In Vitro Release Profile of Mitomycin C from Albumin Microspheres: Extrapolation from Macrospheres to Microspheres

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To analyze the *in vitro* release profiles of mitomycin C from albumin microspheres prepared by chemical denaturation in a multiparticulate system, a method to calculate the total cumulative amount of mitomycin C released from a batch of microspheres was developed. Mitomycin C-loaded albumin macrospheres (diameter in mm range) were prepared, and the *in vitro* release kinetics of mitomycin C from individual macrospheres were determined. Then the relationship between the kinetic parameters and the physical parameters (e.g., diameter, weight) was investigated under the assumption that macrospheres and microspheres behave identically. Further, the size distribution of microspheres was measured, and the total cumulative amount of mitomycin C released from albumin microspheres was calculated. The release profiles of mitomycin C from individual macrospheres fitted first-order release kinetics better than spherical matrix kinetics. The calculated initial mitomycin C contents and first-order release rate constants for individual macrospheres were correlated with the weight and reciprocal of surface area of the macrospheres, respectively. The observed *in vitro* release profile for the microspheres agreed with the calculated values. These results suggest that this method is valid for calculating drug release from albumin microspheres.

KEY WORDS: microspheres; macrospheres; mitomycin C; *in vitro* release; macrosphere-to-microsphere extrapolation.

INTRODUCTION

Albumin microspheres have been used as a drug delivery system to target the containing drugs to tumor tissues (1,2) and Kupffer and mast cells (3,4), and to sustain and control the drug release rate (5,6). We have reported that albumin microspheres afforded sustained release of 5-fluorouracil after intraperitoneal injection into Ehrlich ascites carcinoma-bearing mice (7) and for targeting of mitomycin C after intraarterial injection into AH272 liver metastasis-bearing rats (8,9).

The release of doxorubicin from albumin microspheres exhibited first-order (10) or biphasic zero-order release kinetics (11,12). With the lipophilic drugs, nitrofurantoin and prednisolone, Jun *et al.* (13) and Burgess *et al.* (14) reported that the drug release could be characterized by a spherical matrix diffusion model, as proposed by Baker and Lonsdale (15).

In contrast, the release of water-soluble drugs from albumin microspheres was usually characterized by an initial rapid release (burst release) to above 90%, followed by slower release of the remaining drug (16,17). Leucuta (18) also reported that the release of pilocarpine from albumin microspheres was biphasic. Hence, it might be difficult to analyze such drug releases with simple diffusion models or compartment models. Although Leucuta (18) analyzed the initial and terminal release phases of pilocarpine using a diffusion model for a spherical matrix, this method is rather complicated. If the microspheres used in these studies (16–18) were distributed over a size range (diameter), the burst release may have occurred mainly from smaller microspheres. While the analysis of the *in vitro* drug release profiles from individual microspheres of different diameters should clarify this mechanism, it is difficult to measure the small amount of drug released from a single microsphere.

If the drug release from microspheres could be estimated by that from macrospheres, and if the total drug release profiles from the individual albumin microsphere or macrosphere could be characterized by dimensional properties of microsphere or macrosphere, i.e., diameter, surface area, or volume, the drug release profiles from macrospheres could help to analyze not only the overall drug release profiles from microspheres but also the cause of the burst release.

Therefore, mitomycin C-loaded albumin macrospheres with different particle sizes (diameter in millimeter range) were prepared by a chemical denaturation method, and the *in vitro* drug release tests were performed with individual macrospheres. The drug release patterns from the macrospheres were analyzed with the aid of several kinetic models. The release kinetic parameters and dimensional parameters of the macrospheres were extrapolated to microspheres by considering each size distribution parameter. Finally, the calculated and experimental release profiles of mitomycin C from albumin microspheres were compared, and the *in vitro* release profiles from the microspheres were characterized from these results.

