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Preparation and subsequent degradation of poly(L-lactic acid) microspheres suitable for aerosolisation: a physico-chemical study

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Abstract

The encapsulation of nedocromil sodium and beclomethasone dipropionate with microspheres of poly(L-lactic acid) has been studied and preparation conditions optimised for entrapment efficiency and microsphere size suitable for inhalation. DSC was used to characterise the microspheres both in terms of drug/polymer interaction and influence of annealing conditions on the T_g and degree of crystallinity. The absence of molecular interaction was confirmed by FTIR. Incubation of the microspheres in phosphate buffer at 37°C for 8 days demonstrated no chemical degradation of the polymer as evidenced by IR spectral analysis and estimates of percentage crystallinity. Surface morphology (SEM) and internal structure (TEM) were consistent with a homogeneous degradation pattern. © 1997 Elsevier Science B.V.

Keywords: Poly(L-Lactic) Acid; Microsphere; Degradation; Beclomethasone dipropionate; Nedocromil sodium

1. Introduction

The use of microspheres for drug formulation has attracted much interest in recent years. They have been used as a protective measure against oxidation/hydrolysis of active components through entrapment and, more recently, by using biodegradable polymers, have found application in sustained release systems. Until now, very little work has been focused on the use of microspheres for inhalation delivery. A microsphere can be defined as solid, spherical particle ranging from 1–1000 μm in diameter. When applied to inhalation drug delivery, a particle size of 0.5–3 μm is desirable to reach the alveolar sacs where drug dissolution and absorption is most useful. It is therefore important to produce a microsphere delivery system of a suitable size for aerosolisation.

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In order to obtain a sustained release formulation, the polymer used to construct the microspheres must be biodegradable. Moreover, it should be biodegradable over a suitable time period to give a suitable therapeutic effect. Biodegradable polymers such as poly(lactic acid) and poly(glycolic acid) (Redmon et al., 1989; Izumikawa et al., 1991), as well as natural polymers such as albumin and gelatine (Burgess and Carless, 1986) have been used in microsphere construction. The most widely used and studied class of polymer are the polyesters of which poly(lactic acid) is an example. Much work has been done on poly(lactic acid) and its copolymers forming microspherical entrapment systems for a wide range of formulations.

There have been a variety of techniques utilised for the preparation of microsphere systems with emphasis on entrapment efficiency and particle size. The most widely used encapsulation technique uses emulsion formation followed by solvent evaporation (Whateley, 1993). The hydrophobic polymer and drug are dissolved in the 'oil', usually dichloromethane, and the surfactant (to prevent particle aggregation) is dissolved in the aqueous phase. On mixing an emulsion is formed which can be disrupted by removal of the organic phase. This leaves microspheres dispersed in an aqueous medium which can be isolated by centrifugation or filtering. This method has been used successfully for the preparation of a number of hydrophobic drug/polymer microsphere systems. It was found, however, that hydrophilic drugs are not suitable species for entrapment into microspheres using this method.

An alternative preparation for water soluble drugs is the oil in oil emulsion system using acetonitrile and liquid paraffin as the internal and external phases, respectively (Tsai et al., 1986; Jalil and Nixon, 1989). Although an increased entrapment of hydrophilic drugs was observed, isolation and purification of the microspheres from the emulsion proved difficult. In addition, this method produced microspheres of 50 μm or larger which is clearly unsuitable for inhalation formulations. Use of a water in oil in water multiple emulsion has been used with more success for hydrophilic drug entrapment. Isolation and purification are relatively simple using this technique as is forming microspheres sufficiently small for an inhalation application.

Degradation of the microsphere at their intended release site is an important property of the formulation. The nature and rate of this degradation are important properties that need to be monitored and regulated so as to get maximum effect from the microsphere release. The release profile should be reproducible with a constant release rate. The polymer used in the microsphere preparation must obviously be non-toxic and easily expelled by the body. Release of the active component should be reasonably rapid in the lung in order to get maximum uptake before the formulation is naturally expelled.

Pretreatment of the polymer is a factor that can affect its subsequent preparation and degradation behaviour. Most polymers have a glass transition and storage of the prepared microspheres above and below this transition temperature will affect the degradation behaviour (Porter and Ridgway, 1983). The size and shape of the microspheres might also be altered if an annealing procedure is used.

