PHARMACEUTICS, PREFORMULATION AND DRUG DELIVERY

Cyclodextrin Multicomponent Complexation and Controlled Release Delivery Strategies to Optimize the Oral Bioavailability of Vinpocetine

LAURA S.S. RIBEIRO,[1] AMÍLCAR C. FALCÃO,[2] JOÃO A.B. PATRÍCIO,[3] DOMINGOS C. FERREIRA,[4] FRANCISCO J.B. VEIGA[1]

[1]Laboratory of Pharmaceutical Tecnology, Faculty of Pharmacy, University of Coimbra, Rua do Norte, 3000-295 Coimbra, Portugal

[2]Laboratory of Pharmacology, Faculty of Pharmacy, University of Coimbra, Rua do Norte , 3000-295 Coimbra, Portugal

[3]Laboratory of Experimental Investigation, University Hospital of Coimbra, Avenida Bissaya Barreto, 3000-075, Coimbra, Portugal

[4]Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Porto, Portugal

ABSTRACT: In the present work, to maintain a suitable blood level of vinpocetine (VP) for a long period of time, VP-cyclodextrin-tartaric acid multicomponent complexes were prepared and formulated in hydroxypropylmethylcellulose matrix tablets. *In vitro* and *in vivo* performances of these formulations were investigated over a VP immediate release dosage form. Solubility studies were performed to evaluate the drug pH solubilization profile and to assess the effect of multicomponent complexation on VP solubility. The drug release process was investigated using United States Pharmacopeia apparatus 3 and a comparative oral pharmacokinetic study was subsequently undertaken in rabbits. Solubility studies denoted the pH-solubility dependence of VP and solubility improvement attained by complexation. Dissolution results showed controlled and almost complete release behavior of VP over a 12-h period from complex hydroxypropylmethylcellulose-based formulations. A clear difference between the pharmacokinetic patterns of VP immediate release and VP complex-based formulations was revealed. The area under the plasma concentration-time curve after oral administration of complex-based formulations was 2.1–2.9 times higher than that for VP immediate release formulation. Furthermore, significant differences found for mean residence time, elimination half-life, and elimination rate constant values corroborated prolonged release of VP from complex-based formulations. These results suggest that the oral bioavailability of VP was significantly improved by both multicomponent complexation and controlled release delivery strategies. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 96:2018–2028, 2007

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INTRODUCTION

Vinpocetine (VP) is a vincamine derivative that has been used in clinical practice in Europe for nearly three decades for the treatment of disorders arising from cerebrovascular and cerebral degenerative diseases.[1,2] VP is thought to increase the cerebral flow in the ischemic areas of patients with cerebrovascular disease, decrease platelet aggregability in patients with transient ischemic attack or stroke, increase red blow cell deformability in stroke patients, and have neuroprotective abilities and a protective effect against brain ischemia.[3,4]

VP is mainly used as immediate oral dosage forms containing 5 mg of the active ingredient, with a daily dosage regimen that can vary between $5 \, mg \times 3$/day to $20 \, mg \times 3$/day.[5] Unfortunately, the very limited aqueous solubility and wettability of VP can give rise to problems of both formulation and low bioavailability ($\sim$6.7%).[6] Indeed, VP is a poorly water-soluble base-type drug that presents pH-dependent solubility. As the solubility and dissolution relationships in the gastrointestinal tract can be critical for the oral bioavailability of poorly soluble weak bases as VP, because of the possibility of drug precipitation upon entry into the small intestine that may also affect the amount of drug available for uptake through the intestinal mucosa,[7] the use of cyclodextrin (CD) multicomponent complexation with βCD, sulfobutyl ether (SBE)βCD, tartaric acid (TA), and polyvinylpyrrolidone (PVP) has been attempted to overcome such VP solubility and dissolution drawbacks.

One potential method of optimizing the efficacy of drug activity is through the use of rationally designed carrier materials such as CDs. In the past decades, CD complexation has been extensively applied to enhance the solubility, dissolution, and bioavailability of poorly water-soluble drugs. Therefore, the use of CDs as excipients in different dosage forms has received much attention because upon complex formation advantages such as improved bioavailability, reduction of unwanted side effects, or improved stability have often been claimed. The improvement in absorption rate of drugs administered in solid dosage forms has been related to the increase in both solubility and dissolution rates of the complexes as compared with pure drug.[8] More recently, a concern with the amount of CDs in dosage forms due to toxicological considerations, formulation bulk, and production costs encouraged the development of methods that could enhance the complexation efficiency of CDs, by using a third additive such as water-soluble polymers[9,10] and hydroxy acids.[11]

In previous works, we have reported the combined use of both βCD and SBEβCD, water-soluble polymers [PVP and hydroxypropylmethylcellulose (HPMC)], and hydroxy acids (TA) to improve VP solubility.[12,13] Considering that VP has an elimination half-life of 2–6 h[14] and requires chronic administration, a controlled release dosage form could provide increased clinical value over conventional formulations, as a result of improved therapeutic effect and patient compliance by reducing dosing frequency, a more constant or prolonged therapeutic effect, and possible enhanced bioavailability. Then, in an effort to reach better dissolution properties as well as controlled release rate of VP, we have prepared VP-CD-TA multicomponent complexes and an optimal formulation was subsequently designed by the combination of these complexes into HPMC-based hydrophilic tablet dosage forms (Ribeiro et al., submitted for publication).

