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Report

Relation Between Individual and Ensemble Release Kinetics of Indomethacin from Microspheres

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Indomethacin microspheres based on a combination of ethylcellulose and polyethyleneglycol were prepared using the solvent evaporation process. Release profiles of ensemble and individual microspheres were measured. Both were found to follow first-order kinetics, in contrast to what was expected. This was attributed to the fact that all the particles showed the same kinetic pattern and had a greater degree of homogeneity in the payload, mean particle size and shape, and k_1 values than in ensembles of microcapsules.

KEY WORDS: microspheres; indomethacin; cumulative release profile; release, single microsphere; controlled release, first-order kinetics.

INTRODUCTION

The kinetic function governing the release profile of a drug from a single microsphere is determined mainly by a restricted number of parameters such as drug content, particle shape and size, surface area, porosity, and coating polymer properties. Furthermore, the ability of some drugs such as indomethacin to exhibit polymorphism (1) could influence, to some extent, the release kinetics from either a single microparticle or a population of microparticles. Recent reports (2–5) have shown that the overall release profile of an ensemble of microparticles is dependent on the heterogenous distribution of these parameters. Therefore, the profile of the overall release behavior is not indicative of the true release mechanism. Deduction of the underlying release mechanism can be made only by microinvestigation of release from individuals (5). In this work, for the first time, the correlation of individual and global release profiles from microspheres was investigated.

MATERIALS AND METHODS

Materials

Indomethacin was purchased from Sigma (St. Louis, Mo.). Ethylcellulose N-100 (EC) was supplied by Hercules (Wilmington, Del.), and polyethylene glycol 4000 (PEG) and polyvinyl alcohol (PVA) by BDH Laboratory (Poole, U.K.). Methylene chloride was analytical grade (Frutarom, Haifa, Israel) and was used without further purification.

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Methods

Preparation of Indomethacin Microspheres

The general procedure used was based on a solvent-evaporation process developed for poly(*d,l*-lactide) microsphere preparation (6,7). PVA (0.8%, w/v) was first dissolved in hot distilled water and allowed to cool at room temperature; 250 ml of this solution was then transferred to a 600-ml glass beaker. An organic solution containing 20 ml of methylene chloride, 0.6 g of indomethacin, and a mixture of 2 g of polymers, EC:PEG (3:1), was added through a separate funnel to the aqueous phase, stirred at a constant rate of 750 rpm by a Stedi-speed stirrer (Fisher, Pittsburgh, Pa.). The resulting emulsion was then agitated for a given period of time (90 min) during which the methylene chloride evaporated. Individual solid microspheres were formed after total removal of the methylene chloride. The microspheres were separated from the solution by decantation and rinsed three times with 200 ml of water to remove any PVA adsorbed at the microsphere interface and any small particles which might remain in the solution. Finally, after a further aqueous washing, the microspheres were collected by vacuum filtration, eventually sieved, and oven-dried at 60°C for 2 hr, yielding a free-flowing powder. This batch of microspheres was at least triplicated. No deviation in drug content beyond 5–7% was observed between the various batches of similar formulation.

Evaluation of Microspheres

Determination of the Active Ingredient Content in the Microspheres. The microspheres (100 mg) were dissolved in chloroform and assayed spectrophotometrically at 322 nm for indomethacin content, using a calibration curve based on standard solutions in chloroform. EC and PEG did not absurb in chloroform at this wavelength.

Microscopy Studies. Optical (Tiyoda, Tokyo, Japan)

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and scanning electron (Philips Model 505, Holland) microscopy was used to evaluate the drug incorporation and surface characteristics of the microspheres prepared under the various conditions used.

Particle-Size Measurements. Particle size was determined using a Tiyoda projection microscope. Samples of microspheres (at least 200) were dispersed on a slide and their diameter was then sized using suitable objectives.

