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In vitro and in vivo evaluation of carbamazepine-PEG 6000 solid dispersions

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Abstract

The present work extended previous physico-chemical investigations on the effects of solid dispersion on the solubility, the dissolution rate and the pharmacokinetic profile of carbamazepine. Solubility studies showed a linear increase in carbamazepine solubility with the increase of PEG 6000 concentration. There is no marked difference between physical mixtures and solid dispersions for the enhancement of carbamazepine solubility by PEG 6000. Less than 60% of pure carbamazepine was dissolved in 90 min. Physical mixtures (carbamazepine phase III) and solid dispersions (carbamazepine phase II) dissolution rates were higher in comparison of the parent drug. The dissolution of carbamazepine phase III was more pronounced than that evoked by the phase II. The dissolution profiles indicated that the percentage of the drug dissolved was dependent on the proportion of PEG 6000. In solid dispersions there was a remarkable enhancement in the dissolution rates of the drug in the vicinity of the eutectic composition as compared with those of corresponding physical mixtures. Hence, the optimum value for the solid dispersion was $80.5 \pm 1.7\%$ of carbamazepine having dissolved within the first 10 min compared to $40 \pm 1\%$ for the corresponding physical mixtures of the same composition. Statistical analysis of pharmacokinetic parameters confirmed that the carbamazepine:PEG 6000 binary systems displayed higher bioavailability of the drug than the pure carbamazepine. The area under the curve (AUC) values highlighted the evidence that only slight differences in the bioavailability of the drug occur between physical mixtures and solid dispersions prepared at the 80:20 and 50:50 drug:carrier compositions. However, the mean normalized plasma concentrations showed that standard error deviations are rather wide intervals for pure drug and physical mixtures in comparison to solid dispersions. One additional interesting point to consider is the disappearance of the multiple peaks on the individual kinetic curves of the 50:50 solid dispersion composition. Furthermore, our investigations have highlighted the interest of solid dispersions prepared at «near»-eutectic composition as our preliminary data show that the plasma concentration (C_{5h}) of the drug for the 15:85

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dispersed sample containing 150 mg of carbamazepine is not significantly different from that obtained for the 50:50 dispersed sample containing 300 mg of the drug.

Keywords: Solid dispersion; Polymorphs; Eutectic composition; Dissolution; Bioavailability; PEG 6000; Carbamazepine

1. Introduction

Oral bioavailability of drugs is affected by a variety of factors which influence their absorption from the gastrointestinal tract. One determinant factor for absorption is drug dissolution which is influenced by the solubility of the drug in the gastrointestinal fluids. A variety of devices have been developed over the years to improve the release and the dissolution of drugs. The solid dispersion method is one effective approach to achieve ideal therapy for drugs with low aqueous solubility by incorporating them into a water soluble polymer matrix. To this end, polyethylene glycols (PEG) are one of the most widely used carriers to prepare solid dispersions.

Carbamazepine, a drug indicated for the treatment of epilepsy, trigeminal neuralgia, bipolar affective disorder and acute mania, is characterized by a low and an erratic absorption. Several attempts have been applied to augment the bioavailability of carbamazepine including complexation with cyclodextrins (Al-Meshal et al., 1993), solid dispersions with sugar (Attia and Habib, 1985), copolymer as polyvinylpyrrolidone/vinylacetate (Zingone and Rubessa, 1994) or PEG 6000 (El-Zein et al., 1998). We have investigated the physical characteristics and the potential interaction of physical mixed and solid dispersed carbamazepine with PEG 6000 in a previous study (Zerrouk et al., 2001). The results reported in the present study extend and complete other studies (Kobayashi et al., 2000) on the influence of PEG 6000 on the solubility, the dissolution rate and the pharmacokinetics profile of carbamazepine. Thus, the present work concentrates on the influence of the carrier and the dispersion method on the polymorphic behavior of carbamazepine. Moreover, special emphasis is given to the attempt to correlate in vitro and in vivo characteristics of the solid dispersions since only limited and disparate work has been performed on this aspect of solid dispersion technology.

2. Materials and methods

2.1. Materials

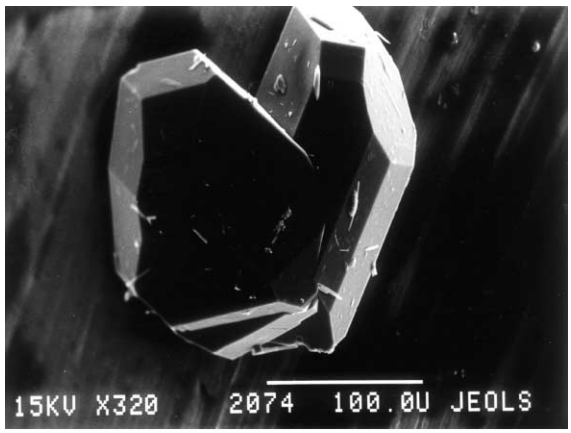
Carbamazepine Form III was supplied by Biocodex (France). The differential scanning calorimetry analysis shows three thermal events (174, 178, 191 °C; Zerrouk et al., 2001). Moreover, optical performed in our laboratory at room temperature have revealed that carbamazepine Form III is composed of agglomerates of prismatic crystals with mean particle length of 45–85 μm estimated from three samples (Table 1). Carbamazepine Form II was prepared by recrystallization of Form III from cyclohexane. The crystals dissolved in boiling cyclohexane were hot filtered before cooling the solution at room temperature for three days to obtain the Form II trigonal crystals. In parallel, the assessment of the polymorphic nature of the drug samples was ascertain by X-ray structure analysis in respect of the powder diffraction profiles of carbamazepine reported by Lowes et al. (1987) and by JCPDS (1983). PEG 6000 was received from BASF (France).

