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Synthesis and characterisation of stimuli-responsive poly(*N*,*N*′-diethylacrylamide) hydrogels

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Abstract

Stimuli-responsive poly(*N*,*N*′-diethylacrylamide) gels were prepared by free radical polymerisation in aqueous solution, using *N*,*N*-methylenebisacrylamide as crosslinking agent. The gels were compared with the corresponding poly(*N*-isopropylacrylamide)-based gels. In particular, the swelling ratio of both gel types including the effect of the crosslinker content, their swelling and deswelling kinetics, their permeability and finally their drug (insulin) storage and controlled release ability were compared. In spite of the similarity in the monomer/crosslinker ratio, the deswelling kinetics and the critical temperatures (ca. 30–32 °C in pure water), some differences could be observed. Compared to poly(*N*-isopropylacrylamide)-based gels, poly(*N*,*N*′-diethylacrylamide)-based gels show a broader phase transition temperature interval, a more pronounced dependency of the swelling ratio on the crosslinker content, slower reswelling kinetics, a higher ingress percentage for dextran standards ranging from 5 to 70 kD, but lower ingress percentages for proteins (BSA, insulin) and much faster drug (insulin) release kinetics. While a non-linear release kinetic was observed in the case of the poly(*N*-isopropylacraylamide)-based gels both in water and in PBS (phosphate buffered saline), this was not the case for the poly(*N*,*N*′-diethylacrylamide)-based gels.

Keywords: Stimuli-responsive hydrogels; PolyNIPAAm; Drug release

1. Introduction

Hydrogels are cross-linked, three-dimensional networks of hydrophilic polymers that may swell, but due to their high degree of crosslinking cannot dissolve when brought into contact with water [1]. As they swell, these gels incorporate large amounts of water and their volume increases drastically. Hydrogels whose hydrophilicity is sensitive to certain environmental stimuli, such as the temperature, the pH, or the ionic strength of the surrounding medium but also light, or magnetic fields, can exhibit pronounced changes in their swelling behaviour, network structure, permeability or mechanical strength in response to such stimuli. Such materials are increasingly discussed for application in fields like controlled drug delivery [2–8], protein absorption [9], immobilisation of enzymes [10–13], recyclable absorbents [9], bioseparation [14], artificial muscles [15], and chemo-mechanical systems among others [16,17].

In the pertinent literature, a number of temperature-responsive hydrogels are described that are swellable below a certain 'critical temperature' (CT) and undergo abrupt changes ('collapse') in volume, as the temperature is increased above the CT. In analogy to the corresponding linear polymer molecules, the thermo-responsiveness of these gels is attributed to a delicate balance of hydrophilic and hydrophobic moieties in the monomeric units [18,19]. One may hence expect a pronounced effect of the chemistry, the size, the degree of crosslinking, the configuration and the mobility of the alkyl side chains on the behaviour and application of the corresponding thermo-responsive hydrogels.

Stimuli-responsive hydrogels have been prepared by creating a three-dimensional network of polymers such as polyethylene oxide, hydroxypropyl cellulose, poly(vinyl alcohol) and derivatives of poly(*N*-substituted acrylamides)

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[8]. Most previous work has been carried out with poly(*N*-isopropylacrylamide)-based gels (polyNIPAAm) either as homogels or as heterogels containing other monomeric species, mostly acrylamide and methacrylates [2,3,9–11,13,18–22]. PolyNIPAAm displays a phase transition when the temperature is increased above approximately 32 °C in pure water [23]. This critical temperature in the physiological range together with the fact the polyNIPAAm is a well-understood and well-characterised material has contributed to the popularity of these gels. However, other chemistries, e.g. gels based on poly(*N*,*N*′-diethylacrylamide), polyDEAAm, can be expected to result in hydrogels that show similar critical temperatures while being more suited to application in the life sciences.

In this paper, polyDEAAm-based hydrogels are prepared and characterised in comparison to the standard polyNIPAAm-based ones in particular in regard to their phase transition, their swelling/deswelling behaviour and their drug release properties.

2. Experimental procedures

2.1. Materials

N-isopropylacrylamide (NIPAAm), *N*,*N*-methylenebisacrylamide (BIS), *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED), ammonium persulfate (APS), dextran molecular weight standards, insulin and bovine serum albumin (BSA) were obtained from Sigma Aldrich Chemical (Buchs, Switzerland) and used as received. *N*,*N*′-diethylacrylamide (DEAAm) was obtained from Polysciences Inc. Europe. Water was purified using an Elix-3 system (Millipore, Bedford, MA).

