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Polarity and Temperature-Dependent Properties of Poly(*N*-isopropylacrylamide) and Poly(*N*,*N*-diethylacryl-amide) Hydrogels Studied by Liquid Chromatography

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Summary: Superporous *N*-isopropylacrylamide (NIPAAm) and *N*,*N*-diethylacrylamide (DEAAm) copolymers with *N*,*N*'-methylenebisacrylamide (MBAAm) were prepared by radical polymerization with the aim to determine their temperature-dependent changes in polarity by liquid chromatography. Superpores were formed by the salt-leaching technique using NaCl as a porogen. Porosities of the hydrogels characterized by water regain and mercury porosimetry, ranging from 81 to 91%, were proportional to the volume of NaCl porogen in the feed. The retention volumes of several phenols decreased with increasing temperature as polarity of the hydrogels decreased. A jump change in solute retention volume was observed at *ca.* 32 °C in PNIPAAm and at *ca.* 35 °C in PDEAAm indicating a change in the mechanism of interaction. The Gibbs energy changes ΔG_{CH_2} were rather low, increasing in the order phenol < benzyl alcohol < ethanol < butan-1-ol. In contrast to the solutes, retention volumes of bovine serum albumin and dextrans were higher at higher temperature confirming thus hydrophobic interactions of the compounds with the studied hydrogels.

Keywords: albumin; dextran; liquid chromatography; polyacrylamides; retention; temperature-sensitive

Introduction

Hydrogels sensitive to temperature, pH, light, pressure and electric field are useful in many applications, such as drug delivery[1] and bioseparation.[2,3] They exhibit interesting changes in swelling properties depending on the stimulus.[4] Thermosensitive hydrogels exhibit phase separation properties in aqueous solutions when the temperature is increased above a certain limit. This phase separation temperature is referred to as the lower critical solution temperature (LCST). Examples of monomers forming materials with thermosensitive properties include *N*-isopropylacrylamide (NIPAAm), *N*,*N*-diethylacrylamide (DEAAm),[5,6] *N*-*tert*-butylacrylamide, *N*-isopropylmethacrylamide (NIPMAAm), *N*-[3-(dimethylamino)propyl] acrylamide, *N*-cyclopropylacrylamide (NCPAm). Thermosensitive properties of these polymers were determined by swelling, turbidimetric or fluorescence measurements if the polymer was modified with a fluorescent probe,[7] by microdifferential scanning calorimetry (DSC), UV spectrophotometry, cryo-electron microscopy, NMR and mechanical behaviour.[8]

Polarity of a polymer, which affects its interaction with solutes, controls properties important for application, such as adsorption of proteins. Polarity of polymers can be studied by fluorescent labelling, determination of ζ-potential, electrophoretic mobility

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and by liquid or solid chromatography. Polarity of porous Porapak and Chromosorb stationary phases was determined by retention indices of Rohrschneider and McReynold polarity probes with respect to standard polarity reference phases for GC, such as squalane and Apolane ($C_{87}H_{167}$).[9] Interaction of phenols with some polymers was measured by high-pressure liquid chromatography (HPLC).[10,11] Mattiason used polyacrylamide monoliths for chromatography of particulate-containing liquids including crude cells and viruses.[12,13] The aim of our investigation was to determine changes of the polarity of poly(*N*-isopropropylacrylamide) (PNIPAAm) and poly(*N,N*-diethylacrylamide) (PDEAAm) hydrogels with increasing temperature and sorption of biopolymers under similar conditions.

Experimental Part

Materials

Monomers NIPAAm, DEAAm and *N,N′*-methylenebisacrylamide (MBAAm) were obtained from Sigma-Aldrich (Steinheim, Germany), methanol (MeOH) was from Lach-Ner (Neratovice, Czech Republic), ethanol (EtOH), propan-1-ol (PrOH) and butan-1-ol (BuOH) from Lachema (Neratovice, Czech Republic), benzyl alcohol (BzOH) from Reachim (Katowice, Poland), phenol (PhOH) from Reactivul (Bucharest, Romania), glucose from Spofa (Prague, Czech Republic), saccharose, and poly(ethylene glycol) (PEG, $M_w = 4 \cdot 10^6$) from Polysciences (Warrington, USA); they were used as received. Bovine serum albumin (BSA) from Sigma-Aldrich, D10, D40, D70, D250, D500 and D2000 dextrans from Pharmacia (Uppsala, Sweden) were used for characterization as 0.1% aqueous solutions. Hydrodynamic diameters of dextrans were evaluated using the Stokes equation

$$D = 0.08506/M_w^{-0.45} \quad (1)$$

where M_w is the molecular weight of dextran.[14,15]

Synthesis

NIPAAm or DEAAm was polymerized with 1.25 or 10 wt.% of MBAAm in the presence of 85 or 90 wt.% of NaCl (250–500 μm), respectively, in 5-ml PE syringe (diameter 11 mm). Composition of polymerization feed is summarized in Table 1. Polymerization was initiated by 0.5 wt.% of AIBN (relative to the monomers) and proceeded for 8 h at 70 °C. After completion of the polymerization, the cylinder was removed from the syringe and NaCl crystals were removed by washing with water leaving large pores (superpores) in the polymer structure.

