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Poly(L-lactic acid) microspheres for pulmonary drug delivery: release kinetics and aerosolization studies

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Abstract

We have previously developed poly(L-lactic acid) (PLA) microspheres containing nedocromil sodium and beclomethasone dipropionate (BDP) for aerosolisation to the respiratory tract (El-Baseir, M.M., Phipps, M.A., Kellaway, I.W., Preparation and subsequent degradation of poly(L-lactic acid) microspheres suitable for aerosolisation: a physico-chemical study. Int. J. Pharm. 151 (1997) 145–153). In this study we have investigated the in vitro release kinetics of these two drugs from PLA microspheres and the deposition of the microspheres in an in vitro lung model (Andersen cascade impactor) following aerosolisation from a dry powder inhaler (Spinhaler®). The in vitro kinetics of drug release revealed a controlled release of nedocromil sodium over 8 days with a burst effect (27–60%, w/w) which varied with the particle size of the microspheres. For BDP entrapped in PLA microspheres, controlled release of BDP occurred over 6 days. BDP release was determined by measuring the shift in the phase transition temperature of dimyristoylphosphatidylcholine (DMPC) liposomes induced by partitioning of the steroid into the lipid bilayers. The residual poly(vinyl alcohol) used as an emulsifier in the production of the microspheres was < 7% (w/w). The in vitro deposition of the microspheres (1.00 ± 0.21 μm) containing BDP from a Spinhaler® to a cascade impactor at a flow rate of 60 l/min, resulted in 20% of the emitted dose deposited on stages corresponding to particles < 3 μm and approximately 42% < 5 μm.

Keywords: Poly(L-lactic acid); Microspheres; Nedocromil sodium; Beclomethasone dipropionate; Acrosolization; Respiratory tract

1. Introduction

Biodegradable microsphere drug delivery systems have shown application for oral and parenteral administration (e.g. Okada and Toguchi, 1995). The controlled release of drug has been the

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objective of such studies. As drug delivery systems, biodegradable microspheres have offered several advantages over conventionally administered medicaments. First, patient compliance with the dose regimen is assured for parenteral depot therapies and the blood levels are controlled by the implant. Second, the first pass effect inherent in the peroral route is eliminated. In addition, the new biosynthetic proteins and peptides can be administered and operations to remove the implanted dosage form after drug release would be avoided. The use of such a system for administration of a contraceptive steroid has been reported by Gresser et al. (1978) and Beck et al. (1979). Biodegradable microspheres were a successful system for controlling the release of adriamycin (Ike et al., 1991).

Biodegradability and biocompatibility of polyesters such as poly(lactic acid), poly(glycolic acid) and their copolymers was established from work undertaken on surgical grafts and implants (Chu, 1982; Fredericks and Melveger, 1984; Hay et al., 1988). The degradation products are mainly carbon dioxide and water excreted via the kidney (Wood, 1980; Yolles and Sarton, 1980).

Biodegradable polymer microspheres are currently of interest as controlled-release pulmonary drug carriers. Pulmonary deposition of aerosolized microspheres may provide the opportunity for the prolonged delivery of a systematically active agent with the drug protected from enzymatic hydrolysis. Microspheres can be produced to meet certain morphological requirements such as size, shape and porosity by varying the process parameters. Microspheres are less susceptible to the effect of hygroscopic growth within the airways. However, the morphology of the lung is such that to achieve effective drug deposition it is necessary to control the particle size to approximately 2–3 μm.

The objectives of this paper were to study the release kinetics of nedocromil sodium and beclomethasone dipropionate (BDP) from PLA microspheres and to study the deposition of these microspheres generated from a Spinhaler® using an Andersen cascade impactor (eight stages) operating at flow rates of 28.3 and 60 l/min.

2. Experimental

2.1. Materials

Poly(L-lactic acid), molecular weight 2000 Da, was obtained from Polyscience (Warrington PA). Poly(vinyl alcohol) (PVA), 87–89% hydrolysed 85–140 kDa, was supplied by Aldrich (Gillingham UK). Nedocromil sodium was a gift from Fisons (Loughborough, UK). Beclomethasone dipropionate was supplied by Sigma (Poole, UK), dimyristoylphosphatidylcholine (DMPC) from Nippon Oil and Fats (Amagasaki, Japan), and dichloromethane, methanol, acetonitrile and chloroform all of HPLC grade, were supplied by Fisons (Loughborough, UK).

