

Effect of solvent removal technique on the matrix characteristics of polylactide/glycolide microspheres for peptide delivery

R. Jeyanthi¹, B.C. Thanoo, R.C. Metha², P.P. DeLuca^{*}

College of Pharmacy, University of Kentucky, Rose Street, Lexington, KY 40536-0082, USA

Received 5 February 1995; accepted 16 August 1995

Abstract

Porous salmon calcitonin (sCT)-loaded PLGA microspheres (size range 35–140 μm) of varying matrix characteristics were prepared by an aqueous emulsification process using either a temperature gradient (Tmp) or dilution (Dil) of the continuous phase (CP). **The Tmp technique resulted in microspheres with a hollow internal core and a porous wall.** The core size and thickness of the porous wall were dependent on the temperature gradient used. A rapid ramp of temperature from 15 to 40°C resulted in a large core and a thin wall, while a gradual temperature rise resulted in a smaller core. **The Dil technique produced microspheres with a uniform, honeycomb like pore structure without a core,** pore size being dependent on the dilution volume used. The specific surface area was higher and bulk density lower for microspheres prepared by the Tmp technique while there was no significant difference in the peptide load (3.2–4.5%) between both techniques. **A rapid removal of CH_2Cl_2 was observed in case of the Tmp technique while the Dil technique facilitated a slower and gradual CH_2Cl_2 removal.** Residual CH_2Cl_2 was approximately < 10–20 ppm for microspheres prepared by the Tmp technique while the levels were 20–130 ppm for microspheres produced by the Dil technique. Higher retention of methanol (~15–50%) was observed in the droplet formation stage with the Tmp technique, subsequent removal of which affected the core size. In the Dil technique, very low levels of methanol (<2%) were retained in the droplets soon after dispersion resulting in a uniform porous structure without core. Slower removal of methanol from the microspheres was partly responsible for the core formation.

Keywords: Solvent removal technique; Matrix characteristics; Polylactide/glycolide microsphere; Peptide delivery

1. Introduction

The internal pore structure of biodegradable polymeric delivery systems plays an important role in the release characteristics of the entrapped agents [1–4]. This is especially true for the copolymers of dl-lactide-glycolide which degrade by a ‘bulk’ homogeneous

hydrolytic process. Matrix structure is one of the factors that determines the capacity of hydration of these hydrophobic polymers and hence will influence both diffusional and erosional release [5,6]. In previous studies [7] with peptide-loaded PLGA microspheres, several factors such as polymer molecular weight, cosolvent concentration, peptide concentration, dispersed phase/continuous phase (DP/CP) ratio and solvent removal method were found to influence the matrix porosity and hence the surface area of the microspheres. Surface area decreased with increase in polymer molecular weight, while it was higher at lower

^{*} Corresponding author. Rose Street, Lexington KY 40536-0082.

¹ Present address: Department of Dental Research, Bldg. 2832, Ernie Pyle Road, Fort Meade, MD 20755, USA.

² Present address: ISIS Pharmaceuticals, 2292 Faraday Avenue, Carlsbad, CA 92008, USA.

DP/CP ratio. Porosity increased with increase in both peptide and CH_3OH concentrations in the DP and the combination of CH_3OH and peptide seemed to be critical in pore formation. In the present study, the effect of two solvent removal techniques on the matrix characteristics and solvent residues of sCT-PLGA microspheres was evaluated. Solvent removal was performed either by using a temperature gradient or by CP dilution.

2. Experimental

2.1. Materials

Poly (dl-lactide-co-glycolide) (50:50, M_w 30 000) (PLGA) was obtained from Boehringer Ingelheim Inc., Germany. Salmon calcitonin (sCT) was supplied by Bachem Inc., Torrance, CA. All other chemicals used were of analytical reagent grade.

2.2. Methods

Preparation of sCT microspheres

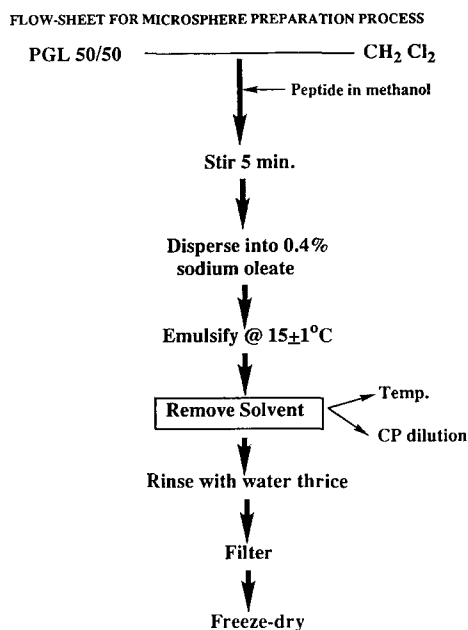
The microspheres were prepared by an aqueous emulsification solvent removal technique [6]. Briefly, the method involved the dispersion of sCT/PLGA in a CH_2Cl_2 - CH_3OH cosolvent system and subsequent dispersion into an aqueous continuous phase consisting of 0.4% (w/v) sodium oleate. Microspheres were hardened upon removal of the solvents. Scheme 1 outlines the preparation process. The microspheres obtained from these preparation techniques were in the size range of 35–140 μm .

Temperature gradient (Tmp) technique

Following dispersion of the polymer-peptide organic solution into the CP and the initial extraction of solvents at $15 \pm 1^\circ\text{C}$ during droplet formation, subsequent removal of solvents was achieved by increasing the temperature of the continuous phase to $40 \pm 1^\circ\text{C}$ using different gradients. The CP volume was maintained constant throughout the preparation process.

