Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 329 (2007) 103–109
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Characterization of chondroitin sulfate and its interpenetrating polymer network hydrogels for sustained-drug release

Shiu-Chih Wang [a], Bing-Hung Chen [a], Li-Fang Wang [a,*], Jenn-Shing Chen [b]

[a] *College of Life Science, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, ROC*
[b] *Department of Applied Chemistry, National University of Kaohsiung, Kaohsiung 811, Taiwan, ROC*

Received 10 May 2006; received in revised form 17 July 2006; accepted 21 August 2006
Available online 1 September 2006

Abstract

The goal of this work was to utilize the chondroitin sulfate (CS) based hydrogels for a drug delivery matrix. CS is a good structure/disease-modifying anti-osteroarthritis drug (S/DMOAD). However, the readily water-soluble nature limits its application as a solid-state drug-delivery vehicle. In this study, two methods were used to prepare CS hydrogels: directly crosslinking CS with poly(ethylene glycol) diglycidyl ether (EX-810) abbreviated as CS-EX or forming an interpenetrating polymer network named CS-EX-IPN. The CS-EX-IPN hydrogel was carried out by sequentially crosslinking reaction between CS and EX-810 in one phase and acrylic acid and di(ethylene glycol) diacrylate (DEGDA) as a counter phase. The swelling percent, cross-section morphology, and effective crosslinking density of hydrogels were characterized. The values of compression modulus and effective crosslinking density of CS-EX-IPN were ∼3.6-fold higher than CS-EX. We also characterized the release of a model drug, diclofenac sodium (DS) and a model protein, bovine serum albumin (BSA), from CS-EX and CS-EX-IPN. The similar release profiles of DS were observed in the both hydrogels but slower release rate of BSA occurred in CS-EX-IPN. The release profiles of the two model drugs fit in a diffusion-controlled mechanism. The D_{eff} values are in the order of 10^{-5} for DS and 10^{-7} for BSA.

Keywords: Diclofenac sodium; Bovine serum albumin; Chondroitin sulfate; Hydrogels; Interpenetrating polymer network

1. Introduction

Chondroitin sulfate (CS) is an important structural component in connective tissues and cartilage. It is a copolymer of D-glucuronic acid and sulfated *N*-acetyl-D-galactosamine in C4 or C6 and belongs to the glycosaminoglycans (GAGs), which are primarily located on the surface of cells or in the extracellular matrix. CS can be degraded by anaerobic bacteria, namely *Bacteroides thetaiotaomicron* and *B. ovatus*, which are resident in the large intestine (Saylers, 1979). This characteristic suggests that CS is a potentially good candidate for use as a colon-targeted drug carrier. Moreover, CS is a good S/DMOAD (structure/disease modifying anti-osteroarthritis drug). Orally administrated CS reduces the pain in osteroarthritis patients (Volpi, 2005), especially over long periods, in comparison with diclofenac sodium (Morreale et al., 1996; Ronca et al., 1998; Rovetta et al., 2004). Since the highly water soluble character limits its application as a solid dosage form in human physiological conditions, the use of CS in controlled release systems is still controversial (Rubinstein et al., 1992; Sintov et al., 1995, 1996). In previous work, we have prepared a polyelectrolyte complex of CS with Ca^{+} to retain CS in a solid form for use in a drug-delivery system. The release of diclofenac sodium has been notably sustained (Tsai et al., 2005). However, the complication arising from the physiological response to calcium ion during a carrageenan-induced edema test showed that the anti-inflammatory activity of CS–Ca ionotropic hydrogel was not as promising as nascent CS itself when diclofenac sodium was loaded. In the present study, we tried to find an optimum condition for the crosslinking reaction between the hydroxyl groups of CS and the epoxide rings of EX-810 to obtain a hydrogel with better dimensional stability.

Much effort has been devoted to developing pH-sensitive matrix systems for oral delivery. The hydrogel based on complexation between poly(*N*-vinyl pyrrolidone) (PVP) and polyacrylic acid (PAA) has been one of them because of the stabilization of hydrogels through the hydrogen bonds between

* Corresponding author. Tel.: +11 886 7 3121101 2217; fax: +11 886 7 3125339.
E-mail address: lfwang@kmu.edu.tw (L.-F. Wang).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.08.041

the carboxylic acid groups on PAA and carbonyl groups on the PVP (Jin et al., 2006). Herein, the interpenetrating polymer network (IPN) was also prepared using acrylic acid-*co*-*N*-vinyl-2-pyrrolidone crosslinked with DEGDA as the counterpart. We hypothesized that the CS hydrogel prepared by IPN results in a stronger gel and better pH sensitivity than CS-EX. In the present study, the physical properties between the two systems were characterized such as the sol percent, compression modulus, effective crosslinking density, and morphology. Two model drugs, diclofenac sodium and BSA, were tested to pursue their applicability in drug delivery systems.

