Indomethacin-Loaded Microspheres: Design and Preparation by a Multiple-Emulsification Technique and Their *in Vitro* Evaluation

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A new oral controlled-release drug delivery system was developed with two polymers using a multiple-emulsification technique. Powdered drug was dispersed in methyl cellulose sol, which was emulsified in ethyl cellulose solution in ethyl acetate. The primary emulsion thus formed was reemulsified in aqueous medium. During this phase, discrete microspheres were formed under optimized conditions. The size distribution of the microspheres was investigated, and scanning electron microscopy revealed the surface topography of the microspheres. The *in vitro* drug release followed first-order diffusion-controlled dissolution. More than 85% of the drug was released over 6 hr at pH 6.2 for all dissolution batches.

KEY WORDS: controlled release; microspheres; multiple emulsification; ethyl cellulose; methyl cellulose; indomethacin; scanning electron microscopy; dissolution profile.

INTRODUCTION

Indomethacin is a nonsteroidal antiinflammatory agent and inhibitor of prostaglandin synthesis (1). However, gastrointestinal irritation and CNS side effects as a result of rapid drug release from conventional dosage forms necessitate the formulation of a controlled-release dosage form of indomethacin. A multiple-unit controlled-release drug delivery system is expected to reduce the peak indomethacin concentration in plasma without a marked loss of bioavailability and thus the incidence of side effects.

Multiple emulsions, used as controlled-release delivery systems (2,3), possess the disadvantages of liquid dosage forms and are prone to degradation. The solid microspheres are devoid of these problems. Morris and Warburton (4,5) first proposed the preparation of three-ply-walled w/o/w microcapsules using a multiple-emulsification technique.

In the present investigation a novel procedure is developed for the preparation of controlled-release microspheres. Two polymers blended in different proportions are used to reduce the time required to congeal the precipitated polymers. The polymers used are ethyl cellulose (EC), a water-insoluble polymer, and methyl cellulose (MC), a water-soluble polymer. The addition of a water-soluble substance to an insoluble polymer serves to adjust the water permeability of the latter polymer (6,7). The release kinetics of indomethacin from the prepared controlled-release drug delivery system were determined *in vitro*.

MATERIALS AND METHODS

Chemicals. Indomethacin (Indian Pharmacopoeia) was received by the courtesy of Cipla Limited, Bombay; ethyl cellulose (BDH, England), methyl cellulose (BDH, England), ethyl acetate (E. Merck, India), potassium dihydrogen phosphate (E. Merck, India), and sodium hydroxide (E. Merck, India) were obtained commercially and used as received. All chemicals were of analytical grade. The drug was passed through a U.S. standard No. 80 sieve (177 μm) (8).

Preparation of the Dosage Form. The method of preparation of microspheres using two polymers was developed using a double-emulsification technique. The ratios of the polymer blends of methyl cellulose (MC) and ethyl cellulose (EC) were 2:1, 1:1, and 1:2. The drug, MC, and EC were at the weight proportions of 1:2:1, 1:1:2, 1:1:1, and 2:1:1. Stirring speeds were 600 and 1000 rpm.

Four grams of methyl cellulose, for the batch at a drug: MC:EC ratio of 1:2:1, was dissolved in 60 ml of warm glass-distilled water under stirring. Two grams of indomethacin (IMC), previously dried and sieved, was incorporated slowly into methyl cellulose sol under stirring. Two grams of ethyl cellulose was separately dissolved in 90 ml of ethyl acetate. Ethyl cellulose was gradually added into ethyl acetate and the speed of the stirrer was adjusted to prevent frothing and formation of ethyl cellulose lumps. The MC–IMC dispersion was slowly poured into the EC solution and was emulsified by vigorous stirring at 1500 rpm. Stirring continued for 30 min to form a thick primary emulsion.

The primary emulsion was then reemulsified in 400 ml chilled aqueous medium, containing 3 ml polysorbate 80 as the emulsifier. However, because polysorbate 80 enhanced the leaching out of indomethacin from the core, polysorbate 80 was subsequently deleted. Soft microspheres could be obtained by optimizing the stirring speed, the rate at which the primary emulsion was added, and the temperature of the external continuous phase. Stirring speeds, as mentioned above, were 600 and 1000 rpm. The primary emulsion was poured into the chilled aqueous medium in a fine continuous stream. After 3–5 min, the system was cooled to 10°C by placing ice cubes externally around the preparation vessel and stirred for 20 min. The soft spheres obtained were congealed by cooling to 5°C or below. After 1 hr of continuous stirring, the external medium was replaced with fresh aqueous medium. Stirring continued for another 2 hr to remove the organic solvent completely by evaporation. Finally, to recover the prepared microspheres, the supernatant was decanted. The microspheres were filtered, kept at 4°C overnight to harden, and air-dried for 24 hr. All other batches were prepared accordingly. Microspheres were dried to a constant weight in a vacuum dessicator. Reproducibility of the methodology was evaluated by preparing replicate batches and determining the yield and percentage of drug embedded.

