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Inulin hydrogels. I. Dynamic and equilibrium swelling properties

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Abstract

Inulin hydrogels were developed as potential new carriers for colonic drug targeting. This work describes the dynamic and equilibrium swelling properties of the prepared inulin hydrogels. The influence of various parameters on these properties were assessed, in particular the degree of substitution and feed concentration of methacrylated inulin and varying concentrations of the initiators of the polymerisation reaction. As these hydrogels were developed as colon-specific drug delivery systems, also the effect of pH, ionic strength and esterases were investigated. The results suggest that the rate of water transport into the inulin hydrogels is quite high (mean swelling time < 1.2 h) and that the hydrogels exhibit anomalous dynamic swelling behaviour. The equilibrium swelling of the hydrogels is influenced by the degree of substitution and feed concentration of methacrylated inulin, by the initiator concentration, and by the ionic strength and an acidic pH of the swelling solvent. Esterase activity and pH values of the swelling solvent commonly encountered in the small intestine and the colon on the contrary do not affect equilibrium swelling. By means of differential scanning calorimetry, the glass transition temperature of dry methacrylated inulin hydrogel samples was determined. The mobility of the hydrogel chains seemed to be affected by both the degree of substitution and the feed concentration of methacrylated inulin. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Inulin; Colon-specific hydrogels; Dynamic and equilibrium swelling; Glass transition temperature

1. Introduction

Hydrogels are defined as networks of hydrophilic polymers which can absorb a significant amount of water ($> 20\%$ of their dry weight) without dissolving or loosing their structural integrity. The network is often formed by covalently cross-linked polymers, but ionic bonds, physical entanglements, crystallites, hydrogen bonds and van der Waals forces can also lead to water swellable materials (Peppas and Mikos, 1986; Park et al., 1993). Hydrogels have been studied for a broad range of medical and pharmaceutical

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applications: contact lenses, wound dressings, implant materials, controlled drug delivery systems.

In previous work, the development of semisynthetic hydrogels based on inulin was described (Vervoort et al., 1997). Inulin is a naturally occurring polysaccharide found in many plants such as garlic, onion, artichoke and chicory (Van Loo et al., 1995), and, belonging to the group of the gluco-fructans, it consists of β 2–1 linked fructose molecules, most of them having a glucose molecule at one end of the chain (Roberfroid, 1993). By reaction of inulin with glycidyl methacrylate, methacryloyl groups could be introduced in the inulin chains; aqueous solutions of this methacrylated inulin were converted in crosslinked hydrogels by free radical polymerisation.

This paper describes the characterisation of two key properties of the prepared methacrylated inulin hydrogels, namely swelling kinetics and equilibrium degree of swelling. The effect of various parameters (degree of substitution and feed concentration of methacrylated inulin, concentration of initiating species, pH, ionic strength, esterase activity) on these properties was investigated and a correlation was made with the glass transition temperatures of dry hydrogel samples.

2. Materials and methods

2.1. Materials

Chicory inulin (Raftiline HP; average degree of polymerisation between 22 and 25) was kindly provided by Orafti (Tienen, Belgium). *N*,*N*-dimethylformamide, ammonium persulfate (APS), sodium dihydrogen phosphate, dipotassium hydrogen phosphate, citric acid and sodium chloride were supplied by UCB (Leuven, Belgium). Glycidyl methacrylate (96%) and isopropanol (anhydrous; 99 + %) were purchased from Acros (Geel, Belgium) and 4-dimethylaminopyridine, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (99%) (TMEDA) and carboxyl esterase were obtained from Sigma (St. Louis, MO). Sodium citrate was supplied by Merck (Darmstadt, Germany) and hydrochloric acid 1 N by Chem-lab (Lichtervelde, Belgium).

