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Solvent evaporation, solvent extraction and spray drying for polylactide microsphere preparation

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Summary

Polylactide microspheres containing vitamin D_3 (D_3) as a model drug were prepared by solvent evaporation, solvent extraction and spray drying. In this work, these three different preparation methods were compared and the extent to which each can affect the properties and characteristics of microspheres was identified. The experimental conditions yielding the best results were chosen within each method. Solvent extraction proved to be the better of the two traditional methods. It resulted in particles that were more regular in shape, smaller, with a narrower size distribution and higher porosity. Spray drying yields results equivalent to those of solvent extraction with the advantage of a higher encapsulation efficiency and shorter duration for the process of preparation. Moreover, the dissolution profile of microspheres prepared by spray drying demonstrated that more gradual release of drug was promoted.

Introduction

Microspheres are defined as porous microparticulate drug delivery systems promoting the controlled release of drugs. They represent a polymeric matrix system containing the drug uniformly distributed throughout the polymer matrix (Wheatley and Langer, 1987).

Several methods have been used in the preparation of microspheres of different biodegradable polymers (Beck et al., 1979; Leong et al., 1986; Sato et al., 1988; Okada, 1989; Morimoto and Fujimoto, 1985), including both natural and synthetic polymers. The selection of a suitable method of preparation depends on the properties of both polymer and drug and can affect the characteristics of microspheres (Jalil and Nixon, 1989; Sato et al., 1988).

The main purpose of this paper was to compare three different techniques used for the preparation of polylactide microspheres loaded with a lipophilic drug. The three methods selected for our investigation were: emulsification by solvent evaporation, emulsification by solvent extraction and spray drying.

The first technique is the most widely used for the preparation of polylactide microspheres (Beck et al., 1979) and involves the emulsification of an

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organic solvent solution of poly(DL-lactide) (PDLLA) and drug in an aqueous medium containing an emulsifier. Subsequent evaporation of the solvent leads to the formation of microspheres.

Solvent extraction (Sato et al., 1988) is a modification of the first technique where the emulsion, consisting of an organic solution of polymer and drug in an aqueous medium, is poured into a diluent phase which is miscible with the polymer solvent but immiscible with the polymer. As a consequence, the solvent migrates from the polymer microdroplets into the diluent phase producing solid microspheres.

The spray drying technique has seldom been used in the preparation of microparticles (Cady et al., 1989; Wang et al., 1990). A solution of polymer and drug in a solvent is sprayed through the nozzle of the spray-drier apparatus. The solvent evaporates very quickly leaving solid microparticles.

Each method necessitates particular experimental conditions to be met. PDLLA of a high molecular weight racemic variety was used to prepare microspheres in the three methods. This polymer is a non-toxic, non-tissue reactive biodegradable material that has been used for surgical sutures (Wise et al., 1979), prosthetic devices (Albizzati et al., 1989), and in controlled release drug delivery systems (Beck et al., 1979; Jalil and Nixon, 1989; Conti et al., 1990) precisely because of these advantageous characteristics.

Vitamin D_3 (D_3) was chosen as a lipophilic model drug (Conti et al., 1990). D_3 and its metabolites and synthetic derivatives are effective in the therapy of a number of chronic bone diseases such as osteoporosis (Slovik et al., 1981; Gallagher et al., 1982). Furthermore, recent investigations have highlighted the antitumoral activity of its fluorinated synthetic derivatives (Abe et al., 1981; Tanaka et al., 1982; Eisman and Frampton, 1984).

Materials and Methods

Poly(DL-lactide) (PDLLA) (Res 206R Mol. Wt 109 000, i.v. 1) was supplied by Boehringer Ingelheim. Vitamin D_3 crystals for biochemistry, methanol, ethanol and methylene chloride HPLC grade, were supplied by Merck-Bracco. All other chemicals used were of reagent grade. Millipore membrane filters of 1.2 and 0.2 μm pore size were employed. The bi-distilled water used was filtered through 0.22 μm Millipore membrane filters.

Microsphere preparation

Microspheres were prepared using the three different methods. The process conditions followed in each of the three were assessed experimentally.

