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Smart polymers as surface modifiers for bioanalytical devices and biomaterials: theory and practice

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Smart, or responsive polymers can reversibly change their state of aggregation, thus switching from water-soluble to insoluble state, in response to minor changes in temperature, pH or solvent composition. Grafting of these polymers to solid surfaces imparts the surfaces with controllable wettability and adsorption behaviour. The review summarizes the theoretical models and the results of physical measurements of the conformational transitions in grafted polymer chains and polymer brushes. Primary attention is paid to the grafting density and the length and spatial arrangement of grafted chains, the role of polystyrene, organosilane or alkanethiol sublayers and their effects on adsorption of proteins and adhesion of cells. The key applications of grafted smart polymers such as cell culture and tissue engineering, cell and protein separation, biosensing and targeted drug delivery are surveyed.
The bibliography includes 174 references.

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I. Introduction

Among the diversity of materials and devices used in biotechnology and medicine, special place is occupied by structures of the solid framework – polymer shell type in which the polymer is chemically bonded to the solid surface. These structures combine the mechanical and structural properties of solid supports with the physicochemical properties and the biocompatibility of polymeric modifiers. Examples of materials and devices of this type are composite sorbents for bioseparation,[1–4] substrates for animal cell culture,[5–8] particles for targeted drug delivery and gene transfection,[9,10] microfluidic devices [11] and biosensors.[12] In the last decade, several comprehensive reviews [13–15] and numerous original papers on this subject were published.

Smart polymers,[16,17] which enable the control over the physicochemical properties of surfaces by varying the temperature, pH or composition of the aqueous solution in contact with the surface, are interesting modifiers for these materials and devices. On the other hand, smart tinted glasses, which reversibly change the light transmission coefficient under the action of minor electrical voltage (2.5 V), are widely used in technology.[18]

The review summarizes the most significant achievements in the field of bioseparation and bioanalysis as well as animal cell culture and tissue engineering using smart composite materials and devices. Despite the increasing

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number of studies in this field, they usually do not cover the physicochemical behaviour of grafted polymers, parameters of polymer – solvent interactions or details of binding of biomacromolecules to smart polymer coatings or smart polymers as such and dissociation of the resulting complexes. There is a certain gap between the physicochemical investigations and biomaterial applications of smart polymers; therefore, an empirical approach predominates in the implementation of controlled adsorptive and adhesive interactions of these materials with biomacromolecules and cells. Attempts to integrate the obtained practical results into an established system of views on the interaction of proteins and biological cells with synthetic polymers are rare. This is noted in the publication that is considered below in detail.[19]

Most often, theoretical description of the conformational transitions in grafted polymer layers goes ahead of experimental data acquisition and, hence, at least, approximate comparison of the results obtained by different methods and published by different authors is required. The time span covered by these publications is rather broad, ranging from the 1980s up to now. It is noteworthy that many results obtained twenty years ago are far from being outdated; they contribute to better understanding of the surface phenomena involving smart polymers and deserve due attention. The present review does not claim to provide an exhaustive analysis of the whole diversity of structures and properties of smart polymer composites but aims at an integrated consideration of the functional properties of smart polymer coatings in conjunction with their structures and physicochemical characteristics, summation of the basic theory predictions, experimental results and key practical achievements and, hence, at introducing the reader to this vigorously developing field of science and technology.

II. The main types of smart polymers and the key achievements in the field of biofunctional smart polymer composites

Smart polymers are water-soluble high-molecular-weight compounds that can reversibly change their state of aggregation by being converted from the hydrophilic (soluble) to hydrophobic (insoluble) form in response to minor changes in the temperature, pH or composition of the aqueous solution.[16,20] Furthermore, water-insoluble polymers with photochromic and electrochromic properties[21,22] as well as polymers responsive to changes in the external conditions are often classified as smart. The most well-known water-soluble smart polymers are poly(*N*-isopropylacrylamide) (PNIPAA) and *N*-isopropylacrylamide (NIPAA)-based copolymers,[23] poly(*N*-vinylcaprolactam) (PVCL) and polymers of some other *N*-substituted vinylamides;[24–26] block copolymers formed by polyethylene oxide (PEO) and polypropylene oxide (PPO) like PEO – PPO – PEO (in particular, Pluronic$^{(R)}$)[27,28] and polypeptides with repeating amino acid sequences VPGVG typical of elastins — mammal connective tissue proteins.[29]

These phase transitions take place in water in quite narrow temperature ranges: within one degree Celsius for PNIPAA (at 32 °C) and elastin-like polypeptides (at 28 °C). Cross-linked smart polymers form gels that are also capable of reversible transitions from the swollen to collapsed state.[30,31] Also, smart polymers can be grafted or adsorbed on solid surfaces, which, as a result, acquire the ability to substantially change their physicochemical properties (first of all, wettability and adsorptivity) in response to minor changes in the environmental conditions.

Materials of this type were first developed and studied by the research team headed by Professor Okano (Japan) in the 1990s. Later, these engineered solutions were repeatedly reproduced in various modifications and upgraded by both the same research team and many other teams. The principal discovery of Okano and co-workers was the possibility of animal cell adhesion, growth and culturing on solid substrates chemically modified by PNIPAA brushes at temperatures above the temperature of conformational transition of the grafted polymer. Moreover, it was found that, on cooling, the obtained cell cultures can be detached from the surface of PNIPAA-containing substrates with retention of viability to a much higher extent than in the case of traditional trypsin and ethylenediaminetetraacetic acid (EDTA) treatment.[32] Subsequently, this procedure was used to culture various sorts of animal cells, in particular, eye cornea epithelial cells, which formed confluent mono- and multi-layer cultures; in this form, they could be detached from the substrate by inducing the conformational transition of grafted PNIPAA and subsequently transplanted into patients with injured cornea.[33] Other types of cells cultured in this way and transplanted into living organisms in order to restore damaged tissues were also described.[34] Markvicheva *et al.*[35] studied culturing of hybridoma cells in hydrogels obtained by thermal deposition of PVCL.

Okamura *et al.*[36] used solid surfaces with grafted smart polymers to separate various animal cell lines. Specific antibodies were adsorbed at 37 °C on the surface of a polypropylene membrane modified by grafted PNIPAA, which was then brought in contact with a cell suspension containing two cell lines. After non-specifically bound cells were washed away, the specifically bound cells were detached at 10 °C, *i.e.*, under conditions of swelling of the grafted polymer. Ivanov *et al.*[8] demonstrated the possibility of specific adhesion of animal cells as a result of complex formation between their surface glycoproteins and boronate-containing polymers, including those grafted on solid substrates. The possibility of cell release by competitive elution with monosaccharides or by shifting pH may underlie new methods for cell separation and culturing.

An early application of the surface-bound smart polymers for protein isolation and purification was reported by Galaev *et al.*[37] By controlling the conformational transitions of PVCL adsorbed on Sepharose containing the Cibacron Blue 3G dye, it is possible to govern the enzyme lactate dehydrogenase sorption and desorption. The enzyme and PVCL are bound to this dye; therefore, transition of adsorbed PVCL from surface-linked globules (at 40 °C) to swollen and more mobile coils (at 23 °C) results in the desorption of the specifically bound enzyme due to competing interaction of PVCL with the dye. In particular, the recovery of lactate dehydrogenase from a muscle tissue extract by the described temperature-controlled elution resulted in a 90% retention of enzyme activity with a 17-fold purification from other proteins. Subsequently, metal chelate affinity precipitation of proteins using smart polymer reagents has come in use.[38]

The effect of temperature on the chromatographic retention of steroidal drugs and amino acid and peptide derivatives on silica gels modified by PNIPAA brushes and NIPAA copolymer brushes was studied in the series of

works of Kanazawa *et al.*[39,40] The temperature rise, which induces PNIPAA dehydration and the conformational transition in the polymer brushes, leads to increased retention of these sorbates, which provides a unique opportunity of chromatographic analysis of phenylthiohydantoin amino acid derivatives in a purely aqueous (salt-free) medium using a temperature gradient.[40] The temperature-controlled adsorption of proteins in microfluidic devices using a surface chemically modified by a grafted PNIPAA layer with an average thickness of 4 nm provided preconcentration and programmed release of proteins by local temperature change in the microscopic fragments of the systems.[11] This principle was implemented for controlled adsorption – desorption of streptavidin and PNIPAA conjugates able to link to immune complexes and then to be adsorbed, on heating, on the PNIPAA-containing surface of the microfluidic device.[41]

The above examples illustrate the broad range of existing and potential applications of the surface-grafted smart polymers in medicine, preparative biochemistry and microbioanalysis methods. Therefore, the structure, properties and behaviour of grafted layers of smart polymers became the subjects of both theoretical and experimental fundamental studies.

III. Modelling of polymer brush structure during conformational transitions

By polymer brushes are meant assemblies of polymer chains attached to a solid or another surface *via* terminal groups and arranged so close to one another that the adjacent chains interact and adopt a more extended structure than the same chains in a free solution.[42,43] The theoretical modelling of the equilibrium structures of polymer brushes and their conformational transitions upon a change in the polymer – solvent interaction parameter is the subject of numerous publications,[42–48] performed to a large extent in Russia (see the studies of Birshtein and co-workers).[44–46]

This Section gives an account of the basic views on the structure and behaviour of polymer brushes, the knowledge of which is important for understanding of the mechanisms of controlled adsorption and adhesion of biopolymers and cells. Consider the simplest model of an electrically neutral polymer brush located on a planar surface and consisting of chains of thickness a equal to the monomer unit length with the degree of polymerization n and the surface area per chain $a^2\sigma$, where $\sigma > 1$ is equal to the ratio of the average area per chain to chain thickness.[45,46] The structural characteristics of the grafted polymer layers were determined in these studies in accordance with the Flory – Huggins theory based on representing the free energy of the chain in the grafted layer as the sum of two terms

$$\Delta F = \Delta F_{el} + \Delta F_{conc}$$

where ΔF_{el} is the free energy of elastic deformation, ΔF_{conc} is the free energy of interaction of chain units with the environment. Since ΔF_{el} and ΔF_{conc} are functions of n, σ, layer thickness H and the Flory – Huggins parameter χ, the minimization of ΔF with respect to H results in the following asymptotic expressions for the equilibrium value H in different solvents:

for a good solvent,

$$H \sim n^{3/5}\left(\frac{1}{2}-\chi\right)^{1/5}, \quad \chi < \frac{1}{2} \tag{1}$$

for a theta-solvent,

$$H \sim n^{1/2}, \quad \chi = \frac{1}{2} \tag{2}$$

and for a poor solvent,

$$H \sim n^{1/3}\left(\chi-\frac{1}{2}\right)^{-1/3}, \quad \chi > \frac{1}{2} \tag{3}$$

In the simplest case of isolated chains, their size coincides with the size of free chains in solution at the same χ. It is worth noting that theories considered in the cited publications[45,46] do not take into account the interaction of chains with the bearing surface, *i.e.*, they do not envisage a chain collapse as a result of adsorption of chain segments on the surface. This imposes some limitations on the predictive power of the model, as an isolated chain is theoretically almost fully collapsed on the adsorbing surface even at a moderate free energy of binding of the monomer unit: $2kT$ and more.[49] Real systems, however, can be characterized by layer thicknesses of tens of nanometres, which considerably exceeds the chain thickness even at very low equilibrium polymer concentrations (0.01 mg ml^{-1}).[50]

In order to pass from asymptotic solutions to more realistic ones and present the dependences of H on χ as continuous functions at different n values, Ivanov and Zubov[51] supplemented the above expressions for H in good and poor solvents with proportionality factors $k_1 = 1.5$, $k_2 = 0.7$, respectively

$$H \approx k_1 n^{3/5}\left(\frac{1}{2}-\chi\right)^{1/5}, \quad \chi < \frac{1}{2} \tag{4}$$

$$H \approx k_2 n^{1/3}\left(\chi-\frac{1}{2}\right)^{-1/3}, \quad \frac{3}{5} > \chi > \frac{1}{2} \tag{5}$$

which provide, at $\chi \approx 0.5$, the H values close to the asymptotic solution of expression (2) for a theta-solvent,

$$H \approx n^{1/3}\left(\chi-\frac{1}{2}\right)^{-1/3}, \quad \chi > \frac{3}{5} \tag{6}$$

The dependences of H on n obtained with the use of the above proportionality factors are shown in Fig. 1. As can be seen, the changes in the thickness of a grafted polymer layer on going from a poor to a good solvent are most pronounced for chains with a high degree of polymerization. It is also noteworthy that the transfer of the polymeric layer from a poor solvent to a theta-solvent ($\chi = 0.5$) is accompanied by a relatively small increase in the equilibrium layer thickness H, whereas on going into a solvent with $\chi = 0.4$, the H value increases to a greater extent.