Particle Size Analysis. Particle size distribution was determined by passing the microspheres through a set of U.S. standard sieves, Nos. 16, 18, 20, 25, 35, 40, and 50. The

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Table I. Reproducibility of the Manufacturing Process

IMC:MC:EC ratio	Stirring speed (rpm)	Yield (% ± SD)[a]	Size range (μm)	Drug content (% ± SD)[a,b]
1:2:1	1000	96.77 ± 0.93	500–1000	24.25 ± 0.02
	600	96.56 ± 0.74	500–1000	24.40 ± 0.02
1:1:2	1000	94.88 ± 0.74	420–1000	23.85 ± 0.02
	600	94.69 ± 0.65	500–1000	24.10 ± 0.01
1:1:1	1000	95.75 ± 0.58	297–841	32.35 ± 0.01
	600	96.60 ± 0.86	500–1000	32.45 ± 0.02
2:1:1	1000	97.64 ± 0.97	297–841	48.50 ± 0.62
	600	97.52 ± 0.93	500–1000	48.65 ± 0.02

[a] Mean results of six batches.
[b] Microspheres were of sieve size 875 μm.

sieves were shaken by a standard sieve shaker having double gyratory and vibratory movements. The microspheres retained by different sieves were further analyzed by a photographic counting method using a particle size analyzer to determine the average size of the maximum number of particles remaining on a particular sieve size.

Content Uniformity. One hundred milligrams of pulverized microspheres was accurately weighed and transferred to an Erlenmeyer flask containing phosphate buffer, pH 6.2. The flask was shaken for 10 hr and diluted to 100 ml with the same solution. An aliquot was withdrawn, diluted, and assayed spectrophotometrically at 320 nm (9).

Scanning Electron Microscopy. The microspheres were fixed on aluminum mounts with the help of a specified fixative. The photomicrographs were taken on a ISI-60A scanning electron microscope after coating the samples with a gold layer using a polar vapor coater unit.

In Vitro *Dissolution Profile.* The *in vitro* indomethacin release profile was studied according to USP XXII specifications using the dissolution apparatus described. Nine hundred milliliters of dissolution fluid was used and the basket was covered with a No. 100 mesh nylon cloth to prevent loss of microspheres. The basket rotation speed was 100 ± 5 rpm. Aliquots of 5 ml were withdrawn at half-hour intervals and replenished with the same fresh volume. Phosphate buffer of pH 6.2 (9) was used as the dissolution medium.

RESULTS AND DISCUSSION

Several variables altered the formation of discrete microspheres, namely, the core-to-coat ratio of the drug–methyl cellulose–ethyl cellulose slurry, consistency of the primary emulsion formed, temperature during microsphere formation, rate at which the primary emulsion was added, and stirring speed during addition of the primary emulsion. If the primary emulsion was too thin, the size of the microspheres and yield were reduced. Yield was low because of

