Encapsulation of Diclofenac Sodium with Acidic Copolymer Hydrogels Based on PEG/Poly(*N*-isopropylacrylamide-*co*-2-acrylamido-2-methyl-1-propanesulfonic acid) Semi-Interpenetrating Network Using *in Situ* Loading Technique

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ABSTRACT: A pH- and temperature-responsive semi-interpenetrating copolymer PEG6000/poly(NIPA-*co*-AMPS) (PEG/AMPS-*co*-NIPA SIPN), for short PEG SIPN, was made by ammonium persulfate-initiated suspension copolymerization of *N*-isopropylacrylamide, 2-acrylamido-2-methylpropanesulphonic acid, and *N*,*N*′-methylene-bis-acrylamide (MBAA; crosslinker) in the presence of PEG6000. The PEG SIPN copolymer matrices containing nanostructures made in the high-temperature copolymerization resulted in channels for PEG and facile migration of drugs. In drug encapsulation or drug-loading process, one can easily ignore or pay less attention to the interaction between a drug and its encapsulation materials; however, the ignored interactions may induce problems in drug properties or the release behavior in use. Sodium diclofenac (DFNa) precipitates as the carboxylic acid form in an acidic environment, and it is challenging to encapsulate sodium diclofenac in such an acidic matrix without precipitation of the sparingly soluble acid form of DFNa on the surface of the polymer substrate. To avoid bulky precipitation in drug loading, an *in situ* loading technique was developed for producing gel spheres with DFNa uniformly distributed in the polymer matrix. The technique is based on fast polymerization of spherical droplets of a pregel solution in which the drug is dissolved. Diffusion-loading prodrugs were made in comparison with *in situ* loading prodrugs in thermal, release kinetics, and release behavior. Drug release profiles (in pH 7.4 phosphate buffer) show that the new drug loading technique gives controlled release during a period of about 7 days at 37°C. By contrast, gel spheres loaded with sodium diclofenac using the conventional diffusion technique produced almost total release of the drug within about 24 h. The thermal stability of sodium diclofenac, the PEG/AMPS-*co*-NIPA SIPN, and the prodrugs made with the SIPN and sodium diclofenac was studied. A near zero-order release kinetics was found in the in vitro release of sodium diclofenac with *in situ* loading PEG SIPN prodrug. We have, for the first time, studied sodium diclofenac release behavior from the PEG SIPN hydrogel systems. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 2217–2231, 2009

Key words: sodium diclofenac; *in situ* loading; semi-interpenetrating network; PEG; *N*-isopropylacrylamide; 2-acrylamido-2-methylpropanesulfonic acid; thermal property

INTRODUCTION

As a pH- and temperature-responsive functional copolymer system, poly(ethylene glycol)/poly(2-acrylamido-2-methyl-1-propane sulphonic acid-*co*-*N*-isopropylacrylamide) semi-interpenetrating network PEG/(AMPS-*co*-NIPA) SIPN copolymers have been systematically studied elsewhere.[1–6] PEG/(AMPS-*co*-NIPA) SIPN hydrogels (PEG-SIPN) are acidic polymer matrices in association with copolymer composition.[3] AMPS-based hydrogels, as a low-friction hydrogel layer that is fixed on the surface of a medical device such as a catheter, becomes a lubrication layer and reduces the friction.[7,8] AMPS-*co*-NIPA copolymers are able to be effectively used to recover lysozyme, which is an industrial useful enzyme in microbiological engineering (recovery $>$ 90%) from egg whites by thermoprecipitation at room temperature.[9]

The strong interactions between drug and polymer matrix sometimes are ignored in drug encapsulation or loading processes. We need to address the problems of drug–polymer interaction and explore ways to avoid or lessen the greater extent of interactions between drug and its carriers. We selected diclofenac sodium (DFNa) in the present work as a model drug, an easily precipitated drug on acidic environment, to demonstrate how significant interactions between drug and the encapsulation materials (here, they are copolymer gels) and what solution or

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technique can be used as such situation to avoid deterioration of drug and drug release behaviors at low pH environments. DFNa is a strongly polar sodium salt that has been widely used as a nonsteroidal anti-inflammatory agent in the treatment of acute and chronic rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis.[10,11] The solubility and stability of DFNa in aqueous solutions strongly depend on temperature and pH. At low pH, DFNa will precipitate.[12–14]

A number of methods have been reported for loading DFNa in polymer matrices.[15–18] To reduce the precipitation of DFNa and to yield a uniform dispersion if loading DFNa to an acidic polymer matrix as AMPS-based copolymer gels, we decided to use a direct loading technique(DLT)—*in situ* loading, in which a uniform dispersion of DFNa in pregel solution was rapidly polymerized as a suspension of droplets in an immiscible suspension medium, to result in a uniform dispersion of the drug in polymer gel particles. In DLT, a rapid gelling/solidification of polymer carrier and good dispersion of drug molecules in reaction medium are vital to succeed. Also, gelling/solidification strongly depend on polymerization temperature and time. Increasing temperature will reduce the reaction time and increase the risk of drug and polymer decomposition. We preferred to lower the temperature as low as 70°C to avoid potential decomposition but, at this temperature, there is the disadvantage of requiring a longer time to complete the copolymerization and crosslinking reactions. For precipitation of drug, the longer the reaction time, the greater the extent for DFNa particles of sediment will be. Such sediment leads to a nonuniform distribution of drug in the gel.

In the present work, the drug-loading methodologies were taken into account with acidity of AMPS[2] in association with the precipitation[13,14,19] of DFNa in the presence of acidic aqueous solution and acidic copolymer component. The direct loading temperature was carefully selected by investigation of decomposition of copolymers and drug. The in vitro drug release behaviors were studied associated with copolymer compositions in the current work. Our study aims were to develop a novel and simple method to load DFNa directly into a pH- and temperature-sensitive PEG/poly(AMPS-*co*-NIPA) SIPN hydrogel system and to testify the drug release patterns at 37°C in association with copolymer composition (PEG% and AMPS/NIPA ratio), loading ways (diffusion loading or *in situ* loading), crosslinker concentration, and pH effect. In general, suspension polymerization requires the densities of the aqueous mixture of comonomers-drug and the suspension medium to be similar. The suspension medium must be liquid at the polymerization temperature, non-/or less toxic, with a boiling point well above the polymerization temperature, and thermally stable and readily available. Two media were used initially: (a) Methyl benzoate[20] with boiling point 199°C, density 1.09 g/cm^3, and LD50 = 1350 mg/kg for oral administration to rats (Sigma B0893). (b) Food grade soya bean oil, which consists primarily of the glycerides linoleic, oleic, palmitic and stearic acids. The density of soya bean oil was measured as 0.919 g/cm^3 at 22°C. The drug release patterns of sodium diclofenac (DFNa) loaded to PEG/poly(AMPS-*co*-NIPA) SIPN hydrogel was evaluated in the in vitro in the present work.

EXPERIMENTAL

Materials

Poly(ethylene glycol) with average molecular weight about 6000 g/mol (PEG6000) was supplied by BDH, 2-acrylamido-2-methyl-1-propane sulfonic acid (AMPS; Merck) and *N*-isopropylacrylamide (NIPA, Acros, Belgium) were both synthesis grade (≥99%) materials. *N*,*N*′-methylene-bis-acrylamide (MBAA; Sigma, St. Louis, MO) was electrophoresis reagent-grade material and was used as crosslinker. Ammonium persulfate (APS; Ajax, Auburn, NSW, Australia) used as initiator was >98% pure. Diclofenac sodium (reagent grade) was supplied by Sigma. The surfactant Brij 30 (Triethyleneglycol monolauryl ether, n Ca. 4-Mr ca. 320) was supplied by SERVA (Heidelberg, Germany) as stabilizing agent. Methyl benzoate[21] was from ACROS (Geel, Belgium). Petroleum spirit (AR) was used to remove methyl benzoate or soya bean oil from the prodrugs. All the other chemicals are analytical grade and used without further purification.

pH measurement

pH measurement was conducted using SevenEasy Mettler Toledo pH meter (Mettler-Toledo, Columbus, OH), which was calibrated with pH 4.0, 7.0, and 10.0 standards before use.

Preparation of pH 7.4 buffer and pH 1.0 simulated gastric liquid

Fresh pH 7.4 phosphate buffer solution (PBS) was prepared via dissolving 1.183 g of KH_2PO_4 and 10.898 g of $Na_2HPO_4{\cdot}12H_2O$ into 1 L of milli-Q water then the final pH of PBS was adjusted by 1*M* NaOH or 1*M* HCl. PH 1.0 simulated gastric liquid (SGL; 0.1 mol/dm^3 HCl solution) was prepared as in the literature.[22]

Morphology and analysis of surface element distribution

Scanning electron microscopy (SEM) was used for morphological study and energy dispersive X-ray spectrometry (EDX) was conducted for surface element distribution analysis on the dried copolymer gels and prodrugs using a Philips XL30 scanning electron microscope. The primary beam energy was set to 20 keV for all measurements. The parameters used in EDX analysis were zero tilt angles for sample, 46.4° take-off angle between sample and detector, and time constant 35×10^{-3} s.

Determination of in vitro release of drug

In vitro release was measured by the use of a SHIMADZU UV 2101 PC UV–VIS scanning spectrophotometer (Shimadzu, Kyoto, Japan). Fresh pH 7.4 PBS and pH 1.0 SGL were maintained at 37°C ± 0.5°C before use. Approximately 40 mg of dried prodrug was put into each of several screw-capped vials, and 20 mL of PBS or SGL was pipetted to each of the vials and recorded the starting time for drug releasing. At various time intervals, the solution in the vials was removed for analysis by UV–vis spectrophotometer, and 20 mL of fresh solution was compensated to each vial to continue the release. The experimental details are given in the results and discussion.

