Controlled-Release Formulations for Hydroxy Urea and Rifampicin Using Polyphosphate-Anion-Crosslinked Chitosan Microspheres

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ABSTRACT: Physically crosslinked microspheres for the controlled delivery of rifampicin and hydroxy urea have been prepared with sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP) anion crosslinkers. Chitosan with a constant degree of deacetylation (75 wt %) and a constant molecular weight (1134 kg/mol) has been found to be useful for the controlled release of selected drugs. The microspheres prepared with the SHMP anion crosslinker are more hydrophobic and compact in their shape and size than the microspheres prepared with the STPP anion crosslinker. The SHMP-anion-crosslinked microspheres show optimum loading at pH 3, whereas the STPP-anion-crosslinked microspheres show optimum loading at pH 4. The STPP-anion-crosslinked microspheres are suitable for the controlled release of rifampicin, but the SHMP-anion-crosslinked microspheres are suitable for the controlled release of hydroxy urea. The drug-release characteristics of the physically crosslinked chitosan microspheres are explained with respect to their size and the ionic interactions of the encapsulated drugs with the polymer matrices and anion crosslinkers. The initial burst release of the loaded drugs is Fickian in nature and follows first-order kinetics, but the controlled step of the drug release shows a non-Fickian nature and follows zero-order kinetics. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 1942–1956, 2007

Key words: biopolymers; chitosan; drug delivery systems

INTRODUCTION

Chitin is most abundant naturally occurring polysaccharide and is isolated from exoskeletons of crustaceans, shrimps, and crabs.[1,2] Chitosan is the most common derivative of chitin and is produced by its partial deacetylation.[3,4] It becomes soluble in aqueous media at low pHs when the degree of deacetylation (DDA) is about 50 wt %. Chitosan is nontoxic,[5] biodegradable, compatible, and mucoadhesive and has shown the ability to enhance drug absorption;[6] hence, chitosan and its derivatives have been found useful in various applications.[1] In biomedicine, chitosan has been found to be attractive for intra-oral drug-delivery systems to reduce the frequency and amount of drug administration.[7] The efficacy of delivery systems depends on the properties and size of the encapsulant, so chitosan beads and microspheres for drug-delivery systems have been prepared with nontoxic crosslinkers.[8] Chitosan delivery systems have been prepared either by chemical crosslinking with dialdehydes such as glutaraldehyde and glyoxal[9–11] or by physical crosslinking with multivalent anions such as tripolyphosphate,[5,12] citrate,[13] sulfate,[14] and polyphosphate.[15] Because chemical crosslinking by glutaraldehyde has sometimes caused irritation to mucosal membranes,[16–19] ionic crosslinking by electrostatic interactions has been found useful to avoid these undesirable side effects.[20] Because chitosan is a weak polybase with a positive charge number of thousands,[21] anions with sufficient charge numbers are effective for the crosslinking of chitosan through electrostatic interactions.[22,23] Among the available multivalent anions, the sodium tripolyphosphate (STPP)[24] and sodium hexametaphosphate (SHMP)[15] anion crosslinkers are nontoxic and efficient in forming chitosan gels by ionic interactions.[25] The properties of ionically crosslinked chitosan are influenced by electrostatic interactions between the anion crosslinkers and chitosan.[26] Because the interactions between chitosan and anion crosslinkers depend on the molecular structure of the anions,[21] efforts have been made to control these interactions by the variation of the charge density of anion crosslinkers[7] and by the use of chitosan in solutions of different pHs.[27] The charge density on anion crosslinkers has been varied with the solution pH[7] or with different concentrations of anion crosslinkers.[21] In addition to the types and concentrations of anion crosslinkers, the physical properties of chitosan, such as the molecular weight and

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DDA,[28,29] also control the interactions between chitosan and anion crosslinkers.[30] These properties of chitosan contribute significantly to modifying the surface morphology of microparticles. An increase in the molecular weight of chitosan increases the viscosity of a solution of chitosan, and this helps in the formation of microparticles with strong walls by the interactions of anion crosslinkers.[8] The shape of microparticles has also been shown to depend on the molecular weight of chitosan.[31] The ionic interactions control the formation of beads, degree of swelling, and loading and release of drugs.[32] In these investigations, chitosan microspheres have been prepared with STPP and SHMP anion crosslinkers, and the effects of the electrostatic interactions between the anion crosslinkers and chitosan have been investigated. The degree of hydrophobicity in chitosan microspheres has been studied to correlate the hydrophobicity with the loading and release behavior of the crosslinked microspheres. The effects of various other experimental conditions on the controlled release of hydroxy urea and rifampicin from STPP- and SHMP-crosslinked chitosan microspheres have been studied.

EXPERIMENTAL

Materials

Chitosan was obtained from Sigma–Aldrich Chemical Co. (Milwaukee, WI) and purified as reported in an earlier communication.[15] STPP and SHMP were received from Loba Chemie (Mumbai, India) and from Ranbaxy, Ltd. (Mumbai, India), respectively, and used without further purification. Hydroxy urea and rifampicin were received as gift samples from Sarabhai Pharmaceuticals (Ahmedabad, India) and Lupin, Ltd. (Aurangabad, India), and used after recrystallization. The other chemicals used in the experimental work were analytical-reagent-grade and were used as received.

Determination of the molecular weight of chitosan

The molecular weight of the chitosan samples was determined viscometrically with an Ubbelohde-type viscometer and with the following equation:[33]

$$[\eta]_{25^\circ C} = kM_v^{\alpha}(\text{Acetic acid}) \qquad (1)$$

where $[\eta]_{25^\circ C}$ is the intrinsic viscosity at 25°C; M_v is the viscosity-average molecular weight; and k and α are 1.81×10^{-3} cm^3/g and 0.93, respectively, at 25°C.

Deacetylation in chitosan

The procured chitosan sample (DDA = 48 wt %) was deacetylated through the refluxing of 0.5 g of chitosan in 50 mL of a 40 wt % sodium hydroxide solution[34] at 80°C for about 7 h. The samples were finally washed with hot and cold water and dried at 30°C in a vacuum oven.

Elemental analysis of chitosan

The elemental analysis of the chitosan samples was carried out with a Heraeus Carlo Erba 1108 elemental analyzer (Milano, Italy), and the data were used to verify the DDA value of chitosan, as determined by the potentiometric method.

Determination of the DDA value of chitosan

The DDA value of the chitosan samples was determined by the potentiometric titration of chitosan (20 mL of 0.01*M* HCl) with 0.1*M* NaOH and with the following equation:[9]

$$\text{DDA (wt\%)} = \frac{203Q}{1 - 42Q} \times 100, \qquad Q = \frac{N\Delta V}{m} \qquad (2)$$

where Q is the partition quotient, m is the mass of the chitosan sample, N is the strength of the sodium hydroxide used for titration, and ΔV is the volume of alkali consumed in the titration of amino groups present in the chitosan samples.

Turbidimetric titration of mixtures of chitosan and the anion crosslinkers

To determine the pH of the maximum interactions between STPP and SHMP anion crosslinkers and chitosan,[35–37] the STPP or SHMP crosslinker (0.1 g/L) and chitosan (0.25 g/L) were dissolved in 20 mL of 0.1*M* HCl and titrated with 0.1*M* NaOH, and the transmittance of the solutions at different pHs was recorded with a UV–vis spectrophotometer (λ_{max} = 420 nm). The degree of ionization of chitosan was determined by the titration of the dissolved chitosan (20 mL of 0.1*M* HCl) with alkali.[35]

Preparation of the physically crosslinked chitosan microspheres

The STPP- and SHMP-crosslinked chitosan microspheres were prepared by the dissolution of a calculated amount of chitosan (0.5 g) in 20 mL of a 2 wt % acetic acid solution under vigorous stirring for about 3 h at room temperature. To obtain the microspheres, the viscous solution of chitosan was blown through a nozzle as fine droplets into a trough containing 250 mL of a 5% methanol solution of NaOH (0.1*M*). The chitosan microspheres that settled in the

trough were separated after 30 min and washed with distilled water. The STPP- and SHMP-anion-crosslinked chitosan microspheres were prepared with different concentrations of the crosslinkers ranging from 2 to 12 wt % in a vessel containing pure chitosan microspheres. The crosslinking of the chitosan microspheres with the STPP anion crosslinker was carried out at pH 4.5 and that with the SHMP anion crosslinker was carried at pH 5.0 at 25°C for 6 h. The microspheres with 0.2 wt % cations and anions were prepared through the mixing of these additives in solutions of chitosan and with a similar procedure.