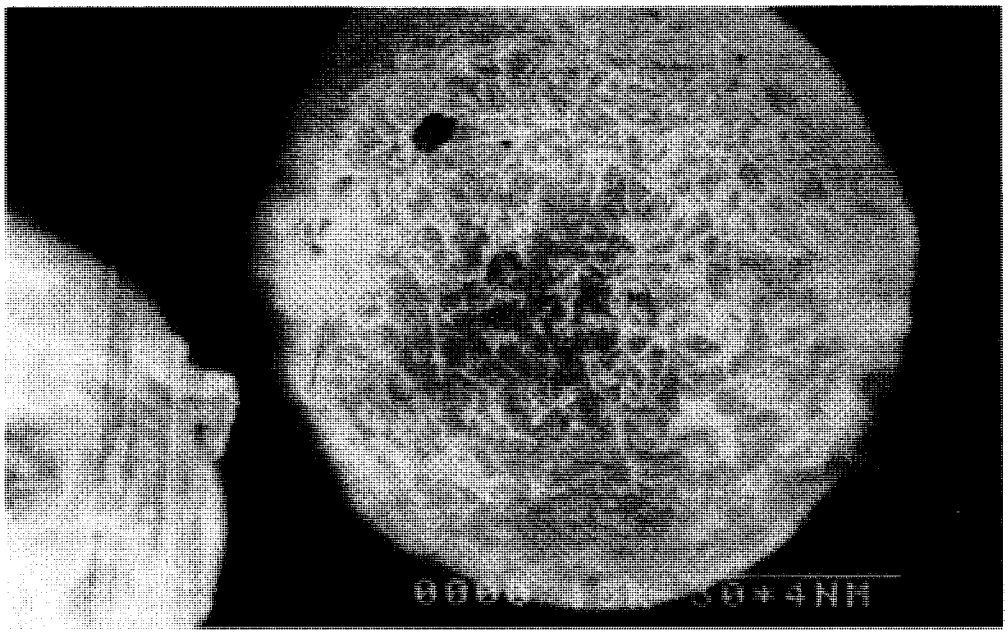

Fig. 1. Scanning electron micrograph of indomethacin microspheres before dissolution.

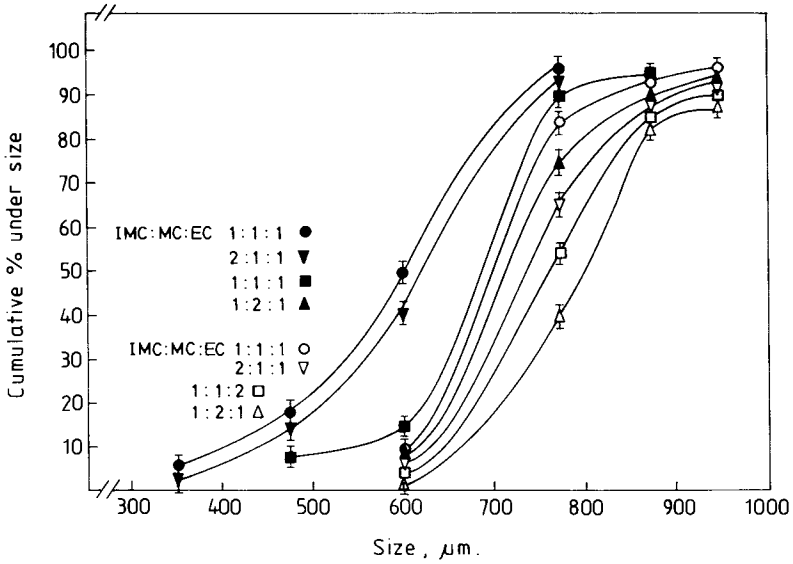

Fig. 2. Size distribution of indomethacin microspheres.

adherence of the polymers and drug to the wall of the vessel. The external temperature of the vessel must be below 10°C after formation of embryonic microspheres to prevent evaporation of ethyl acetate, resulting in the formation of irregular microspheres. A slow pouring rate of the primary emulsion increased the size of the microspheres, whereas quick pouring produced a multiple emulsion instead of microspheres. A multiple emulsion also resulted with a high stirring speed, but a low stirring speed caused agglomeration of the microspheres. Optimization and proper control of all these variables were essential for the formation of discrete and spherical microspheres. Further cooling of the system was necessary to congeal the soft spherical beads.

The results in Table I illustrate the physical and pharmaceutical properties of the indomethacin-loaded microspheres. The coefficient of variation was within 5% in all cases. Figure 1 shows a scanning electron micrograph of the prepared microspheres. The microspheres displayed a

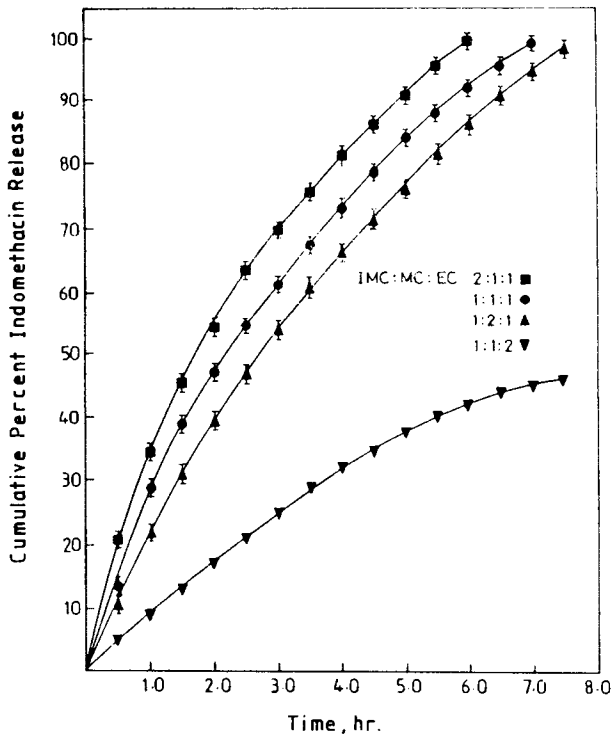

Fig. 3. Effect of core coat ratio on the *in vitro* drug release profile at a particle size of 875 μm.

