

Chitosan-Gelatin-Pectin Composite Films from Polyion-Complex Hydrogels

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Preparation and properties of composite films from gelatin, chitosan and pectin hydrogels are presented. Ionic interactions between positively charged gelatin and negatively charged pectin produce reversible physical hydrogels with homogeneous molecular arrangement that improve water resistance but not alter thermal stability relative to single polymer gels. The effect of preparation conditions to the swelling rate and drug delivery of chitosan-gelatin-pectin films were also studied. Resulting interspersed permanent chemical hydrogel showed a decreased swelling attitude with the increased dosage of crosslinker. However, further the films improved higher permeation in simulated colonic fluid compared to the other media. The amount of bovine serum albumin released from the films at different time intervals was estimated by UV spectrophotometric method at 279 nm. The dissolution profile and *in vitro* release kinetics showed that chitosan-gelatin-pectin films were promising for controlled delivery of the drug. Formulation containing pectin, chitosan and gelatin may be suitable as a coating formulation for colon targeted drug delivery.

Key Words: Pectin, Composite films, Swelling rate, Bovine serum albumin, Colonic delivery.

INTRODUCTION

The past few years have witnessed rapidly expanding interest in renewable feedstocks and marine food processing wastes as sources of biomolecules with potential to replace synthetic polymer¹ in fabricating materials with biocompatibility, bioactivity, biodegradability for unique application². Due to biomolecules have become more available, ever-increasing demand for high-performance "natural" matrices for biomedical and pharmaceutical applications such as organ regeneration and tissue engineering³, wound dressing⁴, medical suture line, artificial limb, controlled drug delivery systems^{5,6}, films, contact lenses and capsules for oral ingestion⁷, has stimulated design of smart matrices able to sense external changes (pH, temperature, ionic strength) and trigger release of active and bioactive compounds.

Pectin is a non-toxic water soluble gel-forming polysaccharide containing carboxylic acid groups. It is extracted from different sources, such as apple, pomelo, orange peel and so on. Pectin which exists in plant cell wall, the main unit is galacturonorhamnan and methylation half of lactose hyaluronic acid, is a kind of natural water soluble gather anion polysaccharide and can carry signal molecules and transmit many biological materials and also can serve as a kind of new biomedical materials. The properties of gelling have been identified as early as hundreds of years ago, but the production of

commercial pectin only began in the early 20th century and the production of domestic pectin began in the 1980's.

Because of the -COOH containing in the pectin molecules and the -NH₂ containing in the chitosan molecules, they will react each other to generate chitosan/pectin (CS/PT) polyelectrolytes compound hydrogels, whose structure appear in the corresponding response varying with environmental pH, ionic strength and temperature, *etc.* Thus, they commonly can be used to the preparation of the film and the pH sensitivity microspheres and also can be used for food, health care products, drug loading and slow-release, *etc.* The three dimensional net shaped microporous structure gels will be composited with chitosan, gelatin and pectin, because of the functional group, which will have a lot of excellent performance.

This research selects chitosan, gelatin and pectin as the research object and the effect of crosslinking agent, pH value, ionic strength, temperature on swelling rate are studied and the effects of the performance of drug release are also investigated.

EXPERIMENTAL

Pectin, gelatin and chitosan (Deacelation degree 80 %) were purchased from Fuda chemical Co., in China; Glutaraldehyde (GA, 50 wt % in water) and glacial acetic acid were purchased from Across, Morris Plane, NJ; bovine serum albumin (BSA); buffer solution (pH = 1.70, 4.00, 7.4, 9.18). All commercially

solvents and reagents were used without further purification. All other chemicals were of analytical grade.

Preparation of films: A series of films with 5.6 wt. % total solid but different composition were generated mixing gelatin, pectin, and chitosan in different amount. Chitosan, gelatin and pectin (CS-Gel-PT) films were prepared in hot water (60 ± 0.5 °C containing 1 wt % acetic acid) with vigorous stirring (1000 rpm) for 0.5 h. After complete interdispersion of the solution, glutaraldehyde (2 wt %) was added to the chitosan, gelatin, pectin solution, to get a series of different glutaraldehyde concentration solutions, under thorough and continuous mixing (550 rpm) one hour to crosslink the gelatin and form the hydrogels.

