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Dual-stimuli-responsive hydrogels based on poly(*N*-isopropylacrylamide)/chitosan semi-interpenetrating networks

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Abstract

The synthesis and characterisation of semi-interpenetrating polymeric networks obtained by the radical-induced polymerisation of *N*-isopropylacrylamide in the presence of chitosan using tetraethyleneglycoldiacrylate as the crosslinker is described. The influence of the degree of crosslinking and that of the ratio of chitosan to poly(*N*-isopropylacrylamide) on the "pH/temperature induced" phase transition behaviour and swelling characteristics of the hydrogel system are investigated. The ability of the same system to act as a controlled release vehicle for pilocarpine hydrochloride is evaluated.

Keywords: Hydrogels; Drug delivery; Chitosan; Poly(*N*-isopropylacrylamide)

1. Introduction

The inherent ability of hydrogels to display dramatic changes in properties in response to external stimuli renders them useful for many biomedical applications (Griffith, 2000; Nguyen and West, 2002; Hoffman, 2002; Martellini et al., 1998; Peppas et al., 2000; Qiu and Park, 2001; Jeong and Gutowska, 2002; Miyata et al., 2002; Kikuchi and Okano, 2002; Gupta et al., 2002). The impetus for the development of dual-stimuli-responsive hydrogels is provided by the need to develop vehicles for the controlled release of actives under "site-of-action determined" conditions; the development of delivery vehicles that respond to localised conditions of pH and temperature represents one of the challenges currently addressed by the controlled release community (Serres et al., 1996; Sahoo et al., 1998; Galaev and Mattiasson, 1999; Yoo et al., 2000; Kim et al., 2002).

Dual-stimuli-responsive hydrogels may be prepared by combining poly(*N*-isopropylacrylamide), PNIPAAm—a widely studied temperature-sensitive polymer (Winnik, 1990; Makino et al., 2001; Chee et al., 2001)—with another stimulus-sensitive polymeric component. Several dual-stimuli-responsive hydrogel systems have been studied to date: these may be based on copolymers (Kubota et al., 2001; Bokias and Hourdet, 2001; Berlinova et al., 2001; Diez-Pena et al., 2002; Yildiz et al., 2002); interpenetrating polymer networks (IPNs) (Kurisawa and Yui, 1998; Ribelles et al., 1999; Ju et al., 2001; Liu and Fan, 2002; Zhang and Peppas, 2002); or interpolymer

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complexes (Peniche et al., 1998; Lowman and Peppas, 1999; Peniche et al., 1999). However, considerable improvements in our appreciation of the factors that determine mechanical strength, biocompatibility and biodegradability is needed before these materials can be usefully employed in biomedical applications.

Chitosan ((1,4)-2-amino-2-deoxy-β-D-glucan; CS)—a biocompatible, biodegradable, and antigenic pH-sensitive cationic biopolymer—represents a promising candidate for employment in the design of controlled delivery systems (Aide et al., 1997; Kaman, 2000; Paul and Sharma, 2000; Sato et al., 2001; Nunthanid et al., 2001; Puttipipatkhachorn et al., 2001; Lillo and Matsuhiro, 1997; Wang et al., 2001; Kurita, 2001; Bayramoglu and Arica, 2002). Nonetheless, dual-stimuli-responsive hydrogels that combine the properties of chitosan with those of poly(*N*-isopropylacrylamide) have received very little attention (Wang et al., 2000, 2001). In this paper, we report on the synthesis and characterisation of semi-interpenetrating polymeric networks (semi-IPNs) obtained by the radical-induced polymerisation of *N*-isopropylacrylamide (NIPAAm) in the presence of chitosan; tetraethyleneglycoldiacrylate (TEGDA) is used as the crosslinker. The influence of the degree of crosslinking and that of the ratio of chitosan to poly(*N*-isopropylacrylamide) on the "pH/temperature induced" phase transition behaviour and swelling characteristics of the hydrogel system, are considered. The ability of the same system to act as a controlled release vehicle for pilocarpine hydrochloride is also examined.

