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INVESTIGATION OF PROGESTERONE LOADED POLY(*D,L*-LACTIDE) MICROSPHERES USING TMDSC, SEM AND PXRD

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Abstract

Poly(*d,l*-lactide) microspheres with progesterone loadings of 0, 10, 20, 30 and 50% w/w were manufactured using an interrupted solvent evaporation process. Spherical microspheres with loadings close to the theoretical values were produced. The glass transition of the polymer could be identified by a step change in the heat capacity measured by TMDSC. Progesterone was found to plasticise the glass transition temperature at contents of 20% w/w or less. At a 30% loading, cold crystallisation of progesterone was seen indicating that an amorphous form of the drug was present; these microspheres were found to exhibit a pitted surface. TMDSC of the 50% progesterone samples suggested that most of the drug was present as crystals. This was supported by the SEM and PXRD results.

Keywords: poly(*d,l*-lactide), progesterone, PXRD, SEM, TMDSC

Introduction

Temperature modulated differential scanning calorimetry (TMDSC) is a development of conventional DSC that uses a sinusoidally oscillating temperature programme [1]. Benefits of this technique have been shown to include separation of overlapping thermal transitions, improved identification of thermal processes and more accurate measurement of heat capacity [2–4]. In this study, the tech-

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nique has been used for the characterisation of a polymer of pharmaceutical significance. In recent years there has been much interest in the use of drug loaded polymer microspheres as a mechanism for controlled release. Microspheres can be delivered to the site of interest where they will release the drug over an extended period of time. Polylactide microspheres have received particular attention because of the biodegradable, biocompatible and non-toxic nature of this polymer [5]. In the body, the polymer degrades into lactic acid, which can then be metabolised. The gradual erosion of the polymer allows the drug to be released. Polylactide has been used as a matrix for delivery of compounds such as anticancer agents and vaccines [6, 7]. In this study, progesterone has been used as a model drug in the production of poly(*d,l*-lactide) microspheres with a range of drug loadings. It has previously been found that the physical form of progesterone in microspheres changes with drug loading [8, 9]. Hence, it is important to characterise any such changes as the release rate, and bioavailability, of the drug will be affected by its physical state. Any changes to the form of the drug may also influence the stability of the product. In this study, the morphology of progesterone loaded microspheres has been investigated by TMDSC. The results are supported by data from scanning electron microscopy (SEM) and powder X-ray diffraction (PXRD).

Experimental details

Materials

Poly(*d,l*-lactide) of M 99800 and progesterone (minimum 99%) were supplied by Sigma (Poole, UK). Dichloromethane, PVA (98% hydrolysed, M 22000) and Tween 80 were supplied by BDH (Poole, UK). Ethanol was laboratory grade. Indium (99.999%), tin (99.999%) and aluminium oxide (99.9%) were supplied by Aldrich (Gillingham, Dorset, UK). Cyclohexane (99.9%) and *n*-octadecane (99.9%) were supplied by Reidel-de Haen (Seelze, Germany).

Preparation of microspheres

Progesterone loaded microspheres were manufactured using a process based on the solvent evaporation method of Benita *et al.* and Benoit *et al.* [8 ,9]. The *d,l*-PLA and progesterone were dissolved in 10 mL of dichloromethane. Progesterone contents of 0, 10, 20, 30 and 50% w/w were used, the total mass of *d,l*-PLA and drug being 500 mg in each case. The solutions were poured into 125 mL of water containing 2% PVA. The resulting emulsion was stirred at a constant rate at ambient temperature and pressure, to allow evaporation of the dichloromethane and formation of microspheres. The process was interrupted after 4 h and the aqueous phase containing PVA was replaced with water to reduce the formation of free drug crystals in the aqueous phase. The solvent evaporation was

then allowed to continue for further 16 h. The microspheres were collected by filtration, washed with water and, once dry, were sieved using a 250 μm sieve. They were then further dried at 200 mbar and 30°C for 88 h to remove any residual solvent. The microspheres were then stored at 20°C in a desiccator containing diphosphorus pentaoxide until characterisation. The yield of microspheres was determined by dividing the mass of the sample after sieving by the mass of raw materials. Particle size was measured using a laser diffractometer (Malvern Particle Sizer, 2600c, Malvern Instrument Ltd. Malvern, UK). For each mean particle size and span measurement, 15 mg of microspheres were suspended in 13 mL of an aqueous solution containing 0.1% Tween.

