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Effect of Surfactant Agents on the In Vitro Release of Insulin from DL-PLA Microspheres

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ABSTRACT

This paper describes the effect of surfactants on the in vitro release profile of bovine insulin from DL*-polyacetic acid (DL-PLA) microspheres prepared by the double-emulsion technique. The surfactants, Tween®-80 and Span®-60, were included in the water and oil phases, respectively, of the first emulsion. The presence of these agents affected the burst effect but not the subsequent release phase. The latter is more controllable by PLA molecular weight selection, with slower release being achieved as the molecular weight increases.*

INTRODUCTION

The therapeutic uses of peptides and proteins have increased in recent years and have created the necessity of developing new delivery systems for administration. Use of these drugs in the treatment of hormone-dependent diseases requires, in general, daily parenteral injection.

The preparation and controlled release of drugs from polyacetic acid (PLA) and PLGA systems is of current interest because these polymers, when formulated as microspheres, could avoid this disadvantage.

The technique most often used to produce water-soluble drug microspheres is the double-emulsion water/oil/water (W/O/W) approach (1). Modifications in the manufacturing process can give rise to different release profiles (2-5), focused in general on decreasing the burst effect and attainment of a sustained release profile. The use of surfactant agents in the manufacture of peptide and protein microspheres has been studied (6,7). Surfactants, depending on the HLB, were shown to modify drug entrapment efficiency and the release profile.

The aim of this work was to study the effect of surfactant agents included in the first emulsion on the microsphere characteristics and release profile of insulin from PLA microspheres, made by the double-emulsion technique.

MATERIALS AND METHODS

Polymer

DL-Polylactic acid (DL-PLA) was obtained by the ring-opening reaction described by Kulkarni et al. (8), using DL-lactide (Aldrich Chemical Co.) with tetraphenyl tin (Merck) as the catalyst. Molecular weights were determined by gel permeation chromatography (GPC) on a Waters® chromatograph with four columns arranged in a series of different pore size (Ultrastyragel) and using tetrahydrofuran (Merck) as solvent. Polystyrene monodisperse standards (Tokyo Soda Ltd.) were used to calibrate the system.

Microsphere Preparation

Microspheres were prepared as has been described in a previous paper (6). Briefly, the bovine insulin (B-Ins, Sigma) was dissolved in a 30% acetic acid (Merck) solution, sonicated with the PLA dissolved in methylene chloride (output 4; 50 W, ultrasonic probe TDI) for 1 min. The first emulsion was then poured into 400 ml of aqueous 0.13% w/v polyvinylalcohol (PVA) of molecular weight 30,000–70,000 (Sigma) and stirred at 8000 rpm (Ultra-Turrax T25) at 5°C for 5 min, then at 250 rpm for 2 hr (room temperature—RT) to evaporate the organic solvent. The microspheres were collected by filtration and dried in a vacuum for at least 24 hr.

Insulin Content in the Microspheres

The microspheres (20 mg) were dissolved in 2 ml 90% acetonitrile (Merck) aqueous solution and B-Ins was extracted with 5 ml 0.05 N hydrochloric acid (Merck). The aqueous solution was filtered through a 0.45-μm filter (Millipore® HA). B-Ins in the aqueous solution was assayed by high-performance liquid chromatography (HPLC, Waters), under the following conditions: Deltapack-C^{18} at RT; mobile phase was a solution of 74 vol of sodium sulfate anhydrous 0.2 M (Merck), adjusted to pH 2.3 with phosphoric acid (Merck) and 26 vol of acetonitrile; flow rate was 1 ml/min and $\lambda = 214$ nm.

Microsphere Morphology

Particle size was measured by laser diffraction (Malvern) by suspending the microspheres in 0.9% NaCl and employing bath sonication to prevent microsphere aggregation. The shapes and surface characteristics of the dried microspheres were examined by scanning electron microscopy (Philips EM 400 STEM).

In Vitro Release Assay

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

RESULTS AND DISCUSSION

To study the effect of surfactant agents in the first emulsion during the preparation of B-Ins micropheres, four batches were made. The process conditions were 10% w/w of B-Ins and PLA concentration of 470 mg/ml in the first emulsion with 400 ml of aqueous 0.13% w/v PVA as the external water phase.

