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CHARACTERISATION OF BIODEGRADABLE MICROSPHERES CONTAINING DEHYDRO-ISO-ANDROSTERONE

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ABSTRACT

Poly-DL-lactide (PLA) and poly-DL-lactide-co-glycolide (DL-PGA) micromatrices (Pharmazomes^{TM}) containing dehydro-isoandrosterone (DHEA), a weak androgen, were prepared by a solvent evaporation process. Micromatrices with increasing drug loading as well as increasing polymer molecular weight were prepared. The morphology of these systems depended on the drug loading, the polymer molecular weight and polymer composition. Increasing the drug loading or polymer molecular weight resulted in increasingly irregular microparticles being formed. DSC thermograms did not reveal the presence of crystalline DHEA in micromatrices containing 10 to 50% DHEA loading. However crystalline DHEA was observed in microspheres heated to above the glass transition temperature of the polymer. X-ray analysis of 30% DHEA micromatrices established the presence of crystalline DHEA in the micromatrices. The percent release of DHEA from the micromatrices, into 40% ethanol at 37^{o}C, increased

with increasing DHEA loading. The dissolution of DHEA from drug-polymer compressed discs of constant surface area was proportional to the square root of time indicating matrix controlled release.

INTRODUCTION

Biodegradable polymers of lactic acid and glycolic acid are currently used as carriers for sustaining the release of drugs. The contraceptive steroids; progesterone and norethisterone have been successfully encapsulated to give controlled delivery systems using poly-DL-lactide and poly-DL-lactide-co- glycolide polymers respectively[1,2]. Of the five antiinflammatory steroids; hydrocortisone acetate, cortisone, cortisone acetate, prednisolone and prednisone, microencapsulated with DL-PGA, only the release of hydrocortisone acetate was found to be appreciably retarded.[3] The drug-polymer ratio, the polymer material and the processing variables have been shown to affect the morphology of these systems[3-6] which in turn may affect the release rate of the drug. The state in which the drug exists in the microspheres can also significantly influence the release rate of the drug[7]. Where microspheres are produced by a solvent evaporation procedure, depending on the solubility of the drug in the solvent, the state in which the drug is present in the polymer matrix may range from total solubilisation to the drug being deposited in the original crystal form. Progesterone was found to form a metastable molecular dispersion in poly-DL-lactide matrix[7], while crystalline hydrocortisone was observed in poly-Dl-lactide microspheres[8]. In this report DHEA-loaded PLA and DL-PGA microspheres were prepared and their characteristics as well as release properties were studied.

EXPERIMENTAL

Materials

Poly-DL-lactide of molecular weights 2,000, 16,000 and 109,000 as well as poly-DL-lactide-co-glycolide 50:50 of intrinsic viscosity 0.5 dl/g or mol. wt. 12,000 (Boehringer Ingelheim) were used. Methylcellulose (BDH) was used as the emulsifier. Acetone, chloroform and dichloromethane (CH_2Cl_2) were of Analar grade.

Preparation of microspheres

Microspheres were formed by a solvent evaporation method. The drug (as a percentage of the polymer weight) and the polymer were dissolved in the casting solvent and emulsified in the aqueous phase. Before solvent evaporation was complete, the external phase was replaced with distilled water and agitation continued to complete solvent evaporation. The microspheres were then filtered, washed and dried.

Evaluation of the microspheres formed:

Microscopy:

A scanning electron microscope (Hitachi S500) was used to examine the surface properties of the microspheres.

A hot stage microscope (Reichert) was used to detect the presence of crystalline drug in the polymer micromatrices.

Differential Scanning Calorimetry (DSC):

A Mettler 3000 calorimeter at a heating rate of 10^{o}K/min (in a nitrogen atmosphere) was used to detect changes in polymer and/or drug properties.

X-ray diffraction analysis:

X-ray diffraction patterns were obtained using nickel filtered copper radiation (Philips).

Assay of Drug Content:

A weighed amount of microspheres was dissolved in 1ml of CH_2Cl_2 and 11 mls of ethanol was added to precipitate the polymer. After centrifugation and dilution, the DHEA content was assayed by HPLC (Waters Millipore) under the following conditions: μ-bondapak C18 reverse column; 35:35:30 acetonitrile : methanol : water at a flow rate of 1ml/min as eluent and 211 nm UV detector.