Determination of progesterone content

Progesterone loaded microspheres (5 mg) were dissolved in 0.8 mL of dichloromethane. The solution was then made up to a 10 mL volume with ethanol to precipitate the polymer. The resulting suspension was centrifuged at 10000 rpm for 10 min (J2-HS centrifuge, Beckman-RIIC Ltd, High Wycombe, UK). The supernatent was then analysed using a UV spectrophotometer to measure the absorbance at 249 nm (UV-VIS spectrophotometer 554, Perkin Elmer Ltd., Beaconsfield, UK). The progesterone content was then calculated using a progesterone calibration curve.

TMDSC, TG, SEM and PXRD methods

TMDSC analyses were made using a TA Instruments MDSC 2920 with refrigerated cooling system (RCS) (TA Instruments, Leatherhead, UK). The DSC cell was purged with 30 mL min^{-1} nitrogen and the RCS was purged with 150 mL min^{-1} nitrogen or helium as required. The instrument was calibrated for temperature using cyclohexane, *n*-octadecane, indium and tin. Indium was used to calibrate enthalpy values. A one point heat capacity calibration constant was determined using aluminium oxide measured at 45°C. This temperature was chosen as it was the centre of the region of interest for the heat capacity signal. Progesterone was analysed as received using conventional DSC with a heating rate of 1°C min^{-1}. The *d,l*-PLA raw material and microsphere samples were analysed using a heating rate of 1°C min^{-1}, a modulation period of 40 s and a modulation amplitude of ±0.5°C which should result in heating with some cooling. Hermetic pans (Perkin Elmer, Beaconsfield, UK) were used; the sample and reference pans being matched by mass to within ±0.02 mg for each experiment. Small sample masses of between 1.880 and 3.968 mg were used to prevent thermal lags developing in the sample. All temperature, enthalpy and heat capacity values presented are the average of three experiments unless otherwise stated.

The mass loss on heating was determined using thermogravimetry (TGA 2950, TA Instruments, Leatherhead, UK). The TGA was calibrated for tempera-

ture using indium and for mass using the manufacturer's instructions. The sample was heated in open pans, using a nitrogen purge and a heating rate of 10°C min^{-1}. The particles were examined using a scanning electron microscope (SEM XL20, Philips Electronoptics, Eindhoven, Netherlands). X-ray analyses were performed on the 20, 30 and 50% w/w microspheres and on the pure progesterone sample. Powder diffraction patterns were taken using a Siemens D500 diffractometer employing the Bragg Brentano geometry. The instrument was fitted with a pre-sample monochromator and a scintillation counter. The radiation was $CuK_{\alpha 1}$ (λ=1.5406 Å). The incident and receiving slits were 0.3 and 0.05° respectively. Data were collected over the range 2θ range, 3 to 35° using a step size of 0.05° and a counting time of 10 s per step. Samples were presented to the beam in a flat, silicon wafer sample holder.

Results and discussion

Table 1 details the yield, drug content, particle size and the mass loss on heating for each sample. The yield of microspheres was calculated as the mass after sieving divided by the mass of raw materials and was found to be over 77% in each case. The drug loadings were found to be close to the theoretical values indicating that the manufacturing process had successfully prevented the formation of free drug crystals. In some cases the loading was slightly larger then the expected values. This could be explained by assuming that the small amount of raw material lost during the manufacturing process was mainly polymer. Particle size analysis showed that most microspheres were in the size range of 100 to 130 μm. There was no trend in particle size with drug loading. Thermogravimetric measurements on heating produced mass losses of 0.6% or less. Although the TG has shown that the mass loss was small, it can not determine whether the loss was due to residual dichloromethane or water.

