

## The Effect of Polyethylene Glycol on Drug Content, Particle Morphology, and Carbamazepine Release Profiles of Sustained Release Microspheres prepared from Cellulose Acetate Butyrate

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**SUMMARY.** Blends of cellulose acetate butyrate (CAB) and polyethylene glycol 1500 (PEG 1500) were used to prepare sustained release carbamazepine-loaded microspheres by the emulsion-solvent evaporation method. The effect of CAB molecular weight (MW 30000 or 70000) and addition of PEG 1500 to the formulations on drug content, particle size, and CBZ release rate were evaluated using a 2<sup>2</sup> factorial design. The CBZ encapsulation efficiency (%) and the drug content varied from 65 to 70% and from 20 to 24% (w/w), respectively. The mean particle diameter varied from 300 to 1400 µm. The addition of PEG 1500 to the formulations led to an increase in surface porosity due to the diffusion of this polymer towards the external phase of the emulsion. The absence of PEG 1500 in the particles was confirmed by FTIR. The statistical analysis revealed that the release rate of carbamazepine was significantly increased when CAB (MW 30000) and PEG 1500 were used to prepare the microspheres.

**RESUMEN.** “Efecto del Polietilenglicol sobre la Carga de fármaco, Morfología y Perfil de Liberación de Carbamazepina en Microesferas de Liberación Prolongada de Acetobutirato de Celulosa”. El objetivo del presente trabajo ha sido el de evaluar el efecto del peso molecular de acetobutirato de celulosa (ABC) (PM 30000 y 70000) y la presencia de polietilenglicol 1500 (PEG 1500) en el tamaño de microesferas preparadas a partir de mezclas de dichos polímeros mediante el método de emulsificación- evaporación del disolvente y cargadas con carbamazepina. Asimismo, se evaluó el perfil de liberación del fármaco a partir de las formulaciones, empleando un diseño experimental 2<sup>2</sup>. Las formulaciones presentaron un tamaño medio entre 300 y 1400 µm, y una eficiencia de asociación y carga de fármaco entre 65-70% y 20-24%, respectivamente. La difusión del PEG para la fase externa de la emulsión resultó en una mayor porosidad de las partículas. El análisis estadístico de los resultados pone de manifiesto un aumento significativo en la liberación de carbamazepina a partir de los sistemas conteniendo ABC con peso molecular de 30000 y conteniendo el PEG 1500 en la fase interna de la emulsión.

### INTRODUCTION

In the last decade, the interest in polymer blending has grown mainly due to the fact that a material property can be modified without undertaking the synthesis of a new compound. In the pharmaceutical field, polymeric blending has been exploited as a way to modify the release properties of drugs from matrix delivery systems. Matrix properties of microspheres such as swelling, permeability, and also biodegradation can be varied in order to obtain a proper drug release profile <sup>1,2</sup>. In several instances, the modification of the release profile of a drug is achieved when a second polymer presenting a hydrophilic nature is added to the microsphere formulation, which is generally based on hy-

drophobic polymers. It has been demonstrated that the presence of a hydrophilic polymer in the matrix system increases the water content of particles and/or induces pore formation improving both dissolution and diffusion of the drug <sup>1,3</sup>.

Cellulose esters such as cellulose acetate, cellulose acetate propionate, and cellulose acetate butyrate are commercially available in many different molecular weights and with different substitution degrees, and can be used as excellent hydrophobic film-formers, suitable for encapsulation purposes. In particular, cellulose acetate butyrate (CAB) is a water insoluble polymer that has been employed to prepare direct-compression matrices and has been used as

**KEY WORDS:** Carbamazepine, Cellulose acetate butyrate, Microspheres, Polyethylene glycol, Polymeric blends.

**PALABRAS CLAVE:** Acetobutirato de celulosa, Carbamazepina, Mezclas poliméricas, Microesferas, Polietilenglicol.

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a semi-permeable membrane for osmotic pumps<sup>4</sup>. Also, this polymer has been used to obtain sustained release microparticles using the emulsion-solvent evaporation method. The drug release from CAB microparticles has been shown to be affected mainly by the particle size, the molecular weight of the polymer, and the drug to polymer ratio in the formulation<sup>5-7</sup>.