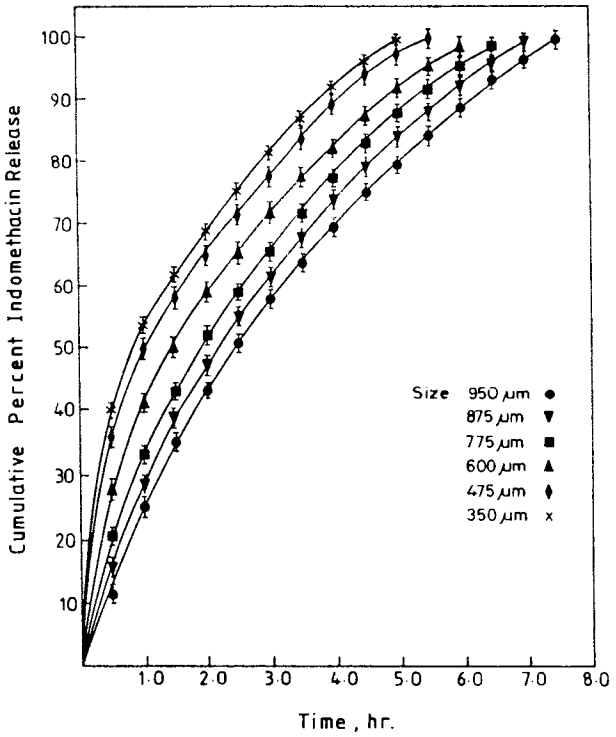

Fig. 4. Drug release profile at different sizes of indomethacin microspheres. Core: coat ratio, 1:1:1.

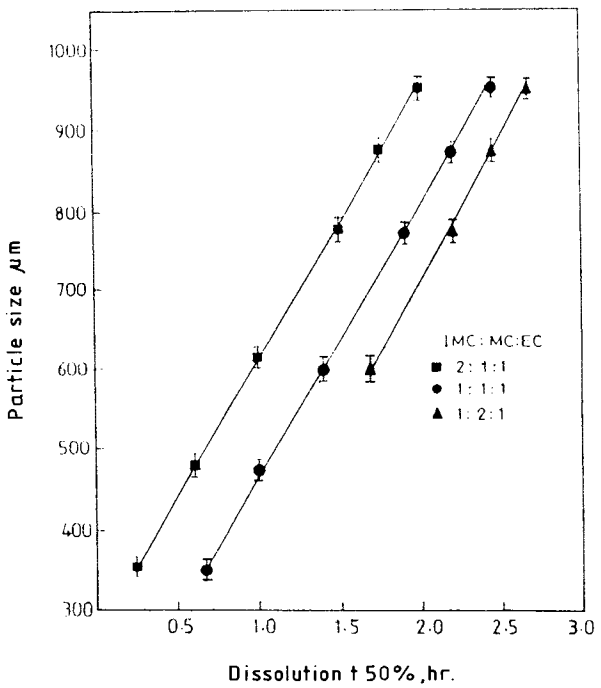

Fig. 5. Effect of the particle size of indomethacin microspheres on the dissolution $t_{50\%}$.

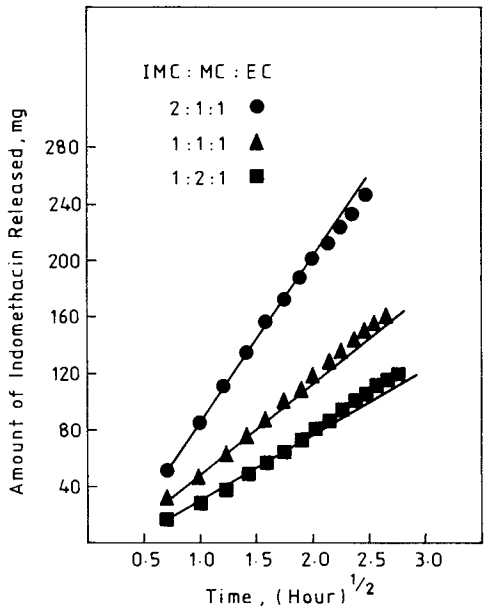

Fig. 6. Release of indomethacin versus square root of time curves of indomethacin microspheres of size 875 μm.

smooth surface with channels. These channels may facilitate drug diffusion from the microspheres during dissolution.