Table 1 Results of analysing microsphere samples for drug content, yield, particle size and TG mass loss

Progestrerone loading/	Drug content[a]/	Yield/	Particle size[b]/	Span	TG mass loss[c]/
% w/w		%	μm		%
0	–	80.8	114.5±3.0	0.53±0.02	0.6±0.1
10	11.0±0.2	77.4	111.1±1.6	0.53±0.04	0.5±0.1
20	19.6±0.5	84.2	111.5±2.3	0.50±0.01	0.4±0.1
30	30.1±0.2	89.0	126.2±1.4	0.50±0.08	0.4±0.1
50	51.3±0.5	83.6	104.1±2.6	0.53±0.08	0.4±0.1

[a] Measured by UV spectroscopy (n=20), [b] D[v, 0.5] (n=9), [c] Measured from ambient temperature to 120°C (n=6)

The conventional DSC analysis of the progesterone, run as received, showed a narrow endotherm with a peak temperature of 130.3±0.3°C and enthalpy of 83.5±1.1 J g^{-1} (n=4). The temperature corresponds well with the melting range of 127 to 130 °C given in the Merck index for the melting temperature of the α-form of progesterone [10]. The enthalpy is close to the value of 80 J g^{-1} (using the conversion 1 cal=4.1868 J [11]) given by Theeuwes *et al.* [12]. The *d,l*-PLA was analysed using TMDSC and produced the total heat flow, heat capacity and phase information shown in Fig. 1a. The total heat flow signal is equivalent to the heat flow produced by a standard DSC method at the same underlying heating rate. For the *d,l*-PLA sample the total heat flow shows an endotherm with an onset of 43.6 ±0.1°C. This transition can be identified as the glass transition of the polymer by the presence of a step change in the heat capacity signal, which had an onset at 44.4±0.1°C. The ability of TMDSC to separate the step in heat capacity

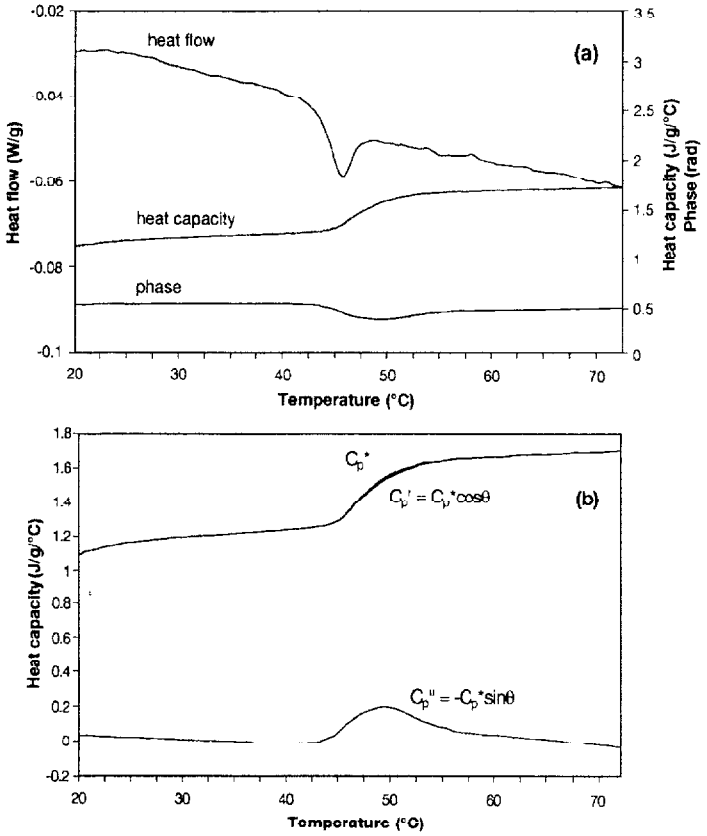

Fig. 1 (a) Total heat flow, complex heat capacity and phase lag, θ, measured by TMDSC for poly(*d,l*-lactide) as supplied (b) Results of phase correction of the complex heat capacity, where C_p^*=complex heat capacity, C_p'=reversing heat capacity and C_p''=kinetic heat capacity