Lot 1, without surfactants, was made as a control batch, and lots 2, 3, and 4 were made containing mixtures of Tween®-80 and Span®-60 in the water and the organic phase, respectively, to achieve HLBs of 6, 7, and 8.

The microsphere characteristics, as seen in Table 1, showed no important differences in the production yield nor mean particle sizes. However, a higher encapsulation efficiency was obtained for lot 1 made without surfactants. The scanning electron micrographs of microspheres from lots 1 and 3 (HLB 7) are shown in Fig. 1.

The presence of surfactants was shown to affect the in vitro release profiles of B-Ins (Fig. 2). Microspheres prepared with surfactants showed a higher initial release ("burst effect"), with between 10% and 17% released

Table 1

Microspheres Characteristics Made with DL-PLA (M_w 27,000) Containing 10% w/w of B-Ins and 470 mg/ml PLA Concentration: HLB Effect

Batch	Production Yield (%)	EE[a] (%)	HLB	dmv (μm)
1	77.4	74	—	38.8
2	77.8	55	6	38.8
3	81.6	42	7	37.2
4	75.5	52	8	39.6

[a]Encapsulation efficiency.

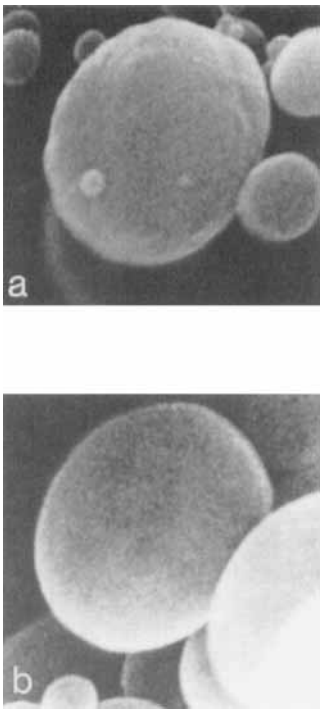

Figure 1. Scanning electron micrograph of B-Ins DL-PLA microspheres: (a) without surfactant; (b) with surfactant (HLB 7).

in the first 24 hr, compared with approximately 7% with lot 1. The surfactant-containing formulations continued to release drug over day 2. However, for the remainder of the test period, no further release was observed. In contract, lot 1 (surfactant-free) continued to release B-Ins from day 2 to day 7 but at an extremely slow rate. After day 7, only 8% of the incorporated drug was released. However, at day 20 (termination of the study), this figure had risen to 28%.

In view of these results, further batches of microspheres were made with the aim of improving the release profiles.

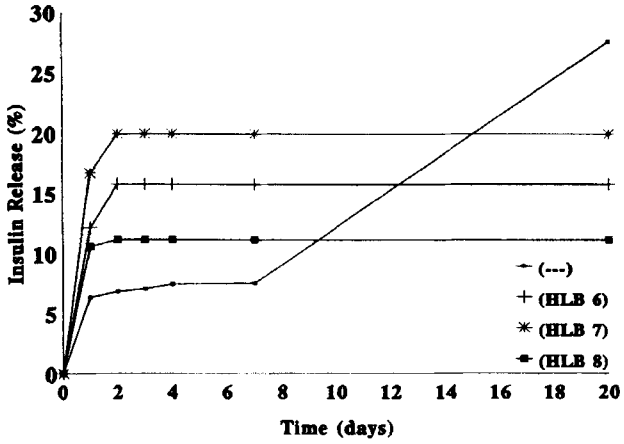

Figure 2. Cumulative release of B-Ins from DL-PLA (M_w 27,000) microspheres. HLB effect.

Some authors (9,10) have claimed that the loading dose, and others (11,12) that the polymer concentration in the oil phase, could affect the release rate of a drug from microspheres. Therefore, two further batches of microspheres were prepared (lots 5 and 6) with the same DL-PLA (M_w: 27,000) but increasing the loading dose to 15% and using a mixture of Tween-80 and Span-60 to give an HLB 7. The ratio of water/methylene chloride and the polymer concentration were also increased.

The microsphere characteristics are shown in Table 2 and the release profiles in Fig. 3. Batch 6, with the lower concentration, presented a high burst effect and after 10 days had released 45%, while lot 5 with higher PLA concentration gave a reduced burst effect and at the 10th day had release 35%. Both provided no further release for at least the following 10 days.

