Insolubilization of Sodium Chondroitin Sulfate by Forming a Semi-Interpenetrating Polymer Network with Acrylic Acid: A Potential Carrier for Colon-Specific Drug Delivery

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ABSTRACT: To reduce the highly hydrophilic property of chondroitin sulfate (ChS), a semi-interpenetrating polymer network (semi-IPN) of chondroitin sulfate/polyacrylic acid (PAA) was prepared as a drug carrier by crosslinking acrylic acid with diethyleneglycol diacrylate. The swelling properties of the semi-IPNs with different concentrations of crosslinking agent were correlated. The moisture sorption profiles were evaluated using differential thermal analysis. Ketoprofen was used as a drug probe to evaluate the performance of the drug released from the semi-IPN matrices. The prepared semi-IPNs demonstrated significant swelling reduction properties with both gastric and intestinal fluids compared with those of both the pure ChS and the ChSAA blend without the crosslinking agent. The amount of accumulated drug released from the semi-IPNs was less than 30 wt % at pH 1.2 and up to 80 wt % at pH 7.4. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 85: 114–122, 2002

Key words: chondroitin sulfate; swelling; semi-interpenetrating polymer network; polyacrylic acid; colon-specific drug delivery

INTRODUCTION

It has been recognized that polysaccharides are a potential candidate for colon-specific drug delivery because of the fermentation resulting from microbial enzymes in the large intestine and their ability to create physical and diffusion barriers, thus providing oral sustained release. In 1996 Hovgaard and Brøndsted[1] reviewed the current applications of polysaccharide for colon-specific drug delivery from three approaches: simple or macromolecular prodrugs, special coatings, and matrices.

Chondroitin sulfate (ChS) consists of D-glucuronic acid linked to *N*-acetyl-D-galactosamide.[2] Salyers and coworkers[3,4] reported that periplasmic enzymes are probably responsible for ChS breakdown: apparently an outer membrane receptor binds ChS and brings it into contact with enzymes such as chondroitin sulfatelyase. They also explained that chondroitinase ABC degraded the polysaccharide chain by β-elimination reaction, leading to the corresponding sulfated unsaturated disaccharides.[4–6] ChS is close to the endogenous substances, which can be degraded by colonic microflora. Moreover, many clinical studies demonstrate the anti-inflammatory activity of orally administered ChS on osteoarthritic patients with improvement of articular functions

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and reduction of pain.[7] Because of the many promising characteristics of ChS, it has been investigated as a matrix material for colon-specific drug delivery.[8–10] However, the readily water soluble natural ChS renders it unable to sustain a drug release. Rubinstein et al.[8–10] developed a method to reduce the hydrophilicity of ChS by crosslinking it with diaminododecane. They reported that crosslinked ChS-based matrices containing indomethacin retained the drug efficiently for more than 10 h at pH 7 and released it substantially through the biodegradation of ChS in colonic medium. Bourie et al.[11] modified Rubinstein's experiment by crosslinking ChS with different diaminoalkanes $H_2N—(CH_2)_x—NH_2$ (x = 6, 8, 10, 12). Instead of using indomethacin, these investigators used more water-soluble theophylline as a model drug. However, they concluded that crosslinking with diaminoalkane-type bifunctional compounds was not as promising as suggested in the work of Rubinstein et al.[8–10]

Interpenetrating polymer network (IPN)–structured hydrogels containing polysaccharides have been reported as a good method for drug-released matrices.[12,13] Here, the semi-interpenetrating polymer network of chondroitin sulfate and polyacrylic acid will be adapted to overcome the hydrosolubility of pure ChS. The merits of this design are (1) to maintain the structural conformation of ChS for preserving the characteristic degradability by bacterial residents in the colon, attributed to the unaltered chemical structure of ChS, and (2) to perform as a pH-sensitive hydrogel ascribed to the functional carboxylic acid groups. In this study, the swelling properties of semi-IPNs were studied in different contents of diethylene glycol diacrylate. Ketoprofen was used as a model drug to evaluate the performance of the drug released from semi-IPNs.

