An Investigation into the Surface Deposition of Progesterone on Poly (d, l-) Lactic Acid Microspheres Using Micro-Thermal Analysis

Paul G. Royall,[1,2] Vivienne L. Hill,[1] Duncan Q. M. Craig,[1,3] Duncan M. Price,[1] and Mike Reading[1]

Received September 12, 2000; accepted December 12, 2000

KEY WORDS: atomic force microscopy; microspheres; micro-thermal analysis; modulated temperature DSC; poly(d,l-lactide); progesterone.

INTRODUCTION

Polylactic acid microspheres are well established as delivery vehicles for a range of low molecular weight and proteinaceous drugs (1–3). However, there is arguably a paucity of information available regarding the physical characteristics of these spheres, with few studies (4) addressing the issue of the manner in which drugs are distributed through the polymeric matrix. Following earlier studies (5,6) we recently investigated the use of modulated temperature DSC (MTDSC) as a means of monitoring the distribution of progesterone within poly(d,l-lactide) spheres using a range of loadings up to 50% w/w (7). By monitoring the thermal response of the spheres we obtained strong evidence for the drug being present as a separate amorphous phase at 30% w/w loading and as a distinct crystalline phase at 50% w/w loading. Complementary SEM studies showed marked changes in the surface morphology of the spheres at these two concentrations, leading to the suggestion of surface drug deposition. However, it was not possible at that stage to definitively establish the location of the drug using the methodologies available.

A recent advance within the thermal analysis field has been the introduction of micro-thermal analysis (micro-TA, 8–10). This method is based on the same principles as conventional AFM but involves the replacement of the probe tip with a Wollaston wire loop. The apex of the wire is etched away to leave the silver filament exposed, resulting in a higher resistance in this section of the tip. Application of a voltage therefore results in Joule heating, hence one may apply a thermal signal to highly specific regions of a sample. In addition, the technique allows isothermal measurement of thermal conductivity by rastering over the surface and measuring the tip resistance. The technique has attracted considerable interest, particularly within the polymer science field, as it represents a unique method of performing thermal analysis on highly specific regions of a sample without the necessity of heating the material in its entirety. Early studies on pharmaceutical materials have also yielded highly encouraging results (11,12). The ability of micro-TA to perform localised thermal analysis suggests that the method may be a means of establishing whether the progesterone is indeed present on the surface of the aforementioned microspheres. The objective of the present study was therefore to investigate the use of micro-TA as a novel means of characterising the surface of the PLA microspheres, with a particular view to obtaining further evidence for the presence or absence of progesterone on the exterior of the spheres.

Methods

Poly(d,l-lactide) microspheres containing 0%, 30% and 50% w/w progesterone were manufactured using the procedure and materials outlined in an earlier investigation (7). Topographical and surface thermal measurements were conducted using a 2990 Micro-Thermal Analyzer (TA Instruments Inc., New Castle, DE). Calibration was performed using benzoic acid by melting and recrystallizing the material on a glass slide to produce an homogeneous flat surface. The probe was placed onto the surface of the benzoic acid and an initial current of 10nA passed through the Z-axis piezo. The probe was heated at 20°C/s with a temperature modulation of 2°C and a frequency of 5Hz. The downward probe deflection, representing the melting of the sample, was taken as the calibration point (122.2°C).

Isothermal sample measurement took place by placing the spheres on double-sided adhesive tape on a microscope slide. The probe was lowered onto the surface of a sphere to a height of 1-2 Angstrom above the surface, with this distance being kept constant via a Z-axis piezo feedback loop. For isothermal imaging a force equivalent to 10nA of sensor deflection (approximately 100nN) was applied and the tip rastered over an area of 20μm × 20μm using a rate of 50μm/s with a resolution of 300 lines. Localised thermal analysis experiments were performed using the same protocol as that described for the calibration. Once clear of the sample, the tip was heated to 450°C to burn off any material that may have adhered to the probe. Each experiment was repeated at least six times. HSM studies were conducted using an Olympus BX50 microscope fitted with a Mettler hot stage. A scanning rate of 1°C/minute was used for each study, with indium being used to check the calibration.