Solvent evaporation Solutions of 5% PDLLA and different amounts of D_3 (5, 10, 20 and 30% of PDLLA weight) in a mixture of CH_2Cl_2 and $CHCl_3$ (1:1 w/w) were prepared at room temperature to form the dispersed phase. Glycerol with 0.02% Tween 20 was used as the continuous phase. The dispersed phase was added dropwise to the continuous phase at a ratio of 1:20 and emulsified using an Ultraturrax model T25 equipped with an S25N dispersing tool, at 13 500 rpm. The emulsion was stirred for 3 min starting at 0°C and ending at 35°C. The manner of agitation was changed after 3 min, the emulsion being stirred, using a nitrogen impeller, for 2 h at 38°C to allow the evaporation of organic solvents. The suspension obtained was filtered through a 1.2 μm Millipore membrane, then rinsed twice with a solution of 15% isopropanol in water to remove solvent and surfactant residues. The microspheres collected by filtration were dried and stored under vacuum.

Solvent extraction The same procedure as that of the preceding section was followed until the emulsion had been formed. The emulsion obtained was poured into a 15% isopropanol solution in water (diluent phase) at a 1:1 ratio between the continuous and diluent phases. This mixture was stirred using a nitrogen impeller for 2 h at 35°C in order to allow the extraction of solvents from the polymer droplets. Microspheres were collected by filtration through a 1.2 μm Millipore membrane, rinsed twice with a solution of 15% isopropanol in water and dried under vacuum.