The incomplete protein release over 20 days could be due to the high adsorption capacity of the protein to the low molecular weight PLA (3,13,14). Although we have checked that this phenomenon can happen with BSA and

Table 2

Characteristics of Microspheres Made with DL-PLA of Different Molecular Weights

Batch	PLA M_w	Phase Ratio	Production Yield (%)	EE[a]	dmv (μm)
5	27000	1:6	89.6	69.1	24.2
6	27000	1:9	90.1	78.4	17.1
7	75000	1:3	69.2	76.3	26.9

[a]Encapsulation efficiency.

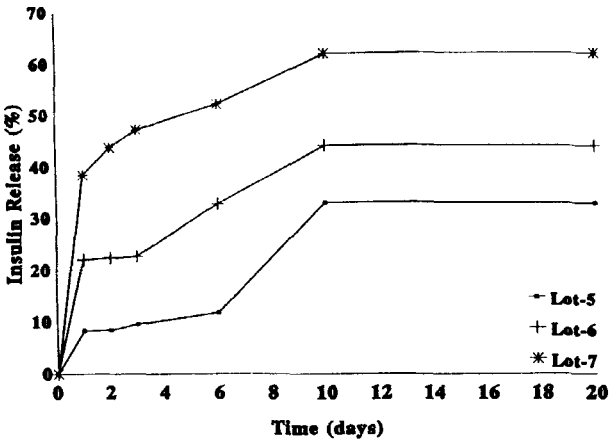

Figure 3. Release profile of B-Ins from DL-PLA microspheres. Lots 5 and 6 with DL-PLA of M_w 27,000, and lot 7 with DL-PLA of M_w 75,000.

insulin, with the latter it is less important. A final lot (lot 7) was made using the same conditions as lot 5, but with PLA or M_w 75,000. Its characteristics are shown in Table 2 and the release profile in Fig. 3. The release rate was faster, the burst effect higher, and the total amount released was larger. However, after 10 days, the polypeptide release ceased, at least between days 10 and 20, as with the other lots.

CONCLUSIONS

In summary, the insulin is well encapsulated in PLA microspheres but the release profile is far from optimal for a sustained-release product. The effect of surfactant agents included in the first emulsion stage may play a role in changing the burst effect but do not seem to control the subsequent release. The release of B-Ins was incomplete although the percentage released increased with the PLA molecular weight, probably due to less protein adsorption.

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REFERENCES

1. Y. Ogawa, M. Yamamoto, H. Okada, T. Yashiki, and T. Shimamoto, Chem. Pharm. Bull., 36(3), 1095 (1988).
2. T. Heya, H. Okada, Y. Tanigawara, Y. Ogawa, and H. Taguchi, Int. J. Pharm., 69, 69 (1991).
3. R. Bodmer, T. Kissel, and E. Traechslin, J. Control. Rel., 21, 129 (1992).
4. M. J. Alonso, R. K. Gupta, C. Min, G. R. Siber, and R. Langer, Vaccine, 12(4), 299 (1994).
5. H. Okada, Y. Doken, Y. Ogawa, and H. Toguchi, Pharm. Res., 11(8), 1143 (1994).
6. I. Soriano, A. Delgado, R. V. Diaz, and C. Evora, Drug Dev. Ind. Pharm., 21(5), 549 (1995).
7. Y. Hayashi, S. Yoshioka, Y. Aso, A. L. Wan Po, and T. Terao, Pharm. Res., 11(2), 337 (1994).
8. R. K. Kulkarni, K. C. Pani, C. Newman, and F. Leonard, Arch. Surg., 93, 839 (1966).
9. M. S. Hora, R. K. Rana, J. H. Nunberg, T. R. Tice, R. M. Guilley, and M. E. Hudson, Pharm. Res., 7(11), 1190 (1990).
10. I. Yamakawa, Y. Tsushima, R. Machida, and S. Watanabe, J. Pharm. Sci., 81(8), 808 (1992).
11. S. Cohen, T. Yoshioka, M. Lucarelli, L. H. Hwang, and R. Langer, Pharm. Res., 8(6), 713 (1991).
12. H. Jeffery, S. S. Davis, and D. T. O'Hagan, Int. J. Pharm., 77, 169 (1991).
13. H. Sah and Y. W. Chien, Drug Dev. Ind. Pharm., 19(11), 1243 (1993).
14. I. Soriano, M. Llabrés, and C. Evora, submitted for publication.