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Magnetic Microcapsules for *in Vitro* Testing as Carrier for Intravascular Administration of Anticancer Drugs: Preparation and Physicochemical Properties[1)]

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The preparation of anticancer agent-loaded microcapsules magnetically responsive to applied magnetic fields is described, together with some biophysical and biochemical data. The ethylcellulose-walled microcapsules containing magnetite (Fe_3O_4, as a ferrofluid) and anticancer agents had a diameter of 0.2—0.6 μm (mean diameter, 0.308 μm). The retention of these microcapsules by magnetic fields was measured in an *in vitro* model of the human circulation system. The apparatus provided steady flow at various rates through various types of horizontally mounted glass capillary tubes. The retention of magnetically responsive microcapsules was also evaluated in terms of the Reynolds number.

The microcapsules prepared in this experiment were caught and held with high efficiency at specific sites within a large artery model by using external permanent or electric magnets, and further, the captured microcapsules did not aggregate irreversibly when the electric field was removed.

Keywords——microcapsule; ethylcellulose; ferrofluid; human circulation system; Reynolds number; drug carrier; multiple emulsion method; FT-207; targeting

Though efforts at cell-specific targeting of cancer chemotherapeutic agents are directed towards the exploitation of biochemical differences between normal and diseased cells, target cell specificity has not been achieved in most cases, and numerous side effects are often apparent with generalized systemic drug administration. Systemic drug therapy is an undesirable way to attack local disease. Localization of chemotherapeutic agents to specific sites would reduce the required dose of a given agent while still achieving effective local concentrations of drug.[2)]

Yatvin *et al.*[3)] suggested a way of combining liposomes as drug carriers with local hyperthermia to achieve preferential local release of drug in a target area. Meanwhile, Kato *et al.*[4)] reported that ferromagnetic microcapsules containing mitomycin C prepared by a coacervation technique could be conducted by an external magnetic field. However, their microcapsules were unsatisfactory as a carrier for use in the human circulation system in that they are too large in size (mean diameter: 307.9 ± 34.5 μm).

In this study, ethylcellulose-walled microcapsules containing magnetite (Fe_3O_4, as a ferrofluid) and anticancer agents were prepared by a multiple emulsion method presented in our previous paper.[5,6)] The retention of these microcapsules by a magnetic field was measured by an *in vitro* apparatus modeled on the human circulation system. The apparatus provided steady flow at various rates throuth these types of horizontally mounted glass capillary tubes (0.58, 0.94 and 1.33 mm). Furthermore, retention of magnetically responsive microcapsules was also evaluated in terms of the Reynolds number, and some biophysical and biochemical properties were examined.

Experimental

Preparation of Microcapsules——Ethylcellulose 0.5 g (as a wall material) and egg lecithin 0.5 g (as an emulsifier) were dissolved in benzene (10 ml). Then 1-(2-tetrahydrofuranyl)-5-fluorouracil (FT-207) (30 mg), magnetite (1.0 ml) and 30 (v/v)% formalin (0.5 ml) were dissolved in 1 (w/v)% gelatin solution (5 ml). This drug solution (5 ml) containing magnetite was emulsified uniformly in polymer solution (10 ml) in a water-immiscible volatile solvent (benzene) to form a water-in-oil (w/o)-type emulsion by the use of a magnetic stirrer. A 1 (w/v)% pluronic F-68 aqueous solution (150 ml) containing Na_2CO_3 (1.5 g) was used as an encapsulating dispersion solution, and the above dispersion (the w/o-type emulsion) was added to this aqueous solution under agitation in a Chemistirrer. At this point a complex (w/o)/w-type emulsion was formed (the w/o-type emulsion was further dispersed in aqueous solution). The temperature was kept at 30 °C and agitation was continued for 2 h. The polymer solvent benzene was gradually lost by dissolution into the aqueous phase and subsequently evaporated from the surface of the aqueous mother liquid, leaving a rigid polymer film around the drug solution. The large microcapsules separated from the suspension by centrifugation at 500 rpm were removed and the supernatant was centrifuged at 3000 rpm for 30 min. The small microcapsules thus obtained were spherical grains having a mean diameter of 0.3 μm. The procedure of microcapsule preparation is schematically shown in Fig. 1.