Polyethylene glycols are hydrophilic polymers which can be used as plasticizer in the formulation of solid dosage forms. The addition of polyethylene glycols to membranes prepared from cellulose esters makes them smoother and less compact. Furthermore, these polymers have been used to change the release rate of drugs from microspheres. Bezemer *et al.*<sup>2</sup> have demonstrated that the release profile of the encapsulated model protein lysozyme can be tailored by variation of the composition of PEG block, when this polymer is covalently bonded in the hydrophobic poly(butylene terephthalate). In other approach, Yeh *et al.*<sup>8</sup> verified that when blends of PEG 8000 and poly acid lactide-co-glycolide were used to prepared ovalbumin-loaded microspheres, the release burst effect and latency time were reduced due to the formation of pores and channels after dissolution of the polyethylene glycol present in the matrix structure followed by the extraction of encapsulated protein.

In this context, the aim of this work was to evaluate the effect of the addition of polyethylene glycol on the morphology of CAB microspheres and, consequently, on the release profile of carbamazepine (CBZ). CBZ is an anticonvulsant drug characterized by a low and irregular gastrointestinal absorption due to its low water solubility. Besides, multiple doses are generally required in order to maintain plasma levels in the therapeutic range over a full 24-h period, leading to the fluctuations of the CBZ plasma concentration and the appearance of adverse effects. The development of sustained-release formulations, then, has been carried out to minimize these inconveniences of CBZ administration and to improve the patient's compliance<sup>9,10</sup>. The microspheres were prepared by the emulsion-solvent evaporation method and characterized by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR). The effect of two different formulation variables, molecular weight of CAB, and the addition of PEG 1500 to the formulation on drug content, particle size, and CBZ release rate was verified using a factorial design.

## MATERIALS AND METHODS

### Materials

Carbamazepine (CBZ) was obtained from Nortec (batch 99063003 Brazil). Cellulose acetate butyrate MW 30000 (38% butyryl and 13.5% acetyl content) and cellulose acetate butyrate MW 70000 (37% butyryl and 13.5% acetyl content) were purchased from Aldrich Chem. Co., (Milwaukee, WI, USA). Mineral oil (Delaware, Brazil), Span 80 (Beraca, Brazil), and PEG 1500 (Synth, Brazil) were used as received. Acetone and n-hexane (E. Merck, Darmstadt, Germany) were of analytical grade and acetonitrile (Tedia, USA) was of HPLC grade.

### Preparation of microspheres

CBZ-loaded microspheres were prepared by an oil-in-oil emulsion method<sup>11</sup>. Briefly, 100 mL acetone solution of CAB (MW 30000, CAB30; MW 70000, CAB70) or acetone solution of CAB/PEG 1500 mixtures containing CBZ (internal phase) were emulsified in 500 mL of mineral oil containing 2% (w/v) Span 80 (external phase) using magnetic stirring. The emulsion was continuously stirred for 24 h at room temperature to allow the acetone evaporation from the internal phase. The microspheres produced were filtered, washed three times, each with 50 mL of n-hexane, and then dried under reduced pressure at room temperature for 4 h. The drug to polymer ratio and the total polymer concentration in the internal phase were 1:2 and 5.0 % (w/v), respectively, for all tested formulations. When the CAB/PEG 1500 mixture was employed the CAB to PEG ratio was 9:1, as previously reported<sup>12</sup>.

