ELSEVIER

Thermochimica Acta 248 (1995) 259–269

thermochimica acta

Thermoanalysis of microspheres

C. Dubernet

Laboratoire de Physico-chimie – Pharmacotechnie – Biopharmacie, URA CNRS 1218, 5 rue J.B. Clément, F92296 Châtenay-Malabry Cedex, France

Received 7 March 1994; accepted 17 March 1994

Abstract

This paper describes different cases in which using DSC allows us to obtain more information about the internal structure of microspheres, especially concerning the nature of the interaction between the polymer matrix and the encapsulated drug. A rapid survey of the processes involved in the manufacture of microspheres points to situations in which thermal analysis is able to provide interesting information.

Keywords: Drug; DSC; Glass transition; Microsphere; TGA

1. Introduction

When Chemical Abstracts are searched combining microspheres and thermal analysis, no more than seven papers are located. Indeed, very few papers deal with thermal analysis as a main goal. However, as seen in this paper, thermal analysis (and particularly DSC) is a very interesting tool in investigating microspheres. Unfortunately, when carried out, DSC scans are often too readily interpreted which may lead to mistaken conclusions.

Why carry out DSC? Microspheres are generally made of polymers. They are then characterized by a glass transition T_g, and eventually by a melting endotherm if the polymer is a crystalline one. Polymers are very variable materials. The operative process needed to produce microspheres from the raw material is able to modify the organization of the polymer chains in the solid state, leading to some mechanical discrepancies between the various forms, and even to different behaviours during ageing. Such transformations will have immediate consequences for both the T_g event and the crystallization of the polymer. The use of DSC will then

0040-6031/95/$09.50 © 1995 – Elsevier Science B.V. All rights reserved
SSDI 0040-6031(94)01947-9

be entirely justified. The problem is still more complex when a drug is encapsulated in the polymer matrix. According to the encapsulation procedure (detailed below), the drug may be either dissolved or physically dispersed in the initial step of the process. If the drug is initially dispersed and remains in this form during the overall process, the situation is quite simple: the drug will be physically suspended in the polymer matrix. However, if the drug is initially dissolved, three opportunities may occur: firstly the drug may be finally dissolved in the polymer, leading to a solid solution (SS). Secondly, the drug may remain molecularly dispersed in the polymer, but with interactions between the drug molecules and the polymer chains too weak to lead to a stable state. This is a metastable molecular dispersion (MMD): the interactions between the drug molecules are strong and the molecules will diffuse through the polymer network and crystallize. The rate of diffusion of the molecules depends on the matrix viscosity and may be so low that recrystallization would take years! Thirdly, the drug may crystallize during the course of microsphere preparation: it will then be physically dispersed in the polymer matrix in the form of a crystalline dispersion. Because these three states, just described, will be different in terms of stability during ageing, or in terms of drug release characteristics, it is essential to differentiate them.

Concerning the SS, the drug molecules and the polymer develop strong interactions between each other, leading to a plasticization of the polymer. The consequences in DSC are a lower T_g and the absence of the fusion event of the drug. For the MMD, the drug remains in the molecular state only because the viscosity of the medium is so high that drug diffusion, and therefore crystallization, is inhibited. In an ideal case, the drug molecules and the polymer chains do not interact with each other, leading to conserved polymer characteristics (T_g). As long as the polymer remains in the vitreous state, crystallization will not occur in the few weeks or months following the microsphere preparation. However, as soon as the matrix is in the rubbery state, the viscosity breaks down which allows crystallization to occur readily. Accelerated ageing can be carried out through annealing experiments. These consist of maintaining the MMD at a temperature very close to the T_g of the polymer, reducing the viscosity and allowing a much higher diffusion rate of the dispersed drug molecules. That portion of drug molecules exceeding the solubility of the drug in the polymer will then be able to crystallize.