from any associated endotherm makes the technique very useful for the study of pharmaceutical materials. Samples of pharmaceutical interest often contain a range of substances such as drugs, excipients, lubricants etc. and hence can yield complex thermal DSC profiles. By clearly identifying glass transitions, TMDSC offers the opportunity to make more accurate interpretation of such traces. The glass transition measured for *d,l*-PLA was lower than many other reported values which typically range from 50 to 57°C [13–15]. However, the underlying heating rate used in this study was much slower than the usual 10 to 20°C min^{-1} used in conventional DSC experiments and this would cause a lower transition temperature to be measured.

The phase lag signal, θ, measures the lag between the modulated heat flow and heating rate signals and can be used to determine when there is a kinetic component to the heat capacity signal. When this is observed, the phase lag is used to separate the measured (complex) heat capacity into the in and out-of phase components. The in-phase component, C_p', is termed the real or reversing heat capacity, while the out-of phase component, C_p'', is thought to relate to kinetic processes [16]. Figure 1b shows the phase corrected heat capacity for the *d,l*-PLA raw material. The reversing (in-phase) heat capacity was found to be almost identical to the complex heat capacity in the glass transition region. Thus, in this instance the complex heat capacity can be used directly for characterisation of the samples without this extra deconvolution step. The similarity of the complex and in-phase heat capacities during the glass transition has been previously noted for a spray dried lactose sample [17].

Figures 2, 3 and 4 show the total heat flow, complex heat capacity and phase lag for each of the microsphere samples. The curves have been separated on the y-axis in each figure to aid presentation. The drug free microspheres showed an endotherm in the total heat flow at 46.2±0.5°C. The onset in the heat capacity signal was 48.3±0.3°C. The higher temperatures measured for the microspheres compared to the raw material indicate that the manufacturing process influences the physical properties of the polymer.

As the drug loading was increased to 10 and 20%, the onset of the change in the heat capacity was seen to decrease to 42.3±1.1°C and 38.5±1.3°C, respectively. The absence of any melting peak indicates that no progesterone crystals were detected within the microspheres by this method. It has been suggested by Benoît *et al.* that progesterone has little mutual miscibility with PLA [18]. This was based on the evidence that 23% w/w progesterone loaded microspheres contained crystalline progesterone after heat treatment at 110°C. Such results suggest that the progesterone was initially trapped as a metastable molecular dispersion rather than as a true solid solution [19]. It is possible to trap a drug with little mutual miscibility with the polymer as a metastable molecular dispersion due to the nature of the evaporation process [20]. However, in this study, the plasticisation of the polymer glass would indicate that the progesterone is present as a true molecular dispersion or solid solution for loadings of 20% or less. This distinc-

tion in molecular dispersion types is extremely important for pharmaceutical formulations; a drug in a metastable state may exhibit low stability over a long time period, which could lead to problems maintaining the 2–3 years shelf life required for most pharmaceutical products. Any processing of the sample that included heating above the glass transition temperature of the polymer could allow the drug to crystallise. This would then cause changes to many important sample properties such as release rate, flow and compaction properties.