### Microsphere characterization

#### Encapsulation efficiency and CBZ content

The CBZ content was determined after dissolving 30 mg of microspheres accurately weighed in acetonitrile. The CBZ concentration was determined by high performance liquid chromatography (HPLC). A HPLC system (Shimadzu, USA) equipped with a UV/VIS detector, two pumps, and a data workstation were used. The analysis was carried out using a Supelcosil LC-18 column (250 x 4.6 mm i.d.; 5 µm; Supelco, USA) and the CBZ was detected by UV absorption at 283 nm. The mobile phase consisted of acetonitrile/water (45/55; v/v), and the flow rate was adjusted to 1.5 ml/min. The encapsulation efficiency of CBZ was calculated as being the difference between the amount of drug initially added to the formulation and the amount

found in the microspheres after HPLC analysis. Finally, the CBZ content in the microspheres (% w/w) was estimated. All the analyses were carried out in triplicate.

#### *Morphological examination of microspheres and particle size determination*

The morphological examination of microspheres was carried out using a scanning electron microscope (Jeol JSM-5800) after the coating of the samples with gold under vacuum. The internal structure was visualized after transversal section of particles and further coating with gold. The arithmetic mean diameter was determined after the Ferret diameter measurement of at least 300 particles had been visualized on micrographs obtained by SEM.

#### *Fourier transform infrared spectroscopy (FT-IR)*

The FT-IR analysis of microspheres was carried out on a Perkin-Elmer Mod. 16PC FTIR. The spectra were obtained in the transmission mode from KBr tablets containing 1% (w/w) of CBZ or microspheres over the 4000-400  $\text{cm}^{-1}$  wave number range. When the microspheres were analysed, the theoretical concentration of CBZ in the KBr tablets was in the order of 0.2 % (w/w).

#### *In vitro CBZ release evaluation*

*In vitro* release studies were carried out in 900 ml of 1% (w/v) sodium dodecyl sulfate solution maintained at  $37^\circ \pm 0.5^\circ \text{C}$  using a standard USP XXIII apparatus with paddle stirring at 75 rpm (PharmaTest equipment, model PTWS3 connected to a spectrophotometer HP 8452A, USA). Colorless hard gelatin capsules (size 00) were filled with microspheres corresponding to 200 mg of CBZ and were placed into the dissolution media. Samples were withdrawn at regu-

lar time intervals for 12 hours and were assayed spectrophotometrically at 284 nm. The samples were immediately returned to the dissolution vessels after analysis. Statistical analysis was performed on the area under the curve (AUC) calculated by the linear trapezoidal method.

#### **Factorial design of experiments**

A  $2^2$  factorial design was performed in order to evaluate the effect of the independent variables on the drug content, the particle size, and the AUC obtained from CBZ release profiles. The independent variables were the CAB molecular weight and the addition of PEG 1500 to the internal phase of the emulsion. Table 1 discloses the independent variables and their levels which were investigated in the preparation of the microspheres. The data were evaluated by analysis of variance (ANOVA).

## **RESULTS AND DISCUSSION**

### **Microsphere characterization**

#### *Encapsulation efficiency and drug content*

The encapsulation efficiency of CBZ and the drug content in the microspheres varied from 65 to 70% and from 20 to 24% (w/w), respectively (Table 1). The statistical analysis demonstrated that the CBZ content in the microspheres decreased significantly when the CAB70 was employed in the formulation ( $F_{\text{cal}} > F_{\text{tab}}$ ,  $\alpha = 0.05$ ), but it was not influenced by the addition of PEG 1500, which, in turn, indicates that the molecular weight of the polymer is the main parameter governing the drug encapsulation (Table 2).

#### *Morphological examination and particle size determination*

Spherical and compact particles with a

Formulation (n = 3)	Independent variables		Encapsulation efficiency (%)	CBZ content % (w/w)	Particle mean diameter ( $\mu\text{m}$ )	AUC (% released/ min)
	CAB MW	PEG 1500				
F1	30000	No	72.05 $\pm$ 1.63	23.91 $\pm$ 0.68	373.4 $\pm$ 87.6 424.3 $\pm$ 78.2 378.6 $\pm$ 75.6	8349 12261 12353
F2	70000	No	60.81 $\pm$ 4.41	20.28 $\pm$ 1.54	1086.6 $\pm$ 66.1 1469.0 $\pm$ 147 733.3 $\pm$ 158	4026 7859 1838
F3	30000	Yes	70.08 $\pm$ 4.38	23.37 $\pm$ 1.42	498.0 $\pm$ 191 527.1 $\pm$ 117 503.1 $\pm$ 125	49992 59694 53088
F4	70000	Yes	62.19 $\pm$ 2.04	20.73 $\pm$ 0.71	1301.0 $\pm$ 162 1190.0 $\pm$ 203 1206.3 $\pm$ 253	5114 7322 6275

