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Optimization of a Dissolution Method in Early Development Based on IVIVC Using Small Animals: Application to a BCS Class II Drug

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ABSTRACT

The aim of the present study was to develop optimal dissolution conditions for a BCS Class II drug in early development. The model drug efavirenz was formulated in two dosage forms: a classical immediate-release suspension and modified-release cubosomes. Dissolution studies were carried out in classical and biorelevant media adapted to early development using animal models to confirm the in vitro findings. The percentage drug dissolved was calculated in all media, and the percentage absorbed was calculated from in vivo data. Level A IVIVC was investigated in all media, and the best one selected based on the highest r^2 value. Optimal dissolution media that could be used as starting points for further development are 0.5% SLS in water for cubosomes and fasted-state simulated intestinal fluid (FaSSIF) for suspensions. The predicted plasma profiles show appropriate contour, and the internal prediction error was at most 12% for the predicted parameters C_{max} and *AUC*. Thus, a simple approach was used for developing IVIVC based on animal in vivo data, which can be used as a quick and simple alternative tool in early development stage depending on which dissolution method can be optimized even before human studies.

KEYWORDS: Efavirenz; biorelevant dissolution; BCS Class II; level A IVIVC; time scaling; prediction error.

INTRODUCTION

Development of a suitable dissolution method is always a challenge in early development before any human in vivo data are available. Either standard techniques and media or methods that are more sophisticated are selected based on previous knowledge or on hypothesis. Animal data, especially from small animals such as rats, can be obtained in early phases with adapted formulations to assist in selecting the best formulation strategy and to optimize the dissolution method. For example, BCS Class II drugs are poorly water soluble and highly permeable, dissolution being the rate-limiting step for in vivo absorption. Often formulation strategies such as solid dispersions, lipid formulations, SMEEDS, and cubosomes are developed to increase bioavailability of these drugs. For classical BCS Class II compounds, the outcome and predictability of the dissolution method depends not only on the formulation but also on the dissolution medium and conditions. For example, a study of the effect of change in pH and surfactant concentration on Class II drug release from formulations is recommended. Alternatively, simulated gastric and intestinal media at fasted and fed states are commonly used as biorelevant media for in vivo prediction for Class II drugs (*1, 2*). As numerous options exist, selection of the right dissolution media and conditions becomes a prerequisite in setting up the in vitro dissolution studies for dosage forms of Class II drug in early development.

Efavirenz (EFV), a Class II antiviral drug, was used as a model drug. EFV exhibits low solubility in a classical immediate-release (IR) formulation such as a tablet or suspension, thus a lipid-based drug delivery system such as a cubosome was developed to improve its solubility. After the oral administration of cubosomes, the lipid component undergoes enzymatic hydrolysis due to various lipase enzymes, and its lipolytic products enhance the solubility of BCS Class II compounds. It was also reported (*3, 4*) that a small quantity of lipid enhances gall bladder contractions, which facilitates increased dissolution of coadministered poorly soluble compounds. These considerations indicate that the use of dissolution testing based on classical media could lead to misinterpretation of the in vivo outcome of cubosomal drug delivery systems.

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IVIVC is used to develop a meaningful relationship between the in vivo behavior of a formulation and in vitro drug release of the same formulation. Establishing IVIVC allows the use of in vitro release profiles as surrogates for in vivo studies (*5*). In the early development stage before human studies, IVIVC could be used to select the best in vitro dissolution conditions and to develop the optimal formulation. The selection could be based on available in vivo data or on a simple animal model.

IVIVC of BCS Class II compounds for dissolution method development is more significant because any changes in vitro would be reflected in absorption, and thus optimized media would be useful for predicting in vivo behavior of the drug. The goal of the present study was to develop optimal dissolution conditions for EFV in a classical IR suspension and a modified-release formulation such as a cubosome, which was designed to optimize availability of the drug, using IVIVC.