EXPERIMENTAL

Materials

Sodium chondroitin sulfate (ChS oral grade; Lot No. OC-97112) was obtained from Tohoku Miyagi Pharmaceutical Co. (Tokyo, Japan). Diethyleneglycol diacrylate and sodium persulfate were purchased from Aldrich (Milwaukee, WI). Ketoprofen and chondroitinase ABC were purchased from Sigma (St. Louis, MO). Acrylic acid (Janssen Chimica, Tokyo, Japan) was distilled before use. Four dextran standards from Polysciences (Warrington, PA) were used for GPC calibration.

Preparation of Semi-Interpenetrating Polymer Networks

ChS (1 g) was dissolved in 3 mL of double-distilled water. The solution was stirred at room temperature until clear. Acrylic acid (1 g) and diethyleneglycol diacrylate (DEGDA; 2.5, 5, 10, 20, and 30 wt % with respect to acrylic acid) were added, followed by the addition of 0.1 g of sodium persulfate. The solution was stirred to a viscous haze at room temperature (~ 2 h) and then placed into a water bath at 75°C for 30 min. The reaction mixture was poured into petri dishes and dried at room temperature under a hood to remove the solvent. The resulting hydrogels (henceforth denoted as ChSAA-2.5, ChSAA-5, ChSAA-10, ChSAA-20, and ChSAA-30) were cut into 10 × 10-mm squares with a thickness of 2–3 mm, and further dried in a vacuum oven (60°C) for 4 days. The specimens were kept in a dry box for further study. The semi-IPNs containing the drug were prepared by adding ketoprofen (0.5 g) dissolved in ethanol (1.5 mL) into acrylic acid monomer. Until the solution became clear, it was mixed with ChS solution and then polymerized as mentioned above.

In a separate experiment, a ChSAA blend without the addition of crosslinking agent was prepared using the method described above, except that double-distilled water (10 mL) was used and the solution was deoxygenated using an alternate connection of the polymerization reactor to a vacuum and nitrogen gas. Without this deoxygenated procedure, the polymerization would not have succeeded.

Thermal Stability

The thermal stability of PAA, ChS, and a mixture (1/1) of ChS and PAA was recorded on a Perkin–Elmer System 2000 FTIR (Perkin Elmer Cetus Instruments, Norwalk, CT). Samples (1 wt % in double-distilled water) were cast on CaF_2 crystals. Until most of the solvent was removed at room temperature, the samples were placed into a vacuum oven and dried at 60°C for 4 days. The spectra were recorded at different temperatures using a sample holder with a temperature controller. Sixteen scans were signal averaged at a resolution of 2 cm^{-1}.

The thermal stability of ketoprofen in semi-IPNs was determined using a Perkin–Elmer DSC

7. Samples of about 10 mg were heated from 50 to 150°C with a heating rate of 10°C/min under N_2 purge.

Swelling Measurements

A 10 × 10-mm piece of square film (~ 200 mg) was swollen in simulated gastric test solutions, pH = 1.2 (0.2*M* HCl/0.2*M* glycine) or in simulated intestinal buffer solutions, pH = 7.4 (0.2*M* phosphate buffer). At predetermined time intervals, the films were weighed after removal of excess surface liquid by light blotting with a laboratory tissue and returned to the buffer media until no additional weight gain was observed. The swelling percentage was expressed as follows:

$$\text{Swelling } (\%) = \frac{W_s - W_d}{W_d} \times 100\%$$

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

Moisture Sorption by DSC

A differential scanning calorimeter (DSC 7, Perkin–Elmer), controlled by a Perkin–Elmer TAC 7 with an automatic cooling apparatus, was used to record the DSC thermograms of the semi-IPNs. Indium and water standards were used as calibrants. To determine the rate of water uptake, samples (~ 10 mg) were accurately weighed and placed into aluminum volatile sample pans into which double-distilled water (10 μL) was added. Samples were held at room temperature for different time periods before analysis. After moisture sorption, the samples were scanned at 5°C min^{-1} from −30 to 30°C. The enthalpies corresponding to the quantities of absorbed water and total water were determined separately and the fraction of absorbed water to total water was calculated.

