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TESTING OF "IN VITRO" DISSOLUTION BEHAVIOUR OF MICROPARTICULATE DRUG DELIVERY SYSTEMS

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SUMMARY

No official dissolution method exists concerning microparticulate drug delivery systems. The purpose of this work is the evaluation of different dissolution methods commonly used to test the *in vitro* release behaviour of microparticulate drug delivery systems. The influence of different environmental conditions, as stirring speed, ionic strength and presence of surfactant, on drug release is also evaluated.

Four dissolution methods, based on different equipments (USP dissolution test apparatus, rotating bottle apparatus, shaker incubator, recycling flow through cell), are performed on the same batch of indomethacin loaded poly-D,L-lactide (PDLLA) microspheres prepared by spray drying. The results obtained with the methods tested show the influence of in vitro dissolution method employed and of the environmental conditions on drug release profile .

INTRODUCTION

Microparticulate drug delivery systems have been widely studied in these years (1, 2). They are polymeric systems able to provide a modified release of drugs and they can be administered by different administration routes: oral (3, 4), parenteral (5, 6, 7), transmucosal (8, 9). The last two administration routes require use of biodegradable and biocompatible polymers such as the polyesters of hydroxycarboxylic acid.

As for all modified release products the release rate of drug from a microparticulate drug delivery system is a very important parameter to be assessed

and evaluated. This parameter basically depends on the polymer the system is made of and on the manufacturing processes followed, which affect microsphere characteristics.

In vitro dissolution test is the first step to evaluate drug release behaviour of a microparticulate drug delivery system. It allows to establish the most suitable dosage form design and to study batch to batch variability.

Since no official *in vitro* dissolution method specifically designed for microparticulate drug delivery systems exists, several dissolution methods have been applied to test microparticulate drug delivery systems. This work aims to evaluate and to compare different dissolution methods usually applied in testing microparticulate drug delivery systems. Furthermore, our attention is focused on those modified release systems intended for parenteral administration, whose dissolution test last for a long period of time (e.g. systems based on polylactide).

For this reason the investigation is performed on indomethacin loaded poly-D,L-lactide (PDLLA) microspheres prepared by spray drying and intended as potential injectable formulation.

Four dissolution methods based on different equipments (USP dissolution test apparatus with paddle stirring elements, rotating bottle apparatus, shaker incubator and recycling flow through cell) are evaluated. Each apparatus has its own geometry and size, along with its characteristic type of agitation, depending on which the dissolution conditions are set.

The influence of some environmental conditions, i.e. agitation, presence of surfactant and ionic strength, on drug release is evaluated for each dissolution method.

MATERIALS

Poly-D,L-lactide (PDLLA) Res 202, Mw 16000, 0.2 dl/g inherent viscosity, was supplied by Boehringer Ingelheim (Boehringer Ingelheim, Ingelheim am Rhein, Germany).

1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid (indomethacin), pKa 4.5, solubility in pH 7.4 phosphate buffer 250 μg/ml, was supplied by Res Pharma (Res Pharma, Trezzo d'Adda, Italy).

Methylene chloride, chloroform, KH_2PO_4, Na_2PO_4 and NaCl analytical grade, were purchased from Merck Bracco (Merck-Bracco S.p.A., Milano, Italy).

1.2 μm, 0.45 μm, 0.22 μm Millipore membrane filters were supplied by Millipore (Millipore S.p.A., Milano, Italy).

Polysorbate 20 was purchased from ATLAS (ATLAS EUROPOL S.p.A., Ternate, Italy).

METHODS

Microsphere Preparation and Characterization

Indomethacin loaded microspheres were prepared by spray drying method described in a previous work (10). A single batch of microspheres was used

whose actual drug content was 31.25 % . Microsphere drug content was determined by UV spectrophotometry, with a UV spectrophotometer Perkin Elmer model C632-001 (Perkin Elmer, Norwalk, CT, USA) at 320 nm , after dissolving a weighed amount of microspheres in CH2Cl2.

Microsphere shape was detected by scanning electron microscopy (SEM) with a Cambridge Stereoscan 200 apparatus at 40 Kv (Cambridge Instruments Ltd, Cambridge, United Kingdom).

