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Controlled delivery of drug from pH sensitive chitosan/poly (vinyl alcohol) blend

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ABSTRACT

The blends of chitosan (CS) and poly(vinyl alcohol) (PVA) react with tetraethoxy silane (TEOS) to give network structure and their selective crosslinking give hydrogel properties. The swelling properties against different media are changed by varying the amount of PVA in CS/PVA blend. The degree of swelling in water is decreased with increasing amount of PVA. The most significant behavior of these blends is their response against pH, exhibiting low swelling in acidic and basic pH conditions and maximum swelling at neutral pH. This unique behavior along with the biocompatibility of the components made them suitable for oral delivery of enzymes and medicines. Dexamethasone has been selected as a model drug. The released profile of dexamethasone loaded CS/PVA complex showed 9.37% of drug release over a period of 2 h in simulated gastric fluid and its transfer to simulated intestinal fluid showed consistent release of remaining drug up to 7 h.

1. Introduction

The blends of hydrophilic polymers are sensitive to the condition of the surrounding environment and this property has been exploited in many fields such as medicine, pharmacy, biotechnology etc. (Khurma, Rohindra, & Nand, 2006; Sokker, Ghaffar, Gad, & Aly, 2009; Tang, Du, Li, Wang, & Hu, 2009). These blends are obtained from the macromolecular networking either by physical (complexation or by aggregation which gives entangled networks) or by chemical (covalently) links. (Berger, Reist, Mayer, Felt, & Gurny, 2004). These covalent links are developed by the addition of crosslinkers such as gluteraldehyde, formaldehyde, genipen, N,N′-methylenebis(acrylamide) or by ionizing radiations (Alvarez-Lorenzo & Concheiro, 2002; Rodrigues, d, Azambuja, & Castagno, 2007; Yang, Su, Leu, & Yang, 2004; Zhao et al., 2003). The crosslinkers that were used need a purification step during manufacturing to remove the unreacted crosslinker which is sometimes very difficult (Berger et al., 2004). Although, the components of the hydrogel are biocompatible, but the inherent toxicity of most of the crosslinkers limit their applications to medical and pharmaceutical fields.

Chitosan, a natural amino functionalized polysaccharides, is a copolymer of β-[1 → 4]-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-2-deoxy-D-glucopyranose (Berger et al., 2004). It has many characteristic properties such as biocompatibility, biodegradability, antimicrobial activity, wound healing and film forming ability. Owing to these attributes, CS is extensively applied in biomedical and pharmaceutical industries as a component of hydrogel (Cho, Han, & Ko, 2000; Kim, Shin, Kim, & Kim, 2005; Liang, Liu, Huang, & Yam, 2009; Pillai, Paul, & Sharma, 2009; Queiroz et al., 2003; Wang et al., 2000; Yang et al., 2010).

PVA is a cheap, non-toxic water soluble polyhydroxy polymer. It has a film forming ability and provides porous and hydrophilic interfaces with body tissue. Due to the ease of chemical modification and simple chemical structure, PVA is widely used in many biomedical applications including contact lenses, artificial muscle, burn wound dressing and vocal cord reconstruction (Khurma et al., 2006; Park & Kim, 2006; Rodrigues et al., 2007).

The strong secondary interactions between the polymer chains of CS and PVA give physically crosslinked complex. The CS and PVA have also been crosslinked by using genipen, glutaraldehyde, formaldehyde, electron beam, borate, tripolyphosphate etc. to give crosslinked complexes (Liang et al., 2009). The CS/PVA complexes exhibited different responses to external stimuli such as pH change (Wang, Turhan, & Gunasekaran, 2004), temperature (Tang, Du, Hu, Shi, & Kennedy, 2007) and the electrical charges (Kim, Park, Kim, Shin, & Kim, 2002). Berger et al. has concluded that CS and PVA complexes are used to prepare biocompatible drug delivery system if pH controlled release is not required (Berger et al., 2004).

