Kinetics of *In Vitro* Drug Release from Chitosan/Gelatin Hybrid Membranes

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ABSTRACT: *In vitro* studies of controlled release from chitosan/gelatin hybrid membranes were carried out using drugs of different molecular weight. It was found that release of urea, 5-fluorouracil (5-Fu), sodium benzoate, sodium salicylate, sodium mandelate, and sulfacetamide sodium followed zero-order kinetics after a short time lag. Variation of the diffusion coefficient, permeation coefficient, and degree of hydration with crosslinking and varying weight percent of gelatin in membrane matrices were studied in detail by using 5-Fu as a model drug. The diffusion coefficient and permeation coefficient of 5-Fu are dependent on the degree of hydration of the swollen membrane. The transport process of drug molecules in the hydrogel membrane is presumed to be predominantly of the pore mechanism. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 68: 1751–1758, 1998

Key words: chitosan/gelatin hybrid membrane, drug release, swelling ratio, degree of hydration, diffusion coefficient

INTRODUCTION

Chitosan [β(1–4)-2-amino-2-deoxy-D-glucose] is a natural polyaminosaccharide derived from *N*-deacetylation of chitin [β(1–4)-2-acetamide-2-deoxy-D-glucose]. Depending on the degree of deacetylation, chitosan is a copolymer of *N*-acetyl-D-glucosamine and glucosamine units, but the glucosamine units predominate. The deacetylation process destroys the structural regularity of the chitin macromolecule, makes the polymer soluble in dilute acid solutions, and yields a rubbery hydrogel in water.

Due to their good biological activity, biocompatibility, and biodegradability, chitosan and its derivatives have attracted attention because of their potential utility as biomedical polymers.[1,2] It has been reported that these biomolecules have applications as anticoagulants, wound-healing accelerators, wound dressings, artificial bone, and immobilized enzyme, and in drug delivery systems.[3–6]

Among all the controlled-release techniques, the use of membranes is the most promising due to their ability to maintain constancy in the drug delivery profiles. Solute transport through hydrogel membranes is generally described in terms of two mechanisms: the pore mechanism and the solution-diffusion or partition mechanism.[7,8] These mechanisms may not operate exclusively, but one may be expected to predominate for a given drug/membrane pair.

In the pore mechanism, the solute is presumed to diffuse through microchannels within the membrane matrix. The diffusion rate of the solute is in relation to the average pore size of the membrane, the molecular volume, and the water solubility of the solute. In the hydrogel membrane with high hydration, such as chitosan or gelatin, these water-filled "pores" or channels are changing in size and are not fixed in definite locations.

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Kim and colleagues[9] investigated the controlled release of riboflavin and insulin through crosslinked chitosan/poly(vinyl acetate) blend membranes. Diffusion of solute is presumed to occur primarily via the free-water region in the swollen membrane.

In the partition mechanism, the solute dissolves in the membrane matrix itself and diffuses through the membrane, so the diffusion rate is dependent on the water solubility of the solute and the physicochemical properties of the membrane.

Gelatin and chitosan are natural biopolymers. Their composite material has been applied in medical fields, such as wound dressing,[10,11] and as matrices for the controlled delivery of sustained drugs. Yao and associates[12] reported a novel pH-sensitive hydrogel based on a crosslinked chitosan/gelatin with a glutaraldehyde hybrid polymer network. The gel swells in acidic medium and deswells in alkaline medium. The purpose of the present work is to study the transport mechanism of various drugs permeating the chitosan/gelatin hybrid membrane, and to determine the effect of crosslinking and varying percent concent of gelatin in the membrane matrix on the release rate of drugs. Variation of the diffusion coefficient, permeation coefficient, and swelling ratio with crosslinking and varying percent concent of gelatin in membrane matrix was investigated by using 5-fluorouracil (5-Fu) as a model drug.