Thermal analysis

A Polymer Laboratories model 12,000 differential scanning calorimeter (DSC) and a Rheometric Scientific (formerly Stanton–Redcroft) Simultaneous Thermal Analyzer STA 1500 capable of simultaneously determining the differential thermal analysis (DTA) and thermogravimetry (TGA) profiles of samples were used, respectively, with an air flow of 150 mL/min and heating rate 10°C/min.

Drug loading techniques

Temperature is a key factor for loading strategy because the individual compound of drug and polymers has its own degradation temperature range. It was known that AMPS and NIPA homopolymers degrade at ~ 240°C[23] to 455°C,[2,24–26] respectively. During copolymerization at heating, the heat will or will not induce degradation of the drug, depending on the degree of temperature. It is known from TGA that the first stage of decomposition for DFNa occurs at ~ 260°C.[27] These known data led to the selection of a strategy wherein the polymerization temperature should be below the degradation temperatures of the individual materials and drug DFNa. 100°C was well-below DFNa and copolymer component degradation temperatures; therefore, it was chosen for *in situ* polymerization and drug loading. Second consideration in the loading strategy was relevant to chemistry environment of drug and copolymer compositions. Synthesis of PEG SIPN was reported in our previous work.[3,4] A high PEG% content was expected to result in rapid release of DFNa.[28] From the work we have published[3] that increasing the AMPS concentration increases the lower critical solution temperature of the SIPN gels to temperature above the LCST (32–36°C) of NIPA homopolymer.[29,30] In addition, the solubility of DFNa in water depends on pH, which requires a reasonably low concentration of AMPS in the SIPN gel because of the strong acidity of AMPS.[2] Taking all of the considerations into account, we decided that the gels had to be a high proportion of NIPA relative to AMPS and that the *in situ* drug loading temperature should be 100°C. Third strategy about the drug loading relates to the forms or encapsulations.

Polymer-based controlled release systems usually take two forms: a form of drug reservoir and a form in which the drug is dispersed in the polymer matrix. In the reservoir delivery system, the drug reservoir is enclosed by a rate-controlling polymer membrane, separating drug from the biological environment. In the matrix delivery system, ideally the drug is uniformly distributed in the polymer phase. Diffusion-controlled or diffusion/dissolution-controlled release of drug from reservoir or polymer matrix is the likely mechanism of release. In the present study, the so-called prodrug is a compacted dried drug-polymer gel disc or particle. A prodrug was produced by (1) diffusion of DFNa into preformed PEG SIPN and (2) *in situ* loading during copolymer gel synthesis. However, diffusion loading resulted in much of the DFNa precipitated on the surface of the SIPN gel, a smaller proportion dispersed in the SIPN gel with concentration decreasing with distance from the surface of the gel. The resulting prodrug is referred to hereafter as "DFLS prodrug." The so-called DFLS prodrug is expected to have a release mechanism with a feature of dissolution and diffusion. In the prodrug produced by "*in situ* copolymerization-direct loading system" (DCLS)-prodrug, the incorporated drug is ideally uniformly dispersed as dispersing phases through the polymer matrix, giving a hybrid reservoir-matrix release system which was expected to provide a diffusion-dissolution release mechanism. To reduce the time required to complete conversion of monomers to gel, by accelerating heat transport, the pregel solution was polymerized as small drops. Hereafter, we describe details on prodrug-preparations with two forms of drug release systems.

Preparation of pearl-like prodrug particles using *in situ* loading technique

Because of the greater solubility of DFNa in alcohol than in water, two pregel media were used, namely an ethanol/water mixture and pure water. *In situ* loading of DFNa associated with ethanol/water mixture then was called "DCLSe" system. For high DFNa% prodrugs, for instance, 0.113 g of PEG6000 was dissolved either in 25 mL of 80% (v/v) ethanol/water or 25 mL of deionized water, and 0.49 g of DFNa was dispersed in each solution. APS (40 mg), 0.0894 g of AMPS, and 0.928 g of NIPA(giving a comonomer mixture with 95 mol % NIPA, and about 10 wt % PEG based on total mass of PEG, AMPS and NIPA), with varying MBAA% (4–15%) based on total mass of AMPS, NIPA, MBAA and PEG were dissolved in each solution with stirring, to form the drug/pre-gel mixture. To these mixtures 1–2 mL of Brij 30 was added, and the resulting mixtures were immediately added dropwise to 200 mL of suspension medium preheated to 100°C ± 5°C in a four-necked 500- mL flask with constant stirring. The reaction mixture was maintained at ~ 100°C for about 5 min then allowed to cool, with continuous stirring, to about 60°C. The whole processes took about 30 min. The suspension medium was removed to a waste bottle by filtering the mixture through a stainless-steel sieve, and the prodrug "pearls" were washed with petroleum spirit several times to remove residual methyl benzoate or soya bean oil. The gel-like prodrug particles were vacuum dried at 70°C for about 4 days.

Preparation of blank polymer disc and blank pearl-like copolymer gel particles using the *in situ* loading technique

Pearl-like polymer particles were made using the procedure described previously. In addition, polymer gel discs with the composition specified previously but without DFNa were made in PTFE moulds. A 1.6 mL aliquot of pregel solution was transferred by pipette into a PTFE container with internal diameter 26 mm. The container was closed with a PTFE lid and heated at 70°C in a water bath to form gel. After 2 h (otherwise will be stated), the container with gel sample was rapidly transferred to an ice/water bath and kept at 0°C for about 10 min, then allowed to stand at ambient temperature for 1 h. Finally, the copolymers were dried in vacuum at 70°C for about 4 days.

Preparation of prodrug disc using diffusion loading technique

Similar to Sun et al.,[15] dried blank polymer gel discs (each about 0.3 g mass) were immersed in 5 mL of 80% (v/v) EtOH/water solution containing about 0.1 g of DFNa for 4 days at 37°C. The unabsorbed drug solution was removed by centrifugation, and the swollen gel discs were dried at 60°C to constant weight. In the diffusion loading process, drug migration into the dried gel would have been accompanied by loss of a proportion of the PEG6000 from the gel. Consequently the final masses of PEG6000 and DFNa in the prodrug were not known accurately, and the apparent DFNa wt% (DFNa*%) defined by

$$\mathrm{DFNa}^{*}\% = \left(\frac{M_{d+g} - M_g}{M_g}\right) \times 100 \qquad (1)$$

where M_g and M_{d+g} are the masses of the dried prodrug before and after drug loading, respectively.

RESULTS AND DISCUSSION

Because the decomposition temperatures for polymers of AMPS, NIPA, and PEG (AMPS ~ 240°C, NIPA ~ 420°C, and PEG ~ 400°C, respectively) were well studied elsewhere[31,32] with the use of TGA, we performed the investigation on decomposition of the drug DFNa using TGA while we analyzed the general thermal behaviors of the prodrugs by using DSC. The data are given in Figure 1 and Table I.

Because the stability of DFNa at different pH and temperature[12–14,27,33] is a significant issue in relation to processing DFNa, either pure or in formulation, DFNa before loading to PEG SIPN was studied by the use of TGA and DSC. The thermal gravimetry (TG and DTG) curves revealed mass loss in five steps up to 900°C.[27] The first step (up to 70°C) was attributed to dehydration, and the second step (260–400°C) was ascribed to elimination of HCl, NH, CH, and ½ CO with formation of ½ Na_2CO_3 and a carbonaceous product. The mass loss in the third step (400–545°C) corresponded to thermal decomposition of a benzene ring with Cl groups and the fourth step to thermal decomposition of another ring and elimination of ½ (CO_2, H_2O) from the sodium carbonate with formation of NaCl and a small quantity of carbonaceous residue. The partial mass loss up to 900°C in the last step was ascribed to pyrolysis of the carbonaceous residue and partial loss of NaCl. British Pharmacopoeia1998[34] described DFNa melting at ~ 280°C with decomposition. Cwiertnia et al.[33] used GC-MS to analyze the DFNa decomposition products and five compounds were found[21]: 1-(2,6-dichloro phenyl)oxindole; (2) 1-(2,6-dichlorophenyl)isatin; (3) 2-[(2,6-dichloro-phenyl)amino]benzyl alcohol; (4) *N*-(2,6-dichlorophenyl) anthranilaldehyde[35]; 4-chloro-10H-9-acridinone.