Determination of the size and morphology of the chitosan microspheres

The size and morphology of the pure and STPP- and SHMP-anion-crosslinked chitosan microspheres was determined with scanning electron microscopy (SEM). To record the SEM micrographs, the microspheres were mounted on metal studs with double-adhesive tape and vacuum-coated with gold.

IR and thermal characterization of the chitosan microspheres

The Fourier transform infrared (FTIR) spectra of the STPP- and SHMP-anion-crosslinked and drug-loaded chitosan microspheres were recorded on KBr pellets with a PerkinElmer (Norwalk, CT) 1600 FTIR spectrophotometer. To analyze the effects of the crosslinking and drug loading on the thermal stability of chitosan, thermogravimetric and differential thermogravimetric analysis was also carried out at a heating rate of 10°C/min under a nitrogen atmosphere with a PerkinElmer Pyris Diamond thermal analyzer.

Surface hydrophobicity of the chitosan microspheres

The surface hydrophobicity of the pure, STPP-anion-crosslinked, and SHMP-anion-crosslinked chitosan microspheres was determined with the Rose Bengal dye adsorption technique.[35] To determine the surface hydrophobicity, 100 mg of microspheres of different specific surface areas was kept separately in a 10-mL solution of a hydrophobic dye (0.1*M*) for about 2 h to partition the Rose Bengal dye at the solution–microsphere interface. The amount of the Rose Bengal dye that adsorbed onto the microspheres was determined by the recording of the absorbance (λ_{max} = 549 nm) of the remaining solution at the end of 2 h. The hydrophobicity of the microspheres was calculated from the slope drawn between Q and the available surface area of the microspheres (μm^2/mol) for the adsorption of dye.[36] Q was calculated as follows:

$$Q = \frac{\text{Amount of dye adsorbed (mol/}\mu m^2\text{)}}{\text{Amount of dye available for adsorption (mol/}\mu m^2\text{)}} \quad (3)$$

Degree of swelling in the chitosan microspheres

The degree of swelling in the crosslinked chitosan microspheres was determined by the placement of 100 mg of STPP- (pH 7) and SHMP- (pH 4) anion-crosslinked chitosan microspheres in 20-mL buffered solutions. The increase in the weight of the microspheres ($W_t - W_0$) with respect to the initial weight of chitosan (W_0) was used to calculate the degree of swelling in the microspheres with the following equation:[9]

$$\text{Degree of swelling}(\%) = \left(\frac{W_t - W_0}{W_0}\right) \times 100 \quad (4)$$

Loading of drugs on the phosphate-anion-crosslinked chitosan microspheres

The loading of hydroxy urea and rifampicin on the STPP- and SHMP-anion-crosslinked chitosan microspheres was carried out by the placement of 100 mg of the microspheres in 20-mL buffered solutions of the drugs for 48 h. The loading of the drugs on the STPP-anion-crosslinked microspheres was carried out at pH 4, whereas the SHMP-anion-crosslinked microspheres were loaded at pH 3. The loading of hydroxy urea (λ_{max} = 204 nm) and rifampicin (λ_{max} = 237 nm) was determined by the recording of the solution absorbance with a Shimadzu 1601 PC UV–vis spectrophotometer (Shimadzu Corp., Kyoto, Japan) after the removal of the microspheres from the solution.

Drug release from the crosslinked chitosan microspheres

The release of hydroxy urea and rifampicin from the crosslinked microspheres was analyzed by the estimation of the amount of the drug released at different time intervals from 100 mg of the microspheres in 20 mL of release media. The amount of the drug released at each interval of 10 h was determined by the recording of the absorbance of the solution with replacement for hydroxy urea (λ_{max} = 204 nm) and rifampicin (λ_{max} = 237 nm) with a Shimadzu 1601 PC UV–vis spectrophotometer (Shimadzu Corp., Kyoto, Japan), and it is presented as the release ratio:

$$\text{Release ratio} = (W_t/W_0) \times 100 \quad (5)$$

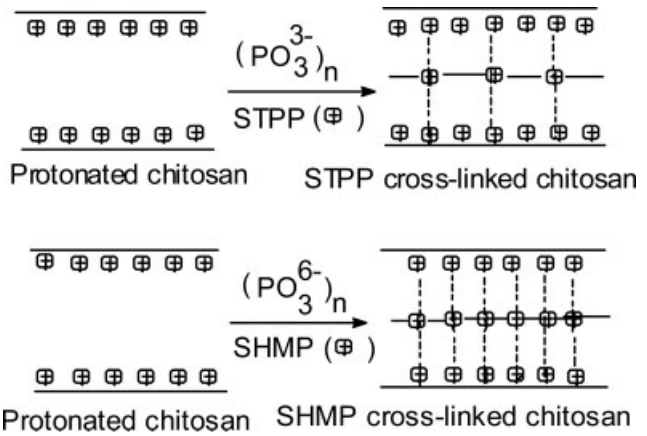
where W_t is the amount of the drug released at a particular time interval and W_0 is the amount of the drug loaded onto the microspheres.

The drug released from the microspheres is also expressed as the burst release and controlled release with the following equations:[15]

$$\text{Burst release } (\%) = \sum \left(\frac{W_t}{W_0} \times 100 \right)_t \quad (6)$$

$$\text{Controlled release } (\%) = \sum \left(\frac{W_t}{W_0} \times 100 \right)_t \quad (7)$$

where $(W_t/W_0 \times 100)_t$ is variable for the burst step of drug release and constant for the controlled step of drug release for a fixed time interval of 10 h.



Scheme 1 Chitosan crosslinking through electrostatic interactions.

RESULTS AND DISCUSSION

Drug-release systems have been prepared for the release of encapsulated drugs in a sustained and controlled manner. The physicochemical properties of polymeric systems[30] play a significant role in controlling the release behavior of delivery systems. The properties of naturally occurring chitosan have shown a dependence on the degree of protonation of amino groups in acidic pH[37] as well as the molecular weight[30,31,38] and DDA[39] of chitosan. The number and distribution of the amino groups on chitosan chains are important factors in controlling the interactions of chitosan with physical crosslinkers in the microspheres.[21] The loading and release characteristics of hydroxy urea and rifampicin have shown specificity for crosslinkers, as observed with STPP- and SHMP-anion-crosslinked chitosan microspheres. Hydroxy urea has shown better loading and release profiles with SHMP-anion-crosslinked microspheres, whereas the loading and release of rifampicin are better with STPP-anion-crosslinked microspheres. The DDA and molecular weight of chitosan[15] influence the loading and release characteristics of hydroxy urea and rifampicin, so chitosan with a constant molecular weight (1134 kg/mol) and DDA (75 wt %) has been used to prepare microspheres for the controlled release of hydroxy urea and rifampicin. The loading and release profiles of hydroxy urea and rifampicin have also shown a significant dependence on the structures and concentrations of the crosslinkers, which control the electrostatic interactions between the chitosan and crosslinkers, as shown in Scheme 1 for crosslinking between chitosan and phosphate anion crosslinkers. These interactions of the chitosan and crosslinkers have been further modified with cations and anions as additives in solutions of chitosan before microspheres have been made. To obtain chitosan with known physical properties, the chitosan samples have been characterized for various parameters before being used in the preparation of microspheres for controlled-release systems.

Molecular weight and DDA of chitosan

The intrinsic viscosity of chitosan was used to determine the molecular weight of chitosan[40] with eq. (1). The molecular weight of chitosan was found to be 1134 kg/mol. The DDA value of alkali-treated chitosan was determined potentiometrically[9] with eq. (2) and was found to be 74.6 wt %. The further verification of DDA of the alkali-treated chitosan samples was conducted with the elemental method of analysis,[41] and DDA was found to be 75.03 wt %. The DDA value determined by the elemental method is very close to the DDA value determined by the potentiometric method; hence, the DDA value for the chitosan samples has been rounded off to 75 wt % and used in these investigations.