**Table 1.** The independent variables, their levels and values of encapsulation efficiency, drug content, mean particle size and AUC obtained from CBZ release profiles for each formulation (n=3).

Source	Drug content			Mean diameter			AUC		
	SS	MS	F	SS (x 10 <sup>4</sup> )	MS (x 10 <sup>4</sup> )	F	SS (x 10 <sup>6</sup> )	MS (x 10 <sup>6</sup> )	F
CAB MW	29.516	29.516	21.94*	152.84	152.84	43.68*	2222.37	2222.37	220.63*
PEG 1500	0.007	0.007	0.005	4.82	4.82	1.38	1514.23	1514.23	150.33*
Interaction	0.730	0.730	0.543	0.275	0.275	0.008	1298.42	1298.42	128.91*
Error	10.76	1.345		27.99	3.49		80.58	10.07	

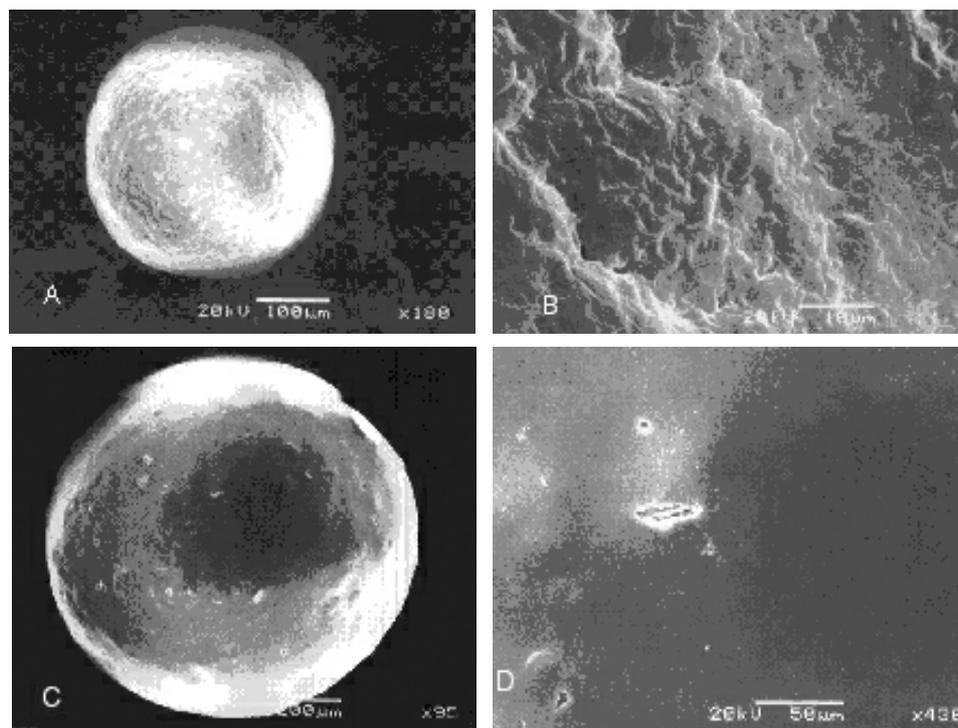
**Table 2.** Summary of analysis of variance results obtained using drug content, the mean particle size, and AUC obtained from CBZ *in vitro* release profiles as dependent variables. CAB molecular weight, 30000 or 70000; PEG addition to the formulations, yes or no;  $F_{tab}$  (DF= 3,8;  $\alpha=0.05$ ) = 4.07; \* significantly for  $\alpha = 0.05$ .

