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INVESTIGATION ON PROCESS PARAMETERS INVOLVED IN POLYLACTIDE-CO-GLYCOLIDE MICROSPHERES PREPARATION

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ABSTRACT

Indomethacin loaded polylactide-co-glycolide (PLGA) microspheres were prepared by emulsification solvent evaporation. The preparation involves several process parameters that can affect the morphological characteristics, the "in vitro" and "in vivo" dissolution behaviour of microspheres.

The evaluation of three process parameters, emulsification stirring rate, emulsifier concentration and dispersed phase to continuous phase ratio was carried out in order to correlate them to some microsphere properties.

Results show that the variables evaluated affect mainly microspheres drug content and, at less extent, particle size.

INTRODUCTION

Microparticulate drug delivery systems made of biodegradable polymers represent suitable systems for the modified release of drugs (1). Moreover, biodegradable and biocompatible polymers allow the formulation of controlled release parenteral dosage forms (2-7).

Several preparation methods have been used to obtain microspheres from different polymers (8-11) . Emulsification solvent evaporation is quite a simple method widely employed in the preparation of microspheres made of biodegradable polymers such as polylactide, polylactide-co-glycolide, poly-ε-caprolactone (8,12). This preparation method involves the emulsification of a dispersed phase, containing the polymer and the drug dissolved in an organic solvent , in an aqueous continuous phase. Subsequent evaporation of the dispersed phase solvent yields solid polymeric microparticles entrapping the drug. The solid microparticles are recovered from the suspension by filtration, centrifugation or lyophilization.

This preparation method involves several process variables that affect the characteristics of the microspheres obtained (13,14), hence the release rate of drug.

In this work three process variables are evaluated in the preparation of indomethacin loaded polylactide-co-glycolide microspheres by an o/w emulsion system. Emulsification stirring rate, emulsifier (PVA) concentration and dispersed to continuous phase ratio are considered as variables and varied one by one in three different steps, while all the other process conditions are kept constant. The influence of the variables on microsphere characteristics (size and drug content) is evaluated.

MATERIALS

Poly(D,L-lactide-co-glycolide) (PLGA) RG506, Mw 98000 Daltons, 0.8 dl/g in $CHCl_3$ intrinsic viscosity, lactide/glycolide ratio 50:50, was supplied by Boehringer Ingelheim (Boehringer Ingelheim, Ingelheim am Rhein, Germany).

1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid (indomethacin) was supplied by Sigma (Sigma Chemical Co., St.Louis, MO 63178, USA).

Polyvinylalcohol (PVA), 87.89 % hydrolized, 85000-146000 Mw, was purchased from Aldrich (Aldrich Chemical Company Inc., Milwaukee, Wis 53233, USA).

HPLC grade acetonitrile, ethanol, methylene chloride and Tween 80 (polyoxyethylensorbitanmonooleat) were purchased from Merck-Bracco (Merck-Bracco SpA, Milano, Italy).

$CH_3COONa.3H_2O$ was supplied by Farmitalia-Carlo Erba (Milano, Italy).

The double-distilled water used was filtered through 0.22 μm Millipore membrane filters.

Millipore membrane filters were supplied by Millipore (Millipore SpA, Milano, Italy).

METHODS

Microsphere Preparation

Microsphere preparation was performed by emulsification solvent evaporation method. Different amounts of PVA were dissolved in hot distilled water and allowed to cool prior to use as continuous phase. The PVA concentrations in water and their corresponding viscosities measured by a Bohlin CS Rheometer model DG 40/50 (shear rate 149.8 sec $^{-1}$) are listed in Table 1. Rheological behaviour of polyvinyl alcohol solutions, at the concentrations tested, resulted to be newtonian. The pH of the continuous phase was always 5.5.

The dispersed phase was constituted by a 5% w/w solution of PLGA and indomethacin (80:20 w/w) in CH_2Cl_2.