EXPERIMENTAL

Materials

Chitosan ($M_n = 1.16 \times 10^6$, degree of deacetylation = 68.8%) and gelatin were used as purchased. Glutaraldehyde (chemical grade) was 28% aqueous solution, while lactic acid and other reagents were analytical grade.

Urea, 5-Fu, sodium benzoate, sodium salicylate, sodium mandelate, and sulfacetamide sodium were kindly provided by First Affiliated Hospital of Anhui Medical University, Hefei, Anhui.

Preparation of Chitosan/Gelatin Hybrid Membrane

Chitosan and gelatin were dissolved in dilute lactic acid solution and the mixture was maintained at 50°C for 3 h. The solution was filtered and cast on a clean glass dish. The dish was kept at 50°C until the solvent was completely removed. The obtained dry membrane was immersed in a mixture of 30% aqueous ammonia and ethyl alcohol at room temperature for 24 h, and then washed with abundant distilled water to remove all trace of alkali.

Preparation of Crosslinked Chitosan/Gelatin Hybrid Membrane

Weighted aqueous glutaraldehyde was added to the mixture of chitosan and gelatin with continuous stirring. After 1 h, the solution was filtered and maintained at 50°C until all bubbles were removed. Other procedures were the same as for preparation of the chitosan/gelatin hybrid membrane. The crosslinking reaction occurred at 50°C. Due to not determining the amount of free amine of gelatine molecules, the crosslink density was represented with mol % GA/CS-NH_2.

Measurements of the Swelling Ratio and Hydration of Membranes

Weighed specimens of the membranes were immersed in distilled water and swollen at 37 ± 0.5°C. The swollen specimens were removed from the water at regular intervals, quickly blotted with filter paper to remove excess surface water, and weighed immediately in a microbalance. The procedure was repeated until a constant weight was obtained. The degree of swelling, Q, was calculated from the following expression:

$$Q = \frac{\text{weight of swollen sample}}{\text{weight of dry sample}} \qquad (1)$$

Degree of hydration, H, is the volume fraction of water in the swollen membrane and is related to the swelling ratio as follows:

$$H = 1 \Big/ \left[1 + \frac{\rho}{\rho_P (Q - 1)} \right] = 1 \Big/ \left[1 + \frac{1}{\rho_P (Q - 1)} \right] \qquad (2)$$

where ρ and ρ_P are the densities of water and membrane matrix, respectively.

Determination of Drug-Release Rate

The release studies were carried out with a reservoir-type device[13] under the following conditions:

