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Effect of preparative parameters on the characteristics of poly (D,L-lactide-co-glycolide) microspheres made by the double emulsion method

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Abstract

The mechanism for drug-release from poly(D,L-lactide-co-glycolide) (PLG) microspheres is generally a combination of the diffusion of the drug and the degradation rate of the polymer. The degradation rate is controlled by the molecular weight and the copolymer composition of PLG. The porosity, of the microspheres, which is dependent on the preparation method used, will also influence the drug-release rate. PLG microspheres containing mannitol^{14}C were prepared by a small-scale w/o/w double emulsion method. As the PLG concentration in the middle phase was increased from 4.3 (w/w) to 43%, the entrapment efficiency of mannitol^{14}C rose from 1 to 25% and the diameter of the microspheres increased from 4.5 to 29 μm while the release rate of mannitol^{14}C decreased. By increasing the volume of the internal aqueous phase the release rate of mannitol^{14}C was increased. When using phosphatidylcholine (PC) as a stabiliser the size of the microspheres decreased from 23 to 16 μm. The presence of PC during preparation lowered the entrapment efficiency of mannitol^{14}C. The results were related to the dynamics of the double emulsion and the porosity of the microspheres.

Keywords: Microspheres; Preparation; Poly(D,L-lactide-co-glycolide); Release; Porosity

1. Introduction

In drug delivery poly(D,L-lactide-co-glycolide) (PLG) microspheres are frequently being considered as drug carriers for future pharmaceutical products. The reason for this is mainly twofold. Firstly the material is biocompatible and degrades in vivo by forming the non-toxic monomers, lactic- and glycolic acids. Secondly, the release rate of entrapped drugs can be controlled by varying the molecular weight and the copolymer ratio.

Furthermore, dispersing the material into microspheres makes drug administration possible by

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injection (Aftabroushad and Doelker, 1994; Morris et al., 1994).

In therapeutic use this drug delivery system may improve the treatments by possible localisation of the drug at the site of action (e.g. in anti-tumor therapy) and by prolonged release of drugs (e.g. in hormone therapy). By the incorporation of sensitive drugs such as peptides and proteins they may be protected against chemical and enzymatic degradation (Morris et al., 1994; Service, 1994; Tice, 1985). In the field of oral administration of vaccines the particulate nature of the microsphere formulations often drastically increases the immunogenic response of entrapped antigens as compared with solutions with free antigens (Morris et al., 1994; O'Hagan et al., 1993).

PLG-microspheres can be prepared by dissolving the polymer together with the dissolved or suspended drug in a volatile organic solvent. The PLG-solution is then emulsified with an aqueous solution forming an oil-in-water (o/w) emulsion. Upon evaporation the volatile solvent is removed resulting in an aqueous suspension of microspheres. For a water-soluble drug the entrapment efficiency may be low due to the partitioning of drug to the continuous external aqueous phase during the evaporation process. Furthermore, the bioactivity of peptides and proteins is often reduced upon contact with organic solvents.

The use of the double emulsion method results in a more favorable process for the entrapment of water soluble substances into microspheres. Briefly, the drug is initially dissolved in a small aqueous aliquot, which is subsequently emulsified with the organic PLG solution forming a water-in-oil (w/o) emulsion. This emulsion is added to an aqueous solution followed by a second emulsification resulting in a w/o/w double-emulsion.

Using the double emulsion method for the entrapment of drugs into microspheres various process parameters will influence the properties of the prepared microspheres. Examples of such parameters are: phase-volumes, emulsification energies, polymer concentration, phase-viscosities, added stabilisers, etc. (Aftabroushad and Doelker, 1994; Schugens et al., 1994; Wang et al., 1991; Yan et al., 1994). When preparing microspheres for therapeutic use it is therefore important to make a systematic investigation on the impact of these parameters on the properties of the microspheres.

In this study we have incorporated small amounts of mannitol^{14}C in PLG-microspheres by a small-scale double-emulsion method. The inner aqueous phase volumes used for dissolving the model drug, mannitol^{14}C was between 50 and 400 μl and the total volume of the double emulsion was approximately 7 ml. The use of a small-scale method is an advantage when incorporating, for example, highly purified antigens. It will be shown by this method how the variation of some experimental parameters will influence the characteristics of the resulting microspheres, i.e. particle size distributions, entrapment efficiency, morphology and drug release kinetics. The PLG concentration in the middle phase of the w/o/w emulsion was varied from 4.3 (w/w) to 43%. Different volumes, 50–400 μl, of the inner aqueous phase were employed. The time interval of the second emulsification was varied from 5 to 80 s. Beside these parameters the effects on microsphere characteristics using phosphatidylcholine (PC) as stabilisers are presented.