Dilution (Dil) technique

Following initial extraction of solvents at $15 \pm 1^\circ\text{C}$ during droplet formation, subsequent solvent removal was achieved by dilution of the CP at $25 \pm 1^\circ\text{C}$. The



Scheme 1.

CP was diluted to predetermined volumes at a constant rate and the temperature was maintained constant at $25 \pm 1^\circ\text{C}$ throughout the solvent removal process. In both cases, final rinses with the CP and water were performed to remove traces of residual solvents.

Analyses of residual solvents

Analyses of residual CH_2Cl_2 and CH_3OH were carried out on microspheres recovered at different stages during preparation. Additionally, these samples were subjected to scanning electron microscopy (SEM) to study the changes in morphology during the different stages of preparation. Wet microspheres were dissolved in dioxane and residual solvents were analysed on a Varian 3500 Gas Chromatograph using a Flame ionization detector. A GS-Q (J&W Scientific) porous layer open tubular (PLOT) column was used to separate the solvents. To avoid the polymer entering into the separating column, the polymer solutions were passed through a precolumn which consisted of a glass injector liner filled with 5% SE-30 on Chromosorb W-HP between two plugs of glass wool. Helium was used as the carrier gas. Residual solvent concentrations were calculated from standard calibration curves and expressed as percentage of their initial concentration in the DP based on dry weight. Individual samples were

dried separately to determine the dry weight of the samples.

Characterization of sCT microspheres

SEM

Approximately 2 mg of microspheres were suspended in 1 ml of 0.1% Tween 80, filtered through a 0.22 μm Poretics polycarbonate membrane filter and placed on an aluminum stub for scanning using a Hitachi model S 800 Scanning Electron Microscope. To study the internal structure, microspheres were sprayed onto a double adhesive tape and fractured on the aluminum stub. The stubs were coated with gold-palladium prior to scanning.

Specific surface area (SSA)

SSA of sCT microspheres (approximately 300 mg) was determined by a BET method using a Micromeritics ASAP 2000. The BET method is based on the adsorption and desorption of nitrogen and krypton gas at the surface and within the pores of the microspheres.

Bulk density (BD)

Tapped BD of the sCT microspheres was determined by measuring the volume occupied by approximately 500 mg of microspheres after 100 tappings or when the volume remains the same for five consecutive tappings. BD was expressed as g/cm^3 using the following equation:

$$\text{BD} = \text{Wt. of microspheres} / \text{Final volume after tapping}$$

Particle size distribution

Size distribution of the microspheres (concentration of 1 mg/ml) suspended in 0.1% (w/v) Tween 80 was determined using a Malvern 2600 Laser Diffraction Particle Sizer.

Peptide load

To approximately 10 mg microspheres placed in a polypropylene tube, 1 ml of 50% (v/v) acetonitrile/phosphate buffer (0.01 M, pH 7.4) was added, sonicated for 15 min, centrifuged and the supernatant assayed for sCT by HPLC on a gradient [7]. The peptide content in the microspheres was calculated from a standard sCT calibration curve and the load was expressed as μg sCT per 100 mg microspheres.

3. Results and discussion

Fig. 1 shows the surface morphology of sCT microspheres prepared by the two solvent removal techniques. Microspheres were well formed and porous irrespective of the solvent removal technique used. Table 1 summarizes the matrix characteristics of microspheres prepared using the two solvent removal

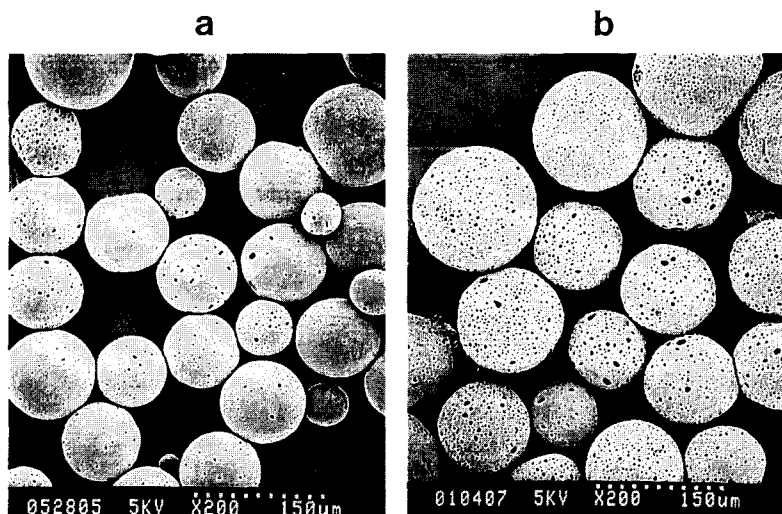


Fig. 1. Surface morphology of sCT microspheres prepared by the two solvent removal techniques: (a) Tmp technique; (b) Dil technique.

