CELLULOSE CHEMISTRY AND TECHNOLOGY

SWELLING BEHAVIOR OF CROSS-LINKED TEREPHTHALOYL THIOUREA CARBOXYMETHYL CHITOSAN HYDROGELS

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Four cross-linked terephthaloylthioureacarboxymethyl chitosan (TTUCM-chitosan) hydrogels were prepared via cross-linking of carboxymethyl chitosan (CM-chitosan) with different concentrations of terephthaloyldiisothiocyanate. The swelling behaviors of the hydrogels at different pH values (4, 7 and 9) and temperatures (25, 35 and 45 ºC), as well as their electrical conductivity, have been investigated. The results reveal that the highest swellability was attained at 35ºC in acidic medium (pH 4), which indicated the dependence of the swellability of the hydrogels on pH and temperature. The results also indicated that the swellability percent of the prepared hydrogels increased with increasing of their cross-linking density, reaching their maximum values at 35ºC. On the other hand, the electrical conductivity of the hydrogels decreased with increasing cross-linking density and increased when rising the temperature up to 45ºC. It is expected that these smart hydrogels may be useful to develop drug delivery systems with improved drug loading capacity and controlled release behavior.

Keywords: carboxymethyl chitosan hydrogels, cross-linking, swellability, pH-dependent, temperature-dependent, electrical conductivity

INTRODUCTION

Hydrogels are cross-linked hydrophilic polymers, with a network structure, which are able to imbibe a large amount of water and are water insoluble.[1-3]For pharmaceutical applications, they are unique carriers for controlled drug delivery. The controlled drug release can be governed by swelling and biodegrading properties.[4-8] The swelling properties of the hydrogels are mainly related to the elasticity of the network, the presence of hydrophilic functional groups (such as –OH, -COOH, -CONH$_2$, -SO$_3$H) in the polymer chains, the extent of the cross-linking and porosity of the polymers. Also, the physical properties of the hydrogels, including their swelling ratio, depend on the balance between attractive and repulsive ionic interaction and solvent mediated effects.[9-11]

A new generation of hydrogels, which absorb a large amount of water very rapidly, has been developed as superporous hydrogels (a three dimensional network of hydrophilic polymers), which swell to equilibrium size in a short period of time,[12-16] due to the presence of interconnected microscopic pores.[17,18]Chitosan is currently receiving a great deal of attention for medical and pharmaceutical applications due to its biocompatibility, biodegradability and non-toxicity.[19]Carboxymethyl chitosan (CM-chitosan), a modified natural amphoteric polyelectrolyte derived from chitosan, has attracted considerable interest in a wide range of biomedical applications, such as wound dressing, antimicrobial agents and blood anticoagulants.[20-23] It has also demonstrated good pH and ion sensitivity due to abundant –COOH and NH$_2$ groups.[24] Hydrogel volume change occurs in response to changing environmental conditions such as temperature,[25,26] pH,[27] solvent composition[28]and electrical stimuli.[29] Due to the specific pH range occurring at physiological, pathological, or subcellular sites, such as stomach, intestine, endosome/lysosome, and tumor sites, pH sensitive hydrogels are the most widely used stimulus in environmentally responsive hydrogels. For this purpose, the most suitable polymers are those bearing weak polyelectrolyte (polyacid, polybase or polyampholyte sequences), pH-sensitive polymers rely on the protonation/deprotonation equilibrium, which depends on the pKa of the acidic and/or basic moieties present in the polymer. Therefore, a pH sensitive polymer can be charged (yielding a swollen state) or uncharged (yielding a hydrophobic/collapsed state) depending on the pH of the environment.[30,31]

Some hydrogels based on CM-chitosan of various degrees of deacetylation and various degrees of substitution were prepared by cross-linking with glutaraldehyde. Their swelling was observed at low pH (<2.0) and also in the pH range of 4.0-13.0; however, deswelling occurred in the pH range of 2.0-4.0. The reason of such behavior is the presence of -NH_2 and –COOH groups ionized to positive and negative charges in a pH dependent manner varying the charge state of the molecule.[32]However, the maximum swellability was obtained at pH 9-10 and upon further increase of pH to 13, a decrease in swellability was observed.[33]CM-chitosan was used as a potential carrier for pH specific delivery of nateglinide after cross-linking using glutaraldehyde in the presence of nateglinide. The pH responsive swelling behavior of the prepared hydrogels was assessed using different pH values (1.2, 6.8 and 7.4). The study indicated very low swelling at pH 1.2 (for the first 2 h) and quick swelling at pH 6.8 (for the next 3 h), followed by linear swelling at pH 7.4 (for the next 7 h) with a slight increase.[34]

In our previous work, we have prepared some super porous hydrogels by cross-linking CM-chitosan with different concentrations of terephthaloylthiourea moieties.[35]In the present work, the effect of both pH and temperature on the swelling properties of the produced hydrogels will be investigated.