No specific interactions between the model drug mannitol^{14}C and the polymer were expected and a low degree of loading was employed. Under these conditions changes in the drug release between different preparations were expected to reflect differences between the polymer matrices obtained.

2. Materials

Poly(D,L-lactide-co-glycolide): copolymer composition 50:50 (PLG), Resomer RG 502 was supplied by Boehringer Ingelheim, Germany. Poly(vinyl alcohol) (13–23 KDa, 87–89 hydrolysed) (PVA) was obtained from Aldrich, USA. Dichloromethane (MC) and dimethylsulfoxide (DMSO) of analytical grade were supplied by Merck, Germany. Solution of Mannitol^{14}C (Dupont NEC-314, 54, 5 Ci/mmol, ethanol:water solution 9:1) was from Dupont NEN. PC (Ovothin 200) was obtained from Lucas Meyer, Germany. All chemicals were used without further purification.

Table 1
Size distributions of microspheres prepared with different PLG concentrations in the oil phase using different internal aqueous phase volumes, $V_{i(aq)}$

$V_{i(aq)}$ (μl)	Diameter[a] (μm) at which indicated fraction (%) of microspheres was larger								
	4.3% PLG			27% PLG			43% PLG		
	10	50	90	10	50	90	10	50	90
50	7.1 (0.1)	4.3 (0.2)	3.1 (0.1)	26 (5)	19 (4)	10 (1)	47 (3)	30 (3)	12 (2)
100	7.6 (0.9)	4.8 (0.2)	3.2 (0.0)	33 (7)	23 (3)	12 (1)	51 (10)	33 (3)	16 (2)
200	8.0 (2)	4.5 (0.5)	3.1 (0.2)	32 (1)	21 (2)	9 (1)	55 (3)	29 (8)	16 (6)
400	8.1 (0.4)	5.0 (0.1)	3.2 (0.1)	31 (2)	22 (2)	12 (1)	50 (14)	29 (2)	16 (4)

[a] Standard deviation given in brackets.

Table 2
Size distributions of microspheres prepared with different concentrations of PC in the oil phase. 27% PLG was used in the oil phase and the internal aqueous volume was 100 μl

Concentrations of PC in the oil phase (%)	Diameter[a] (μm) at which indicated fraction (%) of microspheres was larger		
	10	50	90
0	33.0 (7)	23.0 (3)	11.4 (0.8)
0.1	27.4 (0.0)	16.7 (0.2)	8.1 (0.9)
0.5	24.6 (0.2)	15.9 (0.4)	8.0 (1)

[a] Standard deviation given in brackets.

3. Methods

3.1. Microsphere preparation

A solution of radioactively labelled mannitol (18 μM) in Milli-Q water (internal aqueous phase), 50–400 μl, was emulsified with a solution of PLG in dichloromethane (oil phase), 1 ml, using a polytron homogenizer (Kinematica AG PT 300) at high speed (approximately 15000 rpm for 10 s).

Three PLG concentrations were employed in the oil phase, 4.3, 27 and 43% (w/w). In some preparations 0.1 and 0.5% (w/v) PC was dissolved in the oil phase. The resulting w/o emulsion was then dispersed by using a vortex mixer (Whirlmixer) for 5 s in a 10% (w/v) poly(vinyl alcohol) solution (external aqueous phase), 1 ml, to produce a w/o/w emulsion. In a series of preparations using a PLG concentration of 27%, the time for the second emulsification was varied from 5 to 80 s to study its effect on microsphere size and entrapment efficiency.

The resulting double emulsion was then poured into 5 ml 10% PVA aqueous solution, stirred magnetically for 15–18 h at room temperature to allow solvent (MC) evaporation and microsphere formation. The microspheres were isolated by centrifugation (Eppendorf Centrifuge 5403) at 3000 rpm for 10 min, washed three times in water, and freeze-dried (Super Modulyo, Edwards) overnight under vacuum at −25°C. The final dried microspheres were stored in a desiccator at room temperature. During the time for storage it was checked that the microspheres were stable.

3.2. Size distribution

The freeze-dried microspheres were dispersed in filtered (0.22 μm filter) 0.9% (w/w) NaCl. Volume size distributions were obtained using a multisizer (Coulter, Multisizer II). The results are presented