Chromatography

A glass column (15 × 1 cm), filled with a copolymer and equipped with the heating jacket, which allowed changing temperature of the solutes and hydrogels in the range 16-40 °C. Water was eluent at a flow rate of 0.5 ml/min. BuOH, EtOH, BzOH and PhOH with Taft σ^* constants −0.13, −0.10, 0.22 and 0.60, respectively, and MeOH, PrOH, BSA and dextrans were used as solutes. The retention volumes were measured with the experimental error ±0.01 ml.

Characterization

Water regain of swollen hydrogels was determined by centrifugation.[16] The pore volume of freeze-dried hydrogels was characterized by mercury porosimetry allowing determination of meso- (2–50 nm), macro- (50-1000 nm) and small superpores (1–116 μm). Porosity (p, %) was calculated from pore volume (V, ml/g) determined by mercury porosimetry or water regain according to Equation (2)

$$p = \frac{V \times 100}{(V + 1/\rho)} \quad (2)$$

Table 1.
Composition of polymerization feeds.

Hydrogel	Monomer (g)	MBAAm (g)	NaCl (g)
PNIPAAm	1.58[a)]	0.02	9
PDEAAm I	1.58[b)]	0.02	9
PDEAAm II	1[b)]	0.1	10

[a)]NIPAAm;
[b)]DEAAm.

where ρ is the density of the hydrogel (1.08 g/ml).

Results and Discussion

Polarity and LCST Changes of PNIPAAm and PDEAAm Hydrogels

Three hydrogels were prepared: PNIPAAm crosslinked with 1.25 wt.% of MBAAm, and PDEAAm crosslinked with 1.25 or 10 wt.% of MBAAm. Porosity was achieved with NaCl added in the feed. The hydrogels were transparent at laboratory temperature; however, they became opaque at temperatures higher than 32 °C. Retention volumes of solutes of different polarity, MeOH, EtOH, PrOH, BuOH, BzOH and PhOH, BSA, dextrans and PEG, were determined on hydrogels by liquid chromatography at temperatures in the range 20–40 °C.

Retention volumes of solutes, which are directly proportional to the solute adsorption and its interaction with the polymer, were correlated with Taft σ^* constants reflecting thus changes in the hydrogel polarity. The retention volumes on PNIPAAm decreased with increasing σ^* (Figure 1) while they increased on PDEAAm II; retention on PDEAAm I showed intermediate behavior. Retention volumes on PDEAAm I were constant with increasing σ^* in the range 20–25.5 °C and decreased at higher temperatures. Retention volumes of all solutes on PNIPAAm and PDEAAm hydrogels always decreased with increasing temperature documenting thus decreasing polarity, i.e, increasing hydrophobicity, of both polymers with increasing temperature (Figure 2) as they deswelled in water during heating. Swelling changes were larger for PNIPAAm than for PDEAAm. An abrupt decrease in retention volumes of solutes on PNIPAAm was observed between 30 and 32 °C; a moderate change in retention volumes between 30 and 35 °C was typical of PDEAAm. According to Inomata,[17] linear PNIPAAm and macroscopic hydrogels have a lower critical solution temperature (LCST) in water between 32 and 34 °C. Idziak found that PDEAAm has LCST of *ca.* 33 °C,[18] which is in a good agreement with our results.