2.2. In vitro release study of nedocromil sodium

Twenty milligrams of freeze dried microspheres prepared using the w/o/w double emulsion solvent evaporation method (El-Baseir et al., 1997), were suspended in 20 ml 0.2 M phosphate buffer, pH 7.4, and incubated in a water bath maintained at 37 ± 0.5°C with 30 rpm agitation. Samples were removed at various times and centrifuged at 3000 rpm for 5 min. The supernatants were subjected to fluorometric measurement at 376 nm (excitation) and 590 nm (emission) before the samples were returned to the original flasks. The cumulative percentage drug release was calculated from a calibration curve.

2.2.1. In vitro release study of BDP using 50% (v/v) isopropanol in water as dissolution media

In a series of test tubes, 5-mg samples of PLA microspheres containing entrapped BDP were dispersed in 10 ml of 50% (v/v) isopropanol. The test tubes were incubated in a water bath maintained at 37 ± 0.5°C. After an interval of 0, 2, 24, 50 and 120 h, one test tube was taken, centrifuged at 3000 rpm for 5 min and 20 μl of the supernatant injected onto an HPLC column as previously described (El-Baseir et al., 1997), but with 50% (v/v) isopropanol as solvent. The BDP release was calculated with reference to a calibration curve.

Table 1
The size of (a) microspheres containing nedocromil sodium used in aerosolization studies as determined by laser diffraction and (b) microspheres containing BDP used in aerosolization studies as determined by photon correlation spectroscopy

Batch no.	10% ± S.D. (μm)	50% ± S.D. (μm)	90% ± S.D. (μm)
(a)			
1	2.88 (0.02)	6.14 (0.06)	10.02 (0.05)
2	2.13 (0.01)	3.39 (0.01)	6.18 (0.13)
3	1.67 (0.03)	2.65 (0.01)	5.08 (0.07)
(b)	50% ± S.D. (μm)	95% limits ± S.D. (μm)	
4	1.00 (0.21)	0.89 (0.14) to 1.12 (0.27)	

2.2.2. In vitro release study of BDP using a calorimetric procedure

Multilamellar DMPC liposomes were prepared at a temperature above that of the gel–liquid crystalline phase transition (T_m) of the lipid (Castelli et al., 1994). Hydrated liposome samples were divided into three aliquots and the transition temperature (T_{m_0}) of each determined by DSC (Perkin-Elmer DSC7). A known amount of PLA microspheres with a predetermined BDP content was added to each sample and incubated at 37°C. After intervals of 0, 2, 4 and 6 days samples were taken, sealed in DSC pans and subjected to DSC analysis at a scanning rate of 5°C/min to determine the T_m. The shift in the DMPC transition peak (ΔT_m) was calculated by subtracting the original value (T_{m_0}). The fraction of BDP released for all samples was calculated with reference to the calibration curve equation.

2.3. Residual polyvinyl alcohol (PVA)

A 5-mg microsphere sample was dispersed in 25 ml chloroform and sonicated for 5 min. The formed suspension was filtered through 0.05-μm pore size cellulose filter. The residual PVA retained on the filter was washed with 25 ml of chloroform. The filter paper was dried in air and immersed in 25 ml of water in a flask. The flask was heated to about 90°C to dissolve the PVA. Ten milliliters of this solution were taken and made up to 25 ml with deionized water, 5 ml of 4% (w/v) boric acid and 3 ml of iodine solution (1.27% (w/v) iodine and 2.5% (w/v) potassium iodide in water) were added to the flask followed by final dilution to 50 ml with deionized water. The assay of this sample was carried out at 690 nm. In order to maximise the absorbance of PVA (Finley, 1961) all measurements were carried out at 25°C by placing the flasks in a water bath maintained at 25°C during the time of sampling. The residual PVA was calculated with reference to a calibration curve.

2.4. In vitro deposition studies

The microsphere formulation was prepared by the solvent evaporation method previously described (El-Baseir et al., 1997). The median size of the microspheres studied was obtained using laser diffraction (Malvern 2600) and photon correlation spectroscopy (N4MD Coulter sizer) and ranged from 1 to 6.14 μm (Table 1). Hard gelatine capsules were filled with 20 mg of microspheres and introduced into a Spinhaler®. The deposition profiles were evaluated using an Andersen cascade impactor (eight-stage). One capsule was discharged into the apparatus per determination ($n = 2–6$) and a standardised flow rate of 28.3 or 60 l/min was used throughout. Microsphere materials deposited in the throat and on cascade impactor stages after each actuation were collected and dispersed into 2 ml dichloromethane prior to extraction of the drug either with phosphate buffer or methanol. The assay of the drug was carried out fluorimetrically for nedocromil sodium and by HPLC for BDP. The data were analysed and presented as percentage of the total sample recovered.