Because dissolution rate of poorly soluble drugs is a function of their water-solubility, enhancement of drug solubility is expected to improve its bioavailability. Recently, SBEβCD, a chemically modified CD, became a more interesting option than βCD to achieve complexation because of better physicochemical properties and improved inclusion behavior. Studies had proven that this CD derivative is able to increase drug oral bioavailability.[15,16] Thereby, the present study was undertaken to evaluate, by means of *in vivo* absorption studies in the rabbit model, the feasibility of using VP-βCD-TA and VP-SBEβCD-TA multicomponent complexes in association with HPMC matrix tablets as strategies to improve VP oral bioavailability and also to obtain a prolonged therapeutic effect of the drug as compared with an immediate release formulation.

EXPERIMENTAL

Materials

VP was purchased from Covex (Madrid, Spain). βCD [Kleptose®; molecular weight (MW) 1135]

and (sulfobutyl ether)$_{7M}$ (SBE)βCD (Captisol^{TM}; total degrees of substitution 6.8, MW 2160) were kindly offered by Roquette (Lestrem, France) and Cydex (Kansas City, MO). Polyvinylpyrrolidone K30 (PVP) and TA were purchased from Sigma Chemical Co. (St. Louis, MO). For the preparation of the matrix tablets, Methocel® HPMC K15M CR (Dow Chemical, Midland, MI), monohydrate lactose, and magnesium stearate of pharmaceutical grade were used. All solvents and chemicals used were of high-performance liquid chromatography (HPLC) or analytical grade. Milli-Q water was used to prepare buffer solutions and other aqueous solutions used in this study.

Preparation of VP-CD-TA Multicomponent Complexes

Solid multicomponent complexes, VP-βCD-TA and VP-SBEβCD-TA, with and without PVP, were prepared by the lyophilization method as described in a previous work.[12] Briefly, equimolar amounts of CDs and VP were dissolved in water and in 1.5% (w/v) TA solution, respectively. The two solutions were sonicated for 15 min and mixed for 2 h at 50°C. After filtration through a 0.45-μm membrane filter (PVDF, Tracer®; Teknokroma, Barcelona, Spain), the resulting clear solution was frozen by immersion in an ethanol bath (Shell Freezer, Labconco, Freezone® model 79490) at −50°C and then the frozen solution was lyophilized in a freeze-dryer (Lyph-lock 6 apparatus; Labconco) for 72 h. In the case of VP-CD-TA-PVP multicomponent complexes, equimolar amounts of CDs and VP were respectively dissolved in 0.25% (w/v) PVP and in 1.5% (w/v) TA solution. The resulting solution was mixed and sonicated for 15 min and then heated in an autoclave at 120°C for 20 min. After an equilibrium period of 72 h at room temperature, the resulting solution was filtrated through a 0.45-μm membrane filter and clear solution was frozen by immersion in an ethanol bath at −50°C, and subsequently lyophilized in a freeze-dryer for 72 h.

VP Solubility Studies

The pH solubilization profile of VP was determined in the pH range found in physiological fasted conditions of the human gastrointestinal tract. The buffer solutions used for the pH solubilization profile were the simulated gastric fluid without enzymes, pH 1.2 [United States Pharmacopeia (USP) XXV], 0.05 M phosphate buffer, pH 4.5 (European Pharmacopeia, 4th ed.), 0.05 M phosphate buffer, pH 6.0 (European Pharmacopeia, 4th ed.), simulated intestinal fluid without enzymes, pH 6.8 (USP XXV), and 0.05 M phosphate buffer, pH 8.0 (European Pharmacopeia, 4th ed.). VP solubility studies were conducted by the shake-flask method. Briefly, an excess of VP was added to glass flasks containing 5 mL of each buffer solution described above. The glass flasks were sealed and mechanically stirred at ambient temperature (22 ± 1°C) for 48 h. The pH of the samples was checked periodically during the experiment to ensure maintenance of buffer pH values. All resulting suspensions were filtered through a 0.45-μm membrane filter (La-Pha-Pack®; Langerwehe, Germany) and VP concentrations analyzed spectrophotometrically (UV-1603; Shimadzu, Japan) at 316 nm. All experiments were performed in triplicate.

VP aqueous solubility was evaluated in complexed and uncomplexed forms. Samples were prepared in triplicate by adding 5 mL of deionized water and excess of solid powders into 20-mL glass flasks which were sealed and mechanically stirred at ambient temperature for 48 h. Resulting suspensions were filtered through a 0.45-μm membrane filter and VP concentrations analyzed spectrophotometrically at 316 nm.

