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Microencapsulation of Isoprinosine with Ethylcellulose

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Abstract □ Isoprinosine, an antiviral agent with a bitter taste, has been clinically used up to a maximum of 4 g daily in 4-8 doses. In this investigation, isoprinosine was microencapsulated with ethylcellulose 22 cps, 50 cps and 100 cps by means of polymer deposition from cyclohexane through temperature change. Complete removal of cyclohexane from the microcapsules was necessary, since ethylcellulose-coated microcapsules obtained from cyclohexane medium were heavily solvated with cyclohexane and formed lumps even after drying. The displacement of cyclohexane by *n*-hexane during isolation of microcapsules (Method III) or the freezing of the final-washed microcapsules before drying (Method II) provided the dried products which were more discrete microcapsules than those which were simply dried in the air overnight (Method I). Method III was especially the most effective procedure in preparing finer and more discrete microcapsules. The drug-release from microcapsules was influenced by the ratio of core to wall, the viscosity grade of ethylcellulose and the overall microcapsule size. The release rate was adequately fitted to both the first-order and the diffusion-controlled processes. It is therefore possible to design the release-controlled microcapsules with ethylcellulose of different viscosity along with various core to wall ratio.

Keywords □ Isoprinosine, ethylcellulose, release-controlled microcapsules, *in vitro* drug release, microencapsulation.

Isoprinosine is a 1 : 3 molar complex of inosine and p-acetamidobenzoate salt of N,N-dimethylamino-isopropanol[1,2]. Isoprinosine has been used in the treatment of viral hepatitis[3], herpes simplex[4,5], herpes zoster[4,5], viral infectious respiratory disease, viral encephalitis, eruptive viral disease and influenza[6].

Isoprinosine has some disadvantages in oral administration; it generally has to be administered rather frequently as 4-8 times per day up to 4 g daily and on top of this, it has a bitter taste[1,2]. Therefore, in this study, microencapsulation technique was applied to prolong the *in vitro* release of isoprinosine and mask a bitter taste.

EXPERIMENTAL METHODS

Materials

Isoprinosine (Methisoprinol, NPT-10381, Inosiplex; Newport Pharmaceutical Int. Inc., USA) of an average particle size of 127 μm was used. Particle size reduction and drug classification were accomplished using a ball mill (Kukje Scientific Instrument Co., Seoul, Korea) and a series of standard K.P. sieves. Ethylcellulose (N-type) had an ethoxy content of 47.5-49.0% by weight. Ethylcellulose N-22 and N-50 were purchased from Hercules Inc. (Wilmington, Del., USA), and N-100 was purchased from Dow Chemical Co. (Midland, Michigan, USA). The viscosity values of ethylcellulose N-22, N-50 and N-100 as 5% (w/w) solution in toluene-ethanol (80 : 20 w/w) were 22, 50 and 100 cps, respectively. Cyclohexane (Shimakyu's Pure Chemicals, Osaka, Japan) and *n*-hexane (Oriental Chemical Ind. Co., Seoul, Korea) were used. All other chemicals used were of analytical reagent grade.

Instruments

UV spectrophotometer (Hitachi Model 200-20,

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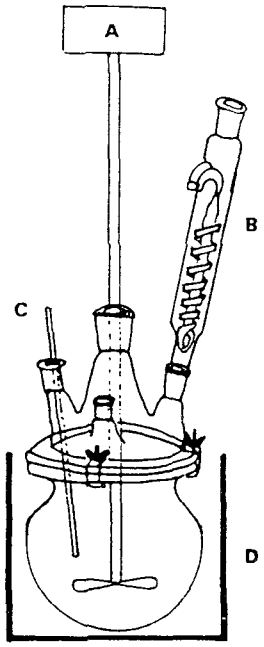


Fig. 1. Microencapsulation apparatus.
A: variable speed motor, B: reflux condenser, C: thermometer, D: water bath.

Hitachi Ltd., Tokyo, Japan), K.P. standard sieves and dissolution tester unit (Hanson Research Corp., Model NO. 64-700-006, Northridge, Calif., USA) fitted with driving control motor and thermostatic bath were used.

Microencapsulation procedure

Method I: Isoprinosine microcapsules were prepared by the reported method of Kim *et al.*[7,8].

