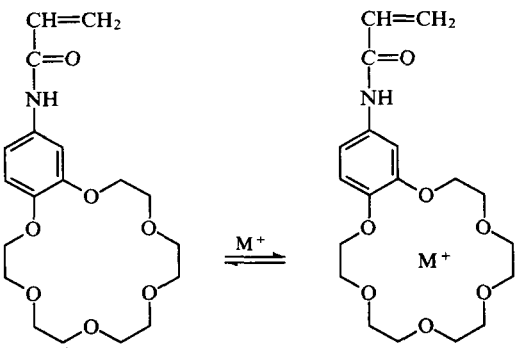
increase in temperature.[227] However, when the leuco-nitrile ligands are incorporated in poly-NIPAA-TH, the critical temperature for the collapse increases from 30 °C in the absence of UV irradiation to 32.6 °C (under UV irradiation). At 32 °C, UV irradiation induces the reversible transition of the gel from the collapsed to the swollen state, whereupon the degree of swelling of the gel changes almost by a factor of 10.[228]



On the other hand, the change in the hydrophilicity of the azobenzene chromophore on UV irradiation is insufficient to induce the collapse. The poly-NIPAA gel containing 10.8 mol% of the chromophore does not collapse, but is gradually dehydrated when the temperature is raised. UV irradiation induces an increase in the degree of swelling by only ~25%,[226] but the change in polarity within the hydrogel and the associated change in its permeability to substrates make it possible to regulate the activity of the enzymes immobilised in polyacrylamide gels containing photosensitive ligands.[229-231]

Another approach involves the use of heat derived from the absorbed light to raise the temperature of the TH, which leads to the collapse of the gel. Thus illumination of poly-NIPAA-TH containing Cu-chlorophyllin as the chromophore, with the power of the illumination exceeding 85 mW, at 31.5 °C results in the collapse of the gel.[232]

The change in the hydrophobicity of the acceptor molecule needed for the isothermal transition of the polymer from one state to another may be induced not only by UV irradiation but also by the introduction of various additives.[226,233] Thus the copolymer of NIPAA with the crown-ether **9** (11.6 mol%) has a LCT of 31.5 °C. In the presence of Na^+, the LCT increases to 33.0 °C, whilst in the presence of K^+ it increases to 38.9 °C. Thus the introduction of Na^+ and K^+ cations leads to the dissolution of the copolymer at 32 °C, the copolymer being insoluble in their absence. At 37 °C, this effect is induced only by the addition of K^+ cations. A similar effect has been observed also for poly-NIPAA-TH containing 12 mol% of the crown-ether. The thermoresponsive hydrogel which has collapsed at 33.8 °C swells by a factor of 10 in the presence of 30 mM potassium sulfate.



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The thermoresponsive hydrogel can be caused to pass from one state to another also under the influence of the products of the reaction catalysed by the enzyme immobilised in the TH. For example, the TH of the NIPAA–acrylic acid copolymer with immobilised urease collapses at 32.4 °C. When urea is added to the collapsed gel (at 33.4 °C), the enzymic reaction results in the formation of ammonia, which increases the pH of the medium. Under these conditions, the LCT of the copolymer increases and the gel swells, its volume increasing by a factor of 7 in 15 min.[234] In a buffer without urea, the TH collapses again.

Rabbit liver esterase hydrolyses the nonpolar ethyl butyrate to more polar products — ethanol and butyric acid. The change in polarity occurring under these conditions is sufficient to induce the swelling of the collapsed poly-NIPAA-TH.[235]

Concanavalin A (a lectin) immobilised in poly-NIPAA-TH can bind various carbohydrates, including the negatively charged dextran sulfate and the neutral methyl α-D-mannopyranoside. Dextran sulfate bound to concanavalin A increases the swelling of the collapsed gel by a factor of 5 as a result of the mutual repulsion of the negative charges on dextran sulfate. The displacement of dextran sulfate by the neutral methyl α-D-mannopyranoside again induces the collapse of the gel. The swelling–contraction cycle can be repeated many times with excellent reproducibility.[235]

