Preparation of Biodegradable Microparticles Using Solution-Enhanced Dispersion by Supercritical Fluids (SEDS)

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Purpose. We have evaluated a new process, involving solution-enhanced dispersion by supercritical fluids (SEDS), for the production of polymeric microparticles.

Methods. The biodegradable polymers, *P*oly (DL-lactide-co-glycolide) : copolymer composition 50:50 (DL-PLG), *P*oly (L-lactide) (L-PLA), *P*oly (DL-lactide) (DL-PLA) and *P*olycaprolactone (PCL), were used for preparation of microparticles using SEDS. Solutions of the polymers in organic solvents were dispersed and sprayed with supercritical CO_2. Extraction of the organic solvents resulted in the formation of solid microparticles. The amounts of highly toxic solvents such as dichloromethane (MC) were reduced in the process.

Results. Microparticles were obtained from all polymers. The mean particle size and shape varied with the polymer used. The morphology of the particles was strongly affected by the choice of polymer solvent. Discrete spherical microparticles of DL-PLG were produced with a mean volumetric diameter of 130 μm. The microparticles of the L-PLA were almost spherical, and their size increased from 0.5 to 5 μm as the density of supercritical CO_2 decreased. PCL formed microparticles with diameters of 30–210 μm and showed a strong tendency to form films at high pressure.

Conclusions. The SEDS process appears a promising method for production of microparticles from biodegradable polymers without the use of toxic solvents.

KEY WORDS: supercritical extraction; SEDS; poly (d,l-lactide-co-glycolide); poly caprolactone; biodegradable microparticles; poly (L-lactide); poly (DL-lactide).

INTRODUCTION

Supercritical fluids offer considerable promise as extraction media for the formation of microparticles of drugs and pharmaceutical excipients (1–4). Pioneering studies on the production of microparticles of biodegradable polymers using different supercritical fluid extraction methods have been reported in the literature (5–7). There are two main reasons for using this technique. Firstly, the selective solvating power of supercritical fluids makes it possible to separate a particular component from a multicomponent mixture. This is because small changes in pressure or temperature near the critical point can greatly modify the density and, hence, the solubilising power of the supercritical fluid. This particular property of supercritical fluids has been utilized for purification and separation purposes in food processing and distillation industries as well as in analytical chemistry applications for many years (5,8–10). Decaffeination of tea and coffee beans and purification of polymers from residual solvent and monomers are examples (1). Secondly, the solvents and the anti-solvent gas involved in the process can be recycled, thus minimising waste. This is interesting from both environmental and economical points of view. In addition, the supercritical extraction process is a one-step process compared to conventional extraction.

Carbon dioxide (CO_2) is one of the most commonly used supercritical fluids because of its relatively low critical temperature and pressure (Tc = 31.1°C, Pc = 73.8 bar). The low critical temperature of CO_2 makes it attractive for processing heat-sensitive flavours, pharmaceuticals and labile lipids. In addition, CO_2 is non-toxic, non-flammable, inexpensive and has a relatively high dissolving power compared to other supercritical fluids such as N_2 specially within temperature ranges between 34–40°C.

In this study, we have explored the use of a new process involving solution-enhanced dispersion by supercritical fluids (SEDS) (11–12) for the preparation of microparticles of several biodegradable polymers. These particles are intended for use in the controlled release of drugs, particularly proteins and peptides, from pharmaceutical preparations (6,13–15). The following polymers were used: Polycaprolactone (PCL), Poly (D,L-lactide-co-glycolide): copolymer composition 50:50 (DL-PLG), Poly (L-lactide) (L-PLA) and Poly (DL-lactide) (DL-PLA). The polymer solutions were initially dispersed and atomized by the supercritical CO_2 using the unique nozzle construction of the SEDS process under investigation. The organic solvents of the polymers, which are soluble in the supercritical CO_2, were extracted, and solid microparticles formed. The particles were characterised by differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and particle size measurement. In addition, attempts were made to reduce or eliminate the use of toxic organic solvents such as dichloromethane (MC) which are commonly used in conventional emulsion-based and alternative supercritical fluid extraction processes for producing microparticles (5–7,16,17). Microparticles produced by supercritical gases whit the aerosol solvent extraction system processes contain less than 30 ppm residual organic solvent (16,17), which is within the regulatory limits in the pharmaceutical industry.