2.2. Synthesis of methacrylated inulin

The synthesis and characterisation of methacrylated inulin (MA-IN) was described in a previous paper (Vervoort et al., 1997). Briefly, 50 g of dried inulin was dissolved in 200 ml of *N*,*N*-dimethylformamide. 4-Dimethylaminopyridine was added as catalyst in a concentration of 10 mol.% versus fructose units. After dissolution of the catalyst, glycidyl methacrylate was added in an amount depending on the desired degree of substitution (DS is the amount of methacryloyl groups per 100 fructose units). After a reaction time of 72 h at room temperature, MA-IN was precipitated and washed in isopropanol, dissolved in Milli-Q water and dialysed for 10 days at 4°C against the same solvent. MA-IN was eventually recovered by lyophilisation. MA-IN with five degrees of substitution was prepared: DS = 4.4, 8.1, 12.1, 15.4 and 22.3.

2.3. Preparation of MA-IN hydrogels

MA-IN solutions (16, 22 and 27% w/w) were prepared in 0.5 M phosphate buffer pH 6.5. After adding 17.5 μmol/ml APS and 39.4 μmol/ml TMEDA buffer, the mixture was divided over molds and free radical polymerisation took place at room temperature for 2.5 h, resulting in crosslinked hydrogels with a diameter of 10 mm and a height of 2–3 mm. After polymerisation, the hydrogels were removed from the molds and washed in demineralised water for at least 14 days to remove unreacted MA-IN and initiating compounds. After washing, the hydrogels were dried at room temperature till constant weight.

2.4. Dynamic and equilibrium swelling study

The dynamic and equilibrium swelling study was carried out by immersing dehydrated hydrogels of known weight (W_d) in demineralised water at 37°C. At regular time intervals, the hydrogels were removed from the water, blotted with tissue paper to remove free surface water and the weight of the swollen hydrogels (W_s) was recorded. The weight swelling ratio (q) was calculated according to Eq. (1):

$$q = \frac{W_s}{W_d} \quad (1)$$

When the hydrogels reached a constant mass, i.e. when no water sorption did occur anymore, the swelling ratio was considered as the equilibrium swelling ratio (q_{eq}).

The effect of ionic strength, pH and esterase activity on hydrogel equilibrium swelling was studied by soaking the hydrogels either in sodium chloride solutions with ionic strength 0.010, 0.088 and 0.166 or in solutions (ionic strength 0.166) of pH 1.2 (0.1 N HCl), pH 5 (0.05 M citrate buffer) and pH 7.4 (0.05 M phosphate buffer). The last buffer was also used to determine the effect of esterase activity (1 or 10 U/ml) on equilibrium swelling.

2.5. Determination of glass transition temperature

Dehydrated inulin hydrogels were ground in a mortar and the powders were stored in a desiccator for more than 1 month until use.

Differential scanning calorimetry measurements were carried out using a Perkin Elmer DSC-7 differential scanning calorimeter (Perkin Elmer, CT) equipped with a liquid nitrogen subambient accessory. Approximately 20 mg of MA-IN hydrogel powder was transferred into stainless steel pans which were hermetically sealed. The samples were cooled to -40°C and heated from -40°C to 180°C at a heating rate of 5°C/min. The glass transition temperature (T_g) was determined as the temperature at the midpoint of the endothermic rise, measured from the extension of the pre- and post-transition baselines with the DSC-7 analysis program of Perkin Elmer (CT).

3. Results and discussion

3.1. Dynamic swelling study

The uptake of water in hydrogels was analysed according to the power law expression (Ritger and Peppas, 1987):

$$M_t/M_\infty = kt^n \quad (2)$$

with n a diffusional exponent which is indicative of the mechanism of solvent transport in the polymer system, k a kinetic constant incorporating characteristics of the polymeric system, M_t the mass of solvent sorbed at time t and M_∞ the mass of solvent sorbed at equilibrium. For cylindrical samples, solvent uptake in the dry hydrogel is considered to be Fickian, i.e. diffusion controlled, if $n = 0.45$, relaxation controlled if $n = 0.89$ and anomalous, i.e. diffusion and relaxation controlled, if $0.45 < n < 0.89$.