2.2. Hydrogel synthesis

Hydrogels were prepared by free radical polymerisation in aqueous solutions of NIPAAm or DEAAm, in the presence of BIS as crosslinking agent. APS was used to initiate the reaction and TEMED was used as an accelerator. The well-known *W*/*C* nomenclature was used to characterise the composition of the gels [24], even though this is far from perfect due to the differences in the mass of the two monomers. In this nomenclature, *W* represents the mass of the combined monomers per 100 mL of water and *C* the mass of crosslinker expressed as a percentage of the total amount of monomer plus crosslinker. Table 1 summarises the composition of the gels prepared in this investigation. For the polymerisation, monomer, cross-linker and water were mixed together in a glass vessel at room temperature (27 °C) for 2 h, under N_2 atmosphere. Then radical starter and accelerator were added and they were mixed together for 5 min. Afterwards, the solution was poured into the moulds and kept at room temperature for at least 24 h during which time the polymerisation took 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 putative unreacted material. The water was exchanged several times during this period.

Table 1
Composition of the prepared polyNIPAAm and polyDEAAm gels

Component	Gel composition ($W \times C$)[a]		
	10×4	10×2	10×1
Monomer[b]	2.4 g	2.45 g	2.475 g
BIS	0.1 g	0.05 g	0.025 g
H_2O	25 ml	25 ml	25 ml
APS	7.5 mg	7.5 mg	7.5 mg
TEMED	4.87 μl	4.87 μl	4.87 μl
Dry mass (polyNIPAAm)	0.144 g	0.144 g	0.100 g
Dry mass (polyDEAAm)	0.173 g	0.222 g	0.104 g

[a] W is the weight in grams of the combined monomers per 100 mL of water and C is the mass of crosslinker expressed as a percentage of the total amount of monomer plus crosslinker.
[b] NIPAAm or DEAAm.

2.3. Measurements of the characterisation parameters

Measurements were repeated at least three times and average values reported in the corresponding figures. The deviation between the measurements was less than 10%. The swelling ratio (SR) of the gels was measured gravimetrically in distilled water in the temperature range from 22 to 45 °C for the polyNIPAAm and from 22 to 50 °C for the polyDEAAm gels. Before the measurement, the gel was incubated in distilled water for at least 24 h at the indicated temperature. The SR was then calculated using the following expression,

$$\mathrm{SR} = W_s/W_d$$

where W_s is the weight of water in the swollen gel at equilibrium at the given temperature and W_d is the dry weight of the gel, Table 1. Gels were dried in vacuum overnight at 40 °C.

The deswelling kinetics (Water Retention, WR) were determined as follows. Gels samples were equilibrated in water at room temperature and were at $t=0$ quickly transferred into hot distilled water (50 °C for polyNIPAAm and 42 °C for polyDEAAm). The deswelling kinetics were measured gravimetrically. The weight changes of each gel were recorded every 10 min for at least 1 h. The water retention (%) was then calculated as:

$$\mathrm{WR} = 100 \times (W_t - W_d)/W_s$$

where W_t is the weight of the gel at a given moment during the measurements.

The reswelling kinetics (Water Uptake, WU) of the gels were measured gravimetrically at 20 °C for polyNIPAAm and at 15 °C for polyDEAAm gels after incubating the samples in hot water for a few hours. The weight changes of

gel were recorded every 10 min for at least 1 h. The water uptake (%) was calculated as

$$WU = 100 \times (W_t - W_d)/W_s$$

The experimental conditions for the measurement of the swelling ration, the deswelling and the reswelling kinetics are summarised in Table 2.

2.4. Molecular ingress and release

Dextran standards with molar masses ranging between 5 and 70 kD and two different proteins, BSA and insulin (molar masses 66 and 5.7 kD, respectively) were used as model permeants for the purpose of the estimation of molecular ingress into the gels. For such measurements the fully swollen hydrogels were placed at room temperature (i.e. $T < CT$) in solutions (0.9% NaCl + 0.01% sodium azide as bactericide) containing 1 mg/mL of the probe molecules. Known volumetric amounts of the gels were incubated in a known volume of the probe molecule solution at room temperature and the concentration of the probe molecule in the external solution was evaluated initially and after 3 and 24 h by gel permeation chromatography. For this purpose, samples were injected into a liquid chromatograph system (pump: HITACHI L-7110, Detector: KNAUER, RI K-2300) equipped with a Shodex protein KW-804 column and a KW-G guard column. Samples were filtrated before the injection into the column.

