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USE OF SURFACTANTS IN POLYLACTIC ACID PROTEIN MICROSPHERES

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ABSTRACT

This paper describes the effect on the in vitro release profile of poly(D,L-lactic) acid (DL-PLA), and poly(D,L-lactic-co-glycolic) acid (D,L-PLGA), microspheres containing BSA as a model protein prepared by the double emulsion technique, due to the addition of surfactant agents in the first emulsion (W/O) made using two type of homogenizer. In the first stage D,L-PLA microspheres were prepared with a mixture of Span-40® and Tween-80® to get HLB 6 and 7, W/O ratio in the first emulsion 1:3 and two types of homogenizers (High pressure homogenizer and Sonicator) against microspheres without surfactants. The microspheres made with high pressure homogenizer presented less BSA trapping efficiency and higher burst effect than those made with sonicator which showed a very slow release rate. In the second stage D,L-PLA and D,L-PLGA microspheres with and without surfactant agents and using sonicator were compared. The BSA release from D,L-PLGA microspheres was continuous, but batches with HLB 6 and without surfactant presented a initial release of 55 % of the incorporated BSA while batch with HLB 7 showed less burst effect and slower release rate. The D,L-PLA microspheres released a percentage of incorporated drug at the beginning of the in vitro assay and then stopped.

INTRODUCTION

The increasing use of proteins in therapy has created a demand for suitable formulations in which to administer these substances. Most peptides and proteins should not be taken orally as they are known to lose potency in the digestive tract; they may be administered intravenously, subcutaneously, intramuscularly or intranasally all of which allow therapeutic levels to be reached in a relatively short space of time. The treatments for which these therapeutic agents are prescribed require stable levels over prolonged periods which has led to the development of sustained release systems generally based on biodegradable polymers of which polylactic acid and its polylactic-glycolic co-polymers are the most often used (1-5) in the manufacture of cylinders and microspheres. Microspheres are the systems to which most attention has been given as they are easier to administer.

The release of proteins from this type of formulation is complex, and seems to occur in three phases, the first, burst effect, the second, the formation of pores in the matrix and the third, polymer degradation (6,7). If the second and third phase can be superimposed on one another, release will be continuous; if not, a time lag may ensue until the polymer begins to break down (3,6,7). This lag time may also occur at the outset of release (8).

To achieve a sustained release all the time, modifications of the various stages of the manufacturing process have been attempted. The most often used technique is that of the double emulsion W/O/W (1), variations in release caused by variations in internal aqueous, internal oleous and external aqueous phase (9,10) have been studied additives (4) and fat acids have been added to form esters and surfactant agents (11).

The aim of this paper was to study if the addition of surfactant agents to the preparation of the first emulsion (W/O) to obtain microspheres by the double emulsion technique modifies the characteristics and the in vitro release profile of DL-polylactic acid (DL-PLA) and poly(lactide-co-glycolide) (DL-PLGA) microspheres containing albumin (BSA) as model protein. In addition, two kinds of homogenizer were evaluated for the preparation of the first emulsion.

MATERIAL AND METHODS

Polymer synthesis.- DL-PLA and DL-PLGA were obtained by the ring-opening reaction described by Kulkarni et al (12). Tetraphenyl tin and stannous octoate (both from Sigma) were used for the respective catalyses.

Polymer characterization.- Molecular weights were determined by gel permeation chromatography (GPC) on a Waters® chromatograph with four columns in a row of different pore size (Ultrastyragel) and tetrahydrofuran (Merck) as solvent. Polystyrene monodisperse standards (Tokyo Soda Ltd) were used to calibrate the system. The composition of the copolymer was determined by 1H-NMR (13,14) using a Bruker AMX spectrometer at 400 MHz. Samples were dissolved in CDCl3 and gently heated. The composition given in moles of lactic-glycolic acid was measured from the relationship of the areas of the signals obtained at δ 4.82 (methylene group of the glycol acid unit) and δ 5.21 (methine group of the lactic acid unit). The samples were prepared by dispersing 15 mg of copolymer in CDCl3 and gently heating. The relative proportions of lactic acid-glycolic acid units (LA-GA) and glycolic acid-glycolic acid (GA-GA) were assessed by 13C NMR (15) on a Bruker AMX at 100.61 MHz using DMSO-d6 as solvent. Signals at 166.7 and 166.8 ppm were associated with the carbonyl groups of the LA-GA and GA-GA units, respectively; the relative intensities of these signals determined the relative proportions of the LA-GA and GA-GA units.

