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Research paper

Poly(ethylene carbonate) as a surface-eroding biomaterial for *in situ* forming parenteral drug delivery systems: A feasibility study

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ABSTRACT

To evaluate the technical feasibility of poly(ethylene carbonate), PEC, for injectable *in situ* forming drug delivery systems, the physical properties of PEC solutions were characterized. The solubility of PEC was investigated in different solvents, and the Hildebrand solubility parameters and Flory–Huggins interaction parameters of PEC were determined. By turbidity titration, the experimental ternary phase diagram of water–NMP/DMSO–PEC was constructed. NMP solution required more water to precipitate PEC compared to DMSO solution. The dynamic viscosity of PEC solution increased at lower temperature, higher polymer concentration and longer aging time. Differential scanning calorimetric (DSC) measurements confirmed only weak physical interactions in the system after aging, and the physical aging effect was thermo-reversible. Release of NMP from PEC formulations was twofold slower than that of DMSO at similar concentrations. The morphology of PEC depots after injection into aqueous solution was studied using scanning electron microscopy (SEM). A DMSO formulation of bovine serum albumin displayed less burst release than a NMP formulation. In summary, our investigations demonstrate that *in situ* depot forming systems can be obtained from PEC solutions. Moreover, a solution of PEC in DMSO would be preferred over NMP due to the reduced burst release.

1. Introduction

Injectable drug delivery systems have received considerable attention during the last decades due to distinct advantages [1,2]. In particular, injections are less invasive than implantations, and improved patient compliance can be expected, and controlled drug release for local or systemic applications can be attained. Most importantly, *in situ* forming depots do not rely on sophisticated equipment for manufacturing such as is required for emulsions [3–5], liposomes [6,7], micelles [8] and micro- or nano-particles [9,10].

An *in situ* forming drug delivery system can be defined as a liquid formulation generating a solid or semi-solid depot after subcutaneous injection [2]. According to the mechanism of solidification, *in situ* forming devices can be classified into four categories, namely: (1) thermoplastic pastes, (2) *in situ* cross-linked systems, (3) *in situ* precipitation systems and (4) solidifying organogels [1,2].

Many studies have addressed the *in situ* precipitation of polymers as described by Dunn and coworkers in recent years [11]. These systems are usually formulated by dissolving the polymer in water-miscible organic solvents, such as *N*-methylpyrrolidone (NMP), in which the drug is incorporated either as a solution or a suspension. Upon exposure to an aqueous environment, the water-miscible organic solvent diffuses from the polymer–drug suspension into the surrounding medium, while water diffuses into the organic solvent phase. Due to the water-insolubility, the polymer precipitates into a solid or semi-solid depot trapping or encapsulating the drug within the polymeric matrix at the site of injection.

Numerous patents and literature references describe this technology in detail [12,13]. Polymers such as poly(lactic acid) (PLA), poly(lactic acid-co-glycolic acid) (PLGA) and their derivatives [14–17], such as poly(lactic acid)-co-poly(ethylene glycol) (PLA–PEG) were investigated. In addition, poly(acrylic acid) (PAA) and its derivatives [18] were studied, e.g. poly(methacrylic acid) (PMA)–PEG, also for the delivery of plasmid DNA [19]. These biomaterials showed some drawbacks; for instance, the accumulation of acidic degradation products generated during the hydrolysis of PLA and its derivatives. Moreover, the control of drug release from such devices turned out to be especially challenging for hydrophilic macromolecules such as peptides and proteins: frequently, non-linear release profiles with high drug bursts were observed [19,20].

Thus, we hypothesized that novel polymers with a distinctly different degradation mechanism, namely surface erosion, could be advantageous for *in situ* forming parenteral devices, since PEC

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was reported to show surface degradation under *in vivo* conditions [21–23]. PEC was synthesized using ethylene oxide and carbon dioxide at 170 °C [21]. Scheme 1 (Supplementary materials) depicts the molecular structure of PEC, which is a unique polymer showing surface degradability induced by superoxide radical anions O_2^-, generated by inflammatory cells in living tissues [22–24]. Drug delivery systems based on PEC protect biologically active drugs from degradation and enable sustained release [25]. As the drug is released upon degradation of the device surface (microspheres), no aggregation or denaturation of protein drugs was observed.

The preparation of microspheres from PEC is not straightforward due to their thermo-elastic properties. The formulation of injectable *in situ* forming depots was investigated as an alternative, since in this case polymer solutions in organic solvents are administered. Therefore, the physical properties of PEC solutions were investigated, and the technical feasibility of producing *in situ* forming devices based on PEC was evaluated.

2. Experiments

2.1. Materials

Poly(ethylene carbonate) was provided by Novartis Pharma AG, Basel, Switzerland (Product No.: 3009594, Batch: 94904 (MW, 283,500 Da) and 96906 (MW, 247,800 Da)); *N*-methylpyrrolidone (NMP), 2-pyrrolidone, ethanol, chloroform, dimethylformamide (DMF) and *n*-hexane were purchased from Acros (Geel, Belgium); Dimethyl sulfoxide (DMSO) was from Merck (Darmstadt, Germany); 1,4-dioxan and cyclohexanone were from Sigma–Aldrich (Taufkirchen, Germany). Bovine serum albumin (MW, 66 kDa, Cohn Fraction V) was obtained from Sigma (Steinheim, Germany). All solvents were of analytical grade and were used without further purification.

2.2. Determination of PEC solubility in organic solvents

The solubility of PEC in different solvents listed in Table 1 was determined gravimetrically according to Lamber and Peck [14] and Shively et al. [15]. Excess amounts of PEC (500 mg) were added into each solvent (300 μL) in Eppendorf vials (1.5 mL), and after sealing the vials, the mixtures were shaken on a Thermomixer (Heidolph Elektro, Germany) at 25 °C for 24 h. A known weight of sample, free of solids, was then added to another vial and washed with water eight times and freeze-dried for 48 h. The weight of dried sample was recorded. Each sample was prepared in triplicate, and mean values of the PEC weights were given.

2.3. Construction of ternary phase diagrams

This measurement was performed as reported by Shively et al. [15]. PEC solutions in NMP and DMSO with various concentrations from 1% to 60% (w/w) were prepared, respectively. PEC solutions were allowed to equilibrate and/or dissolve completely for at least 24 h. Polymer solutions and water were kept in a thermostatted water bath at 37 °C and at room temperature (22 °C), respectively, for 30 min. Water was added to the polymer solutions in 20 μL aliquots until polymer precipitation was observed. This operation was repeated until the precipitate did not redissolve any more within 2 h. The total amount of water dropped into the solution to cause permanent precipitation was recorded. Each sample was investigated in triplicate. The composition of the precipitate was plotted in the ternary phase diagrams.

