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Effect of Polymerization Coadjuvants on Nanocapsule Elaboration and Triamcinolone Entrapment

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ABSTRACT

The physical and chemical properties of tramcinolone acetonide nanocapsules, prepared by interfacial polymerization of isobutyl-2-cyanoacrylate at the oil/water interface, have been studied. The joint effect of monomer and oil in organic phase on the size and encapsulation efficiency has been analyzed. Polymer coating around the oil droplets causes an important variation in droplet size and encapsulation efficiency. The release of drug from the nanocapsules was also investigated in vitro.

INTRODUCTION

Recently some authors have developed drug carriers based on emulsion-polymerization procedures. As result of this process they have obtained colloidal particles less than 1 μm diameter (nanoparticles).

Biodegradable nanoparticles can be made from alkylcyanoacrylate monomers, which have bioadhesive properties and are potential drug delivery systems (1). The drug is dissolved or entrapped inside the polymeric matrix or adsorbed to the surface of the particles (2). Drug release may be controlled by degradation of the polymeric matrix, desorption of the drug from the wall, or molecular diffusion from the polymer (3). Two limiting factors determine the amount of drug entrapment in nanoparticles: drug solubility in aqueous phase and solubility in the polymeric matrix (4).

Difficulties associated with the entrapment of lipophilic drugs inside nanoparticles were overcome by Al-Khouri et al. (5) using an interfacial polymerization process leading to spheres of 250 nm. Florence et al. (6), using the same interfacial polymerization method, obtained spherical nanoparticles. This new colloidal carrier showed a capsular structure formed by a poly-

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mer envelope surrounding a central oil cavity. Moreover, active ingredients are dissolved in an oil drop covered with a polymer film.

Different authors analyzed the behavior of morphometric parameters of polyalquilcyanoacrylate nanocapsules from monomer and oil variations. In this way El-Salmaligy et al. (7) studied the formation mechanism of polymethylcyanoacrylate nanocapsules and reported that an increase in monomer concentration leads to an increase in the size of these particles, which was attributed to an increase in the thickness of the wall of the nanocapsules. Rollot et al. (8) noted a significant decrease in size of polyisobutylcyanoacrylate nanocapsules when the amount of monomer in organic phase increased, and they attributed these results to a reduction in the interfacial free energy in the biphasic system. Moreover, no significant size difference in polyisohexylcyanoacrylate nanocapsules was observed by Choinard et al. (9).

The aim of this paper was to study the influence and interaction of certain factors—monomer concentration, active drug amount, and oil/water ratio—on morphometrical parameters and encapsulation efficiency of a typical lipophilic substance, triamcinolone acetonide, in nanocapsules of isobutylcyanoacrylate.

EXPERIMENT

Materials

Triamcinolone acetonide (TCA) and isobutyl-2-cyanoacrylate (IBCA) were obtained from Sigma (Saint-Louis, USA). Mygliol 812 was purchased from Roig Farma (Barcelona, Spain). Poloxamer 188 (Surfoxid® 7068) was generously supplied by Tenneco (Barcelona, Spain). All reagents used were of analytical grade. Water was twice distilled from a permanganate solution using a Pyrex apparatus.

Preparation of Nanocapsules

Polyisobutylcyanoacrylate (PIBCA) nanocapsules containing triamcinolone acetonide (TCA) were prepared according to the interfacial polymerization technique described by Al-Khouri and co-workers (5). A lipophilic phase, containing different amounts of drug (0.1–2.4 mg/mL), Mygliol 812 (0.5–3 %), and IBCA (10–100 μL/10 mL), dissolved in 10 mL of absolute ethanol, was added under mechanical stirring at a constant flow rate (0.3 mL/min), using a peristalt pump, to 25 mL of an aqueous phase containig 0.25% of nonionic surfactant (Poloxamer 188). The nanocapsules were formed immediately by polymerization of monomers on the water/oil interface. After polymerization (1 h) the colloidal suspension of nanocapsules was concentrated by evaporation under vacuum and the final volume of 10 mL was filtered through fritted glass (9-15 μm).

