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Preparation and characterization of chitosan nanopores membranes for the transport of drugs

XingYi Li*, KaiHui Nan, Hao Chen, Yu Xu

Institute of Biomedical Engineering, School of Ophthalmology & Optometry and Eye hospital, Wenzhou Medical College, 270 Xueyuan Road, Wenzhou 325027, China

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ABSTRACT

In this paper, a novel chitosan nanopores membrane was developed by selective dissolution of its composition. Polyethylene glycol (PEG) as the porogen was selected to generate the nanopores structure of chitosan membrane. As the observation with scanning electron microscopy (SEM), we could find that the PEG content was greatly influenced on the structure of chitosan membrane. As the PEG content was larger than 50%, the chitosan nanopores membrane could successfully developed. Differential scanning calorimeter (DSC) measurement revealed that the PEG component could not be completely dissolved from the membrane and there was presence the possible interaction (hydrogen bond) between two components. Water adsorption test suggested that the obtained membranes have the great capacity of water adsorption ranging from $162.4 \pm 22.5\%$ to $321.5 \pm 6.5\%$. *In vitro* degradation experiment showed that the obtained chitosan membranes have good biodegradability in the lysozyme solution. The permeability test was performed with two model drugs: vitamin B12 (non-ionic water-soluble drug) and sodium sulfamerazine (ionic water-soluble drug). And the results showed that these two drugs have significant differences in the permeability, indicating that chitosan nanopores membranes can potentially be used to the transport of drugs with controlled diffusion manner.

1. Introduction

In recent years, the natural polymers such as dextran, chitosan, alginate and heparin have been widely applied in the biomedical and pharmaceutical filed(Thanou et al., 2001; Di Martino et al., 2005; Hwang et al., 2009; Li et al., 2009; Bhattarai et al., 2010). Because of their favorable biocompatibility and biodegradability as well as non-toxicity property, films or membrane made from these polymers showed a great range of potential applications: preparation of bioactive and biomimetic coating, preparation of drug release carrier and development of anti-adhesive films (Jeon et al., 2009). More recently, the application of membrane for modulating the release behavior of islet encapsulation have drawn considerable attention. The drug or cell-loaded chambers sealed with permeable membrane are capable of releasing the molecule of interest for a long period of time. The membrane could form the barrier between the surrounding and the inside of the chamber, yet modulating the release behavior of drugs (Okano et al., 1990; Santos et al., 2008; Zhang et al., 2008).

Chitosan, the only cationic polysaccharide in nature, is assembled with N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues. As the product of deactylation of chitin, chitosan could only be dissolved in some acidic solution when pH value was below 6 (Thanou et al., 2001; Di Martino et al., 2005; Bhattarai et al., 2010). Numerous studies have demonstrated that chitosan showed the excellent film-forming capacity with good mechanical properties (Li et al., 2010b). On the other hand, membrane with pore structure is very important for the various applications (Madihally and Matthew, 1999; Ho et al., 2004). For chitosan based films or membrane, there are several methods developed to fabricate porous membranes, such as freeze-gelation method and CO_2-in-water (C/W) emulsions (Lee et al., 2007). The so-called freeze-gelation method is an ideal substitute for the traditional freeze-drying method due to its energy-saving. The membrane developed by this method is an asymmetric structure with pore size in micro-meter scale. Recently, the porogen agents such as silica particles, calcium carbonate ($CaCO_3$), etc. were employed to develop the porous chitosan based membranes with easy control pore size by modulation of particle size (Zeng and Ruckenstein, 1998; Gümüdereliolu and Agi, 2004; Liu et al., 2005; Chao et al., 2006). Santos et al. (2008) employed such a technique to prepare porous chitosan membranes using silica particle as the porogen agent and the obtained asymmetric membrane showed well drug permeability with two model drug (sodium sulfamerazine and sulfametoxipyridazine). Many other applications such as adsorption of bacterial endotoxins, concanavalin and model proteins like humanserum albumin (HSA) and

* Corresponding author. Tel.: +86 577 88833806; fax: +86 577 88833806.
E-mail address: lixingyi_1984@yahoo.cn (X. Li).