Table 1
The transition temperature and enthalpy changes of PEC formulation.

Solvent	Concentration (%)	Tune (days)	Peak (°C)	ΔH (J/g)
NMP	9.62	7	42.922	8.601
XMP	9.62	20	46.169	13.270
NMP	24.21	7	46.804	18.853
DMSO	10.41	7	44.866	0.333
DMSO	20.2	7	45.033	0.544
DMSO	20.2	20	45.200	6.283

2.4. Investigation into the rheological properties of the polymer solutions

The rheological properties of polymer solutions were studied using a plate-cone rotational viscosimeter, HAAKE RheoStress 1 (Thermo Fisher Scientific Inc., Karsruhe, Germany), sensor: C35/1 Ti L05 005, slit 0.053 mm. The influence of polymer concentration, solvent and polymer molecular weight on the rheological properties was examined at 20 and 37 °C, respectively, whereby the temperature was controlled by a thermostat. The relationship of shear stress and shear rate for polymer solutions was obtained by varying the shear rate from 0 to 25 s^{-1}. The slope of the rheogram represents the viscosity of the polymer solution. To investigate the effect of aging on viscosity, polymer solutions were aged in sealed glass tubes at 20 °C. Measurements were taken at predetermined intervals by removing equal amounts of polymer solution from the glass tube and loading them directly into the viscosimeter without further treatment.

2.5. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to evaluate the thermal behavior of PEC solutions using a DSC 7 (Perkin Elmer) apparatus. Samples, 5–12 mg, were weighed and sealed in standard aluminum pans and then scanned over a temperature range from 30 to 70 °C at a heating rate of 20.00 °C/min. Indium and gallium were used as standards for the calibration. Each sample was measured in triplicate, and average data are presented.

2.6. Solvent release rate from PEC depots

The solvent release experiments were performed in 15-mL tubes containing 10 mL of phosphate-buffered saline (PBS) (0.01 M, pH 7.2). PEC stock solutions were prepared at a concentration of 10% (w/w) for the NMP solution and 10% and 15% (w/w) for the DMSO solution. Then, 300 mg of polymer solutions was injected into 10 mL of PBS buffer (0.1 M, pH7.4) through an 18-gauge needle, 1.2 × 25 mm, and the vials were kept at 37 °C. The aqueous incubation medium was completely replaced with equal volumes of fresh PBS at predetermined time intervals. The amount of solvent released into the PBS at different incubation times was quantitated using reversed-phase high performance liquid chromatography (RP-HPLC), performed on Waters instruments equipped with a UV detector (Waters, Eschborn, Germany). A Nucleosil RP C-18 column (150 × 4 mm, 5 μm particle size, 100 Å pore size) was used for the separation. Each sample was filtered through a 0.2-μm PTFE membrane filter (Fisher brand, Fisher Scientific GmbH, Nidderau, Germany) before injection. The analysis conditions were as follows: the elution of DMSO was carried out by simply rinsing the column with distilled water for 10 min. The eluent was monitored by measuring the UV absorbance at 197 nm [26]. In the case of NMP, acetonitrile/water (85/15, v/v) with 0.1% trifluoroacetic acid (TFA) was used as eluent, and the absorbance was monitored at 263 nm [27]. The flow rate for detecting both solvents was 0.8 mL/min. The amounts of solvent were determined with the aid of standard calibration curves. Data analysis was performed using the Millenium^{32} software Version

3.05 (Waters, Eschborn, Germany). Tests were carried out in triplicate, and average values of the results were plotted.

2.7. Scanning electron microscopy (SEM)

SEM was used to investigate the internal and external surface morphology of PEC depots after injection into water. Then, 300 mg of NMP and DMSO solutions with various concentrations of PEC was injected into 10 mL of PBS buffer and stored in a rotating thermostat, RotaTherm (Liebisch, Bielefeld, Germany) at 20 rpm and 37 °C for 24 h. After washing carefully with distilled water three times and freeze-drying for 7 d, the samples were sputter-coated with gold. A SEM instrument Cam SCAN Series 4 (Cam SCAN, USA) was used to investigate the morphology of the samples.

2.8. Release of bovine serum albumin from PEC depots under in vitro conditions

The *in vitro* release studies of bovine serum albumin (BSA) from PEC depots were conducted in phosphate-buffered saline (PBS) (0.01 M, pH 7.4) at 37 °C. To prepare the BSA containing formulation, 300 mg of stock polymer solution was mixed with BSA (1% w/w) at room temperature. The mixture was injected into 10 mL of PBS buffer (0.01 M, pH 7.4) in a 15-mL plastic vial through an 18-gauge needle, 1.2 × 25 mm. The vials were kept in a rotating thermostat, RotaTherm (Liebisch, Bielefeld, Germany) at 20 rpm and 37 °C. Spherical matrices were formed as the polymer solidified, with reproducible diameters of approximately 4 mm. The release medium was replaced at predetermined time intervals, and the BSA concentration in the release medium was quantitated using a protein assay. Briefly, 25 μL of samples was pipetted into 96-well plates, and 200 μL of bicinchonic assay reagents was added. After incubation at 37 °C for 40 min, the absorbance was measured at 570 nm using a plate reader. BSA concentrations were determined using a standard calibration curve. Each sample was independently measured in triplicate.

3. Results and discussion

3.1. Solubility and solubility parameters of PEC

Polymer solubility parameters, specifically the Hildebrand or one-component solubility parameter, can be used to estimate the miscibility of polymers and solvents [14,28]. Thus, we evaluated the Hildebrand solubility parameters of PEC for the selection of appropriate solvents. The solubility parameters of low-molecular weight compounds can be measured directly by the heat of vaporization, while Hildebrand solubility parameters of polymers are taken as equal to those of their good solvents.

A list of solvents, with gradually increasing Hildebrand solubility parameter [29] (listed in Table 1, Supplementary materials), was investigated in this study. The solubility of PEC is illustrated in Fig. 1. PEC was found to be soluble in those solvents with a solubility parameter (δ) ranging from 9.3 to 14.7 $cal^{1/2}/cm^{3/2}$ and had the highest solubility at $\delta = 10\ cal^{1/2}/cm^{3/2}$.

Among the solvents studied here, the Hildebrand solubility parameters of both NMP and DMSO lie within the solubility parameter (δ) ranges of PEC. Moreover, NMP is classified as a Class 2 solvent (ICH Guidelines) and is actually used in commercial drug depots [11,30]. DMSO has the additional advantage that it dissolves many proteins [31]. Some proteins retained their biological activity after recovery from DMSO solution [32,33]. Both NMP and DMSO have been used extensively for injectable depot formulation [20,34]. For further evaluation of appropriate solvents, Flory–Huggins solubility parameters were calculated to take the thermodynamics of diluted polymer solutions into consideration [29].

