Drug Development and Industrial Pharmacy, 23(3), 259-265 (1997)

RESEARCH PAPER



# pH-Dependent Cellulosic Microspheres Containing Cefuroxime Axetil: Stability and In Vitro Release Behavior

M. Cuña,* M. L. Lorenzo-Lamosa, J. L. Vila-Jato, D. Torres, and M. J. Alonso

*Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Santiago de Compostela, 15704 Santiago de Compostela, Spain*

## ABSTRACT

*Cefuroxime axetil (CA) was encapsulated in pH-dependent cellulosic microspheres with the final aim of masking taste while assuring its release into the intestinal cavity. The polymers selected were: CAT (cellulose acetate trimellitate) and two types of hydroxypropylmethylcellulose phthalate, HPMCP-55 and HPMCP-50. The CA-loaded CAT and HPMCP-55 microspheres were obtained by a solvent extraction procedure, whereas the encapsulation of CA into HPMCP-50 microspheres was only achieved by a solvent evaporation technique. All the formulations displayed pH-dependent release profiles, releasing their total content in 30 min when exposed to an aqueous medium of pH 6.0. Analysis of the encapsulated molecule by HPLC revealed that a problem of compatibility arises between CA and CAT, leading to the formulation of a high amount of CA impurities. By contrast, a minimum amount of impurities was detected upon encapsulation of CA within HPMCP, this amount being lower for HPMCP-55 than for HPMCP-50. Finally, the taste-masking test carried out for the formulation made of HPMCP-55 evidenced the efficacy of the polymer coating in preventing the release of CA in an acidic medium and thus masking its taste.*

*To whom correspondence should be addressed.

## INTRODUCTION

In the past, enteric coatings were reserved for drugs that cause gastric irritation or are destroyed by acid or gastric enzymes; however, these coatings have recently found application in achieving delayed drug release (1–4) and taste-masking of drugs (5–8). The special feature of these polymers is that they are insoluble in strong acid media but dissolve at pH values in the range of 4.5–7.0. The pH sensitivity depends on the presence of carboxyl groups which are nonionized at low pHs but dissociate at pHs higher than 4.5 (9). Therefore, the enteric coatings withstand prolonged contact with gastric fluids but dissolve rapidly in the mild acidic-to-neutral environment of the small intestine. This pH-dependent dissolution behavior can be also of great benefit in coating bitter drugs, thus preventing their release when suspended in an acidic medium and, therefore, their unpleasant taste.

Various polymers are available for use in enteric coatings, especially acrylic and cellulosic derivatives. Among these, acrylic polymers such as Eudragit L and S have a limitation due to the reactivity of their acid groups and their possible interaction with basic drugs (10,11). On the other hand, despite the wide application of cellulosic and acrylic polymers in pan or fluidized-bed coating procedures, the efficacy of these techniques in achieving a homogeneous shell around the drug particles is sometimes limited (5).

In view of all these limitations, microencapsulation using enteric cellulose derivatives was proposed in the present work in order to achieve a double objective: taste-masking and protection of the active molecule against degradation in the stomach. Cefuroxime axetil (CA), the orally absorbed prodrug of cefuroxime, was selected as a drug model. Three enteric cellulose esters were selected as coating materials: cellulose acetate trimellitate (CAT, soluble at pH 5.0) (12) and two varieties of hydroxypropylmethylcellulose phthalate (12,13), HPMCP-50 (soluble at pH 4.8) and HPMCP-55 (soluble at pH 5.2). These varieties differed on the percentage of phthalic acid in their structure: 24% in HPMCP-55 and 33% in HPMCP-50. The predecessor of these materials, cellulose acetate phthalate (CAP), soluble at pH 6.5 (12), has been widely used as an enteric coating material in the field of microencapsulation (14–17). However, the advantage of the selected polymers, with respect to CAP, is that they allow the encapsulated drug to release at a lower pH, a fact that is crucial when the drug is absorbed in the upper part of the intestinal tract. With this fact in mind, it is surprising that, in spite of the interest of these polymers, information about their use in microencapsulation has been quite limited. Therefore, the main aim of the present work has been to develop adequate techniques for the encapsulation of CA within the selected polymers and to evaluate the efficacy of this approach in order to control the release of CA and mask its taste while preserving its stability.