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bovine serum albumin (BSA) were also investigated (Ruckenstein and Zeng, 1998; Zeng and Ruckenstein, 1999). According to the previous study, for the membrane-based drug delivery system, the porous structure such as pore size, porosity and tortuosity were greatly effect on the release behavior of inset encapsulation (drugs). Numerous studies have demonstrated that the chitosan macroporous asymmetric membranes could efficiently influence the release behavior of encapsulated drugs. However, the application of chitosan nanopore membrane in control drug release had not been investigated. Herein, in this paper, the chitosan nanopore membrane was first developed by the selective dissolution of PEG component from chitosan blend films, then ability of control of releasing was investigated.

2. Materials and methods

2.1. Materials

Chitosan (with 86% degree of deacetylation (DD)) with ~200 kDa was supplied by Sigma–Aldrich (USA). Polyethylene glycol (PEG; Mn ~2000) was purchased from Sigma–Aldrich (USA). Lysozyme was bought from the Amresco (USA). Vitamin B12 and sodium sulfamerazine were purchased from WenZhou Chemical Reagents Co. LTD (China). All other chemicals used in this paper were analytic grade. Distilled water from Milli-Q water system was used to prepare the aqueous solutions.

2.2. Preparation of chitosan nanopores membrane by selective dissolution of PEG component

Initially, chitosan (1 g) was dissolved in 1% acetic acid solution (99 ml) under magnetic stirring at room temperature to form a 1% polymer solution. The resulting chitosan solution was filtered for the further usage. PEG solution was obtained by dissolving calculated weight of PEG into a certain water solution. Subsequently, 20 ml of membrane-formation solutions, regardless of weight ratio of chitosan/PEG (25%, 50%, 75% and 100%), were poured into a glass frame model and dried at 60 °C for 1 day. The obtained series of chitosan/PEG blend membranes were neutralized with a 2% aqueous NaOH solutions for 30 min after the drying, washed with distilled water to neutral (pH = 7) and peeled from the glass frame model. After that, the obtained series of chitosan/PEG blend membranes were immersed into a hot water bath (85 °C) for 12 h to select dissolution of PEG component and to generate the nanoporous structure. Finally, the obtained chitosan nanopores membranes were immersed in a 20% glycerol solution for 15 min, washed with distilled water and allowed for drying at room temperature. The treatment of chitosan nanopores membranes with glycerol solution was to plasticize the membranes, increasing their processability and to avoid the closure of the porous structure during the drying process. The chitosan nanopores membranes were named S_1, S_2, S_3 and S_4 for chitosan/PEG weight ratio of 25%, 50%, 75% and 100%, respectively.

2.3. Characterization

2.3.1. Weight loss of chitosan membrane

Weight loss of series of chitosan membranes were detected by weighting the membrane pieces before and after the hot water bath treatment. The weight loss of dissolvable part was calculated by following Eq. (1):

$$\text{Weight loss}\,(\%) = \frac{W_0 - W_1}{W_0} \times 100 \quad (1)$$

Where W_0 is the original dry weight of chitosan membrane and W_1 is the dry weight after the hot water bath treatment.

2.3.2. Morphological analysis

The morphological characterization including surface and cross section of membranes were performed by scanning electron microscopy (JSM-5900LV, JEOL, Japan). Samples were placed at cabinet drier for 24 h before observation. The cross section of membranes was obtained by cutting membrane with liquid nitrogen treatment.

2.3.3. Thermal properties

The thermal properties of various chitosan membranes were characterized by a differential scanning calorimeter (DSC, NETSCZ 200, Germany). The purified and dried samples were used for DSC test. Samples were first heated from 20 °C to 100 °C under nitrogen atmosphere at a heating rate of 10 °C/min, and reheated to 100 °C at the same rate after quenched to 20 °C, at last sample was cooled to 20 °C again at the cooling rate of 10 °C/min.

2.3.4. Water adsorption study

According to our previous report (Li et al., 2010b), water absorption of various chitosan nanopores membranes was measured by weighing the membrane pieces before and after placing in pH 7.4 phosphate buffer solution. Each chitosan membrane was cut into portions of 1 cm^2 (1 cm × 1 cm), weighed and subsequently immersed into phosphate buffer solution for 24 h. Finally, membranes were taken from the medium and weighed after removal of the surplus surface water using filter paper. The percentages of water absorption were calculated by following Eq. (2):

$$\text{Water absorption}(\%) = \frac{W_{24} - W_0}{W_0} \times 100 \quad (2)$$

Where W_{24} is the weight of wet membrane at 24 h and W_0 is the original film weight at zero time, respectively.