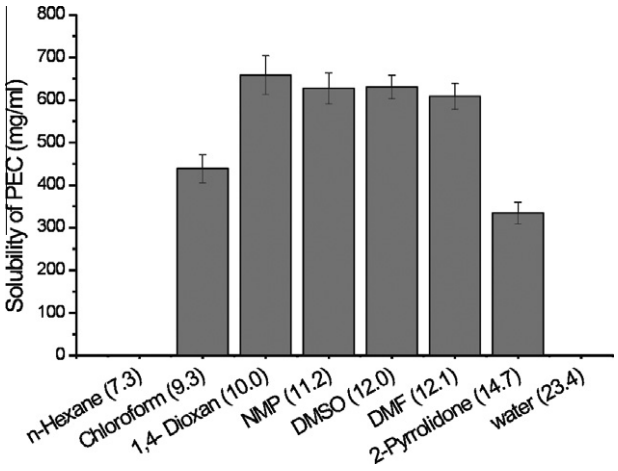

Fig. 1. Solubility of PEC with a molecular weight of 247.8 kDa. The number after each solvent name is the Hildebrand solubility parameter (dimension: $cal^{1/2}\ cm^{3/2}$) of this solvent.

Flory–Huggins theory is based on the assumption that long-chain polymer molecules occupy the sites of a "lattice" and solvent molecules occupy the single site. In this theory, a polymer–solvent interaction parameter (Flory–Huggins parameter, χ) was proposed. It represents a dimensionless quantity characterizing the difference in interaction energy of a solvent molecule immersed in a pure polymer compared with in a pure solvent. A good solvent has a low χ value. When Flory–Huggins parameters approach zero, maximum solubility of the polymer is achieved. The Flory–Huggins interaction parameter (χ) usually contains both enthalpic (χ_h) and entropic (χ_s) contributions. This can be expressed as Eq. (1). The enthalpic contribution of the Flory–Huggins interaction parameters (χ_h) was calculated using Eq. (2)

$$\chi = \chi_h + \chi_s \tag{1}$$

$$\chi_h = \frac{V_1}{RT}(\delta_1 - \delta_2)^2 \tag{2}$$

$$\frac{\delta_1^2}{RT} - \frac{\chi}{V_1} = \left(\frac{2\delta_2}{RT}\right)\delta_1 - \frac{\delta_2^2}{RT} - \frac{\chi_s}{V_1} \tag{3}$$

where subscripts 1 and 2 represent solvent and polymer, respectively, V_1 denotes the molar volume of solvent (cm^3/mol), R is the gas constant, T is the absolute temperature, and δ is the Hildebrand solubility parameter.

Empirically, χ_s was usually found to lie between 0.2 and 0.6. In our cases, it was assumed that the entropic contribution of the Flory–Huggins interaction parameter (χ_s) is negligible [15,35]. The left side of Eq. (3) was plotted versus the solubility parameter of the solvent. From the y-intercept, and the experimentally obtained solubility parameter of PEC ($10.0\ cal^{1/2}/cm^{-3/2}$), χ values of NMP–PEC were calculated as 0.41 ($\chi_h = 0.23$, $\chi_s = 0.18$) at 25 °C, while χ values of DMSO–PEC were 0.59 ($\chi_h = 0.48$, $\chi_s = 0.11$). Although many factors influence the accuracy of the Hildebrand solubility parameter of polymers and also affect the calculation of Flory–Huggins parameters, the observed trends will remain the same as Shively reported [15]. The calculated Flory–Huggins parameter can be used to estimate the miscibility of solvents and polymers.

NMP was found to be a better solvent than DMSO due to the smaller Flory–Huggins parameter. It was therefore predicted that more water or other precipitant would be needed for NMP to induce phase inversion compared with DMSO.

3.2. Construction of ternary phase diagrams

The dynamics of the interactions taking place between the polymer solution and non-solvent for the formation of *in situ* depots was similar to the formation of a polymer membrane caused by non-solvent induced phase inversion. In our case, the solvent diffused from the polymer solution into the non-solvent environment, which was miscible with the solvent. The diffusion of solvent thereby reduced the solubility of the polymer and eventually induced a phase inversion to a solid or semi-solid polymer-rich matrix. This diffusion also promoted the formation of the morphology of both surface and inner polymeric depots.

Numerous investigations have demonstrated that the dynamics of non-solvent induced phase inversion can be quantified in terms of ternary phase diagrams [37,38]. Ternary phase diagrams characterize the solubility of a polymer in a solvent, the phase inversion of polymer solutions, and the interactions among polymer, solvent and water (non-solvent) [36]. Titration using distilled water was reported to result in the same visual properties as using buffer solutions [15]. The observed precipitation reported here was obtained using distilled water as the precipitant.

The experimental ternary phase diagrams were constructed using two PECs with different molecular weights as a function of solvents and temperature. In experimental ternary phase diagrams (Fig. 2), the parts above the precipitation curve were single phase (polymer solution), while parts below the precipitation curve were two phases (precipitated polymers and dissolved polymers). The curve was the critical curve of phase inversion.

Analysis of these figures suggested that more water was needed to precipitate PEC at 37 °C than at 22 °C. At the same time, the amount of water required for the NMP solution was higher than that for the DMSO solution. These results are compatible with the prediction from Flory–Huggins interaction parameters. PECs with higher molecular weight need less water for phase inversion. The figures also suggested that the required water amount decreased as the concentration of PEC increased.

Requiring more water to precipitate the polymer could lead to longer solidification times for depot formation. All of these factors could possibly affect both the inner and surface morphology of *in situ* forming parenteral depot systems [37,38].