Fig. 1 represents the swelling ratio as a function of time for hydrogels prepared from MA-IN with varying degree of substitution (A) and feed concentration (B). The initially dry, glassy hydrogels became rubbery and transparent upon swelling, except for hydrogels prepared from a 27% w/w solution of MA-IN DS 22.3 which became a little opaque. This could indicate that in these hydrogels phase separation occurred (Gehrke and Lee, 1990). Eq. (2) was applied to the initial stage of swelling and the diffusional exponent n was calculated from the slope of plots of $\ln M_t/M_\infty$ vs $\ln t$ (Table 1). Since $0.45 < n < 0.89$ for all hydrogels tested, solvent transport into the MA-IN hydrogels is considered to be anomalous which is common in glassy hydrogels that become rubbery upon swelling (Gehrke and Lee, 1990). All hydrogels attained equilibrium swelling within the 48 h period of the experiment, irrespective of degree of substitution and feed concentration. To characterise the swelling rate, the mean swelling time (MST) was calculated according to Eq. (3) (Möckel and Lippold, 1993):

Table 1
Swelling behaviour of MA-IN hydrogels of different composition (mean ± S.D., $n = 3$)

Hydrogel composition (DS; feed concentration (%w/w)	Diffusional exponent n	Mean swelling time (h)
4.4; 27	0.60 (±0.01)	0.66 (±0.18)
8.1; 27	0.62 (±0.02)	0.61 (±0.15)
12.1; 27	0.65 (±0.07)	0.50 (±0.03)
12.1; 22	0.65 (±0.01)	0.70 (±0.06)
12.1; 16	0.64 (±0.02)	0.44 (±0.06)
15.4; 27	0.64 (±0.03)	1.01 (±0.04)
22.3; 27	0.66 (±0.01)	1.12 (±0.08)