The coating vessel used was 800 m*l*-capacity round bottom flask fitted with a flanged **4** ports cover. Through the center port, a 2-blade stirrer was connected to the chuck of variable speed motor. The remaining ports were used to provide the coating vessel with a reflux condenser, a thermometer and an entry point. The lower part of the vessel was heated by immersing in water bath with a temperature control unit. The microencapsulation apparatus is shown in Fig. 1.

To prepare a batch of microcapsules, 600 m*l* of cyclohexane was added to the reaction vessel stirred with a speed of about 250 rpm and heated to 60℃. An adequate amount of ethylcellulose in respect of the ratio of core to wall was added to the heated cyclohexane, and the temperature was raised to 70℃ over 20 min. Core material, isoprinosine (6 g) was then added and the temperature was raised to 80-81℃ over 1 hr at a continuous stirring rate of about 500 rpm. After being maintained at this temperature for 1 hr, the product was continuously stirred and slowly cooled to 35℃ over 3 hr, and then was allowed to sediment at room temperature. The excess cyclohexane was decanted, and the product was washed 3 times with 300 m*l* of cold cyclohexane (10 ℃), filtered and allowed to dry in the air overnight.

Method II: The microcapsules prepared by Method II were washed with cyclohexane as in Method I, froze at −20℃ for 1-2 hr and dried in the air below 10℃.

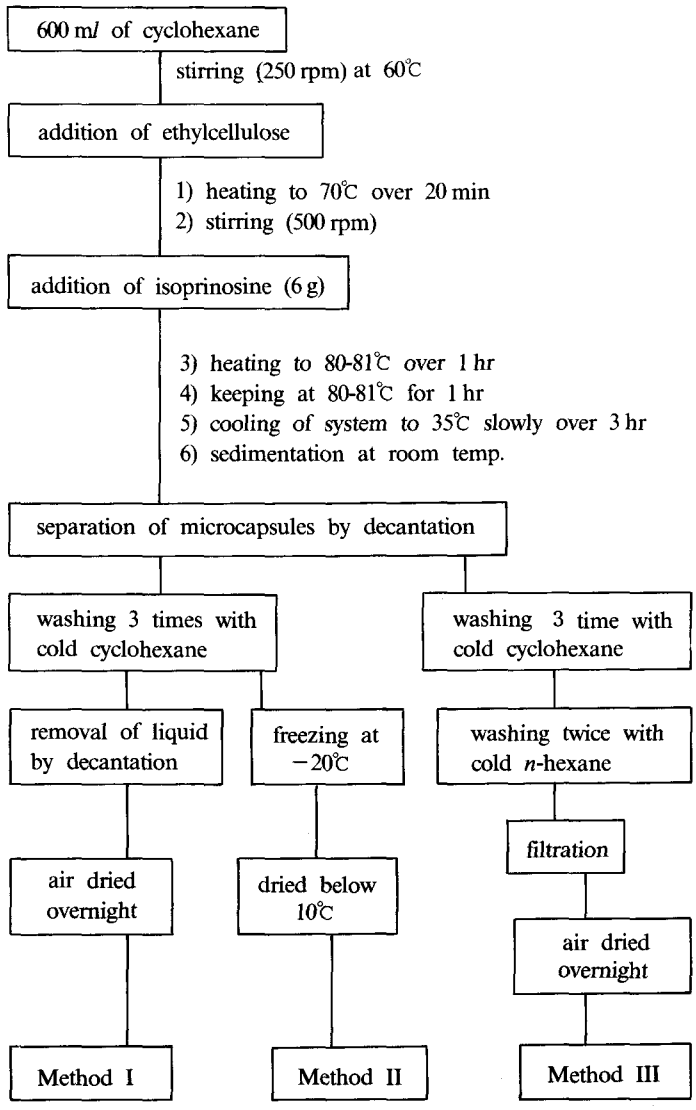
Method III: This method is a modification of the technique of Moise *et al.*[9,10]. The microcapsules prepared by Method I were washed once with 100 m*l* of cold cyclohexane and then twice with 200 m*l* of *n*-hexane. The microcapsules were then filtered and dried in the air overnight. Scheme 1 shows the encapsulation procedures for isoprinosine.