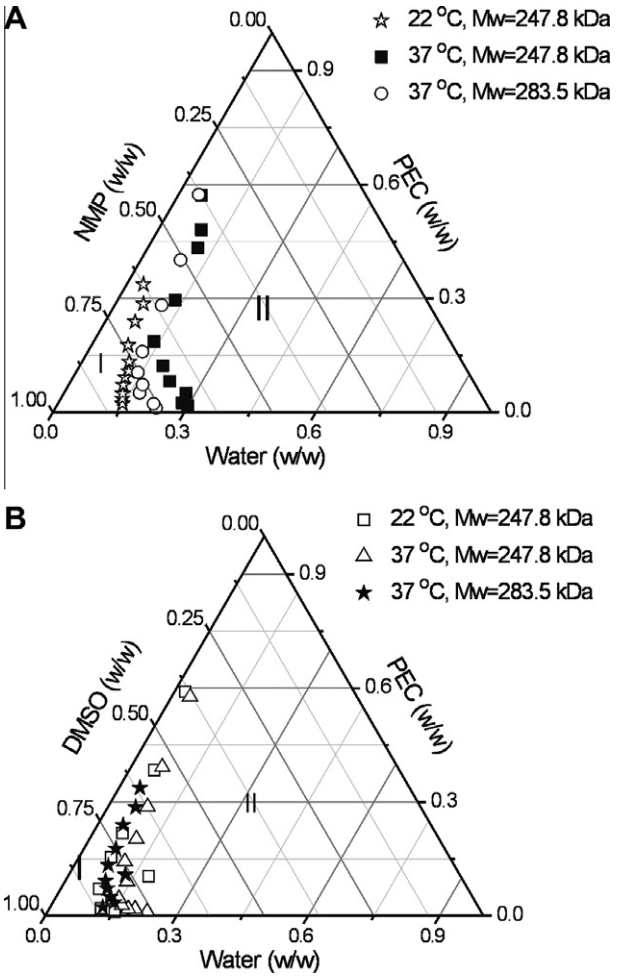

Fig. 2. Experimental ternary phase diagram of PEC precipitation as a function of molecular weight and temperature. (A) NMP solution; ☆ represents NMP solution at 22 °C, the molecular weight of PEC is 247.8 kDa; ■ represents NMP solution at 37 °C, the molecular weight of PEC is 247.8 kDa; ○ represents NMP solution at 37 °C, the molecular weight of PEC is 283.5 kDa; (B) DMSO solution; □ represents DMSO solution at 22 °C, the molecular weight of PEC is 247.8 kDa; △ represents DMSO solution at 37 °C, the molecular weight of PEC is 247.8 kDa; ★ represents DMSO solution at 37 °C, the molecular weight of PEC is 283.5 kDa; the area I above precipitation points (curve) is one phase area (polymer solution); the area II below precipitation points (curve) is two phases area (precipitated polymers and dissolved polymers).

3.3. Rheological properties of PEC formulations

The solid or semi-solid depots were formed via phase inversion which occurred by the exchange of solvent and non-solvent across the interface between the polymer solution and the environmental solution. The viscosity of the polymer solution was believed to affect the exchange rate of solvent and non-solvent during phase inversion [39–41]. Thus, the rheological characterization can be considered as an important parameter to influence the depot-formation kinetics [28]. Moreover, proper viscosity is critical for the injection of the polymer solution through a syringe needle. The viscosity of the solution must be low enough for injection, high enough for stabilizing suspensions of insoluble drugs and yet stable enough for storage [42].

The relationship between the shear stress and the shear rate of an NMP solution as a function of PEC concentration (Fig. 3A) demonstrated that shear stress increased with increase in shear rate in a linear fashion. Here, we ignored the intercept of all regression lines, and PEC solutions can be described as a Newtonian fluid. The slope of the regression line was considered as the dynamic viscosity of the PEC solution.

The effects of PEC molecular weight, solvents and temperature on the rheological properties are shown in Fig. 3B. PEC solution with a molecular weight of 283.4 kDa in NMP showed a higher dynamic viscosity than one with a MW of 247.8 kDa, and a lower dynamic viscosity than the solution in DMSO with the same PEC at the similar concentration (11.15%). Kranz et al. also found a lower dynamic viscosity of PLA solution in NMP compared to DMSO, and the polymer concentration positively influenced the dynamic viscosity [28]. This was attributed to NMP being a better solvent than DMSO for polymer dissolution, similar to our observations. The dynamic viscosity of the PEC solution was higher at 20 °C than at 37 °C, and a decreased dynamic viscosity of polymer solution with increasing temperature was also reported by others, due to the increased chain mobility at higher temperatures [41,43].

The dynamic viscosity of a PLGA solution in some solvents increased during storage [16,44] similar to PEC (Fig. 4). The NMP solution showed a higher increase in dynamic viscosity than the DMSO solution at the same concentration, which can be explained by the formation of three-dimensional polymer–solvent complex structures [44,17]. A "gel-like" structure formed as a consequence of increasing the dynamic viscosity of PEC; however, after heating the aged PEC solution to 60 °C, the dynamic viscosity returned to normal.