Effect of the solution pH on the chitosan–crosslinker interactions

The interactions of chitosan with STPP and SHMP anion crosslinkers show a dependence on the solution pH[30] that is due to the variation in the degrees of ionization of the chitosan and STPP and SHMP anion crosslinkers. Chitosan is a weak base (pK_a = 6.2) and shows a high degree of ionization below pH 4.5 (Fig. 1), whereas STPP (pK_a = 9) and SHMP (pK_a = 5) are weak and strong acids and ionize completely at pHs 4.5 and 5.0, respectively. The solution pH varies the degree of ionization of the chitosan and phosphate anion crosslinkers, which controls the electrostatic interactions between the chitosan and phosphate anion crosslinkers. The variation in the turbidity (100% transmittance) of mixtures of chitosan and phosphate anion crosslinkers with the solu-

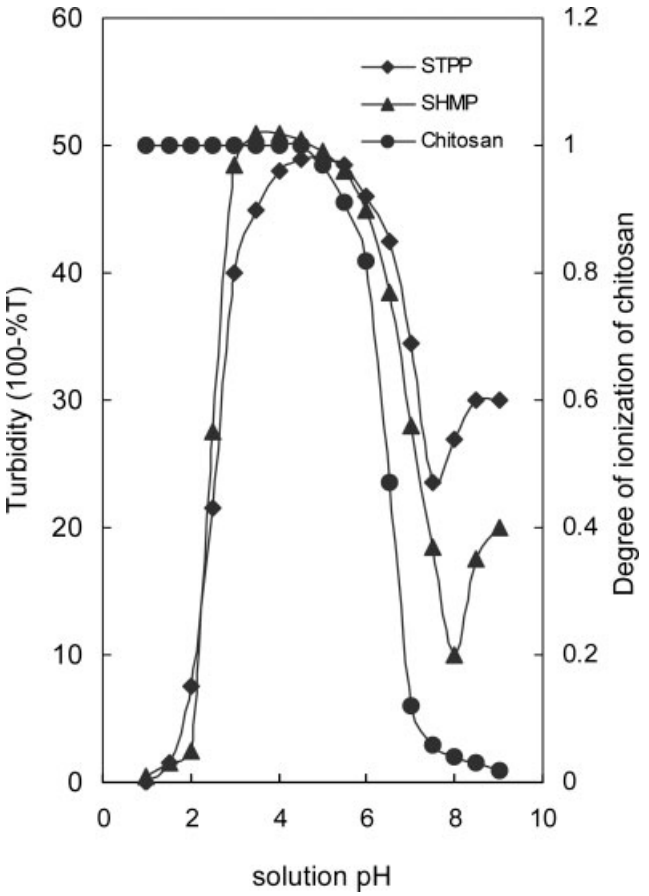


Figure 1 pH-dependent turbidity of mixtures of chitosan and STPP and SHMP anion crosslinkers and degree of ionization of pure chitosan.

tion pH (Fig. 1) gives an indication of the variation in the electrostatic interactions between the chitosan and phosphate anion crosslinkers. The mixtures of the chitosan and phosphate anion crosslinkers show maximum turbidity within a pH range of 3–5 (Fig. 1), which is considered a suitable range for preparing physically crosslinked microspheres for the controlled release of drugs.

Phosphate-anion-crosslinked chitosan microspheres and their characterization

The STPP- and SHMP-anion-crosslinked chitosan microspheres were prepared within the optimized pH range to ensure maximum interactions between the chitosan and phosphate anion crosslinkers. To analyze the effect of the phosphate anion concentration, the microspheres were prepared with different concentrations of the STPP and SHMP anion crosslinkers (Table I). To provide complete crosslinking for chitosan, the pure chitosan microspheres were kept in solutions of STPP and SHMP anion crosslinkers for more than 6 h so that the phosphate anions were successful in the penetration of the chitosan microspheres to maximize electrostatic interactions with protonated amino groups of chitosan and to maintain electroneutrality in the microspheres. The concentration of the phosphate anion crosslinker was varied from 2 to 12 wt % to control the interactions between the chitosan and phosphate anions (Table I). The concentration variation of the crosslinkers allowed the maximum participation of the amino groups of chitosan (DDA = 75 wt %, weight-average molecular weight = 1134 kg/mol) in physical crosslinking with added phosphate anion crosslinkers. The interactions of the crosslinker in the chitosan microspheres controlled the size of the microspheres and their physical appearances (Table I and Fig. 2). The STPP-anion-crosslinked microspheres were larger than the microspheres prepared with the SHMP anion crosslinker (Table I). This was due to the high charge density on the SHMP anion crosslinker in comparison with the STPP anion crosslinker; hence, the SHMP-anion-crosslinked chitosan microspheres were bound more strongly than the

TABLE I
Physical Characteristics of STPP- and SHMP-Anion-Crosslinked Chitosan Microspheres

Microsphere type	Crosslinker concentration (wt %)	Size (μm)	Degree of swelling (wt %)	Hydrophobicity (mol/μm^2)
STPP-crosslinked				
CH-0	0	157.5	399	0.041
CHPP-1	2	121.4	315	0.051
CHPP-2	4	109.8	299	0.071
CHPP-3	6	78.7	280	0.095
CHPP-4	12	57.0	210	0.107
SHMP-crosslinked				
CHMP-1	2	103.5	120	0.076
CHMP-2	4	67.3	97	0.115
CHMP-3	6	48.5	85	0.112
CHMP-4	12	27.7	77	0.192

The molecular weight of CH was 1134 kg/mol, and DDA was 75 wt %. The pH was 7 (STPP) or 4 (SHMP).

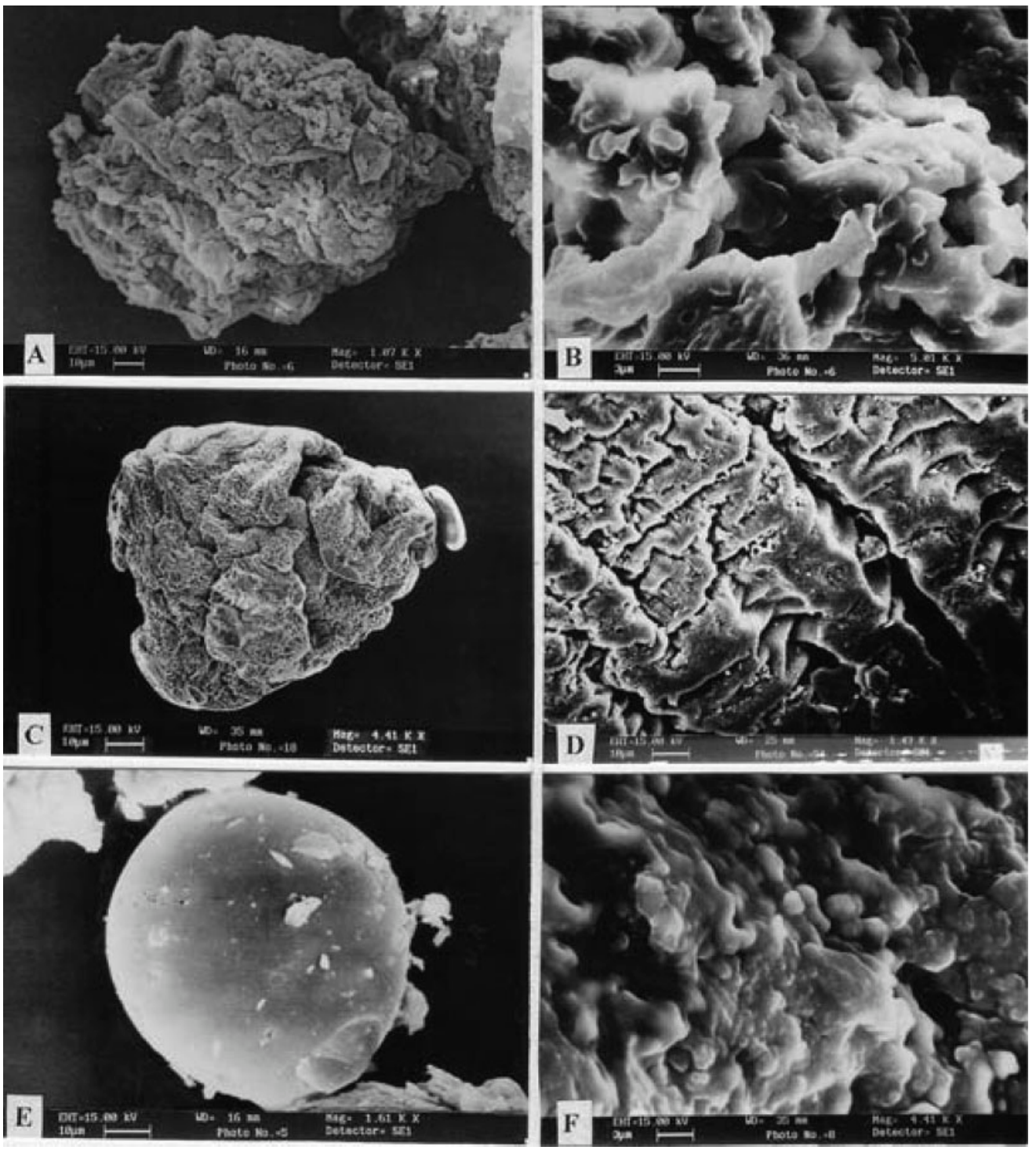


Figure 2 SEM photographs of (A) pure chitosan, (C) STPP-anion-crosslinked chitosan microspheres, (E) SHMP-anion-crosslinked chitosan microspheres, and (B,D,F) their morphologies.