Insulin was used as model drug in the release experiments. Dry gels were immersed in 10 mL of a 0.1% solution of insulin in PBS (phosphate buffered saline, pH 7.2, 0.1 M, 0.9% NaCl). The gels were left to soak in the solutions for 3 days under mild agitation at room temperature. This permitted the hydrogels to swell. Then, the gels were transferred into warm (37 °C) water for the release experiments. The amount of insulin released into the medium was determined using a Lambda 20 UV-spectrophotometer (Perkin–Elmer, Norwalk, CT) at 274 nm (calibration curve 0–1 mg/mL). The insulin release was calculated according to

$$\text{Insulin Release} = M_t/M_{in}$$

Where M_t is the amount of insulin released at time t and M_{in} is the amount of insulin initially present in the gel at time 0.

3. Results and discussion

3.1. Swelling ratio and kinetics

PolyNIPAAm is expected to have a critical solution temperature in pure water between 32 °C and 34 °C according to the literature [25–28]. For polyDEAAm polymers a critical solution temperature in the same region, i.e. around 30 °C is given [29,30]. Whereas polyNIPAAm macromolecules typically have a very sharp phase transition within a fraction of a degree centigrade, the turbidity curves published for polyDEAAm show a phase transition spanning several degrees centigrade [31].

Fig. 1 shows the swelling ratio of polyNIPAAm and polyDEAAm hydrogels with a $W \times C$ composition of 10×4 as a function of the temperature. The observed behaviour is reminiscent of that of the linear polymers of the same type as discussed above. In the case of the polyNIPAAm gel the swelling ratio decreases sharply once the critical temperature (CT) is passed and drops from 14 to 3 between 30 and 35 °C. The point of inflection of the swelling ratio versus temperature curve is observed at 32 °C, i.e. at a value that is identical with the point of inflection of the typical turbidity curves recorded for linear polyNIPAAm molecules. The change in the swelling ratio occurs over a much broader temperature range in the case of the polyDEAAm gel. A first effect is already observed at 25 °C. In addition, the swelling curve of polyDEAAm includes a point of inflection around 30 °C followed by a gradual further reduction of the swelling ratio that tapers off to a value of 2 at 38 °C. In spite of the similar W/C composition, i.e. presumably a similar degree of cross-linking, the swelling ratio at low temperature ($\ll$CT) is twice as high in the case of the polyNIPAAm gel (14) compared to the polyDEAAm one (6.2). At elevated temperatures ($\gg$CT) the differences become less pronounced, however, with swelling ratios of approximately 3 and 2 the polyNIPAAm gel still shows a 50% higher residual 'swelling ratio' than the polyDEAAm gel.

These differences in the swelling behaviour can presumably be related to the different chemistry of the monomeric side chains, i.e. the isopropylamino and diethylamino groups, Fig. 2. According to Otake et al., the temperature-induced collapse of thermo-responsive hydrogels in water and the thermo-precipitation of the corresponding linear polymer aqueous solutions are induced by an aggregation of the polymer segments due to the

Table 2
Conditions adjusted for the deswelling/reswelling experiments

	PolyNIPAAm		PolyDEAAm	
	Temperature (°C)	Time	Temperature (°C)	Time
SR	22–45	24 h	22–50	24 h
WR	50	10–60 min	42	10–60 min
WU	20	10–60 min	15	10–60 min

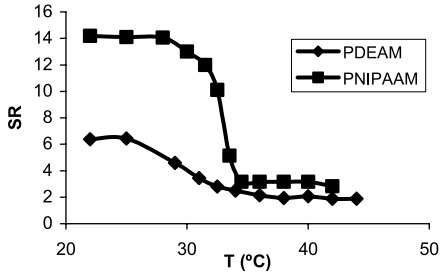


Fig. 1. Comparison of the swelling ratio as a function of the temperature for a polyNIPAAm and a polyDEAAm gel. Composition of both gels, $W \times C = 10 \times 4$.

hydrophobic interaction [32–34]. Generally, the strength of the hydrophobic interaction is proportional to the number of water molecules that form the hydrophobic hydration and increases with temperature (entropy effect). It can be therefore being presumed that the gel whose hydrophobic group has a larger hydrophobic surface (contact) area undergoes phase transition at lower temperatures. Since the side chains of the polyDEAAm units contain one carbon atom more, this would explain the earlier onset of shrinkage in the polyDEAAm-based gels, but also the lower swelling ratio (lower tendency for water uptake) for a given monomer/crosslinker ratio observed in these gels.