THEORETICAL

The cumulative amount of drug release, q_i, from the ith sphere, where the spheres are numbered from the smallest to the largest, may be expressed as a function, f, as follows:

$$q_i = f(\alpha, \beta, \gamma, \ldots) \quad (1)$$

where α, β, and γ are independent parameters. If the function, f, can be replaced by linear kinetics, Higuchi's diffusion equation (19) and/or simple polynominal expressions, independent parameters such as α, β, and γ become rate constants, diffusion coefficients, and so on. Total cumulative amount of drug release, Q, from n spheres is the sum of q_i as follows:

$$Q = \sum_{i=1}^{n} q_i \quad (2)$$

In the case of the release of mitomycin C from individual albumin microspheres, q_i could not be obtained because

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of low assay sensitivity to mitomycin C. Therefore, an equation showing the release kinetics from individual albumin macrospheres was investigated under the assumption that individual microspheres showed the same release kinetics as individual macrospheres. Also, kinetic parameters obtained for macrospheres would relate dimensional and physical parameters such as their diameter, surface area, weight, and density. Under these assumptions, the release characteristics for macrospheres can be extrapolated to the microspheres, and the total cumulative amount of drug released, Q, for microspheres can be calculated using a distribution function of their dimensional and physical parameters.

MATERIALS AND METHODS

Materials

Bovine serum albumin (fraction V) was obtained from Seikagaku Kogyo Co., Ltd. (Tokyo). Mitomycin C was kindly supplied by Kyowa Hakko Co., Ltd. (Tokyo). *n*-Octane and 25% glutaraldehyde were purchased from Wako Pure Chemicals (Osaka, Japan). In this experiment, 5% glutaraldehyde was prepared by diluting 25% glutaraldehyde with 1/15 *M* phosphate buffer (pH 7.4). All other reagents used were commercial reagent-grade.

Preparation of Mitomycin C-Loaded Albumin Macrospheres and Microspheres

Mitomycin C-loaded albumin macrospheres (Types A and B) and microspheres (Types C and D) were prepared by a chemical denaturation method. Bovine serum albumin (270 mg) was dissolved in 1.0 ml of 1/15 *M* phosphate buffer (pH 7.4). Mitomycin C (30 mg) was homogeneously mixed and dispersed in this resulting solution at 0°C. Then 5% glutaraldehyde (0.6 ml) was added to the mitomycin C-suspended in bovine serum albumin solution. About 30 sec later, the mitomycin C-suspended bovine serum albumin solution was dropped into 100 ml of *n*-octane with and without Span 80, emulsified with a glass stirrer, and then allowed to solidify at 0°C for 1 hr. After solvent removal, the spheres were washed three or four times with diethyl ether and stored in a desiccator until use. Detailed conditions for the concentration of Span 80 and stirring speed are shown in Table I.

The spherical shape of the microspheres and macrospheres was confirmed with a scanning electron microscope (X-650, Hitachi Co., Ltd., Tokyo) and stereoscopic microscope (model BH, Olympus, Tokyo), respectively (Fig. 1).

Table I. Conditions for the Concentration of Span 80 and Stirring Speed at Preparation of Types A, B, C, and D Spheres

Type	Concentration of Span 80 (v/v, %)	Stirring speed (rpm)
A	0.2	150
B	—	150
C	0.2	400
D	1.0	700

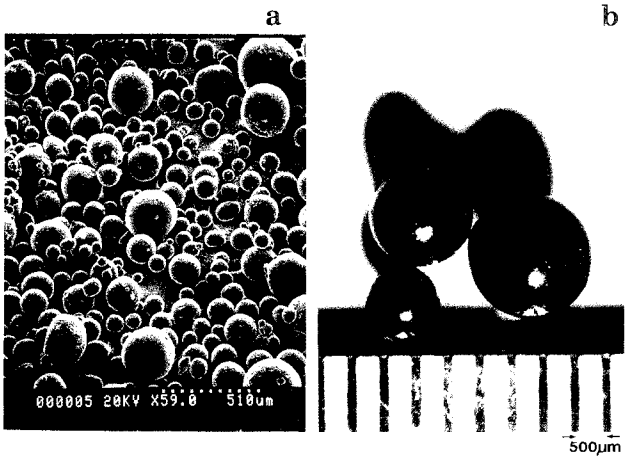

Fig. 1. (a) Scanning electron micrograph of microspheres and (b) stereoscopic photograph of macrospheres.

Measurement and Calculation of Dimensional and Physical Parameters of Spheres and Determination of Distribution Function of Microspheres

The weight and diameter of individual macrospheres were measured with a semimicro-chemical balance and micrometer (M110-25, Mitsutoyo MFG. Co., Ltd., Tokyo). The other dimensional and physical parameters (surface area, volume, density) of the macrospheres were calculated from the weight and diameter. The diameter of individual microspheres was measured by a microscopic method. Microphotographs of microspheres were taken with a scanning electron microscope and their apparent diameters were measured on the photographs. The distribution of weight, W_i, and surface area, S_i, of the microspheres was determined in triplicate by measuring 100 spheres for each determination.