MATERIALS AND METHODS

Materials

PCL (inherent viscosity (i.v.) approximately 1.13), three grades of DL-PLG (i.v. approximately 0.63, 0.78 and 1.07), L-PLA (i.v. approximately 1.00) and DL-PLA (i.v. approximately 0.60) were purchased from Birmingham polymers, Inc., USA. Poly (vinyl alcohol) (13–23 kDa, 87–89 hydrolysed) (PVA) was obtained from Aldrich, USA. Dichloromethane, Ethyl acetate, Acetone, Hexane, Isopropanol, and Methanol of analytical grade were purchased from Merck, Germany. All chemicals were used without further purification.

Microsphere Preparation

A schematic diagram of the SEDS apparatus used in this study for formation of microparticles is shown in Fig. 1. The

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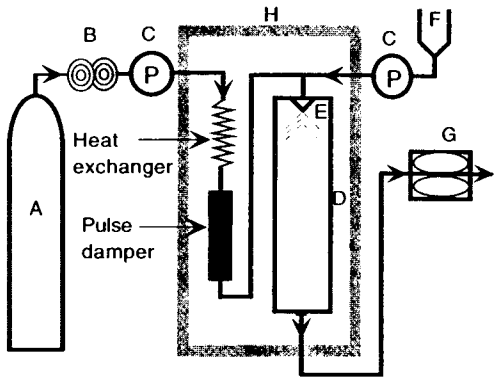


Fig. 1. Apparatus for the SEDS gas anti-solvent precipitation process. A, CO_2 cylinder; B, cooler; C, high pressure pump; D, vessel; E, nozzle; F, polymer solution; G, back pressure regulator; H, oven.

apparatus was purchased from Bradford Particle Design BPD Ltd. (Bradford, United Kingdom). Briefly, a suitable anti-solvent gas, in this case CO_2 was used, is fed from source (A) to a cooler (B), in order to ensure the liquefaction of the gas and to prevent cavitation. The CO_2 is then fed by a conduit from the cooler to a high pressure pump (C). From there it is pumped to the high pressure vessel (D) via a nozzle (E). A saturated solution of polymer, in a suitable organic solvent, is drawn from source (F) by a conduit to the high pressure pump (C) and is fed to the high pressure vessel (D) via nozzle (E). The supercritical CO_2 leaves the high pressure vessel and flows to the back pressure regulator (G) which controls the pressure discharge in the system. The organic solvent is extracted into the supercritical fluid, resulting in the formation of solid microparticles in the vessel (D) (18). The microparticles are collected from the vessel and stored in a desiccator at room temperature, pending analysis.

During particle formation, the pressure and temperature were fixed at 130, 185 bar respectively at 35, 40 °C for all polymers except for DL-PLG. Because of the low glass transition temperature (Tg) of DL-PLG, the temperature range for processing these particles was 33 to 38°C. The relevant physical properties of the polymers used in the study are given in Table I. The CO_2 flow rate was kept at 21 ml/min and the polymer solutions had a flow rate of 0.13 ml/min during all experiments. The concentration of all the polymers was 2.3% (w/v). The process parameters for microsphere production from the different polymers are summarised in Table II. At the end of each experiment, the microparticles were flushed with CO_2 at 18 ml/min for 20 minutes to remove any possible residual solvent.

Table I. Inherent Viscosity, Glass Transition (Tg), and Melting (Tm) Temperatures of the Polymers

Polymer	Inherent viscosity (dL/g)[a]	Tg (°C)[a]	Tm (°C)[a]
PCL	1.13	−65–−60	58–63
DL-PLA	0.60	55–60	
L-PLA	1.00	60–65	173–178
DL-PLG (50:50)	0.63, 0.78, 1.07	45–50	

[a] According to the manufacturer.

