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PREPARATION AND BIOLOGICAL EVALUATION OF ETHYLCELLULOSE MICROSPHERES CONTAINING TOLMETIN

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ABSTRACT

Tolmetin microspheres were prepared by the coacervation process from the ethylcellulose. Microspheres were obtained both in presence and without protecting colloids, such as polyisobutilene (PIB) or ethyl-vinylacetate copolimers (EVA). The effect of these agents on the preparation, drug content, wall thickness, surface morphology, drug dissolution and re-

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lease from microspheres, were evaluated. The dissolution rate analysis was carried out also in the presence of a surfactant (Tween 80) at different pH values.

In addition, microspheres containing Tolmetin as a core material were submitted to biological tests, in comparison with the free drug, to evaluate upon experimental models the antipyretic activity and the gastric tolerability.

INTRODUCTION

Microincapsulation is at present one of the methodologies used to control drugs properties. The inclusion of the thin particles of a substance in the polymeric wall of microspheres, microscopic sizing packages as containers, lead to change the availability of the core material and to alter some of its physical properties, like solubility, chemical reactivity, color, taste and particle shape, besides of protect the core from light, heat and oxidation effects.

Such an ability adds itself, in pharmaceutical formulations, to the possibility of slowing the drug release, as well as to prolong its therapeutic activity (1-4).

Many different coating materials and processes can be used in the microincapsulation, and several reviews have been published in the last years (5-6).

Tolmetin is a non-steroidal antiinflammatory agent (NSAID) being a pyrrole-acetic acid derivative, which showed a remarkable analgesic and antiinflammatory efficacy in man (7-9). As well as other NSAIDs, Tolmetin displays a marked irritating effect on gastrointestinal mucosae.

This paper reports the preparation of Tolmetin loaded microspheres with ethylcellulose and attemps to observe the influence of the use of polyisobutylene or ethylene-vinyl acetate copolymer upon microcapsule size distribution, drug content, wall thickness, surface morphology and dissolution parameters that control the drug relase rate from the coating polymer. In addition, biological results regarding the activity and tolerability of microencapsulated Tolmetin, with respect to the free drug, are reported.

EXPERIMENTAL SECTION

Materials

Tolmetin was obtained as dihydrate sodium salt from Aldrich (U.S.A.) and was used as free acid. Ethylcellulose (EC), N-Type (Hercules Incorporated, Delaware,USA), has an ethoxyl content of 47.5 to 49.0% and the viscosity of a 5% w/w solution in toluene-ethanol (80/20 w/w) was 100 cps. Polyisobutylene (PIB), with a molecular weight of 300.000 was obtained from BASF (Ludwigshafen, Germany) and ethylene-vinyl acetate copolymer (EVA), vinyl acetate content 33%, from Aldrich (USA). Cyclohexane was of reagent grade. All other ingredients and solvents used were of pharmaceutical grade.

Methods

Preparation of the microspheres

The preparation was based on the method of Miller et al. (10) and Jalsenjak et al. (11). Three hundred milliliters of a cycloexane solution containing 0 or 3% w/w of

protective colloid, i.e. PIB or EVA, were placed into a 1 L three-necked round-bottomed flask equipedd with a Heidolph mod. RZR 2000 stirrer, a thermometer and a reflux condenser. With stirring at 300 rpm, 0.75g of EC were added at room temperature and the temperature was raised to 66°C over 90 min. The core material, Tolmetin, was then added (1.5 g) and over a period of 60 min the temperature was further raised to 80°C. After being maintained at this temperature for 60 min and with continued stirring, the system was cooled to 35°C in 120 min, then cooled rapidly with ice to 25°C and stirred for further 20 min. The obtained microspheres were recovered by decantation, and the product was washed three times with 200 ml aliquots of cyclohexane (10°C), after a 60 min agitation, then filtered and air dried overnight. Yields were always greater than 95% .

Classification of microspheres

The different size of microspheres in the various obtained batches were separated into five fractions by sieving on a mechanical shaker using a range of standard sieves (200-1000 μm) and a shaking time of 10 min.

Dissolution studies from microspheres

Drug release from microspheres was studied by the rotating paddle method specified in F.U.I. (IX Edn.) (12).

Samples (25 mg) of the selected particle size ranges were introduced in different solution media [pH 4 or pH 7.4 (intestinal liquids)] and kept at 37± 0.5°C. A stirring

speed of 100 rpm was maintained as constant in all experiments. At suitable intervals, a 2 ml aliquot was removed and filtered (0.22 μm Millipore membrane). An equivalent quantity of dissolution medium was added into the dissolution vessel immediately after each sample drawing, to maintain the original volume. Dissolution studies were carried out also in the presence of a small amount of surfactant (Tween 80, 0.02%)in order to improve the wetting of microspheres.

The concentration of Tolmetin was determined spectrophotometrically at 320 nm. It was confirmed that the surfactant contained in the medium did not disturb the optical absorption of the drug at 320 nm in the present assay.

