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The formation and characterization of hydrocortisone-loaded poly((±)-lactide) microspheres

M. CAVALIER, J. P. BENOIT* AND C. THIES†

Laboratoire de Pharmacie Galénique et Biopharmacie, Université Paris-Sud, Chatenay-Malabry, France

The solvent evaporation process has been used to form hydrocortisone-loaded microspheres from poly((±)-lactide) (PLA) and a lactide-glycolide copolymer (65/35). Methylene chloride was the casting solvent. Partially hydrolysed (88%) poly(vinyl alcohol) and methylcellulose were used as aqueous phase emulsifiers. Methylcellulose was preferred, because it gave stable emulsions as the amount of hydrocortisone being encapsulated increased whereas poly(vinyl alcohol) did not. With methylcellulose as the emulsifier, a broad size range of spherical microspheres containing up to 50% (w/w) hydrocortisone could be prepared. Thermal and X-ray analyses established that poly((±)-lactide) microspheres containing hydrocortisone retained thermal events characteristic of both materials. This is evidence that such microspheres contain, to some extent, crystalline hydrocortisone domains dispersed in a PLA matrix. But most of the encapsulated drug was molecularly dispersed in the PLA glass. The stability of hydrocortisone in microspheres was evaluated in different storage conditions: no degradation of drug was found. The release of hydrocortisone from 250–350 μm diameter microspheres into agitated 37 °C water (nitrogen atmosphere) was determined by HPLC analysis. The microspheres evaluated had initial hydrocortisone payloads of 12 to 47% (w/w). The rate of drug release increased as the initial drug payload carried by the microspheres increased. The release data are not adequately described by zero order, first order, or square-root-of-time release kinetics. Drug release from microspheres that contain 12% (w/w) hydrocortisone approached a plateau value well below the amount of drug actually carried by the microspheres. This is particularly true for hydrocortisone encapsulated in lactide-glycolide polymer.

Several reports have disclosed the use of the solvent evaporation process to prepare drug-loaded microspheres from poly((±)-lactide) (PLA), a biodegradable carrier (Beck et al 1979; Benita et al 1984). The procedure consists of dissolving the polymer in methylene chloride, a volatile solvent. A drug is then either dissolved or suspended in the solution and the resulting mixture is emulsified in an aqueous phase that contains an emulsifier. The solvent is allowed to evaporate thereby giving drug-loaded microspheres. Although the procedure is conceptually simple, a number of factors affect the nature of the microspheres obtained. These include the solubility of the drug in the casting solvent and aqueous phase, the drug–polymer ratio, the nature of the polymer used as the carrier, and the aqueous phase emulsifying agent. This paper describes how several variables affect the formation of hydrocortisone-loaded PLA microspheres, and characterizes their properties.

† On sabbatical leave from Biological Transport Laboratory, Washington University, Saint-Louis, MO 63130, USA.
* Correspondence.

MATERIALS AND METHODS

Materials

Poly((±)-lactide) (PLA) (Southern Research Institute, Birmingham, Ala) had an inherent viscosity of 1·14 dl g^{-1} (30 °C; 0.5 g PLA dl^{-1} chloroform). A 65 mole percent lactide and 35 mole percent glycolide (65/35 L/G) copolymer was supplied by M. Vert, Laboratoire des Substances Macromoléculaires, Rouen, France. Methylene chloride (CH_2Cl_2) (J. T. Baker Chemical Co, Phillipsburg, NJ), hydrocortisone (Sigma Chemical Co, St Louis, MO), partially hydrolysed (88%) poly(vinyl alcohol) (PVA) (Vinol 205, Air Products & Chemicals, Allentown, PA) and methylcellulose (400 & 10 cps Grades, Dow Chemical Co, Midland, Mich.) were used as received.

Methods

Hydrocortisone-loaded microspheres were prepared by the continuous solvent evaporation process (Benita et al 1984). The steroid–polymer–methylene chloride mixtures used were emulsified (240–270 rev min^{-1}) in water containing a polymeric emulsifier, and the methylene chloride was then evaporated at

22 °C to give solid steroid-loaded microspheres, 50 to 150 μm in diameter. Since hydrocortisone is only partially soluble in methylene chloride, all the solvent phases used contained dissolved steroid and undissolved crystals.