Table II. The Solvent Composition and the Pressures and Temperatures Employed During Microparticle Formation

Polymer	Solvent composition[a]	T (°C)	Pressure (bar)
PCL	MC, A, I (1.1:6.2:2.7)	35, 40	130
	MC, MeOH (9:1)	35, 40	185
DL-PLA	A, E, H (1:7.8:1.2)	35, 40	130, 185
L-PLA	MC, A, I (3.3:6.5:0.2)	35, 40	130, 185
DL-PLG (i.v. 1.07)	MC, MeOH (9:1)	34	185
DL-PLG (i.v. 0.78)	A, E, I (4:5.6:0.4)	33, 38	130, 185
DL-PLG (i.v. 0.63)	A, E, I (4:5.6:0.4)	33, 38	130, 185
	MC, MeOH (9:1)	33	185

[a] Abbreviations: see Table III.

Particle Size Measurement

The particles were suspended in 2 ml of 5% (w/v) PVA in water and sonicated for 20 seconds prior to particle size measurement. Size volume distributions were obtained using a laser diffractometer (LS 230, Coulter, USA). The results are presented as mean values of triplicate samples ± one standard deviation. Two-way ANOVA analysis with replication was employed to investigate the dependency of the microparticle size distribution on the processing parameters. ANOVA is a powerful statistical technique which can be used to separate and estimate the different causes of variation. We used two-way ANOVA to test for a significant effect on particle size due to a controlled factor (pressure and temperature in our case), It can also be used to estimate the variance of an uncontrolled factor. The test was based on at least two batches under each of the four different process conditions.

Morphology of Microspheres

Surfaces and cross-sections of representative microparticles were coated with gold—palladium (metallization) (JFC 100, Ion sputter, Jeol, Japan) in an argon atmosphere at room temperature. The external and internal morphology of the particles was then examined using scanning electron microscopy (JSM, T 330, Scanning microscope, Jeol, Japan).

Differential Scanning Calorimetry (DSC)

3–6 mg samples were placed in sealed aluminium sample pans and scanned at a rate of 5°C/min in a differential scanning calorimeter (TC 3000 Mettler, Hightstown, NJ, USA) to determine the glass transition-, crystallisation- and melting temperatures of the polymers, before and after the SEDS process.

RESULTS AND DISCUSSION

In this study we investigated the applicability of the SEDS process for production of microparticles of biodegradable polymers. The optimal flow rates of CO_2 (21 ml/min) and the polymer solutions (0.13 ml/min), and the optimal concentration of the polymers in solution (2.3% w/v) were determined in preliminary studies and then kept constant for all polymers throughout this study. All polymer solutions were saturated by adding small amounts of organic non-solvent (isopropanol or methanol). The use of saturated polymer solutions enhanced

Table III. Environmental Acceptability of Solvents Used in These Experiments. The Ranking is Based on The LD 50 Data and Side Effects Related to the Solvents

Solvent	Abbreviation	Environmental acceptability
Acetone	A	+ + +
Ethyl acetate	E	+ + +
Hexane	I	+ + +
Isopropanol	H	+ +
Methanol	MeOH	+
Dichloromethane	MC	−

[a] + + +, highly acceptable; + +, generally acceptable; + = minimally acceptable; −, not acceptable.

particle formation for all polymers after spraying into the supercritical CO_2. This is probably due to a more rapid solidification rate than when using unsaturated polymer solutions. The solvent compositions for the different polymers were chosen with regard to the solubility of the polymer and environmental aspects related to the toxicity of the solvents (see Table III). The 10, 50 and 90% quintiles of the size distributions of the microparticles are listed in Table IV.

DL-PLG

In general, the organic solvents used for dissolving DL-PLG are acceptable from an environmental point of view. The DL-PLGs with i.v.s of 0.78 and 0.63 were dissolved in a mixture of ethyl acetate, acetone and isopropanol. However, since the DL-PLG with an i.v. of 1.07 was not soluble in these solvents, it was dissolved in dichloromethane and methanol (9:1).

In contrast to earlier studies (5,6), we succeeded in producing discrete microparticles from DL-PLG with supercritical fluid technology by using the SEDS process under investigation. The particle formation properties of the three DL-PLGs investigated (i.v. 1.07, 0.78 and 0.63) varied with the inherent viscosity of the polymer. The SEDS product from the DL-PLG polymer with the highest i.v. (1.07) was forming a bead of a network-like powder in which particles were connected through solid bridges. The particles were compact and nearly spherical in shape (Fig. 2a).

The microparticles obtained using DL-PLG with i.v. 0.78 were discrete and spherical in shape (Fig. 2b). The particle size distribution with this DL-PLG sample was not affected by varying experimental parameters such as pressure and temperature (Table IV).

