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Parameters affecting in-liquid drying microencapsulation and release rate of cefaclor

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Abstract

An in-liquid drying microencapsulation method for producing sustained release cefaclor (CF) microspheres has been developed. Sieved fractions of CF monohydrate particles together with polyvinylpyrrolidone (M_W ~40 000) were dispersed in a solvent mixture of dichloromethane with cyclohexane (40:60 v/v) containing dissolved ethylcellulose (EC; 48–49.5% ethoxy content). Encapsulation was effected by adding the dispersion to a stirred aqueous medium saturated with CF. The resulting microspheres (collected overnight and oven-dried at 40°C) were examined for size and surface features by scanning electron microscopy, and for solid-state interactions and phase changes using differential scanning calorimetry (DSC), thermogravimetric analysis and powder X-ray diffractometry. The rate and extent of CF release in aqueous medium was measured at 37°C using the USP rotating basket method at 100 rpm. The size, degree of sphericity, and rate of CF release of the microspheres were all shown to depend on the CF to EC mass ratio and the stirring speed employed in encapsulation. Higher EC to CF mass ratio and lower stirring speed afforded microspheres with slower release rate of CF. The rate of CF release from the microspheres fit the simplified Higuchi's planar model equation, and for the samples prepared at various EC:CF ratios, exhibited a strong correlation with drug loading ($r = 0.98$; $n = 4$ (mean values); $p < 0.05$). DSC studies on the samples indicated an absence of molecular interactions between EC and CF in the microspheres. The results demonstrate that the in-liquid drying method, if appropriately optimized, could be used to produce sustained-release CF microspheres with the desired release pattern and physical properties.

Keywords: In-liquid drying microencapsulation; Drug-polymer ratio; Stirring speed; Cefaclor-ethylcellulose microspheres; Sustained release

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1. Introduction

In antibiotic therapy, maintenance of adequate drug level in the blood or plasma for the required dosing period is crucial to the treatment of bacterial infections. However, this can be difficult to attain, particularly with the β-lactam antibiotics, since they undergo rapid elimination from the systemic circulation following administration. This problem is of particular concern in paediatric patients whose rate of antibiotic elimination is normally faster than that in adults (Harvey, 1990). While frequent dosing should, in principle, be able to sustain the required antibiotic level, this may be difficult to achieve in practice, primarily because of compliance difficulties, and may lead to sub-therapeutic drug levels and poor control of the infection.

Aimed at improving antibiotic therapy, the present study has examined the feasibility of employing a continuous in-liquid drying microencapsulation technique (Thies, 1992) to develop an oral sustained-release preparation of cefaclor (CF) for once-daily administration. CF (chemically designated as 3-chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid) is a broad-spectrum cephalosporin antibiotic widely prescribed for treating otitis media in paediatric patients (Huls et al., 1992). Frequent dosing is typical for this class of antibiotics, which requires three times daily oral administration. It can, therefore, be envisaged that formulation of an oral sustained-release preparation of this antibiotic, particularly one suitable for use in paediatrics, will greatly enhance patient compliance. From a physicochemical perspective, CF is amphoteric ($pK_a = 1.5$, 7.17 (carboxyl, amino)) and exists as a monohydrate in the solid state; the anhydrous form melts at about 220°C with decomposition (Lorenz, 1980). The drug is moderately soluble in water (~10 mg/ml) and very slightly soluble in most organic (non-aqueous) solvents (< 0.5 mg/ml). This large solvent-dependent difference in solubility renders CF a particularly suitable drug candidate for microencapsulation with ethylcellulose (EC) using an in-liquid drying technique. The specific objectives of the present investigation were to assess the influence of process variables on the morphology, physical properties and drug release characteristics of the encapsulated products; and to identify the major process variables governing drug release rate.

2. Materials and methods

2.1. Reagents and materials

CF monohydrate (98% purity) and polyvinylpyrrolidone (M_W ~40 000) were purchased from Sigma (St. Louis, MO). Cyclohexane, hexane, and chloroform were of analytical grade and supplied by BDH, UK, Malinckrodt, USA, Labscan, Ireland, respectively. EC (48–49.5% ethoxy content; 7 mPa/s) was obtained from Riedel-de Haën, Germany. All water used was double distilled.

2.2. Preparation of CF microspheres

Sieved fractions (250–355 μm) of accurately weighed CF monohydrate particles (1–3 g) were dispersed together with polyvinlypyrrolidone (PVP; M_W 40 000; 1.2 g) in a solvent mixture (40 ml) of either dichloromethane (DCM) with cyclohexane (CHX) (40:60 v/v) or DCM with hexane (HX) (50:50 v/v) containing dissolved EC (48–49.5% ethoxy content; 1–3 g). The compositions of the mixtures were so chosen that their densities approximated to that of the aqueous external phase. This was to ensure that the internal phase (containing the dispersed particles) remained well dispersed in the external phase during the encapsulation process. Encapsulation was effected by adding the dispersion to an aqueous saturated solution of CF (400 ml) stirred continuously by means of a motor-driven propeller (Heidolph RZR 2051) at a defined speed (700–900 rpm) in a 1-l reaction vessel at 20 ± 1°C. The aqueous encapsulation medium was saturated with CF to minimize the dissolution of the CF particles and to maximize their incorporation into the EC. The resulting microspheres were collected overnight by vacuum filtration and oven-dried at 40°C for 48 h prior to analysis.