2. Materials and methods

2.1. General remarks

Chitosan (MW = 150,000; 84.5% deacetylated) and all other chemicals were obtained from Sigma–Aldrich and used as purchased. Phosphate buffer solutions (PBS) were prepared from citric acid and disodium orthophosphate according to a literature procedure (Wode, 1980). Elemental analysis was performed using a Carlo-Erba CHN 1106 Elemental Analyser. FTIR spectra (KBr discs) were recorded using a Perkin-Elmer Paragon 1000 FTIR Spectrometer. A Unicam α Helios UV-Vis spectrophotometer was used for the study of phase transitions and for determining drug release profiles.

2.2. Semi-IPNs preparation

To a stirred solution of chitosan (5 ml; 2%, w/w) in dilute acetic acid (1%, w/w) were added to *N*-isopropylacrylamide and TEGDA followed by a mixture of ammonium persulphate and sodium bisulphite (1:3, w/w); reagent ratios used are presented in Table 1. The resulting solution was poured into glass moulds (50 mm × 50 mm × 3 mm) and kept at 20 °C

Table 1
Mixing ratios (w/w) and final composition (w/w) of the hydrogels

Hydrogel	Initial		Final	
	CS/NIPAAm	TEGDA/NIPAAm (100×)	CS/NIPAAm	TEGDA/NIPAAm (100×)
H1	0.25	7.5	0.24	6.8
H2	0.67	7.5	0.27	5.0
H3	0.25	12.5	0.28	13.5
H4	0.67	12.5	0.44	10.3
H5	0.20	10.0	0.22	10.7
H6	0.79	10.0	0.26	6.8
H7	0.43	6.4	0.27	5.3
H8	0.43	13.5	0.46	15.4
H9	0.43	10.0	0.38	9.6
H10	0.43	10.0	0.36	9.3
H11	0.43	10.0	0.38	9.9
H12	0.43	10.0	0.36	9.8
H13	0.43	10.0	0.38	9.6

for 24 h. The hydrogel that formed was washed repeatedly by soaking in deionised water, dried at 40 °C (12 h; vacuum) and finely ground.

2.3. Phase transition studies

The phase transition behaviour of the synthesised hydrogels (placed in 5 mm quartz cuvettes) was studied by considering the optical transmittance of the system as a function of temperature; studies were conducted at 480 nm in the temperature range 20–40 °C. For purposes of comparison, a sample of chitosan and one of crosslinked PNIPAAm (10% TEGDA) were also monitored.

2.4. Swelling measurements

For swelling experiments, a series of PBS (pH ranging from 2 to 10) were prepared by adding appropriate quantities of disodium orthophosphate and citric acid. The swelling properties were determined gravimetrically. In addition, the influence of pH on PBS uptake was monitored over time and over a range of temperatures within the 20–40 °C limits. The mass equilibrium degree of swelling, SD_{eq} (%), and the rate of swelling, k (min^{-1}) were calculated using:

$$SD_{eq} = \frac{m_{eq} - m_0}{m_0} \times 100, \quad kt = -\ln(m_{eq} - m)$$

where m_0 is the initial sample weight (g), m_{eq} is the sample weight at swelling equilibrium (g), m is the sample weight at time t (g) and t is the time (min).

2.5. Controlled release experiments

The controlled release experiments were performed using hydrogels that had been loaded with 30% (w/w) pilocarpine hydrochloride. Tablets (30 mg, 13 mm diameter) of each hydrogel were pressed from dried samples using a stainless-steel pellet die (8 t, 10 min). The in-vitro release profile of pilocarpine was obtained spectrophotometrically: a drug-loaded tablet was placed into phosphate buffer solution (80 ml; pH 7.2; 37 °C; shaking water bath) and the time-profile of pilocarpine release was determined by absorbance measurements (222 nm). Calibration was performed using a series of PBS containing known amounts of pilocarpine (concentrations range 0.001–0.02%, w/w). Blank experiments, using polymer-only tablets, confirmed that the hydrogels did not contribute to the 222 nm absorption.