It is the intention of this paper to investigate the preparation and subsequent behaviour of a series of microspheres made from poly(L-lactic acid) incorporating both a hydrophilic drug (nedocromil sodium) and a hydrophobic drug (beclomethasone dipropionate). An important consideration is the size of the final microsphere and modifications of the preparation method will be demonstrated to obtain microspheres of a suitable size for aerosolisation. Microsphere degradation experiments, in phosphate buffer, will be presented using various analytical procedures to assess the performance of the microspheres. The effect of annealing on the microsphere will also be presented.

2. Materials and methods

2.1. Materials

Poly (L-lactic acid) $_{w}$ 2000) was obtained from Polyscience. Polyvinyl alcohol (87–89%,

hydrolysed), was supplied by Aldrich; beclomethasone dipropionate was supplied by Sigma, and nedocromil sodium was supplied by Fison. All solvents were of HPLC grade and supplied by Fison.

2.2. Preparation of microspheres

2.2.1. Preparation of poly(L-lactic acid) microspheres containing nedocromil sodium

Nedocromil sodium (ca. 30 mg) in deionised water (0.5 ml) was added to poly(L-lactic acid) (PLA) (300 mg) in dichloromethane (5 ml) and stirred at 24 000 rpm (Ultra-turrax KI T25) for 5 min. Poly(vinyl alcohol) (PVA) (20 ml, 2% w/v) in deionised water was added and stirred at the same speed for a further 5 min. The w/o/w double emulsion was magnetically stirred for 7.5 h at room temperature to allow evaporation of the organic phase. The microspheres were isolated using centrifugation at 2000 rpm and washed with cold deionised water. The microspheres were freeze dried at -60°C for 24 h and stored over silica gel until use.

2.2.2. Preparation of poly (L-lactic acid) microspheres containing beclomethasone dipropionate (Jeffery et al., 1991)

Beclomethasone dipropionate (9 mg) and PLA (90 mg) were dissolved in dichloromethane (1.5 ml). This solution was added to a solution of PVA in deionised water (2% w/v, 12 ml) and stirred at 8 000 rpm for 5 min. The o/w emulsion was stirred for 5 h at room temperature to allow evaporation of the organic phase. The microspheres were isolated using centrifugation at 3000 rpm and washed with cold deionised water. The microspheres were freeze dried and stored as before.

Blank PLA microspheres were prepared using the same method with omission of beclomethasone dipropionate.

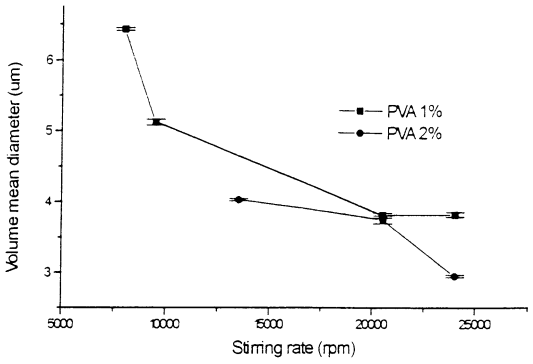

Fig. 1. Effect of stirring rate and PVA concentration on microsphere volume mean diameter.

Table 1
Effect of stirring rate and phase ratio on volume mean diameter for nedocromil sodium-loaded microspheres

Stirring rate (rpm)	Phase ratio (organic:aqueous)	Volume mean diameter (μm)
9500	1:4	4.18
9500	1:5	5.42
13 500	1:4	2.96
13 500	1:5	3.19
24 000	1:4	3.82
24 000	1:8	5.07

2.3. Microsphere treatment

Annealed samples were stored above the glass transition temperature of the polymer (60°C and 110°C) for 24 h then left to cool for 24 h prior to analysis. Degraded samples were prepared by suspending PLA microspheres (10 mg) in phosphate buffer (0.2 M, pH 7.4, 10 ml) at 37°C for 1–8 days. The microspheres were recovered using the procedure outlined above.