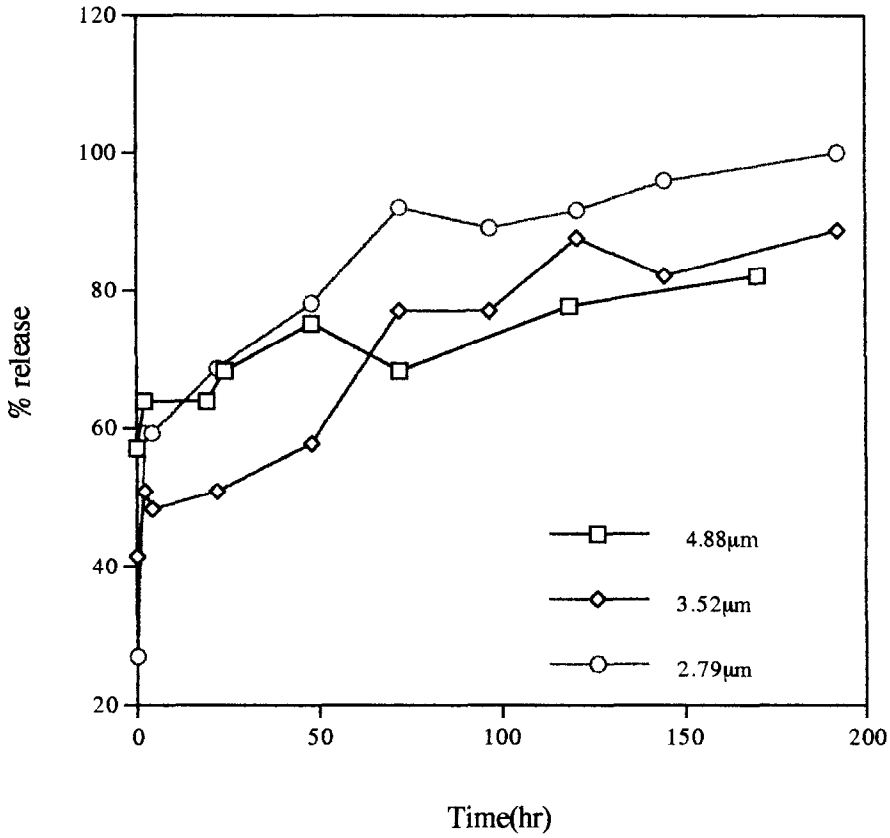

Fig. 1. The effect of microsphere size on the release profile of nedocromil sodium ($n = 2$). Entrapment efficiencies for 4.88 ± 0.07-, 3.52 ± 0.02- and 2.79 ± 0.02-μm microspheres were 11.22 ± 0.72, 9.92 ± 1.42 and 5.42 ± 0.19% (w/w) and data range maxima were 4.59, 4.67 and 9.24%, respectively.

3. Results and discussion

For in vitro investigations of the rates of release of drug from a carrier, the goal is to mimic the expected in vivo conditions as close as possible. The pH should therefore be adjusted to 7–7.4 to allow physiological comparability. High pH can enhance the drug release rate (Jalil and Nixon, 1990) and the rate of hydrolytic degradation of the polymer is also affected by changes in pH (Makino et al., 1985). In vitro release kinetics of nedocromil sodium exhibited a biphasic pattern, characterised by an initial variable and rapid release phase followed by a period of continuous slow release. The initial phase was due to release of drug adsorbed or located near the surface of the microspheres and was found to be affected by the size of the microspheres. For 2.79-μm microspheres, 27% of the drug was immediately released (Fig. 1) and an even greater release of drug (42–60%) was observed within the first 5 min for microspheres of larger size (3.52 and 4.88 μm diameter). The initial rapid release of a bioactive agent from a polymeric system such as microspheres has always been a problem with controlled release delivery systems (Kwong et al., 1986). For all the tested batches, 80–100% of the drug was released over the 8-day test period.

