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5-Aminosalicyclic acid permeability enhancement by a pH-sensitive EVAL membrane

Ming-Jium Shieh, Ping-Shan Lai, Tai-Horng Young*

Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, Taipei 10016, Taiwan, ROC

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Abstract

A pH-sensitive membrane for colon-specific drug delivery was synthesized by the covalent bonding of glycine on the poly(ethylene-co-vinyl alcohol) (EVAL) membrane via isocyanation of surface hydroxyl groups and subsequent conversion to activated ester. The processes of surface modification would not change the membrane structure under the observable detection sensitivity of the scanning electron microscopy. Both the EVAL membrane and the glycine-immobilized EVAL membrane appeared as fairly dense structures almost without any holes existing in the membrane. Permeation of 5-amoinosalicylic acid (5-ASA) through the prepared membranes was studied at pH 2.0 and 7.4 at 37 °C. Regardless of the EVAL membrane and the glycine-immobilized EVAL membrane, the 5-ASA permeation at pH 2.0 was very conspicuously small, which agrees with the application of colon-specific drug delivery that drug is protected in the acidic environment. In contrast, the relative values of the 5-ASA permeation through the EVAL membrane and the glycine-immobilized EVAL membrane after 24 h at pH 7.4 and 2.0 were 6 and 41 times, respectively. Clearly, the significant increase in the 5-ASA permeability of the glycine-immobilized EVAL membrane is suitable for local treatment of ulcerative colitis. Furthermore, the mechanism of 5-ASA permeation through the EVAL membrane and the glycine-immobilized EVAL membrane at pH 2.0 and 7.4 was discussed. This study shows the 5-ASA permeability enhancement by the EVAL and the glycine-immobilized EVAL membrane in the neutral environment is ascribed to totally different mechanisms. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: EVAL membranes; pH-sensitive; 5-ASA permeation

1. Introduction

In the recent years, the development of drug delivery systems capable of selective release of drug in the colon has received much attention [1,2]. For example, 5-amoinosalicylic acid (5-ASA) was developed for treating ulcerative colitis [3], which is a disease of the colonic mucosa of unknown etiology. The optimum oral 5-ASA delivery system in such a case requires technologies that protect the drug through the stomach and small intestine and then distribute the drug to act topically in the lumen of colon. Hydrogels have been used widely for the preparation of drug delivery systems with physically or chemically modulated responses, because the water can freely enter the swollen gel to act as a transport agent for drug. Generally, the pH-sensitive hydrogels are based on anionic polymers, such as acrylic or methacrylic acids, which are water-impermeable at low pH, but get swollen by the change of pH of the medium [4–8].

To our knowledge, poly(ethylene-co-vinyl alcohol) (EVAL) has not been used so far for the preparation

* Corresponding author. Tel.: +886-2-23123456x1455; fax: +886-2-23940049.
E-mail address: thyoung@ha.mc.ntu.edu.tw (T.-H. Young).

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of the specific delivery of drugs to the colon for local treatment. Clinically, EVAL with appropriate biocompatibility was used in hemodialysis [9]. In our laboratories, EVAL membranes have been studied intensively over the past 10 years for different biomedical applications, such as plasma protein separation [10] and cell culture [11,12]. In the present work, we report the preparation of a glycine-immobilized EVAL membrane and the permeation of 5-ASA through the membrane at pH 2.0 and 7.4 for the development of a pH-sensitive membrane. We selected immobilization of glycine on the EVAL membrane because glycine is a natural and cheap amino acid. We demonstrated that the glycine-immobilized EVAL membrane is a potential material for the specific delivery of 5-ASA to the colon for local treatment.

2. Experimental

2.1. Materials

EVAL (E105A, containing ca. 56 mol% vinyl alcohol) was kindly supplied by Kuraray Co. (Japan) and was used as received. Ethanol was purchased from Showa Chemical (Japan). Water was double distilled and de-ionized before use. In this study, analytic-grade hexamethylene diisocyanate (HMDI, TCI, Japan), 4-dimethylaminopyridine (DMAP, Lancaster, England), *N*,*N*-dimethylformamide (DMF, Fisher, USA), disuccinimidyl oxalate (DSO, Fluka, Switzerland), glycine (Lancaster, England), and acetone (Tedia, USA) were used to modify the surface of EVAL membranes to result in the conversion of the membrane to highly pH-sensitive. Also, 5-aminosalicyclic acid (5-ASA) was purchased from Sigma (USA) and used as the model drug for colon-specific delivery.