2. Materials and methods

2.1. Materials

Sodium chondroitin sulfate (CS, oral grade, Lot No. OC-01009) was purchased from Maruha Corporation (Tokyo, Japan). Acrylic acid (AA), ammonium persulfate (APS), di(ethyleneglycol) diacrylate (DEGDA), *N*-vinyl-2-pyrrolidone (NVP), and poly(ethylene glycol) diglycidyl ether (EX-810, $M_w = 512$) from Aldrich (Milwaukee, WI) were used as received without further purification. Bovine serum albumin (BSA), chondroitinase ABC and diclofenac sodium (DS) were obtained from Sigma (St. Louis, MO). Potassium dihydrogenphosphate, disodium hydrogenphosphate, potassium chloride were obtained from Fluka (Buchs, Switzerland) and used as received. Dye reagent for protein assay was purchased from Bio-Rad Laboratories, Inc. (Hercules, CA).

2.2. Synthesis of CS-EX and CS-EX-IPN

The synthesis of CS-EX was adapted from (Jensena et al., 2002). Briefly, 30 wt% of CS in 1 M NaOH solution was crosslinked with EX-810, using a molar ratio of 0.6 relative to CS for 24 h at room temperature. In another experiment, the 0.5 molar ratio of acrylic acid (AA) and *N*-vinyl-2-pyrrolidone (VP) relatively to the repeating units of CS and 5 mol% of DEGDA with respective to AA and VP were added into the CS and EX-810 solution as stated above. The catalytic amount of ammonium persulfate and tetramethylethylene diamine was added to initiate the chain polymerization of vinyl monomers at room temperature.

2.3. Sol percent and swelling measurement

The as-prepared hydrogel was cut into 8 mm disks with a thickness of 3 mm and dried in a vacuum oven to a constant weight, W_p, and then extracted with 50 ml of DD water for 7 days and dried in a vacuum oven to a constant weight, W_e. Data were averaged for three disks and the sol percent was calculated by the following equation:

$$\text{sol percent}\,(\%) = \left[\frac{W_p - W_e}{W_p}\right] \times 100\% \quad (1)$$

The dried samples were swollen in 0.1 M phosphate buffer at pH 7.4, simulated gastric fluid, and DD water. The swollen samples were weighed after removal of excess surface liquid by light blotting with a laboratory tissue and returned to the buffer until no additional weight gain was observed. The swelling percentage was expressed as follows:

$$\text{swelling}\,(\%) = \left[\frac{W_s - W_e}{W_e}\right] \times 100\% \quad (2)$$

where W_s is the weight of the swollen sample and W_e is the weight of the extracted sample. Each experiment was done in triplicate.

2.4. Effective crosslinking density (Chiu et al., 2002)

Effective network density of hydrogels was calculated from the modulus of elasticity in mechanical compression at the equilibrium swelling state at pH 7.4 phosphate buffer for 1 day. The specimens were cut into an 8 mm diameter piece by cock borer. The equilibrium heights of swollen gel were recorded and the elastic modulus in compression was measured by a Hounsfield H5K-S universal testing machine (Hounsfield, England). The deformation rate was 0.1 mm/min and the extent of the deformation was set at 1/5 of the initial height of a swollen hydrogel. The equilibrium modulus of elasticity was determined from Eq. (3):

$$\frac{F}{A} = -G(\lambda - \lambda^{-2}) \quad (3)$$

where F/A is the compressive stress applied, G the modulus of elasticity, and $\lambda = l/l_0$, where l and l_0 are the equilibrium heights of deformed and original gels, respectively. The number of effective crosslinking density was obtained from Eq. (4):

$$\nu_e = \frac{G}{RT v_{2s}^{1/3} \langle a \rangle_0^2} \quad (4)$$

where ν_e is the effective network density in mol/m^3, R the gas constant, T the absolute temperature, v_{2s} the polymer volume fraction of a hydrogel at equilibrium swelling, and $\langle a \rangle_0$ is the isotropic dilation factor and is approximated to $v_{2r}^{1/3}$ (where v_{2r} is the polymer volume fraction at the relaxed state).

2.5. Scanning electron microscopy (SEM)

A sample equilibrated in double distilled water, pH 7.4 phosphate buffer or simulated gastric fluid for 24 h at room temperature, was taken off and frozen in liquid nitrogen immediately. The frozen specimen was fractured with a sharp scalpel to obtain a cross-sectional interior of the hydrogels that would reveal their interior structure. The samples were transferred to a freeze drier and freeze-dried to remove the imbibed water completely. The sample was observed using a JEOL-JSM5300 scanning electron microscope after coating with gold.

2.6. Measurement of in vitro drug release

Since BSA was hydrolysed in the basic condition during the preparation of hydrogels, BSA was loaded into hydrogels by absorption after the hydrogel extraction in DD water for 7 days.

