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# Effect of loading parameters on theophylline release from polystyrene beads

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## Summary

Various parameters of preparation of theophylline loaded by solvent swelling on polystyrene beads were evaluated. The resulting samples were characterized by X-ray photoelectron spectroscopy, differential scanning calorimetry, X-ray diffractometry, electron scanning microscopy and wettability test. The release of drug was carried out in pH 7.5 buffer medium and a biphasic process was seen. An initial phase was adequately described by a cube-root of undissolved drug equation and a terminal phase by a square-root of time equation. Drug–polymer systems, washed with water to remove the surface drug before the release study, exhibit a diffusion mechanism controlled by the Higuchi equation.

## Introduction

Incorporation of a drug by swelling of a polymeric matrix is a well known technique to obtain a controlled release preparation (Korsmeyer and Peppas, 1981; Gyselinck et al., 1983; Peppas and Franson, 1983; Stefton et al., 1984). Lippold and Lütschg (1978) studied the inclusion of kavain in a series of organic insoluble crosslinked carriers; polystyrene beads were not fully investigated because of their low degree of swelling in water.

Since drug location in the polymer has proved to play a major role in the drug release process (Lee, 1985), the aim of the present work is to evaluate the influence of various loading parameters on the distribution of theophylline in the polystyrene microspheres. The parameters considered were volume of drug solution, temperature of evaporation of chloroform during the drying phase, time of contact between suspended polymer and drug solution, and rinsing with water of the microspheres after the loading phase.

Physical characterization of the theophylline–polystyrene systems has been carried out using X-ray diffractometry, X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and wettability test to determine the exact location of drug molecules loaded on the polystyrene beads.

Two possible kinds of kinetics of drug release were considered: the diffusion of the drug through the polymeric matrix and the dissolution of surface-deposited crystalline particles of drug. The applicability of the models using the square-root

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of time equation (Higuchi, 1963) and the cube-root of undissolved drug equation (Hixson and Crowell, 1931) to the mechanism of drug release was examined.

## Materials and Methods

*Materials*

The polystyrene crosslinked with 1% divinylbenzene (Bio-Beads SX-1, Biorad, Italy) was used as received. The microspheres have a particle size range of 35–75 $\mu$m determined by mercury porosimetry (mod. 200, Carlo-Erba Strumentazione), and a swelling volume in chloroform and water of 8.1 mg/ml and 0.2 mg/ml, respectively. Theophylline was supplied by Farmitalia Carlo-Erba. Solvents and buffers were analytical grade.

*Polymer loading*

*Procedure A.* The powdered polymer was loaded with a volume of chloroform solution of the drug smaller than the maximum swelling capacity of the polymer. 8 ml of a chloroform solution of theophylline (6 mg/ml) were poured slowly onto 1 g of powdered polymer under continuous mixing in a mortar. After swelling the loaded polymer was dried to constant weight in an oven at 60 °C for 2 h, subsequently deaggregated with an 80 $\mu$m sieve and mixed.

*Procedure B.* The powdered polymer was suspended in an excess volume of the drug solution. 1 g of the polymer was suspended in 25 ml of a chloroform solution of theophylline (6 mg/ml). The resultant system was stirred at room temperature for a predetermined time and filtered. The loaded polymer was dried in an oven at a predetermined temperature until constant weight and treated as in Procedure A.

Furthermore 500 mg of the samples, prepared by this technique, were placed on a porous filter and rinsed with 250 ml of distilled water. The polymer was dried at 60 °C for 2 h and after trituration passed through an 80 $\mu$m sieve. The preparative conditions and composition of the formulations are reported in Table 1.

*Preparation of physical mixture*

Physical mixture was prepared by simply mixing 40 mg of theophylline, recrystallized from chloroform, and 1 g of polystyrene possessing the same particle size range (35–75 $\mu$m).

TABLE 1

*Preparative conditions and composition of the formulations*

| Formulation no. | Procedure of preparation | Time of suspension (days) | Temperature of drying (°C) | Drug content (mg/g) | |
|---|---|---|---|---|---|
| | | | | not washed | washed |
| 1 | A | – | 60 | 40 | – |
| 2 | B | 1 | 60 | 28 | 4 |
| 3 | B | 3 | 60 | 32 | 5 |
| 4 | B | 7 | 60 | 37 | 13 |
| 5 | B | 7 | 4 | 37 | 7 |
| 6 | B | 7 | 40 | 37 | 11 |
| 7 | B | 7 | 80 | 37 | 15 |

*Analysis of theophylline content*

The drug content of loaded polymer was checked by repeated extraction with chloroform in a Soxhlet apparatus. Theophylline was assayed spectrophotometrically at 271 nm (Perkin Elmer 559 spectrophotometer).