Thermal analyses of the microspheres were carried out with a Rigaku Simultaneous Differential Thermal–Thermal Gravimetric Analyzer (DTA/TGA) (Rigaku, Inc, Tokyo, Japan) and a Perkin-Elmer Model 4C Differential Scanning Calorimeter (DSC) equipped with a thermal analysis data station (Perkin-Elmer Corp., Norwalk, Conn.). DTA samples were heated at 10 °C min^{-1} in an air or nitrogen atmosphere. DSC samples were heated at 20 °C min^{-1} in a nitrogen atmosphere. X-ray powder diagrams of microspheres were obtained with a Norelco X-ray diffraction unit (3 h copper Kα radiation) by Prof. L. Gulbranson, Washington University, St Louis, MO.

Microsphere chlorine contents were determined by Galbraith Laboratories, Knoxville, Tenn.

The rate of release of steroid from four microsphere samples into water at 37 °C was determined under nitrogen in a 500 ml round bottom flask equipped with a stirrer. The volume of release medium was fixed at 250 ml. The weight of microspheres used (30–100 mg) was adjusted to keep the drug concentration in the extraction medium below 20% of the saturation solubility of water (28 mg/100 ml at 25 °C) (Merck Index 1968) if all of the hydrocortisone carried by the microspheres was released. Aliquots (5 ml) of extraction medium were drawn at known times and subjected to HPLC analysis with a Spectra-Physics 8000 Liquid Chromatograph equipped with a 254 nm detector (Spectra-Physics, Santa Clara, Calif.). The elution solvent was a 25/75 (v/v) mixture of water and methanol. The flow rate was 2 ml min^{-1}. Under these operating conditions, the retention time of the steroid was 100 s.

The steroid content of microspheres was determined by dissolving them in methylene chloride; the PLA was precipitated by addition of excess ethanol and the resulting clear solution was subjected to the same HPLC assay procedure used to obtain the steroid release data. The steroid contents reported are mean values of duplicate determinations.

RESULTS AND DISCUSSION

Table 1 lists several features of 12 microsphere runs carried out with 0·27% PVA as the aqueous phase emulsifier. For these runs, the steroid/polymer ratio charged to the reactor varied from 0·15 to 0·56. Spherical microspheres were produced consistently when this ratio was <0·4, but products were irregularly shaped when the ratio increased to 0·56.

Column 5 of Table 1 shows that the steroid content of the isolated microspheres ranged from 4·2 to 21·6% which is well below the expected values if all that initially added to the solvent phase had remained in it. Column 7 shows that 37·5 to 71·3% of the steroid partitioned to the aqueous phase during preparation of the microspheres. Steroid in the aqueous phase was a mixture of well-defined crystals and dissolved drug, the amount that partitioned remaining essentially constant as the steroid/polymer ratio varied from 0·23 to 0·56 (Column 6).

The twelve runs listed in Table 1 include triplicate runs from one procedure and duplicate runs from two other procedures. The consistency of results

Table 1. Data on microsphere preparations made with 0·27% PVA as the aqueous phase emulsifier.

Material charged to reactor				Location of drug at end of experiment		
Drug (g)	Polymer	Polymer (g)	Drug/polymer ratio*	Content in microspheres (%)	Wt in aqueous phase (g)	Fraction in aq. phase (%)**
0·15	PLA	1·0	0·15	4·2	0·107	71·3
0·23	PLA	1·0	0·23	8·8	0·134	57·2
0·28	PLA	1·0	0·28	12·0	0·144	51·8
0·28	PLA	1·0	0·28	12·2	0·142	50·7
0·28	PLA	1·0	0·28	11·9	0·145	51·8
0·28	65/35 LG	1·0	0·28	13·3	0·127	45·3
0·28	65/35 LG	1·0	0·28	13·0	0·130	46·4
0·28	PLA	0·93	0·30	13·5	0·136	48·6
0·28	PLA	0·93	0·30	13·3	0·139	49·6
0·28	PLA	0·70	0·40	16·6	0·140	50·0
0·40	PLA	1·0	0·40	20·5	0·150	37·5
0·28	PLA	0·50	0·56	21·6	0·142	50·7

* Wt drug/wt polymer, g/g.
** Fraction of drug initially added to CH_2Cl_2 phase that partitioned to the aqueous phase.

obtained demonstrates the reproducibility of the manufacturing process.