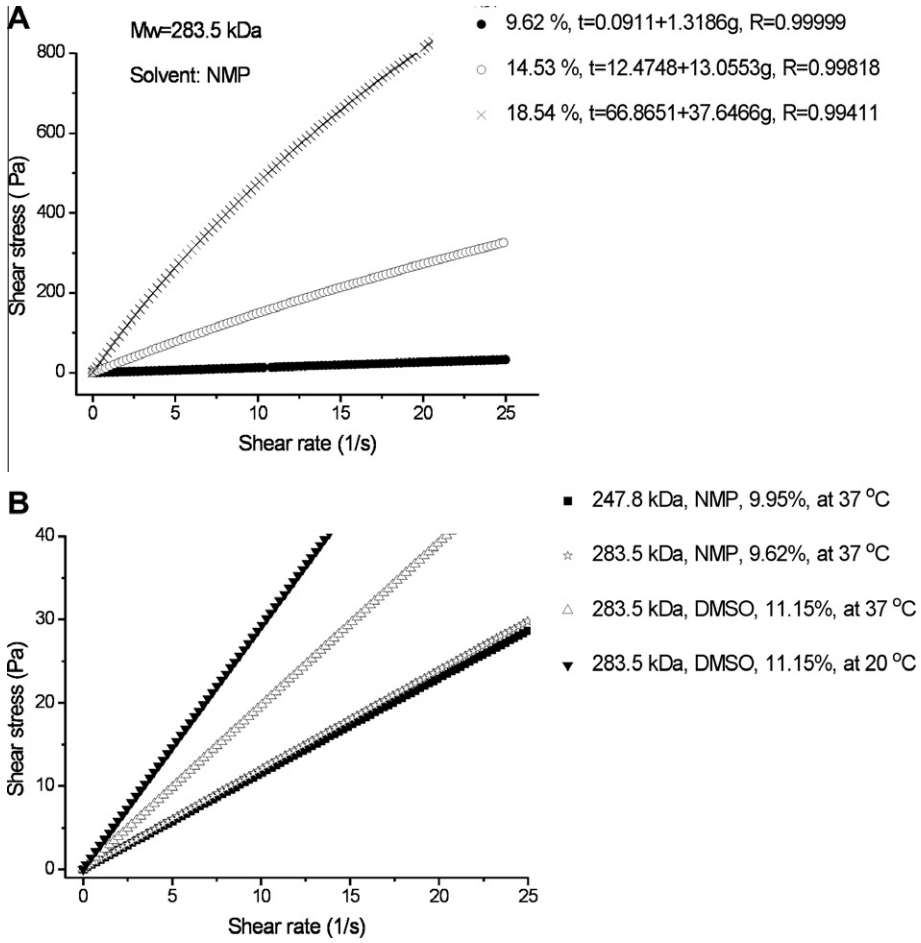

Fig. 3. Relationship of shear stress and shear rate. (A) Effect of PEC concentrations (NMP solution), the equations in legend are linear regression results of the curves, *R* is correlation coefficient; ● represents 9.62% of NMP solution; ○ represents 14.53% of NMP solution; × represents 18.54% of NMP solution; (B) effects of PEC molecular weight, solvents and temperature. ■ represents 9.95% of NMP solution at 37 °C, the molecular weight of PEC is 247.8 kDa; ☆ represents 9.62% of NMP solution at 37 °C, the molecular weight of PEC is 283.5 kDa; △ represents 11.5% of DMSO solution at 37 °C, the molecular weight of PEC is 283.5 kDa; ▼ represents 11.5% of DMSO solution at 20 °C; The molecular weight of PEC is 283.5 kDa.

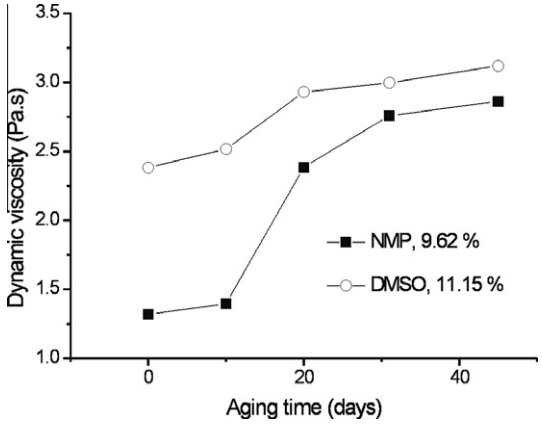

Fig. 4. Dynamic viscosity of PEC (MW = 247.8 kDa) solution of NMP and DMSO as a function of aging time. ■ represents 9.62% of NMP solution; ○ represents 11.15% of DMSO solution.

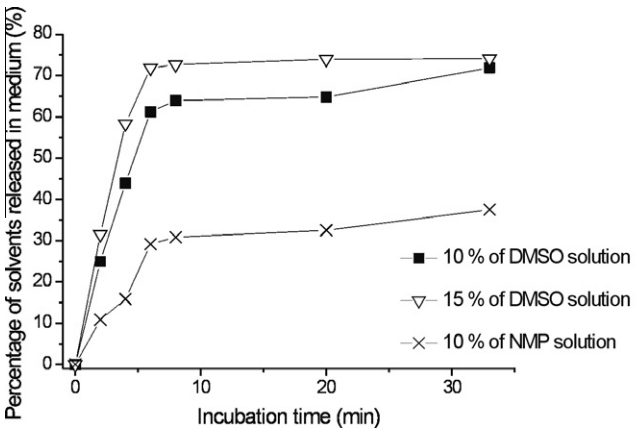

Fig. 5. Solvents release as a function of incubation time in medium. ■ represents 10% of DMSO solution; ▽ represents 15% of DMSO solution; × represents 10% of NMP solution.

3.4. Differential scanning calorimetry

To confirm the thermo-reversible formation of a physically crosslinked structure in the PEC solution with aging, DSC was used to measure the thermal behavior of PEC formulations. An endothermic peak appeared at around 45–50 °C for the aged solution, and solutions with higher concentration and longer aging time displayed a more distinct endothermic peak. However, the enthalpy changes were relatively small (8.6–18.9 J/g, as shown in Table 1). The DMSO solution showed a smaller ΔH than the NMP solution. PEC concentration and aging time positively affected the enthalpy

peaks. After aging for 7 d, NMP solution at concentration of 24.21% showed the peak at 46.8 °C and ΔH was 18.853 J/g, while at 9.95% the peak was at 42.9 °C and ΔH was 8.601 J/g. After aging for 20 d, at 9.95% the peak moved to 46.169 °C and ΔH increased to 13.270 J/g. This small enthalpy peak suggested the formation of a three-dimensional gel-like structure in the aged solution, possibly induced by random aggregation of polymer chains or other physical interactions. Both fresh and re-heated aged samples showed no endothermic peak, indicating thermo-reversibility of this "gel-like" structure. These results are compatible with the increase in thermo-reversible dynamic viscosity during aging.

Among the basic intermolecular forces, hydrogen bonds typically have binding energies of 10–40 kJ/mol, while van der Waals interactions show binding energies of about 1 kJ/mol for complex fluids [42,45]. Okamoto and Guenet reported that solvents can induce the formation of helical conformations of polymer chains in alkyl and alkoxy substituted poly(2,5-dialkyl-*p*-phenyleneethylnylene) (PPE) and poly(methyl methacrylate) (PMMA) systems [46,47]. Jeong reported the formation of hydrogen bonds in an aqueous gelation system for the PEG–PLGA–PEG copolymer [48]. Venkatraman et al. have also reported the small enthalpy changes of physical aging structure in PLGA/benzyl benzoate (BB) system [42]. Therefore, the thermo-reversible "gel-like" structure in aged PEC solution can be explained by the results of physical interactions caused by the solvent or the entanglement of long linear PEC molecular chains.

3.5. Solvent release rate from injectable in situ forming depot of PEC

The release or diffusion rate of solvents from polymer depots can play a critical role in the formation of polymer depots at the