*Morphological analysis*

Microspheres were observed under an optical microscope with polarized light (Zeiss mod. Universal) or poured onto a plate, sputter-coated with gold and examined by a scanning electron microscope (Stereoscan 604, Cambridge, U.K.).

*Differential scanning calorimetry (DSC)*

Thermal analysis was carried out with a differential scanning calorimeter (Mettler DSC 20, TA 3000) under a nitrogen flow, indium as a calibration standard (156.6 °C) and a heating rate of 10 °C/min.

*X-Ray diffractometry*

The powdered theophylline–polystyrene systems were exposed to Cu-K$\alpha$ radiation in a wide-angle X-ray diffractometer (Philips, PW 1050/70) over a range of $2\vartheta$ from 4° to 35°.

*X-Ray photoelectron spectroscopy (XPS)*

The sample was prepared by pressing a suitable

amount of powder onto a foil of pure indium (99.999% purity, Goodfellow U.K.) and placed in an XPS-AES instrument (Physical Electronic Inc. PHI Mod. 548) using Al-K$\alpha$ (1448.6 eV) as anode material. A base pressure of $3 \times 10^{-7}$ Pa was obtained in the analysis chamber. After smoothing and subtraction of the background the peak intensities were calculated by digital integration.

*Solid–water contact angle*

Solid–water contact angles were measured with a wettability tester (Lorentzen-Wettre, Sweden). Small drops of distilled water were placed on the surface compact by a microsyringe and, after stabilization, the magnified image of drops were projected onto a screen. The contact angle values were derived from the height and length of the drops image via a trigonometric relationship. At least 6 replications were carried out.

*Dissolution rate measurements*

900 ml of a phosphate buffer (38.8 mM), pH 7.5, were placed in a rotating paddle apparatus (USP XXI) at 150 rpm and maintained at 37 ± 0.2 °C. An accurately weighed sample of loaded polystyrene was placed in a capsule and introduced to the bottom of the vessel. At predetermined time intervals a 5.0 ml portion of the solution was sampled out, filtered through a 0.45 $\mu$m pore size Millipore filter and assayed spectrophotometrically at 271 nm. The same volume of fresh buffer was then added to the dissolution medium. Sink conditions were always maintained. Each experiment was performed in triplicate and the mean experimental results were reproducible within 5%.

## Results and Discussion

*Physical state of theophylline loaded in the polystyrene*

The physical state of the theophylline loaded in the polystyrene beads has been assessed by X-ray diffraction and differential scanning calorimetry.

The X-ray diffraction spectra of the powdered polystyrene, of theophylline recrystallized from chloroform, of formulations 1 and 2 are shown in

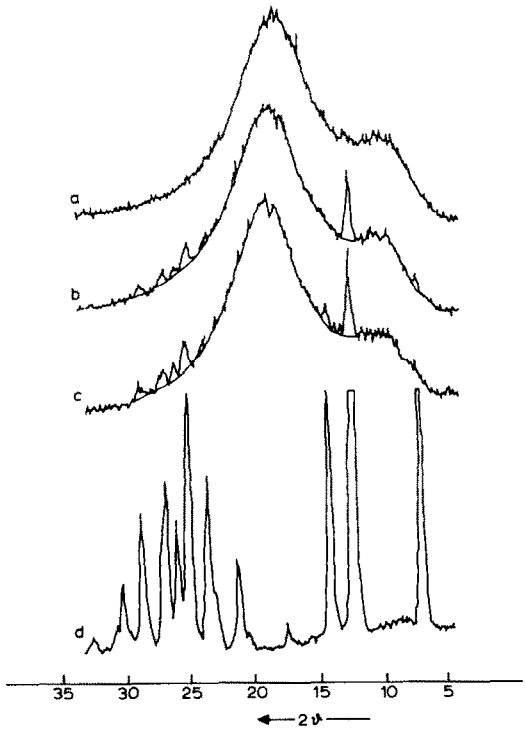


Fig. 1. X-Ray diffraction spectra of: (a) polystyrene; (b) formulation 1; (c) formulation 2; (d) theophylline recrystallized from chloroform.