2.4. In vitro degradation test

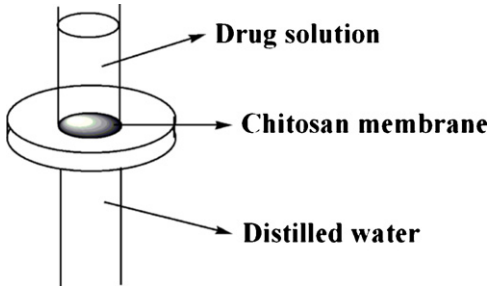
In vitro degradation study of chitosan membranes were performed in 5 ml phosphate-buffered solution (PBS, pH = 7.4) at 37 °C containing 1.5 μg/ml of lysozyme. Briefly, each chitosan membrane was first cut into portions of 1 cm^2 (1 cm × 1 cm), weighed and subsequently placed in degradation medium for periodical study. Lysozyme solution was refreshed daily to ensure continuous enzyme activity. Samples were removed at predetermined time interval (1, 2, 3 and 4 weeks), and rinsed with distilled water, finally dried under vacuum and weighed. The degree of *in vitro* degradation was expressed by the weight remaining as following Eq. (3):

$$\text{Weight remaining}(\%) = \frac{W_t}{W_0} \times 100 \quad (3)$$

Where W_0 is the dry weight before degradation test and W_t is the dry weight at predetermined time t.

2.5. Drug permeability test

The permeability test of two model drugs, vitamin B12 (non-ionic water-soluble drug) and sodium sulfamerazine (ionic water-soluble drug), were determined using a diffusion cell made of two glass compartments with 5 ml of capacity, as shown in Scheme 1. The membrane area was 0.785 cm^2. Chitosan membranes were first swollen in water solution for 2 h before the drug permeability test. The upside cell was filled with 0.5 ml drug solution with concentration at 5 mg/ml for vitamin B12 and sodium sulfamerazine, respectively. The downside cell was filled with 5 ml of distilled water with magnetic stirring at 200 rpm. And the system was kept at 37 ± 0.5 °C during the test. Aliquots of 1 ml from the



Scheme 1. Sketch of standard apparatus used for the drug permeability test.

Table 1
Thickness of chitosan nanopores membrane made from various weight ratio of chitosan and PEG (chitosan/PEG weight ratio ranging from 25/75 to 100/0).

Sample codes	Weight ratio (chitosan/PEG, w/w)	Thickness (μm)
S_1	25/75	19.9 ± 1.4
S_2	50/50	23.1 ± 1.8
S_3	75/25	27.6 ± 1.2
S_4	100/0	30.4 ± 0.8

downside cell were withdrawn at specific time intervals and subsequently replaced with 1 ml of fresh distilled water. The collected samples were stored at −20 °C for further analysis to establish the *in vitro* diffusion profile of drug. HPLC (SHIMADZU LC-20AD, Japan) and Lambda 35 UV–vis spectrometer (Perkin Elmer®, Inc, Singapore) were employed to determine the concentration of vitamin B12 and sodium sulfamerazine, respectively. HPLC analysis was performed on a reversed phase C18 column (4.6 × 250 mm, 5 μm, Grace, USA) at room temperature. The mobile phase was phosphate buffer (pH = 7.4)/methanol (70/30, v/v), filtered through a 0.22 μm Millipore filter and degassed prior to use. The flow-rate was 1.0 ml/min and the effluent was detected by SPD-M20A detector at 360 nm. UV–vis analysis was performed at 255 nm to determine the concentration of sodium sulfamerazine.

3. Results and discussion

3.1. Preparation of chitosan nanopores membrane

Previous studies have demonstrated that the pore size of membrane was greatly influenced the release behavior of islet encapsulation (Santos et al., 2008; Zhang et al., 2008). In this paper, PEG/chitosan blend film was first prepared by casting/solvent evaporation method, and then the PEG component was extracted by hot water to induce the nanopores structure of chitosan membrane. All obtained membranes were transparent after drying at room temperature (data not shown). However, the membrane with chitosan/PEG weight ratio of 100/0 and 75/25 had slightly yellowish color in water solution, while the membrane with chitosan/PEG weight ratio of 50/50 and 25/75 became opalescence as placing in water solution. Furthermore, it also clearly observed that the obtained membrane became more brittle as the PEG content was larger than 50%. As depicted in Table 1, the thickness of obtained chitosan membrane was ranging from 19.9 ± 1.4 μm to 30.4 ± 0.8 μm.