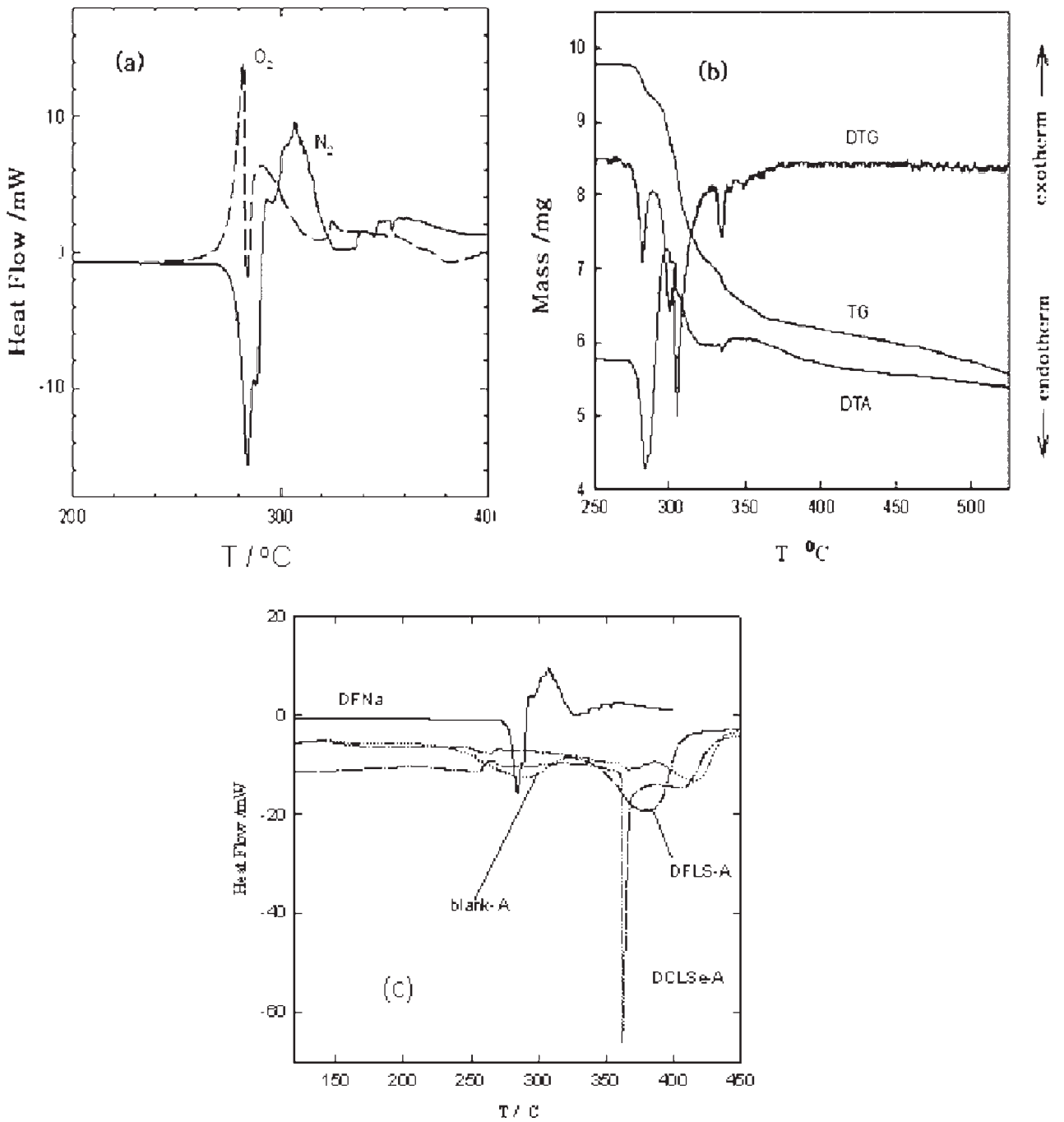

Figure 1 Thermal analysis using DSC or TGA methods: (a) DSC scans for DFNa. Curve "O_2" and curve "N_2" indicate the presence of oxygen or nitrogen. Heating rate was 10°C/min. (b) Thermal gravimetry data, including DTG, TG, and DTA curves for the thermal decomposition of diclofenac in N_2 at heating rate 10°C/min. (c). DSC scans for DFNa, blank SIPN gel A (blank–A), DFLS-A (4.8 wt % of apparent DFNa), and DCLSe-A (28.7 wt % of DFNa). All polymer and prodrugs contain 4.2 wt % MBAA. Copolymer network consists of 95 mol % NIPA and 10 wt % PEG. Sample mass was 9–10 mg in DSC scans.

Detailed investigation of DFNa was conducted in the present work. Figure 1(a) shows that in the presence of O_2, the first exothermic peak at 282°C might correspond to oxidation of the DFNa to 1-(2,6-dichlorophenyl)isatin soon after melting. The second exothermic peak at 291°C might be due to the formation of 2-[(2,6-dichlorophenyl)amino]-(9CI)-benzoic acid. In the presence of N_2, the first endothermic peak at 282°C [TGA, Fig. 1(b)] or 284°C [DSC, Fig. 1(a)] might correspond to the cyclization of DFNa soon after melting to the structure 1-(2,6-dichlorophenyl)oxindole. The main exothermic peak at 307°C [from DSC, Fig. 1(a)] or 305°C [TGA, Fig. 1(b)] might be due to the formation of 2-[(2,6-

TABLE I
Thermal Properties of Diffusion-Loaded Prodrugs

Thermal property	DFLS-A	DFLS-B	DFLS-C
T_g (°C)	156 ± 3.1	171 ± 3.5	[a]
$T_{dp(1)}$ (°C)	252 ± 5.5	255 ± 4.6	253 ± 8.4
$T_{dp(2)}$ (°C)	374 ± 0.8	390 ± 1.3	378 ± 2.0
DFNa*%	4.8 ± 0.7	0.01 ± 0.0	4.5 ± 0.3

The prodrugs of DFLS-A, DFLS-B and DFLS-C contain 4.2, 8.1, and 15.0 wt % of MBAA, respectively. MBAA wt% based on total mass of AMPS, NIPA, MBAA, and PEG. Copolymer network consists of 95 mol % NIPA and 10 wt % PEG.

[a] Glass transition not clearly discernible in the DSC scan. Subscripts dp(1) and dp(2) represent first and second decomposition peak, respectively. DFNa*% as apparent percentage of DFNa.

dichloro-phenyl) amino]benzyl alcohol. The third peak at 337°C [TGA, Fig. 1(b)] might be due to the formation of *N*-(2,6-dichlorophenyl) anthranililaldehyde. These studies revealed that during rapid heating (10°C/min), except for the dehydration of DFNa at around 70°C, the drug was thermally stable below 250°C under oxygen and nitrogen, and the rapid heating below 250°C for the *in situ* loading of DFNa to form prodrug was unlikely to have caused significant decomposition of the drug. The issue of the stability and possible deactivation of DFNa at low pH and high temperature has been addressed in a number of studies.[13,27,33,36–38]

Contrary to other workers' conclusions,[13,39] Palomo et al.[14] found that the acid form of DFNa did not undergo intramolecular cyclization in acidic solutions. In the prodrugs [Fig. 1(c)], there exist components of AMPS, NIPA units, PEG, and DFNa, and the individual components might have interactions of which we were not aware. After the drug was loaded in the SIPNs, the drug-SIPN could be a "composite-like" structure. We were more interested in the overall thermal behavior of the prodrugs rather than the decomposition feature of the DFNa. Prodrugs in Table I were from diffusion loading and the value of DFNa*% was given from eq. (1). In Table II, the drug content DFNa% was taken from the percentage of loaded drug weight to whole prodrug weight. The temperature position for first principal endotherm at a significantly lower temperature than that (289°C) for the blank gels found elsewhere,[2] indicating that the loaded DFNa affects more or less on the thermal behavior of the prodrugs. When comparing DFNa*% values in Table I to the DFNa% data in Table II, we realized that the lower DFNa*%, particularly DFLS-B, in Table I might be attributable to the loss of PEG molecules that occurred in the diffusion loading because the determined value of DFNa*% based on eq. (1) became smaller. The other finding was that the glass transition temperature for prodrug depended mainly on the copolymer components and PEG chains in the network. The higher glass transition temperatures of diffusion-loaded prodrugs (see Table I) than those of *in situ*-loaded prodrugs (Table II) were also probably caused by the lower PEG% (diffusion loading results in consequence of PEG loss from prodrug matrix). We know that PEGs have low glass transition temperatures because of its free rotation of C—O bond and higher free volume.[40] Table II also shows, similarly, the first principal endotherm temperature occurred at a significantly lower temperature than for the blank gels.[3]

The significant difference in the thermal behavior of the prodrugs was in the shape of the endotherm within the 350–450°C. It was worth noting that the sharp endotherm at ~ 280°C for DFNa was not found in both prodrugs within the same temperature range. However, the much stronger endotherms at temperature >380°C for both prodrugs than that for blank SIPN gel A (blank-A) were believed from two overlapped endotherms: one from SIPN copolymer network[2] and the other from the encapsulated or dispersed DFNa in the polymer matrix. The *in situ* loaded sample (DCLSe-A, with 28.7 wt % of DFNa) may decompose over a more narrow temperature range than the diffusion loaded sample (DFLs-A), consistent with a more uniform dispersion of the drug in the *in situ* loading copolymerization matrix.

Figure 2 shows thermal behaviors of *in situ* loaded prodrugs with different PEG6000 contents at 5 wt % of DFNa level (much lower than drug level from direct loading in prodrug DCLSe-A of [Fig. 1(c)] and Table II). It is apparent that the PEG content (<20 wt %) of the gel has no significant effect on the

TABLE II
Thermal Properties of *In Situ* Loaded Prodrugs Formed in the *In Situ* Copolymerization from Aqueous Ethanol Pregel Solutions

Thermal property	DCLSe-A	DCLSe-B	DCLSe-C
T_g (°C)	129 ± 1.0	143 ± 2.8	[a]
$T_{dp(1)}$ (°C)	251 ± 4.9	256 ± 0.7	257 ± 5.0
$T_{dp(2)}$ (°C)	281 ± 7.8	322 ± 2.8	325 ± 1.4
$T_{dp(3)}$ (°C)	362 ± 0.2	351 ± 1.2	348 ± 1.1
DFNa%[b]	28.6 ± 0.85	27.8 ± 0.28	26.4 ± 0.65

The prodrugs of DCLSe-A, DCLSe-B, and DCLSe-C contain 4.2, 8.1, and 15.0 wt % of MBAA, respectively. MBAA wt% based on total mass of AMPS, NIPA, MBAA, and PEG. Copolymer network consists of 95 mol % NIPA and 10 wt % PEG.