The extracted and dried CS-EX and CS-EX-IPN disks were weighted as W_e and then immersed into a 10 wt% BSA solution in DD water for 2 days. The swollen hydrogels were taken out and dried in a vacuum oven at 60 °C until a constant weight was obtained as W_b. The BSA loading amount in the hydrogel disk was calculated from the weight difference between W_b and W_e. Each weighed disk was put into 5 ml of pH 7.4 phosphate buffer shaken at 37 °C. At a certain interval, the 5 ml solution was removed and replaced with a fresh phosphate buffer. The amount of released BSA was determined spectrophotometrically using a Bio-Rad protein assay. This method relies on binding a dye (Coomassie Brilliant Blue) to arginyl and lysyl residues of a protein. The BioRad dye solution was diluted by four-fold with DD water. To 50 μl of known or unknown BSA solution in 96 wells, 200 μl of Bio-Rad dye diluted reagent was added and mixed by shaking for 5 min. The absorbance was measured at 595 nm by an ELISA reader (Thermo, Finland). The calibration curve was obtained from measuring the known concentration of BSA, and covered a range of BSA concentrations from 0 to 150 μg/ml and remained linear in this range. Each data point was averaged from six wells.

In the other experiment, the DS concentration of 1 wt% relative to hydrogels was added before polymerization. The 8 mm diameter disk was cut and put into 10 ml of pH 7.4 phosphate buffer and shaken at 37 °C (Thermostat oscillator, Cherng Huei, RB-60, Taiwan). At a certain interval, the solution was removed and replaced with 10 ml of fresh phosphate buffer. The absorbance of DS was measured at 271 nm by a UV spectrophotometer (Shimadzu UV-160A, Japan). The DS release studies were carried out in triplicate. The results were presented in terms of the cumulative release as a function of time:

$$\text{Cumulative amount released (wt\%)} = \left(\frac{M_t}{M_\infty}\right) \times 100 \qquad (5)$$

where M_t is the amount of BSA or DS released from the hydrogels at time t and M_∞ is the amount loaded in the hydrogels.

The effective diffusion coefficients, D_{eff}, were calculated assuming that the drug release was controlled by a diffusion model, using the equation below (Larsen and Balazs, 1991; Leach and Schmidt, 2005):

$$\frac{M_t}{M_\infty} = 4\left(\frac{D_{eff}t}{\pi\delta^2}\right)^{1/2}, \qquad \frac{M_t}{M_\infty} < 0.6 \qquad (6)$$

where D_{eff} is the effective diffusion coefficient of the drugs in the hydrogels and δ is the hydrogel thickness after 1 day of swelling.

3. Results and discussion

3.1. Synthesis of CS-EX and CS-EX-IPN hydrogels

In the previous publication (Tsai et al., 2005), we had used 4 wt% CS in 0.1 M NaOH solution to react with EX-810 (a molar ratio of 0.2 relative to CS) in order to keep the solution soluble and form ionotropic complexation with Ca^{2+}. To prepare a CS-EX hydrogel, the higher CS concentration was needed to react with the EX-810 crosslinker. CS gels were not formed

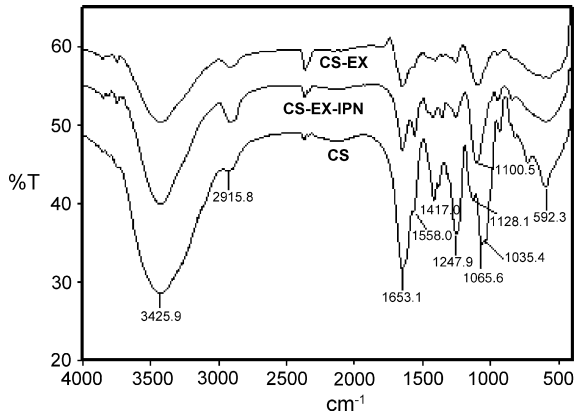

Fig. 1. FTIR spectra of CS, CS-EX, and CS-EX-IPN.

when the CS wt% was below 10 wt%. Gels disintegrated before they reached equilibrated hydration when CS wt% was in the range of 10–20 wt%. Therefore 30 wt% of CS was used to prepare CS-based hydrogels. The reaction between CS and EX-810 is not clear but is likely to involve the CS hydroxyl groups to act as nucleophiles and attack the epoxide ring on EX-810 as similarly stated in the crosslinking reaction between hyaluronic acid (HA) and EX-810 (Tornihata and Ikada, 1997). In order to make a stronger hydrogel, the gel was also prepared by an interpenetrating polymer network of CS and EX-810 in one phase; acrylic acid-*co*-*N*-vinyl-2-pyrrolidone crosslinked with DEGDA as another phase. Fig. 1 shows the FTIR spectra of CS, CS-EX, and CS-EX-IPN, respectively. The representative absorption peaks for nascent CS can be adapted from the publication (Cael et al., 1976): OH and NH stretching (3426 cm^{-1}), CH_2 stretching (2916 cm^{-1}), amide I (1653 cm^{-1}), COO^- antisymmetry stretching (1559 cm^{-1}), COO^- symmetry stretching (1417 cm^{-1}), SO_4^- related modes (1248 cm^{-1}), and C–O stretching (1088, 1074 cm^{-1}). The slight shift of C–O stretching into the higher frequency of 1100 cm^{-1} was observed. There is no significant difference in the IR spectra between CS-EX and CS-EX-IPN, except for the notable increasing intensity at 2916 and 1559 cm^{-1} because of the introduction of PAA.