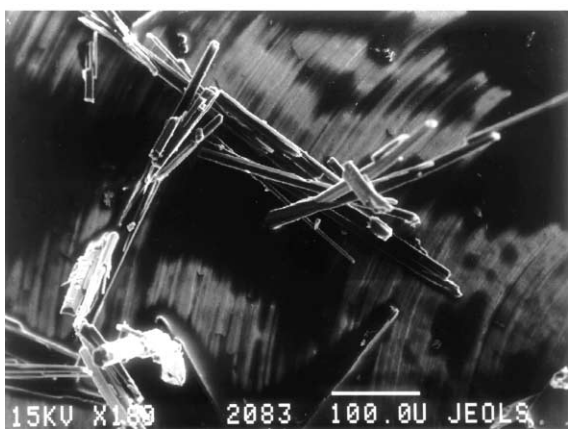
2.2. Methods

2.2.1. Preparation of solid dispersions

Solid dispersions of carbamazepine with PEG 6000 were prepared by fusion and crystallization of the melted mixtures according the procedure previously described by Sekiguchi and Obi (1961). Briefly, each mixture was melted in a metallic vessel heated at 200 °C in an oil bath. Immediately after fusion, the samples were poured onto a metallic plate and kept at ambient temperature and then ground in a mortar. The powder samples of the physical mixtures investigated in the present experiments were chosen to be comparable to those of the solid dispersions used in the in vitro and in vivo studies. The mean diameter was in the range of 250 μm to a maximum diameter of 450 μm.

Table 1
Scanning electron micrographs of carbamazepine Form III (top) and Form II (bottom)





2.2.2. Solubilization properties of PEG6000

The effect of different PEG 6000 concentrations on the equilibrium solubilities of carbamazepine in distilled water at 25 °C were carried out by adding an excess of drug (50 mg) into a screw-capped glass vial containing 50 ml of hydrochloride solution 0.1 M (HCl, pH 1.2) and various amounts of the carrier. The samples placed on a shaker were agitated at 25 °C for 24 h previously determined to be adequate time for equilibration. At the end of this period, an aliquot of each solution was withdrawn and filtered through a 0.45 μm pore size Millipore membrane filter. The assay of carbamazepine was determined spectrophotometrically at 285 nm, a wavelength at which PEG 6000 does not interfere. Similarly, the solubility of carbamazepine from the physical mixtures and the solid dispersions was determined as described above for pure drug after agitation in vials with excess drug with mixed or dispersed drug with PEG 6000.

2.2.3. Dissolution studies

In vitro release was evaluated using a conventional dissolution test. Dissolution studies were carried out first on pure carbamazepine (Form II, Form III and dihydrate) and secondly on dispersions of PEG 6000 at various weight ratios (5–80% in carbamazepine) and with the corresponding physical mixtures. Each test was carried out in a 1000 ml dissolution medium at 37 °C ($n = 6$) with a 6 flasks-dissolution apparatus (Dissolutest-Prolabo USP XXII connected to a UV-VIS Shimadzu model U.V 1201 spectophotometer). The dissolution media used was HCl 0.1 M (pH = 1.2). An accurately weighted quantity of each sample equivalent to 20 mg of carbamazepine filled into capsules was subjected to the test. Samples were taken at appropriate time intervals each 5 min for the first h, then each 15 min until 2 h. The volume of the dissolution medium was kept constant throughout the run by replacing the removed samples with an equal quantity of freshly solution. The aliquots collected at various intervals of time were analyzed spectrophotometrically at wavelength of 285 nm after suitable dissolution with 0.1 M HCl. Under these experimental conditions PEG 6000 did not interfere with the spectrophotometric assay.

2.2.4. Constant surface area dissolution studies

Discs containing the melt of carbamazepine and PEG 6000 were prepared according to the rotating method of Ford and Rubinstein (1977). Weighted quantities of PEG 6000 and carbamazepine were melted at 200 °C and poured into upturned aluminium vials covers (2 cm internal diameter). The excess of the melted sample was sliced away to leave a planar flat surface before the discs were stuck centrally to the agitation system onto the lower surface of the dissolution apparatus. In these conditions, only one side of the disc surface was available for dissolution. The discs were immersed 2.5 cm from the flask bot-

tom. The dissolution medium used was HCl (pH 1.2) at 37 ± 1 °C. The rotation speed of the disc holder was 50 rpm. The amount of carbamazepine dissolved was monitored at 285 nm. The intrinsic dissolution rates expressed in mg mm^{-1} cm^{-2} were calculated from the initial portion of the dissolution using a linear regression procedure. The derived rate was divided by the surface area of the exposed sample (3.194 cm^2).

2.2.5. In vivo study

Twelve White New-Zealand rabbits, 2.65 ± 0.3 kg, were randomly divided into two groups to investigate the 80:20 and the 50:50 carbamazepine:PEG 6000 w:w composition. Twelve h before the experiments started the animals were fasted but had free access to water. Each of 6 animals received 300 mg carbamazepine from each formulation according to a three-treatment randomised crossover schedule. One-week washout period was allowed between two successive dosings. Blood samples, obtained from the marginal vein by individual venous puncture were collected in tubes. After oral administration, blood samples were collected predose (-15 min) and 1, 2, 3, 4, 5, 6, 8, 10, 12 and 16 h postdose. The samples were centrifugated at 3500 rpm for 20 min. The plasma obtained was maintained at -20 °C and stored until analyzed for carbamazepine using the ADX carbamazepine assay which utilizes fluorescent polarization immunoassay technology (FPIA).

2.2.6. Pharmacokinetic data analysis

Pharmacokinetic parameters were estimated from a two-compartment model from the individual plasma concentrations versus time profiles. The maximum plasma concentrations (C_{max}) of carbamazepine and the respective time (T_{max}) were obtained from the raw data of plasma concentrations versus time. The area under the plasma carbamazepine concentration versus time curve from zero time to the last experimental point ($AUC_{0\rightarrow 16h}$) was calculated by means of a combination of the regular trapezoidal and the logarithmic trapezoidal rules. The total area under the plasma drug concentration versus time curve ($AUC_{0\rightarrow\infty}$) was calculated by adding to the $AUC_{0\rightarrow 16h}$ value the area from the last experimental time point to infinite time, estimated by logarithmic-linear decline. The terminal disposition half-life ($t_{1/2}$) was calculated as the ratio 0.693/b where b represents the terminal disposition rate constant estimated by logarithmic-linear regression.