RESULTS

The surface topographies for the spheres containing 0, 30 and 50% w/w progesterone are shown in Figure 1. Examination of the topologies indicate a similar trend to that observed using SEM (7). In the earlier study, the unloaded spheres had smooth surfaces, the 30% spheres showed a 'honeycomb' surface structure which coincided with evidence from the MTDSC studies for the drug being present in an amorphous form, while the 50% spheres had a 'plate-like' surface struc-

[1] The School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK 1. IPTME, Loughborough University, Loughborough LE11 3TU, UK.

[2] Present addresses: a. Department of Pharmacy, School of Health and Life Sciences, King's College London, Franklin-Wilkins Building, 150 Stanford Street, London SE1 8WA; b. Imperial College of Science & Technology, Exhibition Road, London, SW7 2AZ, UK.

[3] To whom correspondence should be addressed. (e-mail: duncan.craig@qub. ac. uk)

0724-8741/01/0300-0294$19.50/0 © 2001 Plenum Publishing Corporation

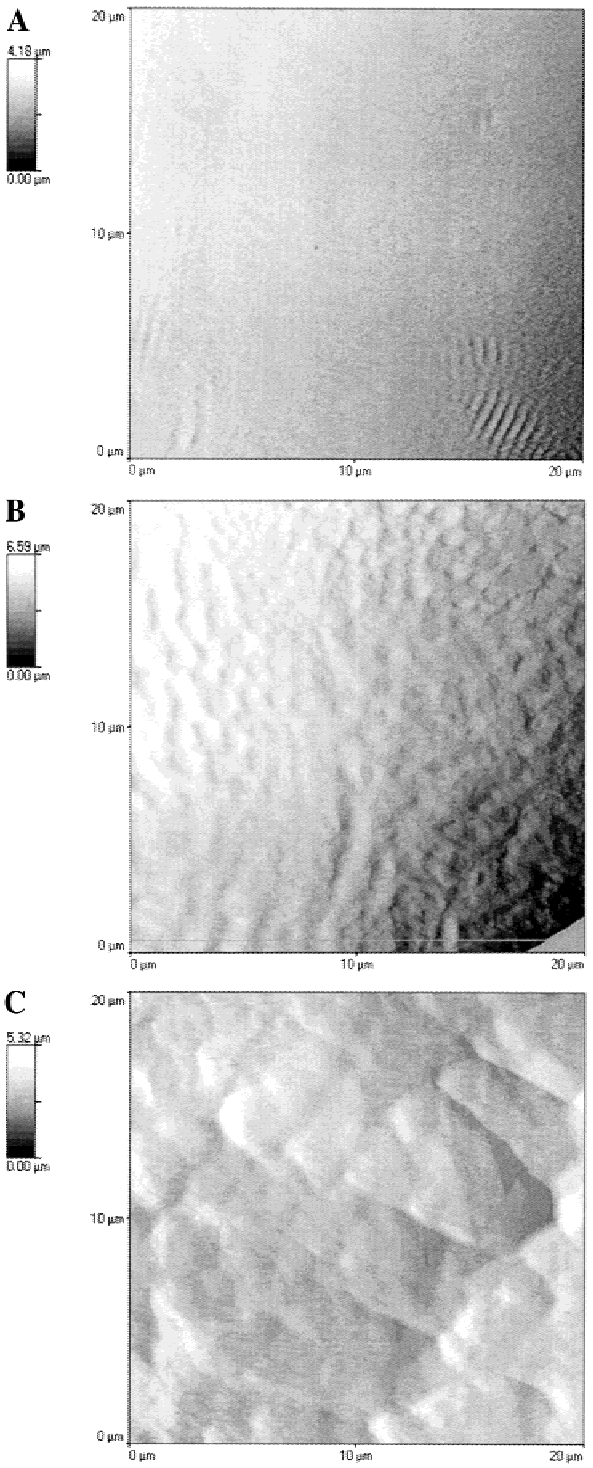

Fig. 1. Surface topologies of polylactic acid microspheres containing a) 0% w/w, b) 30% w/w and c) 50% w/w progesterone.