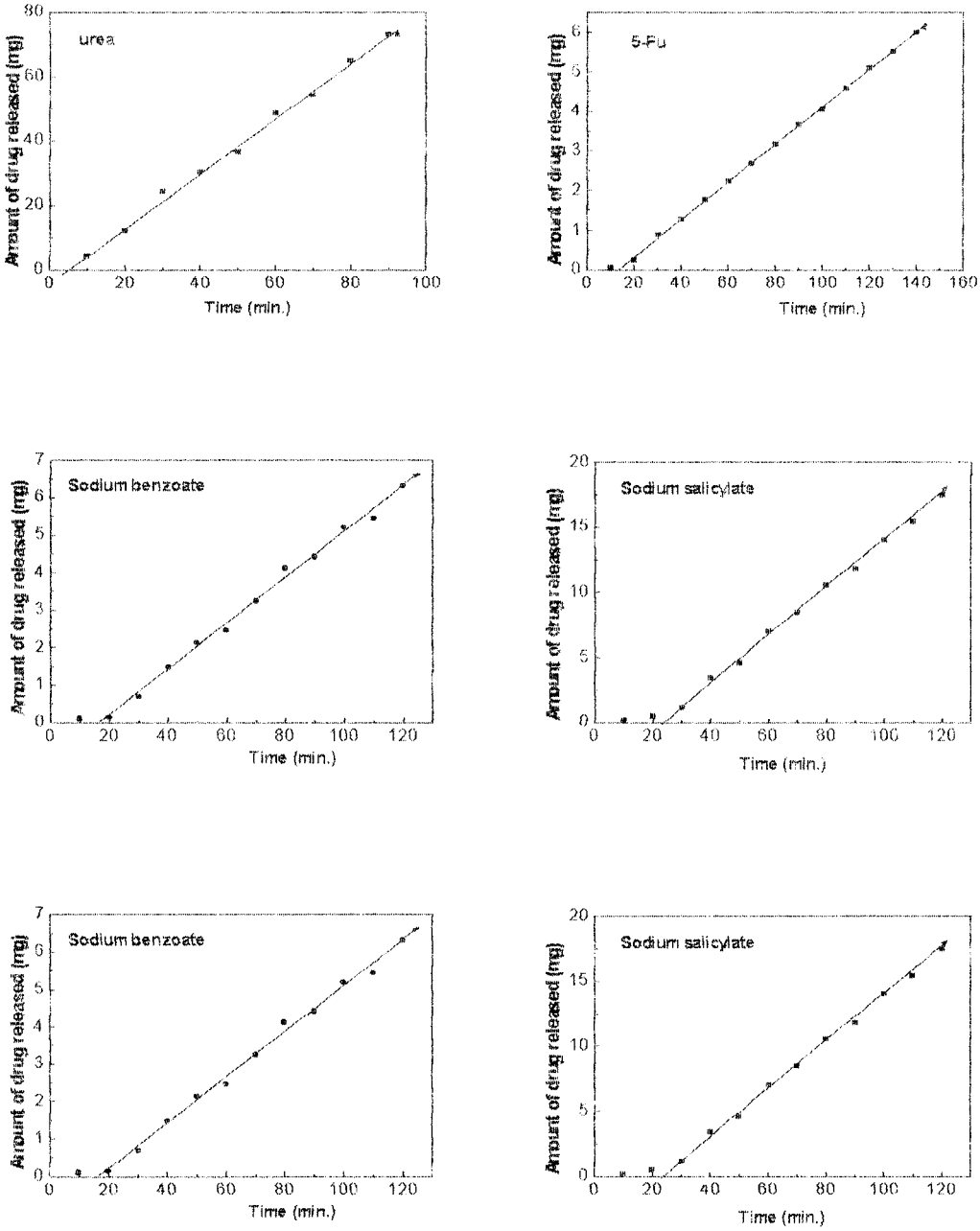

Figure 1 Plot of total amount of drugs released through chitosan/gelatin (1 : 1, w/w) hybrid membrane in a release cell with stirring at 37 ± 0.5°C.

container of saturated drug, continuous stirring, and periodical renewal of the release medium. The device containing 1 g solid drug was placed into a glass beaker with 50 mL water and then stored at 37 ± 0.5°C with continuous stirring. The extractant was withdrawn at suitable time intervals and the drug concentration was measured by an ultraviolet (UV)-2100 spectroscope. The extractant was renewed after each measurement. All experiments were in triplicate and the average values were plotted.

Determination of Permeation Coefficient

The swollen membrane was fixed in a quartz cell as a partition membrane. The given volume of permeated solution was poured into the left compartment of the cell and an equal volume distilled

Table I Diffusion Coefficients and Permeation Coefficients of All the Drugs Studied Through the Chitosan/Gelatin (1 : 1) Hybrid Membranes

Drug	Molecular Weight	Thickness (mm)	t_0 (min)	Diffusion Coefficient ($\times 10^{-7}$ cm^2/s)	Permeation Coefficient ($\times 10^{-6}$ cm^2/s)
Urea	60	0.517	5	14.8	31.6
5-Fluorouracil	130	0.518	14	5.32	4.64
Sodium benzoate	144	0.494	17	3.98	10.3
Sodium salicylate	160	0.524	23	3.31	6.42
Sodium mandelate	174	0.479	25	2.54	5.86
Sulfacetamide sodium	236	0.485	29	2.25	2.65

water into the right. The cell was placed in a constant temperature bath of 37 ± 0.5°C. The dialysis procedure was carried out with continuous stirring. The concentration of drug in both chambers was determined by means of a UV-spectrophotometer. The permeation coefficient was calculated by an equation found in the literature.[14]