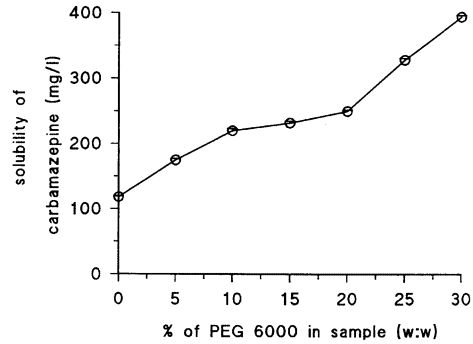


Fig. 1. Effects of increasing weight fractions of PEG 6000 on the dissolution of carbamazepine. Each point represents the means ± S.E.M. of six experiments.

2.2.7. Statistics

The differences in the solubility of carbamazepine from physical mixtures and solid dispersions with PEG 6000 were evaluated by two-sample t-test. The effects of the various weight fractions of PEG 6000 on the dissolution rate of carbamazepine was evaluated by one-way ANOVA. Differences in pharmacokinetic parameters were tested statistically using one-way ANOVA. In all tests, a probability value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Solubility determinations

Fig. 1 shows the solubility phase diagram representing the effect of increasing the concentrations of PEG 6000 on the apparent solubility of carbamazepine in water at 25 °C. The enhancement of the absolute amounts of the dissolved drug was approximately linear. A similar feature of an A_L-type solubility phase diagram was found at 37 °C. These results are in accordance with the well

established formation of soluble complexes between water-soluble polymeric carriers and poorly soluble drugs (Mura et al., 1996). However, no enhancement of the solubility of a poorly water-soluble drug such as norfloxacin has been observed in the presence of PEG 6000 (Guyot et al., 1995). Moreover, a reduced solubility of carbamazepine in the presence of polyvinylpyrrolodone acetate at lower concentrations of the polymeric carrier have been reported and attributed to the formation of insoluble complexes (Zingone and Rubessa, 1994).

For all the physical mixtures and solid dispersions tested at different weight ratio, an increase in carbamazepine solubility was found as the concentration of PEG 6000 is augmented in the binary system. On the other hand, it should be noted that no marked difference between physical mixtures and solid dispersions for enhancement of the ability of PEG 6000 to solubilize carbamazepine was observed within the whole range studied (Fig. 2). This result is consistent with some findings performed with drugs such as etoposide or norfloxacin demonstrating the lack of difference between the solubilitization ability of PEG 6000 on the drugs released from physical mixtures and dispersion solids (Shah et al., 1995; Guyot et al., 1995). On the contrary, the aqueous solubility of etoposide dispersed in PEG 8000 was significantly higher in comparison to the physical mixture (Du and Vasavada, 1993). Others investigations have shown that increasing PEG 6000 content beyond the optimal drug-carrier composition leads to a decrease of the solubilization of piroxicam (Bhattacharyya et al., 1993).

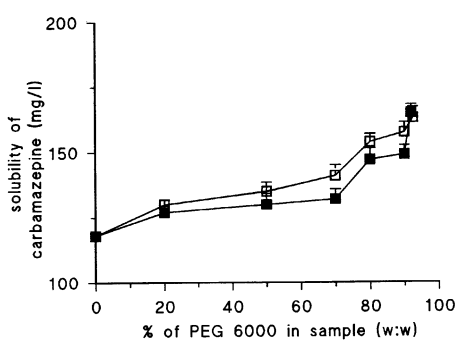


Fig. 2. Effect of PEG 6000 on solubility of carbamazepine from carbamazepine-PEG 6000 systems: physical mixtures (□) and solid dispersions (■) taking account the content of carrier in the samples. Each point represents the means ± S.E.M. of six experiments.

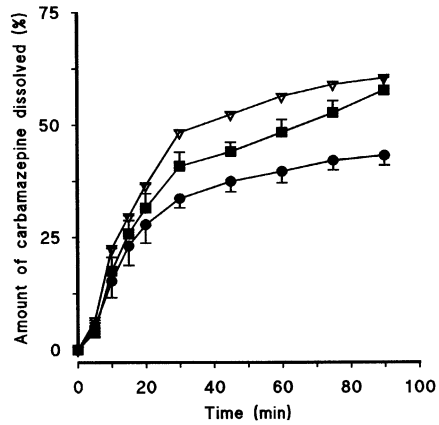


Fig. 3. Dissolution profiles in an hydrochloride solution 0.1 M (pH 1.2, 37 °C) for carbamazepine polymorphs (Form II: ●; Form III: ■) and dihydrate (▽). Each point represents the means ± S.E.M. of six experiments.

3.2. Dissolution studies

Our experimental approach has taken into account the data from our previous physico-chemical investigations demonstrating that carbamazepine exists as Form III in physical mixtures and Form II in solid dispersions (Zerrouk et al., 2001). The dissolution profiles of carbamazepine Form II, Form III and dihydrate are illustrated in Fig. 3. Thus, the present study supplement the data reported by Kobayahi et al. (2000) showing that the dissolution rate of carbamazepine Form I was higher than that of Form III. Analysis of Fig. 3 shows an initial step (up 20 min) characterized by the lack of any difference between the dissolution rates of Form III and Form II. This result is consistent with the view that anhydrates of carbamazepine convert to dihydrate crystals in water and at high relative humidity (Laine et al., 1984; Kobayahi et al., 2000). In the following step, the 30–75 min range, the dissolution of the dihydrate obtained with Form III was significantly more pronounced than that evoked by Form II (Fig. 3). A possible cause for the differences in the hydratation rates of the

two forms may arise from their dissimilar crystal morphology (Table 1).

