A DRUG-LOADED GEL BASED ON GRAFT RADICAL CO-POLYMERIZATION OF N-VINYLPYRROLIDONE AND 4-VINYLPYRIDINE WITH CHITOSAN

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A drug-loaded gel based on chitosan was prepared by radical graft co-polymerization of N-vinylpyrrolidone and 4-vinylpyridine with chitosan and cross-linking by N,N'-methylene-*bis*-acrylamide. The structure and properties of the hydrogel were characterized by FTIR, XRD and SEM. The surface and morphology of the gel were observed using SEM. The swelling behavior of the prepared hydrogel, with the optimal amount of gelator, was investigated. Also, the *in vitro* controlled release behavior of doxocyline, as model drug, from the prepared hydrogels, was studied. The release profiles of doxocyline from the hydrogels were determined by UV-Vis absorption measurement under different conditions. The results showed that the new hydrogels exhibit a pH and temperature-responsive swelling ratio, while the pH and temperature were also found to strongly influence the drug release. In addition, the release mechanism of the drug was analysed by fitting the amount of the drug released to the Higuchi, Korsmeyere-Peppas and Hixon-Crowell kinetic models. The drug release occurred through the non-Fickian diffusion mechanism and the release profile was best fitted with the Higuchi square root model.

Keywords: chitosan, co-graftting, N-vinylpyrrolidone, 4-vinylpyridine, gel, drug loaded, controlled release

INTRODUCTION

The modification of natural polymers is a promising method for the preparation of new materials. This enables introducing special properties and enlarging the field of potential applications of these abundant biopolymers.[1-3] Chitosan is a linear cationite type natural polyaminosaccharide, produced by N-deacetylation of chitin. Chitosan consists of β-(1,4)-2-amino-2-deoxy-D-glucosamine and β-(1,4)-N-acetyl D-glucosamine units.[4-7]

The non-toxicity, biocompatibility, biodegradation and bone adhesion of chitosan confirm its suitability for transportation of genes and drugs in medicine and biotechnology, and for stabilization of antibacterial metal nanoparticles. However, the solubility of chitosan only in acidic environment limits its use as a carrier in controlled release of some drugs.

The hydrophilicity, swelling and other properties of chitosan have been improved by cross-linking it with several cross-linkers and investigated in diverse fields, such as tissue engineering,[8] wound-healing,[9] wound dressings,[10] oral controlled release drug delivery,[11] photodynamic therapy,[12] dyes and metals removal,[13] drug encapsulation,[14] blood anticoagulations,[15] and bioadhesives implantations.[16] The amino groups of chitosan enable this carbohydrate to form Schiff-base bonding with aldehyde compounds, and introduce different hydrophilic-hydrophobic monomers into the backbone, which could afford dynamic behavior to the obtained hydrogels.[3] In addition, these hydrogels are reversible and sensitive to chemical stimuli, such as pH, and can be disrupted under different conditions.

In the present work, we aimed to develop a special drug release matrix for stomach specific drug delivery of hydrophilic small molecule drugs, offering stimulation of the intestinal mucosa. We prepared drug-loaded polymer networks composed of chitosan-PVPr-graft-co-P4VP copolymer cross-linked by N,N'-methylene-*bis*-acrylamide (MBAA), which showed slow drug release behavior in simulated intestinal fluid and at low pH. The effect of various preparation parameters on the release performance of the model drug was investigated. In addition, doxocyline as model drug was loaded into the hydrogel and the drug release profile was fitted with different kinetic models – zero order, first order, Higuchi square root law, Korsmeyere-Peppas model and Hixson-Crowell cube root model.

EXPERIMENTAL

Materials

Chitosan (CS) was obtained from Sigma Aldrich, with a deacetylation degree of 87-90% and average molecular weight of 35 kDa. Acetic acid (Glacial), ethanol (95%), acetone (residue analysis, ≥99.9%), diethyl ether (containing 1 ppm BHT as inhibitor, anhydrous, ≥99.7%) and MBAA were purchased from Sigma Aldrich. 4-Vinylpyridine (95-96%), and N-vinylpyrrolidone (≥99%) from Acros Organics were distilled just before use. 2,2-Azobisisobutyronitrile (AIBN) was purified by recrystallization with 95% ethanol. The solvents obtained from Sigma Aldrich were purified by distillation, according to conventional methods. Doxycycline hyclate (Dox), $C_{22}H_{24}N_2O_8 \times HCl \times 0.5H_2O \times 0.5C_2H_6O$, also was obtained from Sigma Aldrich.