The temperature dependences of retention volumes of all solutes on PNIPAAm were similar (Figure 2). However, a different situation was found for PDEAAm. The solutes were not so strongly bonded to

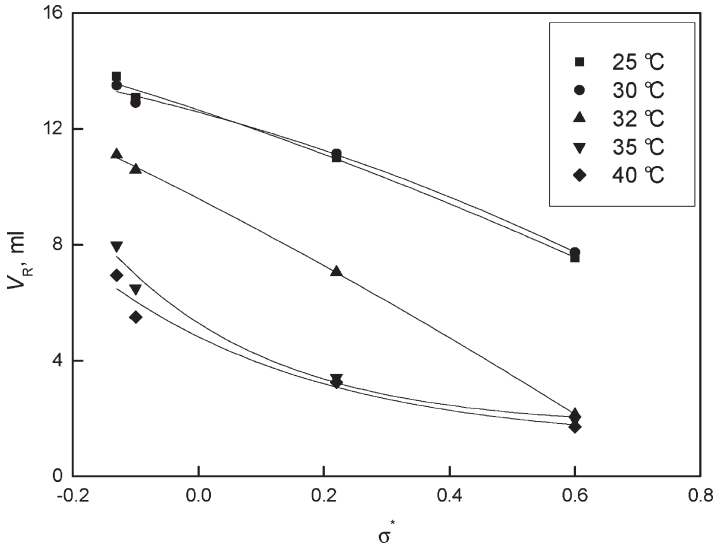

Figure 1.
Correlation of solute retention volume on PNIPAAm with Taft σ^* constants.

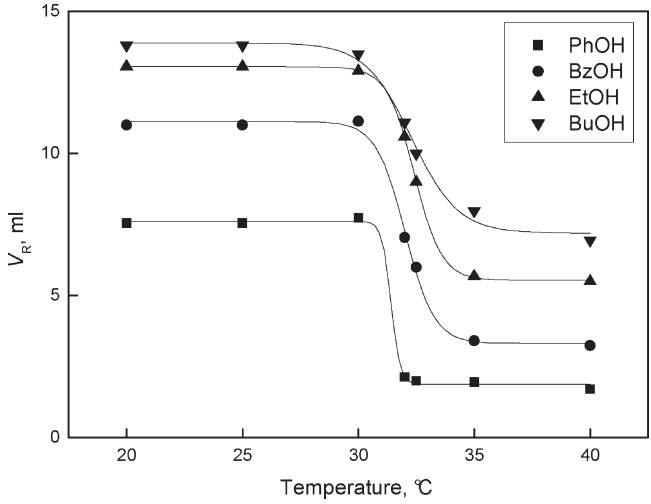

Figure 2.
Temperature dependence of retention volumes of solutes on PNIPAAm.

PDEAAm II than to PNIPAAm. While BuOH and EtOH were more retained by PDEAAm II than by PNIPAAm, PhOH and BzOH were strongly retained by both of them. Low-crosslinked PDEAAm I exhibited intermediate behaviour. This supports the view that PNIPAAm is more polar than PDEAAm. A decrease in the retention volume of BuOH, a non-polar solute, with increasing temperature was only moderate, as it was not strongly bonded to PDEAAm II. It should be noted that the crosslinking degree affected the retention volumes. The retention volumes of solutes on PDEAAm II (10 wt.% MBAAm) were lower then those on PDEAAm I (1.25 wt.% MBAAm) or PNIPAAm (1.25 wt.% MBAAm).

Retention of BSA on PNIPAAm and PDEAAm Hydrogels

The retention volume of bovine serum albumin (BSA) measured at 20 and 40 °C increased from 3 to 24 ml with increasing temperature (Table 2). It indicates that BSA was more strongly bonded at 40 than at 20 °C. Moreover, BSA was more strongly bonded on PDEAAm than on PNIPAAm. The interactions increased in the series PNIPAAm < PDEAAm I < PDEAAm II. The opposite trend was observed for other low-molecular-weight solutes: the retention volume decreased with increasing temperature (Figure 2). Low retention of BSA at 20 °C in comparison with other measured solutes can be explained by its low polarity.

Table 2.
Temperature dependence of retention volume V_R (ml) of bovine serum albumin and phenol on hydrogels.

Solute	Temperature (°C)	PNIPAAm	PDEAAm I	PDEAAm II
BSA	20	6.85	2.9	8.56
BSA	20	7.42	3.3	–
PhOH	20	10.94	15.26	12.29
BSA	40	9.37	21.25	10.95
BSA	40	10.41	24.17	–
PhOH	40	6.05	2.23	9.12
BSA[a)]	20	0	0	–
BSA	20	6.76	–	–
BSA	20	6.86	–	–

[a)]Without injection of BSA.

These findings support the view that the solutes other than BSA, such as BuOH, PhOH, EtOH and BzOH, interact with the hydrogel via polar, e.g. H-bonds, but BSA through hydrophobic interactions. When the temperature decreased from 40 to 20 °C, the retention volume of measured solutes also decreased to the original value 6.8 ml. Retention of PEG on PNIPAAm decreased with increasing temperature, but was constant on PDEAAm.

