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# Influence of the synthesis conditions and ionic additives on the swelling behaviour of thermo-responsive polyalkylacrylamide hydrogels

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**Abstract**

Temperature-responsive poly(*N*,*N*-diethylacrylamide) and poly(*N*-isopropylacrylamide) hydrogels were synthesised by free radical polymerisation at room temperature with different crosslinker and constant initiator/accelerator concentration. At low crosslinker concentration transparent or translucent gels were obtained, while the gels produced at high crosslinker concentration were opaque. Whereas little difference could be observed between these gels in regard to the temperature of collapse, the swelling/deswelling behaviour showed discrepancies, in that the opaque gels (higher degree of crosslinking) showed a lower swelling ratio, but more efficient water release and more pronounced relative water uptake (reswelling). Low molecular weight additives (potassium salts) had an effect on the on the critical temperature and the swelling ratio; the strength of the observed effect corresponded to the position of the anion in the Hoffmeister series. For most salts the critical temperature was found to decrease ('salting out' effect) almost linearly with increasing salt concentration. A linear relationship could be established between the change in critical temperature of the gels and the 'Viscosity *B* Coefficient' of the added anion. Low concentrations of KI showed a 'salting in' effect for all investigated gels, while low amounts of KCl showed such an effect only in the case of the poly(*N*,*N*-diethylacrylamide) gels. The 'salting in' effect was accompanied by an increase in the maximum swelling ratio below the critical temperature. In a cytotoxicity test with Jurkat cells the poly(*N*-isopropylacrylamide) gels, but less the poly(*N*,*N*-diethylacrylamide) gels negatively influenced the morphology, if not the number and viability of the cells, after a contact time of 6 h.


*Keywords:* Thermo-responsive polyalkylacrylamide hydrogels; Swelling; Opaque

## 1. Introduction

Thermo-responsive hydrogels have gained considerable attention in the medical and life sciences due to their ability to swell or deswell abruptly in response to a small change in the temperature of their environment [1,2]. One of the best-studied thermo-responsive materials is poly(*N*-isopropylacrylamide) (polyNIPAAM) [3]. In contact with water polyNIPAAM shows an abrupt property (solubility) change in the vicinity of 32 °C [4–7]. Below this critical temperature (CT), the polymer chains are excessively hydrated and form expanded structures. Above the CT, the chains rapidly dehydrate (collapse) and aggregate to form compact structures. The ubiquiteousness of polyNIPAAM as material for thermo-responsive structures tends to cloud the fact that other thermo-responsive polyalkylacrylamides exist and that these molecules—lacking for example the H-atom in the side chain—may be more suited for certain applications in the life sciences. One of these structures is poly(diethylacrylamide) (polyDEAAM), which shows a CT around 30 °C [8]. However, for this molecule and in particular its hydrogels, little can be found in the literature.

The CT is an inherent characteristic of a particular polymer molecule, which depends in a not yet fully understood manner on the balance of hydrophobic and hydrophilic regions within the monomeric units. The CT can be influenced via additives to the polymer backbone (co-polymers) or to the environment (co-solutes). An effect of the molar mass and in the case of linear oligomeric polyDEAAM of the tacticity on the CT has also been observed [9]. In the case of hydrogels, copolymerising

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NIPAAM with different monomers has been reported to cause shifts in the CT, as well as the swelling degree and the abruptness of the volume change during phase transition [10–12]. Increasing the crosslinker density may possibly also affect the critical temperature.

Apart from these molecular aspects, there have been many attempts to control the thermally induced reversible collapse of *N*-alkylacrylamide—mainly polyNIPAAM—gels through co-solutes, for example through the use of mixed solvents [12,13], the addition of salts [14–16], or the addition of surfactants [12,16,17]. Park and Hoffman [18] were the first to demonstrate that the addition of NaCl to the aqueous environment can lower the CT in the case of a non-ionic polyNIPAAM hydrogel. They monitored the effect for a series of sodium salts and concluded that the anion and not the cation is responsible for the change in phase transition and that the effect of a given anion correlates roughly with its position in the Hoffmeister series. Similar observations were made by Dhara et al. [15], who found that in a series of alkali halides, the efficiency of lowering the CT is $I < Br < Cl < F$, independent of the cation.

The effects of salts on the transition temperature can be explained by an effect on the water structure. Liquid water has a decided structure, which distinguishes this solvent from most others. Any thermodynamic description of a dissolution process in water must hence take entropic effects caused by changes in the water structure into account. In fact, one contribution to the occurrence of a CT is the fact that the water around hydrophobic parts of a molecule is more ordered than bulk water and entropy can be increased when a part of this 'ordered water' is released into the bulk upon direct association of the hydrophobic surfaces during chain collapse [19,20]. Salts may influence (strengthen/weaken) the water structure. They also interact with the bulk water and decrease the entropy through hydration. All of these effects influence the temperature at which the hydration of the polymer chains become unstable and collapse of the chains occurs.

In a previous paper [21], we discussed thermo-responsive polyDEAAM gels compared to polyNIPAAM gels as potential candidates for drug delivery vehicles. In this article, we investigate further the influence of synthesis conditions on the appearance of the gels and on the swelling behaviour as a function of the temperature. The second subject of the present article is the investigation of the effect of additives (inorganic salts) on the phase transition and the swelling ratio of the gels. Finally, a first estimate of the biocompatibility of the gels is made.