Table 1
Matrix characteristics of microspheres prepared using two solvent removal techniques

Batch No.	Solvent removal process	SSA (m ² /g)	Bulk density (g/cm ³)	Peptide load (%)	Surface morphology	Residual CH ₂ Cl ₂ (ppm)
1 ^a	Temp. 15–40°C (rapid)	8.8	0.07	3.56	Porous surface; small core, thick porous wall	18
2	Temp. 15–40°C (rapid)	5.6	0.05	4.25	Porous surface; large core, thin porous wall	< 10
3	Temp. 15–25–37–40°C (gradual)	10.1	0.05	3.20	Porous surface; medium core, thick porous wall	< 10
4	Temp. 15–25°C (60 min)–40°C (step-wise)	6.4	0.07	3.38	Porous surface; medium core, thick porous wall	< 10
5	Dilution 1.5 ×	4.9	0.10	4.23	Porous surface; no core, honeycomb matrix	21
6	Dilution 2.5 ×	5.2	0.12	4.14	Porous surface; no core, honeycomb matrix	129

^a17.6% w/w CH₃OH concentration; all other batches 7.0% CH₃OH (w/w).

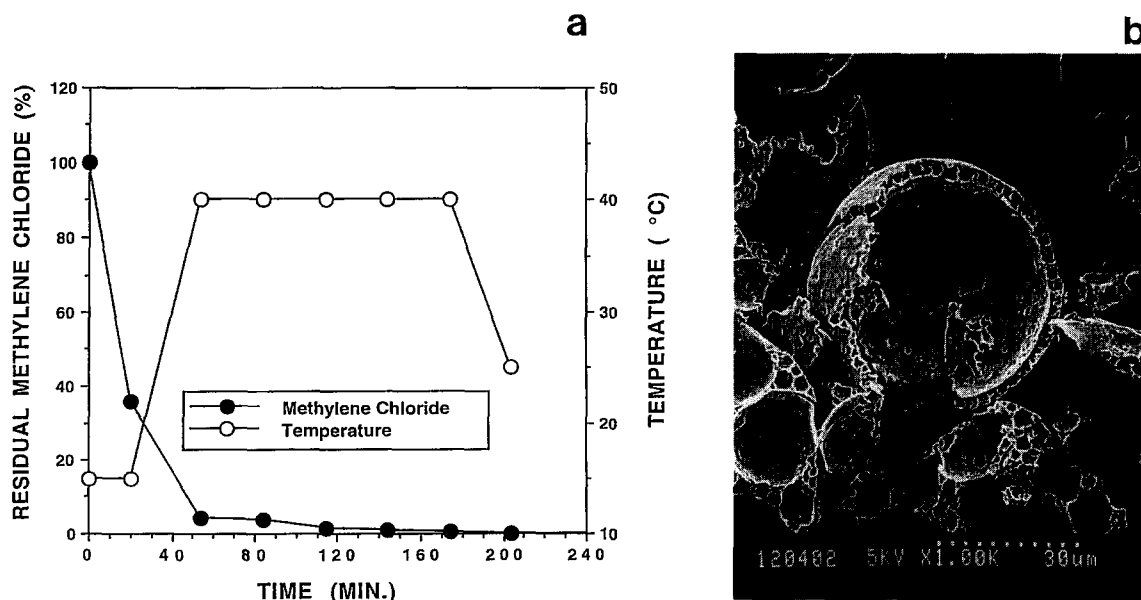


Fig. 2. CH₂Cl₂ removal profile (a), and internal pore structure (b) of batch 2 sCT microspheres prepared by the Tmp technique. Temperature raised rapidly from 15–40°C.

techniques. SSA was higher (5–10 m²/g) and bulk density lower (0.05–0.07 g/cm³) with the Tmp technique while the Dil technique resulted in SSA of approximately 5 m²/g and a bulk density of ~0.10. The solvent removal technique did not seem to affect peptide load although both batches by the Dil technique were above 4% while three of the four batches by the Tmp technique were below 4%. Microspheres prepared by the Tmp technique were porous on the surface with

a hollow core and a porous wall. The Dil technique produced porous microspheres with a uniform honeycomb matrix without a core. Residual CH₂Cl₂ was appreciably low (10–20 ppm) with the Tmp technique while the levels were 20 and 129 ppm with the Dil technique. A closer observation of the microsphere morphology and the residual solvent levels at different stages during the preparation process was undertaken to explore the solvent removal effect.