3. Results and discussion

Semi-interpenetrating network hydrogels based on poly(*N*-isopropylacrylamide) and chitosan were prepared by the free radical copolymerisation of NIPAAm and TEGDA crosslinker, in the presence of chitosan. The degree of crosslinking and composition in the obtained hydrogels were determined by elemental analysis, Table 1. For the same initial amount of chitosan (i.e. H7, H8, H9), an increase in the crosslinking ratio led not only to a higher crosslinking density but also to an increase in the quantity of chitosan that becomes immobilised within the three-dimensional network.

With very few exceptions, the CS to NIPAAm ratio of the materials was found to be lower than that expected on the basis of the amount of monomer employed for the synthesis. These differences become more pronounced at low crosslinking densities or at higher CS content: for an initial chitosan ratio varying from 0.20 to 0.80, the proportion of CS in the final materials was found to be no greater than 0.46. A likely explanation is that, under the imposed conditions, the efficient crosslinking of NIPAAm was impeded due to the limited availability of reactive functionalities at high concentrations of chitosan, polymerisation in acidic solution leads to the formation of loose three-dimensional networks.

In addition to the spectral features that are typical of PNIPAAm and chitosan (3360–3400 cm^{-1} broad; 1600 cm^{-1} amidic; 1550, 1375 and 1080 cm^{-1}), the FTIR spectra of the hydrogels exhibited new bands that are characteristic of the crosslinked network (1750 and 1180 cm^{-1}). In accord with expectation, the intensity of the 1750 cm^{-1} band, which is assigned to TEGDA ester groups, increased with increased crosslinking density; an increase in the intensity of the 3360–3400 cm^{-1} absorption band was also evidenced with increasing CS to NIPAAm ratio.

Phase transition studies, Figs. 1 and 2, in aqueous solution (pH 2.41) revealed that the onset of the phase transition temperature for samples of PNIPAAm that had been crosslinked in the absence of chitosan (10%

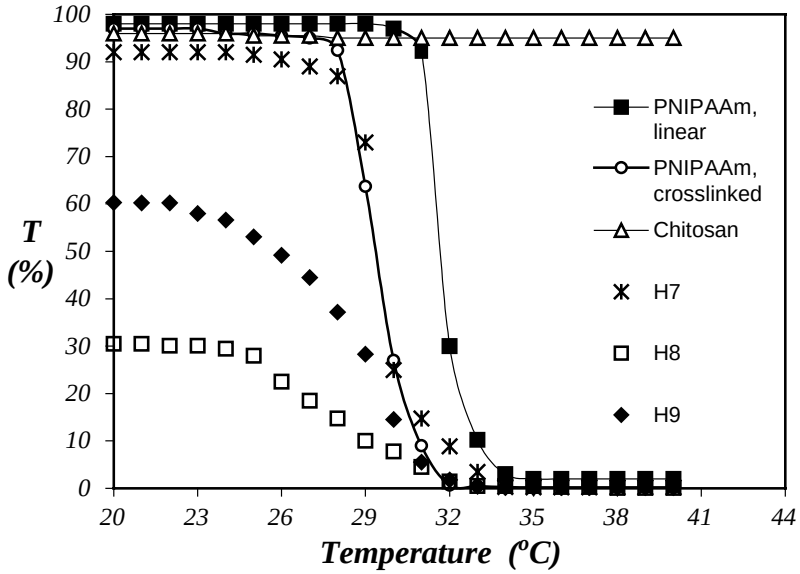

Fig. 1. Transmittance (T, %) vs. temperature for chitosan/NIPAAm hydrogels.

TEGDA) is lower (ca. 28 °C) than that of the parent polymer (ca. 31 °C; Winnik, 1990); the crosslinked polymer retains the good optical transparency characteristics of its non-crosslinked congener ($T = 98\%$ at 20 °C; Chee et al., 2001).