2.2. Membrane preparation

EVAL was dissolved in a co-solvent containing 40 vol.% water and 60 vol.% ethanol to form a 15 wt.% EVAL homogeneous solution at 60 °C. This solution was dispersed uniformly on a glass plate (ca. 120 μm) at 60 °C, and then was immediately placed in an oven at 60 °C until the casting solution became a solid membrane. The thickness of the membrane was 16 ± 1 μm.

2.3. Surface modification

Prior to the chemical modification, HMDI, DMAP and DMF were dried on molecular sieves. Immobilization of glycine on the EVAL membrane was carried out in the following procedure, as reported previously [13]. As shown in Fig. 1, briefly, the EVAL membrane was immersed in HMDI (10% v/v) in the presence of DMAP (0.5% w/v) for 4 h and then was washed thoroughly with acetone. In this procedure, the hydroxyl group of membrane surface was isocyanated by HMDI. The isocyanated EVAL membrane was further immersed in 1 N aqueous NaOH solution to hydrolyze the surface isocyanate group. Subsequently, the amino group of the aminoalkylated membrane surface was converted to the activated ester in DMF containing a prescribed amount of DSO for 1 h. After the activation reaction, a glycine coupling reaction was performed in an aqueous solution of prescribed amount of glycine at pH 9 for 1 h. All the reactions were carried out at room temperature and under a nitrogen atmosphere. After the immobilization, the glycine-immobilized EVAL membrane was sufficiently washed with water for removal of adsorbed glycine.

2.4. Scanning electron microscopy

The morphology of the membrane was examined using a scanning electron microscope (SEM). The freeze-dried sample was gold coated and viewed with a SEM (S-800, Hitachi, Japan) at 20 kV.

2.5. Attenuated total reflection/Fourier transform infrared spectroscopy

The surface-modified EVAL membranes were analyzed by attenuated total reflection (ATR)/Fourier transform infrared (FTIR) spectroscopy employing a Nicolet Impact 410 spectrophotometer provided with an ATR device. All spectra were taken by 40 scans at a nominal resolution of 4 cm^{-1}.

2.6. X-ray photoelectron spectroscopy (XPS)

XPS analysis of the prepared membranes was performed using an ESCA PHI 1600 photoelectron spectrophotometer (Physical Electronics; USA) with

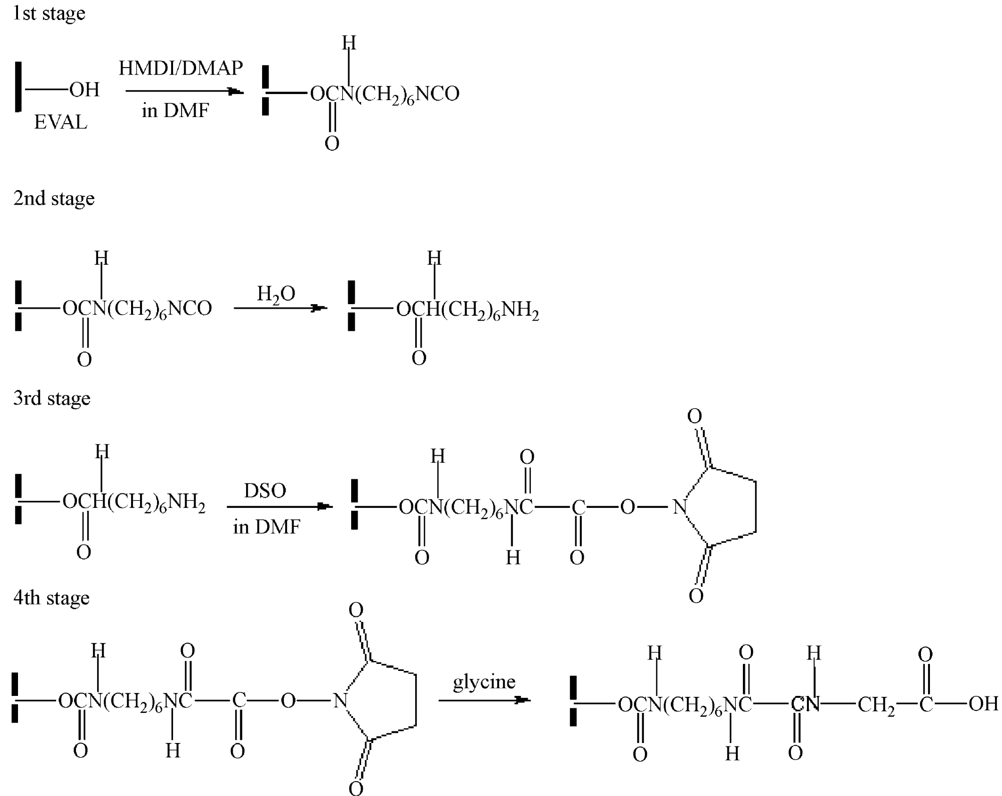

Fig. 1. Schematics of glycine immobilization on the EVAL membrane.

a magnesium anode (Mg Kα = 1253.6 eV). EVAL, aminoalkylated EVAL and glycine-immobilized EVAL membranes were irradiated with photons from a soft X-ray source with a well-defined energy. The angle between the sample and electron detector was 45°. The survey scan was from 0 to 1000 eV to find the atoms of surface. The relative atomic percentage of each element at the surface was estimated from peak areas using the standard software provided with the instrument.

