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Formation and In-vitro Evaluation of Theophylline-loaded Poly(methyl methacrylate) Microspheres

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Abstract—Theophylline-loaded poly(methyl methacrylate) (PMM) microspheres were prepared by the solvent evaporation method. Increasing the drug to polymer ratio increased both the mean particle size of the microspheres and the release rate. Polyethylene glycol (PEG) 4000 was used to improve the release rate of theophylline from the microspheres. No marked effect was observed on particle size distribution of the microspheres as a function of PEG concentration but there was a pronounced effect on drug release. The different particle sizes of microspheres prepared from the same drug to polymer ratio showed no significant difference in drug content, indicating that the ratio between theophylline and PMM remained practically constant regardless of the size of microspheres. Release characteristics of the microspheres were influenced by drug to polymer ratio, the amount of PEG incorporated and the particle size of microspheres. The release rate was slightly higher in simulated gastric fluid than in simulated intestinal fluid. The release profiles of the drug were modified by mixing microspheres of different formulations in different ratios.

Theophylline has been widely used as a bronchodilator for the treatment of chronic asthma. The bronchodilator effect of theophylline increases with serum concentration over a range of 5 to 20 $\mu g\ mL^{-1}$ but, at a plasma level above 20 $\mu g\ mL^{-1}$ there is an increased risk of toxic effects (Ogilvie 1978). The desirability of maintaining theophylline plasma levels within this narrow range had led to the development of several sustained-release formulations.

Several studies have been reported on in-vitro dissolution and pharmacokinetics of sustained-release theophylline (Anderson et al 1983; Simons et al 1984; El-Yazigi & Sawchuk 1985; Macheras et al 1987). However, few reports have mentioned the preparation of the dosage form. Theophylline microcapsules prepared from ethylcellulose using ethylene-vinyl acetate (EVA) copolymer as a coacervation-inducing agent have been described (Lin & Yang 1985). In this study, various concentrations of EVA copolymer were used and the effect of its concentration on the micromeritic properties such as particle size, density, porosity, wall thickness and surface topography of the microcapsules along with the corresponding drug release behaviour were studied. Theophylline has also been prepared in the form of a drug-resin complex and coated with paraffin or encapsulated with ethyl cellulose by various methods. One of these products was subsequently coated with paraffin to achieve a wide range of release rates (Motycka et al 1985).

Poly(methyl methacrylate) (PMM) has been used extensively as a prosthetic material in dental and mandibular corrections (Hodosh et al 1968, 1969; Castelli et al 1971) and as a bone cement in hip joint reconstruction (Charnley & Pusso 1968). Implanted methyl methacrylate polymers appear to be well tolerated if the implants are monomer-free and under a certain threshold size (Bischoff & Bryson 1964).

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The aim of this paper is to describe the preparation of PMM microspheres containing theophylline and to investigate some of the process parameters affecting the properties and the in-vitro dissolution of theophylline from the microspheres.

Materials and Methods

Materials

Materials were obtained from commercial sources: Poly(methyl methacrylate) low molecular weight (Aldrich Chemical Co., Wisconsin, USA); polyethylene glycol (PEG) 4000 (Maruishi, Osaka, Japan); theophylline, magnesium stearate, mineral oil, hexane and dichloromethane (Wako Pure Chemical, Osaka, Japan). Theophylline was pulverized and passed through a sieve of 200 mesh before use.

Preparation of microspheres

PMM microspheres were prepared by the solvent evaporation process. PMM (2·25 g) and PEG 4000 were dissolved in 15 mL of acetone and various amounts of theophylline were added to this solution. The mixture was then dispersed into 90 mL of mineral oil containing 0·5% of magnesium stearate and 10 mL of hexane. The mixture was kept stirred at 950 rev min^{-1} for about 1h until the acetone had evaporated. The PMM solidified and enveloped the theophylline, forming microspheres which were separated from the vehicle by centrifugation at 2000 rev min^{-1} for 15 min. The microspheres were washed three times, with 80 mL of hexane for each wash, to remove any adsorbed mineral oil and collected by filtration. They were then dried in a vacuum at room temperature (25°C). The concentration of PEG was varied from 5 to 30% of polymer weight and the initial theophylline loading was varied from 1–5 g. Data for each formulation of microsphere were obtained from duplicate experiments.

Microsphere size distribution

Dried microspheres were sized by passing through a nest of

small standard sieves #18 (850μm), 24 (710μm), 28 (590μm), 36 (425μm), 48 (297μm) and 80 (177μm). The microspheres that passed through one sieve and retained on the subsequent finer sieves were collected and weighed. The mean particle size of each fraction was taken as the arithmetic mean size of the apertures of the retaining and preceding screen.