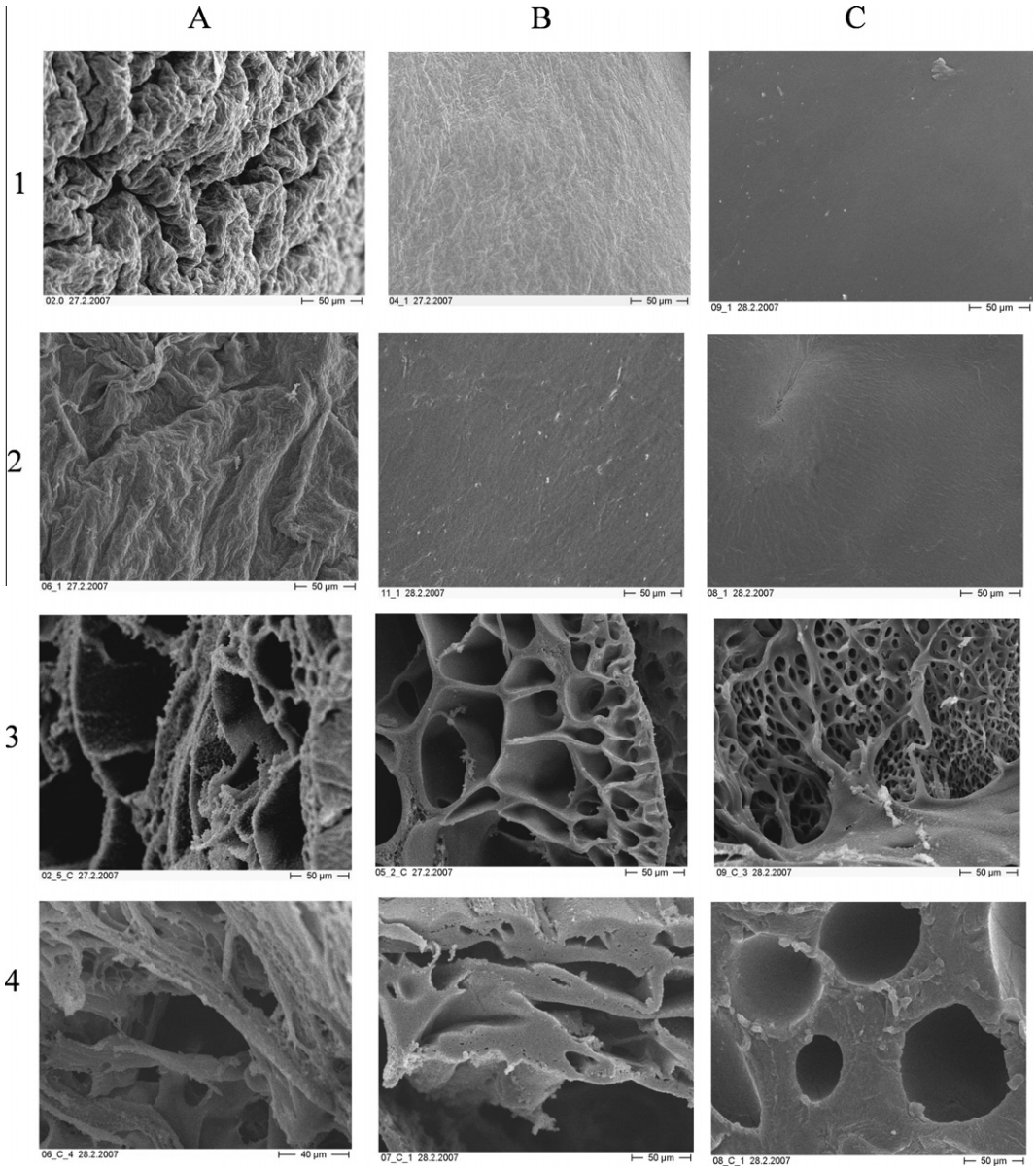

Fig. 6. Scanning electron micrographs of NMP and DMSO depots. (A1) Surface skin of depots from 2.98% NMP solution; (B1) surface skin of depots from 9.62% NMP solution; (C1) surface skin of depots from 14.53% NMP solution; (A2) surface skin of depots from 2.98% DMSO solution; (B2) surface skin of depots from 11.15% DMSO solution; (C2) surface skin of depots from 16.12% DMSO solution; (A3) cross-section of depots from 2.98% NMP solution; (B3) cross-section of depots from 9.62% NMP solution; (C3) cross-section of depots from 14.53% NMP solution; (A4) cross-section of depots from 2.98% DMSO solution; (B4) cross-section of depots from 11.15% DMSO solution; (C4) cross-section of depots from 16.12% DMSO solution.

injection site and hence control initial drug release from *in situ* forming devices [28,16].

Fig. 5 shows solvent release into PBS buffer as a function of time. The release rate of DMSO was double that of NMP. While only about 30% of NMP was released into PBS at 8 min, 65% and 73% of DMSO were released at 8 min for PEC concentrations of 10% and 15%, respectively. In the case of NMP, even at 33 min, there was still more than 60% of NMP in the depots. These results can be explained by the lower water uptake rate [17] and better solvent–PEC affinity of NMP than DMSO. Moreover, linear PEC molecules with such high molecular weight can also reduce the molecular motion.

3.6. Morphology of PEC in situ form depots

Depot morphology was investigated to characterize the surface and internal structure and to obtain an insight into their effect on drug release characteristics [37,49]. A decrease of the initial drug release (drug burst) was thought to correspond with a more dense morphological structure [43]; thus, the morphology of depots was studied by scanning electronic microscopy (SEC). Various concentrations of NMP and DMSO solutions were prepared, and 300 mg of PEC solutions was injected into 10 mL of PBS buffer. After incubation at room temperature for 24 h, the depots were washed with distilled water and freeze-dried for 7 d.

Fig. 6 shows the morphologies of PEC depots prepared from NMP and DMSO solutions with different concentrations. The surface structure of both depots became smooth and dense with increasing PEC concentrations, while the internal structure changed from macro-voids to less porous structures, especially in depots made from DMSO solutions. This suggested that the phase inversion rate was reduced by increasing the PEC concentration [37], possibly caused by reduced water influx rates. Similar results were reported for membrane forming systems [50,51]. The reduced water influx rates can be caused by different effects, including higher dynamic viscosity, lower system diffusivities, thicker skins and increased system hydrophobicity, all of which may contribute to the phase inversion dynamics [37]. As for the less porous structure of depots from DMSO solution, one can assume that due to a higher dynamic viscosity of DMSO solutions, less water is required to precipitate PEC. Moreover, the faster release of DMSO from PEC depots relative to NMP suggested more rapid formation of depots from DMSO solution [15]. The phase inversion is probably caused by a nucleation and growth mechanism [38]. The faster formation of a semi-solid polymer layer (skin) on the surface of the depots became an effective barrier to restrict mass transfer into the interior of depots and slowed the phase separation process. Therefore, it was expected that the initial drug release from PEC depots made from NMP solution would be higher than from DMSO solution.

