Chitosan and Chitosan–PEO Blend Membranes Crosslinked by Genipin for Drug Release

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ABSTRACT: Crosslinked chitosan and chitosan/poly(ethylene oxide) (PEO) blend membranes were prepared using the solution casting technique for eleutherococcus sentisocus (ES) and vitamin B_{12} release. A naturally occurring and nontoxic crosslinking agent, genipin, was used to form the chitosan and chitosan/PEO blend networks. The permeability of vitamin B_{12} through the membranes was investigated. The effect of crosslink density of the membrane on the drug release behavior was assessed. The drug release rate decreased with increasing crosslink density, and was very sensitive to crosslink density at low crosslink densities. PEO was introduced into the membrane to adjust the mesh size of the membrane and to control the drug release rate by PEO molecular weight and amount used. The drug release rate was sensitive to medium pH. For ES release from crosslinked chitosan carriers, the release rate was controlled by both the mesh size of the membrane and the molecular interactions between chitosan and ES. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 436–444, 2006

Key words: drug release; chitosan; membrane; small-angle X-ray scattering

INTRODUCTION

Controlled drug release technology emerged during the 1980s as a commercially sound methodology of extending existing ways of administering pharmaceutical therapies.[1] Conventional dosage forms often lead to wide swings in serum drug concentrations.[2] The safety and therapeutic efficacy of current treatments may be improved if their delivery rate, biodegradation, and site-specific targeting can be predicted, monitored, and controlled.

In recent years, considerable research efforts have been directed towards the development of safe and efficient drug delivery systems with the use of polymers as agents for the controlled release of drugs from various types of formulated products, such as tablets, implants, and adhesive strips. Evidence of the high degree of interest in the design of such dosage forms is provided by number of reviews[3–5]and books[6–8] that has been concerned with these subjects. The release of drugs, absorbed or encapsulated by polymer, involves their slow and controlled diffusion from or through polymeric material. Drugs covalently attached to biodegradable polymers or dispersed in a polymeric matrix of such macromolecules may be released by erosion or degradation of the polymer. Therapeutic molecules, complexed by polymers, may also be released from gels by diffusion.

Chitosan with excellent biodegradable and biocompatible characteristics is a naturally occurring polysaccharide. Because of its unique polymeric cationic character, gel and film-forming properties, chitosan can be a suitable matrix, available in different forms, for sustained release of various drug formulations. Up to now, conscious of the limiting effects of the poor mechanical properties of chitosan upon its application, drug delivery formulations based on chitosan (such as films, beads, microspheres, etc.) have been prepared by chemical crosslinking with glutaraldehyde, etc.[9–11] However, the chemical crosslinking agents may induce toxicity and other undesirable effects, for example, chemically synthesized glutaraldehyde can cause irritation to mucosal membranes due to its toxicity.[12,13]

In our previous work,[14] we have already reported that the chitosan and chitosan/poly(ethylene oxide) (PEO) blend films crosslinked by genipin are pH-sensitive and have desirable mechanical properties. These characteristics and film's nonpoisonous nature[15] make it ideal for development as a controlled-release drug delivery system. In the present study, the potential for such development was investigated. Vitamin B_{12} and eleutherococcus sentisocus (ES) were used as drug models.

ES is an herbal medicine. In recent years, there has been a marked revival in the use of herbal medicine for the treatment of a wide range of ailments.[16] ES has

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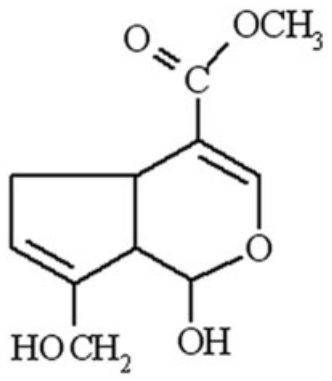

Figure 1 Schematic of the chemical structure of genipin.

been widely used in a number of countries, including People's Republic of China, Japan, Korea, Russia, and USA for treating heart disease, diabetes, and blood circulatory problems.[17] For such treatments, controlled ES release may be particularly important to improve therapeutic efficacy and safety. In this report, we mainly study the effects of crosslink density and the mesh size of the membrane, and molecular interactions between the drug carrier and the drug on the drug release rate.

EXPERIMENTAL

Materials

Chitosan was purchased from Fluka, UK. The molecular weight of chitosan was measured by means of gel permeation chromatography. $M_w = 3.0 \times 10^5$, $M_n = 5.8 \times 10^4$, and $M_w/M_n = 5.5$. The degree of deacetylation of the chitosan was 85%, which was determined by means of Fourier transform infrared spectroscopy (FTIR). PEO with molecular weight of 20,000 g/mol (HPEO) was obtained from BDH Laboratory Supplies, UK. Poly(ethylene glycol) powder with viscosity average molecular weight of 600 g/mol (LPEO) was obtained from the Aldrich Chemical, UK. Genipin was obtained from Challenge Bioproducts, Taiwan. Its chemical structure is shown in Figure 1. Vitamin B_{12} (cyanocobalamin, ~98%) was obtained from Fluka Chemical, UK. ES was kindly provided by the Institute of Chinese Medicine, People's Republic of China. Acetic acid and phosphate-buffered saline (PBS) were obtained from Aldrich Chemicals, UK. All chemicals were used without further purification.