The size range of microparticles of the DL-PLG with the lowest i.v. (0.63) was almost the same as that of the DL-PLG with i.v. 0.78 but the particles were porous and irregular in shape (see Fig. 3a). Two-way ANOVA testing indicated no significant differences between the median diameters of microspheres of DL-PLGs with i.v. 0.78 and 0.63 prepared under varying conditions (see Table IV).

To study the effect of the solvent on particle formation, DL-PLG with an i.v. of 0.63 was also dissolved in dichloromethane : methanol (9:1) at 185 bar and 33°C. The particles obtained from the dichloromethane : methanol solution (Fig. 3b) were smaller in size and had a more spherical shape then those prepared from acetone : ethyl acetate : isopropanol (Fig. 3a).

One of the difficulties of using amorphous polymers is the expanded range of interaction between supercritical CO_2

Table IV. The Mean Volumetric Particle Size Distributions of Microparticles Prepared from Biodegradable Polymers Under Varying Processing Conditions

Pressure (bar) Polymer, T (°C)	Diameter (μm) of microparticles					
	130			185		
	10%	50%	90%	10%	50%	90%
PCL, 35°C	13.7	106	121.2	141.6	189	1045
PCL, 40°C	4.9	27	49.7	145.5	212	585
DL-PLA, 35°C	37	135	234	75	216	444
DL-PLA, 40°C	45	178	265	32	105	199
DL-PLG (0.78)[a], 33°C	24	140	220	21	130	221
DL-PLG (0.78)[a], 38°C	32	126	199	21	136	202
DL-PLG (0.63)[a], 33°C	—	—	—	95	151	182
DL-PLG (0.63)[a], 38°C	110	173	240	38	158	197

[a] Inherent viscosity.

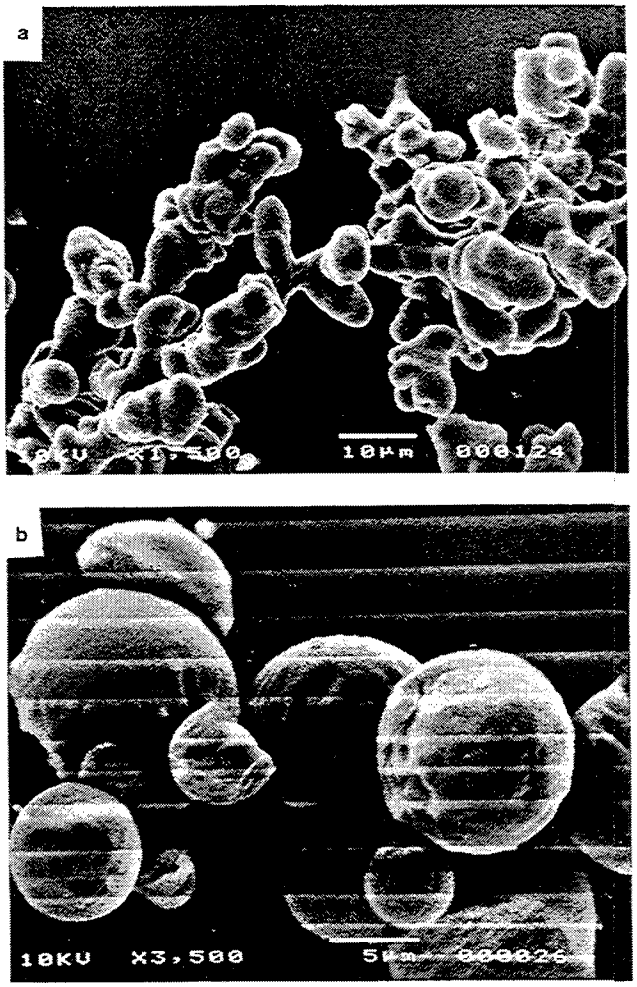


Fig. 2. Representative SEM pictures of: (a) DL-PLG (i.v. 1.07) prepared at 185 bar and 34 °C, (b) DL-PLG (i.v. 0.78) prepared at 185 bar and 38°C.