Dissolution experiments were duplicated and were closely reproducible.

Determination of Microspheres Content

To determine the total drug content in the various size fractions of microspheres, 1.25 mg samples were dissolved in 50 ml of methanol, which dissolved off all the coating materials used. The resulting solutions were diluted and assayed spectrophotometrically at 313 nm, using a calibration curve based on standard solutions in methanol.

EC did not absorb in methanol at this wavelenght.

Microscopic Studies

The surface topography of the microspheres were investigated with scanning electron microscopy (SEM). Microcapsule

samples were mounted onto stubs using double sided adhesive tape and vacuum-coated with gold film (10 A) by a Polaron Sputter Coater E 5100 and analyzed by a SEM Philips mod. 500 apparatus.

Fluorescence microscopy experiments were carried out by a Reichert-Jung mod. Diastar apparatus, equipped with a exciter filter BG 12 plus KB 418 and a barrier filter OG 515.

Determination of Wall Thickness

The wall thickness of the microspheres was calculated from the drug content, particle size and the relative densities of the wall material and core material. If the particles are assumed to be uniform, smooth and spherical, the average wall thickness is given by Madan's equation [1] (13):

$$\text{wall thickness} = \frac{Ww}{W - Ww} \times \frac{\sigma}{\sigma w} \times \frac{d}{6} \qquad [1]$$

If W= weigth of microspheres, Ww is the weight and σw the density of the wall material, σ is the density of Tolmetin and d is the mean diameter of drug particles. The density of the microspheres, the wall material (Ethylcellulose) and core material (Tolmetin) were calculated from the displacement volume of a known weight of ethylcellulose and drug using cyclohexane as a displacement fluid by picnometer. The calculated value are 1.11 g/cm ^{3} for the wall material and 1.75 g/cm ^{3} for Tolmetin at 25°C.

Biological studies

Animals

Male albino New Zealand rabbits (2.3 ± 0.2 Kg) obtained from Charles River (Calco, Como) were used. Animals were kept in a temperature-controlled room (21 ± 2 °C) with a 12 h light-dark cycle and 60% humidity.

Gastric ulceration assay

Rabbits were fasted overnight and the compounds were administered orally as 0.5% w/v suspensions in carboxymethyl-cellulose (CMC, 10 ml/Kg).18 h later all the rabbits were sacrificed (sodium pentobarbital, 50 mg/Kg, i.v.). The stomach was removed, cut along the greater curvature and the lesions on the gastric mucosa counted by visual examination under 3x magnification. All lesions were counted regardless of size. The dose producing gastric lesions in 50% of animals (UD_{50}) was determined according to the method of Litchfield and Wilcoxon (14).

Antipyretic activity

Hyperthermia was induced in rabbits by i.v. injection of 10 µg/kg of bacterial endotoxin from Escherichia Coli, purchased from Sigma Chemical Co (St. Louis, U.S.A.).

Drugs were administered orally in CMC as above, 120 min after the injection of pyrogen. Rectal temperature variations was measured at 30 min intervals for 5 h.

RESULT AND DISCUSSION

The preparation of microspheres obtained by coacervation using EC as coating material requires a carefull attention to details of the procedure in order not to have a product containing largely aggregated masses of the starting materials (15). Furthermore, it has been reported the importance of a protective colloid as PIB in forming individually film-coated core particles as opposed to aggregates (16-19). Moreover, we thought to be interesting to study also the effect of EVA on the preparation of Tolmetin-loaded EC microspheres.

The presence of these agents, like shown by the results in Tab.I, causes a decrement in the particle size, more remarkable for PIB. Whilst the presence of the coacervating agent did not significatively influence the drug content neither within the different batches nor in the overall microspheres preparation. The coacervation agents were used at a concentration of 3% w/w (8, 20). The wall thickness of the microspheres produced with or without a coacervation-inducing agent increased in the order PIB <none <EVA.

Scanning electron micrographs of microspheres are presented in Fig.1 . The surface of the microspheres prepared without any coacervation inducing agent was rough and irregular (fig.1a).

In the case of PIB the surface was smooth, and many small holes were apparent (fig.1b), with EVA, the surface was smooth with a few small holes (fig.1c).

TABLE I.

Effects of coacervation-inducing agents on microspheres formation.

Coacervation inducing agent[a]	Tolmetin content (%)	Wall thickness (μm)	Sieve fraction (%) (μm)				
			200-355	355-500	500-710	710-1000	<1000
None	66.7	15.16	19.30	16.00	17.30	20.10	27.30
PIB	67.2	14.63	23.20	43.60	28.94	3.15	1.11
EVA	66.3	15.57	14.06	33.01	29.04	13.14	10.75

[a] The concentration of coacervation-inducing agent was 3% w/w.