The optical transparency of chitosan in solution ($T = 97\%$; 2%, w/w; in 1%, w/w, CH_3COOH) is not affected by temperature, but when present in the three-dimensional network of PNIPAAm, this biopolymer impacts upon both the onset and the

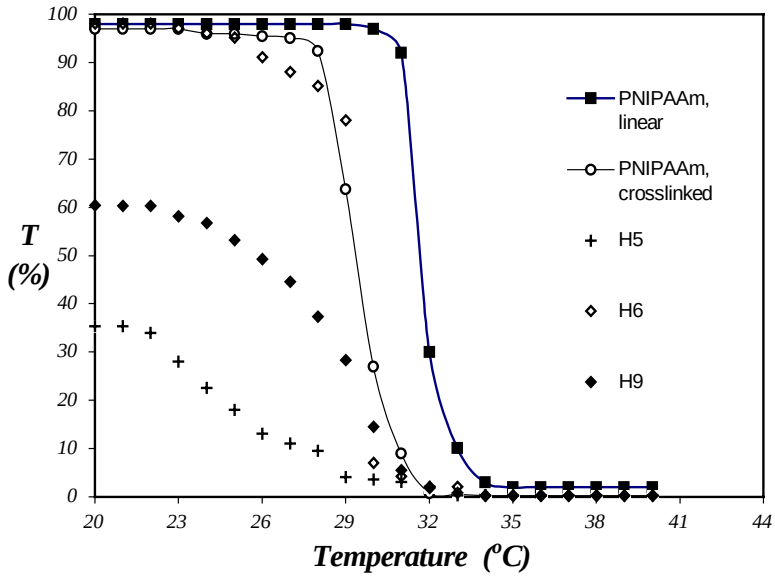

Fig. 2. Transmittance (T, %) vs. temperature for TEGDA/NIPAAm hydrogels.

intensity of the hydrogel phase transition. As the CS to NIPAAm ratio is increased (H7 < H9 < H8), the optical quality of the hydrogel is seen to deteriorate. In parallel, the temperature range for the phase transition becomes less well defined with its onset moving to lower temperatures; a similar behaviour has previously been reported for CS networks containing interpenetrated PNIPAAm (Wang et al., 2000).

The highly crosslinked Hydrogel H5, which contains a minimal amount of chitosan (Fig. 2) was seen to exhibit the lowest onset of the phase transition (23 °C), suggesting that crosslinking density is the dominant parameter influencing the phase transition process.

The swelling behaviour of the hydrogels has been studied over a range of pH values (2–10) and temperatures (20–40 °C); the effect of temperature on the equilibrium degree of swelling (SD_{eq}) at pH 2.41 is summarised in Fig. 3. For crosslinked PNIPAAm (10% TEGDA, no chitosan present), the equilibrium degree of swelling is seen to decrease progressively with increasing temperature until a plateau is reached just above the phase transition temperature; a de-swelling of crosslinked PNIPAAm was observed. A possible explanation is that the PNIPAAm segments collapse with increasing temperature because the hydration capability of the gel is suppressed due to the progressive replacement of the intermolecular, polymer–water hydrogen bonded interactions by intramolecular hydrogen bonds involving PNIPAAm alone.

In marked contrast to the behaviour of simple PNIPAAm networks, hydrogels containing interpenetrating chitosan were seen to exhibit maximum swelling capacity at temperatures that were close to the phase transition temperature of pure PNIPAAm; the magnitude of this effect was found to be dependant on the CS to NIPAAm ratio. It is worth noting that Wang et al. (2000) have reported that in water/ethanol, temperature had little effect upon the swelling behaviour of a CS network, while for a full-IP CS/NIPAAm network, the same workers reported an abnormal swelling behaviour, which they attributed to the stress cracking of the gels (Wang et al., 2001). It appears that two competitive phenomena determine the behaviour of the gel: as the temperature rises, the hydration capacity of chitosan increases whereas that of PNIPAAm decreases as segments collapse due to the temperature-controlled conformational transition. The maximum value for the equilibrium degree of swelling was observed with the least crosslinked hydrogel (H7), which also contains the lowest proportion of chitosan. The degree of crosslinking also plays an important role in determining the swelling behaviour