EXPERIMENTAL

Materials

Chitosan with a degree of deacetylation of 88% and molecular weight of 2.0×10^5 was purchased from Acros Organics, New Jersey, USA. All other chemicals and reagents were of analytical grade, from Aldrich, and were used as received.

Preparation of carboxymethyl chitosan (CM-chitosan)

CM-chitosan was prepared following the method reported previously,[36] where chitosan (10 g), sodium hydroxide (13.5 g) and solvent isopropanol (100 ml) were suspended in a flask to swell and alkalize at room temperature for 1 h. The monochloroacetic acid (15 g) was dissolved in isopropanol, and added to the reaction mixture dropwise within 30 min and reacted for 4 h at 55°C. Then, the reaction was stopped and isopropanol was discarded. Ethyl alcohol (80%) was added and the solid product was filtered and rinsed with 80-90% ethyl alcohol to desalt and dewater, and was vacuum dried at 50°C. The degree of substitution of CM-chitosan was determined by pH-titration[37] and was found to be 0.75.

Synthesis of terephthaloylthiourea cross-linked CM-chitosan (TTUCM-chitosan) hydrogels

Four different predetermined amounts of terephthaloyl dichloride were dissolved in 20 ml methylene chloride and were added dropwise to solutions of ammonium thiocyanate of appropriate concentrations in 20 ml methylene chloride. The molar ratio of terephthaloyl dichloride to ammonium thiocyanate was 1: 2, respectively. Polyethylene glycol-400 (1 ml) was added dropwise to each reaction mixture as a phase transfer catalyst. After stirring for 2 h at room temperature, the white precipitate of the formed ammonium chloride was removed by filtration. Each filtrate (terephthaloyldiisothiocyanate, Scheme 1A) was added to CM-chitosan solution (5.06 g, 20 mmol, in 200 ml of water). The reaction mixture was stirred at 60°C for 2 h, cooled, and the homogenous cross-linked hydrogels formed (Scheme 1B) were submerged in methanol for 24 h for dewatering to yield yellowish white products. The dewatered hydrogels were filtered and dried at 60°C to constant weights. The ratios of the reactants were varied as shown in Table 1 to give four new hydrogels which were designated as TTUCM-chitosan-1, TTUCM-chitosan-2, TTUCM-chitosan-3, and TTUCM-chitosan-4, according to their increasing degree of cross-linking, respectively. A detailed description of the cross-linking process is reported in our previous work.[30]The data of elemental analysis, FTIR spectra, ^{13}C-NMR spectra, X-ray diffraction patterns and SEM photographs of the prepared hydrogels are in good agreement with those previously reported in our earlier work.[35]

Measurements

Elemental analyses of the hydrogels were done on a Perkin-Elmer (Model 2410 series II) C, H, N, S Analyzer (USA) at the Micro-analytical Center, Cairo University (Egypt).

Determination of the swellability of the hydrogels in various swelling media: a known weight of the dry hydrogel sample was immersed in a buffer solution of pH 4, 7 and 9 at different time intervals, and different temperature (25°C, 35°C and 45°C), until equilibrium swelling was reached. The swollen sample was then removed from the immersion media, quickly wiped with filter paper to remove the droplets on its surface and reweighed. The percent swelling was calculated using the following equation:

Swelling (%) = $[(W_1 - W_o)/W_o] \times 100$,
where W_o is the weight of the dry hydrogel and W_1 is the weight of the swollen hydrogel. Swelling measurements were made in triplicate, and the error was estimated to be within 1%.