STPP-anion-crosslinked ones. However, these interactions between the chitosan and phosphate anion crosslinkers varied in the presence of added anions and cations and influenced the physical and controlled characteristics of the chitosan microspheres. The microspheres prepared with cations (Na^+ and Fe^{3+}) were more compact than the microspheres prepared with anions (CO_3^{2-} and PO_4^{3-}). The addition of anions increased the force of repulsion with the phosphate anions; hence, these microspheres were less compact than the microspheres with the cations. However, the addition of ionic species influenced the electrostatic interactions more significantly in the microspheres prepared with the STPP anion cross-

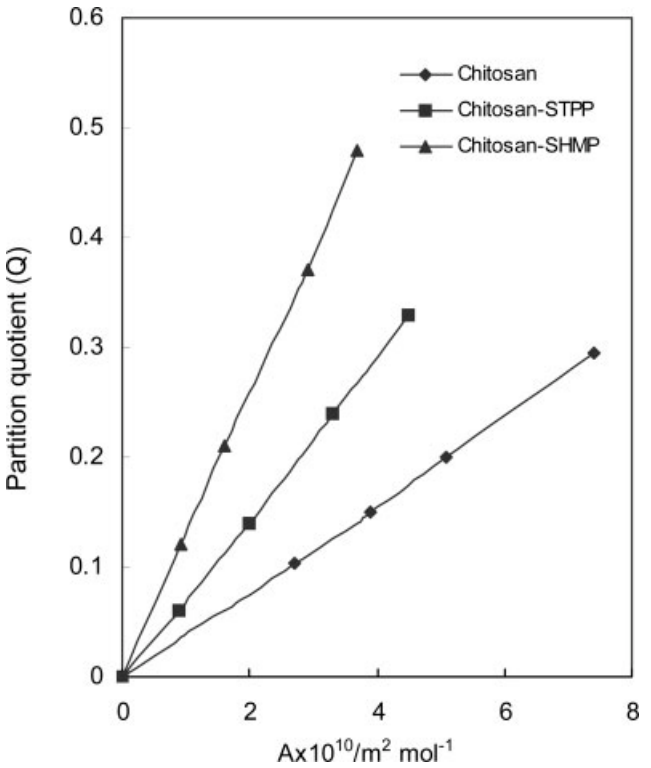


Figure 3 Surface hydrophobicity of (A) pure chitosan, (B) STPP-anion-crosslinked chitosan microspheres, and (C) SHMP-anion-crosslinked chitosan microspheres.

linker than in the microspheres prepared with the SHMP anion crosslinker. The SHMP-anion-crosslinked microspheres showed a smooth morphology [Fig. 2(F)] in comparison with the pure [Fig. 2(B)] and STPP-anion-crosslinked chitosan microspheres [Fig. 2(D)], as is clear from SEM micrographs.

Hydrophobicity of the phosphate-anion-crosslinked chitosan microspheres

The crosslinking in the pure chitosan microspheres (CH-0) with the STPP (CHPP) and SHMP anion crosslinkers (CHMP) shows significant variation in the surface hydrophobicity in the chitosan microspheres, as is clear from the data shown in Table I and Figure 3. The amount of Rose Bengal dye adsorbed per unit of area of the chitosan microspheres (CH-0) is low (0.041 mol/μm^2) in comparison with that for the microspheres crosslinked with the STPP (CHPP) and SHMP anion crosslinkers (CHMP). The hydrophobicity in the microspheres increases with an increasing degree of crosslinking in the phosphate-anion-crosslinked microspheres (Table I), but the increase in the hydrophobicity is more with the SHMP-anion-crosslinked microspheres than with the STPP-anion-crosslinked microspheres (Table I and Fig. 3); this is an indication of stronger interactions between the chitosan and SHMP anion crosslinker than between the chitosan and STPP anion crosslinker. This variation in the degree of hydrophobicity is due to the high charge density on the hexametaphosphate anions in comparison with the tripolyphosphate anions used for crosslinking with chitosan. These interactions are also responsible for the variation in the size and surface morphology of the microspheres (Table I and Fig. 2). The effect of surface hydrophobicity can be ultimately observed in the degree of swelling and controlled characteristics of the crosslinked chitosan microspheres in comparison with the pure chitosan microspheres. The variation in the hydrophobicity of the chitosan microspheres with the concentration and type of phosphate anion crosslinker is useful for controlling the loading and release characteristics of physically crosslinked chitosan microspheres.

Swelling in the phosphate-anion-crosslinked chitosan microspheres

The degree of swelling in STPP and SHMP-anion-crosslinked chitosan microspheres has been studied at pHs 7 and 4, respectively, at a constant temperature (37°C). The degree of swelling in the pure chitosan microspheres (CH-0) is high (399 wt %) in comparison with the microspheres prepared with the phosphate anion crosslinkers (Table I and Fig. 4). The pure chitosan microspheres show a decreasing trend in the degree of swelling after 20 h, which has been attributed to the degradation of the chitosan

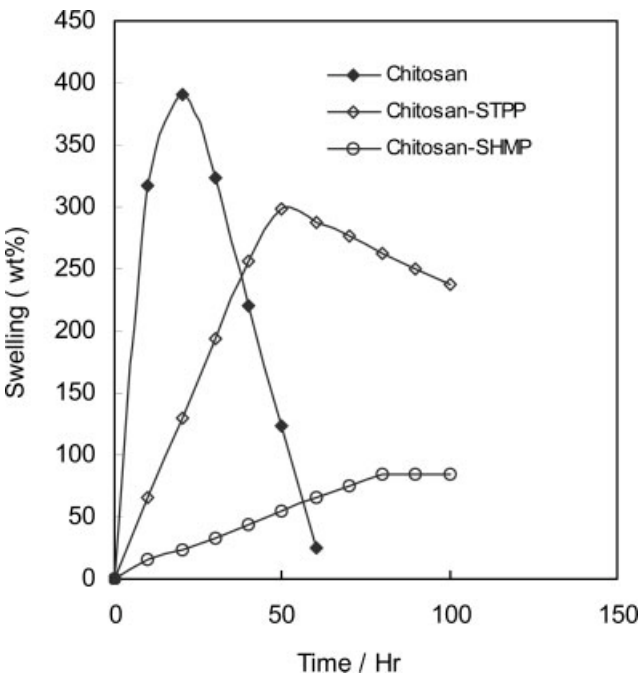


Figure 4 Degree of swelling in (A) pure chitosan, (B) STPP-anion-crosslinked chitosan microspheres, and (C) SHMP-anion-crosslinked chitosan microspheres.

microspheres. The swelling in the microspheres decreases with an increasing degree of crosslinking in chitosan microspheres prepared with different concentrations of the phosphate anion crosslinkers (Table I). However, the swelling is low in the SHMP-anion-crosslinked chitosan microspheres in comparison with the microspheres prepared with the STPP anion crosslinker (Table I and Fig. 4), and this is due to the difference in the electrostatic interactions in the chitosan microspheres prepared with the phosphate anion crosslinkers. The SHMP-anion-crosslinked chitosan microspheres have strong electrostatic interactions in comparison with the STPP-anion-crosslinked chitosan microspheres. These characteristics of the crosslinkers ultimately control the solution degradability of the microspheres, as is clear from the data shown in Table I and Figure 4. The microspheres prepared with the SHMP anion crosslinker are more stable and show a slow rate of swelling in comparison with the STPP-anion-crosslinked chitosan microspheres (Fig. 4). The SHMP-anion-crosslinked chitosan microspheres are stable even after achieving the maximum degree of swelling. The trend of swelling in the crosslinked microspheres controls the trend of drug release from the chitosan microspheres. The encapsulated drug from the microspheres is released either by a diffusion process or by a combination of diffusion and degradation of the chitosan matrices. However, to obtain a controlled and sustained delivery system for a reasonable period of drug release, the degradation of the delivery systems must be slow, and the drug must be released by a diffusion mechanism. Because electrostatic interactions between chitosan and phosphate anion crosslinkers are pH-dependent, the degree of swelling in these microspheres is more pH-sensitive than that in chemically crosslinked chitosan microspheres. The STPP-anion-crosslinked chitosan microspheres show a greater degree of swelling at a low pH than the SHMP-anion-crosslinked chitosan microspheres, and this is due to the low degree of ionization of the STPP anion crosslinker in comparison with the SHMP anion crosslinker within a pH range of 3–5; hence, the difference in the degrees of swelling in the microspheres in solutions of different pHs is due to the difference in the electrostatic interactions in the phosphate-anion-crosslinked chitosan microspheres. In addition to these factors, the degree of swelling in chitosan microspheres is also dependent on the types of ionic additives used in the fabrication of the microspheres. These ionic species modify the electrostatic interactions between the chitosan molecules and phosphate anion crosslinkers and increase the degree of swelling in the presence of anionic additives (CO_3^{2-} and PO^{3-}) but reduce the degree of swelling when cationic additives (Na^+ and Fe^{3+}) are used in the fabrication of the phosphate-anion-crosslinked chitosan microspheres. However, the charge densities on added ionic species also play a significant role in the degree of swelling in chitosan microspheres.