smooth surface were produced when CAB70 was used as the encapsulating polymer (Figs. 1C and 1D). In contrast, the surface of CAB30 microspheres was rougher and the spherical form of the particles was lost (Figs. 1A and 1B). Usually, when the emulsion-solvent evaporation method is used, the increase in the solvent elimination rate during microencapsulation leads to the formation of rougher particles<sup>13</sup>. In this case, a faster solvent elimination was provided by the lower viscosity of the CAB30 solution facilitating its diffusion from the internal phase of the emulsion, and hence affecting the surface properties of the particles.

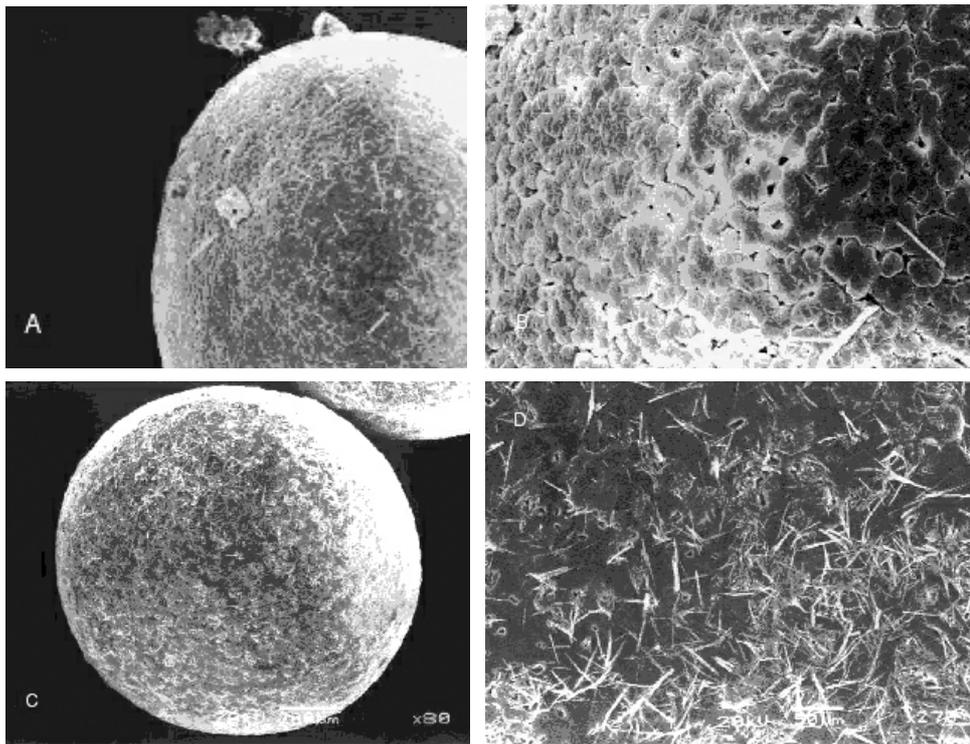
The PEG 1500 addition in the internal phase of the formulations led to the formation of particles with a more porous surface (Fig. 2). In addition, a sponge-like matrix structure was observed when microspheres were prepared from

CAB30 (Figs. 2A and 2B). The increased porosity observed for the microspheres could be attributed to the elimination of the PEG 1500 from the internal phase during the microencapsulation process since this polymer is partially soluble in mineral oil. This interpretation is supported by the formation of large pores inside of the particles as can be observed in the micrographs obtained from cross-sectioned microspheres (Figs. 3A and 3B). Specific domains of PEG 1500 are most likely to be formed in the internal phase of the emulsion before its migration towards the external phase.