Thermal analysis is therefore a useful tool in investigating the nature of the dispersion of drugs in microspheres. However, it may also be used to calculate the solubility of the drug in the polymer [1]. For this purpose, as a first step, microspheres of varying drug concentrations (different encapsulation ratios) have to be prepared. The heat of fusion of the crystallized drug may be measured by DSC. The heats of fusion obtained are then plotted against the encapsulation ratios. The drug concentration corresponding to a zero heat of fusion (no crystals present) should equate to the drug solubility in the polymer. However, it should be emphasized that such a solubility is determined at the melting point of the drug and not at the ambient temperature, where there may be a quite different solubility.

The following review will be devoted to examples from the literature illustrating how DSC may be used, firstly to check the polymer characteristics after micro-

sphere formation, and secondly to determine the nature of the drug dispersion in the polymer matrix. Some information about the encapsulation processes is, however, necessary.

2. Summary of microencapsulation procedures

Microspheres can be defined as spherical solid particles with an internal matrix structure. They are most frequently obtained from a polymer solution which is dispersed by appropriate means into droplets. The microsphere formation is based on a phase separation process occurring in the dispersed droplets, leading to precipitated solid polymeric spheres. Many reviews deal with microencapsulation processes and the parameters influencing the microsphere properties. The goal of this summary is not to write a new review but to underline the key steps of the main procedures used to prepare microspheres. The aim is to be able to predict the physicochemical changes in either the polymer or the drug which may be expected.

2.1. Solvent evaporation process

This process is probably the most famous one. It consists of the following steps: a solution of the polymer, in an organic solvent in which the drug will be incorporated, is dispersed in a continuous phase containing surface active agents. Stirring is maintained until complete evaporation of the organic solvent has occurred [2–4]. When the continuous phase is water, methylene chloride is the most appropriate solvent for the polymer solution because of both its low boiling point (around 40°C) and its slight solubility in water. Such properties lead to a precipitation rate of the polymer which is neither too slow nor too fast. In the case of an oily continuous phase acetonitrile can be used, and evaporation is performed at 50°C.

The rate of polymer deposition will vary according to the polymer solubility in the organic solvent and to the rate of solvent evaporation. In the case of a slow process, the polymer molecules will have time to rearrange which will not be the case in a fast deposition process. At the same time, according to the drug solubility in the organic phase, the saturation concentration of the drug may be reached at very different times from the beginning of the evaporation process, according to the initial drug : polymer ratio. For instance, in the case of a low initial drug content the drug will only precipitate after a rather long period when the polymer phase has become very viscous; on the contrary, in the case of a high initial drug content, drug precipitation will occur in a rather fluid polymer phase.

A variant of this method consists of extraction of the volatile organic solvent by dilution of the emulsion into a diluting phase, miscible with both the organic solvent and the continuous phase, but not miscible with the polymer (isopropanol is an example). The advantage is that such a procedure can be carried out at a temperature lower than the boiling point of the organic solvent. Because of the high extraction rate, the microspheres thus formed are frequently porous.

2.2. *Spray drying* [2,4,5]

In this case, a polymer solution containing the drug to be encapsulated is spray dried through the nozzle of the spray dryer system. In such a case, the nature of the organic solvent and the values of the inlet and outlet temperatures will influence the rate of the polymer and drug deposition in a similar manner as pointed out in the previous section. However, solvent evaporation is rapid compared to solvent evaporation processes, leading to highly porous particles.

Other procedures are also described such as phase separation by the addition of non-solvents, interfacial polymer deposition, in situ polymerization or even melting methods. Even if the processes differ, the rationale in carrying out DSC will be the same: checking the physical state(s) of polymer and the drug in relation to the drug content and the time of storage.

3. The characterization of unloaded microspheres by DSC

Microspheres can be formulated for varying purposes, namely, for oral administration, chemoembolization, or subcutaneous implant. If a large variety of polymers are candidates, polylactic acid (PLA) and its copolymers, polylactide–co-glycolide (PLCG), are the most extensively investigated polymers for the parenteral route because of their good histocompatibility and biodegradability.