2.4. Microsphere characterisation

The microspheres obtained were characterised using particle size (Malvern 2600 laser sizer for larger particles (>1 μm) and photon correlation spectrophotometry, N4MD Coulter, for smaller particle sizing (<3 μm), scanning electron microscopy (SEM) (EM400 STEM), transmission electron microscopy (TEM), differential scanning calorimetry (DSC) (Perkin Elmer DSC7), thermogravimetric analysis (TGA) (Perkin Elmer TGA7) and Infra red spectroscopy (IR) (Perkin Elmer 1600 series FTIR spectrophotometer).

Table 2
Effect of drug/polymer ratio on entrapment efficiency, mean size and percentage yield for loaded microspheres

Drug	Drug/polymer ratio	Entrapment efficiency (%)	Stirring rate (rpm)	Mean size (μm)	Percentage yield (%)
Beclomethasone dipropionate	1:10	52.4	20 500	1.4	53.5
	1:5	48.0	20 500	0.5	41.9
	1:5	70.0	8 000	1.0	60.28
	1:2	82.8	8 000	0.8	63.3
Nedocromil sodium	1:10	5.4	24 000	2.8	26.4
	1:3	7.9	24 000	8.6	22.3
	1:2	3.9	24 000	6.1	23.2
	1:1.5	2.2	24 000	3.1	14

2.5. Drug entrapment in microsphere efficiency

2.5.1. Nedocromil sodium

The microspheres (ca. 10 mg) were dispersed in dichloromethane (3 ml) and made up to 25 ml with methanol. The samples were shaken and centrifuged at 3000 rpm for 5 min. The supernatant was subjected to fluorometric measurement at excitation wavelength 376 nm, emission wavelength 590 nm and the drug content assessed from a calibration curve.

2.5.2. Beclomethasone dipropionate

The microspheres (ca. 5 mg) were dispersed in dichloromethane (100 μl) and made up to 5 ml with methanol. After 24 h the sample was centrifuged at 3000 rpm for 5 min. Then, 20 μl was injected onto a reverse phase Zorbax 5 μm column with acetonitrile/water (3:2) eluant flowing at 0.75 ml/min. A UV detector at 254 nm recorded the absorbance and the concentration of drug was assessed using a calibration curve.

3. Results and discussion

Stirring rate and PVA concentration (Fig. 1) influence the size distribution of the formed microspheres. Increased PVA concentration and increased stirring rate reduce the size of the microspheres. This supports the observations of Mumper and Jay (1992) and indicates the possibility of production of microspheres of a suitable size for aerosolisation. All the concentrations of PVA and all stirring rates gave a particle size suitable for aerosolisation where 90% of the particles are < 10 μm (Gupta and Hickey, 1991). The decrease in microsphere size when PVA concentration is increased from 1–2% may be due to improved packing at the emulsifier surface and hence giving protection against droplet coalescence. It is clear that a small mean size is preferable and by increasing the stirring rate and PVA concentration to 24 000 rpm and 2%, respectively, it is possible to produce microspheres where 90% of the particles are < 4.2 μm.

Table 1 displays the same effect of stirring rate with particle size and also shows that, by keeping the aqueous phase as small as possible prior to emulsion formation, a smaller microsphere can be

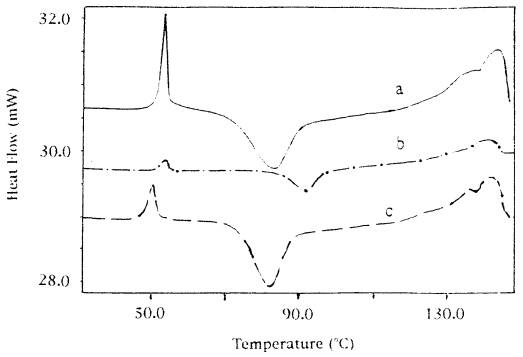

Fig. DSC spectra of (a) nedocromil sodium-loaded microspheres, (b) beclomethasone dipropionate-loaded microspheres, and (c) blank microspheres.