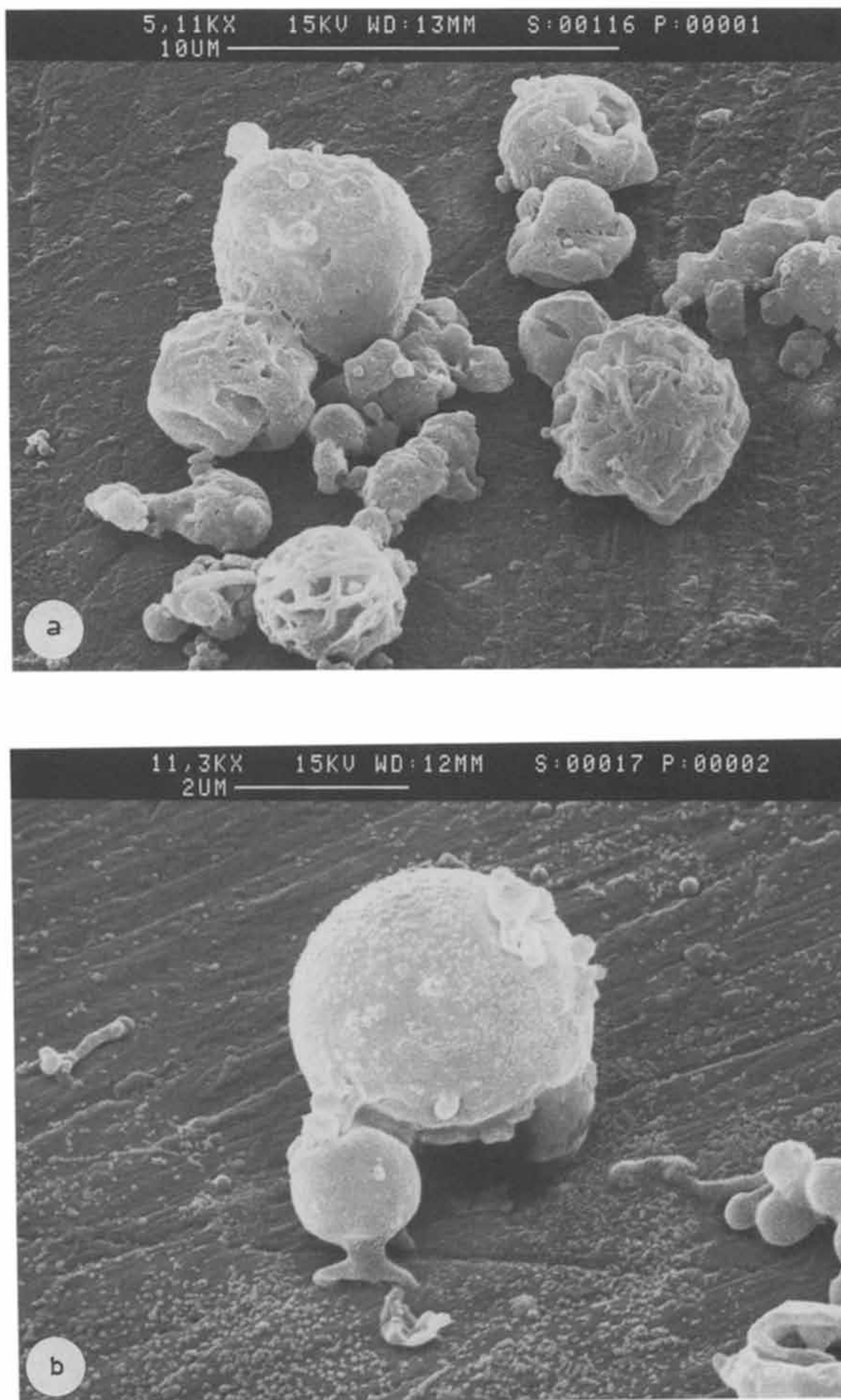

Fig. 1. Photomicrographs of PDLLA microspheres prepared by (a) solvent evaporation; (b) solvent extraction; (c) spray drying.

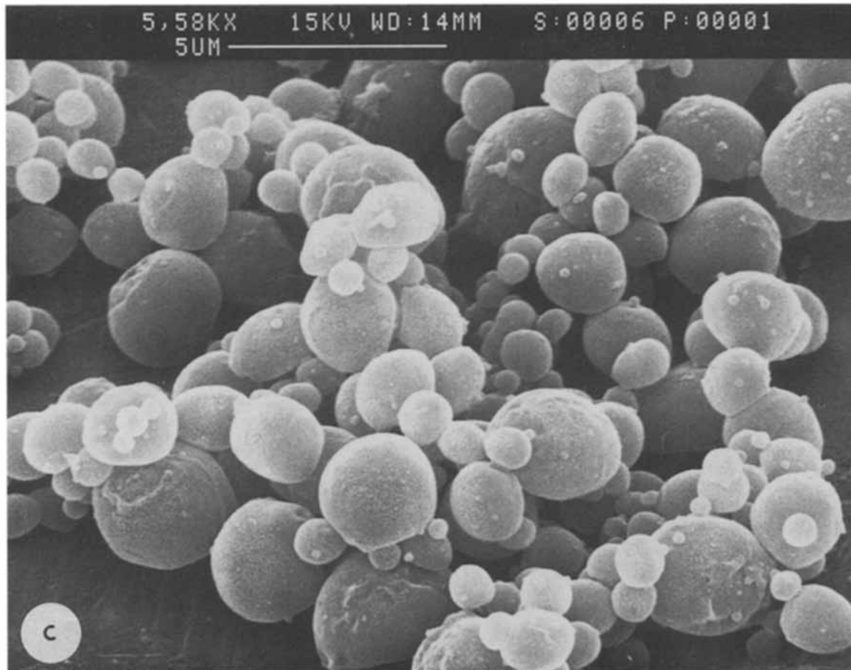

Fig. 1. (c).

Spray drying Solutions containing 0.75% of PDLLA and different amounts of D_3 (5, 10, 20 and 30% of PDLLA weight) in a mixture of $CH_2Cl_2/CHCl_3$ (1:1 w/w) were prepared at room temperature. They were sprayed through the nozzle of a mini spray dryer model BUCHI 190 with an inlet temperature of 51°C and a spray-flow pressure of 5 bar. The solid microspheres that had precipitated into the bottom collector (34°C outlet temperature) were harvested and kept under vacuum.

Microsphere characterisation

Optical microscopy The shape and size of all batches of microspheres were monitored during the process under an optical microscope (Laborlux Leitz model K) with transmitted light at a magnification of 500 ×.

Scanning electron microscopy The microspheres were characterized by scanning electron microscopy. A Cambridge Stereoscan 200 electron microscope was used. A small amount of microspheres was suspended in ethanol, sonicated for 15 min and a drop of the resulting suspension was placed on the sample holder, dried and observed under the electron microscope.