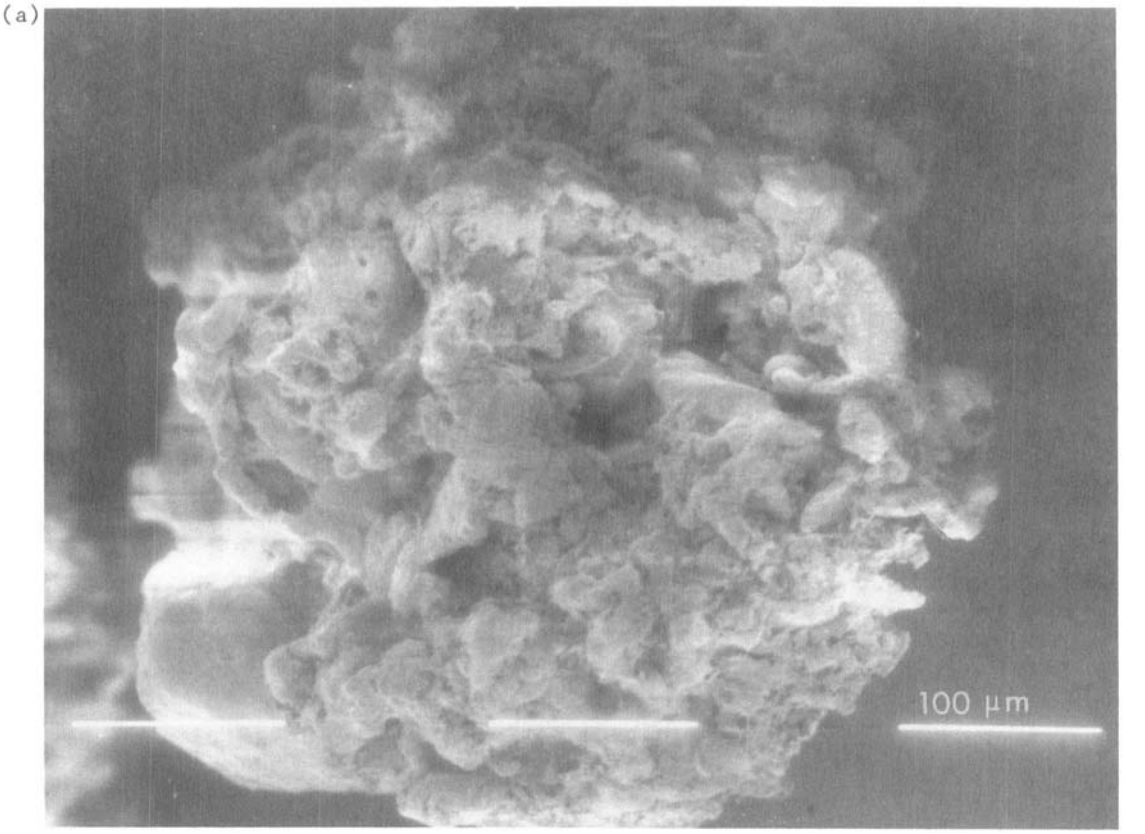
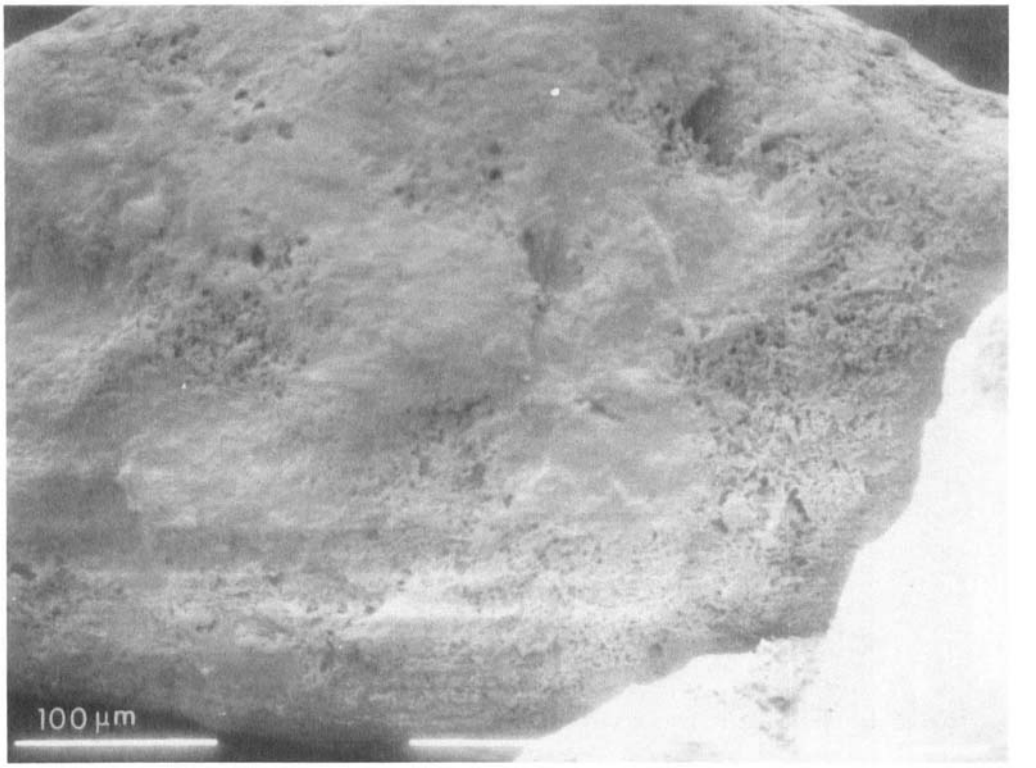


Fig. 1 - Scanning Electron Micrographs of microspheres surfaces.
(a) without coacervation-inducing agent, (b) PIB.
(c) EVA.