The aim of the present study is to develop a pH sensitive blend based on chitosan and PVA using a non toxic crosslinker. In this work, the aim is to synthesize a pH sensitive CS and PVA complex for drug delivery system. Silane coupling agents such as TEOS has been selected as crosslinker due to its acceptance in biomaterials applications (Rasool, Yasin, & Akhter, 2008; Rasool, Yasin, Heng, & Akhter, 2010). The developed CS/PVA complexes showed maximum swelling at neutral pH on contrary to acidic and basic pH

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conditions. This behavior and biocompatibility of the components made them suitable for controlled delivery of drugs. Dexamethasone is widely used as anti-inflammatory agent and it has potential for suppressing both acute and chronic inflammatory response after device implantation (Galeska et al., 2005). It is glucocorticoid that exerts diverse inhibitory effects on several inflammatory mediated responses (Pires et al., 2005). Dexamethasone has been selected as a model drug for loading and its released behavior has been studied in simulated gastric and intestinal fluids. UV spectroscopy technique has been used to measure the amount of the drug.

2. Materials and methods

2.1. Materials

CS (product number C3646 having degree of deacetylation ca 75%; bulk density 0.15–0.30 g/cm^3, viscosity >200 cP) and poly (vinyl alcohol)(M_w: 146,000–186,000; 98–99% hydrolyzed) are received from Sigma–Aldrich. Analytical grade TEOS, acetic acid, sodium hydroxide, hydrochloric acid and methanol (99.7%) are purchased from Sigma–Aldrich (Milwaukee, WI) and are used as received. Dexamethasone was obtained from Tabros Pharma, Karachi, Pakistan.

2.2. Synthesis of blend

CS is dissolved in acetic acid (0.5 M) in a glass reactor fitted with magnetic stirrer. Varying amounts of PVA (4, 8 and 10%) are added in deionized water at 80 °C and heated for 1 h to make clear solution. For CP4 blend, 0.96 g of chitosan is dissolved in 40 mL of acetic acid and mixed with 0.04 g of PVA in 20 mL of water. The pH of the CS/PVA mixed solution is ~3.5 at 27 °C. TEOS is added to this blended solution under constant stirring. After 1 h, the resultant solution is poured into plastic dishes for drying at room temperature. After drying, the obtained films are vacuum dried at 60 °C. The CP4, CP8 and CP10 codes are used to represent the CS/PVA blends containing 4, 8 and 10% PVA, respectively.

2.3. IR analysis

The IR spectra are recorded at room temperature on FTIR spectrophotometer (Nicolet, 6700) from Thermo Electron Corporation, USA. Attenuated total reflectance technique with diamond crystal is used at scanning range of 4000–400 cm^{-1} (200 average scans) and resolution of 4.0 cm^{-1}.

2.4. Scanning electron microscopy

The surface morphology of the blends is examined under SEM, Model JEOL JSM 6490 (LA). Samples are prepared by swelling in distilled water and then frozen in liquid nitrogen. Freeze-drying of samples is done using LGJ-10 Freeze-Dryer. The images are analyzed at different magnifications.

2.5. Thermal gravimetric analysis

The thermal behavior of the samples is studied with Mettler Toledo, TGA/SDTA851^e instrument under nitrogen atmosphere (50 mL/min). The sample (6–8 mg) is heated at a rate of 20 °C/min from room temperature to 600 °C.

2.6. Swelling experiments

Swelling experiments of the hydrogel are performed using following procedure. A known amount of sample (~50.0 mg) is immersed in a vial containing swelling solvent (100 mL) in a temperature controlled bath at desired temperature. The swollen weight of the sample is determined at given time after removing the excess surface solution. The samples are immersed again in the same solution until equilibrium swelling is achieved. The swelling of the sample is calculated by using the following equation:

$$\text{Swelling(g/g)} = \frac{(W_s - W_d)}{W_d}$$

where W_d is the dry weight and W_s is the swollen weight of the sample at time t (Rasool et al., 2008, 2010; Reis et al., 2008).

The pH response of the samples was studied in non-buffer and buffer solutions. Non-buffer solutions of pH (1.0–13.0) were prepared from the standard stock solutions of HCl (0.1 M) and NaOH (0.1 M). Buffer solutions were prepared by standard method. The swelling response of hydrogels was also investigated in sodium chloride and calcium chloride solutions at different concentrations ranging from 0.05 to 1.0 mol/L. The swelling is calculated by using the above equation.