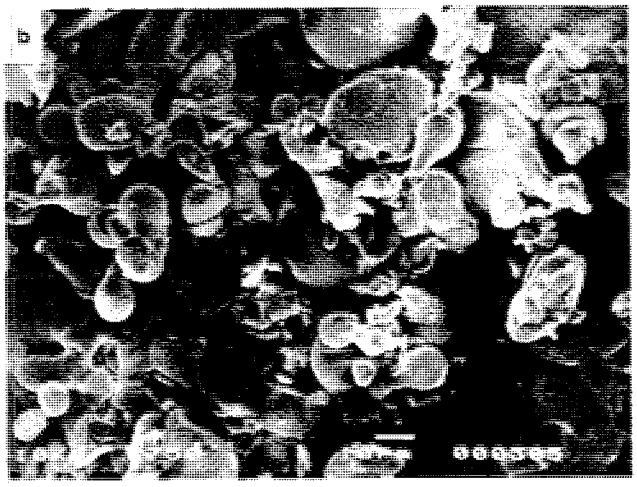
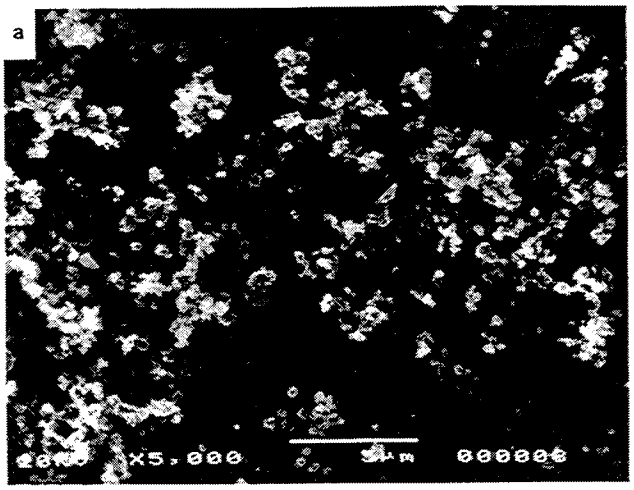


Fig. 3. SEM pictures of DL-PLG (i.v. 0.63) prepared under the same preparation conditions (185 bar and 33°C) with two different solvent compositions: (a) acetone: ethyl acetate: isopropanol (4:5.6:0.4), (b) dichloromethane: methanol (9:1).



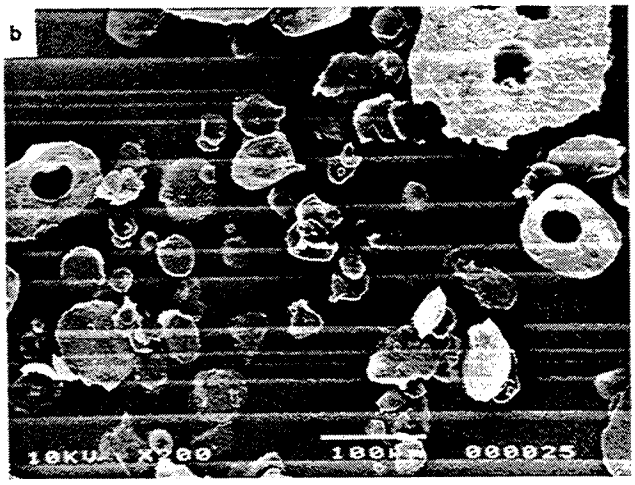


Fig. 4 SEM pictures of: (a) L-PLA microparticles prepared at 180 bar and 35°C, (b) DL-PLA particles prepared at 130 bar and 40°C.

and the polymer. At high pressure, and also supercritical CO_2, is relatively soluble in amorphous materials. As a result, a partial extraction of the polymer in the lower molecular range is obtained (5). A strong tendency to form a film at the base or on the wall of the vessel was also observed for the amorphous polymers. Actually, for the polymers forming discrete particles, the production yield is mainly reduced due to the adsorption of material to vessel surfaces. In the preparations described in this paper typical yields were 50–60%, however, processing higher amounts of material will increase this yield.

L-PLA

L-PLA is highly soluble in toxic organic solvents such as MC and chloroform, and in more expensive organic solvents such as hexafluoroisopropanol. The L-PLA microparticles produced by other research groups using supercritical fluid extraction methods produced from solutions in MC (3,6,14). In this study we have endeavored to substitute and reduce the amounts of toxic organic solvents. We used 30% MC to dissolve L-PLA: much less than in previous studies (3,5,6,13,15). Most of the MC was replaced by acetone. A small volume of isopropanol was also added to the solution in order to obtain a saturated solution of L-PLA.