The distribution functions were determined using a general polynominal equation as follows:

$$W_i = a_w \cdot i^3 + b_w \cdot i^2 + c_w \cdot i + d_w \quad (3)$$

$$\log S_i = a_s \cdot i^3 + b_s \cdot i^2 + c_s \cdot i + d_s \quad (3')$$

where a_w, b_w, c_w, d_w, a_s, b_s, c_s, and d_s are coefficients depending on the microspheres used.

In Vitro Release Experiment

After measuring the weight and diameter of an individual macrosphere, it was added to 3.5 ml of pH 7.4 phosphate buffer at 37°C and magnetically stirred at 150 rpm. At predetermined intervals, 3.0 ml of supernatant was withdrawn to measure the drug release and replaced with fresh buffer.

In the case of microspheres, a 30-mg sample was added to 30 ml of the buffer. At predetermined intervals, 10 ml of supernatant was withdrawn for assay. The same volume of fresh buffer was added to continue the experiment.

The determination of mitomycin C was done by HPLC (LC-6A, Shimadzu, Kyoto, Japan) as follows: 1.0 ml of sample mixed with 1.0 ml of methanol was centrifuged at 16,000 rpm (M-150, Sakuma Seisakusyo, Co., Ltd., Tokyo) and 20 μl of the supernatant was injected on HPLC. HPLC conditions were as follows: column, 4.6 × 250-mm stainless-steel

column packed with Nucleosil $5C_{18}$ (Macherey Nagel, West Germany); mobile phase, 0.01 *M* phosphate buffer (pH 6.0):methanol (6:4); flow rate, 0.8 ml/min; and detection, UV, 360 nm (SPD-6A, Shimadzu).

RESULTS

Release Behavior of Mitomycin C from Macrospheres

Figure 2 shows three examples for the time course of the cumulative amount of mitomycin C released from single macrospheres (Nos. 10, 16, and 20). These release profiles were analyzed using first-order release kinetics or spherical kinetics. Correlation coefficients (r) by the first-order kinetics for single macrospheres 10, 16, and 20 were 0.999, 0.996, and 0.998, respectively, and those by the spherical matrix kinetics were 0.997, 0.991, and 0.996. The *in vitro* release profiles for all single macrospheres tested in the present study fitted first-order kinetics ($0.930 \leqq r \leqq 0.9998$) better than spherical matrix kinetics ($0.910 \leqq r \leqq 0.998$). Furthermore, first-order release kinetics can be readily extrapolated from macrospheres to microspheres. Therefore, first-order release kinetics were used for the following analysis. Hence, the cumulative amount of mitomycin C released from the ith macrosphere could be expressed as follows:

$$q_i = M_{oi}[1 - \exp(-k_i \cdot t)] \tag{4}$$

where M_{oi} and k_i are the calculated initial mitomycin C content and first-order release rate constant of the ith macrosphere. The total cumulative amount of mitomycin C released could be expressed from Eqs. (2) and (4) as follows:

$$\sum_{i=1}^{n} q_i = \sum_{i=1}^{n} M_{oi}[1 - \exp(-k_i \cdot t)] \tag{5}$$

Tables II and III show dimensional and physical parameters (diameter, surface area, volume, weight, and density) and independent kinetic parameters (M_{oi} and k_i), respectively.

In the next step, relationships between the dimensional parameters and the kinetic parameters of mitomycin C release were investigated. Figure 3 shows the correlation between M_{oi} and W_i (V_i, volume of ith macrosphere) and between k_i and $1/S_i$ for Types A and B (no Span 80) albumin macrospheres ($r \geqq 0.998$). These correlations were better than those between k_i and diameter ($r \leqq 0.987$) and k_i and volume ($r \leqq 0.995$) (data now shown). Types A and B macrospheres showed different M_{oi} and k_i values against the W_i and S_i as follows.