A similar behaviour is observed when the two gel types are prepared with a lower degree of crosslinking, Fig. 3. The relative crosslinker concentration affects the value of the possible swelling ratio, but not the general shape of the curve, the broadness of the phase transition, or the transition temperature. A lower degree of crosslinking, as expected, leads to a higher swelling ratio. The effect is especially pronounced in the case of the polyDEAAm gels at temperatures below CT. PolyNIPAAm gels consistently show a sharper phase transition and a higher swelling ratio compared to the corresponding polyDEAAm gels. The reason for this consistently observed behaviour, which mirrors that of the corresponding linear polymers in solution, can only be speculated upon. The more hydrophobic side chain group should enhance the hydrophobic interactions between the polymer segments. In the case of a thermo-responsive gel, this will influence the phase transition. However, at present the direct verification of the experimental results by a mathematical calculation of the phase diagram is not possible. The interested reader is referred to the seminal review by Shibayama and Tanaka for a detailed discussion of the phenomena related volume

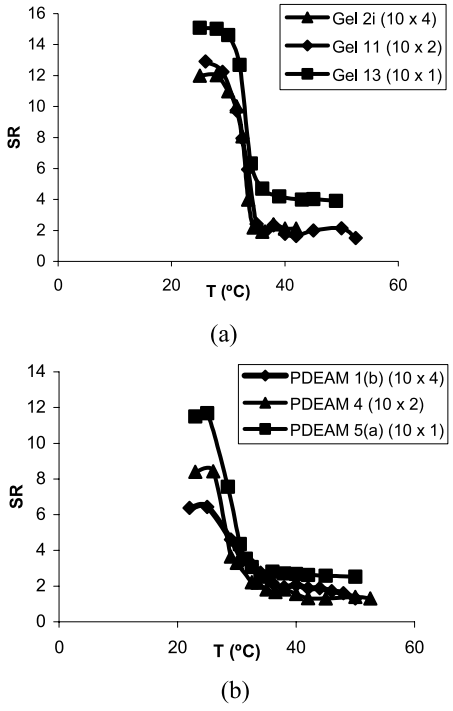


Fig. 3. Temperature dependence of the swelling ratio of (a) polyNIPAAm and (b) polyDEAAm gels prepared using different ratios of monomer/-crosslinker. Measurements were repeated at least three times and average values are reported in the figure. The deviation between the measurements was less than 10% of the average value.

transition of polymer gels [35]. Whereas a reduction of the crosslinker concentration from 10×4 to 10×1 nearly doubles the swelling ratio below CT in the case of the polyDEAAm gels a much smaller effect (increase ca. 20%) is observed in the case of the polyNIPAAm gels.

When the expulsion of water from the collapsed gels is followed at 50 °C (polyNIPAAm) and 42 °C (polyDEAAm) respectively, Fig. 4, it is evident that both gel types respond quickly and in a very similar manner. 80% of the stored water is released within 10 min, after which no further loss of water can be observed. If we presume the collapse of the gels above the critical temperature to be due to a destruction of the solubilising H-bridges at that temperature followed by an enforced interaction of the 'naked' hydrophobic groups in order to increase the entropy of the system, i.e. an effect mainly driven by the surrounding water molecules and less so by the solubilised polymer chains, the similarity in the deswelling kinetics of the two gels types is to be expected as long as both gels are placed

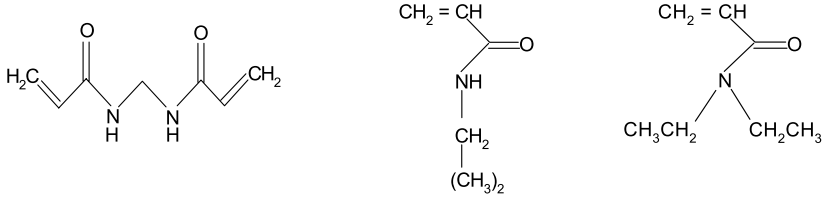


Fig. 2. Chemical structures of (a) *N*-isopropylacrylamide, (b) *N,N'*-diethylacrylamide, (c) *N,N*-methylenebisacrylamide (crosslinker).