Findings of SEM analysis can be confirmed by means of fluorescence microscopy (FM). By considering that the drug is fluorescent, unlike EC and coacervating agents, from Fig. 2 it is evident, besides of the occurred coating, that microspheres obtained in the presence of EVA (fig. 2c) reduce more uniformely.

(b)



(c)

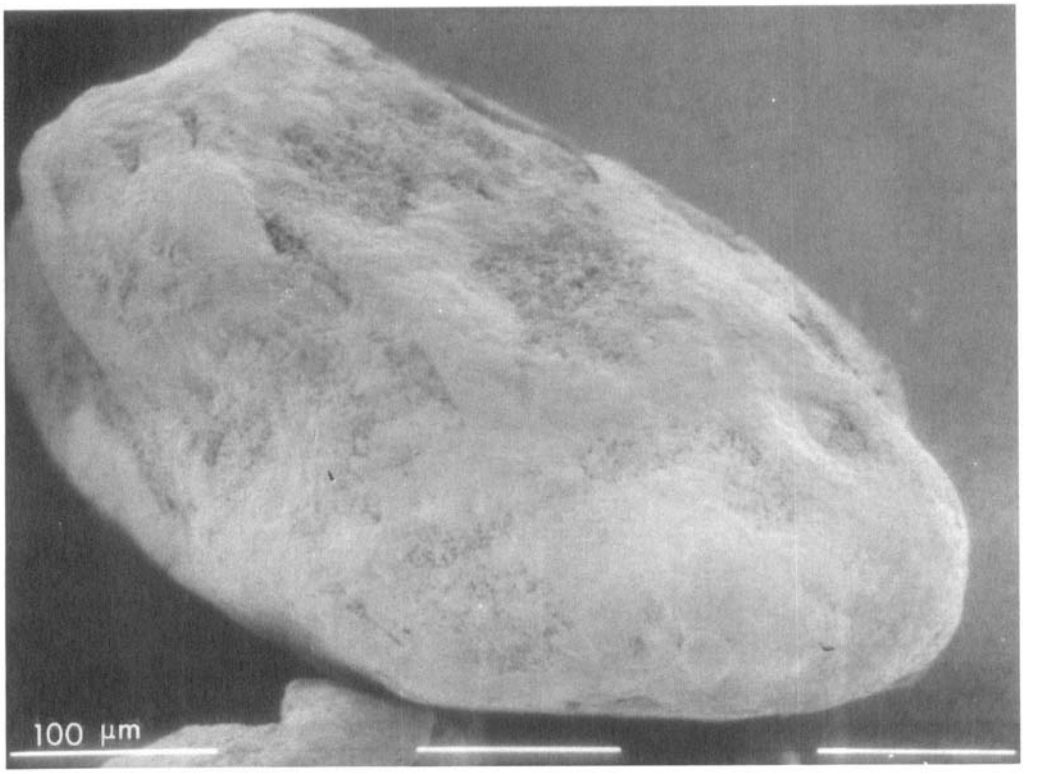


Fig. 1 Continued

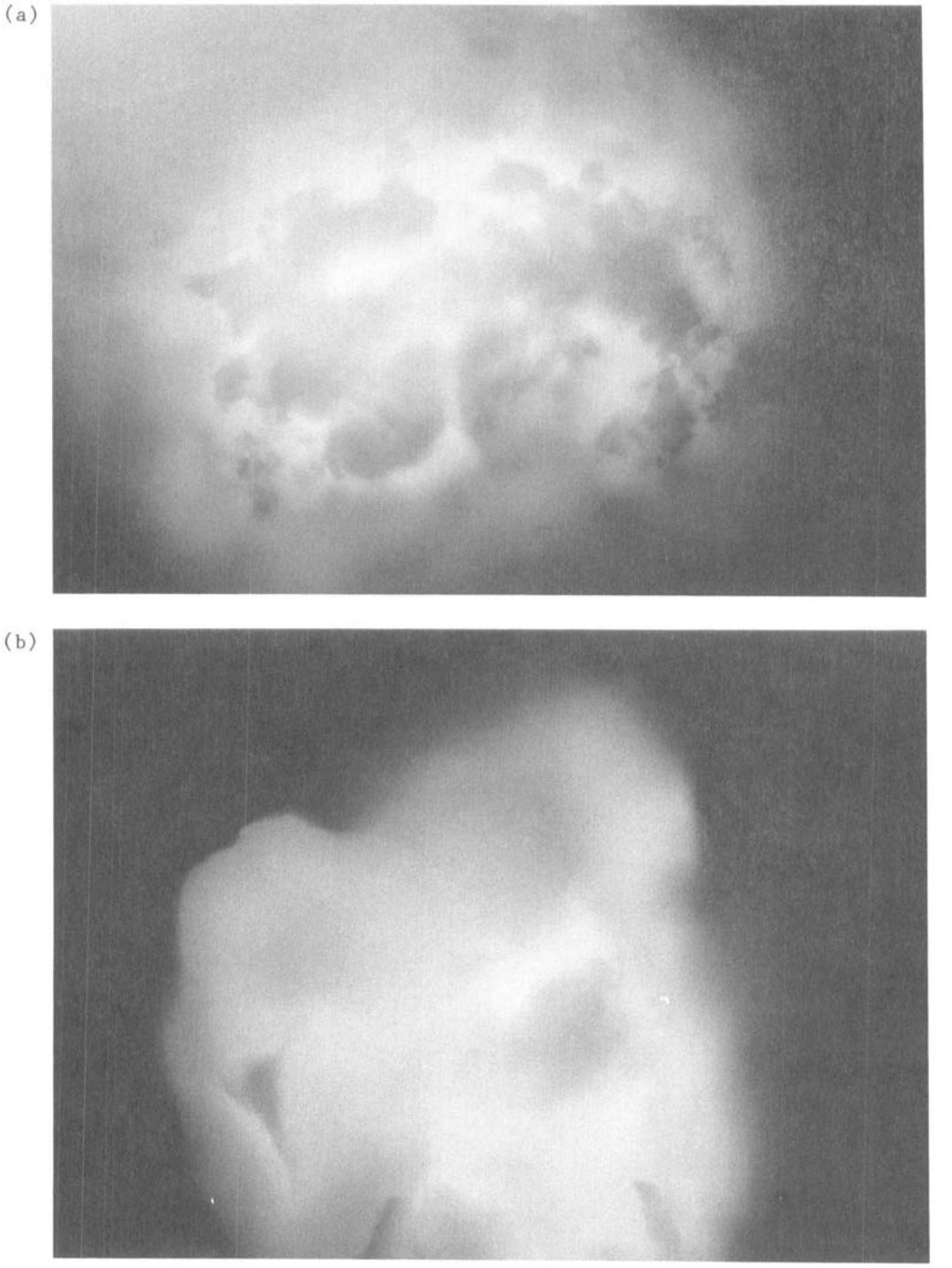


Fig. 2 - Fluorescence microscopy of mirrospheres and uncoated Tolmetin.
(a) without coacervation-inducing agent, (b) PIB, (c) EVA, (d) uncoated Tolmetin.

(c)

(d)