Microsphere preparation.- Microspheres were prepared by a modification of Ogawa's method (1): BSA (158 mg) was dissolved in water together with the more hydrophilic surfactant and the polymer (630 mg), [DL-PLA (Mw = 27,000) or DL-PLGA (Mw = 53,000)] with the more lypophilic surfactant agent were dissolved in methylene chloride to make a water-in-oil (W/O) first emulsion; the polymer and BSA solutions were homogenized either with a high pressure homogenizer (HPH) or a sonicator for 1 min and then poured into 400ml of aqueous 1% Polyvinylalcohol (PVA) and the solution was stirred at 8000 rpm at 5°C for a few seconds to make a (W/O)/W emulsion which was then stirred for 2 hours at 250 rpm (room temperature) to evaporate off the methylene chloride. The microspheres were collected by filtration and dried in a vacuum for 24 hours.

The albumin content of the microspheres was determined by dissolving them in methylene chloride and extracting the albumin in water. The concentration of BSA was measured by the Lowry spectrophotometric method (16). Pluronic®, Span®40, Tween®80 and PVA of av.mol. wt. 30,000-70,000 were purchased from Sigma Chemical Co. Bovine serum albumin (BSA) came from Merck. All the other chemical were reagent grade.

Particle size.- Microspheres suspended in 0.9 % NaCl solution were measured by a Coulter counter (Coulter® Multisizer II) after bath sonication.

Morphology.- The shapes and surface characteristics of the dried microspheres were examined by scanning electron

microscopy (SEM). To study their surface structure, the microspheres were coated with gold palladium under argon atmosphere.

In vitro release assay.- Microspheres were dispersed in isotonic PBS containing 0.001% Tween®80 and 0.02% sodium azide and kept in a water bath at 37°C. At each time interval 1 ml of the medium was withdrawn, the albumin released and the suspension refilled with 1ml of fresh medium. The release assays were replicated three times.

RESULTS AND DISCUSSION

Preliminary screening made use of different percentages of Pluronic® (HLB = 29), Span®40 (HLB = 6.7), Span®60 (HLB = 4.7) as surfactants and resulted in very low trapping or difficulties in extracting the BSA.

Although HLB in the range of 3 to 6 is recommended (17) for a stable W/O emulsion, because of the polarity of the organic solvent, in view of the preliminary results, a comparative study was designed. DL-PLA microspheres containing mixtures of Tween®80 and Span®60 in the aqueous and organic phase respectively, intended to achieve HLB's of 6, 7 and 8, and 1:20 and 1:10 ratios of water/methylene chloride were prepared using a high pressure homogenizer to obtain the first W/O emulsion and compared with microspheres made without surfactants. The results are shown in Table 1, the trapping efficiency was very low for all batches particularly for batches made with mixtures of surfactants to give an HLB of 8 which was therefore dropped from this study which involved only DL-PLA microspheres prepared with a mixture of surfactants to give HLB 6 and 7, a 1:3 ratio of water/organic solvent and two types of homogenizer and microspheres without surfactants.

The results are set out in Table 2, the average mean of trapping efficacy was 66.7% for microspheres made with the high pressure homogenizer and 89.7% for those made with the sonicator. Size ranged from 23.2 to 35.7 µm. Figure 1 shows microspheres from batches A(without surfactants), C(with) and D(without), E(with) as examples of microspheres made in the HPH and sonicator respectively.

The release profiles are shown in Figure 2; as can be seen, the release of BSA seems to be affected by the presence of surfactants modifying the initial release. When the high pressure homogenizer was used, the microspheres with surfactants showed a higher burst effect, 35% in the first 6 hours, than did those without surfactants, 18%, which presented greater size. Three batches (A, B and C) released BSA during 48 hours and then stopped, never releasing their full complement, at least not within the fortnight that the assay lasted. Of the batches made in a sonicator, E released 20% of the

TABLE 1
Manufacture conditions and percentage of Albumin entrapping for microspheres made with HPH.

Batch	Phases Ratio	HLB	Entrapping efficiency (%)
1	1 : 10	8	0.32
2	"	-----	4.65
3	"	8	6.50
4	"	6	6.87
5	"	7	7.19
6	1 : 20	8	12.70
7	"	7	22.70
8	"	6	16.30

TABLE 2
Effects of HLB and type of homogenizer in BSA entrapping efficiency and mean volume diameter (dmv) of PLA microspheres with a 1:3 ratio water/organic solvent.