Measurement of Physicochemical Properties of the Microcapsules——Microcapsules coated with gold vapor under a high vacuum were observed with a scanning electron microscope (model JSM T-200, JEOL Ltd.) to examine their shape and surface characteristics.

The size distribution and the mean diameter of the microcapsules were determined with a Coulter model N_4 (Coulter Electronics Ltd., Engl.)

The magnetization curve of the microcapsules was examined with a magnetic balance (model MB-11, Shimadzu Seisakusho) as shown in Fig. 2.

The electrophoretic mobility of the microcapsules in physiological saline was measured at 25 °C with a Laser Zee system 3000 (Pen Ken Inc., N.Y., U.S.A.). By means of the Smoluchowski equation (Eq. (1)), the mobility was converted into zeta potential, ξ,

$$\xi = 4\pi\eta u/D \tag{1}$$

where u is the mobility of the microcapsules, and η and D are the viscosity and dielectric constant of the dispersion medium, respectively.

The flow curve of the microcapsules in physiological saline was measured with a Rheomat 30 (Contraves Industrial Products Ltd., Switzerland). First, the relation between shear rate, D and shear stress, τ was measured and second, the apparent viscosity, η, was calculated by use of the following equation (Eq. (2)).

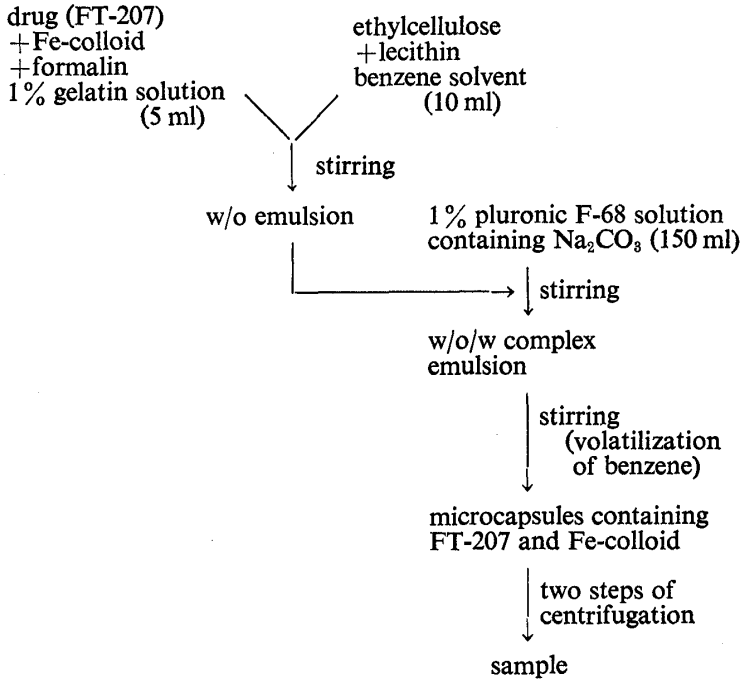

Fig. 1. Schematic Diagram for the Preparation of Magnetically Responsive Microcapsules Containing an Anticancer Drug

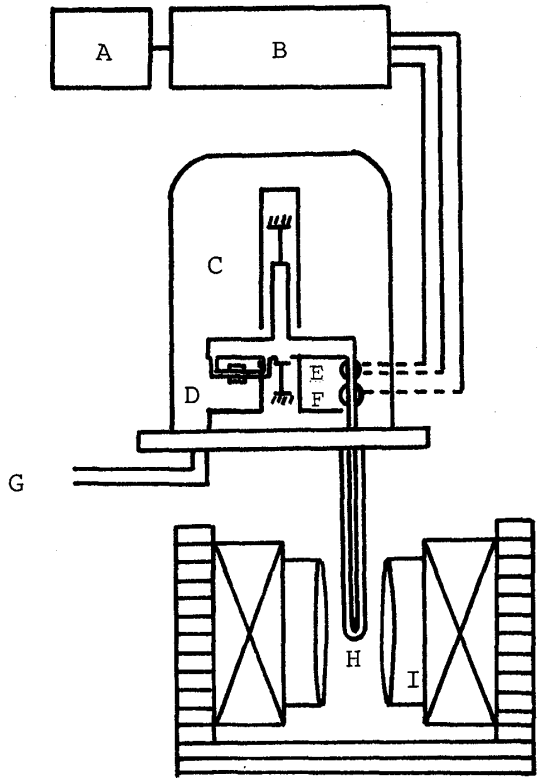

Fig. 2. Experimental Arrangement of Magnetic Balance

A, recorder; B, force measuring amplifier; C, elastic wire; D, rider; E, differential transducer; F, feedback coil; G, vacuum pump; H, sample; I, magnet.