2.7. Preparation of simulated solutions and drug loading

Simulated gastric fluid (SGF, pH = 1.2) and intestinal fluid (SIF, pH = 6.8) are prepared by using published method (Nho, Park, Kim, & Hwang, 2005; Rasool et al., 2010). SGF is prepared by mixing 1 g of NaCl in 3.5 mL of HCl (37%) and diluted up to 500 mL with distilled water. SIF is prepared by mixing 250 mL of KH_2PO_4 (0.2 M) with 118 mL of NaOH (0.2 M).

The dried sample (40 mg) is equilibrated in dexamethasone solution of 25 mg/25 mL of solvent (methanol:water, 1:2). The sample showed swelling in the solvent system and drug is loaded by sorption. The drug loaded samples are placed for drying in dark conditions. The drug loaded sample is placed in a beaker at 37 °C containing SGF solution (100 mL) for 2 h and then it was transferred to SIF solution at 37 °C for further 5 h. Aliquots of 5 mL is taken from vial after every 30 min. 5 mL of fresh solutions were added back into the vial to make up liquid volume. The amount of released dexamethasone is determined spectrophotometrically using a Perkin Elmer Lambda 40 UV–Vis spectrophotometer at 242 nm. SGF and SIF are used as reference standards. Calibration line is drawn using dexamethasone solutions ranging from 2 mg/L to 25 mg/L. The amount of dexamethasone released by the hydrogel is determined using calibration line.

3. Results and discussion

The hydroxyl group of CS and PVA reacts with TEOS to produce crosslinked hybrid polymer network. All the prepared complexes exhibited pH sensitive swelling and their swelling in different media mainly depends upon the PVA contents in the complex.

3.1. Structural analysis

The IR spectra of CS, PVA, CP4 and CP10 are shown in Fig. 1. The CP4 and CP10 spectra confirm the presence of incorporated components. Strong peaks of amide I and amide III are observed at 1653 cm^{-1} and 1322 cm^{-1}, respectively. The amide II peaks ranges from 1510 cm^{-1} to 1570 cm^{-1} which is the characteristic of CS. In addition, the peak at 893 cm^{-1} due to pyranose ring and 1155 cm^{-1} due to saccharine structure also confirmed the existing chitosan moiety (Costa-Júnior, Barbosa-Stancioli, Mansur, Vasconcelos, & Mansur, 2009; Mansur, de Costa, Mansur, & Barbosa-Stancioli, 2009). Broad band between 3500 cm^{-1} to 3250 cm^{-1} shows O—H stretching of inter and intramolecular hydrogen bonds in the complex and its intensity is increased as the amount of PVA increased

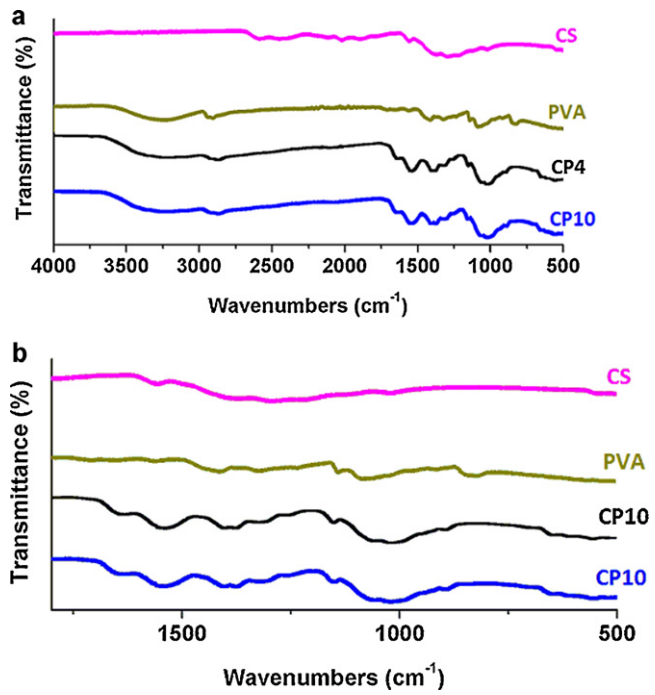

Fig. 1. IR spectra of CS, PVA, CP4 and CP10. (a) 4000–500 cm^{-1} (b) 1800–500 cm^{-1}.

from 4 to 10%. Vibrational band of alkyl group (C—H stretching) is observed from 3000 cm^{-1} to 2840 cm^{-1}.

