Synthesis and Characterization of Biodegradable Interpenetrating Polymer Networks Based on Gelatin and Divinyl Ester Synthesized from Poly(caprolactone diol)

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ABSTRACT: This article describes the synthesis and characterization of interpenetrating polymer networks (IPNs) from hydrophilic and hydrophobic polymers using emulsification technique. Tween 20 (0.001 wt % of gelatin) was employed as emulsifier for the preparation of semi and full IPNs. Gelatin (G), a hydrophilic component was crosslinked by glutaraldehyde (Glu) and divinyl ester (DVE), a hydrophobic component was polymerized/crosslinked using 3 mol % of AIBN as an initiator. Structural characterization was done using FTIR (doublet at 1620 and 1636 cm^{-1}) and NMR (signals in the range of $\delta = 5$–7 ppm), which confirmed the formation of DVE. Several samples were prepared by varying the ratio of gelatin : DVE (w/w) and the Glu concentration. The swelling characteristics (as a function of varying pH maintained using buffers) and degradation behavior (in phosphate buffer saline pH 7.4) of hydrogels was studied to investigate the effect of composition and crosslinker concentration. Percent water uptake decreased from 496 to 181 at pH 7.4 and pH 6.5 in IPNs as the concentration of DVE increased from 0.3 g to 0.7 g per g of gelatin. Semi-IPNs, where DVE was not polymerized, demonstrated higher swelling at pH 7.4 in contrast to pH 6.5 irrespective of Glu concentration. Gelatin hydrogels degraded within 180 h and IPNs degraded within 290 h whereas DVE did not degrade till the study period of 20 days. The formation of IPN was confirmed by thermal characterization (DSC, TGA) and scanning electron microscopy (SEM). Observation of cross-sectional microstructure of disrupted honeycomb of Gx into closely packed fiber-like structure upon interpenetration by SEM clearly suggests the formation of IPN. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 1478–1487, 2009

Key words: interpenetrating polymer networks (IPNs); gelatin; divinyl ester (DVE); poly(caprolactone diol); percent water uptake; thermal characteristics; SEM; degradation

INTRODUCTION

In the current era of drug delivery technologies, hydrogels have made an irreplaceable space because of their unique properties. They are three dimensional, insoluble, crosslinked polymer networks which retain large volumes of water/biological fluid in their swollen structures when placed in water. Additionally, low interfacial tension with surrounding biological fluids and tissues, high mobility of polymer chains at the hydrogel surface prevent protein and cell adhesion. Because of their soft and rubbery nature, they mimic the biological tissues and possess high biocompatibility.[1] No single hydrogel satisfy all the requirements with respect to controlled drug release, mechanical properties, and biodegradation. Evolution of new hydrogel structures to achieve desired properties for particular treatment has gained a great deal of attention. One such kind of system is interpenetrating polymer networks (IPNs), especially hybrid of synthetic and natural polymers, with altered morphologies and synergistic properties of homopolymers, leads to materials with features of both homopolymer components, have addressed great advantages.[2–4] IPNs of well characterized hydrophilic and hydrophobic polymers[5–7] have improved capability of immobilizing the drug and allow drug release by diffusion or dissolution through matrix due to the swelling of hydrophilic chains. However, when such system contains a biodegradable polymer, drug release by diffusion as well as simultaneous degradation of the chain avoids follow-up surgery after the application.

In the present study, IPNs based on gelatin and DVE have been synthesized and characterized. Gelatin, a natural polymer consisting of degradable amino acid chains with low antigenicity is widely used in tissue engineering, coating material for pharmaceuticals, would dressing, adsorbing pad, matrix

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for drug delivery etc. Gelatin-based semi-interpenetrating polymer networks (sIPN) and IPNs are widely reported for drug delivery applications.[8–12] Research in implants in recent years, indicates that the Glu crosslinking has been clinically acceptable at lower concentrations to improve biocompatibility[13] despite having some demerits.[14] PCL, a bioerodable, biocompatible polymer, is used to prepare hydrogels[15–18] as tissue engineering blocks, drug delivery system.[19–21] Polycaprolactone as hydrogel scaffold for cell delivery application,[22] sIPN as drug delivery devices[23] with other polymers have been reported. Its biodegradability is enhanced when it is used as copolymer[24] or blend[25] with other polymers. Recently, porous sIPN films of poly(caprolactone diol) and vinyl modified polycaprolactone with polystyrene film was reported by Lumelsky et al.[26] by emulsification technique. Very recently combination of PCL with gelatin for drug delivery and tissue engineering application has been reported.[27,28]

Earlier articles published from our group were based on IPNs of gelatin and acrylic acid/vinyl pyrrolidone.[29,30] These IPNs possess a biodegradable component (gelatin) and water soluble component (acrylic acid or vinyl pyrrolidone) crosslinked using degradable crosslinker i.e., methylene bisacrylamide, whereas in the present article the authors have chosen IPNs, where both the polymeric chains are biodegradable and highly biocompatible. Gelatin, being highly hydrophilic degrades faster in contrast to DVE, being a hydrophobic counterpart, is proven for its slow degradation. However, IPNs comprising of gelatin and DVE has shown intermediate in degradation in comparison to hydrogels based on gelatin or DVE alone. Thus the above system may find application as drug delivery system for anticancerous drugs in the treatment of solid tumors after excision, where the ideal requirement for implantable devices is not only biocompatibility and biodegradability, but also moderate swelling, low drug burst, and controlled drug release profile. The article describes synthesis and physicochemical evaluation of IPNs based on gelatin and divinyl ester (DVE). The other investigations addressing its drug release kinetics are under progress.

EXPERIMENTAL

Materials

Poly(caprolactone diol) (PCL diol mol wt = 530) (Aldrich, Germany), gelatin A of 300 bloom (Sigma, Germany), methanol (Qualigens, Mumbai, India), glutaraldehyde 25% solution (Thomas Baker, Mumbai, India), glycine (Sisco Laboratories, Mumbai, India), azobis-isobutyrylonitrile (AIBN) (G.S. chemical testing lab and allied industries, Bombay, India), Tween 20 (Sigma, Germany), chloroform (Qualigens, Mumbai, India), phosphate buffer saline pH 7.4 and 6.5, acryloyl chloride (Aldrich, Germany), anhydrous triethylamine (TEA) (Spectrochem Pvt Ltd, Mumbai, India), anhydrous sodium sulfate (Merck Ltd, Mumbai, India). All the chemicals were used as received.