Once formed, all hydrogels were degassed and spread over the bottom of petri dishes (100 mm diameter) or polycarbonate rectangular templates (300 mm length \times 150 mm width) depending on the specific successive analysis. Final films were obtained after evaporation of water in a vacuum oven at 40 ± 0.5 °C for 24 h. Crosslinked films were additionally washed several times with Milli-Q water and then air-dried room temperature for 24 h.

Scanning electron microscopy analysis (SEM): Strips of dry film (10 mm wide \times 30 mm long) were immersed in 30 mL aliquots of 80 wt. % ethanol water solution, with several changes. Then 80 % ethanol was decanted and replaced with several changes of absolute ethanol. Next, strips were removed from absolute ethanol, quickly blotted dry and immersed in liquid nitrogen for 5 min. The frozen strips were cross fractured manually using cold tweezers and the fractured pieces were thawed in absolute ethanol. Finally, the fragments were critical point dried from liquid CO₂ and the dried fragments were glued to specimen stubs with Duco cement, sputter-coated with a thin layer of gold and examined with a Quanta 200 FEG scanning electron microscope, operated in the high vacuum, secondary electron imaging mode.

FT-IR analysis: FT-IR spectra were recorded using a Spectrum One spectrophotometer (NICOLET 200SXV FT-IR, Perkin Elmer, USA) equipped with an Universal Attenuated Total Reflectance (UATR) device for tablet analysis in the spectral region (4500-500 cm⁻¹).

Swelling behaviour: To evaluate the water sorption resistance of the CS-Gel-PT films, square piece of dry samples were weighed (W_i) and then immersed in distilled water at 30 °C with shaking (100 rpm) for up to 24 h. Swollen films were removed from water periodically, blotted dry and weighed (W_f) to track sorption kinetics. The swelling rate (SR) was determined as described by others⁸.

$$\text{Swelling rate (\%)} = \left[\frac{(W_f - W_i)}{W_i} \right] \times 100 \%$$

Dissolution tests *in vitro*: Films samples ($0.5 \text{ g} \pm 0.001$) were accurately weighed and immersed in bovine serum albumin solution (BSA, 0.5 wt %) at 0 °C for 25 h. The swollen films loaded with drug were placed in a vacuum oven and dried under vacuum at 37 °C. The loading amount of drug in the films was calculated from the decrease in the concentration for the BSA solution (279 nm) which was determined using a UV spectrophotometer (UV-2401-PC, Shimadzu, Kyoto, Japan).

The kinetics of drug release were recorded using a Distek™ dissolution 2100A paddle system (500 rpm, 37 ± 0.5 °C) coupled with an UV Hewlett Packard spectrophotometer for detection of BSA and presented using the diffusion equation⁹ as the ratio of the amount of drug released at the time t (M_t)/the total amount (M_{inf}) of the drug release from the films. The concentration of BSA in the samples was calculated based on average calibration curves ($n = 6$). All dissolution studies were performed in triplicate¹⁰.

RESULTS AND DISCUSSION

Optimization of the films: According to previous research in this field, the variables affecting the ultimate swelling capacity of the films. After identification of the more effective parameters in the reaction, the optimum conditions for various experiments are shown in Table-1.

Sample	m_{CS} (g)	m_{PT} (g)	m_{Gel} (g)	V_{GA} (μ L)	V_{CH_3COOH} (μ L)	SR (%)
A	0.3	0.3	0.3	400	1000	743
B	0.2	0.5	0.2	400	1000	463
C	0.5	0.2	0.2	400	1000	319
D	0.2	0.2	0.5	400	1000	418
E	0.40	0.1	0.40	400	1000	344

After synthesizing the films following the conditions propose by the statistical method (five samples), the swelling rate of samples was evaluated. The ratio of C complexes formed membrane swelling degree is the smallest, that is in the pectin/chitosan/gelatine films the isoelectric point of gelatin, -COO⁻ and -NH₃⁺ charge equal, mutual electrostatic attraction between strong, leading to swelling smaller. But the proportion of C is too thick, not easy to form a homogeneous composite membrane. Therefore, select the sample of D in the following composite membrane preparation process.

