Polymer 50 (2009) 3537–3546

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

# Simultaneous and sequential micro-porous semi-interpenetrating polymer network hydrogel films for drug delivery and wound dressing applications

T. Thimma Reddy, Atsushi Takahara*

*Institute for Materials Chemistry and Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan*

ARTICLE INFO

*Article history:*
Received 31 December 2008
Received in revised form
11 May 2009
Accepted 25 May 2009
Available online 10 June 2009

*Keywords:*
Semi-interpenetrating polymer networks
Drug release
Swelling

ABSTRACT

Novel semi-interpenetrating polymer networks (so-called simultaneous SIPNs) of various compositions were synthesized using segmented polyurethane urea (SPUU), *N*-isopropylacrylamide (NIPAM), acrylic acid (AA), and butylmethacrylate (BMA), resulting in an SIPN film denoted as SPUU/poly(NIPAM-co-AA-co-BMA). The resulting simultaneous SIPN films were neutralized by exposure to pH 7.4 phosphate buffer solution (PBS). The neutralized films were dried and then characterized by differential scanning calorimetry (DSC). The DSC results showed that the $T_g$ of the SIPNs depends mostly on SPUU content and on the composition of the acrylate monomers. PNIPAM was incorporated as a second network in one composition of simultaneously prepared SIPN film through a sequential polymerization method (so-called sequential SIPNs). Both simultaneous and sequential SIPN films were examined by scanning electron microscopy (SEM) after freeze drying at their equilibrium states. The SEM study revealed that simultaneous SIPNs had a porous morphology in the absence of BMA, but the porosity disappeared at higher BMA content. The morphology of the sequential SIPN film was almost similar to that of pure PNIPAM. Finally, simultaneous and sequential SIPNs were used for swelling and drug release studies at different pH values and at different temperatures to determine the environmental sensitivity of these gels. Simultaneous SIPNs absorbed more water than sequential SIPNs, but both had a poor rate of water absorption at pH 1.2. In the drug release study, a higher thermosensitivity was observed for sequential SIPNs than for simultaneous SIPNs.



## 1. Introduction

Hydrogels consist of a hydrophilic polymer that forms a three-dimensional network capable of retaining water. Hydrogels are one of the most promising materials for biomedical applications and have great potential for use as wound dressings, contact lenses, and drug delivery systems. Hydrogels prepared from either natural or synthetic sources, however, have poor flexibility in both dry and hydrated states. Hydrogels in the dry state are very brittle due to their high $T_g$ and break easily because there are very few energy-dissipation mechanisms to slow crack propagation. In addition, their crosslinking points are distributed irregularly and the polymer chains between the crosslinking points have different lengths. Stress on the gels cannot be evenly distributed between the polymer chains, and cracks develop easily. The opposite is true for hydrogels in the hydrated state; these gels are too soft, posing significant handling difficulties [1]. There are different ways to improve the mechanical properties of hydrogels, including copolymerization with hydrophobic monomers [2–4] and formation of interpenetrating polymer networks (IPNs) using flexible polymers [5,6]. The formation of IPNs, instead of synthesizing new types of polymers, constitutes the most useful method of improving the properties of the individual components in order to satisfy the requirements of specific applications [7,8].

IPNs are a network of two polymers, at least one of which is crosslinked or synthesized or synthesized and crosslinked in the presence of the other. Microphase-separated hydrophilic and hydrophobic morphologies can be achieved with an IPN and block copolymers. The phase morphology in an IPN is stable to environmental changes because it is fixed by the crosslinks. If one of the networks is a hydrophilic polymer, then the resulting IPN can form a hydrogel. Hydrogels can absorb water because of hydration, which is related to the presence of chemical groups (such as –COOH, $CONH_2$, –CONH–, and $SO_3H$), capillary effects, and osmotic pressure. Depending on the chemical groups, the swelling behavior of hydrogels may change in response to the external environment. These stimuli-sensitive hydrogels can exhibit dramatic changes in their swelling behavior, permeability, or mechanical strength due to changes in pH, ionic strength, temperature, or electromagnetic radiation.

* Corresponding author. Tel.: +81 928022517; fax: +81 928022518.
*E-mail address:* takahara@cstf.kyushu-u.ac.jp (A. Takahara).

0032-3861/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2009.05.062

The most commonly studied environmentally sensitive hydrogels are either pH or temperature sensitive [9,10]. Poly(*N*-isopropylacrylamide) is a well-known thermosensitive hydrogel, noted for its relatively low critical solution temperature of 32 °C in aqueous solution. pH-sensitive hydrogels, meanwhile, contain pendent acidic (e.g., carboxylic and sulfonic acids) or basic (e.g., basic ammonium salts) groups that either accept or release protons in response to changes in pH. For many biomedical applications, it would be preferable if hydrogels could respond to two types of stimuli simultaneously—most importantly pH and temperature—either mutually or independently. Chen and Hoffman [11] first introduced this idea by grafting PNIPAM to poly(acrylic acid) (PAA) chains to achieve a temperature-induced phase separation over a wide range of pH values. Later, many groups attempted to achieve simultaneous pH and temperature sensitivity by copolymerizing the temperature-sensitive NIPAM with other monomers that contained weakly acidic groups such as acrylic acid [10,12–16]. However, the incorporation of pH sensitivity in some cases ruined the thermosensitivity of copolymerized hydrogels, and a high acrylic acid content led to a complete suppression of thermosensitivity. For example, Kobayashi et al. [17] synthesized poly(NIPAM-co-acrylic acid-co-*N*-*tert*-butylacrylamide) (P(NIPAM-co-AA-co-tBAAm)) hydrogels and demonstrated that when the content of acrylic acid reached 10 mol%, the hydrogel did not exhibit thermosensitive properties. Lee et al. [18,19] have made similar observations with acrylic acid. Lee et al. [20] used acrylic acid neutralized to 50 mol% by sodium hydroxide (SA50) as a copolymer with NIPAM. When the content of SA50 reached 16.4 mol%, the thermosensitivity of the resultant hydrogel was extremely weak. Developing hydrogels that are simultaneously sensitive to both temperature and pH or simultaneously sensitive to two temperatures and pH is a challenging task. Chen et al. [13] reported dual temperature and pH sensitivity in hydrogels developed using sequential IPNs of NIPAM and sodium acrylate. Xian et al. [21] and Jian et al. [22] reported sequential PNIPAM/PNIPAM IPN hydrogels with improved mechanical properties.

Improved mechanical and sustained drug release properties of PNIPAM have been achieved by copolymerizing NIPAM with butylmethacrylate (BMA), a hydrophobic monomer [2–4]. BMA has also been used to prepare IPNs with segmented polyurethanes (SPUs). SPUs, because of their excellent mechanical properties and tissue compatibility, have been used widely as a biomedical material [23–25].

In a previous study, we reported the synthesis of SIPNs based on SPUU and PNIPAM (SPUU/PNIPAM), and we showed that the thermosensitivity of these films toward cell adhesion and detachment was favorable for use as a wound dressing [6]. This paper describes the development of pH- and temperature-sensitive hydrogel films prepared from SPUU, NIPAM, AA, and BMA through both simultaneous and sequential semi-interpenetrating polymer networks. In the first step, we prepared SPUU/poly(NIPAM-co-AA-co-BMA) simultaneous SIPNs. To these SIPNs, PNIPAM has been introduced as a second network through the sequential SIPN method. The concept of introducing PNIPAM through the sequential SIPN method is to increase the PNIPAM mass per unit volume to obtain a thermosensitive polymer as well as to provide properties that allow sustained drug release.