3.2. Characterization

3.2.1. Weight loss of chitosan membrane

PEG is a semi-crystalline polymer, which has been widely used as a porogen to prepare porous membrane by the phase-inversion method (Zeng et al., 2004; Courtois et al., 2006). Herein, PEG as the porogen agent was employed to develop a novel chitosan nanopores membrane. As shown in Fig. 1, we could find that the weight loss of membrane significantly decreased with the decrease of PEG content in blend film, indicating that the PEG component could be selected to dissolve from chitosan blend film (Zeng et al., 2004). Meanwhile, we speculated that the possible interaction between chitosan and PEG was not very strong, so that the selective dissolution of PEG component could be occurred. For pure chitosan membrane, it also found that there is also a certain weight loss after the hot water treatment, which might be induced by that a part of chitosan with the low molecular weight could slowly dissolve from the membrane (Zeng et al., 2004; Li et al., 2010b).

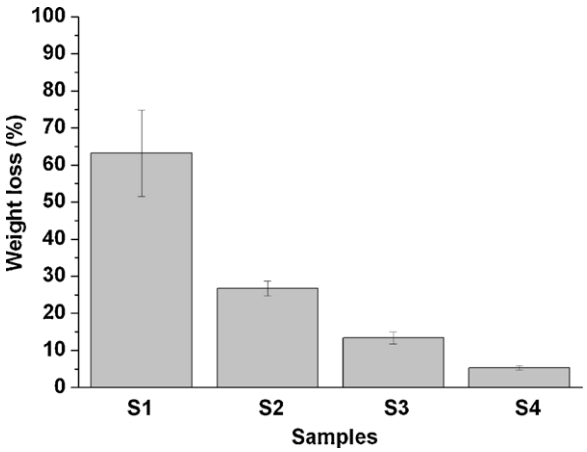


Fig. 1. Weight loss of various chitosan membranes after the extraction with hot water for 12 h.

3.2.2. Morphological analysis

Previous studies have demonstrated that the micro-pore chitosan membrane could be obtained by using PEG as the porogen (Zeng et al., 2004). However, the preparation of chitosan nanopores membrane using PEG as the porogen has not been reported. As depicted in Fig. 2, we could find that the membrane with chitosan/PEG weight ratio of 25/75 and 50/50 had obviously nanopores structure on both surface and cross-section, while the membrane with chitosan/PEG weight ratio of 75/25 and 100/0 did not show any pore structure no matter on surface or in the cross-section, which suggested that the suitable PEG content in membrane was very important for the pore structure of chitosan membrane. According to our previous study, due to the presence of interaction between PEG and chitosan, PEG component could not be dissolved completely from blend film (Li et al., 2010b). Therefore, no pore structure of membrane with chitosan/PEG weight ratio of 75/25 and 100/0 could be observed because of the incomplete dissolve of PEG component.

3.2.3. Thermal properties

The depression of melting point of a crystalline polymer blended with other polymers provides information about their miscibility. Both chitosan and PEG are crystalline polymers; pure PEG melts at about 65 °C and its glass transition temperature is around −60 °C. On the other hand, chitosan undergoes thermal degradation at 270 °C prior to melting (Kong et al., 2010). Thus, the melting point of PEG was monitored to understand the miscibility of the two polymers. As presented in Fig. 3, the melting temperature (T_m) of membranes increased from 53.1 °C to 59.3 °C as the PEG content increasing from 25% to 75%, indicating the presence of possible interaction such as hydrogen-bonding between chitosan and PEG (Li et al., 2010a). Meanwhile, it also implied that the PEG component could not be completely dissolved from the membrane.