[a] Glass transition not clearly discernible in the DSC scan.

[b] DFNa is not apparent drug percentage. DFNa% can be determined by calculation of sum of dried copolymer gel and drug added. All data from minimum two determinations. Subscripts dp(1), dp(2), and dp(3) represent first, second and third decomposition peak, respectively.

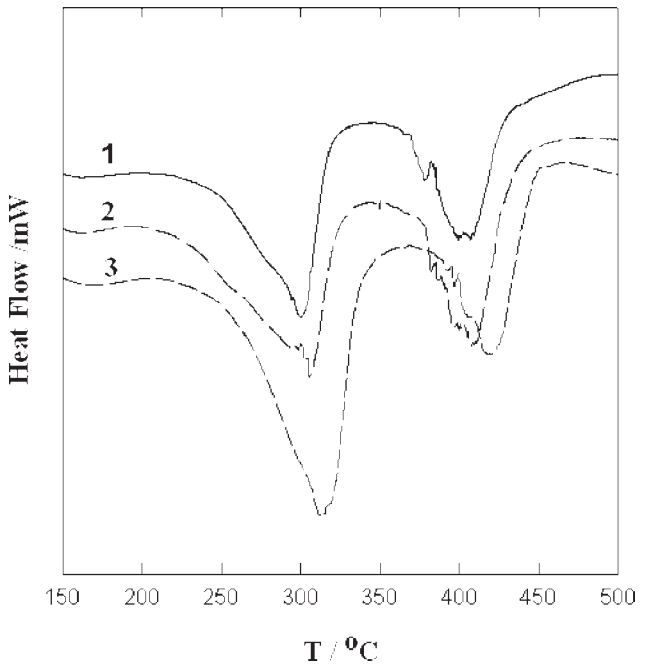

Figure 2 PEG effects on the thermal behavior of a drug *in situ* loading system (5 wt % DFNa). 1, 2, and 3, denote prodrugs with 5, 10, and 20 wt % PEG6000, respectively. Copolymer SIPN gels consist of 95 mol % NIPA, 5 mol % AMPS, and 4.2 wt % MBAA.

thermal behavior. However, both of ~ 300°C peak and ~ 400°C peak were found shifting up about 10°C from 5 wt % PEG to 20 wt % PEG in prodrug matrixes, indicating a markedly PEG crystal melting effects.[2] It is also noteworthy that the prodrugs (Fig. 2, curve 1) with ~ 5 wt % DFNa loading using *in situ* polymerization result in the different thermal behaviors from DFLS-A in Figure 1(c). This finding indicates that dispersion of DFNa in the copolymer network matrix depends on loading methods and where the drug dispersed. The significance of the thermal analysis for DFNa, copolymer matrix, and prodrugs discloses the mutual influences of drug and components in thermal behavior, in the other hand, determines a proper temperature for drug *in situ* loading.

For the drug release kinetics for both erodible and nonerodible polymer matrices[41] can be represented with eq. (2).

$$Q/Q_0 = \alpha t^n \tag{2}$$

where Q and Q_0 are the cumulative amount of drug released and the amount loaded into the matrix, respectively, α is a constant, t is release time, and n is a parameter characterizing the mechanism of transport. The case $n = 1$ corresponds to zero-order release. The most commonly used equation to describe general solute release behaviour of controlled release polymeric devices, particularly for swellable matrix and polydisperse multiparticulate systems, was developed by Ritger and Peppas.[42,43] The equation takes the form as shown in eq. (3).

$$\frac{M_t}{M_\infty} = kt^n \tag{3}$$

where M_t/M_∞ is the fractional solute release after time t, k is a constant, and the exponent n is characteristic of the release mechanism. When $n = 0.5$ the drug release mechanism is Fickian diffusion, $0.5 < n < 1.0$ corresponds to anomalous (non-Fickian) transport, and $n = 1.0$ is also the case of zero-order release.

The solubility of DFNa is strongly pH-dependent.[14,19,44] From spectroscopic (UV, IR) and thermoanalytical studies,[12] it was found that the solubility is about 1 μg/mL at pH = 3. In the present work, the solubility of DFNa in simulated gastrointestinal liquid at low pH, the solubility and the absorbance at 276 nm was negligibly small. Consequently it was not very accurate to obtain a reliable Beer-Lambert plot for these conditions. DFNa in pH 7.4 phosphate buffer PBS exhibited two absorption maxima with λ_{max}= 200 ± 2 nm and 276 ± 1 nm, respectively, in agreement with reported data.[12] For λ_{max} = 276 nm the Beer-Lambert law is obeyed for concentrations up to 115 μmol/L giving $\varepsilon_{276} = 1.06 \times 10^4$ L/(mol cm).

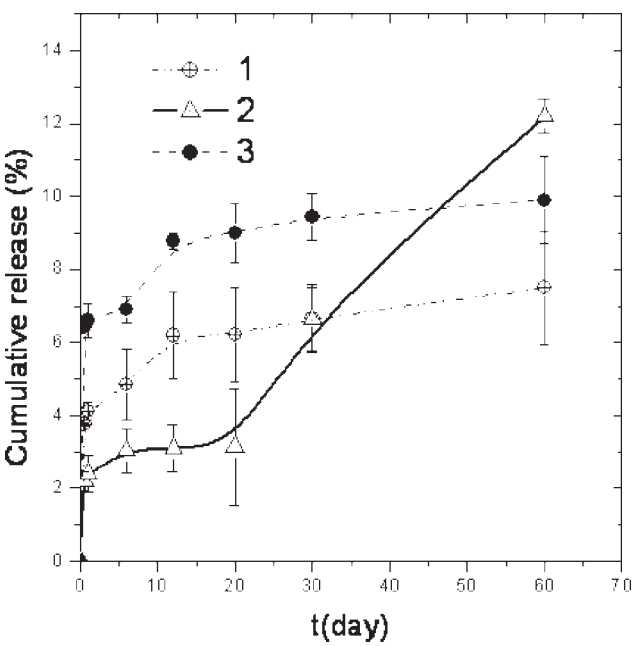

Figure 3 In vitro release profiles in pH 7.4 PBS at 37°C for *in situ* loaded prodrug (DCLSe-series) comprising 5 wt % DFNa, 5 mol % AMPS/95 mol % NIPA, 15 wt % MBAA and varying PEG content denoted as: 1, 5 wt % PEG; 2, 10 wt % PEG; 3, 20 wt % PEG. The prodrugs were made in soya bean oil as suspension medium. Vertical lines are error bars from standard deviation determined from five measurements. Lines through each point are guidelines for viewing.

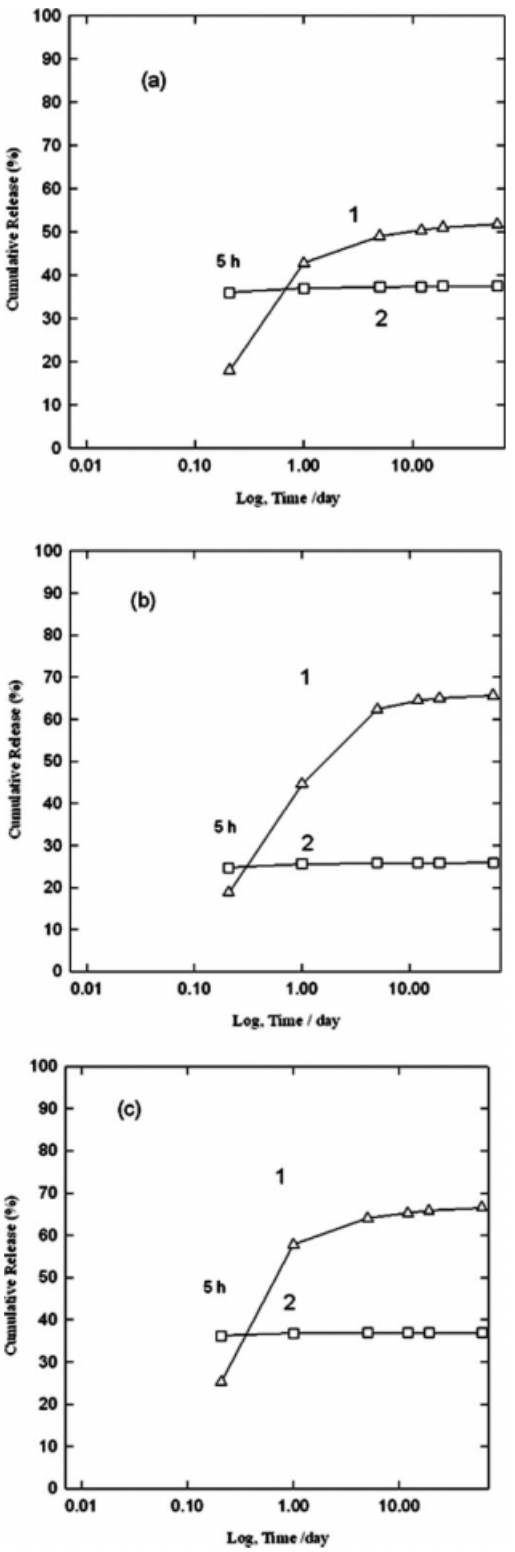

Figure 4 Comparison of release profiles in PBS pH 7.4 between *in situ*-loaded and diffusion-loaded pearl-like prodrugs (a) 1, DCLSe-A prodrug, 2, DFLS-A prodrug; (b) 1. DCLSe-B prodrug, 2. DFLS-B prodrug; (c) 1. DCLSe-C prodrug 2. DFLS-C prodrug. See Tables I and II for the prodrug compositions. The data presented in Figure 4 were average determined from two measurements.

