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Porous microspheres of methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-D,L-lactide) prepared by a melt dispersion method

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ABSTRACT

Surfactant-free biodegradable porous microspheres of methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-D,L-lactide) (MPEG-*b*-PCLDLL) diblock copolymers were prepared by a simple melt dispersion method in water at 80 °C with magnetic stirring. Any organic solvents and surfactants can be neglected for this method. Different CL/DLL ratios in the MPEG-*b*-PCLDLL were investigated for preparation of the porous microspheres. It was found that microsphere sizes decreased and surface pore sizes increased as the increasing DLL ratio. The pores were well interconnected throughout the microsphere matrices for all MPEG-*b*-PCLDLLs. The larger pore sizes can be obtained when the PEG was blended with diblock copolymer before preparation of porous blended microspheres. Possible mechanisms for formation of the porous microspheres with and without PEG blending were also proposed.

1. Introduction

In recent years, interest in porous biodegradable microspheres has increased steadily because of a variety of applications such as controlled-release drug delivery systems [1–6], cell culture substrates [7,8] and adsorbents [9]. The porous microspheres have been prepared by a few methods including water$_1$-in-oil-in-water$_2$ ($W_1/O/W_2$) double emulsion solvent evaporation [1,4–8], oil$_1$-in-water-in-oil$_2$ ($O_1/W/O_2$) emulsion [9] and electrospraying [10]. For controlled-release drug application, drug loading efficiency and drug release rate depend upon the surface pore size of the porous microspheres [11].

Poly(ε-caprolactone) has been interested by researchers for applying in pharmaceutical and medical applications as it is known as a biocompatible and biodegradable polymer. Poly(ε-caprolactone) microspheres have been prepared by a melt dispersion method in poly(ethylene glycol) without any organic solvents and surfactants [12]. However, preparation of porous biodegradable microspheres by the same method in water has not been reported, and it is of utmost interest to develop preparation procedures that avoid non-biocompatible porogenes. The value of porous microspheres, which today are used in medical and pharmaceutical applications, will increase if we can design porous microspheres that free from these chemicals. Methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone) (MPEG-*b*-PCL) and methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide) (MPEG-*b*-PDLL) diblock copolymers are biodegradable and biocompatible polymers that are widely synthesized and investigated for use in various biomaterial applications [13–18].

In this work, we introduce a melt dispersion method for preparing the porous microspheres of methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-D,L-lactide) (MPEG-*b*-PCLDLL). The MPEG-*b*-PCLDLLs with different CL/DLL ratios were molten and dispersed as droplets by stirring force before cooling to solidify. Moreover, influence of PEG, extractable porogen, blending with MPEG-*b*-PCL on microsphere morphology and porous structure was also investigated.

2. Experimental part

2.1. Synthesis and characterization of MPEG-b-PCLDLLs

Methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-D,L-lactide) (MPEG-*b*-PCLDLL) diblock copolymers with different CL/DLL ratios were synthesized by ring-opening polymerization of CL and DLL monomers in bulk at 130 °C for 48 h under a dry nitrogen atmosphere. The schematic diagram of the diblock copolymer preparation and reagents used is shown in Scheme 1. The different amounts of MPEG (0.190, 0.193, 0.195 and 0.200 mmol), CL (100, 95, 90 and 80 mmol) and DLL (0, 5, 10 and 20 mmol) were used to synthesis the MPEG-*b*-PCLDLLs with CL/DLL ratios of 100/0, 95/5, 90/10 and 80/20 (mol%), respectively. The CL monomer (99%, Acros organic) was purified by fractional distillation under reduced

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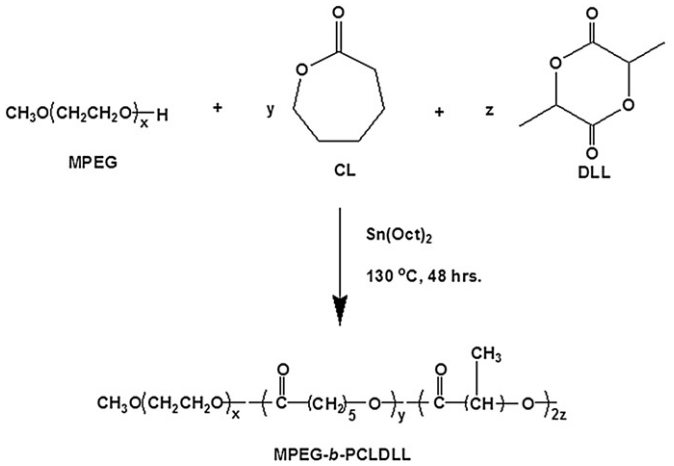

Scheme 1. Polymerization reaction of MPEG-*b*-PCLDLL.

pressure. The DLL monomer was synthesized from D,L-lactic acid (85%, Acros organic) and purified by re-crystallization from distilled ethyl acetate before drying in a *vacuo*. The MPEG with molecular weight of 5000 g/mol (Fluka) and stannous octoate ($Sn(Oct)_2$, 95% Sigma) were used as the initiating system. The molecular weights of all MPEG-*b*-PCLDLLs calculated from comonomer feed ratios and MPEG molecular weight (5000 g/mol) were approximately 65,000 g/mol. Stannous octoate concentration was kept constant at 0.04 mol% (16.2 mg, 0.04 mmol). As-polymerized diblock copolymers were purified by dissolving in chloroform before being precipitating in cool *n*-hexane. Finally, they were dried to constant weight in a *vacuo* at room temperature before characterization and microsphere preparation.