ture, with MTDSC studies indicating that the drug was present in a crystalline form at this concentration. Inspection of the thermal conductivity profiles indicated that these images followed the surface topographies (data not shown). This phenomenon of the conductivity being a mirror of the topography reflects the change in the quantity of sample immediately adjacent to the tip on moving the probe up or down a 'slope' on the sample surface, hence any observed apparent changes in thermal conductivity do not necessarily reflect changes in the fundamental physical or chemical structure of a sample other than surface roughness. The topography dependance of the measured conductivity is a major issue within the field and is the subject of a separate study by our group (12).

Figure 2 shows the localised thermal data for the three systems. For the 50% w/w drug loaded spheres, a clear discontinuity in sensor position is seen at 124 ± 2°C, indicating that sensor penetration increases dramatically at this temperature, which corresponds well to the melting point of progesterone (128°C (7)). It may therefore be concluded that the localised thermal analysis technique has provided strong evidence for the presence of crystalline progesterone on the surface of the spheres at 50% w/w drug loadings. Examination of the 0% w/w and 30% w/w systems indicate a discontinuity at circa 84°C in both cases (onsets 87 ± 9°C and 82 ± 4°C respectively). In these cases, the temperature dependent penetration takes place over a wider temperature range, with the more gradual onset resulting in higher standard deviation values than seen for the systems containing 50% w/w progesterone.

HSM studies were conducted to ascertain whether any change in appearance could be detected over the temperature range under study. Observation of the unloaded spheres during heating showed no marked changes in appearance up to approximately 80°C, at which point the spheres appeared to soften under the weight of the coverslip (Figure 3a). As the temperature was increased to 110°C the spheres were seen to progressively 'flow' on the slide, as indicated in Figure 3b. This almost certainly reflects gravitational forces overcoming the surface tension of the $>Tg$ spheres, as is well known for the collapse of freeze-dried products (13). To our knowledge, however, this process has not been previously described for PLA microspheres.

DISCUSSION

The study has provided clear evidence from the micro-TA studies that the drug is indeed present in crystalline form on the surface of the microspheres at 50% w/w loadings, as suggested in an earlier study. However, the behaviour of the 0% w/w and 30% w/w systems require careful consideration. Intuitively, one may expect the discontinuity for the 0% sample to be associated with the glass transition of the PLA,

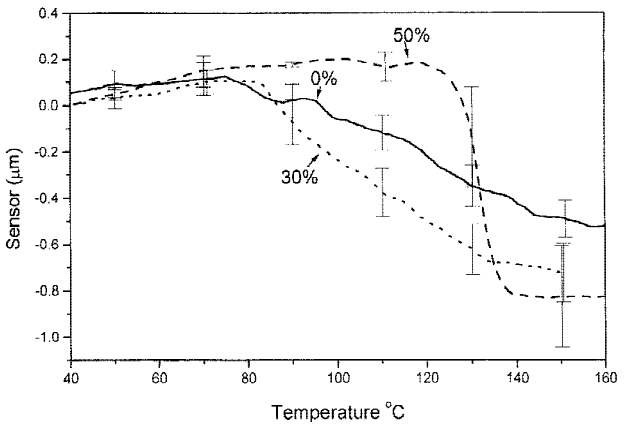

Fig. 2. Localised thermal analysis experiments for polylactic acid microspheres containing 0% w/w, 30% w/w and 50% w/w progesterone.