## 2. Experimental

### 2.1. Materials

*N*-Isopropylacrylamide (NIPAAM), *N*,*N*-methylenebisacrylamide (BIS), ammonium persulphate (APS), *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED) and inorganic potassium salts were from Sigma Aldrich Chemicals (Buchs, Switzerland) and used as received. *N*,*N*′-Diethylacrylamide (DEAAM) was obtained from Polysciences Inc. Europe (Eppelheim, Germany). Water was purified using an Elix-3 system (Millipore, Bedford, MA, USA). For the investigation of the influence of ionic additives on the swelling ratio of the hydrogels, aqueous potassium salt solutions were prepared in the concentration range from 0 to 2 M.

### 2.2. Hydrogel synthesis

The hydrogels were synthesised by free radical polymerisation in aqueous solution (25 mL water) of NIPAAM or DEAAM (2.5 g minus the amount (in gram) of added crosslinker), in the presence of BIS (0.1 g for the 10×4 gels, 0.05 g for the 10×2 gels, 0.025 g for the 10×1 gels) as crosslinking agent (see Scheme 1 for the chemical structures of the monomers and the crosslinker). APS (7.5 mg) was used to initiate the reaction and TEMED (4.87 μL) was used as accelerator. The gel composition was characterised by the *W*×*C* nomenclature [22], where *W* represents the weight in grams of the combined monomers per 100 mL of water and *C* the mass of crosslinker expressed as percentage of the total amount of monomer plus crosslinker. For the polymerisation, monomer, crosslinker and water were mixed together in a glass vessel at room temperature for 2 h ($N_2$ atmosphere). Then initiator and accelerator were added and mixed in for 5 min. Afterwards, the solution was poured into the moulds (25 mL capped centrifuge tubes, TPP, Milan, Italy) and kept at room temperature for at least 24 h to allow the polymerisation to take place. The gels were then removed from the moulds and placed in distilled water at room temperature for at least 2 days in order to remove any unreacted material. The water was exchanged several times during this period. If desired the gels were dried in vacuum at 40 °C until constant weight, but at least over night.

### 2.3. Methods

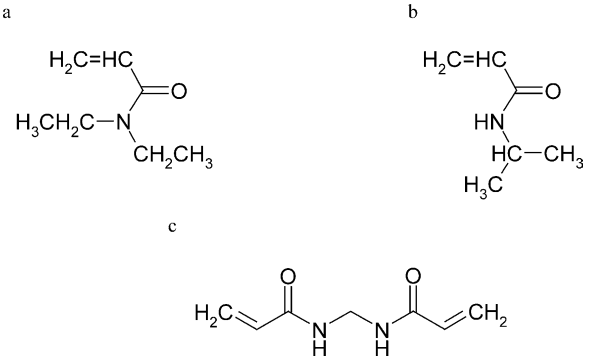
For the measurement of the swelling ratio (SR) a dry gel sample (xerogel, mass 0.1 g unless indicated otherwise) was incubated in the solution of interest for at least 24 h at the indicated temperature. After that time the SR was calculated as [23,24]:

$$\mathrm{SR} = \frac{W_s}{W_d} = \frac{W_w - W_d}{W_d} \quad (1)$$

where $W_s$ is the weight of the liquid in the swollen gel, $W_w$ is the weight of the wet gel and $W_d$ is the dry weight of the gel.

The critical temperature (CT) of the gels was defined as the point of inflection of the resulting SR versus temperature curves (approximated as the value at half height).

The deswelling kinetics (Water Retention, WR) were




Scheme 1. Chemical structures of the two monomers, (a) *N*,*N*-diethylacrylamide (DEAAM) and (b) *N*-isopropylacrylamide (NIPAAM) as well as (c) the crosslinker *N*,*N*-methylenebis-acrylamide (BIS).

determined by transferring gels samples equilibrated in water at room temperature at $t=0$ quickly into hot distilled water (50 °C for polyNIPAAM and 42 °C for polyDEAAM gels). The weight change of each gel was recorded every 10 min for at least 1 h and the WR-value (%) calculated as follows [23,24]:

$$\mathrm{WR} = 100 \times \frac{W_t - W_\mathrm{d}}{W_\mathrm{s}} \tag{2}$$

where $W_t$ is the weight of the gel at measurement time and $W_\mathrm{s}$ refers as in Eq. (1) to the equilibrium weight of the corresponding xerogels swollen at room temperature.

For the measurement of the reswelling kinetics (Water Uptake, WU) xerogel samples were incubated in hot water (>CT) until they reached the equilibrium-swelling ratio (few hours) and transferred into water below the CT of the gel (20 °C for polyNIPAAM, 15 °C for polyDEAAM). The WU-value (%) was calculated according to [23,24]:

$$\mathrm{WU} = 100 \times \frac{W_t - W_\mathrm{d}}{W_\mathrm{s}} \tag{3}$$

All measurements concerning the swelling/collapse of the hydrogels were repeated at least three times and average values are reported in the corresponding figures. The deviation between the measurements was less than 10%.

### 2.4. Cytotoxicity test

The cytotoxicity test was realised with Jurkat cells (ATCC TIB 152), a human T-cell leukaemia line. Cells were cultured at 37 °C and 5% $CO_2$ in RPMI-1640 medium supplemented with 10% heat inactivated foetal calf serum, 1% non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, 1% HEPES, 50 U/mL penicillin and 50 μg/mL streptomycin. Two millilitres of the cell suspension was added to each well of a 12-well cell culture plate (cell density approximately $10^5$ cells/mL) and a 0.5 $cm^3$ piece of either a 10×4 polyNIPAAM or polyDEAAM hydrogel (swollen in PBS, phosphate buffered saline) was added. Samples were prepared in triplicate and observed for 6 h. The trypan blue viability assay was used in regular intervals during that time on aliquots taken from the cultures to assess the viability of the cell populations. A haemocytometer was used to count the average number of cells present in each well. The morphology of the cells was assessed after 6 h by light microscopy. Cell densities and viabilities were compared to data gathered for control cultures performed in the absence of the hydrogel.