## 2. Experimental section

### 2.1. Materials

The synthesis of segmented polyurethane urea (SPUU) and its characterization was reported previously [6,26,27]. Polycaprolactone (PCL), lysine diisocyanate (LDI), and 1,4-butanediamine (BDA) were used in the synthesis and the sample code of SPUU was designated as PCL(1250)(70)LDI-BDA [6]. *N*-Isopropylacrylamide (NIPAM, Wako Pure Chemical, Japan) was recrystallized from toluene/hexane. Acrylic acid (AA) and butylmethacrylate (BMA) (Aldrich Chemical) was used after distillation under vacuum. *N*,*N*′-Methylenebisacrylamide (MBAM) (Kishida Co., Japan), α,α′-azobisisobutyronitrile (AIBN), ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) (Kanto Chemical Co., Inc. Japan), sulfamethoxazole (SF), and phosphate buffer solution (PBS) (pH 7.4) (Aldrich Chemical) were used as received. Dimethylacetamide (DMAc) was purchased from Wako Pure Chem. Co. (Japan). The pH 1.2 NaCl–HCl buffer was prepared in our laboratory.

### 2.2. Simultaneous synthesis of SPUU/poly(NIPAM-co-AA-co-BMA) SIPNs

Samples of SPUU, NIPAM, AA, BMA, MBAM, and AIBN of known weights were dissolved in dimethylacetamide (DMAc). After complete dissolution of all the reactants, nitrogen gas was bubbled through the solution for 30 min, and the total mixture (15 wt% of total solids) was then transferred into Petri dishes. The Petri dishes were kept in a separable flask and heated to 80 °C under controlled vacuum over 5 to 6 h to complete the polymerization of reactive monomers and for simultaneous evaporation of the solvent (Fig. 1). The resulting transparent SIPN films were purified by soaking in water at room temperature for two days, and the water was repeatedly changed to remove impurities. This soaking was followed by 1 day of soaking in water and methanol (3:1) to remove methanol-soluble reactants. The preparation method and reactant feed compositions of the simultaneous SIPNs of SPUU/poly(NIPAM-co-AA-co-BMA) are given in Fig. 1 and Table 1, respectively. The SIPNs were exposed to PBS with pH 7.4 over 5 h to neutralize AA. The neutralized SIPN films were again washed, dried, and used for characterization. The sample codes are listed in Table 1. Disk-shaped samples (8 mm in diameter and 0.2–0.3 mm thick) for swelling and drug absorption studies were cut from the water-equilibrated SIPN films at room temperature and then dried. The yield of gel fraction was determined gravimetrically after extracting the specimen with DMAc. In all the SIPNs, it was found to be around 90%.

### 2.3. Sequential synthesis of SIPNs

Predetermined amounts of the NIPAM monomer and the MBAM crosslinker were dissolved in 1.75 ml distilled water. A known amount of dried SIPN 5 hydrogel synthesized above was immersed into the above monomer and crosslinker solution until almost all the monomer solution was absorbed into the SIPN 5 hydrogel network (usually 30 min). APS and TEMED, redox initiators, were added to the SIPN 5/NIPAM monomer solution to initiate the polymerization of the NIPAM monomer and the crosslinking of the MBAM to form the second PNIPAM network within the original SIPN 5 network. The reaction was carried out for 24 h at room temperature in a sealed polystyrene plastic mold. Subsequently, an SIPN network structure, consisting of SIPN 5 and PNIPAM networks, was formed and labeled as SIPN 5-ipn-PNIPAM. The resulting sequential SIPN was extracted with water for 2 days, with refreshment every few hours, to remove unreacted species. The feed composition ratio of the NIPAM monomer to SIPN 5 is summarized in Table 2. Schematic representation of the formation of the second network of PNIPAM in SIPN 5 is shown in Fig. 2.

### 2.4. Characterization

Differential scanning calorimetry (DSC) thermograms were recorded on a Rigaku DSC. The samples were heated from −100 °C to 170 °C at a rate of 10 K/min under nitrogen (30–40 ml/min gas

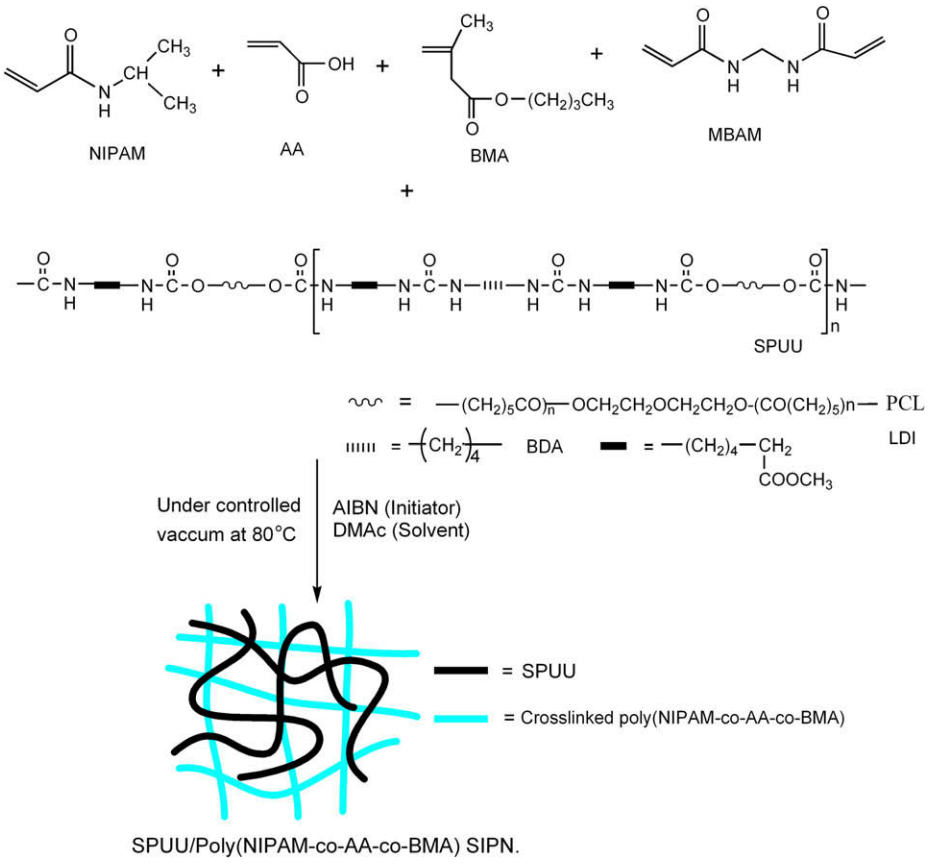


**Fig. 1.** Synthesis of SIPN by simultaneous polymerization method.

flow rate). The sample weights were approximately 5 mg each. Air-dried hydrogels were used for DSC measurements. For the SEM study, the SIPN films were first equilibrated in distilled water at room temperature; swollen films were frozen in defreeze and then freeze dried until all the solvent was removed. The freeze-dried films were fixed on aluminum stubs, coated with gold for 40 s, and observed using a scanning electron microscope.