Preparation of chitosan and chitosan–PEO membranes crosslinked by genipin

Chitosan was dissolved in 1 wt % aqueous acetic acid at room temperature overnight to obtain a concentration of 0.5 wt %/vol %. The viscous chitosan solution was filtered through filter paper to remove any undissolved gel. The clear, lightly yellow chitosan solution was then mixed with a 5% aqueous solution of PEO in different weight percentage ratios and the mixtures were stirred overnight at room temperature. The various wt % of genipin (genipin/chitosan) was dissolved in 3 mL of water and then was added to the chitosan solutions or to the chitosan/PEO mixed solutions under stirring for 30 min at room temperature. After 2 h, the solutions started to turn light blue and became increasingly viscous. The solutions were then immediately cast on glass plates. The crosslinked chitosan and chitosan/PEO blends become dark blue after 1 day. The crosslinked structure of chitosan by genipin can be found in Ref. 18. The thicknesses of the dried films were measured to be 30–60 μm. For all experimental studies, the samples were first treated to remove the residual acetic acid.

Determination of crosslink density of chitosan film

Crosslink density of polymer is defined as following:

$$\rho_c = \frac{1}{M_c} \tag{1}$$

where M_c is the number average molecular weight between crosslinks.

The crosslink density of chitosan was determined by using swelling measurements. The Flory-Rehner equation was used to calculate M_c.[19] The equation is

$$-[\ln(1-u_r) + u_r + \chi u_r^2] = \rho V_O M_c^{-1} u_r^{1/3} \tag{2}$$

where χ is the chitosan–water interaction parameter (0.5917),[20] ρ is the density of chitosan (1.25 g/cm^3), V_0 is the molar volume of water (18 cm^3/mol), and u_r is the volume fraction of chitosan in swollen state. u_r was obtained by the swelling experiment, as $W_d \times 1.003/[W_d \times 1.003 + (W_s - W_d) \times 1.25]$, where W_s and W_d are weights of the membranes in the dry and swollen states, respectively. The various calculated crosslinking densities of chitosan membranes are listed in Table I.

Swelling studies

The volume fraction of water, H, in the swollen membrane was determined by swelling measurements at 23°C. The samples (~0.05 g) were first immersed in water. The immersion time was 48 h. It was confirmed that after a 48-h period, the films had reached their swelling equilibrium. The membranes were withdrawn from water and their wet weights were determined after first blotting with a filter paper to remove surface absorbed water. The volume fraction of water was expressed by the following equation.

TABLE I
Permeability and Diffusion Coefficient of Vitamin B_{12} Through Chitosan Membranes at pH 7.4 and 37°C

Sample	Crosslink density (%)	P (cm^2/s)	D (cm^2/s)	K_d
CS	0	7.04×10^{-7}	3.44×10^{-7}	2.045
	0.0686	5.43×10^{-7}	5.99×10^{-7}	0.906
	0.264	3.12×10^{-7}	2.83×10^{-7}	0.908
	0.985	1.29×10^{-7}	1.257×10^{-7}	1.026
	2.227	1.09×10^{-7}	9.90×10^{-8}	1.101
	4.645	9.57×10^{-8}	8.14×10^{-8}	1.176
	9.220	6.75×10^{-8}	4.63×10^{-8}	1.458
	14.350	4.25×10^{-8}	2.39×10^{-8}	1.779

$$H = (W_s - W_d)/W_s \tag{3}$$

where W_d and W_s are the weights of the samples in the dry and swollen states, respectively. Each swelling experiment was repeated five times and the average value was taken.

Permeation studies

The permeation studies were performed using modified diffusion apparatus, which consisted of two cylindrical half-chambers.[21] The chitosan membrane was placed between the compartments. The effective membrane area was 2.83 cm^2. Each compartment was stirred continuously by externally mounted constant-speed motors. The diffusion apparatus was placed in water bath maintained at 37 °C ± 0.1°C. Membranes were swollen to equilibrium in PBS solution at 37°C before they were placed between the two chambers. Vitamin B_{12} was dissolved in PBS solution to make solutions with concentration of 1 mg/mL.

The donor compartment was filled with 20 mL of a 1 mg/mL vitamin B_{12} solution and the receptor side with 20 mL of the same buffer solution used in the donor compartment but without drug. At predetermined time intervals, 2.5 mL samples were taken and the vitamin B_{12} concentration of the sample was determined using an UV spectrometer by measuring the absorbance at 361 nm and then replaced them after measuring. The permeability, P, was calculated from the UV absorbance data by the following equation:[22]

$$\ln\left(1 - 2\frac{C_i}{C_0}\right) = -\frac{2A}{Vl}Pt \tag{4}$$

in which C_t is the vitamin B_{12} concentration in the receptor cell at time t, C_0 is the initial vitamin B_{12} concentration in the donor compartment, A is the membrane surface area, V is the volume of each cell, l is the membrane thickness, and P is the permeability.