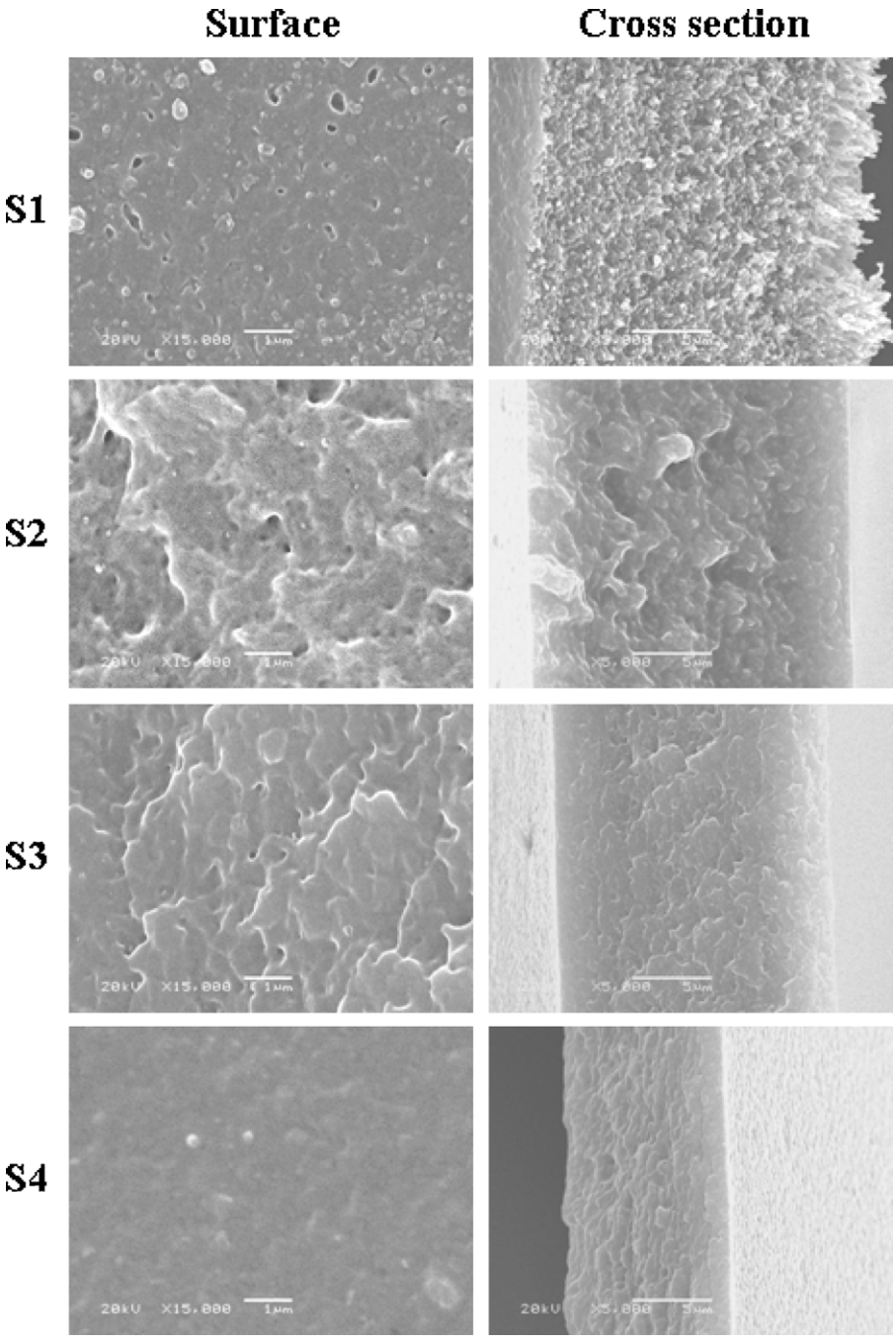


Fig. 2. SEM observation of surface and cross-section of various chitosan membranes.

3.2.4. Water adsorption

Fig. 4 depicts the water adsorption of various membranes, which indicated that all the prepared chitosan membranes have great ability of water adsorption ranging from $162.4 \pm 22.5\%$ to $321.5 \pm 6.5\%$. The dramatic increase in water adsorption with the increase of chitosan content in membrane was observed. As we all known that, chitosan containing primary amine ($-NH_2$) and hydroxyl group ($-OH$) cannot only increase its affinity to water but also form hydrogen bonds with water. Thus, dramatic increase in water adsorption of membrane might be explained by the higher chitosan content in membrane (Li et al., 2010a). As the previous report, water is absorbed into the membranes by two processes: water binding to the materials itself and water being retained in pore space (Chen et al., 2008). Although the obvious nanopores structure (SEM observation) could be found in the membrane with chitosan/PEG weight ratio of 25/75 and 50/50, the water adsorption was lower than that of membrane with chitosan/PEG weight ratio of 75/25 and 100/0. Therefore, we speculated that the improvement in the water adsorption mainly induced by the water bond to the chitosan itself rather than by the water being retained in pore space, so that the membrane with chitosan/PEG weight ratio of 75/25 and 100/0 could adsorb more water than that of membrane with chitosan/PEG weight ratio of 25/75 and 50/50.