Microsphere size was analysed by a light scattering/light blockage apparatus HIAC/ROYCO model 9064 equipped with a MCR 05 sensor (AESSE S.p.A. Milano, Italy).

In Vitro Dissolution Tests

In vitro dissolution tests were performed by the following equipments: USP XXII dissolution test apparatus (Erweka-Apparatebau GmbH, Germany), rotating bottle apparatus Diffutest (Eurand Microencapsulation S.p.A., Cinisello Balsamo, Italy), shaker incubator (Isco Italia, Milano, Italy), modified flow through cell (Millipore S.p.A., Milano, Italy).

Dissolution behaviour of microspheres was tested in two different phosphate buffer solutions whose characteristics are listed in Table 1. The influence of surfactant on release rate of drug was tested by performing the dissolutions tests on the same buffer solutions (Table 1) after addition of 0.1% w/v Polysorbate 20.

Buffer solution 1 (11) represents the actual dissolution conditions for microspheres intended for parenteral administration, while buffer 2 was chosen for its high ionic strength suitable to evaluate the influence of this parameter on drug dissolution behaviour. All dissolution tests were carried out in sink conditions. In all cases the release profiles are reported as mean of three samples (standard deviation ranged between 1.5 and 6.0).

All tests were carried out at 37 ± 0.5 °C.

According to the equipment, the dissolution conditions were assessed as follows:

A - USP XXII Dissolution Test Apparatus (Paddle);
dissolution was performed in covered vessel made of glass, whose nominal capacity was 1000 ml;
dissolution medium volume 1000 ml;
paddle rotation speed 100 rpm and 200 rpm;
B - Rotating Bottle Apparatus;
dissolution was performed in locked, flat bottom bottles made of glass, whose nominal capacity was 100 ml;
dissolution medium volume 100 ml;
rotation speed 29 rpm;
C - Shaker Incubator;
dissolution was performed in closed erlenmeyer flasks whose nominal capacity was 200 ml;
dissolution medium volume 100 ml;

TABLE 1
Compositions, pHs and Ionic Strenghts of Phosphate Buffers Employed as the Dissolution Media

Identification	Composition (g/l)	pH	Ionic strength
1 (F.U.I. IX ed.)	2.38 Na_2HPO_4 + 0.19 KH_2PO_4 + 8 NaCl	7.4	0.190
2	55.51 Na_2HPO_4 + 13.61 KH_2PO_4	7.4	1.273

agitation 60 strokes/min and 120 strokes/min;
D - Modified Recycling Flow Through Cell;
the modified flow through cell is a molded polypropilene cell (30 mm high, 22.6 mm internal diameter), provided with a 1.2 μm membrane filter (Millipore S.p.A., Milano, Italy). The cell is connected to a peristaltic pump (Bellco S.p.A., Mirandola, Italy) by a Pivipol^R (PVC-polyurethane) coextruded tubings (Bellco S.p.A., Mirandola, Italy);
dissolution medium volume 1000 ml;
flow rate 33 ml/min and 17 ml/min.

When methods A, B, C were employed 5 ml of dissolution medium were withdrawn at fixed time intervals and centrifuged (centrifugette 4206, ALC Srl, Milano, Italy). The supernatant (4 ml) was filtered (0.22 μm Millipore membrane) and analysed by UV spectrophotometry at 320 nm. The residual 1 ml of dissolution medium was recollected with 4 ml of fresh buffer and employed to restore the dissolution medium .

When method D was employed 4 ml of phosphate buffer were withdrawn directly by dropping them from the cell and directly analysed as explained above. The dissolution medium was always restored with 4 ml of fresh buffer.

RESULTS AND DISCUSSION

Results of microsphere characterization by SEM and by particle size analysis are shown in Figure 1 and Figure 2 respectively. Microspheres resulted in spherical shaped particles whose mean diameter was 3.14 μm, and 90 % of particles were smaller than 9.73 μm.