Drug Stability by HPLC

Because ketoprofen was added before polymerization, the stability of ketoprofen was checked. HPLC analysis was conducted on a Hewlett–Packard 1050 system (Hewlett–Packard, Palo Alto, CA) containing a quaternary pump, on-line degasser, HP 1100 photodiode array detector, and equipped with a C_{18} column (HP Spherisob ODS-2 column). A 250-mg piece of ChSAA-10 containing 20 wt % ketoprofen was extracted with ethanol (20 mL) for 24 h. The supernatant solution was filtered through 0.45-μm filters (Millipore, Bedford, MA), diluted to the suitable quantity, and eluted with volume ratios of phosphoric acid solution (pH 3.0) : acetonitrile, equal to 55 : 45 (vol %) at 1 mL/min. The eluent was monitored at 254 nm and the column oven was set at 50°C.

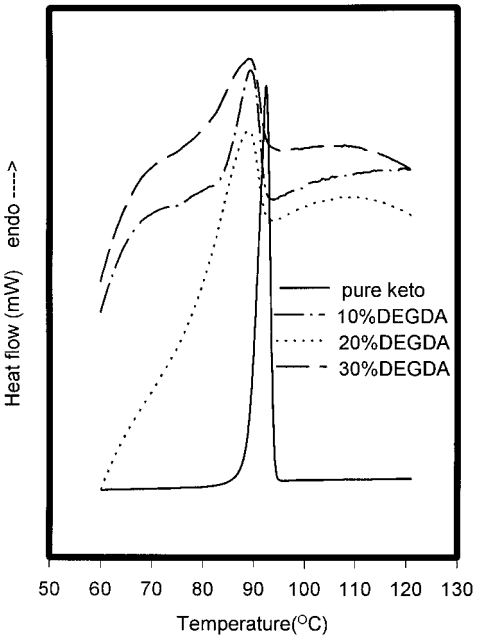

Figure 1 DSC thermograms of ketoprofen in semi-IPNs and in its pure form.

Drug Release

Drug release studies were carried out using the USP basket (apparatus I) method with a dissolution tester made by Hsiang Tai Machinery Industry Co. (Taipei, Taiwan), at a speed of 100 rpm. A 10 × 10-mm piece of square film (~ 200 mg) containing about 40 mg ketoprofen was immersed in 900 mL of simulated gastric acid solutions (pH = 1.2) or simulated intestinal buffer solutions (pH = 7.4 at 37 ± 0.1°C). At a certain interval, 1 mL of solution was withdrawn and replaced with an equal volume of the same dissolution medium. The percentage of drug release was calculated by measuring the UV absorbance in a Shimadzu UV-160A spectrophotometer (Shimadzu, Japan) at 260 nm. The cumulative percentage of ketoprofen released was calculated, using the standard calibration curve for ketoprofen. All experiments were performed in triplicate.