Particle size analysis by instrumental methods A small amount of microspheres was suspended in 50 ml of bi-distilled water, sonicated for 15 min, and analyzed, under continuous stirring, by using a light blockage instrument (HIAC/ ROYCO Model 3000) equipped with an HR60HC sensor (1–60 μm analysis range) and a small-volume sampling probe, and by employing a Coulter Multisizer (Coulter Electronics, Ltd, Luton, U.K.) fitted with a 70 μm orifice tube. The analyses were carried out at six size levels (1.5, 2, 4, 5, 10 and 20 μm) with the HIAC/ ROYCO apparatus and at 16 size levels, between 1.4 and 42 μm, with the Coulter Multisizer. The results are the averages of five withdrawals.

Surface area Surface area was determined on a FlowSorb II 2300 (Micromeritics). The analyses were performed on three batches of microspheres for each preparation method used.

Differential scanning calorimetry (DSC) DSC analysis of polymer, drug, microspheres and drug-loaded microspheres was performed on a DSC Mettler Model TA 3000 equipped with a DSC 20 cell. Samples (5–6 mg) were scanned in pierced aluminium pans, at a heating rate of 5 k/min, in the temperature range 40–250°C under a nitrogen atmosphere.

Drug loading The D_3-loaded microspheres were dissolved in CH_2Cl_2 and, after filtration through a 0.22 μm Millipore membrane, directly analysed by HPLC (Varian Model 9010). A mobile phase comprising methanol/water/ethanol (90:5:5) and a Lichrosorb RP8 Column 250 × 4 (Merck) were used in conjunction with a UV detector at 264 nm (Varichrom U.V.2550, Varian Instrument).

Dissolution tests

Dissolution tests were carried out in a Diffutest Eurand apparatus (Eurand Microencapsulation SpA). Amounts of microspheres, equivalent to a total D_3 concentration of 4 μg/ml, were weighed out in triplicate for each preparation method used, as well as a reference sample consisting of the same amount of D_3. Drug loading, previously measured by HPLC, was almost identical for all samples used in dissolution tests. Microspheres and the reference sample were placed in 100 ml saline phosphate buffer solutions (pH 7.4) and rotated at 37°C for 96 h. A small amount of surfactant (0.2% Tween 20) was added to the buffer to improve the wettability of microspheres. Release patterns were constructed from the D_3 concentrations determined via HPLC assay.

Results and Discussion

Characterization of microspheres

The microspheres detected by scanning electron microscopy were essentially spherical in shape (Fig. 1). The microspheres produced by spray drying revealed a more pronounced spherical shape and greater homogeneity in the size distribution than those produced by solvent evaporation and solvent extraction. Moreover, photomicrographs of microspheres prepared by solvent evaporation demonstrated particles with a rough surface and very large pores.

Table 1 lists the particle size distributions for microspheres prepared by the three methods. Particle counts as determined on both the HIAC and Coulter instruments are expressed as undersize cumulative percentages. The apparently different results obtained using the two instrumental methods, for the same sample, at the lowest size level, depend on the counting characteristics of the instrument employed.

Both instrumental methods emphasize the fact that microspheres produced by solvent evaporation are larger than those prepared via other

TABLE 1

Particle size distribution of PDLLA microspheres

Method	Undersize cumulative distribution (%)				
	1.2–2 μm	< 5 μm	< 10 μm	< 20 μm	< 42 μm
Solvent evaporation					
HR	18.2	68.0	96.4	99.7	–
CM	30.5	85.7	97.9	99.8	100
Solvent extraction					
HR	26.8	98.8	99.6	99.9	–
CM	55.2	96.4	99.7	99.9	100
Spray drying					
HR	18.1	64.7	95.4	99.9	–
CM	32.2	90.2	99.5	99.9	100

Data obtained on (HR) HIAC/ROYCO and (CM) Coulter Multisizer.