### 2.5. Swelling ratio

The neutralized dried-disk samples (8 mm diameter) of known weight were immersed in water and in wound fluids for 1 h at room temperature to allow them to equilibrate. For pH-dependent equilibrium swell samples were immersed in pH 1.2 buffer solution for a day. The swelling ratio of each gel was measured gravimetrically. The weight of the wet sample ($W_s$) was determined after the surface water was blotted with filter paper. The equilibrium swelling ratio was calculated for all the samples by $Q = W_s/W_d$, where $W_s$ is the weight of the swollen sample and $W_d$ is the weight of the dry sample. For each specimen, four independent measurements were made and averaged.

**Table 1**
Composition of monomers used to synthesise SIPNs.

| Sample code | Composition |
|---|---|
| | SPUU/NIPAM/AA/BMA[a] (wt%) |
| SIPN 1 | 00/30/30/40 |
| SIPN 2 | 10/60/30/00 |
| SIPN 3 | 10/40/30/20 |
| SIPN 4 | 10/20/30/40 |
| SIPN 5 | 30/25/30/15 |
| SIPN 6 | 30/35/20/15 |
| SIPN 7 | 30/20/20/30 |
| SIPN 8 | 30/50/20/00 |
| SIPN 9 | 30/70/00/00 |
| PNIPAM | 00/100/00/00 |

[a] In addition, 2 wt% crosslinker (MBAM) and 2 wt% (AIBN) thermal initiator based on the total polymerisable group were added into the above composition. Dimethylacetamide (DMAc) is used as a solvent (15 wt% solids are used for all compositions).

### 2.6. Drug loading

SF was loaded into both simultaneous and sequential SIPNs by using the solution sorption method. A saturated solution of SF was prepared in 50:50 water:ethanol. A known quantity of SIPN 5 and SIPN 5-ipn-PNIPAM films were added to drug solution at room temperature. After 4 h of incubation, the films were taken out and rinsed in the water:ethanol solution to remove any surface-adsorbed drug and then air-dried. Drug-loaded samples were coded as SIPN 5-SF and SIPN 5-ipn-PNIPAM-SF.

Loading efficiency was determined spectrophotometrically at 264 nm after extensive extraction in water. Loading percentage was calculated as

**Table 2**
Concentration of the reactants used in the formation of second network.

| Sample code | SIPN 5 (mg) | NIPAM (mg) | MBAM (mg) | Water (ml) |
|---|---|---|---|---|
| SIPN 5-ipn-PNIPAM | 100 | 350 | 7 | 1.75 |

50 μl of 5 wt% APS solution as an initiator and 10 μl of TEMED as an accelerator were used.

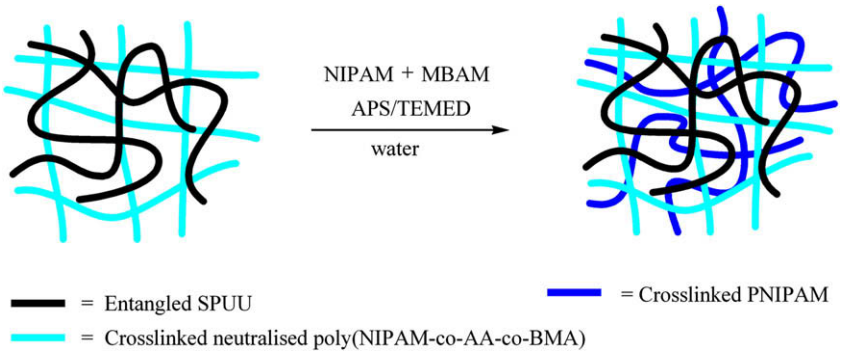


**Fig. 2.** Schematic representation of formation of second network by sequential polymerization method.

$$\%\ \text{Loading} = (W_{\text{drug}})/(W_{\text{polymer}} + W_{\text{drug}})) \times 100.$$

### 2.7. Drug release

Drug release from disk-shaped dry samples was conducted at 37 °C and 22 °C in buffer solution of pH 7.4 and 1.2, respectively. The dried drug-loaded samples were immersed at predetermined times directly in 10 ml fresh buffer solution, pre-equilibrated to 37 °C and 22 °C. Aliquots (0.5 ml) were withdrawn periodically to determine drug concentration and, in all cases, equal volumes of dissolution media were immediately added to maintain a constant volume. SF concentration was determined spectrophotometrically at 264 nm. Absorbance (following correction based on blank samples, i.e., polymer films without the drug) as a function of time was systematically measured and subtracted from the absorbance of drug-loaded films. This measurement took into account any unreacted material that leached to the external solvent during the experiments. Samples were withdrawn until two successive aliquots showed no increase in absorbance. The amount of SF released from the polymer films in the dissolution media, at any given time, was calculated by using standard curves of SF in the corresponding buffer and expressed as the percentage of the total drug content of the investigated films. Experiments were performed in triplicate, and the average value was considered for the purposes of data treatment and plotting.

### 2.8. Statistical analysis

For every test, the data are expressed as means plus or minus the standard deviation ($n = 3$). The statistical analysis was performed with a Students' $t$-test at a 0.05 level.

## 3. Results and discussion

### 3.1. Synthesis of SIPNs

SIPNs of SPUU/poly(NIPAM-co-AA-co-BMA) were synthesized by thermally initiated free-radical polymerization. Table 1 summarizes the composition of the reactants used to prepare SIPNs by simultaneous polymerization. The reaction mixture, dissolved in dimethylacetamide (DMAc), was subjected to thermal heating to transform the reactants into transparent solid polymer films through free-radical-initiated polymerization, as shown in Fig. 1.

The obtained polymer films were thoroughly washed and exposed to an elevated pH to neutralize AA, which allowed a faster rate of water absorption. The integrity of swollen SIPN 2 and SIPN 5 is shown in Fig. 3. SIPN 5 is more flexible than SIPN 2 due to the high content of SPUU in SIPN 5. Earlier SIPNs, based on polyurethane/PNIPAM or polyurethane/PAA, were prepared by photopolymerization followed by thermal heating to remove the solvent [28]. Depending on the feed composition and thickness of the solution, thermal effects are seen during photopolymerization that cause non-homogeneity and defects in the final material. These defects greatly alter the physical properties of the final products [29]. Polyurethane/PBMA SIPNs were also studied extensively to improve their mechanical properties [30].

To the best of our knowledge, there has been no work regarding the synthesis and characterization of SIPNs based on SPUU/poly(NIPAM-co-AA-co-BMA) for drug release and wound dressing applications. Each component of the present SIPN system has its own function when used in wound dressings. For instance, SPUU is used to improve the flexibility of the SIPN system. Both NIPAM and AA have been chosen to achieve adhesion to the wound site and easy removal, and to improve fluid absorption to keep the wound in a moist environment. The last component of SIPN, BMA, was