The examples quoted above demonstrate the extensive possibilities of the employment of 'smart' polymers for the creation of exceptionally simple, reliable, and fairly sensitive sensor systems. However, this does not exhaust all the possibilities. The phase transition in solutions of such polymers has been used by Hoffman and coworkers[237-239] to devise a new method of precipitation immunoassay (PRECIPIA). The essential features of this method are explained in Fig. 7. *N*-Isopropylacrylamide was copolymerised with monoclonal antibodies to the κ-chain of human immunoglobulin G (IgG) modified by *N*-hydroxysuccinimidylacrylamide. The polymeric conjugate was incubated for 1 h at room temperature with the IgG solution being analysed, poly-NIPAA (to facilitate the thermoprecipitation of the conjugate), and monoclonal antibodies to the γ-chain of human IgG labelled with fluorescein or phycoerythrin. The temperature was raised to 45 °C, the resulting polymeric phase was separated by centrifugation and dissolved in a cold buffer solution, and the fluorescence was measured.

This system is not inferior as regards sensitivity to the traditional heterogeneous immunoassay methods, but makes it possible to overcome their significant disadvantage — the need for incubation in the course of many hours due to the diffusional hindrance to the interaction of the antigen with the antibody immobilised on an insoluble matrix. Since the antigen–antibody interaction during precipitation immunosassay takes place in solution, the incubation time can be shortened to only 1 h.[237-239]

6. Controlled delivery of medicinal drugs

An effective medicinal preparation is not simply a chemical substance but one which is supplied at an appropriate time and at an appropriate place in the organism. The creation of systems for the directed transport and controllable delivery of medicinal substances is one of the hot spots in modern pharmacochemistry. A significant role is assigned to polymers in this process.[68,240,241] Above we have already spoken of the industrial employment of reversibly soluble polymers for the formation of a protecting shell around peroral medicines.

The use of hydrogels for the controllable delivery of medicines is based on their ability to undergo a sharp change in the degree of their swelling and hence in their permeability in response to changes in the medium. The pathological processes occurring in the organism are as a rule associated with changes in the pH, temperature, and concentrations of specific substances. Thus it becomes possible to create systems with feedback, where the pathological process initiates the release of the medicinal drug.