Release Studies

The release of DHEA from microspheres containing a theoretical weight of 9mg of DHEA was measured in 40% aqueous ethanol at 37°C. Solubility studies indicated that 40% aqueous ethanol provided sink conditions for DHEA at 37°C. The dissolution tests were carried out in 100mls stoppered flasks in a shaker water bath at 37°C[9]. One ml samples were withdrawn at intervals of time and assayed by HPLC. All samples were replaced with fresh medium.

RESULTS AND DISCUSSION

Micromatrices of increasing DHEA loading in PLA of mol. wt. 2000

Table 1 lists several features of DHEA micromatrices formed using increasing DHEA loading in PLA 2000. The DHEA loading was varied from 10% to 50% of total weight of the polymer. Spherical micromatrices were produced when the DHEA loading was ≤ 30% while at higher DHEA loading microparticles formed were irregularly shaped (fig. 1&2). The drug content in the microspheres was influenced by the agitation rate. At high stirring speed, the rate of solvent evaporation was more rapid resulting in a lower drug loss to the external phase. At both stirring rates when the ratio of the acetone to chloroform used was increased from 1:1 to 3:1, the percentage drug entrapped was

TABLE 1
Data on DHEA micromatrices (MM) prepared with PLA 2,000.

DHEA/g of polymer(g)	solvent system	stirring rate (rpm)	shape of MM	DHEA in MM g/g of polymer	% drug lost
0.10	1:1 A:C*	1500	spherical	0.071	29.0
0.25	1:1 A:C	1500	spherical	0.225	10.0
0.30	1:1 A:C	300	spherical	0.217	27.7
0.30	3:1 A:C	300	spherical	0.222	26.0
0.30	1:1 A:C	1500	spherical	0.280	6.67
0.30	3:1 A:C	1500	spherical	0.281	6.33
0.50	CH_2Cl_2**	1500	irregular	0.440	12.0

* A:C = acetone: chloroform. ** CH_2Cl_2= methylene chloride

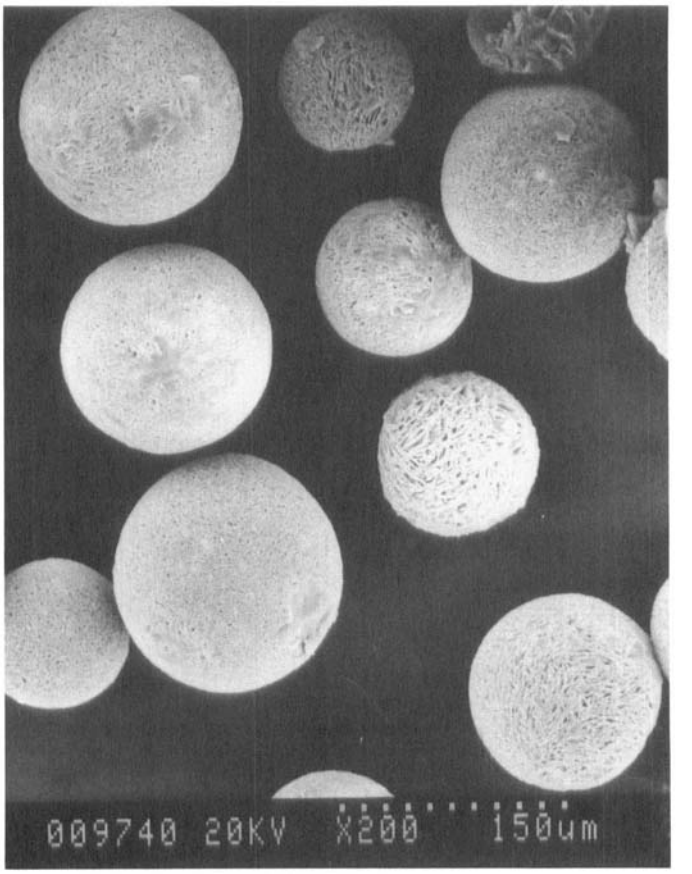

FIGURE 1
Photomicrograph of PLA 2000 micromatrices containing 25% DHEA (w/w of PLA 2,000)