Release Kinetic Experiments

Release-Rate Determination from an Ensemble of Microspheres. Overall release of indomethacin from an ensemble of microspheres (80 mg) was carried out in an experimental system resembling, as much as possible, the system used to determine the release of indomethacin from a single microsphere. Indomethacin microspheres were immersed in 500 ml of phosphate buffer solution, pH 6.5, containing 0.02% polysorbate 20 to ensure sink conditions (8). The sink solutions were agitated at 100 rpm in a thermostated shaker water bath (37°C). Indomethacin release was determined spectrophotometrically at 322 nm (Biochem LKB, Ultraspec 4052 TDS) by pumping the filtered sink solutions through flow cells using a peristaltic pump (Watson-Marlow 302) and automatically recording at given time intervals. Measurements were repeated six times.

Release-Rate Determination from Single Microspheres. The basic procedure previously described (3,5) was used. Small random samples were transferred and spread on glass slides. Single microspheres selected randomly were removed, observed microscopically, and drawn to scale before measurement of release. Some of the microspheres were cut and the internal morphology was examined by SEM.

Individual microspheres were placed in 1-cm narrow rectangular cells containing 0.5 ml of the release medium already described above and allowed to sink to the bottom which was outside the optical beam. The depth at the bottom of the cell which is outside the optical beam was measured and found to range from 0 to 10 mm, very much in excess of the diameter of the microspheres. Therefore the presence of the microsphere in the cell did not interfere with the absorbance of the indomethacin solution. This method was suitable for large microspheres (the particle size of which ranged mainly from 400 to 600 μm) containing enough indomethacin giving measurable absorbance over the full release, which was recorded continuously at given time intervals of 2 min, using the LKB spectrophotometer. Since mixing could not be done externally when the spectrophotometer was in operation, it was decided to place the cell in a continuously revolving circular platform especially designed for this spectrophotometer. This induced convection of the indomethacin solution ensured the homogeneity of the solution with regard to the drug concentration in the cell. Under these conditions, convection caused fairly rapid mixing as checked by intermittently applied external stirring.