A reference control emulsion was prepared in the same way as the nanocapsules, using the procedure described above, but omitting the monomer that forms the coating.

Physicochemical Characteristics of the Nanocapsules

The particle size distribution of nanocapsules and the control emulsion were determined by Photon Correlation Spectroscopy (PCS) using an Autosizer IIC (Malvern Instruments, Malvern, UK) with a helium-neon laser. The signals were processed by Malvern 7032-N multibit correlator (Malvern Instruments). The method of cumulats was selected to perform data analysis. The measurements were carried out at 25°C and at a constant angle of 90°. This apparatus also provides insight into the relative distribution of size by means of a polydispersity index (10). Samples were diluted before measurement with twice-distillated water freshly filtered through a Millipore® membrane.

Determination of Encapsulation Efficiency

Triamcinolone acetonide was determined by high-performance liquid chromatography (HPLC) with a Hewlett Packard HP1090 instrument, using a modification of the U.S.P. method (11). Content of drug in the nanocapsules was calculated by determining the difference between the total drug present in final colloidal suspension, after dissolution of samples in dimethylformamide, and the free amounts of the drug in the aqueous phase. The samples were submitted to an ultracentrifugation process at 40,000 rpm (Beeckman centrifuge). The result of this process was the separation of two phases of colloidal system. Encapsulation efficiency and drug lost during elaboration and purification process were expressed as percentage of drug versus the amount of drug in organic phase.

In Vitro Drug Release from Nanocapsules

The amount of drug released with time from nanocapsule colloidal suspension, TCA emulsion, and TCA solution was determined by a membrane diffusion technique (12). In this liberation model, the dispersion phase

(1 mL of sample diluted with 4 mL of Tris buffer, pH 7.4) of the carrier is separated from a Tris buffer bulk by means of a dialysis membrane (Visking). The system was held at 37°C. The suspension was stirred continuously and at various time intervals 100 μL of bulk solution was analyzed by HPLC. The samples withdrawn were replaced by 100 μL of Tris buffer solution.

RESULTS

In order to obtain information about the physical structure of TCA nanocapsules, morphometrical properties, particle size, and polydispersity were determined. The effects of concentration variations of IBCA, Mygliol, and TCA contents in the oil phase (TCAo) on the size and polydispersity of isobutylcyanocrylate nanocapsule are shown in Table 1. These results suggest that the increase in the percentage of Mygliol added to the organic phase led to an increase in particle diameter. Moreover, an increase in diameter of particles can be observed when IBCA in organic phase increases up to 50 μL/10 mL. For higher amounts of IBCA added to etanolic medium the particle diameter decreased. The amount of triamcinolone acetonide entrapped in the nanocapsules (TCAe) depends on the monomer added. As can be seen in Table 1, the encapsulation efficiency of TCA decreases when IBCA content increases. On the other hand, the increase in nanocapsule size, for a constant amount of IBCA, involves more oil encapsulated, with the same quantity of drug solubilized. Therefore, the increase in entrapped Mygliol produces an increase in encapsulated drug. This agrees with results reported by Choinaird et al. (9).

Results obtained in the present study (Table 1) are only a portion of all possible results of independent variable (Mygliol, IBCA,TCAo) variations. With the aim to study general behavior of the size and the encapsulation efficiency of TCA in IBCA nanocapsules and explain the contradictory results obtained by other authors (7–9), experimental data obtained in this study were treated mathematically by regression to obtain two polynomic equations.