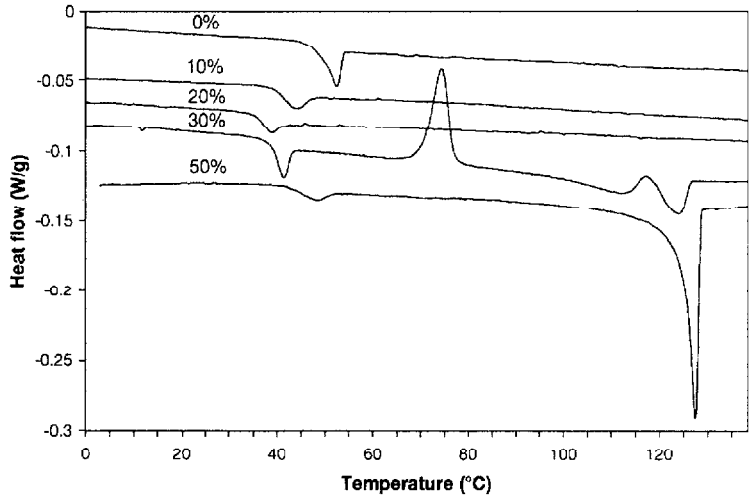

Fig. 2 Total heat flow measured by TMDSC for microspheres at each drug loading

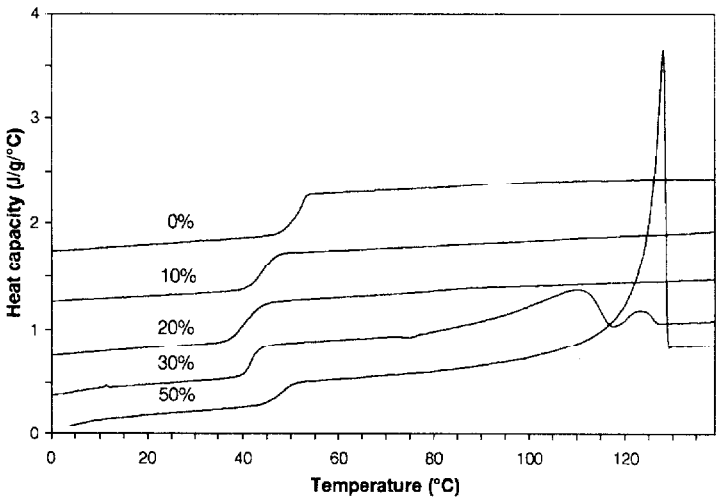

Fig. 3 Complex heat capacity measured by TMDSC for microspheres at each drug loading

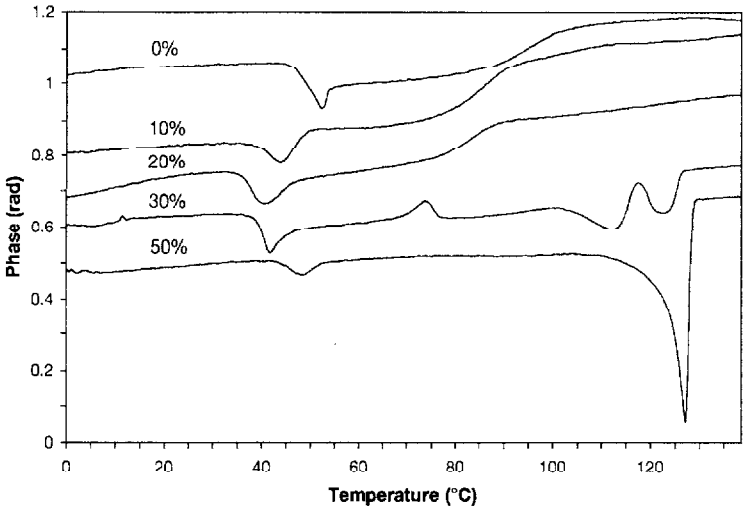

Fig. 4 Phase lag measured by TMDSC for microspheres at each drug loading

When the loading is increased to 30%, there is no further decrease in the glass transition temperature. As the sample is heated, an exothermic peak is observed in the total heat flow with an onset at 70.4±0.1°C. This would suggest that there is limit to the miscibility of progesterone with PLA, which is exceeded at a loading of 30%. The excess drug does not further plasticise the polymer but is present in a different amorphous form that crystallises on heating. The sample then shows two melting transitions. The first is a broad transition which begins soon after the cold crystallisation exotherm and has a peak temperature of 112.0±0.6°C. The second has a peak temperature of 123.9±0.1°C. The peak temperature has been used to characterise the endotherms as a gradual onset of melting (as seen in the first melt) can make determination of the onset temperature difficult. It was found that the combined enthalpies of the two melting endotherms was similar to that of the crystallisation peak, suggesting that there was little initial crystallinity within the sample. However, it is not clear why the amorphous drug should crystallise into two forms with distinct melting points. The lower temperature endotherm is significantly lower than that expected for the β-form, which melts at 121°C [10]. It is perhaps possible that some interaction between the crystals and polymer is causing this effect. The second endotherm was slightly lower in temperature than the pure α-form. This could be due to the presence of the polymer as an impurity in the crystals or to the influence of some molten drug being present as a result of the less stable drug crystals melting prior to this temperature.