The dissolution behavior of pure carbamazepine, and of carbamazepine from physical mixtures and solid dispersions prepared with PEG 6000 in various weight fractions (20, 50, 80 and 92.5%) of the carrier are shown in Fig. 4. The depicted dissolution profiles of the three types of samples can be assigned with the following rank order of percentage of carbamazepine dissolved, being solid dispersion > physical mixture > pure drug. Less than 60% of pure carbamazepine was dissolved in 90 min. In the different binary systems, an increase could be observed in the dissolution rate of carbamazepine with increment in the carrier proportions (Fig. 4). The dissolution rate of carbamazepine measured at 10 min and examined as a function of PEG 6000 concentration is presented in Fig. 5. The established relationship revealed a non linear pattern. Indeed, at lower amounts of PEG 6000, the plots illustrate a slight and progressive increase in the dissolution rates of carbamazepine released from physical mixture and solid dispersion up to the 85:15 and 70:30 ratio of the carrier:drug compositions respectively. These data are in line with those indicating

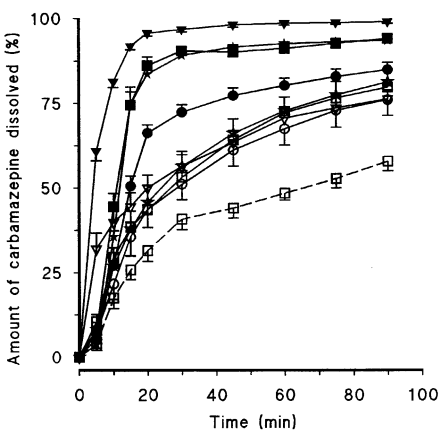


Fig. 4. Dissolution profiles of pure carbamazepine and of carbamazepine from physical mixtures (open symbols) or solid dispersions (solid symbols) with various contents of PEG 6000 as a function of time (min): a — 80:20 w:w (○, ●); b — 50:50 w:w (☆, ★); c — 20:80 w:w (□, ■); d — 7.5:92.5 w:w (△, ▲) are the drug-carrier ratio. Each value represents the mean ± S.E.M. of six experiments.

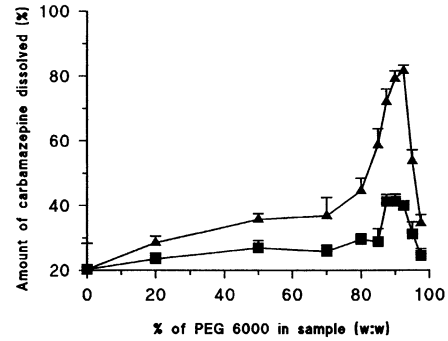


Fig. 5. Line graphs show effects of increasing concentrations of PEG 6000 on the amount of carbamazepine released from physical mixtures (■) and solid dispersions (▲) after 10 min. Each point represents the mean ± S.E.M. of six experiments.

that PEG 6000 is effective in improving the dissolution of poor water-soluble drugs regardless of whether the drug is present as a physical mixture or solid dispersion (Lin and Cham, 1996). This result may arise from the high hydrophilic potency of PEG 6000, one contributing factor to the increase of the wettability of the particles of carbamazepine and to the local enhancement of the drug solubility at the diffusion layer surrounding the particles (Mura et al., 1996). At higher content of the carrier, our results show that the extent of the dissolution rate was closely associated with the ratio of drug to PEG 6000 as suggested by the steep increase in the dissolution of the drug (Fig. 5). The dissolution rates of carbamazepine attains the maximal at weight fractions of 0.9 and 0.925 of the polymer evidencing that these ratio are crucial to obtain the optimal solubilization of carbamazepine. Beyond these proportions of the carrier, dissolution of the solid dispersions was markedly enhanced when compared with physical mixtures. Hence, the values for solid dispersions were 79 ± 2.5 and $80.5 \pm 1.7\%$ of carbamazepine having dissolved within the first 10 min in comparison of 41.2 ± 2.2 and $40 \pm 1\%$ for the corresponding physical mixtures of the same composition (Fig. 5). Since solubilization efficiencies elicited by PEG 6000 on carbamazepine physical mixed or dispersed are not different, it is worthy to mention that an additional phenomenon such as the reduction of particle size reduction or the formation of an eutectic invari-

ant in the rich-PEG region (Zerrouk et al., 2001) may determine the elevated dissolution rate of dispersed carbamazepine. In this regard, data have shown the highest dissolution behavior of drug prepared in solid dispersions with carrier at the eutectic composition (Du and Vasavada, 1993; Margarit et al., 1994; Lheritier et al., 1995). Conflicting data reported that the dissolution of papaverine does not attain a maximal value when prepared with PEG 4000 at the eutectic composition. Above the ratio of 7.5:92.5 drug to carrier the dissolution rate of carbamazepine declines as the PEG 6000 concentration increases (Fig. 5). This feature may reflect the delayed dissolution process of the drug induced by the solubilization of PEG 6000 in excess since the polymeric carrier is characterized by an elevated aqueous solubility.

3.3. Intrinsic dissolution rate study

For carbamazepine Form III, the intrinsic dissolution rate per unit surface area calculated from the initial linear portion of the dissolution plot was 0.0267 mg $min^{-1}cm^{-2}$ ($r = 0.998$) confirming that the dissolution of carbamazepine follows the Noyes-Withney law (Fig. 6). In experiments performed in simulated gastric acid fluid, the dissolution rate of this form was established to be 0.0605 mg min^{-1} cm^{-2} (Kobayahi et al., 2000). The intrinsic dissolution rate of carbamazepine was considered as a reference value to evaluate the dissolution of carbamazepine and PEG 6000 melts.

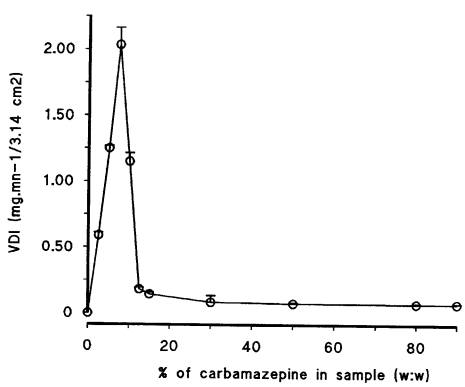


Fig. 6. Line graph shows the effects of increasing concentration of carbamazepine on the intrinsic dissolution rate of the carbamazepine-PEG 6000 melts.