2.7. Swelling behavior

A piece of a known weight membrane was immersed in buffered solution, which was present in a large excess compared to the amount of membrane at pH 2.0 and 7.4 at 37 °C. The swelling followed, and then the swollen membrane was removed from the buffer solution and weighed after the superfluous liquid was carefully wiped with tissue paper. The swelling equilibrium was established until no further weight increase was observed. Therefore, it allowed determination of the swollen membrane being equilibrium with buffered solution of different pH gravimetrically. The swelling degree has been expressed as a relative weight increase (gm of liquid/gm of dry membrane). All the data were averages of six independent experiments.

2.8. Neutralization titration

Neutralization titration studies were carried out to determine the dissociation constant (pK_a) of the glycine-immobilized EVAL membrane. The surface-modified EVAL membrane was immersed in water with some phenolphthalein used as an indicator. Then the surface carboxylic acid of EVAL membrane was neutralized with 0.036 N aqueous NaOH solution. It

allowed the determination of the dissociation constant, pK_a of the surface-modified EVAL membrane by plotting the titration curve [14]. The pK_a of the surface-modified EVAL membrane equal to the hydronium ion concentration at the half-neutralization point was determined to be 6.4.

2.9. Permeability of 5-ASA

Permeation by diffusion of 5-ASA through the prepared membranes was studied at pH 2.0 and 7.4 at 37 °C with 5-ASA concentration of 100 μg/ml. The diffusion experiments were carried out using a two-chamber, well-stirred diffusion cell with a volume of 40 ml each. The membrane was placed between two chambers with 5.3 cm^2 of available membrane area. The stirring speed in each chamber was maintained at approximate 600 rpm using independently controlled motors. Since the permeability measurement was performed under sufficient stirring, the diffusion resistance at the liquid–membrane interface was neglected [15].

The donor of the diffusion chamber was filled with buffer solution containing 5-ASA and the receptor was filled with buffer solution only. The permeability of 5-ASA through the EVAL membrane was monitored by periodically removing 200 μl samples from both the chambers and equal volume of drug-free buffer solution was refilled to avoid errors arising from the resulting volume variation. The concentrations of 5-ASA were analyzed by an UV spectrophotometer (Ultrospec 1000E, Pharmacia Biotech, Sweden) from the peak absorbency at 303 nm. Each experiment was repeated three times and the results were expressed as the mean of the three results. It was noted that the UV standard curve of 5-ASA in pH 2.0 and 7.4 is different because the environmental pH gives 5-ASA positive and negative charges at pH 2.0 and 7.4, respectively [16].

3. Results and discussion

3.1. Membrane morphology

Macroscopically, the EVAL membrane and the glycine-immobilized EVAL membrane appeared transparent. The microscopic analysis of the EVAL membrane showed fairly dense structure, almost without any holes existing in the membrane (not shown here). After surface modification, the glycine-immobilized EVAL membrane still showed similar dense morphology (Fig. 2). Thus, the processes of surface modification did not change the membrane structure under the observable detection sensitivity of the SEM.

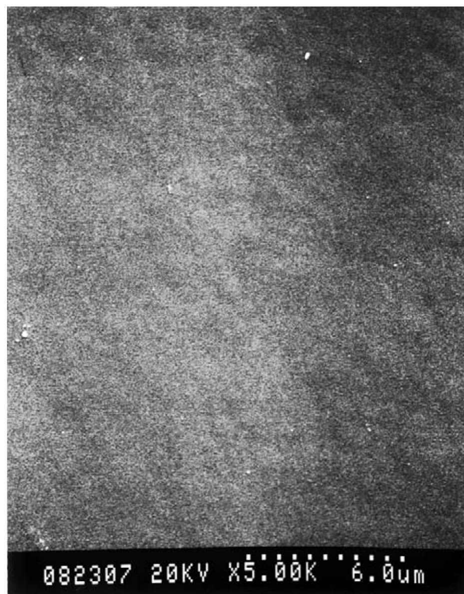

Fig. 2. The SEM photomicrograph of a glycine-immobilized EVAL membrane.