Fig. 1. Comparison of those spectra indicates that theophylline loaded in the polymer has the same crystalline form as that of pure drug, substanti-

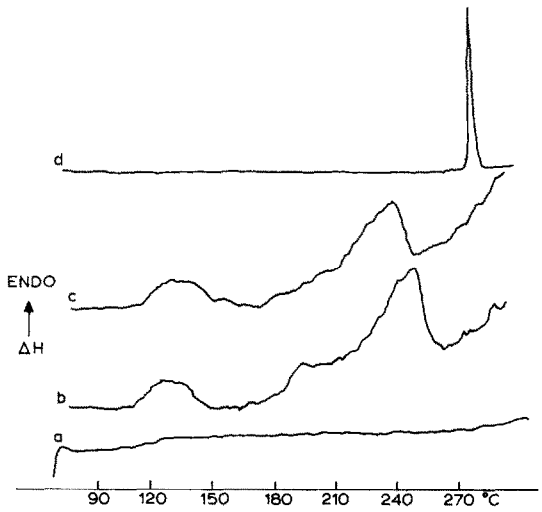


Fig. 2. DSC thermograms of : (a) polystyrene; (b) formulation 1; (c) formulation 2; (d) pure theophylline.

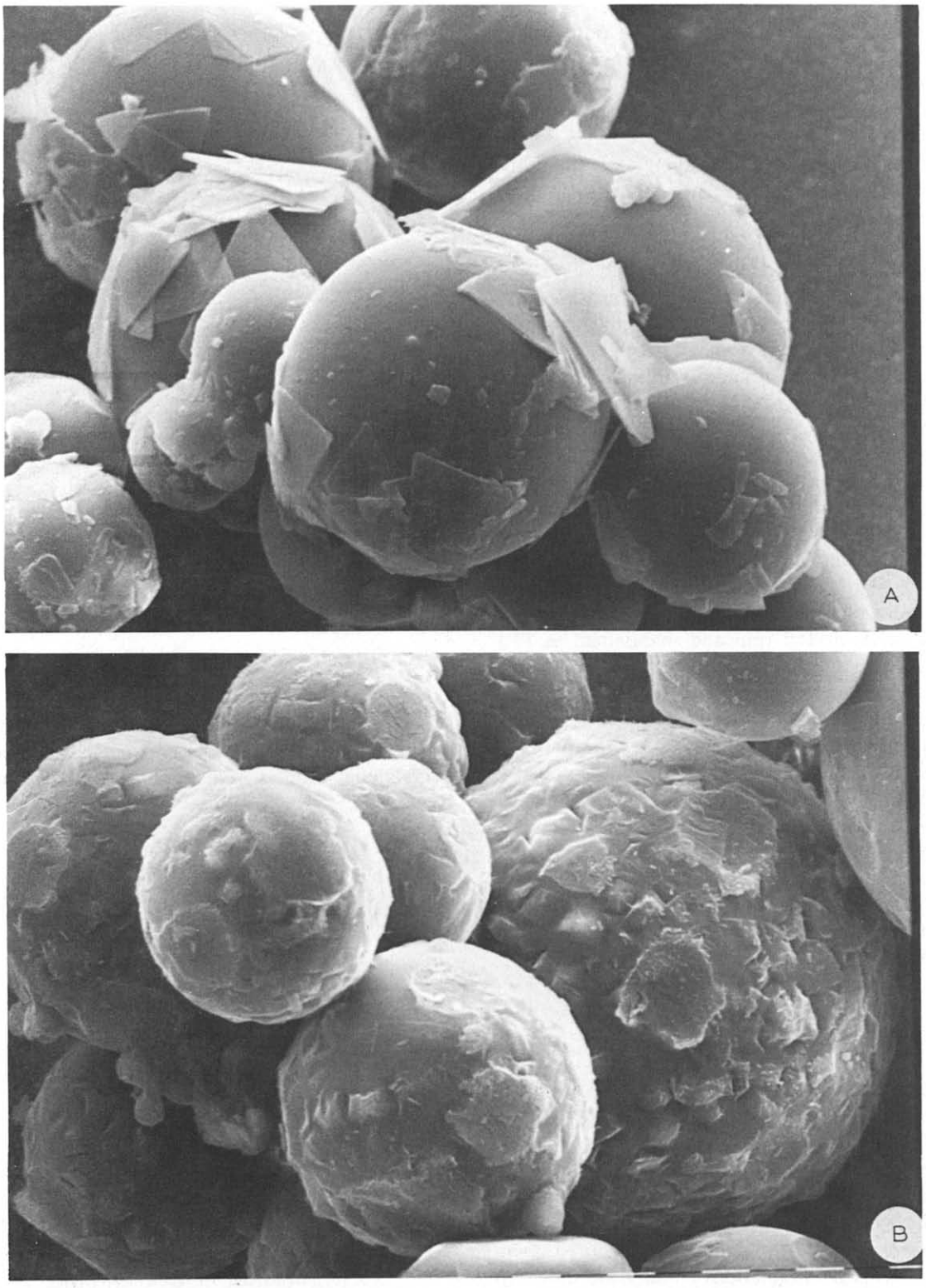


Fig. 3. Scanning electron micrographs of polystyrene microspheres loaded with theophylline (×640). A: formulation 1. B: formulation 2. C: washed formulation 2.

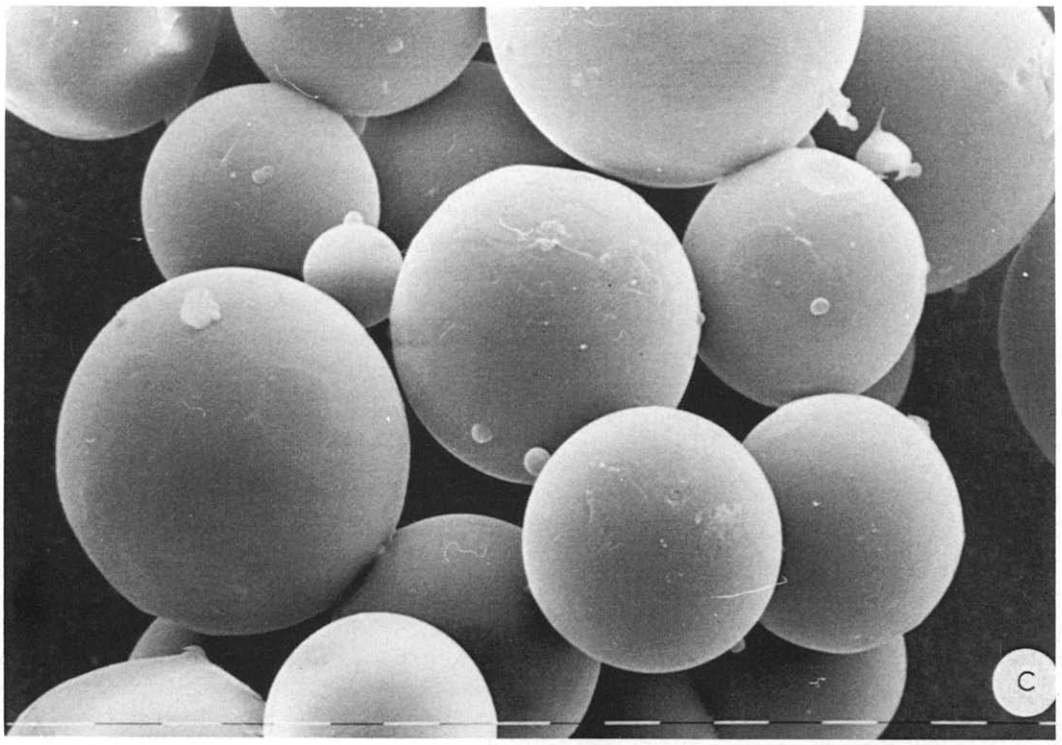


Fig. 3 continued.

ated by the presence of its 3 principal peaks. However, an accurate analysis of the theophylline–polystyrene system is not possible because of the low percentage of drug incorporated in the polymeric matrix.

Typical DSC thermograms of theophylline–polystyrene systems, powdered polymer and theophylline recrystallized from chloroform are reported in Fig. 2 and their relative temperatures and enthalpies of fusion are listed in Table 2. Theophylline, recrystallized from chloroform, melts at 271.4°C with an enthalpy of fusion of 160.1 J/g while polystyrene shows a glass–rubber transition peak at 120°C. The thermograms of samples which had been washed with water, substantiated the disappearance of the endothermic melting peak of theophylline, this was probably because of the low content of the drug in the microsphere.