3.7. In vitro release experiments with bovine serum albumin

The initial release of bovine serum albumin (BSA) from PEC depots is shown in Fig. 7. Within 24 h, almost 100% of the BSA was released from NMP depots, while 25% of BSA was retained in DMSO depots. These results agreed with our assumption based on the physical properties of the PEC solution. Also the results from PLGA and PLG injectable depots showed similar properties with DMSO as the solvent [15]. However, it should be noted that only drug burst can be studied in PEC depots systems under *in vitro* test conditions, due to the unique surface degradation property of this polymer. The complete release profile requires the presence of superoxide radical anions, $O_2^{-\cdot}$, generated by inflammatory cells in living tissues. Hence, these studies need to be conducted under *in vivo* conditions [22]. Further optimization of the release properties and drug delivery *in vivo* are currently in progress.

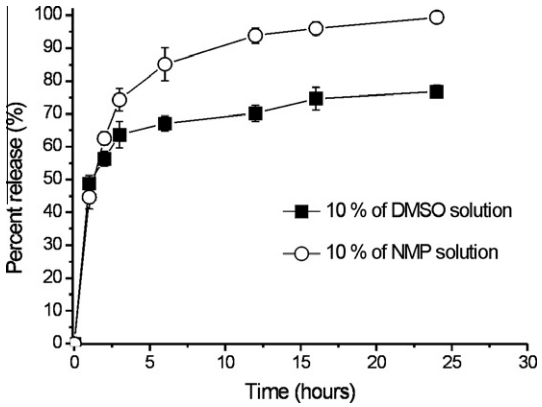

Fig. 7. Release of BSA from PEC solution in PBS. Error bars indicated standard deviation ($n = 3$). ■ represents 10% DMSO solution; ○ represents 10% NMP solution.

4. Conclusions

To evaluate the technical feasibility of using PEC as a surface-eroding biomaterial for the preparation of biodegradable *in situ* forming parenteral drug delivery system, the physical properties of PEC solutions were investigated in detail. This system consists of an injectable liquid formulation based on water-insoluble PEC dissolved in a water-miscible organic solvent, resulting in a depot after injection caused by non-solvent induced phase inversion mechanism.

The solubility and conventional Flory–Huggins parameters of the polymer were found to be useful for predicting the effect of solvents on the physical properties of injectable depots [14,15]. In this study, the solubility of PEC was investigated in various solvents, and the solubility parameter of PEC was also measured. Due to the better solvent–PEC affinity, an NMP-based system showed a lower dynamic viscosity and slower solvent release. Alternatively, depots based on the relatively weak solvent (DMSO) undergo faster solvent dissipation and reduced protein burst release. Specifically, less solvent–PEC affinity causes morphological changes in depots that are favorable for reduced burst release. It is evident that the solvent–PEC affinity plays a critical role in the *in situ* forming depots. Based upon these results, it can be concluded the application of PEC in injectable depots is feasible. The optimization of the formulation and more detailed studies on the release kinetics are in progress.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2010.07.009.

References

[1] A. Hatefi, B. Amsden, Biodegradable injectable in situ forming drug delivery system, J. Control. Release 80 (2002) 9–28.
[2] C.B. Packhaeuser, J. Schnieders, C.G. Oster, T. Kissel, In situ forming parenteral drug delivery systems: an overview, Eur. J. Pharm. Biopharm. 58 (2004) 445–455.
[3] S.S. Davis, L. Illum, Colloidal drug delivery systems: opportunities and challenges, in: E. Tomlinson, S.S. Davis (Eds.), Site-Specific Drug Delivery, Wiley, New York, 1986, pp. 210–224.
[4] L.C. Collins-Gold, R.T. Lyons, L.C. Batholow, Parenteral emulsion for drug delivery, Adv. Drug Deliv. Rev. 5 (1990) 189–208.
[5] A.T. Florence, D. Whitehill, The formulation and stability of multiple emulsions, Int. J. Pharm. 11 (1982) 277–308.
[6] A. Sharma, U.S. Sharma, Liposomes in drug delivery: progress and limitations, Int. J. Pharm. 154 (1997) 123–140.
[7] S.M. Moghimi, Opsono-recognition of liposomes by tissue macrophages, Int. J. Pharm. 162 (1998) 11–18.