The dispersed phase was dropwise added to the continuous phase under continuous stirring and emulsified for 3 min by an IKA Ultraturrax R disperser model T25 equipped with a S25 dispersing tool (IKA R Laboratory Technology, D, Staufen). The temperature during the emulsification step was kept between 20 °C and 25 °C. The emulsion obtained was stirred by a Vibromixer laboratory model (Chemap AG, CH8604, Volketswil, Switzerland) for 1 hour at 38 °C to allow solvent evaporation. The microspheres were recovered by centrifugation and subsequent filtration through 1.2 μm membrane filter. The microspheres obtained were rinsed with 50 ml of a 50% v/v ethanol aqueous solution, dried and kept under vacuum at least 24 hours before analysing.

The three process variable evaluated are:

polyvinyl alcohol concentration in the continuous phase;

emulsification stirring speed ;

dispersed phase : continuous phase ratio.

TABLE 1
PVA Concentrations and Related Viscosities

PVA(% w/w)	Viscosity (Pa.s)
0.5	$3.244\ 10^{-3}$
2	$6.132\ 10^{-3}$
5	$2.762\ 10^{-2}$

TABLE 2
Process Conditions

Variables		Constant
PVA(% w/w)	Stirring speed (r.p.m.)	Disp. ph. : Cont. ph.
0.5	8000-9500-13500-20500	1 : 20
2	8000-9500-13500-20500	1 : 20
5	8000-9500-13500-20500	1 : 20

TABLE 3
Process Conditions

Variable		Constants
Disp. ph. : Cont. ph.	PVA (w/w %)	Stirring speed (r.p.m.)
1 : 20	2	9500
1 : 15	2	9500
1 : 10	2	9500

The experimental design to evaluate the three process variables was set as follows:
- in the first step emulsification stirring speed and PVA concentration were varied while keeping constant the third variable (Table 2);
- based on the results obtained , in terms of minimum variation in particle size and in drug encapsulation efficiency, one PVA concentration and one emulsification stirring speed were selected as constants while the dispersed phase : continuous phase ratio was varied as shown in Table 3.

Table 4 lists the formulations of the indomethacin loaded PLGA microspheres prepared by different experimental conditions.

Microsphere Characterization

Microsphere size was analysed by a light scattering/light blockage apparatus HIAC ROYCO model 9064 equipped with a MCR-05 counter sensor (AESSE SpA, Milano, Italy). Small amounts (3-4 mg) of microspheres were weighed and suspended in 100 ml of 0.1%

TABLE 4
Formulations

Batch #	PVA (w/w %)	Disp. ph. : Cont. ph.	Stirring speed (r.p.m.)
1I	0.5	1:20	8000
2I	"	"	9500
3I	"	"	13500
4I	"	"	20500
5I	2	"	8000
6I	"	"	9500
7I	"	"	13500
8I	"	"	20500
9I	5	"	8000
10I	"	"	9500
11I	"	"	13500
12I	"	"	20500
13I	2	1:15	9500
14I	2	1:10	9500

Tween aqueous solutions. All aqueous solutions used for particle size analyses were previously filtered through 0.22 μm Millipore membrane filters. The samples were ultrasonicated for 2 min and analysed, under continuous stirring, between 1.5 μm and 40 μm. The results are the averages of 3 withdrawals.

The percentage (w/w) of drug entrapped inside the microspheres was detected by HPLC analysis. Indomethacin loaded microspheres were dissolved in CH_2Cl_2, the solution obtained was filtered through a 0.22 μm Millipore membrane and analysed by HPLC Varian model 9010 (Varian instruments, Milano, Italy). A mobile phase of acetonitrile/ 0.1 M CH_3COONa aqueous solution 35:65 v/v and a Lichrosphere 100 RP18 column 125x4 mm (Merck-Bracco SpA) were used in conjunction with a Varichrom UV2550 detector (Varian) at 258 nm.

RESULTS AND DISCUSSION

The influence of the variables is evaluated on particles size, drug loading and yield of production.

The results obtained from granulometric analyses highlight that microsphere size is affected both by emulsification stirring speed and PVA concentration.

The influence of three different PVA concentrations on mean diameter at different agitation speed is represented in Figure 1.

The highest variability in microsphere mean diameter (3-7.5μm), as a function of stirring speed, is observed for the lowest PVA concentration examined.