Briefly, whereas (±)-PLA is essentially amorphous, (−)-PLA is a crystalline polymer characterized by a low biodegradation rate. The crystallinity of the copolymer, PLCG depends upon the molar ratio of the two monomer components in the copolymer chain: copolymers containing less than 70% of the glycolide are amorphous [4]. The DSC profile of such polyhydroxy acids can be found in various works dealing with microspheres, loaded with various drugs. As an example Jalil and Nixon [3] studied three different batches of (−)-PLA, differing in their molecular weight (MW). Such semicrystalline polymers are characterized by both their glass transition temperatures and their melting points, the values of which increase with increase of the MW. According to Jalil and Nixon [3], T_g can vary between around 40 to 60°C and the melting temperature of the crystalline domains between around 130 to 170°C when the molecular weight rises from 2400 to 61 300.

When low MW fractions are mixed with high MW (±)-PLA fractions, for example 2000 and 120 000 Da respectively [6], a unique T_g is found. It decreases with increasing low MW PLA content from 0 to 100%, giving values intermediate between the T_gs of each individual polymer. This observation leads to the conclusion that the two polymer fractions are miscible in all ratios. However, whereas this is perfectly observed in the case of a cast film, the variation in the T_g is less pronounced when the polymer is processed into microspheres. This observation remains unexplained.

Finally, a glass transition may be accompanied by an endothermic overlap, representing the energy necessary to overcome the microstructure of the polymer which had developed during processing. Bodmeier et al. [6] noticed that these peaks

were absent in films containing more than 50% of low MW PLA. This was explained by the fact that during solvent casting, the high MW fractions precipitated rapidly because of a lower solubility in the casting solvent than the lower MW fractions. The films thus obtained were more porous and structured than films essentially composed of low MW PLA.

This observation raises the problem of polymer history. Some authors recognize the need for melt-quenching of samples prior to DSC: they argue that the T_g measured is then much more representative of the polymer itself, and allows the comparison of polymers of different histories. However, in the field of microencapsulation, it is most important to characterize the system soon after microsphere preparation and to follow any changes during ageing. Removing the sample history in such a case will not give information about the future stability of the system. However, such procedures remain of interest as soon as a chemical change in the polymer is suspected, e.g. due to the polymer processing. This was applied to ethylcellulose (EC) microspheres [7] which showed a slightly lower T_g than the raw material. Unfortunately in this case, heating EC to temperatures above its T_g leads to a rapid degradation of the polymer, essentially by an oxidation process. When melt-quenching cannot be performed in safety, annealing experiments may be of greater use. As previously mentioned, they allow the polymer to rearrange at a temperature close to the T_g. In the case of EC, they clearly demonstrated that neither the raw material nor the microspheres were at equilibrium, which explained the difference in T_g levels [7].

In the case of PLA, attention is only paid to T_g and fusion events and these two parameters should be sufficient to compare two samples. In the case of EC, only a T_g was shown which was unable to allow comparison between the raw material and microspheres prepared by solvent evaporation [7]. Indeed, as no difference existed in either MW, or residual solvent content, the T_g was not affected. However, the precise investigation of the thermal events relating to the degradation of EC showed, in a first step, that microspheres were more sensitive to oxygen than the raw material. By comparing the exotherms of decomposition obtained, and calculations of the mass of the polymer and its specific surface area, the authors came to rely upon the modified sensitivity to oxygen as an indication of a chemical change in the polymer, limited to the microsphere surface [7]. Only hypotheses were forwarded as to the nature of this chemical change: some ethoxyl links may have been disrupted during the preparation of the microspheres, principally at the water–oil interface, leading to a decrease in the degree of substitution of EC.