3.2. Surface modification

The chemical composition of the prepared membrane surface was analyzed by ATR/FTIR. Fig. 3 shows the ATR/FTIR spectra of EVAL membrane (A), modified EVAL membrane after isocyanation time of 4 h (B), and of 8 h (C). In contrast with spectra (A), there is a significant new peak at 1682.1 cm^{-1} in spectra (B) and (C), which could be attributed to the formation of the C=O bond. The C=O bond formation was due to the formation of urethane bonds between hydroxyl groups in EVAL membrane surface and isocyanate groups in HMDI, and the peak increased with an increase in the isocyanation time. Therefore, HMDI can be immobilized on the EVAL membrane by this method. However, in our system

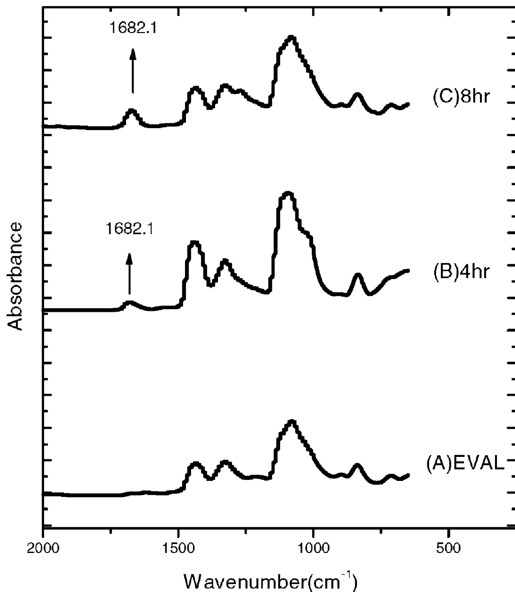

Fig. 3. ATR/FTIR spectra of EVAL membrane (A), modified EVAL membrane after isocyanation time of 4 h (B), and of 8 h (C).

a definite change for glycine immobilized on the EVAL membrane in infrared spectra was not identified due to overlap of absorption bands from urethane bonds between HMDI and EVAL. Therefore, the immobilization of glycine on the EVAL membrane was confirmed by XPS. Table 1 shows the surface ratio of N/C on the EVAL, aminoalkylated EVAL and glycine-immobilized EVAL membranes. Clearly, the surface nitrogen content was in the order of glycine-immobilized EVAL membrane > aminoalkylated EVAL membrane > EVAL membrane, suggesting glycine is immobilized on the EVAL membrane surface. In addition, the amount of glycine immobilized on the EVAL membrane was quantitatively determined by measuring the difference of the absorbency of the solution at 280 nm between that before and after the glycine coupling reaction.

Table 1
XPS results for EVAL, aminoalkylated EVAL and glycine-immobilized EVAL membranes

Membrane	N/C ratio
EVAL	0.005
Aminoalkylated EVAL	0.050
Glycine-immobilized EVAL	0.075

3.3. Swelling behavior

Fig. 4 shows the equilibrium swelling of EVAL and glycine-immobilized EVAL membranes at pH 2.0 and 7.4. The equilibrium swelling was considered to be the maximum hydration degree reached after immersion of six samples in the buffered solution. The equilibrium swelling degree of EVAL membranes did not change significantly at pH 2.0 and 7.4. However, the equilibrium swelling degree of the glycine-immobilized EVAL membranes was greatly increased from 8.9 ± 0.6 to 27.1 ± 2.6 when the pH value was changed from 2.0 to 7.4. Basically, the difference of the equilibrium swelling between these two membranes at pH 2.0 was relatively small, and even the degree of swelling of the glycine-immobilized membranes was slightly less than that for EVAL membranes. Therefore, the sharp change of equilibrium swelling between pH values at 2.0 and 7.4 for the glycine-immobilized EVAL membranes could be useful for the application of colon-specific drug delivery.

3.4. 5-ASA permeability

Fig. 5 shows the time dependence of the cumulative amount of 5-ASA permeation through EVAL membranes at pH 2.0 and 7.4 at 37 °C. The permeation rate of 5-ASA through the EVAL membrane at pH 2.0 was very conspicuously small. It is believed that dense membrane structure led to the low permeation rate of 5-ASA. However, the cumulative amount of 5-ASA permeation through EVAL membranes after 24 h increased about six folds for the pH value change from 2.0 to 7.4. Therefore, besides the membrane structure, there should be an additional repulsion between EVAL and 5-ASA at pH 2.0, which results in being more difficult for the permeation of 5-ASA through EVAL membranes. In fact, 5-ASA would carry positively charged quaternary ammonium groups at pH 2.0 [16], and the same charge would be contained in the protonated oxygen atoms in the hydroxyl group of EVAL by strong acids to give alkyloxonium ions. Therefore, 5-ASA would be electrostatically prevented from adsorbing onto the EVAL. In contrast, the observed increase in drug release suggests no or lower