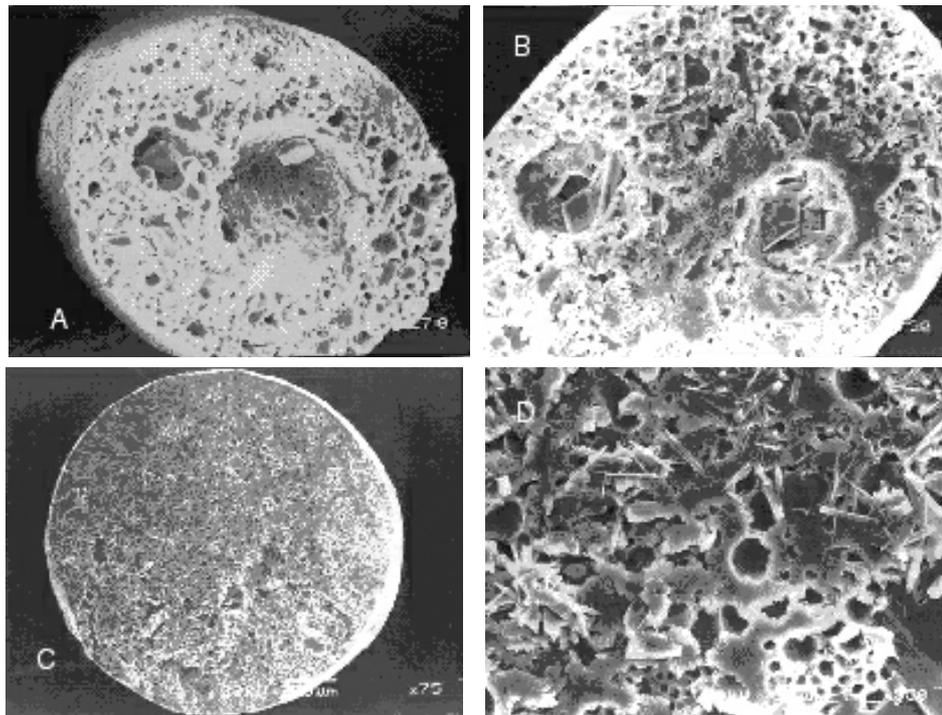
It is also possible to observe the presence of two carbamazepine crystal forms can also be seen in the cross-sectioned microspheres (Fig. 3), which indicates the presence of polymorphs of this drug. Indeed, this important anticonvulsant crystallizes as four different anhydrous



**Figure 1.** SEM micrographs of microspheres prepared without PEG 1500 addition in the internal phase of the emulsion. (A) and (B) CAB30 microspheres (C) and (D) CAB70 microspheres.



**Figure 2.** SEM micrographs of microspheres prepared with PEG 1500 addition in the internal phase of the emulsion. (A) and (B) CAB30 microspheres (C) and (D) CAB70 microspheres.



**Figure 3.** SEM micrographs of microsphere cross sections prepared with PEG 1500 addition in the internal phase of the emulsion. (A) and (B) CAB30 microspheres (C) and (D) CAB70 microspheres.

polymorphs (I, II, III and IV) and one dihydrated form<sup>14</sup>. Commercial anhydrous CBZ has been described as a mixture composed of polymorphs I and III, as major constituents, which correspondingly display a trigonal and a monoclinic structure<sup>15</sup>. Therefore, the presence of CBZ polymorphs forms interior of the particles

is to be expected, since the drug is not completely dissolved in the internal phase of the emulsion during the preparation of microspheres. Since there has been much confusion surrounding the naming of CBZ polymorphs and because morphologically similar trigonal and triclinic forms are difficult to distinguish

from one another without the aid of power X-ray diffraction, the polymorphic differentiation was not undertaken in this work.