Retention of Dextrans on PNIPAAm and PDEAAm Hydrogels

It is interesting to compare interactions of hydrogels and low-polar BSA with those of polar dextrans. A range of D10-D2000 dextrans differing in molecular weight, glucose and saccharose were therefore tested in terms of their retention volume on hydrogels at 20 and 40 °C (Figure 3). Although dextrans are more polar than BSA, their retention volumes on PNIPAAm were higher at 40 °C than at 20 °C. This can be explained by lower difference in polarity of dextrans in comparison with the used solutes. Retention of dextrans on PNIPAAm II increased with increasing temperature similarly as that of BSA. This supports the view that synthetic polyacrylamides are hydrophobic compared with dextrans. It can be thus concluded that both dextrans and BSA were more strongly bonded to PNIPAAm at 40 °C than at 20 °C. Even though PNIPAAm deswelled at 40 °C and the difference in lower and upper exclusion limit decreased from 1.76 to 0.81 ml of eluent, the overall retention volume of D2000 dextran substantially increased from 14.62 to 21.25 ml.

In contrast to PDEAAm I, the retention of dextrans and alcohols on PDEAAm II was higher at 40 °C than at 20 °C. This documents that the properties of PDEAAm substantially changed with increasing cross-linking.

The dependence of retention volumes of dextrans on their molecular weight characterizes the pore size distribution of hydrogels. The retention volumes of D2000–D70 dextrans were almost constant on PNIPAAm as they were excluded from the hydrogel. The retention volume corresponds to the gel exclusion limit. If the molecular weight of dextrans was compared with their dimensions evaluated according to Equation (1), the pore diameter of the hydrogel can be estimated to range from 1 to 58 nm. When the temperature increased to 40 °C, the retention volume decreased. As the retention

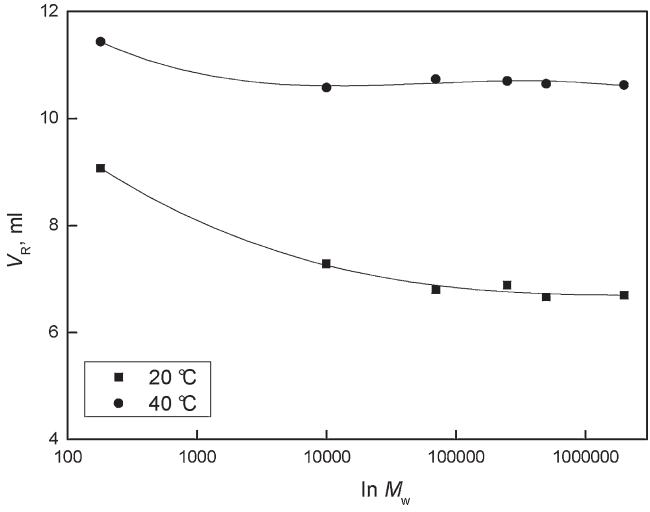

Figure 3.
Retention volume of dextrans on PNIPAAm at 20 and 40 °C.

volumes of D2000–D10 dextrans were constant, the pores are expected to be smaller than 5 nm.

The retention volume of glucose (the smallest measured molecule) was higher than the dead volume of column (1.6 ml). This means that its retention volume consisted of the size exclusion and polar interaction contribution. The retention volume of glucose on PDEAAm I and II also confirmed the presence of pores smaller than 5 nm. In contrast to PNIPAAm, the retention volume of glucose on PDEAAm I and II was lower at 40 °C than at 20 °C. A large difference in the retention volumes of glucose or saccharose and dextrans was observed on PDEAAm II. Their retention volumes on PDEAAm I and II, similarly as on PNIPAAm, were higher at 40 °C than at 20 °C.

Gibbs Energy of Interaction

To compare the hydrogels, the retention volumes of a homologous series of aliphatic alcohols were estimated and the value of Gibbs energy of CH_2 group evaluated (Table 3). The energy of interaction of hydrogels with alcohols (ΔG_{CH2}, kJ/mol) was calculated according to Equation (3):

$$-\Delta G_{CH2} = 4.18 \cdot RT^* \ln S \quad (3)$$

where R is the gas constant, T is the temperature in K and S is the slope of the dependence of retention volumes of solutes on the number of alkyl carbon atoms.

The Gibbs energy of the CH_2 group decreased from PNIPAAm to PDEAAm and with increasing temperature. The Gibbs energy of the interactions increased in the order phenol < benzyl alcohol < ethanol < butan-1-ol. The determined values of Gibbs energy were rather low: 10.1 and 4.5 kJ/mol for PNIPAAm and PDEAAm II, respectively, indicating dehydration of the hydrogels. This confirms that the nature of the polymers changes from hydrophilic to hydrophobic as the Gibbs energy decreases.