FT-IR analysis: FT-IR spectroscopy was used to ensure that the crosslinking reaction between the chitosan, pectin and gelatine had occurred. From the FT-IR spectral interpretation the following result were obtained. The FTIR of chitosan show intense band at 1595 cm⁻¹ corresponding to the functional group -NH₂ bending. The FT-IR of pectin show the band at 1732 cm⁻¹ belongs to the -COOH stretching vibration peak. The absorption peaks at 1539 cm⁻¹ can be assigned to the -NH₂ bending vibration of gelatine. In contrast, after crosslinking, the vibrational band corresponding to primary amino groups at 1590 and 1539 cm⁻¹ weaken and the vibrational band at 1732 cm⁻¹ disappeared (Fig. 1), which prominent bands at 1428 cm⁻¹ was ascribed to -COO-NH₂. These results clearly confirmed that the crosslinking reaction had occurred between the polymers.

SEM analysis: Composite membrane was revealed by scanning electron microscopy. SEM images of frozen-fractured surface in Fig. 2. Composite membrane with smooth surface and uniform has good biocompatibility in the composite membrane between chitosan, pectin and gelatin.

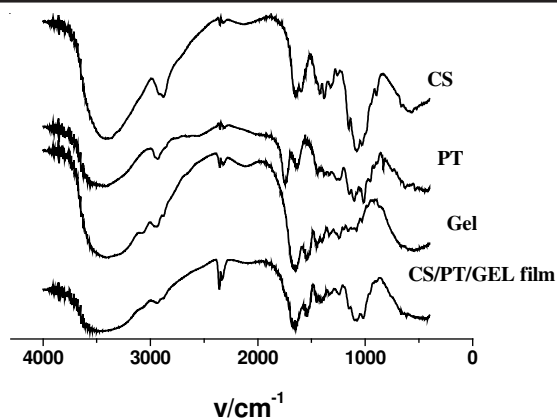


Fig. 1. FTIR of chitosan, pectin, gelatin and composition film

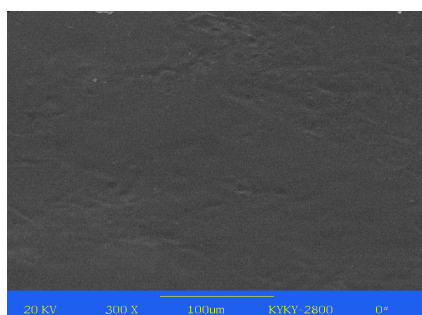


Fig. 2. SEM photograph of composite membrane

Effect of crosslinking agent on the swelling of composite membrane: Membrane swelling ratio reduced with increasing the amount of crosslinking agent in Fig. 3, this is because that the amount of crosslinking agent decreased, not only the molecular chain crosslinking density among chitosan/ gelatin/and pectin is low, but also the chain network structure of hydrogel molecular is loose and free space. Furthermore, there are strong hydrophilic interaction between -OH, -COOH and -NH₂ groups, so the film interior can hold a lot of water and keep the larger degree of equilibrium swelling. However, when the amount of crosslinking agent increased, the crosslinking density of the film increases and the network structure and grid are reduced, therefore, water molecular inward permeability effective channel pore contract, so the equilibrium swelling degree reduced. But when the amount of crosslinking agent is more than 1 mL, the film is more difficult to prepare, so the preparation with the amount of crosslinking agent is 1 mL.

Effect of pH on membrane swelling properties: The pH is an important factor that can strongly influence on the swelling capacity of a film. At first, in order to survey the pH sensitivity of the film, swelling behaviour of the CS-Gel-PT film at 25 °C in water with the pH ranging from 1.40-9.18 was investigated¹¹. The swelling rate of the film decreased in the pH rang between 1.40-7.40, but it is increased with increasing pH from 7.4-9.18. The minimum swelling rate of the film was achieved at pH 7.4 (Fig. 4).