Loading of drugs on the phosphate-anion-crosslinked chitosan microspheres

The anticancer drugs hydroxy urea and antituberculosis rifampicin were loaded onto chitosan microspheres crosslinked with different concentrations of the STPP (Table II) and SHMP anion crosslinkers (Table III). The hydroxy urea and rifampicin on the STPP-anion-crosslinked chitosan microspheres (CHPP) were loaded at pH 4 (Table II), whereas the loading of these drugs on the SHMP-anion-crosslinked chitosan microspheres (CHMP) was carried out at pH 3 because of the difference in the degree of swelling in these crosslinked microspheres (Table

TABLE II
Loading and Release Characteristics of Hydroxy Urea and Rifampicin in STPP-Anion-Crosslinked Chitosan Microspheres

		Drug release (pH 7)		
Drug-loaded microsphere	Maximum loading (wt %)	Burst release (wt %)	Control release (wt %)	Diffusion constant (10^{-12} cm^2/s)
Hydroxy urea				
CHPP-1	24.0	58.3	38.4	29.396
CHPP-2	33.0	42.0	55.4	13.443
CHPP-3	41.5	50.7	49.0	2.171
CHPP-4	48.5	55.6	42.4	0.955
Rifampicin				
CHPP-1	40.0	33.2	65.6	6.491
CHPP-2	43.0	38.5	60.5	2.447
CHPP-3	38.5	55.9	41.7	0.925
CHPP-4	35.0	68.5	30.4	0.466

The molecular weight of CH was 1134 kg/mol, and DDA was 75 wt %. The temperature was 37°C, and the pH was 4 (loading).

TABLE III
Loading and Release Characteristics of Hydroxy Urea and Rifampicin in SHMP-Anion-Crosslinked Chitosan Microspheres

Drug-loaded microsphere	Maximum loading (wt %)	Drug release (pH 4)		Diffusion constant (10^{-12} cm^2/s)
		Burst release (wt %)	Controlled release (wt %)	
Hydroxy urea				
CHMP-1	50.0	51.7	48.0	7.503
CHMP-2	58.0	40.1	59.4	2.627
CHMP-3	66.0	31.5	67.8	0.502
CHMP-4	53.0	43.8	55.6	0.213
Rifampicin				
CHMP-1	32.0	46.6	52.4	1.498
CHMP-2	31.5	58.0	40.8	0.728
CHMP-3	25.0	68.5	30.0	0.324
CHMP-4	19.5	81.8	16.6	0.183

The molecular weight of CH was 1134 kg/mol, and DDA was 75 wt %. The temperature was 37°C, and the pH was 3 (loading).

III). Hydroxy urea and rifampicin show an increasing trend in their loading on the chitosan microspheres prepared with high concentrations of the phosphate anion crosslinkers, but when the concentration of the crosslinker increases beyond a certain concentration, the maximum loading of the drug in these microspheres decreases (Tables II and III). The loading of drugs in chitosan microspheres has been found to be crosslinker-specific; therefore, the overall loading of rifampicin (the maximum loading) is high with STPP-anion-crosslinked chitosan microspheres (Table II) in comparison with SHMP-anion-crosslinked chitosan microspheres (Table III). However, hydroxy urea shows the opposite trend for the maximum loading (Tables II and III). In addition to the swelling properties of the microspheres, the sizes and charge densities of the loaded drugs also play significant roles in the loading and release of the drugs from these microspheres. Hydroxy urea and rifampicin are ionic drugs, and hydroxy urea is smaller in size than rifampicin, so the loading of hydroxy urea in the SHMP-anion-crosslinked chitosan microspheres (CHMP) is high (Table III). Rifampicin is negatively charged and larger in size, so it has more affinity for loading in the STPP-anion-crosslinked chitosan microspheres (Table II). The microspheres with 4 wt % STPP anion crosslinker (CHPP-2) show a maximum loading (43 wt %) for rifampicin (Table II), whereas a maximum loading of hydroxy urea (66 wt %) can be found in the microspheres (CHMP-3) prepared with a 6 wt % concentration of the SHMP anion crosslinker (Table III). Because these drugs are ionic, they influence the chitosan and phosphate anion interactions. Hydroxy urea is less acidic ($pK_a = 10.56$) and is soluble within a pH range of 1–10. The loading of hydroxy urea is low in microspheres with cations (Na^+ and Fe^{3+}) and is high in microspheres with anions (CO_3^{2-} and PO_4^{3-}), and this is due to the difference in the electrostatic forces of repulsion between cations and hydroxy urea and the electrostatic forces of attraction between hydroxy urea and anions in the microspheres. This is the reason for the variation in the loadings of hydroxy urea and rifampicin in microspheres prepared with different concentrations of the STPP and SHMP anion crosslinkers (Tables II and III). The loading of hydroxy urea increases with an increasing concentration of the anion crosslinkers, whereas the loading of rifampicin decreases with an increasing concentration of the phosphate anion crosslinkers. However, the effect of the electrostatic forces of repulsion and attraction between the crosslinkers and drugs is insignificant at high concentrations of the crosslinkers, as is clear from the loading trend observed for hydroxy urea and rifampicin (Tables II and III).

IR and thermal characterization of the drug-loaded, phosphate-anion-crosslinked chitosan microspheres

The FTIR spectra of rifampicin [Fig. 5(A)], hydroxy urea [Fig. 6(A)], and phosphate-anion-crosslinked chitosan microspheres [Fig. 5(B) and Fig. 6(B)] have been recorded and used to verify the loadings of rifampicin [Fig. 5(C)] and hydroxy urea [Fig. 6(C)] on the phosphate-anion-crosslinked chitosan microspheres (CHPP-2 and CHMP-3). The STPP-anion-crosslinked chitosan microspheres after loading with rifampicin show a characteristic peak at 1247 cm^{-1} [Fig. 5(C)], which is initially absent in the STPP-anion-crosslinked chitosan microspheres [Fig. 5(B)]. The absorption peak can be observed at 1253 cm^{-1} in the IR spectrum of pure rifampicin [Fig. 5(A)] and corresponds to —NC— and —CNC— functional groups in pure rifampicin. The rifampicin-loaded

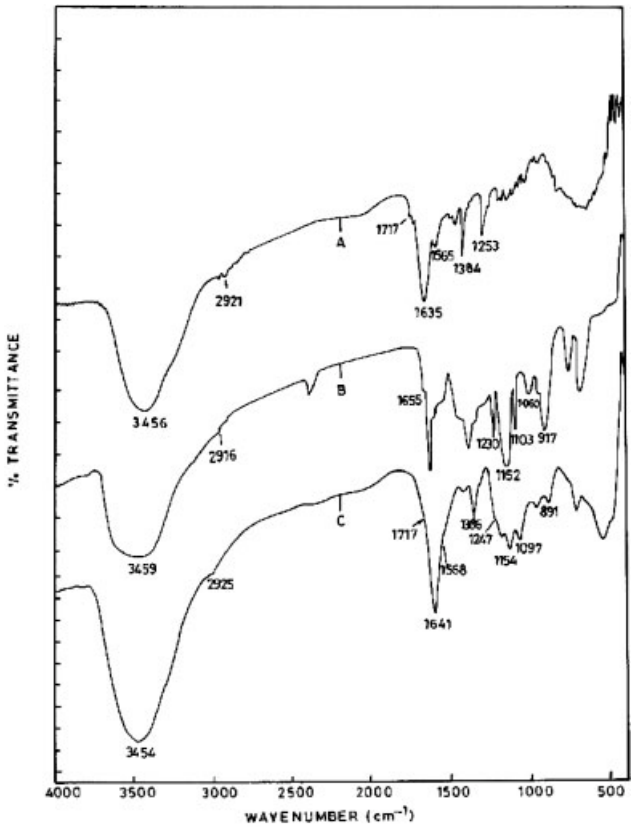


Figure 5 FTIR spectra of (A) rifampicin, (B) STPP-anion-crosslinked chitosan microspheres, and (C) rifampicin-loaded, STPP-anion-crosslinked chitosan microspheres.