## MATERIALS

Cellulose acetate trimellitate CAT (kindly supplied by Eastman Kodak, France)
Two varieties of hydroxypropylmethylcellulose phthalate: HPMCP-55 (Shin-Etsu, Japan) and HPMCP)-50 (kindly supplied by Eastman Kodak, France)
Cefuroxime axetil (donated by Glaxo, Spain)
Highly liquid paraffin (Merck, Germany)
Acetone (Romil, United Kingdom)
Ethanol (Probus, Spain)
Chloroform (Normasolv, Spain)
Petroleum ether (Panreac, Spain)
Span 85 (Fluka, Spain)

## METHODS

### Microencapsulation Methods

Microspheres were prepared by solvent extraction or solvent evaporation techniques, depending on the nature of the organic solvent required to dissolve the polymer. In this respect we observed that HPMCP-55 was readily soluble in acetone, whereas the incorporation of a small amount of ethanol or water was required for the dissolution of CAT and HPMCP-50, respectively.

#### Emulsion–Solvent Extraction in Oil Phase

The CAT microspheres were prepared by a procedure previously reported by Sanghvi and Nairn (18), conveniently modified. Briefly, 1.2 g of CAT and a variable amount of CA (200–300 mg) were dissolved in 10 g of acetone:ethanol (3:1 v/v). This organic phase was then emulsified in 100 ml of highly liquid paraffin at 500 rpm for 1 hr in a round-bottomed kettle equipped with a cover to minimize evaporation. Following this, 100 ml of chloroform were added to the system in order to promote the extraction of the solvent and stirring was continued for additional 15 min. The microspheres

were isolated by filtration, washed with 100 ml of chloroform, and dried under reduced pressure for 24 hr. Formulations were designated CAT(200 mg) and CAT(300 mg) depending on the amount of CA incorporated in the microspheres.

The HPMCP-55 microspheres were prepared by the method described by Garcia-Encina et al. (19). The internal phase consisted of 10 ml of an acetonic solution of HPMCP-55 (1.5 g) and CA (200 and 300 mg). The solution was incorporated into 100 ml of highly liquid paraffin containing sorbitan trioleate (Span 85) 1% w/w. The system was agitated at 500 rpm for 30 min and 100 ml of petroleum ether were added to allow the extraction of the acetone. After stirring for 15 min, microspheres were filtered, washed with 80 ml of petroleum ether, and dried under reduced pressure for 24 hr. Formulations were designated HPMCP-55(200 mg) and HPMCP-55(300 mg) depending on the amount of CA incorporated in the microspheres. One batch of HPMCP-55 microspheres was freeze-dried.

Emulsion–Solvent Evaporation in Oil Phase

For the encapsulation of CA within HPMCP-50 microspheres, we developed a technique based on a solvent evaporation process. HPMCP-50 (1.2 g) and CA (200 mg) were dissolved in 10 g of acetone:water mixture (9:1 v/v). The resulting solution was emulsified in 100 ml of highly liquid paraffin. The system was maintained under agitation at 500 rpm at room temperature for 3 hr. Afterwards, the microspheres were isolated by filtration, washed with petroleum ether, and dried, under reduced pressure, for 24 hr. This formulation was designated HPMCP-50(200 mg).

**Particle Size Analysis**

Microscopic particle size analysis was carried out on 100 microspheres using an optical microscope Olympus BH-2.

**CA Loading**

Microspheres were totally dissolved in phosphate buffer pH 6.0 and the CA content was determined by measuring the ultraviolet (UV) absorbance at 280 nm, following subtraction of the absorbance of blank microspheres at the same wavelength. The percentage of CA encapsulated with respect to the total amount of CA used for encapsulation was taken as the loading efficiency. The amount of CA encapsulated per 100 mg of microsphere was considered as the CA loading.

**Drug Release Studies**

In vitro drug release tests were carried out using Apparatus II of USP XXIII. An adequate amount of CAT and HPMCP microspheres, in order to maintain the system under sink conditions, was added into 500 ml of dissolution medium. The release medium was agitated at 100 rpm, the temperature kept at 37°C, and the system was stirred at 100 rpm. Samples were taken at the appropriate time intervals and replaced by fresh medium. Concentration of CA was determined by measuring the UV absorbance at 280 nm, following subtraction of the absorbance due to the presence of the coating polymers in the release medium. The dissolution media were constituted by phosphate buffer at different pHs: 5.0 and 6.0 for the CAT microspheres, 4.8 and 6.0 for HPMCP-55 microspheres. The medium of pH 6.0 was selected to assure the complete release of CA at the upper part of the small intestine, while the other values of pH were selected based on the pH dissolution intervals of the polymers.