The presence of siloxane bond (Si—O—) resulted by TEOS is confirmed by the absorption peaks at 1020 cm^{-1} and 1080 cm^{-1} (Rasool et al., 2008, 2010). These characteristic peaks are absent in the spectrum of pure CS and PVA. The gel content of the CP4, CP8 and CP10 is around 42% which also confirmed the presence of crosslinked network resulted by TEOS.

3.2. Morphological analysis

The scanning electron microscopy is used to study the morphology of the crosslinked samples. The micrographs of CP4, CP8 and CP10 are shown in Fig. 2. These micrographs show that all samples have porous network structure which is responsible for their swelling. Macroscopically, all the samples appeared transparent. EDS analysis results of thread like structures in the micrographs also shows the presence of silicon which is a confirmatory evidence for the presence of siloxane bond within the hydrogels.

3.3. Thermogravimetric analysis

The thermograms of CS, PVA, CP4 and CP10 are shown in Fig. 3. It can be seen from this figure that the onset of degradation of PVA started from 280 °C and nearly 90% of the polymer was decomposed up to 600 °C. Whereas, in the TG curve of chitosan, the first weight loss from 50 °C to 280 °C is due to the removal of moisture, loss of bound water and dehydration. The main skeleton of the chitosan started degradation around 280 °C. The TG curves of CP4 and CP10 are quite similar to chitosan but their second onset of degradation started much earlier as compared to CS and PVA. CP10 is thermally more stable as compared to CP4. The 10% weight loss of CP4 is at 190.1 °C while it is increased to 233.1 °C in CP10 blend. This increased thermal stability of CP10 is due to the incorporation