Swelling rate in different buffer solution with pH sensitivity is different, this is because of the polar groups such as -COOH, -NH₂, -OH and other else in the films and the difference of the degree of ionization. In the solution of pH 1.20, -NH₂ in CS-Gel-PT composite membrane is protonated into -NH₃⁺, increased the electrostatic repulsion and hydrophilicity,

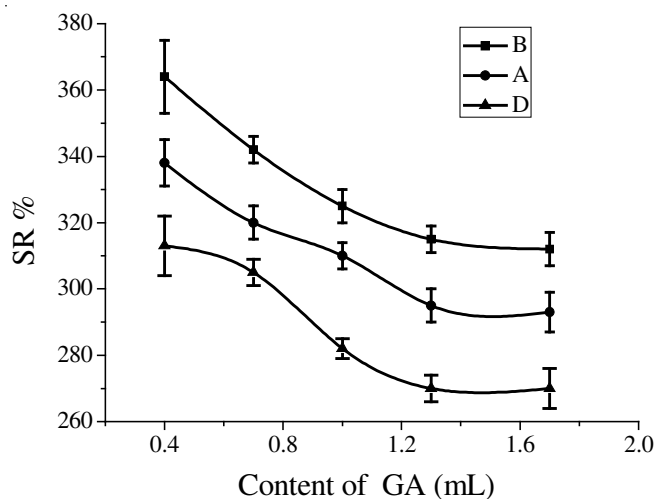


Fig. 3. Effect of crosslinking agent on the swelling of composite membrane

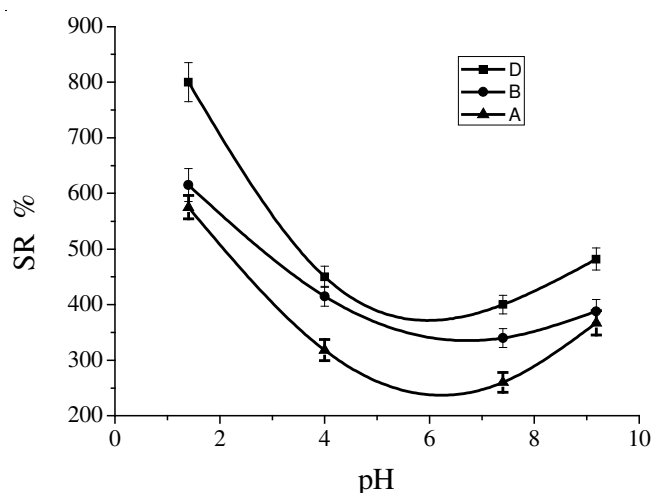


Fig. 4. Effect of pH on membrane swelling properties

leading to more swelling degree. At pH 4, the swelling rate is lower, because that protonation degree and mutual electrostatic repulsion effect were weakened. In pH 7.4 solution, composite membrane was in isoelectric point, thus swelling degree was minimum. In pH = 9.18, amino and hydroxyl hydrogen bonding effects lead to rise in swelling degree.

Effect of salt solution on the swelling rate: In comprise to the deionized water, the swelling of the absorbent in saline solution was significantly decreased. This well-known phenomenon¹², commonly observed in the swelling of composite membrane, can be explained on the basis of osmotic pressure developed due to imbalanced distribution of ions in the polymer network and medium. The ionic attached to the polymer network are immobile and considered to be separated from the external solution by a semi-permeable membrane. While the film was placed in pure water, the maximum osmotic pressure is gained and thus the maximum water uptake is achieved. When the superabsorbent is in NaCl solution, the development of osmotic pressure is much lower due to presence of Na⁺ and Cl⁻ in the external solution. Therefore the swelling is rapidly declined. To investigate the influence of ionic strength on amount of water absorbency, the swelling in different NaCl solutions have been measured. The curve included in Fig. 5 showed that with increasing the ionic strength of the

solution, the swelling capacity of composite membrane was significantly reduced¹³.