At 7.5%, the calculated intrinsic dissolution rate was 0.648 mg min^{-1} cm^{-2} which indicates that the dissolution of carbamazepine is almost 20-fold greater than that of the pure drug. This phenomenon might be ascribed to the elevated dispersion of the drug in the polymeric carrier. Of particular note, the elevated concentration of the polymer in the diffusion layer does not seem of importance or may have only a limited effect on the rate of the dissolution process in accordance to our data discussed above demonstrating no substantial difference in the solubilization effect of PEG 6000 on carbamazepine between the physical mixtures and the solid dispersions. Therefore, the maximal value may be explained to the presence of the carrier over the disc surface playing the role of a wetting agent to the release of fine particles of the drug for which the dispersed system provides a greater surface area. This is supported by our data evidencing the formation of an eutectic mixture within the range 7.5–5% giving probably rise to a fine suspension of readily dissolved carbamazepine (Zerrouk et al., 2001).

Throughout the 7.5–2.5% range, the intrinsic dissolution rate of carbamazepine decreases linearly on increasing PEG 6000 fraction. Apparently, addition of carrier might only dilute the drug and, hence, will not be able to enhance the dissolution rate further.

In the concentration range from 7.5–30% of carbamazepine, there is also a decline in the dissolution of the active drug. This feature is consistent with a putative self-controlled release of carbamazepine. Indeed, inversion in the dissolution process may be explained by the supersaturating behavior of the drug at the diffusion surface layer. This was generally attributed to the depletion of the carrier at the surface of the disc leaving the drug characterized by a lower dissolution rate. Therefore, the limit of the declining zone corresponds to the solubilization action of PEG 6000 carrier on carbamazepine.

Over 30% of carbamazepine, the intrinsic dissolution rate is linear and not different to that of the

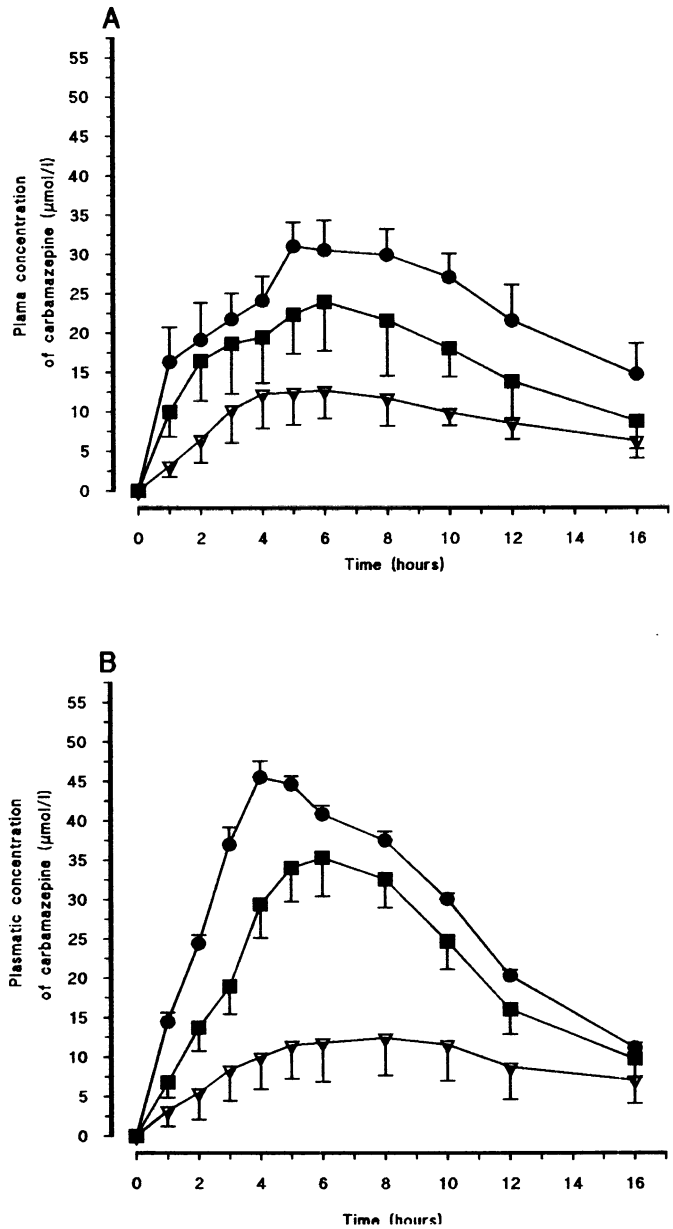


Fig. 7. Plasma levels of carbamazepine in rabbits following administration of pure drug (▽), physical mixed (■) and solid dispersed (●) oral forms. (A) carbamazepine-PEG 6000 w:w ratio is 80:20 (B) carbamazepine-PEG 6000 w:w ratio is 50:50. Each point represents the mean ± S.E.M. of six experiments.

pure drug. Thus, it is clear that at higher contents of the drug, the dissolution rate is scarcely affected by the amount of the active substance dispersed in the binary system formed with PEG 6000.

3.4. Pharmacokinetic study

The profiles of the plasma concentrations of carbamazepine versus time after oral administration of the pure drug, physical mixtures or solid

dispersions are depicted in Fig. 7. At all time intervals, the plasma levels of the physical mixed or dispersed drug with PEG 6000 are higher than those measured for the plain drug.