Mean diameter, at different stirring speeds, varies in a narrow range (2.5-4μm), when higher PVA concentrations are tested (2%, 5%). Probably 2% is the optimal PVA concentration to get a stable emulsion, as further additions do not modify the system.

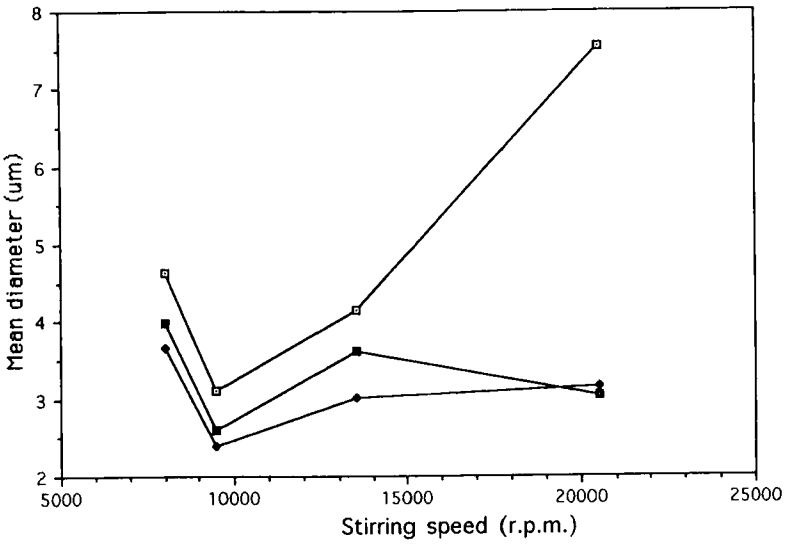

FIGURE 1

Influence of emulsification stirring speed and of PVA concentration on microsphere size .

PVA 0.5% PVA 2% PVA 5%

TABLE 5

Yields of Indomethacin Loaded PLGA Microspheres

Batch #	Actual drug content (%)	Encapsulation efficieny (%)	Yield of production (%)
1I	13.52	67.65	55.35
2I	3.49	17.49	74.34
3I	18.00	90.05	70.40
4I	3.88	19.41	69.44
5I	9.24	46.20	75.97
6I	7.24	36.20	60.00
7I	11.92	59.60	79.11
8I	7.60	38.80	70.39
9I	4.26	21.33	43.60
10I	3.94	19.71	28.66
11I	12.29	61.44	40.00
12I	3.51	17.55	25.70
13I	10.55	52.77	68.15
14I	8.54	42.70	80.65

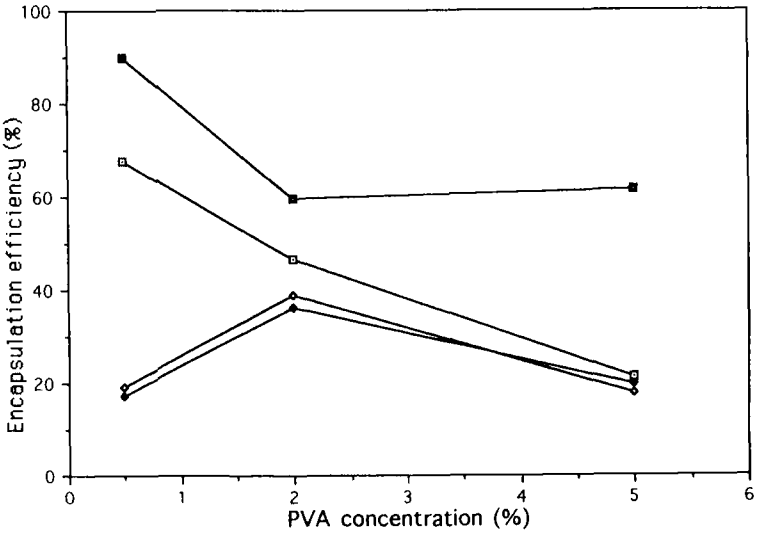

FIGURE 2

Influence of PVA concentration and of emulsification stirring speed on drug encapsulation efficiency.