RESULTS AND DISCUSSION

Effect of Molecular Weight of Drug on Diffusion Coefficient

Figure 1 shows plots of the total amount of drug released versus time for each drug. Each plot has a nonlinear zone which corresponds to the time lag before steady release. When the steady state is reached, the relationship of the amount of drug released (M_t) and time (t) can be given by the following expression[15]:

$$M_t = \frac{PC_0A}{h}\left(t - \frac{h^2}{6D}\right) \quad (3)$$

where P is the membrane permeability, C_0 is the drug reservoir concentration, A is the effective release area of membrane, h is the membrane thickness, and D is the diffusion coefficient.

The linear parameters of all plots in Figure 1 under steady-state conditions were obtained by using line regression. The value of the cross point of the regression line and the X axis is the time lag $\left(\frac{h^2}{6D}\right)$, which is the time it takes the drug to cross the membrane and begin to release. Therefore,

$$D = \frac{h^2}{6t_0} \quad (4)$$

The diffusion coefficients of all drugs in chitosan/gelatin hybrid membrane can be obtained by eq. (4); values are listed in Table I.

All the drugs studied appear to give higher diffusion and permeation coefficients in the swollen membrane. For a hydrogel such as a chitosan/gelatin hybrid material, a large amount of water imbibed by hydrophilic polymer provides the main diffusional pathway for the solute through the swollen membrane. The release rate of the drug depends, therefore, on hydration of membrane and solubility of drug in the hydrous phase of the swollen membrane.

A plot of the diffusion coefficients of the drugs studied, in the swollen membrane versus their molecular weight, is shown in Figure 2. The diffusion coefficient decreases with the increase of molecular weight. Drug release from hydrogel membrane can occur in terms of a solution-diffusion mechanism and/or a pore mechanism, which is dependent on the molecular volume of drug and the swelling ratio of the membrane. In general, the drug transport process is predominantly a partition mechanism in the system comprising a

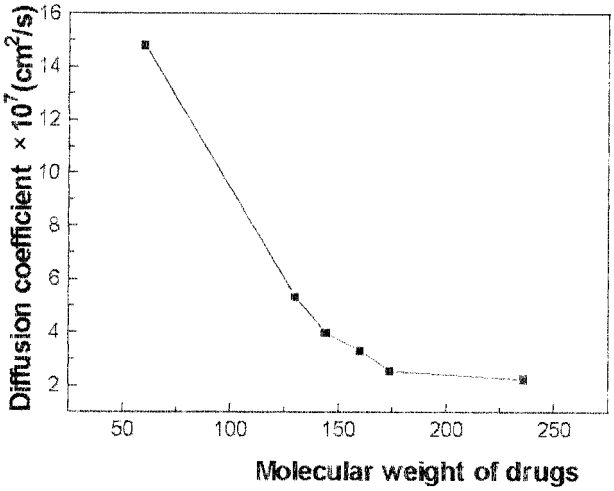

Figure 2 Plot of diffusion coefficient of drugs of different molecular weights through chitosan/gelatin (1 : 1, w/w) hybrid membrane in a release cell with stirring at 37 ± 0.5°C.

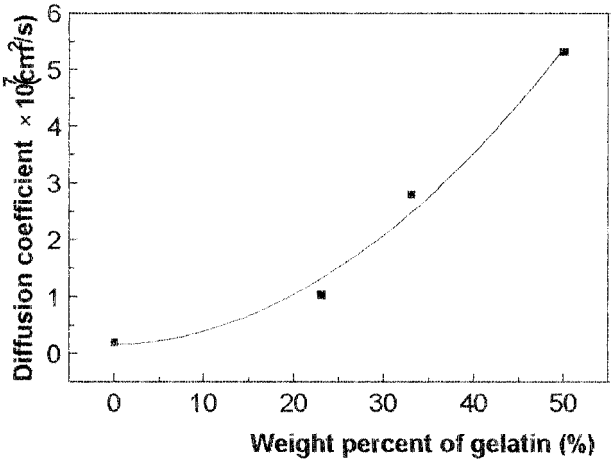

Figure 3 Plot of diffusion coefficient of 5-Fu through chitosan/gelatin (1 : 1, w/w) hybrid membranes versus weight percent of gelatin in a release cell with stirring at 37 ± 0.5°C.