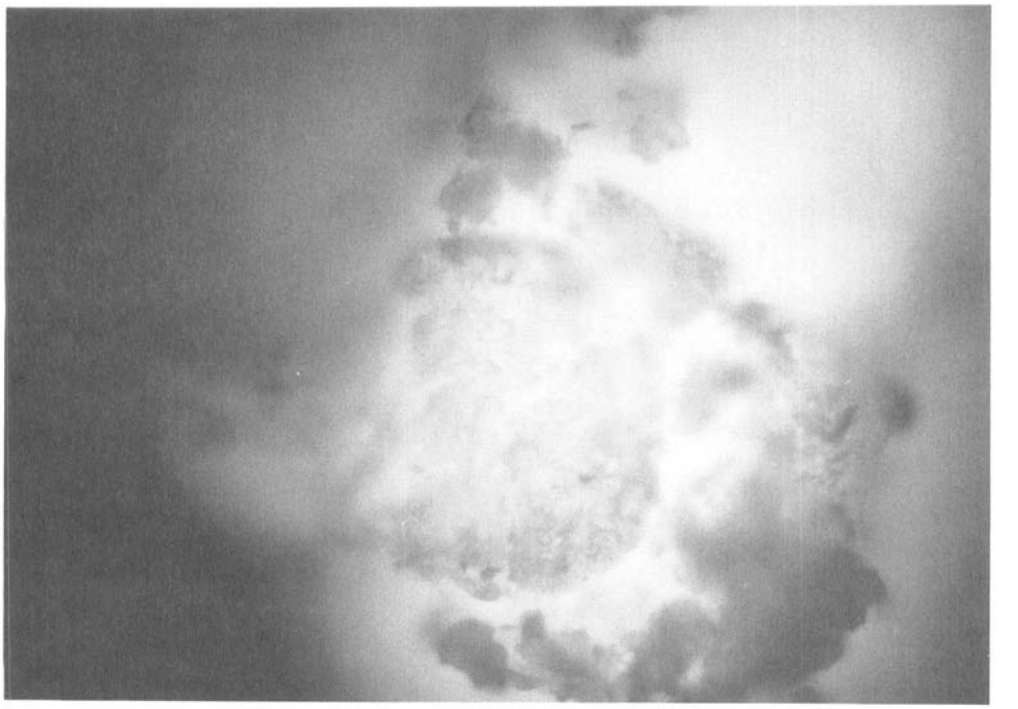


Fig. 2 Continued

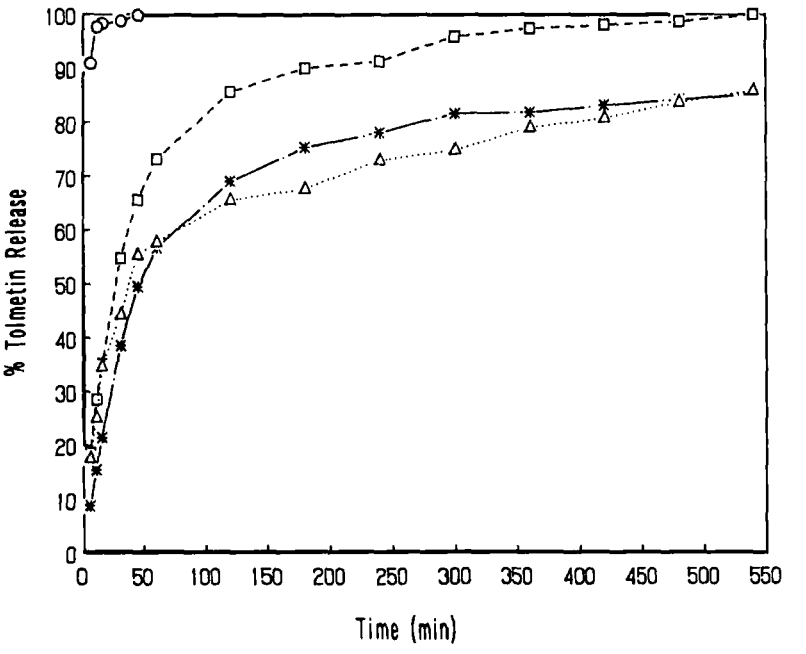


Fig. 3 - Release (%) of Tolmetin from microspheres at pH 7.4 at a temperature of 37 ± Ø.1 °C. -O-: uncoated Tolmetin, -Δ-: without coacervation inducing agent, -□-: PIB, -✱-EVA.

Tolmetin fluorescence (Fig. 2d) with respect to those prepared with PIB or with no coacervating agent, for which areas with dishomogeneous fluorescence are visible (Fig. 2b and 2a). A study on drug dissolution rate was also carried out on EC microspheres, at pH 7.4, in comparison with the free drug; the influence of the coacervating agents upon the drug release from microspheres was in the meantime evaluated (Fig. 3).

The time required to dissolve 50% of Tolmetin from microspheres (t_{50}) was 37.5 min if the microspheres were prepared without a coacervation-inducing agent. When PIB