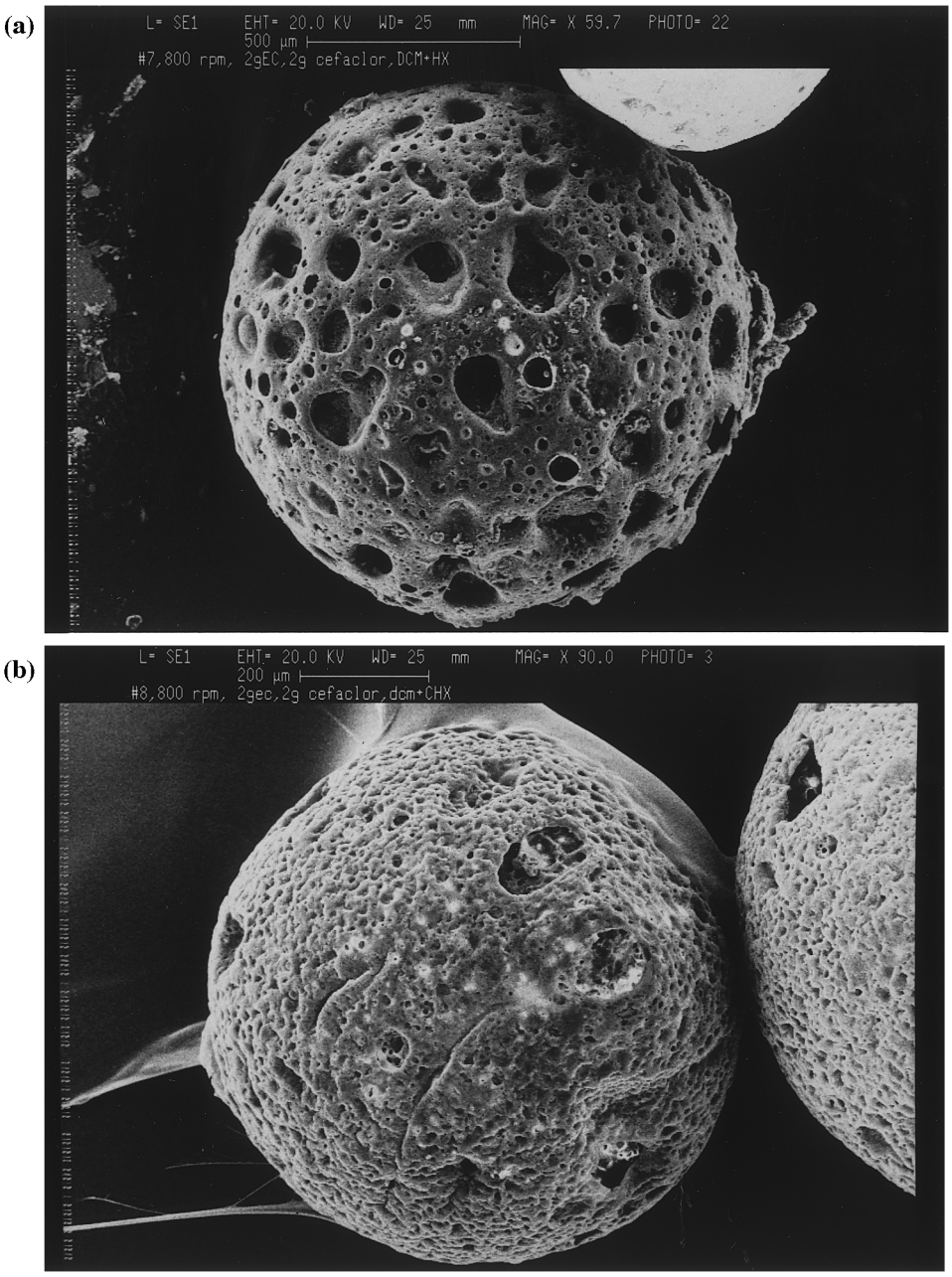


Fig. 1. Scanning electron photomicrographs of representative CF microspheres prepared from (a) DCM and hexane solvent system; and (b) DCM and cyclohexane solvent system.

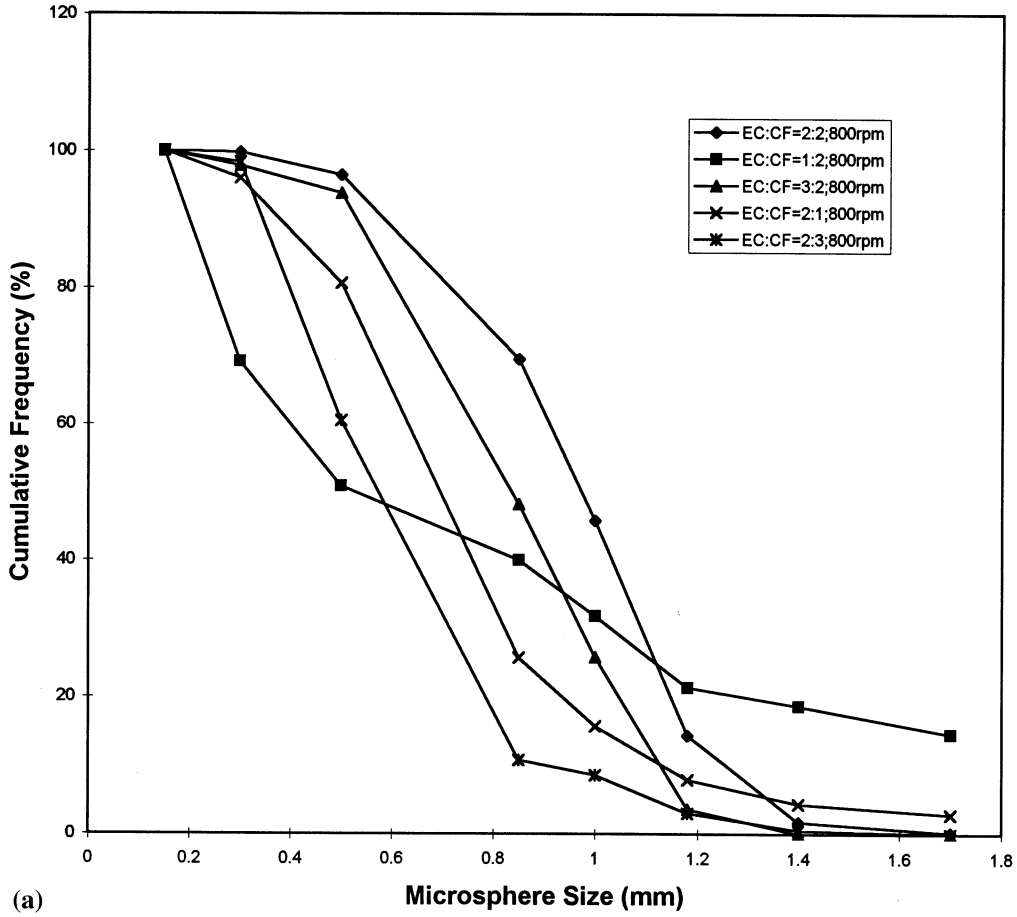


Fig. 2. (a) Cumulative frequency versus size of CF microspheres prepared at various CF:EC ratios and at 800 rpm. (b) Cumulative frequency versus size of CF microspheres prepared at various stirring speeds and at a CF:EC ratio of 2:2.