Microscopy studies

Scanning electron microscopy was used to evaluate surface texture of the microspheres. The microspheres were coated with 150 Å of gold (Eiko IB-3 Ion Coater) and were examined with a scanning electron microscope (Hitachi S-430).

Determination of theophylline content

A sample of about 15 mg of microspheres was weighed accurately and dissolved in 20 mL of dichloromethane. Triplicate samples of 1 mL of this solution were diluted further with 9 mL of dichloromethane and the concentration of theophylline was determined spectrophotometrically at a wavelength of 274 nm (Hitachi Model 200–20). All the assays were carried out in duplicate.

In-vitro dissolution studies

Release of the theophylline from the microspheres was carried out using a USP dissolution assembly (Toyama NTR-5S3), each dissolution beaker contained 1000 mL of simulated intestinal fluid pH 6·8 or simulated gastric fluid pH 1·2 without enzyme. Polysorbate 80 (0·02% w/v) was added to the dissolution fluid to overcome the non-wetting characteristic of the microspheres and to make the solution more closely resemble the surface tension of gastrointestinal fluid. An accurately weighed 100 mg quantity of microspheres was added to each dissolution beaker. The dissolution medium was kept at 37°C and stirred at 100 rev min^{-1}. Three mL aliquots of dissolution medium were taken at predetermined time intervals. An equal volume of dissolution medium was added to maintain volume after each sample was removed. Theophylline in the dissolution medium was determined spectrophotometrically at 274 nm. All the assays were carried out four times.

Results and Discussion

It is well accepted that additives in the formulation and stirring speed during preparation are two factors that will affect the properties of microspheres. It was found that the use of PEG 4000 in the formulation and a stirring speed of 950 rev min^{-1} gave satisfactory results in terms of particle size and surface texture of microspheres. The microspheres were found to be smooth surfaced, spherical, rigid and free flowing.

Microsphere size distribution

Different concentrations of PEG ranging from 5 to 30% did not affect the microsphere size distribution, which was similar to results previously reported (Pongpaibul & Whitworth 1986).

In an attempt to increase the theophylline content of the microspheres, a number of formulations with increasing amounts of theophylline to a constant weight of PMM were prepared. As the amount of theophylline increased from 1 to 4 g, satisfactory microspheres were obtained. When the amount of theophylline was increased to 5 g the stirring speed of 950 rev min^{-1} was not fast enough to disperse the mixture of theophylline and polymer solution in mineral oil.

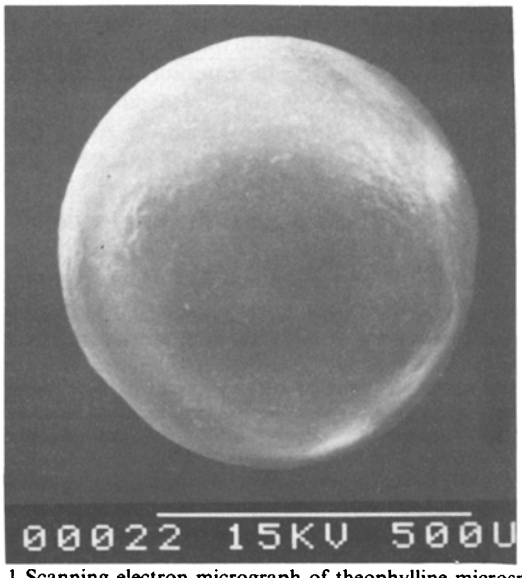

FIG. 1 Scanning electron micrograph of theophylline microsphere prepared from drug to polymer ratio of 2:2·25 and 10% PEG. Bar = 500μm

Increasing the drug to polymer ratio from 1:2·25 to 4:2·25, caused the microsphere size distribution to shift toward the higher particle size. This was due to the fact that the higher concentration of theophylline produced a more viscous dispersion which formed larger droplets in the mineral oil and consequently, larger microspheres.

Microscopy studies

A scanning electron micrograph of theophylline microsphere containing 10% PEG is shown in Fig. 1. As the concentration of PEG was increased to 30% the surface of the microspheres became more porous (Fig. 2).

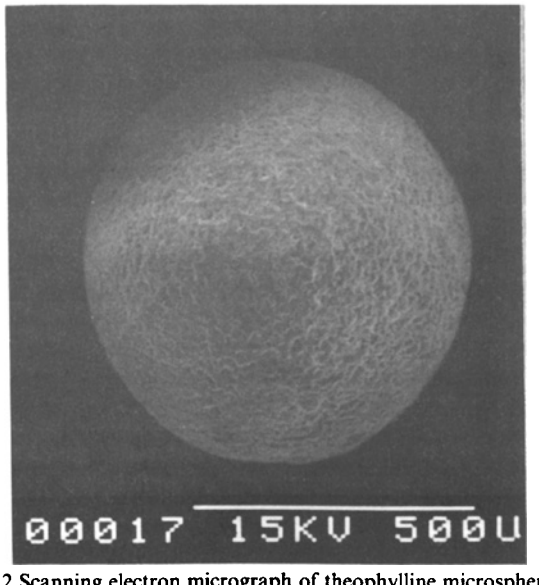

FIG. 2 Scanning electron micrograph of theophylline microspheres prepared from drug to polymer ratio of 2:2·25 and 30% PEG. Bar = 500μm

Table 1. Effect of the process parameters on theophylline loading, % drug entrapped and t50.