STPP-anion-crosslinked chitosan microspheres also show a characteristic absorption band of rifampicin at 1386 cm^{-1} [Fig. 5(C)], which is initially absent in the IR spectrum of the unloaded microspheres [Fig. 5(B)] but is present at 1384 cm^{-1} in the IR spectrum of pure rifampicin [Fig. 5(A)]. The appearance of characteristic peaks in the IR spectrum of rifampicin-loaded microspheres has confirmed the loading of rifampicin on crosslinked microspheres. Similarly, the IR spectrum of hydroxy urea loaded, SHMP-anion-crosslinked chitosan microspheres [Fig. 6(C)] shows the characteristic absorption band of hydroxy urea at 1649 cm^{-1}, which is absent in the IR spectrum of the unloaded microspheres [Fig. 6(B)] but can be observed at 1660 cm^{-1} in the IR spectrum of pure hydroxy urea [Fig. 6(A)]. The presence of the characteristic absorption band of hydroxy urea in the IR spectrum of hydroxy urea loaded microspheres again provides support for the loading of hydroxy urea on SHMP-anion-crosslinked chitosan microspheres.

The thermal stability of sodium phosphate anion crosslinked chitosan microspheres (CHPP-2 and CHMP-3) has been studied by the recording of thermogravimetric and differential thermogravimetric thermograms and compared with thermograms of pure chitosan microspheres (CH-0) as well as microspheres loaded with rifampicin (CHPP-2-REF) and hydroxy urea (CHMP-3-HU) for the variation in the weight loss at different temperatures (Table IV). The temperature of the maximum weight loss increases with the crosslinking of the chitosan microspheres with the STPP anion crosslinker (CHPP-2) and with the SHMP anion crosslinker (CHMP-3), as is clear from the data shown in Table IV. The crosslinking in the microspheres also increases the thermal stability, as is clear from the energy of activation for the decomposition of pure chitosan microspheres (94.3 kJ/mol) and phosphate-anion-crosslinked microspheres (181.9 and 192.3 kJ/mol). The loading of rifampicin on STPP-anion-crosslinked chitosan increases the thermal stability, whereas the loading of hydroxy urea on SHMP-anion-crosslinked chitosan microspheres reduces the thermal stability (Table IV). The variation in the degree of decomposition of the drug-loaded microspheres is due to the variation in the degree of free and bound water[42,43] and due to the variations in the interactions of the loaded drugs with the crosslinked chitosan microspheres. Although the

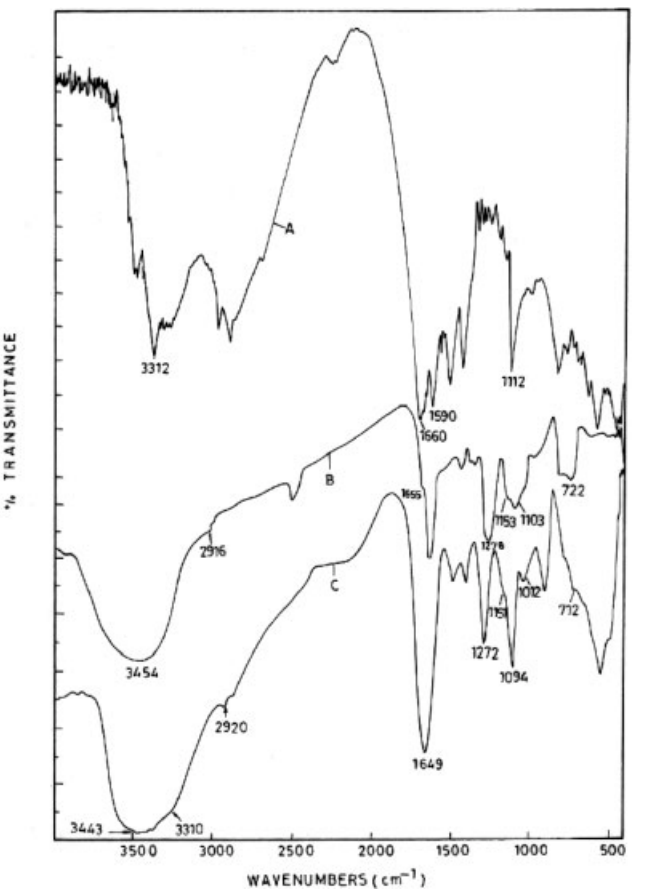


Figure 6 FTIR spectra of (A) hydroxy urea, (B) SHMP-anion-crosslinked chitosan microspheres, and (C) hydroxy urea loaded, SHMP-anion-crosslinked chitosan microspheres.

TABLE IV
Thermal Behavior of SHMP- and STPP-Anion-Crosslinked and Drug-Loaded Chitosan Microspheres

Microsphere	Temperature of maximum weight loss (°C)	Weight loss (wt %) 100°C	Weight loss (wt %) 200°C	Maximum weight loss at 800°C (wt %)	Energy of activation (kJ/mol)
CH-0	172.0	12.0	16.5	86.4	94.3
CHPP-2	193.0	11.7	14.7	71.4	181.9
CHPP-2-REF	264.5	4.3	5.5	62.0	250.5
CHMP-3	212.0	4.0	4.8	54.6	192.3
CHMP-3-HU	175.0	7.1	10.6	52.2	154.7

The molecular weight of CH was 1134 kg/mol, and DDA was 75 wt %.

thermal stability of the SHMP-anion-crosslinked chitosan microspheres upon the loading of hydroxy urea is low (154.7 kJ/mol) in comparison with that of the STPP-anion-crosslinked chitosan microspheres (181.9 kJ/mol), the SHMP-anion-crosslinked chitosan microspheres are more stable (192.3 kJ/mol) than the unloaded STPP-anion-crosslinked chitosan microspheres (181.9 kJ/mol), and this is due to the high degree of crosslinking with the SHMP-anion-crosslinked chitosan microspheres (Table IV).

Drug release from the phosphate-anion-crosslinked chitosan microspheres

The release behavior of drugs from crosslinked microspheres depends on the degree of crosslinking and the extent of the drug loading on the microspheres. The loading trends clearly indicate that rifampicin is loaded more in the STPP-anion-crosslinked (CHPP) microspheres (Table II) and hydroxy urea is loaded more in the SHMP-anion-crosslinked (CHMP) chitosan microspheres (Table III). The release behavior of hydroxy urea and rifampicin from the phosphate-anion-crosslinked chitosan microspheres (CHPP and CHMP) has been analyzed and clearly indicates that the release of hydroxy urea from the STPP-anion-crosslinked microspheres (CHPP) is poor (Table II) because most of the loaded hydroxy urea is released in the burst step of drug release rather than in the controlled-release step, as is clear from the trends shown in Table II. The diffusion constant for the release of hydroxy urea from the STPP-anion-crosslinked microspheres (CHPP) is high, and this is due to the weak interactions of hydroxy urea with the STPP-anion-crosslinked microspheres (Table II). Similarly, the release behavior of rifampicin from the SHMP-anion-crosslinked chitosan microspheres (CHMP) has been examined (Table III), and it is clear that the maximum amount of rifampicin is burst-released in comparison with the amount of rifampicin released in a controlled manner (Table III). These observations clearly suggest that the release behavior of drugs varies with the type of crosslinker and the polymer–drug interactions. To explain these observations, the release behavior of rifampicin and hydroxy urea from phosphate-anion-crosslinked chitosan microspheres (CHPP and CHMP) has been studied as a function of time (Figs. 7 and 8), and the cumulative amounts of rifampicin and hydroxy urea released in the burst-release and controlled-release steps of drug release are shown in Tables II and III. The initial burst release and controlled release of rifampicin (Table II and Fig. 7) and hydroxy urea (Table III and Fig. 8) vary (Figs. 7 and 8) with the concentration of the phosphate anion crosslinkers in the microspheres. The microspheres with a 4 wt % concentration of the STPP anion crosslinker (CHPP-2) show better controlled release of rifampicin (60.5 wt %)

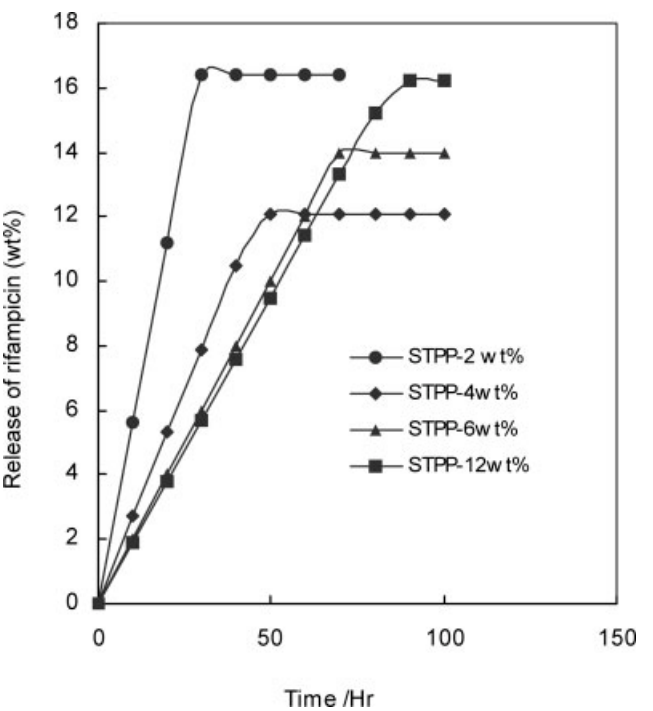


Figure 7 Release of rifampicin from chitosan microspheres with different concentrations of the STPP anion crosslinker.