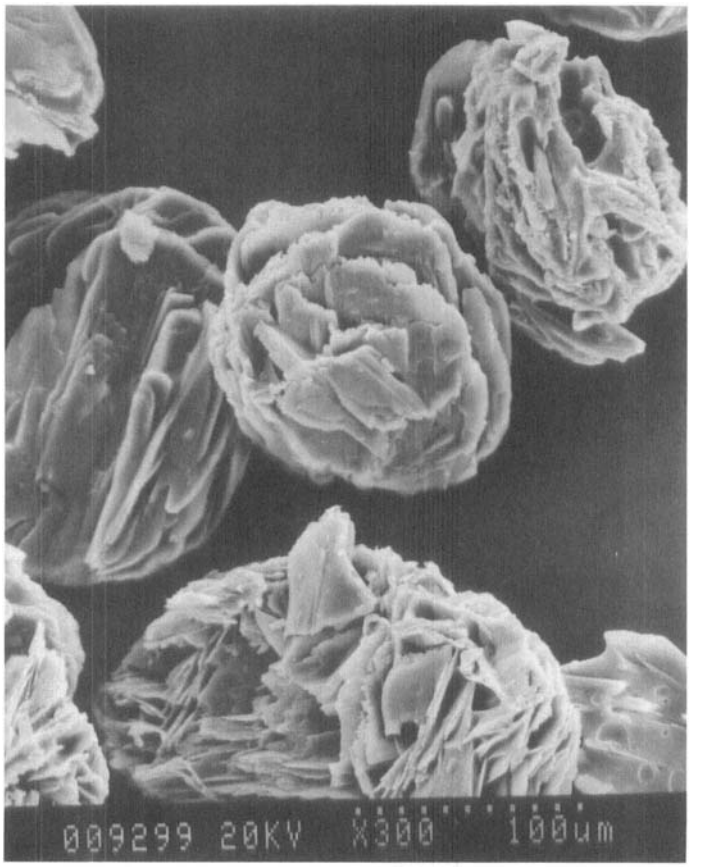

FIGURE 2
Photomicrograph of PLA 2,000 micromatrices containing 50% DHEA (w/w of PLA 2,000)

slightly higher. The % drug lost to the external phase at the high stirring speed ranged from 6.33% to 12.0% for drug loadings from 25% to 50% of total polymer weight. At the 10% loading, the drug loss was higher at 29%. The DHEA which partitioned in the aqueous phase crystallised out as fine needles.

TABLE 2
Data on DHEA micromatrices (MM) prepared with PLA of increasing molecular weight and DL-PGA 12,000.

DHEA/g of polymer(g)	solvent system	PLA mwt	shape of MM	DHEA in MM g/g of polymer	% drug l ost
0.10	1:1 A:C*	2,000	spherical	0.071	29.0
0.10	1:1 A:C	16,000	spherical	0.063	37.0
0.10	CH_2Cl_2**	DL-PGA	spherical	0.062	33.8
0.30	1:1 A:C	2,000	spherical	0.280	6.67
0.30	CH_2Cl_2	16,000	spherical	0.257	14.3
0.30	CH_2Cl_2	109,000	irregular	0.246	18.0
0.30	CH_2Cl_2	DL-PGA	irregular	0.194	35.5

*A:C = acetone: chloroform ** CH_2Cl_2 = methylene chloride

Micromatrices produced from polymers of increasing molecular weight and differing composition.

Micromatrices of 10% and 30% DHEA loading were prepared using PLA of nominal molecular weights 2,000, 16,000 and 109,000 and DL-PGA of nominal molecular weight 12,000. The characteristics of these systems are listed in table 2. At 10% DHEA loading, microspheres prepared using either PLA 2,000 or PLA 16,000 were spherical and had a wrinkled surface while with the DL-PGA, the surface was smooth. The drug lost to the external phase during the manufacture was high in all cases ranging from 29 to 37%. At the 30% drug loading, DHEA lost to the external phase was lower than at the 10% loading and showed increasing loss with increasing polymer molecular weight. The microparticles formed with the 30% loading were spherical when polymer molecular weight was increased to 16,000 but were irregular when PLA 109,000 (fig. 3) or DL-PGA was used.