2.3. Scanning electron microscopy (SEM)

The microspheres were coated with gold in a sputter coater (Polaron SC502 and Polaron CA508, VG Microtech) and examined for morphological features using a scanning electron microscope (Stereoscan 360, Leica Cambridge).

2.4. Determination of microsphere size distribution

Weighed samples were gently sieved (to avoid the build-up of static charges) into various fractions using brass sieves in the size range of 150–1700 μm. The fractions were weighed and expressed in terms of % weight (% frequency) and cumulative % weight (cumulative % frequency). The data were analyzed for normal or log-normal distribution.

2.5. Powder X-ray diffraction (PXRD)

Samples were ground in a mortar with a pestle before being layered into a standard sample holder. Gentle to moderate force was required to grind the raw materials to the appropriate size for the analysis while the microsphere samples required vigorous grinding to attain similar sizes. The analysis was conducted in a Siemens D5000 X-ray diffractometer using CuKα X-rays. The samples were scanned from $2\theta = 1.5$–60° at a speed of 1°/min.

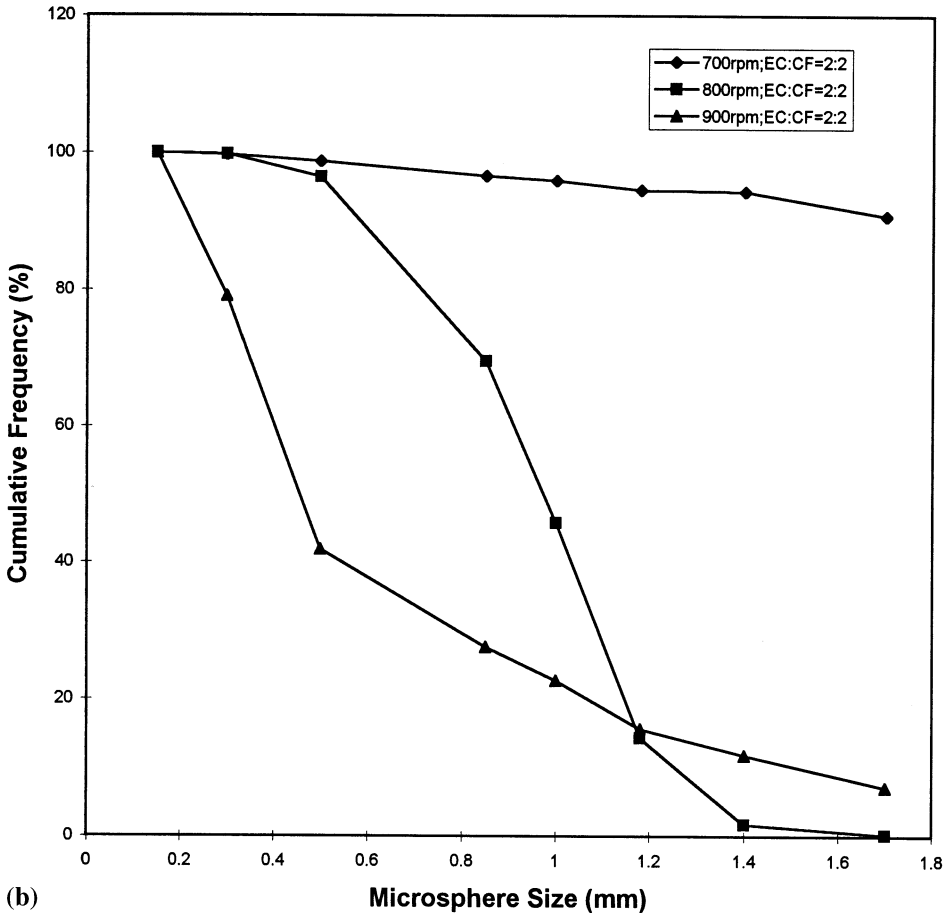


Fig. 2. (*Continued*)

2.6. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

The melting point, T_m, of CF and the glass transition temperature, T_g, of EC in the microsphere samples (2–4 mg) were determined in non-hermetically sealed aluminum pans heated at a rate of 10°C/min in a DSC (Perkin Elmer DSC 6) using nitrogen as the purge gas and indium as the calorimetric standard.

The raw materials (CF monohydrate and EC) and representative microsphere samples were subjected to TGA in a thermogravimetric analyzer (Hi Res TGA 2950, TA). Each weighed sample was heated in a platinum pan at a rate of 10°C/min under nitrogen purge.

2.7. Determination of CF loading in microspheres

Samples (50 mg) were thoroughly ground in a mortar using a pestle. Sufficient water (50 ml) was then added to dissolve the CF present. The undissolved EC was filtered off, and the clear filtrate was made up to 100 ml prior to CF assay by UV spectrophotometry (UV-160A Shimadzu spectrophotometer) at 265 nm.

2.8. Determination of CF release rate

The release rate of CF from microspheres (0.5 g) in water (1 l) at 37°C was determined in triplicate in an automated eight-spindle dissolution tester (Erweka DT 80, Heusenstamm, Ger-

Table 1
Particle sizes of CF microspheres

Preparation conditions		Particle size	
Stirring speed (rpm)	EC:CF ratio (w/w)	Geometric mean diameter, d_g (μm)	Geometric standard deviation, σ_g
700	2:2	—[a]	—
900	2:2	500	2.13
800	2:2	940	1.22
800	1:2	570	3.00
800	3:2	780	1.30
800	2:1	650	1.51
800	2:3	570	1.43

[a] Above upper size limit of sieve (1700 μm) used.