The SEDS process using L-PLA resulted in the formation of microparticles under all the investigated experimental conditions (Fig. 4a). The size distribution of the L-PLA microparticles appeared to be more sensitive to changes in the density of the supercritical CO_2 than to changes in temperature or pressure (see Table V). Increasing the temperature at constant pressure

Table V. Mean Volumetric Particle Size Distribution of L-PLA Microparticles Prepared with Different Densities of CO_2

Pressure (bar)/ temperature (°C)	Diameter[a] (μm) of microparticles			CO_2 density (g/ml)
	10%	50%	90%	
130/35[b]	0.13(0.0)	0.72(0.1)	22(0.3)	0.77
130/40	0.3(0.0)	5.3(0.4)	50(35)	0.69
185/35	0.14(0.0)	0.5(0.1)	13(6.4)	0.85
185/40	0.14(0.0)	0.6(0.0)	41(2.2)	0.82

[a] Standard deviation given in brackets.
[b] Data based on three batches.

decreased the density of the supercritical CO_2 (5,15) and increased the particle size. Similarly, when the density of CO_2 was increased by increasing the pressure from 130 to 185 bar at constant temperature, the particle size decreased.

Two-way ANOVA testing with replication of the size distribution of L-PLA microparticles processed by SEDS indicated a highly significant difference in size distribution between particles produced under different processing conditions. The mean particle size decreased from 5.3 to 0.5 μm when the density of CO_2 was increased from 0.69 to 0.85 (see Table V). Decreasing the density of the supercritical CO_2 decreased its solubilizing power which subsequently decreased the extraction rate and increased the solidification time during particle formation. Particles appear to increase in size when low density supercritical CO_2 is employed. The data presented in Table V are based on at least two batches under each of the different processing conditions. In order to investigate the reproducibility of the SEDS process, a third batch was studied under one randomly selected set of processing conditions (130 bar and 35°C). These data demonstrate the high reproducibility of the SEDS process (see Table V). The small size of the semicrystalline L-PLA as compared with the amorphous polymers in this study shows that microparticles from crystalline polymers precipitate faster than those from amorphous polymers in the SEDS process. The observed production yield of L-PLA was significantly higher than those of the amorphous polymers.

DL-PLA

Using SEDS process the amorphous biodegradable polymer, DL-PLA was sprayed in a mixture of acetone and ethyl acetate into supercritical CO_2 to obtain microparticles. A small volume of hexane was added in order to obtain a saturated solution. The organic solvents used are acceptable from an environmental point of view. After depressurising, a mixture of discrete and agglomerated particles of DL-PLA was retained on the walls and at the base of the vessel. The particles were porous and varied widely in size and shape under all the investigated experimental conditions (see Table IV and Fig. 4b). This may indicate that the CO_2 was acting as a plasticiser. No clear relationship between the particle size distribution and variations in the experimental parameters was observed for DL-PLA.

PCL

PCL was dissolved in a mixture of acetone and MC, and a small volume of isopropanol was added to saturate the solution. The PCL particles obtained were large and very irregular in shape (see Table IV). There was also a tendency for PCL to form a film at the base of the vessel. The difficulties in producing discrete particles with a regular shape from PCL may be explained by its very low Tg (−65 to −60°C). It has previously been shown in earlier studies that semicrystalline PCL swells in CO_2 at higher temperatures and pressures (6). This property may have contributed to the difficulties we encountered in particle formation. No particles were obtained when the process was carried out at high pressure (185 bar). In order to produce particles under these conditions (185 bar; 35 and 40°C), higher proportions of MC were required (90%). MC is less polar than acetone and is therefore more miscible with supercritical CO_2, hence, the solidification rate of the