At a loading of 50% drug, there is now little evidence of plasticisation of the glass transition and no exotherm is seen. The total heat flow signal shows an endotherm with a peak temperature of 127.9±0.4°C and an enthalpy of 40.5±1.5 J g^{-1}. These

results would suggest that little, if any, of the drug is present as a molecular dispersion within the polymer but is mainly present as crystals of the α-form.

Figure 4 shows the phase lag signals for each of the experiments shown in Figs 2 and 3. It was seen that any peaks are small and so would have a negligible effect during the glass transition region. However, the phase change was larger during the melting endotherms, particularly for the 50% sample. In these cases the complex heat capacity includes a kinetic component, the interpretation of which is not clear at present. It was also noted that the 0, 10 and 20% samples showed a baseline step change in phase lag between 70 and 100°C. It is possible that at these temperatures the polymer has softened to such an extent that the microspheres can coalesce. The increased thermal contact between the particles would change the lag between the applied heating rate signal and measured heat flow signal. This affect of sample flow on the phase signal has been seen in polystyrene powder by Schaap [21].

SEM showed that all the samples formed good spheres. The 0, 10 and 20% samples were seen to have smooth surfaces. However, the 30 and 50% w/w microspheres showed rough surfaces at high magnifications (Fig. 5). The 30% sample revealed a pitted surface while the 50% sample showed overlapping scales. Powder X-ray diffraction of the 50% sample showed a diffraction pattern similar to that seen for the pure crystalline drug (Fig. 6a and b). This would suggest that the particles seen on the sphere surface could be due to progesterone crystals.

The TMDSC analyse of this sample had also indicated that progesterone crystals were present. Such surface crystals have been previously reported for high progesterone loadings (68.3%) in other studies [8, 9]. The reason for the formation of the pitted surface seen on the 30% microspheres is not clear at present but similar dimpled or pitted surfaces have also been reported for poly(*d,l*-lactide-co-glycolide) microspheres containing 35% progesterone and quinidine-loaded *d,l*-PLA microspheres prepared at pH 12 [22, 23]. The 20 and 30% samples showed no Bragg diffraction but only a broad diffuse peak in the diffraction profile indicating the absence of any significant crystallinity (Figs 6c and d) which was in agreement with the results of the TMDSC analyses.

The surface effects seen in the SEM for the 30 and 50% microspheres could also provide an explanation for the lack of phase change in the 70 to 100°C temperature range, as was seen for the 0 to 20% drug loaded samples (Fig. 4). It is possible that the surface characteristics prevent increased thermal contact until after the progesterone has melted. A step change is seen in the phase signal during the melting regions. It is also interesting to note that the crystallisation temperature of the amorphous progesterone in the 30% sample coincides with onset of the step in phase seen for the lower loading samples. This could indicate that it is not until the polymer is soft enough to flow that the progesterone can nucleate and crystallise. The possible use of the phase signal to monitor changes in the rheology of the samples warrants further investigation.