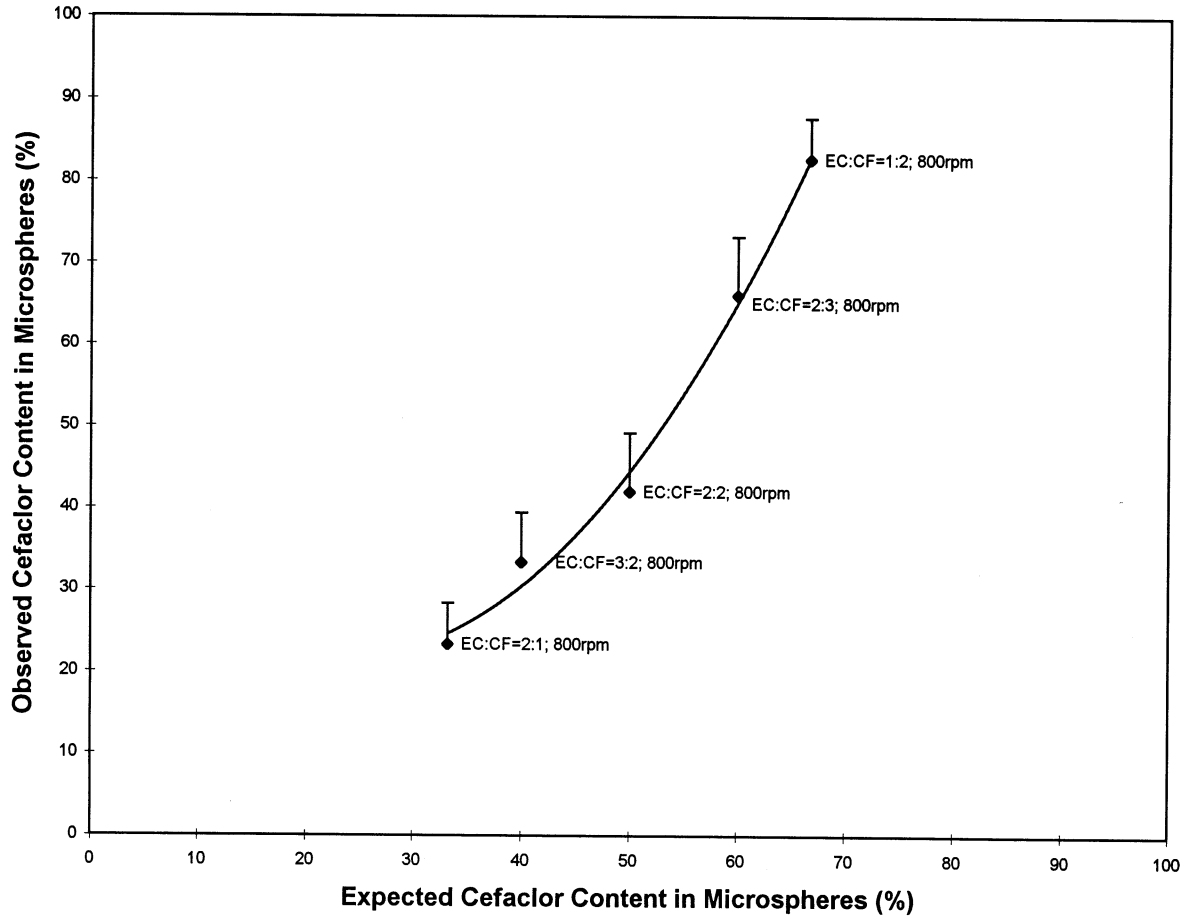


Fig. 3. Observed CF content in microspheres versus expected CF content in microspheres. The vertical bars depict standard deviations of triplicate determinations.

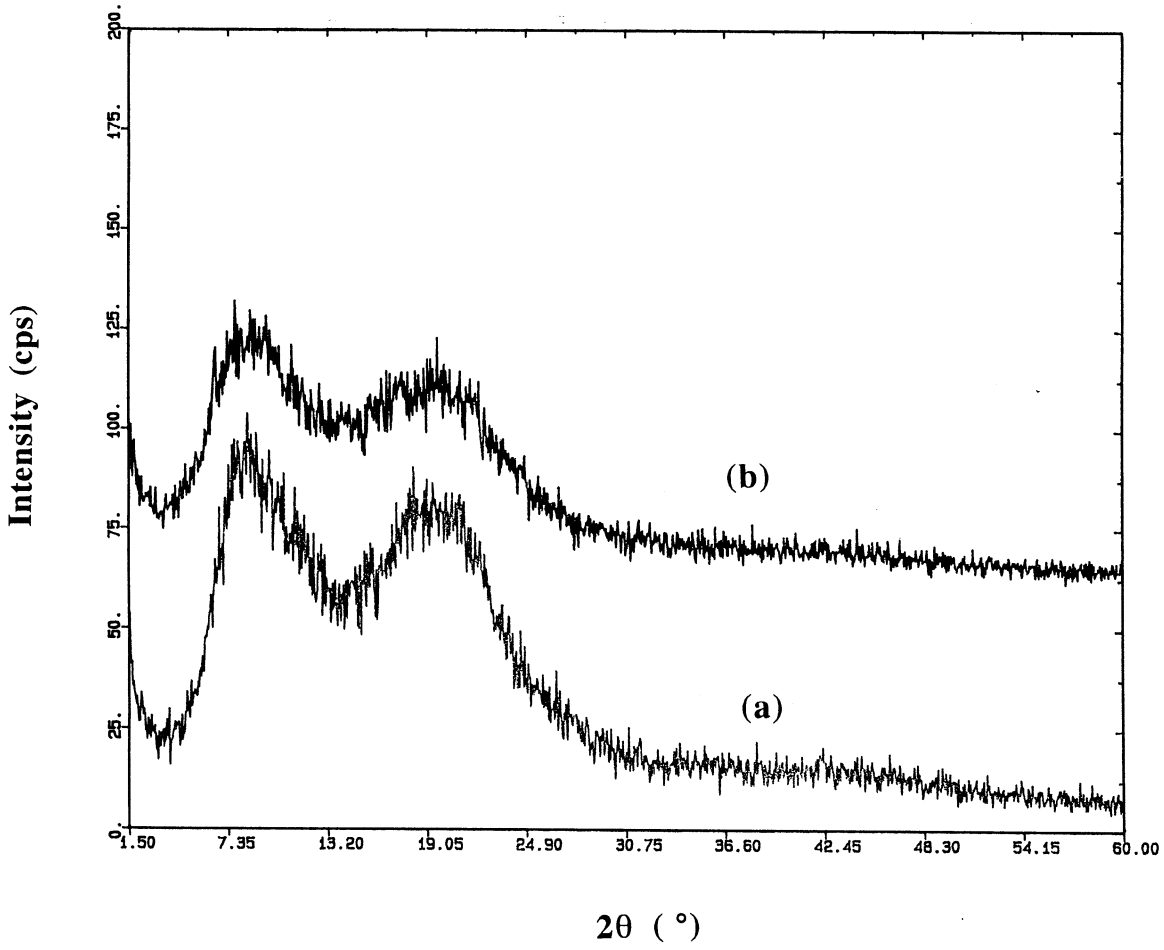


Fig. 4. X-ray diffraction patterns of (a) EC; and (b) ground CF microsphere sample.

many) employing the USP (1990) dissolution method 1 with the basket rotating at 100 rpm. Filtered samples (3 ml) were withdrawn at selected time intervals and analyzed for CF concentrations by UV spectrophotometry at 265 nm.