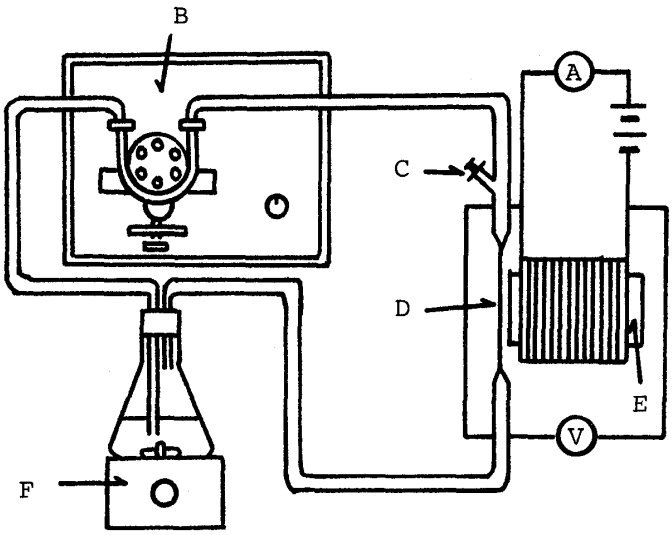

Fig. 3. A Flow Apparatus Used to Measure the Magnetic Responsiveness of the Microcapsules

A, ammeter; B, roller pump; C, injection syringe; D, glass capillary; E, electric magnet; F, magnetic stirrer; V, voltmeter.

$$\eta = \tau / D \qquad (2)$$

In vitro release properties of the drug FT-207 from the microcapsules were examined by a method modified from that described by Meyer *et al.*[7] A cellophane membrane was used because of its extremely small hole diameter. An amount of microcapsules containing 10 mg of FT-207 was suspended in a cellophane tube (diameter 27 mm, volume 20 ml) which was then immersed in 200 ml of physiological saline solution at 37 ± 0.1 °C. At intervals, 2 ml aliquots were withdrawn, diluted suitably, and assayed spectrophotometrically at 270 nm.

Measurement of of the Magnetic Responsiveness of the Microcapsules——The flow apparatus used to measure the magnetic responsiveness of the microcapsules is illustrated in Fig. 3. A 6 (w/v)% dextran 70 (M.W. 70000) solution in physiological saline (0.9% NaCl), corresponding to whole human blood in terms of viscosity, was used as the suspension medium (the flow phase). The flow rates were adjusted to correspond to different levels of human circulation. A roller pump was used to give laminar flow rates between 0.1 cm/s and 15 cm/s in various glass capillary tubes (0.58, 0.94, and 1.33 mm in diameter). Each of these glass capillary tubes was horizontally mounted and passed over a unipolar magnetic field at some point.

Results and Discussion

For the preparation of microcapsules, ethylcellulose and FT-207 were used as the wall-forming material and core material, respectively. Ethylcellulose has various desirable characteristics: safety, stability, hydrophobicity and compact film-forming nature as a water-insoluble polymer.[8] FT-207 is a derivative of 5-fluorouracil (5-FU), a well-known anti-metabolite. This drug, dissolved in gelatin solution for sustained release control, was microencapsulated with ferrofluid.

Scanning electron micrographs of the microcapsules are shown in Fig. 4. The microcapsules were spherical (see Fig. 4a), and their surface was smooth (see Fig. 4b).