Particle size classification of microcapsules

The different sizes of microcapsule particles in the batch were classified into suitable fraction by using a nest of K.P. standard sieves mounted on the electromagnetic laboratory sieve shaker (FRITSCH analysette 3, German).

Dissolution procedure

A known amount of microcapsules containing 100 mg of isoprinosine was placed in a K.P. dissolution basket of aperture size of 420 μm, and its base lined with 74 μm sieve, and then was immersed in 600 m*l* of artificial gastric juice at 37± 0.5℃ in a 1000 m*l*-round bottom flask. With a constant stirring speed of 120 rpm, 4 m*l* of the solution was collected and filtered through a millipore filter (Millipore Corp., Bedford, Mass., USA) fitted with a 0.45 μm pore size membrane between appropriate time intervals. The amount of isoprinosine released was analyzed spectrophotometrically at 260 nm. After each sample was removed, an equivalent amount of fresh dissolution medium was immediately added to the dissolution vessel to retain a constant quantity of medium for drug-release. The following equation was used to compensate for the portion of drug removed from the dissolution vessel after sampling for determining concentration:



Scheme 1. Preparation of ethylcellulose microcapsules.

$$C_{corr,n}=C_{read,n}+\frac{4}{600}\sum_{s=1}^{n-1}C_{uncorr,s} \quad (1)$$

where $C_{corr,n}$ is the corrected concentration of the sample at time t; $C_{read,n}$ is the spectrophotometrically measured concentration at time t; 4/600 regards to the 4 ml of the sample withdrawn from 600 ml of dissolution medium, and $\sum_{s=1}^{n-1}C_{uncorr,s}$ is the sum of the uncorrected concentration of the previous runs.

RESULTS AND DISCUSSION

The technique of polymer separation for coating the solid particles to form microcapsules is apparently easy, but slight changes in the procedure may produce a marked variation in size distribution, shape and fluidity of the final product. Several important factors influencing the size of microcapsules include the stirring rate, cooling rate, washing method, and the size and shape of paddle in relation

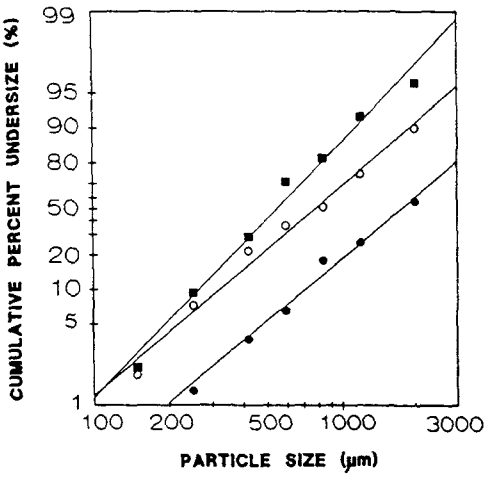


Fig. 2. Log-normal distribution of particle size by weight of microcapsules containing isoprinosine prepared using ethylcellulose 100 cps by Method I (●), II (○) and III (■) with the core to wall ratio of 1 : 1.

to the flask size.

Photomicrographic examination showed that larger microcapsule particles would partly aggregate with the smaller ones. The particle size of isoprinosine-microcapsules prepared with ethylcellulose N-100 by three different methods was found to have log-normal distributions as shown in Fig. 2. The values of R^2 from Method I, II and III were 0.988, 0.985 and 0.995, respectively. The most discrete and smallest microcapsules were obtained by Method III, whereas the largest and aggregated microcapsules were prepared by Method I. From preliminary observations, ethylcellulose heavily solvated with cyclohexane was found to make the microcapsule aggregates even after drying. However, microcapsules in this study were not solvated with *n*-hexane and showed discrete forms. Therefore, ethylcellulose-walled microcapsules washed with *n*-hexane formed the more discrete, finer and well-coated product after drying.

For the characterization and comparison of microcapsules prepared under different conditions, it is useful to examine *in vitro* release as well as *in vivo* release. In general, the release of drug from a matrix can be either *via* a first-order process or a diffusion-controlled process.