The diffusion coefficient, D, of vitamin B_{12} in the various films was evaluated by

$$D = \frac{P}{K_d} \tag{5}$$

in which K_d is the partition coefficient. The partition coefficient can be defined in two ways.[23] The first one is ratio of solute concentration in the membrane to that in the surrounding solution. This can be determined by solute uptake experiments. The membrane previously equilibrated in deionized water was incubated in vitamin B_{12} solution (1 mg/mL, 20 mL) at 37°C. After 48 h, the membrane was taken out from the solution, blotted with a tissue to remove the excess aqueous solution on the membrane surface, and immersed in 20 mL deionized water at 37°C for 48 h. The concentration of vitamin B_{12} released in the solutions (C_2) was determined by UV spectrometer as described earlier. From the known concentration of solute after equilibration with the membrane (C_1), it was possible to estimate the amount of solute absorbed into the swollen membrane. The partition coefficient, K_d, was calculated according to eq. (6)

$$K = \frac{C_2 V}{V_p(C_1 - C_2)} \tag{6}$$

where V and V_p refer to the volume of the buffer and swollen membrane, respectively.

The second one is the ratio of the solute concentration in the membrane standardized by the water volume in the membrane to that in the bulk solution. If we express the former K and later as K', the two partition coefficients are related as follows:

$$K = HK' \tag{7}$$

Preparation of an ES drug carrier

To study loaded drug release from a carrier, sorption and desorption experiments were employed. The membranes, which had previously been kept in equilibrium with water, were immersed in ES drug solution for 2 weeks until they attained equilibrium. The concentration of ES solution was determined by an UV spectrometer at 200 nm. The concentration of ES absorbed by the membrane, C, was calculated by measuring the concentration of ES in the aqueous solution prior to (C_0) and after (C_∞) the sorption experiments, using the expression

$$C = \frac{(C_0 - C_\infty)V}{V_p} \tag{8}$$

V and V_p are the volumes of the aqueous solution and membrane sample, respectively.

In simple sorption and desorption experiments, the solute dissolves and diffuses into the membrane. The fractional mass uptakes are a function of time. The total release can be described according to the following equation:[24]

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{m=0}^{\infty} \frac{1}{(2m+1)^2} \exp\left[- \frac{4D(2m+1)^2\pi^2 t}{l^2} \right] \tag{9}$$

where M_t and M_∞ are the mass release at time t and at infinite time, respectively, l is the membrane thickness, and m is an integer.

At short times it approximates to

$$\frac{M_t}{M_\infty} \approx 4\left(\frac{Dt}{\pi l^2}\right)^{1/2} \tag{10}$$

Small angle X-ray scattering (SAXS) study

Morphology of chitosan/PEO blends and networks were studied by means of SAXS using a the Kratky Compact Small Angle system with a stationary-anode copper-target X-ray tube (wavelength 1.542 Å) at room temperature. The fine-focus X-ray generator was operated at 45 kV and 40 mA. The measured intensity was desmeared and corrected for background scattering and photoelectric absorption in the samples.[25]

RESULTS AND DISCUSSIONS

Vitamin B_{12} release

From the diffusion cell experiments, the permeability, P, of the membranes for vitamin B_{12} was determined using eq. (4). The vitamin B_{12} diffusion coefficient was calculated from the results of the permeability and partition coefficient by using eq. (5). The effects of crosslink density, PEO weight content in crosslinked chitosan/PEO blend, and pH of vitamin B_{12} solution on the B_{12} release behavior were investigated.

Effect of crosslink density on vitamin B_{12} release through chitosan membranes

The permeation of vitamin B_{12} through the crosslinked chitosan membrane with different crosslink density was studied in phosphate buffer (0.1M) of pH.7.4 at 37°C. Table I lists the measured values of permeability, diffusion, and partition coefficients of the membrane with different crosslink densities.

Figure 2 shows the effect of crosslink density on the permeability of vitamin B_{12} through the chitosan membranes. The results shown in Table I and Figure 2

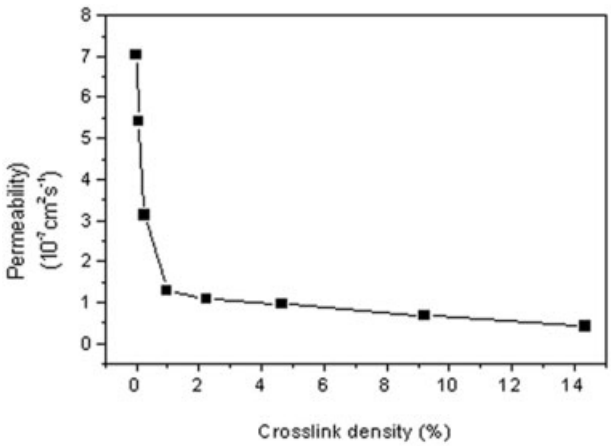

Figure 2 Effects of crosslink density on permeability of vitamin B_{12} through the chitosan membranes at pH 7.4 and 37°C.

indicate that the drug release rate was found to be dependent on the crosslink density. As expected, the permeability decreased with increasing the crosslink density. It can also be clearly observed from Figure 2 that the permeability was particularly sensitive at low degrees of crosslink density, which could provide useful information for controlled drug release.