Equation 1 shows the relation between the size of nanocapsules and the three independent variables fixed previously in the nanocapsule eleboration process (Mygliol 812, IBCA amounts, and TCA contents in the organic phase [TCAo]). Equation 2 shows the relation between TCAe and the same independent variables.

$$\begin{aligned}\text{Size (nm)} = {} & + 199.9113 \\ & - 21.2137\,X - 0.0774\,Y - 0.1533\,Z \\ & + 4.1239\,X^2 - 0.0098\,Y^2 - 0.0000\,Z^2 \\ & + 0.6734\,XY - 0.0275\,XZ + 0.0017\,YZ \\ & + 0.0001\,XYZ\end{aligned}$$

Table 1

Morphometrical Parameters (Average Size, Polydispersity) and TCA Content in the Nanocapsules for Different Experimental Conditions

Independent Variables				Dependent Variables		
IBCA (μL/mL)	Mygliol (%)	TCAo (μg/mL)	Size (nm)	Polydispersity (Q)	TCAe (μg/mL)	TCAe (%)
10	1.0	200	156.2 ± 4.5	0.044 ± 0.024	102.7 ± 15.9	51.3 ± 3.4
25	1.0	200	163.2 ± 3.5	0.146 ± 0.036	98.7 ± 25.6	47.8 ± 6.5
50	1.0	200	171.3 ± 4.5	0.098 ± 0.023	43.4 ± 16.9	23.7± 2.7
100	1.0	200	144.7 ± 9.3	0.039 ± 0.017	41.6 ± 9.0	21.8 ± 4.7
100	1.0	2400	178.1 ± 2.9	0.085 ± 0.004	120.4 ± 12.1	5.0 ± 0.5
100	2.0	2400	189.0 ± 7.8	0.069 ± 0.012	139.6 ± 13.5	6.1 ± 0.6
100	3.0	2400	222.3 ± 14.8	0.064 ± 0.009	139.4 ± 16.5	5.8 ± 0.4
100	2.0	200	197.0 ± 11.5	0.121 ± 0.042	61.2 ± 0.6	29.4 ± 0.8
100	2.0	600	200.5 ± 1.0	0.094 ± 0.026	143.2 ± 8.1	26.3 ± 5.7
100	2.0	1200	190.9 ± 5.3	0.087 ± 0.042	126.4 ± 5.4	10.9 ± 4.4
100	2.0	2400	203.5 ± 10.0	0.089 ± 0.040	178.3 ± 4.5	7.4 ± 1.7
50	0.5	100	171.0 ± 14.0	0.142 ± 0.032	52.9 ± 2.5	53.2 ± 2.6
50	0.5	200	163.3 ± 6.7	0.070 ± 0.008	81.0 ± 3.5	41.3 ± 7.9
50	0.5	300	155.9 ± 4.5	0.095 ± 0.004	84.7 ± 1.7	28.9 ± 6.3

Table 2

Experimental and Predicted Size and TCA Content in Nanocapsules Prepared with the Acceptable Values of Independent Variables Corresponding to Maximum Entrapped Drug

Independent Variables			Dependent Variables					
Calculated			Calculated			Experimental		
IBCA (μL/10mL)	Mygliol (%)	TCAo (μg/mL)	Size (nm)	TCAe (μg/mL)	TCAw (%)	Size (nm)	TCAe (μg/mL)	TCAw (%)
76.3	1.04	52.5	170.3	20.8	31.7	178.1 ± 5.0	21.6 ± 0.7	28.6 ± 1.6

Coefficient of Correlation = 0.9891
Standard Error = 6.768
X = Mygliol 812 (%)
Y = IBCA (μL/10 mL)
Z = TCAo (μg/mL)

$$\text{TCAe (μg/mL)} = +273.6985 - 144.8291X - 4.8247Y + 0.1072Z - 27.6384X^2 + 0.0137Y^2 - 0.0000Z^2 + 2.6433XY + 0.0531XZ - 0.00001YZ + 0.0005XYZ \quad (1)$$

Coefficient of Correlation = 0.9345
Standard Error = 30.667
X = Mygliol 812 (%)
Y = IBCA (μL/10 mL)
Z = TCAo (μg/mL)

$$\text{TCAe (μg/mL)} = +305.3985 - 144.8291X - 4.8247Y - 0.8928Z - 27.6384X^2 + 0.0137Y^2 - 0.0000Z^2 + 2.6433XY + 0.0531XZ - 0.00001YZ + 0.0005XYZ \quad (2)$$

Only a portion of the drug dissolved in organic phase is lost during elaboration process (TCAl) because a decrease of TCA solubility was produced when the etanolic phase was added in Poloxamer solution. Insoluble drug was precipitated in crystal forms and separated in filtration process.