Figure 3 allows a comparison among the different dissolution methods employed in pH 7.4 phosphate buffer with surfactant . Even if the release profiles

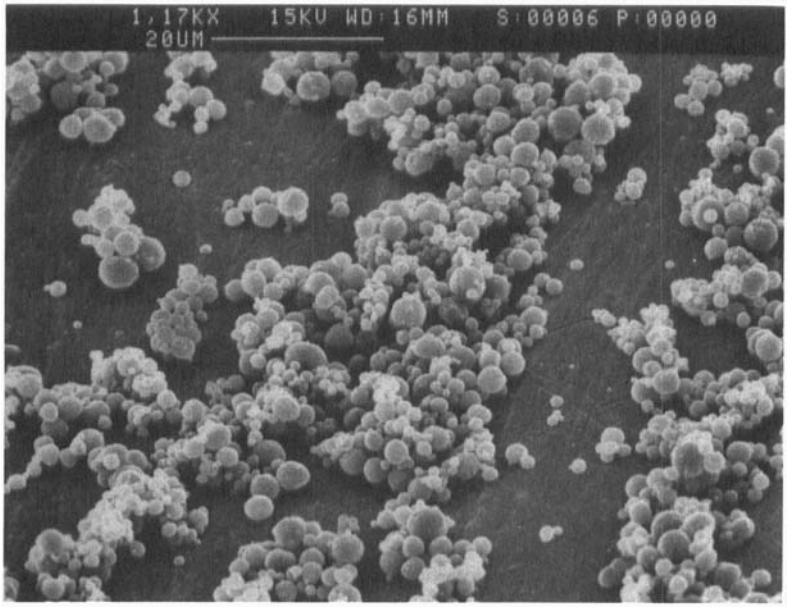

FIGURE 1
Photomicrograph of indomethacin loaded PDLLA microspheres.

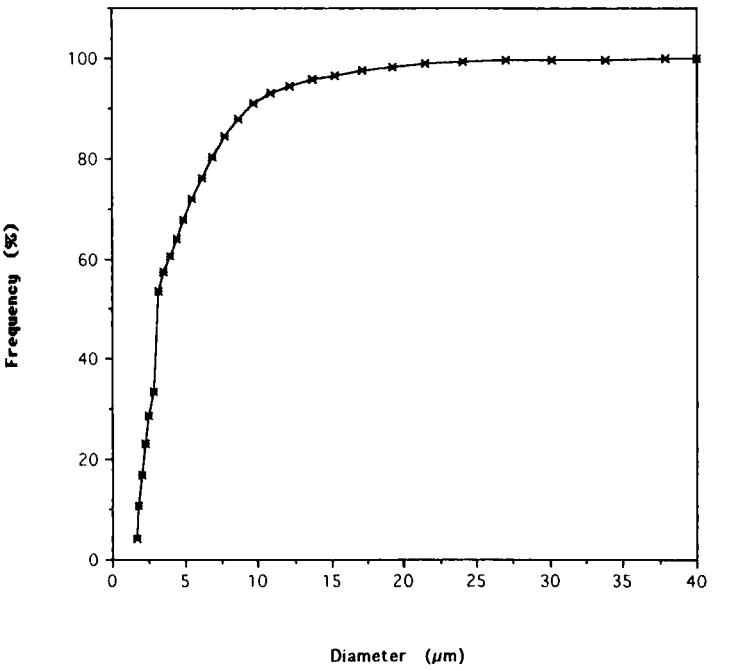

FIGURE 2
Undersize cumulative particle size distribution of indomethacin loaded PDLLA microspheres.