The conformational transitions of closely arranged grafted chains, which form a polymer brush, are much more complicated. Under the theta-conditions, the criterion

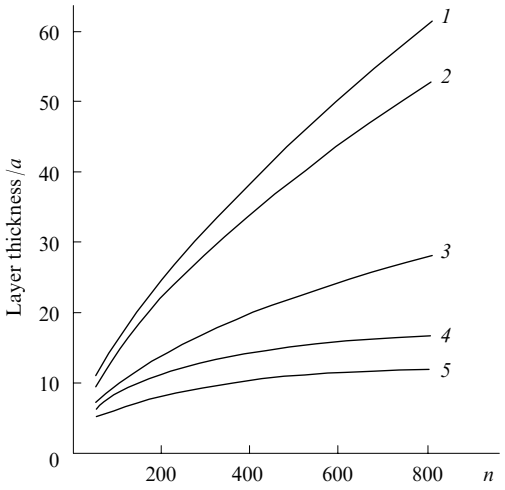


Figure 1. Thickness of a grafted polymer layer (in chain thickness units *a*) *vs*. degree of polymerization for non-interacting chains at different χ.[51]
χ: (*1*) 0.3, (*2*) 0.4, (*3*) 0.5, (*4*) 0.55, (*5*) 0.9.

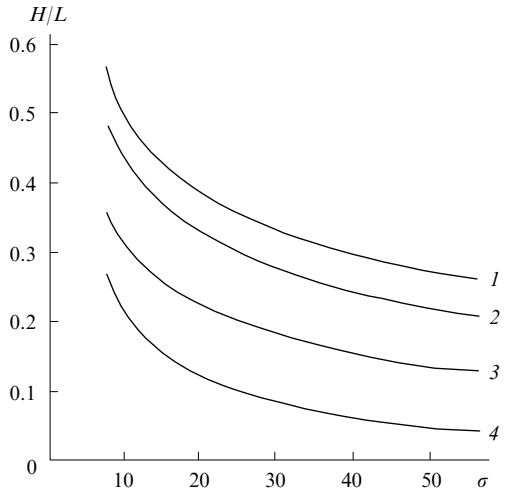


Figure 2. Relative thickness of a grafted polymer layer *vs*. the ratio of average area per chain to the chain thickness for interacting chains.[51]
χ: (*1*) 0.3, (*2*) 0.4, (*3*) 0.5, (*4*) 0.6.

for overlap and interaction of neighbouring chains is determined by the inequality

$$\frac{n}{\sigma} > 1 \tag{7}$$

For the ratio of the equilibrium thickness H of the brush to the contour length of the chain L, the following dependences on χ and σ were derived.[45]

$$\frac{H}{L} \sim \sigma^{-1/3}\left(\frac{1}{2}-\chi\right)^{1/3}, \quad \chi < 0.5 \tag{8}$$

$$\frac{H}{L} \sim \sigma^{-1/2}, \quad \chi = 0.5 \tag{9}$$

$$\frac{H}{L} \sim \sigma^{-1}\left(\chi-\frac{1}{2}\right)^{-1}, \quad \chi > 0.5 \tag{10}$$

In this case, for description of H/L as a continuous function of χ and σ, it is necessary to represent it as linear combinations of functions (8)–(10) for each range of χ and select the appropriate proportionality factors that would ensure continuity of both the function and its derivative (this is necessary to obtain a smooth dependence of H/L on χ predicted by the theory [45]). Ivanov and Zubov [51] obtained the following functions of H/L for three ranges of χ:

$$\frac{H}{L} \approx k_3\left[\sigma^{-1/3}\left(\frac{1}{2}-\chi\right)^{1/3} + k_4\,\sigma^{-1/2}\right], \quad \chi < 0.45 \tag{11}$$

$$\frac{H}{L} \approx \sigma^{-1/2} + (1-k_5\,\sigma)\left(\frac{1}{2}-\chi\right), \quad 0.6 > \chi > 0.45 \tag{12}$$

$$\frac{H}{L} \approx k_6\left[\sigma^{-1}\left(\chi-\frac{1}{2}\right)^{-1} + k_7\,\sigma^{-1/2}\right], \quad \chi > 0.6 \tag{13}$$

Note that the presented approximate expressions (11)–(13) do not provide a perfect coincidence at any n and σ for the points $\chi = 0.45$ and 0.6 but generally they describe the conformational transitions in line with the theory predictions,[45] represent the general trends of these transitions and are valid in the ranges of $100 > \sigma > 6$ and $1 > \chi > 0$. The proportionality factors were found to be $k_3 = 1.3$, $k_4 = 0.4$, $k_5 = 3.6 \times 10^{-3}$, $k_6 = 0.16$, $k_7 = 1.2$.

The dependences of the relative thickness of the polymer layer formed by the overlapping chains as a function of σ for various χ are shown in Fig. 2 and the dependence on χ is presented in Fig. 3. In this polymer layer, the interaction between the segments of different chains results in chain stretching relative to the Gaussian statistic coil conformation not only in good but also in poor solvents. As a result, the conformational transition is less pronounced than in an

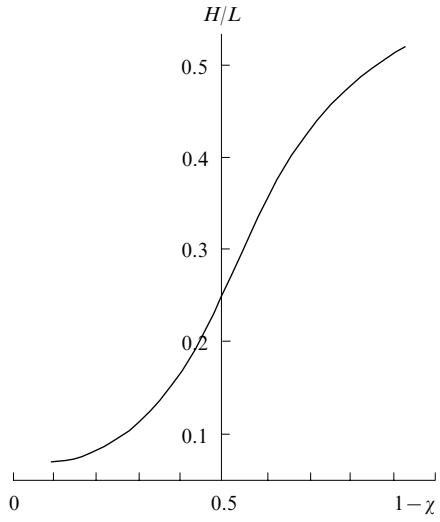


Figure 3. Relative thickness of a grafted polymer layer *vs*. the Flory–Huggins parameter for interacting chains at $\sigma = 16$.[51]

isolated coil and occurs over a broader range of χ values shifted to $\chi > 0.5$. At high grafting density, these effects are enhanced (see Fig. 2).

It is worth noting that the theories considered in the above-cited publications [44 – 47] were developed first of all for the description of the conformational transitions in the polymers soluble or swellable in organic solvents; most often, the Flory – Huggins parameter increases with decreasing temperature, and the thermodynamic quality of the solvent is, correspondingly, deteriorated. In this case, the theory predicts a gradual compression of the polymer brush as a whole without vertical or lateral segregation into separate domains with higher or lower polymer concentration. Upon compression in a poor solvent, the polymer layers become impermeable for a solvent flow, *i.e.*, theoretically, their permeability can be controlled by changing the thermodynamic quality of the solvent.[42, 47]

The conformational transitions in polymer brushes formed by smart polymers in aqueous media represent a substantially different picture. In this case, the Flory – Huggins parameter increases first gradually and then stepwise with temperature rise. The transitions in the smart polymer brushes are described in terms of the two-state model according to which the monomeric units of the polymer exist simultaneously in the non-associated hydrated form and in the form of hydrophobic clusters consisting of several units.[52] These forms are in equilibrium, which distinguishes this model from the models considered above [42 – 47] in which all monomeric units are assumed to be equivalent. Furthermore, the model in question [52] considers the dependence of effective χ value not only on the temperature but also on the volume fraction of the polymer in the brush. The dependence of the effective χ value on the concentration of the soluble polymer was found experimentally for both PEO – PPO – PEO and PNIPAA.[53] A key prediction of the theory [52] is the χ- and σ-dependent bimodal distribution of the volume fraction of the polymer along the normal to the surface. In addition, at high χ and σ values, lateral segregation of the grafted polymer is possible, giving concentrated micelle-like structures separated by spaces with relatively low polymer concentration.[54]

These predictions received qualitative experimental evidence in a study [55] in which high-molecular-weight PNIPAA was grafted on silicon plates *via* free radical polymerization deliberately up to moderate surface densities of grafting. According to atomic force microscopy (AFM) data, at 26.5 °C, a grafted PNIPAA layer was mainly uniform, while at 40 °C, it had a clear-cut cellular structure with an effective size of polymer chain aggregates of ~100 nm (Fig. 4). This structural transition accompanied by dehydration of the grafted polymer layer and a change in the layer weight was detected independently by a quartz crystal microbalance. The theoretical conclusions [52] about a bimodal distribution of the bulk density of PNIPAA segments were confirmed in one more study.[53]

Note once again that calculations carried out by Ivanov and Zubov [51] are rough and mainly serve to illustrate conclusions of earlier theoretical papers. Nevertheless, they have some predictive power, as shown in the subsequent sections of this review. Detailed strict theoretical description of phase transitions in smart polymer and polyelectrolyte brushes has been reported in several papers [52, 54, 56] (see also references cited in the review [54]).

IV. Flory – Huggins parameters, polymer coil dimensions and their temperature dependences for some smart polymers

In order to conceive which temperature ranges are best suited for implementation of the controlled conformational transitions in hydrated polymer brushes, we will consider selected experimental papers describing the determination of the Flory – Huggins parameter in aqueous solutions of smart polymers and in hydrogels. The dependence of χ on temperature for PNIPAA was elucidated in a study dealing with water swelling of the gel of this polymer. The dependence of χ on $1/T$ is nearly linear at $\chi < 0.55$, which corresponds to the temperature range from 15 to 35 °C, passes through theta-conditions at 31.5 °C, has a break at 35 °C and then sharply increases up to $\chi = 0.8 – 0.9$ (Fig. 5).[57] Going back to Figs 1 and 2, note that considerable swelling of a PNIPAA brush ($\chi = 0.4$) requires a temperature of ~21 °C, *i.e.*, well below the phase transition temperature of the soluble polymer.