Synthesis and characterization of DVE

Fifty grams of PCL diol (0.0981 mol) was dissolved in 250 mL of chloroform in a 500-mL round bottom flask. To this 19.089 g (0.189 mol) of triethylamine was added followed by dropwise addition of acryloyl chloride [17.1454 g (0.1886 mol)] and the mixture was stirred continuously. After complete addition, the solution was stirred at 60°C for 20 h. Intermittently TLC was carried out to confirm the formation of DVE using chloroform-methanol (7 : 3) system. Unreacted acryloyl chloride and TEA salts were removed by repeated washing with water and chloroform of 100 mL each, alternatively 250 g of sodium sulfate was added in 10 g increment to the above solution till it becomes clear. Residual chloroform was removed using rotary vacuum evaporator. Formation of vinyl group was confirmed by potassium permanganate test i.e., to 1 mL of aqueous solution of potassium permanganate a drop of DVE was added, immediate decolorization indicated the presence of double bond which was further confirmed by FTIR and NMR technique. FTIR spectra of PCL diol and divinyl ester were recorded using Nicolet 5PC, FTIR spectrophotometer, as a thin film on KBr crystal from 400 to 4000 cm^{-1}. A Brucker AC 300 spectrophotometer at a frequency of 300 MHz was used for recording NMR of PCL diol and divinyl ester using $CDCl_3$ as solvent and tetramethyl silane as an internal standard.

FTIR spectrum of DVE shows the absence of absorption band due to hydroxyl group and presence of doublet at 1620 and 1636 cm^{-1} due to the vinyl groups. In ^{1}H-NMR spectrum of PCL no signal was observed in the range of $\delta = 5$–7 ppm. The signal due to OH was observed at $\delta = 3.7$ ppm. In ^{1}H-NMR spectrum of DVE, the signal due to methine proton was observed at $\delta = 6.01$–6.05 and two vinyl protons (terminal) being different were observed at $\delta = 5.69$–5.73 ppm and 6.24–6.25 ppm. All other signals present in the ^{1}H-NMR spectrum of PCL were seen in the spectrum of DVE except hydroxyl group thereby confirming the structure of DVE (Figures not shown).

Preparation of hydrogels

For the preparation of homopolymers of gelatin and DVE the following procedures were adopted.

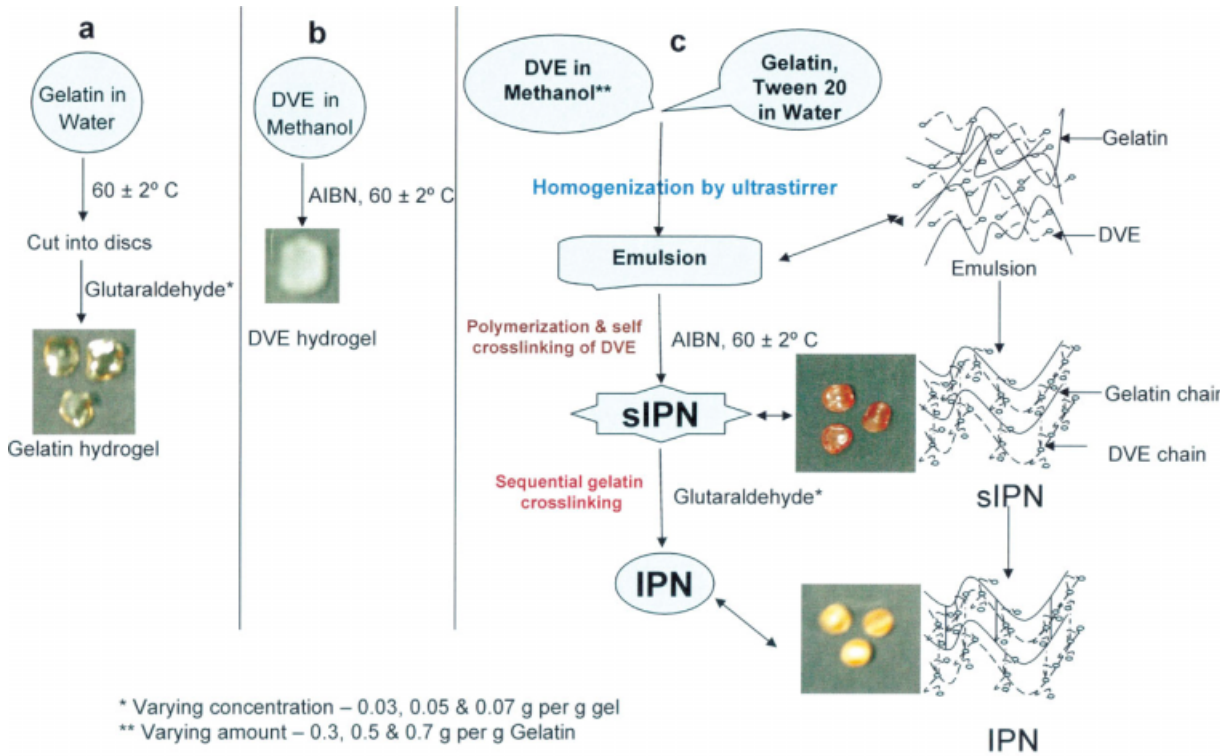
TABLE I
Details of Sample Designation, Feed Composition, and Percent Equilibrium Swelling of Hydrogels at pH 6.5 and 7.4

Hydrogel designation	DVE (g)	Gelatin (g)	Glu (wt % of gel)	DVE – gelatin ratio	% Eq. Swelling	
					pH 7.4	pH 6.5
Dx	1	–	–	1 : 0	80	0
$Gx_{0.03}$	0	2	0.03	0 : 1	517	719
$Gx_{0.05}$	0	2	0.05	0 : 1	430	519
$Gx_{0.07}$	0	2	0.07	0 : 1	425	417

D represents DVE and G represents Gelatin.
Mark x indicates crosslinking of the corresponding polymer. Numerical subscription of G indicates the weight percent of glutaraldehyde used for crosslinking.

Two grams of gelatin solution (dissolved in 20 mL of distilled water after heating at 37°C) after purging with nitrogen for 5 min was poured into silicon sprayed petri-dish and left overnight in an incubator at (60 ± 2)°C. Dried gelatin film was removed and cut into small discs using 7 mm stainless steel punch. Weighed amount of discs were crosslinked with varying concentration of Glu (0.03, 0.05, and 0.07 g per g of gelatin) to get hydrogels $G_{x0.03}$, $G_{x0.05}$, and $G_{x0.07}$ respectively.