Preparation of HPMC Matrix Tablets

HPMC matrix tablets were manufactured by direct compression of the formulation mixtures presented in Table 1 under a pressure of 1000 (F-A) and 4000 kg/cm^2 (F-B to F-D). The respective powders (VP, multicomponent complexes, HPMC K15 M CR, lactose, and magnesium stearate) were blended thoroughly with a mortar and a pestle. Formulation mixtures were weighed and fed manually into the die of an instrumented single-punch tablet press (Specac Ltd., Kent, UK) fitted with 8-mm flat-faced punches. The tablet weight was kept constant, at 200 and 300 mg for the formulations incorporating pure drug or βCD and SBEβCD multicomponent complexes, respectively, by adjusting the amount of lactose used in each formula. The weight variation of tablet formulations is due to MW differences of both CDs, because SBEβCD has a MW of 2160, a significantly higher value than that of βCD (MW 1135). All tablet formulations contained 20 mg of VP or its equivalent.

Table 1. Composition of VP Tablet Formulations

Formulation	Complex or Pure VP (mg)	HPMC (mg)	Lactose (mg)	Mg Stearate (mg)	Total Weight (mg)
F-A[a]	20.0	—	178.0	2.0	200.0
F-B[b]	99.6	60.0	38.4	2.0	200.0
F-C[b]	158.2	90.0	48.8	3.0	300.0
F-D[b]	120.6	90.0	86.4	3.0	300.0

[a]Immediate release formulation of VP.
[b]HPMC-based matrix tablet formulations of VP: B) VP-βCD-TA, C) VP-SBEβCD-TA, D) VP-SBEβCD-TA-PVP, multicomponent complexes.

In Vitro Dissolution Testing

Dissolution tests were performed using USP apparatus 3 (Bio-Dis III extended release tester, Vankel™, Cary, NC), with a pH gradient method, to simulate the conditions of the fasted human gastrointestinal tract. Buffer solutions used for dissolution testing were the same as described above. The pH of the dissolution media and corresponding dissolution durations were set as follow: pH 1.2 for 1 h, pH 4.5 for 0.5 h, pH 6.0 for 2.5 h, and pH 6.8 for 8 h. The pH values and residence times in each row were selected on the basis of literature information of the pH values found in different parts of the human gastrointestinal tract in the fasted state.[17−20] Dissolution testing was performed at $37 \pm 0.2°C$. The vessels were fitted with 250 mL of media and tablet formulations, corresponding to 20 mg of VP, were placed in the dipping tubes which contained a polypropylene bottom screen of 420-μm mesh size. The mesh size of the top screens was also fixed at 420 μm. A standard dip rate of 15 per minute was used and dipping tubes were drained for 1 min before moving to the following media. Sample solutions (3 mL) were collected at specified time intervals from dissolution vessels, using a plastic syringe coupled with a polypropylene tube, which was inserted inside the vessel, and an equal volume of fresh test medium was replaced. Samples were filtered through membrane filters of 0.45-μm pore size (La-Pha-Pack®, Langerwehe, Germany) and analyzed for UV absorption (UV-1603; Shimadzu, Kyoto, Japan) at 316 nm. The cumulative percent of drug released was calculated according to calibration curves for each pH buffer solution and a correction was applied for the cumulative dilution caused by replacement of the sample with an equal volume of fresh medium. All experiments were made in triplicate.

Fit factors (f_1 and f_2) were used for comparing the dissolution patterns because they are very popular methods used to compare dissolution profile data and are recommended for use in a number of FDA guidance documents.[21] For curves to be considered similar, f_1 value should be close to 0 and f_2 value to 100. Generally, f_1 values ≤ 15 and f_2 values ≥ 50, which means an average difference of no more than 10% at the sample time points, ensures equivalence of the curves and thus of the performance of the test and reference products.[22]

In Vivo Study Design

In vivo study was designed as a two-way randomized crossover study, with 2 weeks' dosing interval, with a single oral dosing administration (in six male New Zealand rabbits with an average weight of 3.3 kg) to minimize any residual or cumulative effects of the preceding dose. The study was approved by the local Committee of Laboratory Animal Care from the University Hospital of Coimbra in accordance with the rules and guidelines concerning the care and use of laboratory animals. Rabbits were housed individually in cages under environmentally controlled conditions, on a 12-h light/dark cycle, and were given free access to food and water. The day before each experiment, rabbits were fasted for 14−16 h and provided water ad libitum. They were catheterized in the marginal ear vein for blood sampling before drug administration. All rabbits received two tablet formulations corresponding to 40 mg of VP as a single dose. The relatively high dose was needed to ensure blood levels appropriate for analytical detection and pharmacokinetic analysis.[15] Tablet formulations were administered orally by gently compelling the opening of the rabbit mouth and depressing of the tongue with pincers. Tablets were swallowed intact by pushing them to the back of the pharynx with a flexible gastric tube. After tablet administration, approximately 10 mL of water was given orally with a syringe to facilitate swallowing and to prevent the tablet from sticking to the rabbit throat. Serial

blood samples (1.2–1.5 mL) were drawn from the marginal ear vein before drug administration and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after drug administration, to spiked ethylenediaminetetraacetic acid tubes to prevent clotting and centrifuged (3500 rpm/15 min) immediately to obtain plasma. Plasma samples were then stored at −20°C until analysis.