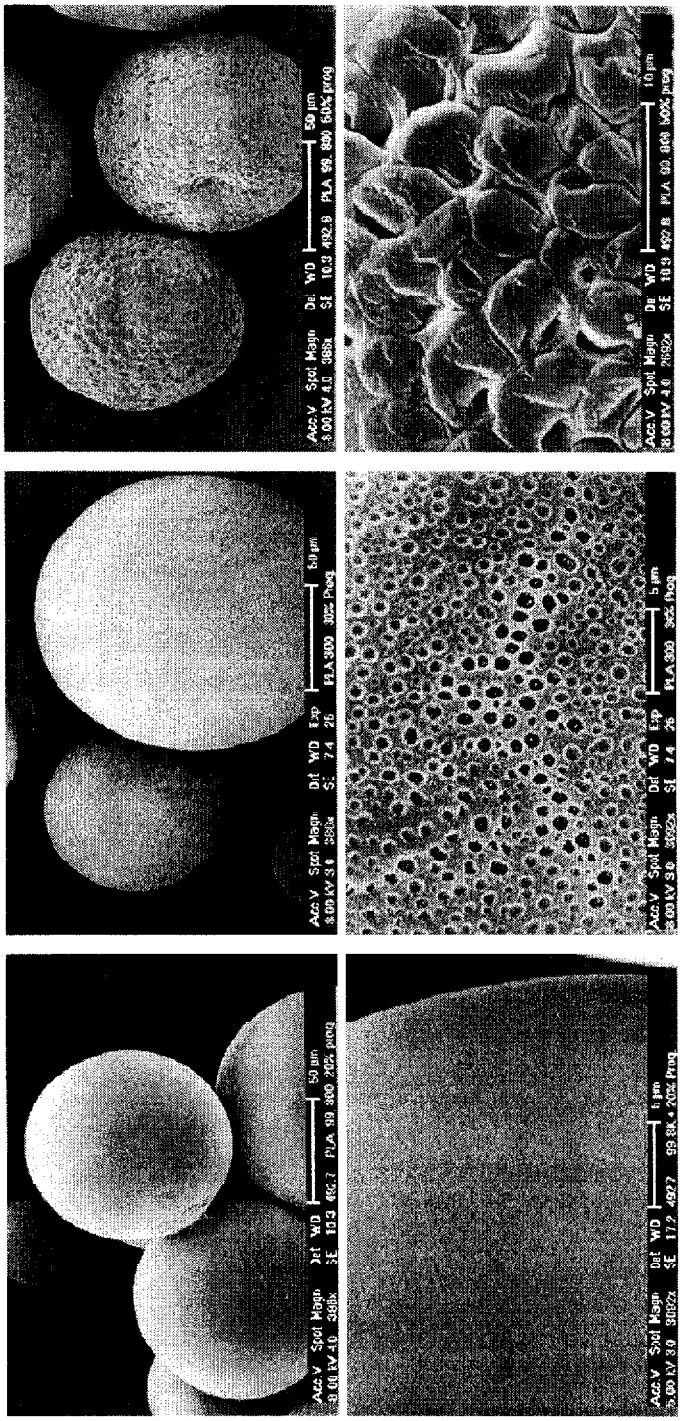

Fig. 5 SEM micrographs for progesterone loadings of: (a) 20 (b) 30 (c) 50%. Two magnifications are shown for each loading