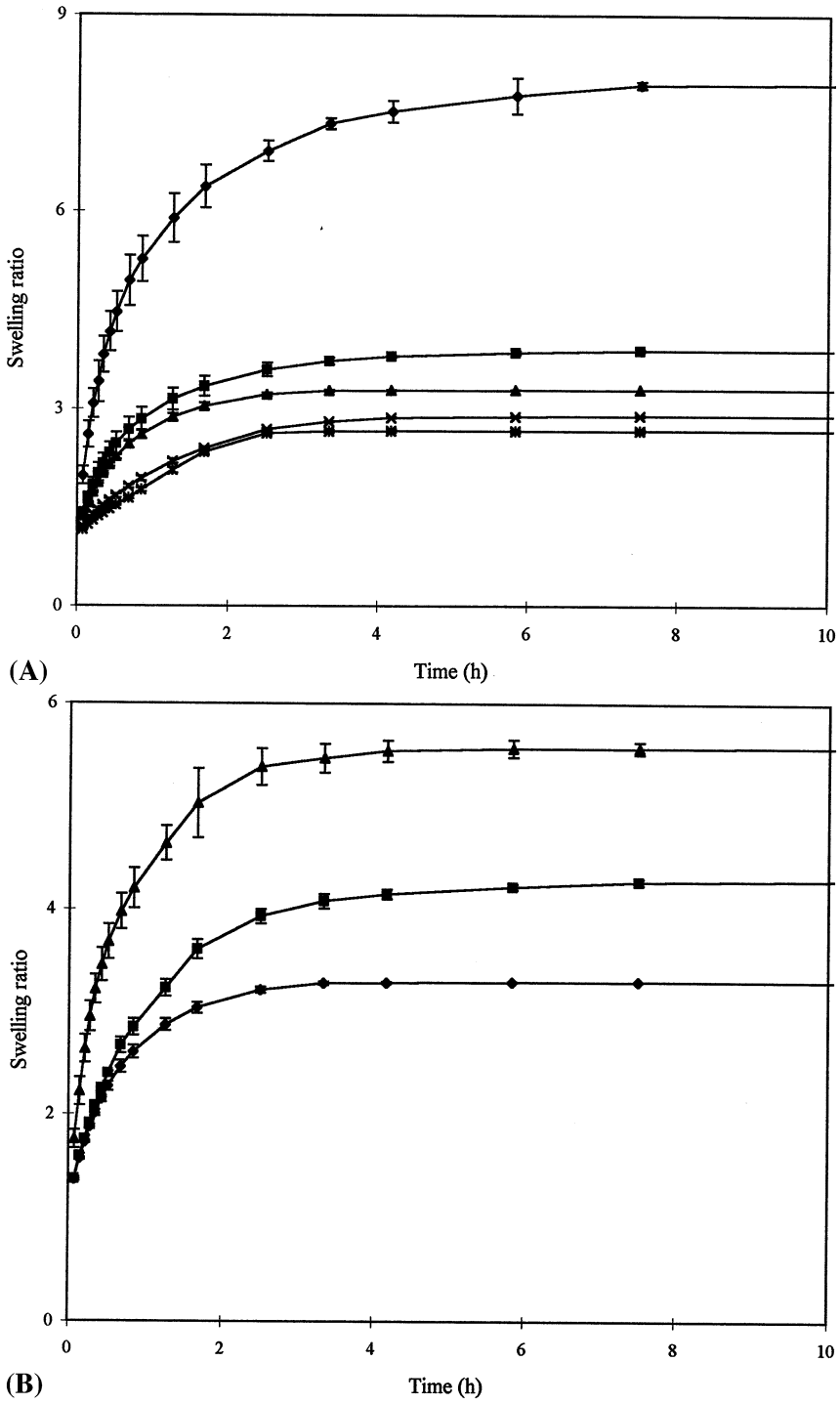

Fig. 1. Swelling ratio as a function of swelling time for hydrogels prepared from a 27% w/w solution of MA-IN DS 4.4 (◆), DS 8.1 (■), DS 12.1 (▲), DS 15.4 (×), DS 22.3 (✱) (A) and for hydrogels prepared from a 16% (▲), 22% (■), 27% (◆) w/w solution of MA-IN DS 12.1 (B). (Each data point represents the mean of three experiments and the error bars indicate the standard deviation from the mean.)

Table 2
Equilibrium swelling ratio of MA-IN hydrogels prepared from a 22% w/w solution of MA-IN DS 12.1 as a function of ionic strength ($\mu_1 = 0.010$; $\mu_2 = 0.088$; $\mu_3 = 0.166$) an pH (ionic strength μ_3) (mean ± S.D., $n = 3$)

Ionic strength			pH (ionic strength μ_3)		
μ_1	μ_2	μ_3	pH 1.2	pH 5.0	pH 7.4
4.29 (±0.02)	4.21 (±0.03)	4.15 (±0.01)	—	4.15 (±0.05)	4.14 (±0.01)

$$\mathrm{MST} = \frac{n}{n+1} k - (1/n) \quad (3)$$

with k and n having the same meaning as in Eq. (2).

The MST values, listed in Table 1, indicate that the swelling rate of the inulin hydrogels is quite high. However, hydrogel swelling slows down for hydrogels with a degree of substitution from 15.4 on. This can be explained by the high degree of cross-linking of these hydrogels, causing a high elastic contractility of the polymer network, which counteracts the swelling process.