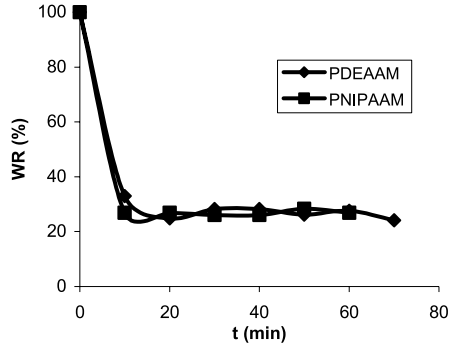


Fig. 4. Deswelling kinetics of a polyNIPAAm gel (at 50 °C) and a polyDEAAm gel (at 42 °C). Composition in both cases: 10×4. Measurements were repeated at least three times and average values are reported in the figure. The deviation between the measurements was less than 10% of the average value.

into an aqueous environment well above the critical temperature.

When the reswelling kinetics are recorded at 20 °C (polyNIPAAm) and 15 °C (polyDEAAm), respectively, some differences can be observed, Fig. 5. Under the same conditions, polyNIPAAm reswells faster than polyDEAAm. PolyNIPAAm absorbs 95% of the water within the first 8 min and has reached equilibrium (>99%) within the first 10 min. PolyDEAAm requires 10 min to absorb 70% of the equilibrium water and at least 30 min to reach full equilibrium. This may be attributed to the differences in the hydrophobicity of the DEAAm and NIPAAm side chains. If the hydrophobic interactions between the DEAAm chains are stronger, slower reswelling kinetics—and, incidentally, a lower swelling ratio for a given crosslinking degree (see also Fig. 1)—are to be expected.

3.2. Molecular ingress and release

The accessibility/permeability of the gels to molecules of differing sizes was estimated using 4 dextran standards (5, 15, 40, and 70 kD) and two proteins (insulin, 5.7 kD and BSA, 66 kD). Fully swollen gels equilibrated with buffer

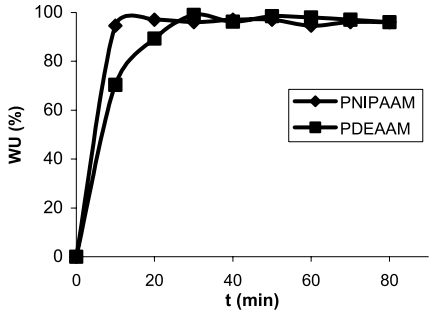
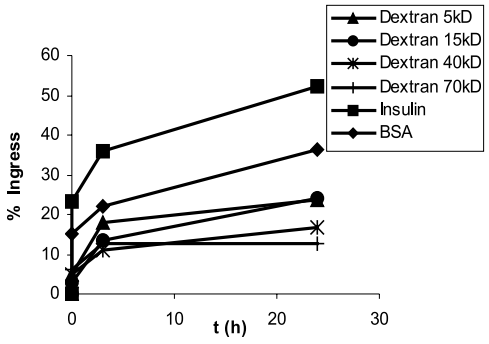


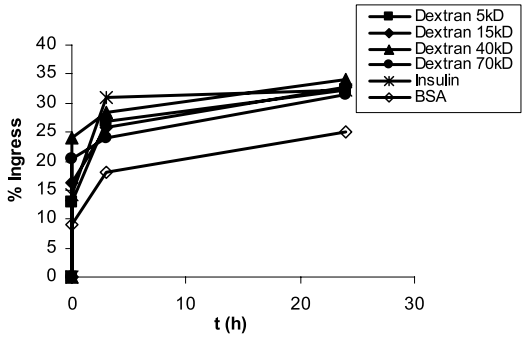
Fig. 5. Reswelling kinetics of a polyNIPAAm gel (at 20 °C) and a polyDEAAm gel (at 15 °C). Composition in both cases: 10×4. Measurements were repeated at least three times and average values are reported in the figure. The deviation between the measurements was less than 10% of the average value.

were placed into solutions containing the probe molecules at a temperature below CT ('room temperature'). Then the ingress of the molecules into the gels was followed chromatographically (gel filtration) over a period of 24 h, Fig. 6. All gels included in this figure had the 10×4 ($W \times C$) composition.