Determination of VP Plasma Levels

Frozen samples were allowed to thaw at room temperature. Rabbit plasma (0.5 mL), test sample or free plasma spiked with known amounts of VP, along with 50 µL of 0.35 µg/mL hydroxyprogesterone acetate solution [internal standard, (IS)], were deproteinized by the addition of 1.5 mL of acetonitrile following vortex mixing at high speed for 30 s and centrifugation for 10 min at 5300 rpm. The clear supernatant was transferred to a new glass tube having 150 µL of 1 N hydroxide sodium solution. After equilibrating, 3.5 mL of n-hexane was added and the extraction was performed by vortex mixing for 1 min. The tubes were centrifuged (5300 rpm/3 min) and the organic layer was transferred to another set of clean tubes to be back-extracted with 1.5 mL of 0.05 N sulfuric acid solution (vortex mixing for 1 min). Tubes were centrifuged (5300 rpm/3 min), the organic layer was discarded and the aqueous phase transferred to a clean tube containing 100 µL of 1 N hydroxide sodium solution and back-extracted with 3.5 mL of n-hexane. After mixing (1 min) and centrifuging (5300 rpm/3 min), the organic phase was recovered to a clean tube to be evaporated to dryness at 40°C under vacuum. The dried residue was reconstituted with 120 µL of mobile phase and 100 µL was injected into the chromatographic system. The extraction efficiency of VP was determined by comparing the peak height of extracted samples to those of nonprocessed quality control (QC) samples at three different concentration levels of 10, 50, and 100 ng/mL ($n = 5$) and was found to be in the range of 90.7–98.3%.

Drug concentration levels were determined using a reversed-phase HPLC method. Briefly, quantitative analysis was performed on an HPLC chromatograph (model 1050; Hewlett-Packard) equipped with an injection valve of 20-µL sample loop (model 7125; Rheodyne, Cotati, CA), a stationary phase of LiChrospher 100 RP18 (250 × 4.6 mm, 5 µm; Merck, Darmsdadt, Germany) fitted with a LiChrospher RP18 guard column

(4 × 4 mm, 5 µm; Merck). The mobile phase consisted of a mixture of acetonitrile–0.01 M sodium phosphate buffer solution containing 5 mM heptane-1-sulfonic acid sodium, and 0.2% (v/v) triethylamine, for which pH was adjusted to 5.5 with 1% (v/v) phosphoric acid solution (55:45). The mobile phase was degassed by passing through a 0.45-µm membrane filter (PVDF, Tracer®, Teknokroma) before use. The volume injected was 100 µL which was eluted isocratically with a flow rate of 1.0 mL/min at 30°C, with detection performed with ultraviolet absorption at a wavelength of 230 nm. The extraction and HPLC method resulted in symmetrical peak shape and good baseline resolution for both VP and IS, with retention times of approximately 8.5 and 10.3 min, respectively.

Stock solutions (0.1 mg/mL) of VP and IS were prepared by dissolution in acetonitrile and kept at 4°C. Standard solutions ($n = 4$) of VP in human plasma (5, 10, 25, 50, 100, and 150 ng/mL) were prepared daily from stock solutions by spiking the suitable volume of various working solutions diluted in the mobile phase and extracted and analyzed as described above. Peak height ratios of each VP to IS were measured and the calibration curves of three consecutive days were obtained from the least-squares linear regression. The average regression line was used to calculate the respective concentrations of VP in the plasma samples. The linearity of detector response was assessed for extracted plasma standards over the range of 5–150 ng/mL. The calibration curve of VP exhibited an excellent linearity and a correlation coefficient of 0.9989.

The intra-day precision of the method (coefficient of variation) was assessed, on a single day, by extracting and analyzing free plasma spiked with known amounts of VP appropriate working solutions yielding concentrations of 10, 50, and 100 ng/mL (QC samples, $n = 5$). The intra-day precision was 6.3% (10 ng/mL), 3.4% (50 ng/mL), and 1.5% (100 ng/mL). The inter-day precision (coefficient of variation) was determined for the same QC samples ($n = 5$) on three consecutive days and was found to be 7.2% (10 ng/mL), 3.9% (50 ng/mL), and 2.1% (100 ng/mL). Accuracy, expressed as the mean % [(mean measured concentration)/(theoretical concentration)] × 100, was determined simultaneously as intra- and inter-day precision in the same QC samples ($n = 5$). The measured values varied between 97.4–104.5% (intra-day) and 93.1–106.2% (inter-day). These results confirmed the precision and accuracy of the method.