8000 r.p.m. 9500 r.p.m. 13500 r.p.m. 20500 r.p.m.

Continuous phase viscosity has a low influence on mean particle size when 8000, 9500, 13500 r.p.m. stirring speeds are tested.

The lowest mean diameters and the lowest influence of medium viscosity are obtained at 9500 r.p.m. stirring speed for all systems evaluated.

High variability in yield of production and encapsulation efficiency are found, as shown in Table 5. Yield of production decreases drammatically for PVA concentrations above 2% (9I-12I), mainly due to technical problems (e.g. excessive foaming) rising during microsphere preparation.

Figure 2 shows how encapsulation efficiency varies as a function of PVA concentrations at different stirring speeds.

Emulsification stirring speed affects more encapsulation efficiency at extreme PVA concentrations (0.5%,5%).

When low amounts of PVA (0.5%) are added to the continuous phase, the encapsulation efficiency results to be highly dependent on emulsification stirring speed i.e. encapsulation efficiency varies between 17.5% and 90%.

Minimum influence of stirring speed is highlighted for 2% w/w PVA concentration.

13500 r.p.m. corresponds to the stirring speed yielding the best results in terms of encapsulation efficiency, indipendently of PVA concentration.

The effect of the dispersed phase/continuous phase ratio has been studied in those conditions where the two variables previously evaluated have the lowest influence on mean diameter (9500 r.p.m.) and on encapsulation efficiency of the systems (PVA 2%).

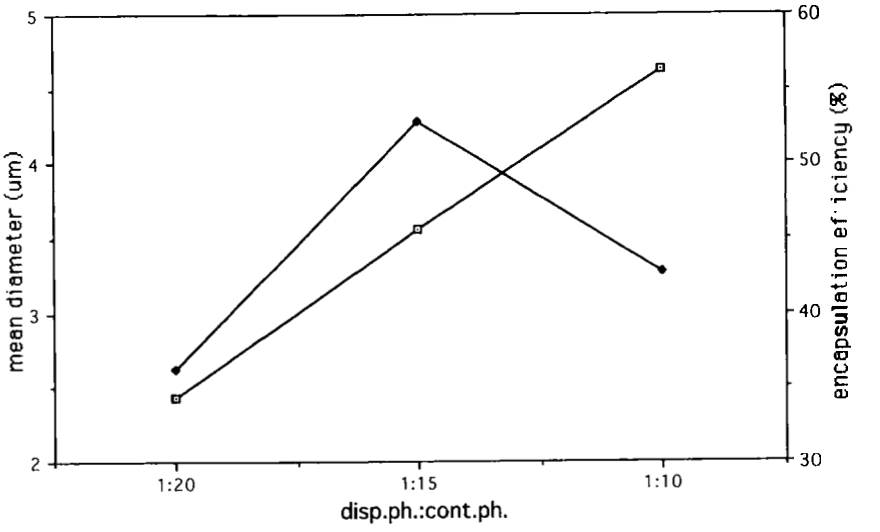

FIGURE 3

Influence of dispersed phase/continuous phase ratio on microsphere size and drug encapsulation efficiency.

mean diameter encapsulation efficiency (%)

Figure 3 shows that as the dispersed phase /continuous phase ratio increases microsphere mean diameter increases linearly, always being below 5 μm; the same increase is not found for drug encapsulation efficiency.

Satisfactory encapsulation efficiency is always achieved and yield of production is kept above 60% (Table 5: 2I, 13I, 14I).

CONCLUSIONS

The two microsphere characteristics evaluated (size and encapsulation efficiency) are differently affected by emulsification stirring speed, PVA concentration and dispersed phase/continuous phase ratio.

Since the mean diameters are always very small (below 8 μm) it can be concluded that the variables evaluated do not affect a potential parenteral application of microspheres.

Encapsulation efficiency results to be greatly influenced by two variables evaluated.

The system seems to be less affected both in size and encapsulation efficiency when 2% w/w PVA concentration is used in the continuous phase; this probabaly corresponds to the condition of highest emulsion stability.

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