The cumulative amount of drug released increased with decreasing mean particle diameter as a result of increased surface area in contact with the dissolution media. Therefore, it may be possible to control the rate of drug release within the airways by using microsphere preparations of a

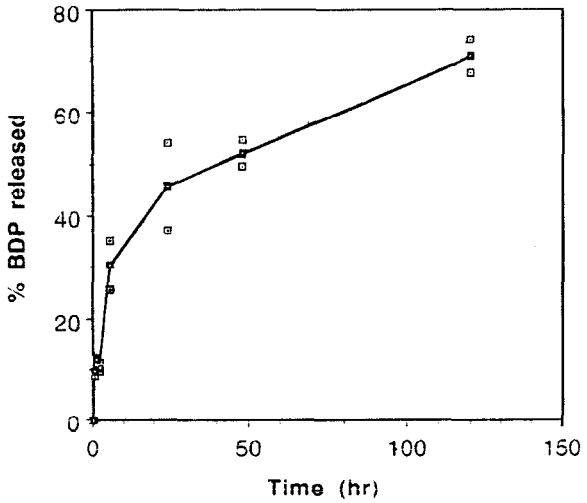

Fig. 2. Cumulative% BDP released versus time using isopropanol (50%) as a dissolution medium at 37°C ($n = 2$), showing data range for each time point. The entrapment efficiency of BDP was 88.9 ± 10.8% (w/w).

selected particle size distribution. The mean particle size of the microspheres can be regulated during the manufacturing process by controlling the stirring rate (El-Baseir et al., 1997).

The release profile of nedocromil sodium was found to follow a square root of time dependent mechanism as defined by the Higuchi equation ($Q = kt1/2$) were Q is the cumulative release of drug, k the release rate constant and t the time period. The regression coefficient (r) was > 0.93 for all batches tested. One factor which was not examined was the effect of drug-loading concentrations on the release kinetics of the nedocromil sodium. It has been shown by others that high loading effects the release mechanism. Microspheres containing high drug concentrations release drug faster than those with a low entrapment (Benoit et al., 1984; Erden and Celebi, 1996). The low percentage of nedocromil sodium entrapped, made it difficult to investigate the relationship between release rate and drug content. The preceding results have shown, however, that a long-acting delivery system may be achieved by applying PLA microsphere technology.

Microspheres showed much higher entrapment levels of the lipophilic drug BDP compared with the more hydrophilic nedocromil sodium (El-Baseir et al., 1997) The dissolution study is an essential part in the development of microsphere dosage forms. The low aqueous solubility of BDP makes it difficult to create sink conditions in an aqueous release medium. Initial experiments were therefore conducted using 50% isopropanol as the dissolution medium.

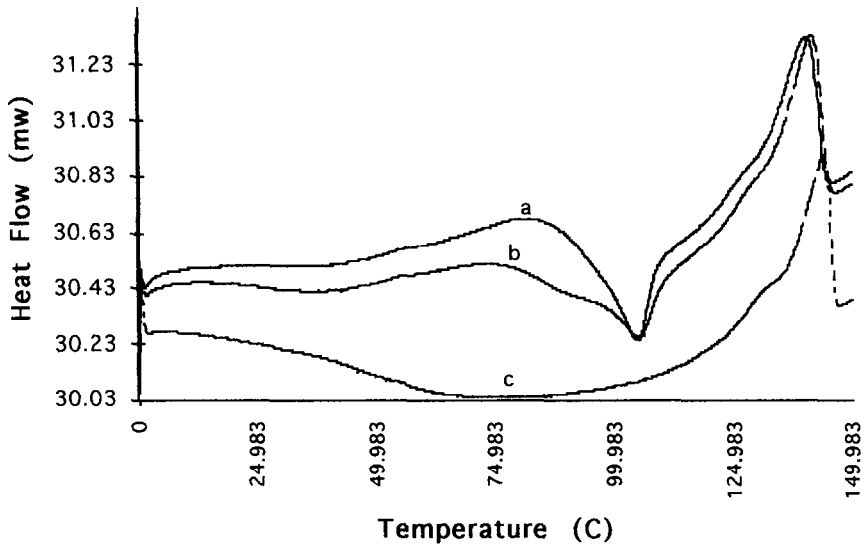

Fig. 3. DSC thermograms of microsphere after (a) 0.5, (b) 5 and (c) 120 h incubation in 50% (v/v) isopropanol at 37°C.