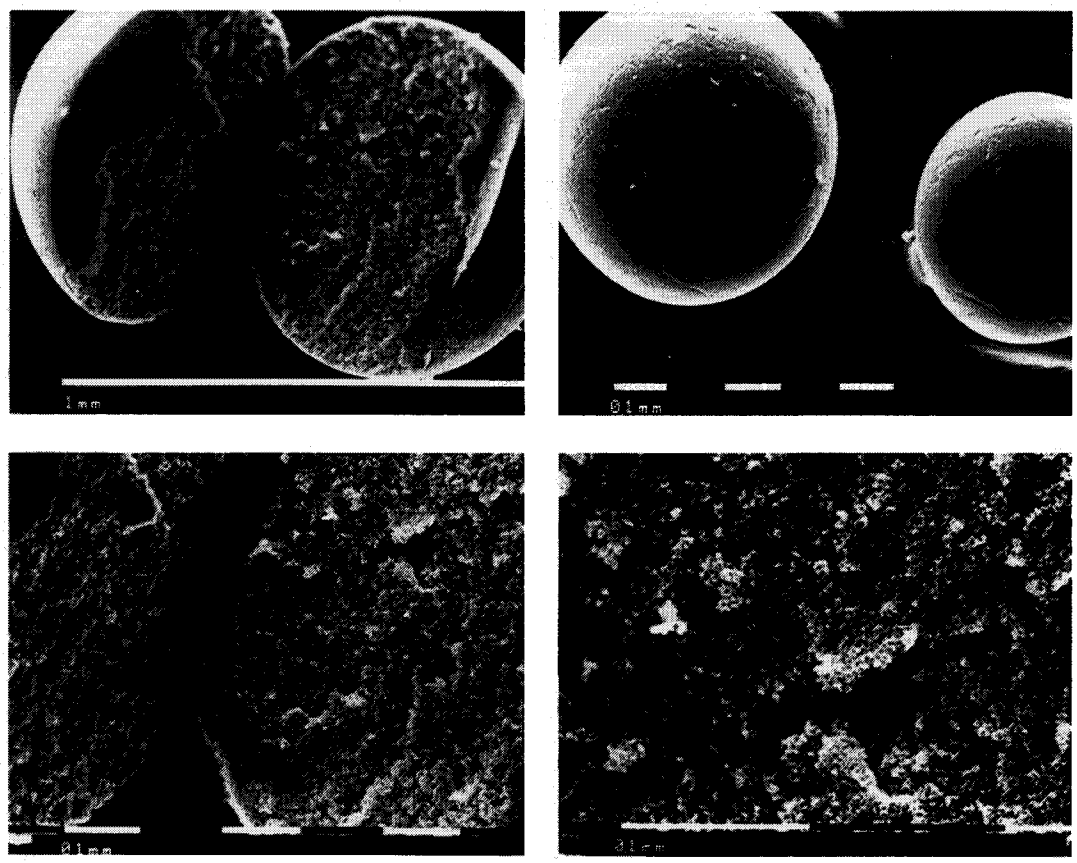

Fig. 1. SEM cross-sectional views of an individual indomethacin microsphere [22.5% (w/w) content] at different magnifications *before* drug release.

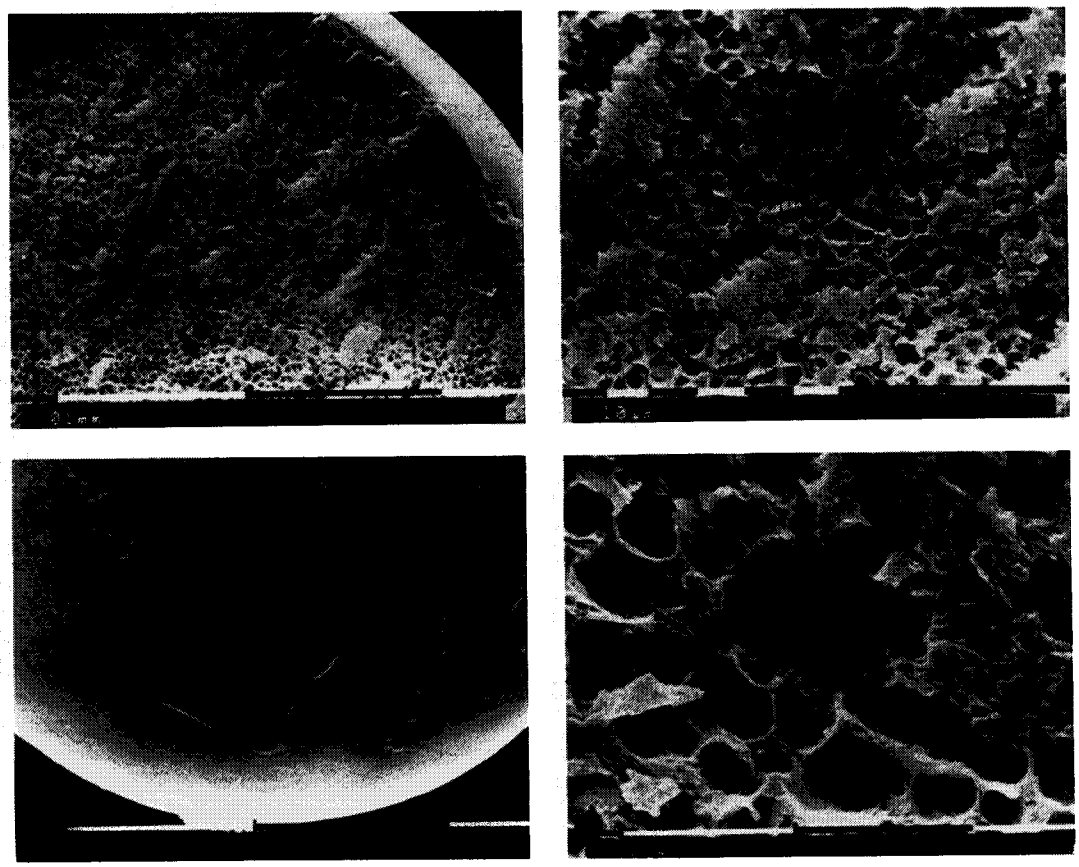

Fig. 2. SEM cross-sectional views of an individual indomethacin microsphere [22.5% (w/w) content] at different magnifications *after* drug release.