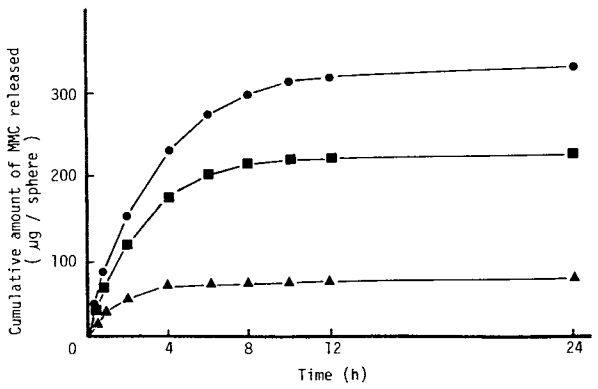

Fig. 2. *In vitro* typical mitomycin C release from individual albumin macrospheres: (●) No. 10; (▲) No. 16; (■) No. 20. (See Tables II and III.)

Table II. Dimensional and Physical Parameters of Individual Mitomycin C-Loaded Albumin Macrospheres

No.	Weight (mg)	Diameter (mm)	Surface area (mm^2)	Volume (mm^3)	Density (mg/mm^3)
Type A					
1	0.57	0.916	2.635	0.402	1.42
2	0.89	1.042	3.409	0.592	1.50
3	0.96	1.083	3.683	0.665	1.44
4	1.24	1.186	4.417	0.873	1.42
5	1.50	1.226	4.720	0.964	1.56
6	1.58	1.303	5.331	1.158	1.36
7	2.81	1.623	8.266	2.235	1.26
8	3.54	1.713	9.214	2.631	1.35
9	5.80	1.905	11.40	3.622	1.60
10	6.33	2.216	14.19	5.030	1.26
Type B					
11	0.20	0.678	1.440	0.163	1.25
12	0.38	0.802	2.021	0.270	1.41
13	0.78	0.998	3.120	0.520	1.50
14	1.03	1.104	3.830	0.705	1.46
15	1.16	1.177	4.352	0.850	1.36
16	1.80	1.341	5.650	1.260	1.43
17	3.26	1.656	8.620	2.378	1.37
18	3.56	1.727	9.370	2.699	1.32
19	3.60	1.717	9.260	2.650	1.36
20	5.84	2.004	12.62	4.214	1.39
21	6.86	2.053	13.24	4.530	1.51
22	8.92	2.357	17.45	6.852	1.30

In the presence of Span 80 (Type A),

$$M_{oi} = 5.06 \times 10^{-2} W_i \tag{6}$$

$$k_i = 3.15/S_i \tag{7}$$

In the absence of the surfactant (Type B),

$$M_{oi} = 3.86 \times 10^{-2} W_i \tag{6'}$$

$$k_i = 4.3/S_i \tag{7'}$$

Therefore, the cumulative amount of mitomycin C released from individual microspheres could be extrapolated by these equations for macrospheres, and also to microspheres if the mechanism of the drug release from microspheres is the same.

The mean diameter of the microspheres can be modified by several conditions such as stirring speed, nature or concentration of surfactants, and the kind of outer phase during the preparation of the microspheres. In the present experiments, the diameter of microspheres prepared with span 80 was 30–200 μm, and that without the surfactant 200–700 μm. The optimum diameter of albumin microspheres for targeting of antitumor agents was ≤200 μm (20,21). Hence, the total cumulative amount of mitomycin C released from Types C and D microspheres was subsequently analyzed using only

Table III. Independence Kinetic Parameters (M_{0i} and k_i) in Individual Mitomycin C-Loaded Albumin Macrospheres

No.	M_{0i} (μg/sphere)	k_i (hr^{-1})
Type A		
1	30.8	1.21
2	42.8	0.92
3	47.0	0.89
4	60.7	0.73
5	82.0	0.63
6	90.7	0.57
7	157.0	0.38
8	170.7	0.35
9	296.1	0.27
10	316.0	0.26
Type B		
11	10.6	3.05
12	14.2	2.10
13	32.2	1.29
14	36.5	1.16
15	44.9	0.92
16	69.4	0.75
17	124.1	0.55
18	131.4	0.54
19	148.9	0.43
20	228.1	0.39
21	269.4	0.29
22	340.4	0.26

the relations obtained from the release data of the macrospheres prepared with the surfactant (Type A) as below.