The permeability and release rate of hydrogels are influenced by the type of solute and the mesh size of the membrane.[2] The mesh size is the space between neighboring chains in the polymer network, and can be considered as an indication for the available space in the membrane for solute diffusion.[26,27] Crosslinking a polymer generally leads to a reduction in permeability to solutes. The permeability reduction may be due to a number of causes, such as interactions between the solute and chains. In the case of hydrogels, the reduction in mesh size by increasing crosslink density is likely to be an important factor. According to the free volume theory,[28] crosslinking a polymer influences the diffusion coefficient through thermodynamics of the polymer–solvent interactions and the specific hole volume of polymer. The addition of chemical crosslinks to a polymer will obviously inhibit the segmental and molecular motion of the chains that arise due to thermal fluctuations. Consequently, the free volume in a crosslinked polymer is expected to be less than that in a noncrosslinked polymer.[28,29] This anticipated behavior is substantiated by the fact that crosslinking increases polymer density. Furthermore, it can be assumed that loss of free volume associated with the formation of crosslinking reduces the hole free volume, which dictates solvent transport.

According to the free volume theory, for hydrogel the permeability and release rate through the membrane are influenced by the crosslink density, which can be directly explained by the degree of equilibrium

swelling achieved by the network under experimental conditions. The effect of crosslink density on the equilibrium of network swelling was studied in our previous work.[14] The reduction in water uptake by the membrane accompanies the increase in crosslinked density.

On the basis of the free volume theory, Yasuda et al.[30,31] have developed the theory for solute diffusion through hydrated polymer membrane. The permeability and release rate are therefore dependent on solute size and the degree of membrane hydration. The normalized solute diffusion coefficient that is the ratio of the solute diffusion coefficient in the swollen gel membrane, D_{gel}, to that in pure water, D_{water}, was expressed as follows:

$$\frac{D_{gel}}{D_{water}} = B(V_d) \exp\left(- \frac{\pi r_s^2 l(1/H - 1)}{V_l} \right) \qquad (11)$$

Here, r_s, is the Stokes hydrodynamic radius, l is the characteristics length, V_l is average free volume of water, and H is the volume fraction of water in the gel membrane. $B(v_d)$, which is constant, denotes the probability of a diffusion species of volume v_d a mesh. The linear dependence in the relation between ln (D_{gel}/D_{water}) and $1/(H - 1)$ is expected by eq. (11).

Reinhart and Peppas investigated the influence of crosslinking on diffusive properties.[32] It was concluded that the term $B(v_d)$ depends upon the size and shape of the mesh formed by the crosslinked chains The linear relationship between ln (D_{gel}/D_{water}) and $1/(H - 1)$ was not existed. Recently, the new analysis equation based on free volume theory for crosslinked poly(vinyl alcohol) was presented by Mastuyama[22] as follows

$$\frac{D_{gel}}{D_{water}} = K \exp\left(- \frac{\pi r^2(1/H - 1)}{V_l} \right) \qquad (12)$$

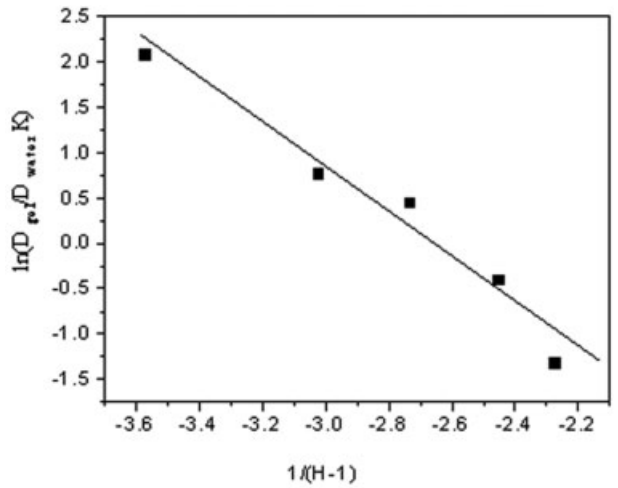

Figure 3 Relation between ln ($D_{gel}/D_{warter}K'$) and $1/(H - 1)$.

TABLE II
Effect of PEO Content on the Permeation of Vitamin B_{12}

Sample	Genipin content (%)	P (cm^2/s)	D (cm^2/s)	K_d
CS	0	7.04×10^{-7}	3.44×10^{-7}	2.045
CS/LPEO10	0.5	4.06×10^{-7}	6.33×10^{-7}	0.641
CS/LPEO20		9.81×10^{-7}	1.56×10^{-6}	0.652
CS/LPEO30		1.17×10^{-6}	1.2×10^{-6}	0.981
CS/HPEO10		1.96×10^{-6}	2.89×10^{-6}	0.678
CS/HPEO20		3.86×10^{-6}	5.07×10^{-6}	0.761
CS/HPEO30		8.56×10^{-6}	1.06×10^{-5}	0.808

In this equation, the partition coefficient was introduced as the probability of a diffusing species finding a mesh. Figure 3 gives the plot of ln ($D_{gel}/D_{water}K'$) against $1/(H - 1)$ for crosslinked chitosan membrane. The linear relationship was obtained. Therefore, eq. (12) is suitable for treating our results.