RESULTS AND DISCUSSION

The investigation of the release profile of an active material from single microspheres requires measureable absorbance over the full release process. Therefore, indomethacin which has a relatively high molar extinction coefficient, yielding an absorbance of 0.185 for a concentration of 10 μg/ml, was selected. The manufacturing conditions were fixed to produce batches of large spherical microspheres having a high payload [measured content, 22.5% (w/w)] and a particle size ranging from 200 to 600 μm, with an arithmetic average diameter of 340 ± 97 μm (SD). In preliminary work, indomethacin microspheres were prepared on the basis of EC alone. However, the indomethacin release rate was too slow and incomplete. Therefore PEG was added to the EC micromatrix to enhance the release rate and enable more complete release of the drug (9).

The internal and external morphology of the individual microspheres is shown before and after drug release in Figs. 1 and 2, respectively. The porous nature of the matrix is clearly observed and points to the release mechanism being via these pores.

The release data of the microsphere population were found to fit first-order kinetics (Fig. 3) far more closely than Higuchi or other equations by a nonlinear regression procedure which is able to distinguish between various possible kinetic models (10).

Since Hoffman and his colleagues (2,3) predicted that apart from special cases, the overall profile would differ from the underlying release kinetics, it was expected that another profile shape would emerge from the kinetic analysis of the individuals. The release data of the individual microspheres are presented in Fig. 4. As expected for multiparticulate systems the profiles exhibited are heterogeneous. Since polymorphism could affect drug release, the ability of indomethacin to exhibit polymorphism (1) which might influence the release kinetics was examined. A differ-

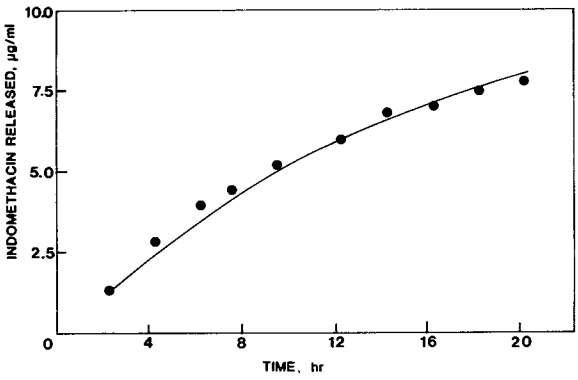

Fig. 3. Global release plots of an ensemble of indomethacin microspheres and their fit to first-order release according to the nonlinear regression procedure (10) (χ^2 = 1.5 and first-order constant = 6.5×10^{-2} hr^{-1}).

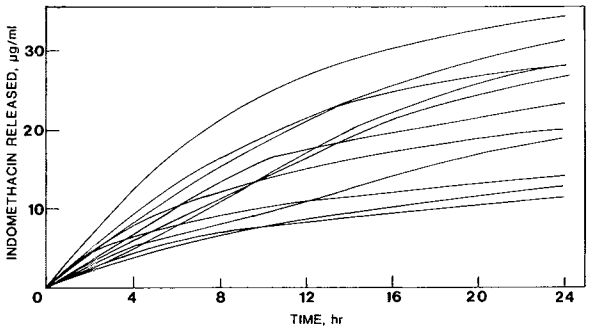

Fig. 4. Individual microsphere release curves of indomethacin expressed as µg/ml.

ential scanning calorimetry (heating cycle of 70–180°C) of pure indomethacin, empty microspheres, and indomethacin loaded microspheres was carried out using the method described in the literature (1) to characterize the nature of the drug encapsulated in the microspheres. In the case of the melting phase transition of pure indomethacin, a unique sharp endotherm was observed at 160°C, corresponding exactly to the melting point of indomethacin, whereas no thermal event was observed in either the empty microspheres or the indomethacin-loaded microspheres. This was evidence that no crystalline or polymorph indomethacin domains were present in the microspheres. The indomethacin was present in either a molecular dispersion or a solid-state solution in the EC:PEG microspheres.