3.2. Sol percent and swelling measurement

Soluble polymers, residue monomers and crosslinkers were extracted before the equilibrated study. The sol percents of CS-EX and CS-EX-IPN were 35.7 ± 2.7 and 15.9 ± 2.0, respectively. The IPN preparation of hydrogels reduced 20% of sol materials. The high sol percentage of CS-EX is in contradiction to the hydrogel made from hyaluronic acid (HA) and EX-810 (Tornihata and Ikada, 1997). In this report, when the water content was lower than 80%, the sol percent of crosslinked hydrogels was close to 0. The water content used to prepare CS-EX was 70%, lower than 80% reported in HA and EX-810. The different functionality of CS from HA is sulfate groups that may result in the higher water solubility of CS-EX if the two hydrogels have the same crosslinking density. The equilibrated swelling of CS-EX and CS-EX-IPN in D.D. water and the different pH values of 1.2 and 7.4 is listed in Table 1. As can be seen, the CS-EX-

Table 1
Swelling test of hydrogels at different conditions

Hydrogel	Swelling (%)		
	DD water	pH 1.2 solution	pH 7.4 solution
CS-EX	4346 ± 50	2052 ± 32	2265 ± 66
CS-EX-IPN	3030 ± 143	1419 ± 56	1615 ± 15

IPN hydrogel shows lower swelling percents than the CS-EX hydrogel in all three tested media. The highest swelling was obtained in DD water because CS is an anionic polysaccharide. In the buffer conditions, the counterions with CS may weaken the repulsion among polyelectrolytes, leading to a decrease in the swelling percentage. Due to the carboxylic functional groups of the hydrogels, the swelling at pH 7.4 is higher than that at pH 1.2.

3.3. SEM morphology

The hydrogels equilibrated in DD water, pH 7.4 phosphate buffer solution, or pH 1.2 simulated gastric fluid for 24 h were quickly frozen with the use of liquid nitrogen as the cryogen. Cryofracturing of the frozen specimens was done to reveal their interior structure. This method has been reported to conserve delicate structures of biological samples (Xin et al., 2004). Hence, we also assume that the cross section structures of our samples were not damaged by this technique. The microstructure of the hydrogels was investigated with a scanning electron microscopy (SEM). The hydrogels appeared as a macroporous matrix, as shown in Fig. 2. The large pores on the CS-EX hydrogel had diameters ranging from 50 to 200 μm in DD water, while smaller pores observed in pH 1.2 simulated gastric fluid showed diameters of 10–100 μm (Fig. 3). However, serious salt deposition on the interior pores was found in pH 7.4 phosphate buffer (Fig. 4), which interfered with the pore size measurement. In contrast, different interior morphologies on CS-EX-IPN were clearly observed. The interior pores formed in CS-EX-IPN were smaller than those in CS-EX. The smooth surface formed in pH 1.2 simulated gastric fluid had pore diameters of 15–33 μm. It is of interest to see that salt deposition on CS-EX-IPN was not as serious as in CS-EX. The prevention of salt deposition from the CS-EX-IPN surface may be attributed to its more hydrophobic character. The pore sizes were in the range of 10–50 μm. The CS-EX-IPN hydrogel had better three-dimensional structures and resulted in smaller pore sizes than the CS-EX hydrogel. Both hydrogels are pH-sensitive because of the different morphologies observed in the buffer solutions of pH 1.2 and 7.4. In conclusion, these water-filled channels observed in SEM are expected to be the conduits for the transport of guest molecules during loading and release.

3.4. Compression modulus and effective crosslinking density

The mechanical property of a hydrogel is strongly related to its swelling degree and reflects on the crosslinking density of the