4. The characterisation of drug-loaded microsphere by DSC

As already mentioned, DSC is of greatest interest in situations where the drug is solubilized at any point of the process before polymer deposition. The most interesting example in the literature is the encapsulation of progesterone. It was found that progesterone was not detectable by DSC in (±)-PLA microspheres loaded with 22.3% or even 31.7% of the drug [8]. This observation indicates that

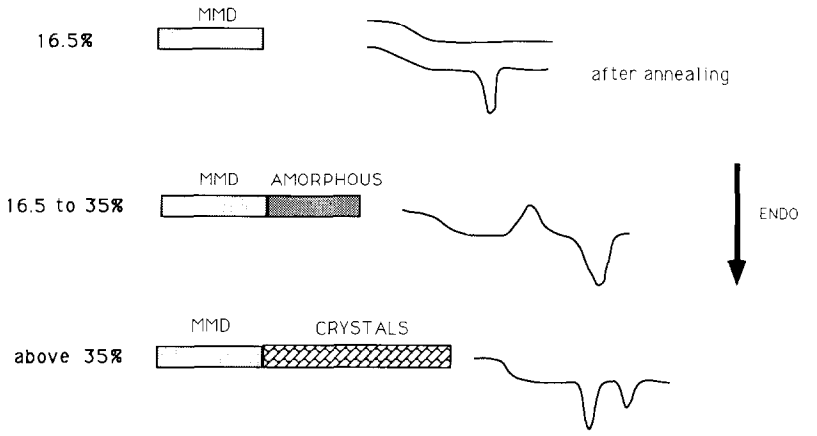

Fig. 1. Schematic representation of DSC scans obtained with progesterone-loaded PLCG microspheres.

progesterone was present in a molecular dispersion or a solid solution state in the PLA. For a drug loading of 68.3%, the melting endotherm of the drug could be observed; simultaneously the T_g event disappeared, probably because of a lack of sensitivity of the apparatus due to the low proportion of polymer in these microspheres. It was proposed that interactions should exist between (±)-PLA and progesterone, at least in the presence of methylene chloride [9]. Indeed, addition of PLA to a concentrated solution of progesterone in methylene chloride caused this solution to change from a turbid system to an optically clear solution. In such a situation, the existence of a SS was not unlikely; however, the annealing of 23% progesterone-loaded microspheres at 110°C led to partial recrystallization of the drug indicating that progesterone was in a MMD [9]. In this study, fluctuations in the melting points of the drug were found, without any comment from the authors. In reality, progesterone was certainly present as two forms (α and β) in the microspheres, as was found later in PLCG microspheres obtained by solvent evaporation [10]. Above a drug loading of 35%, the drug spontaneously crystallized (α and β forms) during the evaporation process. Below 16.5%, the drug was in a MMD, and recrystallized after annealing at 80°C. Finally, between 16.5% and 35%, part of the drug was in an amorphous state and recrystallized during the DSC heating cycle, giving rise to an exotherm before the melting transition of the drug (Fig. 1). Undoubtedly, the presence of the polymer influenced the crystallization process of the drug. Microspheres of (±)-PLA obtained by spray-drying led to similar observations. Whereas progesterone crystallized predominantly in the α form when spray-dried alone, the β form was dominant when the drug was spray-dried in combination with the polymer [11].

The MW of the polymer may also play a role in the drug crystallization process. Low MWs are associated with low viscosity of the casting solution, especially at the point of drug precipitation. More stable forms of drug aggregates may be favoured.

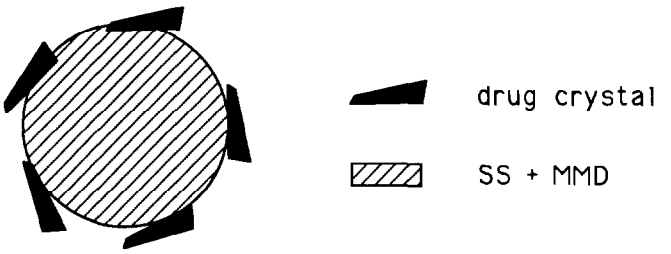

Fig. 2. Schematic representation of the structure of 16–22% ibuprofen-loaded ethylcellulose microspheres.