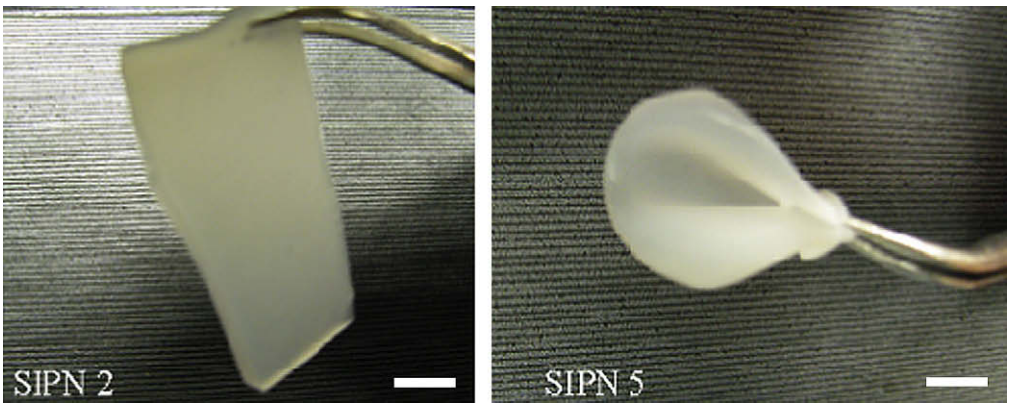


**Fig. 3.** A photograph of swollen SIPNs (scale bar = 0.5 cm).

**Table 3**
Glass transition temperatures, $T_g$, of crosslinked PNIPAM, SPUU and SIPNs in dry state.

| Sample code | $T_g$ (°C) |
|---|---|
| PNIPAM | 138 |
| SIPN 2 | 160 |
| SIPN 4 | 145 |
| SIPN 6 | 136 |
| SIPN 7 | 135 |
| SIPN 8 | 150 |
| SIPN 9 | 114 |
| SPUU | −39 |

incorporated to check the formation of a skin-like barrier by the SIPN network, which may be useful to control microorganism infections as well as to control drug release. BMA may also act as an internal plasticizer in the NIPAM copolymer system.

### 3.2. Characterization

DSC measurements have been used to determine the effect of PBMA and SPUU on glass transition temperature ($T_g$) of simultaneous SIPN systems. It is known from the literature that the two $T_g$s are indicative of a phase-separated structure, with the $T_g$s of individual components often shifted toward each other, indicating a partial mixing of the networks [28]. $T_g$s of crosslinked PNIPAM, SPUU, and some of the SIPNs in the dry state are summarized in Table 3. The $T_g$s of crosslinked PNIPAM ($T_g = 139$) are very high, typical of acrylic polymers, whereas the $T_g$s of SPUU [6,28] and PBMA [30] are −39 and 24, respectively. However, when an SIPN has been prepared from NIPAM, AA, BMA, and SPUU, the $T_g$ varies depending on the compatibility or miscibility of SPUU and poly(NIPAM-co-AA-co-BMA). A $T_g$ for more compatible SIPNs has been observed at lower temperatures while the $T_g$ for less compatible SIPNs has been observed at higher temperatures. The $T_g$ of SIPN would also depend on the content of BMA, the internal plasticizer, in the system. The compatibility between SPUU and poly(NIPAM-co-AA-co-BMA) could be explained based on inter- and intramolecular hydrogen bonding. To understand the compatibility and plasticity of SPUU and PBMA in the SIPN system, we have selectively chosen some samples for the DSC study.

The DSC results (Table 3, Figs. 4 and 5) show that the $T_g$s of PNIPAM, SIPN 2, and SIPN 4 are 138, 160, and 145, respectively. The high $T_g$ of SIPN 2 may be due to more crosslinking in the presence of AA, while the relatively low $T_g$ of SIPN 4 compared with SIPN 2 may be due to internal plasticization by PBMA. A decrease of $T_g$, due to compatibility between SPUU and poly(NIPAM-co-AA-co-BMA), has not been observed when a low content of SPUU was used. However, this can be clearly seen when a high content of SPUU was used. From Fig. 5 and Table 3, it is evident that the $T_g$s of SIPN 7, SIPN 6, SIPN 8, and SIPN 9 are 135, 136, 150, and 114, respectively.

Except for SIPN 8, all show a lower $T_g$ than PNIPAM. Depending on inter- and intramolecular hydrogen bonding between SPUU and the components of the copolymer system (such as NIPAM, AA, and BMA), the compatibility varies, thereby a change in $T_g$ is observed. For instance, in the case of SIPN 9, the $T_g$ is low because of the higher compatibility between SPUU and PNIPAM through intermolecular hydrogen bonding; thereby the $T_g$ of this particular SIPN system is reduced. In the case of SIPN 8, the higher $T_g$ may be due to high crosslinking density due to the presence of AA as well as by intramolecular hydrogen bonding between PNIPAM and PAA. However, in the cases of SIPN 6 and SIPN 7, a relatively low $T_g$ was observed compared to SIPN 8 due to internal plasticization by PBMA. The shift of the $T_g$s suggests that the SIPNs are compatible. It should also be noted that due to the small SPUU content in the SIPNs (10 wt%,