Gel preparation

An amount of 1.5 g of chitosan was dried at 40-50 °C for 24 hours and then suspended in 150 mL of 2% CH_3COOH. After 1-2 hours of mixing, 2 mL of freshly distilled monomers were added to the solution in a 1:1 mol ratio and continuously stirred for 30 minutes.[3] The reaction medium was coupled to a 65 °C water thermostat and the reaction was performed in inert gas-N_2 environment with 0.15 mol/L AIBN concentration, for 2 hours. Following this period, the solution was brought to pH = 10-11 by adding 1M KOH, after which the product precipitated. The graft copolymer was filtered, washed 2-3 times with water, alcohol and acetone (to remove poly-4-vinylpyridine, poly-N-vinylpyrrolidone and the monomers, it was washed with diethyl ether for 2 days in Soxhlet apparatus). The graft copolymer sample was dried to a constant weight at 40-50 °C. The yield was 78-86%.

An amount of 0.20 g of *graft-CS* was dissolved in 3 mL of acetic acid (0.1%, v/v) solution. The mixture was stirred with a heater/magnetic stirrer to ensure that the *graft-CS* was completely dissolved. Then, the solution was heated to 70 °C. After that, different amounts of MBAA as gelator were added to the mixture. As a typical hydrogel preparation, a cross-linker solution was prepared by dissolving 0.02 g of MBAA in 3 mL of water-ethanol (40%:60% w/w) and then added to the *graft-CS* solution. The amount of MBAA was varied from 0.020 to 0.028 g in different hydrogels. Variable weight ratios of *CS*/cross-linker *(*w/w *%)* were obtained by the addition of the cross-linker solution including different amounts of MBAA (10%, 14% w/w, with respect to *CS*) to the *CS* solution, and gelation occurred within 30 min at 70-80 °C. The prepared hydrogels were washed and centrifuged with ethanol and distilled water, respectively. Finally, the hydrogels were dried under vacuum.

Characterization

The FTIR spectra of the samples were recorded in the 4000-500 cm^{-1} scan area, with 4 cm^{-1} spectral resolution, by the KBr pellet method, in an AVATAR 370 (Thermo Nicolet Corporation, USA). Thermogravimetric investigations (TGA) were carried out using a Thermogravimetric Analyzer (NETZSCH, STA 409 PG.4.G Luxx, USA), in 50 mL min^{-1} nitrogen environment, in the temperature range from 20 °C to 400 °C at a rate of 10 °C min^{-1}. The X-ray diffractograms of chitosan and reaction products were obtained on Bruker Advance D8 equipment (Germany). The diffractograms comprised (2θ) 0.020 imaging, scattering rates ranged from 3 to 800, at a scanning speed of 2.0 min^{-1}, accelerated tension of 40 kV and intensity of 35 mA. Morphological surface images of *CS*, the hydrogel and *graft-CS-Dox* were obtained by a scanning electron microscope (SEM, JSM-6390, Japan). The UV-visible analysis of *Dox* was carried out using a UV-visible Spectrophotometer (Perkin Elemr Lamda-850), in the absorbance mode, in the wavelength range of 200-800 nm.

Swelling properties, gelation, drug loading

Swelling measurements were carried out in distilled water, in simulated intestinal buffer of pH 6.8 and in simulated gastric buffer of pH 1.4 (7 mL of HCl, 2 g of NaCl and 100 mL of distilled water) at room temperature. A known weight of dry sample was placed into a teabag and immersed into aqueous medium. After a while, the bag was taken out and hung for 5-10 min to eliminate excess unabsorbed water, and then weighed. The degree of swelling was calculated using the relation $(W-W_0)/W_0 \times 100$, where W and W_0 are the weight of the swollen and dry samples, respectively.

Gelation time was measured using the tube inversion technique.[16] Briefly, 3 mL of the prepared *graft-CS* solution was transferred to a test tube and maintained in a water bath preheated at 20 and 37 °C.

The flowability of two samples was observed by inverting the tube at regular intervals and the time when the hydrogel ceased to flow was recorded as the gelation time. The gelation times for each sample were measured in triplicate.