Pharmacokinetics and Statistical Data Analysis

The pharmacokinetic parameters extracted from the plasma data were calculated using noncompartmental analysis (WinNonlin®, version 1.1) and included the maximum plasma concentration (C_{max}), the time to reach the maximum plasma concentration (t_{max}), the whole area under the drug plasma concentration-time curve ($AUC_{0-\infty}$), the mean residence time (MRT), the elimination half-life ($t_{1/2}$), and elimination rate constant (λ_z). The relative bioavailability of formulations B, C, and D was calculated taking as reference formulation A, through the ratio of the respective $AUC_{0-\infty}$ values. Data are presented as mean values ± standard deviation.

Statistical analysis of the pharmacokinetic parameters (C_{max}, t_{max}, $t_{1/2}$, MRT, λ_z, and $AUC_{0-\infty}$) was performed using GraphPad Prism® version 4.0 software, by one-way analysis of variance and followed by Tukey's post test for multiple comparison analysis. A value of $p \leq 0.05$ defined the statistical equivalence.

RESULTS AND DISCUSSION

VP Solubility Studies

Solubility and pH-solubility profiles are particularly useful means of identifying compounds likely to have absorption and distribution problems,[23] especially if drugs present pK_a values in the physiological range, because the apparent solubility may change greatly with changes in pH of the environment.[18] Because the rate and extent of drug release from most controlled release systems are influenced by the pH of the dissolution medium for drugs with pH-dependent solubility and this dependency of drug release on pH may lead to additional inter and intra-subject variability in drug absorption,[24] VP solubility was examined as a function of pH over the range typically encountered under fasted physiological conditions of the human gastrointestinal tract.

In the pH range studied (1.2–8.0), VP showed an approximately linear relationship between the logarithm of the solubility and pH (Fig. 1). Over this range, the solubility was significantly enhanced by a reduction in pH, as expected, because of the weak base character ($pK_a = 7.31$) of the drug and a drastic decreased solubility was observed with higher pH values. Indeed, the estimated solubility values for each pH solution studied were 24.16 ± 0.04 mg/mL at pH 1.2,
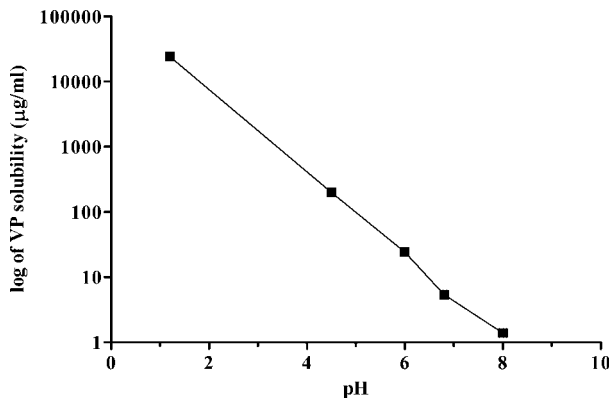

Figure 1. VP pH solubilization profile in the pH range of 1.2–8.0. Each point represents mean values ± SD.

199.14 ± 4.49 µg/mL at pH 4.5, 24.23 ± 0.25 µg/mL at pH 6.0, 5.38 ± 0.53 µg/mL at pH 6.8, and 1.39 ± 0.15 µg/mL at pH 8.0. Hence, VP solubility was increased by a factor of approximately 17,400 considering both extreme pH values tested.

The solubility studies reported clearly illustrate the impact of pH on VP solubility and dissolution. From the solubility values obtained, it is predictable that the extent of VP dissolution in the gastric environment will be high. Oppositely, under the pH values generally found in the upper regions of the gastrointestinal tract, the solubility and dissolution of pure VP will not be sufficient for complete dissolution of the doses habitually administered. However, it is expected that this drawback may be overcome by multicomponent complexation of VP with CDs and TA, because resulting complexes have demonstrated to have better solubility and dissolution performances in simulated intestinal fluid (pH 6.8) (Ribeiro et al., submitted for publication).

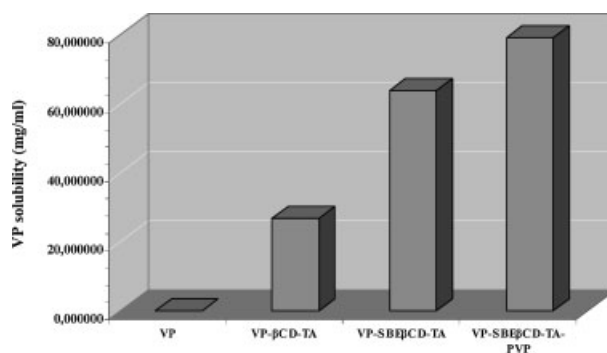VP solubility measurements in deionized water are schematically represented in Figure 2. As

Figure 2. VP aqueous solubility obtained from the uncomplexed form and lyophilized multicomponent complexes. Results are presented as mean values.