3.3. In vitro degradation behavior study

In order to investigate the biodegradability of obtained membranes *in vitro*, membranes were placed in contact with the

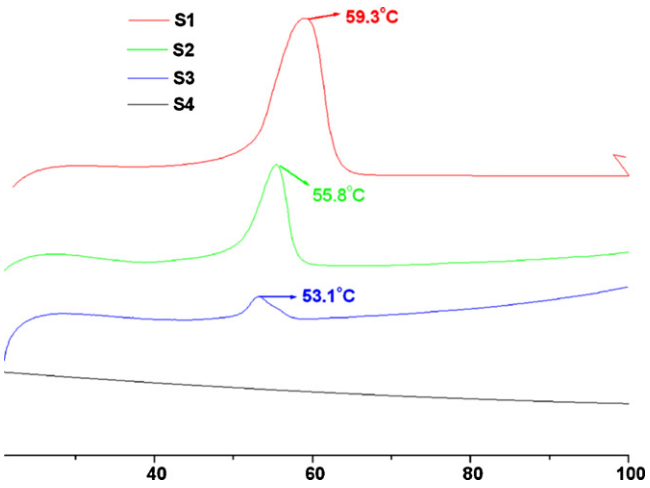


Fig. 3. Differential scanning calorimeter (DSC) spectra of chitosan membrane with various PEG contents (0% (S4), 25% (S3), 50% (S2) and 75% (S1)).

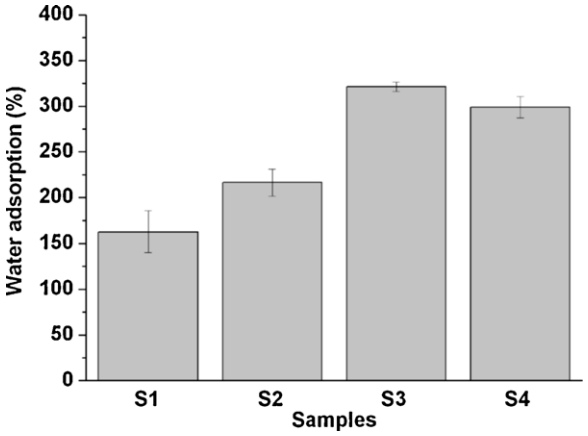


Fig. 4. Water adsorption of chitosan membrane with various PEG contents (0% (S4), 25% (S3), 50% (S2) and 75% (S1)).

lysozyme in PBS solution with concentration at 1.5 μg/ml. It is well known that, lysozyme is able to hydrolyze chitosan and hyaluronidase, which is found in plasma and in various tissues, is able to cleave hyaluronan (Picart et al., 2005). The degree of *in vitro* degradation was expressed by the weight remaining of membrane at specific time interval. As presented in Fig. 5, we could find that

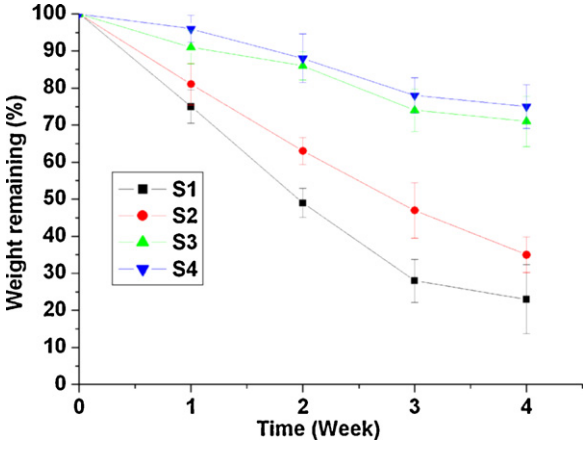


Fig. 5. *In vitro* degradation behavior of chitosan membrane with various PEG contents (0% (S4), 25% (S3), 50% (S2) and 75% (S1)).