Copolymer compositions were measured by ^{1}H NMR spectrometry using a Bruker Advance DPX 300 ^{1}H NMR spectrometer. $CDCl_3$ was used as a solvent at room temperature, and

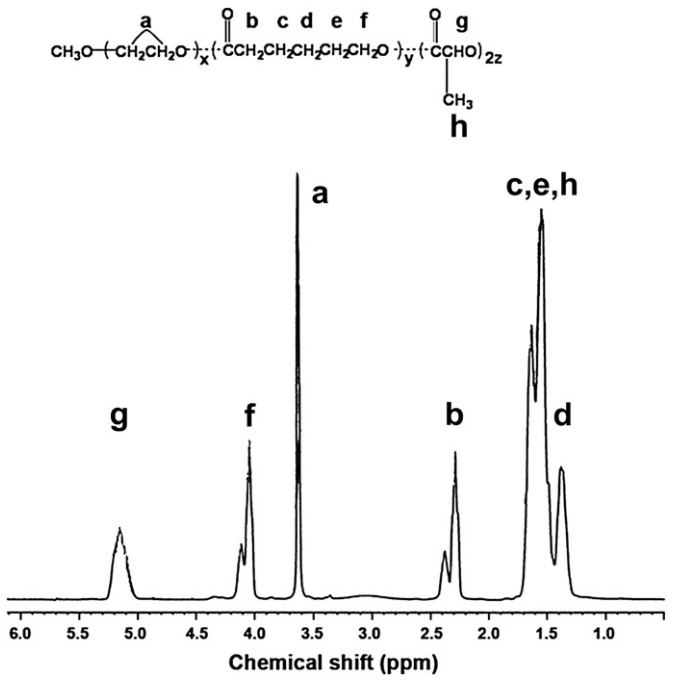

Fig. 1. ^{1}H NMR spectrum of MPEG-*b*-PCLDLL with CL/DLL ratio of 80/20 (mol%) in $CDCl_3$ used as solvent at room temperature.

Table 1
Copolymer compositions and $\overline{M}_n$ of the MPEG-*b*-PCLDLLs.

MPEG-*b*-PCLDLLs (CL/DLL mol%)	CL/DLL (mol%)		EO/CL/DLL (mol%)		$\overline{M}_n$ (g/mol)		MWD[c]
	Feed ratio[a]	^{1}H NMR[b]	Feed ratio[a]	^{1}H NMR[b]	^{1}H NMR[b]	GPC[c]	
100/0	100/0	100/0	20/80/0	18/82/0	62,300	61,400	1.9
95/5	95/5	94/6	20/76/4	19/76/5	64,000	62,200	1.9
90/10	90/10	91/9	20/72/8	18/75/7	64,600	62,100	1.8
80/20	80/20	81/19	20/64/16	19/66/15	63,200	61,600	1.9

[a] Calculated from comonomer feed ratios.
[b] Calculated from ^{1}H NMR spectra.
[c] Obtained from GPC curves.

tetramethylsilane was used as the internal standard. Number-average molecular weight ($\overline{M}_n$) and molecular weight distribution (MWD) were determined by gel permeation chromatography (GPC) using a Waters 717 plus Autosampler GPC equipped with an Ultrastyragel® column and a refractive index detector operating at 40 °C and employing universal calibration. THF was used as the solvent at a flow rate of 1 mL/min. The $\overline{M}_n$ was also measured from the ^{1}H NMR spectrum using the MPEG molecular weight as a reference. Thermal properties were characterized by non-isothermal differential scanning calorimetry (DSC) using a Perkin–Elmer Pyris Diamond DSC. For DSC, the sample (~10 mg) was heated at the rate of 10 °C/min under helium flow to observe their melting temperatures (T_m) and heats of melting (ΔH_m).

2.2. Preparation and characterization of porous microspheres

Surfactant-free porous microspheres of the diblock copolymers were prepared by the melt dispersion method. This method was explained as follows. Approximately 0.5 g of the MPEG-*b*-PCLDLL was molten and dispersed in 150 mL of distilled water at 80 °C with magnetic stirring for 30 min before cooling to room temperature. The stirring speeds of 600–900 rpm were investigated. The resulted microspheres were recovered by centrifugation before freeze-drying. The porous microspheres of MPEG-*b*-PCL/PEG blends were produced as the same method using stirring speed of 800 rpm. Dichloromethane was used as a blended solvent and evaporated before microsphere preparation. The MPEG-*b*-PCL/PEG blended ratios of 10/1 and 10/2 (w/w) were investigated.