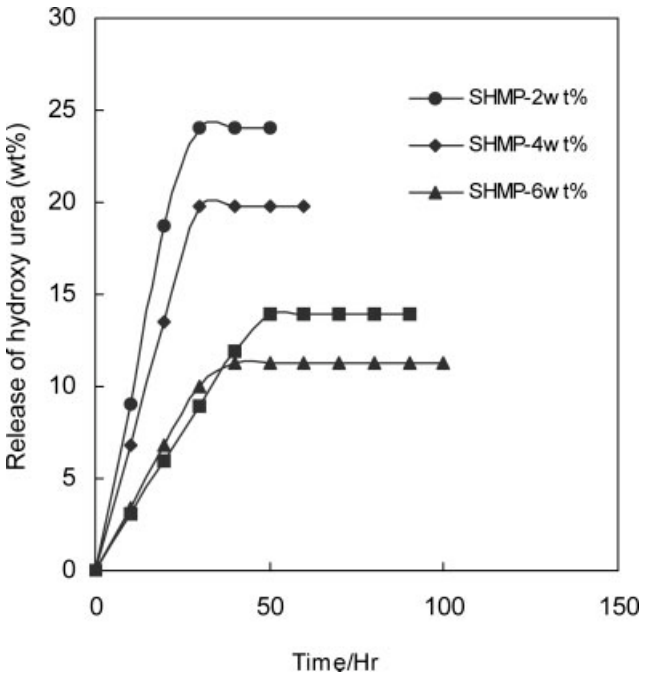


Figure 8 Release of hydroxy urea from chitosan microspheres with different concentrations of the SHMP anion crosslinker.

within a period of 50 h than microspheres prepared with other concentrations of the crosslinker (Table II and Fig. 7). Although microspheres with a 2 wt % concentration of the STPP anion crosslinker (CHPP-2) release more rifampicin in a controlled manner (65.6 wt %), the time of controlled release is shorter (40 h) in comparison with that of microspheres with a 4 wt % concentration of the STPP anion crosslinker (Table II and Fig. 7); hence, microspheres with a 4 wt % concentration of the STPP anion crosslinker are more useful for prolonging and sustaining the delivery of rifampicin. The microspheres with high concentrations (greater than the weight percentage) and low concentrations (lower than the weight percentage) of the STPP anion crosslinker are not suitable because these microspheres either release more rifampicin in a burst-release manner or release rifampicin in a controlled manner for shorter times (Table II and Fig. 7). This is due to the difference in the degree of crosslinking, which influences significantly the degree of swelling and drug diffusion from the microspheres. The release behavior of hydroxy urea from chitosan microspheres (CHMP) with different concentrations of the SHMP anion crosslinker has also been analyzed (Table III and Fig. 8) to compare the composition of the microspheres for the optimized release of hydroxy urea with the composition of the microspheres optimized for the release of rifampicin. When the concentration of the SHMP anion crosslinker is increased from 2 (CHMP-1) to 6 wt % (CHMP-3), the initial burst release of hydroxy urea shows a decreasing trend, whereas the controlled release of hydroxy urea shows a continuously increasing trend. The microspheres with a 6 wt % concentration of the SHMP anion crosslinker (CHMP-3) show the maximum controlled release of hydroxy urea (67.8 wt %) within a period of 60 h and show the minimum burst release of hydroxy urea (31.5 wt %) within a period of 40 h (Table III and Fig. 8). However, when the concentration of the crosslinker is further increased (>6 wt %), the controlled release of hydroxy urea decreases to 56.6 wt %, and the burst release of hydroxy urea increases to 43.8 wt % (Table III and Fig. 8). The high concentration of the SHMP anion crosslinker (6 wt %) for the controlled release of hydroxy urea (Table III and Fig. 8) is due to the smaller size of hydroxy urea in comparison with rifampicin, which requires a low crosslink molecular weight in the microspheres; otherwise, the release of hydroxy urea is faster because of the high crosslink molecular weight in the microspheres if a low concentration of the SHMP anion crosslinker (<6 wt %) is used. The microspheres at a high concentration of the SHMP anion crosslinker (>6 wt %) release the maximum amount of hydroxy urea in a burst-release manner because of a decrease in the rate of swelling in these microspheres. Because rifampicin is larger in size than hydroxy urea,

TABLE V
Release of Hydroxy Urea and Rifampicin in Solutions of Different pHs from Optimized Microspheres of STPP and SHMP Anion Crosslinkers

	Release of hydroxy urea from CHMP-3 microspheres			Release of rifampicin from CHPP-2 microspheres		
Solution pH	Burst release (wt %)	Controlled release (wt %)	Diffusion constant (10^{-12} cm^2/s)	Burst release (wt %)	Controlled release (wt %)	Diffusion constant (10^{-12} cm^2/s)
1	51.6	48.0	1.836	61.5	37.9	25.054
3	40.2	58.8	1.363	50.0	49.2	12.203
4	31.5	67.8	0.366	43.0	56.4	7.351
5	47.2	52.0	0.272	40.6	58.5	4.570
7	69.5	29.2	0.238	38.5	60.5	1.982
8	82.3	16.2	0.229	69.2	29.4	1.316

The molecular weight of CH was 1134 kg/mol, and DDA was 75 wt %. The temperature was 37°C.

TABLE VI
Release of Hydroxy Urea and Rifampicin from STPP- and SHMP-Anion-Crosslinked Microspheres with Ionic Additives

	Release of hydroxy urea from CHMP-3 microspheres			Release of rifampicin from CHPP-2 microspheres		
Ion (0.2 wt %)	Burst release (wt %)	Controlled release (wt %)	Diffusion constant (10^{-12} cm^2/s)	Burst release (wt %)	Controlled release (wt %)	Diffusion constant (10^{-12} cm^2/s)
Blank	31.5	67.8	0.502	38.5	60.5	2.447
Na^+	34.7	64.0	0.405	24.9	73.2	1.764
Fe^{3+}	41.6	57.4	0.212	33.7	64.8	0.729
CO_3^{2-}	50.0	48.6	4.340	43.0	56.0	14.522
PO_4^{3-}	59.5	39.5	18.286	49.6	49.6	45.409

The molecular weight of CH was 1134 kg/mol, and DDA was 75 wt %. The temperature was 37°C.

microspheres with a low concentration of the STPP anion crosslinker are suitable for the controlled release of rifampicin (Table II and Fig. 7).