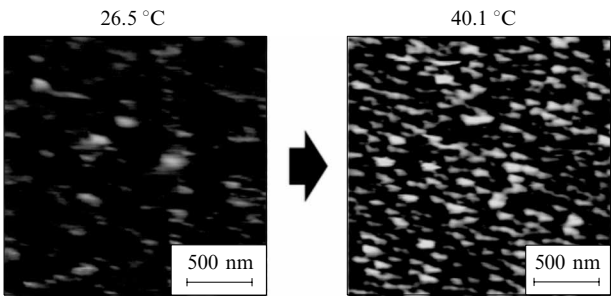


Figure 4. AFM images of the surface microstructure of a grafted PNIPAA-modified silicon plate obtained in water in a semicontact mode.[55]

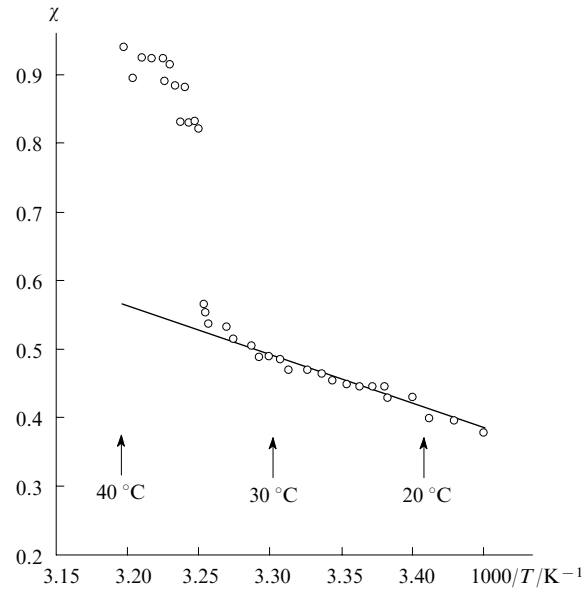


Figure 5. Temperature dependence of the Flory – Huggins parameter for the PNIPAA hydrogel.[57]

The phase transition of the soluble high-molecular-weight PNIPAA [with a molecular weight (M) in the range from 1.6×10^6 to 25×10^6 g mol^{-1}] was studied by static and dynamic light scattering at low polymer concentrations ($\sim$13 mg litre^{-1}).[58,59] It was found that, as the temperature increases from 20 to 30.5 °C, the size of the swollen polymer coils gradually decreases and arrives at theta-conditions at 30.6 °C.[59] Above these temperature, both the hydrodynamic radius and the radius of gyration of the coils sharply decrease, the chain hydrophobicity increases, and space fluctuations of the polymer concentration (Δc), caused by chain association and dissociation, are enhanced. This brings about an increase in light scattering intensity proportional to the mean-square fluctuation of the concentration $\langle\Delta c^2\rangle$.[60] The PNIPAA phase transition was also studied by differential microcalorimetry, where the endotherm maximum, which determines the lower critical solution temperature (LCST), was located at 32.2 °C, which corresponded to the initial segment of the temperature dependence of the light scattering intensity.[61] At temperatures above the LCST, association of polymer chains becomes significant, submicrometre insoluble polymer particles are formed and then flocculate to give a precipitate. The inflection point in the temperature dependence of the light scattering (or light absorption) can be referred to as the flocculation point by analogy with the flocculation point of the submicrometre particles of cross-linked PNIPAA.[62]

The temperature dependences of the hydrodynamic radius of the coil and the light scattering of polymer solutions were also studied for another smart polymer — poly(*N*-vinylcaprolactam) with M from 10^5 to 23×10^5 g mol^{-1} (see Ref. 63). Similarly to the temperature dependences derived for PNIPAA, the hydrodynamic radius of the coils of high-molecular-weight PVCL in water decreases with increasing temperature, passes through theta-conditions at 29.3 °C and then decreases up to LCST (31.0 °C); at this temperature, a phase transition takes place (Fig. 6). Simultaneously, the light scattering intensity sharply increases because of coil association giving insoluble particles.

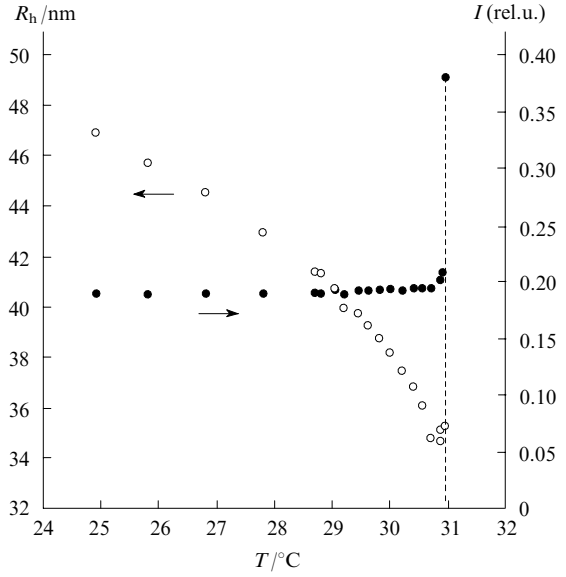


Figure 6. Temperature dependences of the hydrodynamic radius of PVCL coils (R_h) and the light scattering intensity (I) of an aqueous solution of PVCL.[63]

Approximately the same trends were found[64] for PVCL with $M = 4\times10^6$ g mol^{-1}. Laukkanen *et al.*[65] found that the lower critical solution temperature of PVCL, determined by microcalorimetry, increases with decreasing molecular weight from 31 °C ($M = 1.5\times10^7$ g mol^{-1}) to 37.5 °C ($M = 21\times10^3$ g mol^{-1}), which is especially evident in relation to PVCL with moderate molecular weights. It was also shown that PVCL, like PNIPAA with charged end groups, forms stable colloid suspensions at temperatures above LCST; this attests to a hydrophilic nature of their surface. Despite the apparent similarity of the phase transitions in PNIPAA and PVCL, the temperature dependence of the specific heat capacity for PNIPAA in the phase transition region sharply increases to reach higher values (18 J g^{-1} K^{-1}) than the similar dependence for PVCL (3 J g^{-1} K^{-1}); furthermore, it is extended to higher temperatures. The change in the volume of the aqueous solution taking place upon phase transition is also greater in the case of PNIPAA. This implies that this polymer binds more water; therefore, more energy is required to destroy the hydration shell and more water molecules are released.[65] Less hydrated PVCL has a higher Flory–Huggins parameter than PNIPAA [$\chi = 0.52$, found at 20 °C for a PVC hydrogel cross-linked by the bis(*N*-vinylpyrrolidone) reagent, 1% relative to the monomer].[66] As opposed to sharp compression of the PNIPAA gel upon temperature rise (see Fig. 5), the PVCL gel is compressed gradually, which is reflected as a change in the degree of swelling from $\sim$2 at 20 °C to $\sim$0.3 at 60 °C.[66] A higher degree of hydration of PNIPAA macromolecules and the related sharp phase transitions of the soluble polymer are, apparently, partly responsible for the fact that surface-grafted PNIPAA is also being extensively investigated.

V. Temperature-dependent transitions in the surface-grafted smart polymers: physicochemical characteristics

The systematic research of the conformational transitions of the surface-grafted PNIPAA started in the 1990s when these transitions were found to influence the adhesion, culturing and harvesting of animal cells.[32–34] Flat glass plates were treated successively with solutions of dimethyldichlorosilane and styrene–aminomethylstyrene copolymer (78 : 22 mol.%) to introduce aminomethyl groups into the substrate. After that, either PNIPAA with the carboxyl terminal group ($M = 13200$ g mol^{-1}) or NIPAA–acrylic acid copolymer (97 : 3 mol.%) was chemically attached to the obtained surface; in both cases, water-soluble carbodiimide was used as the condensing reagent.[32]

Water wetting of PNIPAA-modified surfaces was studied by measuring the contact angles at various temperatures. The obtained dependences of the advancing and receding contact angles are presented in Fig. 7. It can be seen that both methods of polymer attachment gave surfaces with a strongly temperature-dependent wettability in the temperature range of 20–30 °C, *i.e.*, somewhat below the phase transition temperature of PNIPAA (32 °C). In our opinion, the shift of the conformational transition temperature of the surface-grafted polymers is attributable to their adsorptive interaction with the hydrophobic sublayer of polystyrene, which is characterized by a contact angle of

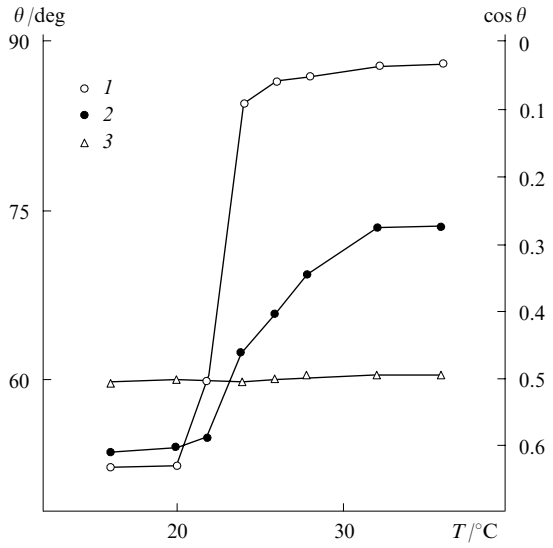


Figure 7. Temperature dependences of the advancing contact angle for surfaces chemically modified by PNIPAA grafting *via* the terminal group (*1*) and multipoint binding (*2*) and the receding contact angle for terminal group grafting (*3*).[51]

80° (see Ref. 67). In the case of relatively weak hydration of PNIPAA ($\chi = 0.45-0.5$) corresponding to temperatures of 25–30 °C (see Fig. 5), the polymer can be adsorbed on polystyrene by means of hydrophobic interactions, so that its desorption requires the condition $\chi < 0.4$, which corresponds to fuller chain hydration and can be achieved at $T < 20$ °C.

It is noteworthy that the advancing contact angle responds to the presence of hydrophobic fragments on the surface, while the receding contact angle is responsive to hydrophilic fragments;[68] therefore, the very weak temperature dependence of the receding angle (see Fig. 7) implies the presence of hydrated polymer over the whole considered temperature range, which is in line with the hydrophilic nature of PNIPAA suspensions above the LCST.[65] In view of this, the increase in the advancing angle with increasing temperature can be attributed, most of all, to outcrops of hydrophobic polystyrene sublayer upon reorientation and aggregation of PNIPAA chains. While discussing the different patterns of dependences shown in Fig. 7, Takei *et al.*[67] arrived at the conclusion that the multipoint grafting of the NIPAA–acrylic acid copolymer retards the polymer dehydration and polymer chain aggregation.

In our opinion, only the second part of this statement seems to be partly true. Theoretical consideration of adsorption of an isolated chain by irreversible binding of segments predicts almost complete collapse of chains on the solid surface to form some small loops,[49] which are poorly mobile and, hence, are not subject to aggregation. In the more realistic stage-by-stage many-chain adsorption, macromolecules adsorbed at late stages form large loops and tails that do not directly contact the solid surface, densely covered with the chains adsorbed in early stages. In this case, the number of long segments is moderate and decreases in proportion to $s^{-11/5}$, where s is the loop length.[69] These segments can aggregate with small loops. The presence of reactive groups in the loop and tail segments of the chemisorbed chains was confirmed experimentally.[2, 70, 71] In all probability, the multipoint chemisorption of the polymer results in a denser surface coating than the attachment by the terminal group and leads to the restricted mobility of adjacent chains, so that the reversible outcrop of submicrometre areas of the hydrophobic sublayer becomes less likely (Fig. 8).

An AFM study of the conformational transitions of PNIPAA, grafted *via* the terminal group by photoinitiated polymerization using iniferters, demonstrated[72] that these temperature-dependent transitions change both the effective thickness and micromechanical properties of the grafted polymer layer. This is in line with the results obtained by Harmon *et al.*,[73] who studied the temperature dependences of the elastic modulus in weakly cross-linked PNIPAA gels. In the temperature range of 30–34 °C at 2.4 mol.% of the cross-linking agent, the elastic modulus sharply increased from 10 kPa to ~1 MPa. When the cross-linking agent content was 4.8 mol.%, this transition took place at lower temperature (24–30 °C) and the elastic modulus reached ~10 MPa at $T > 32$ °C.