The following Eq. (8) could be elucidated from Eq. (5) by Eqs. (6) and (7):

$$\sum_{i=1}^{n} q_i = \sum_{i=1}^{n} 5.06 \times 10^{-2} W_i [1 - \exp(-3.15/S_i \cdot t)] \tag{8}$$

The distribution functions, W_i and S_i, were determined by using 100 microspheres. Figure 4 shows the distribution of W_i and log S_i for Types C and D microspheres. Batch-

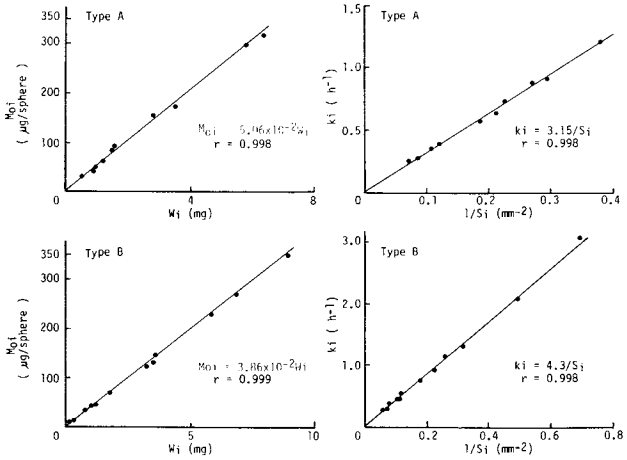

Fig. 3. Relation between M_{oi} and W_i or k_i and S_i for albumin macrospheres of Types A and B.

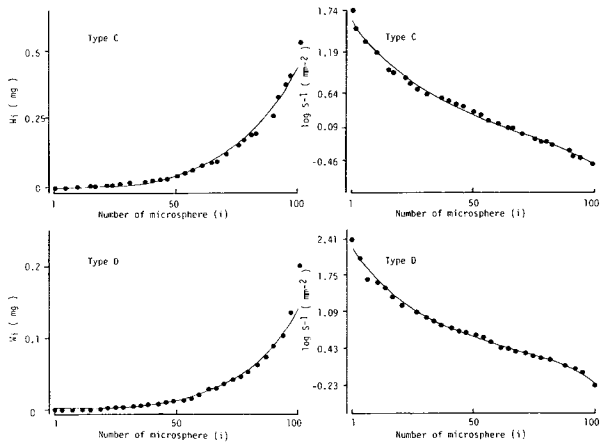

Fig. 4. Weight or surface area distributions of albumin microspheres of Types C and D.

to-batch variation was markedly low (data not shown). The distribution functions were obtained by curve-fitting to Eqs. (3) and (3′). Distribution functions of W_i and S_i are as follows.

In the case of Type C,

$$W_i = 7.47 \cdot 10^{-7} i^3 - 3.85 \cdot 10^{-5} i^2 + 9.19 \cdot 10^{-4} i - 1.01 \cdot 10^{-2} \tag{9}$$

$$\log S_i = -2.28 \cdot 10^{-6} i^3 + 4.60 \cdot 10^{-4} i^2 - 4.28 \cdot 10^{-2} i + 1.546 \tag{10}$$

In the case of type D,

$$W_i = 2.34 \cdot 10^{-7} i^3 - 1.02 \cdot 10^{-5} i^2 + 1.76 \cdot 10^{-4} i - 3.38 \cdot 10^{-5} \tag{9′}$$

$$\log S_i = -3.87 \cdot 10^{-6} i^3 + 7.51 \cdot 10^{-4} i^2 - 6.12 \cdot 10^{-2} i + 2.322 \tag{10′}$$

Finally, the total cumulative amount of mitomycin C released was obtained by substituting W_i and S_i values in Eqs. (9) and (10) or Eqs. (9′) and (10′) to Eq. (8).

The observed values of the cumulative amount of mitomycin C released from Types C and D microspheres were compared with the calculated values obtained as above. Figure 5 shows the comparison of the calculated and observed time courses of total cumulative amount of mitomycin C released from Types C and D microspheres. A good agreement was found between them, which suggested that this calculation method for the drug release from albumin microspheres is valid and useful.