## 3. Results and discussion

### 3.1. Influence of synthesis conditions on the appearance and phase transition of the gels

In this investigation, the polymerisation of DEAAM- and NIPAAM-based hydrogels was initiated by ammonium persulphate $(NH_4)_2S_2O_8$ in the presence of BIS as crosslinker. Technically, the polymerisation was carried out at room temperature as the reaction vessel was neither heated nor cooled. Gels of both chemistries were prepared containing varied amounts of crosslinker, in particular gels with a $W\times C$ composition of 10×4, 10×2, and 10×1. At least three gels of each type and composition (crosslinking degree) were prepared and investigated.

Thermo-responsive hydrogels and especially polyNIPAAM gels prepared at room temperature are normally described as transparent (or nearly transparent) in the swollen state, but to become opaque when the temperature is increased above the critical temperature (CT) due to the formation of a denser and more heterogeneous structure upon collapse. The gels prepared in this study deviated from the expected behaviour, Table 1. Only the 10×2 and 10×1 polyNIPAAM gels were transparent as expected, while the 10×4 polyNIPAAM gels were opaque even below CT. In the case of polyDEAAM, the 10×1 gels were translucent rather than transparent, while the 10×2 and 10×4 gels were opaque even below CT. When the temperature was increased above the CT, all gels collapsed and became opaque.

Permanently opaque stimuli-responsive hydrogels have been obtained before, e.g. when phase separation took place during gel synthesis and a heterogeneous structure was formed [25,26]. This will, e.g. be the case when the synthesis of the thermo-responsive gel takes place at temperatures above CT [22,27]. According to Suzuki et al. [28], high synthesis temperatures may lead to the formation of monomer clusters at the initial reaction stage, which become more compact and rigid than the remainder of the network due to the high degree of crosslinking that occurs within the cluster. These insoluble clusters are then incorporated into the gel network during the subsequent gelation process and cause permanent opaqueness of the gels [29].

Polymerisation at temperatures above CT a priori does not apply to the gels considered here, which were prepared

at room temperature, i.e. below CT. One possible reason for the observed phenomenon is, off course, a temperature increase during the reaction. Monitoring the temperature during polymerisation would obviously have been beneficial, since the reaction was known to be exothermic. The experimental set up, however (the polymerisation took place in capped centrifuge tubes as moulds), did not permit insertion of a thermo probe for temperature monitoring during the reaction. Still, since mould geometry and initiator concentration was identical in all cases, while the concentration of polymerisable groups was at least very similar, putative differences in the reaction temperature alone cannot explain the differences in the appearance of the gels as a function of only the crosslinker density. Since the highly crosslinked gels were opaque, it is possible that in such cases diffusional mass transfer was hindered early on in the reaction and as a consequence regions of higher crosslinking density formed within the gels. The fact that the polyDEAAM gels showed opaqueness already at lower crosslinking degree than the polyNIPAAM ones may be due to the lower CT-value of these hydrogels, but also to the more pronounced hydrophobicity of the monomer and the ensuing stronger tendency for the formation of monomer clusters in water.

### *3.2. Swelling behaviour of the gels as a function of the composition*

The critical temperature (CT) at which the gels collapsed, was determined as the point of inflection of the corresponding swelling ratio (SR) versus temperature curves. As had already been found in previous experiments with gels of similar composition [21], the swelling ratio (SR) as a function of the temperature of the polyDEAAM and polyNIPAAM hydrogels prepared for this investigation showed the point of inflection in the expected range, i.e. $30 \pm 2$ °C for the polyDEAAM gels and $32 \pm 1$ °C for the polyNIPAAM ones. This was independent of the degree of crosslinking or the dry mass of the gel. The transition was consistently broader in case of the polyDEAAM gels compared to the polyNIPAAM ones. For a given gel chemistry, the SR at temperatures below CT became smaller with increasing degree of crosslinking. For a similar ($W \times C$) composition, the polyDEAAM gels consistently showed a lower SR than the corresponding polyNIPAAM gels.

Table 1
Appearance of the polyNIPAAM and polyDEAAM gels as a function of the crosslinker concentration

| Hydrogel | Gel composition | Appearance (below CT) |
|---|---|---|
| PolyDEAAM | 10×4 | Opaque |
| | 10×2 | Transparent |
| | 10×1 | Transparent |
| PolyNIPAAM | 10×4 | Opaque |
| | 10×2 | Opaque |
| | 10×1 | Translucent |

The reduced swelling ratio observed at higher crosslinking had to be expected [16]. The lower SR of the polyDEAAM gels, but also their broader transition temperature interval, had previously been linked to the higher hydrophobicity of the involved monomeric units, due to their slightly larger hydrocarbon side chains [21]. However, in view of the results presented here, it is also possible that the inhomogeneities proposed for the microstructure of the opaque gels are responsible. Such clusters characterised by a high degree of crosslinking may determine and limit the overall swelling ability of the gel. While this will not necessarily affect the temperature dependency of the phase transition per se, it may be responsible for the broader phase transition and the lower degree of swelling observed for the opaque 10×2 polyDEAAM (SR = 8.7) gels as opposed to the transparent 10×2 polyNIPAAM ones (SR = 12).