The relationships between UV absorbance and DFNa concentration in pH 7.4 PBS are expressed by eq. (4).

$$\mathrm{ABS}_{276\ \mathrm{nm}} = 1.062 \times 10^{-2} C + 1.118 \times 10^{-2} \qquad (4)$$

where C is the concentration in μmol/L.

The data plotted in Figure 3 shows that the release behavior of DFNa was significantly affected by PEG content in these prodrugs. The influence of PEG on the release profile was not straightforward because increasing PEG in general increases the potency of hydrogen bonding, hydrophilicity and the free volume of the copolymer network, and, increasing PEG also modifies the 3D structure of the gels and the interaction between copolymer network and drug, consequently, these factors induce high hydration to the gel and allow faster motion of water and DFNa molecules between release mediums and copolymer network. PEG effects as found in current research might be the explanation for the release profile of the prodrugs as seen in Figure 3 that prodrugs with 10 wt % PEG (curve 2) differed qualitatively in release profiles from the prodrugs with 5 and 20 wt % PEG. Moreover, after ~ 20 days the 10 wt % PEG prodrug gave a near zero-order release kinetics, with $M_t/M_\infty = 0.002t$, $n = 1.0$, $k = 0.002$, $R^2 = 0.98$, indicating that this PEG SIPN may be a promising candidate as an ideal drug carrier for DFNa.

When the *in situ* copolymerization loading system (DCLSe) and diffusion loading system (DFLS) in Figure 4(a–c) are compared, it is not surprising that DFLS prodrugs have much greater burst release for the first 5 h (marked with 5 h in each profile) and gave almost total release of the drug within about 24 h as compared with the corresponding DCLSe

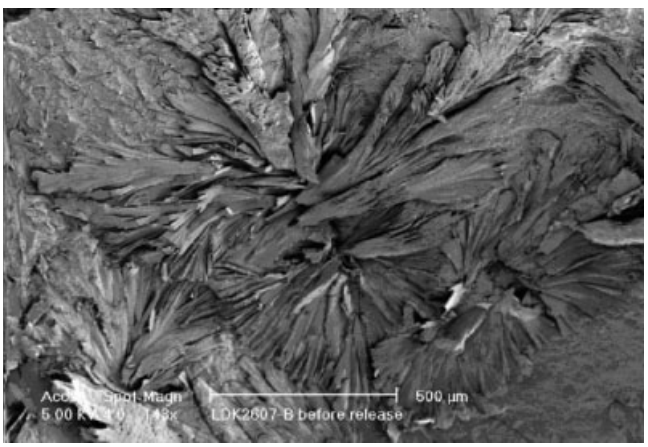

Figure 5 An example of DFNa precipitate on the prodrug made using diffusion loading method. The copolymer composition for the prodrug matrix consists of 8.1 wt % MBAA, 10 wt % PEG, 5 mol % AMPS and 95 mol % NIPA.

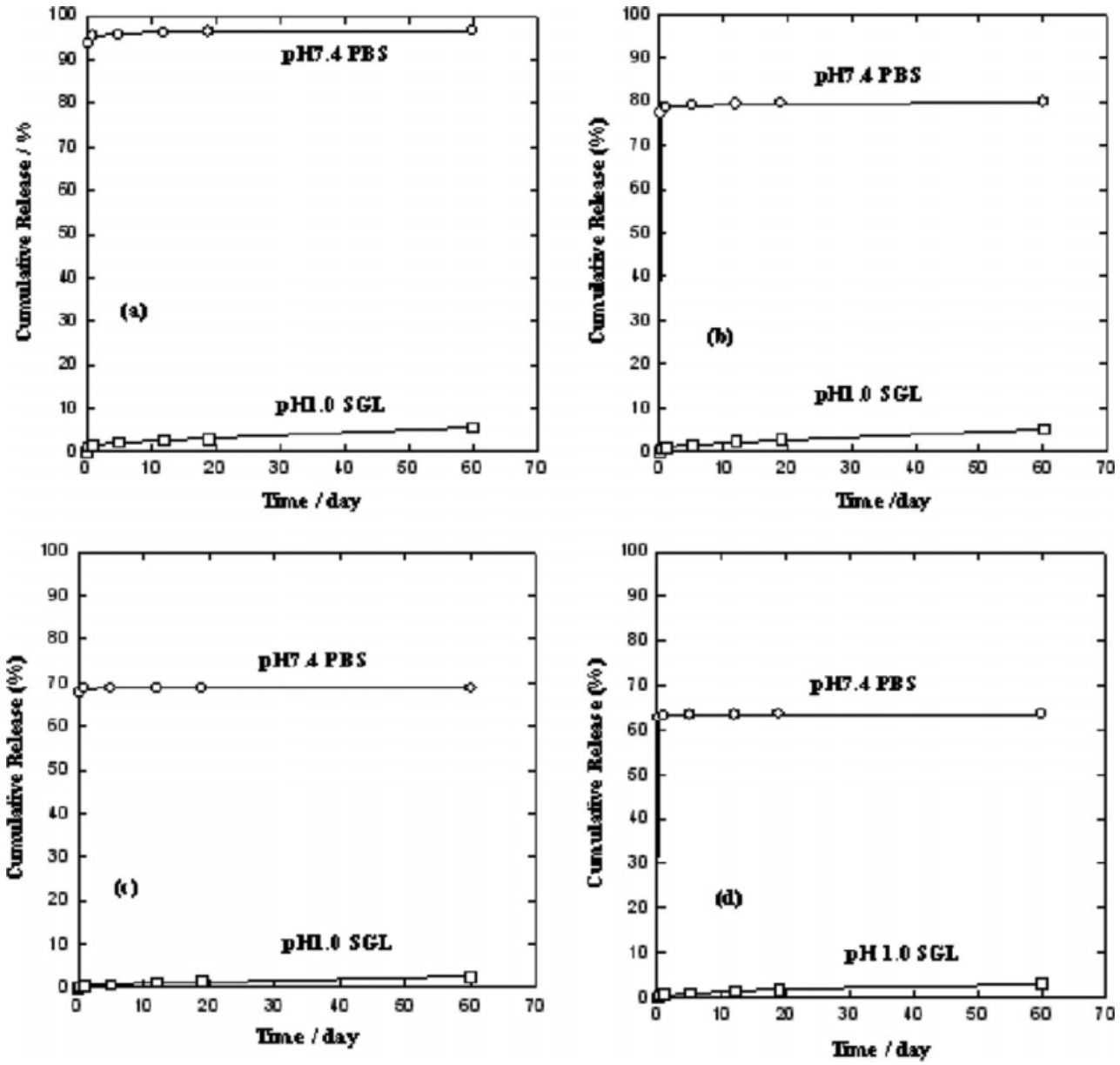

Figure 6 Cumulative release (%) profiles for pearl-like diffusion-loaded prodrugs in pH 7.4 PBS and pH 1.2 SGL. Polymer matrix compositions: (a) 100 mol % NIPA homopolymer gel; (b) 10 wt % PEG-100 mol % NIPA SIPN gel; (c) 10 wt % PEG-10 mol % AMPS-90 mol % NIPA SIPN gel; (d) 10 wt % PEG-5 mol % AMPS-95 mol %NIPA SIPN gel.

prodrugs, indicating that diffusion loading makes more drug molecules be absorbed on the surface or shallow layers of the polymer matrix. In contrary to the diffusion loading, *in situ* loading prodrugs such as DCLSe prodrugs had greater cumulative release (%) than those of DFLS prodrugs after the first burst time (5 h). Therefore, *in situ* loading DCLSe prodrug as a release system or drug reservoir demonstrates a much promising sustained release than DFLS system does because the *in situ* loading system, as expected, had lower burst release and greater cumulative release level as time increased. The new drug loading technique (*in situ* loading DCLSe) provided seemly well-controlled release profile during a period of 7 days at 37°C. In contrary, the diffusion-loaded prodrugs gave unfavored patterns as higher burst release occurring, this outcome was consistent with a surface excess distribution of DFNa (see SEM micrographs in Fig. 5). Figure 5 showed the local morphology of the surface of the prodrug (5 mol % AMPS in comonomer ratio) after the blank dried gel had been loaded with DFNa using diffusion. The crystalline structures precipitated on the acidic prodrug surface were confirmed to be DFNa using EDX analysis (not published here). Other areas of the surface showed different morphologies, and the nonuniformity of the surface structure was apparent. The crystalline DFNa on the surface is most likely the

