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RESEARCH PAPER

Encapsulation Study of 6-Methylprednisolone in Lipid Microspheres

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ABSTRACT

The search for new pharmaceutical forms that provide greater security and efficiency is one of the main research activities of pharmaceutical technology. This paper concerns a detailed study of the encapsulation of a glucocorticoid with anti-inflammatory action (6-methylprednisolone) in lipid microspheres, one of the systems used for the transport and delivery of lipid-soluble-type drugs. The method used, in common with previous experiments, was that of the solubilization of the constituents of the oil phase (refined soybean oil, soybean lecithin, cholesterol, and the active agent) and, separately, of the aqueous phase of the emulsion (Tween 80, glycerol, and distilled water). The two phases were then mixed by mechanical shaking, and the micelles were homogenized and filtered through membranes of 1.20-μm pore diameter. To determine the percentage encapsulated of the active agent, the first step was the separation of the encapsulated fraction from the free fraction in the medium by molecular exclusion chromatography or filtration in gel. A dextrane (Sephadex G-50 fine) gel was used for the absorbent or stationary phase, and distilled water was used for the eluant, or mobile phase. The determination of the free glucocorticoid in the hydroethanol solution by ultraviolet spectrophotometry at 243 nm permits us to calculate the percentage taken by the microspheres. The formula composed of soybean oil and lecithin in the ratio 1:1.1 (w/w), cholesterol (0.50 g/100 ml), Tween 80 (0.25 g/100 ml), and glycerol (0.63 g/100 ml) produced the greatest quantity of encapsulated active agent: 84.91 ± 4.45%.

***Key Words**: Drug transport and delivery systems; Fat emulsions; Glucocorticoids; Lipid microspheres; 6-Methylprednisolone.*

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INTRODUCTION

Glucocorticoids are a group of drugs that are essential in current therapeutical use, though their clinical application has so far been limited, mainly due to the numerous side effects. With the aim of reducing these as far as possible, the molecule can be associated with a suitable carrier capable of transporting it directly to the biophase, thus preventing its interaction with other cells or tissues (1).

One of these microparticle-sized vector systems is that of lipid microspheres (2). These are oil/water (O/W) emulsions with an internal phase diameter that is always less than 0.5 μm. Structurally, they are composed of fatty micelles made up of vegetable oils surrounded by a phospholipidic layer, immersed in a hydrosoluble fluid. Among their advantages, with respect to other drug transport and delivery systems, are:

1. Great stability, ensuring the effectiveness of the product over a prolonged period
2. Extensive clinical experience in the use intravenously administered fat emulsions in total parenteral nutrition
3. Large-scale production, of great importance for production by the pharmaceutical industry

The prototype of this form of microspheres was Intralipid® (3). The diameter of its micelles is less than 0.5 μm, similar to that of quilomicrons (0.03–0.50 μm). After administration there are no apparent signs of toxicity, as the molecules are completely biodegradable. Another characteristic of lipid microspheres is their high level of stability; they can remain effective for 2 years if stored at 4°C, or even at room temperature (4). The stability is related to the tensioactive action of the lecithin or phosphatidylcholine, a substance that forms a barrier and prevents the coalescence of the lipid drops, in addition to creating a surface potential that keeps the micelles individualized by forces of repulsion.

Among the main encapsulated liposoluble active agents are nonsteroid (5) and steroid (6) anti-inflammatory drugs, bronchial and vasodilatory prostaglandins (7), analogues of prostacyclines (8), antagonists of tromboxane (9), coenzyme Q (10), and cytostatic agents (4,11).

After standardizing methods of obtention, separation, evaluation, and extraction of the active agents from the lipid microspheres (12,13), we considered the encapsulation of a glucocorticoid with anti-inflammatory action such as 6-methylprednisolone.

MATERIALS AND METHODS

Table 1 gives the different formulations prepared, listing their composition, the soybean oil (SO):soybean lecithin (SL) ratio (w/w) and the concentration (g/100 ml) of the different constituents used: refined soybean oil (Roig Farma), soybean lecithin (J. Escuder), cholesterol (Merck), Tween 80 (Glyco Ibérica), and glycerol (Roig Farma). The concentration of 6-methylprednisolone was constant in every formulation, at 0.005 g/100 ml.