The degree of crosslinking, if not the presence of heterogeneities may also influence the swelling and deswelling kinetics. When the deswelling behaviour of gels was investigated, all investigated gels behaved in a similar manner, Fig. 1. The major part of whatever water is lost is shed within the first 10 min. In case of the polyDEAAM chemistry, Fig. 1(a), the gels that appeared opaque, i.e. those with a 10×2 and 10×4 composition, showed rapid and rather similar behaviour, losing 75%, respectively, 60% of water within the first 10 min. The 10×2 polyDEAAM gel reaches equilibrium during that time, while the 10×4 polyDEAAM gel keeps losing water, reaching almost 80% water release within the next 60 min. The translucent 10×1 polyDEAAM gel showed significantly less efficient water release. A similar tendency is observed in case of the polyNIPAAM chemistry, Fig. 1(b). Here the opaque (10×4) polyNIPAAM gels rapidly lose approximately 80% of the initially stored water, whereas water release is less pronounced in case of the transparent 10×2 and 10×1 polyNIPAAM gels (40 and 50%, respectively). Interestingly, the less crosslinked 10×1 polyNIPAAM gels show more pronounced water release than the 10×2 polyNIPAAM gels in this case, indicating that crosslinking may not be the most determining factor for water release from such gels. Increasing the deswelling temperature, e.g. in the case of the polyDEAAM gels from 42 to 50 °C makes no difference in the deswelling kinetics, as long as the temperature is well above CT, data not shown.

The reason for the more complete water release in case of the opaque gels can at present only be speculated upon. It is possible that water release is especially pronounced in the areas of higher crosslinking density. The experimental set up unfortunately prevented a more detailed investigation of the deswelling (and reswelling, see below) kinetics within the first 10 min. However, the rapid water release observed in all cases argue against the formation of a dense, water-impermeable skin layer as a consequence of the increase in the environmental temperature and gel collapse occurring from the outside to the inside of the structure as has been

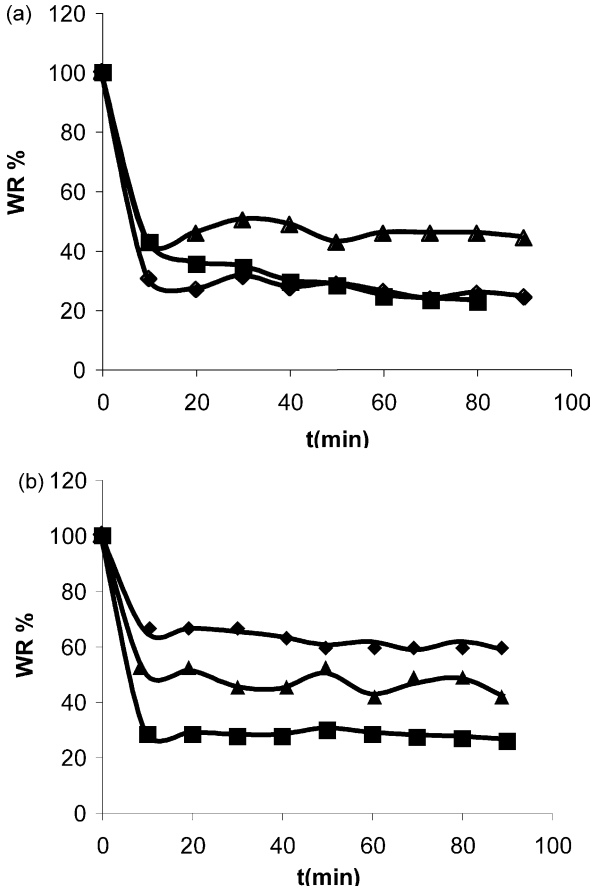



Fig. 1. (a) Deswelling kinetics at 42 °C of polyDEAAM gels with different gel composition (■ 10×4, ♦ 10×2, ▲ 10×1). (b) Deswelling kinetics at 50 °C of polyNIPAAM gels with different gel composition, (■ 10×4, ▲ 10×2, ♦ 10×1).

proposed by others [30,31]. There is also little evidence of further water release in the form of 'bursts' as a result of further shrinkage, concomitant increase in internal pressure, and subsequent breakage of the skin layer.

If we consider the reswelling kinetics of the gels, Fig. 2, a possible effect of the microstructure on the behaviour can again be observed, as the opaque gels show much more complete water uptake than the transparent/translucent ones. In other words, opaque polyDEAAM and polyNIPAAM xerogels hydrated above CT reach similar water contents during 'reswelling' below CT as the corresponding xerogels directly immersed into cold water ($W_s$). In the case of the translucent gels only a fraction of this water is imbibed, i.e. the water uptake of these gels during 'reswelling' stays below the equilibrium $W_s$-value of the corresponding dry hydrogels directly immersed into cold water. This is especially pronounced in the case of the 10×4 opaque polyNIPAAM gel, Fig. 2(b), where water uptake reaches 100% of $W_s$ within 10 min, while the transparent 10×2 and 10×1 polyNIPAAM gels show only an uptake corresponding to 30% of $W_s$. This effect only manifests itself in comparison to a xerogel swollen in cold water. If the wet gels are put through repeated collapse-reswelling cycles, the effects are fully reversible.