In this study, *Dox* was selected as model drug. Vacuum dried hydrogel samples (0.10 g) were placed in an aqueous solution of the drug (0.05 g *Dox* dissolved in 3 mL of distilled water) separately at a constant temperature of 25 °C for 12 h under sufficient stirring. During this period, the *Dox* diffused into the hydrogel network. Then, the *Dox* loaded hydrogel based on *CS* was kept in a dialysis tube (molecular weight cut-off: 20,000). Then, the dialysis bag containing the drug-loaded hydrogel was placed in 200 mL of buffer solution at 37 °C. At specific given time intervals, 1 mL of the release medium was removed and replaced with 1 mL of fresh buffer solution. Then, the samples were filtered and the concentration of released drug was measured by a UV spectrophotometer. Moreover, the standard calibration curve of the absorbance as a function of drug concentration was studied on the UV spectrophotometer. Drug release was studied for the hydrogel with 10% and 14% cross-linking in pH 1.5, pH 6.8 and distilled water.

Encapsulation efficiency and *in vitro* drug release

The loading capacity (milligrams of *Dox* entrapped per 100 milligrams of dried drug-loaded gels, %) of *CS* graft gels (*CS-g*) was determined as follows: the drug-loaded *CS-g* gel was ground to fine power and a known amount of it was immersed into 100 mL of HCl aqueous solution for 24 h under magnetic stirring. Then, the solution was filtered and used to determine the absorbance (A) of the drug contained in it by a Perkin Elmer Lambda 35 UV/vis Spectrophotometer at 280 nm. A quantitative calibration curve was obtained based on the absorbance of the drug in standard solutions of various concentrations at 280 nm. The Beer–Lamberts relationship upon which the calibration curve is based can be written as A = kC, where k is the slope of the curve, C is the concentration of the standard solution. The amount of the drug entrapped can be then calculated. The average loading capacity of the *CS-g* gel obtained from the measurements was about 5.8 mg *Dox* per 100 mg *CS-g* gels (5.8%).

The release profile of the model drug *Dox* from the drug-loaded polymers was determined in distilled water, pH 1.5 and pH 6.8 buffer solutions.[17,18] All the studies were carried out in triplicate. Both the mechanism of swelling of the hydrogels and the mechanism of drug release from the drug-loaded hydrogels were determined by using the equation ($M_t/M_\infty = kt^n$) provided by Ritger and Peppas.[19,20] In the equation, M_t/M_∞ is the fractional swelling/release of drug in time '*t*', '*k*' is a constant characteristic of the drug-polymer system, and '*n*' is the diffusion exponent characteristic of the swelling/release mechanism. Different parameters of the drug release kinetics from the drug-loaded hydrogels were determined. The maximum amount of drug release and the initial rate of drug release were calculated by using the equation $t/C_t = \alpha+\beta t$. Here, C_t is the amount of drug released at time t, $\beta = 1/C_{max}$ is the inverse of the maximum amount of released drug, $\alpha = 1/(C_{max})^2$, $k_{rel} = 1/r_o$ is the inverse of the initial release rate, and k_{rel} is the constant of the release kinetics.[21] Different kinetic models were applied to the drug release data obtained for the release of the drug from the drug-loaded hydrogels in different media. To find out the mechanism of drug release from the hydrogels, the data were fitted with different mathematical models, *i.e.* zero order, first order, Higuchi square root law, Korsmeyere-Peppas model and Hixsone-Crowell cube root model.[22]

RESULTS AND DISCUSSION

In our previous study, we obtained the optimal reaction conditions for graft radical copolymerization of N-vinylpyrrolidone and 4-vinylpyrrolidone into *CS* macromolecule.[3] The combined effects of the major parameters of the graft radical copolymerization were studied systematically. The grafting degree of the monomers to *CS* (G) and the dependence of grafting effectiveness (E) upon temperature, amount of *CS*, concentration of initiator, solvent ratio, reaction time and concentration of monomers were studied. The results revealed that G = 258% and E = 17-19% were maximum at 60-70 °C, within 150 min, in the graft copolymerization of 0.35 g *CS* with 2.4 mol/L of N-vinylpyrrolidone and 0.2 mol/L of 4-vinylpyridine in the presence of 75 mL of 2% CH_3COOH as solvent, and the degree of homopolymerization was estimated at minimum 18-20%. In typical synthesis, MBAA as cross-linker was successfully prepared. In fact, MBAA as a cross-linking gelator with two reactive double chemical bond functional groups allows the formation of bridges between polymer chains for the preparation of the three-dimensional network. Then, the new hydrogels based on *CS* chains can form after homogeneously mixing *graft-CS-PVPr-co-P4VP* and MBAA by radical cross-linkages among the $–CH_2-$ groups of PVPr, the $-NH_2$ groups of *CS* and the –CH< groups on P4PV. Therefore, hydrogels with 10 and 14 wt% of cross-linker were prepared at the end of the mixing process. A possible mechanism for the grafting reaction is illustrated in Figure 1.