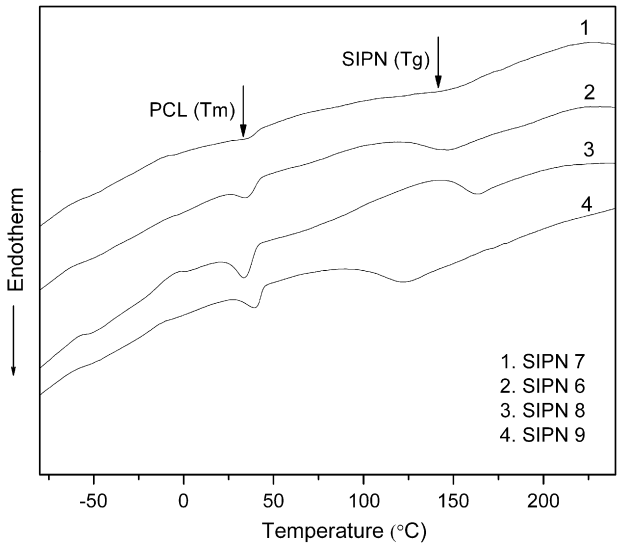


**Fig. 5.** DSC thermogram of high SPUU content SIPNs in dry state.

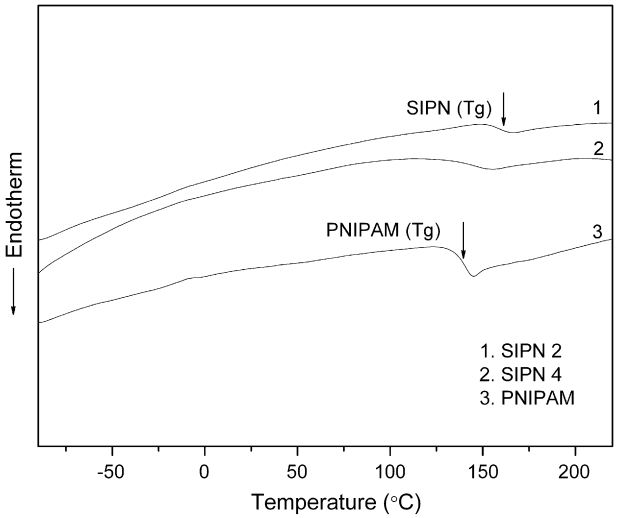


**Fig. 4.** DSC thermogram of PNIPAM and low SPUU content SIPNs in dry state.

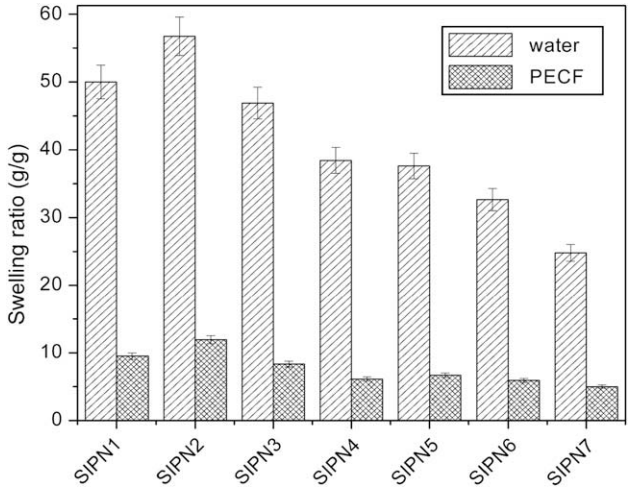


**Fig. 6.** Water equilibrium swelling ratio of SIPNs at 23 °C.

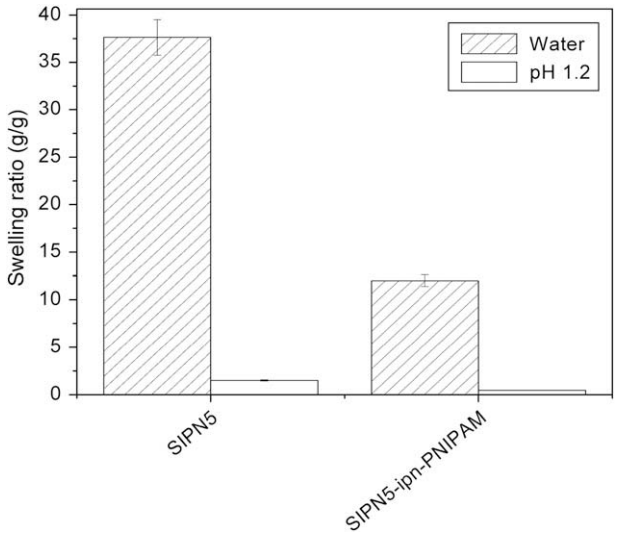


**Fig. 7.** Swelling ratio of SIPNs in water and pH 1.2 buffer at 23 °C.

Fig. 4), the soft-segment $T_g$ and melting ($T_m$) peak have not been detected. When 30 wt% of SPUU was used, although the $T_g$ of the soft segment in some cases was not clear, the $T_m$ was detected (Fig. 5) and exactly matched pure SPUU. The dehydrated SIPNs of greater SPUU content are flexible, whereas SIPNs of lower SPUU content are hard and glassy at room temperature. After reaching equilibrium in water at room temperature, all of the networks were soft, highly swollen, and flexible depending on SPUU content (Fig. 3).

### 3.3. Swelling of hydrogels

The equilibrium swelling of simultaneous SIPNs in water and wound fluid [31] was determined at room temperature, with the results presented in Fig. 6. In order to simulate the absorption behavior of SIPNs to wound fluids, pseudo-extracellular fluid (PECF) solution (pH 7.4) was used as the wound fluid. Fig. 6 shows the uptake of water and the PECF solution by the SIPN gels after 1 h of immersion at room temperature. Apparently, the SIPNs absorbed more water than the PECF solution. This may be due to the fact that the ionic strength of the medium significantly affects the swelling capacity of ionic gels (ionic strength of PECF is 0.48 M). It is also observed from Fig. 6 that the swelling ratio of the gels also depends on the hydrophilic and hydrophobic components of SIPNs. For instance, a higher swelling ratio was observed when a low SPUU content was used in the SIPN system. In contrast, a lower swelling ratio was observed at a high content of SPUU. It has also been observed that when SPUU and PAA content are constant, the swelling levels decreased with an increase in PBMA content. We have also conducted swelling kinetics, which revealed that all simultaneous SIPNs reached equilibrium within 15 min (data not shown). Swelling of simultaneous SIPN 5 and sequential SIPN 5-ipn-PNIPAM in water and at pH 1.2 were also conducted at room

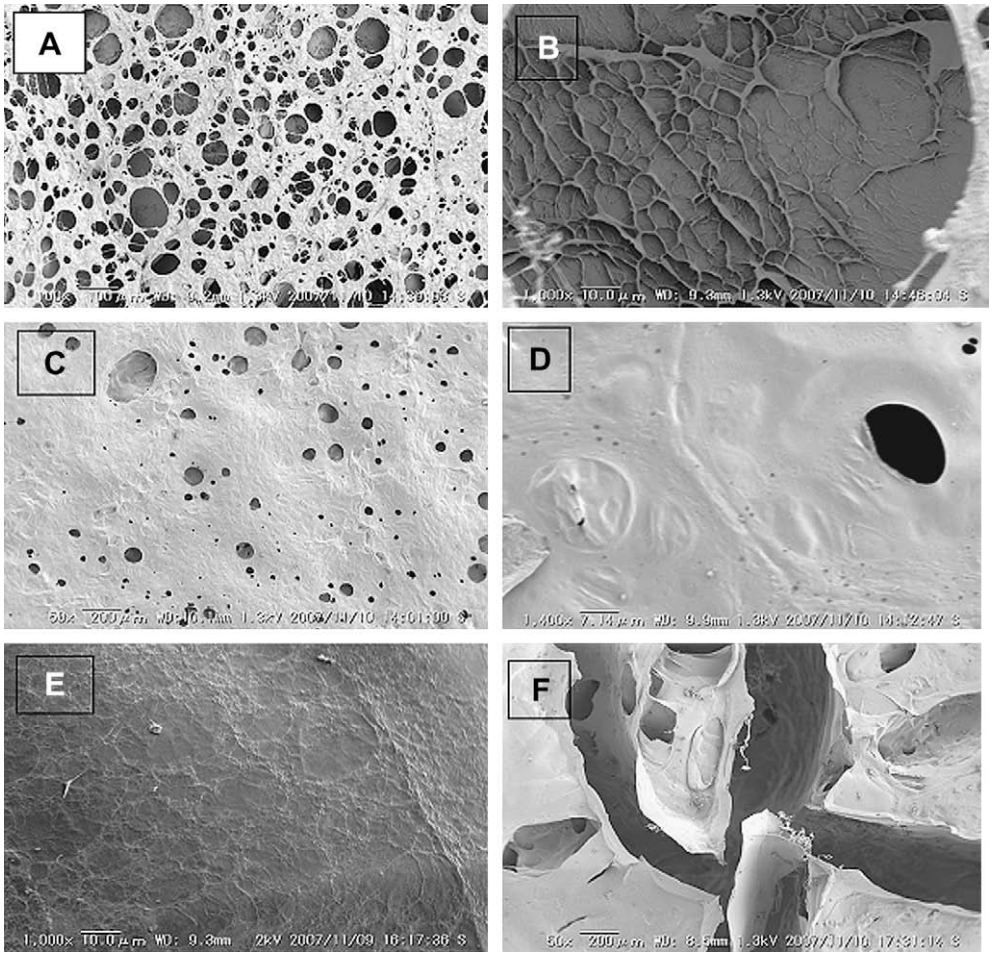


**Fig. 8.** SEM micrographs of freeze-dried samples (A) SIPN 2 (100×), (B) SIPN 2 (1 k×), (C) SIPN 3 (50×), (D) SIPN 3 (1.4 k×), (E) SIPN 4 (1 k×), (F) SIPN 1 (50×).