MATERIALS AND METHODS

Materials

EFV was obtained as a gift sample from Lupin Pharmaceuticals, Inc. (Pune, India). Carboxymethyl cellulose (CMC) sodium was obtained from Oxford Laboratories (Mumbai, India). Sodium taurocholate, pepsin, pancreatic lipase, hydrochloric acid, maleic acid, acetic acid, and potassium dihydrogen phosphate were purchased from Loba Chemie Pvt. Ltd. (Mumbai). Lecithin was obtained from New Modern Chemicals (Mumbai). All other reagents and chemicals were of analytical grade and were used as received. Double-distilled water was used for all analytical work.

Animals

Male Wistar rats (250–300 g) were divided randomly into three groups of six animals each. All animals were housed under standard conditions with free access to water and laboratory diet ad libitum. All animal care and experimental studies were approved by the Institutional Animal Ethical Committee of Sinhgad College of Pharmacy, Pune, India, constituted under CPCSEA Ministry of Environment, Forest and Climate Change, Government of India.

Formulation of Sustained-Release Cubosomes

Sustained-release (SR) cubosomes were prepared by a sonication method. Fifteen milliliters of monoolein was gently melted in a water bath at 70 °C, and 4 g of drug was dissolved in it. The solution was then injected dropwise into a preheated Poloxamer 407 solution (1 mL Poloxamer 407 in 88 mL of water) at 70 °C and subjected to mechanical stirring at 1500 rpm for 5 min. Dispersions were cooled to room temperature and then ultrasonicated at maximum power of 120 W (UCB-40, Spectrolab, India) for 1 min (*6*). After equilibration for 24 h, cubosome dispersions were obtained. The formulation that had the smallest particle size with the best entrapment efficiency was selected as the optimized formulation and was used in the present study (*7*).

Formulation of Efavirenz IR Suspension

Sodium CMC 1.5% w/v, 0.5% SLS, and 4 g drug were triturated together with 50 mL of water to form a smooth paste. The mixture was transferred to a 100-mL measuring cylinder, and the volume was adjusted to 100 mL with distilled water.

Determination of Solubility

Before the dissolution medium was selected, solubility studies were done using a shake-flask methodology. The saturation solubility of EFV was determined in pH 6.8 and pH 7.4 phosphate buffers; water; 0.1 N HCl; 0.5%, 1%, and 2% sodium lauryl sulfate (SLS); simulated gastric fluid (SGF); $SGF_{sans\ pepsin}$; fasted-state simulated intestinal fluid (FaSSIF); fed-state simulated intestinal fluid (FeSSIF); and lipolytic media with 800 USP lipase units and with 2× 800 USP lipase units. Ethanol and methanol solubility was also performed as high-solubility references. All media were prepared, and an excess amount of EFV was added to 5 mL of each medium placed in vials, which were then shaken on a mechanical shaker. After 48 h of shaking, a 1-mL aliquot was taken from each sample and filtered through 22-µm Whatman filter paper No 41. Absorbance was measured in the range of 200–400 nm on a UV–vis spectrophotometer, and the solubility of drug in each medium was calculated.

In Vitro Drug Release Study

Dissolution studies were conducted in a dissolution apparatus using the Labindia mini paddle device (250-mL vessel). The mini paddle is based on the USP paddle setup with dimensions scaled down by exactly one third (*8*). This setup was used to study doses and formulations for animals, keeping the shape and volume of the dissolution apparatus proportional to those used for human formulations. The composition of the dissolution medium used for all formulations was based on the GI physiological conditions in rats (*9*).

A dialysis method was used to evaluate the in vitro release of cubosomes using a Labindia mini paddle at 50 rpm and 37 ± 0.5 °C (*10, 11*). A cubosome formulation (equivalent to 10 mg of EFV) was placed in a dialysis bag (cellophane membrane, molecular weight cutoff 10,000–12,000, Hi-Media, India), which was then sealed at both

ends. The dialysis bag was dipped into the receptor compartment containing the dissolution medium. At selected intervals, 1-mL aliquots were withdrawn from the release medium and replaced with the same amount of medium to maintain sink conditions.

The in vitro release of the IR suspension was done using a Labindia mini paddle at 50 rpm and 37 ± 0.5 °C. The IR suspension (equivalent to 10 mg of drug) was added with a glass syringe at the center of the dissolution vessel (*10, 11*). At selected intervals, 1-mL aliquots were withdrawn from the release medium and replaced with the same amount of medium. The same procedure was used in all dissolution media (n = 6). The samples were analyzed by UV–vis spectrophotometer at 247 nm, and the amount of drug release at various times (%CDR) was calculated. A graph of %CDR versus time (min) was plotted. Dissolution was carried out under all dissolution conditions using all the media listed above (n = 6).