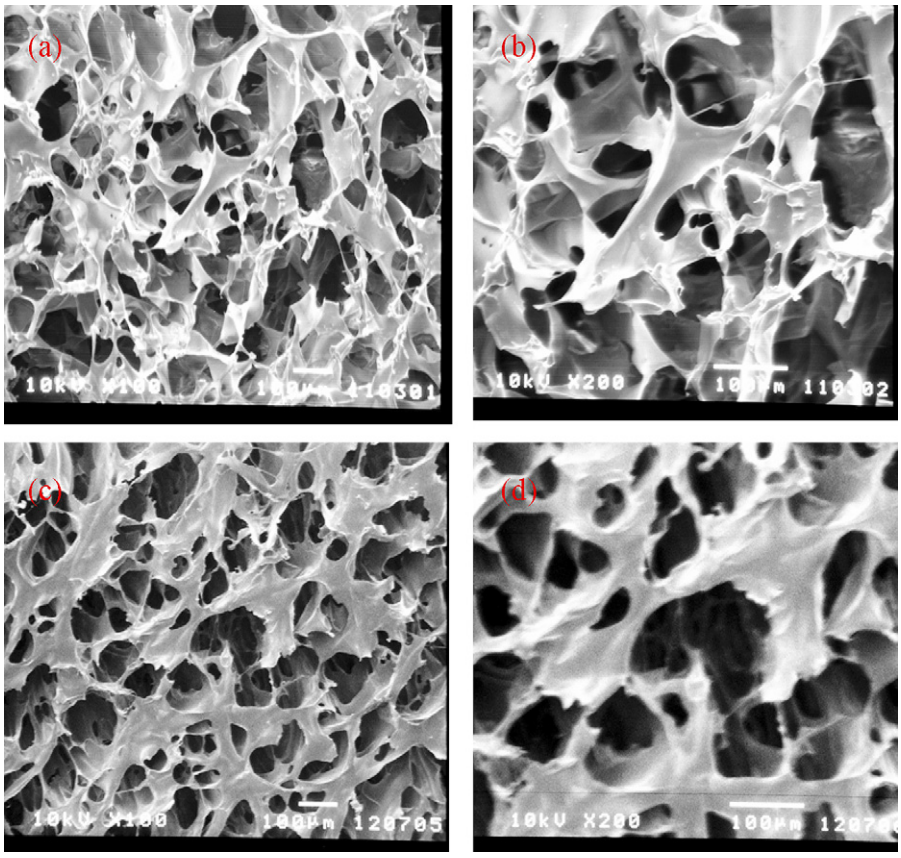

Fig. 2. SEM micrographs of CS-EX-IPN (a and b) and CS-EX (c and d) in DD water using two magnifications.

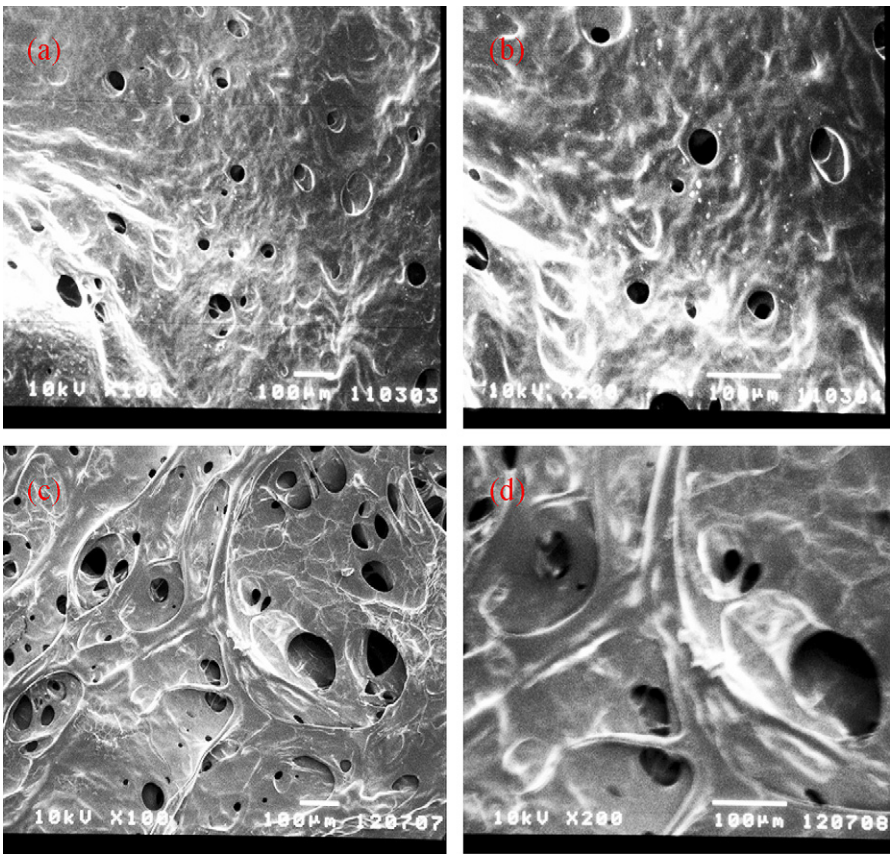

Fig. 3. SEM micrographs of CS-EX-IPN (a and b) and CS-EX (c and d) in the pH 1.2 solution using two magnifications.