Some interesting differences can be observed. For a given kind of probe molecule, i.e. the series of dextrans on one side and the two proteins on the other, the smaller molecules show a higher ingress rate than the larger ones. This may reflect real differences in the accessible inner gel volume as a function of the size of the entering molecule. Between the two molecule classes, however, there is not strict relationship between size and ingress. For example, BSA (66 kD) shows higher ingress than even the 5 kD dextran standard for the polyNIPAAm gel. This may still be explained by the more compact structure of the protein compared to the dextrans. However, the dextran standards then show higher ingress into the polyDEAAm gel than into the polyNIPAAm one. In the case of the polyNIPAAm gels, after 24 h the 5 and 15 kD dextrans reach an ingress of approximately 25% and appear to still increase their ingress, while the 40 and 70 kD dextrans reach 15 and 12% ingress,



(a)



(b)

Fig. 6. Ingress of four dextran standards (5, 15, 40, and 70 kD) as well as two proteins (insulin: 5.7 kD, BSA: 66 kD) as a function of the equilibration time for (a) a 10×4 polyNIPAAm and (b) a 10×4 polyDEAAm gel. Measurements were repeated at least three times and average values are reported in the figure. The deviation between the measurements was less than 10% of the average value.

respectively. In the case of the 70 kD dextran, no further ingress is observed after the first three hours. In the case of the 10×4 polyDEAAm gels, almost all investigated molecules after 24 h show an ingress of about 32%, which still seems to be increasing.

Proteins seem in general more prone to enter the polyNIPAAm gel than the polyDEAAm one, as insulin after 24 h reached an ingress value of 50% in the case of the polyNIPAAm gel compared to only 30% in the case of the polyDEAAm one (BSA: 35% versus 23% after 24 h). Again the more hydrophobic environment provided by the polyDEAAm gel may be responsible for the lower tendency of the two blood proteins to enter this type of hydrogel.

When a gel with a lower cross-linking degree (composition 10×1) is chosen, Fig. 7, ingress reaches slightly higher proportions, especially for the smaller molecules. For some of the larger ones lower ingress values are observed in some cases. The most noticeable difference is that for the 10×1 gels, maximum ingress is generally already observed after 3 h, whereas in the case of the 10×4 gels significant ingress still takes place between 3 and 24 h.

The ability of the gels to release a drug upon stimulation was investigated using insulin as model drug. The gels (polyNIPAAm and polyDEAAm, both 10×4) were equilibrated in a solution containing 0.1 mg/mL of insulin. Then the gels were transferred to a 37 °C buffer solution ('physiological temperature', >CT, collapse of the structure) and the insulin release was followed photometrically, Fig. 8. In the case of the polyNIPAAm gel, there are two stages of release. During the first stage insulin slowly diffuses out, reaching 40% of release in 24 h. During the second stage, insulin release reaches almost 90% within the next 5 h. In the case of the polyDEAAm gel, insulin release is fast and linear and reaches 80% within the first 10 h. For both gels close to 90% of the insulin initially present within the gel matrix are released after the gel had been forced to undergo temperature induced phase transition.

The reason for the non-linear behaviour observed in the case of the polyNIPAAm gel can at present only be speculated upon. It is especially surprising in view of the highly similar water release kinetics shown in Fig. 4, where 80% of the water was squeezed out of the gels within 10 min. The differences in the insulin release are not due to the release environment (phosphate buffered saline, PBS), as a very similar behaviour was observed for release in water, Fig. 9. The difference may be due to the 'collapse kinetics'. PolyNIPAAm undergoes phase transition very quickly and within a fraction of a degree centigrade. It is possible that as a result of this phenomenon a very tight skin forms around the polyNIPAAm gel, through which water,