By the Higuchi equations for diffusion-controlled transport in a homogeneous polymer matrix, the release of drug from microcapsules holds the following relationship:

$$Q=[D(2A-C_s)C_st]^{1/2} \quad (2)$$

where Q is the amount of drug released per unit area exposed after time t; and D, A and C_s are the diffusivity, initial concentration and solubility of the drug in the matrix, respectively. If the matrix is granular and the drug is released by leaching through a capillary network, Q, the amount of drug liberated per unit surface area of matrix into the external medium in time t is given by:

$$Q=[\frac{\varepsilon}{\tau}D(2A-\varepsilon C_s)C_st]^{1/2} \quad (3)$$

where D is the diffusivity of the drug in the leaching medium, ε is the porosity and τ is the tortuosity of the matrix. In the homogeneous case, drug-release is directly proportional to the square root of time. Equation 2 is more commonly expressed as follows:

$$Q'=K't^{1/2} \quad (4)$$

If Q′ is the amount of drug released from an area of film, S, $Q'=QS$ and $K'=KS$ where K is the release rate constant given by:

$$K=[D(2A-C_s)C_s]^{1/2} \quad (5)$$

Equation 3 also describes the case of the granular matrix if the value of K remains constant throughout the leaching process. K then is given by:

$$K=[\frac{\varepsilon}{\tau}D(2A-\varepsilon C_s)C_s]^{1/2} \quad (6)$$

For a first-order release, the data can also be expressed by the following first-order equation:

$$\log(A-Q')=\log A-\frac{K't}{2.303} \quad (7)$$

where Q′ is the amount of drug released from an area of film, S; and (A−Q′) is the drug content of the microcapsules at time t.

All sets of data from the release test were plotted as a function of time according to the two relationships considered previously[11,12], i.e. Q′ versus square-root of time and ln (A−Q′) versus time where Q′ is the amount of drug released and A is the original amount of drug present in the matrix. The data were fitted using linear regression to generate release rate constants.

Table I. *In vitro* release rate constant of isoprinosine from microcapsules coated with different viscosity grade of ethylcellulose

Grade of Ethycellulose	Core : Wall	Higuchi's square-root of time		First-order	
		K', $mg \cdot h^{-1/2} \pm$ S.E.	R^2	K', $(h^{-1}) \pm$ S.E.	R^2
22 cps	2 : 1	73.8± 5.43	0.963	0.881± 0.0198	0.996
	1 : 1	71.1± 3.19	0.986	0.587± 0.0117	0.997
50 cps	2 : 1	67.3± 1.88	0.994	0.523± 0.0117	0.996
	1 : 1	61.9± 1.85	0.994	0.376± 0.0076	0.997
100 cps	2 : 1	59.7± 1.72	0.994	0.343± 0.0123	0.991
	1 : 1	51.2± 0.79	0.998	0.258± 0.0058	0.995

The microcapsules were coated with 22, 50 and 100 cps of ethylcellulose with the core to wall ratios of 2 : 1 and 1 : 1. The size range of microcapsules was 250-420 μm.

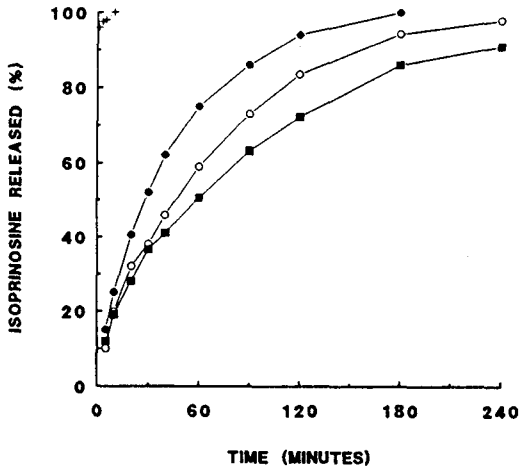


Fig. 3. The release of isoprinosine from uncoated drug (VII), and microcapsules coated with 22 (●), 50 (○) and 100 (■) cps of ethylcellulose with the core to wall ratio 1 : 1. The size range of microcapsules was 250-420 μm.