Effect of PEO content in crosslinked chitosan/PEO blends on vitamin B_{12} release

The improvement in the mechanical properties of chitosan film was studied with adding PEO, and it was found that the degree of water wettability increased with increasing PEO content.[14] In this study, the effect of the PEO content in membranes on the permeation of vitamin B_{12} was examined. Table II shows experimental results.

Figure 4 shows that the permeability of vitamin B_{12} through the membranes prepared by blending chitosan with PEO was much higher than through the chitosan membrane alone. The permeability of vitamin B_{12}, for instance, increased with increasing weight ratio of PEO in crosslinked chitosan/PEO membranes. It was observed from a comparison between

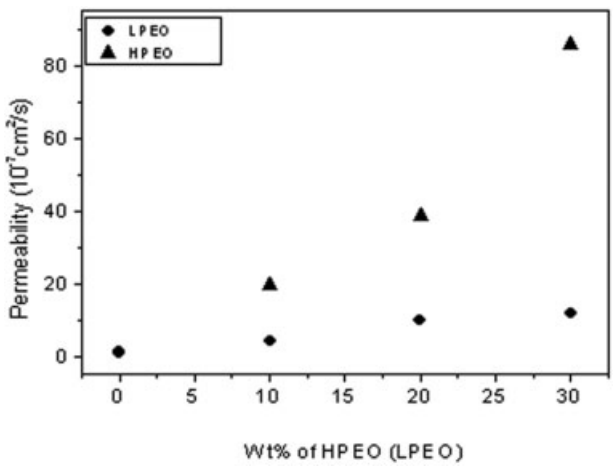

Figure 4 Effects of PEO content on permeability of Vitamin B_{12} through crosslinked chitosan/PEO membranes.

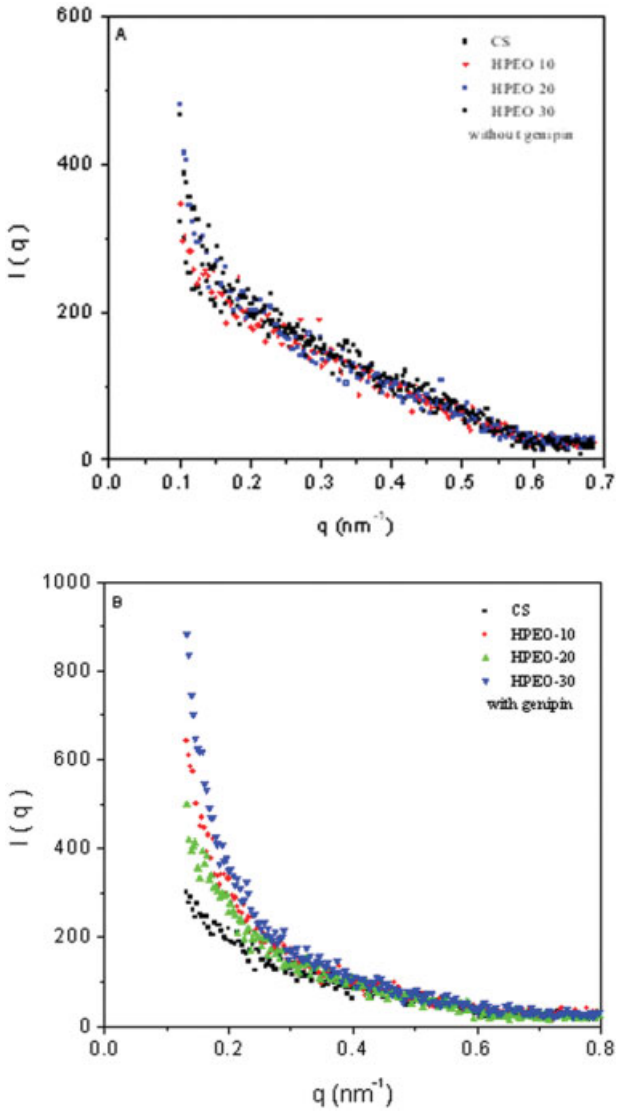

Figure 5 Scattered intensity versus scattering vector (q) for (A) chitosan/HPEO blends and (B) the blend networks. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

crosslinked chitosan/LPEO and chitosan/HPEO membranes that the rate of drug permeation of crosslinked chitosan/HPEO membranes was higher than that of chitosan/LPEO membranes.