The gelation time of the prepared hydrogels was assessed by the tube inversion method. Interestingly, the gelation time varied between 2

min (for 14% gelator) and maximum 5 min (for 10% gelator), as a function of cross-linker concentration. In fact, the cross-linking interaction sites in the system increased with the increase in the concentration of the gelator, so gelation occurred rapidly. In addition, the hydrogels showed a fixed shape, with higher flexibility and toughness after the addition of 14 wt% gelator. Also, the new hydrogels with radical cross-linkages displayed pH-sensitivity. In fact, pH-sensitive hydrogels can conveniently change their volume in response to the environmental stimuli of different pH values. Due to this, these hydrogels have been extensively investigated as important drug carriers.[23]

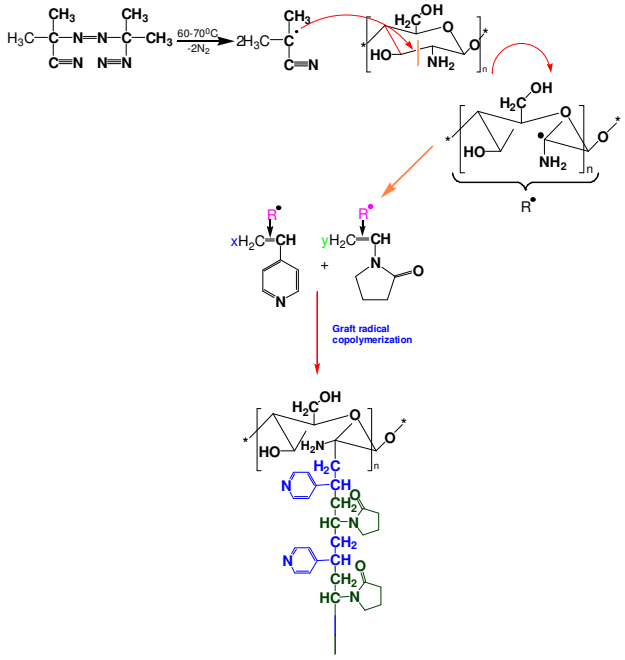


Figure 1: The radical co-graft polymerization mechanism of vinyl monomers onto *CS* macromolecules

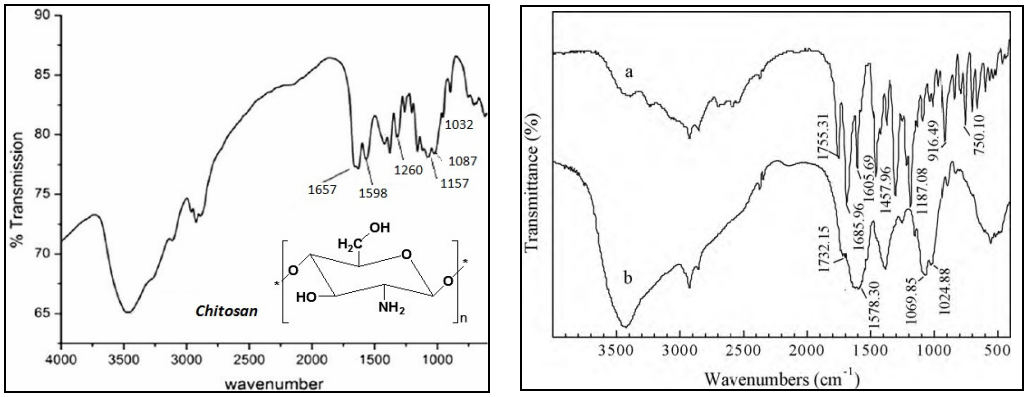


Figure 2: FTIR spectra of *CS*, *graft-CS-Dox* (a) complex and *graft-CS* (b)

The FTIR spectrum of *CS* (shown in Fig. 2) is characterized by absorptions around 1657 cm^{-1} (amide I), 1598 cm^{-1} (bending vibration of $-NH_2$ groups) and 1260 cm^{-1} (stretching vibration band of C–N groups). The absorption bands at 1157 cm^{-1} (asymmetric stretching vibration of the C–O–C bridge), 1087 and 1032 cm^{-1} (skeletal vibration involving C–O stretching) are characteristic of its saccharide structure.[24,25] Compared with the spectrum of *CS*, a part of the bending vibration of $-NH_2$ groups shifted from 1598 cm^{-1} to about 1578 (Fig. 2a) and 1578 cm^{-1} (Fig. 2b), accompanied with an increase in the intensity of the spectra of the *CS* gel cross-linked by MBAA, respectively. This shift and increased intensity may be ascribed to the interaction between MBAA and *CS*.