When the initial steroid/polymer ratio was increased to 0·6 or more, emulsions formed with 0·27% PVA as the emulsifier were unstable. This caused formation of large aggregates; microspheres were not isolated. Stable emulsions and microspheres were formed if methylcellulose was dissolved in the aqueous phase either alone or with PVA. Table 2 summarizes the features of 9 microsphere runs made with initial steroid/PLA ratios ranging from 0.6 to 1.0 and methylcellulose-400 dissolved in the aqueous phase. Microspheres isolated from runs made with 0·3% methylcellulose-400, or 0·3% in admixture with 0·27% PVA as the aqueous phase emulsifier, were non-spherical. Fig. 1 illustrates the particle geometry obtained. Similarly shaped particles were obtained with methylcellulose-400 concentrations as low as 0·05%. A mixture of 0·05% of it with 0·27% PVA gave spherical microspheres (Fig. 2). Methylcellulose-400 alone gave a stable emulsion, but its high molecular weight and accompanying high solution viscosity led to the formation of distorted particles. Addition of PVA to a dilute methylcellulose-400 solution altered its solution rheology properties enough to allow formation of spherical microspheres. Methylcellulose-10, alone or in combination with PVA, also produced spherical microspheres when the initial steroid/PLA ratio was 0·6 or more. A mixture of 0·05% methylcellulose-10 and 0·27% PVA permitted production of spherical microspheres when the initial steroid/polymer ratio was raised to 1·5, but Fig. 3 shows that large, rod-like hydrocortisone crystals were attached to the surface of such particles. They grew as solvent evaporation progressed.

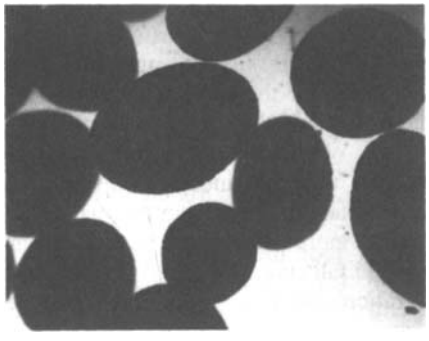

FIG. 1. Photomicrograph (×100) of hydrocortisone-loaded (49% w/w) PLA microspheres fabricated with an aqueous phase emulsifier of 0·27% PVA and 0·33% methylcellulose-400.

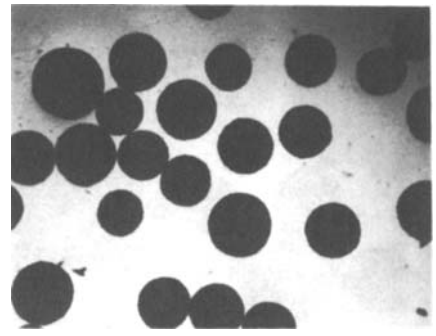

FIG. 2. Photomicrograph (×100) of hydrocortisone-loaded (49% w/w) PLA microspheres fabricated with an aqueous phase emulsifier of 0·27% PVA and 0·05% methylcellulose-400.

For the runs listed in Table 2, 0·036 to 0·171 g of steroid partitioned to the aqueous phase as the microspheres were fabricated (column 7). The steroid present in the aqueous phase as a mixture of dissolved drug and crystal represented 3·6 to 25·2% of the drug initially added to the solvent phase (column 8). This is lower than the values reported in

Table 2. Data on microsphere preparations made with methylcellulose (MC) as a component of the aqueous phase emulsifier.

Material charged to reactor			Emulsifier		Location of drug at end of experiment		
Drug (g)	PLA (g)	Drug/PLA ratio*	PVA Concn (%)	MC Concn (%)	Drug in microspheres/(%)	Drug in aqueous phase (g)	Fraction in aqueous phase/(%)**
0·60	1·0	0·6	—	0·33	32·0	0·130	21·7
0·80	1·0	0·8	—	0·33	38·6	0·171	21·4
1·00	1·0	1·0	—	0·33	49·0	0·036	3·6
0·60	1·0	0·6	0·27	0·28	34·1	0·083	13·8
1·0	1·0	1·0	0·27	0·28	46·5	0·114	11·4
1·0	1·0	1·0	0·27	0·30	45·7	0·159	15·9
1·0	1·0	1·0	0·27	0·33	46·7	0·124	12·4
0·60	1·0	0·6	0·27	0·05	31·0	0·151	25·2
1·00	1·0	1·0	0·27	0·05	47·0	0·114	11·4

* Wt drug/wt PLA, g/g.
** Fraction of drug initially added to CH_2Cl_2 phase that partitioned to the aqueous phase.