Figure 5 shows that the microcapsules prepared in this study are distributed in a

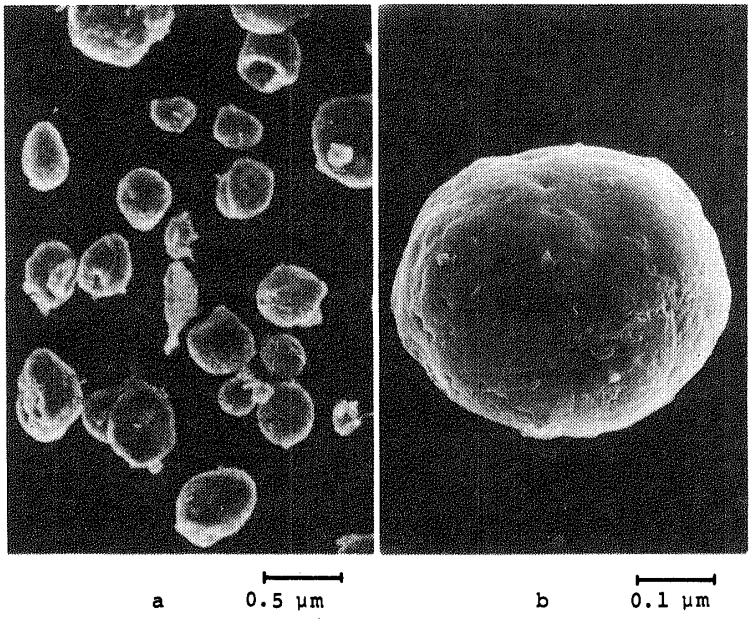

Fig. 4. Scanning Electron Micrographs of Microcapsules

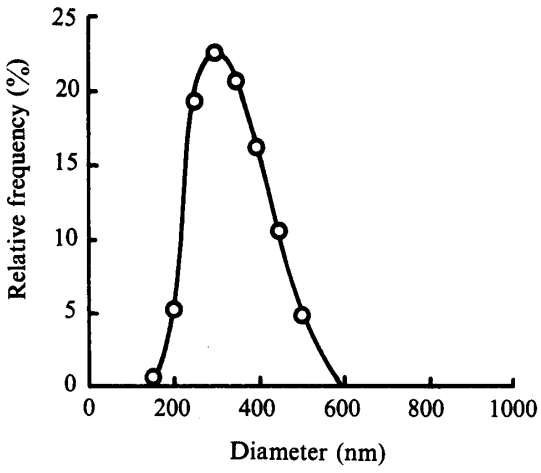

Fig. 5. Size Distribution Curve of Microcapsules Determined with a Coulter (Model N_4)

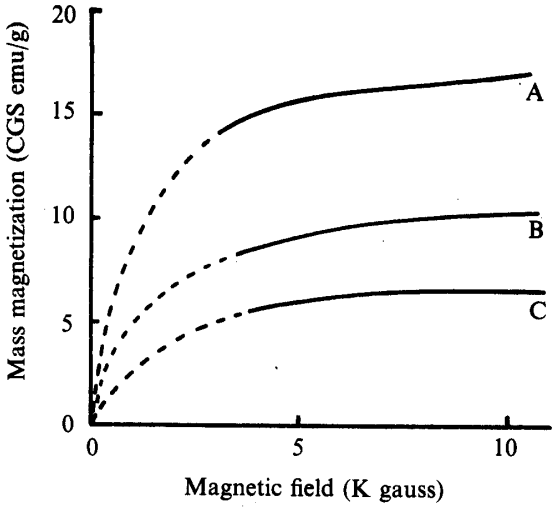

Fig. 6. Magnetization of Microcapsules Containing Various Volumes of Ferrofluid under an External Magnetic Field

A, microcapsules containing 1 ml ferrofluid; B, microcapsules containing 0.75 ml ferrofluid; C, microcapsules containing 0.5 ml ferrofluid.

relatively narrow range of diameter. The mean diameter was calculated to be 308 nm. By scanning electron microscopy, these preparations were seen to consist of small capsules ranging from 0.2 to 0.6 μm in diameter. If large microcapsules, *i.e.* more than 1 μm, are injected into the bloodstream, they are unlikely to be able to pass through capillary blood vessels. Therefore, in terms of size, the microcapsules prepared here should not cause

microvascular embolization.

Generally, ferromagnetic materials are readily magnetized in a magnetic field. The microcapsules prepared in this work were also magnetized in an external magnetic field. As shown in Fig. 6, the mass magnetization of the microcapsules was dependent on the total amount of ferrofluid contained in them. From this result, it appears that microcapsules containing large amounts of ferrofluid are readily magnetized in a low magnetic field.