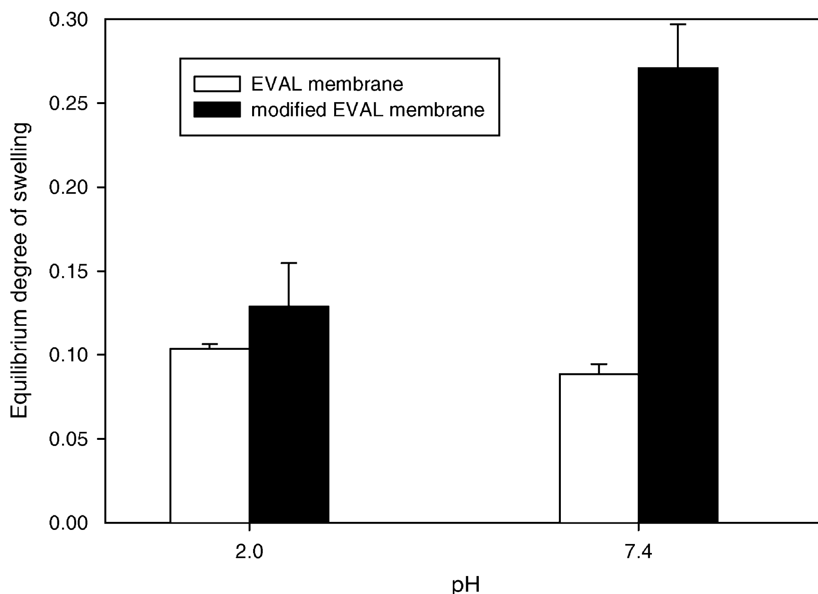

Fig. 4. Equilibrium degrees of swelling of EVAL and glycine-immobilized EVAL membranes at pH 2.0 and 7.4.

electrical repulsive force between EVAL and 5-ASA at pH 7.4. This is because, 5-ASA dissociates to a carboxylate ion, being the salt of a weak base with negative charge [16], but EVAL exists as neutral at pH 7.4. Therefore, the resistance for the permeation of 5-ASA through EVAL membranes is relatively low at pH 7.4 compared to that at pH 2.0.

Permeation by diffusion of 5-ASA through glycine-immobilized EVAL membranes was also studied at pH 2.0 and 7.4 at 37 °C and plotted versus time in

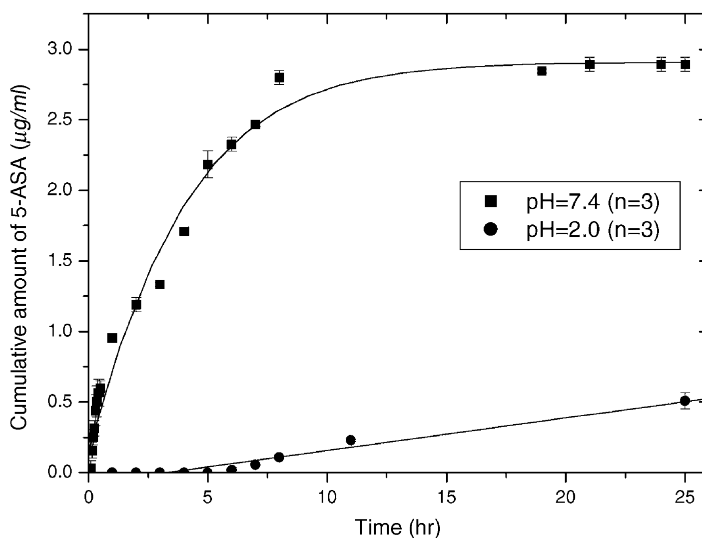

Fig. 5. Cumulative amount of 5-ASA permeation through EVAL membranes at pH 2.0 and 7.4.