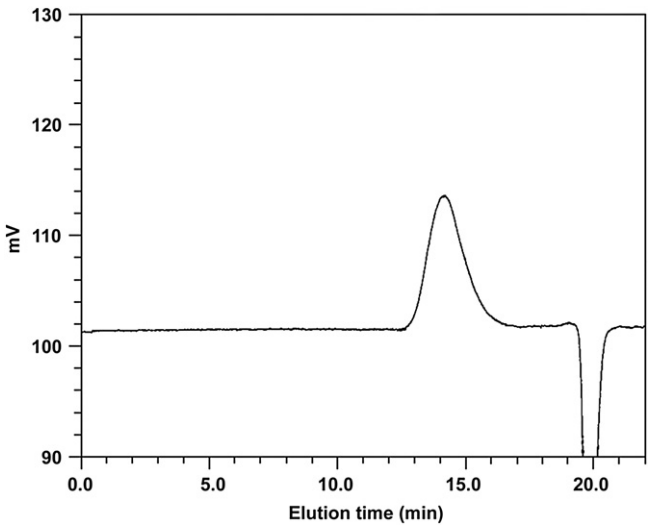

Fig. 2. GPC curve of MPEG-*b*-PCLDLL with CL/DLL ratio of 80/20 (mol%).

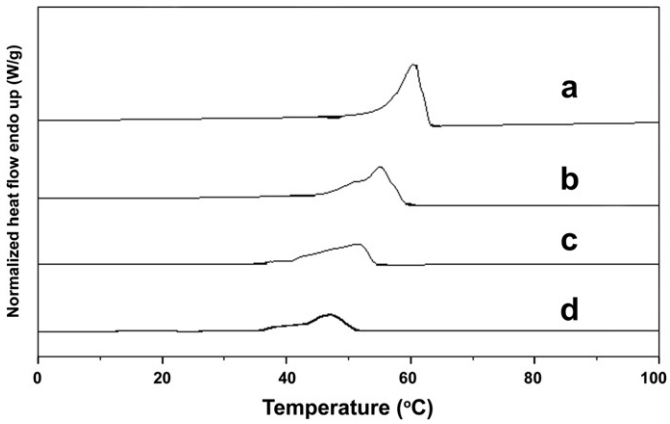

Fig. 3. DSC thermograms of MPEG-*b*-PCLDLLs with CL/DLL ratios of (a) 100/0, (b) 95/5, (c) 90/10 and (d) 80/20 (mol%).

Chemical structures of the microspheres were measured by Fourier transform infrared (FTIR) spectroscopy using a Perkin–Elmer Spectrum GX FTIR spectrophotometer with air as the reference. The resolution of 4 cm^{-1} and 32 scans were chosen. FTIR spectra were collected by using a KBr disk method. Particle sizes and size distributions of the microspheres were determined by light-scattering analysis using a Coulter LS230 light-scattering particle size analyzer at 25 °C. Morphology and porous structures of the microspheres were analyzed by scanning electron microscopy (SEM) using a JEOL JSM-6460LV SEM. Before SEM measurement, the microspheres were sputter coated with gold for enhancing surface conductivity. The average surface pore sizes were measured from several SEM images counting a minimum 100 surface pores using smile view software (version 1.02).

Table 2
Thermal properties and average particle sizes of the MPEG-*b*-PCLDLLs.

MPEG-*b*-PCLDLLs (CL/DLL mol%)	T_m[a] (°C)	ΔH_m[a] (J/g)	Particle size[b] (μm)
100/0	61	108.3	97 ± 52
95/5	55	85.2	68 ± 38
90/10	51	65.1	55 ± 40
80/20	47	43.8	50 ± 34

[a] Measured from DSC thermograms.
[b] Measured from light-scattering analysis.

3. Results and discussion

3.1. Characterization of MPEG-b-PCLDLLs

All purified MPEG-*b*-PCLDLLs had percent yields higher than 90%. The copolymer compositions of the MPEG-*b*-PCLDLLs were determined from their ^{1}H NMR spectra, using the peak area ratios corresponding to the methylene protons of ethylene oxide (EO, repeating units of MPEG block) at $\delta = 3.6$–3.7 ppm, the ε-methylene protons of CL at $\delta = 4.0$–4.2 ppm and the methine protons of DLL at $\delta = 5.0$–5.3 ppm. The ^{1}H NMR spectrum of MPEG-*b*-PCLDLL with CL/DLL ratio of 80/20 mol% is shown as an example in Fig. 1 and the all calculated compositions of CL/DLL and EO/CL/DLL (mol%) are given in Table 1. As expected, the calculated copolymer compositions are similar to the comonomer feed ratios, indicating that the synthesis reactions proceeded to near-quantitative conversion.