**Stability Studies**

The stability of the encapsulated drug and its compatibility with the polymers used to prepare the microspheres were determined by high-performance liquid chromatography (HPLC) using an adaptation of the British Pharmacopeia method (20). The solvent employed in the chromatographic analysis of the samples was the methanol/phosphate buffer pH 5.7 (20/30 v/v). The work was divided into two parts:

- Compatibility of CA with cellulosic polymers and acetone: The CA and the cellulosic polymers were dissolved in acetone, in the proportions employed for the preparation of the microspheres, and kept for 1 hr at room temperature. Then samples were injected into the chromatographic system for the analysis of impurities.
- Assay of CA in the developed formulations: The CAT, HPMCP-50, and HPMCP-55 microspheres were dissolved in the solvent and injected into the chromatographic system to determine CA content and possible impurities and/or degradation products.

### Taste-Masking Test

The formulation of HPMCP-55 microspheres containing 200 mg of CA was selected for a taste-masking blind test. The HPMCP-55 blank microspheres and a standard formulation with fruit taste were used as controls. Microspheres were suspended in citrate buffer (pH 4.0) containing sacarose and fruit aroma proportioned to that of the standard formulation. Numerical scores from 0 to 3 were assigned by the four panel members in order to evaluate the taste masking of the formulations tested: 0, tasteless; 1, acceptable taste; 2, slightly bitter; and 3, very bitter taste.

## RESULTS AND DISCUSSION

The CAT, HPMCP-50, and HPMCP-55 are pH-sensitive polymers which do not dissolve in the stomach but become more soluble throughout the intestine. Therefore, CA microspheres elaborated with these polymers should prevent the hydrolysis of the molecule in the acidic medium of the stomach and facilitate the release of the drug in the small intestine, thus permitting its absorption. Similarly, it was expected that, by suspending these microspheres in an acidic medium prior to oral administration, the taste of the drug would be masked.

The methods we selected for the microencapsulation of CA have in common the emulsification of a polymer solution containing the drug in an immiscible external phase, the highly liquid paraffin. Polymer precipitation within the droplets occurred through the removal of the polymer solvent, thus leading to the formation of solid microspheres upon complete removal of the solvent. The solvent in the internal phase and the method of solvent removal differed depending on the solubility properties of the polymers. The HPMCP-55 was readily soluble in acetone whereas in the case of CAT and HPMCP-50 it was necessary to incorporate ethanol or water to the internal phase in order to increase their solubility. More specifically, CAT was dissolved in acetone:ethanol (3:1 v/v) and HPMCP-50 in acetone:water (9:1 v/v).

In addition, it was found that the method of solvent removal and the nature of the solvent-extracting agent were determined by the polymer employed. Thus, the formation of microspheres made of CAT and HPMCP-55 was accomplished by a solvent extraction technique using two different extracting solvents: chloroform and petroleum ether, respectively. The use of chloroform as an extracting solvent did not permit the formation of HPMCP-55 microspheres, due to the partial solubility of this polymer in this type of solvent. However, it was found that petroleum ether did not dissolve the polymer and it was therefore used as extraction solvent.

In the case of HPMCP-50, the elimination of the solvent, and therefore the formation of microspheres, was only achieved by slow evaporation of the solvent. Initially, the feasibility of microencapsulation with HPMCP-50 by the solvent extraction technique was investigated, but no microspheres were obtained. The use of chloroform, petroleum ether, and *n*-hexane as extracting agents led to a rapid precipitation of the polymer and the formation of aggregates. Consequently, the solvent evaporation method was selected as the most appropriate technique for the formation of HPMCP-50 microspheres.

Table 1 shows the mean size, loading efficiency, and CA loading of the different formulations prepared by the described techniques. The smaller size of the CAT, with respect to the HPMCP, microspheres could be explained by the different nature of the organic solvent (acetone:ethanol vs. acetone) and its dispersability in the paraffin oil.

***Table 1***

*Mean Diameter, Loading Efficiency, and CA Loading of Microspheres Made of CAT, HPMCP-50, and HPMCP-55*

| Formulation | Mean Size ± *SD* (μm) | Loading Efficiency ± *SD* (%) | Drug Loading ± *SD* (%) |
|---|---|---|---|
| CAT (200 mg)[a] | 67 ± 37 | 73.61 ± 3.63 | 10.93 ± 0.48 |
| CAT (300 mg) | 88 ± 32 | 62.68 ± 7.50 | 13.53 ± 1.42 |
| HPMCP-50 (200 mg) | 222 ± 73 | 82.08 ± 1.38 | 12.03 ± 0.18 |
| HPMCP-55 (200 mg) | 265 ± 111 | 78.25 ± 0.74 | 9.45 ± 0.08 |
| HPMCP-55 (300 mg) | 251 ± 95 | 70.08 ± 5.61 | 12.29 ± 0.87 |

[a]Amount of cefuroxime axetil in the formulations.