Similarly 1 g of DVE was dissolved in 1 mL of AIBN in methanol and purged for 5 min with nitrogen and kept undisturbed in an incubator at (60 ± 2)°C, overnight. The formed gel was washed several times with methanol to remove unreacted DVE. The gels were further dried to a constant weight under vacuum. Feed composition and hydrogel designation are given in Table I Homopolymers of DVE and gelatin (crosslinked with Glu) were designated as Dx and Gx, respectively. The numerical suffix to D represents the amount of DVE to 1 g of gelatin whereas numerical suffix to G represents the Glu concentration used for the crosslinking process. The symbol x in D and G denotes the crosslinking. For example D in the formulae denotes free DVE and Dx denote crosslinked DVE. Similarly G represents free gelatin and Gx denotes crosslinked gelatin. Schematic representation of preparation of hydrogels was depicted in Scheme 1(a,b).



Scheme 1 Schematic representation of preparation of Gelatin hydrogel (a), DVE hydrogel (b) sIPN and IPN from DVE and Gelatin (c). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
Details of Sample Designation, Feed Composition, and Percent Equilibrium Swelling of sIPNs at pH 6.5 and 7.4

Group designation	sIPN designation	DVE (g)	Gelatin (g)	Glu (wt % of gel)	DVE – gelatin ratio	% Eq. Swelling	
						pH 7.4	pH 6.5
Group A	$Dx_{0.3}G$	0.6	2	–	0.3 : 1	*	*
	$Dx_{0.5}G$	1.0	2	–	0.5 : 1	*	*
	$Dx_{0.7}G$	1.4	2	–	0.7 : 1	**	**
Group B	$D_{0.3}Gx_{0.03}$	0.6	2	0.03	0.3 : 1	705	663
	$D_{0.3}Gx_{0.07}$	0.6	2	0.07	0.3 : 1	409	382
Group C	$D_{0.5}Gx_{0.03}$	1.0	2	0.03	0.5 : 1	593	558
	$D_{0.5}Gx_{0.07}$	1.0	2	0.07	0.5 : 1	371	342
Group D	$D_{0.7}Gx_{0.07}$	1.4	2	0.03	0.7 : 1	539	504
	$D_{0.7}Gx_{0.07}$	1.4	2	0.07	0.7 : 1	323	290

D represents DVE and G represents Gelatin.
Mark x indicates crosslinking of the corresponding polymer. Numerical subscription of D indicates the amount of DVE in g used. Numerical subscription of G indicates the weight percent of glutaraldehyde used for crosslinking.
* sIPNs degraded with in few h without any swelling.
** sIPN degraded in 24 h without any swelling.

Preparation of sIPNs

For the preparation of sIPNs, gelatin (2 g) was soaked in 20 mL of double distilled water for half an hour and heated at 37°C for complete dissolution. To this solution Tween 20 (0.001 wt % of gelatin) was added and designated as part A. The nonaqueous phase containing AIBN (3 mol % of DVE) in 10 mL of methanol was designated as Part B. In 1 mL of above solution weighed amount of DVE was dissolved and remaining 9 mL of part B was kept aside. Nine milliliters of AIBN in methanol (Part B) was added to aqueous gelatin solution with stirring at 22,000 rpm using an ultra-stirrer. This was followed by the addition of 1 mL of DVE in methanol drop by drop and stirring was continued for 5 min at 26,000 rpm to get an emulsion and purged with nitrogen for 5 min. The emulsion, thus formed was immediately poured into a silicone sprayed glass petri-dish and left overnight undisturbed in an incubator at (60 ± 2)°C. For the preparation of sIPN in which DVE was non-crosslinked, initiator AIBN was not added. Opaque sIPN films, thus formed were washed several times with distilled water, methanol and cut into circular discs of 7 mm diameter and dried under vacuum. sIPN samples having non-crosslinked gelatin with the ratio of 0.3 : 1, 0.5 : 1, 0.7 : 1 of DVE : gelatin have been denoted as $D_{x0.3}G$, $D_{x0.5}G$, $D_{x0.7}G$, respectively, whereas sIPN samples of DVE (non-crosslinked) and gelatin crosslinked with varying amounts of Glu have been denoted as $D_{0.3}G_{x0.03}$, $D_{0.3}G_{x0.07}$, $D_{0.5}G_{x0.03}$, $D_{0.5}G_{x0.07}$, $D_{0.7}G_{x0.03}$, and $D_{0.7}G_{x0.07}$, respectively. In all these samples Dx and Gx represents crosslinked DVE and gelatin, respectively. The numerical suffix to Dx represents the ratio of DVE to gelatin (w : w) and numerical suffix to Gx represents the amount of Glu used for crosslinking. The details of feed composition and sample designations are given in Table II and the preparation of sIPNs and IPNs is shown in Scheme 1(c).

Preparation of full-IPNs

Dry sIPN discs ($D_{x0.3}G$, $D_{x0.5}G$, $D_{x0.7}G$) of ~ 0.5 g were treated with varying amounts of Glu (0.03/0.05/0.07 g per g disc) (20 mL) for 6 h at room temperature to form IPNs. The excess Glu was quenched with 10 mL of 0.05*M* glycine solution in water, followed by repeated washings with double distilled water till no detectable amount of Glu was found using spectroscopy. The IPN samples were designated as $D_{x0.3}G_{x0.03}$, $D_{x0.3}G_{x0.05}$, $D_{x0.3}G_{x0.07}$, $D_{x0.5}G_{x0.03}$, $D_{x0.5}G_{x0.05}$, $D_{x0.5}G_{x0.07}$, $D_{x0.7}G_{x0.03}$, $D_{x0.7}G_{x0.05}$ and $D_{x0.7}G_{x0.07}$ where $G_{x0.03,\ 0.05,\ 0.07}$ represents the crosslinker concentration of Glu and $D_{x0.3,\ 0.5}$ and $_{0.7}$ represents the amount of DVE to 1 g of gelatin. The details of feed composition and sample designations are given in Table III.