expected from previous work,[13] VP solubility in multicomponent form was found to be higher than pure VP. In fact, VP solubility in uncomplexed form (≈ 5 µg/mL) increased to approximately 80 mg/mL in VP-SBEβCD-TA-PVP multicomponent complex, which corresponds to a 16,000-fold-higher increase on VP aqueous solubility. The improved VP solubility observed as a consequence of multicomponent complexation was greater for VP-SBEβCD-TA lyophilized complexes compared with VP-βCD-TA lyophilized complex, clearly denoting the superior solubilization and complexation efficiency of SBEβCD. In addition, solubility improvement of VP may be associated with the high energetic amorphous state after complexation in VP from VP-SBEβCD-TA lyophilized complexes as confirmed previously by X-ray diffraction studies performed.[12]

In Vitro Dissolution Testing

In vitro dissolution testing serves as an important tool for characterizing the biopharmaceutical quality of a product at different stages in its life cycle. In early drug development, *in vitro* dissolution properties are supportive for choosing between different alternative formulation candidates for further development and for evaluation of active ingredients.[25] The therapeutic benefit of a number of drugs administered in a traditional dosage form is sometimes limited by physiological barriers, undesirable physiochemical drug properties, or issues of drug toxicology. In such cases, the development of drug delivery systems that produce a modified *in vivo* drug release is a common research aim. By manipulating the release of drugs from its dosage form, such restrictions may be overcome and an improvement in therapeutic effect observed.[26] The present *in vitro* dissolution study was designed to theoretically investigate the influence of drug release properties from immediate and controlled release formulations, the dissolution of the different dosage forms, and the release performance of drug particles from multicomponent complexes through the hydrated gel layer of HPMC tablet formulations.

The release profiles of VP from an immediate release formulation (A) and from hydrophilic HPMC-based matrix formulations (B, C, D) are presented in Figure 3. Because of the basic nature of VP, the release of the drug from formulation A was immediate in the first hour of the dissolution testing (pH 1.2) and thus there was no more VP available to proceed on the following steps of
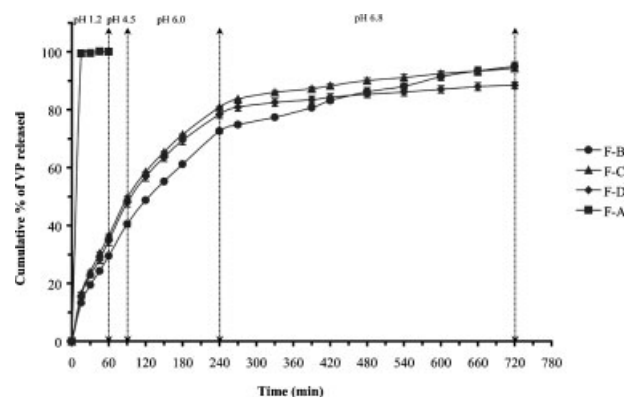

Figure 3. Dissolution profiles of the immediate release formulation (F-A) and multicomponent complex-based HPMC matrix formulations (F-B, F-C, and F-D) of VP. Each point represents mean values $\pm$ SD.

the dissolution experiment. Based on previous solubility studies, a drastic decrease was expected on VP solubility and dissolution at higher pH values; therefore, multicomponent complexation was attempted to improve the dissolution performance of the drug over a pH range that simulates the one found in the fasted gastrointestinal tract. Hence, hydrophilic HPMC-based matrix formulations B, C, and D, containing the drug in the form of VP-CD-TA multicomponent complexes, were designed to overcome the VP solubility drawback at higher pH values and to extend drug release for a longer period of time without the risk of precipitation. The dissolution profiles of these formulations clearly indicated a controlled release pattern over 12 h of the experiment, because HPMC tablet formulations swelled upon contact with the dissolution media and a gel layer was formed on their surface. This gel retarded further ingress of fluid and subsequent drug release. Nevertheless, drug release was nearly complete in the end of the dissolution experiment, almost certainly because of higher solubility and dissolution performances of VP after multicomponent complexation. Consequently, there existed a clear difference in the *in vitro* drug release characteristics of hydrophilic HPMC-based matrix formulations B, C, and D as compared with VP immediate release formulation A, which in turn, was expected to provide a different *in vivo* drug release.

The results of the mathematical comparison of hydrophilic HPMC-based matrix formulations B, C, and D, by applying f_1 and f_2 fit factors, revealed a convergence of the dissolution profiles of these formulations (Table 2) and demonstrated the

Table 2. Fit Factor Values Obtained for Formulations B, C, and D

Fit Factor	F-C/F-B[a]	F-C/F-D[a]	F-B/F-D[b]
f_1	8.4	4.5	4.1
f_2	58.3	72.0	63.0

[a]Taking formulation C as reference.
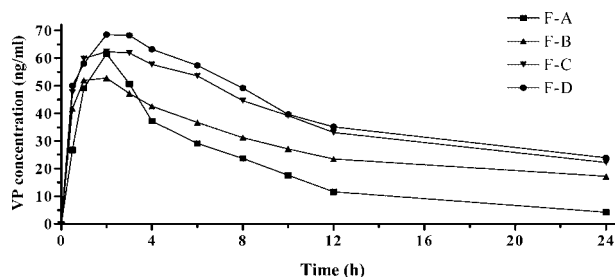[b]Taking formulation B as reference.