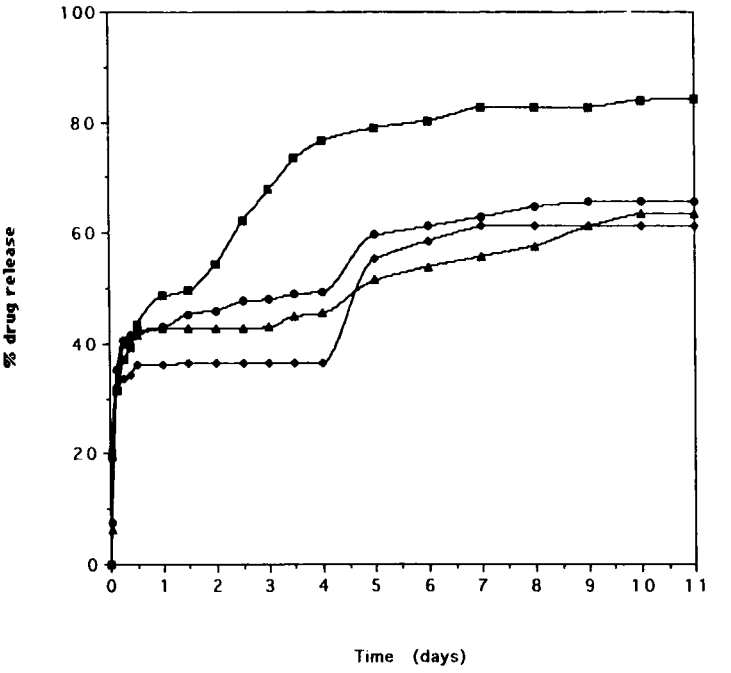

FIGURE 3

Comparison of Indomethacin "in vitro" dissolution profiles obtained by method A ● , method B ▲, method C ◆ , and method D ■ , in pH 7.4 phosphate buffer and in presence of surfactant.

obtained by methods A, B, C are quite similar, the slowest drug release rate is obtained by method C; this corresponds to the very mild agitation achieved with this equipment. The stronger agitations achieved both with USP dissolution test apparatus (method A) and rotating bottle apparatus (method B) lead to similar dissolution profiles; sligthly faster release rates are obtained by method A when compared with method B. The fastest release rate is achieved by flow through cell (method D).

Three variables have been evaluated by performing the dissolution tests: agitation, presence of surfactant and dissolution medium ionic strength.

The effect of agitation in increasing drug release rate is confirmed by Figure 4 that shows the in vitro dissolution profiles obtained at different stirring speeds (100, 200 rpm) with method A in pH 7.4 phosphate buffer and in presence of Polysorbate 20. The release profiles in the first 12 hours are almost superimposed (Fig 4a). After this period of time an increase of about 20% of the amount of