Formulation	Drug:polymer ratio	PEG (%)	Drug loading (% ± s.d.)	Drug entrapped (%)	t50 (h)
1	1:2·25	10	30·83 ± 0·09	99·97	>8
2	2:2·25	10	42·98 ± 0·38	94·04	>8
3	3:2·25	10	51·94 ± 0·19	94·14	2·55
4	4:2·25	10	58·14 ± 0·59	94·11	1·25
5	2:2.25	5	43·28 ± 1·08	89·36	>8
6	2:2·25	10	41·78 ± 1·21	94·04	>8
7	2:2·25	15	41·07 ± 0·18	92·59	1·80
8	2:2·25	20	41·38 ± 1·16	83·69	1·20
9	2:2·25	30	39·86 ± 1·01	91·28	0·50

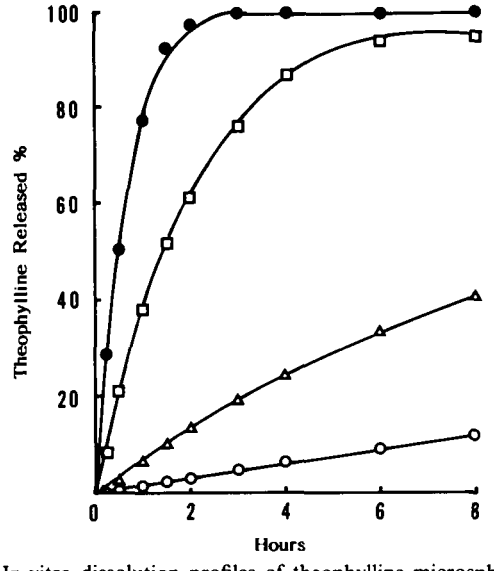

FIG. 3 In-vitro dissolution profiles of theophylline microspheres. Mean microsphere size of 508 μm in simulated intestinal fluid. Each point represents the average of four determinations. All microspheres were prepared from drug to polymer ratio of 2 : 2·25 and PEG 5% ○; 10% △; 20% □ and 30% ●.

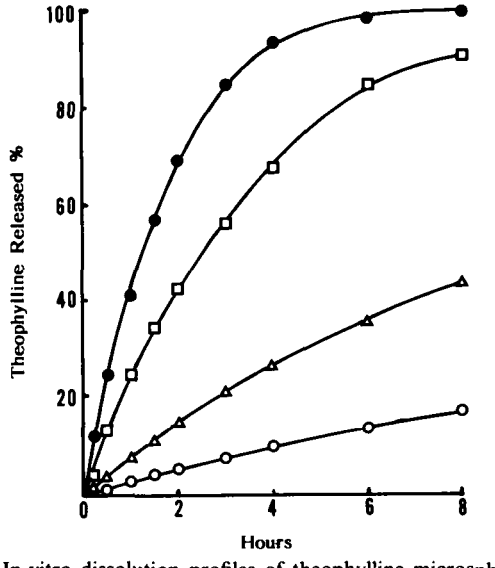

FIG. 4 In-vitro dissolution profiles of theophylline microspheres. Mean microsphere size of 780 μm in simulated intestinal fluid. Each point represents the average of four determinations. All microspheres were prepared from 10% PEG and drug to polymer ratio of, 1 : 2·25 ○; 2 : 2·25 △; 3 : 2·25 □ and 4 : 2·25 ●.

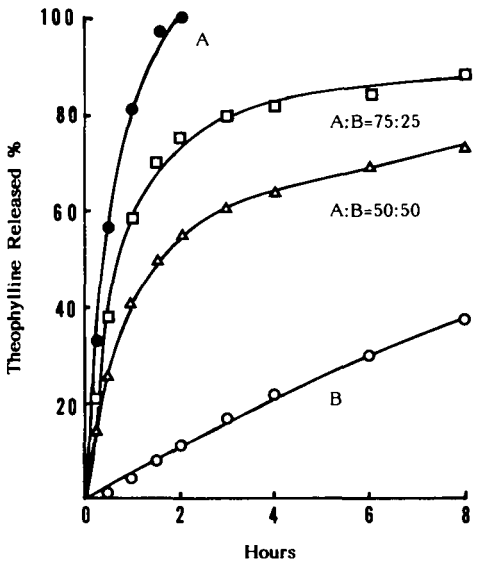

FIG. 5 In-vitro dissolution profiles of mixed theophylline microspheres of different batches with different ratios, in simulated intestinal fluid, mean microsphere size of 780 μm. Each point represents the average of four determinations.