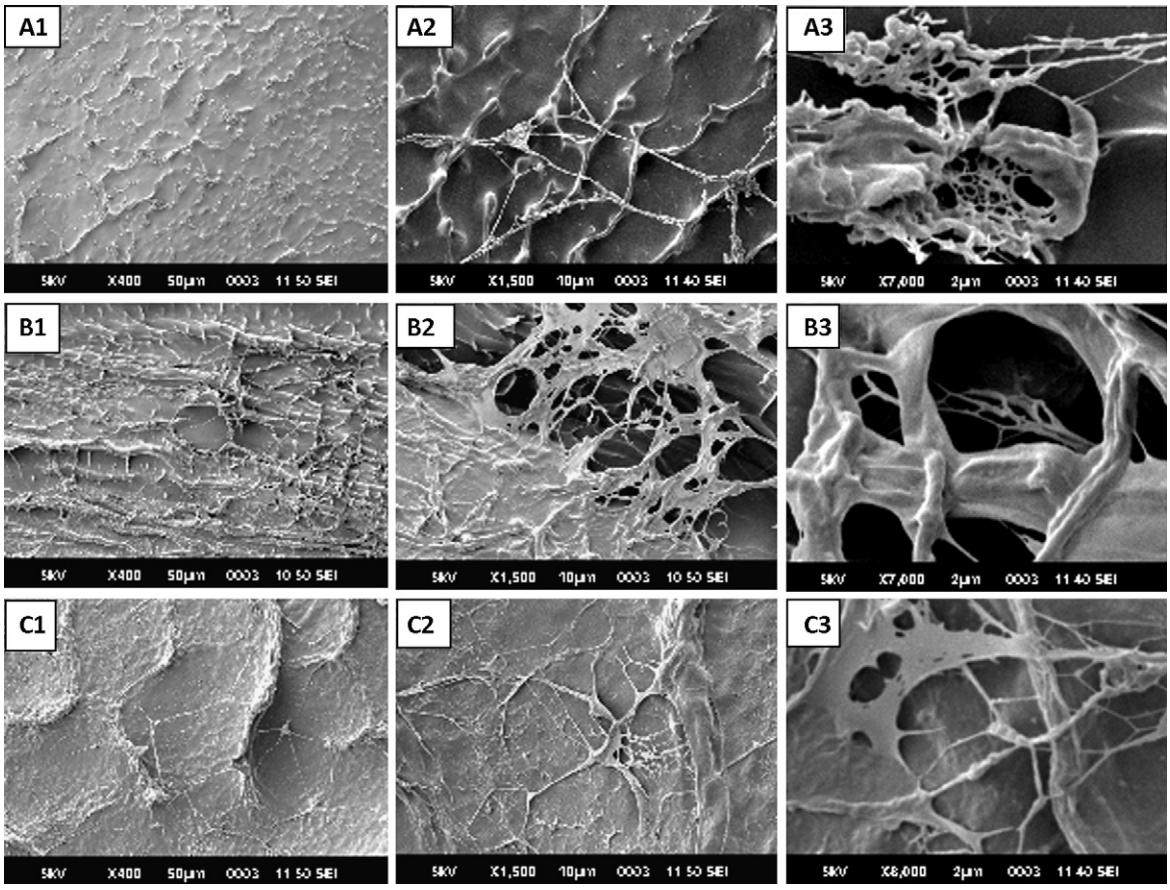

Fig. 2. SEM micrographs of CP4 (A1 ×400), (A2 ×1500) and (A3 ×7000), CP8 (B1 ×400), (B2 ×1500) and (B3 ×7000) and CP10 (C1 ×400), (C2 ×1500) and (C3 ×8000).

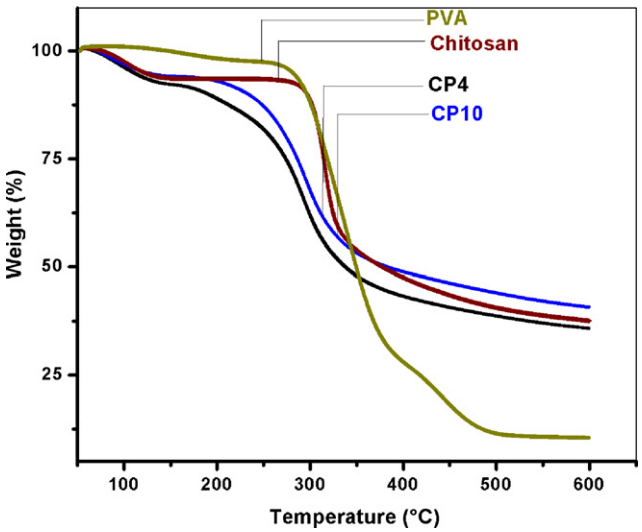

Fig. 3. Thermograms of CS, PVA, CP4 and CP10.

of more —OH groups during crosslinking reactions which increase its stability as compared to CP4.

3.4. Swelling response in water

Time dependent swelling response of CS/PVA complexes in deionized water is shown in Fig. 4(a). This figure shows a linear increase in the swelling of all the samples with time and an equilibrium in swelling is reached around 6 h. The increased amount of PVA from 4 to 10% in the samples decreases the swelling. CP4 has higher swelling at all time interval and reached the maximum swelling (770.4 g/g) after 6 h. Whereas, at same time interval, CP10 (which contains 10% PVA) showed 447.4 g/g. This decrease in swelling with increase in PVA is mainly due to the increase in hydrophobic contents and the formation of compact complex structure.

Generally, the swelling of a material is caused by the diffusion of solvent into its structure from extracellular matrix. The mechanism of solvent diffusion is determined by the following equation:

$$F = kt^n$$

where 'k' is the swelling rate constant which exhibits the characteristics of polymer network and water, 'F' is the fractional swelling at time 't' (min) and 'n' is the swelling or diffusion exponent. The values of 'n' and 'k' parameters were obtained from the swelling data of CS/PVA complexes in water. Fig. 4(b) shows the plot of ln F vs ln t and the values of diffusion parameters are given in Table 1. All complexes show non-Fickian diffusion mechanism. The value of 'n' shows a linear diffusion response with the increase of PVA content in the complexes. All the complexes have same crosslinking density but different amounts of PVA. This reveals that the amount of PVA affects the diffusion parameters of the samples. The value of 'n' indicates the mechanism of diffusion, transport or release mechanism (Brannon-Peppas & Peppas, 1990). Fickian and Case II diffusion having 'n' value equal to 0.5 or 1, respectively. Non-Fickian (anomalous diffusion), when it is between 0.5 and 1 and if the value of n is greater than 1, then it is super Case II diffusion (Francis, Kumar, & Varshney, 2004; Swarnalatha, Gopi, Kumar, Selvi, & Sekaran, 2008).

Table 1
Diffusion parameters of CP4, CP8 and CP10 blends.