3.2. Equilibrium swelling ratio

Fig. 2 illustrates the equilibrium swelling ratio of MA-IN hydrogels prepared from MA-IN with different degree of substitution and feed concentration (A) and prepared with various concentrations of radical generating compounds (B). Degree of substitution and feed concentration clearly influence equilibrium swelling of the inulin hydrogels. A high degree of substitution (more reactive vinyl groups) results, upon polymerisation, in a hydrogel network, which is characterised by a high network density; the large number of intermolecular cross-links restricts network expansion upon swelling (Brøndsted et al., 1995; Perera and Shanks, 1996). A decrease in polymer concentration (feed concentration), i.e. increasing dilution, promotes intramolecular cross-linking, because the probability of bond formation between different chains is proportional to the probability that these bonds lie in the same small volume-element (James and Guth, 1947; Yeh et al., 1995). Hence, less chains are incorporated in a hydrogel network of low feed concentration (increased sol fraction) and the resulting network exhibits restricted rigidity because of the limited amount of intermolecular cross-links formed. Consequently, hydrogels of low feed concentration exhibit less restriction to swelling and are characterised by a higher q_{eq}. Besides degree of substitution and feed concentration of MA-IN, the concentration of the radical generating compounds of the polymerisation reaction also influences equilibrium swelling of the inulin hydrogels (Fig. 2(B)). With increasing concentration of initiators, more radicals are formed in the initiation step of the polymerisation reaction. These free radicals attack the π-bonds of MA-IN creating active centres on the sugar polymer which can subsequently react with remaining methacryloyl groups to form inter- or intramolecular cross-links. The formation of more active centres implies that less unreacted vinyl groups are left to link the inulin chains. Consequently, the resulting hydrogels have less restraining force to swelling and higher q_{eq} values.

As already mentioned, inulin hydrogels were developed as potential new oral colon-specific drug delivery systems and since pH and ionic strength vary along the gastro-intestinal tract (Friend, 1991; Johnson et al., 1993; Kinget et al., 1998), q_{eq} was also determined as a function of these two parameters. Hydrogels prepared from 22% w/w solutions of MA-IN DS 12.1 were used for these experiments and their equilibrium swelling was determined in solutions of various pH (ionic strength 0.166): pH 1.2 (to mimic the stomach), pH 5 (to mimic the upper small intestine) and pH 7.4 (to mimic the lower small intestine) and in sodium chloride solutions with an ionic strength of 0.010, 0.088 and 0.166 (to mimic the range of ionic strengths along the gastro-intestinal tract). The obtained q_{eq} values are reported