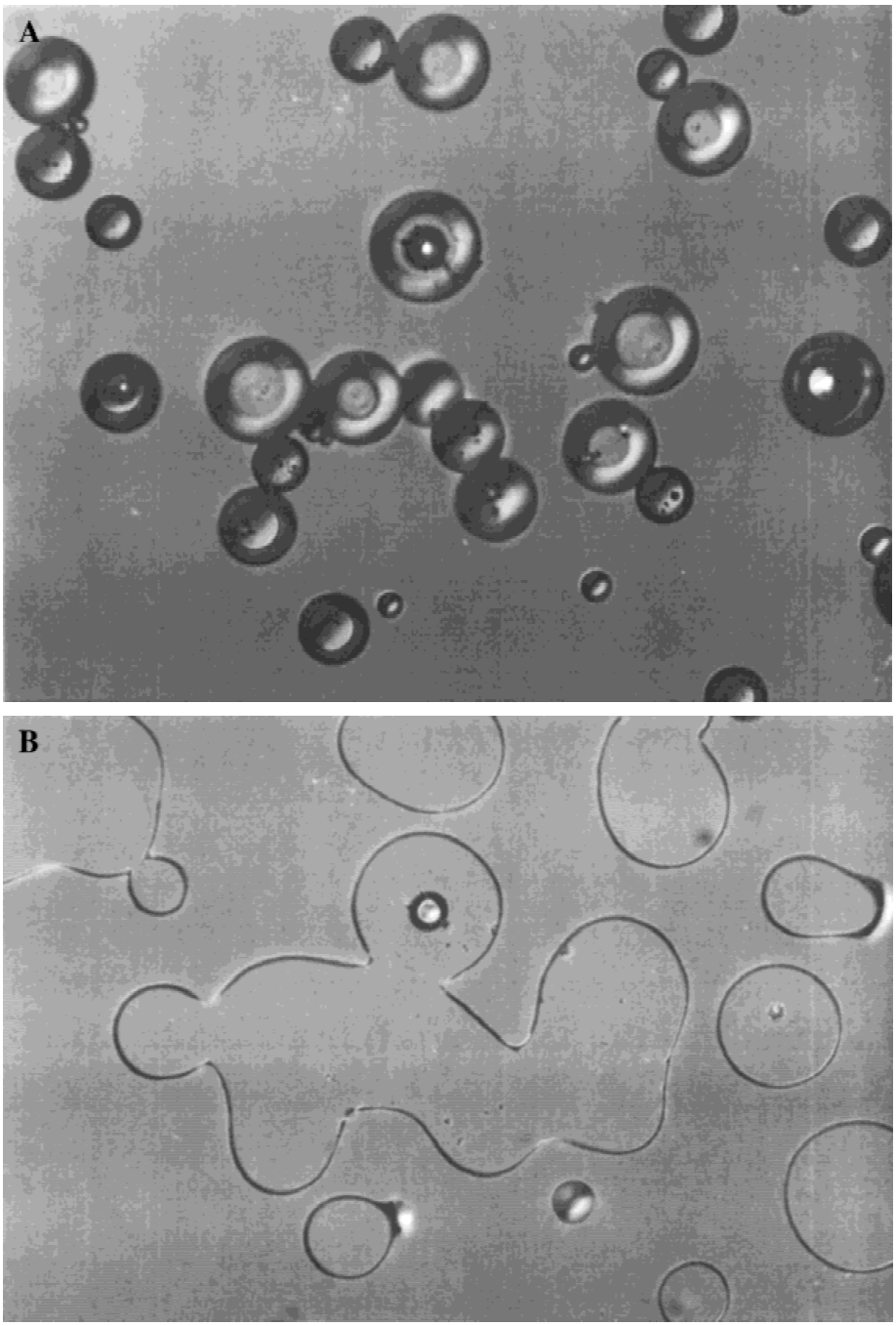

Fig. 3. Hot stage microscopy studies for unloaded microspheres a) 80°C and b) 110°C.

at least for the unloaded systems. The similarity in localised thermal behaviour of the 30% w/w systems does not in itself contradict this. Previous studies (15) have indicated that the glass transition of progesterone is circa 5°C, hence even if the 30% w/w loaded spheres do have an amorphous surface progesterone layer, this layer will be well above *Tg* at the penetration temperature and may not contribute greatly to the thermomechanical response. However, it is noted that the 30% w/w systems showed greater temperature dependent penetration than did the unloaded spheres, possibly due to plasticization of the PLA by the drug. It should also be noted that the absence of any evidence for progesterone recrystallisation is almost certainly a function of the rapid heating rate used.