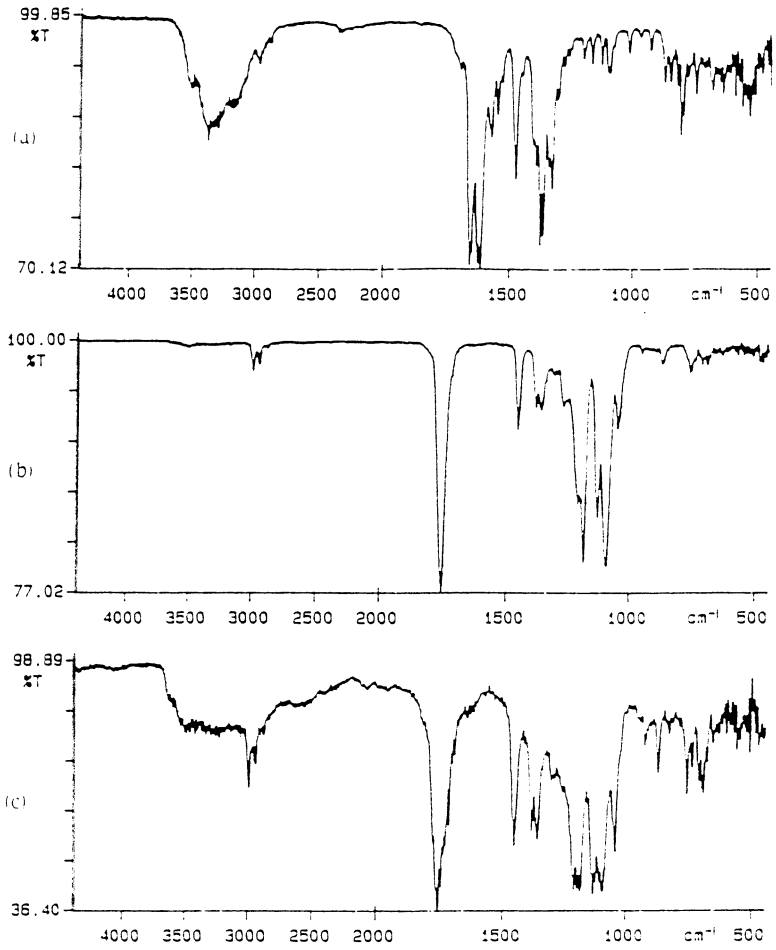

Fig. 3. IR spectra of (a) nedocromil sodium, (b) nedocromil sodium-loaded microspheres, and (c) pure poly(L-lactic acid).

produced. This is as expected for the hydrophilic drug, nedocromil sodium, as there is less opportunity for crystal growth and reorganisation in a less polar emulsion.

The microspheres formed with beclomethasone dipropionate (hydrophobic model drug) also resulted in microspheres of a suitable size for aerosolisation. Table 2 compares the entrapment efficiency, mean size and percentage yield for beclomethasone dipropionate and nedocromil sodium-loaded microspheres as a function of drug/polymer ratio. By increasing the concentration of beclomethasone dipropionate in the internal phase, the entrapment efficiency was increased to a maximum of 83% when 1:2 drug/polymer ratio was used. Stirring rate was seen to have little effect on the final size of the microspheres with a maximum mean size of 1.4 μm. This is still a suitable size for aerosolisation. By contrast, attempts to entrap nedocromil sodium resulted in microspheres with a very poor entrapment efficiency. An optimum efficiency of 7.9% was found for a drug polymer ratio of 1:3 which is very poor compared to the beclomethasone microspheres. This trend is expected due to the increased solubility of nedocromil sodium in the final phase. It is expected that a large proportion of the drug will be washed away during preparation.

Table 3
DSC results from annealed microspheres at different temperatures

Sample	Annealing temperature (°C)	Time (h)	T_m (°C)	ΔH_{fus} (J g^{-1})	Degree of crystallinity
PLA	0	0	127.9	41.1	0.20
	60	24	131.7	52.9	0.26
Nedocromil sodium	0	0	141.5	38.2	0.19
Loaded	60	24	141.3	29.9	0.20
Microspheres	110	24	140.9	52.0	0.26

The mean sizes were also larger than those for beclomethasone dipropionate demonstrating the increased degree of difficulty in microsphere preparation when a hydrophilic drug is to be entrapped.