Jones *et al.*[74] grafted PNIPAA on gold plates by atom transfer radical polymerization (ATRP) and obtained grafted polymer layers several tens of nanometres thick. The surface-initiated polymerization of NIPAA gave rise to polymer brushes. An inherent feature of the ATRP method is equilibrium between the active form of growing macroradicals and the inactive form, *viz.*, a carbochain polymer containing a terminal halogen atom, the equilibrium being shifted towards the inactive form. Under the action of a reducing transition metal (usually, monovalent copper) complex, this alkyl halide is converted to a growing macroradical, which is readily transformed back into the inactive form.[75] Therefore, first, the probability of radical recombination is low and, second, the resulting polymer chains have approximately the same length, because the growing macroradicals pass from the active into inactive form at equal frequency. Therefore, the surface-initiated atom transfer radical polymerization usually affords uniform polymer brushes with minor thickness variation.[76] An AFM study of PNIPAA brushes in water showed a decrease in the thickness from 29 to 11 nm as the temperature increased from 25 to 35 °C.[74]

A similar approach to the synthesis of grafted PNIPAA was used to obtain brushes with a variable molecular mass and grafting density on glass plates with a spray-deposited gold layer.[77] The polymer layer became thicker following increase in the amount of immobilized bromoisobutyric acid ester, the source of radicals initiating the polymerization. Varying this value gave polymer brushes with different PNIPAA grafting densities and an average area per chain of 2.3 to 19 nm^2. The polymer brushes of various thickness (from 10 to 97 nm) obtained at the maximum grafting density had different contact angles ranging from 66 to 83° at 25 °C. Upon the temperature rise in the range of 29–33 °C, all the grafted layers showed a stepwise increase in the contact angle by only 5–10°, related to dehydration and conformational transitions in PNIPAA. The fact that the heat sensitivity of the coating was relatively weak (*cf.* Fig. 7) can be attributed to the coating high density and uniformity and, hence, poor accessibility of the alkanethiol sublayer for water wetting.

Considerable differences between the micromechanical and adhesive behaviours of PNIPAA brushes at temperatures above and below the phase transition temperature of

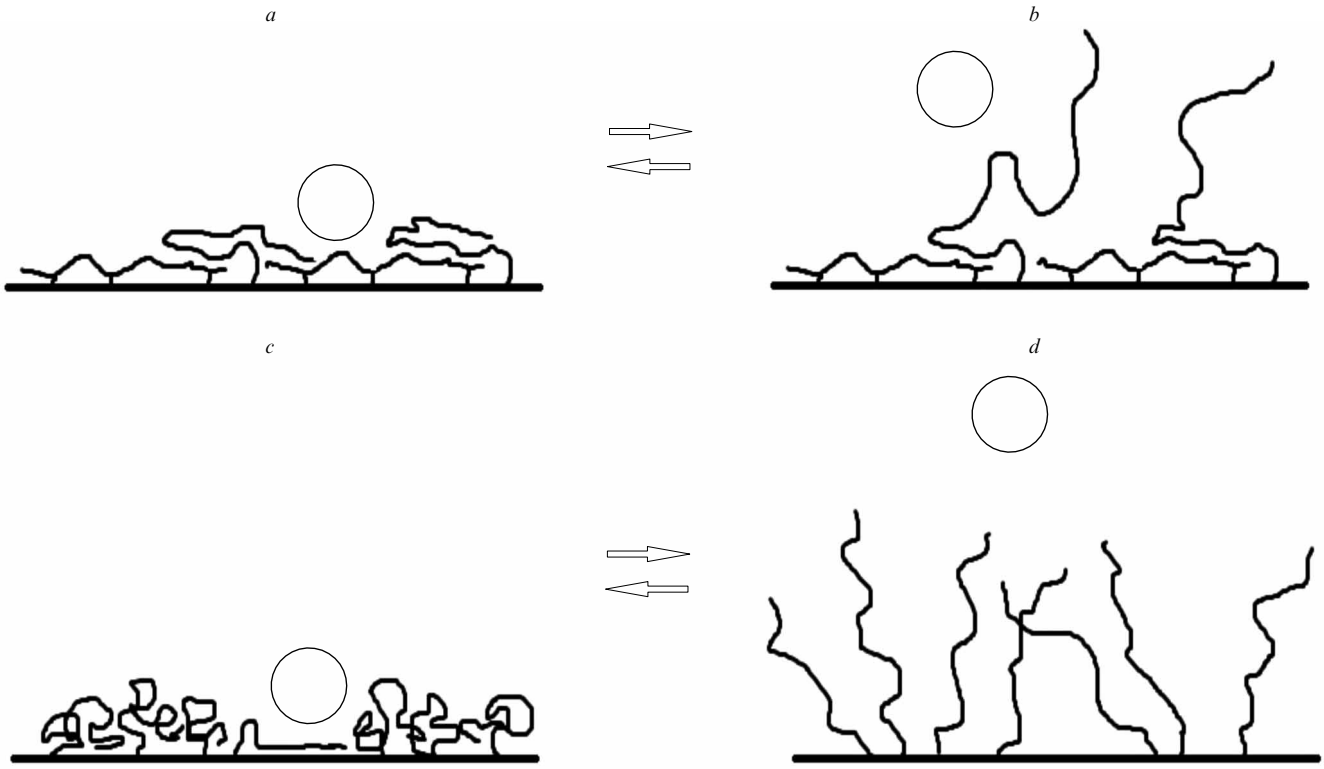


Figure 8. Scheme of conformational transitions in smart polymers chemisorbed on the surface (*a*, *b*) and grafted *via* the terminal group (*c*, *d*).
High mobility of grafted chains (*c*, *d*) and the capability for association with one another promote partial outcrop of the hydrophobic areas of the support surface at elevated temperature and enable thermally controlled adsorption of proteins (*c*). Surface-attached chain segments, which are highly collapsed in chemisorbed layers (*a*, *b*), protect proteins from the direct contact with the support, and conformational transitions of rare large loops and tails located in the same layers affect little the adsorption of proteins. The circle marks the position of the protein macromolecule.

this polymer were detected by measuring the repulsion forces between the plates with grafted PNIPAA and mica plates arranged in parallel, *i.e.*, using the surface-force measurements (Fig. 9). At 26 °C, the repulsion forces arise at distances of ~250 nm corresponding to the contour length of PNIPAA chains (M = 26 3000 g mol^{-1}) and far exceeding the thickness of a dry grafted layer (125 nm). This attests to a substantial swelling of the polymer in water at 26 °C. Further movement of the parallel surfaces towards each other resulted first in a gradual increase in the repulsion forces and then in their sharp growth at distances of ~120 nm. At a temperature of 36 °C, the repulsion forces follow a quite different dependence on the distance between the plates (see Fig. 9): the forces are weak at 120 – 250 nm distances but increase very sharply at distances of < 105 nm. This is caused by the fact that the grafted polymer is dehydrated and has a compact structure; the number of segments exposed to the solution beyond this compact structure is relatively small; therefore, they do not hamper moving of the plates towards each other. On further decrease in the distance, a contact of the mica plate with the dense collapsed polymer layer is accompanied by the sharply increasing repulsion. The described experiment was carried out at a maximum PNIPAA grafting density and an average area per chain of 2.3 nm^2, which corresponds to $\sigma \approx 5$.

The compression of the grafted PNIPAA layer with temperature rise from 17 to 37 °C was also measured by ellipsometry in an aqueous medium.[78] The layer thickness gradually decreased from 83 to 50 nm without a sharp step at the phase transition temperature of soluble PNIPAA

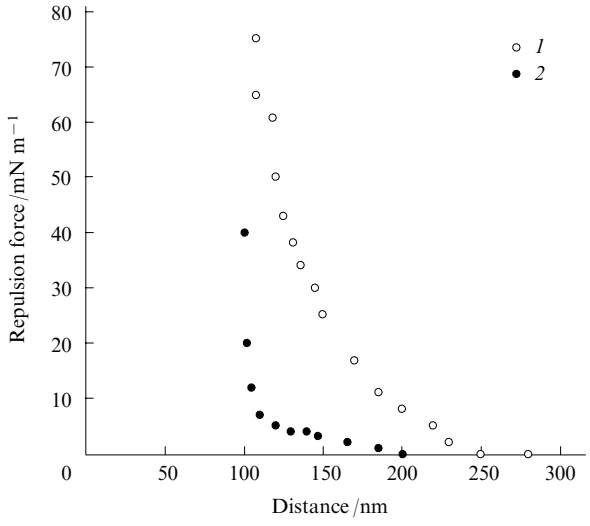


Figure 9. Dependence of the repulsion force between a PNIPAA brush and a mica plate located parallel to each other on the distance between them at 26 (*1*), 36 °C (*2*).[77]

(32 °C). Lowering of the temperature was accompanied by a reversible increase in the grafted layer thickness. The results are in agreement with predictions of the theories considered above [45, 46] about a broadening of the temperature range of conformational transitions in the grafted polymer layers with interacting chains. Moreover, the reported [77, 78] 1.5 – 2.5-fold change in the layer thickness is comparable with the predictions of calculations [51] for high grafting density, although it is somewhat lower than the theoretical values: the states of the system with $\chi = 0.45$ and 0.9 at $\sigma = 5$ are described by $H/L = 0.52$ and 0.17, the values calculated from Eqns (11) and (13), *i.e.*, they correspond to a ~3-fold change in the polymer layer thickness. Here it should be borne in mind that, first, expressions (11) and (13) are approximate and, second, real systems are characterized by broad variations of the σ and H/L values, which may account for the indicated deviations. As noted above, the repulsion forces measured by Plunkett *et al.*[77] arose at distances equal to the chain contour length, *i.e.*, many chains existed in the most extended (or nearly most extended) conformations.

A detailed study of the structure of the PNIPAA grafted layer was reported in the paper [53] devoted to a neutron reflection study of the conformational transitions in grafted layers. Graft polymerization on a gold plate surface was carried out by the ATRP method. The first step of the synthesis led to mixed chemisorbed monolayers of dodecanethiol and 11-mercaptoundecan-1-ol taken in variable ratios, then the alcohol groups were acylated with 2-bromopropionyl bromide, and then NIPAA polymerization was carried out in the presence of a catalyst and CuBr. This gave grafted PNIPAA with the molecular weight of 13×10^3 to 230×10^3 g mol^{-1} and a grafting density of 0.01 to 0.54 chains per nm^2 or, correspondingly with an average area per chain of 100 to 1.9 nm^2 ($\sigma \approx 4$). The distribution of the segments of grafted PNIPAA along the normal to the surface is bimodal (Fig. 10), which is consistent with the predictions of the theory.[52] Increase in the temperature around the LCST of poly(*N*-isopropylacrylamide) induces polymer dehydration and conformational transition and compression of the grafted layer.

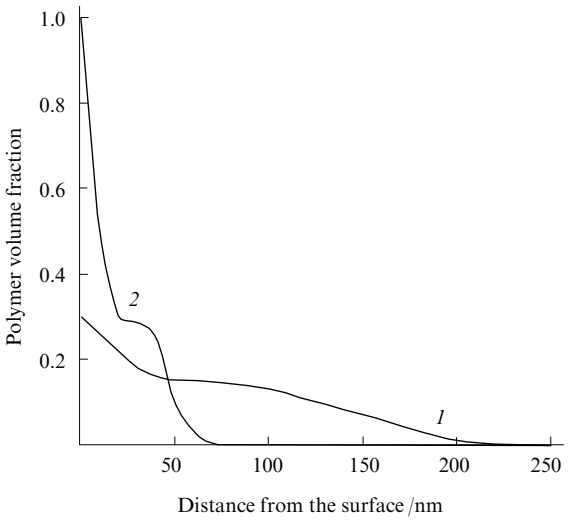


Figure 10. Volume fraction of PNIPAA ($M = 230 \times 10^3$ g mol^{-1}) grafted on a gold plate *vs.* the distance from the surface for grafting density of 0.08 chains per nm^2 (12.5 nm^2 per chain, $\sigma \approx 27$) at temperatures of 20 (*1*) and 39°C (*2*).[53]

To summarize the foregoing, it can be concluded that the thermoresponsivity of the grafted PNIPAA layers studied using a broad range of physicochemical methods involves compression of the layers, increase in their rigidity and deterioration of wettability taking place upon increase in the temperature in the range close to the LCST of the free polymer. It is typical that the densest and most homogeneous layers of grafted polymers show the least clear-cut changes in the surface properties, possibly, because of more pronounced shielding of the solid support surface. Therefore, before turning to consideration of the interactions of grafted polymer layers with proteins, pay attention once again to the possible role of organosilane or alkanethiol sublayers both in these interactions and in the mechanism of temperature-dependent transitions of grafted PNIPAA.