Characterization

Water uptake

Swelling studies of hydrogels, sIPNs, and IPNs were carried out in phosphate buffer saline (pH 7.4) and phosphate buffer (pH 6.5) at 37°C gravimetrically. For this purpose ~ 10 mg of each disc was taken in 2 mL of buffer solution of varying pH in a glass vial with a rubber cap and kept in an incubator

TABLE III
Details of Sample Designation, Feed Composition, and Percent Equilibrium Swelling of IPNs at pH 6.5 and 7.4

Group designation	IPN designation	DVE (g)	Gelatin (g)	Glu (wt % of gel)	DVE – gelatin ratio	% Eq. Swelling	
						pH 7.4	pH 6.5
Group E	$Dx_{0.3}Gx_{0.03}$	0.6	2	0.03	0.3 : 1	496	524
	$Dx_{0.3}Gx_{0.05}$	0.6	2	0.05	0.3 : 1	376	363
	$Dx_{0.3}Gx_{0.07}$	0.6	2	0.07	0.3 : 1	326	343
Group F	$Dx_{0.5}Gx_{0.03}$	1.0	2	0.03	0.5 : 1	320	343
	$Dx_{0.5}Gx_{0.05}$	1.0	2	0.05	0.5 : 1	263	255
	$Dx_{0.5}Gx_{0.07}$	1.0	2	0.07	0.5 : 1	221	220
Group G	$Dx_{0.7}Gx_{0.03}$	1.4	2	0.03	0.7 : 1	262	283
	$Dx_{0.7}Gx_{0.05}$	1.4	2	0.05	0.7 : 1	201	174
	$Dx_{0.7}Gx_{0.07}$	1.4	2	0.05	0.7 : 1	181	149

D represents DVE and G represents Gelatin.
Mark x indicates crosslinking of the corresponding polymer. Numerical subscription of D indicates the amount of DVE in g used. Numerical subscription of G indicates the weight percent of glutaraldehyde used for crosslinking.

undisturbed. At different time intervals, the discs were taken out from the medium, blotted with filter paper and weighed. Every time fresh solution of buffer was replaced to the vial. Percent water uptake was calculated using the equation:

$$\text{Percent water uptake} = W - W_o/W_o \times 100$$

where W = weight of the swollen sample W_o = weight of the dried sample.

Degradation studies

For degradation studies, a known weight of hydrogels, sIPNs, and IPNs was immersed in phosphate buffer saline pH 7.4 at 37°C and the change in weight was recorded at regular intervals till it degraded completely. For this purpose, ~ 10 mg of each disc was put into phosphate buffer saline (2 mL) and kept in an incubator at 37°C. At different time intervals the samples were taken out, blotted with filter paper, and weighed. After every reading, the samples were immersed in a fresh medium and the procedure was repeated till the samples degrade completely or disintegrate and was not possible to weigh. The experiments were carried out in triplicate and the mean values were taken to plot degradation time versus weight (Fig. 2).

Morphological characterization

Scanning electron microscope (Gemini, Zeiss, Germany) was used for the morphological characterization of hydrogels. For this purpose, gels were swollen to equilibrium in PBS (pH 7.4) for 24 h, washed several times with distilled water, quickly frozen in liquid nitrogen, and freeze dried using a freeze drier (Christ, Germany, Alpha 1–2) at −52°C for overnight. Then transverse sections were cut using a cold knife and the cross-sectional morphology was observed at 45° angle and surface morphology at 90° angle by changing the sample stage on the scanning electron microscope.

Thermal characterization

Differential scanning calorimetry (DSC) scans of hydrogels, sIPN, and IPNs were recorded using 2920 Modulated DSC (TA instruments). A heating rate of 5°C/min and a sample size of 5 ± 1 mg was used in each experiment. Thermal stability of hydrogels, semi-IPNs, Full IPNs was investigated by recording thermogravimetric (TG)/derivative thermogravimetric (DTG) traces in nitrogen atmosphere. TG/DTG traces were recorded using 5 ± 1 mg of samples at a heating rate of 20°C/min from 50° to 850°C using TA 2100 thermal analyzer.

RESULTS AND DISCUSSION

An extensive study related to IPNs based on gelatin and poly(acrylic acid) (PAA) or poly(vinyl pyrrolidone) (PVP)[29,30] as drug carriers has been carried out in our laboratory and their application as drug carrier has either been reported or is being under investigation. The above said IPNs belong to biocompatible polymers but the synthetic components used are classified as water soluble and not-degradable. Furthermore, these systems demonstrated higher drug burst and longer residence time on *in vivo* implantation studies.[31] Therefore, the authors replaced PAA or PVP by divinyl ester in the present study. On the basis of the composition of IPNs (gelatin : DVE), these systems can be

classified as degradable as well as biocompatible and the detailed study is described as follows.

Initial studies and physical characterization of gels

In the beginning of the experimentation an attempt was made to crosslink gelatin using poly(ethylene glycol) (PEG) dialdehyde (mol wt 1000) because of its accepted biocompatibility. However, no stable gels were obtained and the handling of the films was difficult because of high hydrophilicity of PEG (data not shown). Therefore to achieve the stable gelatin gels Glu was employed as a crosslinker. Moreover, initial formulation studies on preparation of DVE hydrogel revealed that 3 mol % of AIBN was sufficient for optimum polymerization. DVE, being insoluble in water, simple mixing of aqueous and nonaqueous phase separated into two different layers, thus emulsification technique (using Ultraturrax, T18, Basic, IKA, Germany) was used. Dx was transparent, brittle in nature whereas Gx was brown in color. IPNs were yellowish brown in color whereas sIPNs of gelatin (non-crosslinked) were white in color.

Swelling behavior

Almost all samples reached equilibrium within 24 h. Since the swelling rate of hydrogels depends on composition (gelatin : DVE w : w), crosslinker concentration and pH of the medium, swelling characteristics are discussed in the following text as a function of these parameters.