If one compares water uptake and release kinetics for the polyNIPAAM gels, Figs. 1(b) and 2(b), it appears that the gels reach the respective equilibria within the first 10 min. In the case of the polyDEAAM gels, Fig. 2(a), the opaque 10×4 gel also shows more pronounced water uptake than the translucent 10×1 gel. Moreover, while the water release, Fig. 1(a), reached equilibrium within the first 10 min, only the 10×4 polyDEAAM gel reaches equilibrium during water uptake within a similar time frame, Fig. 2(a). In the case of the translucent 10×1 gel, reaching the water uptake equilibrium required at least 20 min. The behaviour of the 10×2 gel is somewhat erratic during water uptake, which after the first 10 min seems to occur almost linearly throughout the experiment. This behaviour can at present not be explained.

The following three steps are proposed to occur in succession during (re-)swelling of a (dry) gel sample in water [32]: (1) Water molecules diffuse into the polymer network, (2) the hydrated polymer chains relax, (3) the polymer network expands into the surrounding liquid. Depending on the rate-determining step, swelling can be described by different equations. If solvent uptake (step 1) is rate determining, swelling can be described by the second

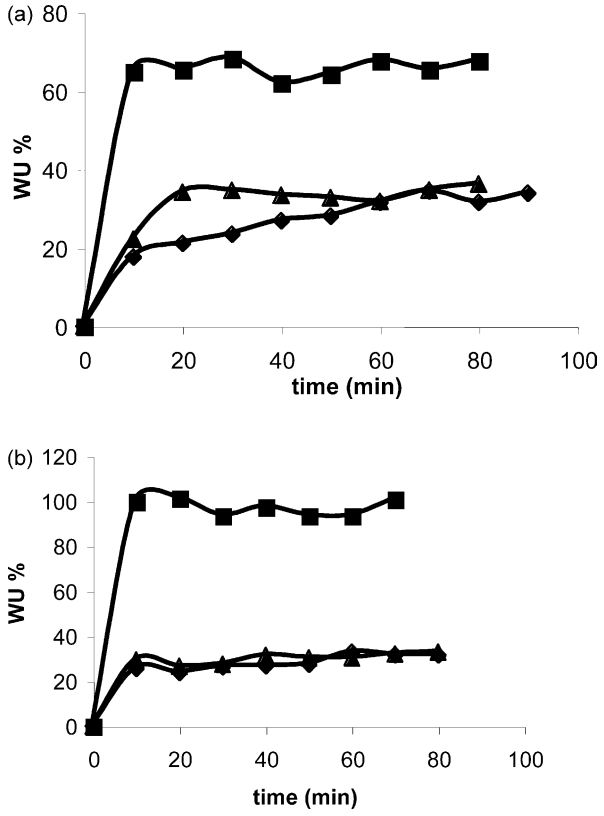



Fig. 2. (a) Reswelling kinetics at 15 °C of polyDEAAM gels with different gel composition (■ 10×4, ♦ 10×2, ▲ 10×1). (b) Reswelling kinetics at 20 °C of polyNIPAAM gels with different gel composition (■ 10×4, ♦ 10×2, ▲ 10×1).

Fick law [33]. In such cases, the total amount of solvent imbibed by the polymer increases roughly in proportion to the square root of time at the early swelling stages.

As the polymer chains become hydrated, polymer–polymer interactions are disrupted. If the macromolecular relaxation rate with hydration is much slower than the diffusion rate of water in the gel, the relaxation process (step 2) becomes rate limiting for swelling. In such a case, during swelling the hydrogel can be envisioned as divided into a glassy core and an already expanded (swollen) outer region, separated by an interface [34]. The interface (swelling front) proceeds from the outside towards the inside of the gel at a constant rate. As a result and in the case of a slab gel the amount of absorbed water increases linearly with time [35]. Finally, when gel expansion dominates the process (step 3), the swelling kinetics are governed by collective diffusion [30,36].

To investigate the mechanism of swelling from a dry gel sample, the swelling ratio of such gels ($<$CT) was analysed as a function of the time for $0 \leq (M_t/M_\infty) \leq 0.6$. The data were fitted to the following equation:

$$\ln\left(\frac{M_t}{M_\infty}\right) = \ln K + n \ln t \tag{4}$$

where $M_t$ and $M_\infty$ are the total amount of water absorbed by the gel at time $t$ and at the equilibrium state, respectively, $K$ is a constant compiling the various characteristic parameters of the gel and the value of $n$ should indicate the rate determining step of the swelling mechanism (Fickian diffusion of water or not). Fig. 3 shows the resulting plots for the investigated 10×4 gels, the corresponding values for $K$ and $n$ are compiled in Table 2. The 10×4 gels polyDEAAM and polyNIPAAM gels were chosen for the sake of comparability, since such gels were opaque for both gel chemistries. The values determined for $n$ indicate that swelling in this case is rate limited by Fickian diffusion of water into the gel. Moreover, the values determined for $n$ and $K$ were close to the experimental values obtained by Lee et al. [37,38] in water.