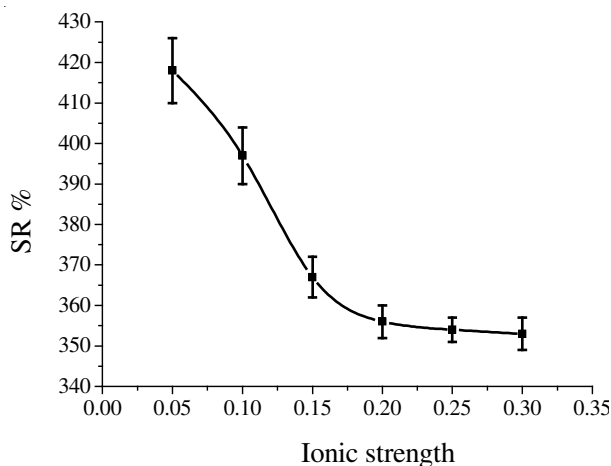


Fig. 5. Swelling rate of the CS-Gel-PT film in different NaCl solution

Drug release kinetics from CS-Gel-PT composite membrane: The D as drug-loaded film carrier, was accurately weighed three part composite membrane of D. The drug loading rate were 6.98×10^{-3} , 6.82×10^{-3} and 7.06×10^{-3} g/g, after immersing in 0.5 % (w %) bovine serum albumin solution for 34 h in room temperature. The release kinetics of BSA from composite membrane in pH 1.2, 4.0, 7.4 and 9.18 buffer solutions are presented in Fig. 6. The sample of D in pH 1.2 solution rapidly disintegrated ($t_{90\%}$, 4 h), whereas those in other pH solution remained intact in aqueous medium. In pH 4-7.4 solutions, show relatively short release times ($t_{90\%}$, 10-14 h). A longer release (34 h) was recorded for the pH 9.18 buffer solution. This is attributed to a hydrophobic barrier limiting access of water and dissolution of the drug. According to Peppas¹⁴, there are three primary mechanisms by which the release of active agents can be controlled *i.e.*, erosion, diffusion and swelling followed by diffusion. Erosion may take place via hydration by hydrolysis of the bulk, the polymer being slowly degraded starting at the periphery of the film. Diffusion can occur through the unhydrated polymer matrix but will generally be facilitated as the polymer gradually swells in contact with the body fluids. Therefore, composite membrane as a carrier for colonic drug delivery has been demonstrated and was useful to achieve the best pH-dependent colonic drug delivery.

Conclusion

Chitosan-gelatin-pectin (CS-Gel-PT) composite films are of interest for use as excipients in controlled drug delivery systems. Hydrophobic interactions are believed to enhance the stability of interactions of chitosan, gelatin and pectin. It is suggested that the release of drug is controlled by diffusion, or by swelling followed by diffusion, depending on both the degree of crosslinking.

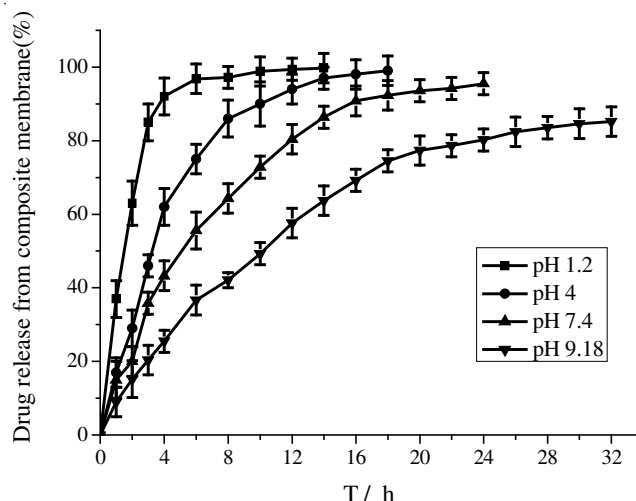


Fig. 6. Drug release kinetics from CS-Gel-PT composite membrane in different pH buffer solution

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