Effect of Glu concentration

Glu, a crosslinker connects the free amino groups present in the adjacent chains of the gelatin and form Schiff base to cure it. When the amount of crosslinker increased, crosslink density increases and thus reduces the water uptake. It has been anticipated that all the amino groups are not being utilized for crosslinking with Glu. Our previous studies[29] have shown that 1% of Glu is sufficient to crosslink all the amino groups of gelatin. However in the present study, authors have employed Glu concentration ranging from 0.03 to 0.07 wt % of gelatin thus hypothesizing that many of the amino groups will remain free and account for swelling of the network. The decrease in water absorption capacity when Glu concentration increased from 0.03 to 0.07% (from $G_{x0.03}$ to $G_{x0.07}$) is evident due to the increase in the crosslinker concentration making the hydrogel network tight and suppressing the water absorption capacity. For sIPNs at pH 7.4 the decrease in water uptake is slightly higher (42%) (From 705 to 409 for $D_{0.3}G_{x0.03}$ to $D_{0.3}G_{x0.07}$) (group B, Table II). The samples prepared using varying amounts of DVE had water uptake of (32 $\pm$ 2)% and it decreased with increasing concentration of Glu. This indicates crosslinking of gelatin chains is dependent on the Glu concentration and independent of DVE amount used. This further supports that there is no interaction of Glu with the other component polymer, DVE. As expected for each fixed amount of DVE, increasing Glu from 0.03 to 0.07 g decreases the water uptake upto 34% (group E - 496 to 320 for $D_{x0.3}G_{x0.03}$ to $D_{x0.3}G_{x0.07}$) at pH 7.4 for IPNs. Similar kind of trend was observed for other set of IPNs (group F and G).

Effect of DVE concentration

The earlier studies based on IPNs made from two highly hydrophilic systems showed 60% of drug burst.[32] However in the present study the authors want to study the release of antineoplastic drugs, where a lower drug burst is an essential requirement for the treatment of cancerous tumors. It is anticipated that the high drug burst not only kills the target cells but also the healthy cells in vicinity due to their high toxicity. Thus a combination of hydrophobic and hydrophilic components has been chosen to synthesize IPNs, and to evaluate them for drug loading and release kinetics. Since the above work is not the purview of the article and is not being discussed here.

To understand the effect of DVE concentration on the swelling of sIPNs, the systems have been categorized into groups A, B, C, and D. Group A comprises systems where gelatin is free and DVE is polymerized/self-crosslinked. These systems degraded within a day and no swelling was observed. The reason for the above behavior is due to the solubility of non-crosslinked gelatin chains. Thus it is clear that the swelling is not due to the DVE component but due to the gelatin chains. Group B, C, and D depict sIPN systems where gelatin was crosslinked and DVE was free. From group B to D, DVE was fixed to 0.3, 0.5, and 0.7 g per g of gelatin, respectively, and for gelatin crosslinking, Glu concentration of only 0.03 and 0.07 wt % has been employed. Increasing DVE ratio to gelatin i.e., 0.3 : 1, 0.5 : 1, 0.7 : 1, from 0.3 to 0.7 (group B to D, Table II) decreases the swelling and % swelling observed was 705, 593, 539 respectively, (100 $>$ 84 $>$ 76%, if 705 is taken as 100%) at pH 7.4 for fixed Glu concentration of 0.03 g. Almost similar trend in water uptake was observed for other hydrogel samples too. The decrease in percent swelling is lower in comparison to IPNs. The higher water uptake in case of sIPN is due to the free DVE chains which does not affect the swelling of the crosslinked gelatin chains. It is also notable that hydrogel of gelatin with same Glu concentration ($G_{x0.03}$) shows lower

equilibrium swelling (517, which is approx 27% lower) compared with its counterpart $D_{0.3}G_{x0.03}$ (705) which indicates free, hydrophobic, flexible chains of DVE in sIPN that does not allow breaking of gelatin chain (gives strength to gelatin chains to increase the chain stability), whereas it was not possible with $G_{x0.03}$. The results obtained from the above sIPN system clearly explain that the system comprising of hydrophobic and hydrophilic chains can be called true sIPNs due to following reason: when gelatin was free, group A system collapse totally without any swelling and degraded within a day, whereas when gelatin was crosslinked and DVE was free, the system shows higher swelling and slow degradation in contrast to systems where both the chains are made of hydrophilic polymers. For example in our earlier work comprising of gelatin and acrylic acid (AA) sIPNs, when AA was not crosslinked, it leached out of the system, thus making sIPNs comparable to a hydrogel system.[29]

The elaborate method for the synthesis of IPNs has been discussed in the experimental section. IPNs have also been categorized into three groups (Group E, F, and G). When the DVE ratio to gelatin was increased, water uptake (at pH 6.5 and 7.4) decreased. This is due to the higher crosslink density of self-crosslinked DVE in between the gelatin chains. For example for a fixed Glu concentration of 0.03 wt % of gelatin (Group E–G), at pH 7.4, equilibrium water uptake decreased as DVE concentration increased : 496 ($D_{x0.3}G_{x0.03}$) > 320 ($D_{x0.5}G_{x0.03}$) > 262 ($D_{x0.7}G_{x0.03}$). If we consider 496 as 100% equilibrium swelling, it decreased to 65% in $D_{x0.5}G_{x0.03}$ and 53% in $D_{x0.7}G_{x0.03}$. Almost similar trend was observed for other samples prepared using Glu concentrations (0.05 or 0.07) at pH 7.4 as well as at pH 6.5. Another interesting observation was that the percent equilibrium swelling of IPNs did not show any significant difference at pH 7.4 and at pH 6.5. The following trend was observed when water uptake was compared in hydrogels

$$Dx < IPN < Gx < sIPN$$

i.e., Dx showed the lowest and s-IPNs showed the highest water uptake.

Effect of pH

Table I shows the percent water uptake in hydrogels at pH 7.4 and 6.5. Crosslinked DVE (Dx) did not show any swelling at pH 6.5 even after immersion for an infinite time. On the other hand, at pH 7.4, 80% swelling was noticed within 20 days. The differences in the swelling behavior at pH 6.5 and 7.4 could be due to the difference in ionic strength,