Electrical conductivity of the hydrogel was measured by inserting a Pocket Conducting Meter (HANNA8733) in a cylinder containing a swollen sample in distilled water (prepared by immersing about 0.2g sample in 50 ml distilled water for 24h). The conductivity measurements were taken at 35ºC and 45ºC.[38]

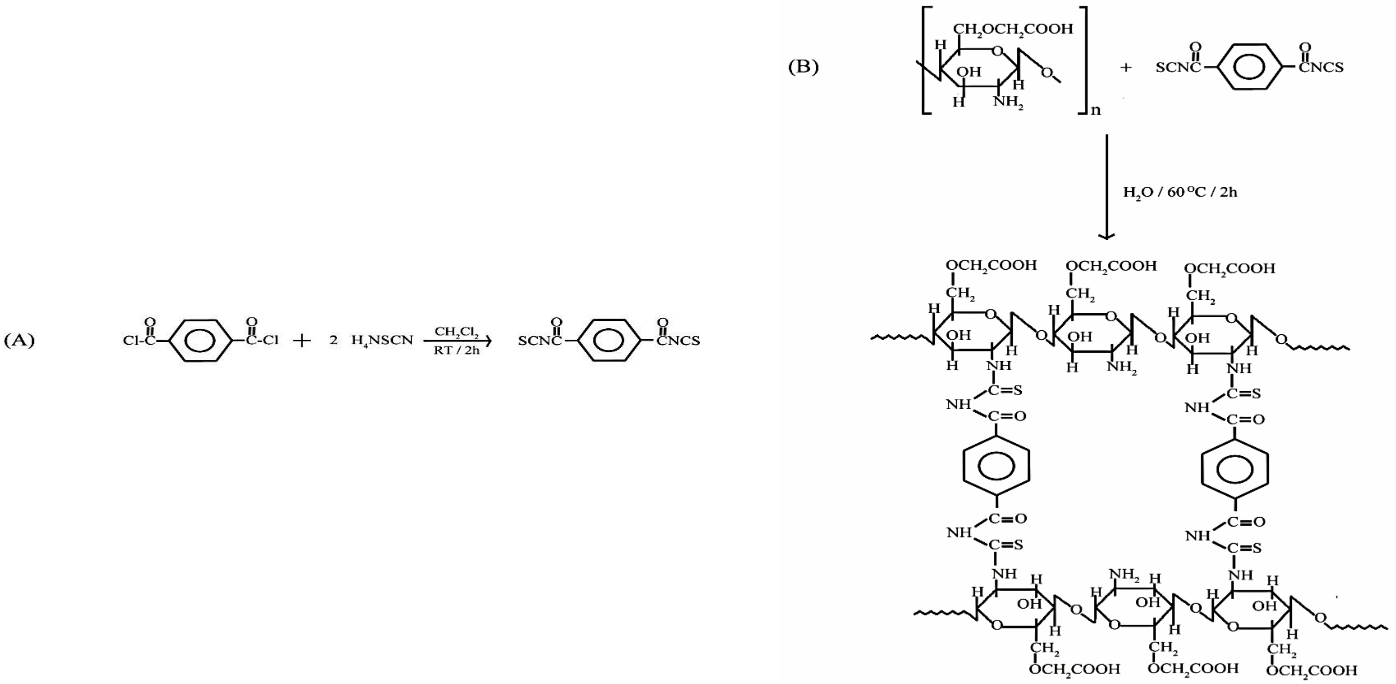
RESULTS AND DISCUSSION

Amphoteric polyelectrolyte hydrogels possessing both positive and negative charges are interesting synthetic analogs for proteins. It is known that materials undergoing continuous or discontinuous volume phase transition, in response to solvent composition, pH, salt concentration, temperature, have received much attention recently due to their scientific technological importance. Among these systems, pH and temperature-responsive hydrogels have been extensively studied in the biomedical field, because these two factors can be easily controlled and are applicable in both *in vitro* and *in vivo* conditions.[39-42]

Effect of pH variation on the swellability of cross-linked TTUCM-chitosan hydrogels