The decrease of the values of the enthalpy of fusion in not-washed samples is due to a partial amorphism of theophylline while the widening of the endothermic melting peak of formulation 1 and 2 can be explained due to the highly dispersed form of the drug (Colombo et al., 1986).

*Surface analysis of theophylline–polystyrene systems*

Scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and wettability

TABLE 2

*Temperature and enthalpies of fusion of theophylline – polystyrene systems*

| Formulation no. | Melting temperature (°C) | Enthalpy of fusion (J/g) | Crystallinity (%) |
|---|---|---|---|
| 1 | 243.4 | 138.8 | 87 |
| 2 | 240.1 | 115.1 | 72 |
| 3 | 240.0 | 111.2 | 70 |
| 4 | 234.8 | 111.8 | 70 |
| 5 | 243.0 | 116.5 | 73 |
| 6 | 244.9 | 127.8 | 80 |
| 7 | 238.1 | 112.4 | 70 |

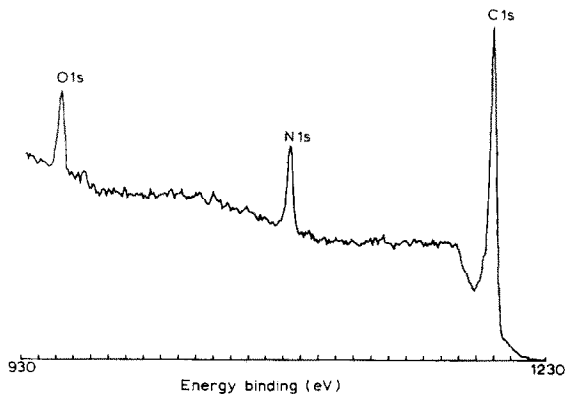


Fig. 4. X-Ray photoemission spectrum of formulation 4.

tests were used to analyze the composition of the surface of the loaded polystyrene.

Scanning electron micrographs of crosslinked polystyrene microspheres loaded with theophylline are shown in Fig. 3. In formulation 1, obtained by Procedure A, the presence of crystals of theophylline on the surface of the beads is clearly seen. Formulation 2, prepared by Procedure B, shows more surface area of the bead covered by microcrystals of theophylline while the same sample, if washed with distilled water, has no apparent surface crystals.

A typical XPS spectrum of theophylline–polystyrene system is depicted in Fig. 4.

The oxygen (1S), nitrogen (1S) and carbon (1S) peaks are clearly identified. Their intensities were used to determine the atomic composition of the surface layers (about 500 nm) of the loaded polymeric particles (Carli and Garbassi, 1985). The nitrogen peak is univocally attributed to the drug molecule; thus the comparison between the experimental atomic N/C ratio and calculated one for a homogeneous intramacromolecular drug distribution permits to identify the drug location in the polystyrene microspheres

As listed in Table 3 all formulations showed high values of experimental N/C ratios attributable to a large excess of the drug on the surface of the polymeric beads. The same formulations, washed with water, are devoid of drug molecule on the surface indicating that the small amount of loaded theophylline is distributed in the inner core of the microspheres.

Contact angle data are reported in Table 4. Theophylline shows a water contact angle (45°) much lower than that of the polymer (91°); thus it is possible to apply the following equation (Cassie and Baxter, 1944):

$$\cos \vartheta = f_1 \cdot \cos \vartheta_1 + (1 - f_1) \cos \vartheta_2 \qquad (1)$$

where $\vartheta$ is the contact angle of the drug/polymer system, $\vartheta_1$ of the pure drug and $\vartheta_2$ of the pure polymer are determined; the surface fraction ($f_1$) of the pure polymer coated by the drug can be derived. A high percentage of polystyrene surface coated by theophylline is obtained by both loading procedures while samples washed with water possess contact angles around 90° indicating again the absence of drug on the surface of the polymer.