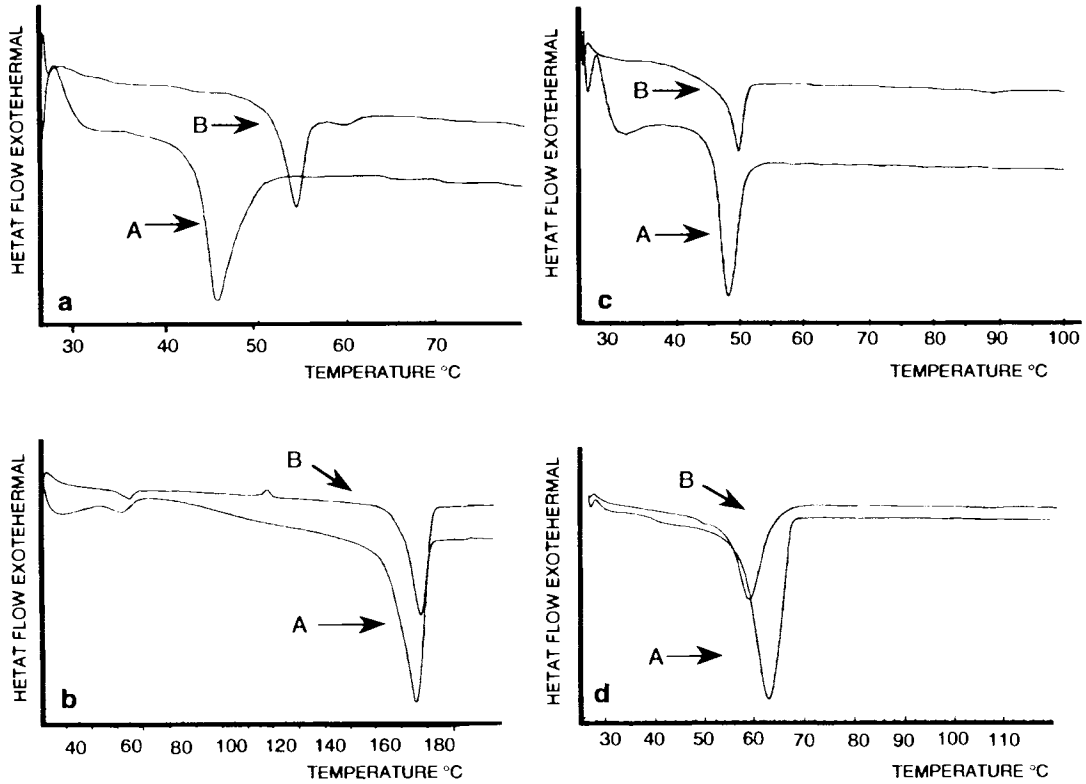


Fig. 5. The DSC thermograms for: (a) DL-PLA, (b) L-PLA, (c) DL-PLG, (d) PCL. Curve A indicates the DSC scan of the untreated (original) polymer and curve B indicates the DSC scan of the microparticles produced by the SEDS process. The microparticles (a, b, d) were produced at 185 bar and 40°C. The DL-PLG microparticles (c) were produced at 185 bar and 33°C.

polymer was increased and consequently the formation of the polymer film was inhibited.

Differential Scanning Calorimetry (DSC)

The DSC thermograms for the polymers before and after the SEDS process indicated a higher degree of homogeneity and purity for the processed polymers (i.e. they had narrower Tg and Tm peaks) than for the unprocessed polymers (Fig. 5). This was expected from the extractive properties of the supercritical CO_2. Varying the process parameters did not affect the thermal properties of the microparticles. In contrast to earlier findings(5,7,14), the SEDS process did not cause fractionation of the polymers. However, the DSC thermogram of DL-PLA showed an increase in Tg from 46°C for the unprocessed polymer to 54°C for the microparticles (Fig. 5a). The phase transitions in Fig. 5 are listed in Table I.

CONCLUSIONS

SEDS can be used for production of microparticles of biodegradable polymers. The ability to precipitate these polymers is the first step towards reaching the goal of incorporating drugs into such microparticles for controlled drug delivery. Great care must be exercised in the selection of suitable solvents and operating conditions in order to optimise the formation of well defined microparticles. Changing the process parameters (temperature and pressure) did not result in any clear differences in the size distribution of microparticles prepared from PCL, DL-PLA and DL-PLG, although further modification of the SEDS process is required in order to optimise the properties of the microparticles. In contrast to the results from these polymers, the size of microparticles prepared from the semicrystalline L-PLA was dependent on the density of the supercritical CO_2. Thus, although microparticle formation from amorphous polymers by supercritical extraction processing is more difficult than that from crystalline polymers such as L-PLA, we were able to produce discrete particles of a relatively uniform size from at least one of the DL-PLG polymers. The results of this study also show that the use of toxic solvents can be greatly reduced when the SEDS process is used for the preparation of microparticles from commonly used polymers.