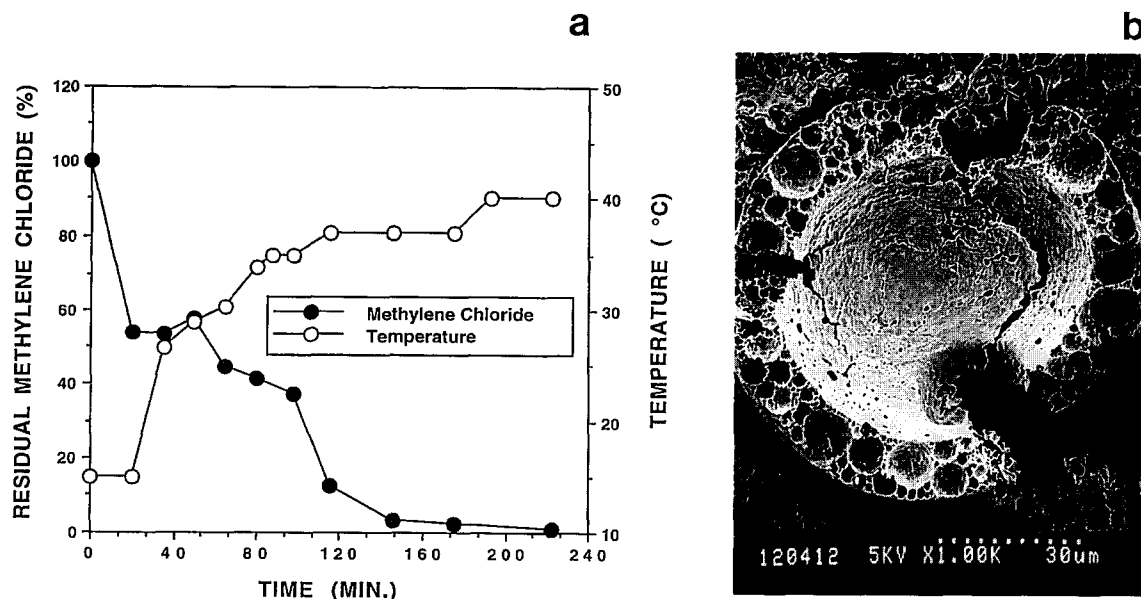


Fig. 3. CH₂Cl₂ removal profile (a), and internal pore structure (b) of batch 3 sCT microspheres prepared by the Tmp technique. Temperature raised gradually from 15–27–37–40°C.

3.1. Solvent removal profiles and internal structure

Temperature gradient technique

Fig. 2a shows the solvent removal profile of sCT-PLGA microspheres utilizing a rapid ramp of temperature from 15 to 40°C. A significant drop in residual CH₂Cl₂ levels was observed soon after dispersion of droplets into the continuous phase and again when the temperature was raised to 40°C. This resulted in microspheres with a very thin wall and a large hollow core (batch 2; Fig. 2b). When the temperature was ramped to 27°C rapidly, followed by a gradual increase to 37°C and finally to 40°C, the drop in residual CH₂Cl₂ levels was also gradual but continuous (Fig. 3a). This resulted in a thicker wall and a smaller core (batch 3; Fig. 3b). Solvent removal profile by a step-wise increase of temperature from 15 to 25 to 40°C after a lag phase of 60 min at 25°C (Fig. 4a) caused a more uniform drop in CH₂Cl₂ levels. This resulted in a more porous interior and smaller core (batch 4; Fig. 4b). SSA was higher in the microspheres prepared with a gradual temperature increase than the step-wise increase since the pores were greater in number and more uniformly distributed throughout the wall in the former case.

Fig. 5a and b shows the solvent removal profile and the internal structure of sCT microspheres (batch 1)

prepared using a cosolvent concentration of 17.6% instead of 7% used in previous batches. The temperature profile was similar to batch 2. Although there was no significant difference in CH₂Cl₂ removal profile between the two batches, there seemed to be a marked difference in the internal morphology. Higher CH₃OH concentration resulted in smaller core and a more uniform and porous internal structure. SSA was also higher for batch 1 due to a wider distribution of pores. The Tmp technique essentially resulted in microspheres with a variable-sized hollow core. The core size could be modified by changing the temperature gradient; the slower the gradient the smaller the core.

Dilution technique

Following dispersion of the polymer/peptide solution, there is a rapid saturation of CH₂Cl₂ in the CP. To continue solvent removal by an extraction process, dilution or replacement of the CP was required. In the Dil technique, the solubility of CH₂Cl₂ in the CP was exploited to ensure solvent removal. It was found that while solubility of CH₂Cl₂ in water was only about 2%, it was ~4.4% in 0.4% sodium oleate (pH 10). Fig. 6a shows the solvent removal profile of sCT microspheres prepared using the Dil technique. The initial volume of CP used for dispersion was twice that used in the Tmp process. Subsequent removal of CH₂Cl₂ was achieved

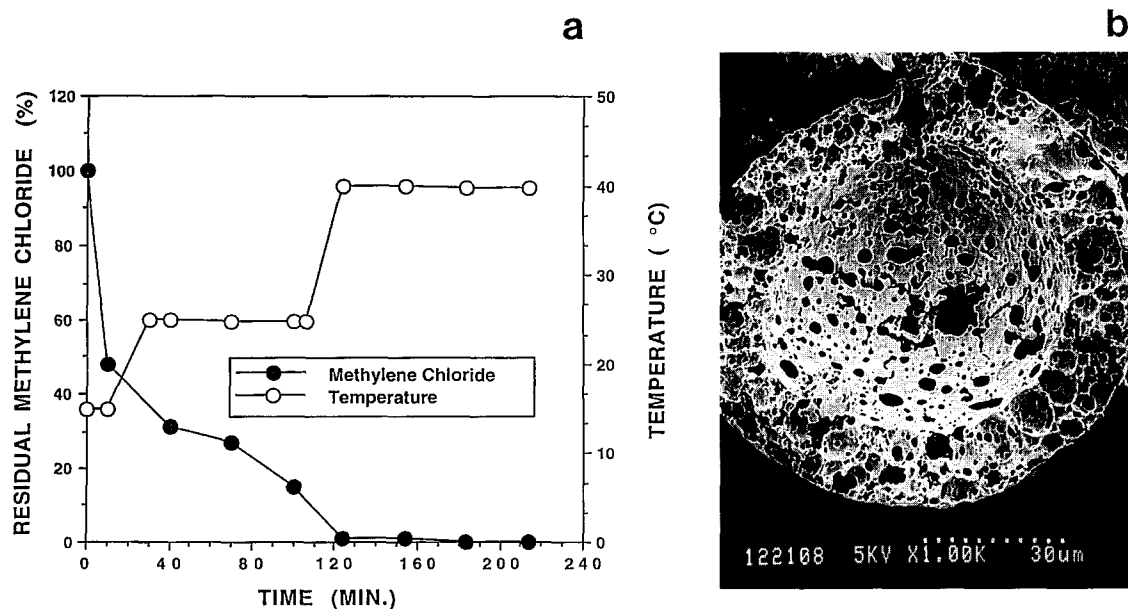


Fig. 4. CH₂Cl₂ removal profile (a), and internal pore structure (b) of batch 4 sCT microspheres prepared by the Tmp technique. Temperature raised step-wise from 15–25–40°C.