In contradistinction, the reswelling kinetics were investigated for gels that had already been hydrated at elevated temperatures, i.e. for wet gels that were presumably in a glassy state (chains aggregated by strong hydrophobic interaction) [39]. For such gels, the diffusion rate of water in the gel, but also polymer relaxation processes may influence the swelling kinetics. In this case, the water uptake of both the polyNIPAAM and the polyDEAAM hydrogels was proportional to time during the early stages. This indicates that the reswelling process is mainly determined by the relaxation of the hydrated polymer chains and that this step (step 2) is rate-determining for reswelling below CT. The comparatively slow reswelling kinetics of the polyDEAAM gels compared to the deswelling kinetics may be due to the fact that in this case the process of breaking the hydrophobic interaction between the aggregated chains required for rehydration is slow compared to the reaction (hydrophobic attachment) required in the deswelling case, where the hydrated chains initially are much more mobile [39]. Moreover, since polyDEAAM is more hydrophobic than polyNIPAAM, rehydrating aggregated polyDEAAM chains needs more time then in the case of polyNIPAAM.

### 3.3. Influence of inorganic salts on the swelling kinetics of the hydrogels

Most application of thermo-responsive hydrogels will occur not in pure water, but in a more complex environment. Cosolutes and especially salts from this environment may influence the temperature but also the kinetics of gel collapse and reswelling. Fig. 4 shows the influence of three inorganic salts (KI, KOH and KCl) at different concentration on the CT of the 10×4 polyNIPAAM gels, Fig. 5 the influence on the CT of the 10×4 polyDEAAM gels. The 10×4 polyDEAAM and polyNIPAAM gels were again chosen for this investigations as both were opaque. Fig. 6 summarises the changes in the swelling ratio versus temperature curves of the 10×4 polyNIPAAM and the 10×4 polyDEAAM gels at different KCl concentration.

As Figs. 4 and 5 show, the critical temperature of the gels decreases as a result of the addition of the salts ('salting out' effect), except for low amounts ($<$0.5 M) of KI in case of the polyNIPAAM gels and of low amounts ($<$0.5 M) of both KI and KCl in case of the polyDEAAM gels, where the salt addition slightly elevates rather than depresses the CT ('salting in' effect). As a consequence, in the case of the polyNIPAAM gels (Fig. 6(a)) the swelling ratio versus temperature curves are shifted towards lower temperatures as increasing amounts of KCl are added. Other than that, however, the curves show little changes in their general shape, e.g. in the abruptness of the transition or the maximum degree of swelling. In particular, roughly the same SR values are reached in salt solution as in pure water.

In the case of polyDEAAM, Fig. 6(b), the picture is similar for those KCl concentrations that show a salting out effect, namely 1.0 and 1.5 M. In these cases the curve is mainly shifted to lower temperatures but the general shape remains similar. At lower KCl concentrations, i.e. 0.25 and 0.5 M, the salting in effect manifests itself by a shift of the curve towards higher temperatures. Concomitantly in the presence of 0.5 M KCl the maximum swelling ratio is increased to almost twice that of pure water. A certain increase in the swelling ratio, albeit less pronounced, is also

Table 2
Values of $n$ and $K$ determined for the water uptake of 10×4 polyNIPAAM and polyDEAAM below CT

| Hydrogel | $n$ | $K$ |
|---|---|---|
| PolyNIPAAM | 0.3618 | 0.3030 |
| PolyDEAAM | 0.1183 | 0.4315 |

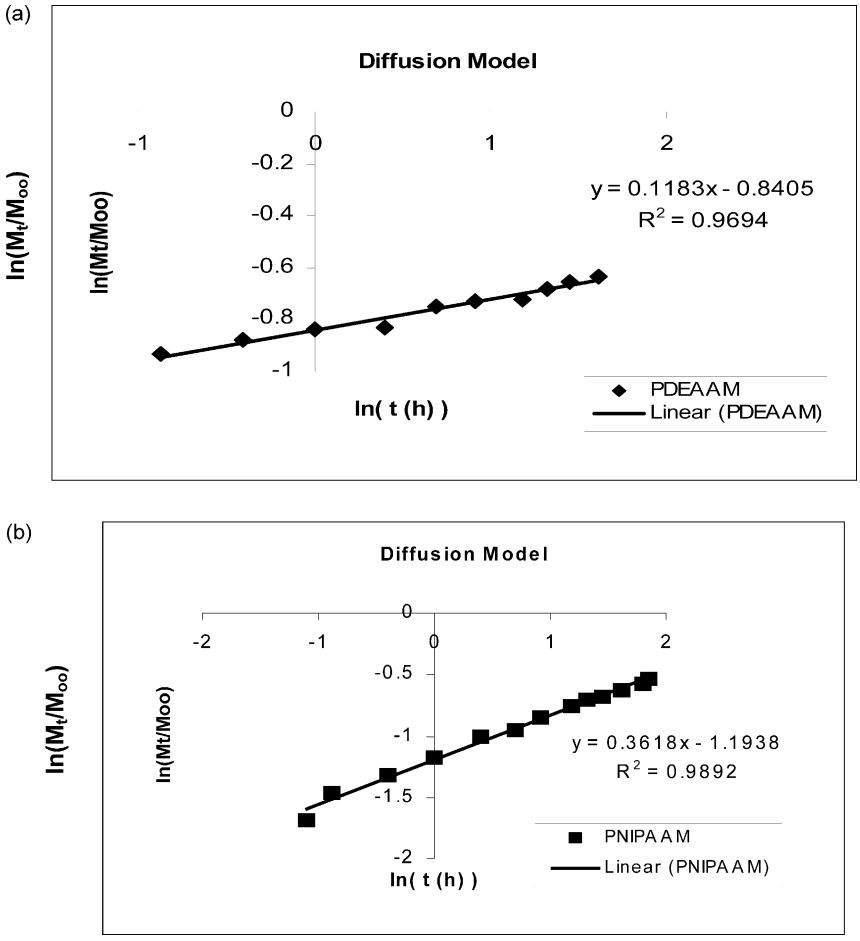



Fig. 3. (a) Double logarithmic plot of the development of the swelling ratio with time for 10×4 polyDEAAM gels at 20 °C in water. An initially dry gel sample was used. (b) Double logarithmic plot of the development of the swelling ratio with time for 10×4 polyNIPAAM gels at 20 °C in water. An initially dry gel sample was used.

observed for KCl concentrations of 1.0 M ('salting out' conditions), but not at 0.25 M ('salting in' condition).