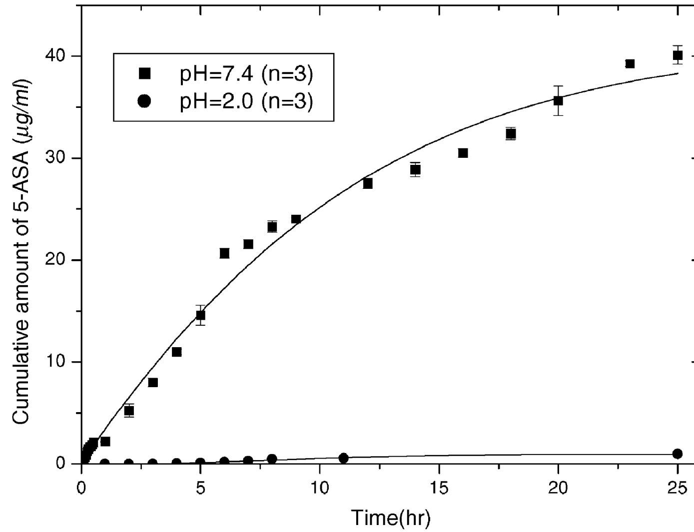

Fig. 6. Cumulative amount of 5-ASA permeation through glycine-immobilized EVAL membranes at pH 2.0 and 7.4.

Fig. 6. It is obvious that the cumulative amount of 5-ASA in the receptor at pH 2.0 was very low. As with the unmodified EVAL membrane data, the cumulative amount of 5-ASA permeated across modified and unmodified EVAL membranes at pH 2.0 appeared to be virtually the same, which agrees with the application of colon-specific drug delivery that drug is protected in the acidic environment. In contrast, there was a much higher cumulative amount of 5-ASA permeated at pH 7.4 relative to at pH 2.0. The cumulative amount after 24 h increased about nearly 41 folds as the pH value changed from 2.0 to 7.4. Such a high cumulative amount in the first 24 h suggests that the glycine-immobilized EVAL membrane is an alternative potential material for the 5-ASA rapidly permeating to the colon for local treatment. This indicates that the modified EVAL membrane can provide different permeability for 5-ASA at different pH values.

In addition, prior to immobilization of glycine on the EVAL membrane, permeation of 5-ASA through aminoalkylated EVAL membranes was studied at pH 2.0 and 7.4 at 37 °C (Fig. 7). As with the unmodified EVAL membrane data, the cumulative amount of 5-ASA permeated across aminoalkylated and unmodified EVAL membranes at pH 7.4 appeared to be similar, however, there was a higher cumulative amount of 5-ASA permeated at pH 2.0 relative to at pH 7.4. This result indicates that the aminoalkylated EVAL membrane and the glycine-immobilized EVAL membrane have different pH sensitivity for the permeability of 5-ASA, suggesting that immobilization of glycine on the EVAL membrane plays an important role on the application of colon-specific drug delivery.

According to the dissociation mechanism of glycine [17] and titration curve of the glycine-immobilized EVAL membrane (not shown here), the pK_a value of carboxylic group on the modified EVAL membrane surface is about 6.4. Therefore, similar to the unmodified EVAL membrane, 5-ASA could not easily permeate the modified EVAL membrane at pH 2.0 because of an electric repulsion between the cationic 5-ASA [16] and the positively charged groups contained in the polymers. Likewise, theoretically, 5-ASA also could not easily permeate the modified EVAL membrane at pH 7.4 because of an electric repulsion between the anionic 5-ASA [16] and the negatively charged groups contained in the polymers. At this point, however, it is evident that the modified EVAL membrane could provide improved permeability of 5-ASA in the neutral environment. Therefore, it is interesting to investigate the mechanism of the enhancement of 5-ASA permeability. There may be two possible mechanisms for the enhancement of 5-ASA permeability in

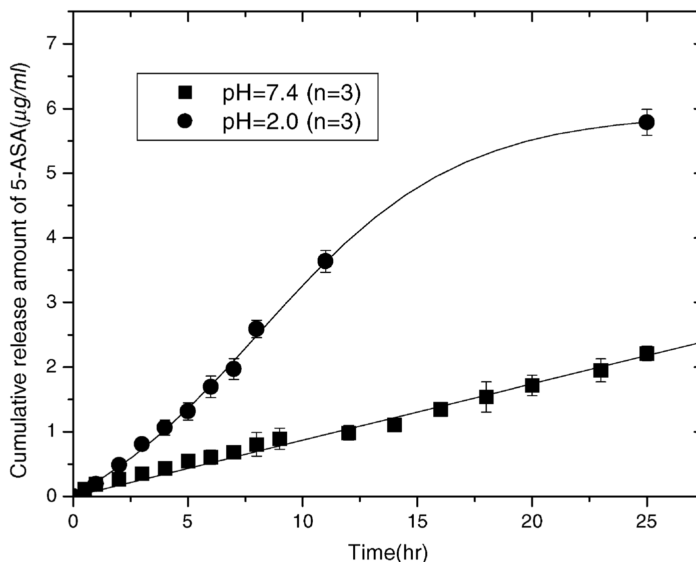

Fig. 7. Cumulative amount of 5-ASA permeation through aminoalkylated EVAL membranes at pH 2.0 and 7.4.