Chitosan superporous hydrogels showed a higher swelling percent in acidic environment than in basic environment, since the amino groups in the chitosan molecules are ionized to NH_3^+ ions in acidic aqueous media and these cationic charges in the gel phase act as repulsive forces between polymer molecules. Moreover, ionization also caused an increase in ionic osmotic pressure. These two factors and the capillary wetting of interconnected open pores of superporous hydrogels were responsible for a higher degree of swelling in acidic pH.[34]The CM-chitosan hydrogel has also demonstrated good pH and ion sensitivity in an aqueous solution due to abundant –COOH and $–NH_2$ groups.[19] The amphoteric hydrogel can act as a polyanionic or polycationic hydrogel depending on the pH value. In the CM-chitosan hydrogel, the amino groups ($-NH_2$) are protonated to become $-NH_3^+$ in acidic solution, while the carboxylic groups on it remain as -COOH, so it behaves as a polycationic hydrogel that swells in acidic medium. Conversely, the carboxylic groups in the CM-chitosan hydrogel are ionized to $–COO^-$ in basic solution, but the amino groups remain in their original uncharged form to give a polyanionic hydrogel, which swells in basic medium.[44] The cross-linking of different concentrations of terephthaloylthiourea moieties with CM-chitosan chains forms terephthaloylthiourea carboxymethyl chitosan (TTUCM-chitosan) superporous hydrogels,[37] with a higher degree of hydrophilicity, which exhibit a higher degree of swelling at various pH values 4, 7 and 9, than that of CM-chitosan (Figs. 1-3). It was found that the swellability of TTUCM-chitosan superporous hydrogels in acidic pH 4 was higher than that at pH 7 and 9. This may be attributed to the fact that in acid medium protonation of the NH, C=O and C=S groups of the thioureacross-linking moiety takes place, thus increasing the number of mobile ions inside the hydrogel network, leading to a large osmotic pressure, which causes the gel to swell. It is clear that the increase in swelling is attributed mainly to the free water content and to the ionic repulsion between the similar charges. Free water has good mobility since it has no interaction with the polymer chains; it increases by increasing the molar ratio of the hydrophilic groups inside the hydrogel.[45]The swelling kinetics and time-dependent swelling behaviors of the investigated hydrogels (TTUCM-chitosan-1, TTUCM-chitosan-2, TTUCM-chitosan-3, TTUCM-chitosan-4) in a pH 4 buffer solution at 25, 35 and 45 ºC are shown in Figs. 1-3. All swelling behavior is plotted on the average of three trials. It was found that all hydrogels swelled rapidly and reached equilibrium within 2h, and the swelling ratio increased with increasing cross-linking density from TTUCM-chitosan-1 to TTUCM-chitosan-4.This is due to the increase of the molar ratio of the hydrophilic groups (thiourea moiety) inside the gel. The structure of the superporous hydrogel with large numbers of pores connected to one another to form capillary channels was favorable for easy diffusion of the swelling medium into the polymeric matrix.[46]

On the other hand, the swellability of the hydrogels at pH 7 and pH 9 is shown in Figs. 1-3. Their swellability is lower than that at pH 4 since at pH > 4 the dominant charges in the gel are the unprotonated carboxylic groups ($-COO^-$) and swellability was attributed to osmosis and the repulsive interaction of the negative charges.



Scheme 1: Synthesis of TTUCM-chitosan hydrogels

Table 1
Synthesis and characterization of TTUCM-chitosan hydrogels with different degrees of cross-linking

Samples	Terphthaloyl dichloride (mmol)	Amm. thiocyanate (mmol)	CM-chitosan (mmol)	Elemental analysis (%)				Cross-linking degree (%)
				C	H	N	S	
CM-chitosan	-	-	20	43.82	5.63	5.53	-	-
TTUCM-chitosan-1	1.25	2.50	20	43.90	4.91	5.71	1.83	11
TTUCM-chitosan-2	2.50	5.00	20	43.98	4.78	5.95	3.27	22
TTUCM-chitosan-3	5.00	10.00	20	44.49	4.58	6.25	5.42	44
TTUCM-chitosan-4	10.00	20.00	20	45.37	4.41	7.76	8.58	88

The water penetrated inside the gel was bound by hydrogen bonds with the unprotonated (-COO$^-$) groups and thiourea moiety, and thus it was not free.