The percentage of the polymeric surface coated by the drug decreases with increase in time of suspension (samples 2, 3 and 4) or with the increase in the temperature of evaporation of the

TABLE 3

*Surface N/C atomic ratio of theophylline–polystyrene systems*

| Formulation no. | $N/C \times 10^3$ | |
|---|---|---|
| | experimental | calculated |
| 1 | 150 | 10.4 |
| 2 | 145 | 7.3 |
| 3 | 272 | 8.3 |
| 4 | 143 | 9.8 |
| 5 | 86 | 9.8 |
| 6 | 145 | 9.8 |
| 7 | 146 | 9.8 |

TABLE 4

*Surface wettability of theophylline–polystyrene systems*

| Formulation no. | Solid/water contact angle (degrees) | Polymeric surface fraction coated by the drug |
|---|---|---|
| 1 | 78 | 0.31 |
| 2 | 73 | 0.43 |
| 3 | 77 | 0.34 |
| 4 | 78 | 0.31 |
| 5 | 69 | 0.53 |
| 6 | 72 | 0.39 |
| 7 | 78 | 0.31 |

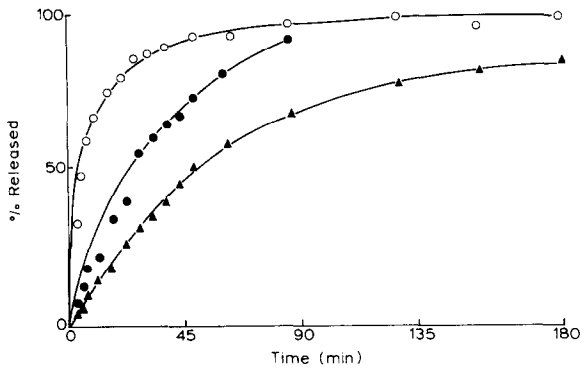


Fig. 5. Dissolution rate profile: ▲, formulation 1; ○, formulation 2; ●, physical mixture.

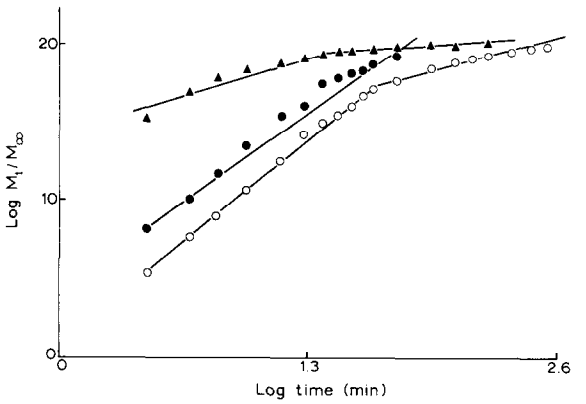


Fig. 6. Double logarithmic plots of theophylline release: ○, formulation 1; ▲, formulation 2; ●, physical mixture.

chloroform (samples 5, 6, 7) during the preparation of the loaded microspheres.

*Influence of various loading parameters on dissolution properties of theophylline loaded on polystyrene beads*

*(a) Volume of drug solution*

The dissolution rate data of formulations 1 and 2, prepared by procedures A and B consisting of different volumes of drug solution, are shown in Fig. 5.

Drug release from polymeric matrices can be described by plotting the logarithm of the amount of drug released versus the logarithm of time (Peppas, 1984). The slope of this plot is a parameter which characterizes the drug release mechanism.

As can be seen from Fig. 6, two different slopes are clearly seen for formulations 1 and 2 while the physical mixture shows a linear behavior. However, their initial release phase can be considered to be similar.

The statistical analysis can be done by plotting the percentage of undissolved theophylline ($M$) versus time according to two different models.

$$\sqrt[3]{100} - \sqrt[3]{M} = k_1 \cdot t \qquad (2)$$

is relative to the dissolution of the free drug

TABLE 5

*Least-squares parameters of two mathematical models for theophylline release from formulations 1 and 2*

| Formulation | Interval time (min) | Eqn. 3 | | | | Eqn. 2 | | | | $F$-test (3–2) | $F_{crit}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $r^2$ | $F$ [a] | $k_2$ | $\Sigma \Delta^2/df$ | $r^2$ | $F$ [a] | $k_1 \times 10^2$ | $\Sigma \Delta^2/df$ [b] | | |
| 1 | 0– 45 | 0.9931 | 644.9 | 9.68 | 4.27 | 0.9998 | 2239 | 2.18 | 1.25 | 3.36 | 3.35 |
| 2 | 0– 25 | 0.9653 | 68.3 | 15.01 | 28.4 | 0.9695 | 78.1 | 6.80 | 1.88 | 15.10 | 9.01 |
| Phys. mixture | 0– 60 | 0.9937 | 785.6 | 13.78 | 9.69 | 0.9923 | 640.4 | 3.56 | 4.69 | 2.07 | 3.68 |
| 1 | 45–360 | 0.9657 | 83.1 | 3.14 | 12.51 | | | | | | |
| 2 | 25–180 | 0.9776 | 129.8 | 1.48 | 1.04 | | | | | | |

df = Degree of freedom.