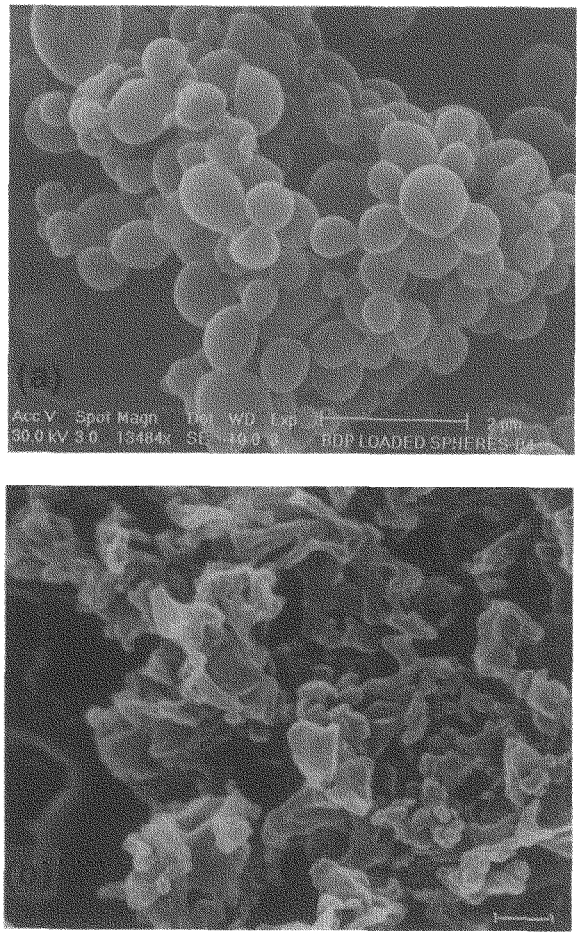

Fig. 4. Scanning electron micrographs of PLA microspheres containing BDP. (a) Prior to incubation in 50% (v/v) isopropanol (scale bar, 2 μm). (b) After 120-h incubation in 50% (v/v) isopropanol (scale bar 1, μm).

Fig. 2 shows the in vitro release profile of BDP from PLA microspheres conducted over 120 h in 50% isopropanol at 37°C. Seventy-five percent (w/w) of BDP was released during this time. The physicochemical (DSC, Fig. 3) and morphological (SEM, Fig. 4) studies of the microspheres during dissolution indicated dramatic changes in the microsphere's structure due to the plasticization effect caused by isopropanol on the polymer, leading to loss in mechanical strength and microsphere deformation.

DSC traces of the microspheres during incubation in 50% isopropanol (Fig. 3), show a gradual decline in the exothermic peak due to crystallisation of the PLA. This was not the case when PLA-microspheres were incubated over 8 days in phosphate buffer (El-Baseir et al., 1997). Nevertheless, sustained release of BDP over 120 h was possible.

Steroids are among the agents shown to disorder lipid bilayers (Lawrence and Gill, 1975). Interaction between a variety of lipophilic drugs and liposomes indicated depression of the phase transition temperature (T_m) of the phospholipid (Bonina et al., 1994). An alternative method was therefore developed based on determining induced changes in T_m in order to study the release kinetics of BDP in a phosphate-buffered saline solution (PBS, pH 7.4) which did not result in any solvent-induced morphological changes in the microspheres. Using DMPC liposomes as a sink for released BDP permitted release rates to be determined by reference to changes induced in the liposome suspensions and recorded on DSC traces. The selection of DMPC as a model membrane was due to its phase transition temperature of 23°C being below the temperature of the drug release studies (37°C) and the glass transition temperature of PLA ($\sim$55°C). No untoward changes in the physical properties of the microspheres was therefore expected during the drug release studies.

The calorimetric heating curves of DMPC liposomes (0.5%, w/v) in the presence of different mole fractions of BDP is shown in Fig. 5. Increasing BDP in the DMPC bilayer led to a marked effect on the phase transition behaviour (broadening of the transition peak and a shift in T_m to a lower value). This effect is similar to that shown for the interaction between tolmetin and DMPC liposomes (Castelli et al., 1994) and may be explained in terms of the fluidifying effect of introducing the lipophilic drug molecule into the ordered structure of the lipid bilayer. Such effects are manifested by a reduction in T_m (Jorgensen et al., 1991). A linear relationship was obtained between the concentration of BDP and the percent change in the transition temperature of the DMPC liposomes (Fig. 6). This suggests that at the concentrations of steroid examined, the drug