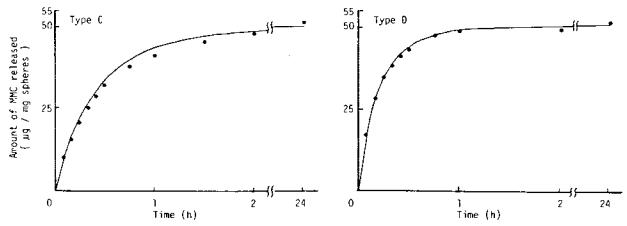

Fig. 5. Comparison between calculated and observed values for *in vitro* mitomycin C release from albumin microspheres of Types C and D. (●) Observed value: (——) calculated value.

DISCUSSION

The statistical properties of drug release from various microparticles should be clarified by using theoretical models and various physicochemical experiments for investigation of the *in vivo* drug disposition from these particles. Gross *et al.* (22) used a statistical model of the total cumulative amount of drug release from individual microparticles to assess the relation between individual and ensemble release kinetics from Eudragit RS microcapsules with a diameter range between 180 and 250 μm (23). Since the *in vitro* mitomycin C release from the albumin microspheres was characterized only as a burst release and sustained release thereafter, the profiles could not be shown as simple mathematical models and must reflect the size distribution of the microspheres. To address this question, the *in vitro* mitomycin C release from individual albumin macrospheres was investigated. The *in vitro* release of mitomycin C from individual macrospheres proceeded by first-order kinetics (Fig. 2). The mitomycin C release from individual microspheres could be extrapolated from the data obtained with the macrospheres, since the mitomycin C content per unit weight of sphere and first-order release rate constant per unit surface area of sphere were constant (Fig. 3). These results suggest that the mitomycin C release from albumin microspheres with various diameters can be evaluated by a set of first-order release kinetics. The better fit of the release profile of mitomycin C from albumin microspheres to first-order kinetics than the spherical matrix model suggests that the drug diffusion rate in the albumin microspheres is very low compared to the partition rate from the spheres to the sink solution and that the drugs in the core and peripheral regions of the spheres release at the same rate. The results also suggest that the initial rapid release was mainly from the smaller microspheres and was not due to the release of the drugs on the surface of albumin microspheres.

The different relationship between W_i and M_{oi} or k_i and $1/S_i$ obtained from Type A (with Span 80) and Type B (without Span 80) macrospheres (Fig. 3) is difficult to understand. Physicochemical interactions between albumin and mitomycin C, water–oil interfacial phenomena at emulsification, or the solubility of mitomycin C in albumin solution at preparation of Types A and B albumin macrospheres might be different with and without Span 80.

The approach in the present paper may also be applied to estimate other drug release rates from microspheres under the assumption that the relations such as W_i and M_{oi} or k_i and $1/S_i$ were the same in macrospheres and microspheres. If the drug release kinetics from spheres obeyed diffusion models as shown by Higuchi (19) or Baker and Lonsdale (15), the mechanism of the drug release might be explained theoretically in detail. Physicochemical approaches to determine the mechanism of drug release described by Ishizaka *et al.* (24) and Yaacobi *et al.* (25) might be improved using this method.

Finally, the total cumulative amount of mitomycin C released from albumin microspheres could be expressed using a distribution function as shown in Eq. (3). As the particle distribution of microspheres prepared in this study was broad, Eq. (3) might be suitable in this case. The size distribution of the albumin microspheres did not fit the normal, log-normal, or Rosin-Rammler (26) distribution functions (data not shown). Moreover, the observed *in vitro* mitomycin C release profiles were similar to the calculation values in Types C and D. This result indicates that the concentration of Span 80, 0.2 and 1.0%, as shown in Table I, during preparation of Types C and D microspheres had little, if any, influence on the relations between W_i and M_{oi} or k_i and $1/S_i$. The same equation [Eq. (8)] may be applied to calculate the total cumulative amount of mitomycin C released from microspheres in this concentration range of Span 80.

In conclusion, this calculation method for the drug release from albumin microspheres serves to predict drug release profiles and to clarify the release mechanism, especially when using a batch of albumin microspheres of variable size.

NOMENCLATURE

i	Subscript for sphere number ($i = 1 \ldots n$) from the smallest to the largest
q_i	Cumulative amount of drug released from the ith sphere (μg/sphere)
Q	Total cumulative amount of drug released from n spheres (μg/mg spheres)
W_i	Weight of the ith sphere (mg)
S_i	Surface area of the ith sphere (mm^2)
M_{oi}	Initial content of drug in the ith sphere (μg/sphere)
k_i	First-order release rate constant of the ith sphere (hr^{-1})

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