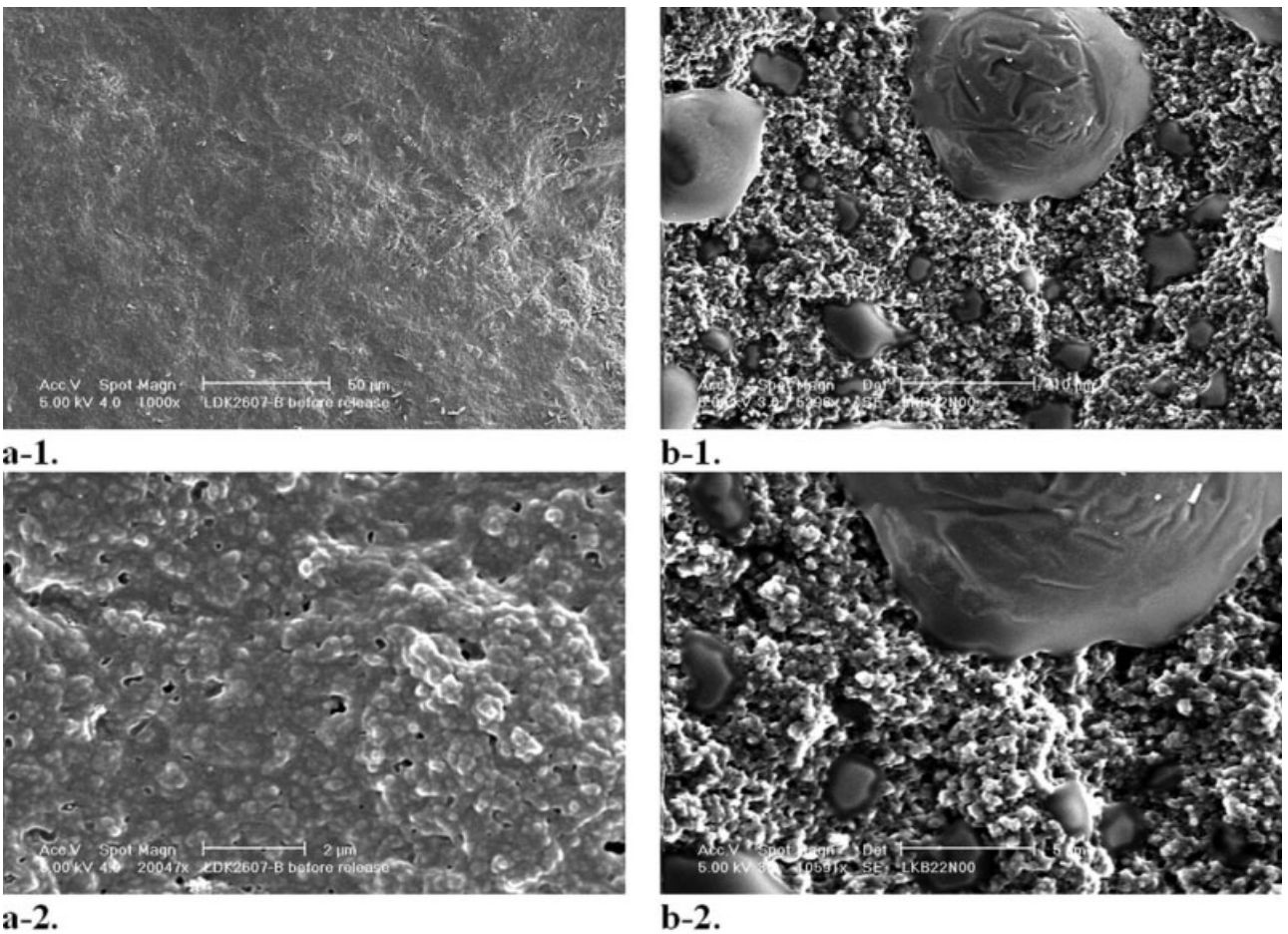

Figure 7 DFLS-B disc with composition of the polymer matrix consists of: 8.1 wt % MBAA, 10 wt % PEG, 5 mol % AMPS and 95 mol % NIPA. Before release in the left column (a-1, a-2); after 60-day release in right column in pH 7.4 phosphate buffer at 37°C (b-1, b-2). All were taken from prodrug surface.

source of the high burst release of DFNa found previously (see Figs. 3 and 4).

Generally, for the diffusion loading system (DFLS) prodrug, most of drug molecules have been precipitated on the gel surface because the SIPN gels contain acidic $-SO_3H$ groups in AMPS units and the DFNa on the surface of the prodrug is facile to dissolve in medium and generated a burst release in a short period of time after immersion in the medium. Subsequent release was a diffusion-controlled process initiated from inside gel network. In the case of *in situ*-loaded (DCLS) prodrug, there was also a period of burst release, but much lower in extent, because of the outmost dispersed DFNa molecules becoming exposed to the surrounding medium. Since most DFNa molecules were in the matrix rather than on the surface of the matrix, drug release following the burst depends predominantly on drug diffusion within the polymer matrix and on the interactions between drug and polymer network. Releases of DFNa and PEG as semi-interpenetrating molecules from prodrug to external environment are diffusion-controlled (see also Fig. 3), and hydrogen-bond between DFNa and PEG6000 slows the motion of DFNa that the drug release kinetics may also depend partly on the PEG6000 concentration in the gels. External stimuli such as pH or temperature can increase or decrease the DFNa release rates and the kinetics of release on pH and temperature are not discussed in the present work.

Release profiles are shown in Figure 6 for effects of varying media (pH 1.0 SGL and pH 7.4 PBS, respectively) in association with prodrug compositions. For the same prodrug, the cumulative released DFNa % in SGL was extremely low. For all of the diffusion-loaded prodrugs in Figure 6, the amount of DFNa released during the burst release period (1 day) is significantly (noted that cumulative release was 60–90% in pH 7.4 PBS for the first day), and all prodrugs in this system showed the greater burst for the prodrugs containing only NIPA [Fig. 6(a)] than for the prodrugs containing 5 mol % [Fig. 6(d)] or 10 mol % AMPS [Fig. 6(c)]. It worthy noting that both polymer matrices [see Fig. 6(a,b)] contain 100 mol % NIPA and no AMPS, the matrices are almost neutral in pH. Thus, there should be not significant

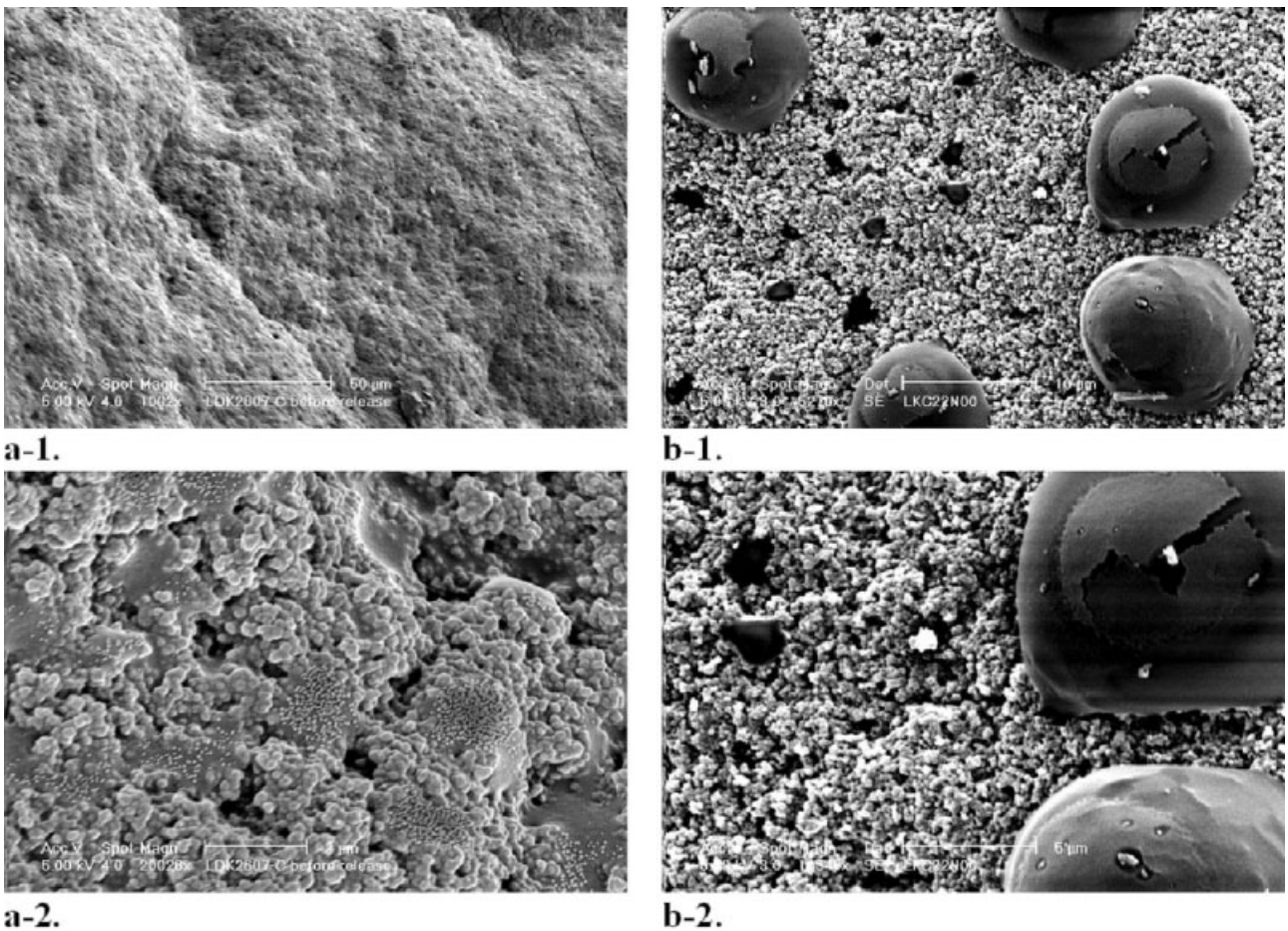

Figure 8 DFLS-C disc with composition of the polymer matrix consists of 15 wt % MBAA, 10 wt % PEG, 5 mol % AMPS and 95 mol % NIPA before release (a-1, a-2); after 60-day release in pH 7.4 phosphate buffer at 37°C (b-1, b-2).