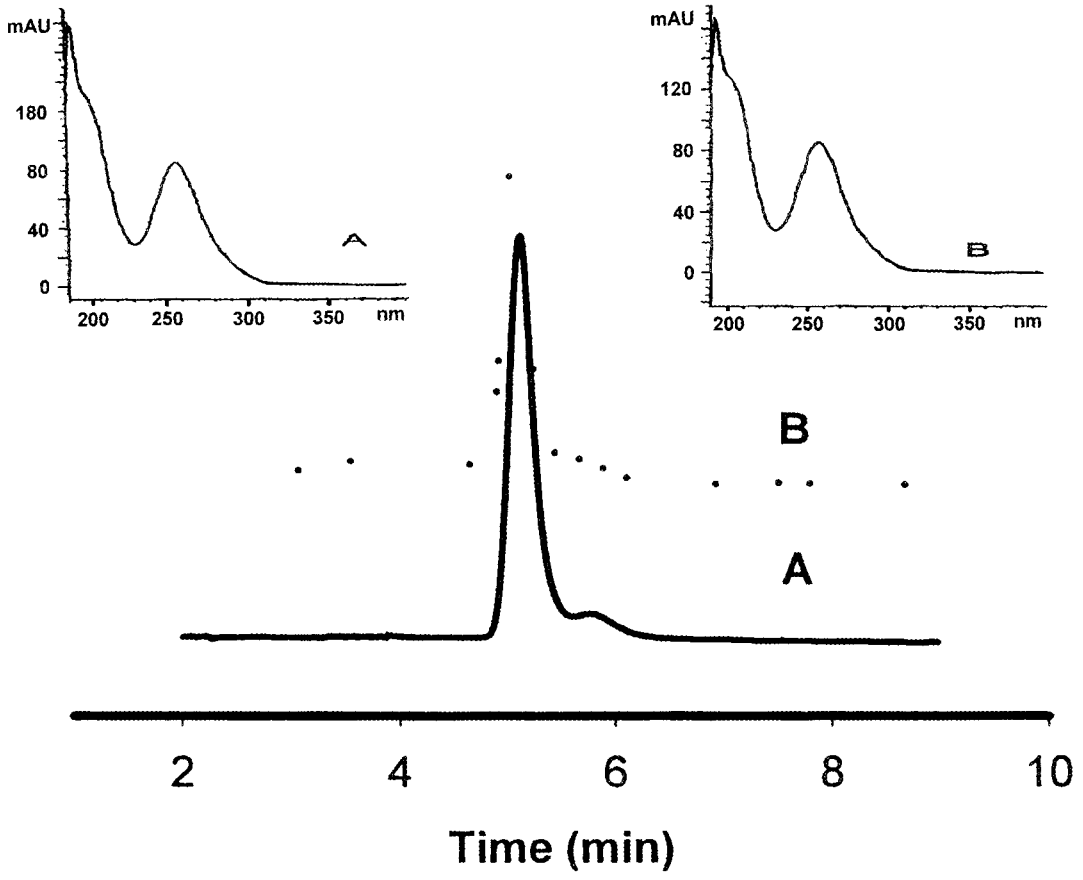

Figure 2 HPLC profiles of (A) pure ketoprofen and (B) the extracted ketoprofen from ChSAA-10 containing 20 wt % of the drug.

The drug release from ChSAA-10 was also performed in another way because of the high cost of chondroitinase ABC. ChSAA-10 (4 mg) was suspended in 40 mL of pH 7.4 phosphate buffer solutions. At certain intervals, 1 mL of solution was withdrawn and replaced with an equal volume of the same dissolution medium. The solvent was removed by free drying *in vacuo,* and ketoprofen was extracted with 1 mL of acetone for 3 h. Supernatant acetone solutions were then withdrawn and evaporated by a rotary vapor evaporator. The drug was dissolved in ethanol (1 mL) and analyzed as stated previously. A similar experiment was carried out in the presence of 2 units of chondroitinase ABC. All experiments were performed in triplicate.

Gel-Permeation Chromatography

The molecular weight was measured using a Waters Model 501 pump (Waters Associates, Milford, MA) equipped with a Shodex sugar KS-G and a KS-804 column and a HP 1047 refractive index detector. An aqueous solution of 0.05*M* NaCl was used as a mobile phase at a flow rate of 1 mL/min at 50°C. The column setting was calibrated using four monodisperse dextran standards.

RESULTS AND DISCUSSION

Stability of Drug and Semi-IPN Matrices

Because the drug was added before the polymerization of semi-IPNs, the thermal stability of ketoprofen was confirmed. Figure 1 shows the thermograms of ketoprofen carried out by DSC. The melting peak of pure ketoprofen was recorded at 91°C and depressed into 88°C in semi-IPN matrices. Although the peak attributed to the melting peak of ketoprofen became broad in the semi-IPNs, it is readily believed that most of the ketoprofen was stable during the polymerization of acrylic acid at 75°C. Moreover, the stability of the