Permeability of the membrane is controlled by the morphological features of the materials, which can alter the mesh size of the membrane.[29] In this study, the morphology of chitosan/PEO blends and networks was examined by SAXS. Figures 5 and 6 show the scattered intensity against scattering vector for uncrosslinked and crosslinked chitosan/HPEO and chitosan/LPEO films, respectively. For an ideal two-phase system, the scattered intensity is proportional to the mean square electron density fluctuation. If there is nonphase separation in a system, in which the electron density is the same, the scattered intensity only represents for background scattering. For a phase-separated system, there is a difference in the electron densities, which results in higher scattering intensity. A comparison between Figures 5(A) and 5(B) shows that the scattered intensity increased for the chitosan/HPEO and chitosan/LPEO systems crosslinked by genipin. This indicates that crosslinking results in phase separation.

As can be seen in Figures 5(B) and 6(B), the scattered intensity further increased with increasing HPEO or LPEO content in the crosslinked chitosan/HPEO or in LPEO system. The stronger the intensity, the greater phase separation was. The larger phase separation in the crosslinked chitosan/PEO blend will lead to reduce molecular interaction between the chitosan and PEO. This fact has been proved by the previous stud-

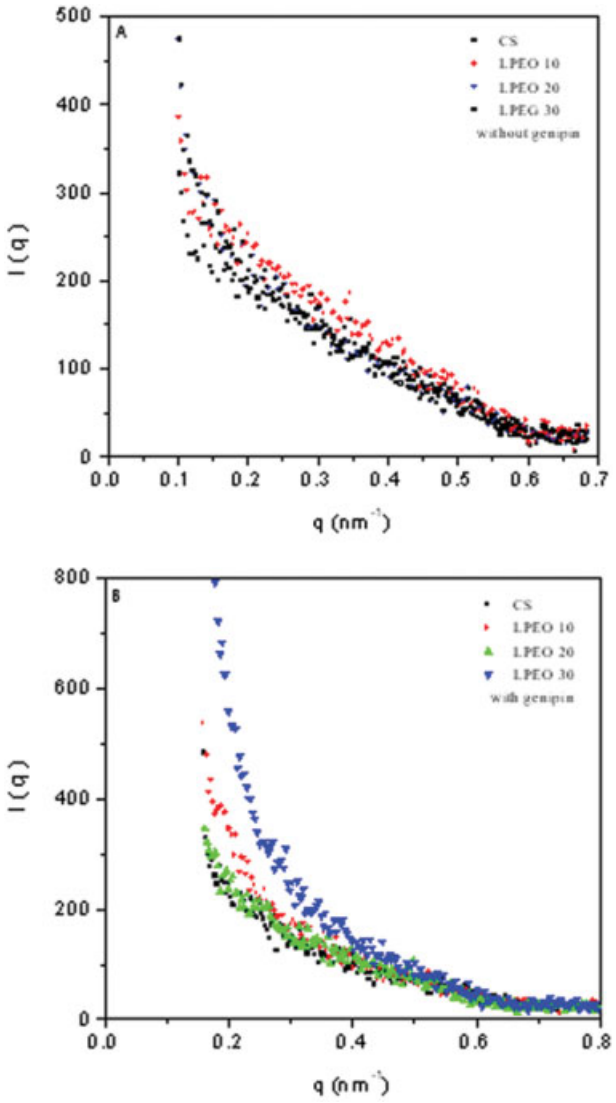

Figure 6 Scattered intensity versus scattering vector (q) for (A) chitosan/LPEO and (B) the blend networks. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE III
Permeability, Diffusion, and Partition Coefficients of Vitamin B_{12} in Solution with Different pHs

pH	P (cm^2/s)	D (cm^2/s)	K_d
7.4	0.957×10^{-7}	0.868×10^{-7}	1.123
5.5	1.26×10^{-7}	0.981×10^{-7}	1.284
4.3	1.81×10^{-7}	1.243×10^{-7}	1.456
3.8	2.91×10^{-7}	2.042×10^{-7}	1.425

ies for the stability of crosslinked chitosan/PEO film in water.[14] These results indicated that after immersing the film in water some of the PEO dissolved. The lower HPEO or LPEO content the better was the stability. The stability of the crosslinked chitosan/LPEO film was better than that of the crosslinked chitosan/HPEO film because of the effect of different molecular weight of PEO. The low stability gives a rise of larger mesh size for the chitosan/HPEO membrane. From the aforementioned discussions, it can be concluded that PEO content and different molecular weight of PEO used in the chitosan/PEO blend network can alter the mesh size of the membrane and control drug release rate.

Effect of solution pH on vitamin B_{12} release

The permeation of a drug through crosslinked chitosan (crosslink density: 4.645%) membranes was studied in PBS solutions with different pHs (3.8, 4.3, 5.5, and 7.4) at 37°C to evaluate of the role of solution pH on the permeation of vitamin B_{12}. Table III shows the effect of pH on the permeability and the diffusion coefficient of vitamin B_{12} through membrane. These clearly show an increase in release rate of vitamin B_{12} when the pH of buffer system become acidic. This result agrees with that of equilibrium swelling nature according to the pH condition.[14] The membrane at low pHs exhibited a high-swelling ratio due to the fact that the repulsive force between the same charges of molecules caused greater intermolecular distances and hydrophilicity. The swollen membrane was expected to have a large-sized mesh, and hence higher rate of drug permeation at lower pH could be attributed to the changes in the mesh size.