The effect of the composition of the organosilane sublayer on the temperature dependences of the contact angles on planar surfaces bearing grafted PNIPAA has been studied.[79] The silicon plates were simultaneously chemically modified with (3-aminopropyl)trimethoxysilane and the trimethoxysilyl reagent containing a hydrophobic perfluoroalkyl substituent. After acylation of the aminopropyl groups with bromoisobutyryl bromide and graft polymerization of NIPAA by the ATRP mechanism, the authors obtained a set of supports with different micro- and nanoheterogeneity of the surface depending on the ratio of silylating reagents. Contrary to expectations, they found that the contact angles on the surfaces containing a relatively small amount of a perfluorinated modifier are smaller than those on the PNIPAA-bearing surfaces without this modifier. The temperature dependence of the contact angle was much sharper in the former case: the contact angle changed by more than 50°, while in the absence of the modifier, the angle changed by only 25°. Typically, as in the study cited above,[78] the conformational transition of grafted PNIPAA took place over a rather broad temperature range — from 25 to 40 °C. While interpreting the obtained results, the authors suggested that in the presence of the modifier, the grafted PNIPAA is oriented on the surface as a result of association of hydrophobic fragments of PNIPAA chains with perfluoroalkyl groups, the polar amide groups being exposed to the aqueous phase. At higher temperature, the conformational transition of PNIPAA is accompanied by association of grafted polymer chains with one another and partial outcrop of the highly hydrophobic sublayer, similarly to what is shown in Fig. 4 and as a scheme in Fig. 8 *c*,*d*. This phenomenon should be taken into account while considering the adsorption of proteins on the PNIPAA-containing surfaces, because the protein binding can theoretically take place both on segments of grafted dehydrated PNIPAA and on the hydrophobic sublayer, similarly to the adsorption on reverse-phase alkylsilyl or perfluoroalkyl [80] sorbents.

VI. Temperature-dependent transitions of the surface-grafted smart polymers and their interactions with proteins

The interaction of synthetic polymers with proteins became the subject of systematic studies back in the 1980s in relation to the development of biomaterials and implants.

A practical purpose of these works was to devise materials with minimized adsorption activity (high inertness or resistance) towards a broad range of biopolymers, first of all, proteins. The principal condition of the surface resistance to protein adsorption is the absence of strong interactions between the protein molecules and the surface of the synthetic material in the physiological medium. The electroneutrality of polymers rules out ionic interactions, while the polarity of functional groups makes it possible to increase water wettability (hydrophilicity) of the material and, hence, to decrease the free energy of the polymer/solution interface as the driving force for the adsorption of dissolved organic compounds. For reference, the free energies of the polymer/water interface are only 3.7 and 3.1 mJ m^{-2} for cellulose and polyvinyl alcohol, respectively, whereas for polyethylene and nylon, these values are 53 and 42 mJ m^{-2} (see Ref. 81). The adsorption of bovine serum albumin from a phosphate buffer (pH 7.4) on the surface of various electroneutral organic materials is substantially enhanced with increase in the interface free energy.[82] A number of neutral polymers — Teflon, nylon, polyvinyl chloride — were found to obey similar dependences in the adsorption of other proteins — human serum albumin, immunoglobulins IgG and IgM, α_2-macroglobulin[83] and fibrinogen.[84] These trends were generally confirmed, although, for example, adsorption of IgG on polystyrene or polydimethylsiloxane decreased with a decrease in the material wettability, which could be due to different surface roughness of the supports.[85]

Grafted polyethylene oxide is one of the best studied protein-resistant synthetic polymers, which is readily hydrated in aqueous media and has a highly flexible and mobile polymer chain. Without going into details of the molecular mechanisms of the surface resistance to protein adsorption, which has become the subject of theoretical investigations[86,87] and lively discussion,[88] note that both the deformation of PEO hydration shell and restriction of the number of possible polymer chain conformations upon contact with protein molecules may induce repulsion.[88] The competing adsorption of grafted PEO segments on the adjacent areas of the solid surface also hampers the adsorption of proteins, both the chain length and grafting density being important factors.[87] According to a theory,[87] high grafting density ensures lower equilibrium amounts of the adsorbed protein, whereas long segments exposed to the solution considerably slow down the adsorption. It was shown experimentally that many brush and chemisorbed coatings based on PEO[1,6,89] and other neutral water-soluble polymers (dextrans,[90] N-substituted polyacrylamides[1,91]) do indeed have high resistance, which reduces the proteins adsorption on solid supports by factors of tens.

In view of the foregoing, one could expect that dehydration of smart polymers at temperatures above the LCST would enhance their adsorption capacity for organic compounds including proteins. In reality, answer to the question of whether proteins are able to attach to smart polymers existing in the dehydrated insoluble (for gels and particles) or collapsed state (for grafted chains) is still not quite unambiguous. Ivanov *et al.*[92] and independently Lakhiari *et al.*[93] investigated chromatography of standard proteins (lysozyme, immunoglobulin,[92] albumin[93]) on the porous glass or silica gel coated by chemisorbed NIPAA-based copolymers and concluded that dehydration and conformational transition of the grafted polymer influence the protein adsorption only to a minor extent, the yield of proteins applied on the column is nearly quantitative and elution occurs with a short retention time, which passes a maximum at about 35 °C , *i.e.*, slightly above the LCST of soluble PNIPAA.[92] The adsorption resistance of these supports might be caused by structural features of the chemisorbed layers of the modifying polymers (see Fig. 8 *a*,*b*), *i.e.*, by the dense coating of the support by the NIPAA copolymer[92] containing rare loops and tails the transitions of which do not affect significantly the protein adsorption.

The influence of the hydrophobic-hydrophilic balance in amino acid molecules on their interaction with PNIPAA gels has been studied.[94] It was found that adsorption of amino acids with hydrophobic side chains, such as valine, and leucine, increases to a much higher extent on going to temperatures above the LCST than in the case of more hydrophilic glutamic and aspartic acids, apparently, due to more pronounced hydrophobic interactions of the former with the dehydrated PNIPAA segments. During chromatography of steroidal hormones on silica gel with grafted PNIPAA, the retention of hydrophobic testosterone was much stronger than the retention of more hydrophilic hydrocortisone[39] both below and above the LCST. Grabstain and Bianco-Peled[95] investigated the adsorption isotherms of bovine albumin, ovalbumin and lysozyme on cross-linked PNIPAA microparticles. The swollen microparticles (25 °C) sorbed markedly more proteins per unit weight of dry polymer than the same particles in the collapsed state at 37 °C; in the latter case, the difference between the contents of adsorbed proteins was insignificant. However, calculation of the amount of adsorbed lysozyme for unit weight of a wet gel resulted in a concentration of the adsorbed protein being 1.5 – 2.5 times higher in the collapsed gel. In the authors' opinion, this is caused by hydrophobic interaction of the protein with the dehydrated polymer; in this case, the adsorption sites are mainly located on the surface of microparticles, unlike the swollen gels in which the internal space is more accessible for protein molecules. Evidently, the volume compression effects hamper the strict interpretation of the obtained adsorption data.

Analysis of the catalytic activity of some enzymes immobilized in smart polymer gels is of interest as an independent experimental method for investigation of smart polymers. Eremeev and Kazanskaya,[96] who studied the properties of chemotrypsin embedded in the PNIPAA gel at temperatures below and above the phase transition temperature of this polymer, observed a 2 – 5-fold reversible decrease in the catalytic activity of chemotrypsin in the temperature range of 30 – 50 °C. The assumptions about a role of diffusion restrictions for substrate molecules and steric restrictions hampering their interaction with the enzyme active site were ruled out considering the dependences of the catalytic activity on the content of enzyme in the gel and the results of experiments with the irreversible proteinase inactivator. Adequate explanation is only nonspecific association of chemotrypsin with the dehydrated PNIPAA, resulting in a partial reversible enzyme inactivation. Temperature dependences of the same type were also obtained for immobilization of the enzyme urease.[97] Morisada *et al.*[98] investigated sorption of serum albumin in cross-linked PNIPAA particles and in similar particles containing tertiary and quaternary alkylamino groups, which are positively charged under the experimental con-

ditions. No protein adsorption took place on neutral particles at either 25 or 40 °C, whereas anion exchange particles were more adsorptive, the adsorption being higher at 25 °C than at 40 °C, which was attributed to compression of particles and lower accessibility of their pores at elevated temperature.

The above data provide the conclusion that switching from the hydrated to dehydrated state of surface-grafted or cross-linked PNIPAA chains, most often, increases only slightly and, in some cases, does not increase at all their adsorption interactions with proteins, as opposed to amino acids and steroids. This is attributable to the fact that the hydrophobic groups of proteins are mainly located inside the macromolecules and, therefore, they are less accessible. Typically, a considerable enhancement of these interactions with temperature rise was attained by using the NIPAA copolymer with the more hydrophobic butyl methacrylate (BMA) monomer, instead of PNIPAA, to chemically modify the silica gel surface.[39] It was noted above that the presence of hydrophobic groups in organosilane sublayers can strongly affect the wettability of the PNIPAA-modified surface[79] and the temperature dependence of the wettability. Apparently, a similar effect is induced by the presence of BMA in the smart polymer, providing more pronounced control over the surface free energy and the related protein adsorption upon variation of the temperature.

Huber's paper[11] is among the most cited publications demonstrating the possibility of temperature-controlled adsorption and desorption of proteins on the grafted PNIPAA surface. The authors performed the chemical grafting of PNIPAA into a microfluidic device bearing alkanethiol groups using chain transfer to these groups in the radical polymerization of NIPAA in a contacting solution. The possible reaction mechanisms are discussed in more detail by Ivanov *et al.*[99] The process gave a grafted PNIPAA layer with a surface area per chain of ~ 10 nm^2 (*i.e.*, not very dense, $\sigma \approx 20$). Using the surface-force measurements, the effective thickness of the PNIPAA layer was found to be ~ 100 nm at temperatures of 20 and 23 °C and to decrease to 50 nm at > 26 °C. The PNIPAA grafting prevented the irreversible adsorption of proteins, in particular myoglobin, at 20 °C on the surface of silicon nitride, a basic material of the microfluidic device.

A study by ellipsometry has demonstrated that adsorption of serum albumin on polymer-modified silica surfaces decreases 10 – 15-fold as compared with the initial non-modified surfaces (25 °C) but increases 20 – 30-fold as the temperature is raised to 55 °C. A 3 μl micro-hot plate device was heated with heat being transferred by arrays of 10 to 50 μm wide gold or platinum heater lines deposited on a thin silicon nitride layer. Heating of the heater lines to 35 °C lasted for less than a microsecond, which is much shorter than the time required for the formation of the protein adsorption layer. Fluorescein-labelled myoglobin was taken as the protein sorbate to study the temperature-dependent adsorption. Using fluorescence microscopy, it was demonstrated that the protein adsorption layer is formed directly above the lines heated to 35 °C. As soon as in a second after heating was terminated, myoglobin was desorbed and diffused to the contacting aqueous solution. Thus, grafted PNIPAA enabled the fabrication of a high-response microfluidic circuit for temperature-controlled protein adsorption and desorption. This design opened up the way for developing a series of controlled analytical devices,[8, 100 – 102] including those enabling controlled binding and detachment of viable animal cells.[8, 100]

The adsorption of myoglobin on silica gel chemically modified with PNIPAA was studied in detail.[103] The adsorption of this protein on silica gel modified with trimethoxy-3-mercaptopropylsilane $[(MeO)_3Si(CH_2)_3SH]$ was fairly high (90 mg g^{-1} or 0.3 mg m^{-2});[103] hence, this sublayer did not itself prevent myoglobin and other proteins from adsorption. As a result of PNIPAA grafting, *i.e.*, owing to the formation of a polymer coating, the amount of protein adsorbed on silica gel decreased 15 – 30-fold. A temperature decrease from 35 to 9 °C resulted in myoglobin release from polymer-coated silica gel into the contacting solution with a ~ 2-fold decrease in the adsorbed amount of the protein. One more heating and cooling induced again binding and release of the protein. Particular attention is attracted by the pattern of adsorption isotherms of myoglobin, which were extrapolated, in the region of low protein concentrations, to the same value of ~ 1 mg g^{-1}. This attests to myoglobin adsorption on the residual outcropped regions of the support surface occurring to the same extents at 9 and 35 °C (Fig. 11). Therefore, it was concluded that thermally dependent protein binding occurs particularly on the dehydrated PNIPAA chains. In view of the statements at the beginning of this section and at the end of the previous one, it appears likely that the temperature-dependent reversible protein adsorption occurs first of all on PNIPAA segments that adjoin the hydrophobic sublayer at temperatures above the LCST and are subject to the most pronounced reorientation as a result of hydration on cooling (see Fig. 8 *c*).