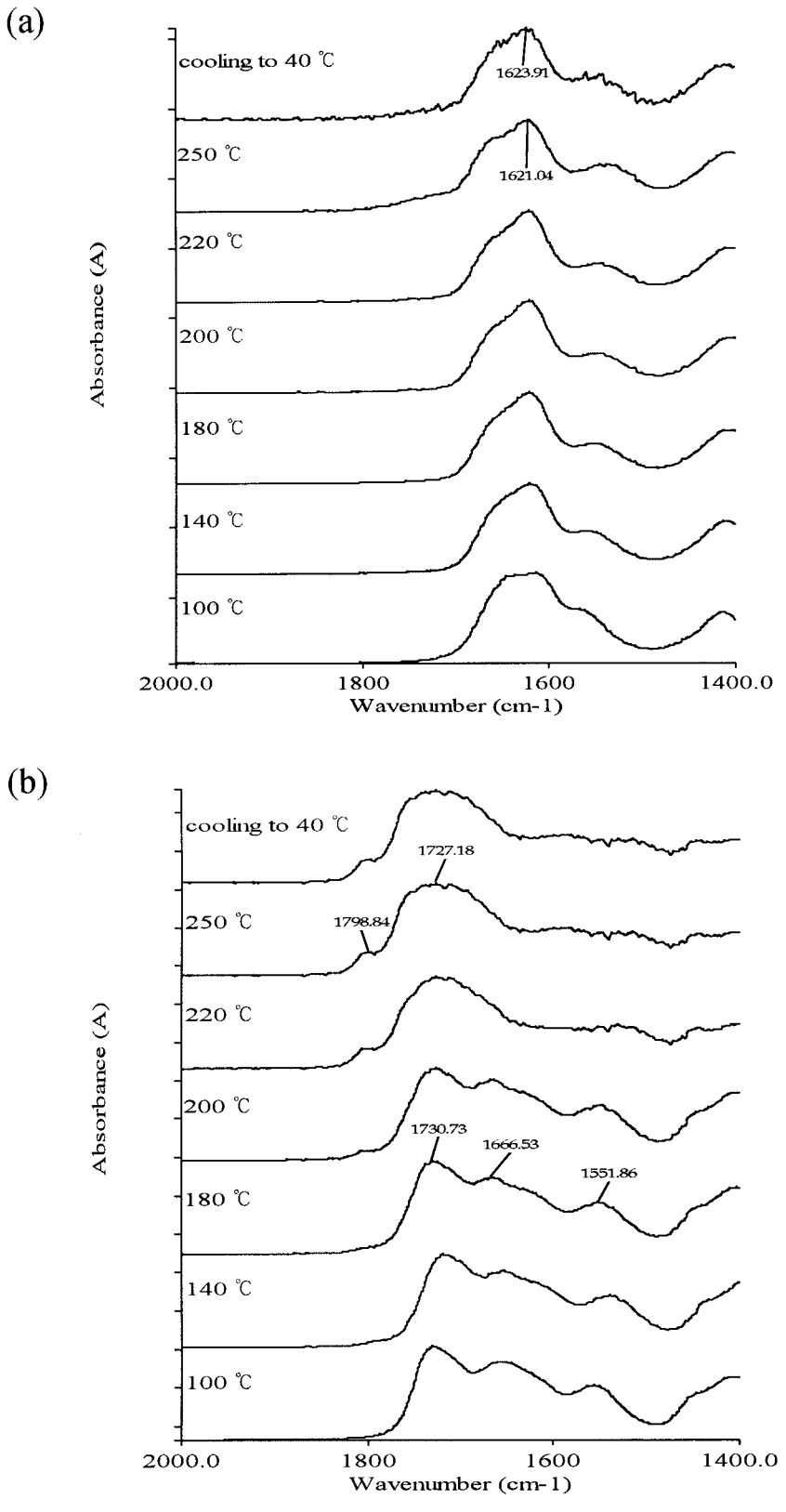

Figure 3 FTIR spectra in the region of 2000–1400 cm^{-1} for (a) pure ChS and (b) ChS–PAA at different temperatures.

drug after polymerization was also confirmed by HPLC. The extracted solution of ChSAA-10 containing 20 wt % of ketoprofen by ethanol was recorded. The same retention time of the extracted solution as that of pure ketoprofen (curve A) appeared at 5.05 min, as shown in Figure 2. The converted UV spectrum was also analogous to that of pure ketoprofen, as shown on the upper right corner of Figure 2. Both DSC and HPLC analyses provided the same result, that ketoprofen is stable during the polymerization. The recovered drug concentration calculated from the standard calibration curve of ketoprofen was about 85.6%.