[8] X. Zhang, J.K. Jackson, H.M. Bert, Development of amphiphilic diblock copolymers as micellar carriers of taxol, Int. J. Pharm. 132 (1996) 195–206.
[9] L. Chen, R.N. Apte, S. Cohen, Characterization of PLGA microspheres for the controlled delivery of IL-I for tumor immunotherapy, J. Control. Release 43 (1997) 261–272.
[10] M.S. Espuelas, P. Legrand, J.M. Irache, C. Gamazo, A.M. Orecchinioni, J.Ph. Devissaguet, P. Ygartua, Poly(caprolactone) nanospheres as an alternative way to reduce amphotericin B toxicity, Int. J. Pharm. 158 (1997) 19–27.
[11] R.L. Dunn, J.P. English, D.R. Cowsar, D.P. Vanderbilt, Biodegradable in-situ forming implants and methods of producing the same, US Patent 4938,763, 1990.
[12] R.L. Dunn, A.J. Tipton, G.L. Southard, Biodegradable polymer compositions for implants, Eur. Pat. Appl. 539751 A1 19930505, 1993.
[13] R.L Dunn, N.L. Krinick, M.S. Cox, K.R. Josephs, Liquid polymer delivery system comprising biodegradable, water-insoluble polymer, PCT Int. Appl. WO 9621427 A1 19960718, 1996.
[14] W.J. Lambert, K.D. Peck, Development of an in situ forming biodegradable poly-lactide-co-glycolide system for the controlled release of proteins, J. Control. Release 33 (1995) 189–195.
[15] M.L. Shively, B.A. Coonts, W.D. Renner, J.L. Southard, A.T. Bennett, Physico-chemical characterization of a polymeric injectable implant delivery system, J. Control. Release 33 (1995) 237–243.
[16] L. Wang, S. Venkatraman, L. Kleiner, Drug release from injectable depots: two different in vitro mechanisms, J. Control. Release 99 (2004) 207–216.
[17] W.Y. Dong, M. Koerber, V.L. Esguera, R. Bodmeier, Stability of poly (D,L-lactide-co-glyolide) and leuprolide acetate in-situ forming drug delivery systems, J. Control. Release 115 (2006) 158–167.
[18] B.O. Haglung, J. Rajashree, K.J. Himmelstein, An in-situ gelling system for parenteral delivery, J. Control. Release 41 (1996) 229–235.
[19] F.A. Ismail, J. Napaporn, J.A. Hughes, G.A. Brazeau, In situ gel formation for gene delivery: release and myotoxicity studies, Pharm. Dev. Technol. 5 (2000) 391–397.
[20] M. Körber, R. Bodmeier, Development of in situ forming PLGA drug delivery system, I: characterization of a non-aqueous protein precipitation, Eur. J. Pharm. Sci. 35 (2008) 283–292.
[21] M. Acemoglu, F. Nimmerfall, S. Bantle, G.H. Stoll, Poly(ethylene carbonate)s, part I: syntheses and structural effects on biodegradation, J. Control. Release 49 (1997) 263–276.
[22] G.H. Stoll, F. Nimmerfall, M. Acemoglu, D. Bodmer, S. Bantle, I. Mueller, A. Mahl, M. Kolopp, K. Tullberg, Poly(ethylene carbonate)s, part II: degradation mechanism and parenteral delivery of bioactive agents, J. Control. Release 76 (2001) 209–225.
[23] M. Dadsetan, E.M. Christenson, F. Unger, M. Ausborn, T. Kissel, A. Hiltner, J.M. Anderson, In vivo biocompatibility and biodegradation of poly(ethylene carbonate), J. Control. Release 93 (2003) 259–270.
[24] M. Acemoglu, Chemistry of polymer biodegradation and implications on parenteral drug delivery, Int. J. Pharm. 277 (2004) 133–139.
[25] O. Lambert, O. Nagele, V. Loux, J.-D. Bonny, L. Marchal-Heussler, Poly(ethylene carbonate) microspheres: manufacturing process and internal structure characterization, J. Control. Release 67 (2000) 89–99.
[26] W. Thumm, D. Freitay, A. Kettrup, Determination and quantification of dimethyl sulphoxide hy HPLC, Chromatographia 32 (1991) 461–462.
[27] J.A. Shea, S.C. Cook, W.F. Shamrock, J. Segretario, Quantitation of residual *N*-methylpyrrolidone in losoxantrone hydrochloride by reversed-phase high-performance liquid chromatography, J. Chromatogr.Sci. 36 (1998) 187–190.
[28] H. Kranz, R. Bodmeier, Structure formation and characterization of injectable drug loaded biodegradable devices: in situ implants versus in situ microparticles, Eur. J. Pharm. Sci. 34 (2008) 164–172.
[29] A.F.M. Barton, Solubility parameters, Chem. Rev. 75 (1975) 731–753.
[30] H. Kranz, G.A. Brazeau, J. Napaporn, R.L. Martin, W. Millard, R. Bodmeier, Myotoxicity studies of injectable biodegradable in-situ forming drug delivery systems, Int. J. Pharm. 212 (2001) 11–18.
[31] G. Houen, The solubility of protein in organic solvents, Acta Chem. Scand. 50 (1996) 68–70.
[32] T.G. Park, H.Y. Lee, Y.S. Nam, A new preparation method for protein loaded poly(D,L-lactic-co-glycolic acid) microspheres and protein release mechanism study, J. Control. Release 55 (1998) 181–191.
[33] C.L. Stevenson, J.L. Leonard, S.C. Hall, Effect of peptide concentration and temperature on leuprolide stability in dimethyl sulfoxide, Int. J. Pharm. 191 (1999) 115–129.
[34] R.G. Strickley, Solubilizing excipients in oral and injectable formulations, Pharm. Res. 21 (2004) 201–230.
[35] G.M. Bristow, W.F. Watson, Cohesive energy densities of polymers. Part I: cohesive energy densities of rubbers by swelling measurement, Trans. Faraday Soc. 54 (1958) 1731–1741.
[36] L. Yilmaz, A.J. McHugh, Analysis of nonsolvent–solvent–polymer phase diagrams and their relevance to membrane formation modelling, J. Appl. Polym. Sci. 31 (1986) 997–1018.
[37] P.D. Graham, K.J. Brodbeck, A.J. McHugh, Phase inversion dynamic of PLGA solutions related to drug delivery, J. Control. Release 58 (1999) 233–245.
[38] B.F. Barton, J.L. Reeve, A.J. McHugh, Observation on the dynamic of nonsolvent-induced phase inversion, J. Polym. Sci. Polym. Phys. Ed. 35 (1997) 569–585.
[39] S. Yang, Z.Z. Liu, Preparation and characterization of polyacrylnitrile ultrafiltration membranes, J. Membr. Sci. 222 (2003) 87–98.
[40] J.H. Kim, K.H. Lee, Effect of PEG additive on membrane formation by phase inversion, J. Membr. Sci. 138 (1998) 153–163.
[41] Q.Z. Zhang, P. Wang, Y.N. Yang, Rheological and thermodynamic variation in polysulfone solution by PEG introduction and its effect on kinetics of membrane formation via phase-inversion process, J. Membr. Sci. 279 (2006) 230–237.
[42] L. Wang, L. Kleiner, S. Venkatraman, Structure formation in injectable poly(lactide-co-glycolide) depots, J. Control. Release 90 (2003) 345–354.
[43] J.R. DesNoyer, A.J. McHugh, The effect of pluronic on the protein release kinetics of an injectable drug delivery system, J. Control. Release 86 (2003) 15–24.
[44] L. Wang, S. Venkatraman, L.H. Gan, L. Kleiner, Structure formation in injectable poly(lactide-co-glycolide) depots II: nature of gel, J. Biomed. Mater. Res. B Appl. Biomater. 72 (2005) 215–222.
[45] R.G. Larson, The Structure and Rheology of Complex Fluids, Oxford University Press, New York, 1999.
[46] W.H. Huang, S. Matsuoka, T.K. Kwei, Y. Okamoto, Aggregation and gelation of fully conjugated rigid-rod polymers: poly(2,5-dialkyl-1,4-phenylenethylene)s, Macromolecules 34 (2001) 7166–7171.
[47] A. Saiani, J. Spevacek, J.M. Guenet, Phase behavior and polymer/solvent interactions in thermoreversible gels of syndiotactic poly(methyl methacrylate), Macromolecules 31 (1998) 703–710.
[48] B. Jeong, Y.H. Bae, S.W. Kim, Thermoreversible gelation of PEG–PLGA–PEG triblock copolymer aqueous solutions, Macromolecules 32 (1999) 7064–7069.
[49] K.J. Brodbeck, J.R. DesNoyer, A.J. McHugh, Phase inversion dynamic of PLGA solutions related to drug delivery. Part II. The role of solution thermodynamics and bath-side mass transfer, J. Control. Release 62 (1999) 333–344.
[50] A.J. McHugh, D.C. Miller, The dynamics of diffusion and gel growth during nonsolvent-induced phase inversion of polyethersulfone, J. Membr. Sci. 105 (1995) 121–136.
[51] R.M. Boom, I.M. Wienk, Th. Van den Boomgaard, C.A. Smolders, Microstructures in phase inversion membranes. Part 2. The role of a polymeric additives, J. Membr. Sci. 73 (1992) 277–292.