The heterogeneity in the release profiles of the individual microspheres should be attributed to the difference in particle size among the microspheres. It should be emphasized that the drug content as a percentage (w/w) does not differ from one microsphere to another but the total amount of drug incorporated into a microsphere would be dependent upon the particle size as shown below.

The volume of a microsphere having a mean diameter of 340 µm will be $(\pi/6)\ (340\ 10^{-4})^3\ cm^3 = 2.0579 \cdot 10^{-5}\ cm^3$. The density of the microsphere is close to the density of the polymers, 1.15 g/cm³. This would make the mass of the microsphere $1.15\ 10^6 \times 2.0579.10^{-5} = 23.66$ µg. The measured content of indomethacin in the microsphere was 22.5% (w/w); therefore the maximum total amount of indomethacin which could be released from a single microsphere of 340 µm in 0.5 ml of buffer is $23.66 \times 0.225 = 5.27$ µg, equivalent to a final concentration of 10.5 µg/ml. By analogy, single microspheres with a diameter of 200, 400, 500, and 600 µm should release, in 0.5 ml of buffer, a maximum total amount of indomethacin of 1.08, 8.67, 16.93, and 29.26 µg, yielding final concentrations of 2.16, 17.34, 33.86, and 58.52 µg/ml, respectively. These results clearly indicate that different release profiles are obtained from single microspheres in spite of the narrow particle-size distribution as confirmed by Fig. 4.

The method of matching the degree of fit of these individual data to various possible kinetic equations is specified by Benita *et al.* (11). It was found that nearly all the individual release profiles fitted most closely a first-order equation (Table I). The starkness of the heterogeneity, illustrated by the tripled value of the amount released at 24 hr, is explained by the presence of at least two variable parameters in the population [Eq. (1)] but is less pronounced than in microcapsules (3).

$$m(t) = m_\infty(1 - e^{-kt}) \quad (1)$$

where $m(t)$ and m_∞ are the quantities released at times t and ∞, respectively, and k is the first-order constant.

Gross *et al.* (4) showed that the only case where individual and cumulative release data can be exponential is when all the particles share the same first-order release constant.

This is expected on elimination of one of the parameters, the payload; hence the plot reflects Eq. (2) behavior and k variation among the individuals:

$$m(t)/m_\infty = 1 - e^{-kt} \quad (2)$$

It should be emphasized that when the individual release data are presented, rather, as the percentage of drug released versus time, all of them show almost the same kinetic pattern (Fig. 5). This was confirmed by the closeness of the first-order constants and the χ^2 values of most of the systems in Table I.

Table I. Statistical Fit to the First-Order Equation for Individual Microspheres, the Release Profiles of Which Are Presented in Fig. 4

Microsphere sample no.	First-order constant value k_1 ($min^{-1} \times 10$)	χ^2 test of fit	df	P value
1	0.90	2.0	9	0.990
2	1.00	4.5	12	0.980
3	0.90	3.2	12	0.980
4	0.89	6.3	9	0.400[a]
5	0.76	0.7	10	0.999
6	0.77	5.0	9	0.900[a]
7	1.10	2.0	8	0.990
8	0.47	1.8	12	0.999
9	0.66	1.7	12	0.999
10	0.69	2.7	12	0.999
11	1.10	2.3	11	0.995
12	1.00	4.0	10	0.940[a]

[a] These values, although not acceptable, were nearer to first order than other kinetic equations.