drug of higher molecular weight and a membrane with low hydration; whereas with the increase of the swelling ratio of membrane, the pore mechanism predominates in the transport of a drug with low molecular weight.

With the chitosan/gelatin hybrid membrane with higher hydration, these drugs may release from the swollen membrane predominantly by the pore mechanism. Due to the existence of a large amount of water in the swollen membranes, the hydrogen bonds of extro- or intermolecular molecules are largely destroyed, and free-water regions similar to a "hole" channel in the swollen membrane are formed, which is of benefit to drugs diffusing through the swollen membrane. Therefore, the transport of drug in the hydrogel membrane can occur by molecular diffusion and/or bulk flow, depending on the hydration and the external conditions.

Physically the pores of the swollen polymer membrane are different from a non-swollen pore membrane, for which pores of fixed sizes and location are assumed. In the swollen hydrogel membrane, these water-filled "pores" or channels fluctuate in size and are not fixed in definite locations. As a consequence of the plasticizing effect of water in the swollen membrane, the macromolecular segments exhibit enhanced mobility so that the size, shape, and location of the pores or channels may continuously change.[16] The transport of solute molecules through the swollen membranes is dependent on the probability that the permeant molecule finds at its location such a hole, which relates to the molecular volume of the solute and the average pore size in relation to the hydration of the swollen membrane. It is obvious that the probability of a small molecule crossing the swollen membrane is higher than that of macromolecules; so the diffusion coefficients and permeation coefficients of drugs through the chitosan/gelatin hybrid membrane decrease with the increase of molecular weight.

Effect of Membrane Gelatin Weight-Percent Variation on Permeability

By using 5-Fu as a model drug, the influence of variation in weight percent of gelatin in the chitosan/gelatin hybrid membrane on the release rate and diffusion coefficient were studied; results appear in Figure 3 and Table II, respectively. The diffusion coefficient and release rate of 5-Fu increase with the increase of gelatin in the hybrid membrane.

Gelatin, whose molecules include a large amount of carboxyl and amine groups, is a water-soluble polymer, readily miscible with chitosan. Due to hydrogen bonds that occur between the functional

Table II Permeability and Swelling Data for Chitosan/Gelatin Hybrid Membranes and Their Crosslinked Membranes

Material Chitosan/ Gelatin (w/w)	Thickness (mm)	GA/CS-NH_2 (mol %)	Diffusion Coefficient ($\times 10^{-7}$ cm^2/s)	Permeation Coefficient ($\times 10^{-7}$ cm^2/s)	Swelling Ratio	Degree of Hydration
10 : 3	0.550	—	1.04	2.86	2.91	0.720
2 : 1	0.463	—	2.81	3.82	3.14	0.743
1 : 1	0.518	—	5.32	4.64	3.42	0.767
1 : 1	0.345	2.0	4.87	2.40	3.26	0.756
1 : 1	0.372	4.0	4.46	1.62	3.15	0.748
1 : 1	0.390	6.0	1.99	1.18	3.05	0.740
1 : 1	0.364	8.0	1.14	0.94	2.96	0.733
Chitosan	0.500	—	0.21	1.70	2.58	0.677