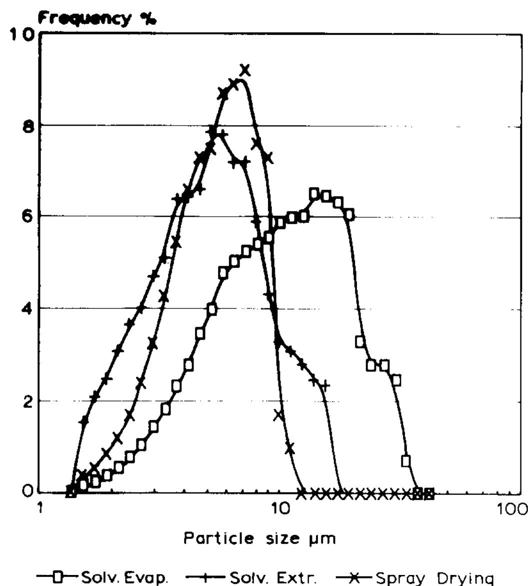

Fig. 2. Particle size distribution by volume, determined using a Coulter Multisizer for PDLLA microspheres prepared by the three different methods.

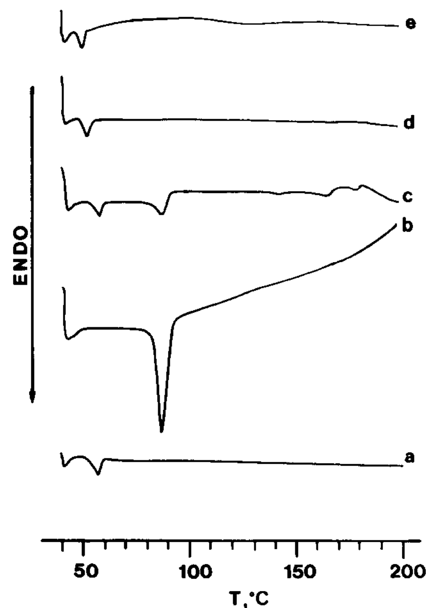

Fig. 3. Thermal behaviour of: (a) PDLLA (Mol. Wt 109 000); (b) vitamin D_3; (c) physical mixture; (d) blank PDLLA microspheres; (e) vitamin D_3-loaded PDLLA microspheres.

methods. With the HIAC instrument, only 68% of the particles produced by solvent evaporation are $\leqslant 5$ μm, while 98.8% of those prepared by solvent extraction are $\leqslant 5$ μm. Spray-dried microspheres showed the best particle size distribution to be between 5 and 10 μm, as demonstrated by the distribution-by-volume measurements with the Coulter Multisizer (Fig. 2). The experimentally determined specific surface areas of PDLLA microspheres prepared using the three methods are listed in Table 2.

The values obtained are greater compared to those of non-porous solid spheres of approximately equivalent mean diameter. The results suggest the porous nature of microspheres prepared by the evaluated methods.

TABLE 2

Specific surface area of PDLLA microspheres

Microsphere preparation method	Surface area (m^2/g)
Solvent evaporation	5.44
Solvent extraction	9.85
Spray drying	6.33
Nonporous microparticles (2.5 μm diameter)	2.4

DSC showed no differences between the polymer (Fig. 3a) and the blank microspheres (Fig. 3d). The drug's melting peak present in the DSC scan for the physical mixture (Fig. 3c) no longer appears in that for the drug-loaded microspheres (Fig. 3e). This suggests that the drug becomes physically dispersed in an amorphous form inside the polymeric matrix structure.

Yields and drug loading

Table 3 lists the production yield and encapsulation efficiency of each process. The three methods assessed differ greatly in the final production yields. The best yields were obtained via solvent evaporation, whilst considerable loss of material occurs during spray drying. This factor probably depends on the technical characteristics of the apparatus used: much of the powder adhering to the cyclone walls is lost during spray drying. This preparation method proved to be the fastest: in 1 h, approx. 1 g of product can be prepared by

TABLE 3

Production yield and encapsulation efficiency of PDLLA microspheres produced by three different methods (each sample represents the average of three batches)