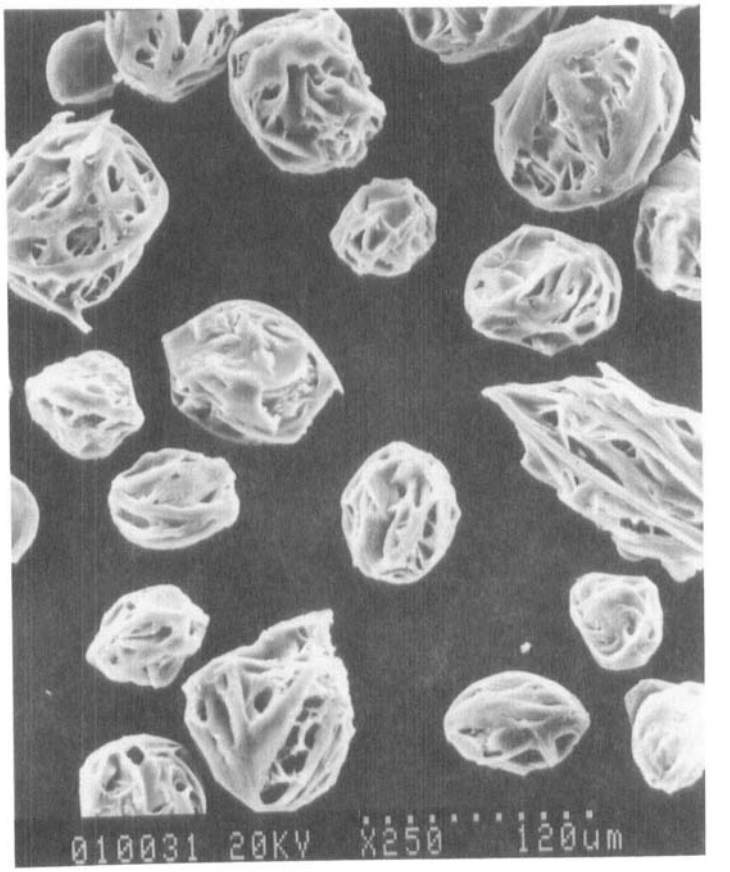

FIGURE 3
Photomicrograph of PLA 109,000 micromatrices containing 30% DHEA (w/w of PLA 109,000)

Characterisation of the DHEA present in the microspheres

The systems listed in table 1 were characterised by DSC. The DHEA used was crystalline with a melting point at 152.4°C, while PLA 2000 showed no detectable thermal event when heated from 30°C. The glass transition (T_g) temperature of PLA 2,000 lies in the range 5°C-25°C[10]. DSC thermograms of the samples listed in table 1 showed no detectable crystalline DHEA (figure 4). The endotherms observed at 70-80°C