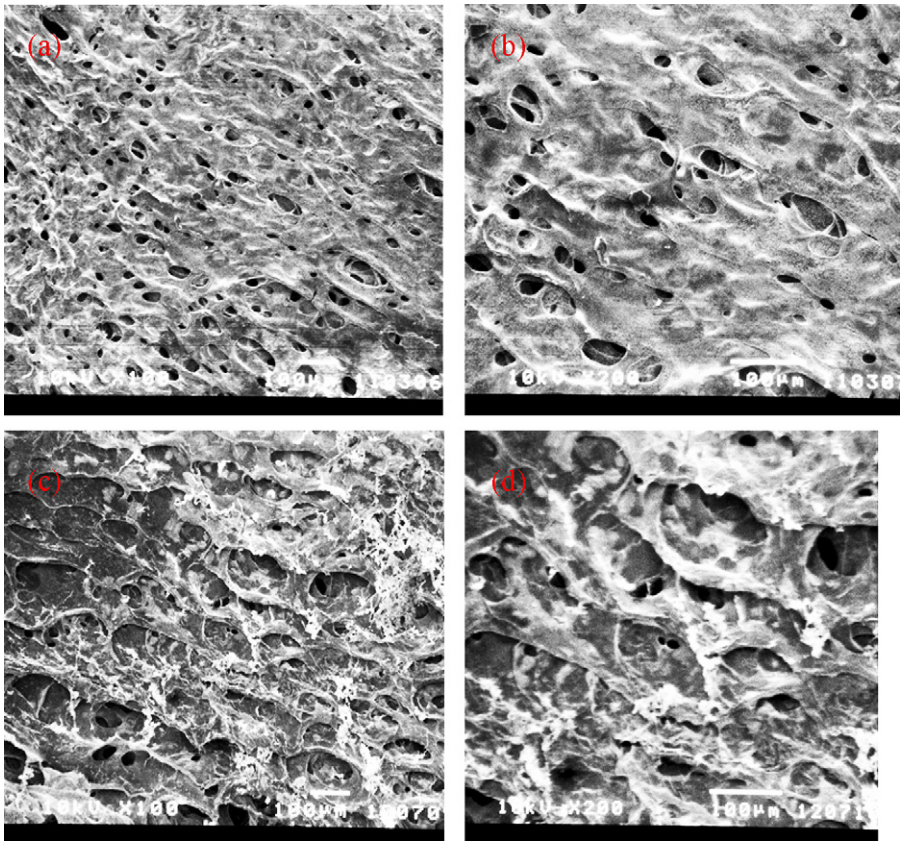

Fig. 4. SEM micrographs of CS-EX-IPN (a and b) and CS-EX (c and d) in the pH 7.4 solution using two magnifications.

Table 2
Compression modulus and effective diffusional coefficient of hydrogels measured at pH 7.4 phosphate buffer

Hydrogel	Compression moulus G ($\times 10^3$ N/m^2)	Crosslinking density ν_e (mol/m^3)	D_{eff}, BSA ($\times 10^{-7}$ cm^2/s)	D_{eff}, DS ($\times 10^{-5}$ cm^2/s)
CS-EX	1.24 ± 0.10	4.26 ± 0.33	5.40 ± 0.87	1.31 ± 0.29
CS-EX-IPN	5.92 ± 0.86	15.8 ± 3.64	3.67 ± 1.20	0.90 ± 0.11

hydrogels. The crosslinking density was calculated based on the theory of rubber elasticity (Peppas and Merrill, 1977), as network chains in all the hydrogels used in the experiment follow the Gaussium statistic model (Lee et al., 2004). The availability of the Gaussium model in our hydrogels was checked to see if the log plot between compression modulus (G) and swelling degree (Q) fell into a linear relationship (Lee et al., 2004). Fig. 5 shows a good linear relationship in the plot of log G versus log Q. Therefore the crosslinking density was calculated for the hydrogels swollen in the pH 7.4 solution using Eqs. (3) and (4). The crosslinking density of CS-EX-IPN was 3.6-fold larger than CS-EX as indicated in Table 2.

3.5. In vitro drug release

The BSA loading percents in the swollen hydrogels were ~8% and ~4%, respectively, to CS-EX and CS-EX-IPN. The low loading percents may be due to the electrostatic repulsion between BSA and CS, both having negative charges, or the large size of BSA itself. A slightly higher loading percent (9%) of BSA into CS-EX has been reported (Jensena et al., 2002). Fig. 6 compares the release profiles for BSA from CS-EX and CS-EX-IPN in the pH 7.4 phosphate buffer solutions. As shown, ~60% and ~80% of the BSA released from CS-EX-IPN and CS-EX occurred within 6 h, respectively. A lower amount of the BSA was released due to the lower swelling degree of CS-EX-IPN, as expected. In the other experiment, the release behavior of a small molecule, diclofenac sodium, was also tested. As seen in Fig. 7, a similar release rate was observed in CS-EX and CS-EX-IPN at the beginning and then a slightly faster release rate was found in CS-EX after 1.5 h. A 100% release amount of DS was obtained at ~5 h. A similar system of sequential interpenetrating polymer network (IPN) of poly(vinyl alcohol) (PVA) and poly(acrylic acid) (PAA) has been prepared and crosslinked

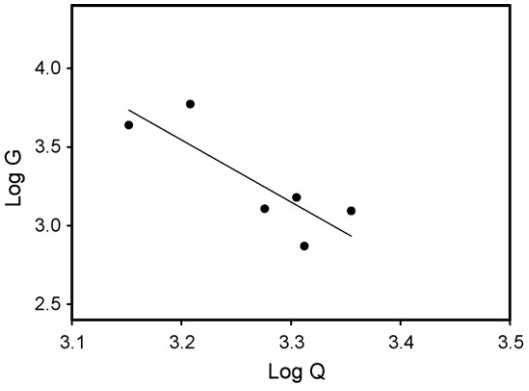

Fig. 5. log G vs. log Q plot of hydrogels (data are taken from the three tested media).