The chain microstructures of PCLDLL blocks are reflected in the fine structures of the ^{1}H NMR spectra. The appearance of multiple resonances for the same proton can be attributed to the presence of different monomer sequences and therefore slightly different chemical environments in the copolymer chain. The α-CH_2 and ε-CH_2 protons in the CL units are seen to be particularly sensitive to

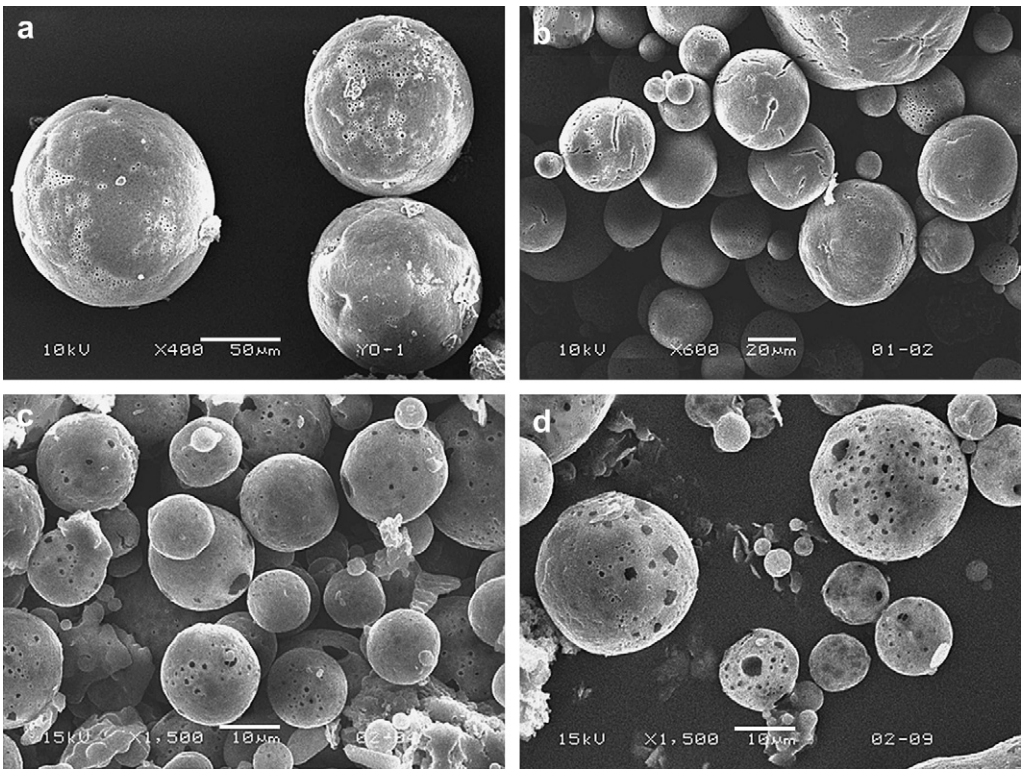

Fig. 4. SEM micrographs of porous MPEG-*b*-PCLDLL microspheres with CL/DLL ratios of (a) 100/0, (b) 95/5, (c) 90/10 and (d) 80/20 (mol%). Scale bars: 50, 20, 10 and 10 μm for (a), (b), (c) and (d), respectively.

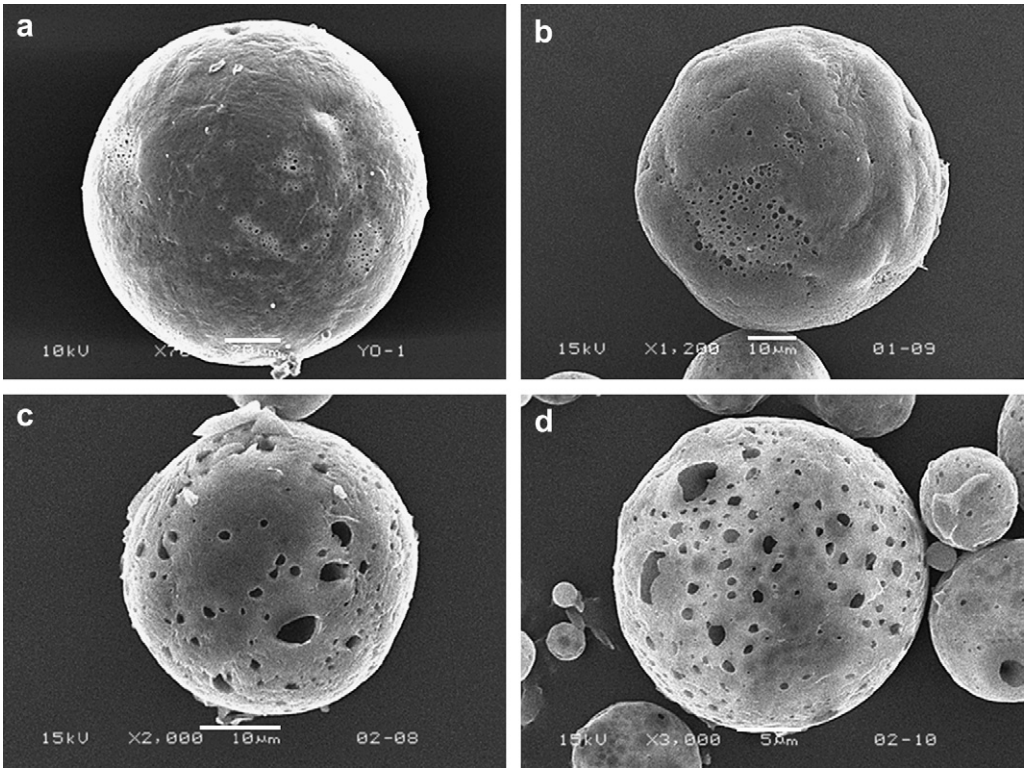

Fig. 5. Expanded SEM micrographs of porous MPEG-*b*-PCLDLL microspheres with CL/DLL ratios of (a) 100/0, (b) 95/5, (c) 90/10 and (d) 80/20 (mol%). Scale bars: 20, 10, 10 and 5 μm for (a), (b), (c) and (d), respectively.

this. As shown in Fig. 1, the bands at 2.4 and 4.1 ppm, corresponding to the α-CH_2 and ε-CH_2 protons in the CL units, respectively are split into quite distinct triplets adjacent to one another suggesting randomization of the CL units in the copolyester blocks.