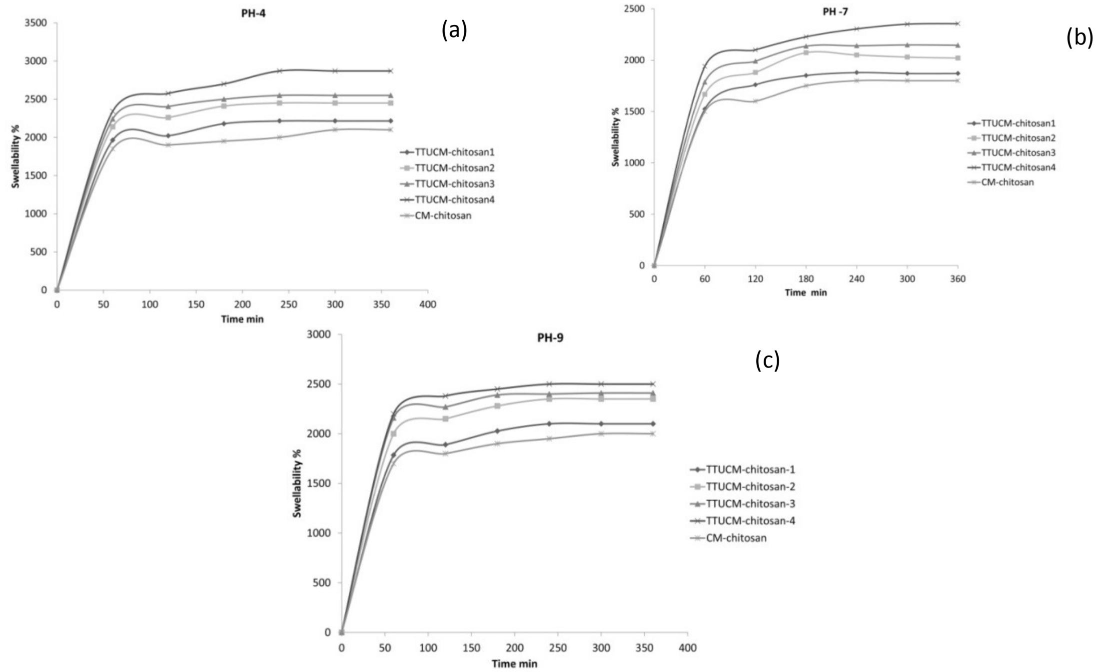


Figure 1:Effect of pH change on the swellability percent of cross-linked TTUCM-chitosan hydrogels (a) pH4;(b) pH7;(c) pH9, at 25 ºC

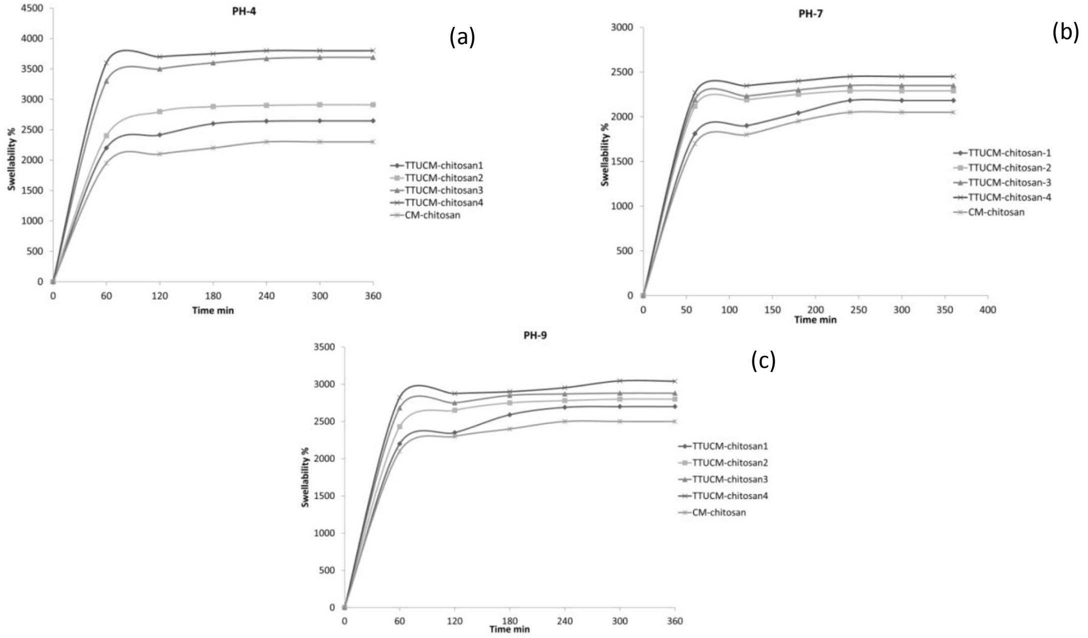


Figure 2: Effect of pH change on the swellability percent of cross-linked TTUCM-chitosan hydrogels (a) pH4;(b) pH7;(c) pH9, at 35ºC