Table 1

Formulations Prepared for the Encapsulation Study of 6-Methylprednisolone in Lipid Microspheres

Formula No.	Composition[a]	Ratio SO/SL (w/w)	Concentration (g/100 ml)					
			SO	SL	CH	TW	GL	6MP
1	SO-SL-CH-TW-GL-6MP	1:1.8	0.685	1.25	0.50	0.50	0.63	0.005
2	SO-SL-CH-TW-GL-6MP	1:1.8	0.685	1.25	0.50	0.25	0.63	0.005
3	SO-SL-CH-GL-6MP	1:1.8	0.685	1.25	0.50	—	0.63	0.005
4	SO-SL-TW-GL-6MP	1:1.8	0.685	1.25	—	0.50	0.63	0.005
5	SO-SL-GL-6MP	1:1.8	0.685	1.25	—	—	0.63	0.005
6	SO-SL-CH-TW-GL-6MP	1:1.5	1.345	1.70	0.50	0.50	0.63	0.005
7	SO-SL-CH-TW-GL-6MP	1:1.5	1.345	1.70	0.50	0.25	0.63	0.005
8	SO-SL-CH-GL-6MP	1:1.5	1.345	1.70	0.50	—	0.63	0.005
9	SO-SL-CH-TW-GL-6MP	1:1.1	1.345	1.25	0.50	0.50	0.63	0.005
10	SO-SL-CH-TW-GL-6MP	1:1.1	1.345	1.25	0.50	0.25	0.63	0.005
11	SO-SL-CH-GL-6MP	1:1.1	1.345	1.25	0.50	—	0.63	0.005

[a]SO: soybean oil; SL: soybean lecithin; CH: cholesterol; TW: Tween 80; GL: glycerol; 6MP: 6-methylprednisolone.

The method used to obtain lipid microspheres carrying the active agent is based on the technique described by Mizushima et al. (6) for the production of Intralipid 10%, with the following modifications, affecting both the constituents of the formulation and the apparatus used (13). Basically, there are three stages:

1. *Solubilization* of the constituents of the oil phase (soybean oil, lecithin, cholesterol, and 6-methylprednisolone) and, separately, of the aqueous phase (Tween 80, glycerol, and distilled water).
2. *Emulsification*, or mixing of the two phases, using a mechanical mixer (Omni mixer; Sorvall, model 230) at an initial velocity of 10000 rpm for 0.5 min, followed by 4000 rpm for 15 min.
3. *Homogenization*, or refining: The emulsion obtained is filtered through Millipore® membranes of 1.20-μm pore diameter.

To determine the percentage of encapsulated 6-methylprednisolone, it is first necessary to separate the fraction corresponding to the active agent solubilized in the lipid microspheres from the free fraction in the medium, and then to quantify it. This is carried out by means of molecular-exclusion chromatography or gel filtration: the sample (2 ml of the fat emulsion) is placed in a column (K 15/30; Pharmacia Fine Chemicals) that contains a dextran gel (Sephadex G-50 fine; Pharmacia Fine Chemicals) to act as the stationary phase. This is then eluted through a mobile phase (distilled water). The process is monitored with the aid of a UV/visible spectrophotometer (Perkin–Elmer; Hitachi; model 124) equipped with a continuous flow cell. The wavelength selected was the one that presented maximum absorbance of the active agent (243 nm).

The dextrane retains the free 6-methylprednisolone due to its smaller molecular size, while the microspheres, being larger, pass through the channels of the gel and, by gravitational force, are first to leave the column, producing the first spike (A) in the spectrum (Fig. 1). The free active agent is pulled along the column by the eluant, and a second spike (B) is recorded in the spectrum. The volume of this fraction is recorded, and it is solubilized with a sufficient quantity of ethanol 96% (volume/volume—v/v) (Panreac) to obtain an 80% (v/v) hydroalcohol solution, which is spectrophotometrically evaluated at 243 nm. The difference between the quantity of 6-methylprednisolone present in each formulation (0.005 g/100 ml) and that free in the medium represents the quantity encapsulated in lipid microspheres. The values thus obtained are submitted to one-way variance analysis (ANOVA) to evaluate the existence of significant differences between the means of the samples compared.

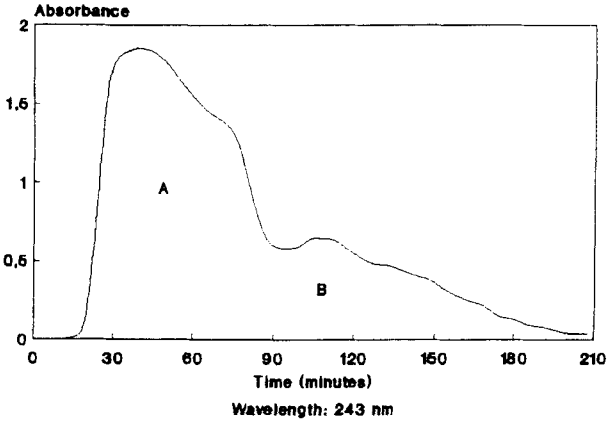

Figure 1. Separation of lipid microspheres carrying 6-methylprednisolone. See text for discussion of A and B.