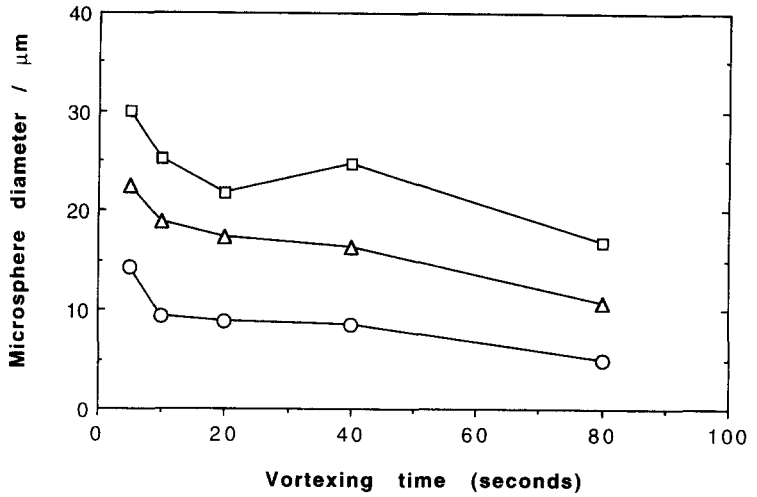

Fig. 1. Size distributions of microspheres prepared with 27% PLG in the oil phase produced using different vortexing times in the second emulsification. Size at which: 10% of the particles was larger (□), 50% was larger (△) and 90% was larger (○).

as mean values with their standard deviations from measurements on duplicate samples from two different batches.

3.3. Morphology of microspheres

The external and internal morphology of some preparations were analyzed by scanning electron microscopy (SEM). Carefully dried microparticles were cross-sectioned with a razor blade. Surfaces and cross-sections of microparticles were coated with gold–palladium (metallization) under an argon atmosphere at room temperature. The external and internal morphology was then examined with a SEM (Jeol-JSM-T 330 Scanning microscope).

3.4. Entrapment efficiency

PLG microspheres (10 mg) were dissolved in 1 ml DMSO. Scintillation liquid (10 ml) was added to the solution. The amount of mannitol entrapped in microspheres was calculated by measuring the radioactivity (cpm) in the container using a liquid scintillation analyzer (Packard, 1900 CA). The results are presented as mean values with their standard deviations from duplicate samples from two batches. The entrapment efficiency (%) was calculated by:

$$\frac{\text{measured radioactivity per batch microspheres}}{\text{initially added radioactivity in the internal aqueous phase}} \times 100$$

3.5. Release experiments

In vitro release studies were carried out to investigate the effects of polymer concentration, internal water phase volume and presence of PC on the release kinetics of mannitol^{14}C. Freeze-dried microspheres (35–75 mg) were dispersed in 10 ml PBS (phosphate buffered salts, pH 7.4) in small plastic containers and incubated on a shaking table (TiterTek, Flow Laboratories) at 37°C. At different time intervals aliquots of 1 ml were carefully taken from the acceptor medium and replaced by 1 ml of fresh buffer. Prior to sampling the containers were centrifuged at 2600 rpm for 20 min to prevent microsphere contamination of the samples. The radioactivity of the aliquots were measured and the cumulative release of mannitol^{14}C was calculated. The monitoring of drug release was continued until cessation of the release.