Figure 4. VP plasma concentration-time profiles after oral administration of VP immediate release formulation (F-A) or hydrophilic HPMC-based matrix formulations containing VP multicomponent complexes (F-B: VP-βCD-TA, F-C: VP-SBEβCD-TA, and F-D: VP-SBEβCD-TA-PVP). Each point represents mean values.

equivalence of *in vitro* performances of formulations containing VP-βCD-TA, VP-SBEβCD-TA, and VP-SBEβCD-TA-PVP multicomponent complexes ($f_1 \leq 15$ and $f_2 \geq 50$). This fact may have relevant pharmaceutical usefulness, in the case of formulation C and D containing respectively VP-SBEβCD-TA and VP-SBEβCD-TA-PVP multicomponent complexes, because the latter complex incorporates a higher amount of VP, due to the enhanced complexation efficiency of SBEβCD toward VP in the presence of PVP, and consequently a decrease of approximately 13% of SBEβCD in the solid dosage form.[12,13,27]

Pharmacokinetics and Bioavailability Studies

The mean VP plasma concentrations obtained after a single oral dose administration of the different formulations were plotted versus time in Figure 4 and related pharmacokinetic parameters are presented in Table 3. The absorption of VP from the immediate release formulation (F-A) was rapid reaching the maximum plasma level of 61.52 ng/mL after 2 h. VP in this formulation showed a relatively high elimination half-life (6.38 h) when compared with the reported value of 2.33 h in humans,[6] which can be due to the high administered dose of the drug, but that is in the limit value of 2–6 h reported by Wichert and

Rohdewald.[14] However, when compared with hydrophilic HPMC-based matrix formulations B, C, and D, the levels of VP plasma from formulation A decreased quickly. This rapid decrease was probably caused by a decrease of absorption at the intestinal level because of the lack of the drug in its dissolved form, which is strongly influenced by the pH in the intestine.[28] No statistical differences in the time values to reach the maximum plasma concentration between VP immediate release formulation (F-A) and HPMC-based matrix formulations (F-B, F-C, and F-D) formulated with multicomponent complexes could be observed ($p > 0.05$). Nevertheless, extended release patterns were evident for the absorption of VP from the latter formulations. This is likely due to the slow release rate of VP under a prolonged period through the hydrated gel of the HPMC-based matrix formulations. Therefore, despite the initial rapid increase in plasma level of VP from hydrophilic HPMC-based matrix formulations, a relatively constant plasma level was

Table 3. Mean (±SD) Pharmacokinetic Parameters and Relative Bioavailability of VP (40 mg) in Six Rabbits, after the Oral Administration of VP Immediate Release Formulation (F-A) or Hydrophilic HPMC-Based Matrix Formulations Containing VP Multicomponent Complexes (F-B, F-C, and F-D)

Formulation	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)	λ_z (h^{-1})	MRT (h)	Relative Bioavailability (%)
F-A[a]	61.52 ± 10.54	2.0	518.35 ± 76.47	6.38 ± 1.41	0.1130 ± 0.0227	9.74 ± 2.76	—
F-B[b]	50.90 ± 6.18	1.5	1086.37 ± 248.69	15.46 ± 4.44	0.0484 ± 0.0156	23.02 ± 6.74	209.58
F-C[c]	64.17 ± 5.10	2.0	1416.26 ± 236.92	14.67 ± 3.55	0.0497 ± 0.0131	21.86 ± 5.25	273.22
F-D[d]	71.19 ± 6.81	2.5	1499.89 ± 150.69	14.85 ± 2.11	0.0475 ± 0.0069	22.01 ± 3.19	289.96

[a]Immediate release formulation containing pure VP.
[b]Controlled release formulation containing VP-βCD-TA multicomponent complex.
[c]Controlled release formulation containing VP-SBEβCD-TA multicomponent complex.
[d]Controlled release formulation containing VP-SBEβCD-TA-PVP multicomponent complex.

maintained for a longer time, reflecting the *in vitro* release characteristics.

Despite similar *in vitro* release profiles of VP from formulations B, C, and D, the *in vivo* study revealed differences in the values of C_{max} between the formulations. Indeed, the C_{max} value obtained for formulation B was significantly lower than the ones obtained for formulations C ($p < 0.05$) and D ($p < 0.001$); this difference was probably related to the distinct solubility performances of βCD and SBEβCD complexes, discussed previously. Conversely, MRT, $t_{1/2}$, and λ_z values were comparable for all three formulations ($p > 0.05$) and significantly different from the same parameters of formulation A ($p < 0.001$). Formulations B, C, and D produced a 2.2- to 2.4-fold increase in MRT values compared with formulation A, clearly suggesting a longer maintenance of VP in plasma and hence prolonged release in the rabbit model. Upon comparing $t_{1/2}$ values, we found that for controlled release matrices, the values were more than double those for the immediate release formulation (F-A). Because $t_{1/2}$ values should be similar for the same substance, the differences encountered here are due to a prolonged absorption phase in the controlled release matrix formulations (F-B, F-C, and F-D), where there is a prolonged continuous introduction of VP into the bloodstream.[29]