Method and sample no.	Yield (%)	Theoretical drug content (%)	Encapsulation efficiency (%)
Solvent evaporation			
1	92.2	4.7	20.2
2	94.8	9.0	10.6
3	96.5	16.6	5.5
4	88.0	22.6	14.2
Solvent extraction			
1	53.7	4.7	20.2
2	69.3	9.0	12.9
3	69.3	16.6	6.9
4	82.1	22.6	11.4
Spray drying			
1	38.9	4.7	58.3
2	40.3	9.0	26.7
3	45.7	16.6	14.3
4	34.2	22.6	22.0

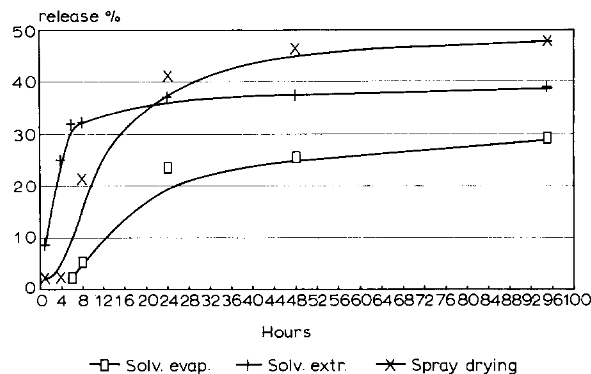

Fig. 4. In vitro dissolution of vitamin D_3 from PDLLA microspheres.

spray drying, whereas a period of at least 4 h is required to yield an equal amount of product with the other methods under our experimental conditions.

Table 3 lists the data obtained on four different concentrations of drug added to the polymer for each preparation method. The amount of drug loaded appears to be affected by the preparation method. Without exception, the highest encapsulation efficiencies were achieved via the spray drying method, with which a maximum encapsulation efficiency of 58.3% is reached, as compared with 20% for the other methods. Encapsulation efficiencies within each method are not significantly correlated with the amount of D_3 added during the preparation process (theoretical drug loading). In contrast, the lowest theoretical drug loadings usually correspond to the highest level of encapsulation efficiency. Theoretical drug loadings above 22.6% always resulted in lower encapsulation efficiencies and in considerable loss of drug.

TABLE 4

Characteristics of microspheres prepared by three different preparation methods

Characteristic	Solvent evaporation	Solvent extraction	Spray drying
Shape	Irregular	Spherical	Spherical
Median diameter (μm)	2.9	2	2.6
Surface area (m^2/g)	5.44	9.85	6.33
Average yield of production (%)	92.8	68.6	39.7
Average encapsulation efficiency (%)	12.6	12.8	30.3
Time for preparation (h)	4	4	1
Relative proportion (%)			
8 h	5	32	20
24 h	22	36	40
96 h	29	39	49

Dissolution tests

The dissolution profiles of microspheres produced by the three different methods (Fig. 4) demonstrate a difference in the behaviour of each sample analyzed. Greater initial release is observed for microspheres prepared by solvent extraction (32% of drug released in the first 4 h), while almost no burst effect and a more gradual release, over 96 h, was found in the case of spray-dried and solvent-evaporated microspheres. In all cases, D_3 release exceeded 96 h in duration. These data are consistent with the higher porosity of the microspheres by emulsion solvent extraction.

Conclusion

Table 4 summarizes the characteristics of microspheres obtained by the three different preparation methods. Between the two traditional methods, solvent evaporation and solvent extraction, the latter results in microspheres with better morphological characteristics. Both methods achieve the same encapsulation efficiency and require the same processing time, however, microspheres prepared by solvent extraction are more regular in shape, smaller, with a narrower size distribution and greater porosity.

Spray drying gives results equivalent to those of solvent extraction, with respect to particle shape, size and size distribution, nevertheless, it attains the highest encapsulation efficiency and requires the shortest duration of the preparation procedure. Taking all of the above factors into account, it was concluded that spray drying is a very satisfactory technique for the preparation of vitamin D_3-loaded polylactide microspheres.

The different characteristics of the microspheres produced by the three methods described in this investigation influence the dissolution behaviour of the microspheres. The greater surface area of the solvent-extracted microspheres leads to a rapid initial rate of release of drug, while more gradual release is observed for microspheres prepared via the other two methods. The low rate of degradation of high molecular weight PDLLA contributes to the slow release of drug over a period of 96 h.

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