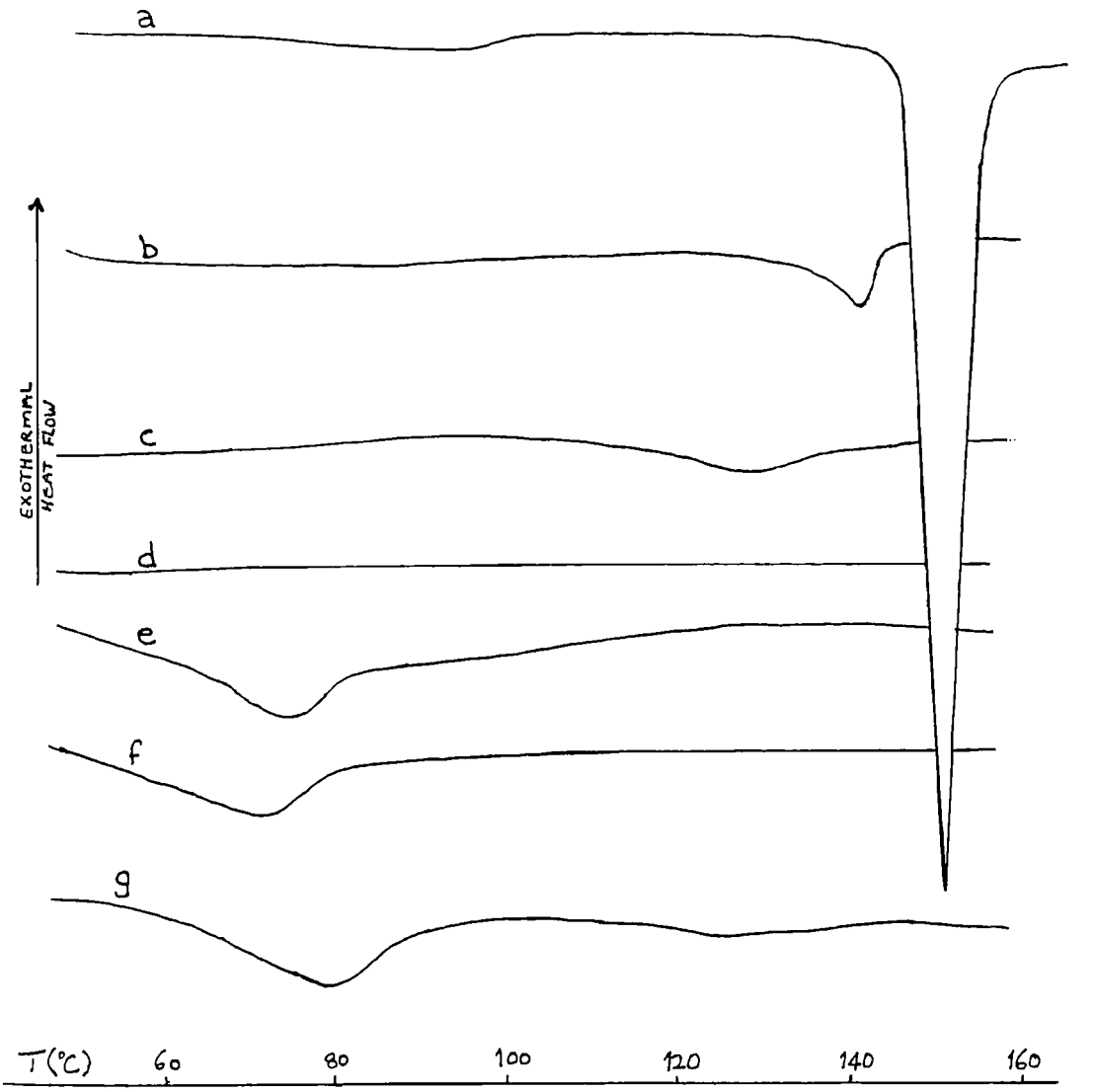

FIGURE 4

DSC thermograms of (a) DHEA starting material, mechanical mixes of DHEA and PLA 2,000 containing w/w (b) 50% DHEA (c) 30% DHEA, PLA 2,000 micromatrices containing (d) 10% DHEA (e) 25% DHEA (f) 30% DHEA and (g) 50% DHEA w/w of polymer.

disappears when samples are rescanned. These probably indicate the presence of residual solvent in the micromatrices. DSC scans of both a 30% and a 50% DHEA physical mixture in PLA 2000 showed an endotherm at 127.5°C and at 141.2°C respectively, suggesting the presence of crystalline DHEA. The x-ray diffraction patterns obtained from 30% DHEA micromatrices showed the presence of crystalline DHEA, not evident by DSC.

When the micromatrices were heated on the hot stage microscope, DHEA crystals were visible at temperatures ≥ 60°C, when the polymer had melted. Fig. 5 shows the DHEA crystals in a 30% DHEA in PLA 2000 micromatrix after being heated to 75°C and flash cooled to room temperature.

Micromatrices containing 30% and 50% w/w of PLA 2000 were scanned to 75°C by DSC and cooled to 50°C at a rate of 5°C/min. On rescanning to 180°C, an endotherm at 117-120°C was observed for the 30% samples while two endotherms at 125°C and at 135°C were observed. With pure DHEA an exotherm at 108.1°C and two endotherms at 140.6°C and 152.3°C were observed on rescanning, suggesting that DHEA exists in an amorphous state and at least two polymorphic forms. It is therefore possible that during microsphere formation, DHEA is partly solubilised in the polymer, and partly deposited in the micromatrices as both amorphous and crystalline domains. When the micromatrices are heated to 75°C, the solubilised and/or amorphous DHEA crystallise giving the thermal events detected at 120°C-135°C. On storage at room temperature for one year, the DHEA in the 50% micromatrices, but not in the 30% samples, crystallised out sufficiently giving an endotherm at 130°C. Both