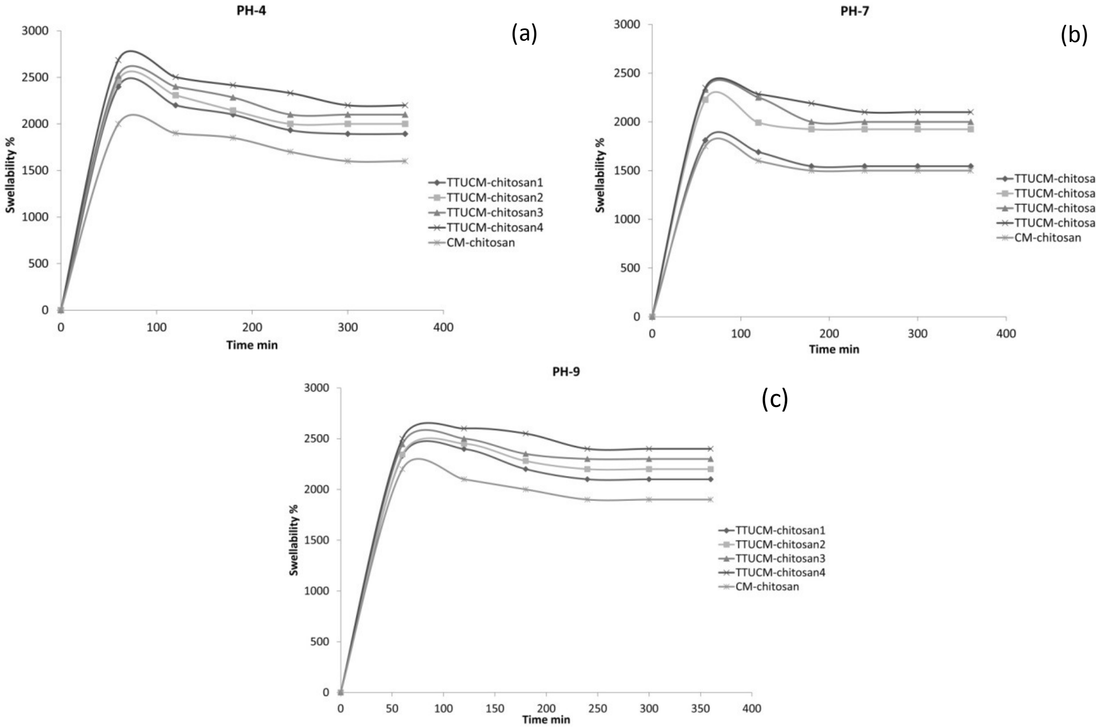


Figure 3: Effect of pH change on the swellability percent of cross-linked TTUCM-chitosan hydrogels (a) pH4;(b) pH7;(c) pH9, at 45ºC

The degree of swelling at pH 9 was higher than that at pH 7due to the increased number of the unprotonated carboxylic groups. In all cases, the degree of swelling increased with increasing the cross-linking density of the hydrogels.

Temperature sensitivity of the swellability of cross-linked TTUCM-chitosan hydrogels

The swelling ability of the TTUCM-chitosan hydrogels as a function of temperature at 25 ºC, 35 ºC and 45 ºC at pH 4, 7and 9 are shown in Figs. 4-6, respectively. It was found that the swellability increased with temperature up to 35 ºC and then decreased at 45 ºC. From kinetic examinations of the swellability with releasing temperature,it was found that the swellability rate increased with elevating temperature up to 35 ºC.This may be due to the increase in the mobility of the charged ions and consequently the increase of the electrostatic interaction between them. As a result the osmotic pressure inside the hydrogel increased so that the swellabilityalso increased. The swelling ratio seemed to increase in time until it reached equilibrium after about 3 h. When rising the temperature to 45 ºC, the swelling ability decreased over time until it attained equilibrium in about 3h. These results can be explained by the fact that the TTUCM-chitosan hydrogels contain hydrophilic groups in the polymer structure, which form intermolecular hydrogen bonds with the surrounding water at a low temperature (below the hydrogel phase transition temperature). Hence, water penetrating into the hydrogel is in a bound state at low temperature (below the hydrogel phase transition temperature), while at higher temperature water molecules gain an enthalpy during the increase of temperature and the hydrophilic groups in the hydrogel turn into intramolecular hydrogen bonds under these conditions. As a consequence, these two results make the water molecules inside the hydrogel change from a bound state to a free state, with subsequent release from the hydrogel, thus causing shrinking to the hydrogel, which increased over time and reached equilibrium after a certain period. This phenomenon makes the swelling ratios of the hydrogels decrease rapidly at the hydrogel transition temperature (Figs. 1-4). Moreover, in all cases the degree of swelling increased with increasing the cross-linking density due to the increase of hydrophilicity of the hydrogel.[47]