The molecular weights of the copolymers were determined by GPC and ^{1}H NMR and the results are reported in Table 1. It was found that $\overline{M}_n$ values of all the MPEG-*b*-PCLDLLs from GPC and ^{1}H NMR are lower than the calculated $\overline{M}_n$ values (~65,000 g/mol) from the comonomer feed ratios. This may be interpreted in terms of degradation side reactions such as transesterification taking place. The all MPEG-*b*-PCLDLLs gave similar unimodal GPC molecular weight distributions as an example of them is shown in Fig. 2.

Thermal properties of the MPEG-*b*-PCLDLL are carried out by means of DSC thermograms as illustrated in Fig. 3 and summarized in Table 2. The T_m and ΔH_m of MPEG-*b*-PCLDLLs decreased when the DLL units were copolymerized and increased its ratio.

3.2. Preparation and characterization of porous microspheres

The melt dispersion process for preparation of porous microspheres from MPEG-*b*-PCLDLLs was developed by melting and dispersion the copolymers in water at 80 °C with simple magnetic stirring. The temperature selected is suitable for melting the copolymers as it is higher than the T_m of all copolymers and lower

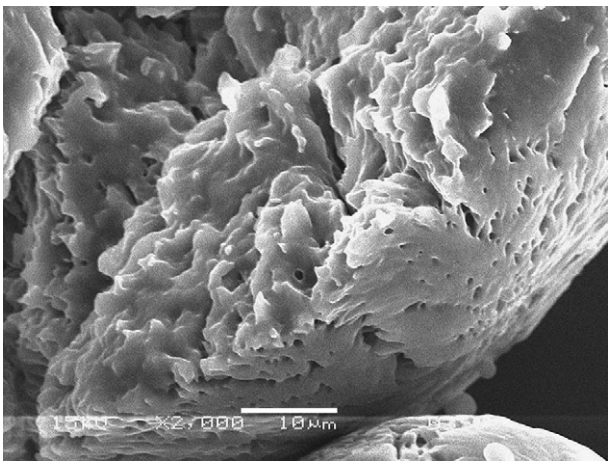

Fig. 6. Expanded SEM micrograph of cross-section of porous MPEG-*b*-PCLDLL microspheres with CL/DLL ratio of 95/5 (mol%) (bar = 10 μm).

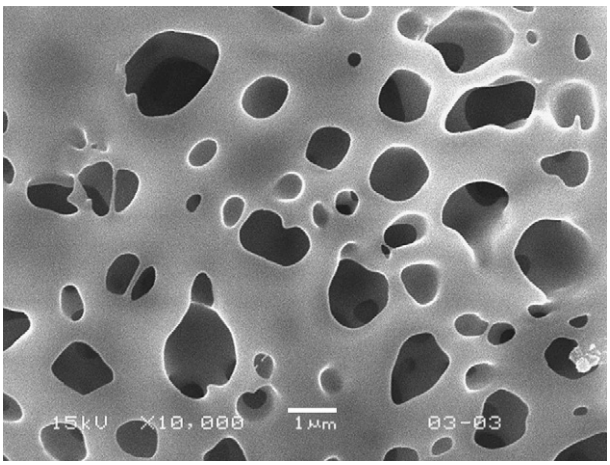

Fig. 7. Expanded SEM micrograph of surface of porous MPEG-*b*-PCLDLL microspheres with CL/DLL ratio of 80/20 (mol%) (bar = 1 μm).

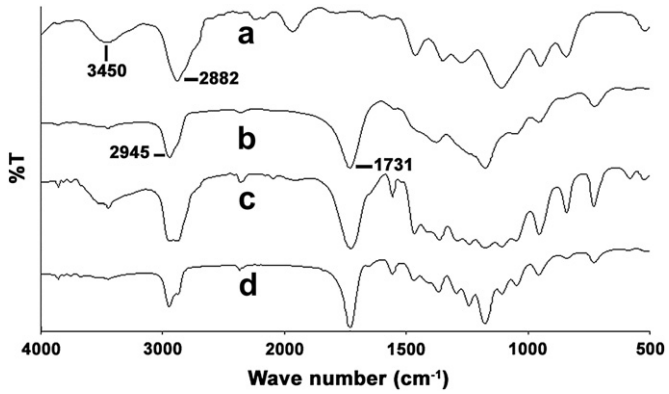

Fig. 8. FTIR spectra of (a) PEG, (b) MPEG-*b*-PCL, (c) 10/2 (w/w) MPEG-*b*-PCL/PEG blend and (d) porous blended microspheres with 10/2 (w/w) MPEG-*b*-PCL/PEG ratio.