In Vivo Pharmacokinetic Study

Pharmacokinetic studies were carried out in rats that were fasted overnight (12 h). Drug formulations were administered by gavage. A tube was carefully inserted into the esophagus of conscious rats, and the corresponding dose poured into the stomach through the tube. An EFV dose of 40 mg/kg at a concentration of 40 mg/mL was investigated. After administration, blood samples (100 µL) were collected from the tail veins at 0, 1, 2, 3, 4, 6, 8, 24, and 30 h. Plasma was deproteinized with 200 µL of carbonate buffer (0.1 M sodium carbonate–sodium bicarbonate, pH 9.4). The concentration of drug was determined by RP–HPLC, and a plasma concentration–time profile was obtained (*7*).

Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated from this data using PK solver software (*12*). The best pharmacokinetic model for absorption kinetics was chosen in each case. Cubosome in vivo data fit a two-compartment model, and the suspension followed a one-compartment model after extravascular administration. The pharmacokinetic parameters (micro constants) C_{max}, AUC, K_e, T_{max}, terminal half-life, and V_d/F were calculated. The estimated pharmacokinetic model, as indicated above for absorption kinetics, allows the calculation of the fraction of dose absorbed (fab) at each in vivo time point (*13, 14*).

Level A IVIVC

An IVIVC was developed by plotting the in vitro parameter (% drug release) versus the in vivo parameter (% fab). Regression values for all the media were calculated. In the case of poor and nonlinear relationships, Levy plots were drawn. The time for certain percentages of drug dissolved in vitro is on the x axis, while the time for similar percentages to be absorbed in vivo is depicted on the y axis (*15, 16*). The Levy analysis allows correction for different rates or lag times observed between in vivo and in vitro data and leads to a final 1:1 relationship between in vitro and in vivo time points. In addition to the correlation coefficient (r^2), an additional parameter, such as %fab at the first time point, can be taken into account to select the best relationship between in vivo and in vitro data, because it would give correct indication of in vitro or in vivo lag times.

Simulated In Vivo Profile from In Vitro Data

Based on the pharmacokinetic model determined for both formulations in the early stage, a simulated plasma profile was calculated from in vitro dissolution data using a microconstant. The simulated plasma profile was estimated based on a superposition principle. At each time point, a percentage of the dose absorbed and individual kinetics are calculated, and the final profile corresponds to the sum of all the individual time points. Superposition based on the PK model, as described by Aiache (*17*), was used to estimate the plasma profiles.

Evaluation of the Predictability of IVIVC

For validation of IVIVC, a prediction error for the pharmacokinetic parameters AUC and C_{max} was calculated from the predicted plasma profile with the following formula (*18*):

$$\%PE = \frac{(observed\ value - predicted\ value)}{observed\ value} \times 100$$

RESULTS

Determination of Solubility in Dissolution Media

The solubility of EFV was determined in pH 6.8 phosphate buffer; pH 7.4 phosphate buffer; 0.5%, 1%, and 2% SLS in water; SGF; $SGF_{sans\ pepsin}$; FeSSIF; FaSSIF and lipolytic medium; water; and 0.1 N HCl. Maximum solubility was observed in media containing SLS, whereas lowest solubility was found in phosphate buffers in the absence of surfactants.