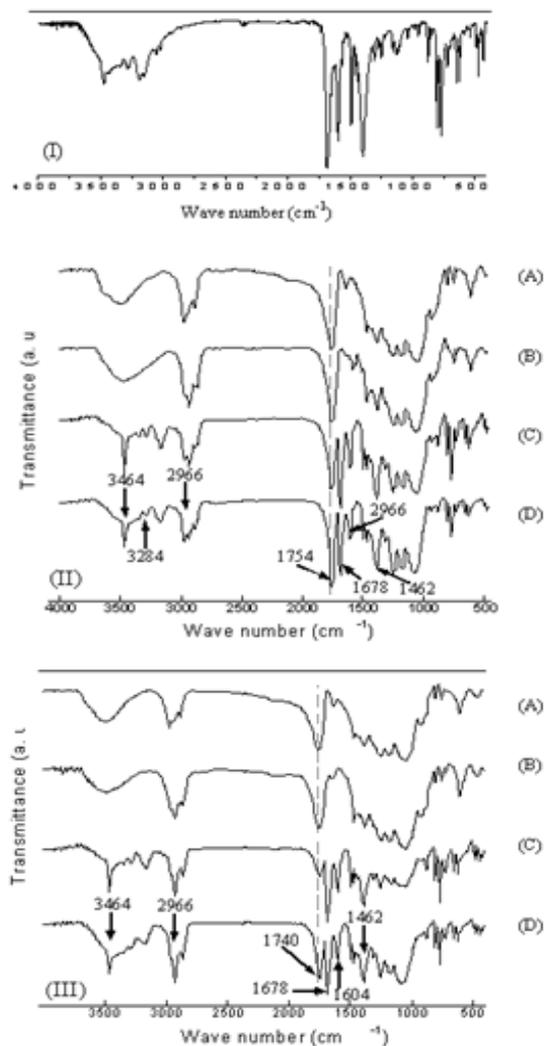
It should be noted that when PEG 1500 was added to the formulation it was possible to observe the presence of needle-like crystals of CBZ on the surface of the microspheres. Conversely, microspheres which were prepared without PEG 1500 displayed crystal-free particle drug surface (Figure 2). This result suggests that PEG 1500 affects the location of the drug in the particle. Most likely, after migration from the internal phase, the PEG acts as a co-solvent in the external phase solubilizing an important fraction of CBZ. After acetone evaporation, the excess of CBZ precipitates from the external phase and its crystals are adsorbed onto the surface of the microspheres.

The mean diameter of the particles varied from 300 to 1400  $\mu\text{m}$ , according to the formulation tested (Table 1). Release profiles of the drugs from microspheres are recognized as being dependent on the particle size. In most cases, the smaller the mean particle diameter, the shorter the diffusion path for drug release, and consequently, the faster the release rate<sup>16</sup>. Hence, a statistical analysis was carried out to verify the effect of the independent variables studied on this microsphere feature. As can be observed in Table 2, the particle size increased significantly when the cellulose acetate butyrate with the highest molecular weight was used ( $F_{\text{cal}} > F_{\text{tab}}$ ;  $\alpha = 0,05$ ). The effect of the polymer molecular weight on particle size has been attributed to an increase in the internal phase viscosity. The higher viscosity makes the polymer solution more resistant to fragmentation into small droplets[0], which, in turn, leads to the generation of a coarser emulsion and, subsequently, to the formation of larger microspheres. This result is in agreement with the literature related to the use of cellulose esters to prepare microspheres by the emulsion technique: the larger the polymer molecular weight, the larger the microspheres<sup>17</sup>. On the other hand, the analysis of variance revealed that the addition of PEG 1500 to the formulations did not affect statistically the size of the microspheres ( $F_{\text{cal}} < F_{\text{tab}}$ ;  $\alpha = 0,05$ ).

#### Fourier Transformed-Infra Red spectroscopy

The FT-IR spectra obtained from microspheres are shown in Fig. 4. The presence of CBZ in the microspheres can be demonstrated by several peaks (Figure 4C and 4D). The peaks