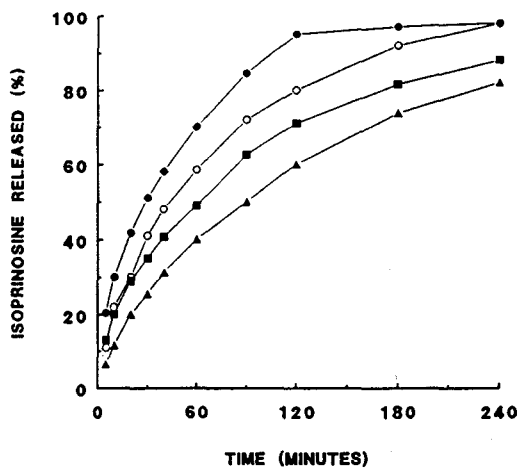


Fig. 4. The release of isoprinosine from microcapsules coated with ethylcellulose 100 cps with the core to wall ratios of 4 : 1 (●), 2 : 1 (○), 1 : 1 (■) and 1 : 2 (▲), respectively. The size range of microcapsules was 420-841 μm.

Fig. 3 shows the release of isoprinosine from the equivalent size range of microcapsules prepared with three different viscosity grades of ethylcellulose (22, 50 and 100 cps). The release rate constants determined from the plots based on EQ (4) and EQ (7) are listed in Table I. The release rate constants from microcapsules coated with 22, 50 and 100 cps of ethylcellulose determined on the basis of EQ (4) were 71.1, 61.9 and 51.2 $mg \cdot h^{-1/2}$, and those according to EQ (7) were 0.587, 0.376 and 0.258 h^{-1}, respectively. These results exhibit a retardation in isoprinosine-release with an increase in the viscosity grade of ethylcellulose. The uncoated drug, isoprinosine itself dissolved completely within a few minutes. From this fact, it can be deduced that this microencapsulation may possibly provide a method for release-controlled isoprinosine microcapsules.

Fig. 4 shows the release of isoprinosine from microcapsules with different ratios of core to wall. The rate constants determined from the plots based on EQ (4) and EQ (7) are listed in Table II. The release rate constants from microcapsules prepared with the ratios of core to wall; 4 : 1, 2 : 1, 1 : 1 and 1 : 2 according to EQ (4) were 67.5, 59.6, 48.8 and 44.0 $mg \cdot h^{-1/2}$, and those from EQ (7) were 0.526, 0.350, 0.224 and 0.183 h^{-1}, respectively. As expected, the isoprinosine-release from microcapsules increased with the ratio of core to wall; the percentages

Table II. *In vitro* releases rate constant of isoprinosine from ethylcellulose-coated microcapsules with different core to wall ratios

Core : Wall	Higuchi's square-root of time		First-order	
	K', $mg \cdot h^{-1/2} \pm$ S.E.	R^2	K', $(h^{-1}) \pm$ S.E.	R^2
4 : 1	67.5± 1.11	0.998	0.526± 0.0291	0.976
2 : 1	59.6± 1.77	0.994	0.350± 0.0073	0.997
1 : 1	48.8± 1.03	0.996	0.224± 0.0071	0.991
1 : 2	44.0± 1.09	0.994	0.183± 0.0033	0.997

The microcapsules were coated with ethylcellulose 100 cps with the core to wall ratios of 4 : 1, 2 : 1, 1 : 1 and 1 : 2, respectively. The size range of microcapsules was 420-841 μm.

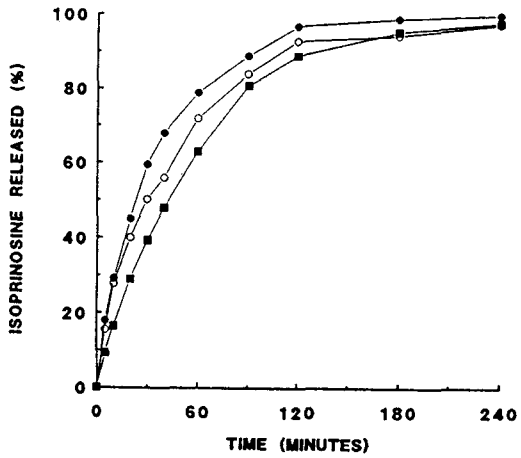


Fig. 5. The release of isoprinosine from microcapsules coated with ethylcellulose 100 cps with the core to wall ratio of 2 : 1. The size range of microcapsules was 149-250 (●), 250-420 (○) and 420-841 μm (■).

of isoprinosine released were 40.0, 49.0, 58.5 and 70.0% for the core to wall ratios of 1 : 2, 1 : 1, 2 : 1 and 4 : 1, respectively after 1 hr. It is thus reasonable to expect that wall thickness increases with a decrease in the ratio of core to wall. Therefore, it is thought that isoprinosine-release can be controlled at a desired rate by varying the ratio of core to wall as well as the viscosity grade of ethylcellulose in the preparation of microcapsules.