Examining the results obtained from the individual analysis, we found for the group of rabbit fasted with the 80/20 carbamazepine/PEG 6000 composition that C_{max} extented between 10.2 and 20.4 μmol l^{-1}; 20.1 and 39 μmol l^{-1} and 26.5 and 41 μmol l^{-1} for pure drug, physical mixed and solid dispersed forms respectively. Similarly the data for the 50/50 composition were as follows: pure drug (6.5–18.6 μmol l^{-1}); physical mixture (21.2–41 μmol l^{-1}) and solid dispersion(44.5–55 μmol l^{-1}). Estimates for the pharmacokinetic parameters of carbamazepine calculated for the three dosage forms are given in Table 2. The statistical analysis of the data (C_{max}, $AUC_{0\rightarrow 16h}$)

Table 2
(A)

Composition 80/20	Pure drug	Physical mixture	Solid dispersion
C_{max}	13.9 ± 1.55	27 ± 2.5 (1)**	33.8 ± 2.4 (1)*** (2)ns
(μmol l^{-1})	(10.2–20.4)	(20.1–39)	(26.5–41)
T_{max}	5.2 ± 0.75	5.5 ± 0.67	6.3 ± 0.92
(h)	(3–8)	(3–8)	(4–10)
$AUC_{0\rightarrow 16}$	114.5 ± 13.2	265.5 ± 19.6 (1)*	365 ± 35.5 (1)*** (2)ns
(μmol l^{-1} h^{-1})	(110.7–203.3)	(207–328)	(248–500.2)
$AUC_{0\rightarrow \infty}$	270.5 ± 49.4	346 ± 37.6 (1)ns	794.5 ± 106.8 (1)** (2)*
(μmol l^{-1} h^{-1}))	(175.5–478.9)	(237–438)	(407–991.8)
$T_{1/2\ el}$	12.6 ± 3.4	13.9 ± 7.25 (1)ns	13.9 ± 7.25
(h)	(4.45–28)	(5.12–50)	(5.12–50)

(B)

Composition 50/50	Pure drug	Physical mixture	Solid dispersion
C_{max}	13.5 ± 2.31	27 ± 3 (1)**	47.8 ± 2 (1)*** (2)ns
(μmol l^{-1})	(6.5–18.6)	(21.2–41)	(44.5–55)
T_{max}	6.4 ± 1.2	5.8 ± 0.7	5.2 ± 0.7
(h)	(4–10)	(4–8)	(4–8)
$AUC_{0\rightarrow 16}$	144.9 ± 22.15	338.4 ± 33.15 (1)*	445 ± 9 (1)***, (2)ns
(μmol l h^{-1})	(77.5–192.3)	(255–416.5)	(426–478)
$AUC_{0\rightarrow \infty}$	258 ± 35	419 ± 47 (1)ns	527 ± 9 (1)**, (2)*
(μmol l h^{-1}))	(175.5–478.9)	(302–544)	(497–558)
$T_{1/2\ el}$	11.25 ± 1.12	5.6 ± 0.34	5 ± 0.2
(h)	(8.9–15.5)	(4.7–6.4)	(4.8–5.8)

Mean bioavailability parameters of carbamazepine obtained in rabbits after single oral doses of pure drug or carbamazepine- PEG 6000 physical mixtures or solid dispersion in rabbits: (A) carbamazepine-PEG 6000 ratio was 80:20 w:w; (B) carbamazepine-PEG 6000 ratio was 50:50 w:w. Each values is the mean ± S.E.M. of experiments in six rabbits. (1) represents the differences between physical mixture or solid dispersion group and pure drug (2) represents the differences between physical mixture and solid dispersion; ns no significant differences between groups, $*P<0.05$, $**P<0.01$, $***P<0.001$.

Plasmatic concentrations of carbamazepine at C_{5h} and C_{8h} obtained in rabbits after single oral doses of the drug. All animals received carbamazepine under pure powder or carbamazepine. PEG 6000 physical mixtures (PM) or solid dispersions (SD): carbamazepine-PEG 6000 ratio was 50:50 or 15:85 w:w. Each values is the mean ± S.E.M. of experiments in seven rabbits. (1) represents the differences between carbamazepine (150 mg)-PEG 6000 (15:85) and carbamazepine (150 mg) -PEG 6000 50:50 data; (3) represents the differences between carbamazepine (150 mg)-PEG 6000 (15:85) and carbamazepine (300 mg) -PEG 6000 50:50 data. ns no significant difference between groups, $*P<0.05$, $**P<0.01$, $***P<0.001$. Ten (seven alive at the end of the experiments) white New-Zealand rabbits, 2.45 ± 0.5 kg, were used. Each of animals received two hard-gelatin capsules containing 150 (2 × 75) or 300 (2 × 150) mg carbamazepine from each formulation according to a eight-treatment randomised crossover schedule. Four days washout period was allowed between two successive dosings. The experiments take place over four weeks

confirmed that the physical mixtures and solid dispersions have higher bioavailability than the drug itself regardless of the carbamazepine/PEG 6000 composition. Therefore the enhanced bioavailability of carbamazepine in the presence of PEG 6000 appears to be related to the increase in solubility and dissolution rate of the drug.

Contrary to what was expected no change in the bioavailability of carbamazepine in rabbits between physical mixtures and solid dispersions was observed in the present study. The AUC values highlight the evidence that only slight differences in the bioavailability of carbamazepine were found among the two binary systems (Table 2). Detailed results show no statistically significant difference between the $AUC_{0\rightarrow 16h}$ values of physical mixture and solid dispersion of the 80/20 composition. For the 50/50 composition, the solid dispersion exhibited only a low increment of the bioavailability ($AUC_{0\rightarrow 16h}$) over the physical mixture ($P < 0.05$), whereas this difference was not statistically significant for the extrapolated AUC. These data are in line with the results of El-Zein et al. (1998). The physical mixture dissolves but CBZ may be absorbed in the lower part of the gastrointestinal tract. However, differences between the two binary systems remained since the analysis of the mean normalized plasma concentrations showed that standard error deviations are rather wide intervals for pure drug and physical mixture in comparison to solid dispersion.

One major finding of the present investigations was the lack of multiple peaks on the individual kinetic curves of the 50/50 solid dispersion composition as exemplified in Fig. 8. The disappearance of the peaks is probably indicative of the rapid and high level in carbamazepine dissolution enhancement. Indeed, this behaviour may counteract the interrupted character of carbamazepine absorption that is caused by the lower solubility of the drug in the group of rabbits given the physical mixtures (80/20 and 50/50) or the solid dispersion at the 80/20 composition (Fig. 8). The possibility of an elimination of carbamazepine from blood serum occurring in two phases to explain the presence of these peaks as suggested by Alexishvili et al. (1997) is, however, difficult to reconcile with the demonstrated ability of the 50/50 solid dispersion composition to normalize the individual kinetic curves.