DSC analysis of PLA showed two endothermic transitions corresponding to the glass transition (58°C) and the melting transition (127°C). These data are in accordance with results previously reported by Pitt and Gu (1987). On formation of microspheres, the glass transition is observed to shift to 49°C and the melt to 140°C. In addition, an exothermic transition is observed at 81°C corresponding to a recrystallisation event (Fig. 2). This is indicative of the semi-crystalline nature of the polymer. Encapsulation of nedocromil sodium and beclomethasone dipropionate resulted in no change of shape in the DSC profile (Fig. 2). This is a strong indication that there is no physical or chemical interaction between polymer and drug. Both TGA and DSC showed no indication of latent solvent residual in any of the samples.

Fig. 3 shows IR spectra for PLA microspheres, nedocromil sodium and nedocromil sodium-loaded microspheres. The polymer displays characteristic carbonyl and alkyl stretching bands at 1747 cm^{-1} and 2900 cm^{-1}, respectively. Nedocromil sodium displays characteristic carbonyl stretches, unsaturated carbon–carbon stretches and amide carbonyl stretches/amine bending at 1657 cm^{-1}, 1627 cm^{-1} and 1574 cm^{-1}, respectively. The PLA bands are observed in the loaded microspheres with no spectral shift but the nedocromil sodium bands are not observed. This is due to the low entrapment of the drug in the microsphere. The lack of spectral shift in the polymer bands indicate a weak or no interaction between the polymer and drug. A physical mixture of nedocromil sodium and PLA showed both PLA and drug bands to have no spectral shift and hence confirming the absence of an interaction between the two species. The IR spectra for PLA microspheres, beclomethasone dipropionate and beclomethasone dipropionate-loaded microspheres showed that the polymer bands are still clearly evident in the loaded microsphere spectrum as is the carbonyl stretch of the drug at 1606 cm^{-1}. This gives strong evidence for the absence of an interaction between the microsphere and drug.

Table 3 shows the results of annealing the microspheres at 60°C and 110°C for 24 h. The degree of crystallinity was calculated by using the enthalpy of fusion value calculated by Jamshidi et al. (1988), for a 100% crystalline sample of PLA (203.4 J g^{-1}). The ratio of the calculated enthalpy and this value can be converted to a percentage crystallinity for each sample. It was observed that the crystallinity of the sample increases after the annealing process and the glass transition and recrystallisation event are not evident. This is consistent with the expected behaviour of a polymer after annealing at a temperature above its glass transition temperature. Mobility of molecules within the sample are increased and so a conformationally more stable morphology is achieved. When the annealing process is over a shorter time period (Table 4), a clear glass transition is observed (Fig. 4). Annealing was performed within the instrument for 5 min prior to subsequent scans. The glass transition and melting transition both decreased with increasing annealing time. After an initial annealing for 5 min a glass transition is observed similar to that re-

Table 4
DSC results from microspheres after successive 5-min periods of annealling at 160°C

Scan number	T_g (°C)	T_m (°C)	ΔH_{fus} (J g^{-1})	Degree of crystallinity
1	—	146.03	36.01	0.18
2	55.66	145.31	20.48	0.10
3	54.34	145.00	18.8	0.09
4	52.95	144.75	17.43	0.08

ported by Masinde (1985). The degree of crystallinity is seen to reduce with each subsequent scan. This is due to the fast cooling rate of the instrument which encourages amorphous precipitation from the melt. This is expected to slowly improve with subsequent heating and cooling.

The DSC thermal profiles for blank microspheres before and after degradation in phosphate buffer at 37°C are shown in Fig. 5. The transition temperatures and enthalpies are presented in Table 5. The glass transition temperature is seen to decrease with increased exposure to buffer. The size of this transition also decreases. This is probably due to partial annealing of the polymer as, during the degradation experiment, the temperature was held at 37°C which is not far from the glass transition temperature. This explains the drop in the recrystallisation enthalpy which indicates less recrystallisation of the polymer. The melting point is seen to increase over the first 2 days and then stabilise to a constant value and is probably due to recrystallisation of the polymer at the microsphere surface. The percentage crystallinity is shown to increase after incubation, supporting the afore mentioned theory.