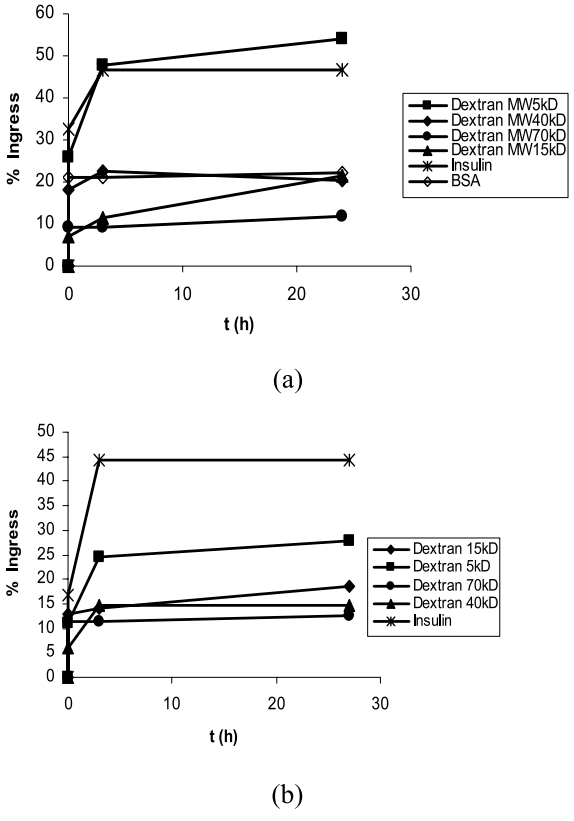


Fig. 7. Ingress of four dextran standards (5, 15, 40, and 70 kD) as well as two proteins (insulin: 5.7 kD, BSA: 66 kD) as a function of the equilibration time for (a) a 10×1 polyNIPAAm and (b) a 10×1 polyDEAAm gel. Measurements were repeated at least three times and average values are reported in the figure. The deviation between the measurements was less than 10% of the average value.

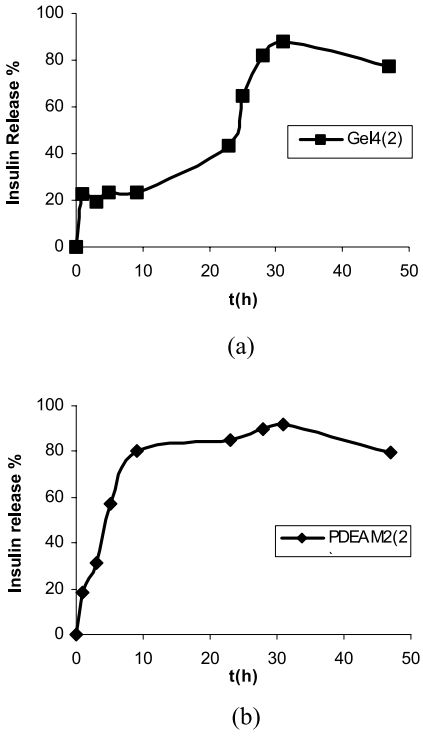


Fig. 8. Insulin release from (a) a 10×4 polyNIPAAm gel and (b) a 10×4 polyDEAAm gel at 37 °C, release medium is PBS (phosphate buffered saline, pH 7.2, 0.1 M, 0.9% NaCl). Measurements were repeated at least three times and average values are reported in the figure. The deviation between the measurements was less than 10% of the average value.

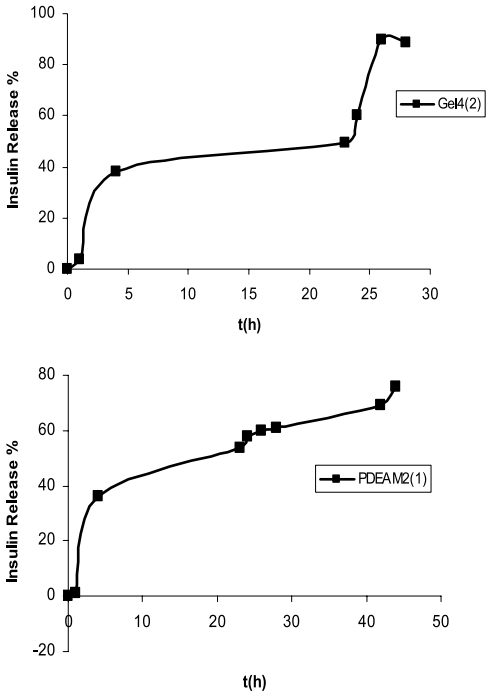


Fig. 9. Insulin release from (a) a 10×4 polyNIPAAm gel and (b) a 10×4 polyDEAAm gel at 37 °C, release medium is distilled water. Measurements were repeated at least three times and average values are reported in the figure. The deviation between the measurements was less than 10% of the average value.

but not necessarily the much larger insulin can pass. Since the collapse is much less abrupt in the case of polyDEAAm, the 'skin' in this case would form more gradually and in the presence of outflowing water. This may possibly help to maintain in the collapsed part of the gel pores large enough for insulin exit by molecular diffusion. The second stage observed during insulin release from the polyNIPAAm gels would according to this hypothesis occur once the entire gel has reached the higher temperature (full collapse) and further association of the collapsed chains has lead to a coarser structure, similar to the ageing and further aggregation observed with time for linear polyNIPAAm molecules. It is also possible that due to the stronger hydrophobic interaction in the collapsed polyDEAAm gel (more hydrophobic side chains) a more porous 'skin' is formed as the result of a more pronounced 'bundling' of the polymer segment in the collapsed state. However, as pointed out above, this is simply a speculation and the effect obviously needs further investigation, before a complete explanation can be given.