Figures 1, 2, and 3 show the release profiles of CAT, HPMCP-55, and HPMCP-50 microspheres, respectively. The common feature in these profiles is that they are highly influenced by the pH of the release medium. At the lower limit of the dissolution pH of the polymers very little CA was released, while at the higher pH most of the drug was released in less of 30 min and the complete disappearance of the microspheres was observed. This dissolution behavior appeared to be adequate, allowing the complete release of CA into the small intestine and thus providing adequate opportunity for drug absorption. It can also be observed that, at the lower pH limit, HPMCP microspheres totally prevented the release of CA, whereas CAT microspheres released 50% of their content between 30 and 60 min. Therefore, HPMCP-50 and -55 microspheres appeared to be more suitable than CAT microspheres as pH-sensitive delivery systems for CA.

Table 2 shows the results of the compatibility studies between the CA and the polymers employed in the microencapsulation process in the presence of acetone. The chromatographically identified hydrolysis products of the drug are cefuroxime, $\Delta^2$ isomers, and β-sulfoxides. Results showed that, following the exposure of CA to an acetonic solution of CAT, the percentage of impurities, with respect to the total product recovered, was very high. In particular, the amount of $\Delta^2$ isomers was high, a fact that showed the usefulness of this polymer

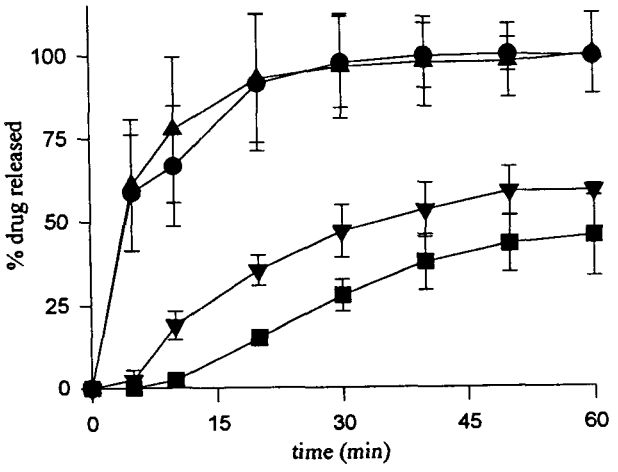


**Figure 1.** Cumulative release of CA from CAT microspheres, prepared with different CA quantities (200–300 mg), in two release media (pH 5.0–6.0): ●, CAT(200 mg) at pH 6.0; ■, CAT(200 mg) at pH 5.0; ▲, CAT(300 mg) at pH 6.0; ▼, CAT(300 mg) at pH 5.0

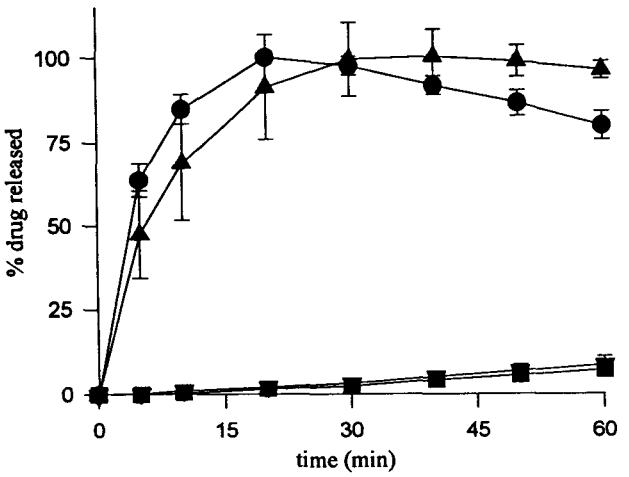


**Figure 2.** Cumulative release of CA from HPMCP-55 microspheres, prepared with different CA quantities (200–300 mg), in two release media (pH 5.2–6.0): ●, HPMCP-55(200 mg) at pH 6.0; ■, HPMCP-55(200 mg) at pH 5.2; ▲, HPMCP-55(300 mg) at pH 6.0; ▼, HPMCP-55(300 mg) at pH 5.2

for the encapsulation of CAT. On the other hand, the low levels of impurities observed upon contact of drug with HPMCP-50 and -55 indicated an adequate compatibility between them.