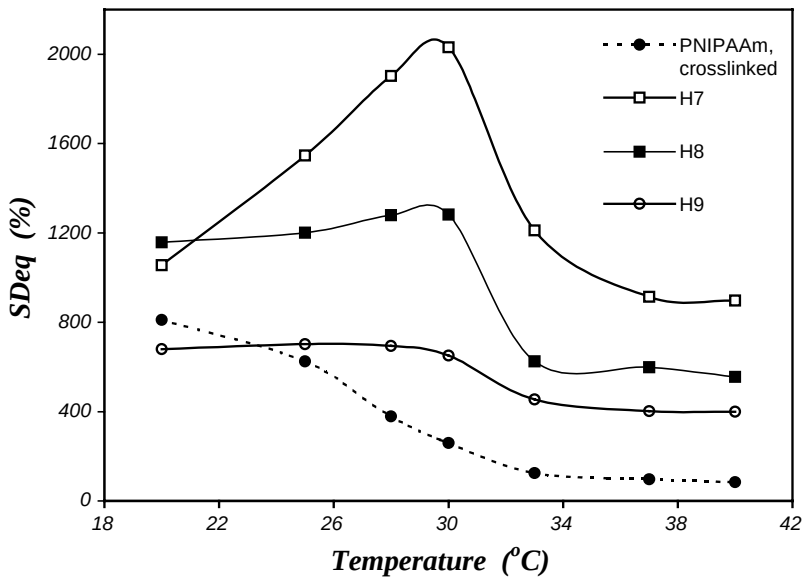

Fig. 3. Equilibrium degree of swelling (%) as a function of temperature.

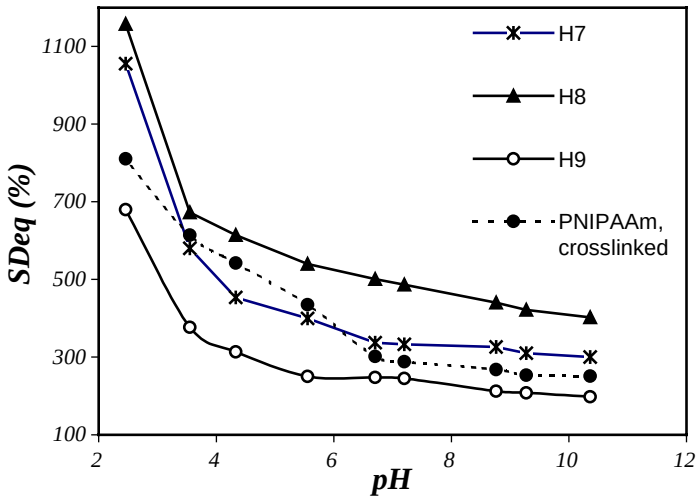

Fig. 4. Equilibrium degree of swelling (%) as a function of pH.

of these materials: H8, which has a higher CS to NIPAAm ratio than H7 and the highest crosslinking density, shows only a moderate increase in SD_{eq}; H9, which has a higher crosslinking density than H7 but a lower CS to NIPAAm ratio than H8, exhibits a relatively flat SD_{eq} profile. It appears that the temperature dependant swelling behaviour is the result of a fine balance that is influenced by both the degree of crosslinking and the CS to NIPAAm ratio.

The presence of chitosan in the crosslinked network was seen to facilitate increased water uptake (up to 2100%). This is especially true in acidic conditions, under which the de-acetylated amino groups of chitosan become ionised; electrostatic effects may be of significance (Vachoud et al., 2000; Berth and Dautzenberg, 2002). Below LCST, a small increase in temperature facilitates the reorganisation of the macromolecular chains and the diffusion of water.

The relationship between equilibrium swelling and pH, measured at 20 °C, is unveiled in Fig. 4. All hydrogels are seen to exhibit very low water uptake at high pH values, but this increases markedly with increasing acidity; neutral and basic conditions appear to be of little significance to the observed degree of swelling. The data reveal that this behaviour becomes more pronounced as the crosslinking density of the hydrogels is increased, presumably because of the reduced mobility of the chitosan chains; at pH < 4.5 the deacetylated amino groups of chitosan become protonated effecting an increase in osmotic pressure (Vachoud et al., 2000; Berth and Dautzenberg, 2002).

In Figs. 5 and 6, the variation in the swelling rate of the hydrogel as a function of CS to TEGDA ratio is presented for acidic and basic conditions, respectively.