Percentages of drug entrapment were in the range of 10—20%. Figure 7 shows the zeta-potential distribution of the microcapsules calculated from the mobility data by using the Smoluchowski equation. In this study, the surface potential of the microcapsules was estimated as the zeta potential. The zeta potential of the microcapsules in physiological saline was -10.4 mV.

It is reported that microcapsules move in an electric field if they contain an aqueous solution of polyelectrolytes even though the microcapsule membrane bears no electric charge.[9] As the microcapsules prepared here contain aqueous gelatin (isoelectric point, 4.8) solution as a polyelectrolyte, it seems that the zeta-potential reflects the sign of the electric

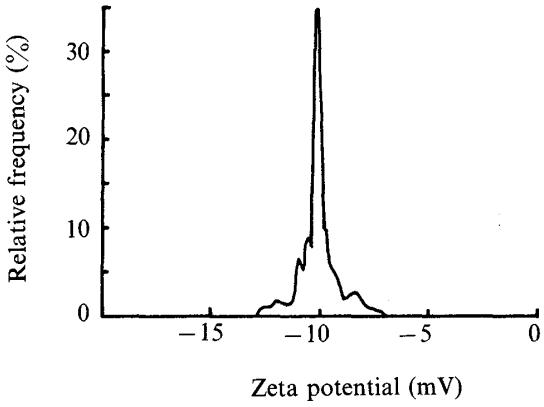

Fig. 7. Distribution and Histogram of Zeta Potential of Microcapsules in Physiological Saline

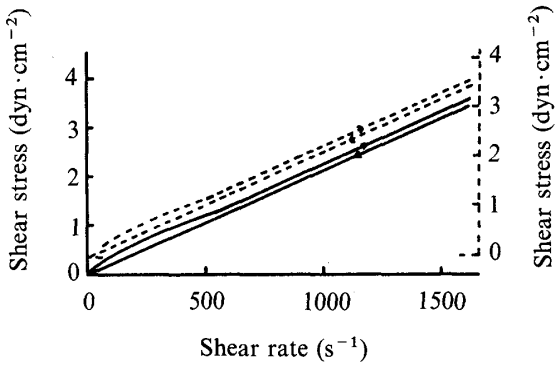

Fig. 8. Shear Stress *vs.* Shear Rate Curves of Microcapsule Suspensions in Physiological Saline with 6% Dextran 70 (See the text for details)

——, sample microcapsules; – – – –, resuspended microcapsules.

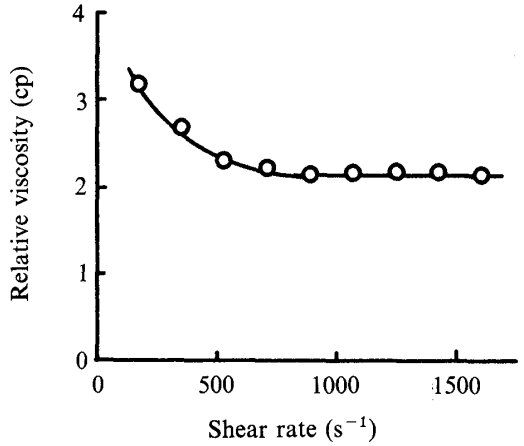

Fig. 9. Plots of Relative Viscosity *vs.* Shear Rate for Microcapsule Suspensions in Physiological Saline with 6% Dextran 70

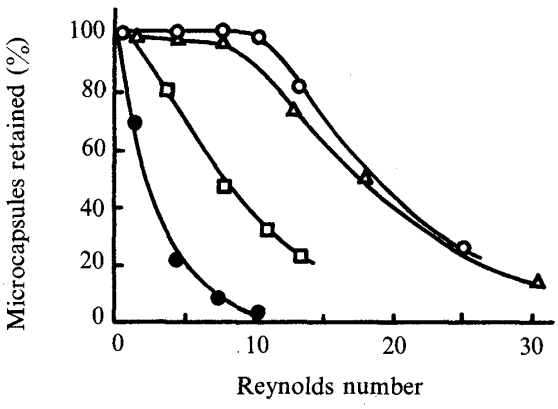

Fig. 10. Retention of Microcapsules Flowing in Physiological Saline with 6% Dextran 70 in Various Magnetic Fields

○, 2800 gauss; △, 2020 gauss; □, 1500 gauss; ●, 840 gauss.

charge on the polyelectrolyte.