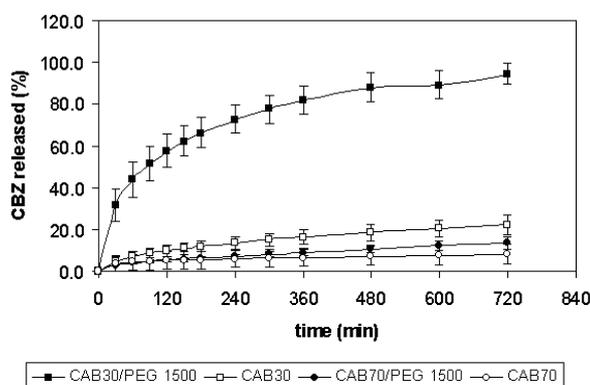
located at 3464  $\text{cm}^{-1}$  and 3284  $\text{cm}^{-1}$  represent the N-H group and linked N-H groups of the drug, respectively. The latter probably relates to the formation of hydrogen bonds between the  $\text{NH}_2$  of CBZ and C=O of CAB30. The other CBZ peaks are related to the C=O at 1678  $\text{cm}^{-1}$ , C=C of aromatic ring at 1604  $\text{cm}^{-1}$ , and C-H at 2966 and 1462  $\text{cm}^{-1}$ . However, it should be noted that the typical PEG 1500 peaks at 1450  $\text{cm}^{-1}$ , 1110  $\text{cm}^{-1}$ , and 960  $\text{cm}^{-1}$  were not seen on the spectra of the microspheres prepared with the addition of this polymer (Fig. 4D). This result is consistent with the hypothesis that PEG 1500 diffuses towards the external phase of emulsion, and thus is absent in the final structure of the particle.



**Figure 4.** FT-IR spectra obtained from (I) Carbamazepine, (II) CAB30, and (III) CAB70 microspheres. (A) Pure CAB, (B) unloaded microspheres, (C) CBZ-loaded microspheres prepared without PEG 1500 addition and (D) CBZ-loaded microspheres prepared with PEG 1500 addition in the internal phase of the emulsion.

### ***In vitro* release studies**

CBZ release profiles for all tested formulations are shown in Fig. 5. In this study, sink conditions were assured by the addition of 1% (w/w) sodium dodecyl sulfate to the release medium, because CBZ solubility was nearly twelve times greater than the concentration of the drug after its complete release from the microspheres. As can be observed in Fig. 5, the use of a CAB 30/PEG 1500 blend provided a faster rate of drug release, attaining nearly 100% over 12 hours. In contrast, extremely slow release rates were obtained for microspheres prepared from CAB70 or CAB30 without the addition of PEG 1500. In order to compare the rates of drug release from the microspheres, a statistical analysis was performed using the AUC values shown in Table I. As can be seen in Table 2, the calculated F value was greater than the critical F value ( $F_{crit} = 4.07$ ;  $\alpha = 0.05$ ) for both dependent variables and for the interaction between them. The value of least significant difference obtained through application of the Tukey test was 8300.58. When this value was used to compare the AUC values demonstrated in Table 1, it was found that the release rate was not affected when only the CAB molecular weight was changed, even though the particle size was statistically reduced for microspheres prepared from CAB with a smaller molecular weight. On the other hand, the addition of PEG 1500 as a second polymer led to a significant increase in drug release from the CAB30 microspheres, while the particle size did not change. However, this effect was not verified for microspheres prepared from CAB70 indicating an interaction between the factors studied. These results can be associated with the increase in the porosity of the particle caused by the presence of PEG 1500 in the internal phase of the emulsion, mainly when CAB 30 is used as a matrix formation polymer.



**Figure 5.** CBZ release profiles from microspheres.

### **CONCLUSIONS**

The results demonstrated that when PEG 1500 was blended with CAB30 in the internal phase of the emulsion, the drug content and the mean particle size the CBZ were not affected, but the release was significantly increased. However, this polymer was not found in the matrix structure of microspheres. PEG 1500 was eliminated from the internal phase of the emulsion during the microencapsulation process leading to the formation of large pores inside the particles. Thus, PEG 1500 can be used to modify the release of drugs from cellulose ester matrices; however, the magnitude of the effect produced was also dependent on the molecular weight of the cellulose derivate, since the effect of PEG 1500 addition on CBZ release profile was not observed when CAB70 was used to produce microspheres.

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