The size distribution of microspheres was influenced by the drug–polymer ratio and stirring speed (Table I and Fig. 2). At a constant speed, as the polymer content increased, the particle size also increased, possibly because of the thicker consistency of the primary emulsion. At a higher stirring speed, smaller microspheres were obtained.

Figure 3 represents the dissolution profile of indomethacin-loaded microspheres. At lower pH's, e.g., pH 1.2, the release of indomethacin was insignificant because of poor drug solubility in acidic medium. Similar plots were obtained with other particle sizes. These plots revealed the pseudo-first-order release kinetics of the drug from the microspheres. An increase in the ratio of the polymers decreased the release profile of indomethacin, because of increased barriers to drug diffusion. Further, an increase in the amount of water-soluble methyl cellulose increased the drug release rate, possibly because of more channels being available for diffusion. However, at even higher levels of ethyl cellulose,

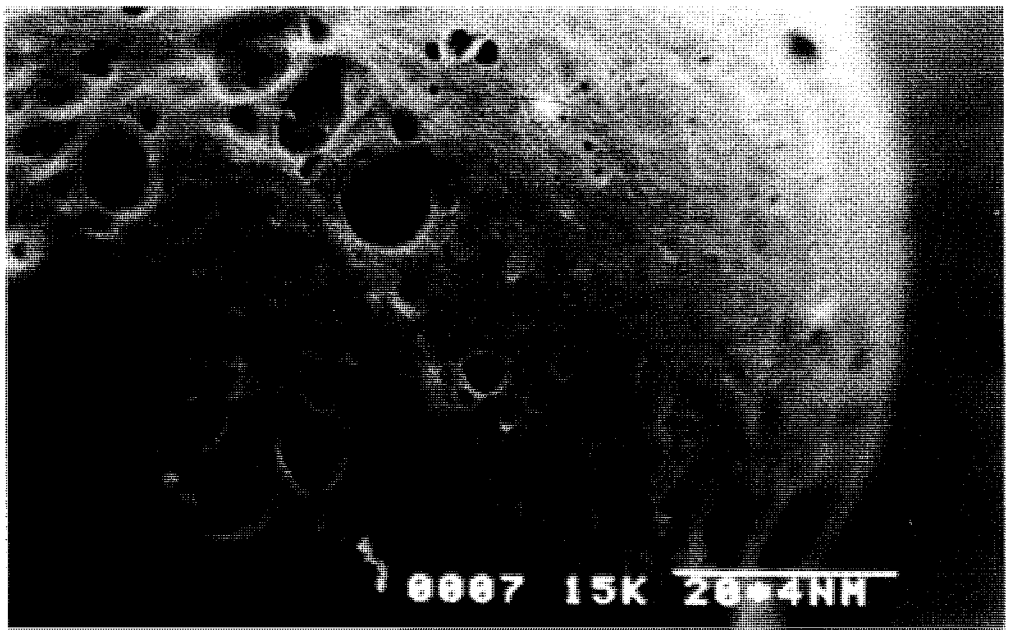

Fig. 7. Scanning electron micrograph of indomethacin microspheres after dissolution.

drug release decreased below acceptable rates. Consequently batches of formulation with a drug:MC:EC ratio of 1:1:2 were discarded.

The drug release was inversely proportional to the size of the microspheres (Fig. 4). This result might be attributed to the decreased diffusional path length and increased effective surface area of the microspheres. There was a nearly linear relationship between dissolution $t_{50\%}$ and particle size over the range tested (Fig. 5). The amount of indomethacin released (Q) versus the square root of time were plotted according to the Higuchi equation (10) (Fig. 6). The nearly linear plots in the initial phase suggested a diffusion-controlled release mechanism, but in the later phase, a deviation from this diffusion-controlled behavior occurred. This nonlinearity in the plot arose only when the concentration of drug remaining in the matrix fell below the saturation value (11).

Figure 7 is a photomicrograph of the microspheres after the dissolution process. The size of the channels on the surface had increased considerably, showing empty holes, while the size and shape of the microspheres were intact. Therefore, the microspheres might have the nature of a monolithic device rather than a reservoir device. The combined results suggest that the dissolution process of indomethacin from the microspheres was diffusion controlled. However, the solubility of methyl cellulose in the aqueous medium affected the first-order diffusion-controlled release mechanism. The deviation of the observed plot from a linear Higuchi plot might have been due to the influence of methyl cellulose.

The production of microspheres by this technique was simple and reproducible. By proper combination of different batches of microspheres having varied drug–polymer ratios and particle sizes, desirable drug-release profiles were achieved, and these double-polymeric microspheres may prove useful for the prolonged release of indomethacin and drugs in general.

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