PAA was stable at temperatures below 180°C and formed anhydride thereafter by FTIR studies.[14] As shown in Figure 3(a), the FTIR spectra of ChS remain intact at temperatures up to 220°C and become noisier, when the temperature was increased into 250°C. This is caused by the oxidation of ChS, given that an exotherm at 225°C was observed in the DSC thermogram (data not shown). After introducing PAA to ChS at the weight ratio of 1 to 1, the frequency at 1666–1620 cm^{-1} attributed to the amide ν_{co} vibration band of ChS disappeared at 220°C, as indicated in Figure 3(b). The anhydride peak shown at 1799 cm^{-1} decidedly appeared at 200°C. This explained that ChS became less stable after blending with PAA at high temperatures.

Swelling Properties of Semi-IPN Matrices

The swelling properties of semi-IPNs were investigated at both pH 1.2 and pH 7.4 buffer solutions, to simulate the gastric and intestinal conditions, and the results are shown in Figure 4(a) and (b), respectively. The swelling percentages at pH 7.4 are almost an order higher compared with those of the same semi-IPNs at pH 1.2, implying their swelling property is highly dependent on pH values. Obviously, the higher content of crosslinking agent resulted in the lower degree of swelling in both conditions.

The water sorption was also evaluated by DSC studies. The scans display a broad endotherm between −20 and −5°C, which was attributed to the water absorbed by the sample, besides the main endotherm ascribed to the melting of free water around 0°C. The weight percentages of absorbed water to the total water of these semi-IPNs calculated from DSC thermograms are plotted in Figure 5. The peak intensity of the absorbed water increased with time and became

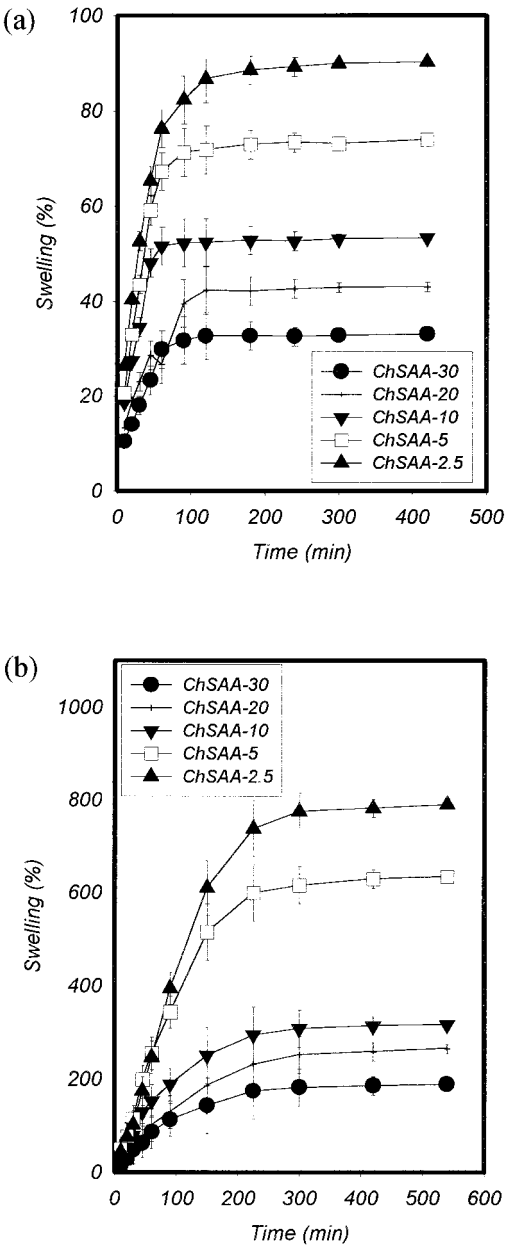

Figure 4 Swelling percentage for semi-IPNs in (a) pH 1.2 and (b) pH 7.4 buffer solutions.

saturated at around 155 min. This could be correlated to the swelling behavior, where the uptake of water was at around 200 min. The weight percentage of absorbed water to the total water decreased with the amount of the crosslinking agent in semi-IPNs. The slopes of curves in Figures 4(b) and 5, implying the rate of water absorption, are slower in the DSC study than those of swelling measurements in the pH 7.4 phosphate buffer solutions.