Theophylline content

As expected, the higher the drug to polymer ratio, the larger the amount of theophylline incorporated into the microspheres. Table 1 lists nine formulations of microspheres used in this study. The microspheres of formulation 1–4 were 780 μm in diameter, prepared from different drug to polymer ratios, and those of formulation 5–9 were 508 μm diameter, prepared from different concentrations of PEG. The drug content of microspheres prepared from various concentrations of PEG decreased from 43·3 to 39·9% as the concentration of PEG increased from 5 to 30%. However, as the drug to polymer ratio was increased from 1 : 2·25 to 4 : 2·25, the drug content increased from 30·9 to 58·1%.

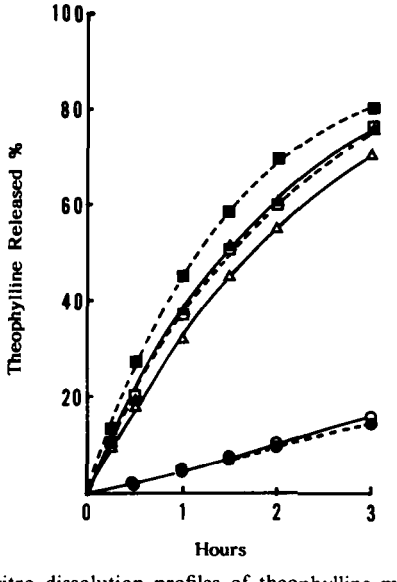

FIG. 6 In-vitro dissolution profiles of theophylline microspheres containing different concentrations of PEG in simulated intestinal fluid (solid line) and simulated gastric fluid (broken line). Mean microsphere size of 780 μm and all microspheres were prepared from drug to polymer ratio of 2 : 2·25 and PEG 10% ○, ●; 15% △, ▲ and 20% □, ■. Each point represents the average of four determinations.

Theophylline content in various microsphere size ranges of 780, 650 and 508 μm of an individual microsphere batch were determined. No significant variation in the content was observed, indicating that the ratio between theophylline and PMM remained practically constant regardless of the particle size distribution of the various microspheres in one batch.

In order to know the efficiency of the process the percent drug entrapped was calculated using:

$$\% \text{ Drug entrapped} = \frac{\text{Dry microsphere weight} \times \text{Theophylline content}}{\text{Initial theophylline loading}} \times 100$$

The percent theophylline entrapped for all formulations was found to be in the range of 83·7–99·95%. The high percentage of theophylline entrapped was due in part to the insolubility of theophylline in mineral oil which was used as the encapsulating medium.

In-vitro dissolution

The release of theophylline from different formulations was measured for 8 h. During the release experiments the microspheres remained intact. Fig. 3 shows the release profiles of theophylline from PMM microspheres (508 μm) for different PEG concentrations. The higher the PEG concentration, the faster the release rate. This was due to the porous surfaces of the microspheres (Fig. 2) and the water soluble property of PEG which formed channels on dissolution and allowed the release of drug from the microspheres. A similar result was obtained with different drug to polymer ratios. Increasing the theophylline content enhanced its release rate from the microspheres (Fig. 4). The increase in theophylline content reduces the concentration of PMM in the microsphere matrix thus facilitating drug transport through the pores and channels of the microspheres towards the sink condition.

According to Higuchi (1963) the release kinetics of dispersed drug from spherical pellets should follow the square root of time up to 50% release but deviation would occur toward the end of the leaching process. It appeared that all the formulations prepared with different drug to polymer ratios fitted a $t_{\frac{1}{2}}$ release expression. This indicated that the microspheres exhibited a non-erodible matrix release mechanism.

As expected, the release of the drug from smaller particle sizes of the microspheres prepared from the same drug to polymer ratio was faster than from the larger size. Since there was no significant difference in drug content, it can be concluded that the rapid drug release is due to the greater surface area subjected to dissolution. The release profiles were also modified by mixing microspheres of different formulations with different ratios (Fig. 5). Formulation A and B were prepared with drug to polymer ratios of 2:2·25 and PEG concentrations of 30 and 10%, respectively. With formulation A the theophylline release was too fast and with formulation B it was too slow. When microspheres of formulation A and B were mixed in the ratios of 50:50 and 75:25, the percent theophylline released from the microspheres progressively increased over that of formulation B alone.

A comparison of the release in simulated gastric fluid versus intestinal fluid shows that the release of the drug in gastric fluid is similar or slightly faster than the release rate in intestinal fluid (Fig. 6). The oral route of administration should therefore be satisfactory for these microspheres.

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