Evaluation of the swelling rate of TTUCM-chitosan hydrogels at different temperatures and medium pH

The rate of swelling of TTUCM-chitosan at different temperatures (25, 35 and 45ºC) was evaluated for the parent CM-chitosan and the four TTUCM-chitosan hydrogels using different medium pH (4, 7 and 9). The experimental data were fitted to Eq. (1), using Origin Pro 2015 software, since the swelling was found to be approximately proportional to the square root of time:

$$S = k_s t^{½} \qquad (1)$$

where$S$ is the fraction swelling at time t, k_s is a rate constant which for matrix diffusion is approximated by: $k_s = 2AC_o\left(\frac{D_{app}}{\pi}\right)^{0.5}$, where A is the surface area available for diffusion (assumed to be constant), C_o is the initial concentration and D_{app} is the apparent diffusion coefficient.[48]

The derived swelling rate constants (k_s) are given in Table 2 for the 1 to 6 h swelling periods. It is seen that for all examined temperatures, the swelling rate constant of the prepared hydrogels is higher than that of the parent CM-chitosan, and this behavior is also observed for all examined medium pH values 4, 7 and 9. It is observed that for the prepared hydrogels the swelling rate constant increased by increasing the cross-linker content rising from TTUCM-chitosan-1 to TTUCM-chitosan-4. All hydrogels showed higher k_s values in acidic medium (pH 4) than that in neutral medium (pH 7) and basic medium (pH 9). It appeared from the results that the swelling rate constant increased by rising the temperature up to 35ºC and then started to decrease upon further increase in the temperature, as shown by the k_s values at 45ºC (Table 2).

Electrical conductivity of CM-chitosan and TTUCM-chitosan hydrogels

The results of electrical conductivity revealed that the magnitude of electrical conductivity for the highly soluble CM-chitosan is greater than that for the prepared superporous hydrogels. This is attributed to the large amount of NH_3^+ and COO^-mobile ions on CM-chitosan, compared to the hydrogels, as indicated in Table 3.

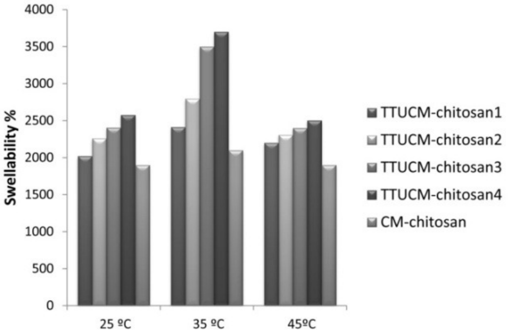


Figure 4: Temperaturedependence ofcross-linked TTUCM-chitosan hydrogels at pH 4 after 120 min

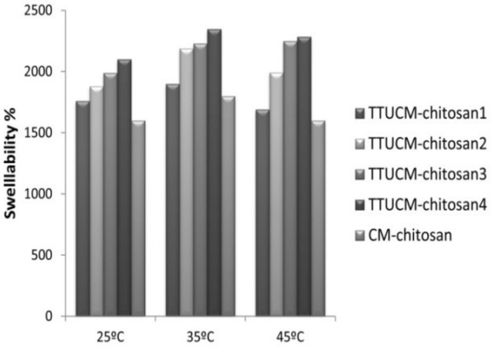


Figure 5: Temperaturedependence of cross-linked TTUCM-chitosan hydrogels at pH 7 after 120 in