Solubility of drug in Poloxamer 188 (0.25%) aqueous solution (TCAw = 31.7 μg/mL ± 2.3) was determined according to the method of Block et al. (13). In optimal conditions of encapsulation, loss of product was not produced and both phases of colloidal suspension are saturated with the TCAo. In these conditions TCAe in equation 2 can be substituted by (TCAo - 31.7) to obtain equation 3.

$$\text{TCAe (μg/mL)} = +305.3985 - 144.8291X - 4.8247Y - 0.8928Z - 27.6384X^2 + 0.0137Y^2 - 0.0000Z^2 + 2.6433XY + 0.0531XZ - 0.00001YZ + 0.0005XYZ \quad (3)$$

X = Mygliol 812 (%)
Y = IBCA (μL/10 mL)
Z = TCAo (μg/mL)

In order to calculate the theoretical amounts of the independent variables used to obtain the optimal condition for the maximun drug entrapment, TCAo in equation 3 was derived from two independent variables (IBCA and Mygliol content in organic phase). The values predicted by the model are in good agreement with experimental data obtained under these conditions (Table 2).

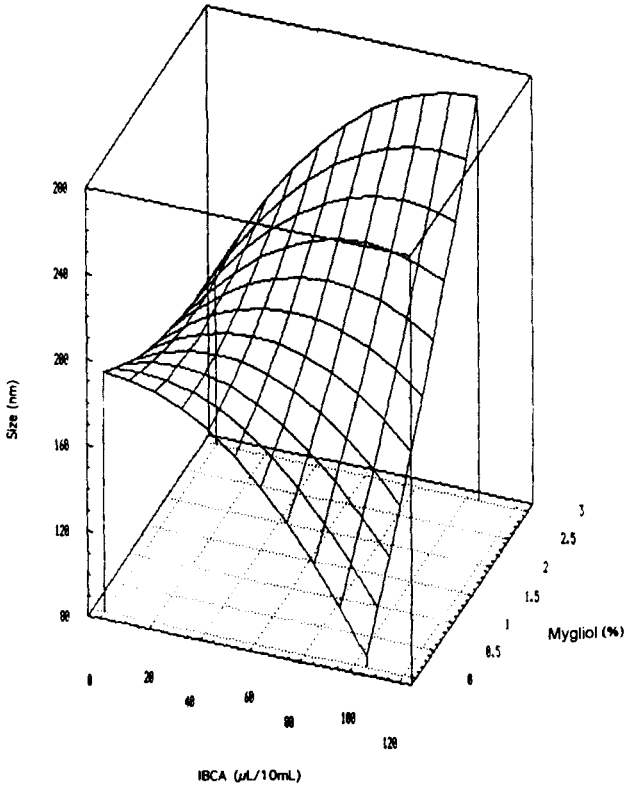

Figure 1. Response surface diagram for the size of PBCA nanocapsules with IBCA and Mygliol 812 variations in organic phase (TCAo = 52.5 μg/mL).

Table 3

Sizes of Nanocapsules, Nanospheres, and Colloidal System Obtained in Assays 1,2,3,4

Assay	Sediment	Colloidal System	Nanocapsules (sobrenadat)	Nanospheres (sediment)
1	+	156.2 ± 4.4	161.2 ± 5.8	85.8 ± 10.2
2	++	163.2 ± 3.5	175.3 ± 8.2	91.6 ± 9.6
3	++	171.3 ± 9.3	180.6 ± 6.0	74.2 ± 12.0
4	++++	144.7 ± 9.3	161.9 ± 4.6	80.3 ± 10.6

DISCUSSION

Physical Characteristics of Nanocapsules

Figure 1 shows the three-dimensional response surface diagram corresponding to graphic representation of equation 1 for optimal TCAo calculated (52.5 μg/mL). It can be observed that the joint effect of IBCA and Mygliol concentrations in organic phase on the particle size response was not a proportional response to IBCA and Mygliol concentrations variations, since the particle size depends on the portion of the surface response considered in the graph and on the IBCA/Mygliol ratio. It can also be observed that for different concentrations of Mygliol, variations of IBCA produced different behavior in nanocapsule sizes.