A similar conclusion was drawn by Fukumori *et al.*,[104] who implemented adhesion of endothelial cells to PNIPAA-modified glass and polystyrene surfaces *via* their binding to the pre-adsorbed fibronectin — a high-molecular-weight intercellular matrix glycoprotein. Fibronectin was adsorbed at 37 °C on relatively thin grafted PNIPAA layers (< 4 nm) but was not adsorbed on layers of the same polymer of 7 nm or greater thickness. The authors believe that grafted PNIPAA chains are promoted to be dehydrated near the polystyrene surface owing to the hydrophobic properties of the support.[104]

This conclusion is evidenced by the dependences of the serum albumin and fibronectin adsorption on the PNIPAA grafting density, which were investigated by Xue *et al.*[19] The number of functional groups initiating the ATRP was controlled by varying the ratio of the organosilyl reagents taken for the chemical modification of flat glass or silicon plates, namely, organotrichlorosilane bearing a short oligoethylene glycol (6 – 9 monomer units) and 11-(2-bromo-2-methylpropionyl)dodecyltrichlorosilane, which initiates polymerization. This resulted in the fabrication of a range of supports with PNIPAA grafting densities of 0 to 0.2 chains per nm^2, which implies that the average surface area per chain is 5 nm^2 and $\sigma \approx 10$ (M from 2.7×10^4 to 4.8×10^4 g mol^{-1}). The PNIPAA grafting on the mixed organosilane sublayer had virtually no influence on the protein adsorption at 23 °C, which amounted to 0.2 – 0.3 mg m^{-2} for conditions of hydrated PNIPAA chains. Meanwhile, the adsorption of proteins at 37 °C depended substantially on the PNIPAA grafting density when the density was low and corresponded to an intermediate state between a brush and isolated chains; in particular, for albumin this value reached 0.9 mg m^{-2}.

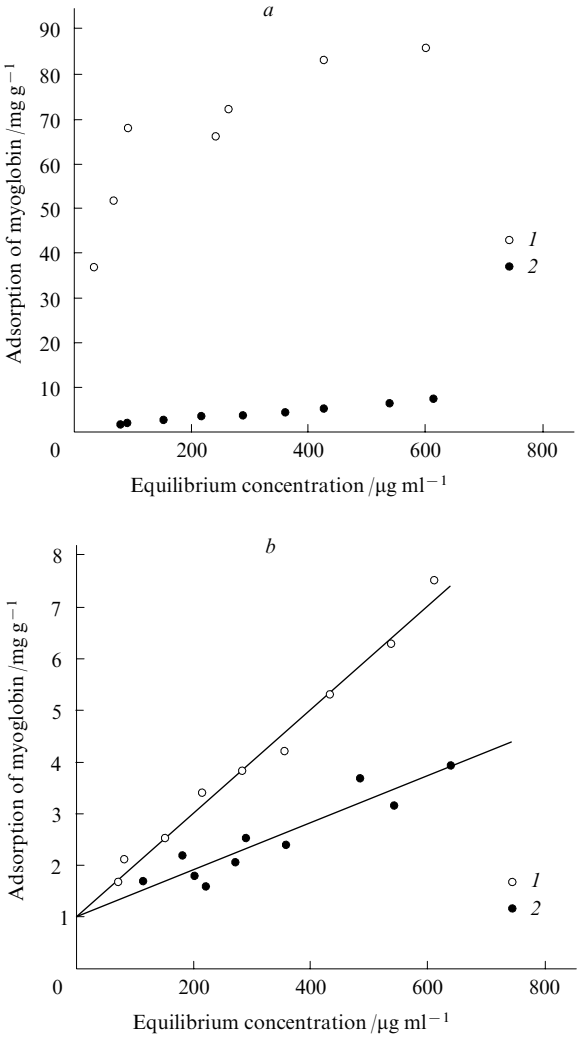


Figure 11. Adsorption isotherms of myoglobin on the silica gel Kromasil 100 (*1*) and on the same silica gel with grafted PNIPAA (*2*) from a 10 mM phosphate buffer solution containing 0.1 M NaCl (pH 6.0) at 35 °C (*a*) and myoglobin adsorption isotherms on silica gel with grafted PNIPAA at 35 (*1*) and 9 °C (*2*) (*b*).[103]

The authors attributed this phenomenon to interaction of PNIPAA chains with the protein molecules that partly penetrate into the polymer layer, because at high PNIPAA grafting density, this interaction is weak or vanishing. In our opinion, at relatively low grafting densities, surface micro- and nanoheterogeneities increase, which promotes adsorption of grafted PNIPAA segments at temperatures above the LCST on the hydrophobic sublayer containing a dodecyltrichlorosilane derivative and desorption of the segments below the LCST, and this accounts for the variations of the protein adsorption like those reported in several papers [11, 103, 104] and illustrated in Fig. 8 *c*,*d*.

VII. Temperature-dependent transitions in the surface-grafted smart polymers: adhesion and detachment of biological cells and tissues

Development of methods for animal cell culturing on PNIPAA-modified surfaces [32–34] has transformed into a separate research area of biological materials science comprising diverse methods of grafting of PNIPAA and NIPAA-based copolymers, culturing of diverse cell lines and preparation of morphologically and functionally variable confluent cultures. The most popular methods for PNIPAA coating of tissue culture polystyrene plates or modified flat glasses include electron beam-induced polymerization of NIPAA in an organic solvent and plasma polymerization.[105] The former technique gives an ultrathin layer of cross-linked PNIPAA gel the thickness of which on the polystyrene plate increases with increasing concentration of NIPAA and ranges from 15 to 30 nm,[106] while the layer thickness on a (3-methacryloylpropyl)trimethoxysilane-modified glass plate varies from 3 to 9 nm.[104] It is noteworthy that according to Akiyama *et al.*[106] and Fukomori *et al.*,[104] adsorption of fibronectin and the subsequent adhesion of endothelial cells take place only in the case of very thin (15 nm) PNIPAA layers having high hydrophobicity: at 37 °C and thicknesses of 15 and 30 nm, the contact angles were 78° and 65°, respectively, and the latter layers were not active towards cell adhesion. The macroscopic PNIPAA gels chemically cross-linked with *N*,*N*′-methylenebis(acrylamide) with a contact angle of 50° were not adsorption- and adhesion-active at 37 °C either,[106] which is in line with the results of other publications.[92, 93, 98] A decrease in the thickness of a grafted PNIPAA layer on polystyrene to <15 nm increased the adhesive activity but brought about the problem of incomplete detachment of confluent layers of cultured cells on cooling,[104] so that the optimal polymer layer thickness was 15–20 nm.

A cross-linked temperature-responsive coating is also formed upon plasma polymerization of NIPAA. The PNIPAA grafting gives in this case thicker layers of cross-linked gel (73 nm at 20 °C and 64 nm at 40 °C in water) with much lower contact angles (34° at 20 °C and 40° at 40 °C).[107] It is evident that the surface-grafted gels obtained by plasma polymerization undergo much less sharp structural transitions than polymer brushes, in which the grafted layer thickness can change 2–3-fold and the contact angle can change by 25–35° (see Figs 7–9). Nevertheless, by culturing endothelial cells at 37 °C, one can obtain a confluent monolayer culture, which can further be detached from the substrate by lowering the temperature to 25 °C.[108]

It is worth noting that the interest in the preparation of the above-described ultrathin gels is due, first of all, to the possibility of their mass production under standard conditions.[104] Meanwhile, it was shown that relatively simple synthetic approaches, *e.g.*, solvent casting of non-cross-linked NIPAA copolymers with *tert*-butylacrylamide on tissue culture polystyrene plates, ensure both efficient cell adhesion and growth and subsequent detachment of the resulting cultures, however, in the presence of proteins such as collagen and laminin promoting cell adhesion.[109] Unlike PNIPAA gels obtained by plasma polymerization, in this case, the thickness of the polymer coating is not crucial for cell adhesion and division and can vary in the range from 30 nm to 2 μm.[110] The polystyrene plates coated by a deposited PNIPAA layer were used for culturing mesenchymal stem cells, which remained undifferentiated in the obtained monolayer cultures, as indicated by the composition of the surface cellular receptors in the cultures detached from the substrate at reduced temperatures.[110] Similar results were independently obtained in another study.[111] Targeted differentiation of stem cells holds great promise

for therapeutic applications; the possibility of obtaining monolayer cell cultures that can be differentiated, if required, appears attractive.

A moderate number of publications are devoted to cell adhesion on the PNIPAA brushes obtained by graft polymerization by the ATRP mechanism.[19, 112] The authors of the above-cited paper [19] studied the adhesion of 3T3 fibroblasts on PNIPAA-containing surfaces with different polymer grafting densities. Like in the adsorption of proteins, cell adhesion on PNIPAA brushes with rather high grafting density (0.2 chains per nm^2, $\sigma \approx 10$) at 37 °C was lower than that observed at low grafting density. Moreover, in the former case, cells had a round shape, while in the latter case, they showed a tendency towards focal adhesion, indicating involvement of interactions between the extracellular matrix and the actin cytoskeleton, which are needed for cell migration and division.

It is important that detachment of single cells and confluent cell cultures from the temperature-responsive polymer substrate taking place on lowering the temperature is caused not only by mechanical lifting resulting from polymer hydration and swelling but also by the spatial reorientation of cellular receptors, signal transduction and the subsequent metabolic processes in the cells. This is indicated, in particular, by the fact that the cell detachment is suppressed upon inhibition of tyrosine phosphorylation in the cell — a process involved in signal transduction from cellular receptors, integrins. The cell detachment was also retarded upon stabilization of F-actin, a key component of cytoskeleton essential for cell mobility and compression during division.[113] Finally, detachment of cells can be suppressed by inhibiting the adenosine triphosphate synthesis.[105] The last-mentioned fact indicates that cells spend their own energy for the detachment. One more piece of evidence indicating a contribution of cell metabolism energy to the detachment of cells is that in some cases, cells are detached more completely at room temperature than at 5 – 10 °C,[113] despite better swelling of PNIPAA at low temperatures.