The interaction between *Dox* and *CS* was investigated as shown in Figure 2a. The drug is bound mainly through hydrogen bonding between $–OCOCH_3$, –COOH on *Dox* and -OH, NH on *CS*. This is evidenced by the major intensity decrease of the infrared bands at 1578 cm^{-1}, as well as the shift of the stretching vibration of >C=O from the ester group on *Dox*, and the skeletal vibration involving C–O stretching on saccharide from 1755, 1087 and 1032 cm^{-1} to 1732, 1069 and 1024 cm^{-1}, respectively. This negative shift signifies that intermolecular hydrogen bonding did occur between *Dox* and *CS*. These results reveal that electrostatic interaction occurs between the anionic COO^- and the cationic NH_3^+. Similar electrostatic interaction was observed by Anh and others.[26-29]

The SEM images of freeze-dried hydrogels based on *CS* (Fig. 3b) clearly show the presence of large quantities of interconnected pores, indicating a porous structure. Interestingly, the properties of the gelator, such as the structure and steric effect of the molecule, played an important role in determining the microstructure of the hydrogels. Thus, the pore diameters were observed in the range of 80-600 nm for the hydrogel with 10% gelator and of 40-350 nm for the hydrogel with 14% gelator. Therefore, by comparing *CS* with the hydrogels with different amounts of gelator (10% and 14%), it could be seen that the pore size decreased and the pore density increased with the content of MBAA as cross-linker agent. So, the amount of cross-linker has a significant influence on the pores.

The XRD analysis of the cross-linked polymer revealed the presence of a broad amorphous peak of low intensity. Similar amorphous nature of grafted polymers has been recorded by the XRD analysis of some biopolymers.[30] It is known that some crystalline phase could be observed in the *CS* macromolecule, which is essentially amorphous. Also, the chemical modification of the polymer macromolecule – the conjunction of alkyl or aromatic groups to the content, and then the conversion to a salt form – affects its crystallinity. For this purpose, X-ray diffractograms of *CS* and the cross-linked graft copolymer *CS-g-PVPr-co-P4VP* were recorded and are provided in Figure 4. The two diffraction peaks at $2\theta=6^0$ and 19.7^0 levels for *CS*, characterized by the crystal domains, are shown in the figure. These crystalline phases have been formed due to the hydrogen bonds between the amine groups. In the diffractogram of *CS-g-PVPr-co-P4VP-10%MBAA*, the peaks are clearly visible. The inclusion of pyrrolidone and pyridine groups and also the cross-linking of *CS* macromolecule leads to an increase in crystallinity up to 28-29%.[31]

The swelling ratios of the hydrogel samples with different content of MBAA as gelator are shown in Figure 5. According to this figure, the swelling capacity of the hydrogels significantly decreased with higher content of cross-linking agent. In fact, higher cross-linker concentration causes more cross-linking sites and less free hydrophilic $-NH_2$ groups in *CS* and thus, a compact structure. Consequently, a hydrogel with higher cross-linking density holds less water than that with lower cross-linking density. Interestingly, greater swelling capacity was observed when 10% of cross-linker concentration was used.