by gradual dilution of the CP to 1.5 times the initial volume. Following dilution, final traces of solvents were removed by two CP replacements. The temperature was maintained at $25 \pm 1^\circ\text{C}$ throughout the solvent removal process. It was interesting that this subtle

change in the solvent removal technique resulted in microspheres with a uniform, honeycomb-like pore structure without a hollow core (batch 5; Fig. 6b). When the CP was diluted to 2.5 times the initial volume, the removal of CH₂Cl₂ was slightly slower in the 20–

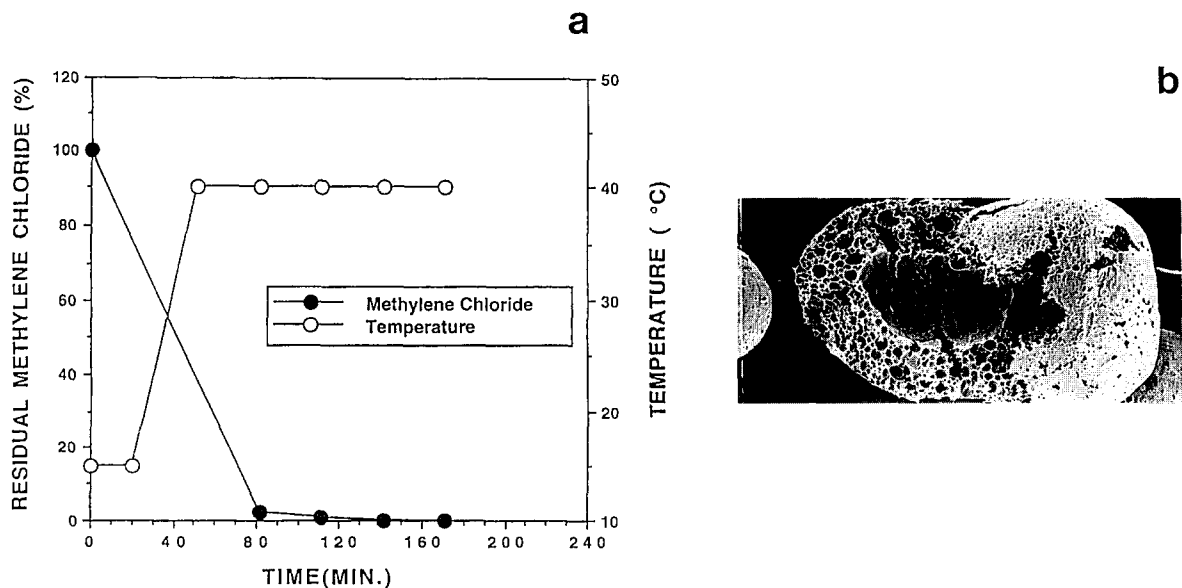


Fig. 5. CH₂Cl₂ removal profile (a), and internal pore structure (b) of batch 1 sCT microspheres prepared by the Tmp technique. Temperature raised rapidly from 15–40°C. Cosolvent concentration: 17.6 w/w% CH₃OH/CH₂Cl₂.

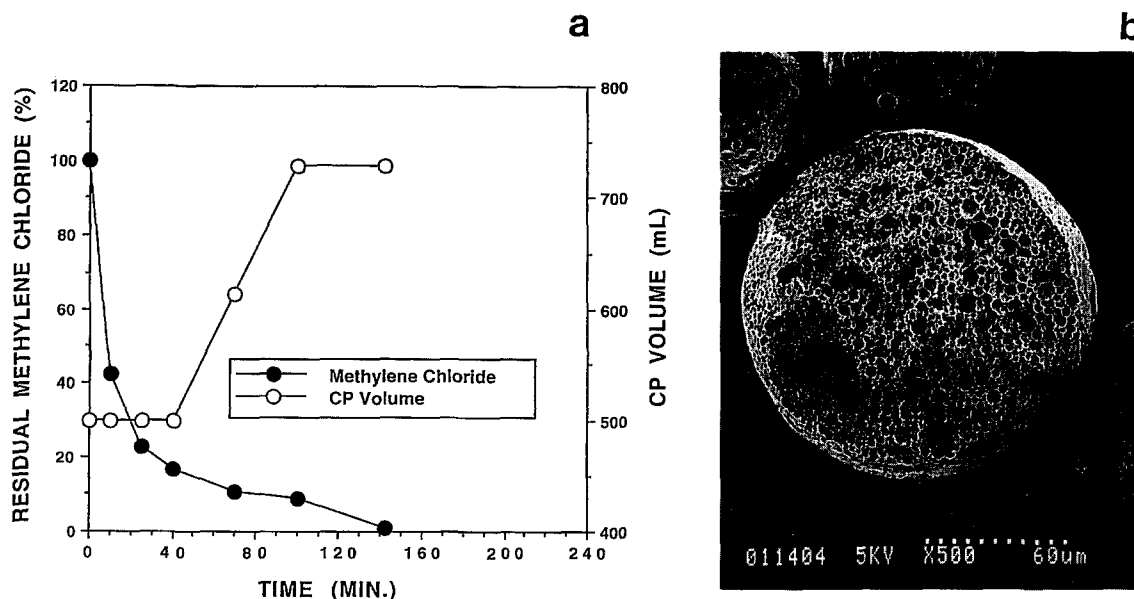


Fig. 6. CH_2Cl_2 removal profile (a), and internal pore structure (b) of batch 5 sCT microspheres prepared by the Dil technique. CP dilution: $1.5\times$.