The change in the CT of the investigated polyNIPAAM and polyDEAAM gels strongly depends on the salt concentration, while the effect of a given salt increases in the following order: KI < KCl < KOH, which is in accordance with the Hoffmeister series [40], but also with the results obtained by other authors [41,42]. In this context, the viscosity *B* coefficient has been proposed as a measure for the water structure enhancing or breaking ability of ions [43]. An ion with a positive viscosity *B* coefficient is a structure maker and tends to enhance hydrophobic interactions (in our case lowering the CT), while an ion with a negative viscosity *B* coefficient is a structure breaker

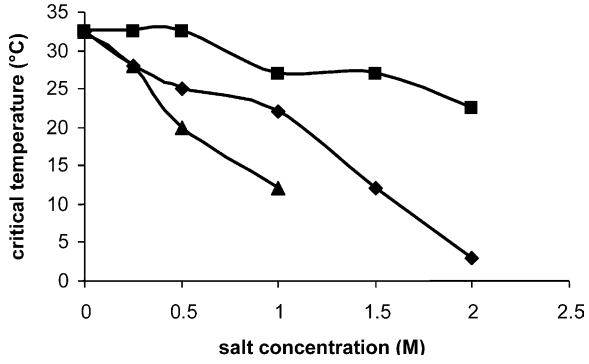



Fig. 4. Influence of potassium salts at different concentrations on the CT of 10×4 polyNIPAAM gels (■ KI, ♦ KCl, ▲ KOH).

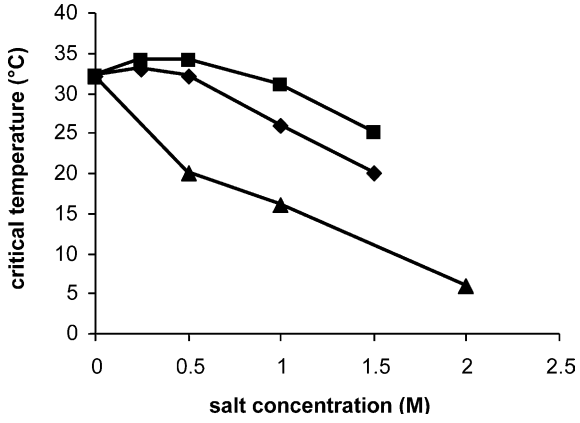



Fig. 5. Influence of potassium salts at different concentrations on the CT of 10×4 polyDEAAM gels (■ KI, ♦ KCl, ▲ KOH).

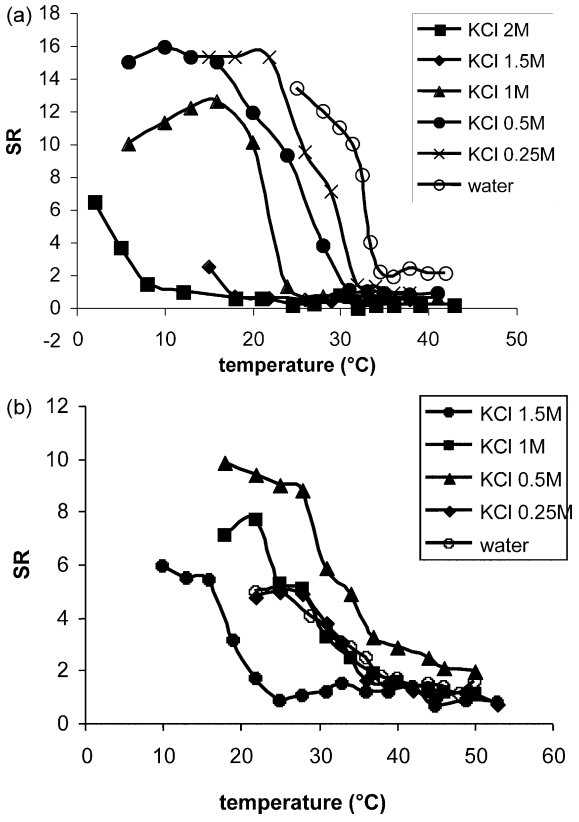



Fig. 6. (a) Influence of different KCl concentrations on the swelling ratio versus temperature curves of 10×4 polyNIPAAM gels. (b) Influence of different KCl concentrations on the swelling ratio versus temperature curves of 10×4 polyDEAAM gels.

and tends to stabilise hydrophobic hydration (in our case increasing the CT). Fig. 7 shows the relationship between the viscosity *B* coefficients of the anions employed in this study and the change in transition temperature ($\Delta_{CT}$) of 10×4 polyDEAAM gels to which 0.5 and 1.0 M of the corresponding salt had been added. $K_2SO_4$ was added in this experimental series to extend the application of the viscosity *B* coefficient for estimation of the salting out ability to a divalent anion. The effect of a 1 M $K_2SO_4$ concentration on the CT could not be determined. An extrapolation of the relationship shown in Fig. 7 shows that the value would have been below 10 °C.