Essentially a measure of the water diffusion rate, the reported values were higher in the acidic environment. In both cases, rates are at a maximum for lightly crosslinked systems with a high CS to TEGDA ratio or for highly crosslinked networks with low CS to TEGDA ratio. Within the range considered, it seems that at low pH values, hydrogel swelling is much faster in a looser network (high CS to TEGDA ratio). By contrast, under alkaline conditions, the hydrogels swell at a faster rate when the PNIPAAm network is highly crosslinked and contains a minimal amount of chitosan.

In an effort to investigate the ability of the prepared PNIPAAm/chitosan hydrogels to act as vehicles for the controlled release of actives, pilocarpine hydrochloride was incorporated in the system and its cumulative discharged amount was monitored spectrophotometrically (Fig. 7). It has been reported that release from CS matrices is highly dependent on the structure of the drug (Puttipipatkhachorn et al., 2001). For PNIPAAm/chitosan hydrogel tablets loaded with 30% pilocarpine hydrochloride, the release process was found to be fast, with most of the drug being released in the first 40 min. Although the release

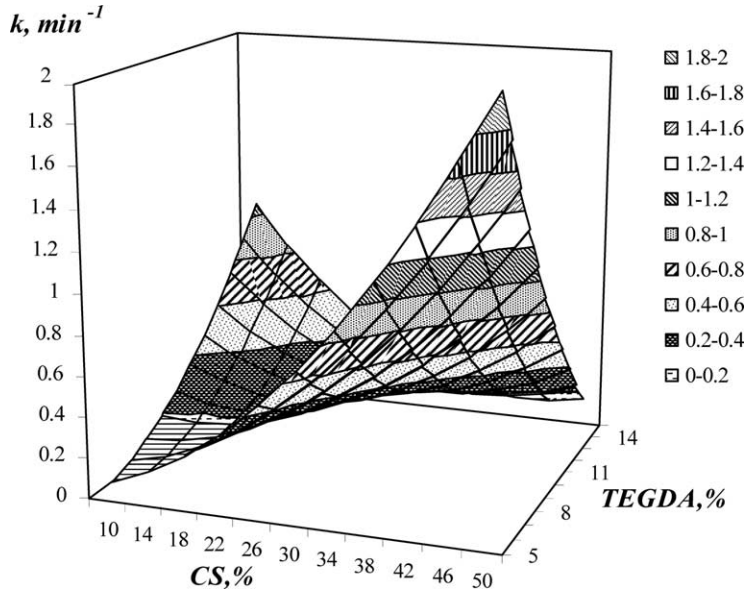

Fig. 5. Swelling rate (k, min^{-1}) of hydrogels at pH 2.41, plotted as a function of chitosan (CS) and crosslinker (TEGDA) content.

profiles of the hydrogels under consideration are quite similar, the more highly crosslinked hydrogels (H3 and H8) exhibited the fastest release characteristics. As the main interactions that determine the retention of the pilocarpine salt are expected to involve the chitosan molecule, it would be reasonable to assume that the loading degree of the hydrogel will be influenced by the amount of this biopolymer present within the