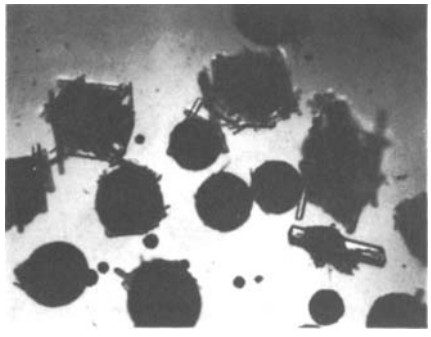

FIG. 3. Photomicrograph (×100) of hydrocortisone PLA microspheres formed with an initial steroid/PLA ratio of 1·5 (w/w). The aqueous phase emulsifier was 0·27% PVA and 0·05% methylcellulose-400.

Table 1 because much more steroid was present in the system. The amounts of steroid in the aqueous phase reported in Table 2 are more scattered than those in Table 1, but the mean value is only 10% less. Over the range of conditions examined, it does not appear that the steroid partitioning to the aqueous phase is strongly influenced by such parameters as the nature and amount of aqueous phase emulsifier, the initial steroid/polymer ratio in the solvent phase, or the polymer dissolved in the solvent phase. It is hypothesized that the slight reduction in mean weight of partitioned steroid recorded in Table 2 is caused by methylcellulose in the aqueous phase.

Several PLA microsphere samples loaded with 12·6% steroid were characterized by thermal and X-ray analyses. A DSC scan for a sample stored at 22 °C for 14 weeks after manufacture had two well-defined thermal events. One occurred at 56 °C, the other at 208–209 °C. The 56 °C event is characteristic of drug-free PLA and was attributed to the glass transition (Tg) of PLA. Since the presence of steroid did not alter the Tg of PLA, the drug is assumed to have little solubility in PLA.

The event at 208–209 °C has little energy associated with it (1·9 cal (8J) g^{-1} microspheres) and disappeared when the microspheres were heated to 250 °C, quench-cooled, and reheated. The X-ray powder diagram of these microspheres taken before heating has d-spacings characteristic of crystalline hydrocortisone. Thus, the 208–209 °C event is attributed to steroid crystal domains located in the microspheres. The 208–209 °C fusion temperature is significantly lower than the 228 °C fusion point characteristic of well-defined crystals of the drug, and indicates that its crystal domains in the microspheres were imperfect. The low energy of fusion suggests that relatively little steroid was present in crystalline form, much of it being molecularly dispersed in the PLA glass. These observations were unexpected because the initial PLA–solvent–steroid mixture used to form the microspheres was turbid and contained undissolved crystals which were thought to be trapped in the final microspheres to give a significant fusion event at 228 °C. Direct observation throughout the solvent evaporation process revealed that this did not occur because the steroid crystals initially present in the methylene chloride migrated spontaneously into the aqueous phase when the methylene chloride was emulsified in the aqueous phase. The crystalline steroid domains in the isolated PLA microspheres were formed by crystallization of solubilized drug that occurred as solvent evaporation progressed. Since the viscosity of the PLA–steroid–solvent solution increased steadily as solvent evaporation progressed, the steroid crystal domains nucleated and grew in a progressively more viscous medium. This was assumed to hinder the development of defect-free crystal domains and the extent of steroid crystallization. (These observations and comments relate to microspheres that contained 12·6% steroid. Microspheres with larger steroid contents would undoubtedly have a more pronounced steroid fusion event which would fall closer to 228 °C. In such cases, the crystallization would occur sooner in the process due to the larger amount of drug present.)