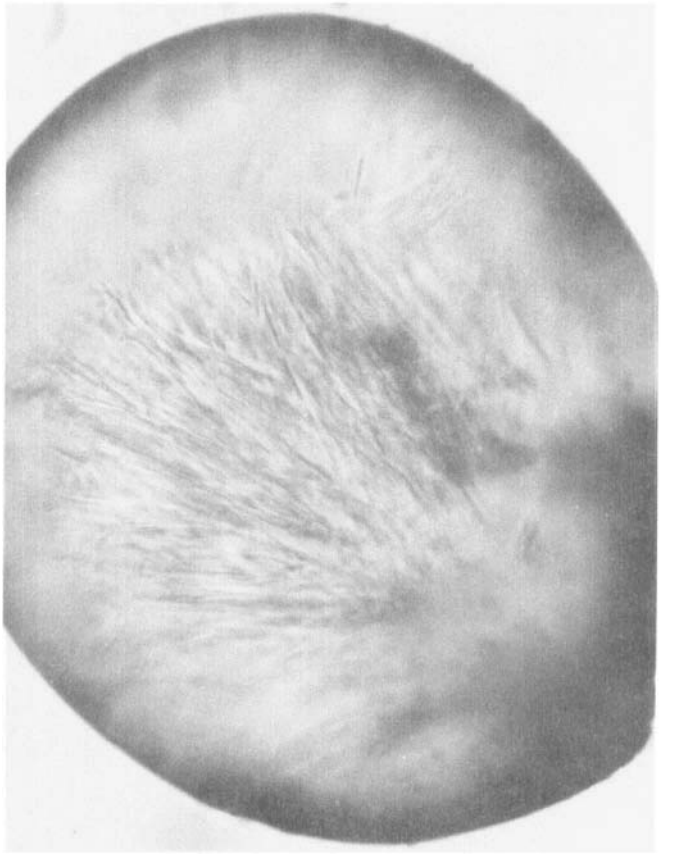

FIGURE 5
Photograph of a 30% DHEA in PLA 2,000 micromatrix after heating to 75°C and flashed cooled to room temperature (X 40).

differential thermal and x-ray diffraction analyses showed the absence of crystalline progesterone in progesterone-loaded (23%w/w) PLA microspheres formed by a solvent evaporation procedure[7]. In contrast hydrocortisone loaded (12.6% w/w) PLA microspheres were found to contain crystalline hydrocortisone as evidenced by both thermal and x-ray analyses[8].

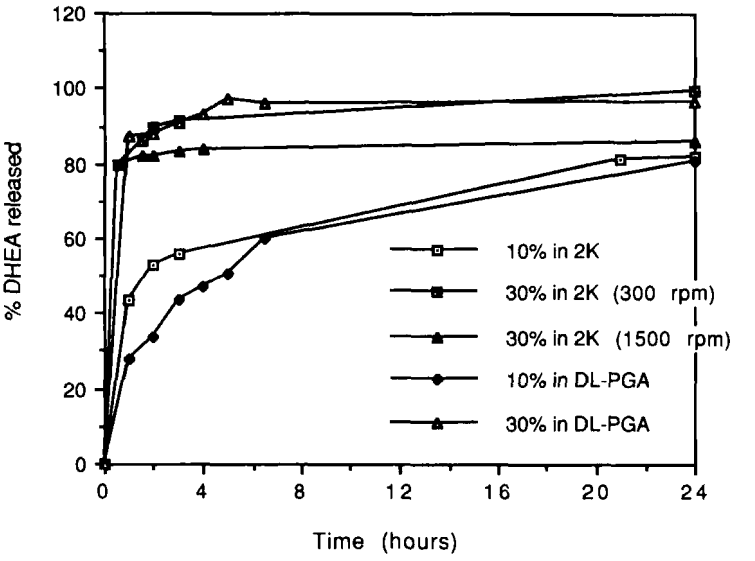

FIGURE 6
Release profiles of DHEA from DHEA-loaded PLA 2,000 and DL-PGA microspheres.

DSC analysis of the DHEA containing PLA micromatrices prepared in table 2 did not indicate the presence of crystalline DHEA. The presence of thermal events at 55°C and 57°C respectively were observed for the 30% DHEA microspheres in the 16,000 and 109,000 polymers representing the glass transition temperatures of the polymers. Glass transition temperatures of 57°C and 60°C were observed for the pure PLA 16,000 and PLA 109,000 respectively, i.e., the drug decreased the T_g of both polymers.