It is well known that the surface of most biological cells has a net negative charge in aqueous media. It is thought that normal human red blood cells have a zeta potential of about -15 mV under physiological conditions, while the surface potential of old red blood cells immediately before being metabolized is lower than that of fresh ones.[10] Accordingly, the surface potential of microcapsules would play an important role in whole human blood, because aggregation and metabolism depend on the variation of surface potential.

Figure 8 illustrates the flow curves of microcapsule suspensions of constant volume concentrations in 6 (w/v)% dextran 70 physiological saline solution. As can be seen in this figure, when the shear rate is greater than 500/s, the flow curve of the microcapsule suspensions is Newtonian.

Further, the flow curve of the resuspended microcapsules magnetically collected was the same as that of the sample suspensions. That is to say, the resulting microcapsules were found not to have been aggregated.

The viscosity behavior of the microcapsule suspensions at lower shear rate was evaluated in terms of the relation between relative viscosity and shear rate (see Fig. 9). From Fig. 9, it is clear that the microcapsule suspension possesses structural viscosity at lower shear rate (below 500/s). The above-mentioned viscosity behavior of the microcapsule suspension is quite similar to that of red blood cell suspension.[9] It is important that when a microcapsule suspension is intravascularly injected, normal blood flow should not be altered at the site of carrier localization.

The retention of microcapsules by a magnetic field at various Reynolds numbers is shown in Fig. 10. The dimensionless Reynolds number, Re, is defined as:

$$\mathrm{Re} = Dv\rho/\eta \tag{3}$$

where D is the mean diameter of the capillary, v is the rate of settling, ρ is the density of the dispersion medium and η is the viscosity of the medium.

We evaluated the magnetic responsiveness of the microcapsules at known Reynolds number in intravascular flow, because all factors on normal blood flow may be described by Eq. (3). It is said that the Reynolds numbers of blood flow in humans are about 0.002, 0.02, and 12 for capillaries, small arteries, and arteries, respectively. From Fig. 10, full retention of the flowing microcapsule suspension in the circulation system *in vitro* was achieved in the applied magnetic field. The generated retention curves were similar to those presented by Senyei *et al.*[11] for magnetically responsive albumin microspheres. This result suggests that

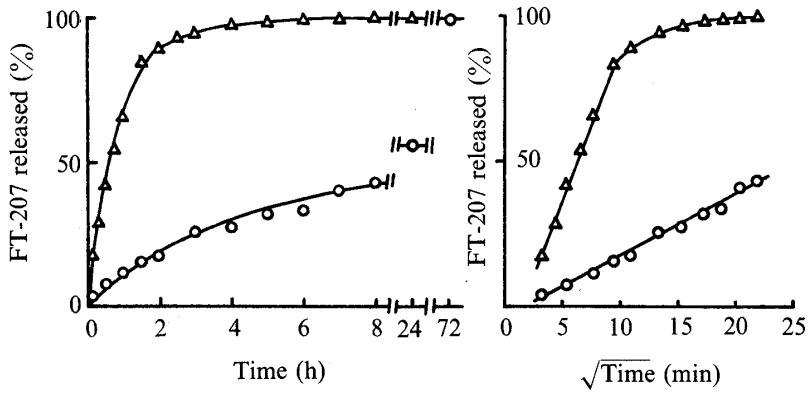

Fig. 11. Release Pattern (a) and Release Percent–Square Root of Time Plots (b) of FT-207 from Microcapsules in Physiological Saline through a Cellophane Membrane

○, microcapsule suspension; △, free drug solution.

microcapsules injected intravascularly can be localized at a target site *in vivo* by means of an externally applied magnetic field.

In vitro release properties of the drug FT-207 from microcapsules through a cellophane membrane are shown in Fig. 11a. Free (nonencapsulated) drug FT-207 was also processed in the same manner for the control. The amount of FT-207 released from the microcapsules at 5 h was 33.1% ($n=3$) while nonencapsulated drug was completely released at this time.

Consequently, sustained release of FT-207 from microcapsules was clearly observed in the *in vitro* assay. Further, Fig. 11b shows a plot of percent FT-207 released as a function of square root of time for microcapsules and control free drug. The plot for microcapsules was linear over a wide range. This release pattern was also found to apply to the Higuchi equation[12] developed to define the release from wax matrices.

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References and Notes

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