Drug Release from Semi-IPN Matrices

The stability of the drug load in the GI tract was tested *in vitro* in pH 1.2 buffer solutions. The fast drug-released profiles of ChS and ChSAA and less than 30% of the drug released from ChSAA semi-IPNs are observed in Figure 6(a). The released ketoprofen from pure ChS and ChSAA prepared without DEGDA illustrated a burst effect and those from semi-IPNs reached about 80% of their drug load within 7 h at pH 7.4, as shown in Figure 6(b). The higher content of DEGDA results in the lower rate of drug release, apparently because of the decrease of swelling property. However, the drug-sustained property was not so successful as that reported in the literature.[8–10] The high concentration of drug release in these semi-IPNs might be attributable to the lower molecular weight of noncrosslinked polyacrylic acid or the leaching out of ChS, which was primarily confirmed by GPC analysis. The solution withdrawn from the drug-dissolution media showed an identical molecular weight distribution peak with pure ChS. The number- and weight-averaged molecular weights of ChS, calculated from the calibration curve of dextran standards, were 58,200 and 95,300, respectively. The evidence of the leaching of ChS (including noncrosslinked PAA) was also observed in the swelling studies. The weight loss was about 37.8% for ChSAA-30 and 55.3% for ChSAA-5 after the samples were redried at the end of the swelling experiments.

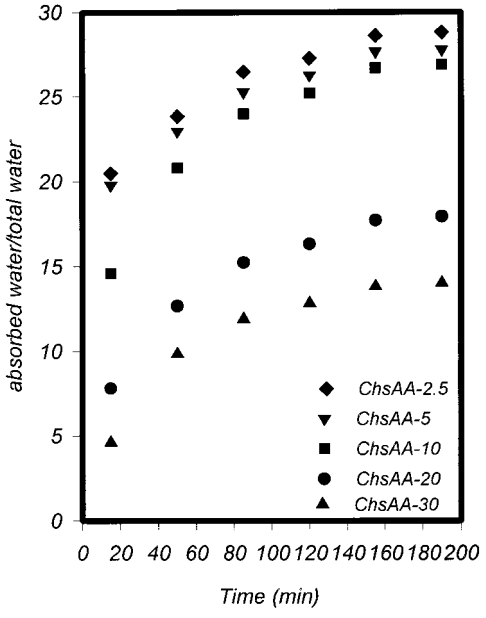

Figure 5 Weight percentage of absorbed water to total water versus time for semi-IPNs.

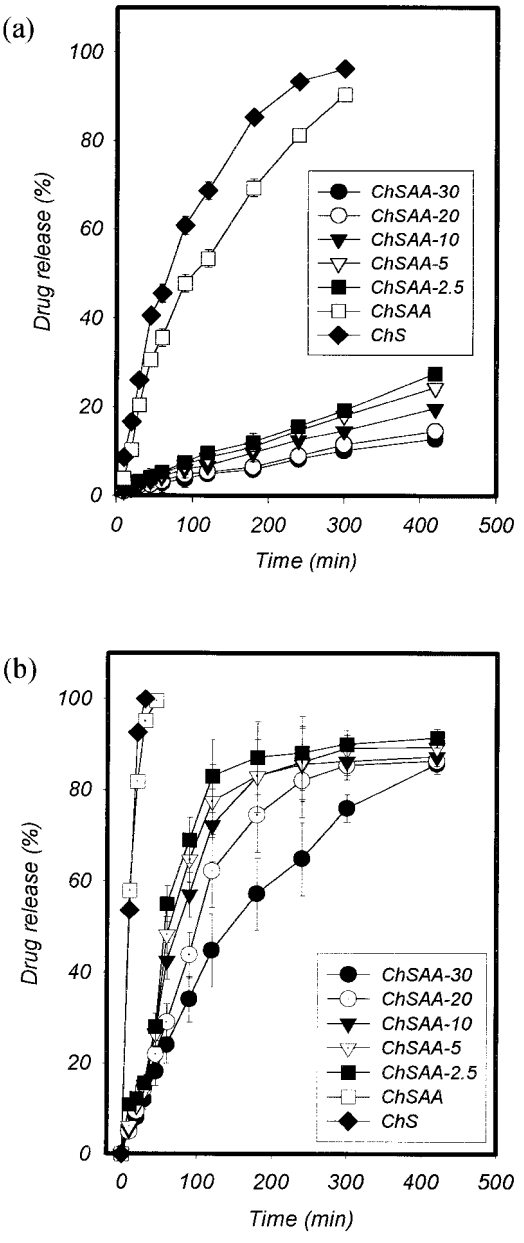

Figure 6 Ketoprofen-released profiles from semi-IPNs in (a) pH 1.2 and (b) pH 7.4 buffer solutions.