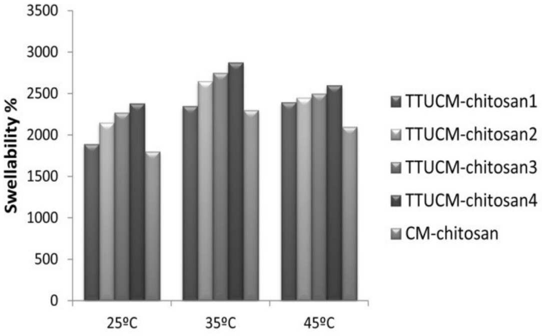


Figure 6: Temperaturedependence of cross-linked TTUCM-chitosan hydrogels at pH 9 after 120 min

Table 2
Swelling rate constant (k_s) of CM-chitosan and TTUCM-chitosan hydrogels

Sample	k_s (min$^{-0.5}$) at 25°C			k_s (min$^{-0.5}$) at 35°C			k_s (min$^{-0.5}$) at 45°C		
	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9
TTUCM chitosan-1	143.9	121.3	135.3	173.8	134.1	172.4	134.5	105.7	144.9
TTUCM-chitosan-2	159.2	132.2	151.9	189.2	150.2	182.1	140.2	130.5	150.4
TTUCM-chitosan-3	166.1	139.2	157.4	240.5	147.0	189.0	146.5	137.4	156.3
TTUCM-chitosan-4	183.5	150.0	163.1	250.4	156.0	197.2	156.0	144.0	163.8
CM-chitosan	133.8	115.7	127.9	148.2	115.5	161.2	115.5	102.3	131.2

Table 3
Electrical conductivity of CM-chitosan and TTUCM-chitosan hydrogels

Hydrogel samples swollen in distilled H_2O	Conductivity (mS/cm) at 35 ºC	Conductivity (mS/cm) at 45 ºC
CM-chitosan	0.50	0.52
TTUCM-chitosan-1	0.35	0.37
TTUCM-chitosan-2	0.29	0.30
TTUCM-chitosan-3	0.24	0.26
TTUCM-chitosan-4	0.20	0.21

It was noticed that the conductivity decreased upon incorporation of the cross-linker, which attacked the amino groups and thus decreased the number of mobile ions inside the gel. The measured conductivity increased by rising the temperature due to the increase in the movement of the mobile ions through the gel and its magnitude decreased with increasing the cross-linking density from TTUCM-chitosan-1 to TTUCM-chitosan-4.

CONCLUSION

Four hydrogel samples based on CM-chitosan cross-linked with various contents of terephthaloylthiourea moieties were successfully prepared via reacting CM-chitosan with various amounts of terphthaloyldiisothiocyanate cross-linker. The cross-linking process takes place through the reaction of both the isothiocyanate groups of the cross-linker with the amino groups of CM-chitosan. The amount of terphthaloyldiisothiocyanate with respect to CM-chitosan was varied to produce four terphthaloylthiourea cross-linked CM-chitosan samples (TTUCM-chitosan hydrogels designated as TTUCM-chitosan-1, TTUCM-chitosan-2, TTUCM-chitosan-3, TTUCM-chitosan-4, according to their increasing degree of cross-linking as follows:11%, 22%, 44% and 88%, respectively, based on their sulphur contents, which were determined by elemental analyses. Their structures were confirmed by elemental analyses, FT-IR, ^{13}C-NMR, SEM and X-ray diffraction. It was found that the swellability of TTUCM-chitosan superporous hydrogels at acidic pH 4 was higher than at pH 7 and pH 9. This may be attributed to the protonation of the NH, C=O and C=S groups of the thioureacross-linking moiety in acidic medium, which increased the number of mobile ions inside the hydrogels network leading to a large osmotic pressure which causes the gel to swell. Kinetic examinations of the swellability revealed that the swellability rate increased with rising temperature up to 35 ºC and decreased at 45 ºC.This may be due to the increase in the mobility of the charged ions and consequently the increase of the electrostatic interaction between them up to 35 ºC. However, rising the temperature to 45 ºC makes the water molecules inside the hydrogel turn from a bound state to a free state, with subsequent release from the hydrogel.This causes the hydrogel to shrink, which is intensified over time, reaching equilibrium after a certain period. The degree of swelling increased with increasing cross-linking density due to the increase in the hydrophilicity of the hydrogel. On the other hand, the electrical conductivity of the hydrogels is smaller than that of the parent CM-chitosan and decreases with increasing cross-linking degree.

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