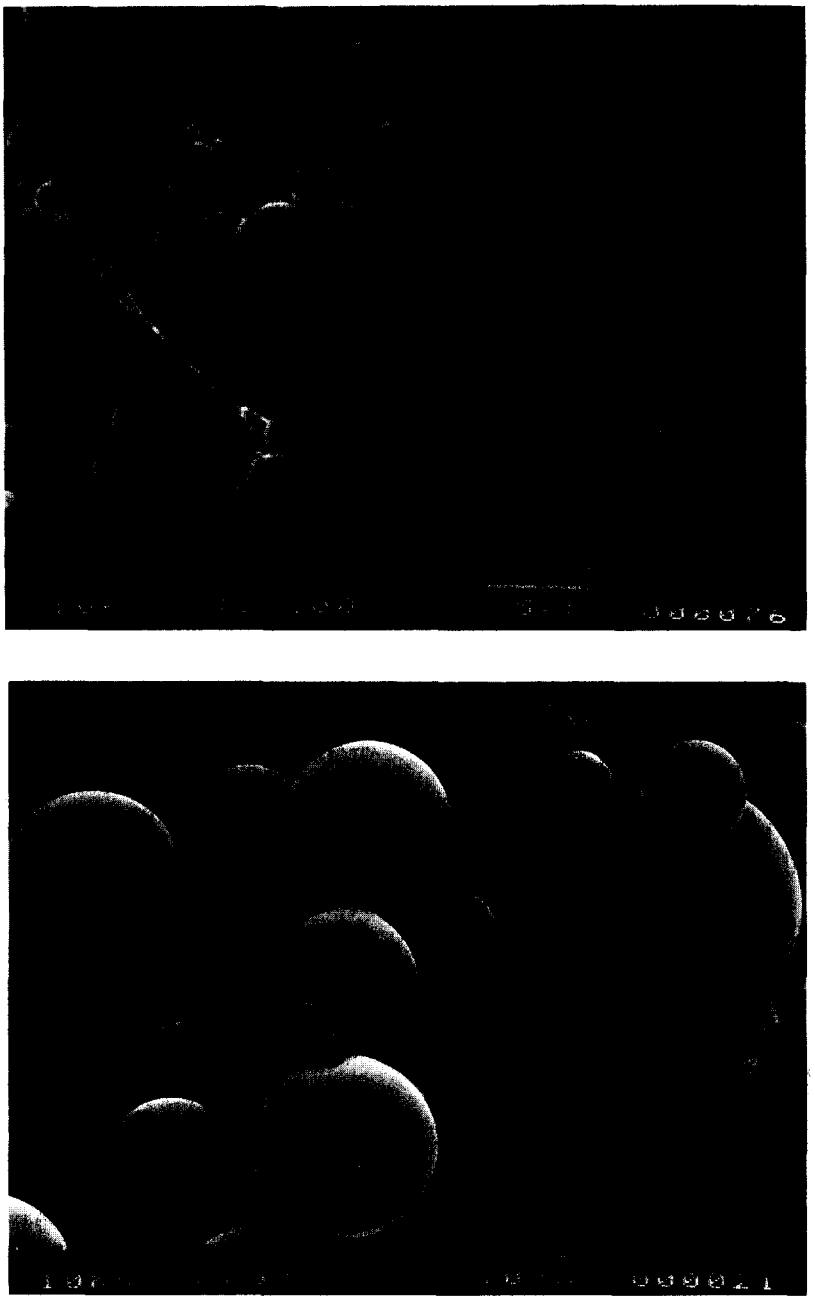

Fig. 2. Representative SEM pictures of microspheres prepared using: (a) 4.3% PLG in the oil phase and (b) 43% PLG in the oil phase. The internal aqueous phase volume was 100 μl.

4. Results and discussion

4.1. Particle size distribution

Table 1 shows how the particle size distributions were affected when using different concentrations of PLG in the oil phase at varying internal water phase volumes. The particle size increased linearly with increasing polymer concentration. The results are in agreement with earlier findings using a similar method of preparation (Yan et al., 1994). The observed relationship is a combination of differences in the PLG contents of the emulsion droplets and a viscosity effect. For example, the median diameter of microspheres prepared at a PLG concentration of 43% was approximately six times larger than for microspheres prepared with 4.3% PLG in the oil phase. By simple calculation, a tenfold difference in PLG contents between oil droplets of the same size would result in a twofold difference in microsphere diameter. The remaining size-difference is probably accounted for by a viscosity effect. The higher viscosity of the oil phase with the higher polymer concentration leads to a less effective emulsifying process resulting in larger droplets producing larger microspheres. Changing the volume of the internal aqueous phases did not affect the size distribution of the microspheres. Thus, the increase in internal aqueous volume from 50 to 400 μl did not affect the size-determining emulsification process.

In Table 2 it is shown how the presence of PC affects the size of microspheres prepared by the present method. The size reduction obtained with PC was expected owing to its emulsifying and stabilising effects. The lower concentration of PC used was sufficient for stabilizing the emulsion. Increasing the concentration of PC from 0.1 to 0.5% in the oil phase did not result in a further size reduction. When employing a concentration of 4.3% PLG in the oil phase, the presence of 0.1% PC reduced the size of microspheres by 40% but when 0.5% PC was used the size was reduced by 60%. Obviously the smaller emulsion droplets obtained using a lower PLG concentration results in a larger interfacial area requiring a higher amount of PC to be stabilised. Thus the effect of PC on the size of microspheres depends on the PLG concentration employed.

Using the double emulsion method for producing microspheres, the second emulsification is decisive for the size of the microspheres. Hence, the intensity and the time of emulsification can be used for controlling size. In Fig. 1 it is shown how the size distribution of microspheres prepared with 27% PLG in the oil phase depends on the vortexing time.

4.2. Morphology of microspheres

Representative SEM pictures of the external surfaces of PLG microspheres are shown in Fig. 2. For all preparations reported in this paper the microspheres were spherical in shape and very few pores were observed on the external surfaces. At the cross-sections of the microspheres spherical holes were observed (see Fig. 3). From their size, 0.1–5 μm, these holes were probably remnants from the internal aqueous phase droplets. From Fig. 3 it can be seen that the number of holes increased with increasing internal phase volume used in the preparation of the microspheres.