[a] Analysis of significance of the linear model.

[b] Data obtained by recasting the equation in form $M = [\sqrt[3]{100} - k_1 \cdot t]^3$

particles (Hixson and Crowell, 1931) and

$$100 - M = k_2 \cdot \sqrt{t} \qquad (3)$$

relative to a process controlled by the diffusion of drug through a polymer matrix (Higuchi, 1963).

The statistical significance of these equations was carried out using an $F$-test as suggested by Bamba et al. (1979).

It is interesting to observe that the two models used for the initial release phase of all 3 samples, are statistically undistinguishable. However, the composition of the physical mixture is constituted by separate crystals of theophylline and polymeric microspheres; its release process can be reasonably attributed to the dissolution of the crystalline particles and a cube-root equation can be conveniently assumed (Table 5).

In this regard, formulation 1 shows the slowest $k_1$ value because of largest sizes of the drug crystals present on the surface of the microspheres as seen by scanning electron microscopy (Fig. 3A). In contrast, formulation 2 presents a $k_1$ value higher than that of the physical mixture because of the increase of the surface area of the drug (Fig. 3B) in contact with the dissolution medium.

The second release phase of formulations 1 and 2 can be described by a diffusion mechanism and the $k_2$ values are quite similar (Table 5). It can be concluded that the loading techniques used with different volumes of drug solution do not lead to significant differences in the release patterns, but they allow varying amounts of drug to be entrapped in the polymeric matrix.

*(b) Time of contact between suspended polymer and drug solution*

Double logarithmic plots of these samples suggest a binary release process with an initial phase which ends at 25 min attributed to the dissolution of superficial drug particles. The second part of the release pattern can again be described by a diffusion mechanism (Table 6).

Formulation 4, obtained after 7 days, shows a decrease in percentage released theophylline at any time considered in the initial phase (Fig. 7) and this can be ascribed to a different surface area of the polymeric microspheres covered by the drug as demonstrated by different values of contact angles. The second part of the release pattern can be again described by a diffusion mechanism (Table 6).

TABLE 6

*Release data of formulation obtained with different times of contact between suspended polymer and drug solution*

| Formulation no. | Eqn. 2 | | | Eqn. 3 | | |
|---|---|---|---|---|---|---|
| | $r^2$ | $F$ | $k_1 \times 10^2$ | $r^2$ | $F$ | $k_2$ |
| 2 | 0.9695 | 78.1 | 6.80 [a] | 0.9653 | 68.3 | 15.03 [b] |
| 3 | 0.9840 | 122.7 | 5.39 [a] | 0.9184 | 32.4 | 2.35 [b] |
| 4 | 0.9533 | 46.8 | 3.40 [a] | 0.9060 | 32.1 | 2.40 [b] |
| 2 washed | – | | | 0.9554 | 115.3 | 2.71 [c] |
| 3 washed | – | | | 0.9870 | 261.8 | 2.30 [c] |
| 4 washed | – | | | 0.9620 | 162.8 | 0.79 [c] |

Interval times (min): [a] 0–25; [b] 25–360; [c] 0–360.

In order to analyze the release process of theophylline included in the microsphere, the samples were rinsed with distilled water. Their physical characterization by contact angle measurements, DSC analysis and SEM clearly demonstrate the absence of drug particles on the surface of the microsphere. XPS analysis depicts for washed formulation 4 the absence of theophylline in the 500 nm surface layers of the microsphere while the other two samples present a drug concentration higher than that of a homogeneous distribution in the same surface layer.