Batch	HLB	Homogenizer	Entrapping (%)	dmv (μm)
A	----	H.P.H.	65.60	35.70
B	6	"	66.00	25.70
C	7	"	68.70	23.20
D	----	Sonicator	92.70	27.60
E	6	"	86.30	26.90
F	7	"	90.30	28.90

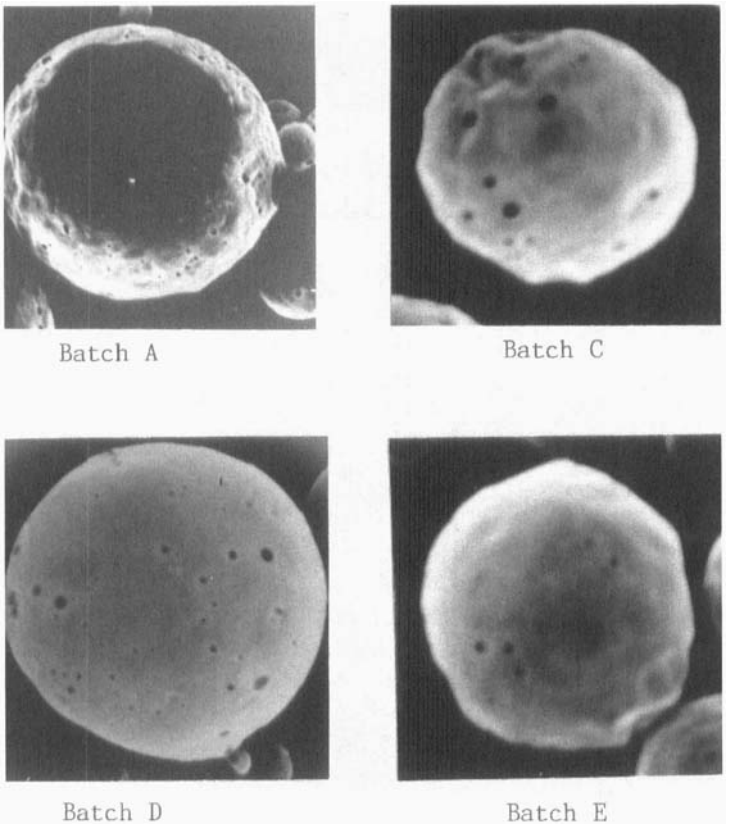

FIGURE 1
Scanning electron micrograph of BSA DL-PLA microspheres: batch A (without surfactants) and batch C (with, HLB 7), prepared with High pressure homogenizer; batch D (without) and batch E (with HLB 6) prepared with Sonicator.

BSA in the first 72 hours, stopped, and D and F released 1.5 and 6% over 48 h before stopping.

As can be seen from Figure 1, there were no important differences in the shape of the microspheres; those made with the high pressure homogenizer, could be rougher and might have had more free drug close to the surface which was released at once.

From these assays it is difficult to distinguish what role is played by the surfactant agents in albumin release kinetics although they would appear slightly to favour release.

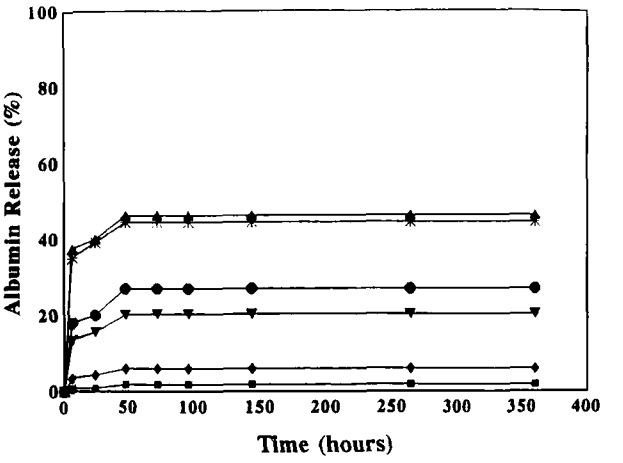

FIGURE 2
Cumulative release of BSA from DL-PLA microspheres. Effects of HLB and type of homogenizer. HPH: lot. A (●) without surfactants, lot. B (▲) HLB=6, lot. C (✱) HLB=7. Sonicator: lot. D (■) without surfactants, lot. E (▼) HLB=6, lot. F (♦) HLB=7.

Quite apart from the presence of surfactant agents, the slow, almost imperceptible, release of these lots of microspheres may be due to the DL-PLA having a relatively low Mw and, as has been suggested elsewhere (18), protein release may be incomplete thanks to the formation of bridges between the end alcohol groups of the polymer, which are in inverse proportion to its molecular weight, and to the odd pairs of protein donor atoms. This phenomenon had previously been observed by Bodmer et al. (7) for DL-PLGA copolymers (75/25) with molecular weights of under 40,000. Other authors (6,19) have suggested that, for a sustained release to be possible, phases 2 and 3 in which it is supposed to occur must be superimposed. If this is not the case, there is a time lapse (non-release) until the polymer begins to be degraded and drug release resumes.

The molecular weights of the microspheres made with DL-PLA, batches were determined at the end of the release assay and proved not to have been modified and the structure of the microspheres, examined under an electron microscopy, remained unaltered, clearly indicating that in these cases release phases 2 and 3 had not been superimposed but that BSA may have been restrained by weak unions with the polymer.