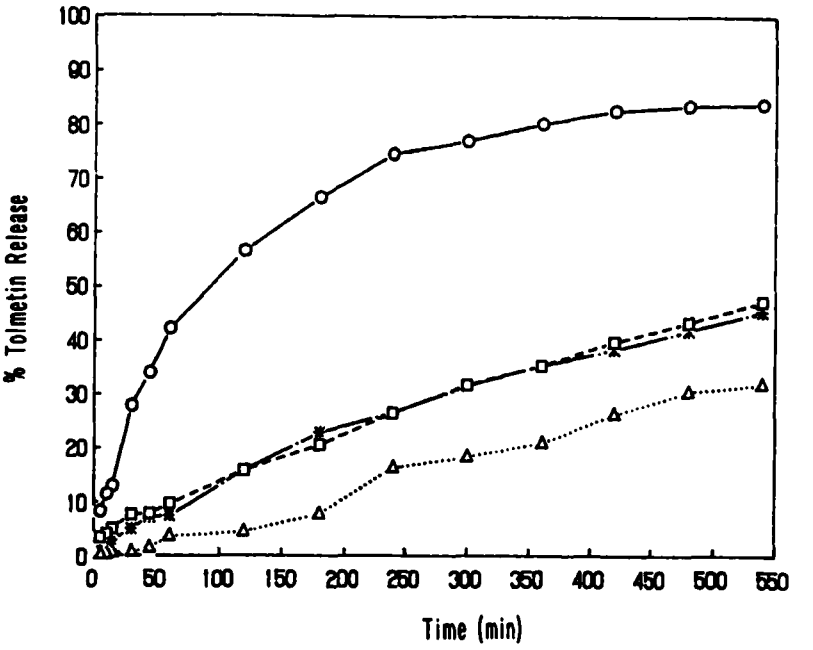


Fig. 4 -- Release (%) of drug from microspheres at pH 4 at a temperature of 37 ± 0.1 °C.
-O-: uncoated drug, -Δ-: without coacervation-inducing agent, -□-: PIB, -✱-: EVA.

and EVA were used the t_{50} values were 26.5 and 45.1 min respectively; unencapsulated Tolmetin showed a t_{50} of about 2.5 min. Changes in t_{50} values are imputable to the different wall thichness and uniformity of coating, as seen by SEM and FM. These result are in accordance to reports of Takamura et al. (21), Nixon et al. (22), which indicated that the dissolution rate of core material from microspheres was related to variables such as wall thickness, porosity, density and other wall characteristics.