4.3. Entrapment efficiency

Increasing the PLG concentration in the oil phase increased the entrapment efficiency of mannitol^{14}C, Table 3. The major fraction, 64–83% (depending on the extent of entrapment) of mannitol was recovered in the external aqueous phase of the double emulsion. Thus, the dominating loss of mannitol must be due to transport of droplets of the internal aqueous phase to the external aqueous phase. As the double emulsion is formed, progressive loss of the organic solvent eventually leads to solidification of microspheres causing cessation of this transport. With an increase in the PLG concentration of the oil phase the time for reaching solidification will be shorter. Also, the increased viscosity in the oil phase caused by the increased PLG concentration will decrease the loss transport of mannitol^{14}C and contribute to

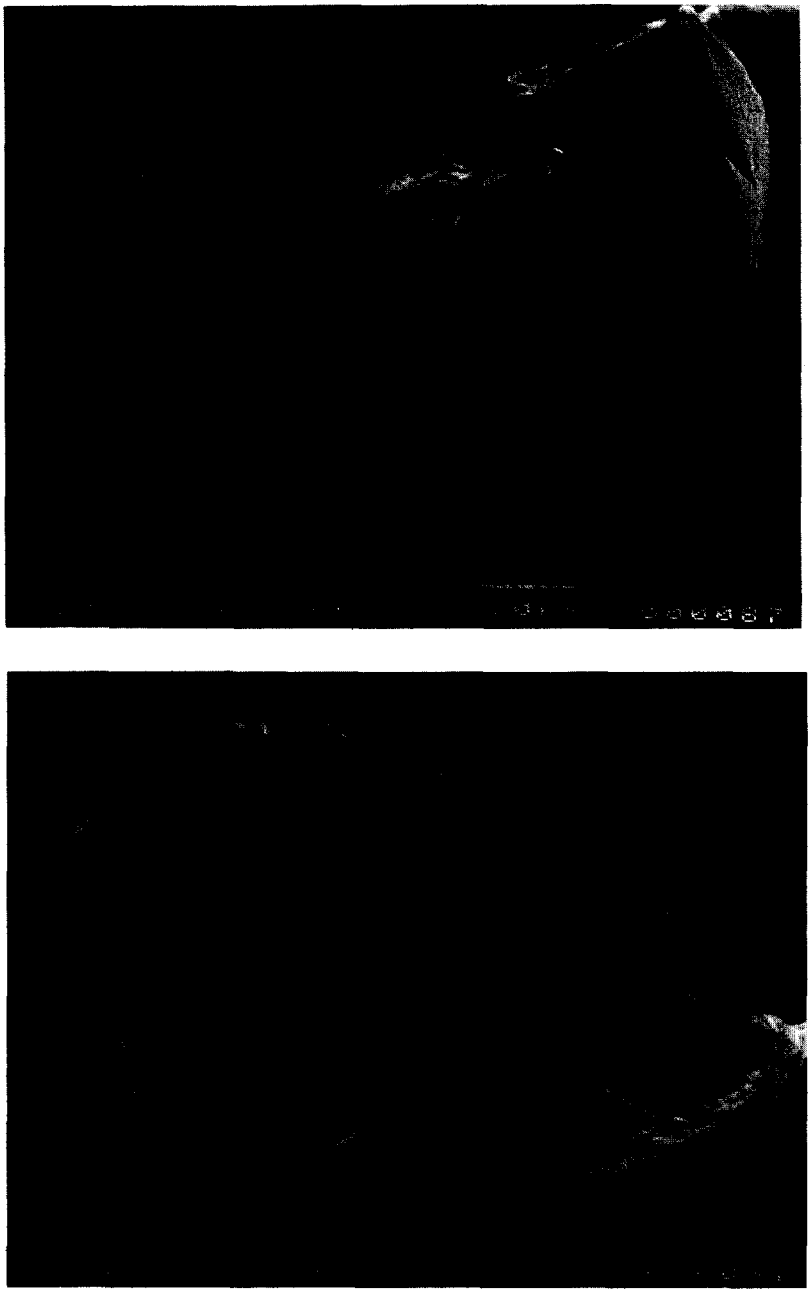

Fig. 3. Representative SEM pictures showing the crossections of microspheres prepared with different internal aqueous phase volumes: (a) 50 μl; (b) 100 μl; (c) 200 μl; and (d) 400 μl. The PLG concentration in the oil phase was 43%.

the enhanced entrapment efficiencies. The time for reaching solidification of the microspheres is therefore critical for the entrapment efficiency of the double emulsion method. By changing the