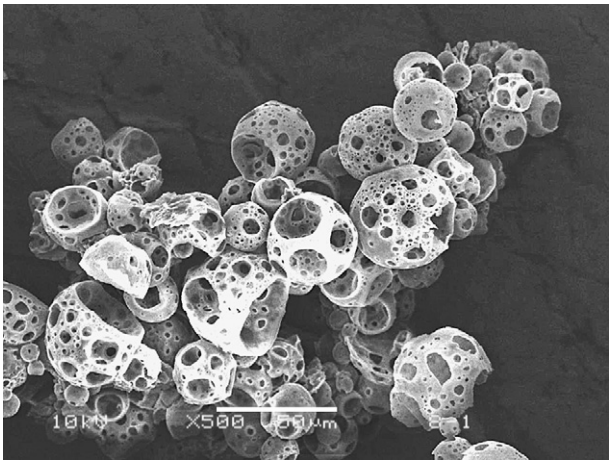

Fig. 10. SEM micrograph of porous blended microspheres of 10/2 (w/w) MPEG-*b*-PCL/PEG blend (bar = 50 μm).

than the boiling point of water medium. Lowering the temperature to 70 °C did not make clear melt dispersion for the copolymers. Microsphere sizes obtained from light-scattering analysis slightly decreased as the DLL ratio increased as reported in Table 2. This may be explained that the MPEG-*b*-PCLDLL with higher DLL ratio had lower melt viscosity in water at 80 °C because of its lower T_m and ΔH_m values. Then, the MPEG-*b*-PCLDLL with higher DLL ratio can be easier disrupted to smaller droplets during melt dispersion process before solidification. Furthermore, the stirring speed which induces shearing force to break down the molten copolymers into small droplets was investigated, which includes the speeds of 700, 800 and 900 rpm at 80 °C. In our study, the magnetic stirring speed of 800 rpm is found the most appropriate for microsphere preparation. The molten polymer could not be broken to form droplets when the stirring speed was lower than 800 rpm. In addition, almost all molten polymers were stuck at the wall of glassware during melt dispersion step when higher stirring speed than 800 rpm was applied.

Microsphere morphology was investigated from SEM micrographs as shown in Fig. 4. All microspheres were nearly spherical in shape with rough surfaces. Their expanded micrographs are illustrated in Fig. 5 and the surface pores can be detected. The pores in the microspheres may be generated from sublimation of imbibed water during the freeze-drying step. The water molecules were trapped in the microsphere matrix during the melt dispersion process. The sizes of surface pores significantly increased with the DLL ratio (Fig. 5). This may be expected that the higher

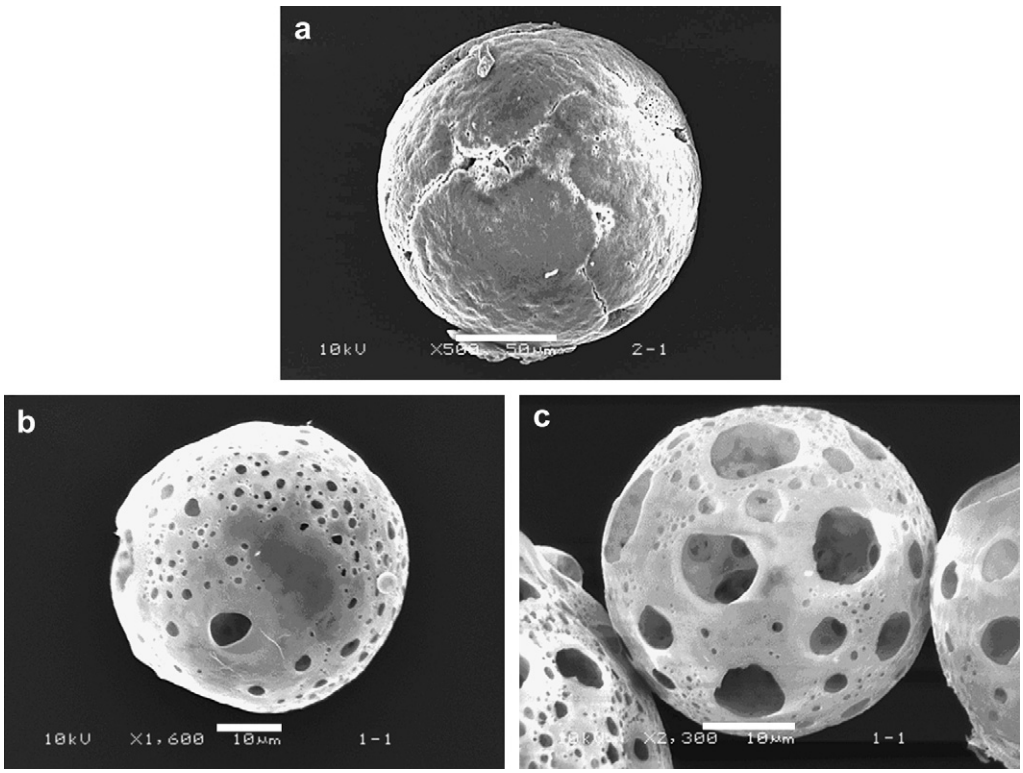

Fig. 9. SEM micrographs of external morphology of porous microspheres with MPEG-*b*-PCL/PEG blended ratios of (a) 10/0, (b) 10/1 and (c) 10/2 (w/w). Scale bars: 50, 10 and 10 μm for (a), (b) and (c), respectively.