glycine-immobilized EVAL membranes. The first possibility is that the 5-ASA in the glycine-immobilized EVAL membrane has higher diffusivity in the neutral environment. The diffusivity of drug in the membrane can be calculated by using the time-lag technique [18] as follows:

$$D = \frac{L^2}{6t} \tag{1}$$

where L is the membrane thickness and t is the time-lag for drug appearing in the receptor. Therefore, the diffusivities of 5-ASA were determined for EVAL and glycine-immobilized EVAL membranes at pH 2.0 and 7.4 by measuring the time-lag (not shown here) of the magnified figures (Figs. 5 and 6). The diffusivities of 5-ASA for EVAL membranes at pH 2.0 and 7.4 are 2.7×10^{-11} and 2.0×10^{-9} cm^2/s, respectively. This is consistent with the resistance for the permeation of 5-ASA through EVAL membranes, which is relatively low at pH 7.4 compared to that at pH 2.0. Interestingly, the diffusivities of 5-ASA for EVAL and glycine-immobilized EVAL membranes did not change greatly at the same pH value. The diffusivities of 5-ASA for glycine-immobilized EVAL membranes at pH 2.0 and 7.4 are 3.4×10^{-11} and 2.4×10^{-9} cm^2/s, respectively. Therefore, the modification process used in this study did not change the diffusion resistance of 5-ASA in the membrane. It is suggested that the enhancement of 5-ASA permeability in the glycine-immobilized EVAL membrane at pH 7.4 cannot be ascribed to the higher diffusivity of 5-ASA in the modified EVAL membrane.

The second possible origin of the enhancement of 5-ASA permeability in the glycine-immobilized EVAL membrane is that the swelling degree of the glycine-immobilized EVAL membrane increased at pH 7.4. Since the modified membrane contains immobilized glycine as the ionizable unit and the pH 7.4 of the buffer solution is higher than the pK_a 6.4 of the modified membrane; fast ionization occurs and is accompanied by an increase in charge density. This leads to electrical repulsion between adjacent carboxylate ions and a transition of compact coiled polymer chains to extended ones to swell a dense membrane. Therefore, the surface modification in this study did not change the diffusivity of 5-ASA in the membrane, but significantly increased the permeability of 5-ASA through the membrane.

Based on the above discussion, the permeability of 5-ASA through the membrane at different pH values depends on the interaction between 5-ASA and membrane or the repulsion within the membrane. When 5-ASA and membrane carry the same charge, the electric repulsion between them will lead to low

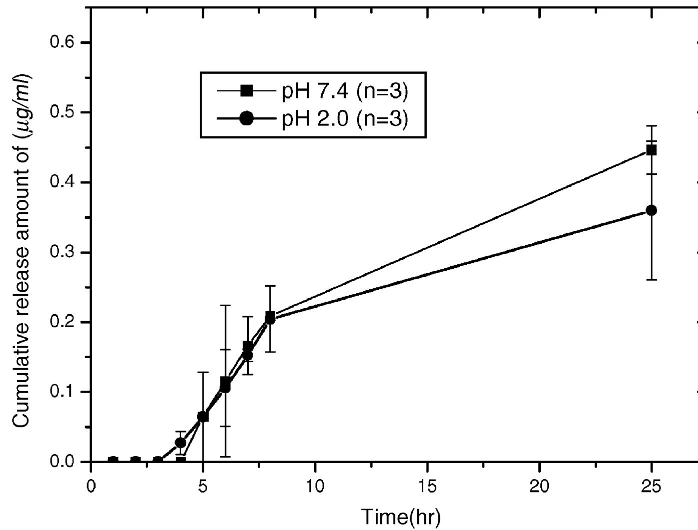

Fig. 8. Cumulative amount of 5-ASA permeation through the thick glycine-immobilized EVAL membranes ($81 \pm 1\,\mu m$) at pH 2.0 and 7.4.

diffusivity and permeability of 5-ASA. However, if there is higher charge distribution within the membrane, the membrane swelling due to electric charge repulsion between adjacent ions will dominate the membrane permeability. Thus, the 5-ASA permeability enhancement by the glycine-immobilized EVAL membrane at pH 7.4 is ascribed to the high membrane swelling degree. Similarly, a higher cumulative amount of 5-ASA permeated across aminoalkylated EVAL membranes at pH 2.0 relative to at pH 7.4 also can be ascribed to the membrane swelling degree. However, theoretically, the surface modification process used in this work cannot change the membrane swelling behavior because surface modification cannot affect the membrane bulk property. Therefore, it is reasonable to attribute such an increased swelling behavior to the immobilization of glycine on a very thin membrane. As shown in Fig. 8, when the membrane thickness was increased from 16 ± 1 to $81 \pm 1\,\mu m$, the glycine-immobilized EVAL membrane could not provide improved permeability of 5-ASA in the neutral environment. That is to say the membrane thickness will have an influence on the pH sensitivity of the modified EVAL membrane. Therefore, the surface modification process on a thin membrane used in this work can modify the membrane swelling property, which in turn can further modify the drug permeability.