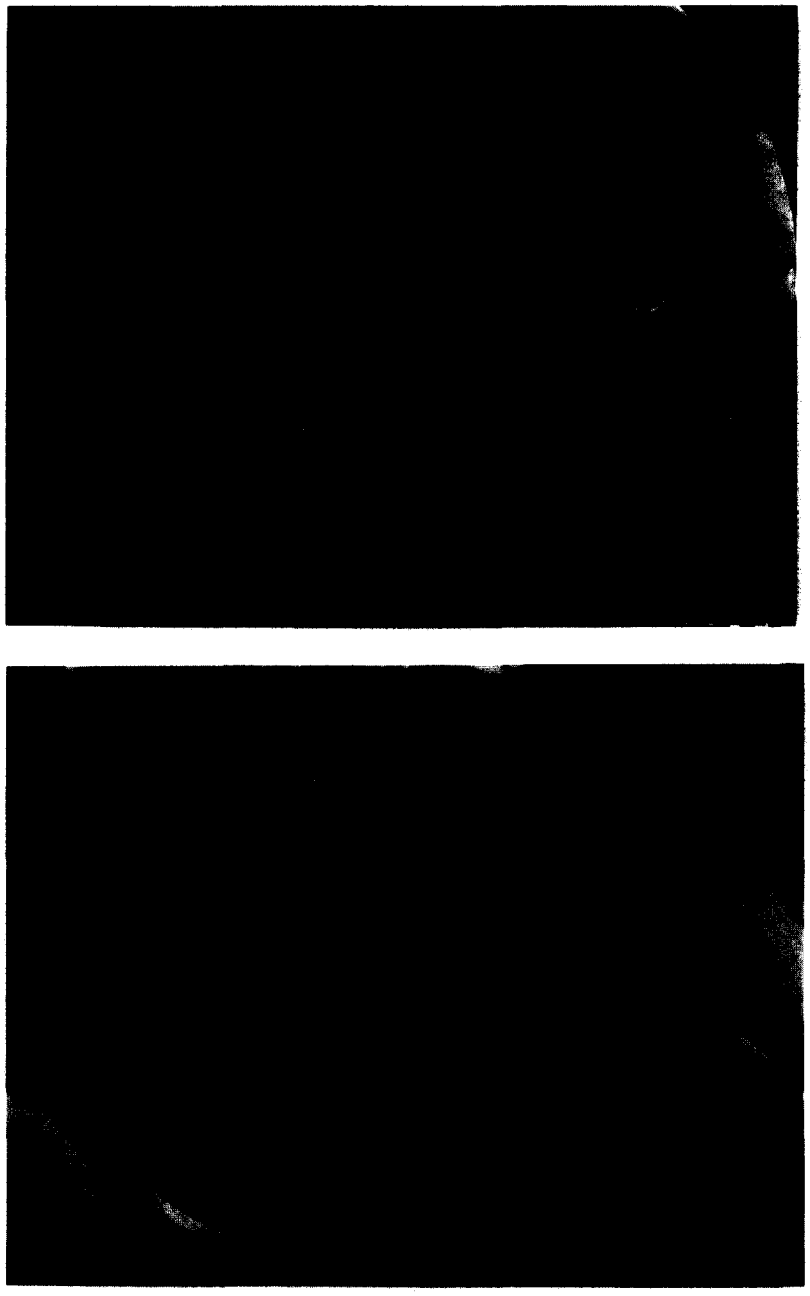

Fig. 3 (C–D).

solubility of the organic solvent in the external aqueous phase or its volume the rate of organic solvent removal can be controlled (Alonso et al., 1993; Bodmeier and McGinity, 1988). It must be born in mind, however, that the rate of solvent removal may also affect the porosity of micro-

Table 3
Entrapment efficiencies of mannitol^{14}C in microspheres prepared with different PLG concentrations in the oil phase using different internal aqueous phase volumes, $V_{i(aq)}$

$V_{i(aq)}$ (μl)	Average entrapment efficiency[a] (%)		
	4.3% PLG	27% PLG	43% PLG
50	1.3 (0.0)	11.0 (2)	19.0 (5)
100	1.6 (0.5)	11.9 (0.4)	26.0 (5)
200	0.8 (0.0)	8.1 (0.1)	25.7 (0.7)
400	0.5 (0.3)	13.9 (0.8)	29.0 (11)

[a] Standard deviation given in brackets.

spheres (Mathiowitz et al., 1990; Albertsson et al., 1994, 1996).

When using 4.3% PLG in the oil phase the entrapment efficiency decreased with increasing volume of the internal aqueous phase. In contrast, when higher PLG concentrations were employed the entrapment efficiency remained unchanged as the inner phase volume was increased (Table 3).

The use of PC as a stabiliser of the emulsion lowered the entrapment efficiency of mannitol^{14}C in microspheres (see Table 4). The presence of PC enhances the emulsification process. Consequently, the water droplets formed from the inner aqueous phase, containing mannitol^{14}C, will be very small. Their diffusion to the external phase will thus be relatively fast, explaining the low entrapment efficiency obtained.