The PLA Tg event had an endothermic peak characteristic of amorphous polymers stored below their Tg (Petrie 1972). This peak was not present when the microspheres were first formed or after 6 days storage at 22 °C. It developed between 6 and 98 days of 22 °C storage. When these microspheres were annealed at 110 °C for 21 h, quench-cooled in liquid nitrogen and subsequently stored at 22 °C, an endothermic peak developed within 3 days storage at 22 °C. The more rapid development of this endothermic peak in heat-treated samples may be due to essentially complete removal of residual methylene chloride during the 110 °C annealing process. TGA scans showed that annealed samples stored for 4 days or more at 22 °C had essentially no weight loss when heated to 250 °C. Unannealed samples had a 2·5 to 5·0% TGA weight loss even after 14 weeks of 22 °C storage. An unannealed sample that carried 12·6% steroid contained 2·74% chlorine after 22 h drying in a vacuum at 22 °C. Extending the drying time to 72 h reduced the chlorine content to 2·56%. If these chlorine contents are assumed to be due solely to residual methylene chloride, the microspheres contain 3·8 and 3·3% of the solvent, respectively. The small reduction in chlorine content caused by

increasing the drying time from 22 to 72 h illustrates the tenacity with which PLA retains methylene chloride at 22 °C when the steroid content of the microspheres is low.

The 110 °C annealing step did not cause a significant change in the 208–209 °C melting event. The steroid crystal domains that existed before heat treatment did not disappear, or become enhanced due to the heat-treatment. No event developed at 228 °C during annealing. The effect of annealing on PLA mol. wt was not determined.

Although annealed samples stored 4 days or more at 22 °C experienced no weight loss, annealed samples stored 0 to 3 days at 22 °C experienced 2–3% TGA weight losses.

The stability of the steroid in PLA microspheres was evaluated with samples stored at −20, 25 and 37 °C. The freshly formed microspheres contained 12·0% drug. After 55 days storage, microspheres kept at −20 °C contained 11·7%, those kept at 25 °C contained 12·2%, those kept at 37 °C contained 11·7% of the steroid. These variations in steroid content are small and are attributed to variations in sensitivity of the assay.

The release of steroid from four drug-loaded microsphere samples into water at 37 °C was examined. The microspheres were a 250–350 μm fraction isolated by sieving a dry microsphere sample. Three of the samples were fabricated from PLA and had initial steroid contents of 12, 31 and 47% respectively. The fourth sample was fabricated from the 65/35 L/G copolymer and had an initial steroid content of 13%. Fig. 4 contains the release curves obtained. The data shown are mean values of duplicate release runs.

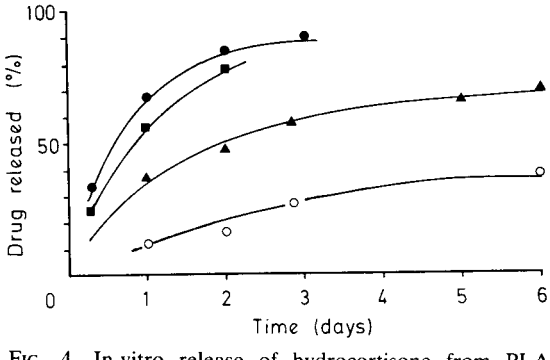

FIG. 4. In-vitro release of hydrocortisone from PLA microspheres into 37 °C water: ○, 13% steroid in 65/35 L/G copolymer microspheres; ▲, 12% steroid in PLA microspheres; ■, 31% steroid in PLA microspheres; ● 47% steroid in PLA microspheres.

All of the curves in Fig. 4 are non-linear. The number of data points is limited, but it appears that none of the curves fits a first order release expression; one sample, 31% steroid in PLA, appears to fit a $\sqrt{t}$ release expression. None of the samples release a major fraction of their drug contents immediately upon immersion in water, but release it over a finite time. Increasing the initial steroid content of the PLA microsphere sample from 12 to 31, and finally 47%, increased the rate of steroid release. Changing the polymer from PLA to 65/35 L/G while fixing the initial steroid content at 12–13%, causes a measurable decrease in rate of drug release. None of the microsphere samples evaluated completely released their steroid contents in the release times evaluated. For three of the four samples evaluated, the rate of release decreased measurably as the release time increased. For the PLA and 65/35 L/G copolymer samples with an initial steroid content of 12–13%, the drug release rate slowed to such a degree after 6 days that it appeared that its complete hydrocortisone release would require several weeks. This assumes that no major structural changes in the microspheres would occur during this time due, for example, to hydrolytic degradation of the polymer. With the 65/35 L/G copolymer significant hydrolytic degradation may occur thereby altering the steroid release rate.

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