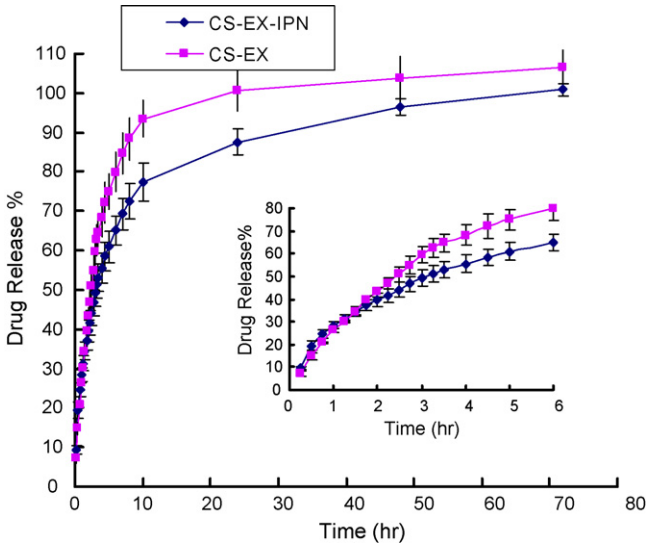

Fig. 6. Drug release profiles from the hydrogels for BSA.

with glutaraldehyde (Kurkuri and Aminabhavi, 2004). The formed pH-sensitive microspheres were used to deliver a model anti-inflammatory drug, diclofenac sodium (DS), to the intestine. The mass of the DS was released at ~200 min if the low concentration of GA was used. Our DS release behavior is analogous to the IPN system using PAA and PVA. However, the

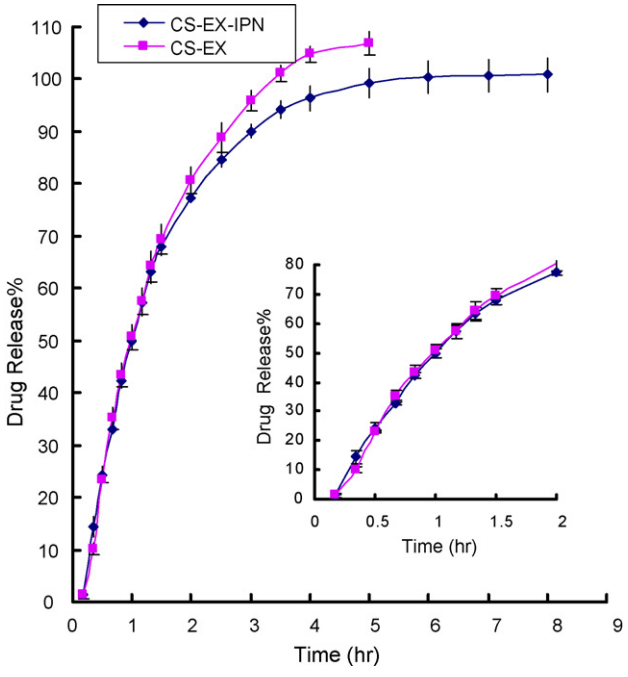

Fig. 7. Drug release profiles from the hydrogels for diclofenac sodium.

increase in the crosslinking density to sustain the drug release is insignificant in a small molecule like DS. Furthermore, the good water solubility of DS itself also accelerates the DS release. Hence, the prolonged release for protein drugs is better than small molecular drugs in these CS-based hydrogels.

The effective diffusion coefficients, D_{eff}, were calculated assuming that the drug released mechanism fits in the diffusion-controlled model. The data are listed in Table 2. The effective diffusion coefficients of a small molecule like DS are 100-fold larger than BSA. The D_{eff} values for BSA released from methacrylate-hyaluronic acid (GMHA) and acrylated polyethylene glycol (PEG) hydrogels are in the range of 0.85×10^{-7} to 4.54×10^{-7} cm/s and decreased with increasing concentrations of GMHA and PEG (Leach and Schmidt, 2005). The D_{eff} value of CS-EX, 5.40×10^{-7} cm/s, is larger than any systems for GMHA-PEG hydrogels but that of CS-EX-IPN is in the range. This implies that varying the vinyl composition of acrylic acid or vinyl pyrrolidone in the preparation of CS-EX-IPN may be an effective method to tune the drug release rate.