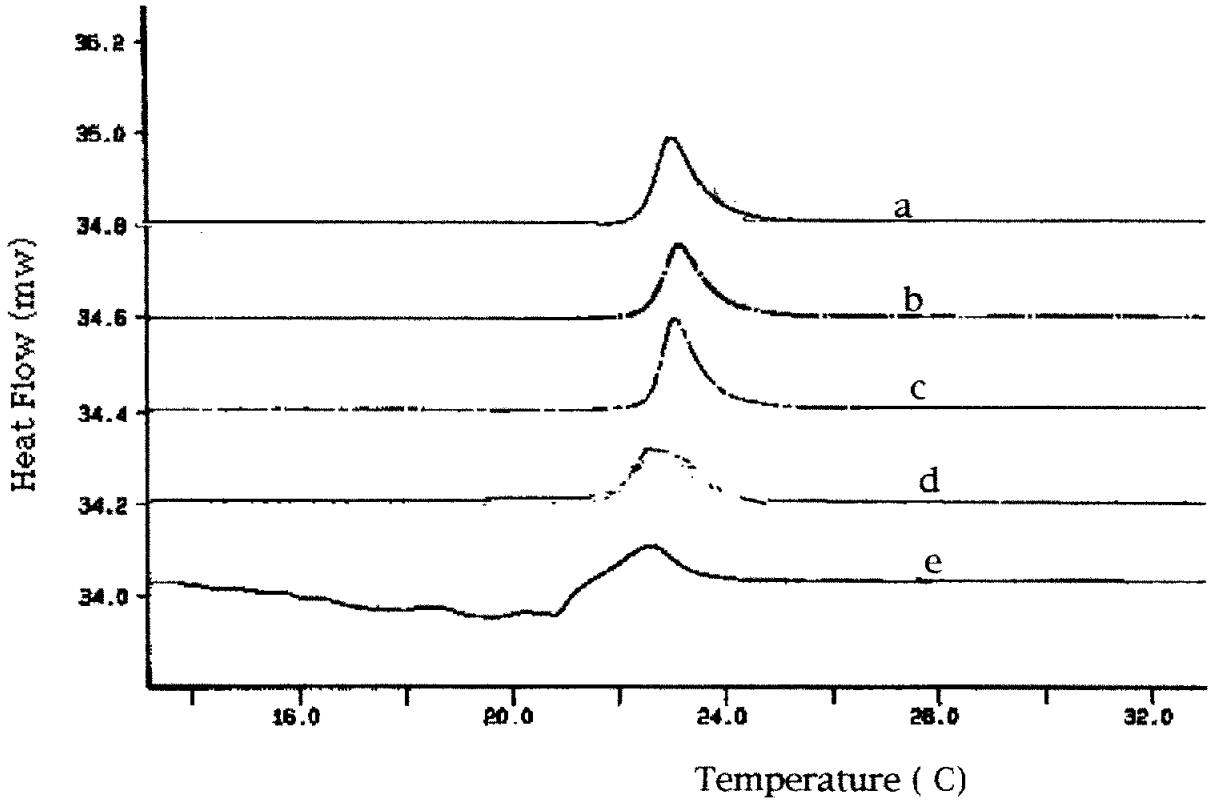

Fig. 5. DSC traces of 0.5% (w/v) DMPC liposomes containing different mole fractions of BDP: (a) 0.0019; (b) 0.003; (c) 0.0098; (d) 0.013; and (e) 0.026 mole fraction.

was well solubilized within the lipid bilayer and saturation levels had not been achieved.

Fig. 7 shows the DSC traces for DMPC liposomes after various contact times with PLA microspheres containing BDP. This demonstrates that BDP release from the microspheres can be followed by the DSC procedure. The phase transition peak of DMPC is successively shifted to lower temperatures as increasing concentrations of BDP are solubilized within the bilayers. Using this assay, it was possible to examine BDP release rates from microspheres containing different BDP mole fractions. Such data would indicate the possibility of sustained release of BDP for over 6 days (Table 2). Experiments were terminated after 6 days due to the expected instability of DMPC liposomes when incubated at 37°C for longer periods. However, not all the loaded BDP could be accounted for in these release studies with only 35–55% (w/w) of the drug content being released. None of the samples released a major fraction of their drug content immediately upon immersion in phosphate-buffered saline. This is in contrast to the results obtained for nedocromil sodium-loaded microspheres (Fig. 1), where a considerable burst effect was observed. Because no release data were available within the first 5 min of testing, the presence of a slight burst effect for BDP loaded microspheres can not be discounted.