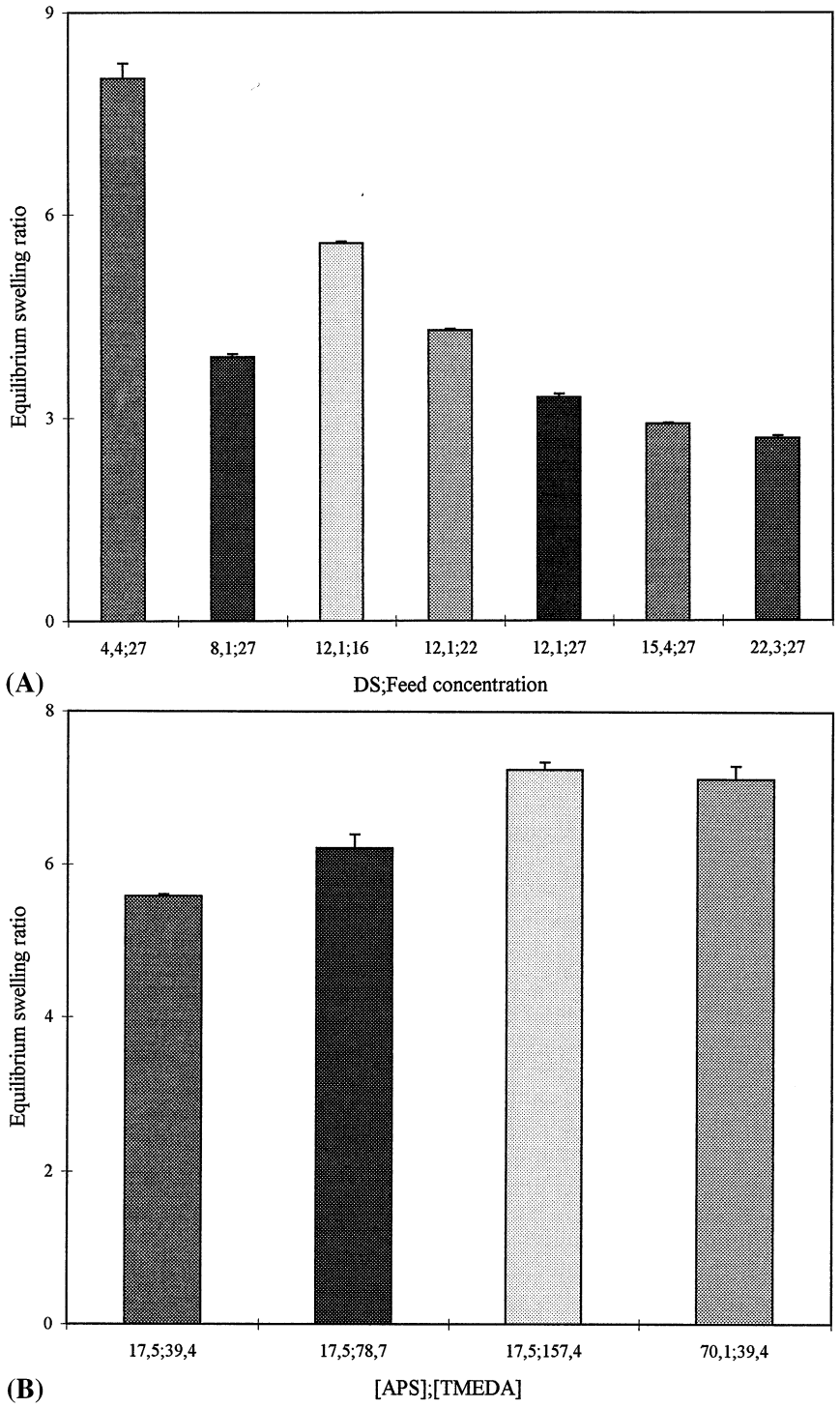

Fig. 2. Equilibrium swelling ratio as a function of DS and feed concentration (% w/w) of MA-IN ([APS] = 17.5 μmol/ml buffer and [TMEDA] = 39.4 μmol/ml buffer) (A) and as a function of concentration (μmol/ml buffer) of the initiating compounds APS and TMEDA for hydrogels of 16% w/w solution of MA-IN DS 12.1 (B). (Each data point represents the mean of three experiments and the error bars indicate the standard deviation from the mean.)

Table 3
Glass transition temperatures (°C) of dry MA-IN hydrogel powder samples as a function of DS and feed concentration

DS 4.4 (27% w/w)	DS 8.1 (27% w/w)	DS 12.1 (27% w/w)	DS 12.1 (22% w/w)	DS 12.1 (16% w/w)	DS 15.4 (27% w/w)
93.0 (± 0.9)	94.5 (± 0.6)	104.5 (± 2.0)	101.2 (± 1.4)	95.9 (± 2.6)	108.2 (± 1.2)

Scanning rate, 5°C/min.
Mean ± S.D., $n = 3$.

in Table 2. As might have been expected for non-ionic hydrogels, the equilibrium swelling was not influenced by pH values commonly encountered in the small intestine and colon (pH 5–7.4). Acidic pH on the contrary, had a significant effect on the inulin hydrogels, since they completely dissolved in the 48 h time period for q_{eq} determination. This can be attributed to hydrolysis either of the β-glycosidic linkages of inulin (Verraest et al., 1995) or of the methacrylate esters in acidic environment. As transit through the stomach in the fasted state is considered to be only 2 h for a solid dosage form (size >2 mm) (Friend, 1991), the degree of swelling of the MA-IN hydrogels under investigation, was also determined in demineralised water before and after immersing the hydrogels for 2 h in 0.1 N HCl. After treatment, q_{eq} increased with 66.1%. This observation suggests that, when the inulin hydrogels are used as oral colon-specific drug delivery systems, premature release of entrapped drug, due to increased hydrogel swelling in the stomach, can occur. However, this premature drug release can be circumvented by enteric coating of the hydrogels.