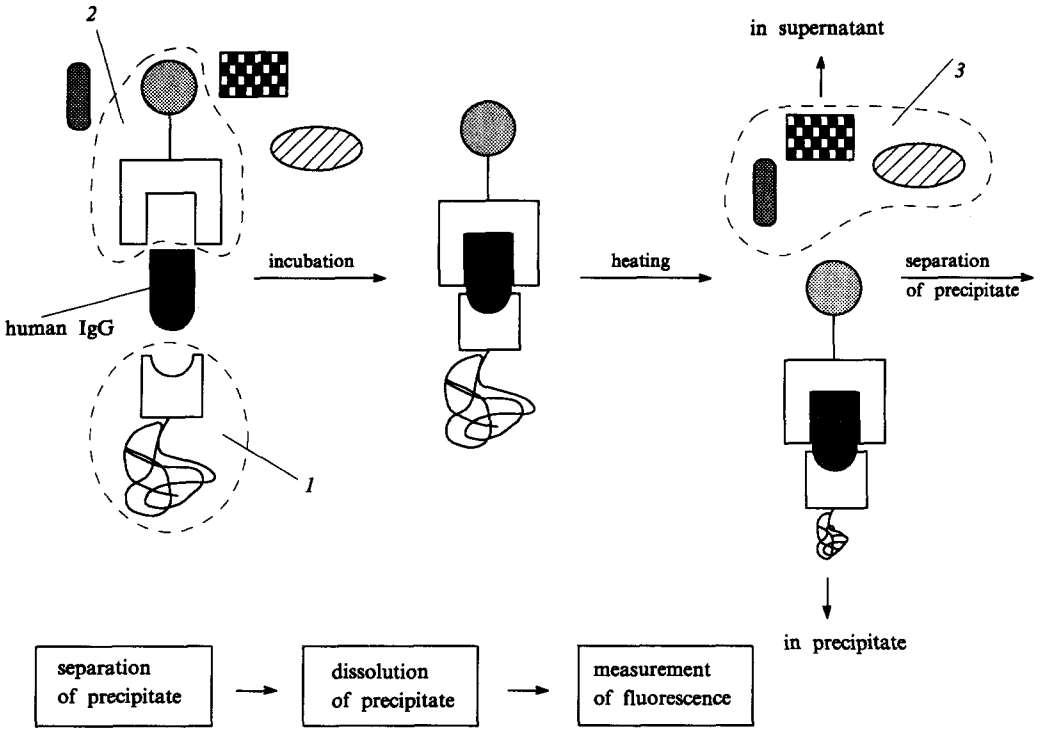


Figure 7. The precipitation immunoassay method.[237] (*1*) Conjugate of poly-NIPAA with monoclonal antibodies to the κ-chain of human IgG; (*2*) monoclonal antibodies to the γ-chain of human IgG with a fluorescent label; (*3*) extraneous proteins.

The gel in the swollen state is 'charged' with the medicinal drug. In the collapsed state, the gel does not give up the drug, diffusion becoming possible only after the swelling of the gel. The transition of the gel from the collapsed to the swollen state may be induced by various factors. Thus the change in the pH of the medium from 5 to 7 leads to the virtually complete arrest of the liberation of caffeine from the gel based on the copolymer of ketyl methacrylate and 2-(*N*,*N*-dimethylamino)ethyl methacrylate.[33] On the other hand, UV irradiation accelerates the diffusion of *p*-aminobenzoate from the poly(2-hydroxyethyl methacrylate) gel containing the azobenzene chromophore.[242] The formation of a charge-transfer complex between triethylamine and the copolymer of 2-hydroxyethyl methacrylate and methacryloyl-β-hydroxyethyl 3,5-dinitrobenzoate increases the swelling of the polymer membrane in water and hence the liberation of Methyl Orange from it.[243] An electric potential of 3–6 V initiates the liberation of edrophonium chloride from the gel based on the butyl methacrylate–2-acrylamido-2-methylpropanesulfonic acid copolymer. In the absence of an electric field, the positively charged medicinal drug is not liberated owing to its interaction with the negatively charged polymer.[244]

The liberation of glucose, insulin, or indomethacin from TH based on the copolymers of NIPAA and alkyl methacrylates ceases completely when the temperature is raised from 20 to 30 °C and is renewed on subsequent cooling to 20 °C. The thin film of the collapsed polymer, formed on the surface of the TH grains when the temperature is raised above the critical value, plays a critical role in the regulation of the diffusion process.[92,244–250] A similar sharp retardation of diffusion as a result of the formation of a collapsed gel film on the surface has been observed also for TH based on interpenetrating polymer networks comprising polyacrylic acid and the acrylamide–butyl methacrylate copolymer.[251] The kinetics of the release of the medicinal drug can be regulated (changing the reaction order from zeroth to first) by altering the nature of the TH, for example by employing interpenetrating polymer networks of poly-NIPAA and poly(methylene glycol ether) of different compositions.[252,253] When the TH collapses rapidly, there is a possibility of the formation of solution-filled cavities, the contents of which are not involved in the exchange with the surrounding medium.[92]

The reversible sorption on the surface of latex particles may be used for the temperature-controlled release of substances. Up to 65% of fluorescence-labelled dextran (M = 70 000), sorbed by the poly-NIPAA latex, is released into the solution on heating. When the temperature is reduced, the entire desorbed dextran is again bound to the latex.[138]