Parameters	CP4	CP8	CP10
n	0.7899 (0.0140)	0.7442 (0.0134)	0.6864 (0.0178)
k	0.0084	0.0119	0.0183
Intercept	−4.7810 (0.0717)	−4.4269 (0.0688)	−4.0016 (0.0910)

3.5. Swelling response in non-buffer media

The prepared CS/PVA complexes exhibit pH sensitive swelling with minimum swelling in acidic and basic conditions and maximum swelling around neutral pH. The change in pH of the external medium also changes the charge balance inside the complex which in turn modifies the degree of association of the polymer chains.

The swelling of CP4, CP8 and CP10 in non-buffer media is shown in Fig. 5(a). This figure shows that all the complexes have low swelling in acidic and basic pH and maximum swelling at neutral pH. The amount of PVA in CS/PVA complexes also affects the swelling as it decreases with increasing amount of PVA. The swelling of all the samples increases steadily up to pH 6; maximum swelling is observed around neutral pH and again decreased in basic pH range. The maximum swelling of 632.3 g/g is exhibited by CP4 at pH 7 and its minimum value of 3.5 g/g is obtained at pH 13. The pK_a value of CS is 6.2 and makes it water soluble when pH is less than 6.2. The amino groups (—NH_2) of CS protonated as ammonium group (—NH_3^+) in acidic medium which results in strong electrostatic repulsion between the polymer chains at low pH. Since counter ions (Cl^-) present in media have to be localized near the polymer chains. So, the subsequent free movement of —NH_3^+ groups is slowed down as they are fixed on the polymer chain and the overall electrostatic potential is neutral. The increased osmotic pressure inside the complex causes less flow of solvent into its structure which in turn results in less swelling. Shibayama et al. has already discussed this behavior in detail in volume-phase transition and related phenomenon of polymer gel (Shibayama & Tanaka, 1993).

At pH > 6.2 or neutral pH, deprotonation of the ammonium groups of chitosan chains occurred resulting in reduced electrostatic repulsion and also changes the inside ionic balance of the complex. Subsequently, intra and inter-chain hydrogen bonding and hydrophobic interactions are observed between polymer chains. Now, the crosslinked swollen network is stabilized through hydrogen bonding between —NH_2 and —OH groups of CS and —OH group of PVA. Consequently, highest swelling is observed due to absorption of more water with hydrogen bonded groups and capillary pores.

Complete deprotonation of —NH_3^+ groups in basic pH range further increases the strength of the hydrogen bonding. As a result, the size of the capillary pores further reduced, resulting in less swelling of the hydrogel.

3.6. Swelling response in buffer media

The swelling response of CS/PVA complexes as a function of buffer pH is shown in Fig. 5(b). All complexes show similar swelling behavior against buffer pH as it was observed in non-buffer media; but the degree of swelling is less as compared to non-buffer media at same pH. The swelling of CP4 is 239.0 g/g at pH 7, whereas, at the same non-buffer pH, it was 632.3 g/g. This lower swelling in buffer solution might be due to its high ionic strength as compared to non-buffer solutions.

3.7. Effect of ionic concentration on swelling

The swelling of hydrogels in NaCl and $CaCl_2$ solutions have been studied. Both salts have same anion (Cl^{-1}) but different cation and charge. The effect of type and concentration of these salts on swelling behavior of CP4, CP8 and CP10 is shown in Fig. 6. Both salts decreased swelling with increased concentration of the