The results of CA stability in the developed formulations correlated with the degradation of the drug following its encapsulation within CAT microspheres (Table 3). Nevertheless, in this study it was also ob-

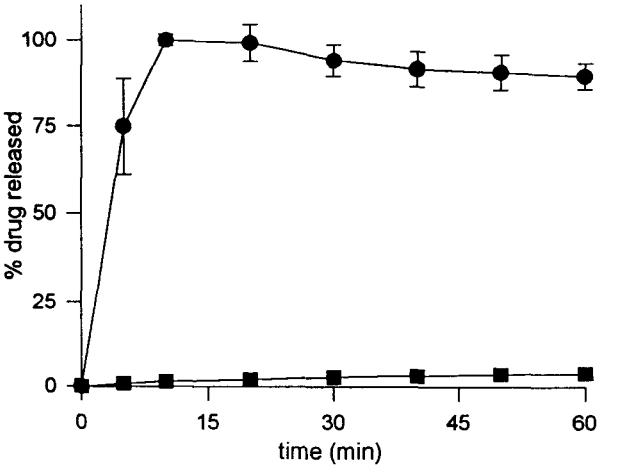


**Figure 3.** Cumulative release of CA from HPMCP-50 microspheres, prepared with 200 mg of CA, in two release media (pH 4.8–6.0): ●, pH 4.8; ■, pH 6.0.

*Table 2*

*Compatibility Study Between CA and the Polymers CAT, HPMCP-50, and HPMCP-55*

| | Impurities[a] | | | | |
|---|---|---|---|---|---|
| | Cefuroxime | $\Delta^2$ | β-Sulfoxide | Other | Total |
| CA standard | 0.2 | 0.5 | 0.1 | 0.7 | 1.5 |
| CA/CAT/acetone | 2.3 | 2.7 | — | 1.8 | 6.8 |
| CA/HPMCP-50/acetone | 1.5 | 0.4 | — | 1.2 | 3.1 |
| CA/HPMCP-55/acetone | 1.5 | 0.4 | — | 0.7 | 2.6 |

[a]Relative percentage of peak area obtained by normalization.

*Table 3*

*Stability of CA Encapsulated in CAT, HPMCP-50, and HPMCP-55 Microspheres*

| | Impurities[a] | | | | |
|---|---|---|---|---|---|
| | Cefuroxime | $\Delta^2$ | β-Sulfoxide | Other | Total |
| CAT (300 mg) | 1.0 | 0.7 | — | 2.9 | 4.6 |
| HPMCP-50 (200 mg) | 3.8 | 1.8 | 0.7 | 1.2 | 7.6 |
| HPMCP-55 (300 mg) | 0.7 | 1.5 | — | 1.2 | 3.4 |
| HPMCP-55 (300 mg)[b] | 0.4 | 0.9 | 0.2 | 0.4 | 1.9 |

[a]Relative percentage of peak area obtained by normalization.
[b]Freeze-drying microspheres.



served that the percentage of CA impurities in the HPMCP-50 microspheres is very high. Since CA was found to be compatible with HPMCP-50 in an acetonic solution for 1 hr, the increased amount of impurities detected in these microspheres could be attributed to the prolonged contact of the drug and the polymer acetonic solution (3 hr) during the microsphere preparation process. To conclude, among the formulations designed, HPMCP-55 microspheres represented the best alternative for ensuring the stability of CA. Additionally, HPMCP-55 microspheres were freeze-dried prior to the analysis of their content and it was observed that the stability greatly improved, probably due to the elimination of solvent traces.

Based on these results, the formulation composed of HPMCP-55 microspheres was selected for a taste-masking test. Results presented in Table 4 clearly indicate that the suspension containing the microspheres is superior in taste properties compared to a standard formulation with fruit taste.

*Table 4*

*Results of Taste-Masking Test*

| Formulation | Scores[a] | | | |
|---|---|---|---|---|
| HPMCP-55 blank | 0 | 0 | 0 | 0 |
| HPMCP-55 (200 mg) | 1 | 0 | 1 | 0 |
| Standard formulation | 2 | 3 | 2 | 2 |

[a]Scores: 0, tasteless; 1, acceptable taste; 2, slightly bitter; 3, very bitter taste.