4. Conclusions

This paper has shown experimentally that the swelling ratio of the gels could be adjusted by altering the content of the crosslinking agent while the critical solution temperature remains unaffected. Also, controlled by external environmental stimuli (temperature), the hydrogels exhibit reversible reswelling and deswelling behaviour. One may simply adjust the crosslinker density and the temperature to control the pore size of the hydrogels in order to allow a given molecule to diffuse in or out of the gel matrix. This property could be useful in the case of drug delivery or for molecular bioseparation.

References

[1] Ramaraj B, Radhakrishnan G. J Appl Polym Sci 1994;52:837–46.
[2] Ogata N. Am Chem Soc Polym Preprints, Div Polym Chem 1998; 39(2):184–5.
[3] El-Samaligy M, Rohdewald P. Int J Pharm 1983;13:23–34.
[4] Park TG. Biomaterials 1999;20:517–21.
[5] Majeti NV, Kumar R, Kumar N. Drug Dev Ind Pharm 2001;27:1–30.
[6] Kim SW, Bae YH, Okano T. Pharm Res 1992;9(3):283–90.
[7] Kost J, Langer R. Adv Drug Deliv Rev 2001;46:125–48.
[8] Yoshida R, Sakai K, Okano T, Sakurai Y. Adv Drug Deliv Rev 1993; 11:85–108.
[9] Kawaguchi H, Fujimoto K, Mizuhara Y. Colloid J Polym Sci 1992; 270:53–7.
[10] Bae YH, Okano T, Kim SW. J Control Release 1989;9:271–9.
[11] Akihiko K, Hideki F. J Ferment Bioeng 1997;84:337–41.
[12] Liu F, Tao G, Zhuo R. Polym J 1993;25:561–7.
[13] Shiroya T, Tamura N, Yasui M, Fujimoto K, Kawaguchi H. Colloids Surf., B: Biointerfaces 1995;4:267–74.
[14] Kim JJ, Park K. Bioseparation 1999;7:177–84.
[15] Low LM, Seetharaman S, He KQ, Madou MJ. Sens Actuators B 2000; 67:149–60.
[16] Beebe DJ, Moore J, Bauer JM, Yu Q, Liu RH, Devadoss C, Jo BH. Nature 2000;404:588–90.
[17] Liu RH, Yu Q, Beebe D. J Microelectromech S 2002;11(1):45–53.
[18] Gan LH, Roshan Deen G, Loh XJ, Gan YY. Polymer 2001;42:65–9.
[19] Zhang XZ, Zhuo RX. Eur Polym J 2000;36:643–5.
[20] Chen S-J, Du F-S, Wu Z-Q, Huang L, Li Z-C, Li F-M. Am Chem Soc, Polym Preprints, Div Polym Chem 1997;38(2):534–5.
[21] Fazila S, Arthur BE. J Polym Sci, A: Polym Chem 1998;36:2095–102.
[22] Zhang X, Zhuo R. Colloid J Polym Sci 1999;277:1079–82.
[23] Hirokawa Y, Tanaka T. J Chem Phys 1984;81:6379–80.
[24] Gehrke SH, Palasis M, Akhtar MK. Polym Int 1992;29:29–36.
[25] Bae YH, Okano T, Kim SW. J Polym Sci, B: Polym Phys 1990;28: 923–36.
[26] Inomata H, Wada N, Yagi Y, Goto S, Saito S. Polymer 1995;36: 875–7.
[27] Inomata H, Goto S, Saito S. Macromolecules 1990;23:4887–8.
[28] Zhang X, Zhuo R, Yang Y. Biomaterials 2002;23:1313–8.
[29] Zhong X, Wang YX, Wang SC. Chem Eng Sci 1996;51:3235–9.
[30] Gan LH, Cai W, Tam KC. Eur Polym J 2001;37:1773–8.
[31] Baltes T, Garret-Flaudy F, Freitag R. J Polym Sci, A: Polym Chem 1999;37:2977–89.
[32] Otake K, Inomata H, Konno M, Saito S. J Chem Phys 1989;91:1345.
[33] Otake K, Inomata H, Konno M, Saito S. Macromolecules 1990;23(1): 283–9.
[34] Heskins M, Guillet JE. J Macromol Sci Chem 1968;A2:1441–55.
[35] Shibayama M, Tanaka T. Adv Polym Sci 1993;109:1–62.