REFERENCES

1. M. A. McHugh and V. J. Krukonis (eds.), Supercritical Fluid Extraction, Butterworth Heinemann, Boston, 1994, pp. 1–26.
2. L. Fredriksen, K. Anton, P. V. Hoogevest, H. R. Keller, and H. Leuenberger. Preparation of liposomes encapsulating water-soluble compounds using supercritical carbon dioxide. *J. Pharm. Sci.* **86**:921–928 (1997).
3. B. Subramaniam, R. A. Rajewski, and K. Snavely. Pharmaceutical processing with supercritical carbon dioxide. *J. Pharm. Sci.* **86**:885–890 (1997).
4. C. A. Eckert, B. L. Knutson, and P. G. Denbenedetti. Supercritical fluids as solvents for chemical and materials processing. *Nature.* **383**:313–318 (1996).
5. J. Bleich, B. W. Müller, and W. Waßmus. Aerosol solvent extraction system - a new microparticle production techniqe. *Int. J. Pharm.* **97**:111–117 (1993).
6. R. Bodmeier, H. Wang, D. J. Dixon, S. Mawson, and K. P. Johnston. Polymeric microspheres prepared by spraying into compressed carbon dioxide. *Pharm. Res.* **12**:1211–1217 (1995).
7. J. W. Tom, G. B. Lim, P. G. Denbenedetti, and R. K. Prudhomme. Applications of supercritical Fluids in the Controlled Released of Drugs. In J. F. Brennecke and E. Kiran (eds.) Supercritical Fluid Engineering Science, ACS Symp. Ser. 514, American Chemical Society: Washington, DC, pp. 238–257 (1993).
8. S. B. Hawthorne. Analytical scale supercritical fluid extraction. *Anal. Chem.* **62**:633–642 (1990).
9. G. Braun, H. Schmitz, K. Kühlein, and R. Steiner, German patent Appl. No. DE 3323940 A1, 1985.
10. P. Hubert and O. G. Vitzhum. Fluid extraction of hops, spices and tobacco with supercritical gases. In G. M. Schneider, E. Stahl, and G. Wilke (eds.), Extraction with Supercritical Gases, Verlag Chemie, Weinheim, 1980, pp. 25–443.
11. M. H. Hanna, P. York, D. Rudd, and S. Beach. A novel apparatus for controlled particle formation using supercritical fluids. *Pharm. Res.* **12**:S141 (1995).
12. B. Y. Shekunov, S. Palakodaty, P. York, M. Hanna, and G. O. Humphreys. Control of particle morphology using Solution Enhanced Dispersion by Supercritical fluids (SEDS). *Pharm. Res.* **14**:S196 (1997).
13. J. Bleich and B. W. Müller. Production of drug loaded microparticles by the use of supercritical gases with the aerosol solvent extraction system (ASES) process. *J. Microencapsulation* **13**:131–139 (1996).
14. P. G. Denbenedetti, J. W. Tom, S. D. Yeo, and G. B. Lim. Application of supercritical fluids for the production of sustained delivery devices. *J. Contr. Rel.* **24**:27–44 (1993).
15. J. Bleich, P. Kleinebudde, and B. W. Müller. Influence of gas density and pressure on microparticles produced with the ASES process. *Int. J. Pharm.* **106**:77–84 (1994).
16. J. Bleich and B. W. Müller. Production of drug loaded microparticles by the use of supercritical gases with the aerosol solvent extraction system (ASES) process. *J. Microencapsulation* **13**:131–139 (1996).
17. F. Ruchatz, P. Kleinebudde, and B. Müller. Residual solvents in biodegradable microparticles. Influence of process parameters on the residual solvent in microparticles produced by the aerosol solvent extraction system (ASES) process. *J. Pharm. Sci.* **86**:101–105 (1997).
18. M. H. Hanna and P. York, UK patent Appl. PCT/GB94/01426, 1994.