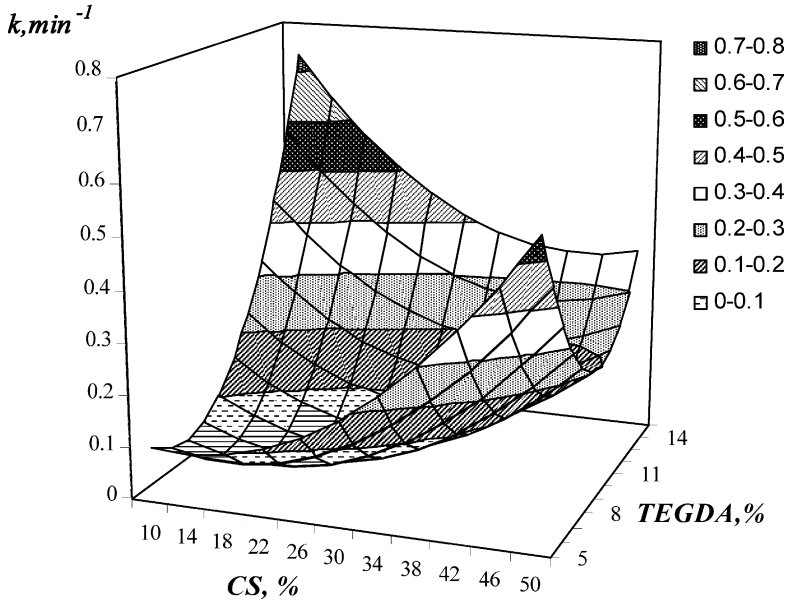

Fig. 6. Swelling rate (k, min^{-1}) of hydrogels at pH 7.21 , plotted as a function of chitosan (CS) and crosslinker (TEGDA) content.

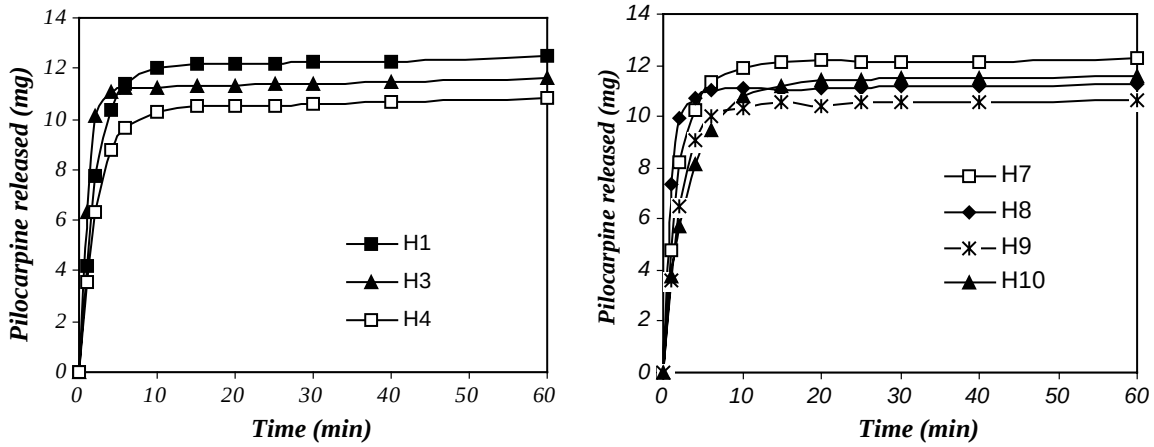

Fig. 7. Release profile of hydrogels at pH 7.21: amount of pilocarpine released (mg) as a function of time (min).

PNIPAAm network; in accord with expectation, hydrogels with a high chitosan content (H4, H8, H9) exhibited some evidence for controlled release.

4. Conclusions

A series of semi-interpenetrating polymer networks was prepared by the free radical polymerisation of *N*-isopropylacrylamide in the presence of chitosan using TEGDA as the crosslinking agent. The structure of the network was seen to be influenced by the quantity of crosslinker and chitosan used: the proportion of chitosan that could be entrapped in the matrix was found to increase as the crosslinking density of the network increased; a maximum CS/NIPAAm ratio of 0.46 was obtained. In addition, as the chitosan content and crosslinking density increased, the phase transition temperature of the CS interpenetrated hydrogels was seen to become less well defined and to shift towards lower temperatures. The incorporation of chitosan into the structure induced pronounced pH sensitivity: the swelling degree of the hydrogel was seen to vary from ∼100 at basic pH to over 2100 at acidic pH. The synthesised hydrogels were also highly sensitive to temperature variations: values for the equilibrium degree of swelling were much higher than for a crosslinked PNIPAAm control, showing a maximum at 29 °C (SD_{eq} over 2000% in acidic buffers). Optical transparency was also found to be a temperature-responsive property of these hydrogels. Finally, a preliminary drug release study involving pilocarpine demonstrated that the system under consideration shows little promise as a controlled delivery vehicle for the delivery of this, positively charged, active; the value of the system for the delivery of neutral or negatively charged therapeutic entities, remains to be investigated.

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