The release-time profiles of CF were analyzed using the following simplified form of Higuchi's equation (Higuchi, 1963):

$$Q_t = \frac{M_t}{A} = [K_t]^{1/2} \quad (1)$$

or,

$$M_t = A[K_t]^{1/2} \quad (1a)$$

and

$$K = \frac{2C_t C_s D\epsilon}{\tau}$$

where Q_t is the amount of drug released (M_t) per unit area (A) in time t from matrix; C_t is the total amount of drug present in the matrix per unit volume; C_s is solubility of the drug in the water-filled pores; D is the diffusion coefficient of the drug in liquid which fills the pores; ϵ is porosity of the matrix; and τ tortuosity of the pores or channels in the matrix. The use of Eq. (1) in the present data analysis carries the following

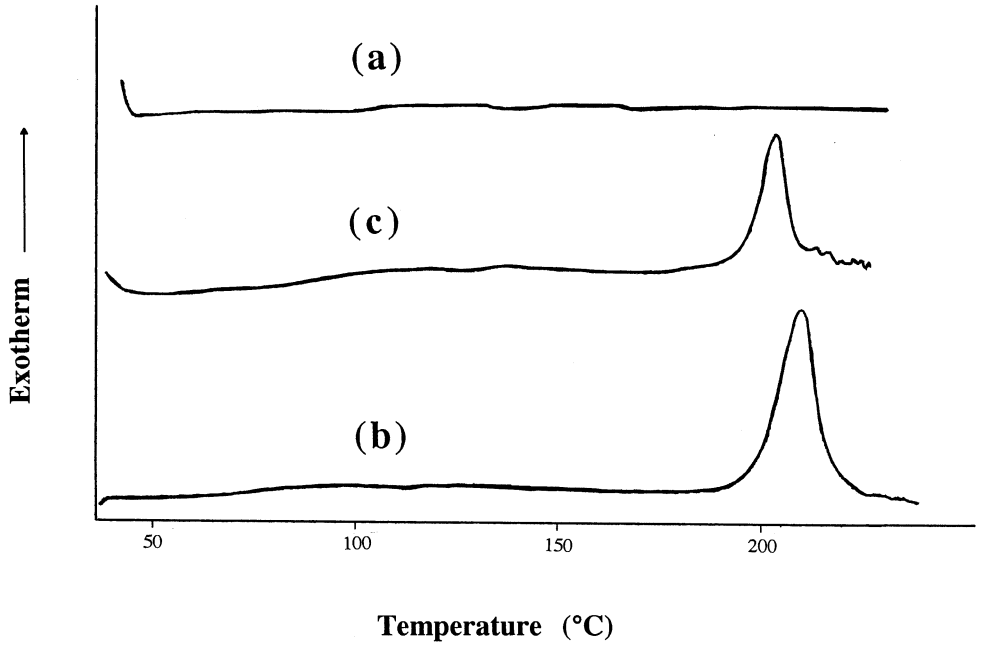


Fig. 5. DSC thermograms of (a) EC; (b) CF monohydrate; and (c) CF microsphere sample.

assumptions: a) the drug particles are uniformly dispersed in the matrix; b) $C_t \gg C_s$; c) the drug concentration is zero at the interface between the matrix and the external medium; and d) the porosity and tortuosity of the matrix and the surface area of drug being exposed during dissolution remain essentially invariant with time (at least in the early stages of dissolution). Thus, a plot of M_t versus $t_{1/2}$ will yield a straight line.

3. Results and discussion

3.1. Microsphere morphology and size distribution

The choice of solvent systems for the internal phase in the present study was guided by their density, boiling point and degree of miscibility with the aqueous external phase. In order to facilitate the encapsulation of the CF particles, the internal phase should preferably have a density comparable to that of the aqueous external phase (to aid uniform dispersion of the internal phase), a boiling point well above room temperature (to allow slow evaporation of solvent) and complete immiscibility with the external phase (to enable emulsion formation and to prevent rapid loss of the organic solvent into the aqueous external phase). Preliminary studies employing two solvent mixtures, namely, DCM-HX and DCM-CHX, indicated that the latter solvent system afforded less porous, and more spherical microspheres (Fig. 1), mostly in the size range of 300–1500 μm, and was therefore employed in the subsequent formulation development.

The particle size data of various microsphere samples approximated well a log-normal distribution, as determined by log-normal probability plots. As shown in Fig. 2a,b and Table 1, the size/size distribution of the microspheres depended strongly on both the EC:CF ratio and the speed of stirring employed in the production. In general, increasing the proportion of EC or reducing the stirring speed increased the size of microspheres. This could be explained by an increased resistance of the dispersed phase to size reduction, since the viscosity of the dis-