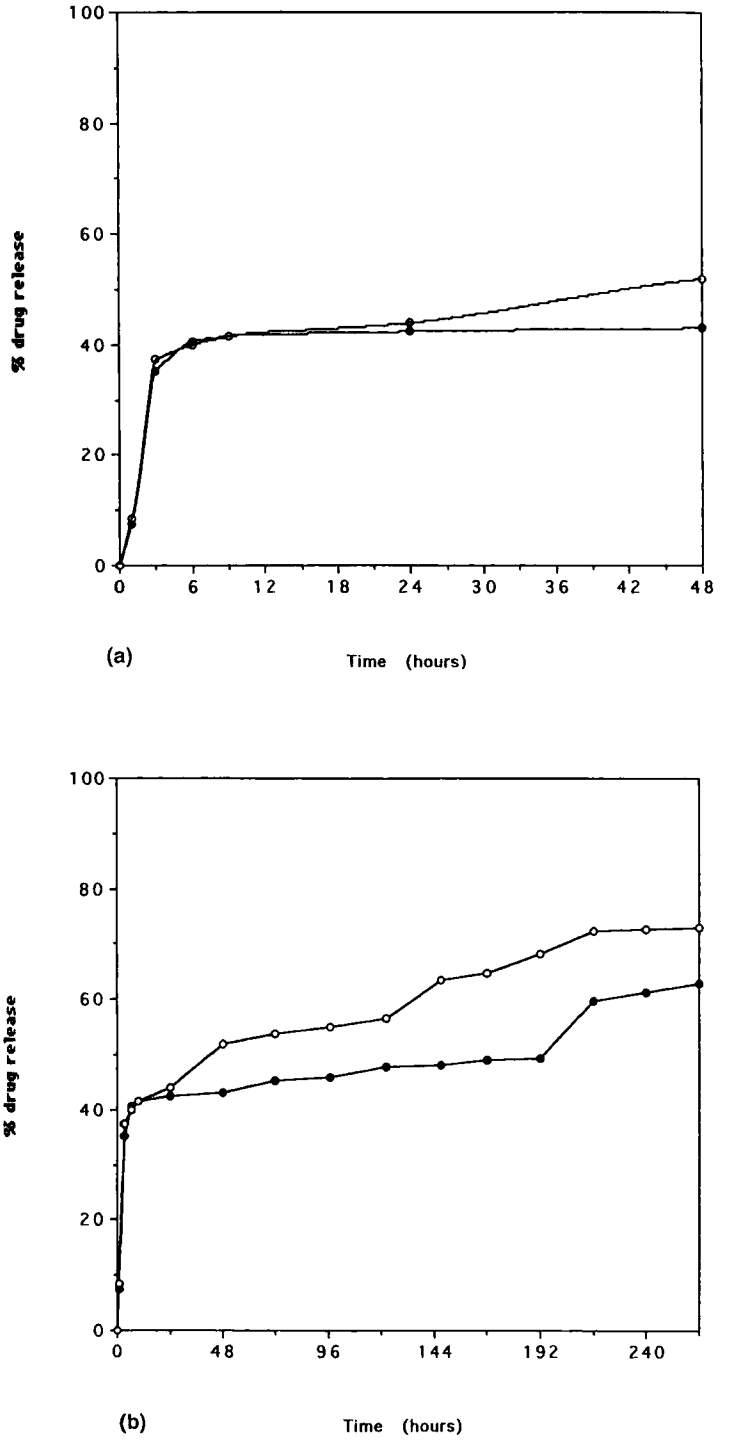

FIGURE 4

Effect of stirring speed on indomethacin "in vitro" dissolution profiles obtained by method A in pH 7.4 (100 rpm •, 200 rpm o): (a) first 48 hours, (b) 264 hours.

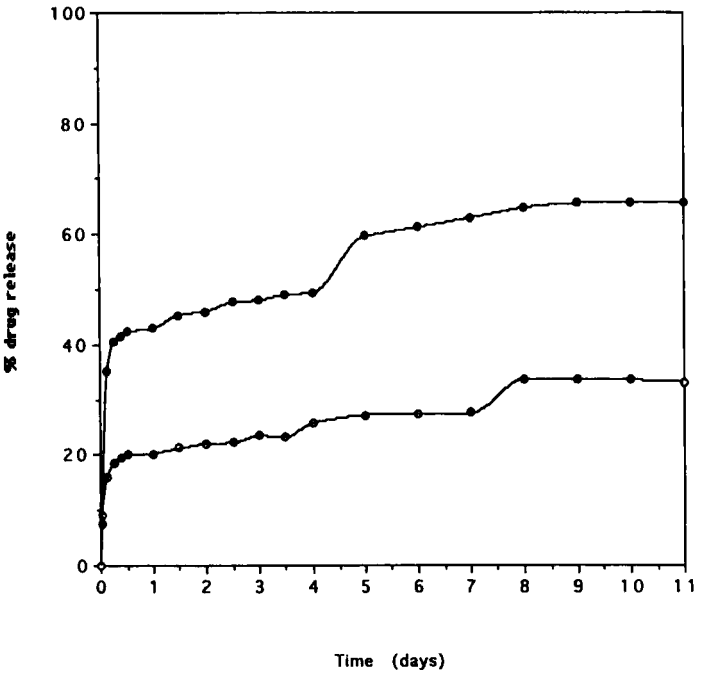

FIGURE 5

Dissolution profiles obtained by method A in pH 7.4 phosphate buffer without surfactant o , and with surfactant ● .

drug released is highlighted when stirring speed is set at 200 rpm for 10 days. This behaviour can be explained by the presence of drug at the surface of microspheres. This amount of drug (about 40% of total) is released during the first 12 hours at a rate independent of agitation. Analogous results are obtained for the others two methods evaluated (C, D) at different agitation conditions.

The influence of surfactant on drug release behaviour, in pH 7.4 phosphate buffer, chosen as example, is shown in Figure 5.

The presence of surfactant doubles the amount of drug released in the first 12 hours averagely for all methods tested .

The effect of ionic strength on indomethacin release rate from PDLLA microspheres is highlighted in Figure 6 for method A (Fig. 6a) and method D (Fig. 6b) . Faster drug release rate is highlighted in buffer 2, compared to buffer 1, when method A is employed. Similar results are obtained for method B and method C. This behaviour may be explained by the faster degradation rate of polymer depending on the medium ionic strength (12, 13). Opposite results are obtained with flow through cell (Fig. 6b).