Dissolution Studies of SR Cubosomes

Dissolution of cubosomes was carried out in all dissolution conditions, and profiles in all media are shown in Figure 1A. In lipolytic media, cubosomes showed faster release as the concentration of surfactant increased. Faster drug release was observed from cubosomes, while

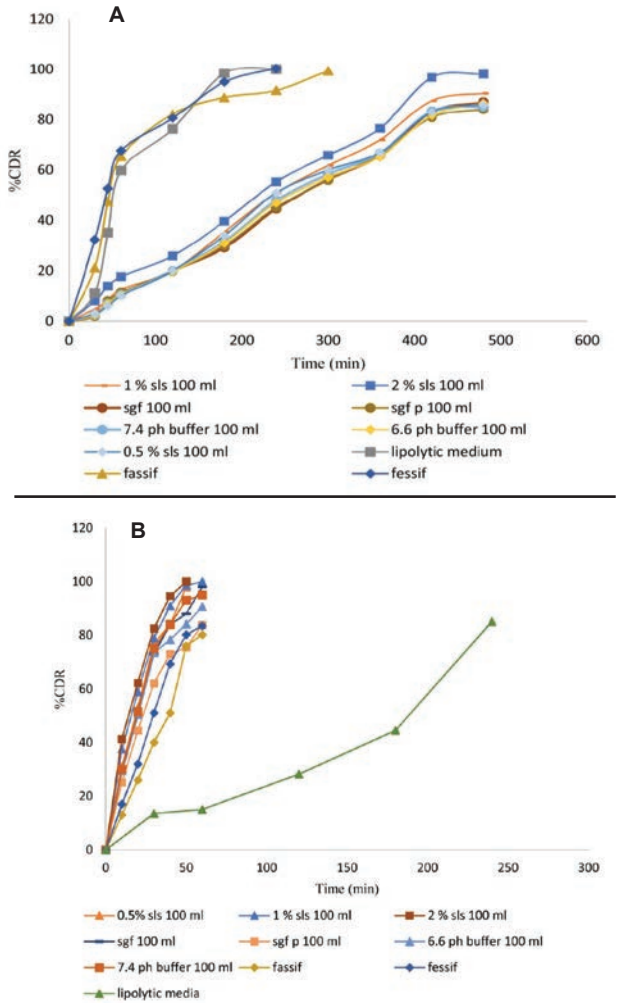

Figure 1. Dissolution profiles of (A) cubosomes and (B) suspensions in all dissolution media.

slower release was observed in phosphate buffer. Using media with volumes other than 100 mL did not result in appreciable differences in the dissolution profiles, so they were not reported.

Dissolution Studies of IR Suspension

Dissolution of a suspension was also carried out under all dissolution conditions, and profiles in all media are shown in Figure 1B.

As the dissolution medium pH increased, faster drug dissolution was observed from the IR suspension. However, the rate and extent of dissolution in the lipolytic media was less.

In Vivo Plasma Profile in Rats

The in vivo plasma concentration–time profiles in rats are shown in Figure 2 with the best-fit model values. C_{max} and *AUC* values are higher for cubosomes (around 180–200% for *AUC* and C_{max}), which indicates improved bioavailability

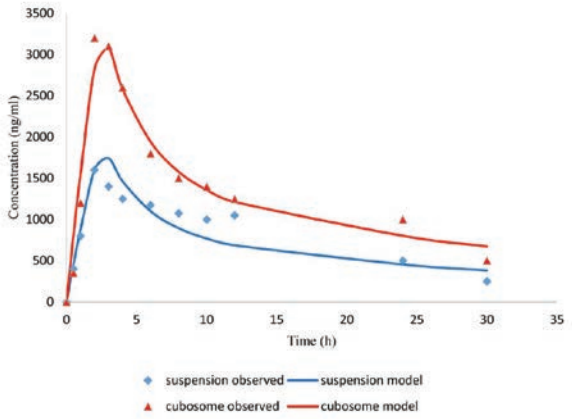

Figure 2. Model fitting of in vivo data for rats.

without major modification of the absorbed (solubilized) fraction. Pharmacokinetic parameters for cubosomes and suspensions are shown in Table 1.