The mechanism of drug released from the polymer matrices was simulated using a simple equation,[15] $M_t = kt^n$, where M_t is the percentage of drug released at time t, k is a release rate constant, and n is an exponent of release. For a planar geometry, the value of $n = 0.5$ signifies a Fickian water-diffusion mechanism, whereas $n = 1.0$ indicates an erosion mechanism. For $0.5 < n < 1.0$, the diffusion mechanism is anomalous,

Table I Kinetic Data of Ketoprofen Released from Semi-IPNs Using the Simple Equation

Semi-IPN	ChSAA-2.5	ChSAA-5	ChSAA-10	ChSAA-20	ChSAA-30
n	0.93	1.13	1.12	1.00	0.89
k	0.94	0.39	0.38	0.48	0.62
r^2	0.88	0.97	0.99	1.00	0.99

where both diffusion and polymer erosion control the overall rate of water uptake. The drug-release profiles at the initial 100 min at pH 7.4 phosphate buffer were used to fit the simple equation. The results are summarized in Table I, where the n values of five semi-IPNs are close to 1. This was also the evidence that the drug-release mechanism was likely to be the erosion control.

Given that the enzymes of microorganisms in the human colon can cleave ChS and activate therapeutics more pronouncedly, chondroitinase ABC was used to pursue whether the breakdown of the glucuronic linkage in ChS of these semi-IPNs increases the rate of drug release. The drug release from ChSAA-10 with and without chondroitinase ABC was carried out in small scale, as stated in the Experimental section. Figure 7 indicates that the addition of enzyme slightly increases the percentage of the drug released, from 70% to 90%. The presence of the enzyme slightly accelerates the rate of drug release but not as dramatically as that reported by Rubinstein's group.[8–10] This may be a result of the unsuccessful formation of template interpenetrating polymer network, prepared by the free-radical polymerization of acrylic acid monomer embedded in ChS. Therefore, the drug release is essentially controlled by the rate of the erosion of ChS, and slightly depends on the enzymatic catalysis. In future studies, the effect of a fully interpenetrating polymer network between PAA and ChS on insolubility and enzymatic degradation will be investigated.

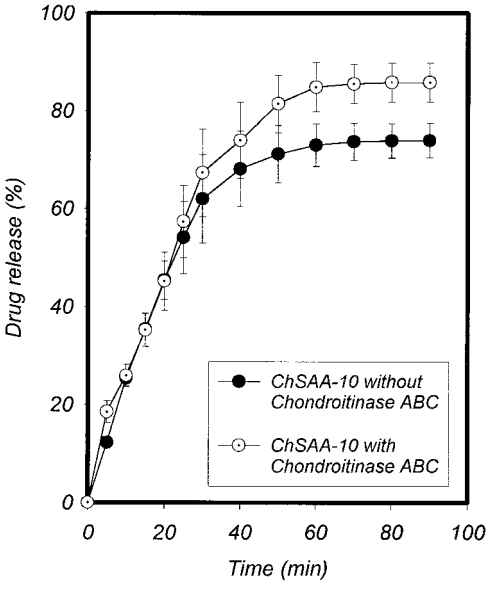

Figure 7 Ketoprofen-released profiles for ChSAA-10 with and without chondroitinase ABC in pH 7.4 buffer solutions.

CONCLUSIONS

The promising semi-IPNs with pH sensitivity and biodegradation were prepared. These semi-IPNs showed significant swelling-reduction properties in ChS in both simulated gastric and intestinal fluids. The release of ketoprofen from the semi-IPNs was sustained up to 300 min in simulated intestinal fluids, likely by an erosion mechanism. Chondroitinase ABC can be used to degrade ChS and slightly accelerate the drug-release rate. Despite the unsuccessful formation of template interpenetrating polymer networks, prepared by the free-radical polymerization of acrylic acid monomer embedded in ChS, the drug-sustained effect of these semi-IPNs is still obtained.

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