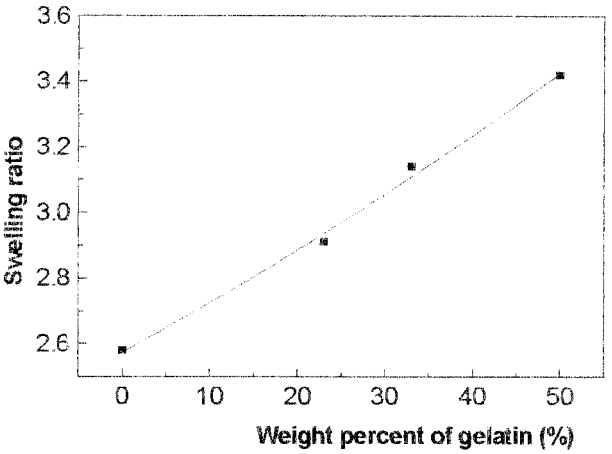

Figure 4 Plot of the swelling ratios of the membranes versus weight percent of gelatin.

groups of chitosan and gelatin, the physically crosslinked composite material is essentially insoluble in a nonacidic aqueous solution. But gelatin being more hydrophilic, both the swelling ratio and permeability increase with weight percent of gelatin in the chitosan/gelatin hybrid membrane, as shown in Figures 4 and 5, respectively. The present data indicates that the hydrogen-bonding interactions between chitosan and gelatin do not lead to a "tighter" network structure but yield a "looser" membrane which exhibits enhanced permeability toward various solutes.

Effect of Crosslink Reaction on Permeability of the Chitosan/Gelatin Hybrid Membrane

Figure 6 shows the total amount of 5-Fu released versus time. Crosslinking a polymer matrix always leads to a reduction in permeability to solutes (results of our experiments appear in Table II). By means of the infrared spectroscopy method, Yao and coworkers[12] confirmed that crosslink network formed via —C═N— formation, due to interaction between the aldehyde groups of glutaraldehyde with amino groups of chitosan and gelatin. As a consequence of the effect of the introduction of junction joints in the hybrid membrane, the macromolecules exhibit reduced mobility to form a "tighter" membrane so that the swelling ratio decreases, thus not aiding the solutes' permeation through the swollen membrane.

Flory[17] has discussed the effect of crosslink density on the equilibrium swelling of polymer networks. Under equilibrium conditions, the swelling ratio in ideal networks decreases nonlinearly with the increased content of crosslinking agent. The percentage volume of water in the hydrogel membrane is affected in turn by the concentration of any crosslinking agent.[7] When the crosslink density is sufficiently high, then permeability may involve a partition process instead of porous transport. However, chitosan/gelatin hybrid membrane is very brittle at a high crosslink density, so only those networks containing 2.0–8.0 mol % of the glutaraldehyde were investigated. The diffusion and permeation coefficients of 5-Fu decrease with the increase of mol % of crosslinking agent added to the polymer matrices, as listed in Table II. The swelling ratio decreases slightly with the increase of crosslink density in the studied range. This indicates that the transport mechanism of 5-Fu, in crosslinked chitosan/gelatin hybrid membrane with low crosslink density, is predominantly of the pore mechanism.

Relationship Between Diffusibility and Degree of Hydration

According to free-volume theory,[8,16] the total amount of pores or channels in a unit volume of membrane represents the free volume through which the transport of permeant predominantly occurs. In polymer hydrogel, effective free volume is essentially the free volume of water, and the transport of solutes is presumed to permeate through the free-water region in the swollen membrane. Therefore, the swelling ratio plays an important role in the transport procedure of solutes in the swollen membrane. With the assumption that the effective free volume in the swollen membrane is equal to the product of the free volume