the degree of *in vitro* degradation was great dependent on the chitosan content in membranes. The result showed that the membrane with chitosan/PEG weight ratio of 25/75 and 50/50 have weight remaining with about 23.5 ± 9.3% and 35.2 ± 4.8% respectively after 4 weeks of incubation, yet the weight remaining for membrane with chitosan/PEG weight ratio of 75/25 and 100/0 was approximately 71.1 ± 6.8% and 75.2 ± 5.9% respectively after 4 weeks of incubation. This might be attributed to the nanopores structure of membrane with chitosan/PEG weight ratio of 25/75 and 50/50, which leads to the lysozyme solution could easily diffuse from surrounding release medium to the inner of membrane, yet resulting in the faster degradation rate. Conversely, for the degradation behavior of membrane with chitosan/PEG weight ratio of 75/25 and 100/0, due to absence of any pore structure, the lysozyme solution could only contact with the surface of membranes instead of diffusing into the inner of membrane, so that the membrane was relatively stable. Although the well degradation behavior of membrane could be gained *in vitro*, the further studies on the *in vivo* degradation experiment should be performed to illustrate the destiny of membrane after *in vivo* application.

3.4. Drug permeability test

In order to evaluate the possibility of developed membrane for control the diffusion on inset molecule, two hydrophilic model drugs (vitamin B12 (non-ionic water-soluble drug) and sodium sulfamerazine (ionic water-soluble drug)) were employed to investigate the ability of control releasing of membrane. The standard apparatus used in for the drug permeability test is schematically shown in Scheme 1. It consists of a couple of glass screw caps and the membrane was fixed between the two glass screw caps. For the

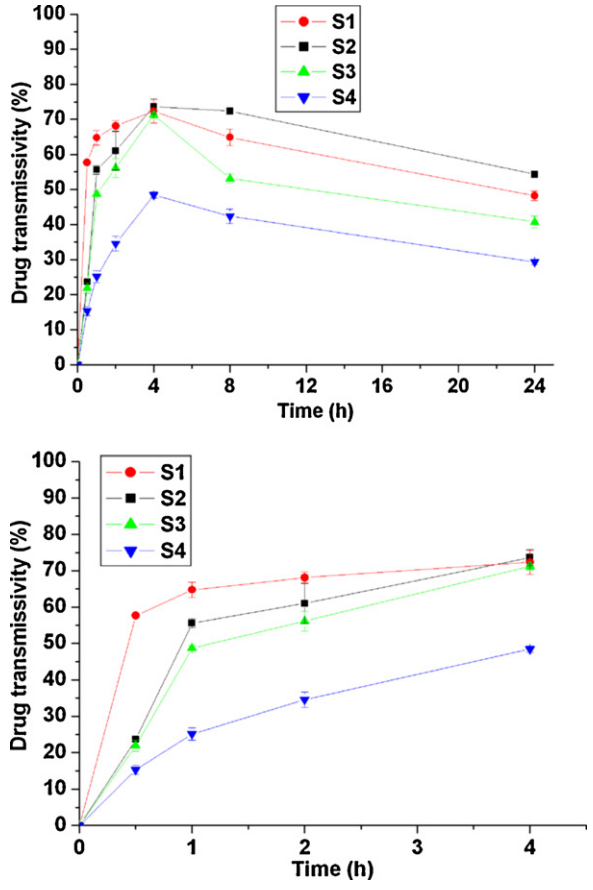


Fig. 6. Diffusion profile of sodium sulfamerazine with concentration at 5 mg/ml.