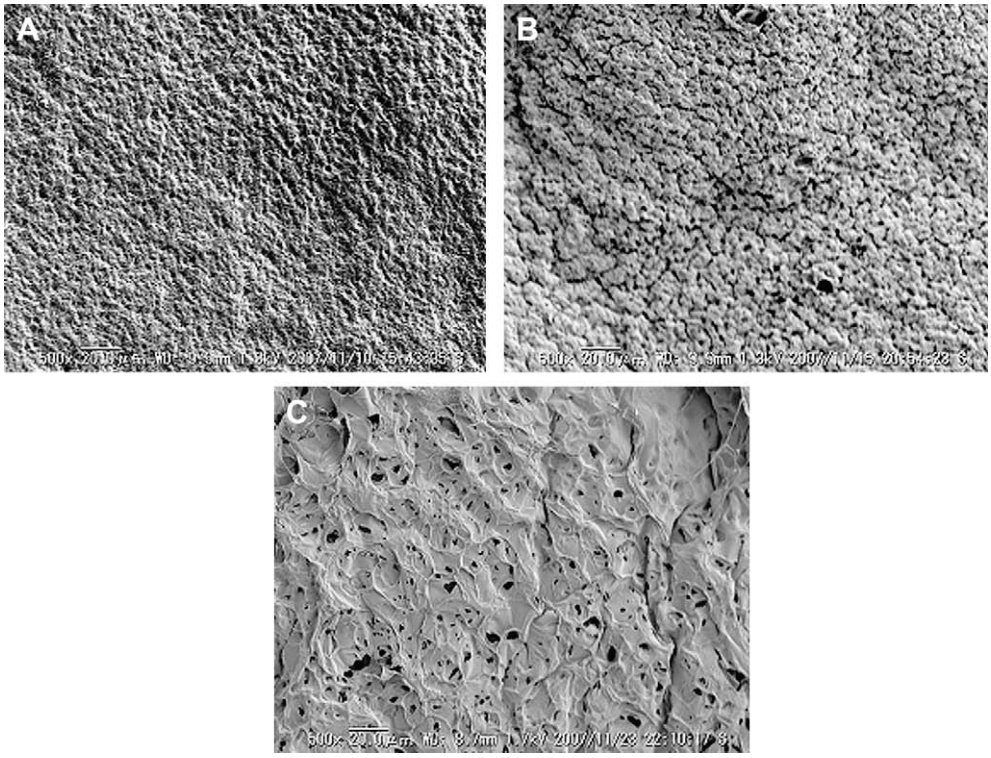


**Fig. 9.** SEM micrographs of freeze-dried samples (A) SIPN 7 (500×), (B) SIPN 5 (500×), (C) SIPN 6 (500×).

temperature, with the results presented in Fig. 7. SIPN 5-ipn-PNIPAM had less water swelling compared with SIPN 5, which may be due to secondary interpenetrating network formation. This reduces the free volume, thereby restricting water penetration into the network structure. However, in both cases the swelling was less at pH 1.2 due to the un-ionization of PAA units.

### 3.4. Interior morphology of hydrogels

Both simultaneous and sequential SIPNs in the swollen state were characterized by scanning electron microscopy (SEM). The structures of SIPN 1, SIPN 2, SIPN 3, and SIPN 4 are presented in Fig. 8. The porous structure of the gels is related to their high swelling levels. The hydrogels of SIPN 2 and SIPN 3, as shown in Fig. 8 (A and C), which shows interconnected porous structures. Fig. 8 (B and D) are the magnified images of Fig. 8 (A and C), show the internal morphology of the pores. Interestingly the pore density decreased with increasing PBMA content, and the pores disappeared when the PBMA content was 40 wt%. This can be attributed to the formation of a skin of PBMA on the hydrogel network. SIPN 2, SIPN 3, and SIPN 4 appear to be tough in the swollen state due to the formation of interconnected pores and by the skin-formation ability of PBMA. However, in the case of SIPN 1, although it forms a porous structure, there is no interconnectivity of the pores due to the absence of SPUU; the gel appears to be broken (Fig. 8 (F)). When a high content of SPUU was used in the SIPN system, the morphology changed from micro-porous to mesoporous, as shown in Fig. 9 (A–C). It is also evident from Fig. 9 (A–C) that the porous morphology varies depending on the AA and BMA content. For instance, in the case of SIPN 7 (Fig. 9 (A)) the porous morphology disappeared and the formation of thick skin was observed, which again may be due to the high content of SPUU and PBMA. However, in the cases of SIPN 5 and SIPN 6, depending on AA content, the varying pore morphology was observed (Fig. 9 (B and C)).

SIPN 5 shows a sponge-like interconnected mesoporous structure (Fig. 9(B)) having carboxyl functionality that can also be used as a template for loading silver nanoparticles to films, resulting in antibacterial activity [31]. The advantages of the present SIPN systems are that the size of the pores and the degree of carboxyl group content can be tailored by varying the composition of the reactants. The meso- and micropores present in the SIPN films facilitate rapid diffusion and confer a high liquid permeability of the reactants through the SIPN gel matrix. The porous morphology of these hydrogels can be used as tissue engineering scaffolds after reacting them with bioactive peptides to induce cell adhesion [32].

The morphology of PNIPAM and the sequential SIPN 5-ipn-PNIPAM hydrogels is shown in Fig. 10 (A and B). The morphology of the normal PNIPAM hydrogel exhibited a homogeneous porous architecture [28] and the SIPN 5-ipn-PNIPAM had a somewhat similar morphology. However, if we look closely at the differences in morphology observed between these two gels, they presumably result from differences in the network moiety and interactions between the first and second networks during the polymerization and crosslinking processes. Uneven mass distribution in the morphology of SIPN 5-ipn-PNIPAM might result from the fact that polymerization and crosslinking may not occur uniformly and simultaneously throughout the system during the formation of the second network, and variation in the NIPAM monomer concentration can cause local variation.

### 3.5. Drug loading

In order to check loading efficiency and release characteristics, we chose two systems in which to examine these properties. Sorption methods were employed to load the drug into simultaneous SIPN 5 and SIPN 5-ipn-PNIPAM. The percentage drug loading in SIPN 5 and SIPN 5-ipn-PNIPAM were 12% and 7%, respectively. SIPN 5 had a higher loading than SIPN 5-ipn-PNIPAM, due to the higher equilibrium swelling of SIPN 5 than SIPN 5-ipn-PNIPAM in the drug solution. Thus,

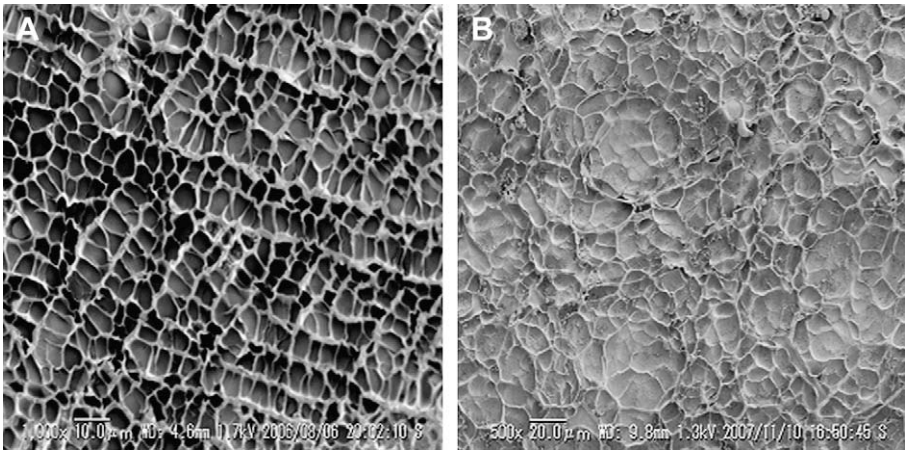


**Fig. 10.** SEM micrographs of freeze-dried samples (A) PNIPAM (1 k×); (B) SIPN 5-ipn-PNIPAM (500×).

the drug loading was influenced by the solubility of the drug as well as the extent of equilibrium solvent uptake by the matrices.