Fig. 5 shows the effect of particle sizes on the isoprinosine-release from ethylcellulose (100 cps)-coated microcapsules, and their rate constants are listed in Table III. The release rate constants from microcapsules with the range of particle sizes; 149-250, 250-420 and 420-841 μm according to EQ (4) were 73.8, 67.3 and 68.6 $mg \cdot h^{1/2}$, and those measured

Table III. *In vitro* release rate constant of isoprinosine from different size of ethylcellulose-coated microcapsules

Particle size	Higuchi's square-root of time		First-order	
	K', $mg \cdot h^{-1/2} \pm$ S.E.	R^2	K', $(h^{-1}) \pm$ S.E.	R^2
149-250 μm	73.8± 5.43	0.963	0.676± 0.0220	0.991
250-420 μm	67.3± 1.88	0.994	0.524± 0.0117	0.996
420-841 μm	68.6± 3.20	0.985	0.460± 0.0086	0.997

The microcapsules were coated with ethylcellulose 100 cps with the core to wall ratio of 2 : 1

on the basis of EQ (7) were 0.676, 0.524 and 0.460 h^{-1}, respectively. As expected, the amount of drug released from microcapsules tended to increase slightly with the decrease in their particle sizes.

It is probable that drug-release from microcapsules is a result of several mechanisms including as leaching, diffusion and erosion compounded by polymer swelling. It is also affected by the presence of air in the coating and drug binding. Whether the drug-release from microcapsules is *via* a first-order process or a diffusion-controlled process was ascertained by the data treatments as such in Fig. 6. Fig. 6 is a typical profile of drug-release from 250-420 μm of microcapsules that contain isoprinosine and are coated with ethylcellulose 22, 50 and 100 cps, respectively. Fig. 6 and Table I-III demonstrate that up to 75% of release all sets of data from the release test fit both of above two equations very well; the R^2 of all sets exceeded 0.96.

In conclusion, the displacement of cyclohexane by *n*-hexane during isolation of microcapsules (Method III) or the freezing of the final-washed microcapsules before drying (Method II) provided the dried products, which were more discrete microcap-

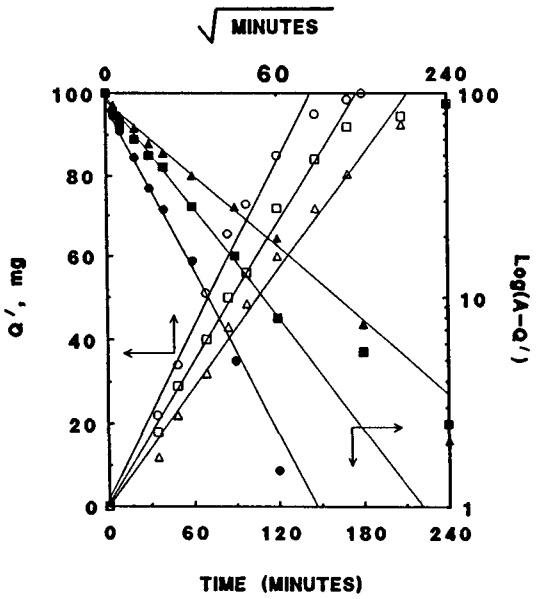


Fig. 6. First-order (closed symbols) and Higuchi square-root of time (open symbols) profiles of release of isoprinosine from microcapsules coated with 22 (●, ○), 50 (■, □) and 100 cps (▲, △) of ethylcellulose with the core to wall ratio of 2 : 1. The size range of microcapsules was 250-420 μm.

sules comparing to those obtained from Method I. Especially, Method III was the most effective for the procedure in preparing finer and more discrete microcapsules. The release of iosprinosine from microcapsules was affected by the ratio of core to wall, the viscosity grade of ethylcellulose and the overall microcapsule size. It is therefore possible to design the release-controlled microcapsules by varying the viscosity grade of ethylcellulose and the ratio of core to wall in the preparation of microcapsules. In addition, the release rate adequately fitted both the first-order and the diffusion-controlled processes.

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