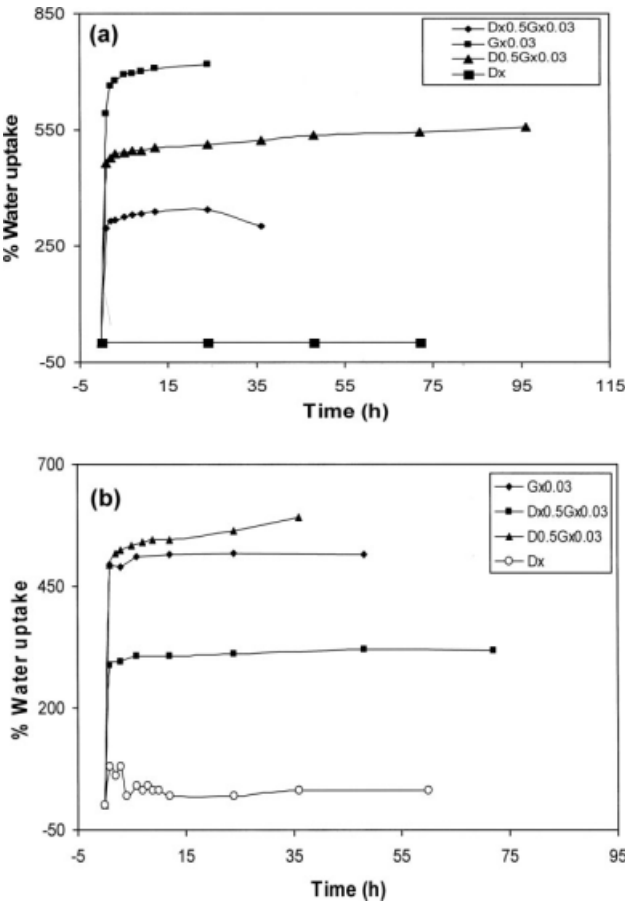


Figure 1 Comparison of water uptake of hydrogel, sIPN and IPN at pH 6.5 (a) and at pH 7.4 (b).

which is further responsible for difference in the osmotic pressure created by the ions inside and outside the system. In contrast, crosslinked gelatin (Gx), where, Glu concentration was increased from 0.03 to 0.07 wt %, the equilibrium water uptake decreased from 719 to 417 at pH 6.5 in comparison to 517 to 425 at pH 7.4. The higher water uptake at pH 6.5 is mainly due to the isoelectric point of gelatin (pH 7–9). Further sIPNs from group B, C, and D show comparatively higher water uptake at equilibrium swelling at pH 7.4 than at pH 6.5 (~ 6–10% lower swelling at pH 6.5). Flexible divinyl ester provides space for the acid groups present in the gelatin chains to ionize and absorb water at pH 7.4. No significant difference in water uptake of IPNs at both the pH could be due to the well crosslinked hydrophobic DVE chains in between gelatin that protects the acid groups to absorb water. The order of water uptake in sIPN at pH 7.4 is as follows:

$$sIPN(DGx) > Gx > IPN > Dx,$$

$$\text{at pH 6.5: } Gx > sIPN(DGx) > IPN > Dx.$$

Representative graphs [Fig. 1(a,b)] show the comparison of water uptake properties in IPNs, sIPNs,

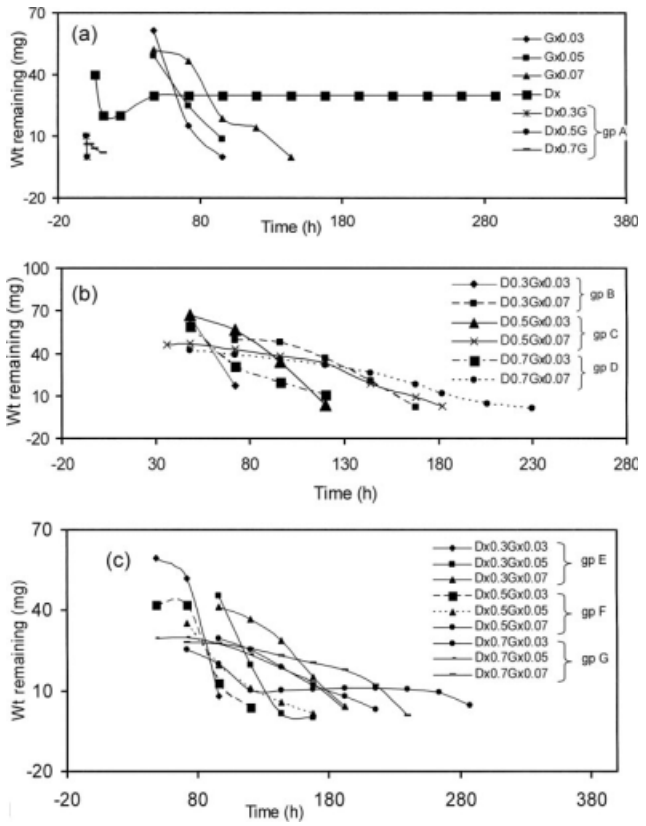


Figure 2 Degradation profiles of hydrogels (a), sIPNs (b), and IPNs (c) at pH 7.4.

and hydrogels. At both pH, Dx shows minimum swelling, Gx and sIPN show maximum swelling and IPN shows swelling intermediate of hydrogels and sIPNs.

Degradation of hydrogels, sIPNs, and IPNs at pH 7.4

Modification of polycaprolactone diol into vinyl macromer increases the degradability. Kweon et al.[33] reported faster and random hydrolytic degradation of vinyl modified polycaprolactone macromer in phosphate buffer saline. In our investigation, DVE, a vinyl modified PCL diol show still faster degradation when it was made into IPN with gelatin network. The reason could be the hydrolytic scission of ester bonds in DVE by the surrounding water molecules absorbed by the hydrophilic gelatin chains. Figure 2(a–c) depicts the degradation profile of hydrogels, sIPN, and full IPN at pH 7.4. The degradation was monitored gravimetrically. Hydrogel of DVE (Dx) did not degrade till the study period of 20 days. With all the samples of Gx, the onset of degradation occurred after 48 h and complete weight loss was seen within 140 h. When Glu concentration increased, for example $G_{x0.07}$ degraded slowly and took longer time for complete degradation [Fig. 2(a)]. Semi-IPNs where gelatin was free and DVE was crosslinked (DxG), the samples degraded within 24 h, suggesting that the water loving groups (e.g. amino group) of gelatin enhanced degradation irrespective of the DVE concentration. However, semi-IPNs where gelatin was crosslinked and DVE was free, the degradation time increased as the Glu concentration increased for a fixed DVE concentration. For example $D_{0.7}G_{x0.03}$ degraded in 120 h and $D_{0.7}G_{x0.07}$ degraded completely in 230 h. Similarly, $D_{0.3}G_{x0.03}$ degraded in 72 h and $D_{0.3}G_{x0.07}$ degraded in 170 h. Thus the sIPN structure having lower Glu concentration allows water to imbibe and supports rapid degradation in *in vitro*. Additionally as the DVE concentration increased, the rate of degradation decreased in sIPN i.e., group B (DVE : gelatin 0.3 : 1) degraded faster than group C (DVE : gelatin 0.5 : 1) and group D (DVE : gelatin 0.7 : 1). As expected, IPNs took longer time to degrade in comparison to sIPNs or hydrogels [Fig. 2(c)]. When the Glu concentration was increased from 0.03 to 0.07%, the degradation was found to be slow and time required for complete weight loss was longer. Further investigation to understand how these sIPNs and IPNs would degrade in biological system is under experimentation.