### 3.6. Drug release

These materials, if used as wound dressings, apart from absorbing wound exudates during the healing process, may also work as reservoirs for suitable drugs for localized delivery or in order to prevent bacterial infection. If they are used for oral drug delivery they should show pH-dependent drug release. Therefore, a study of the pH and temperature dependence of drug release was conducted.

Drug release from these matrices may depend on the composition of the SIPN, such as AA, NIPAM, BMA, and SPUU. SF has been incorporated as a model antibacterial drug in to these matrices and its in vitro drug release at pH 7.4 and 1.2 at 37 °C and 22 °C was studied. Figs. 11 and 12 represent the temperature-dependent drug release from SIPN 5 and SIPN 5-ipn-PNIPAM at pH 7.4. In the case of SIPN 5, the drug release is very rapid and all most all of the drug was released in 3 h at 22 °C. The same release behavior was found at 37 °C but at a little lower rate. The thermosensitivity of PNIPAM was nullified by a high content of hydrophilic PAA [17–20]. It is possible to improve the thermosensitivity of SIPN 5 by incorporating PNIPAM into SIPN 5 through the sequential polymerization method, as described above. Fig. 12 shows the temperature-dependent drug release of SIPN 5-ipn-PNIPAM at pH 7.4. The drug release is almost complete within 4 h at 22 °C, whereas the drug release at 37 °C continues for 24 h. These results clearly indicate the temperature dependence of the drug release. Rapid drug release is advantageous if the drugs are used in wound dressing applications.

In order to check the pH dependence of drug release from these SIPN systems, release studies were carried out at pH 1.2. Fig. 13 shows the drug release from SIPNs at pH 1.2 and 37 °C. The percentage of drug release from SIPN 5 and SIPN 5-ipn-PNIPAM at pH 1.2 was, however, very different from release at pH 7.4. Only 20% and 10% of the drug was released from SIPN 5 and SIPN 5-ipn-PNIPAM, respectively, after 24 h at pH 1.2, whereas complete drug release was seen in 24 h at pH 7.4. The slow release of SF could be related to its poor solubility in acidic buffer as well as to poor swelling of the gels at an acidic pH. At pH 1.2, the network swelling was low and the release was limited to an initial burst. At pH 7 and above, the network became ionized and higher swelling resulted in higher drug release. Hydrogels that are responsive to both temperature and pH can be made by simply incorporating ionizable and thermosensitive functional groups into the same system. Many

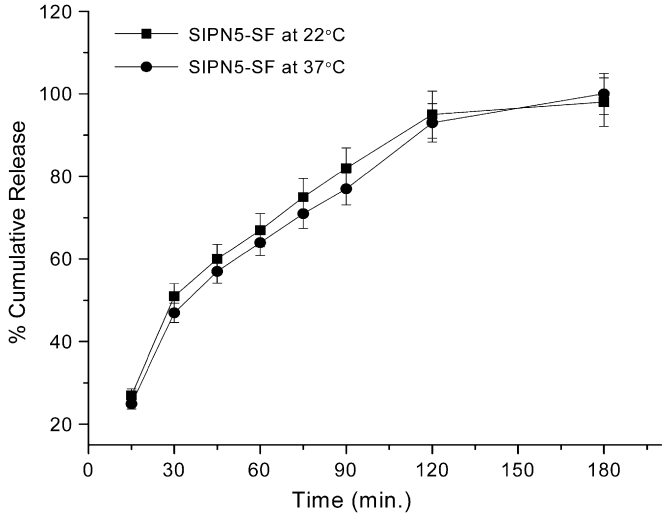


**Fig. 11.** In vitro percent cumulative drug release versus time for SF-loaded simultaneous SIPNs at different temperatures in pH 7.4 PBS.

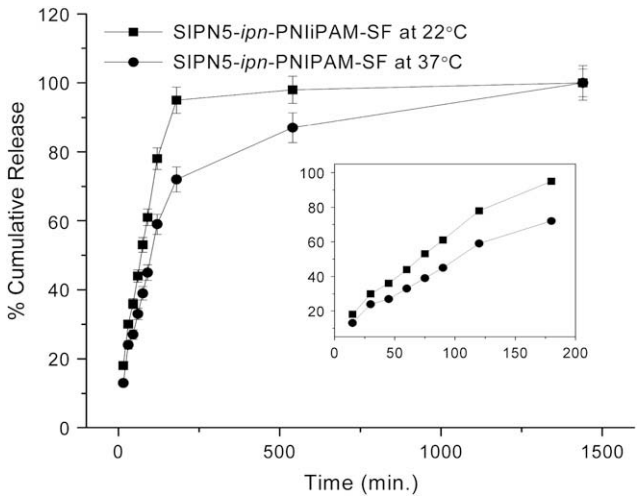


**Fig. 12.** In vitro percent cumulative drug release versus time for SF-loaded sequential SIPNs at different temperatures in pH 7.4 PBS.

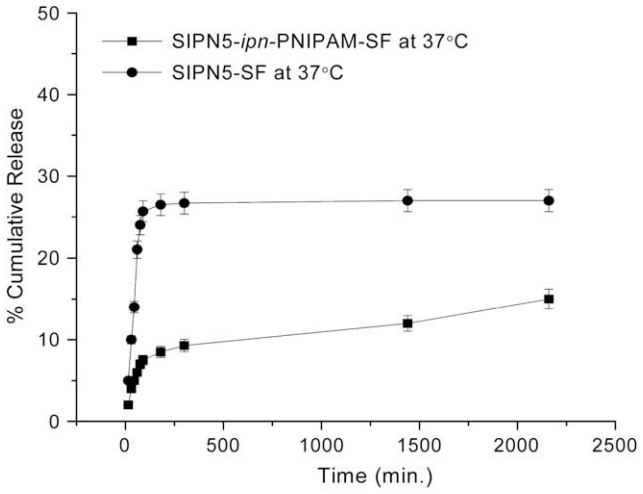


**Fig. 13.** In vitro percent cumulative drug release versus time for SF-loaded simultaneous and sequential SIPNs at 37 °C pH 1.2 buffer.

such systems have been developed to achieve both pH and thermosensitivity in drug delivery applications. For instance, terpolymer hydrogels made of NIPAM, vinyl-terminated polydimethylsiloxane macromer and acrylic acid were used for the delivery of indomethacin and amylase [33,34]. Other terpolymer hydrogels containing NIPAM, acrylic acid, and 2-hydroxyethyl methacrylate were prepared for the pulsatile delivery of streptokinase and heparin, as a function of stepwise pH and temperature changes [35,36]. pH-sensitive hydrogels have also been used in making biosensors and permeation switches [37].