To verify if dissolution rate of encapsulated Tolmetin is influenced by pH variations, we repeated dissolution

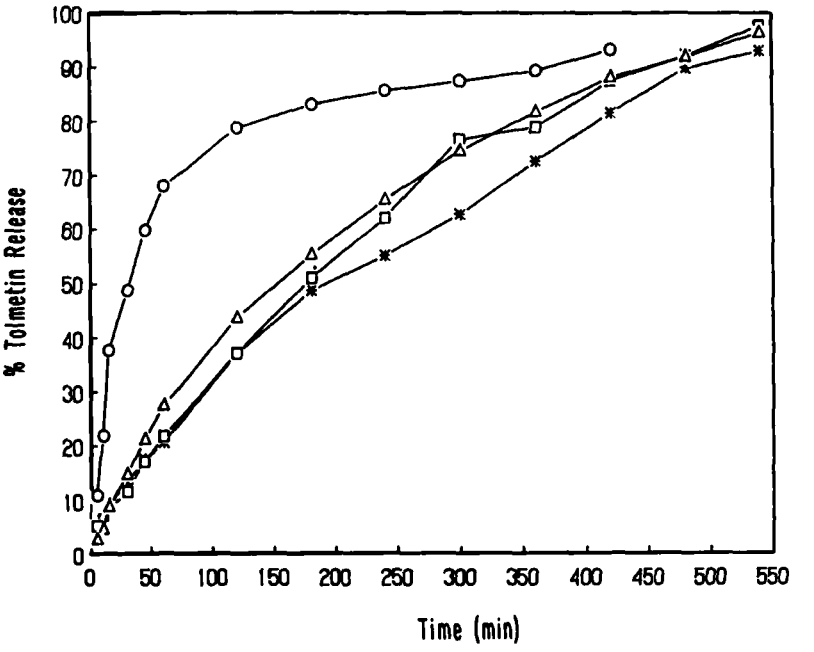


Fig. 5 - Release (%) of Tolmetin from microspheres at pH 4 in presence of Tween 80 (0.02 %) at a temperature of 37 ± 0.1 °C.
-⊙-: uncoated Tolmetin, -△-: without coacervation inducing agent, -□-: PIB, -✱-: EVA.

tests in a pH 4.0 buffer. Such a pH value was chosen by considering the insolubility of the drug in a gastric (pH 1.1) buffer. The analysis at pH 4.0 evidenced a remarkable decrease in the percentage of dissolved drug from microspheres (Fig. 4), as regards the values obtained at pH 7.4. These data suggest the suitability of using a surfactant to increase the wettability of microspheres as well as the solubility of the drug in the dissolution medium (23).

The chosen surfactant was Tween 80. The dissolution assays were thus performed again at pH 4.0 and in the presen-

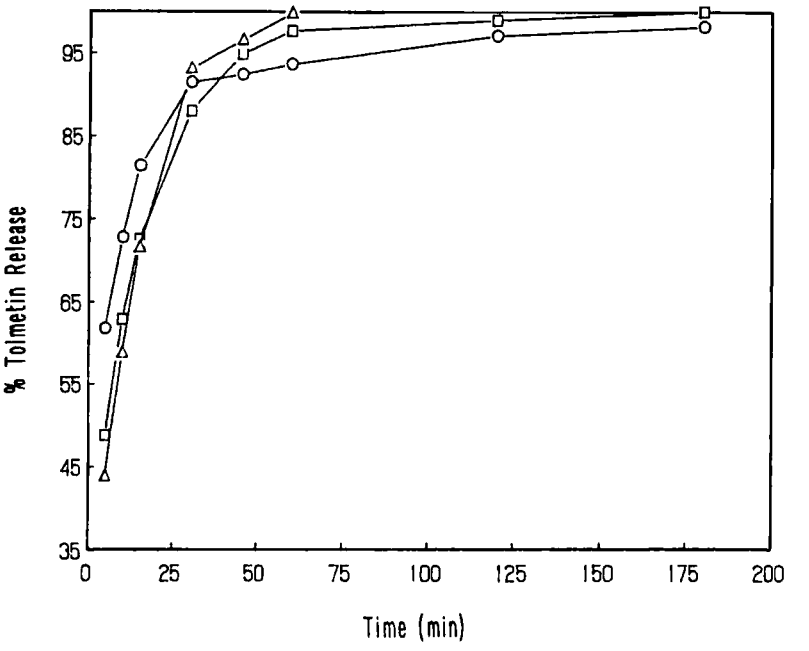


Fig. 6 - Release (%) of drug from microspheres at pH 7.4 in presence of Tween 80 (0.02%) at a temperature of 37 ± 0.1 °C.
-△-: without coacervation-inducing agent, -□-: PIB, -✱-: EVA.