RESULTS

Table 2 gives the results obtained from the spectrophotometric analysis of the different fractions of free 6-methylprednisolone collected after chromatographic separation. The quantity of free active agent (mg/100 ml) is given for each formula, together with the percentage of the free agent and that encapsulated by the lipid

Table 2

Results of the Encapsulation Study of 6-Methylprednisolone in Lipid Microspheres (n = 5)

Formula No.	Free 6MP		Encapsulated 6MP, %
	mg/100 ml	%	
1	1.85 ± 0.52	37.00 ± 5.16	62.99 ± 5.16
2	1.75 ± 0.40	34.99 ± 4.09	65.01 ± 4.09
3	1.60 ± 0.58	31.99 ± 5.83	68.01 ± 5.83
4	2.13 ± 0.36	42.46 ± 3.61	57.53 ± 3.61
5	2.31 ± 0.33	46.19 ± 6.67	53.81 ± 6.67
6	1.86 ± 0.23	37.20 ± 2.35	62.80 ± 2.35
7	1.95 ± 0.11	38.92 ± 1.12	61.08 ± 1.12
8	1.21 ± 0.50	24.20 ± 5.05	75.79 ± 5.05
9	1.13 ± 0.62	22.70 ± 6.18	77.31 ± 6.18
10	0.76 ± 0.49	15.09 ± 4.44	84.91 ± 4.45
11	1.35 ± 0.56	26.92 ± 5.64	73.08 ± 5.64

microspheres. The data given are the means of five values, accompanied by the respective standard deviations.

DISCUSSION

The variables considered in the encapsulation study were the soybean oil:lecithin ratio (w/w) and the presence and concentration of Tween 80 in the formulation. Concerning the first of these variables, the oil:lecithin ratio, it was seen that an increase in the quantity of refined soybean oil (SO), or a decrease in the quantity of soybean lecithin (SL) (reduction in the SO:SL ratio) led to an increase in the percentage of encapsulated 6-methylprednisolone. These data were statistically significant only in the presence of Tween 80.

In the formulations that incorporated 0.50 g/100 ml of Tween 80, these results were obtained: formula 1, with an SO:SL ratio of 1:1.8 (w/w) encapsulated 62.99 ± 5.16%, while formula 9 (SO:SL ratio of 1:1.1, w/w) had a take-up rate of 77.31 ± 6.18% (p = 0.01199). Comparing the percentage obtained with formula 6 (SO:SL ratio of 1:1.5, w/w): 62.80 ± 2.35% with that of formula 9, the results are seen to be similar (p = 0.00176).

The other formulations where the concentration of Tween 80 was 0.25 g/100 ml gave the following results: formula 2 (SO:SL ratio of 1:1.8, w/w) encapsulated 65.01 ± 4.09% of the active agent, compared to formula 10, which encapsulated 84.91 ± 4.45%, with an SO:SL ratio of 1:1.1 (w/w) (p = 0.00021). At the same time, the percentage taken up by formula 7 (SO:SL ratio of 1:1.5, w/w) was 61.08 ± 1.12%. This is in contrast to the results of formula 10, where the results were significantly different, for a value of $p = 7.4854 \cdot 10^{-6}$.

The results of the formulations prepared without Tween 80, differentiated only by the soybean oil:lecithin ratio (w/w) (formulas 3, 8, and 11), were not significantly different.

We also studied the influence of Tween 80 on the percentage of 6-methylprednisolone encapsulated by the microspheres. In general, a reduction in the concentration of this constituent (and in particular, its absence from the formulation) resulted in an increase in the percentage incorporating these vectors, except when the oil:lecithin ratio was 1:1.1 (w/w). However, the results were only significantly different for the comparison of formula 6 (prepared with 0.50 g of Tween 80/100 ml), formula 7 (0.25 g of Tween 80/100 ml), and formula 8 (prepared without Tween 80). In all these, the SO:SL ratio was equal to 1:1.5 (P/P). Formula 6 encapsulated 62.80 ± 2.35%; formula 7 encapsulated 61.08 ± 1.12%, virtually identical to the former; while formula 8 (without Tween 80) increased the percentage of glucocorticoid taken up: 75.79 ± 5.05% ($p = 2.1219 \cdot 10^{-5}$). Similar results were obtained with the formulations prepared with an SO:SL ratio of 1:1.8 (w/w) and variable quantities of Tween 80: formula 1 (0.50 g Tween 80/100 ml) encapsulated 62.99 ± 5.16%, formula 2 (0.25 g Tween 80/100 ml) encapsulated 65.01 ± 4.09%, and formula 3 (prepared without the tensioactive agent) encapsulated 68.01 ± 5.83%. In this latter case, the variance analysis indicated that there were no significant differences between the values studied.

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