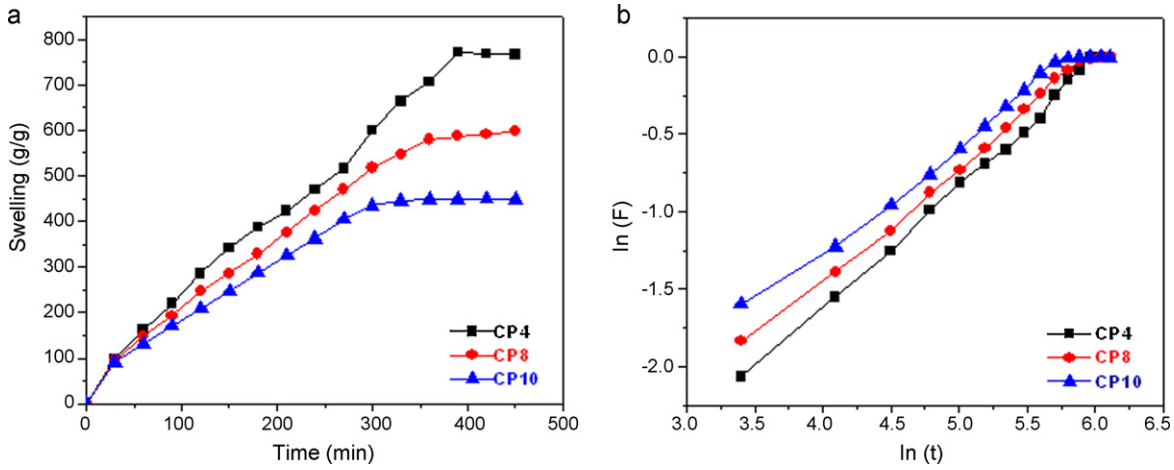

Fig. 4. (a) Time dependent swelling of CP4, CP8 and CP10 in water. (b) ln (*F*) plotted against ln (*t*) for CP4, CP8 and CP10.

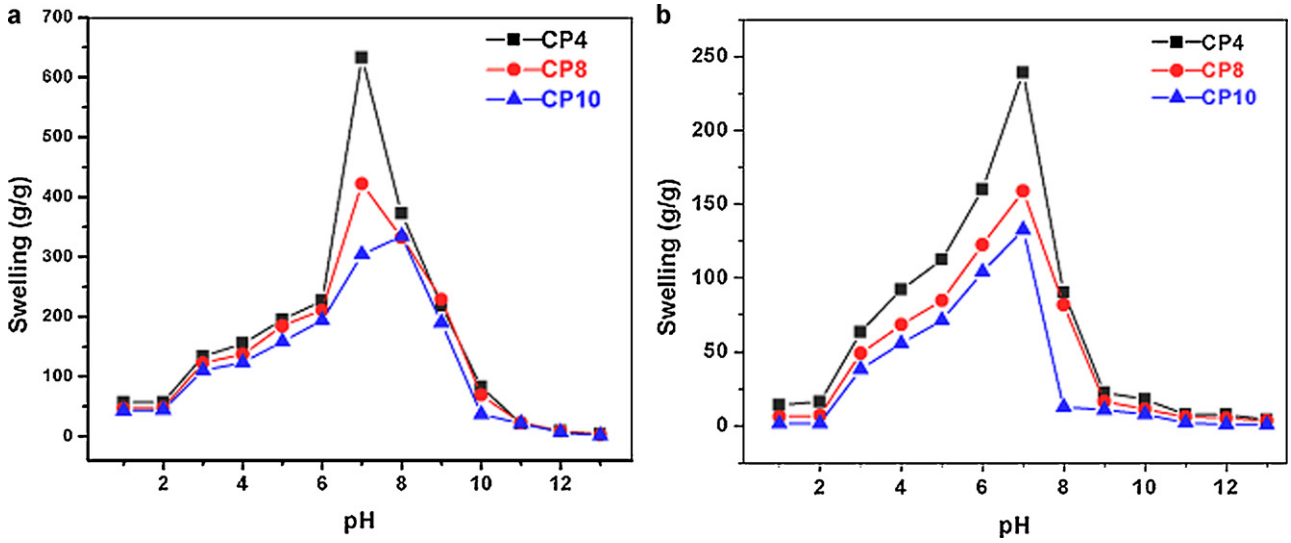

Fig. 5. (a) Swellings of CP4, CP8 and CP10 at different non-buffer pH (1–13). (b) Swellings of CP4, CP8 and CP10 at different buffer pH (1–13).