This was observed in the case of quinidine-loaded PLA microspheres. Increasing the amount of low MW fractions of PLA led to an increase in recrystallization temperatures of the amorphous drug during the DSC run [6]. Certainly drug–polymer interactions must play a role at this point. The transparency of quinidine-loaded films of PLA increased with the proportion of low MW PLA, showing an increase solubility of the drug. Such an observation was not correlated with DSC. It might have been possible to correlate with the measurements of heats of melting, but the heats of fusion of the pure drug relate poorly with the heats of fusion of the encapsulated drug. Because the drug will interact with its polymeric environment, the experimental heat of melting is the result of the theoretical heat of melting of the drug and the heat of mixing [1]. Such an approach has been applied to ibuprofen-loaded EC microspheres [12]. Usually the endotherms of fusion obtained for drug-loaded microspheres are different to those of the melting endotherms of pure drugs. In the case of ibuprofen-loaded EC microspheres, they were indeed diffuse and could be split into 1, 2 or 3 parts according to the encapsulation ratio (between 16% and 57% drug). Scanning electron microscopy (SEM) helped to correlate the different endotherms to different types of crystals, especially crystals at the surface of the microspheres and crystals embedded in the polymer matrix. The fraction of drug soluble in EC was calculated by linear regression from the results of the heats of melting (ΔH_{melt}) versus the encapsulation ratios; it was 10%. From there, the heats of mixing (ΔH_{mix}) were calculated as the difference between the experimental ΔH_{melt} and the expected ΔH_{melt} (calculated from the fraction of drug that ought to be crystallized). Interpretation of the results led to the conclusion that a portion of the drug existed as a MMD for loadings of 16% and 22%. These microspheres could then be described as a matrix consisting of both a SS and MMD, surrounded by drug crystals which had grown towards the aqueous medium [12,13] (Fig. 2).

Similar enlargement of the peak of melting, with lower melting temperatures than the pure drug, were also reported in the case of nifedipine encapsulated in Eudragit microspheres [14]. Undoubtedly, the drug interacted with the polymer, making it difficult to produce quantitative measurements.

5. Specific examples

The examples described so far are idealized, although somewhat complex. Environmental cases are not always so easy to interpret and some will be developed further.

It was previously stated that DSC was not justified in cases where the drug was initially dispersed in the casting solution. However, it is important to verify whether the drug remains dispersed during the overall process. For example, in a study on hydrocortisone-loaded PLA microspheres, it was found that the steroid crystals initially present in the methylene chloride migrated into the aqueous phase during the emulsification step and dissolved [15]. The crystalline domains in the isolated PLA were formed during the evaporation step by precipitation of the solubilized drug. The melting temperatures found during DSC were consequently different from the melting point of the pure drug.

In the solvent evaporation process, the polymer dissolved in an organic solvent, usually methylene chloride. The affinity of polyhydroxy acid (PLA and PLCG) for this solvent is particularly important and it is hard to remove the residual solvent, which may reach 2–5% w/w [16]. Such residual solvent will plasticize the polymer and it will become difficult to differentiate the plasticizing effect of the drug from that of the solvent. The presence of the solvent may be assessed by an evaporation endotherm around 60 to 80°C, and by a weight loss on TGA [10,15]. Generally, annealing experiments are carried out to evaporate the residual solvent, but it may be difficult to precisely analyse the DSC traces performed after annealing where peaks may result from both the annealing process (rearrangement of the polymer chains, drug recrystallization) and the departure of the plasticizing solvent. Certainly such situations have led to mistaken interpretations, especially in terms of polymer organization [9].

It has already been mentioned that a SS and a MMD may be differentiated by their stability on storage. Although in the first case, the T_g must be lowered and in the second case it must not, no conclusion can be made without annealing experiments [17]. When residual solvent is present and modifies the T_g, annealing is still more justified in eliminating the plasticizing effect on the solvent [16]. However, it must not be forgotten that annealing has to be carried out at a temperature close to the T_g of the system: how can a so-called "annealing" performed at 4°C in the case of PLA microspheres be interpreted? [18].