3.5. In vivo bioavailability in comparison with in vitro dissolution parameters

To assess the correlation between the in vivo bioavailability characteristics and the in vitro dissolution parameters of low carrier content carbamazepine:PEG 6000 samples, the C_{max} or $AUC_{0\rightarrow\infty}$ and the times taken to dissolve 20, 50 or 90% of carbamazepine were subjected to linear regression analysis and the correlation coefficient (r) was calculated. The data revealed less valuable correlation for the 80/20 carbamazepine/PEG 6000 composition. On contrary, the relationship for the 50/50 composition was well characterized by a relative quite valuable linear model (C_{max}-physical mixture: $r = 0.996$, solid dispersion: $r = 0.998$; $AUC_{0\rightarrow\infty}$-pure drug: physical mixture: $r = 0.994$, solid dispersion: $r = 0.996$). The linear relationship between pharmacokinetic parameters and the amount of carbamazepine dissolved at different time intervals is summarized in Fig. 9. These results confirm that the dissolution process is the rate limiting step during the absorption of carbamazepine in our animal model.

3.6. In vivo behavior of samples at eutectic composition

In the present study, we address the specific applicability of formulations derived from the eutectic zone. Indeed, the data indicate that in vitro dissolution curves substantially correlate to in vivo bioavailability characteristics for the 50:50 carbamazepine:PEG 6000 composition. However the evidence of such correlation does not allow us to claim that dissolution data obtained for carbamazepine:PEG 6000 solid dispersions may be predictive of in vivo behavior. This has been partially investigated in a comparison between the plasma concentrations at C_{5h} and C_{12h} of various carbamazepine-PEG

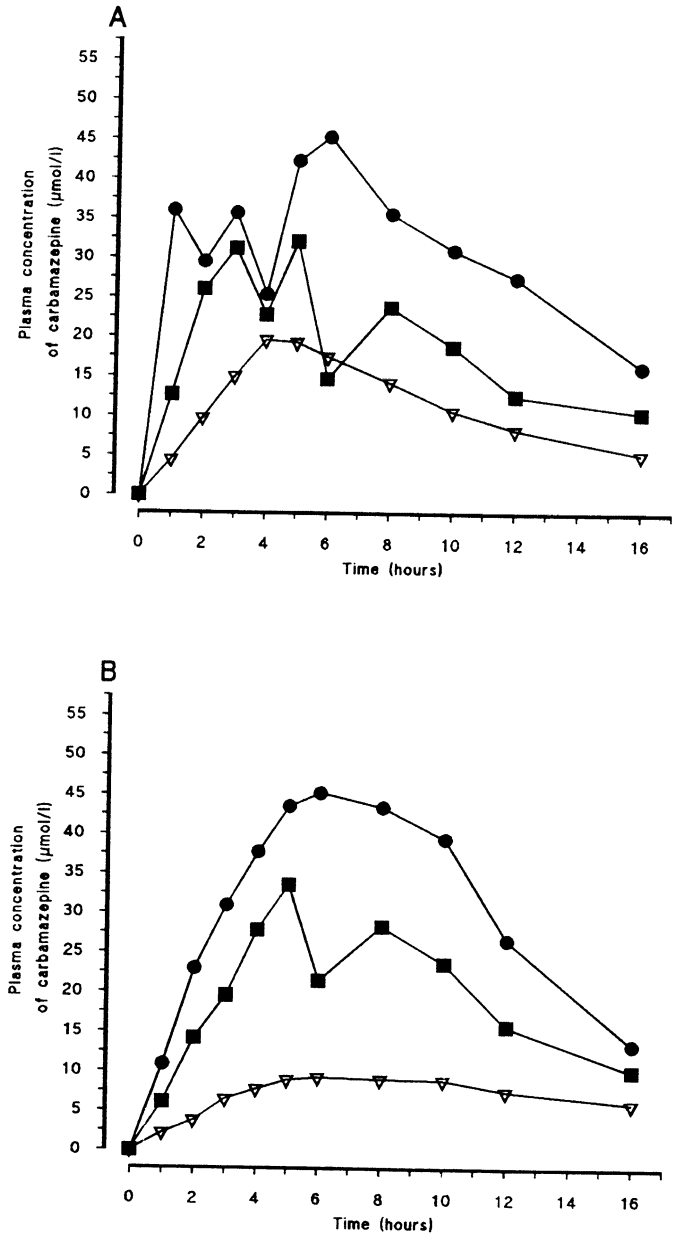


Fig. 8. Plasma levels of carbamazepine in individual rabbits following administration of pure drug (▽), physical mixed (■) and solid dispersed (●) oral forms. (A) carbamazepine-PEG 6000 w:w ratio is 80:20 (B) carbamazepine-PEG 6000 w:w ratio is 50:50.

6000 systems prepared with 150 (50:50 and 15:85) or 300 mg (50:50) of the drug (Table 3). Although the type of experiments performed does not account for all events that can affect the in vivo fate of the the drug, data obtained at C_{5h} show that the plasmatic concentration of the drug for the 15:85 dispersed sample containing 150 mg of carbamazepine is not significantly different to that obtained for the 50:50 dispersed sample containing 300 mg of the drug.