As mentioned above, the size for the prepared microspheres was controlled by the intensity and time used in the second emulsification. On the other hand, if the second emulsification is too intensive, this will result in loss of the double emulsion structure with a concomitant drop in the amount of entrapped mannitol^{14}C. The entrapment efficiencies of mannitol were therefore checked for the preparations subjected to longer vortexing times (27% PLG in the oil phase, vortexing for 5, 10, 20, 40 and 80 s, respectively). The vortexing time had no significant effect on the entrapment efficiency even though the size of the microspheres was reduced by 52% (see Fig. 1). The PLG concentration used in the oil phase for these preparations was 27%. When employing a PLG concentration of 4.3% in the oil phase, however, the integrity of the double emulsion was gradually lost when the vortexing time was increased. This was observed by microscopical observation. Thus, the lower viscosity obtained by using a lower concentration of PLG in the oil phase reduced the stability of the double emulsion.

Table 4
Entrapment efficiencies of of mannitol^{14}C in microspheres prepared with different concentrations of PC in the oil phase using 27% PLG in the oil phase and 100 μl internal aqueous phase volume

Concentrations of PC %	Average entrapment efficiency[a] (%)
0.0	11.8 (0.4)
0.1	7.0 (0.4)
0.5	4.5 (0.2)

[a] Standard deviation given in brackets.

4.4. Release experiments

Mannitol^{14}C was incorporated in microspheres prepared using PLG concentrations of 4.3, 27 and 43%, respectively. The release of mannitol from these preparations is shown in Fig. 4. Since PLG microspheres sometimes display discontinuous drug-release behavior it is important to monitor the release process until 100% of the incorporated substance is recovered. The amounts of recovered mannitol from all release experiments were in close agreement with the values obtained from the entrapment determinations by dissolving the microspheres in DMSO.

A strong burst effect was observed for the microspheres prepared with the lowest PLG concentration. The strong burst effect is probably a result from diffusion of surface-localised mannitol^{14}C. The smaller size of these microspheres leading to a higher area/volume ratio, as compared with the microspheres using higher PLG concentrations in the oil phase, accounts for this effect. For the other two preparations mannitol^{14}C was continuously released.

For microspheres prepared using 43% PLG in the oil phase the volume of the internal aqueous

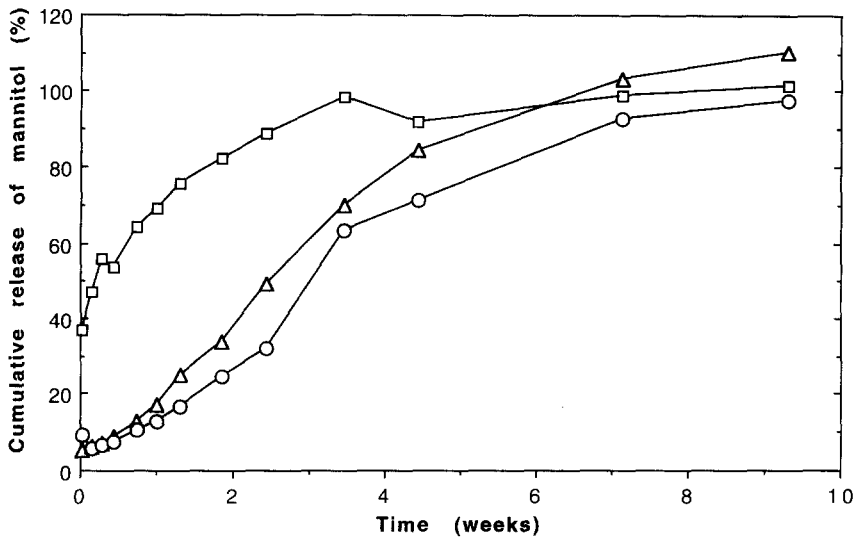

Fig. 4. Release of mannitol^{14}C from microspheres prepared with different concentrations of PLG in the oil phase: 4.3% PLG (□), 27% PLG (△) and 43% PLG (○). The internal aqueous phase volume was 100 μl.

phase was varied. The release of mannitol^{14}C from these preparations is shown in Fig. 5. The release rate increased with increasing internal aqueous volume used at the preparation stage. Empty spaces were created in the microspheres as the water of the internal aqueous phase had been removed by the preparation process (see Fig. 3). In the SEM study it was observed that the porosity increased with increasing internal aqueous phase volume used at the preparation. Thus, there is a correlation between drug-release and the porosity of the microspheres.

Finally the release of microspheres prepared in the presence of PC was studied. In Fig. 6 the release of mannitol from these preparations are compared with the release from microspheres prepared without PC. The presence of PC induced a higher burst effect. This indicates that with PC present during the preparation, the distribution of mannitol within the matrix is modified causing an increased localisation of the incorporated substance at the surface of the microspheres.

Release from biodegradable microspheres is dependent both on diffusion through the polymer matrix and on polymer degradation. At higher loading ratios, pores are created as a result of drug dissolution by penetrating water resulting in increased release rates.