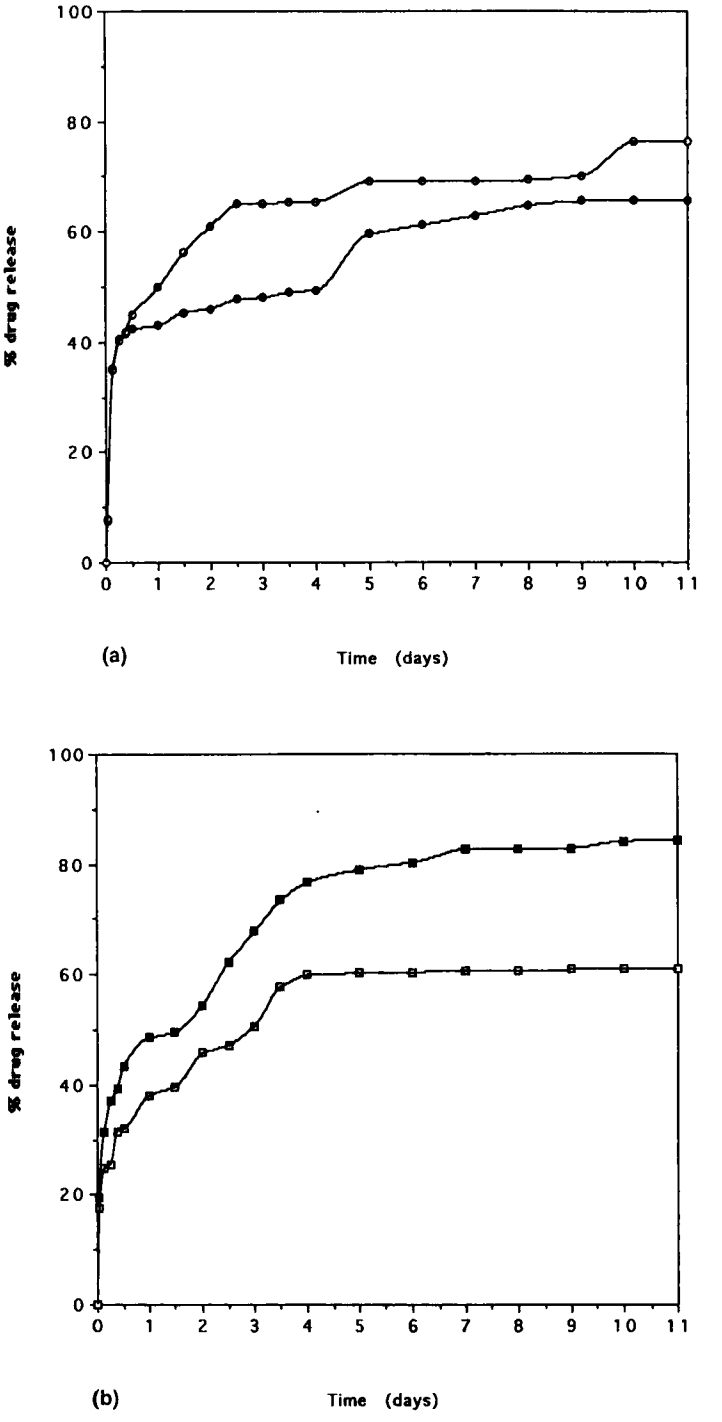

FIGURE 6

Influence of ionic strength on dissolution profiles: (a) method A, buffer1 ●, buffer 2 o ; (b) method D, buffer1 ■, buffer 2 □ .

CONCLUSIONS

The results obtained with all dissolution methods evaluated are always affected by the intrinsic apparatus characteristics and by the environmental conditions (i.e. stirring speed, presence of surfactant, ionic strength). In the case of flow through cell method, the type of agitation that produce intimate contact of the powder with the whole dissolution medium, can explain the significantly different results obtained by this method.

The system tested, based on polylactide, consists in a non wettable powder that aggregates and floats easily when dispersed in aqueous solution. Due to these characteristics and taking into account the results obtained, flow through cell seems to be the most suitable among the in vitro dissolution methods tested for polylactide microparticles intended for parenteral administration. Our results are in agreement with United States Pharmacopeia (14) that recently proposed flow through cell as the official method for extended release dosage forms.

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