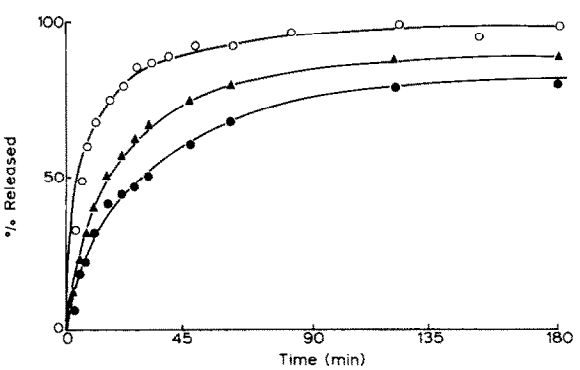


Fig. 7. Theophylline release profiles of formulations obtained with different times of contact between suspended polymer and drug solution: ⊙, formulation 2; ▲, formulation 3; ●, formulation 4.

Their release data fit a square-root equation and a diffusion mechanism can be assumed (Table 6). After 360 min the percentages of drug released for washed formulations 2, 3 and 4 are 52.4, 49.4 and 26.5, respectively; this decrease is due to the different location of theophylline in the microsphere: a maximum time of contact permits the entrapment of the theophylline in the inner core of the microsphere and consequently a slow release of the drug is noticed.

*(c) Temperature of evaporation of chloroform during the drying phase*

This parameter has been studied only on formulation 4, obtained after 7 days of contact between suspended polymers and drug solution (Procedure B). Three samples (5, 6, 7) have been prepared with different temperatures of evaporation: 4 °C, 40 °C and 80 °C, respectively, and their release profiles are shown in Fig. 8.

The physical techniques used do not allow one to notice significant differences among these 3 samples. However, formulation 5 shows the highest surface area of the microsphere covered with theophylline because of its high value of solid/water contact angles (Table 3). Also in this case a binary release mechanism can be postulated. After 30 min the percentages of released drug are 84, 69 and 63 for samples 5, 6 and 7, respectively; this confirms that sample 5 has the highest amount of drug distributed on the surface of the microsphere, which is released by a dissolution mechanism (Table 7).

TABLE 7

*Release data of formulations obtained with different temperatures of evaporation of chloroform during the drying phase*

| Formulation no. | Eqn. 3 | | |
|---|---|---|---|
| | $r^2$ | $F$ | $k_2$ |
| 5 | 0.9907 | 318.1 | 7.21 [a] |
| 6 | 0.9909 | 327.4 | 4.76 [a] |
| 7 | 0.9610 | 73.1 | 3.86 [a] |
| | Eqn. 2 | | |
| | $r^2$ | $F$ | $k_1$ |
| 5 washed | 0.9520 | 77.4 | 2.13 [b] |
| 6 washed | 0.9904 | 564.2 | 1.04 [b] |
| 7 washed | 0.9773 | 195.2 | 1.68 [b] |

Interval times (min): [a] 0–30; [b] 0–360.

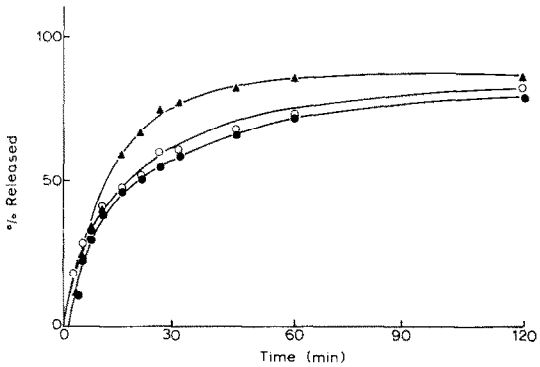


Fig. 8. Theophylline release patterns of formulations obtained with different temperature of evaporation of chloroform during the drying phase: ▲, formulation 5; ⊙, formulation 6; ●, formulation 7.

The diffusion release process of theophylline included in the microsphere is demonstrated in samples washed with distilled water. In this case the absence of drug particles on the surface of the microsphere is recognized by the physical techniques used. Their mathematical parameters are reported in Table 7. Washed sample 5 has the highest percentage of released drug after 360 min, this can be reasonably attributed to the fact that theophylline is more concentrated in the outer layer of the polymeric microspheres.

## Conclusion

In conclusion, methods used to load theophylline in the polystyrene beads produce a system with the drug dispersed on the surface of the polymeric bead and partially entrapped in the polymer network.

The release of theophylline can be controlled by adjusting the time of contact between suspended polymer and drug solution or the temperature of evaporation of the solvent during the loading phase.

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