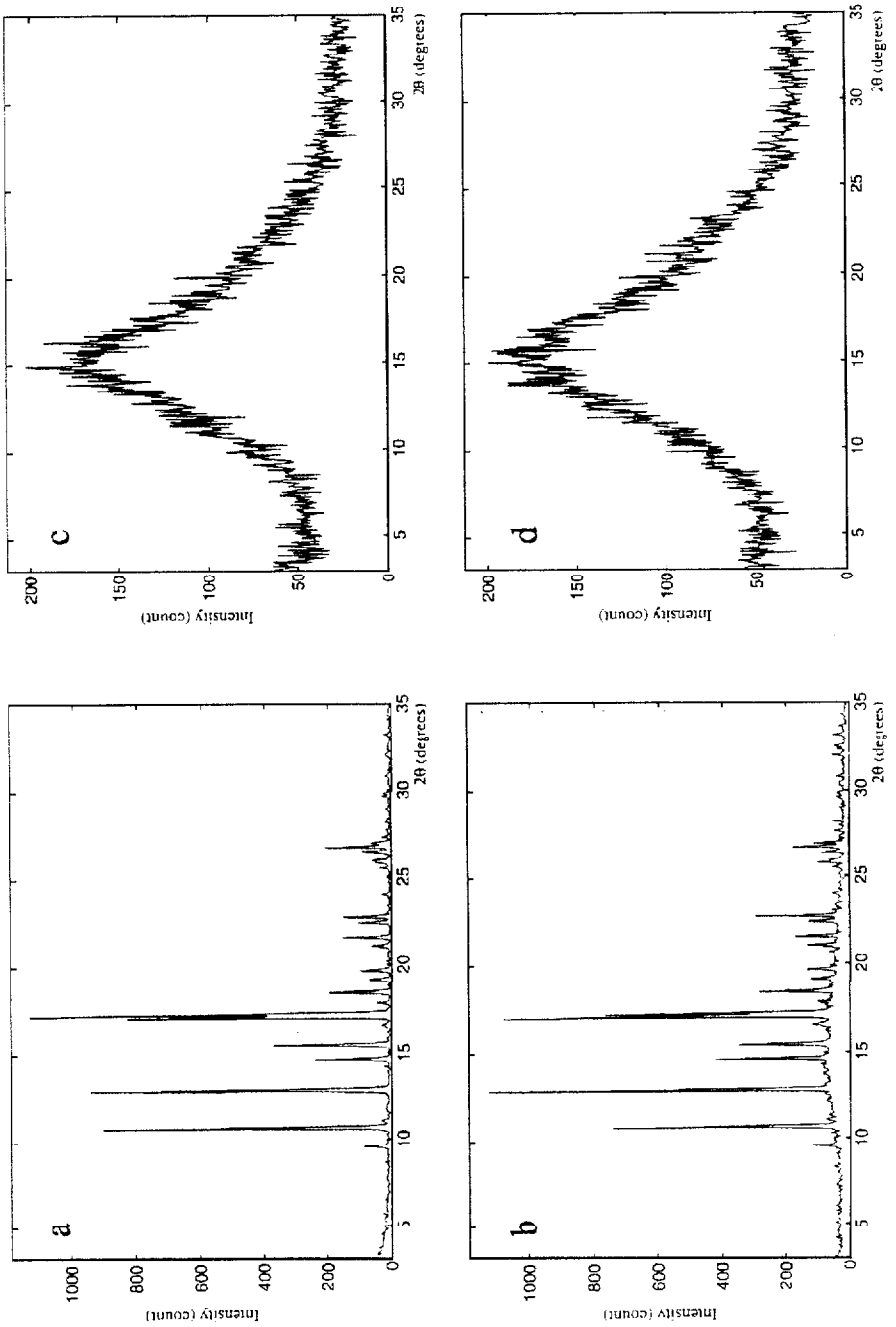

Fig. 6 Powder X-ray diffraction patterns for (a) pure progesterone and (b) 50 (c) 30 (d) 20% progesterone microspheres. λ=1.5406Å

Conclusions

A combination of TMDSC, SEM and PXRD was used to characterise the physical form of progesterone in *d,l*-PLA microspheres. TMDSC allowed the plasticisation of the polymer glass transition at low drug loadings (10 and 20%) to be measured. Such interactions between the drug and polymer indicate that the progesterone is present as a solid solution. At intermediate loadings (30%) the drug was found to crystallise on heating. This is evidence that progesterone has a limited solubility in *d,l*-PLA which can be exceeded at levels of more than 30% drug. PXRD confirmed the initially amorphous nature of the progesterone at this loading. At a 50% loading, all three techniques indicated the presence of progesterone crystals. SEM showed that the solvent evaporation method produced spherical microspheres at all drug loadings. The 30% progesterone microspheres had a pitted surface and at 50% loading drug crystals could be seen. The phase signal produced by the TMDSC technique has also provided some interesting information on the behaviour of the samples on heating and may provide a method for observing softening of the microspheres. The investigation of these samples has shown that TMDSC is a useful tool for characterising the physical form of progesterone in drug loaded polymer microspheres.

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