There is, however, a large discrepancy between the *Tg* values measured using MTDSC and the 'softening' values measured using micro-TA, the former being 48°C and 40°C for the unloaded and 30% w/w systems respectively (7). There are two possible explanations for this. Firstly, the high heating rate may result in superheating of the <*Tg* system such that the glass transition occurs at temperatures above that normally expected. This is well established for DSC studies in that high heating rates may result in increases in *Tg* compared to the value measured on cooling. The second ex-

planation is that the discontinuity is not a direct reflection of the *Tg* itself but is instead an indication of some form of post-*Tg* viscoelastic response. This is also a well-established phenomenon in the thermomechanical analysis field, whereby temperature-dependent deformations are more correctly referred to as softening points, hence the detection of a glass transition is not automatically assumed. It should also be noted that no softening was observed using micro-TA for HPMC at temperatures corresponding to the glass transition (11), hence again it should not be automatically assumed that the changes in mechanical properties associated with *Tg* will necessarily be directly detected by micro-TA in every case.

While either of the two explanations outlined above may be correct based solely on the micro-TA data, both the HSM and MTDSC (7) studies indicate that there is a distinct >*Tg* event that occurs in the same temperature region as the discontinuity seen with the localised thermal studies. In terms of MTDSC, this event is seen in the phase angle ϕ which represents the ratio of the imaginary and real components of the complex heat capacity. Hill et al (7) reported a step increase in ϕ between 70°C and 110°C for the unloaded spheres. For the 30% w/w system, a positive phase peak was seen between 70°C and 75°C, the temperature range at which an exothermic peak was seen in the total heat flow data indicating a recrystallisation process. No such discontinuity was seen over this temperature range for the 50% w/w systems. The interpretation of the phase angle remains a source of debate within the thermal analysis field. However, it is currently believed (14) that the phase lag arises from a combination of sample and instrumental effects. In terms of the glass transition, the value of ϕ is theoretically 0 before the *Tg* region due to the relaxation time of the glass being longer than the temperature modulation period, hence the relaxation processes do not follow the heating signal. Above *Tg,* the molecular relaxation times (τ) are short in relation to the period and hence the structure can follow all the imposed temperature modulations. In the region of *Tg,* however, the phase angle passes through a minimum of up to a few tenths of a radian due to the equivalence of the relaxation times to the applied modulation period, with the midpoint of the transition occurring when $\tau \sim$ period. In practice, however, the phase angle tends to be non-zero above and below *Tg* due to non-ideal heat transfer between the instrument and sample. This effect is dependent on experimental parameters and may be given by

$$\phi = \arctan (mC_p^* \omega/K) \qquad \text{Eq. 1}$$

where m is the sample mass C_p^* is the complex heat capacity, $\omega$is the period and K is an instrument parameter describing the heat transfer characteristics of the system. Hill et al (7) tentatively ascribed the phase angle effect to changes in the physical integrity of the spheres within the pans (i.e., flow effects). We believe that this explanation is correct and that it is now possible to interpret the phase angle behaviour in terms of the change in contact between the sample and the base of the pan as the spheres lose their physical integrity. Such increased contact will result in alterations to the parameter K in Eq. 1 over a fairly narrow temperature range.

Overall, therefore, the evidence for all three techniques supports the concept of there being a distinct softening response circa 80°C. Clearly, it is unlikely that spheres will be exposed to these temperatures post manufacture. However, it should be borne in mind that this softening is almost certainly a kinetic rather than a first order thermodynamic event. Consequently the softening observed here could occur over longer time periods at lower temperatures. Indeed, the previous study (7) highlighted the fact that the *Tg* may be lowered to temperatures in the region of body temperature by the presence of incorporated drugs, hence it is feasible that certain formulations may soften over the period of drug administration within the body.