5% residual range (batch 6; Fig. 7a). However, owing to the higher CP volume, polymer precipitation was faster. This also resulted in a honeycomb matrix without core as shown in Fig. 7b, but the internal pore size was larger than that for microspheres prepared with the lower CP Dilution.

In the Dil process, microspheres remained soft over a longer duration. CH_2Cl_2 was removed slowly and gradually during this period resulting in a uniform, honeycomb-like internal structure. However, pore size seemed to be affected by the extent of CP Dilution. At higher dilutions, pores were larger since removal of

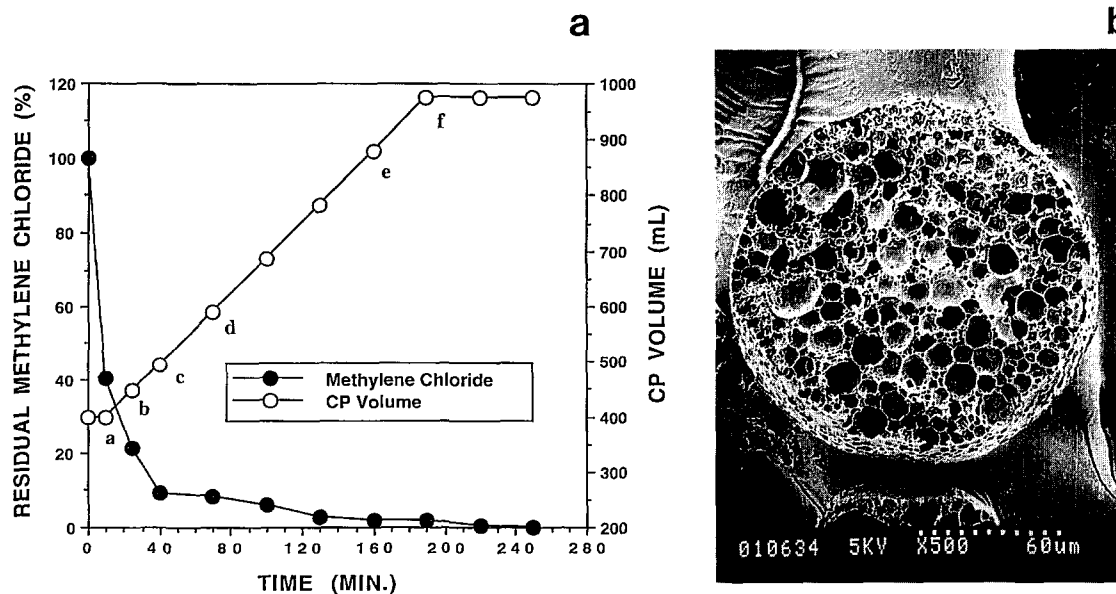


Fig. 7. CH_2Cl_2 removal profile (1), and internal pore structure (b) of batch 6 sCT microspheres prepared by the Dil technique. CP dilution: $2.5\times$. Labels (a–f) on the CP dilution curve indicate stages analyzed for changes in surface morphology in Fig. 8.