Morphological characterization

Figure 3 show the surface (3a–c) and cross-sectional (3d–e) SEM images of simple and IPN hydrogels. SEM of Dx [Fig. 3(a)] shows irregular, thick-walled porous structure whereas that of Gx shows uniform network of spider-web-like structure. Upon phase mixing and interpenetration, IPN (DxGx), shows disrupted network of gelatin along with thick-walled Dx network [Fig. 3(c)]. This is further confirmed by the observation of disruption of honeycomb-like microstructure of Gx [Fig. 3(e)] into closely packed fiber-like structure of DxGx [Fig. 3(f)] in the cross-sectional view whereas nonporous structure was observed in cross-sectional micrograph of Dx [Fig. 3(d)].

Thermal characterization

Differential scanning calorimetry

In the DSC scan of Gx, a broad diffused endotherm was seen in the temperature range of 25–150°C followed by a shift in the base line corresponding to the transition at 195°C. These scans were recorded using modulated DSC and it was observed that the first endotherm is nonreversible whereas the second one is reversible. On this basis we can say that the first endotherm could be due to the loss of water (adsorbed/absorbed) and the second endotherm

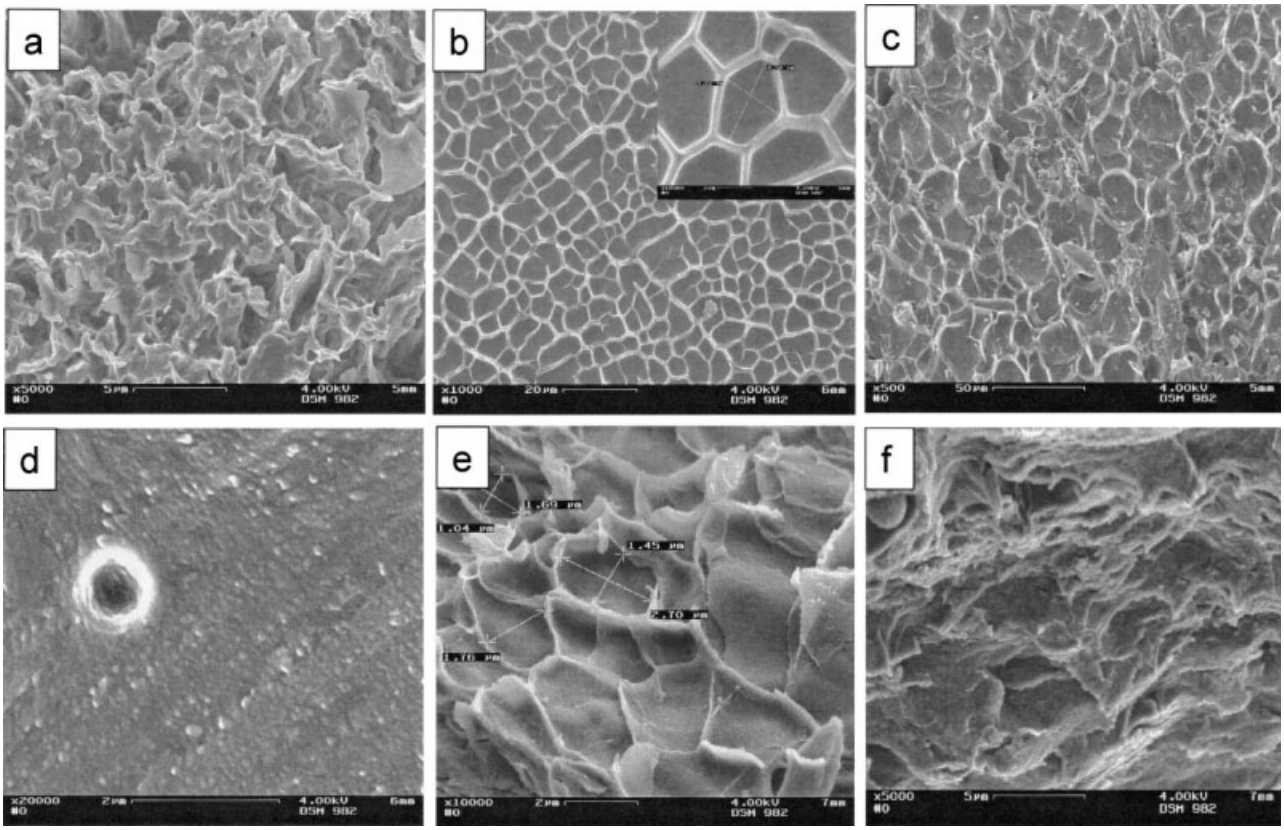


Figure 3 Shows morphology of freeze dried hydrogels by scanning electron microscope. Image 3 a (Dx), 3b (Gx), 3 c (DxGx) are depicting the surface morphology at 90° angle. Image 3d, 3e, and 3f show the cross-sectional morphology of same hydrogels at 45° angle.

could be due to the glass transition whereas in the DSC scan of Dx, an endotherm was observed with a peak at 49°C which was not reversible. In IPNs and sIPNs, broad endotherm due to loss of water was observed similar to that as in Gx. Second transition was observed with a midpoint inflection at 195°C in IPNs whereas in sIPNs, it was at 190°C. This could be due to the restriction of mobility of gelatin chains in IPNs which were absent in sIPNs.