4. Conclusions

In this work, we demonstrated two methods to prepare the CS-based hydrogels. The hydrogel prepared by IPN showed a lesser degree of swelling, a higher compression modulus and crosslinking density than CS-EX. Diclofenac sodium released from the CS-EX and CS-EX-IPN hydrogels was rapid but BSA could be moderately controlled. The release profiles of both drugs fit in the diffusion-controlled mechanism. This preliminary result indicated that these CS-based hydrogels were more effective to sustain the release of large molecules like BSA.

Acknowledgement

We are grateful for financial support from the National Science Council of Taiwan (NSC93-2216-E037-001).

References

Cael, J.J., Isaac, D.H., Blackwell, J., Koenig, J., 1976. Polarized infrared spectra of crystalline glycosaminoglycans. Carbohydr. Res. 50, 169–179.

Chiu, H.C., Hsu, Y.H., Lin, P.J., 2002. Synthesis of pH-sensitive inulin hydrogels and characterization of their swelling properties. J. Biomed. Mater. Res. 61, 146–152.

Jensena, M., Hansena, P.B., Murdanb, S., Frokjaera, S., Florence, A.T., 2002. Loading into and electro-stimulated release of peptides and proteins from chondroitin 4-sulphate hydrogels. Eur. J. Pharm. Sci. 15, 139–148.

Jin, S., Liu, M., Zhang, F., Chen, S., Niu, A., 2006. Synthesis and characterization of pH-sensitive semi-IPN hydrogels based on hydrogen bond between poly(*N*-vinylpryrrolidone) and poly(acrylic acid). Polymer 47, 1526–1532.

Kurkuri, M.D., Aminabhavi, T.M., 2004. Poly(vinyl alcohol) and poly(acrylic acid) sequential interpenetrating network pH-sensitive microspheres for the delivery of diclofenac sodium to the intestine. J. Control Release 96, 9–20.

Larsen, N.E., Balazs, E.A., 1991. Drug delivery systems using hyaluronan and its derivatives. Adv. Drug Deliv. Rev. 7, 279–293.

Leach, J.B., Schmidt, C.E., 2005. Characterization of protein release from photocrosslinkable hyaluronic acid–polyethylene glycol hydrogel tissue engineering scaffolds. Biomaterials 26, 125–135.

Lee, K.Y., Bouhadir, K.H., Mooney, D.J., 2004. Controlled degradation of hydrogels using multi-functional cross-linking molecules. Biomaterials 25, 2461–2466.

Morreale, P., Manopulo, R., Galati, M., Boccanera, L., Saponati, G.B., Occhi, L., 1996. Comparison of the antiinflammatory efficacy of chondroitin sulfate and diclofenac sodium in patients with knee osteoarthritis. J. Rheumatol. 23, 1385–1391.

Peppas, N.A., Merrill, E.W., 1977. Crosslinked poly(vinyl alcohol) hydrogels as swollen elastic networks. J. Appl. Polym. Sci. 21, 1763–1770.

Ronca, F., Palmieri, L., Panicucci, P., Ronca, G., 1998. Anti-inflammatory activity of chondroitin sulfate. Osteoarthr. Cartilage 6, 14–21.

Rovetta, G., Monteforte, P., Molfetta, G., Balestra, V., 2004. A two-year study of chondroitin sulfate in erosive osteoarthritis of the hands: behavior of erosions, osteophytes, pain and hand dysfunction. Drugs Exp. Clin. Res. 30, 11–16.

Rubinstein, A., Nakar, D., Sintov, A., 1992. Colonic drug delivery: enhanced release of indomethacin from cross-linked chondroitin matrix in rat cecal content. Pharm. Res. 9, 276–280.

Saylers, A.A., 1979. Energy sources of major intestinal fermentative anaerobes. Am. J. Clin. Nutr. 32, 158–163.

Sintov, A., Di-Capua, N., Rubinstein, A., 1995. Cross-linked chondroitin sulfate: characterization for drug delivery purposes. Biomaterials 16, 473–478.

Sintov, A., Di-Capua, N., Rubinstein, A., 1996. Cross-linked chondroitin sulfate: characterization for drug delivery purposes. Biomaterials 16, 473–478.

Tornihata, K., Ikada, Y., 1997. Preparation of cross-linked hyaluronic acid films of low water content. Biomaterials 18, 189–195.

Tsai, M.F., Chiang, Y.L., Wang, L.F., Huang, G.W., Wu, P.C., 2005. Oral sustained delivery of diclofenac sodium using calcium chondroitin sulfate matrix. J. Biomater. Sci. Polym. Ed. 16, 1319–1331.

Volpi, N., 2005. Chondroitin sulfate for the treatment of osteroarthritis. Curr. Med. Chem., Anti-inflamm., Anti-allergy Agents 4, 221–234.

Xin, X., Borzacchiello, A., Netti, P.A., Ambrosio, L., Nicolais, L., 2004. Hyaluronic-acid-based semi-interpenetrating materials. J. Biomater. Sci. Polym. Ed. 15, 1223–1236.