Table 3
Blended ratios, particle sizes and surface pore sizes of porous MPEG-*b*-PCL/PEG blended microspheres.

MPEG-*b*-PCL/PEG blended ratio (w/w)	Average particle size[a] (μm)	Average surface pore size[b] (μm)
10/0	97 ± 52	0.7 ± 0.6
10/1	64 ± 38	3.2 ± 2.1
10/2	35 ± 20	9.5 ± 6.7

[a] Measured from light-scattering analysis.
[b] Measured from SEM images.

hydrophilicity of the DLL units induced larger content of imbibed water.

Internal or core morphology of the porous microspheres was investigated from microsphere cross section as an example of which is shown in Fig. 6 for the porous microspheres with CL/DLL ratios of 95/5 (mol%). It can be observed that the all pores were well interconnected throughout the microsphere matrix. This indicated that the water molecules were imbibed throughout the microsphere matrix during the melt dispersion process. In addition, the interconnected pores of microspheres with CL/DLL ratio of 80/20 (mol%) can be clearly observed through its surface pores as shown in Fig. 7. It should be noted that this method is easy, safe and fast for larger scale preparation of porous biodegradable microspheres because of the surfactants, organic solvents and non-biocompatible porogenes can be avoided.

3.3. Preparation and characterization of porous blended microspheres

Porous microspheres made from MPEG-*b*-PCL/PEG blends were also achieved by similar procedure as the MPEG-*b*-PCLDLLs. FTIR spectra of PEG, MPEG-*b*-PCL, MPEG-*b*-PCL/PEG blend and porous blended microspheres are presented in Fig. 8. The FTIR spectra of PEG [Fig. 8(a)] and MPEG-*b*-PCL [Fig. 8(b)] show absorption bands at 3450 and 1731 cm^{-1}, assigned to hydroxyl and carbonyl groups of the PEG and the MPEG-*b*-PCL, respectively. The bands at 2945 and 2882 cm^{-1} in the FTIR spectra of MPEG-*b*-PCL and PEG, respectively are attributed to their methylene groups. The FTIR spectrum of MPEG-*b*-PCL/PEG blend in Fig. 8(c) shows both hydroxyl and carbonyl groups of PEG and MPEG-*b*-PCL characteristics, respectively. The disappearance of the characteristic hydroxyl band at 3450 cm^{-1} of the PEG in the spectrum of porous blended microspheres in Fig. 8(d) may indicate that the blended PEG was leached out during the melt dispersion process. The FTIR spectra of MPEG-*b*-PCL/PEG blend and its porous blended microspheres with MPEG-*b*-PCL/PEG ratio of 10/1 (w/w) showed also similar evidence. This suggested that the blended PEG fraction might be removed out from the microsphere matrices. However, if some residual PEG remains in the microspheres, it may not affect on biomaterial applications due to its biocompatibility character.

The morphology of porous blended microspheres was also analyzed from their SEM micrographs as shown in Fig. 9. All microspheres were nearly spherical in shape. The morphological results indicated that the PEG blending before microsphere preparation by the melt dispersion method induced the larger porous structures as shown in Figs. 9(b), (c) and 10. The average surface pore sizes measured from several expanded SEM micrographs are summarized in Table 3 which indicated that larger pore size can be obtained with the higher PEG blended ratio.

The internal morphology of microspheres was investigated from the SEM micrographs of microsphere cross sections as illustrated in Fig. 11(a) and (b) for 10/0 and 10/1 (w/w) porous blended microspheres, respectively whereas the 10/2 (w/w) porous blended microspheres can be directly observed through its surface pores as