During the growth, adhered cells express the intercellular matrix proteins, which are specific to each cell line and acquire mobility upon conformational or phase transitions in the polymer substrate. During the temperature-initiated detachment of confluent cultures, proteins can be partly adsorbed on the substrate surface, which was confirmed in a number of publications (see, for example, Refs 108 and 113). The matrix proteins mainly remain in the detached monolayer culture, which is beneficial for retention of important membrane proteins, including expressed cell markers.[110, 111] Apart from the above-described monolayer cultures, multi-layer cell cultures were also obtained, in particular, keratinocytes, epidermis cell cultures, which were clinically used for treating burns. In addition, multilayer cultures of cardiomyocites, heart muscle cells, were prepared.[34] The subcutaneous transplantation of these cultures into animals induced the formation of a new tissue incorporating blood vessels. Moreover, the transplantation of double-layer cardiomyocite cultures served to restore the heartbeat function at myocardial infarction.[34]

Subsequently it was shown that transplantation of autologous (*i.e.*, taken from the patient) myoblast cultures resulted in the appearance of intercellular communications between the transplanted and body cell sheets and synchronizing of their electrical potential pulsing as soon as 30 – 45 min after transplantation and in general activation of the injured cardiac tissue repair.[114] Culturing on PNIPAA-coated substrates gave multi-layer mucosal epithelial cell cultures (Fig. 12). One culture was 50 – 60 μm thick and comprised 3 to 5 layers of stratified cells (see Fig. 12 *c*). The resulting autologous cell cultures were successfully used for transplantation and subsequent tissue regeneration, in particular, for treating oesophageal ulcer.[114]

Apart from the above-described methods for animal cell culture on smart polymer coatings, of considerable interest are procedures for cell separation and analysis based on their reversible specific adhesion to functionally active smart polymers. One approach to cell fractionation is the above-mentioned binding to antibodies adsorbed on a polypropylene (PP) membrane coated by plasma-polymerized PNIPAA.[36] This approach was further developed by fabrication of 3D porous substrates from PP fibres for animal cell culturing.[115]

One more approach, which is becoming more and more popular, is controlled cell adhesion on polymer brushes containing immobilized boronic acids. In a number of studies [8, 116 – 118] carried out with the participation of one author of the present review, cross-linked polysaccharide particles and yeast or animal cells were found to bind to these substrates by forming boronic esters with the surface

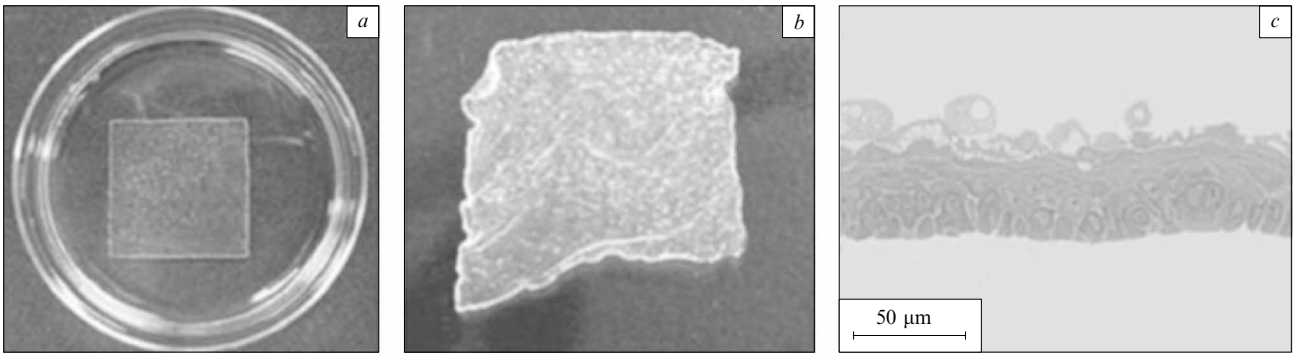


Figure 12. Transplantable oral mucosal epithelial cell cultures.[114]
(*a*) Confluent epithelial cells cultured in square patterns with grafted PNIPAA at 37 °C on polystyrene Petri dishes; (*b*) the same culture detached from the dish at 20 °C without enzyme treatment; (*c*) Toluidine blue staining shows cell stratification in the multi-layered culture.

oligosaccharides of the particles and cells at pH 8 and higher, *i.e.*, when phenylboronic acid (PBA) is partly ionized [pK_a = 9 (see Ref. 119)] and quite a number of reactive phenylboronate anions are present. Both the particles and cells can be detached from substrates by treatment with solutions of sugars and polyols (fructose, sorbitol), possessing high affinity for phenylboronates and competing for bonding to oligosaccharides. The binding constants of monosaccharides and polyols to borates and phenylboronate have been reported.[120] It is noteworthy that animal cells could not only adhere to boronate-containing polymers but also be cultured on glass plates bearing polymer brushes of the indicated composition. The viability of hybridoma cells of the M2139 line adhered on these substrates was confirmed by the MTT assay. The cultured cells could be subsequently detached by a solution of fructose with 70% retention of the viability.[8] The possibility of specific binding of yeast cells on electrodes bearing immobilized boronic acid followed by desorption in the presence of fructose was reported.[121] The immobilization of *m*-aminophenylboronic acid on gold electrodes treated with 10-carboxydecane-1-thiol resulted in the design of a sensor for the recognition of sialic acid (Neu5Ac), which is abundant on the metastatic melanoma cell surface.[122] The proposed method for the detection of this sugar can underlie development of an assay for tumour malignancy.

The adhesion of MCF-7 breast cancer cells to the poly(3-acrylamidophenylboronic) acid brush grafted on silicon nanofibres pretreated with (3-mercaptopropyl)trimethoxysilane (by analogy with methods considered in other papers [11, 116]) has been studied.[100] The cells were attached to the substrate at pH 6.8 and 7.8 and could be detached at pH 7.8 in the presence of 70 mmol litre^{-1} of glucose. It is noteworthy that cell adhesion at pH 6.8 under conditions of low ionization of PBA took place in the presence of 70 mmol litre^{-1} of glucose, *i.e.*, the cells could be detached by merely increasing the pH. The authors attributed this behaviour to the complex formation of sialo-containing oligosaccharides of cell glycocalyx with unionized PBA at pH < 7.[100]

This concept was spread in some other papers [122–124] owing to the fact that free sialic acid, unlike other sugars, reacts with phenylboronic acid at relatively low pH, namely, at pH 4–7. It was stated [124] that the product of reaction of PBA with the glycerol group of Neu5Ac is stabilized by the coordination interaction between the boron atom and the *N*-acetyl group of Neu5Ac. Djanashvili *et al.*[125] argued against this mechanism, pointing out that the reaction at pH < 8 is due to the association of the phenylboronic acid diol with the α-hydroxycarboxylate group of Neu5Ac. The terminal residues of Neu5Ac were attached to the neighbouring saccharide units particularly *via* the α-hydroxyl oxygen atom of the glycoside; hence, this atom cannot be involved in the reaction. The mechanism of binding of Neu5Ac to PBA at pH < 8 remains obscure. The fact of this binding itself looks plausible, as adhesion of desialylated red blood cells on a gold electrode with immobilized PBA at pH 7.4 induces much less pronounced changes in the electrode potential than adhesion of the native cells.[123] Therefore, it is stated in several papers [122–124] that, when interacting with animal cells in neutral and weakly acidic media, boronate-containing reagents selectively bind to sialic acid. Note that adhesion of yeast cells devoid of sialic acid to boronate-containing grafted polymers at pH 7 is indeed weak, unlike adhesion at pH 8–9.[117]

The possibilities for preparative separation of animal cells *via* reversible adhesion to grafted boronate-containing polymers have been demonstrated.[126] The substrates used were wide-pore polyacrylamide cryogels with a pore diameter of up to 100 μm, which is sufficient for penetration of both yeast cells and animal cells inside the substrate. Substrates of this type are widely used for separation and culturing of animal cells.[127–131] The NIPAA copolymer with *N*-acryloyl-*m*-aminophenylboronic acid (1 : 1) was grafted by radical polymerization initiated by the diperiodocuprate (trivalent copper compound), which was used previously for grafting water-soluble monomers on polyacrylamide substrates.[132] The adhesive properties of the cryogel were demonstrated using yeast cells (Fig. 13). It was shown that the surface-dependent cells (Cos 7 line fibroblasts), like surface-independent CC9C10 and D9D4 hybridoma cells, are readily adhered to grafted boronate-containing NIPAA copolymers. The hybridoma cells could be detached from the substrate by elution with a 0.1 M fructose solution (pH 7.2), whereas detachment of fibroblasts required fructose concentration of 0.5 mol litre^{-1}. This substantial difference between the elution conditions can be dictated by different compositions and amounts of the surface glycoproteins in the cells.

Thus, by stepwise elution, it is possible to separate surface-dependent and surface-independent animal cell lines. It is noteworthy that the temperature-dependent elution on heating to 37 °C allowed detachment of only 30%–40% of the cells. A probable reason for this is slow dissociation of boronate glycoprotein complexes. The boronate-containing NIPAA copolymers are known to lose the ability to undergo sharp phase transitions in the presence of mucin glycoprotein, whereas the presence of monosaccharides merely increases their LCST.[120] The mucoadhesive properties of boronate-containing copolymers have been studied by Ivanov *et al.*[133]

After the publication of Srivastava *et al.*,[126] the preparation of thin gels formed by polyvinyl alcohol and boronate-containing polymers was reported.[134] The gels were deposited on a surface of polystyrene plates, and NIH3T3

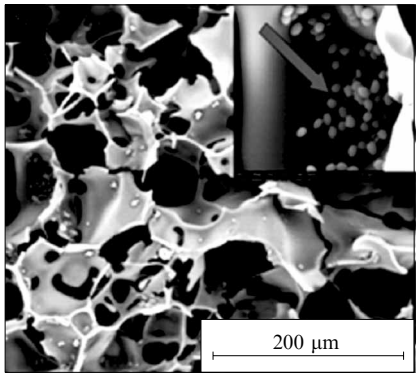


Figure 13. Photomicrograph of the polyacrylamide cryogel with the surface modified by grafted boronate-containing copolymer, which holds yeast cells.[126]
The arrow in the inset points to the cryogel area with high cell density.

line fibroblasts were cultured on the gel surface. In several days, the fibroblasts reached the confluent structure and then the gels were partly dissolved in a 0.2 M fructose solution, and the monolayer culture was separated from the gel. Thus, the researchers found a new method for non-enzymatic detachment of these cell cultures from substrates alternative to the above-described detachment from thermo-responsive PNIPAA-containing surfaces.

VIII. Surface-grafted polymers and gels in the materials and methods of bioanalysis

The sensing elements of modern bioanalytical devices such as surface plasmon resonance (SPR) spectrometers, quartz crystal microbalance (QCM) and precision interferometers, including spectral correlation interferometers (SCI), comprise biomolecular ligands immobilized on solid support surfaces and responsible for specific recognition and binding of analytes, their complexes (for example, immune complexes) or biological cells. The supports perceive weak generated signals and transduce them to stronger signals that can be recorded by electric systems. Examples of such supports (transducers) are thin gold coatings (in the SPR method), quartz crystals (in the QCM method), planar glass plates (in the SCI method) and capillaries. All these materials are capable of strong non-specific sorption of proteins, lipoproteins, phospholipids and other components of physiological fluids and foodstuffs, which decreases the specificity and accuracy of analysis. In this connection and in accordance with the views outlined in Section VI, quite a few attempts were made to endow gold, glass and quartz with adsorption resistance by surface grafting of various hydrophilic polymers, both neutral and zwitter-ionic ones. The latter also considerably decrease the adsorption activity of solid supports.[135]

Previously, PEO- and dextran-containing coatings were used to prevent non-specific sorption of biopolymers on the surfaces of biosensing devices.[90, 136] Recent studies have been concentrated on the controlled graft polymerization of oligoethylene glycol methacrylate (OEGMA)[6, 137] and other related monomers.[138] Atom transfer radical polymerization of OEGMA initiated by a bromoisobutyric ester, which forms a monolayer on the gold plate surface for the surface plasmon resonance, has been reported.[6] The polymerization gives grafted polymer layers with thickness of up to 50 nm, which is virtually unattainable upon chemical immobilization of PEO. Despite the high sensitivity of the SPR method, it did not show adsorption of proteins from a 10% or 100% blood serum or from a fibronectin solution (1 mg ml^{-1}), which attests to high surface resistance of the resulting coating. In the case of PEO (M = 2000 g mol^{-1}) with terminal amino groups immobilized on organosilylated glass plates, the non-specific adsorption of DNA decreased by a large factor, while the yield of DNA hybridization reaction with PEO-immobilized oligonucleotides simultaneously increased fourfold.[139] The decrease in the non-specific adsorption of DNA is needed to suppress noise signals in the design of biosensors tuned for highly selective binding *via* DNA hybridization with immobilized oligonucleotide sets.[140] These sets, called biosensor microarrays, contain a large number of single-type microcells; they are widely used for high throughput screening — simultaneous analysis of a large number of samples.[141] The immobilized specific ligands, including oligonucleotides, can be supported by both polymer brushes and cross-linked microgels, which are used, for example, for immobilization of bacterial cells and the subsequent fluorescence analysis of intracellular process, which can serve for quantification of antibiotics.[142]