Table 3.
Gibbs energies of solute - hydrogel interactions ($-\Delta G_{CH2}$, kJ/mol).

Temperature, °C	PNIPAAm	DEAAm I	DEAAm II
20	10.1	6.5	4.5
40	7.8	7.9	5.5

The mechanism of interaction of solutes with temperature-sensitive PNIPAAm and PDEAAm hydrogels can be explained by the loss of the hydration cover around the amide group during heating resulting in hydrogel deswelling. *N,N*-dialkyl-substituted amides are expected to be more lipophilic than the *N*-monoalkyl-substituted PNIPAAm due to the absence of the hydrogen atom on the amide group which is capable of formation of a hydrogen bond with water. A decrease in retention volumes of all non-polar and polar solutes with increasing temperature shows that H-bonds between water and amide groups break during heating. This is accompanied also by changes in the polymer appearance, from transparent to opaque. If polarity changes during heating, one would expect non-polar solutes to have stronger interactions with hydrogels than polar ones and the retention volume of non-polar solutes increases with temperature; however, this was not observed.

Comparison of Porosity Determined from Retention Volumes, Water Regain and Mercury Porosimetry

It is interesting to compare retention volume of solutes in hydrogels measured by liquid chromatography with pore volumes of hydrogels determined by other methods, such as water regain and mercury porosimetry. Table 4 compares porosities determined by these methods with the volume percentage of NaCl in the polymerization feed. Each method, however, covers different ranges of pore size. While the retention volume of solutes includes both pores of all sizes and swelling of the matrix, water regain reflects the overall porosity of the hydrogel. Because some water was removed from large pores during centrifugation, water regain values were slightly lower than those obtained from

Table 4.
Porosities (vol.%) determined from the retention volume of solutes, water regain and mercury porosimetry.

Hydrogel	NaCl[a] (vol.%)	Retention volume		WR[d]	MP[e]
		20 °C	40 °C		
PNIPAM	80.2	97.5[b]	98.0[b]	92.4	57.8[f]
PDEAAm I	81.7	96.9[b]	97.9[b]	78.6	49.3[f]
PDEAAm II	82.2	94.7[b]	97.7[b]	78.0	28.2[f]
PNIPAM	80.2	87.6[c]	91.8[c]	92.4	22.3[g]
PDEAAm I	81.7	79.8[c]	89.7[c]	78.6	17.7[g]
PDEAAm II	82.2	87.2[c]	88.6[c]	78.0	13.4[g]

[a] NaCl in the polymerization feed;
[b] with glucose;
[c] with D2000 dextran;
[d] WR – water regain;
[e] MP - mercury porosimetry;
[f] superpores;
[g] macro- and mesopores.

retention volumes. The difference between the retention volume of glucose and D2000 dextran reflected then the accessibility of the porous structure. This difference decreased with increasing temperature due to deswelling of the hydrogel. Porosity values roughly corresponded to the volume percentage of NaCl porogen used in the polymerization. Mercury porosimetry, however, provided lower porosities probably due to the hydrogel contraction during drying; moreover, the method determines only pores smaller than 116 μm, while the dimension of NaCl crystals was 250 μm.

Table 4 shows also the effect of temperature on porosity determined from the retention volume of solutes, which was high in PNIPAAm but rather low in PDEAAm I and II; it was comparable with porosities determined from water regain 92 and 78 vol.%, respectively, which is in accordance with the percentage of NaCl porogen in the polymerization feed (80–82 vol.%).

Conclusion

Changes in polarity of PNIPAAm and PDEAAm hydrogels were determined by liquid chromatography. Sorption of BSA was investigated depending on the temperature. The retention volumes of solutes in PNIPAAm crosslinked with 1 wt.% of MBAAm decreased with increasing temperature; a jump change was observed at *ca.* 32 °C. The dependence of the solute retention volumes on the Taft σ^* constants confirmed a decrease in polarity with increasing temperature. Gibbs energy of the interactions ΔG_{CH2} increased in the order phenol < benzyl alcohol < ethanol < butan-1-ol and the values were rather low: 10.1 kJ/mol for PNIPAAm and 4.5 kJ/mol for PDEAAm. The highest energy of interaction of butan-1-ol and PNIPAAm confirmed its rather nonpolar character. BSA and dextrans exhibited higher retention volumes at 40 °C than at 20 °C confirming thus the hydrophobic interactions of these compounds and PNIPAAm at 40 °C. Properties of PNIPAAm and PDEAAm hydrogels might be of high interest in applications such as scaffolds for cell cultivation.

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