Due to the presence of ester bonds in the hydrogel cross-links, the effect of esterase activity on q_{eq} was also investigated since esterases are abundantly present in the small intestine (Friend, 1991). Transit through the small intestine is fairly constant and takes approximately 4 h (Friend, 1991; Kinget et al., 1998). Hence, q_{eq} determination in demineralised water before and after immersion of hydrogels (DS 12.1; feed concentration is 22% w/w) for 4 h in phosphate buffer pH 7.4 containing 1 or 10 U/ml carboxyl esterase. However, no significant difference could be observed in q_{eq} before and after immersion, for neither 1 nor 10 U/ml esterase. Accessibility of the ester bonds to esterase might be sterically hindered by the density of the network. Nevertheless, hydrogels with a rather high swelling ($q_{eq} = 4.30$) and thus a rather loose network, were used for these experiments. Another possible explanation that has to be taken into consideration is shielding of the ester by the methyl group in its vicinity as was reported in the literature to explain the resistance of polymethyl methacrylate to alkaline hydrolysis (Bevington et al., 1958).

Concerning the effect of ionic strength, q_{eq} decreased slightly, but significantly with increasing ionic strength of the swelling solvent (Table 2). Sodium chloride can possibly penetrate the hydrogel upon swelling and can interact with network segments. Stretching or contracting properties of the network can be altered by this interaction. Solutes often have unpredictable effects on the swelling of hydrogels because of their interaction with the hydrogel polymer, which can enhance or disrupt specific interactions between swelling solvent and polymer causing increased or decreased swelling of the hydrogel (Refojo, 1976; Gehrke and Lee, 1990).

3.3. Determination of glass transition temperature

Inulin hydrogels of different compositions (DS, feed concentration) were also characterised by their glass transition temperature T_g (Table 3). T_g increased with increasing degree of substitution of MA-IN: the mobility of the polymer chains is restricted due to an increased rigidity of the network with increasing cross-linking degree caused by a higher degree of substitution of MA-IN (Nielsen, 1969; Hariharan and Peppas, 1996; Giammona et al., 1996). Increased feed concentration also resulted in an increased T_g. As

mentioned above, intramolecular cross-linking is favoured when hydrogels are prepared from more diluted solutions. So hydrogels of lower feed concentration are characterised by a lower cross-linking density and the polymer chains are able to move more freely compared to hydrogels of higher feed concentration. Hence, increasing T_g with increasing feed concentration. The data of the DSC experiments support the data of the equilibrium swelling study: higher degree of substitution or feed concentration of MA-IN yield hydrogels with an increased cross-linking density, resulting in lower q_{eq} and higher T_g values.

4. Conclusion

Water transport in dehydrated inulin hydrogels is regulated by both diffusional and macromolecular relaxational processes, irrespective of the degree of substitution and feed concentration of the hydrogel polymer. The rate of swelling is quite high; the mean swelling time ranges from 0.44 to 1.12 h for all hydrogels tested. Equilibrium swelling of the hydrogels exhibits a reciprocal relationship with the degree of substitution and feed concentration of MA-IN and with ionic strength of the swelling media. Exposure of the gels to swelling media with pH values commonly encountered in the small intestine and the colon or media containing esterases, does not affect the equilibrium swelling ratio, whereas media mimicking the stomach, cause an increase of q_{eq} or even completely dissolve the hydrogels, depending on the exposure time.

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