In conclusion, due to no electrical repulsive force between EVAL and 5-ASA at pH 7.4, 5-ASA permeated through the EVAL membrane at pH 7.4 at a relatively faster rate than at pH 2.0. On the other hand, although there would be electrical repulsive forces between glycine-immobilized EVAL membrane and 5-ASA at pH 7.4, the permeability of 5-ASA is mainly controlled by the repulsive forces within the membrane, not by the repulsive force between membrane and 5-ASA. Therefore, the 5-ASA permeability enhancement by the EVAL and the glycine-immobilized EVAL membrane is ascribed to totally different mechanisms.

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References

[1] R.J. Mrsny, The colon as a site for drug delivery, J. Control. Release 22 (1992) 15.
[2] P.J. Watts, L. Illum, Colonic drug delivery, Drug Dev. Ind. Pharm. 23 (1997) 893.

[3] D.K.Y.B. Morrill, L.S. Eichmeier, R.C. Lanman, M.B. Lanman, D.H. Giesing, S.J. Weir, Pharmacokinetics of 5-aminosalicylic acid from controlled-release capsules in man, Eur. J. Clin. Pharmacol. 48 (1995) 273.

[4] B. Vazquez, M. Gurruchaga, I. Goni, E. Narvarte, J.S. Roman, A pH-sensitive hydrogel based on poly(ethoxy triethylene glycol) monomethacrylate, Polymer 17 (1995) 3327.

[5] E.O. Akala, P. Kopeckova, J. Kopecek, Novel pH-sensitive hydrogels with adjustable swelling kinetics, Biomaterials 19 (1998) 1037.

[6] H. Brondsted, C. Andersen, L. Hovgaard, Crosslinked dextran—a new capsule material for colon targeting of drugs, J. Control. Release 53 (1998) 7.

[7] H. Park, J.R. Robinson, Mechanisms of mucoadhesion of poly(acrylic acid) hydrogels, Pharm. Res. 4 (1987) 457.

[8] M.D. Blanco, O. Garcia, R.M. Trigo, J.M. Teijon, I. Katime, 5-Fluorouracil release from copolymeric hydrogels of itaconic acid monoester, Biomaterials 17 (1996) 1061.

[9] Y. Sakurada, A. Sueoka, M. Kawahashi, Blood purification device using membranes derived from poly(vinyl alcohol) and copolymer of ethylene and vinyl alcohol, Polym. J. 19 (1987) 501.

[10] D.T. Lin, L.P. Cheng, Y.J. Kang, L.W. Chen, T.H. Young, Effects of precipitation conditions on the membrane morphology and permeation characteristics, J. Membr. Sci. 140 (1998) 185.

[11] T.H. Young, J.H. Huang, S.H. Hung, J.P. Hsu, The role of cell density in the survival of cultured cerebellar granule neurons, J. Biomed. Mater. Res. 52 (2000) 748.

[12] T.H. Young, C.H. Yao, J.S. Sun, C.P. Lai, L.W. Chen, The effect of morphology variety of EVAL membranes on the behavior of myoblasts in vitro, Biomaterials 19 (1998) 717.

[13] A. Kondoh, K. Makino, T. Matsuda, Two-dimensional artificial extracellular matrix: bioadhesive peptide-immobilized surface design, J Appl. Polym. Sci. 47 (1993) 1983–1988.

[14] D.A. Skoog, D.M. West, Analytical Chemistry, 5th Edition, Saunders, London, 1990.

[15] K.A. Smith, C.K. Colton, E.W. Merrill, L.B. Evans, Convective transport in a batch dialyzer. Determination of the true membrane permeability from a single measurement, AIChE Symp. Ser. 64 (1968) 45.

[16] American Hostipal Formulary Service, Drug Information, American Society of Hospital Pharmacists, MD, 1993, p. 1838.

[17] A.L. Lehninger, D.L. Nelson, M.M. Cox, Principles of Biochemistry, 2nd Edition, Worth Publishers Inc., New York, 1993.

[18] J. Crank, The Mathematics of Diffusion, Oxford University Press, London, 1975.