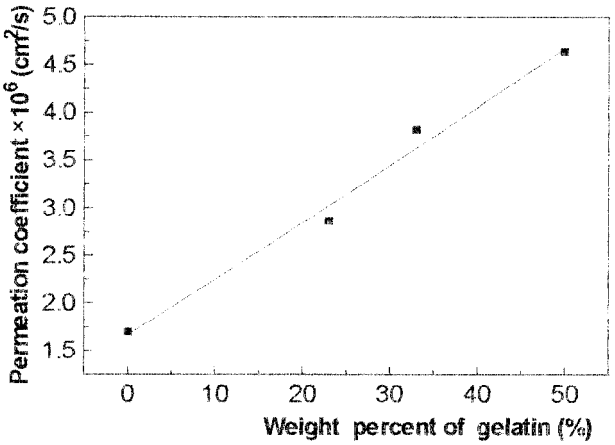

Figure 5 Plot of permeation coefficient of 5-Fu through chitosan/gelatin (1 : 1, w/w) hybrid membranes versus weight percent of gelatin in a release cell with stirring at 37 ± 0.5°C.

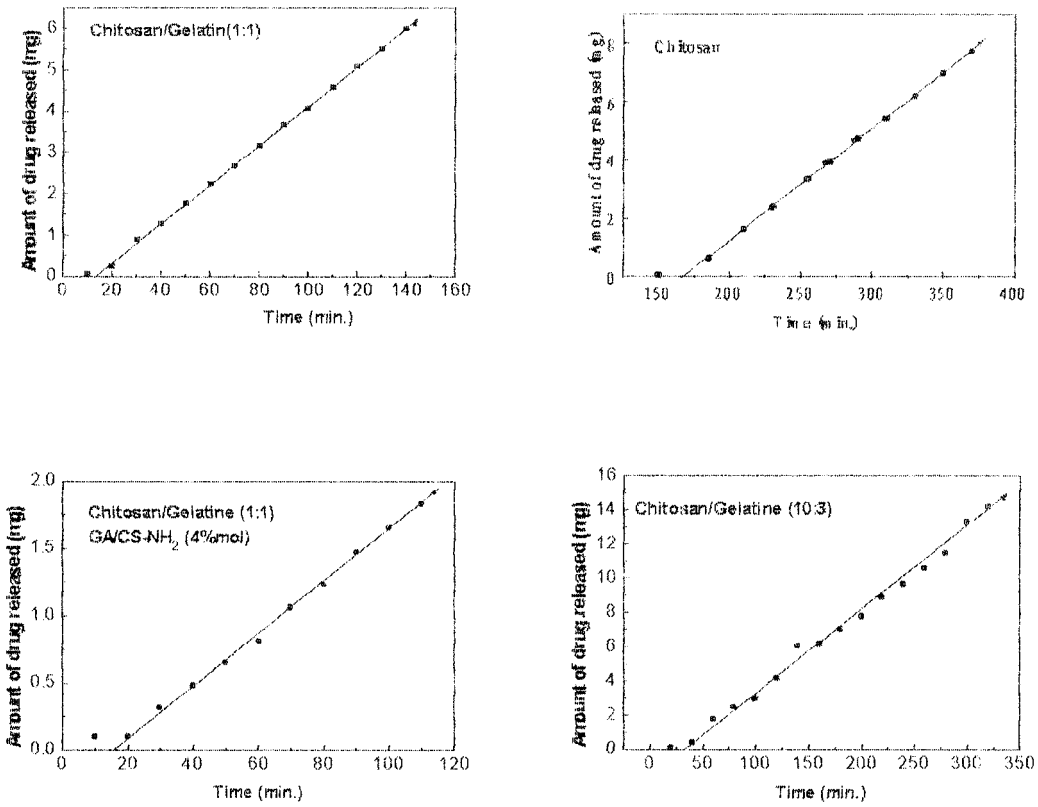

Figure 6 Plot of total amount of 5-Fu through chitosan and crosslinked membranes in a release cell with stirring at 37 ± 0.5°C.

of pure water (V_f) and the degree of hydration (H), Yasuda and Lamaze[8] developed an expression relating the diffusion coefficient (D) and the degree of hydration of the membrane.