precipitation of DFNa on the surface so that the greater burst release cannot be attributed to concentrated distribution of DFNa on the prodrug surface. In this special case, the burst was most likely from the effects of NIPA unit on gel structure during copolymerization and heating. SEM micrographs, as seen in Figures 7–9 or elsewhere,[2] disclosed that the gels made with high mol% NIPA are more "porous" nanostructure (Figs. 7–11) and these prodrugs were all made at temperature 100°C. We all know that

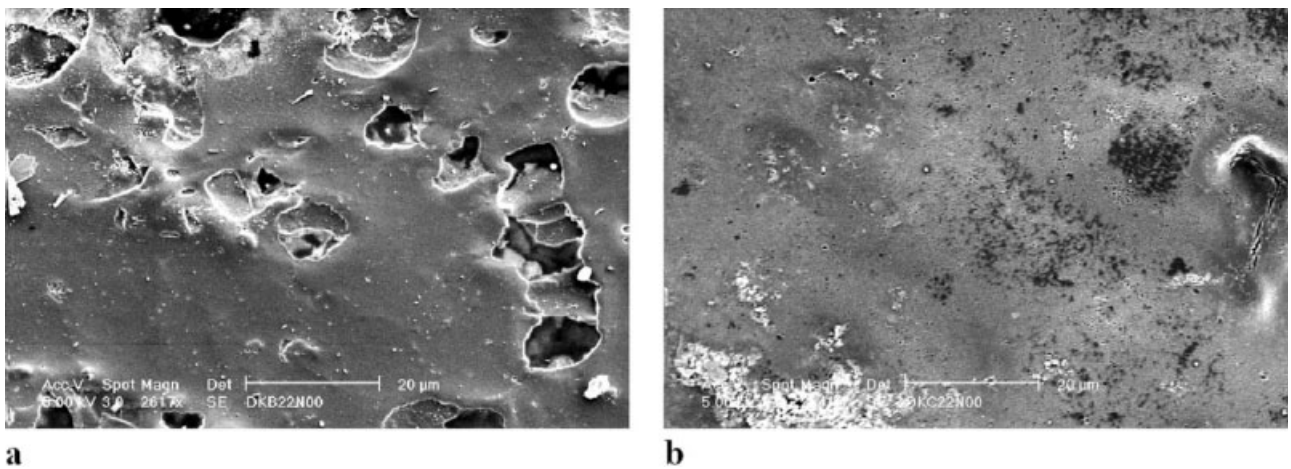

Figure 9 Surface micrographs of *in situ*-loaded prodrug:(a), DCLSe-B disc (MBAA = 8.1 wt %); (b), prodrug-DCLSe-C disc (MBAA = 15.0 wt %). Both prodrugs consists of 10 wt % PEG, 5 mol % AMPS and 95 mol % NIPA. Images were from dried prodrugs after 60-day release in pH 7.4 phosphate buffer at 37°C.

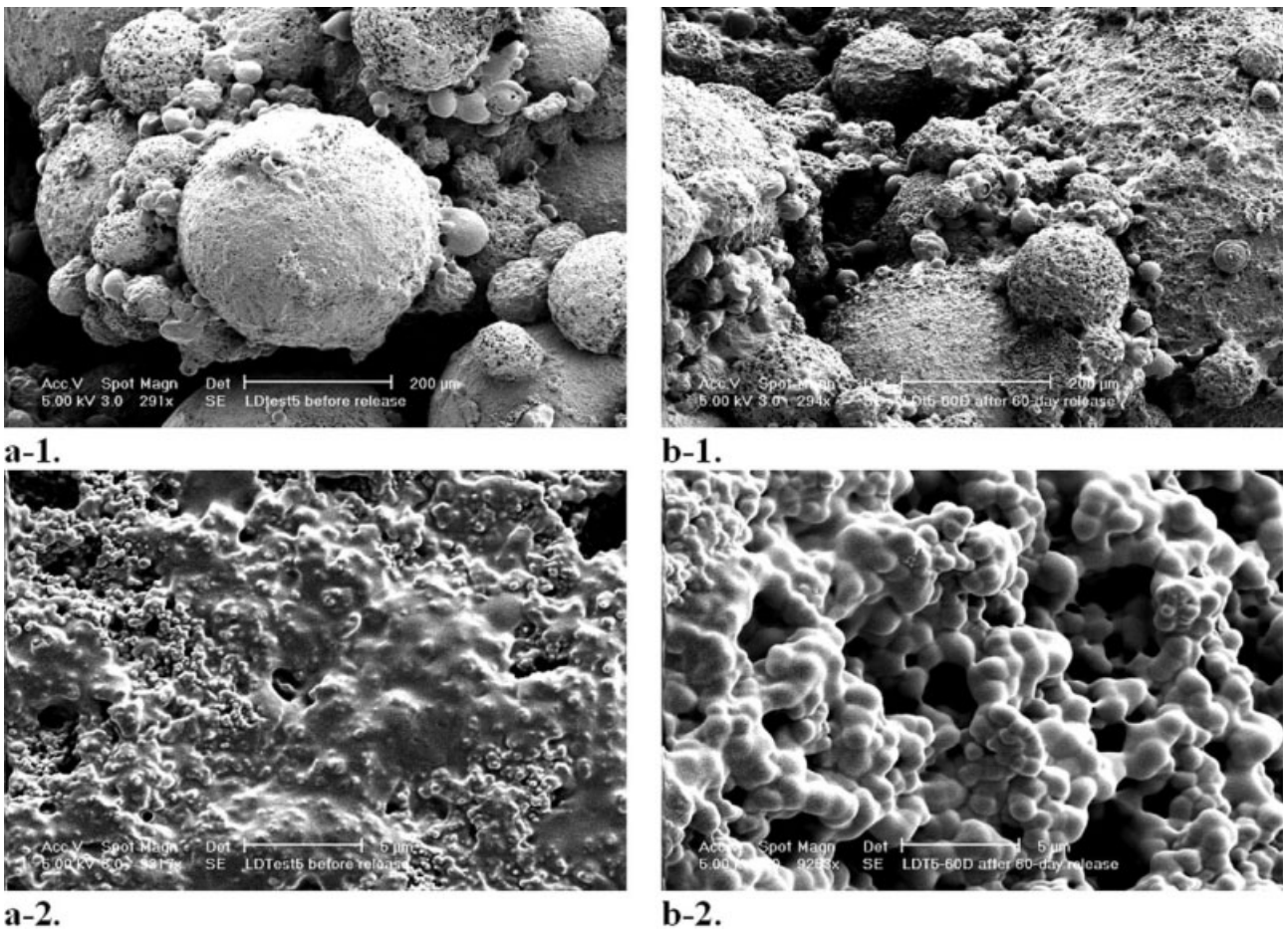

Figure 10 Scanning electron micrographs of *in situ*-loaded pearl-like prodrug consists of 5 wt % DFNa, 15 wt % MBAA, 20 wt % PEG, 100 mol % NIPA, before release (a-1 and a-2); after 60-day release in pH 7.4 phosphate buffer at 37°C (b-1 and b-2).

100°C is much higher than LCST of NIPA chains (normally about 32°C) and the higher-than-LCST temperature causes the NIPA chains collapse to form so-called voids or channels in gel structure.[45] These voids and channels were accessible for facile motion of the drug molecules between the SIPN matrix and external medium, and, provided more connections for drug molecules soluble to/exchangeable with the release medium, subsequently, inducing markedly burst release.

The addition of PEG [see Fig. 6(a,b)] reduces the burst release of prodrugs with zero AMPS content. It seems that PEG inhibits migration of DFNa from the gel, as discussed previously, probably through hydrogen-bonding interactions between NH groups of DFNa and the oxygen atoms of PEG. Not, surprisingly, the burst release for prodrug with 5 mol % AMPS is smaller than that for prodrug with 10 mol % AMPS, in Figure 6d comparing to Figure 6c. This, again, probably is due to more precipitates of DFNa on the lower pH surface of prodrug with 10 mol % AMPS than that on prodrug with higher pH at 5 mol % AMPS (as discussed previously that more DFNa precipitates on prodrug surface give higher burst release).

It seems that *in situ*-loaded prodrug has the advantages that the burst release is smaller than for corresponding diffusion-loaded prodrugs and that the prodrug continues to release DFNa for a longer time. Those are desirable features of prodrugs designed for controlled drug release.