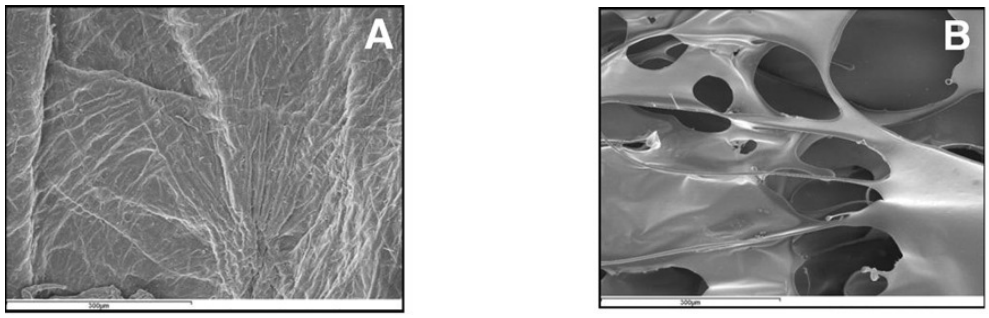


Figure 3: SEM images of *CS* (A) and hydrogel *CS-g-PVPr-co-P4VP-10%MBAA*

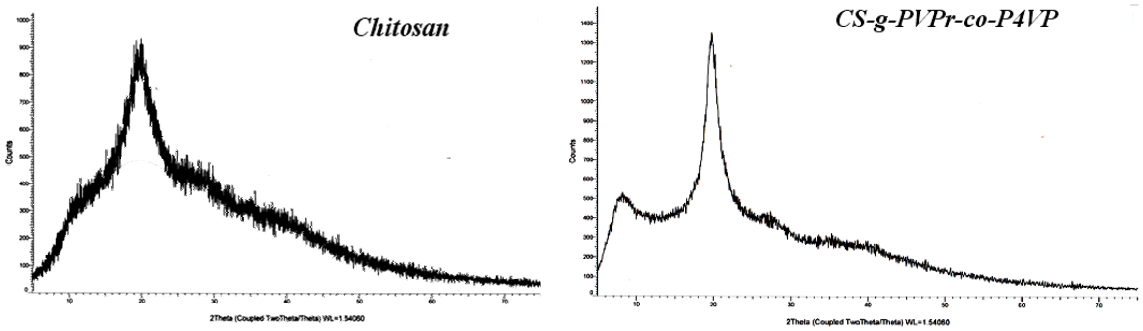


Figure 4: X-ray diffractograms of *CS* and *CS-g-PVPr-co-P4VP-10%MBAA*

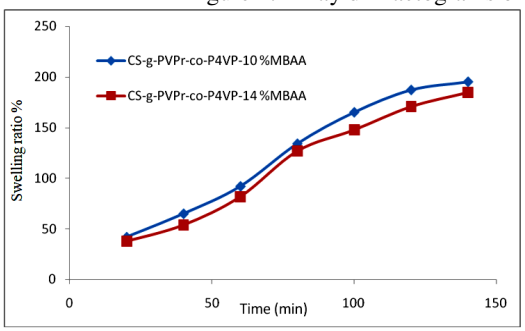


Figure 5: Effect of MBAA amount on swelling ratio of *CS-g-PVPr-co-P4VP* hydrogel in distilled water medium at 25 °C

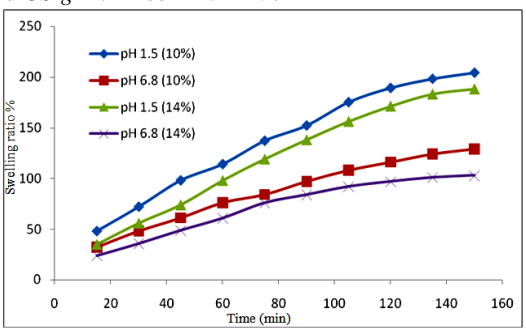


Figure 6: pH-responsive swelling behavior of *CS-g-PVPr-co-P4VP* based hydrogels with 10% and 14% cross-linker

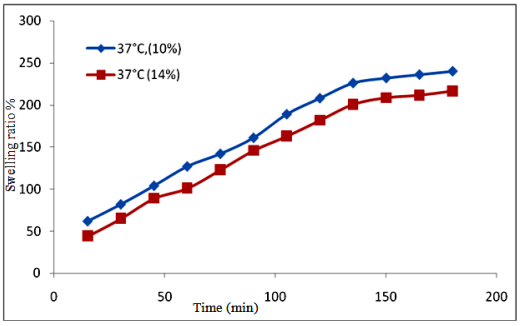


Figure 7: Temperature-dependent swelling of *CS-g-PVPr-co-P4VP* based hydrogel with 10% and 14% cross-linker at 37 °C

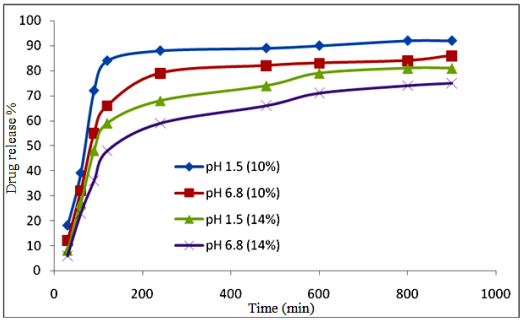


Figure 8: Release profile of *Dox* from graft *CS* hydrogel with 10% and 14% gelator