This behavior can be explained because two types of particles with different densities, also reported by other authors (14,15), were obtained in the final colloidal suspension. Nanocapsules were less dense than the aqueous phase because their main component is an oil: Mygliol 812 (caprylic/caproic acid) with lower density (0.93) than water; and a second type of particle with higher density and lesser diameter (Table 3). Whereas the nanocapsules with lower density were located floating on the aqueous phase of centrifugate, the particles with high density led to a pellet after centrifugation. Moreover, an increase in the monomer content in the organic phase, up 50 μL/10mL, produced an increase in the pellet size, probably due to the increase of nanosphere production and/or a growing of wall thickness nanocapsules, which could produce a density increase, according to results reported by El-Salmaligy et al. (7). Secondary nanospheres obtained in nanocapsule production show a mean size lesser than nanocapsules that could produce a decrease in middle size of the system colloidal final.

Encapsulation Efficiency

The joint effect of IBCA and Mygliol on the encapsulation efficiency was better studied by means of a regression method according to equation 2, the tridimensional representation of which is shown in Figure 2. The effect of IBCA variation on the association efficiency of

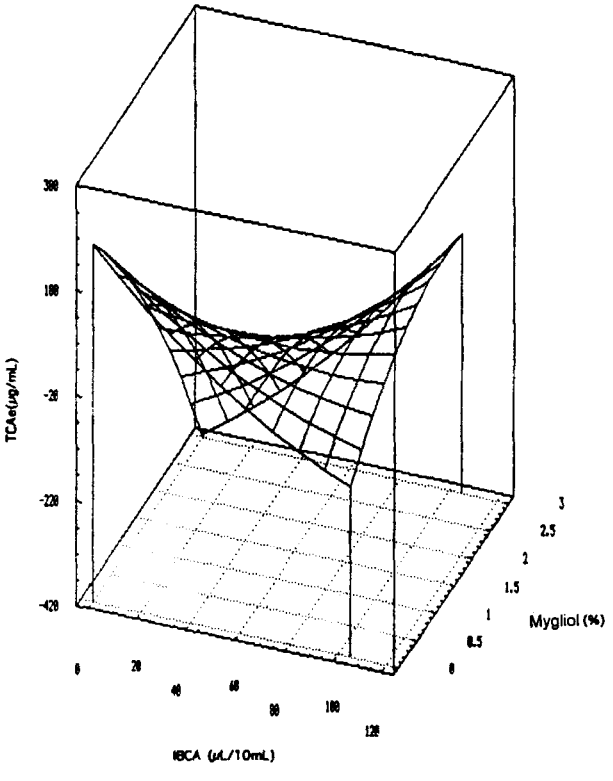

Figure 2. Response surface diagram for the entrapped drug content of PIBCA nanocapsules with IBCA and Mygliol variations in organic phase (TCAo = 52.5 μg/mL).