ES release from a chitosan carrier

The release of ES over time was investigated at 37°C. The diffused mass can be calculated on the basis of the measurement of concentration. Figure 7 shows M_t/M_∞ for released ES versus the time. M_t, the amount of ES sorbate released at time t, was calculated. M_∞, the amount of ES released after infinite time, was taken to be the value at which the equilibrium was attained.

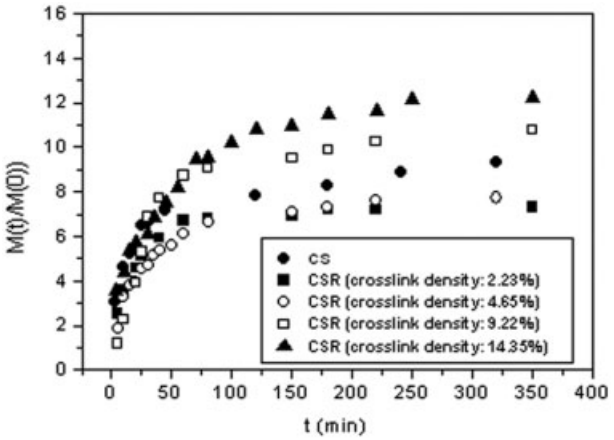

Figure 7 $\phi = M_t/M_\infty$ for released ES versus time.

From the linear part of the curves, the diffusion coefficient, D, was calculated using eq. (10).

Figure 8 shows the diffusion coefficient versus crosslink density. The results indicate that the diffusion coefficient strongly depends on the crosslink density. When the crosslink density was 2.23% or 4.65%, the diffusion rate was slower than that of the uncrosslinked ones. However, when the crosslinking density was increased to 9.22% or 14.35%, the diffusion rate became faster than that of the uncrosslinked ones.

It can also be observed from Figure 7 that the release amount of ES was very small. Even when the release time was 350 min and the crosslink density was 14.35%, only about 15% of the loaded drug was released from the carrier. This suggests that for the carrier (chitosan/ES system), the drug release mechanism is not only regulated by the "pore " mechanism, and that there exist molecular interactions between chitosan and ES. This means that the release rate may be controlled by both the mesh size of the membrane and the interactions between chitosan and ES. To understand the aforementioned diffusion behavior, the

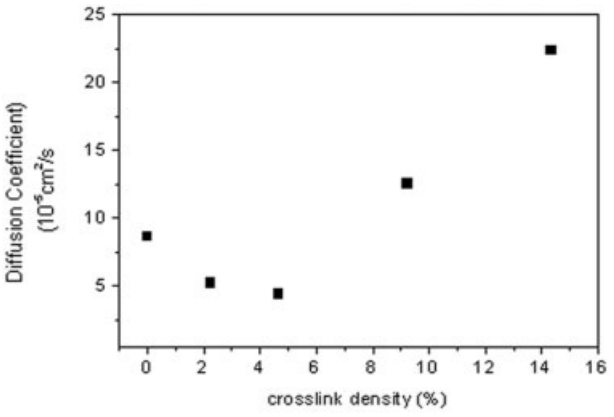

Figure 8 Diffusion coefficient versus crosslink density.

molecular interactions between chitosan and ES was studied by FTIR spectra analysis; the results of which are shown in Figure 9. The frequency of the free amine band of chitosan in chitosan/ES system shifted up from 1558 to 1567 cm^{-1}, confirming that ES interacts with chitosan at the position of amino groups.

For further understanding of the effect of crosslink density on the interactions between ES and the free amino groups of chitosan, chitosan samples with 72 and 95% degree of deacetylation were employed. Figure 10(A) and 10(B) shows FTIR spectra for chitosan/ES systems with 72 and 95% degree of deacetylation, respectively. Table IV gives a comparison of frequency shifts. The results indicate that the molecular interactions were stronger when chitosan with higher degrees of deacetylation was used, which is explained by the greater shifts of the frequency of the free amine band.

The free amino groups of chitosan reacted with genipin, which was confirmed by FTIR and UV spectra analysis.[33] The content of free amino groups of chitosan could be reduced due to crosslinking. According to this, the content of amino group will decrease with increasing crosslink density.

From the FTIR results and the aforementioned analysis, it can be assumed that the interaction between chitosan and ES in chitosan/ES systems may be reduced when chitosan is crosslinked by genipin. For the crosslink densities of 2.23% and 4.65%, the diffusion rate deceased because the mesh size decreased, although because of crosslinking the interaction between chitosan and ES became weaker. In this case, the mesh size is more important than the interaction for controlling the release rate in the chitosan/ES system. However, for the crosslink densities of 9.22% and 14.35%, the drug release rate was more dependent on the interactions in the chitosan/ES system.