CONCLUSIONS

The study has investigated the use of micro-TA in conjunction with MTDSC and HSM as a means of characterising the surface of progesterone-loaded microspheres. Strong evidence for the presence of crystalline progesterone on the surface of the 50% w/w spheres was obtained from localised thermal analysis, while a softening was seen for the unloaded and 30% w/w systems. It is suggested that this softening may be a reflection of flow rather than a direct reflection of the *Tg*. The technique of micro-TA therefore appears to be of considerable potential use as a means of characterising the surface properties of microspheres, although it is necessary to exercise caution in the interpretation of the data obtained. In particular, the study suggests that the technique may be more sensitive to softening at temperatures >*Tg* than to the glass transition itself. This is not in itself a disadvantage but it is clearly essential to know the nature of the thermal event under study in order to further the development and applications of this method.

ACKNOWLEDGMENTS

We would like to thank the EPSRC for financial support for Dr Paul Royall and the BBSRC/Abbott Laboratories Ltd. for financial support for Dr Vivienne Hill

REFERENCES

1. T. L. Whateley. Biodegradable microspheres for controlled drug delivery. In D. R. Karsa and R. A. Stephenson (eds.), *Encapsulation and Controlled Release,* Royal Society of Chemistry, 1993 pp. 54–65.
2. Y. Ogawa. Injectable microcapsules prepared with biodegradable poly(α-hydroxy) acids for prolonged release of drugs. *J. Biomater. Sci. Polymer Edn.* **8**:391–409 (1997).
3. B. R. Conway, J. E. Eyles, and H. O. Alpar. A comparative study on the immune responses to antigens in PLA and PHB microspheres. *J. Control. Release* **49**:1–9 (1997).
4. M. van de Weert, R. van't Hof, J. van der Weerd, R. M. A. Heeren, G. Posthuma, W. E. Hennink and D. J. A. Crommelin, Lysozome distribution and conformation in a biodegradeable polymer matrix as determined by FTIR techniques. *J. Control. Release* **68**:31–40 (2000).
5. J. P. Benoît, F. Courteille, and C. Thies. A physiochemical study of the morphology of progesterone-loaded poly(D,L-lactide) microspheres. *Int. J. Pharm.* **29**:95–102 (1986).
6. S. Benita, J. P. Benoît, F. Puisieux, and C. Thies. Characterisation of drug-loaded poly(d,l-lactide) microspheres. *J. Pharm. Sci.* **73**: 1721–1724 (1984).
7. V. L. Hill, N. Passerini, D. Q. M. Craig, M. Vickers, J. Anwar, and L. C. Feely. Investigation of progesterone loaded poly(d,l-lactide) microspheres using TMDSC, SEM and PXRD. *J. Therm. Anal.* **54**:673–685 (1998).
8. A. Hammiche, M. Reading, H . M. Pollock, M. Song, and D. Hourston. Localised thermal analysis using a miniaturised resistive probe. *Rev. Sci. Inst.* **67**:4268–4274 (1996).
9. M. Reading, D. M. Price, H. M. Pollock, A. Hammiche, and A. Murray. Recent progress in microthermal analysis. *Am. Lab.* **31**: 13–16 (1999).
10. D. M. Price, M. Reading, A. Hammiche, and H. M. Pollock.

Micro-thermal analysis: Scanning thermal microscopy and localised thermal analysis. *Int. J. Pharm.* **192**:85–96 (1999).
11. P. G. Royall, D. Q. M. Craig, D. M. Price, M. Reading, and T. J. Lever An investigation into the use of micro-thermal analysis for the solid state characterisation of an HPMC tablet formulation. *Int. J. Pharm.* **192**:97–103 (1999).
12. P. G. Royall and D. Q. M. Craig. Micro-thermal analysis of a coated tablet: A simple method for the enhancment of the DC thermal image. *J. Pharm. Pharmacol.* **51S**:89 (1999).
13. M. J. Pikal and S. Shah. The collapse temperature in freeze drying: Dependence on measurement methodology and rate of water removal from the glassy phase. *Int. J. Pharm*. **62**:165–186 (1990).
14. J. M. Hutchinson. Characterising the glass transition and relaxation kinetics by conventional and temperature-modulated differential scanning calorimetry. *Thermochim. Acta* **324**:165–174 (1998).
15. J. Kerç and S. Srçiç. Thermal analysis of glassy pharmaceuticals. *Thermochim. Acta* **248**:81–95 (1995).