Release studies in 40% ethanol

DHEA release from the 10% and 30% microspheres were studied in 40% ethanol solution. The release profiles from the PLA 2,000 and DL-PGA microspheres are shown in figure 6. Increasing the drug

loading from 10% to 30% resulted in an increase in the percent DHEA released at each time interval. At the 30% loading, the DHEA release was similar from both systems, while at the 10% drug loading, DHEA release was slower initially from the DL-PGA micromatrices. DHEA release from the PLA 16,000 micromatrices were similar to the release from the PLA 2,000 systems.The DHEA release from the 30% DHEA micromatrices prepared at 1500 rpm was slower than from the corresponding microspheres prepared at 300 rpm. However DSC and x-ray diffraction analyses showed no differences between the two systems.

The dissolution of DHEA in 40% aqueous ethanol was therefore studied from compressed discs of pure DHEA, 30% DHEA in PLA 2,000 physical mixture and 30% DHEA in PLA 2,000 microspheres prepared at stirring rates of 300 and 1500 rpm (figure 7). The dissolution profile of pure DHEA was linear indicating a stable polymorphic form and sink conditions. Drug release from discs containing PLA was greatly reduced, the rates decreasing with time. Initial release was greater from compacts made from microspheres while at later times release fell below that of the physical mixture. The release from compacts of microspheres prepared at the lower stirring speed (300 rpm) was higher, particularly at early times than from compacts of microspheres prepared at 1500 rpm. This was consistent with the release from the uncompressed microspheres. The release profiles were fitted to the equation[11]:

$$W = A \times T^N$$

where W is the weight of drug dissolved, A and N are constants and T is the elapsed time. A value of N = 0.5 is indicative of matrix controlled

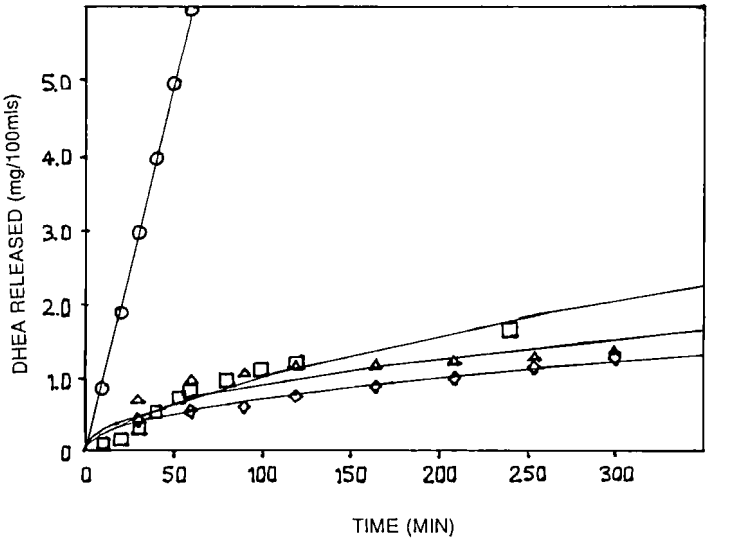

FIGURE 7
Dissolution profiles of DHEA from compressed discs of (O) DHEA (□) 30% DHEA in PLA 2,000 mechanical mix, PLA 2,000 micromatrices containing 30% DHEA prepared using 1:1 acetone:chloroform at (▲) 300 rpm and (◊) 1500 rpm.

release from a planar surface. The amount of DHEA released versus the square root of time plots gave reasonably linear fits for the physical mix and the microspheres prepared at 1500 rpm.

The best estimates of N by nonlinear least squares were 0.66, 0.53 and 0.244 for the physical mixture, microspheres prepared at 1500 rpm and microspheres prepared at 300 rpm respectively. Early time points for the 300 rpm product were systematically above the fitted curve (fig. 7) and the value of A was higher than for either of the other two systems. The constant A in matrix release is dependent on the drug loading, the solubility of the drug, the porosity and tortuosity of the matrix system. Higher initial release and more curved profile would be consistent with a higher energy form of the drug in the microspheres. X-ray diffraction