The most interesting approach has been developed by Hoffman and coworkers.[254,255] The authors used hydrogels sensitive to changes both in the pH and temperature. At 37 °C and pH 1.4 (the conditions within the stomach), the TH based on copolymers of NIPAA with acrylic acid and polydimethylsiloxane are in the collapsed state. Indomethacin or amylase, incorporated in the TH grains, hardly diffuse to the outside. At 37 °C and pH 7.4 (the conditions within the intestine), the TH swells markedly and the substances incorporated in it are rapidly released to the surrounding medium: more than 90% of indomethacin is released after 5 h. Thus a system has been created with directional transport and a prolonged release of the medicinal drug.

The creation of an artificial pancreas, i.e. a device releasing insulin in response to changes in the concentration of glucose, makes it possible to solve serious problems in the treatment of the numerous patients suffering from sugar diabetes. The development of such systems is therefore arousing unflagging interest.[256] Definite advances on these lines have been achieved by employing 'smart' polymers. The enzyme glucose oxidase, which oxidises glucose to gluconic acid, is usually employed as the glucose sensor. This reaction is accompanied by a decrease in pH, which in fact initiates the release of insulin. Technically this is achieved in different ways, for example by employing a membrane consisting of the 60:20:20 2-hydroxyethyl acrylate–*N*,*N*-diethylaminomethacrylate–4-trimethylsilylstyrene copolymer. In the swollen state, such a membrane contains 30% of water at pH 6.3 and 53% at pH 6.15, its permeability to insulin increasing in this case by a

factor of 42. Capsules made of this copolymer and containing glucose oxidase and insulin released insulin approximately five times faster in the course of 2–3 h in the presence of 0.2 M glucose. After the removal of glucose, the rate of release of insulin fell to the initial value.[257]

The swelling of the gel in the presence of glucose has been used in a mechanical device consisting of two compartments. The first compartment contained a glucose-sensitive gel and the second a solution of insulin. The solution of insulin was simply forced out by the swollen gel from the device through a valve.[33]

The complex of poly(vinyl alcohol) with the vinylpyrrolidinone–*m*-acrylamidophenylboronic acid may be used as the glucose sensor instead of glucose oxidase.[157] In this case, the hydroxy-groups of glucose displace the hydroxy-groups of poly(vinyl alcohol), as a result of which the polymeric complex decomposes. When glucose has been removed, the complex is reformed. A communication concerning the creation of an artificial pancreas operating in accordance with a similar principle has appeared recently.[258]

An interesting approach to the employment of 'smart' polymers for the controllable delivery of medicinal drugs has been proposed by Tirrell.[259] Among the numerous polyelectrolytes, polyethylacrylic acid possesses the fairly unique ability to undergo a change in conformation in the physiological pH range 7.4–6.5. The transition from the uncoiled to the compact conformation is accompanied by intensification of the interaction of the polymer with lipid membranes.[260] Poly-ethylacrylic acid, immobilised covalently on the surfaces of the vesicles formed by phosphatidylcholine, induces a reorganisation of the large vesicles on transition to the compact conformation, converting them into more compact micelles. The contents of the vesicles are then released into the external medium.

IV. Conclusion

The author hopes that he has succeeded in demonstrating the extensive possibilities for the employment of 'smart' polymers in various branches of biotechnology and medicine. There is no doubt that the examples presented do not exhaust all the possibilities. This field is developing dynamically judging from the constantly growing number of publications. For example, a communication has appeared quite recently[261] stating that the graft-copolymerisation of NIPAA makes it possible to convert a hydrophilic surface (at a temperature below the LCT) into a hydrophobic one (at a temperature above the LCT). This may be used for the directed transport of microparticles with a medicinal drug in the organism or for the segregation and purification of biomolecules (hydrophobic chromatography). The cells of microorganisms also interact in different ways with hydrophilic and hydrophobic surfaces.

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