TABLE 3
Microspheres elaboraded with DL-PLGA (70 / 30) copolymer. Effects of HLB in BSA entrapping efficiency and mean volume diameter (dmv).

Batch	HLB	Entrapping (%)	dmv (μm)
G	---	69.00	22.50
H	6	58.50	24.90
I	7	67.00	17.50

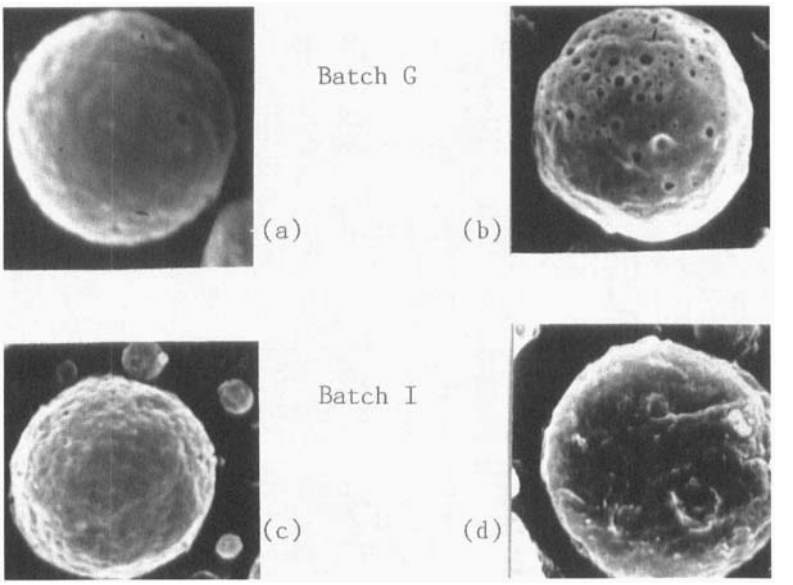

FIGURE 3
Scanning electron micrograph of BSA DL-PLGA (70/30) microspheres: batch G (without surfactants), (a) before and (b) after release assay; Batch I (with HLB 7), (c) before and (d) after release assay.

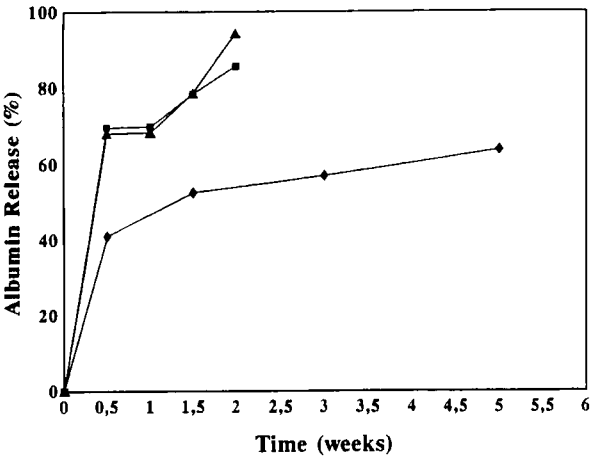

FIGURE 4
Release profile of BSA from DL-PLGA (70/30) copolymer. Effects of HLB in BSA release. Lot. G (▲) without surfactants, lot. H (■) HLB=6, lot. I (♦) HLB=7.

In view of the foregoing, 3 further batches of microspheres were prepared, G, H and I (Table 3) with a DL-PLGA copolymer (70/30) which was more sensitive to hydrolysis and therefore had much faster degradation kinetics than PLA (6,7). Its molecular weight was 53,000, higher than that of the PLA and so it was to be expected that it would contain fewer free alcohol groups and not retain the protein as much.

Figure 3 depicts microspheres from lots G (without) and I (with surfactants) before the completed release assay and afterwards. The release profiles of these lots are shown in Figure 4; the release rate was higher, lots G and H practically completed release in 12 days but with a very high burst effect, of about 55%. Lot I, made of a mixture of Span® 60 and Tween® 80 to give HLB 7, had less burst effect and also a slower subsequent release after 5 weeks it had released 63.4 %. When the release assay was over, the molecular weight of the copolymer in all lots had decreased from its initial 53,000 to approximately 37,000 which seems to indicate that in these cases the phases of immediate release and copolymer degradation are superimposed and release is continuous.

The surfactant agents included in the first emulsion may modify protein release from microspheres made with DL-PLA and DL-PLGA although this study has not been able to identify the exact mechanism. Protein release is a relatively complex process, as commented earlier, and is influenced by many variables; at the

moment, although it is thought that release is fundamentally governed by diffusion and degradation processes, these are not enough satisfactorily to account for the differences observed in the release profiles.

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