Figures 7(b-1,b-2) and 8(b-1,b-2) showed, in comparison with Figures 7(a-1,a-2) and 8(a-1,a-2), separately, that there were some marked changes on the surface of the prodrug after release of DFNa for 60 days. The erosion of the polymer matrix by the release medium is significant. The oval or circular spots on the surface are attributed to PEG6000 migrating from the gel to the exposure surface. (This was also confirmed using EDX scanning during taking images, not presented here.) The loading methods and crosslinking were believed to affect the morphologies of the prodrugs either before or after release of DFNa. For diffusion loading, the crosslinking effects can be compared with Figures 7 and 8. In general, increasing crosslinker concentration results

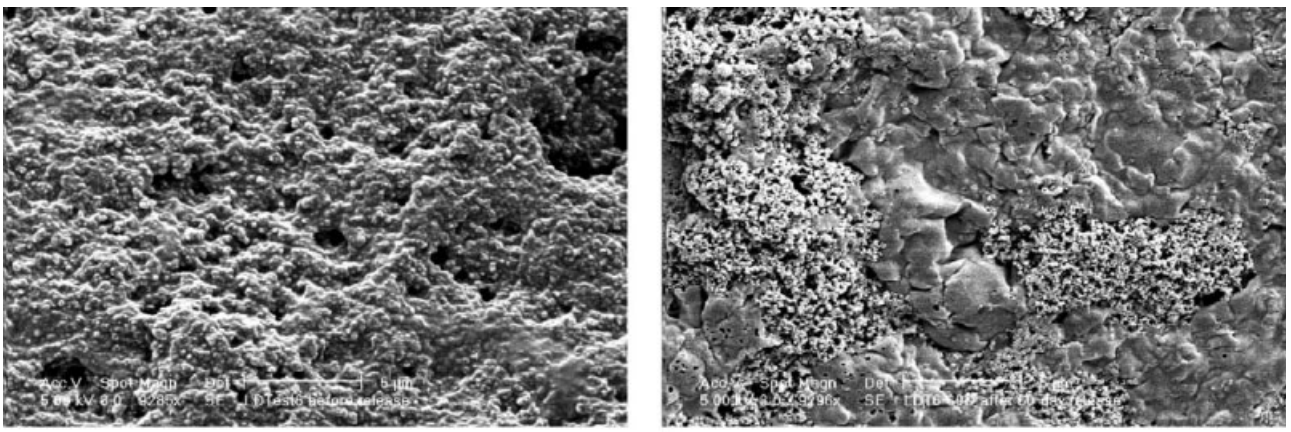

Figure 11 Scanning electron micrographs of *in situ*-loaded pearl-like prodrug with 5 wt % DFNa, 15 wt % MBAA, 10 wt % PEG, 5 mol % AMPS and 95 mol % NIPA, (a) before release; (b) after 60-day release in pH 7.4 phosphate buffer at 37°C.

in smaller and denser nanostructure of copolymer domains. Similarly, the effects of crosslinker concentration on morphology for *in situ* loading can be compared with Figure 9(a,b). It was clear that increasing crosslinker concentration resulted in smaller channels and smoother surface, indicating lower rate for drug transporting. Comparison of (b-1 and b-2) and (a-1 and a-2) in Figure 10 also shows that the surfaces of the prodrug particles were eroded during the release process, and the structure shown in b-2 was mainly consisted of NIPA polymer nanostructure after PEG and DFNa reached out to the medium solution from the prodrug. In general, as seen in Figures 7–11, the surface morphologies of the *in situ*-loaded prodrugs (Figs. 9–11) after release is dramatically different from those of the diffusion-loaded prodrugs (Figs. 7 and 8). Migration of DFNa and PEG from the *in situ*-loaded prodrugs appears to be a more controlled process, which is consistent with the release profiles (see Fig. 4) that indicates a superior performance from *in situ* loading prodrugs to the diffusion-loaded prodrugs. Comparison of Figure 10 with Figure 11, the images showed that the surface morphology of the prodrugs with the similar concentration of comonomers and crosslinker was altered by addition of more PEG in the copolymerization, and the continuity of the surface was disrupted by the erosion of buffer solution in release processes. Comparison of (a) to (b) in Figure 11 reveals no significant changes of the surface structure of the particles. This may explain why the release profiles of the prodrug with 10 wt % PEG and 5 mol % AMPS gave a zero-order release profile (see curve 2 in Fig. 3).

The purpose of using EDX for the prodrugs was to determine whether the drug component of the prodrugs was uniformly distributed in polymer matrices and the relative concentration of loaded DFNa. EDX[46] gives qualitative analysis for the identification of the elements present at the point on the sample surface being struck by the electron beam. Because the EDX detector can be positioned close to the specimen and cover a very small area, it is a good tool for determination of local elemental composition and elemental distribution. The EDX results in Figures 12 and 13 show that the Cl counts which are proportional to chloride mole concentration were

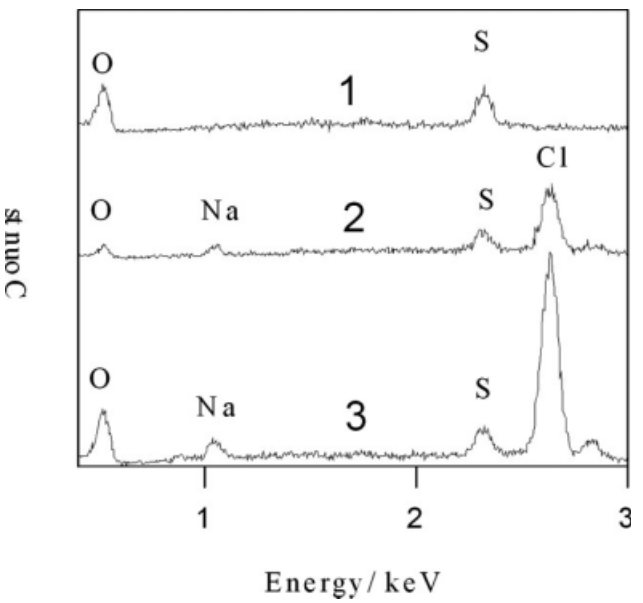

Figure 12 Typical surface EDX spectra of gel disc or prodrug discs: 1, PEG SIPN dried gel disc; 2, diffusion-loaded prodrug; 3, *in situ*-loaded prodrug. O, Na, S and Cl denote oxygen, sodium, sulfur and chlorine, respectively. All matrices consist of 15 wt % MBAA, 10 wt % PEG, 5 mol % AMPS and 95 mol % NIPA.

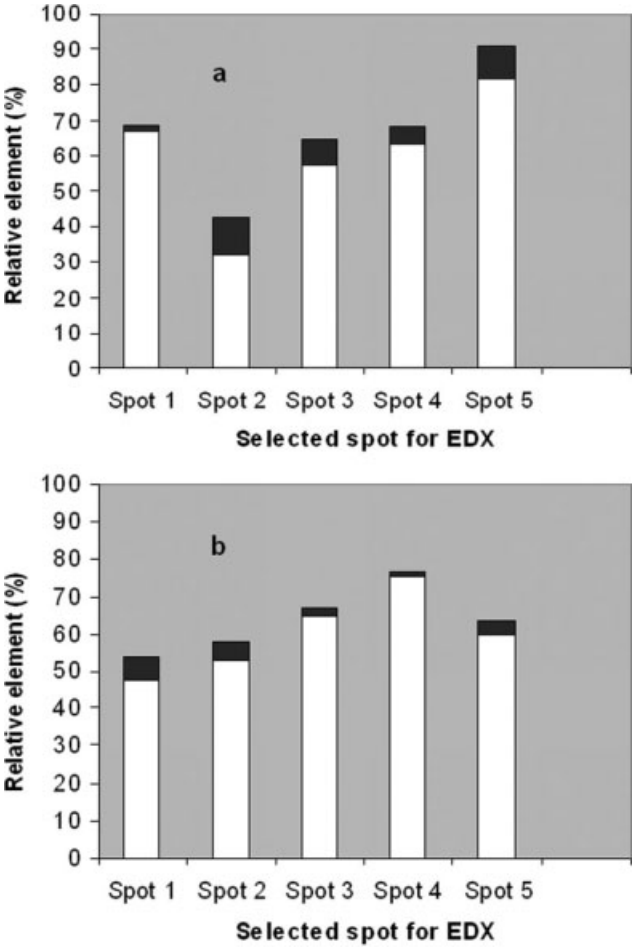

Figure 13 EDX elemental analysis from selected spots on one surface of the (a) diffusion-loaded prodrug disc and (b) on one cross-sectional surface of an *in situ*-loaded prodrug disc. Unfilled column: % Cl; filled column: Na %. Spot 4 was thought to be a region with high drug concentration. The matrix composition consists of 15 wt % MBAA, 10 wt % PEG, 5 mol % AMPS and 95 mol % NIPA.

consistent with the DFNa % in column 4 of Tables I and II, respectively.

Curves 2 and 3 of Figure 12 showed that prodrug discs made by either diffusion or *in situ* loading contain Cl and Na, which originate from sodium diclofenac. It was clear from Figure 13(a,b) that DFNa was not uniformly distributed on the surfaces of the prodrug discs. However, the *in situ*-loaded prodrugs [Fig. 13(b)] showed that the drug distributed more uniformly on the surface than the diffusion-loaded prodrugs [Fig. 13(a)] did.

CONCLUSION

Interactions between drug and drug encapsulation materials (polymer matrix) were discussed in association with thermal properties and drug release behaviors. In a known strong drug-polymer matrix interaction, sodium diclofenac (DFNa) as a model drug was successfully loaded *in situ* in copolymerization to the acidic PEG6000/poly(AMPS-*co*-NIPA) SIPN gels that respond to changes of pH and temperature for a direct encapsulation. PEG6000, crosslinker MBAA and comonomer ratio determine the thermal and morphological properties of the prodrugs associated with DFNa as well as the drug release profiles. An SIPN with 10 wt % PEG6000, and copolymer chains with monomers in the molar proportion 95 NIPA/5 AMPS has properties that are appropriate for the encapsulation of DFNa *in situ* into the SIPN during copolymerization and crosslinking, provides a relatively uniform dispersion of the drug through the gel particles, and is superior to the conventional diffusion-loading method of drug encapsulation.

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