PVA is an emulsifier frequently used in the fabrication of microspheres. The presence of PVA has resulted in drug crystals attached to the surface of the particles (Kwong et al., 1986). The PVA hydrophilic surface layer, however, has the advantage of allowing an almost instantaneous re-dispersion of the microspheres in water (Allemann et al., 1993). Data in Table 3 showed 7% (w/w) or less of PVA was left in the batches of

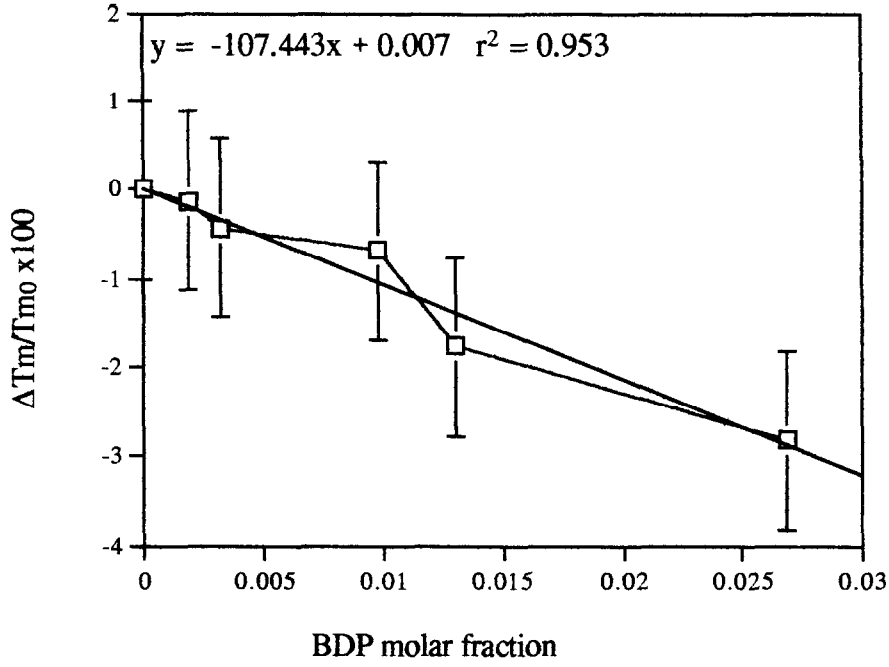

Fig. 6. Calibration curve relating the depression of DMPC lipid bilayer transition temperature (T_m) to the concentration of BDP.

microspheres examined. Less PVA was associated with the batches of microspheres with a mean diameter > 1 μm compared with batches of lower mean size. These results confirm that PVA forms a stable layer at the surface of the particles that is not eliminated during the isolation procedure.

The deposition of the emitted dose from DPIs in the oral cavity can cause discomfort to the patient such as an unpleasant taste, coughing, candidiasis and gastrointestinal tract side effects when swallowed (Johansson et al., 1982). In vitro assessment of deposition is often undertaken using an inertial particle deposition apparatus. For example, several formulations of cromylin sodium and beclomethasone dipropionate have been studied using such equipment (Holzner and Muller, 1995). The Andersen Cascade Impactor (ACI) is an official USP method proposed for aerosol particle size determination (Pharmacopeial Forum, 1992). It provides the greatest resolution in particle size distribution; however, its operation is more resource intensive than some other impactors. At an air flow rate of 28.3 l/min, the ACI permits particles larger than 10 μm to be retained in the throat and fractionation of the residual particles onto eight stages with 50% cut-off diameters of 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7 and 0.4 μm, together with a terminal filter (Molina and Rowland, 1974). Application of the Hinds equation (Hinds, 1982), enables the ACI to be operated at a flow rate of 60 l/min when the eight stages have 50% cut-off diameters of 6.18, 3.98, 3.23, 2.27, 1.44, 0.76, 0.48 and 0.27 μm.

The influence of mean microsphere size on in vitro deposition in an ACI operating at 28.3 l/min was examined for nedocromil sodium loaded PLA microspheres (Table 4). The actuated sample (% of capsule contents leaving the device) increased as the mean microsphere size decreased. This trend was also evident for the fractions depositing in the ACI of < 4.7 and < 3.3 μm. The polydispersity of the microspheres (Table 1) resulted in deposition on stages corresponding to particles with diameters much less than the mean microsphere size. As the plates in the ACI were not precoated with an adhesive material, it is possible that some re-entrainment of microspheres occurred. The appearance of almost one-third of the