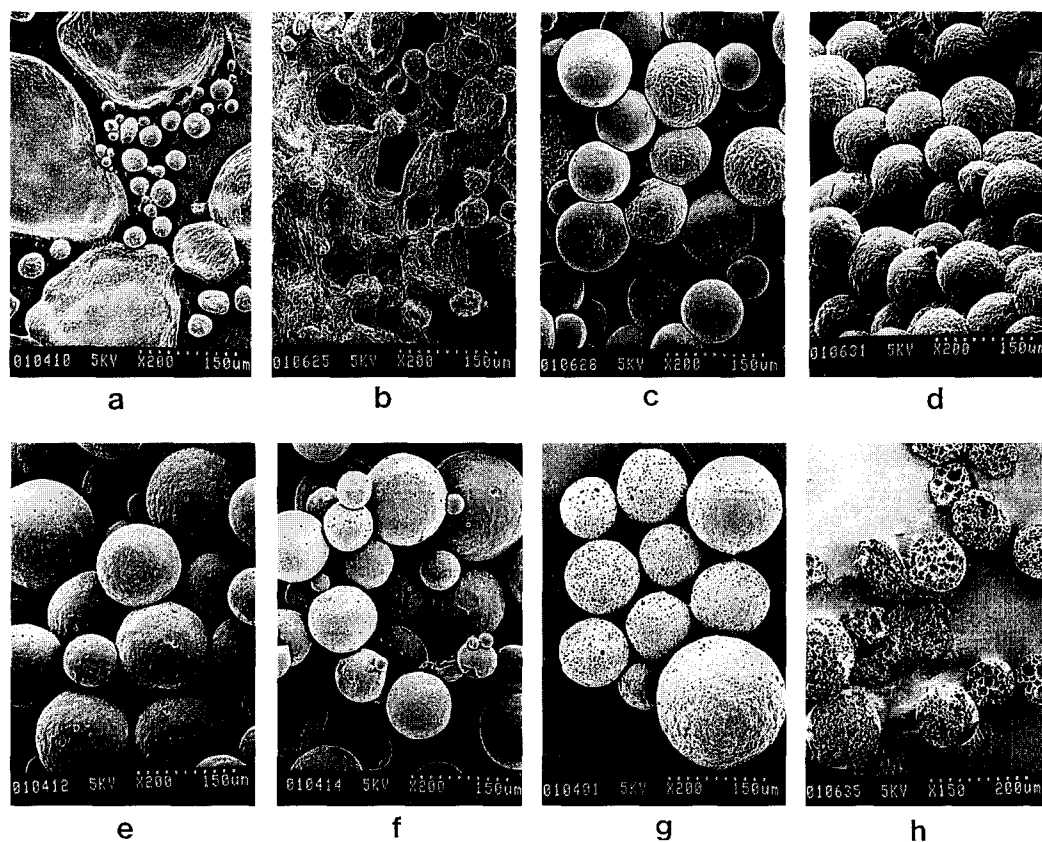


Fig. 8. Surface morphology of batch 6 sCT microspheres during various stages of the preparation process. Samples a–f were removed at the stages indicated in Fig. 7a; (g) freeze-dried microspheres; (h) fractured after freeze-drying.

CH_2Cl_2 was faster and polymer precipitation more rapid.

Evaluation of microspheres at different stages of preparation

A detailed evaluation of the microsphere preparation process was undertaken to evaluate the solvent removal and solidification stage of microspheres. Surface morphology of sCT microspheres (batch 6) prepared by the Dil technique (Fig. 7a) was evaluated during various stages of the preparation process. Fig. 8a–f shows the changes in surface morphology during microsphere preparation. Fig. 8a shows the soft and amorphous surface of microspheres after dispersion of polymer/peptide solution into the CP. Fig. 8b shows that the microspheres were still soft in the early stages of CP dilution. During the intermediate stages of dilution, solidification of microspheres occurred as shown in

Fig. 8c and d. Fig. 8e and f shows the hardening of microspheres during the final stages of CP dilution. Fig. 8g shows the porous microspheres after final rinses with CP and water and freeze-drying. Fractured microspheres (Fig. 8h) revealed a porous interior with a uniform, honeycomb matrix without any core.

In the preparation of sCT microspheres, the dispersed phase solvent consisted predominantly of CH_2Cl_2 with a small addition of CH_3OH as cosolvent for the peptide. Following dispersion, the solvents CH_2Cl_2 and CH_3OH diffuse into the continuous phase immediately. This results in a rapid precipitation of the polymer and partial solidification of the droplets entrapping a large amount of CH_2Cl_2 (~45%) and CH_3OH (~0–20%). Microspheres remained quite soft at this stage (Fig. 8a). Subsequent removal of the residual solvents from the microspheres determined the formation of the internal structure. Microspheres were too

soft to be fractured during these stages. Solidification of microspheres was initiated at a CP dilution of approximately 1.2 times the initial volume and the residual CH_2Cl_2 was about 9%. Microspheres were not sufficiently hardened until the end of dilution due to the presence of small amounts of CH_2Cl_2 . This was evident from the microspheres shown in Fig. 8d and at point d on Fig. 7 when residual CH_2Cl_2 was still 8%. At point e hardening was complete. This corresponds to a CH_2Cl_2 content of 2%. From the CH_2Cl_2 levels and the photomicrographs obtained between points d and e, it was deduced that a CH_2Cl_2 level below 6% was necessary to effect a final surface morphology. During the final rinses with CP and water, traces of CH_2Cl_2 were removed and microspheres were free-flowing after freeze-drying (Fig. 8g).

3.2. Effect of CH_3OH removal on core formation

During the solvent removal step, there is a continuous diffusion of the aqueous CP into and out of the microspheres extracting the solvents in the process. Fig. 9a shows the CH_3OH removal profile of batch 2 microspheres prepared by the Tmp technique and Fig. 9b shows the removal profile for batch 5 microspheres prepared by the Dil technique. Residual CH_3OH levels of 15–50% were maintained during droplet formation with the Tmp technique while microspheres prepared by the Dil technique had <2% CH_3OH remaining during droplet formation. CH_3OH acting as a cosolvent for the polymer will have a direct effect on polymer precipitation. It is interesting to note that a faster removal of CH_2Cl_2 in the Tmp technique was accompanied by a slower removal of CH_3OH and vice versa for the Dil technique. Although approximately the same amount of CH_2Cl_2 (45–50%) was retained during droplet formation stage in both the Tmp and Dil techniques, subsequent removal was affected by the residual CH_3OH concentration. In the Tmp technique, longer retention of CH_3OH and faster removal of CH_2Cl_2 at higher temperature may be partly responsible for the core formation. In the Dil technique, <2% CH_3OH was retained due to higher initial CP volume than that in the Tmp process. Since CH_3OH is highly water-soluble, removal of CH_3OH from the microspheres was quite fast. The kinetics and thermodynamics of microsphere preparation process and their role in the