Table 1. PK Parameters Obtained in Rats (n = 6) by Fitting Data in Best Fit IV Model

PK parameters	Cubosome	Suspension	Ratio % cubosome/ suspension
AUC (ng.h/mL)	51181 ± 867	29015± 588	176
C_{max} (ng/mL)	3200 ± 456	1600 ± 121	200
$t_{1/2}$ (h)	15.6 ± 3.5	11.8± 7.5	-
AUC t (ng.h/mL)	39925 ± 665	24775 ± 374	161
IV model for 40 mg/kg			
A (ng/mL)	1897		
alpha (h-1)	0.329		
B (ng/mL)	1484		
beta (h-1)	0.029		
Quality of fit			
Error %	Cubosome	Suspension	
AUC t	-1	11	
C_{max}	4	-9	
R^2	0.98	0.99	

alpha and beta: Hybrid constants resolved graphically by method of residuals
A and B: Hybrid first-order constants for rapid distribution and slow elimination phase

Absorption was calculated over 2–2.5 h, where the formulations followed zero-order absorption and disposition kinetics. This is in line with the low solubility of the drug. Figure 2 displays the observed value and the fitted data according to the intravenous model as it follows zero-order kinetics. The fit is good for cubosomes because the percentage error for the model used to fit the data results in *AUC* and C_{max} values of -1 and

4%, respectively. This is considered acceptable for a suspension because the percentage errors for the model fit of *AUC* and C_{max} are 11 and -9, respectively, which complies with predictability rules (Table 2).

Table 2. Percentage Prediction Error for PK Parameters

	Cubosome %	Suspension %	Mean %
AUC	2	12	7
C_{max}	-11	2	7

Level A IVIVC

Percentage fab was related to dissolution observed in all media for both formulations. Graphs of % fab versus % drug dissolved were established in each dissolution medium, and points were related using a linear relationship. Regression coefficients were calculated in all cases.

Levy plots for time scaling of cubosomes and suspensions after oral administration in rats are shown in Figure 3.

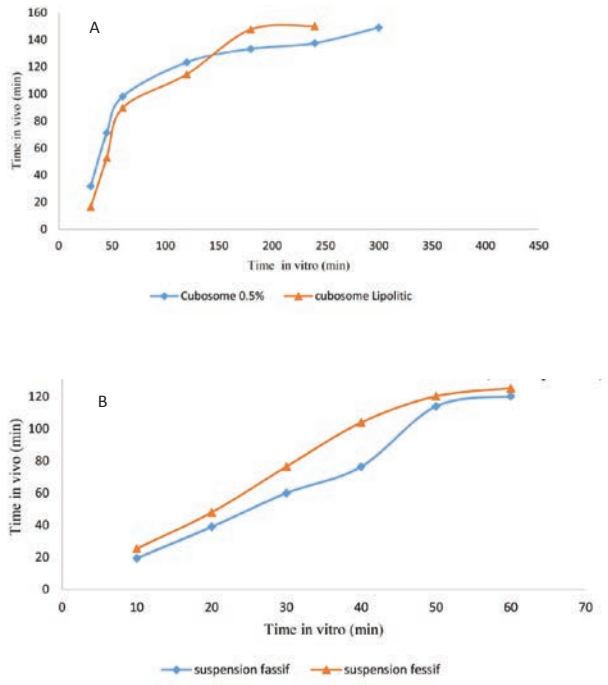

Figure 3. Levy plots for (A) cubosomes and (B) suspensions in best in vivo correlating media.

DISCUSSION

In the selection of a dissolution medium, solubility is the most prominent parameter to be considered because the amount of drug added should completely dissolve in the test medium, which is referred to as sink conditions (*19*). EFV is highly hydrophobic with functional groups like –Cl, cyclopropane, –CF3, and alkyl groups. It also contains =NH–C=O, which makes the enol extended conjugated. Hence, it exhibited the lowest water solubility compared with all other solvents as shown by the log *P* value of 4.6, which indicates low aqueous solubility. The solubility of EFV also increased proportionally with an increase in surfactant concentration. Surfactants increase wettability and decrease interfacial tension, hence they increase the solubility of a hydrophobic drug. The use of low concentrations of surfactant in dissolution media (e.g., 2% SLS) to improve the dissolution rate of hydrophobic drugs is suggested in the FDA guideline (*20*). Media that provide the highest solubility should be selected.

In the lipolytic medium, cubosomes showed faster release, possibly because of the breakdown of monoolein by lipase. The lipolytic product of the degradation of the liquid crystalline structure showed an increase in solubility and faster drug release. As the surfactant concentration was increased, faster drug release was observed from cubosomes. SLS was used as a surfactant in the medium to improve dissolution, which might have caused ion pairing with EFV leading to micellar solubilization and faster dissolution. In all phosphate buffers, cubosomes showed the lowest drug release due to the absence of surfactant in the media. However, there was a visible difference in release in relatively all the media used for dissolution.