The IR spectra for a series of blank PLA microspheres after incubation in phosphate buffer at 37°C for a number of days showed no shift of the main polymer bands over this time period, indicating no chemical degradation of the polymer. The absorbance at 1604 cm^{-1} is due to CO_2 stretching bands as a result of random chain scission at ester groups in the polymer. The pH of the supernatant from the microsphere suspension was found to be constant over the 8 days of degradation. This implies that CO_2 detected in the IR spectrum is of a very low concentration. This is in agreement with Sah et al. (1994) that little or no conversion of ester to carboxylic acid is observed during the degradation process.

It should be noted that the percentage crystallinity of the PLA used in this study was no greater than 20% and remained unchanged within the range $\pm 3\%$ after exposure of the microspheres to phosphate buffer. This confirms the observations of Pitt and Gu (1987).

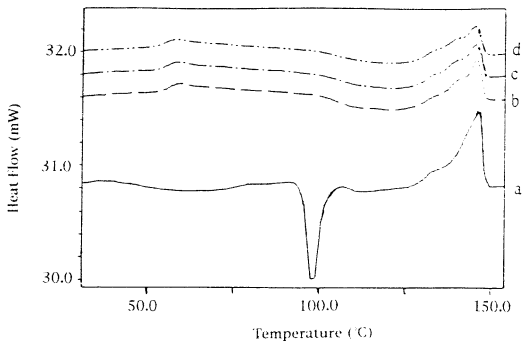

Fig. 4. DSC spectra of blank microspheres (a) 1, (b) 2, (c) 3, and (d) 4 repeat heating and coolings within the DSC instrument.

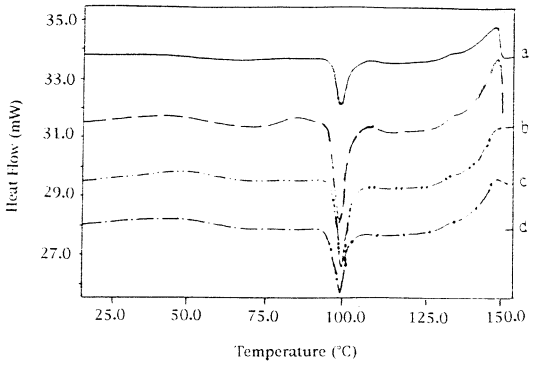

Fig. 5. DSC spectra of blank microspheres after (a) 1 day, (b) 2 days, (c) 4 days, and (d) 8 days incubation in phosphate buffer.

Table 5
DSC and weight loss data after degradation study at 37°C in phosphate buffer

Time (days)	% Weight loss	T_g (°C)	T_m (°C)	ΔH_{fus} (Jg^{-1})	Degree of crystallinity
0	0	49.39	140.03	34.23	16.83
2	11.5	47.06	146.43	40.02	19.68
4	12.0	42.28	145.86	37.27	18.32
8	15.0	41.53	145.16	35.04	17.21

SEM micrographs of microspheres after 0 and 8 days incubation in phosphate buffer show little change to the surface morphology indicative of an even degradation of the microsphere. TEM studies of a cross section of the microspheres after 0 and 8 days incubation in phosphate buffer show no pores or channels through the surface of the microsphere indicating a homogeneous degradation pattern.

4. Conclusion.

A method has been discussed which resulted in the successful entrapment of a hydrophilic and hydrophobic drug into poly(L-lactic acid) microspheres. It was demonstrated that, by adjusting the stirring rate and emulsifier concentration, microspheres can be prepared of a suitable size for aerosolisation and that the polymer and drugs are compatible with each other.

Annealing of the polymer was shown to alter the crystallinity and thermal behaviour of the polymer. Obviously, the crystallinity of the delivery polymer is an important characteristic in a formulation if a reproducibly predictable dose is to be administered over time.

The degradation of the microspheres to release drug was shown to be slow (Table 5). With only 15% of the microsphere dissolved over 8 days, the release rate of drug is too slow for practical application. SEM micrographs, however, show an even degradation with no surface roughening. This will give a predictable degradation rate which is essential for microsphere delivery systems.

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