Both for 0.5 M and for 1 M salt concentration, the relationship between the viscosity *B* coefficient of an anion and its ability to lower CT appears to be linear. This may be due to the fact that the simple inorganic ions used in this study form hydrates exclusively through ion–dipole interactions. When more complex electrolytes capable of secondary interactions are used, their addition may have a less predictable effect on the CT of the gels [17].

### 3.4. Cytotoxicity tests

Jurkat cells are of human origin and can be used to indicate acute cytotoxicity. In our group these cells are for example used to study apoptosis, i.e. programmed cell death. Here they were used in a first estimate of possible cytotoxicity of the hydrogels. For this purpose the cells were cultured for 6 h in the presence of both hydrogel types (triplicate cultures for each gel type in comparison to a similar number of controls). During the culture, the percentage of viable cells in the control samples (no hydrogel) and in samples drawn from the cultures performed in the presence of the hydrogels were determined regularly. In addition, the appearance of the cells was evaluated under the light microscope after 6 h. No significant decrease in viability was observed for the cells cultured in the presence of either hydrogel type compared to the controls. In all cases the viability of the cells was high and 95–98% of the counted cells were viable regardless of whether the came form the control or the hydrogel containing cell cultures. However, the appearance of the cells under the microscope shows putatively significant differences, Fig. 8. In the case of the cells cultured in the presence of the polyDEAAM gel, Fig. 8(c), the cells appear well-rounded and—save for a somewhat increased tendency for aggregation that may be due to the enhanced hydrophobicity of the surface—show little difference in number or appearance to the control cells, Fig. 8(b). The cells cultured in the presence of the polyNIPAAM gel, on the other hand, Fig. 8(d), are less numerous and their morphology differs from that of the healthy control cells. As mentioned, measurements were done in triplicate, albeit with cells and gels form a given batch. Further study with additional gels but also tests that allow a closer evaluation of the cellular response to exposure to the different hydrogel chemistries are necessary before general conclusions should be drawn from these cytotoxicity experiments.

## 4. Conclusions

Thermo-responsive hydrogels prepared below their

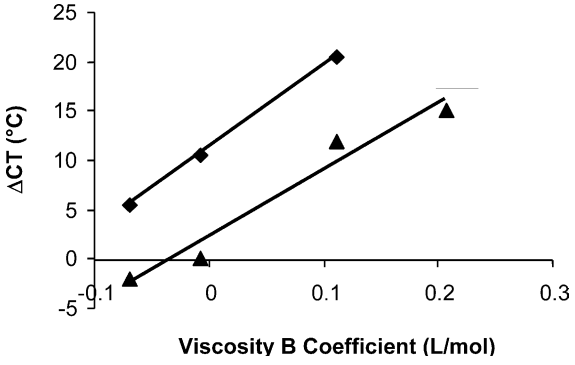



Fig. 7. Correlation between the viscosity *B* coefficient of several anions and the change (reduction) in the CT ($\Delta_{CT}$) of 10×4 polyDEAAM gels to which 0.5 M (▲) and 1 M (♦) of the respective potassium salts has been added. Values fore the viscosity *B* coefficient in water at 25 °C were taken from the literature [14,44], namely: $I^-$ (−0.069), $Cl^-$ (−0.007), $OH^-$ (0.112), $SO_4^{2-}$ (0.208). The viscosity *B* coefficient of $K^+$ is −0.007.

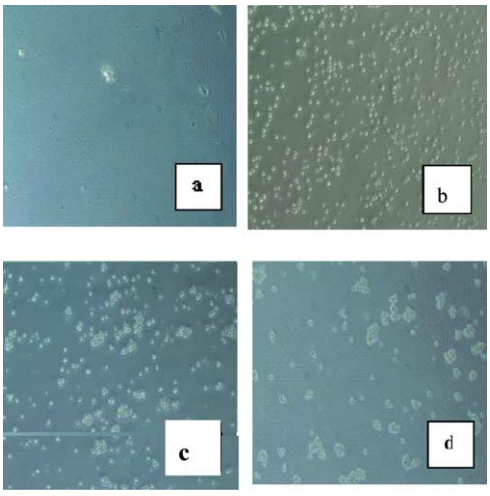



Fig. 8. (a) Hydrogel surface, (b) Jurkat cells (controls), (c) Jurkat cells grown on the polyDEAAM gels after 6 h, (d) Jurkat cells grown on the polyNIPAAM gel after 6 h.

critical temperature (CT) are not necessarily homogeneous in structure and may show a lack in optical transparency. Opaqueness is favoured by a high crosslinker concentration. The swelling behaviour and gel collapse may be influenced by the presence of the heterogeneities, since the opaque gels were found to have a lower maximum swelling ratio below CT, while showing more pronounced relative water uptake and more complete relative water release. The presence of heterogeneities had no influence of the CT itself. The swelling kinetics may be different for a dry xerogel compared to the reswelling of a wet collapsed gel. Inorganic salt were found to influence the phase transition in the expected manner. The change in CT for a given salt concentration correlates with the position of the anion in the Hoffmeister series and in the investigated cases (0.5 and 1.0 M) showed a linear dependency on the viscosity *B* coefficient of the anion. In spite of its broad acceptance, the biocompatibility of polyNIPAAM hydrogels should be estimated for any particular case, as in the experiments described here, adverse effects on cells from a human cell line after as little as 6 h could not be excluded.

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