In general, the rates of diffusion of drug molecules from polymers depend on three factors: the size, shape, and solubility of drug molecules; the mobility of the polymer chains; and the interactions between the drug and the polymer chains. Drug molecules diffuse more slowly in polymers that have a higher $T_g$ because these polymers have less free volume and the mobility of the chains is lower. In contrast, drug molecules diffuse more quickly in polymers with a lower $T_g$. However, when we produce SIPNs by polymerizing monomers (which have a high $T_g$ after polymerization) in the presence of a low-$T_g$ polymer, the resulting SIPN has a $T_g$ between the two, if it is a miscible SIPN system. Therefore, the diffusion of a drug from SIPNs can be changed accordingly [6].

Drug release kinetics was analyzed by plotting the cumulative release data versus time by fitting these data to an exponential equation of the type [38]

$$\left(\frac{M_t}{M_\infty}\right) = kt^n$$

Here, $M_t/M_\infty$ represents the fractional drug release at time $t$, $k$ is a kinetic constant related to the drug–polymer interaction and $n$ is an empirical parameter characterizing the release mechanism. By using the least-squares procedure, we estimated the values of $k$ and $n$ for the two samples at two different temperatures, which are given in Table 4. For cylindrical samples, if $n = 0.45$, the drug diffuses and releases out of the polymer matrix following a Fickian diffusion. If $0.45 < n < 0.89$, an anomalous or non-Fickian-type drug diffusion occurs [38]. If $n = 0.89$, a completely non-Fickian or Case II release is operative. In the present study, the values of $n$ calculated for the two systems at two different temperatures varied between 0.66 and 0.68, indicating a swelling controlled release or non-Fickian-type mechanism. The lower $k$ values for all of the systems indicate a lesser interaction between the polymer and the drug.

**Table 4**
Drug release kinetic parameters.

| Sample code | 22 °C | | | 37 °C | | |
|---|---|---|---|---|---|---|
| | $k$ | $n$ | $r^2$ | $k$ | $n$ | $r^2$ |
| SIPN 5 | 0.0489 | 0.66 | 0.9779 | 0.041 | 0.685 | 0.9822 |
| SIPN 5-ipn-PNIPAM | 0.03 | 0.66 | 0.9977 | 0.023 | 0.686 | 0.9922 |

## 4. Conclusion

New interconnected meso- and micro-porous SIPNs were synthesized by simultaneous and sequential polymerization methods. SIPNs prepared by the simultaneous polymerization method exhibited less thermosensitivity due to a greater content of AA. However, thermosensitivity was improved when PNIPAM was incorporated into simultaneous SIPN 5 by using the sequential polymerization method. In addition, both simultaneous and sequential SIPNs exhibit pH sensitivity. Thus, hydrogels that are responsive to both temperature and pH have been synthesized by simply incorporating ionizable and thermosensitive functional groups into the same system. These hydrogels possess sufficient flexibility in the hydrated state, and therefore these hydrogel films may be promising materials for use in wound dressing and other biomedical applications.

## Acknowledgements

The present work was supported by a Grant in aid for the Global COE program 'Science for Future Molecular systems from the MEXT', Japan. T.T.R. gratefully acknowledges Japan Society for Promotion of Science (JSPS) for the financial support in the form of a fellowship.

## References

[1] Hutmacher DW. J Biomater Sci Polym Ed 2001;12:107.
[2] Okano T, Bae YH, Jacobs H, Kim SW. J Controlled Release 1990;11:255.
[3] Gutowska A, Bae YH, Feijen J, Kim SW. J Controlled Release 1992;22:95.
[4] Okuyama Y, Yoshida R, Sakai K, Okano T, Sakurai Y. J Biomater Sci Polym Ed 1993;4:545.
[5] Baek SH, Kim BK. Colloids Surf A Physicochem Eng Aspects 2003;220:191.
[6] Thimma Reddy T, Kano A, Maruyama A, Hadano M, Takahara A. Biomacromolecules 2008;9:1313.
[7] Katono H, Maruyama A, Sanui K, Ogata N, Okano T, Sakurai Y. J Controlled Release 1991;16:215.
[8] Hoffman AS. J Controlled Release 1987;6:297.
[9] Peppas NA, Bures P, Leobandung W, Ichikawa H. Eur J Pharm Biopharm 2000;50:27.
[10] Jing Z, Nicholas AP. Macromolecules 2000;33:102.
[11] Chen GH, Hoffman AS. Macromol Rapid Commun 1995;16:175.
[12] Bulmus V, Ding Z, Long CJ, Stayton PS, Hoffman AS. Bioconjug Chem 2000;11:78.
[13] Hong C, You-Lo H. J Polym Sci Part A Polym Chem 2004;42:3293.
[14] Chen H, Hsieh YL. J Polym Sci Part A Polym Chem 2004;42:6331.
[15] Kuckling D, Adler HP, Arbdt KF, Ling L, Habicher WD. Macromol Chem Phys 2000;201:273.
[16] Shibayama M, Fujikawa Y, Nomura S. Macromolecules 1996;29:6535.
[17] Kobayashi J, Kikuchib A, Sakaia K, Okano T. J Chromatogr A 2002;958:109.
[18] Yoo MK, Sung YK, Cho CS, Lee YM. Polymer 1997;38:2759.
[19] Yoo MK, Sung YK, Lee YM, Cho CS. Effect of polyelectrolyte on the lower critical solution temperature of poly(*N*-isopropyl acrylamide) in the poly(NIPAM-co-acrylic acid) hydrogel. Polymer 2000;41:5713.
[20] Lee W, Shieh C. J Appl Polym Sci 1999;73:1955.
[21] Xian-Zheng Z, Da-Qing W, Chih-Chang C. Biomaterials 2004;25:3793.
[22] Jian-Tao Z, Shi-Wen H, Si-Xue C, Ren-Xi Z. J Polym Sci Part A Polym Chem 2004;42:1249.
[23] Takahara A, Coury AJ, Hergenrother RW, Cooper SL. J Biomed Mater Res 1991;25:341.
[24] Takahara A, Hergenrother RW, Coury AJ, Cooper SL. Artif Organs 1990;14:87.
[25] Lyman DJ, Metcalf LC, Albo D, Richard KF, Lamb J. Trans Am Soc Artif Int Organs 1974;20:474.
[26] Takahara A, Hadano M, Yamguchi T, Otsuka H, Kidoaki S, Matsuda T. Macromol Symp 2005;224:207.

[27] Thimma Reddy T, Hadano M, Takahara A. Macromol Symp 2006;242:241.
[28] Gutowska A, Bae YH, Jacobs H, Feijen J, Kim SW. Macromolecules 1994;27:4167.
[29] Kim SY, Cho SM, Lee YM, Kim SJ. J Appl Polym Sci 2000;78:1381.
[30] Vilas DA, Priti SP. Bull Chem Soc Jpn 2003;76:1265.
[31] Thimma Reddy T, Takahara A. J Polym Sci Part A Polym Chem, submitted for publication.
[32] Hoffman AS. Adv Drug Deliv Rev 2002;43:3.
[33] Dong LC, Hoffman AS. J Controlled Release 1991;15:141.
[34] Vakkalanka SK, Brazel CS, Peppas NA. J Biomater Sci Polym Ed 1996;8:119.
[35] Brazel CS, Peppas NA. J Controlled Release 1996;39:57.
[36] Hoffman AS. Intelligent polymers. In: Park K, editor. Controlled drug delivery: challenge and strategies. Washington, DC: American Chemical Society; 1997. p. 485–97.
[37] Dong LC, Hoffman AS. J Controlled Release 1990;13:21.
[38] Ritger PL, Peppas NA. J Controlled Release 1987;5:37.