It must be kept in mind that when solubilities are evaluated by DSC they are obtained at the melting temperature of the drug. The problem becomes even more complicated when the drug melts above the polymer melting transition. In such a case, the solubility measured is that of the drug in the molten polymer at the drug melting temperature (far from the solubility of the drug in the solid polymer at room temperature!). This was the case for indomethacin dispersed in EC–poly(ε-caprolactone) microspheres [19]. The crystalline fraction of poly(ε-caprolactone) melts at 60°C and indomethacin at 160°C. Microspheres loaded with 30% drug did not show any melting endotherm of the pure drug, although drug crystals were visible by SEM. After the melting transition of the polymer, the microsphere

structure is lost and existing drug crystals can either dissolve or remain dispersed in the new matrix. The problem was the same with progesterone loaded into polyhydroxybutyrate–polyhydroxyvalerate (PHBV) microspheres [20,21]. The polymer melted at 124°C, giving rise to a broad endotherm. Progesterone melts at 121°C (β form) or 130°C (α form), which may overlap the melting transition of the polymer. As previously described polymers loaded with 5% or 10% drug show drug crystals, but no melting endotherm of the drug was detected. However, when the drug loading reached 30%, a peak corresponding to the melting of progesterone could be observed. At this point, the solubility of the drug in the molten polymer has been surpassed. It is then impossible to precisely quantify the solubility of the drug in the solid polymer, although it was not zero in the case of progesterone and PHBV as assessed by the lowering of the melting temperature of the polymer. The authors were not able to follow the T_g of the polymer with their apparatus because its value was too low (around 5°C) [21].

A last, problematical case is when the encapsulated drug melts below the glass transition of the polymer matrix. In such a case, it is impossible to carry out annealing experiments, unless at a very low temperature compared with the polymer T_g. This can be illustrated by ibuprofen-loaded EC microspheres [12]. Pure ibuprofen melts at 77°C and the T_g of non-loaded microspheres was around 130°C. It was shown that the T_g was lowered to around 105°C for a 3.7% drug loading. Above this ratio, the T_g event could not be localized, essentially because of the broad melting endotherm of the encapsulated drug probably overlapping the glass transition. The plasticizing effect of the drug could not then be followed further. By chance, EC degrades above 165°C and it was noticed that the temperature of the beginning of the oxidation process was lowered when the drug loading was increased. A correlation was established between the lowering of this oxidation temperature and the lowering of the T_g. Hence, the existence of a fraction of SS was demonstrated. Because the encapsulated drug began to melt below 50°C, it was not possible to carry out annealing to allow the eventual additional MMD to recrystallize and then to quantify the amount of SS. Only careful measurement of the heats of melting helped to determine the dissolved fraction, as mentioned above. It is worthwhile underlining that in such a quantitative study, DSC runs were performed at a rate of 5°C min^{-1}, against an usual rate of 10 to 20°C min^{-1} in most studies.

Such a low heating rate allows a precise quantification of drug crystals. It was particularly suitable in the ibuprofen–EC model for differentiating between the amount of drug crystals lying on the surface of microspheres prepared by the solvent evaporation method with two different stabilizing agents (methylcellulose and polyvinyl alcohol) [13]. It could then be shown that the amount of surface crystals was higher in the case of methylcellulose used at equivalent drug loading. Modified solvent evaporation methods coupled with DSC runs led to a better understanding of drug crystal growth at the microsphere surface.