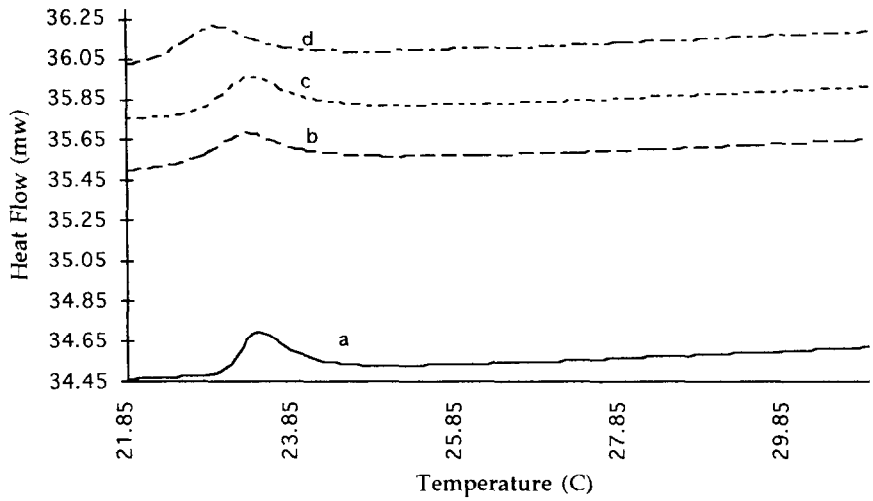

Fig. 7. DSC traces of DMPC transition temperature changes after (a) 0 h, (b) 4 h, (c) 24 h and (d) 147 h liposome contact at 37°C with BDP-loaded microspheres containing 0.049 mole fraction of BDP.

Table 2
The influence of BDP loading concentration on % BDP released after 6 days at 37°C

Drug loading (mole fraction BDP)	% BDP released after 6 days (mean and range)
0.033	55.0 (54.7–55.2)
0.049	43.0 (42.0–43.9)
0.070	35.9 (34.8–36.9)

actuated sample of the small microspheres (2.65 μm mass median diameter) in the throat, would indicate considerable particle aggregation. However, for this sample, approximately 26% of the capsule contents were collected on stages corresponding to particles < 3.3 μm and which may be considered as available for peripheral lung deposition and hence provide the depot for controlled drug release.

BDP-PLA microspheres not only resulted in higher drug entrapment than for nedocromil sodium, but were also of a smaller microsphere size when produced under identical conditions. Flow rate has an influence on both capsule emptying and the deposition profile of the aerosolized particles. An air flow rate of 60 l/min is claimed as ideal for the Spinhaler® (Sumby et al., 1992). For a 1.0-μm Mmd batch of BDP-PLA microspheres, 60% of the capsule contents were aerosolized of which 42 and 20% were deposited on stages corresponding to particles < 3.23 and < 2.27 μm, respectively.

Table 3
The residual PVA in microspheres from batches of different particle size ($\pm$ S.D., $n = 3$)

Batch no.	Particle mean size (nm)	Residual PVA (mg/5-mg microspheres)	Residual PVA% (w/w)
1	843 (34.7)	0.35 (0.04)	7.04 (0.88)
2	847 (61.7)	0.33 (0.01)	6.65 (0.16)
3	559 (16.8)	0.36 (0.02)	7.23 (0.32)
4	1133 (311)	0.27 (0.01)	5.31 (0.17)
5	1121 (517)	0.27 (0.01)	5.31 (0.17)

Table 4
Deposition of PLA microspheres in an Andersen Cascade Impactor

Sample	Mmd ± S.D. (μm)	Flow rate (l min^{-1})	n	Actuated sample ± S.D. (%, w/w)	% Deposition ± S.D.[a]			
					Throat	Stage 0-filter	Stage 2-filter	Stage 3-filter
PLA nedocromil sodium microspheres	6.14 ± 0.01	28.3	3	28.9 ± 17.8	39.8 ± 0.96	60.3 ± 0.95	12.1 ± 7.14	7.28 ± 5.47
	3.39 0.01	28.3	2	49.0	28.4	71.7	26.8	18.3
	2.65 ± 0.01	28.3	3	72.6 ± 26.6	32.8 ± 3.64	67.3 ± 3.65	29.0 ± 1.80	26.4 ± 0.66
PLA–BDP microspheres	1.00 ⊥ 0.21	60.0	6	60.6 ± 14.6	31.1 ± 6.21	68.9 ± 6.23	41.8 ± 4.32	20.0 ± 3.27

[a] % Deposition calculated relative to the actuated sample (emitted dose).

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