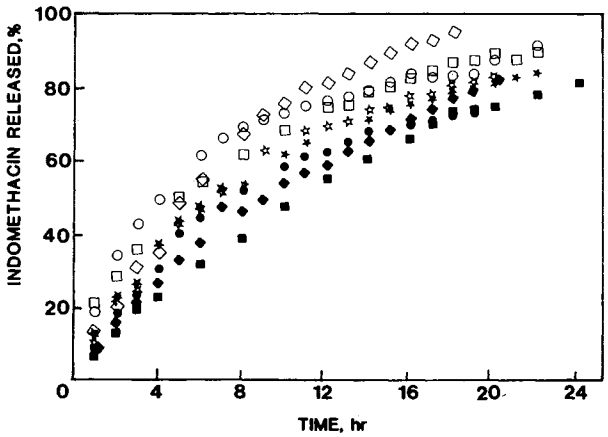

Fig. 5. Individual microsphere release curves of indomethacin expressed as percentage released.

Although an apparent discrepancy between bulk release and single-particle release could be deduced from the different results presented in Figs. 3 and 4 with regard to the indomethacin concentration released (μg/ml), this could be explained as follows: 80 mg of microspheres had a total maximum amount of incorporated indomethacin of 80 × 0.225 = 18 mg, which could be released in 500 ml of buffer solution, yielding a final concentration of 36 μg/ml. Since about 9 μg/ml was released in approximately 24 hr (Fig. 3), that means that about 25% of the total amount of indomethacin present in the microspheres was released.

As previously stated, PEG was added to the EC micromatrix to enhance the release rate and facilitate more complete release of the drug. Actually, the complete release of indomethacin from EC:PEG microspheres will take a long period of time, even weeks. These results might appear to be inconsistent with the kinetic data presented in Fig. 5. The kinetic data of single microspheres (percentage released) reported in Fig. 5 were calculated on the basis of almost the maximum amount of indomethacin released within 24–48 hr and not on the basis of the maximum amount of indomethacin present in the microspheres. This approach was considered valid since it can be observed from Fig. 4 that the indomethacin release rate decreased markedly after 24 hr and it took several days, even weeks, to release the major part of indomethacin incorporated in the microspheres. As an example, based on theoretical calculations, about 50–70% of the total incorporated drug is released within 10 days. This approach was used only to show that the individual microspheres share the same kinetic pattern.

Mathematical summation of the individual release data reported in Fig. 4 was found to fit very closely the first-order equation according to the nonlinear regression procedure accompanied by the χ^2 test ($P > 0.999$, indicating a very high level of statistical significance). A curve quite similar to that described in Fig. 3 was obtained. Furthermore, the first-order constant values of the theoretical curve was 8.2 $\times 10^{-2}$ hr^{-1}, close to the value characterizing the observed global release curve, which was 6.5 $\times 10^{-2}$ hr^{-1}.

These results demonstrated that the individual microspheres randomly sampled truly represented the entire microsphere population. The visual structure observations (Figs. 1 and 2) indicated that indomethacin should be dissolved by penetrating release medium that subsequently diffused out of the microspheres into the sink solution through the pores and channels left by the dissolution of the hydrophilic polymer (PEG) (9). Evidently, since the individual kinetic process is governed by a first-order kinetic equation, the mechanism is neither dissolution controlled nor observant of the conditions governing matrix-type kinetics which might have been expected to operate in such microspheres. The cause may lie in the higher porosity introduced by leached PEG, leading to a first order-dependent concentration gradient. It is proposed that in the case of EC/PEG indomethacin-loaded microspheres, identical release profiles were observed for both cumulative and individual kinetics as a result of homogeneity of the determining parameters in the population (4). Since hitherto there has been no other work to which we can refer for comparison, the question arises whether it is only a special and rare case or a more general case.

This work presents a practical technique for the microinvestigation of individual release from microspheres which still remains to be studied in depth. It also shows that individual microspheres prepared by the solvent evaporation process have a relatively high degree of homogeneity, not only in size, shape, and payload, but also in release-rate constants, and this is the probable reason for the unexpected first-order observance by both the individuals and the ensembles.

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