The effect of pH (pH 1.5 and 6.8) on the swelling ratios of the hydrogels is illustrated in Figure 6. According to this figure, the synthesized hydrogels showed different swelling behaviors in acidic and basic pH solutions. It can be seen that the swelling ratio increased with decreasing pH. These results could be interpreted by the pKa of the amine group in *CS*. The free amine groups of *CS* are protonated only at acidic pH and deprotonated at neutral pH, because the pKa value of the amino group is nearly 6.8. As a result, when the pH is low, the protonation of $-NH_2$ groups causes electrostatic repulsion between the positive charges and dissociation of secondary interactions, which lead to the network expansion and diffusion of more water into the hydrogel network. Meanwhile, at high pH, enhanced hydrogen bond interactions between the *CS* chains due to deprotonation of $-NH_2$ groups lead to reduced swelling. Interestingly, this pH-sensitive behavior of the hydrogels makes them a suitable candidate for controlled drug delivery systems.

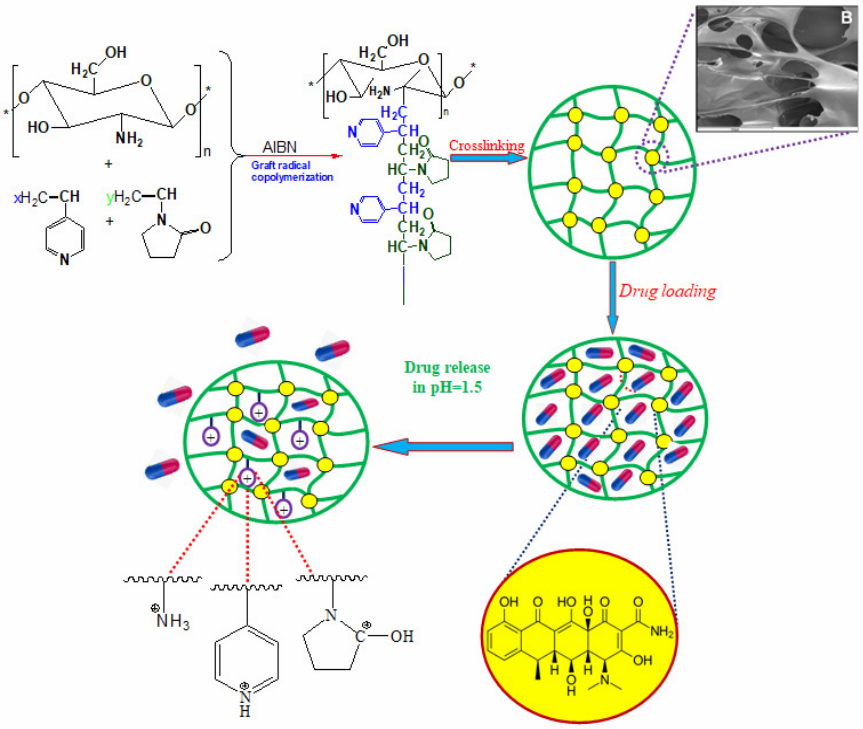
To find out the effect of temperature on the swelling capacity of the synthesized hydrogels, two temperature levels (25 and 37 °C) were

applied in distilled water (*DW*). The results from Figure 7 show that, with increasing the temperature to 37 °C, the water uptake increased outstandingly. In fact, the rising temperature increases the rate of diffusion of the solution to the hydrogels and the adsorption capacity increases.

The release profile of *Dox* from the drug loaded *CS-g-PVPr-co-P4VP* based hydrogels, with 10 and 14% cross-linker, at physiological temperature (37 °C) is shown in Figure 8. It has been observed that the drug released in pH 1.5 buffer solution in higher amounts than in pH 6.8 buffer. This may be due to the higher solubility of the drug in pH 1.5 buffer solution and its swelling ratio. In fact, the different swelling behaviors at various pH values are responsible for the differences of the drug release in the medium. The release of the drug occurred through a non-Fickian diffusion mechanism and the rate of diffusion was higher during the earlier stages of drug release (Table 1).

The kinetic parameters indicate that the maximum amount of drug (C_{max}) was released in pH 1.5 buffer solution at the highest initial release rate (r_o), as compared to the other release medium. It has also been found that the drug release from the hydrogel obeyed all the kinetic models (R^2>0.97), but was best fitted with the Higuchi square root model, with the highest value of the regression coefficient (R^2) (Table 2).