In addition to the properties of the polymer matrix the release depends on the specific properties of the drug molecule such as diffusivity in the core material, solubility, the size of the drug molecule, the distribution of drug in the microsphere matrix and specific interactions with the polymer.

In the present study the release experiments were performed to probe differences in the matrix properties caused by simple modifications of the preparative process. This is the reason why an extremely low contents of mannitol in the microspheres was employed. In addition no specific interactions between mannitol and PLG were expected. A low porosity thus results in longer diffusion distances through the matrix for mannitol and lower the permeability for penetrating water leading to a slow degradation of PLG. The release rate for larger molecules, such as peptides and proteins from microspheres at low drug loading should be dominated by the properties of the polymer material due to their large sizes. For microspheres of low porosity, the polymer barrier must degrade in order to create channels and

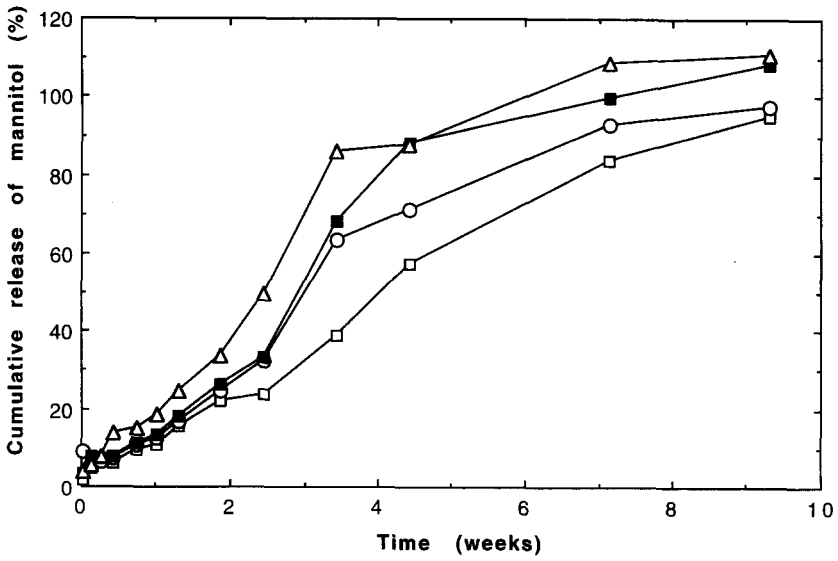

Fig. 5. Release of mannitol^{14}C from microspheres prepared with different volumes of the internal aqueous phase; 50 μl (□), 100 μl (○), 200 μl (■) and 400 μl (□). The PLG concentration in the oil phase was 43%.

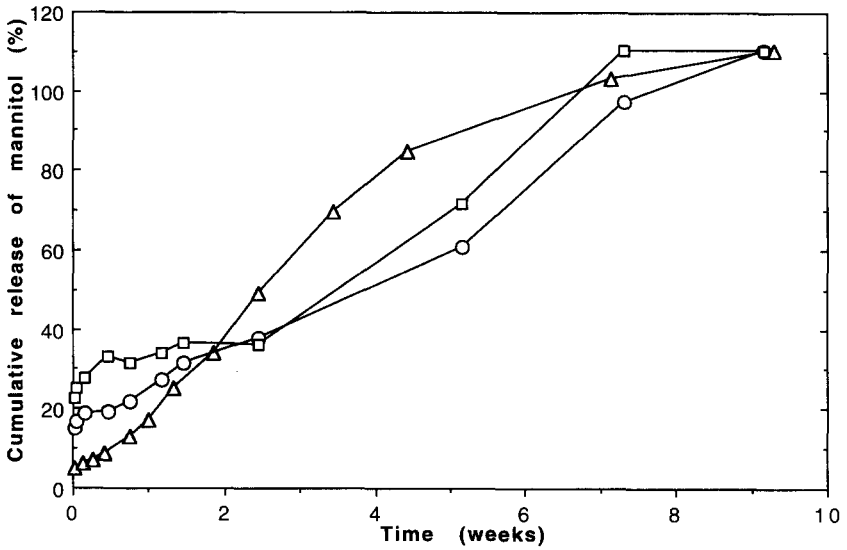

Fig. 6. Release of mannitol^{14}C from microspheres prepared with different concentrations of PC in the oil phase: 0% PC (△), 0.1% PC (○) and 0.5% PC (□). The PLG concentration in the oil phase was 27%.

pores within the matrix large enough for large molecules to be released. The PLG polymer material first swells in an aqueous environment and then degrades at rate, depending on the polymer molecular weight and the copolymer composition (Jalil and Nixon, 1990; Shah et al., 1992).

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