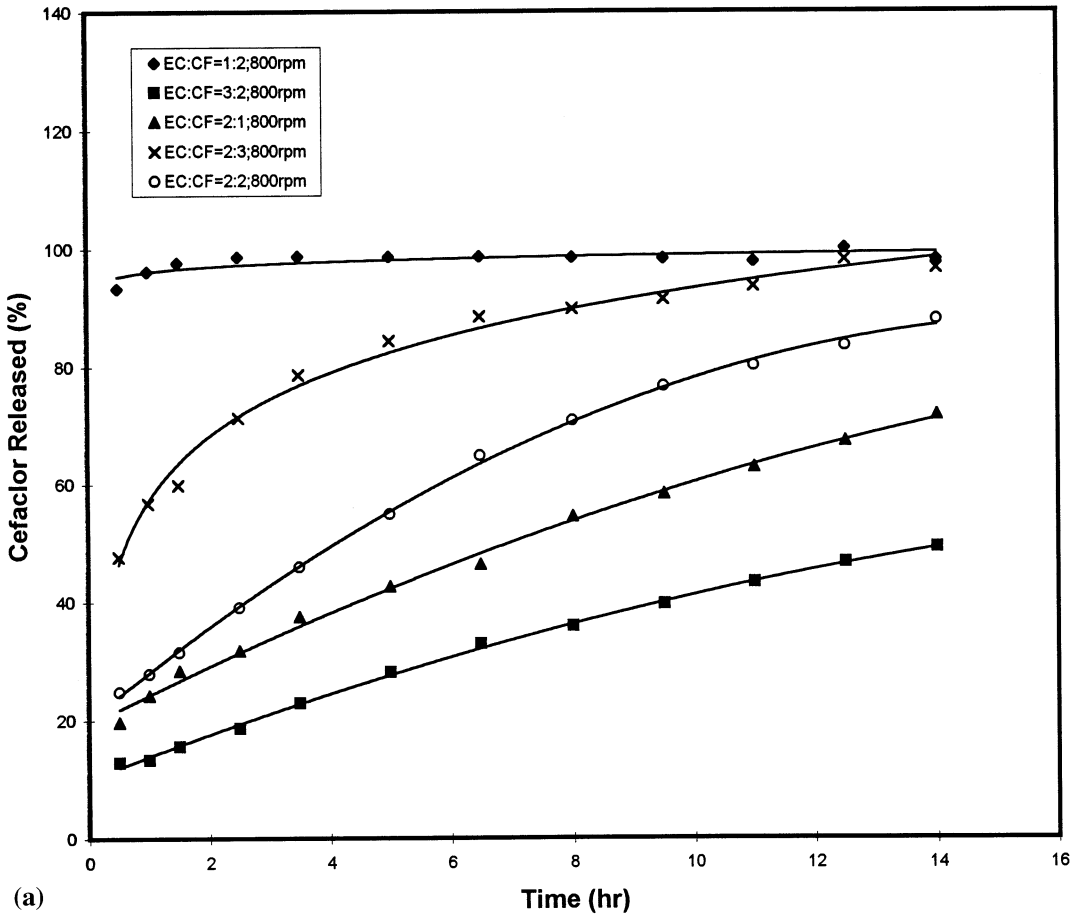


Fig. 6. (a) Release–time profiles of CF from microspheres prepared at various CF:EC ratios and at 800 rpm. Each data point is the mean of triplicate determinations; the coefficient of variation ranges from 7 to 11%. (b) Release–time profiles of CF from microspheres prepared at various stirring speeds and at a CF:EC ratio of 2:2. Each data point is the mean of triplicate determinations; the coefficient of variation ranges from 6 to 13%.

persed phase increased with an increase in EC content or as the stirring speed was reduced.

3.2. Efficiency of encapsulation

The % efficiency of CF encapsulation was calculated by multiplying the ratio of the CF content determined for the microspheres to that actually used in the encapsulation by 100.

The extent of CF encapsulation was closely related to the EC:CF ratio used (Fig. 3), while the percent efficiency of CF encapsulation showed a somewhat different dependence. The samples prepared at EC:CF ratios of 1:2 and 2:3 had an encapsulation efficiency of over 100% (124 and 110%, respectively), reflecting either a partial loss of EC or incomplete coating of CF particles during the encapsulation. The encapsulation efficiency was almost identical (83%) for the samples produced at EC:CF ratios of 2:2 and 3:2 while the sample formulated at an EC:CF ratio of 2:1 had an encapsulation efficiency of 70%.

With the EC:CF ratio being fixed at 2:2, increasing the stirring speed from 700 to 900 rpm during the encapsulation enhanced both the extent and efficiency of encapsulation in a linear manner ($r = 0.99$; $n = 3$; $p < 0.05$), from 38 and 76% to 49 and 98% respectively.