In the Higuchi square root model, the drug released from the hydrogels is directly proportional to the square root of time. The release pattern is described by the Higuchi model, where it is assumed that the loaded drug dissolves firstly from the outer layer of the polymeric device, and when this layer becomes exhausted of the drug, the next inner layer begins to be depleted by dissolution and diffusion through the polymer matrix to the release medium. Thus, the interface between the region containing dispersed drug and the release medium moves into the interior as a front.[32,33] The synthesis reaction and the full mechanism of the drug release are illustrated in Scheme 1.



Scheme 1: Schematic illustration for the preparation of covalently cross-linked hydrogel based on *CS* and drug release of *Dox*

Table 1
Kinetic parameters of *Dox* release from drug-loaded *CS-g-PVPr-co-P4VP* based hydrogel with different gelator content

Release medium	Diffusion exponent, n	Gel characteristic constant, $k\times10^3$	Max. amount of released drug, C_{max} (mgL^{-1})	Constant of release kinetics, $k_{rel}\times10^5$ (S^{-n})	Initial release rate, $r_0\times10^2$ ($mgL^{-1}s^{-1}$)	Diffusion coefficients (cm^2/min)		
						Initial, $D_i\times10^5$	Average, $D_a\times10^5$	Final, $D_l\times10^5$
pH=1.5 buffer (10% gelator)	0.694	17.271	436.28	88.72	158.25	23.451	12.314	22.825
pH=6.8 buffer (10% gelator)	0.702	16.364	372.16	102.04	113.47	25.316	14.732	24.318
pH=1.5 buffer (14% gelator)	0.678	17.012	394.76	92.45	172.28	22.473	10.872	23.657
pH=6.8 buffer (14% gelator)	0.714	16.047	371.70	106.81	102.64	26.012	13.476	25.481

Table 2
Kinetic interpretation of *Dox* release from *CS-g-PVPr-co-P4VP* based hydrogel with different amounts of gelator

Release medium	Zero order [R^2], k_0 (min^{-1})	First order [R^2], k_1 (min^{-1})	Higuichi [R^2], k_H ($min^{-1/2}$)	Korsmeyer-Peppas [R^2], k_{KP} (min^{-n})	Hixon-Crovell [R^2], k_{HC} ($min^{-1/3}$)
pH=1.5 buffer (10% gelator)	0.9838	0.9936	0.9942	0.9932	0.9984
pH=6.8 buffer (10% gelator)	0.9642	0.9613	0.9935	0.9925	0.9938
pH=1.5 buffer (14% gelator)	0.9729	0.9704	0.9924	0.9947	0.9974
pH=6.8 buffer (14% gelator)	0.9612	0.9568	0.9916	0.9912	0.9918

It is anticipated that a low pH causes the -NH_2 groups to become protonated and exist in the form -NH_3^+. Thus, these groups are ionized and their charges repel each other. This force expands the hydrogel, leading to more space among the polymer chains, which facilitates the migration of drug molecules out of the network. Meanwhile, at high pH, both forms of -NH_2 groups are present and the repulsion force decreases and less *Dox* is released. In addition, the obtained results showed that the drug release increased with decreasing content of cross-linking agent. By comparing the hydrogels with different amounts of gelator, it could be seen that the pore size decreased and the pore density increased with the gelator content. In addition, the swelling index was higher for the hydrogel with 10% gelator and resulted in an increased release rate. In fact, for the hydrogel with 14% gelator, the pore size of the porous hydrogel network gets smaller, while the swelling capacity significantly decreases and the hydrogel cannot hold a large amount of water. From these results, it can be concluded that increased porosity aided higher release of *Dox*.

The findings of the study indicate that *CS-g-PVPr-co-P4VP* hydrogels are a good candidate for localized drug delivery. The strong mucoadhesion of *Dox* loaded hydrogels with mucosal membrane can help in the treatment of local infections.

CONCLUSION

In summary, novel pH- and thermo-sensitive hydrogels based on chitosan were prepared and they were successfully used for the release of doxocyline as a model drug. The chitosan-based hydrogels were developed using N-vinylpyrrolidone and 4-vinylpyridine as graft comonomers and N,N'-methylene-*bis*-acrylamide as cross-linking gelator under mild conditions, with the help of covalent chemistry between the amine groups of the chitosan chains and the double bond groups of the cross-linker through radical linkage. The novel hydrogels exhibited pH and temperature dependent swelling characteristics and can be a suitable candidate for drug delivery systems.

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