$$\frac{D}{D_0} = \varphi_{(q)} \cdot e^{Bq/V_f \cdot (1/H - 1)} \qquad (5)$$

where D_0 is the diffusion coefficient of solute in water, $\varphi_{(q)}$ is the probability of occurrence of a pore with a size larger than the molecular volume of the solute in the swollen membrane, B is a constant, q is the cross-sectional area of the solute molecule, and H is the degree of hydration of swollen membrane.

$$\ln\left(\frac{D}{D_0}\right) = \ln \psi_{(q)} - \frac{B_q}{V_f}\left(\frac{1}{H} - 1\right) \qquad (6)$$

The present data in Table II are used to check the applicability of eq. (6) to crosslinked chitosan/gelatin hybrid network, as shown in Figure 7. It can be seen that the plot of diffusion coefficients of 5-Fu in different chitosan/gelatin swollen membrane versus $(1/H - 1)$ appears to follow a linear relationship in agreement with eq. (6). It is interesting that variation of weight percent of gelatin in the membrane and crosslinking with low crosslink density have little influence on the value of $\varphi_{(q)}$ and B, both of which are characteristics of the swollen membrane. This indicates the transport mechanism is not affected by the presence of low levels of crosslinking agent and variation of the proportionality of chitosan and gelatin.

Linearity of the plot in Figure 7 indicates that in all systems studied, the transport of 5-Fu in the different swollen crosslinked chitosan/gelatin hybrid membranes with low crosslink desity is predominantly by the pore mechanism.

CONCLUSIONS

1. The zero-order release of all the drugs studied from covering with chitosan/gelatin hybrid material could be achieved after a short time lag. The diffusion coefficients, permeation coefficients, and release rates all decrease with the increase of the drugs' molecular weight.

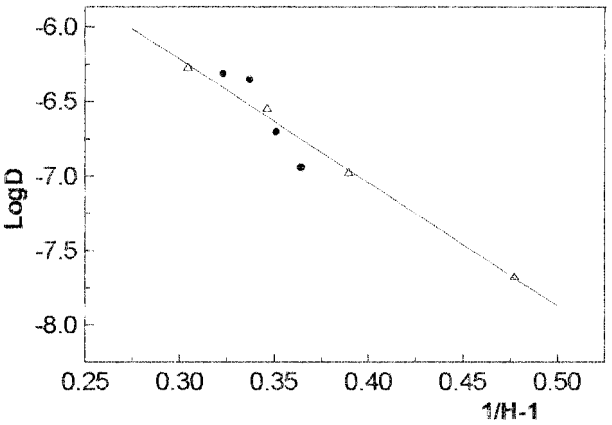

Figure 7 Plot of logarithm of diffusion coefficient of 5-Fu through chitosan/gelatin (1 : 1, w/w) hybrid membranes and its crosslinked membranes versus 1/(H-1); (●) crosslinked membrane; (△) noncrosslinked membrane.

This indicates that the hybrid material has potential value for use as a coating material of sustained drugs.

2. Due to the hydrogen-bonding interaction that occurs between the functional groups of chitosan and gelatin, the physically crosslinked composite material is essentially insoluble in nonacidic aqueous solutions. The swollen hybrid membrane has a higher degree of hydration, as measured by swelling ratio, which can be altered either by crosslinking or by varying the weight percent of gelatin in the membrane matrix.
3. The transport process of the drugs studied through the hydrogel membrane involves not only "molecular flow" (diffusion) but also "bulk flow," in which molecules move as a cluster. Therefore, the transport of solutes is described in terms of solution-diffusion and a pore mechanism. Because of higher hydration of the chitosan/gelatin hybrid membrane, the transport process for these drugs molecules is presumed to be predominantly of the pore mechanism.
4. The diffusion and permeation coefficients of 5-Fu in the hydrogels were found to depend on the degree of hydration of the membrane. The presence of crosslinking agent and the variation in weight percent of gelatin in the membrane matrix affected the diffusion and permeation coefficients because they alter the equilibrium swelling of the hydrogel. The transport mechanism of 5-Fu is little influenced by variation of membrane composition and crosslinking with low crosslink density.

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