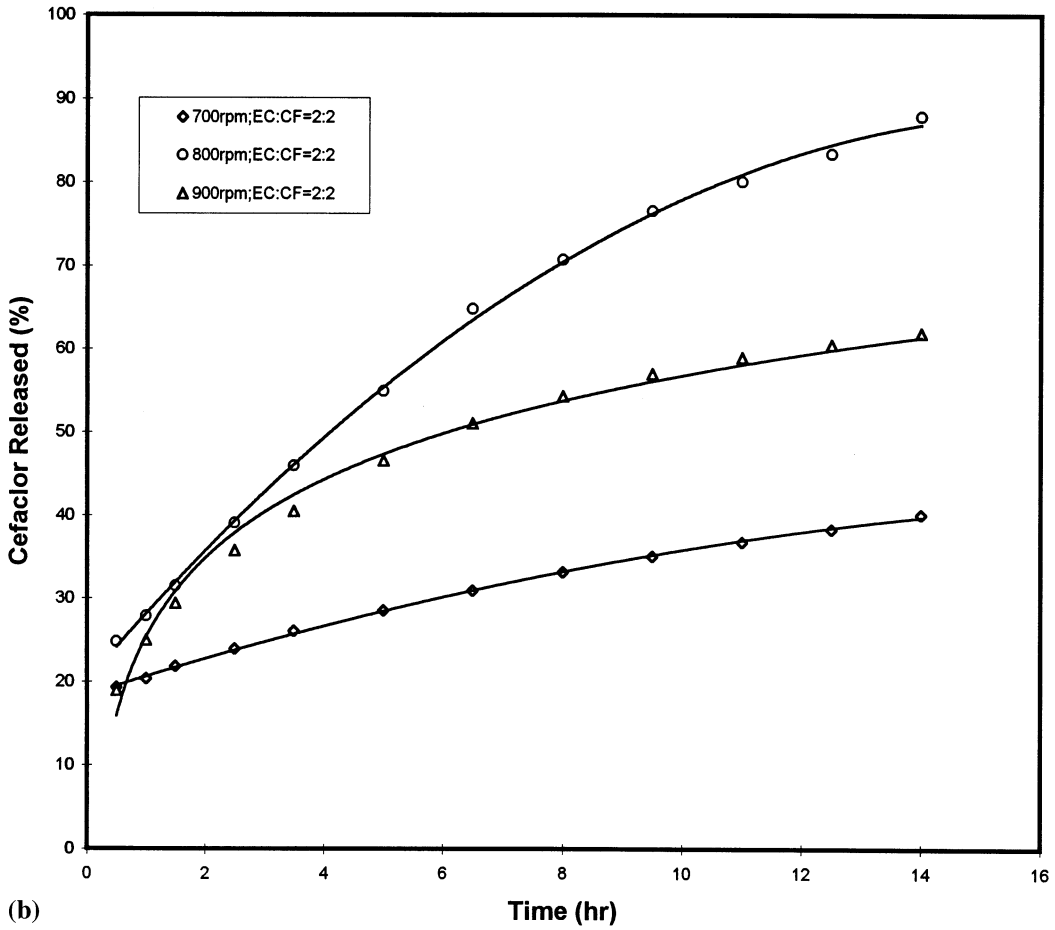


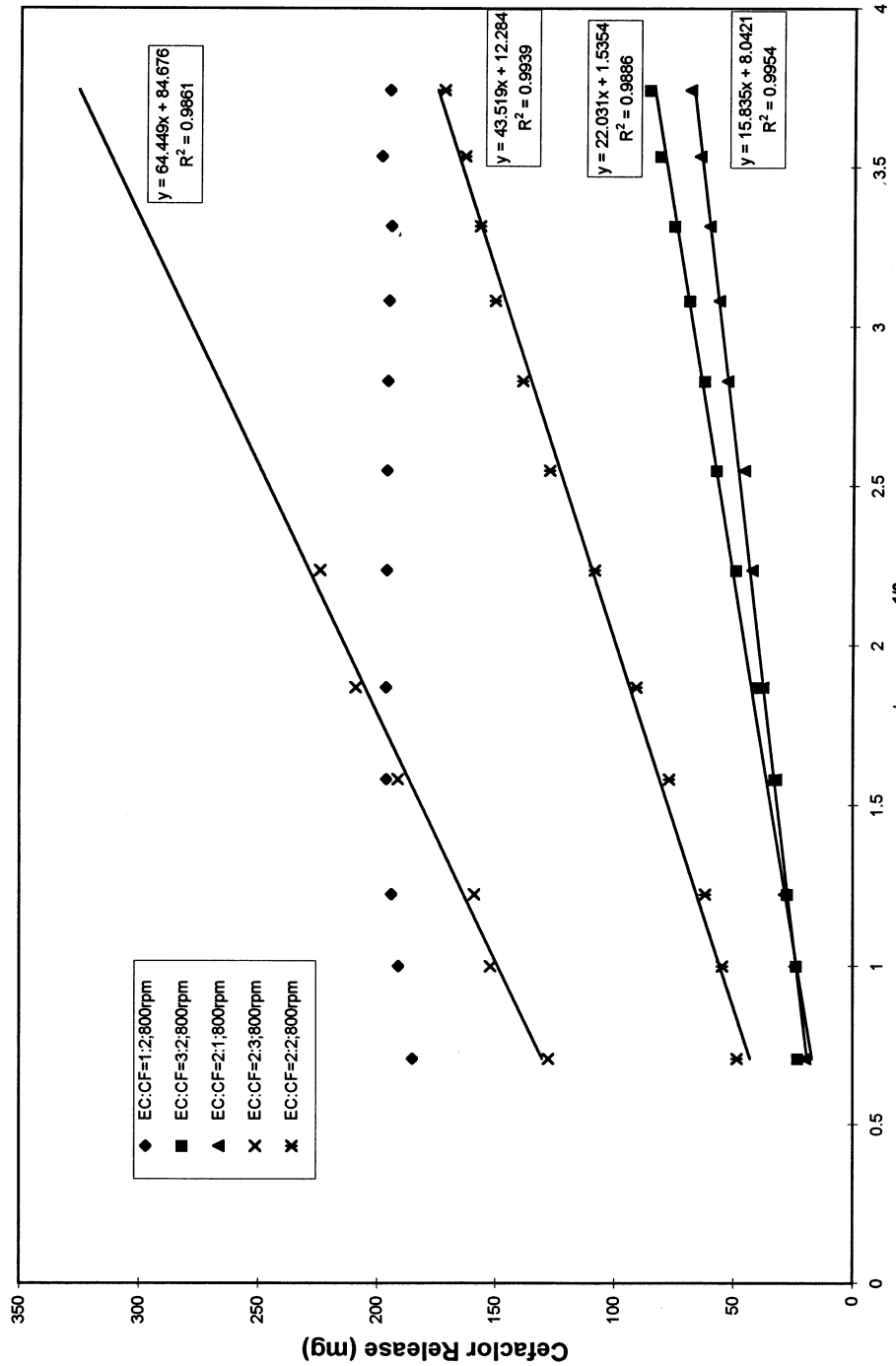
Fig. 6. (*Continued*)

The above observations suggest that for optimal encapsulation of the CF particles, the amount of EC used should be comparable to that of CF, and the agitation rate should be no less than 800 rpm.

3.3. PXRD, DSC analysis and TGA

PXRD studies on pure EC revealed a broad shallow peak reflecting a lack of crystallinity, while pure CF monohydrate showed characteristic diffraction peaks at $2\theta = 6.5$, 13.2, 16.2, 17.2, 17.5, 18.6, 25, 26.8°. Because of the considerable shearing force required to grind the microspheres to the appropriate size for the X-ray diffraction analysis, the ground samples appeared to be X-ray amorphous with a broad shallow peak resembling that of pure EC (Fig. 4).

DSC analysis of the EC material showed a glass transition at around 125–130°C (T_g; Fig. 5a). Pure CF monohydrate exhibited no apparent dehydration peak between 100 and 120°C, but an exothermic peak at 196°C, which was probably due to decomposition of the anhydrate upon melting (Fig. 5b). TGA on the CF monohydrates showed a weight loss of ~0.044% between 40 and 80°C, corresponding to a loss of 1 mol of water from 1 mol of CF monohydrate; and a massive weight loss at ~185°C, corresponding to decomposition of the anhydrate. The microsphere samples displayed thermal changes similar to those of the EC and CF



(a)

Fig. 7. (a) Plot of CF release versus square root of time for microspheres prepared at various CF:EC ratios and at 800 rpm. Each data point is the mean of triplicate determinations; the coefficient of variation ranges from 7 to 11%. (b) Plot of CF release versus square root of time for microspheres prepared at various stirring speeds and at a CF:EC ratio of 2:2. Each data point is the mean of triplicate determinations; the coefficient of variation ranges from 6 to 13%.