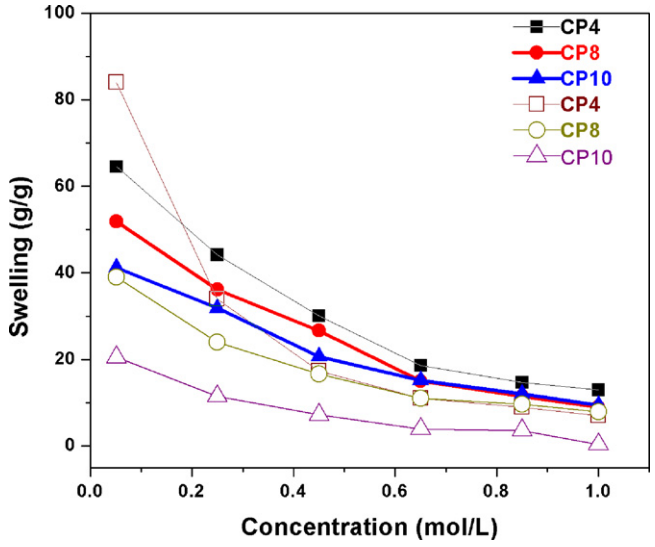

Fig. 6. Swelling of CS/PVA hydrogel in different molar concentrations of NaCl (solid) and $CaCl_2$ (hollow) solutions.

electrolytes. This behavior may be attributed to the increased ionic strength of external solution, which reduces the osmotic pressure difference between the complexes and external salt solution. Therefore, the diffusion of solvent into the complexes is decreased which causes less swelling. Higher swelling is observed in NaCl solution as compared to $CaCl_2$ solution.

3.8. Release analysis of dexamethasone

The CP4 and CP8 are loaded with dexamethasone and its release in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) is studied as a function of time and the results are shown in Fig. 7. This figure shows that release behavior of the drug from both the complexes is the same in SGF and SIF. Approximately 9.4% dexamethasone is released from CP4 in SGF during first 2 h. After 2 h, these drug loaded complexes are placed in SIF. A sustained amount of dexamethasone was released in SIF and after 6 h the amount became constant. This release profile of dexamethasone fulfills the US Pharmacopeia requirement (USP XXIV). According to this, maximum 10% release in SGF and minimum 80% in SIF are acceptable for the oral delivery of drug (Zeitoun, Dib, & Mroueh, 2003).

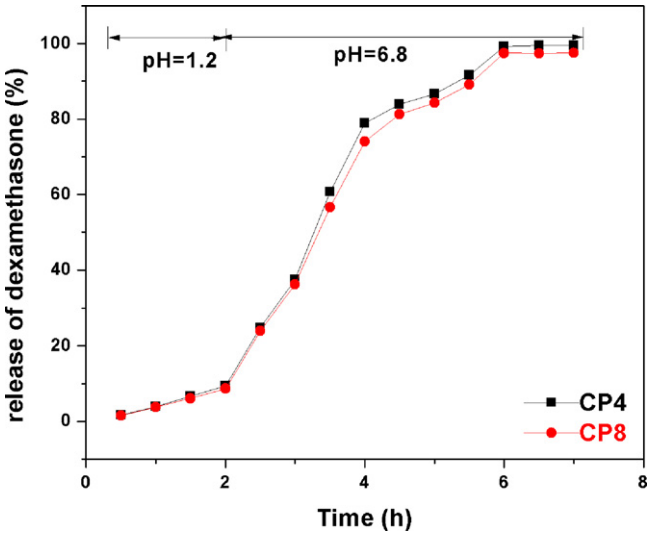

Fig. 7. Release behavior of dexamethasone from CP4 and CP8.

4. Conclusions

A novel pH sensitive CS/PVA blend with varying amount of PVA has been synthesized and crosslinked with TEOS. IR analysis confirmed the presence of siloxane linkage between the incorporated components. The increased amount of PVA in CS/PVA complexes showed higher thermal stability whereas, degree of swelling in water was decreased. Swelling of complexes in deionized water was affected by increasing the amount of hydrophobic contents. CP4 showed maximum swelling of 770.4 g/g in deionized water. Non-Fickian diffusion controlled mechanism was observed in all complexes and the value of diffusional exponent '*n*' ranged from 0.79 in CP4 to 0.68 in CP10. The response against pH that showed low swelling in acidic and basic pH and maximum swelling around neutral pH, make these complexes more suitable for drug delivery systems. This pH sensitivity of the developed complexes could play a significant role in medicine and pharmaceutical applications. CP4 and CP10 hydrogels have been used for drug loading and for release behaviour study. The in vitro analysis of hydrogels loaded with dexamethasone in SGF (pH 1.2) showed 9.4% of dexamethasone release over a period of 2 h. A sustained release of remaining dexamethasone after 7 h was observed in simulated intestinal fluid (pH 6.8).

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