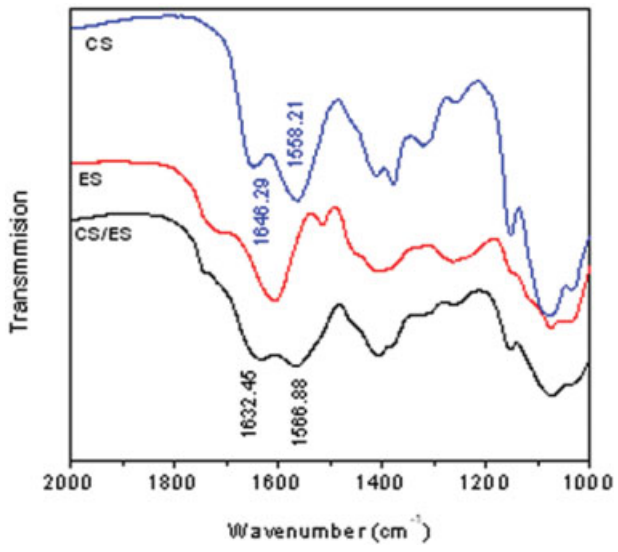

Figure 9 FTIR spectra of chitosan (CS), ES, and CS/ES (DD: 85%). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

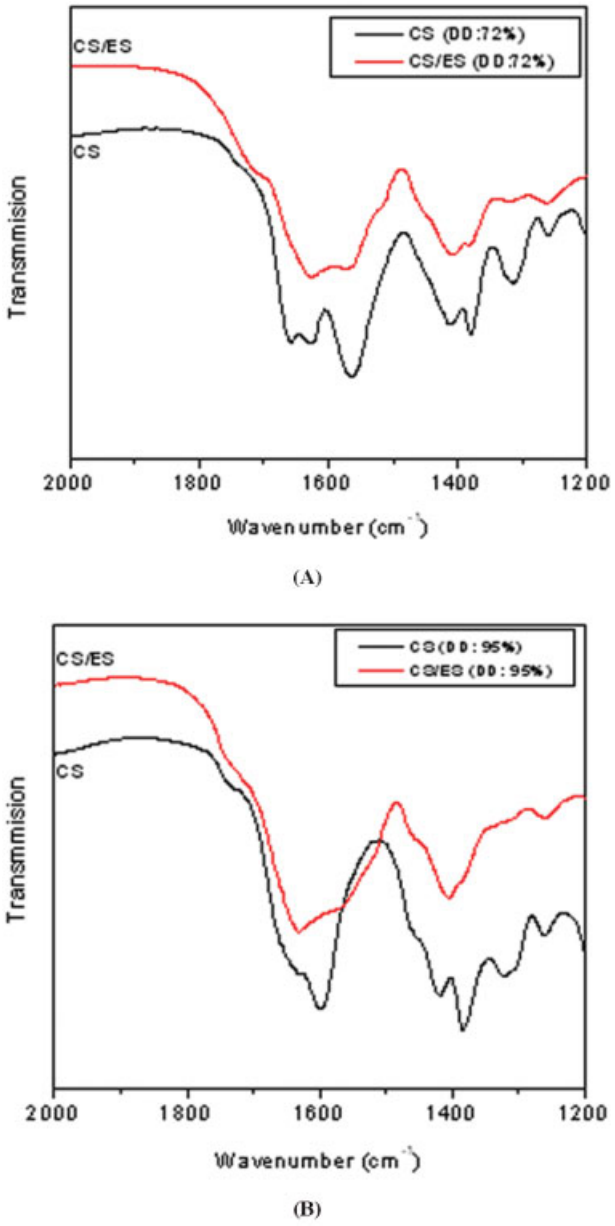

Figure 10 FTIR spectra of chitosan (CS) and CS/ES. (A) DD: 72% and (B) DD: 95%. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CONCLUSIONS

The permeability of vitamin B_{12} through chitosan and chitosan–PEO membranes was investigated. The drug release rate was controlled by the mesh size of the membrane, which can be adjusted by a number of factors, such as crosslinking density, the presence of PEO in membrane, and pH of the medium. Crosslink-

TABLE IV
A Comparison of Frequency Shifts Using Different Degree of Deacetylation of Chitosan

	CS		CS/ES	
DD	Wavenumber (cm^{-1})	Shift (cm^{-1})	Wavenumber (cm^{-1})	Shift (cm^{-1})
72	1563	11	1574	11
	1627	1	1626	1
85	1558	9	1567	9
	1647	−15	1632	−15
92	–	–	–	–
	1599	−32	1631	−32

ing resulted in a decrease of the drug release rate, and they were very sensitive to crosslink density at low crosslinking density. PEO was introduced into the membrane, which can change the mesh size of the membrane and control drug release rate by PEO molecular weight and amount of its content. The drug release rate was sensitive to medium pH and this was in agreement with swelling nature.

ES drug release from the chitosan carriers was affected by the crosslink density of the chitosan films. The drug release rate for the chitosan/ES system was governed by both the mesh size of membrane and specific interactions between chitosan and ES.

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