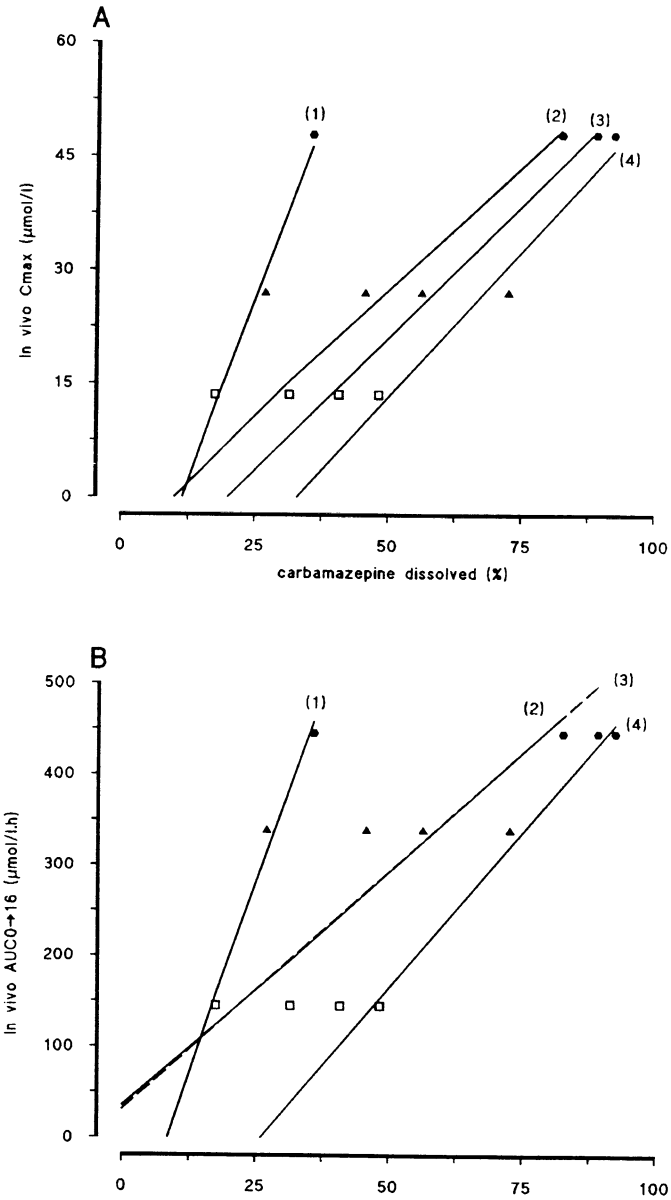


Fig. 9. Linear correlation between the percentage of carbamazepine dissolved in vitro (pure drug: □; physical mixture 50:50: ▲; solid dispersion 50:50: ●) and some pharmacokinetic parameters indicative of the bioavailability of the drug (A: C_{max}; B: AUC $_{0\rightarrow\infty}$). The linear regression of the data points are plotted by solid lines. The corresponding evaluations are as follows: — 1 (time of dissolution = 10 min), C_{max}: $r^2 = 0.996$ P^{**}; AUC $_{0\rightarrow16}$ $r^2 = 0.997$, P^{**}. — 2 (time of dissolution = 20 min), C_{max}: $r^2 = 1$ P^{*}; AUC $_{0\rightarrow16}$ $r^2 = 0.91$, ns. — 3: (time of dissolution = 30 min), C_{max}: $r^2 = 1$ P^{*}; AUC $_{0\rightarrow16}$ $r^2 = 0.998$, P^{***}. — 4: (time of dissolution = 60 min), C_{max}: $r^2 = 0.998$ P^{**}; AUC $_{0\rightarrow16}$ $r^2 = 0.999$, P^{**}.

Table 3

$n = 7$	CBZ:PEG6000 (50:50) CBZ: 2×75 mg[a] (1)		CBZ:PEG6000 (15:85) CBZ: 2×75 mg[a] (2)		CBZ:PEG6000 (50:50) CBZ: 2×150 mg[b] (3)	
Drug formulation	PM	SD	PM	SD	PM	SD
$C_{5h(\mu mol\ l^{-1})}$	9.7 ± 2.55	16.9 ± 1.8	10.5 ± 3.1 (1)ns; (3)* vs PM	37.3 ± 5.6 (1)**; (3)ns vs SD	19.4 ± 4.8 (1)*** vs PM	42.7 ± 2.5 (1)*** vs SD
$C_{8h(\mu mol\ l^{-1})}$	4.5 ± 2	12.5 ± 1.1	7.8 ± 2.4 (1)ns; (3)* vs PM	14.6 ± 3.1 (1)ns; (3)*** vs SD	13.9 ± 4.5 (1)*** vs PM	34.8 ± 3.6 (1)*** vs SD

[a] Pure carbamazepine (2×75 mg): $C_{5h(\mu mol\ l^{-1})}$: 4.7 ± 2.4 μmol l^{-1}; $C_{8h(\mu mol\ l^{-1})}$: 7.3 ± 2.4 μmol l^{-1}.
[b] Pure carbamazepine (2×150 mg): $C_{5h(\mu mol\ l^{-1})}$: 9.4 ± 2.95 μmol l^{-1}; $C_{8h(\mu mol\ l^{-1})}$: 6.6 ± 2.5 μmol l^{-1}.

4. Conclusion

The present studies show the lack of difference between physical mixtures and solid dispersions for the ability of PEG 6000 to enhance carbamazepine solubility. Moreover, the dissolution of carbamazepine monoclinic Form III present in physical mixtures was more pronounced than that evoked by the trigonal Form II included in solid dispersions. The PEG 6000 weight fraction-carbamazepine dissolution rate profile reveals two distinct zones. The first pattern is characterized by a smooth profile for which the analysis of the pharmacokinetic parameters of the corresponding samples argue for comparable bioavailability of the drug released from physical mixtures or solid dispersions with low carrier contents. However, the bioavailability profile displayed by the solid dispersions was more constant and reliable. This feature may have clinical significance since reduced serum fluctuations of carbamazepine lead to fewer dose-related side effects. The second pattern located at the vicinity of the eutectic composition is evidenced by a marked increase in the dissolution of the drug released from solid dispersions. Therefore, it is advisable to take into account physico-chemical investigations in view of their application towards bioavailability study. Our preliminary data suggest that it would be appropriate to prepare solid dispersions with a lower proportion of active substance to obtain optimal dissolution characteristics and pharmacokinetic parameters. The reduction of the dose of carbamazepine could further minimize the in vivo formation of chemically reactive metabolites like 2-hydroxyiminostilbene postulated to be responsible for the idiosyncratic toxicity of carbamazepine (Riley et al., 1989; Ju and Uetrecht, 1999). Hence further experiments need to be conducted to fully investigate the biopharmaceutical interest of solid dispersions with high carrier content.

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