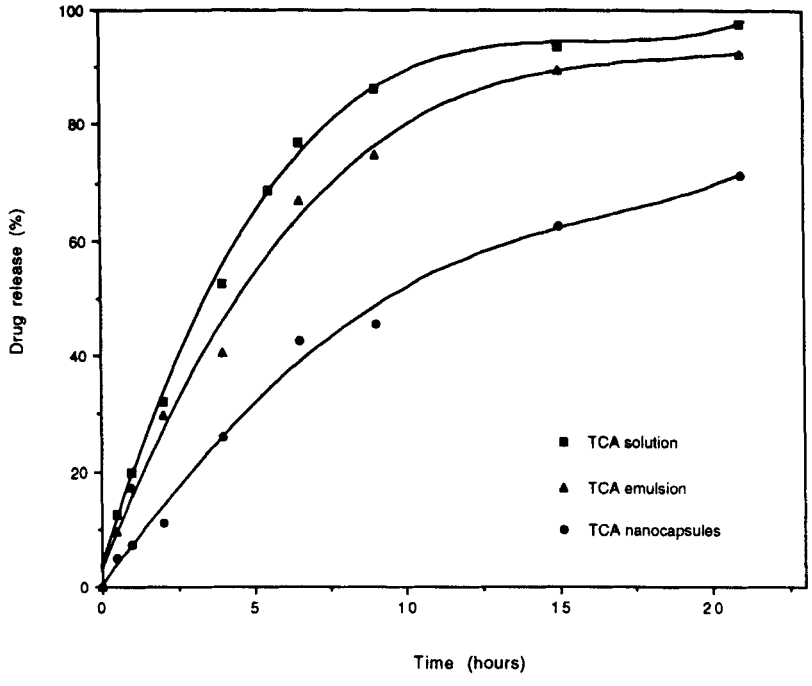

Figure 3. Release of TCA from solution, emulsion, and nanocapsules in buffer bulk, pH 7.4, at 37°C (TCAs = 52.5 μg/mL, IBCA = 73.6 μg/10mL, Mygliol 812 = 1.04 μL/mL).

TCA depends on the Mygliol content. As can be seen in the graph, the behavior of TCAe is opposite at extreme concentrations of Mygliol (0.5% and 3%) in the interval studied. This behavior depends on the Mygliol concentration present in organic phase. The entrapment of TCA in nanocapsules decreased when IBCA increased in organic phase at low oil concentration. On the contrary, a higher Mygliol concentration in organic phase produced an increase in encapsulation of TCA when the IBCA concentration was high. These results may be attributed to the simultaneous formation of two kinds of particles with different proportions of polymer. High-density particles have less TCAe associated and a higher proportion of polyisobutylcyanoacrilate because they were made when the oil concentration was low. When the oil concentrations were too much, an increase of IBCA produced an increase of Mygliol entrapped with more TCA dissolved.

Table 4

Mean Particle Diameters, Drug Entrapment, and Release Rate Constants of Triamcinolone Acetate Nanocapsules

Drug form	Particle Size (nm)	Drug Entrapment (μg/mL)	Drug Entrapment (%)	Release Rate Constant (h^{-1})
Solution	—	34.2 ± 2.6	100	0.1561
Emulsion	223.4 ± 18.5	20.6 ± 3.4	60	0.1221
Nanocapsules	178.1 ± 5.0	21.6 ± 0.7	63	0.1006

In Vitro Drug Release from Nanocapsules

The release of triamcinolone acetonide from PIBCA nanocapsules is characteristically biphasic with an initial fast release phase followed by a second much slower first-order release phase. These results are in accordance with those obtained by Illum et al. (16). Typical release profiles are shown in Figure 3. The profiles of the curves corresponding to nanocapsule suspension (c) were significantly different from those corresponding to drug solution (a) or emulsion (b) in all cases, especially in the first 10 hours. The first-order release rate constants were calculated and are given in Table 4.

Within this period, the transport of the drug across the dialysis bag was much faster with solutions than the triamcinolone acetonide nanocapsules. The release pattern of TCA emulsion showed intermediate results.

CONCLUSIONS

Results obtained suggest that relation between the diameter of particles and the variables studied is nonlinear (monomer and oil concentration in organic phase) and this behavior depends on the IBCA/Mygliol ratio. The same conclusions are suggested for TCA entrapped in nanocapsules. Secondary nanosphere production can explain anomaly behavior of size and encapsulation variations. Finally, the release pattern of TCA nanocapsules is lower than that of the free drug, especially in the first hours.

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