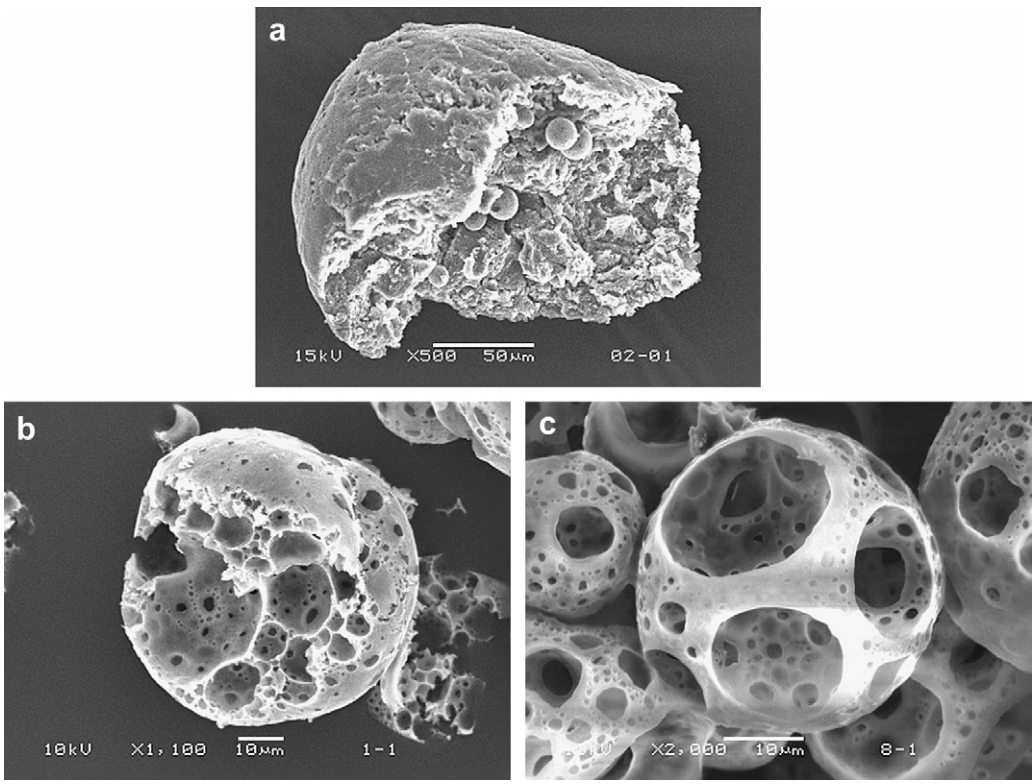

Fig. 11. SEM micrographs of internal morphology of porous microspheres with MPEG-*b*-PCL/PEG blended ratios of (a) 10/0, (b) 10/1 and (c) 10/2 (w/w). Scale bars: 50, 10 and 10 μm for (a), (b) and (c), respectively.

shown in Fig. 11(c). It can be seen that the porous microspheres without the presence of blended PEG was denser for its surface and interior than the 10/1 and 10/2 (w/w) porous blended microspheres.

3.4. Possible mechanisms of porous microsphere formation

For the porous microspheres prepared by the melt dispersion method of MPEG-*b*-PCLDLL, the MPEG-*b*-PCLDLL molten and dispersed in water at 80 °C forms the droplets with magnetic stirring before cooling down to room temperature. It has been reported that the MPEG-*b*-PDLL nanoparticles could be prepared because the MPEG blocks act as the shell to prevent nanodroplet coalescence [18,19]. Thus, the possible mechanism of porous microsphere formation can be proposed that the MPEG block of MPEG-*b*-PCLDLL in our case may also prevent aggregation of the molten MPEG-*b*-PCLDLL droplets during solidification process. In addition, the interconnected pores appeared throughout microsphere matrices were formed from sublimation of the imbibed water during the freeze-drying step. The pore sizes increased as the increasing DLL ratio due to higher hydrophilicity of MPEG-*b*-PCLDLL.

In the case of porous microspheres prepared from MPEG-*b*-PCL/PEG blend, the bigger pore size was obtained than in the case of MPEG-*b*-PCLDLL. The larger interconnected pores of the porous microspheres can be induced by *in situ* leaching out of the blended PEG from the molten blended droplets during the melt dispersion process. Then, the porous blended microspheres containing larger pores can be prepared by using higher blended PEG ratio. The possible mechanisms for porous microsphere formations of melt dispersion – *in situ* leaching out of MPEG-*b*-PCL/PEG blends may be proposed in two ways: (i) the PEG was immediately dissolved and leached out to the external aqueous phase, leaving MPEG-*b*-PCL matrix behind during the melt dispersion step. Then, many pores were formed and fused together before microsphere solidification. (ii) the PEG was molten and swollen with the imbibed water in the molten MPEG-*b*-PCL droplets before dissolving and leaching out to external aqueous phase. Finally, the large pores were obtained. Existence of the imbibed water throughout the microsphere matrix is according to the possible mechanism of porous microsphere formation as described above. Therefore, it can be proposed that the possible mechanism of melt dispersion – *in situ* leaching out method is the second mechanism for the MPEG-*b*-PCL/PEG blend.

4. Conclusion

The MPEG-*b*-PCLDLLs with different CL/DLL ratios were synthesized by ring-opening polymerization of CL and DLL monomers by using MPEG and $Sn(Oct)_2$ as the initiating system. The porous microspheres of MPEG-*b*-PCLDLLs with spherical in shape and rough surface were successfully prepared by the melt dispersion method in water at 80 °C with simple magnetic stirring. The organic solvent, surfactant and porogen were avoided for this method. The microsphere sizes decreased and surface pore sizes increased as the increasing DLL ratio. The pores were well interconnected throughout the microsphere matrices. The porous structure occurred from the sublimation of the imbibed water in the microspheres was the possible proposed mechanism. The porous microspheres containing larger interconnected pores were obtained when the PEG was blended in MPEG-*b*-PCL before microsphere preparation. The proposed mechanism for formation of porous blended microspheres is due to the leaching out process of the PEG from the MPEG-*b*-PCL/PEG blended matrix and probably together with the sublimation of the imbibed water. The PEG is considered as an extractable porogen which is leached out during the melt dispersion process. The pore sizes of the porous microspheres can be controlled either by the ratio of CL/DLL or by the use of PEG to blend with diblock copolymer. These porous biodegradable microspheres might be of interest for use as biomaterials in cell microcarrier and drug delivery applications.

Acknowledgements

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