## CONCLUSIONS

CA was efficiently encapsulated within HPMCP-55 microspheres by a solvent extraction procedure. These microspheres remain intact when exposed to an acidic environment but release their total content within 20 min of exposure to a medium of pH 6.0. Consequently, the HPMCP-55 microspheres represent a useful vehicle in

masking the taste of CA and preventing its release in the stomach. Thus their formulation has potential use as an extemporaneous suspension.

## ACKNOWLEDGMENT

This work was supported by Laboratorios Glaxo Wellcome, S.A., Spain.

## REFERENCES

1. J. N. C. Healey, Enteric coatings and delayed drug release, in *Drug Delivery to the Gastrointestinal Tract*, Ellis Horwood Limited, Chichester, 1989.
2. P. Giundechi, U. Conte, and A. La Manna, Carbamazepine modified release dosage forms, Drug. Dev. Ind. Pharm., 17(13), 1753–1764 (1991).
3. D. Torres, G. García-Encian, B. Seijo, and J. L. Vila-Jato, Formulation and in vitro evaluation of HPMCP-microencapsulated drug–resin complexes for sustained release of diclofenac, Int. J. Pharm., 121, 239–243 (1995).
4. N. B. Dharamadhikari, S. B. Joshi, and N. C. Manekar, Preparation and in vivo evaluation of salbutamol sulphate microcapsules, J. Microencapsulation, 8(4), 479–482 (1991).
5. K. Lehmann, Coating of multiparticulate using polymer solutions, in *Multiparticulate Oral Drug Delivery*, Marcel Dekker, New York, 1994.
6. D. R. Friend, Polyacrylate resin microcapsules for taste masking of antibiotics, J. Microencapsulation, 9(4), 469–480 (1992).
7. G. Weib, A. Knoch, A. Laicher, F. Stanislaus, and R. Daniels, Microencapsulation of ibuprofen by a coacervation process using Eudragit L100-55 as an enteric polymer, Drug Dev. Ind. Pharm., 19(20), 2751–2764 (1993).
8. J. A. Bakan, T. C. Powell, and P. S. Szotak, Recent advances using microencapsulation of taste-masking of bitter drugs, in *Microcapsules and Nanoparticles in Medicine and Pharmacy*, Max Donbrow, New York, 1992.
9. P. Deasy, Core and coating properties, in *Microencapsulation and Related Drug Processes*, Marcel Dekker, New York, 1984.
10. D. B. Beten, M. Gelbcke, B. Diallo, and A. J. Moës, Interaction between dipyramidole and Eudragit S, Int. J. Pharm., 88, 31–37 (1992).
11. H. K. Lee, J. Hadju, and P. McGoff, Propranolol/methacrylic acid copolymer binding interaction, J. Pharm. Sci., 80, 178–180 (1991).
12. Eastman Kodak Publication, N. EFC-202C, October 1994.
13. Shin-Etsu Chemical, Analytical Methods and Specifications of HPMCP, September 1984.
14. H. Takenaka, Y. Kawashima, and S.-Y. Lin, Preparation of enteric-coated microcapsules for tableting by spray-drying technique and in vitro simulation of drug release for the tablet in GI tract, J. Pharm. Sci., 69(12), 1385–1392 (1980).
15. I. Maharaj, J. G. Nairn, and J. B. Campbell, Simple rapid method for the preparation of enteric-coated microspheres, J. Pharm. Sci., 73(1), 39–42 (1984).
16. S.-Y. Lin, Y. L. Tzan, C. N. Weng, and C. J. Lee, Preparation of enteric coated microspheres of *Mycoplasma hyopneumoniae* vaccine with cellulose acetate phthalate. I. Formation conditions and micromeritic properties, J. Microencapsulation, 8(3), 317–325 (1991).
17. J. W. Beyger and J. G. Nairn, Some factors affecting the microencapsulation of pharmaceuticals with cellulose acetate phthalate, J. Pharm. Sci., 75, 573–578 (1986).
18. S. P. Sanghvi and J. G. Narin, Phase diagram studies for microencapsulation of pharmaceuticals using cellulose acetate trimellitate, J. Pharm. Sci., 80(4), 394 (1991).
19. G. García-Encina, S. P. Sanghvi, and J. G. Nairn, Phase diagram studies of microcapsule formation using hydroxypropylmethylcellulose phthalate, Drug. Dev. Ind. Pharm., 18, 561 (1992).
20. *British Pharmacopeia*, 1993, addendum 1994.