Compared with the VP immediate release formulation (F-A), the administration of all hydrophilic HPMC-based matrix formulations increased the $AUC_{0-\infty}$ values of VP, reflecting a greater systemic exposure and, consequently, higher oral bioavailability of the drug. The ratio of mean $AUC_{0-\infty}$ ranged from 2.1 to 2.9 and the difference in $AUC_{0-\infty}$ values between the formulations was found to be statistically different ($p < 0.001$). The more than twofold increase in the mean $AUC_{0-\infty}$ values of formulations B, C, and D, was afforded by a greater VP aqueous solubility and dissolution rate from multicomponent complexes. In turn, this made possible the design and use of controlled release solid dosage forms, with smaller risk of drug precipitation in the upper regions of the gastrointestinal tract, and, consequently, in association to HPMC-based matrix formulations, a maintenance of a more constant VP blood level.

In corroboration with our previous *in vitro* results, there was no significant difference between $AUC_{0-\infty}$ values of formulations C and D ($p > 0.05$) and therefore similar oral bioavailability in rabbits was observed with both formulations.

These formulations were obtained respectively with VP-SBEβCD-TA and VP-SBEβCD-TA-PVP multicomponent complexes. In the latter complex, PVP increased the solubility and complexation efficiency of SBEβCD toward VP and as a result the amount of SBEβCD in solid dosage forms was reduced approximately 13%.[13] Such observation suggests the possibility of decreasing the amount of CDs in solid dosage forms, by the addition of water-soluble polymers to the aqueous complexation media, thereby contributing to the reduction of formulation bulks, toxicity, and costs with CDs, without injury of their pharmaceutical potential, as previously stated by other authors.[30]

Significantly higher $AUC_{0-\infty}$ values were obtained with both formulations C and D relatively to formulation B. The differences stated reflect the same trend observed for C_{max} values for these formulations and hence are probably related to different solubility and efficiency complexation performances of VP observed with both CDs. Thus, the use of VP-SBEβCD-TA multicomponent complexes in the design of controlled release solid dosage forms seems to be a better approach than that of VP-βCD-TA multicomponent complexes.

CONCLUSIONS

The present study demonstrates the suitability of the combined CD multicomponent complexation and controlled release delivery strategies to improve and maintain the oral absorption of VP for a longer period of time.

Solubility is an important property that influences drug liberation and absorption and hence has an important role in drug bioavailability, because for a drug to be absorbed it must be present in the form of an aqueous solution in the absorption site.[31] Therefore, in an effort to achieve better solubility and dissolution properties through all regions of the gastrointestinal tract, controlled release rate, as well as improved oral bioavailability of VP, we have prepared VP-CD-TA multicomponent complexes and then optimal formulations were designed by the combination of these complexes into HPMC-based hydrophilic tablet dosage forms. A comparative *in vitro* dissolution study of the resulting formulations with an immediate release formulation of the drug clearly showed controlled and almost complete release behavior of VP over a 12-h period in the case of HPMC formulations. Conversely, complete dissolution of VP was observed for the

immediate release formulation in the first hour of the dissolution experiment, because of the basic nature and pH-solubility dependence of the drug.

Further biopharmaceutics studies demonstrated that controlled release multicomponent complex-based formulations could dramatically improve VP bioavailability over an immediate release formulation, after oral administration in rabbits. The lower oral bioavailability of VP from the immediate release formulation was attributed to poor solubility at intestinal pH, leading to precipitation of the drug from the dosage form, followed by a slow process of dissolution and hence a limiting step for absorption. However, increasing the solubility of drug such as through multicomponent complexation with CDs and hydroxy acids, as used in the present study, ensured better VP dissolution in the upper regions of the gastrointestinal tract and resulted in higher extent of bioavailability. Furthermore, the administration of these multicomponent complexes assisted by their formulation in HPMC matrix tablets resulted in the maintenance of higher plasma drug levels, thus supporting that not only multicomponent complexes but also additional properties of the carrier material may be responsible for the controlled release and improved oral bioavailability of VP.

The use of experimental animals such as rabbits is common to predict pharmacokinetic parameters of drugs administered by the oral route in humans.[16,32–34] Although VP solubility and dissolution studies were performed in conditions that intended to simulate the pH range found in physiological conditions of the human fasted gastrointestinal tract and *in vivo* studies were performed in the rabbit model, we believe that interspecies comparisons with humans are feasible because the pH values found in the gastrointestinal tract of rabbit vary between 1.9 in the stomach to 6.0–8.0 in the intestine.[35] Considering the relatively high pK_a value of VP (7.31), such differences should not reflect an important variability of VP dissolved in the different regions of the gastrointestinal tract in both species.

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