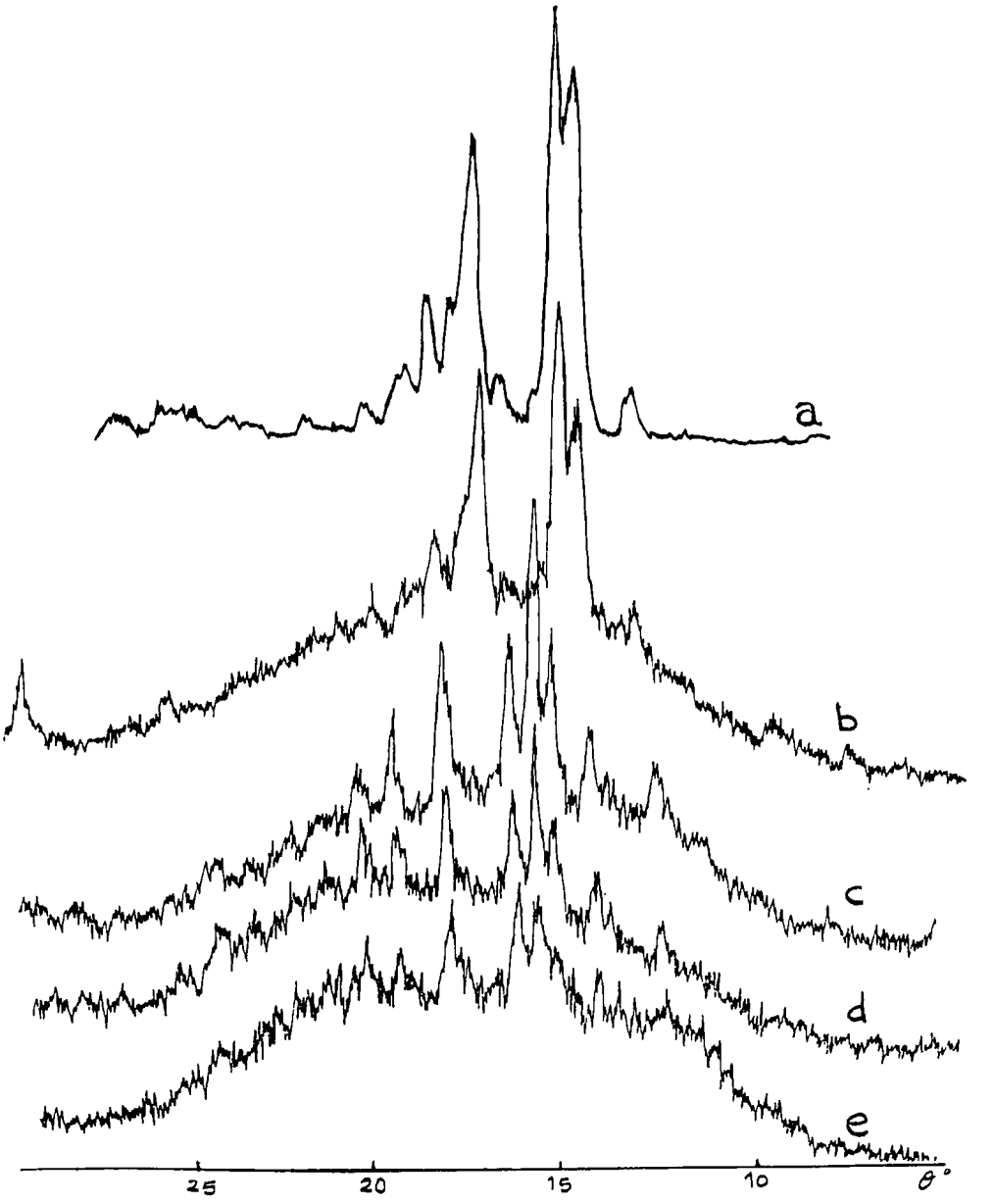

FIGURE 8
X-ray diffraction patterns of (a) DHEA, 30% DHEA in PLA 2,000 (b) mechanical mixture (c) micromatrices prepared at 1500 rpm and the surface of compressed discs of 30% DHEA in PLA 2,000 micromatrices prepared at (d) 1500 rpm and (e) 300 rpm, both after dissolution in 40% aqueous ethanol for 5 hours.

analysis revealed that the microspheres contained a different crystal form than that of the pure drug or the physical mixture (figure 8). Furthermore the scan of the microspheres showed that the 300rpm sample appeared to be the least crystalline. Thus the differences in release obtained between the 300 rpm and 1500 rpm samples are likely a consequence of the different proportions of the higher energy form(s) of the drug present in the systems and/or a difference in tortuosity, both of which in turn are functions of the processing variables used in microsphere production.

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