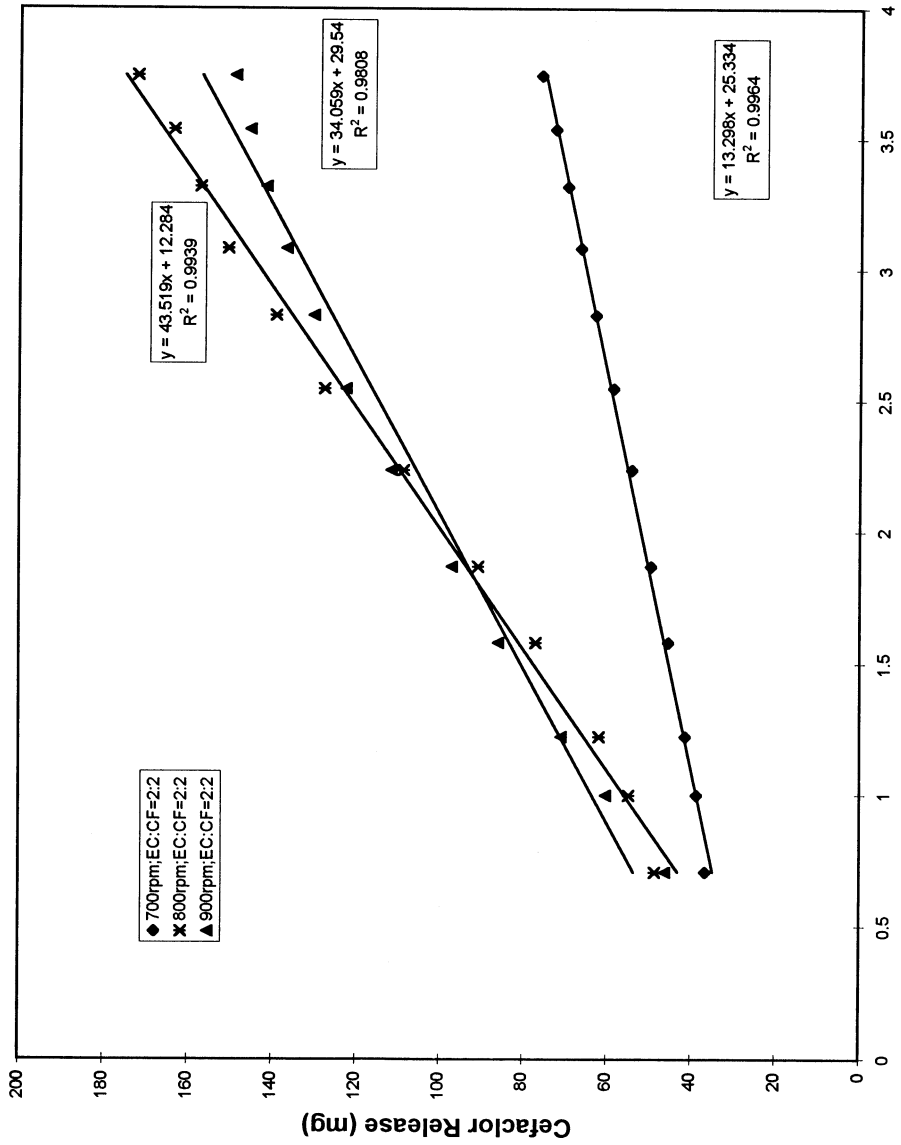


Fig. 7. (*Continued*)

physically mixed together, viz. glass transition of EC at around 125–130°C, partial weight loss of the samples between 40 and 80°C (due to the loss of water) and exothermic decomposition (accompanied by massive weight loss) of the dehydrated CF upon melting (Fig. 5c).

3.4. Release–time profiles of CF from microspheres

The rate of CF release from the microspheres demonstrated marked dependence on both the EC:CF ratio and the stirring speed used in the encapsulation (Fig. 6a,b).

The samples formulated at an EC:CF ratio of 1:2 exhibited the most rapid release rate with complete release occurring in less than 1 h, consistent with incomplete coating with EC. In general, a slow release rate was associated with a higher proportion of EC or a slower stirring speed (700 rpm) being used.

The rate of CF release from all the microsphere samples fit the simplified Higuchi's equation (Eq. (1); Fig. 7a,b), suggesting that the release of CF is controlled by diffusion. The samples prepared at an EC:CF ratio of 1:2 could not be analyzed by Eq. (1) since all the CF was released in less than 1 h. Samples prepared using higher EC:CF ratios exhibited CF release rates which correlated strongly ($r = 0.98$; $n = 4$ (mean values); $p < 0.05$) with drug loading, suggesting that the release of CF can be regulated by varying the EC:CF ratio or the drug loading in the microspheres. A change in stirring speed in the encapsulation brought about a change in drug loading, but no statistically significant linear correlation ($r = 0.55$; $n = 3$ (mean values)) between drug release rate and drug loading was discernable in this case, implying that factors other than drug loading (e.g. porosity) may play a role.

4. Conclusions

A number of conclusions can be drawn from these studies.

Firstly, the sizes/size distributions of the microspheres, degree of CF encapsulation and the rate and extent of CF release depend on both the EC:CF ratio and the stirring speed used in encapsulation.

Secondly, the encapsulated CF particles show no significant physical bonding interactions with the EC in the microspheres, as shown by DSC, thus ruling out the possible involvement of such interactions in the CF release process.

Thirdly, the release rate of CF from the microspheres fit the simplified Higuchi's equation and, for the samples prepared at various EC:CF ratios, correlated strongly with drug loading, suggesting that the release process is diffusion-controlled and the rate of release can be systematically controlled by changing the EC:CF ratio in the encapsulation or the CF loading in the microspheres.

Lastly, the results demonstrate that the in-liquid drying method, if appropriately optimized, can be used to produce sustained-release CF microspheres with the desired release pattern and physical properties.

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