Thermogravimetric analysis

The relative thermal stability of various samples was evaluated by comparing the T_i (initial decomposition temperature), T_f (final decomposition temperature), T_{max} (temperature of maximum rate of mass loss), and percent char yield at 800°C. The results of thermogravimetric analysis are summarized in Table IV. Except Dx all other samples showed initial weight loss upto 200°C, which could be due to the moisture present in the hydrogel samples. Dx showed no such loss because it was synthesized in methanol. Single step degradation was observed with hydrogels and two-step degradation was observed for sIPNs and IPNs. In all the samples (sIPN and IPNs), increasing DVE and Glu concentration increased the T_{max}. IPNs showed higher stability as compared to hydrogels. This could be due to the formation of interpenetration of network structure. If the two crosslinked polymers in IPNs degrade independently then one would have expected char yield based on the additivity rule. However the obtained

TABLE IV
Details of Thermogravimetric Analysis of Hydrogels, sIPNs, and IPNs at Nitrogen Atmosphere (heating rate 20°C/min)

Sample designation	T_i (°C)	T_m (°C)	T_f (°C)	% Char yield
$G_{x0.07}$	293	334	389	18
D_x	393	427	453	01
$D_{0.3x}G$	335	420	453	12
$D_{0.7x}G$	288	328	362	12
	384	421	456	
$D_{0.7}G_{x0.07}$	303	343	433	13
$D_{0.7x}G_{x0.03}$	361	423	456	09
$D_{0.7x}G_{x0.05}$	291	342	370	12
	390	428	466	
$D_{0.7x}G_{x0.07}$	290	339	368	12
	388	423	457	
$D_{0.3x}G_{x0.07}$	289	329	369	14
	385	412	462	

values of percent char were lower than the expected values, indicating the interpenetration of polymer networks. Similar kind of behavior was reported for IPNs based on Gelatin-AA and Gel-PVP.[29,30]

CONCLUSIONS

IPNs based on gelatin and DVE were successfully synthesized by emulsion technique. Percent water uptake in crosslinked gelatin and sIPNs was considerably higher as compared with IPNs, which clearly indicates the role of hydrophobic DVE on designing a new hydrogel with altered swelling and degradation properties. Scanning electron microscopy and thermal characterization of hydrogels clearly show the formation of IPN structure. All samples degrade within 12 days indicating that these hydrogels could be useful as passive targeted drug delivery systems (implants) for the treatment of solid tumor.

References

1. Park, K.; Shalaby, W. S. W.; Park, H. Biodegradable Hydrogels for Drug Delivery; PA, 1993; p 5.
2. Kaur, H.; Chatterji, P. R. Macromolecules 1990, 23, 4868.
3. Gil, E. S.; Hudson, S. M. Biomacromolecules 2007, 8, 258.
4. Wu, W.; Li, W.; Wang, L. Q.; Tu, K.; Sun, W. Polym Int 1996, 55, 513.
5. Li, S.; Yang, Y.; Li, H.; Yang, X.; Xu, H. J Appl Polym Sci 2007, 106, 3792.
6. Shukla, S. A.; Bajpai, A. K.; Kulkarni, R. A. J Appl Polym Sci 2005, 95, 1129.
7. Lim, Y. H.; Kim, D.; Lee, D. S. J Appl Polym Sci 1997, 64, 2647.
8. Konishi, M.; Tabata, Y.; Kariya, M.; Khani, H.; Suzuki, A.; Fukuhara, K.; Mandai, M.; Takakura, K.; Fujii, S. J Control Release 2005, 103, 7.
9. Einerson, N. J.; Stevens, K. R.; Kao, W. J. Biomaterials 2002, 24, 509.
10. Burmania, J. A.; Stevens, K. R.; Kao, W. J. Biomaterials 2003, 24, 3921.
11. Cristina, M. A.; Lopes, M.; Felisberti, I. Biomaterials 2003, 24, 1279.
12. Kurisawa, M.; Yui, N. J Control Release 1998, 54, 191.
13. Jayakrishnan, A.; Jameela, S. R. Biomaterials 1996, 17, 471.
14. Goissis, G.; Marcantonio, E.; Marcantonio, R. A. C.; Lia, R. C. C.; Cancian, D. C. J.; De Carvalho, W. M. Biomaterials 1999, 20, 27.
15. Jiang, Z.; You, Y.; Deng, X.; Hao, J. Polymer 2007, 48, 4786.
16. Wang, B.; Zhu, W.; Zhang, Y.; Yang, Z.; Ding, J. React Funct Polym 2006, 66, 509.
17. Cho, C.-S.; Han, S.-Y.; Ha, J.-H.; Kim, S.-H.; Lim, D.-Y. Int J Pharm 1999, 181, 235.
18. Im, S. J.; Choi, Y. M.; Subramanyam, E.; Huh, K. M.; Park, K. Macromol Res 2007, 15, 363.
19. Huang, M. H.; Li, S.; Hutmacher, D. W.; Schantz, J. T.; Vacanti, C. A.; Braud, C.; Vert, M. J Biomed Mater Res A 2004, 69, 417.
20. Rosenberg, R. T.; Siegel, S. J.; Dan, N. J Appl Polym Sci 2008, 107, 3149.
21. Dhanaraju, M. D.; Gopinath, D.; Ahmed, M. R.; Jayakumar, R.; Vamsadhara, C. J. Biomed Mater Res A 2006, 76, 63.
22. Park, J. S.; Woo, D. G.; Sun, B. K.; Chung, H. M.; Im, S. J.; Choi, Y. M.; Park, K.; Huh, K. M.; Park, K. H. J Control Release 2007, 124, 51.
23. Cho, C.-S.; Ha, J.-H.; Kim, S.-H.; Han, S.-Y.; Kwon, J.-K.; Sung, Y.-K. J Appl Polym Sci 1996, 60, 161.
24. Jenkins, V. F. Polym Paint Colour J 1977, 167, 626.
25. Koleske, J. V. 1978. In Polymer Blends; Academic Press: New York, 1978; Vol. 2, p 369.
26. Lumelsky, Y.; Zoldan, J.; Levenberg, S.; Silverstein, M. S. Macromolecules 2007, EST, 5.7.
27. Wang, Y.; Chang, H.-I.; Wertheim, D. F.; Jones, A. S.; Jackson, C.; Allan, G. A. Coombes Biomaterials 2007, 28, 4619.
28. Salerno, A.; Oliviero, M.; Maio, E. D.; Iannace, S.; Netti, P. A. J Appl Polym Sci 2007, 106, 3335.
29. Burugapalli, K.; Bhatia, D.; Koul, V.; Choudhary, V. J Appl Polym Sci 2001, 82, 217.
30. Singh, D.; Choudhary, V.; Koul, V. J Appl Polym Sci 2007, 104, 1456.
31. Changez, M.; Koul, V.; Dinda, A. K. Biomaterials 2005, 26, 2095.
32. Changez, M.; Burugapalli, K.; Koul, V.; Choudhary, V. Biomaterials 2003, 24, 527.
33. Kweon, H. Y.; Yoo, M. K.; Park, I. K.; Kim, T. H.; Lee, H. C.; Lee, H.; Oh, J.; Akaike, T.; Cho, C. Biomaterials 2003, 24, 801.