Effect of the solution pH and additives on drug release from the microspheres

To analyze the effect of the solution pH on the release behavior of rifampicin and hydroxy urea, microspheres with optimized concentrations of the STPP anion crosslinker (CHPP-2) and SHMP anion crosslinker (CHMP-3) were used in solutions of different pHs ranging from 1 to 8 (Table V). The drug-release behavior of physically crosslinked delivery systems depends on the degree of swelling, which is ultimately controlled by the degree ionization of the crosslinkers in the polymers. The drug-release behavior of physically crosslinked delivery systems in solutions of different pHs is also influenced by the ionic state of the drugs in the crosslinked microspheres when placed in release media of different pHs. At low pHs, hydroxy urea and rifampicin are present in a protonated form like chitosan and interact electrostatically with phosphate anions of the crosslinkers in the microspheres. However, the electrostatic interactions between the phosphate anion crosslinkers and protonated drugs in solutions of different pHs depend on the degree of ionization of the phosphate anion crosslinkers in the chitosan microspheres. The STPP anion crosslinker is ionized more at a high pH than the SHMP anion crosslinker; hence, STPP-anion-crosslinked microspheres (CHPP-2) release rifampicin in a burst-release manner at a low pH but show the maximum release in a controlled manner (60.5 wt %) at pH 7 (Table V). At a high pH (>7), rifampicin is mainly released (69.7 wt %) in a burst manner (Table V). This variation in the release behavior of rifampicin from STPP-anion-crosslinked microspheres is due to the variation in the degree of ionization of the STPP anion crosslinker and chitosan, which controls the electrostatic interactions and degree of swelling in the microspheres, which are optimum at pH 7 for the controlled release of rifampicin (60.5 wt %). The SHMP anion crosslinker is ionized more at a low pH; hence, these microspheres show more controlled release (67. 8 wt %) of hydroxy urea at pH 4, but at a high pH (>4), the release of hydroxy urea in a controlled manner is reduced (Table V). A comparison of the drug-release behavior of hydroxy urea and rifampicin from phosphate-anion-crosslinked microspheres has clearly indicated that microspheres crosslinked with the SHMP anion crosslinker

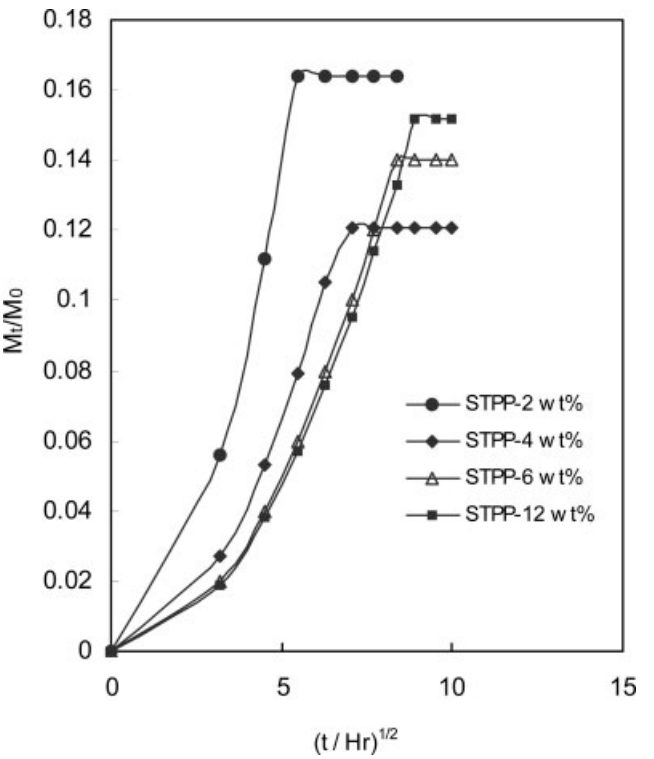


Figure 9 Fractional release of rifampicin from chitosan microspheres with different concentrations of the STPP anion crosslinker.

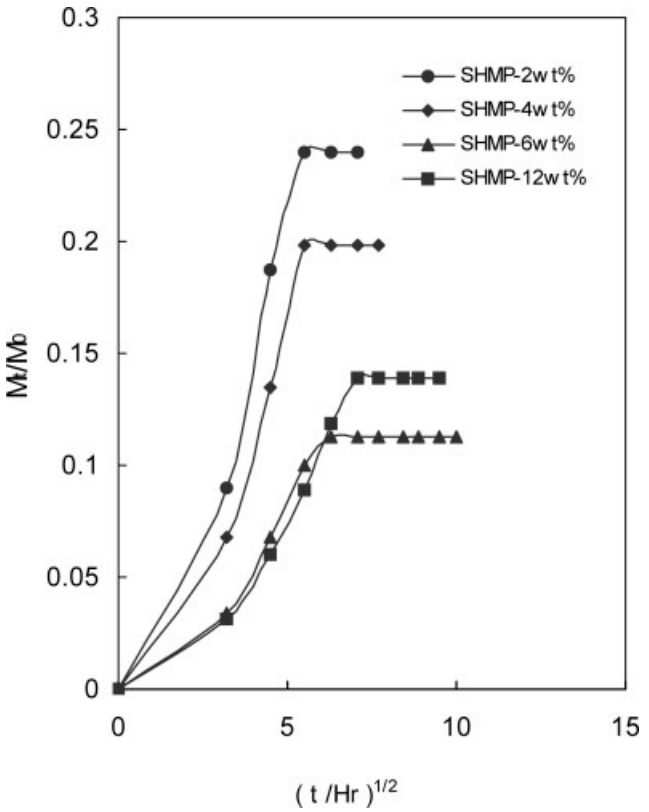


Figure 10 Fractional release of hydroxy urea from chitosan microspheres with different concentrations of the SHMP anion crosslinker.

(CHMP-3) show better controlled release of hydroxy urea at low pHs than STPP-anion-crosslinked chitosan microspheres (CHPP-2), which show better controlled release of rifampicin at pH 7 (Table V). The addition of anionic salts shows a decreasing trend for the controlled release of hydroxy urea and rifampicin from phosphate-anion-crosslinked chitosan microspheres (Table VI) but the addition of cations shows an increasing effect on the controlled release of rifampicin from STPP-anion-crosslinked microspheres and shows a decreasing effect on the controlled release of hydroxy urea from SHMP-anion-crosslinked microspheres (Table VI). The variation is due to the increased electrostatic interactions in STPP-anion-crosslinked chitosan microspheres in comparison with SHMP-anion-crosslinked chitosan microspheres in the presence of cations.

Release kinetics of drugs from the phosphate-anion-crosslinked microspheres

The fractional release of drugs as a function of the square root of time has been used to analyze the release mechanism and order of drug release from phosphate-anion-crosslinked chitosan microspheres prepared with different concentrations of the crosslinkers (Figs. 9 and 10). The initial fractional release (M_t/M_0) of hydroxy urea and rifampicin from crosslinked microspheres varies linearly as a function of the square root of the release time and is indicative of Fickian behavior for drug release.[44] However, the time and amount of the drug released by a Fickian mechanism depend on the type and concentration of the crosslinker used in the microspheres (Figs. 9 and 10). The microspheres with a 4 wt % concentration of the STPP anion crosslinker show low Fickian release of rifampicin (38.5 wt %) in comparison with microspheres prepared at other concentrations of the STPP anion crosslinker (Table II and Fig. 9). The chitosan microspheres with 6 wt % hexametaphosphate anion crosslinker show low Fickian release of hydroxy urea in comparison with microspheres obtained at other concentrations of the SHMP anion crosslinker (Table III and Fig. 10). The initial fractional release of rifampicin and hydroxy urea follows first-order kinetics, but after equilibrium swelling in phosphate-anion-crosslinked microspheres, the fractional release of rifampicin and hydroxy urea is non-Fickian and follows zero-order kinetics[45] (Figs. 9 and 10). The initial drug release has been used to determine the diffusion constant for encapsulated drugs from phosphate-anion-crosslinked chitosan microspheres (Tables II and III). The value of the diffusion constant for drug release from microspheres in solutions of different pHs and from microspheres with different ionic additives has been determined (Tables V and VI). The diffusion constant for the drugs also varies with the concentration and type of crosslinker (Tables II and III). When the concentration of phosphate anion crosslinkers is increased, the diffusion constant decreases because of the increase in the degree of crosslinking. The diffusion constant for the release of hydroxy urea from the SHMP-anion-crosslinked chitosan microspheres is low in comparison with the diffusion constant observed for the release of rifampicin from the STPP-anion-crosslinked microspheres (Tables II and III and Figs. 9 and 10) These variations in the diffusion constant clearly indicate that the SHMP-anion-crosslinked microspheres are highly crosslinked and more compact than the STPP-anion-crosslinked microspheres.

CONCLUSIONS

The controlled-release characteristics of rifampicin and hydroxy urea from STPP- and SHMP-anion-crosslinked chitosan microspheres have been evaluated with chitosan with a constant DDA (75 wt %) and molecular weight (1134 kg/mol). The microspheres prepared with the SHMP anion crosslinker are smaller in size and compact in comparison with the STPP-anion-crosslinked microspheres; hence, the SHMP-anion-crosslinked microspheres show significant controlled release of loaded hydroxy urea in

comparison with rifampicin, whereas the STPP-anion-crosslinked chitosan microspheres are larger in size and are suitable for the controlled release of rifampicin. The loading and release characteristics of the microspheres have also been determined as functions of the degree of crosslinking and the solution pH. The variation in the chitosan phosphate anion interactions with the solution pH has been explained in terms of the degree of ionization of the chitosan and phosphate anion crosslinkers. The microspheres have also been characterized by IR and thermal methods to provide proof for the loading of drugs and to explain the thermal stability of drug-loaded microspheres. Drug release from the microspheres has taken place in Fickian and non-Fickian manners, and encapsulated drugs have shown mixed-release kinetics.

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