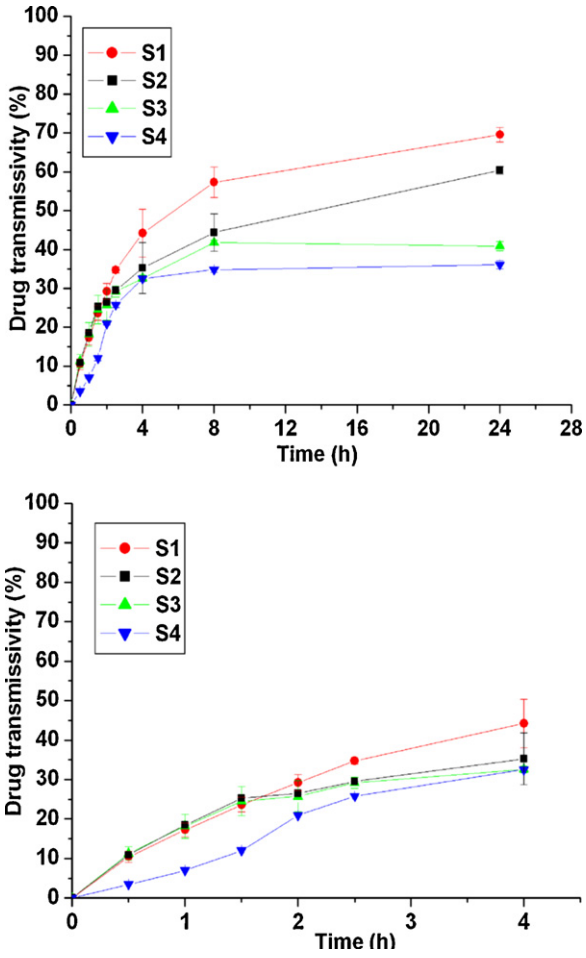


Fig. 7. Diffusion profile of vitamin B12 with concentration at 5 mg/ml.

drug permeability test, the drug water solution was placed on the upside of the container and the release medium was positioned on the downside of the container. And the air bubbles were avoided since they disturb the diffusion process. Fig. 6 depicts the diffusion profile of sodium sulfamerazine, and the results showed the sodium sulfamerazine could quickly transport the chitosan membrane to achieve the diffusion equilibrium within 4 h. However, the diffusion of sodium sulfamerazine was decreased after 4 h, which might be attributed to the instability of sodium sulfamerazine as exposed in the air and light. For the drug diffusion from the membrane, there are two distinctive diffusion mechanisms must be considered: the classic solution-diffusion through the dense polymer layer and the pore-flow mechanism. As in case of pure chitosan membrane (S4), due to absence of any pore structure, we proposed the relative slower diffusion rate might be resulted from the classic solution-diffusion mechanism, in accordance with the report of Akhgari et al. (2006). However, for the nanopores chitosan membrane, due to presence of numerous interconnected nanopores (S1 and S2), drug molecule which was much smaller than the membrane's pore size, could easily transport the membrane by the pore channel, yet resulting in the quick diffusion rate within 1 h. After 24 h, the final sodium sulfamerazine transmissivity reached about 60%, implying that the final diffusion equilibrium was achieved.

Fig. 7 depicts the diffusion profile of the vitamin B12. From Fig. 7, we could find that the vitamin B12 also could quickly transport all the chitosan membranes to achieve the diffusion equilibrium after 8 h test. There was significance difference of diffusion behavior between pure membrane and chitosan nanopores membranes. For chitosan nanopores membranes, the zero order model was best fitted with release kinetic in 4 h experiment with regression coefficients (R^2) of 0.996 (data not shown). Cohen and coworkers prepared micro/nano porous polyelectrolyte multilayer films for controlled drug release (Berg et al., 2006). A Fickian diffusion of the drug was observed with microporous films (300 nm to 2 μm pore size). However, nanoporous films displayed the zero-order release kinetics. For chitosan nanopores membrane, we also could find that the zero-order release kinetics of vitamin B12 (within 4 h) was obtained indicating that pore-flow mechanism might was main manner for vitamin B12 transport. Meanwhile, the diffusion rate of vitamin B12 was much lower than that of the sodium sulfamerazine, which might be attributed to the fact that the possible ionic interaction between chitosan and sodium sulfamerazine, thus facilitating the transport of sodium sulfamerazine. Furthermore, the various molecular weight of two model drugs might also influence the diffusion behavior.

4. Conclusion

In this paper, a novel chitosan nanopores membrane was successfully developed by selective dissolution of PEG component in chitosan membrane. According to the result of DSC test, the PEG component could not be completely dissolved from the blend film, indicating that there was presence the possible interaction between PEG and chitosan. With the SEM observation, we could found that the PEG content greatly influence on the structure of chitosan membrane, while the PEG content in membrane was larger than 50%, the obvious nanopores structure cloud be gained. According to the result of *in vitro* water adsorption test, it found that the obtained chitosan nanopores membranes had the great ability of water adsorption. *In vitro* degradation test revealed that the obtained chitosan nanopores membrane had the excellent biodegradation as placing in the lysozyme solution. *In vitro* permeability test suggested that the prepared chitosan nanopores membrane could well control the diffusion of model drugs (vitamin B1 and sodium sulfamerazine), implying that the chitosan nanopores membranes can potentially be used to the transport of drugs with controlled diffusion manner.

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