Although the conformational transitions of grafted PNIPAA have been described in detail by SPR and QCM methods, the use of smart polymers to modify the transducer surfaces is still not extensive. The reasons are not quite clear; thin polymer hydrogels with specific ligands and/or dyes have been repeatedly studied and used as optical sensors for sugars,[143] amines and alcohols.[144] The enzyme immobilization into thin gels containing pH-sensitive dyes and the subsequent photometric measurement of catalytic processes has turned into a popular method for detection of enzyme substrates and substrate-like compounds.[145] Furthermore, monolayers of amphiphilic polycations with embedded enzymes have been repeatedly used to modify the electrode surfaces for potentiometric recording of molecular recognition processes.[146 – 148] Apparently, graft copolymerization of NIPAA with functionally active monomers, at least ATRP, is faced with certain difficulties, because solvation of copper(I) complexes with amine-containing ligands and, the more so, substitution of these ligands, have a substantial influence on the reductive potential of complexes and, hence, the chain propagation constant.[149]

We would like to note one more widely used bioanalytical method in which the application of smart polymers has not received wide acceptance as yet, namely, capillary electrophoresis (CE). Some water-soluble polymers (both cationic and neutral) that are used as working buffer components for the protein CE prevent their irreversible adsorption on the quartz capillary surface as a result of dynamic modification of the surface.[150] It was shown that the phase transition in solutions of PEO – PPO – PEO type copolymers taking place as temperature increases from room temperature to 38 °C makes it possible to control the viscosity of the mobile phase and, hence, to optimize separation of oligonucleotide and DNA mixtures.[151] In our opinion, solutions of temperature-responsive polymers could be used in the preparative CE to separate the purified compounds from the polymeric modifier by its thermal deposition from solution.

Turning back to smart polymer composites, note that particular researchers' attention was drawn by gold nanoparticles embedded in NIPAA-based polymer hydrogels[152] or in microparticles of these gels.[153] Upon compression of gels induced by heating or a change in the aqueous phase composition, the distribution density of immobilized gold nanoparticles increases, which results in a red shift of the plasmon resonance frequency.[153]

Spectral correlation interferometry has been used[99, 154] to study the conformational transitions of the *N*,*N*-dimethylacrylamide copolymer with *N*-acryloyl-*m*-aminophenylboronic acid (NAAPBA) (9 : 1) grafted on planar glass surfaces. Transitions are caused by enhancement of phenylboronic acid ionization following the increase in the pH from neutral to 9.2 and are accompanied by an increase in the polymer brush thickness, which reversibly decreases as pH is lowered. It is noteworthy that in the ionized state (pH 9.2), the dissolved and grafted copolymers spontaneously bind mucin glycoprotein, which corresponds to the conditions of adhesion of animal cells on similar substrates[8, 118] and attests to the probable mechanism of this adhesion. The

20 nm-thick poly-NAAPBA brushes were recently prepared on quartz crystals and studied by QCM as sensors for glucose.[155] No binding of this polymer to fructose, which is the major competing saccharide, was detected by this method, which allowed for selective measurement of the glucose concentration.

Several alternatives for chemical modification of planar glass surfaces meant for immobilizaion of specific antibodies and application in the SCI method were studied with the goal of optimizing molecular recognition and binding cardiac troponins — regulatory proteins that present interest for diagnosing myocardial infarction and other heart diseases.[156] By using the SCI method, it is possible to compare the thicknesses of immobilized antibody layers directly during their chemical attachment to the activated surface and, hence, to evaluate the efficiency of immobilization. The specific binding of troponins was monitored based on the gain of the adsorption layer thickness, which increased linearly with increasing logarithm of the analyte concentration and amounted to ~3 nm at saturation. The subsequent attachment of the biotin-labelled detecting antibodies induced an additional gain of the adsorbed protein layer thickness. The sensitivity of secondary detection of troponins was markedly increased by using magnetic nanoparticles bearing the same detecting antibodies. The specific binding of these magnetic nanoparticles to antigens can be revealed by using the non-linear magnetization effect.[157] Using this method, picogramme amounts of staphylococcal toxins were determined in a very complicated medium such as neat milk.[158]

Magnetic nanoparticles (MNPs) bearing grafted polymers, mainly PEO, are widely used as contrast agents for magnetic resonance diagnostics[159] and as carriers for targeted drug delivery.[160] A popular method is magnetic separation of animal cells *via* their binding to specific antibodies immobilized on magnetic particles coated by PEO or other polymers.[161] The key applications of MNPs in medicine and physical principles of their use were summarized in the review of Nikoforov,[162] while Pershina *et al.*[163] covered methods of chemical conjugation of MNPs with nucleic acids and their use in bioanalysis and bioseparation. Owing to large specific surface area, MNP can adsorb large amounts of proteins and other organic compounds.[162, 164] The adsorption is more selective if the polymer modifiers bear reactive groups. Recent studies[165, 166] dealing with grafting of metal-chelating polyelectrolytes on MNPs demonstrated the possibility of extracting pharmaceutical drugs and heavy metal ions from physiological fluids for subsequent quantitative analysis.

A large group of publications are devoted to chemical modification of MNPs with smart polymers or incorporation of these particles into microgels. These dispersion systems were developed, first of all, for targeted drug delivery under the action of magnetic field and subsequent drug release in the target organs by local temperature rise. For this purpose, magnetite nanoparticles coated by amorphous silica were obtained and then chemically modified with (3-methacryloylpropyl)trimethoxysilane and copolymerized with NIPAA in the presence of a bifunctional cross-linking agent. This resulted in cross-linked PNIPAA particles of a nearly spherical shape (300–500 nm in diameter) with a magnetite core inside.[167] As the temperature increased from 20 to 50 °C, the obtained composite particles were compressed (the diameter decreased 1.5–2-fold), while on cooling they restored the initial size.

The superparamagnetic properties of magnetite can serve for fast preconcentration and deposition of a particle suspension by using a permanent magnet. Liu *et al.*[168] studied composite particles of this type as thermo-responsive carriers of zinc phthalocyaninesulfonate — a known agent for the photodynamic therapy of cancer. The bulk phase transition of the gel shell consisting of the NIPAA copolymer with *N*-hydroxymethylacrylamide induces release of this agent; this release is minor at temperatures of 25–37 °C and sharply increases at a temperature of 39 °C corresponding to a two- or three-fold decrease in the particle diameter.

The reversible swelling and compression of the polymer shell was also studied for MNPs coated with the thermoresponsive PEO–PPO–PEO block copolymer modified with polyethyleneimine (PEI).[169] As opposed to the results of the above-cited work,[168] the drug release was found to take place on cooling in the range from 35 to 20 °C rather than on heating, *i.e.*, upon swelling of the polymer shell of the carrier particles. Apparently, this difference is attributable to different mechanisms of drug binding: whereas binding to the *N*-substituted polyacrylamide is due to a combination of polar and hydrophobic interactions by analogy with the formation of poly(*N*-vinyllactam) complexes with aromatic compounds,[37, 170, 171] in the case of PEO–PPO–PEO block copolymers, occlusion of organic molecules during the copolymer collapse at elevated temperature and release of these molecules at lower temperature are most likely (Fig. 14).

The PNIPAA-coated magnetic nanoparticles were also studied for magnetic separation of cells. Biotinylated particles were attached to avidin and then to biotin-modified antibodies located on the surface of yeast cells. By temperature-controlled aggregation of particles and application of magnetic field, MNPs with bioaffinity-selected cells were concentrated and separated from the cells devoid of the antibodies.[172] Similar approaches were later also used to separate animal cells. In mouse experiments, neutrophils

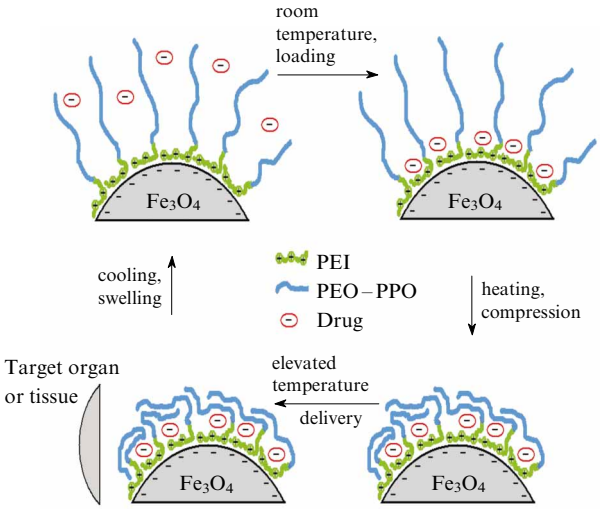


Figure 14. Drug loading of composite magnetic particles and drug release on lowering the temperature.[169]

were separated from macrophages by binding the latter to magnetic particles with a thermo-responsive coating containing immobilized F4/80 antibodies specific to macrophages.[173] The particles aggregated on heating to 30 °C and precipitated together with the bound cells under the action of magnetic field. Thus, the fraction of neutrophils, which are more labile and fragile cells, could be substantially enriched *via* negative selection. The resulting cells were then activated and used to study cytokine secretion processes.[174]

IX. Conclusion

Summarizing the above, we can draw several conclusions. The ability of smart polymers to change their state of aggregation in response to minor changes of the environmental conditions, in particular, temperature, as a result of reversible hydration – dehydration of polymer chains is retained to a large extent for polymers grafted on a surface through a terminal group and, to a lesser extent, for chemisorbed polymers.

The relevant conformational transitions were studied by a variety of physicochemical methods including AFM, surface-force measurements, ellipsometry, neutron reflection and protein adsorption measurements. A general trend is compression of the grafted polymer upon temperature rise accompanied by an increase in its micromechanical rigidity and, in the case of a moderate grafting density, by lateral segregation of the grafted layer. The corresponding theoretical predictions were in some cases qualitatively or semi-quantitatively confirmed.

At high grafting density, the conformational and aggregative transitions of PNIPAA, which is the best studied smart polymer, are less clear-cut than in the case of moderate or low grafting density. Meanwhile, segregation of grafted polymer layers opens up the possibility of controlling adsorptive and adhesive behaviours of the surface by means of reversible partial outcrop of the substrate surface having adsorption-active organosilane or alkanethiol sublayers needed for chemical grafting of polymers. This phenomenon was used for controlled protein adsorption and animal cell adhesion, culture and detachment upon temperature rise. The possibility of controlling adhesive properties of polymer layers was found to depend on both grafted layer thickness and grafting technique. The efficiency of cultured cell detachment is determined by not only the hydration and swelling of the grafted polymer but also by signalling processes and changes in the intracellular metabolism as a result of temperature-dependent reorientation of the surface cellular receptors.

An interesting line of research is reversible adhesion of bacterial and animal cells on boronate-containing polymer brushes, which opens up broad prospects for culturing, immobilization and detachment of these cells under the action of sugars, *i.e.*, under gentle conditions close to physiological ones. The reactions of boronate-containing polymers with surface cellular glycoproteins and intercellular matrix may underlie targeted drug delivery. The use of smart polymers as surface modifiers and recognition elements of biosensors is a prospective and not yet adequately developed area of research. The same is true for smart polymer-coated magnetic particles as drug carriers.

Evidently, the outlined research areas will be vigorously developed in the near future. Generally, the opportunities for designing controlled nano-, micro- and macrosystems based on smart polymers are, beyond doubt, highly promising.

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