6. What about TGA?

TGA is a useful method by which to determine the presence or absence of residual solvent in microspheres prepared by the solvent evaporation method. However, as it monitors the mass loss of a sample during heating, it was applied to determine the temperature at which polyanhydride microcapsules ruptured due to an increase in inner pressure with increasing temperatures [22]. The aim of the study was to evaluate the porosity of the microcapsule wall according to the monomer composition. The mass loss observed was the result of a more or less complete impermeability of the membranes. The idea was to prepare photochemical-controlled release systems which released their content only during exposure to UV light [23]. Such systems are microspheres encapsulating azobisisobutyronitrile (AIBN) able to photochemically emanate nitrogen gas. Microspheres must be impermeable, until a given gas pressure at which they rupture. A TGA study is then a very precious tool by which to simulate the release conditions.

7. Conclusion

As discussed, thermal analysis is particularly suitable for investigating the nature of drug dispersion in microspheres. Although rather simple to interpret in most cases, DSC must however be performed with accuracy if precise information is expected. It should not be used unless the objectives of the study have been previously defined which lead to the choice of appropriate experimental conditions.

References

[1] F. Theeuwes, A. Hussain and T. Higuchi, J. Pharm. Sci., 63 (1974) 427.
[2] B. Conti, F. Pavanetto and I. Genta, J. Microencapsulation, 9 (1992) 153.
[3] R. Jalil and J.R. Nixon, J. Microencapsulation, 7 (1990) 41.
[4] R. Jalil and J.R. Nixon, J. Microencapsulation, 7 (1990) 297.
[5] F. Pavanetto, B. Conti, I. Genta and P. Giunchedi, Int. J. Pharm., 84 (1992) 151.
[6] R. Bodmeier, K.H. Oh and H. Chen, Int. J. Pharm., 51 (1989) 1.
[7] C. Dubernet, J.C. Rouland and J.P. Benoit, Int. J. Pharm., 64 (1990) 99.
[8] J.P. Benoit, S. Benita, F. Puisieux and C. Thies, in S.S. Davis, L. Illum, J.G. McVie and E. Tomlinson (eds.), Microspheres and Drug Therapy, Pharmaceutical, Immunological and Medial Aspects. Elsevier, Amsterdam, 1984.
[9] J.P. Benoit, F. Courteille and C. Thies, Int. J. Pharm., 29 (1986) 95.
[10] J. Benoit, V. Rosilio, M. Deyme, C. Thies and G. Madelmont, Proc. 13th Int. Symp. Contr. Rel. Bioact. Mater., 1986, p. 171.
[11] R. Bodmeier and H. Chen., J. Pharm. Pharmacol., 40 (1988) 754.
[12] C. Dubernet, J.C. Rouland and J.P. Benoit, J. Pharm. Sci., 80 (1990) 1029.
[13] C. Dubernet, J.P. Benoit and F. Puisieux, Eur. J. Pharm. Pharmacol., 37 (1991) 49.
[14] A. Barkai, Y.V. Pathak and S. Benita, Drug Dev. Ind. Pharm., 16 (19900 2057.
[15] M. Cavalier, J.P. Benoit and C. Thies, J. Pharm. Pharmacol., 38 (1986) 249.

[16] J.P. Benoit, G. Madelmont, F. Puisieux and C. Thies, Proc. 12th Int. Symp. Contr. Rel. Bioact. Mater., 1985, 27.
[17] M.C. Bissery, F. Puisieux and C. Thies, Proc. 3rd Int. Conf. on Technological Pharmacy, Paris, Vol. III, 1983, p. 233.
[18] B. Wichert and P. Rohdewald, J. Controlled Release, 14 (1990) 269.
[19] R. Bodmeier and H. Chen, J. Controlled Release, 10 (1989) 167.
[20] N. Gangrade and J.C. Price, J. Micoencapsulation, 8 (1991) 185.
[21] N. Gangrade and J.C. Price, Drug Dev. Ind. Pharm., 18 (1992) 1633.
[22] E. Mathiowitz and M.D. Cohen, J. Memb. Sci., 40 (1989) 27.
[23] E. Mathiowitz, M.D. Cohen and R. Langer, Reactive Polymers, 6 (1987) 275.