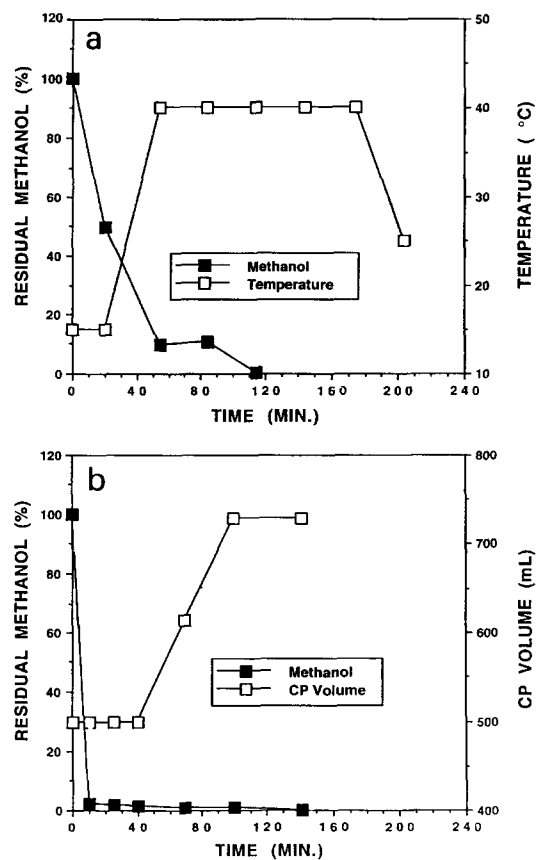


Fig. 9. CH_3OH removal profile of sCT microspheres (a) batch 2 prepared by the Tmp technique — rapid temperature rise from 15–40°C; (b) batch 5 prepared by the Dil technique. CP dilution: 1.5×.

formation of pores are discussed in detail elsewhere [8].

The role played by CH_3OH in core formation was further supported from the data on residual CH_3OH of batch 1. Residual CH_3OH during solvent removal at 40°C in microspheres prepared with 17.6% initial CH_3OH concentration (batch 1) was about 40% (Fig. 10) while it was ~10% for microspheres prepared using 7% CH_3OH (batch 2) with the same temperature gradient. The difference in the core sizes of these two batches can be attributed to the difference in CH_3OH removal. Also, in microspheres of reducing core sizes, there was a corresponding decrease in residual CH_3OH levels.

In summary, the solvent removal technique directly influenced the formation of internal structure in sCT microspheres. The temperature gradient technique resulted in a microsphere matrix with a hollow core,

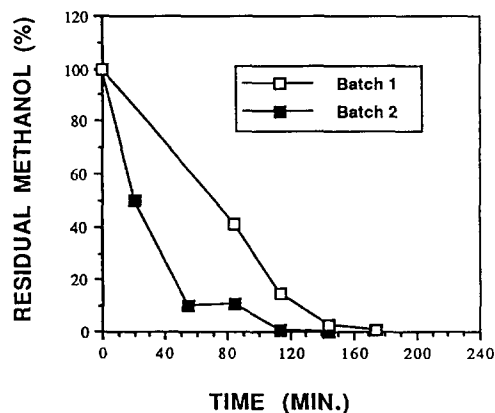


Fig. 10. CH_3OH removal profiles of sCT microspheres of different cosolvent concentrations, prepared by the Tmp technique. Temperature raised rapidly from 15–40°C. Batch 1: 17.6% and batch 2: 7% w/w% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$.

the size of which was dependent on the temperature gradient employed. The dilution technique produced microspheres with a uniform honeycomb like matrix, pore size being dependent on the extent of CP dilution. From the current study, the authors believe that the solvent removal technique is critical in the formation of matrix structure in PLGA microspheres. However, it is possible to control the matrix characteristics of sCT microspheres for controlled release applications by manipulating the solvent removal process.

References

- [1] M. Iwata and J.W. McGinity, Dissolution, stability and morphological properties of conventional and multiphase poly(DL-lactic-co-glycolic acid) microspheres containing water-soluble compounds, *Pharm. Res.* 10 (1993) 1219–1227.
- [2] T. Sato, M. Kanke, H.G. Schroeder and P.P. DeLuca, Porous biodegradable microspheres for controlled drug delivery. I. Assessment of processing conditions and solvent removal techniques, *Pharm. Res.* 5 (1988) 21–30.
- [3] M.P. Redmon, A.J. Hickey and P.P. DeLuca, Prednisolone-21-acetate poly(glycolic acid) microspheres: Influence of matrix characteristics on release, *J. Control. Release* 9 (1989) 99–109.
- [4] N. Gangrade and J.C. Price, Poly(hydroxybutyrate-hydroxyvalerate) microspheres containing progesterone: preparation, morphology and release properties, *J. Microencapsul.* 8 (1991) 185–202.
- [5] R.C. Mehta, R. Jeyanthi, S. Calis, B.C. Thanoo, K.W. Burton and P.P. DeLuca, Biodegradable microspheres as depot system for parenteral delivery of peptide drugs, *J. Control. Release* 29 (1994) 375–384.
- [6] P.P. DeLuca, R.C. Mehta, A.G. Hausberger and B.C. Thanoo, Biodegradable polyesters for drug and polypeptide delivery, in: M.A. El-Nokaly, D.M. Piatt and B.A. Charpentier (Eds), *Polymeric Delivery Systems, Properties and Applications*, ACS Symposium Series 520, Am. Chem. Soc., Washington, DC, 1993, pp. 53–79.
- [7] R. Jeyanthi, S. Calis, R.C. Mehta and P.P. DeLuca, Influence of processing parameters on the porosity of poly(glycolide-co-lactide) microspheres, *Pharm. Res.* 9s (1992) 252.
- [8] W.-I. Li, K.W. Anderson, R.C. Mehta and P.P. DeLuca, Prediction of solvent removal profile and effect on properties for peptide-loaded PLGA microspheres prepared by solvent extraction/evaporation method, *J. Control. Release* (1995) in press.