ce of 0.02 Tween 80 (Fig. 5). Dissolution rates appeared largely enhanced by the surfactant to a t50 of 150.9 min for the encapsulated Tolmetin (in EC microspheres without any coacervating agent) and 37.1 min for the free drug. Microcapsules obtained in the presence of PIB or EVA gave t50 values of 172.5 min and 188.6 min, respectively, in these conditions. The influence of tween 80 as the dissolution rate of Tolmetin from microspheres was also verified at pH 7.4. In this in presence of PIB and EVA the t_{50} values were respectively 6.9 min and 5.9 min whereas

TABLE II

Gastric lesions produced by Tolmetin and microspheres in rabbits after oral administration.

Compounds	Dose [a] (mg/Kg)	no. of animals with gastric lesions	UD_{50} and 19/20 confidence limits
Tolmetin	50	1/6	190 (73-494)
	100	2/6	
	200	3/6	
	400	6/6	
Microspheres[b]	50	0/6	240 (188-386)
	100	1/6	
	200	2/6	
	400	6/6	
Microspheres[c]	50	0/6	270 (188-386)
	100	0/6	
	200	2/6	
	400	5/6	

([a]) as to the amount of free drug administered.
([b]) microspheres without coacervation-inducing agent.
([c]) microspheres prepared in the presence of PIB.

in absence of coacervating agent was about 4 min, as shown in Fig. 6.

Biological tests were carried out to evaluate the ulcerogenic potency (UD_{50}) of Tolmetin carried in microspheres. Results showed that the drug displays a better tolerability when it is coated in the microspheres (Table II), as a consequence of the shorter effective contact time with gastric walls.

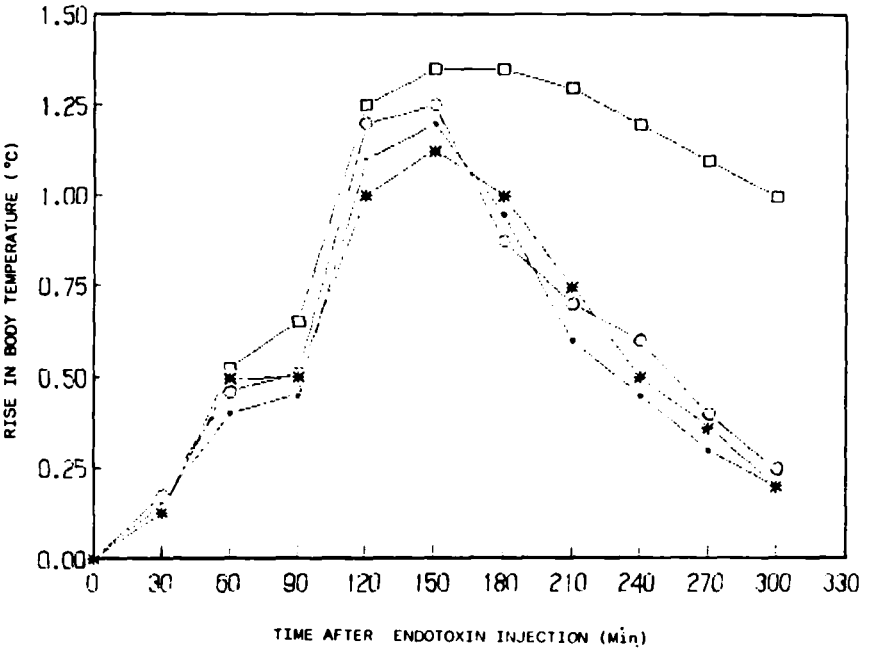


Fig. 7 - Antipyretic activity in rabbit of Tolmetin -O-, and microspheres prepared without -·- or in the presence of a coacervation-inducing agent (PIB) -✱-. Control rabbits -□- received 10 ml/Kg of 0.5 % w/v carboxymethylcellulose time 0. Each point represents the mean (± S.E.) of 3 rabbits. Standard errors (for several values) are not indicated for the sake of clarity.
* $p < 0.05$; ** $p < 0.01$ vs controls.
Student 's "t" test was used for the analysis of variance.

The slight better value of the ulcerogenic index, showed by microspheres prepared in the presence of a coacervating agent (PIB), night be ascribed to the more uniform coating of the resulting microspheres, as SEM studies have showed (Fig.1).

In addition, the antipyretic effect, as reported in Fig. 7, for either Tolmetin or the microspheres prepared without

or in the presence of a coacervation inducing agent (PIB), administered orally at a drug dose of 40 mg/Kg, produced a marked, superimposable fall in body temperature of the rabbits fevered by a bacterial endotoxin-induced pirexia.

ACKNOWLEDGEMENTS

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