For the IR suspension, an increase in medium pH caused faster drug dissolution; however, in lipolytic media, the rate and extent of dissolution were less. This might be due to incompatibility between medium and formulation ingredients, which may have caused precipitation of the drug. In addition, there was no lipid barrier for the lipase enzyme, as observed in the case of cubosomes. As the concentration of surfactant increased, faster drug dissolution was observed from suspension. Again, slower drug dissolution was observed in phosphate buffer. The absence of surfactant in the medium must have retarded drug solubilization.

A zero-order pharmacokinetics model for absorption better fits both the cubosome and suspension data. The only adjustment performed was the absorption extent and the ratio of *AUC*, which is being used as a correction factor (Table 1).

Level A IVIVC

The best in vivo correlating media was selected based on the highest r^2 value. The intercept was also taken into account; a too-strong negative intercept would lead to a long lag time, which does not exist in vivo, denoting a bad relationship.

Among the in vitro conditions, FaSSIF, FeSSIF, and 0.5% SLS in water showed the highest regression values for both formulations. For the suspension, FaSSIF and FeSSIF displayed an acceptable fit with r^2 = 0.9897 and 0.9815, respectively. FaSSIF is a good option for the suspension but leads to a negative intercept for the cubosome, so an alternative medium of 0.5% SLS in water (r^2 = 0.9960) was selected. A priori, the lipolytic media was thought to be the best option for the cubosome; however, the IVIVC was not perfect (low r^2 value). The suspension in lipolytic medium exhibited slower release, and a fine precipitate was observed, possibly because of incompatibility between medium and formulation ingredients.

The Levy plot is biphasic for cubosomes (Figure 3A), which points to the fact that in vitro and in vivo data are not consistent at early time points. This indicates that the exact same pattern was not observed in vitro and in vivo in early stages but was similar afterwards. Other in vivo factors that may have been involved in the process of lipolysis, such as time needed in vitro to start the lipolysis (in vitro lag time), should be accounted for during dissolution condition development. The suspension FaSSIF plot is linear in vitro (Figure 3B), which implies that the in vitro and in vivo data are consistent. There is an almost linear relationship between the in vitro dissolution time for a certain percentage of drug and the in vivo absorption time for the same percentage, which indicates similar in vivo and in vitro patterns.

Based on the previous findings from the Levy plots (Figure 3) and the fact that the linear regression value was highest for the IR suspension after time scaling, FaSSIF was the best medium for an in vitro–in vivo correlation in rats, while 0.5% SLS in water was the best medium for cubosomes.

A comparison of the dissolution of cubosome and suspension in the selected media indicates that the relationship between the in vitro times for suspension and cubosome is not linear but logarithmic (Figure 4). That indicates that the in vitro behavior is not similar for both formulations. This could be due to the nature of the cubosome, which modifies the dissolution rate observed in vitro. However, this difference is not observed in vivo; only the extent is modified, which indicates that the in vitro dissolution media cannot be universal for a drug but is formulation dependent. It also highlights the fact that in in vivo studies, the limiting factor is the extent of drug solubilization, while the rate of solubilization is not so influential.

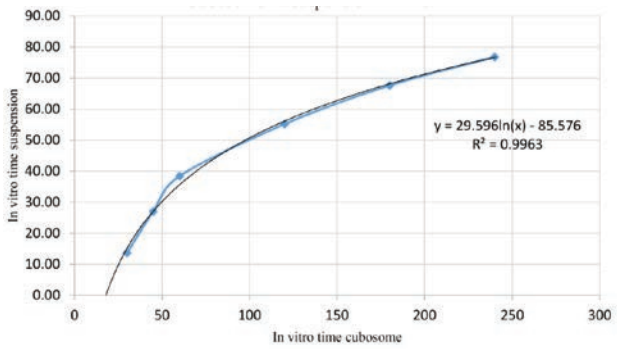

Figure 4. Comparison of cubosome and suspension in vitro dissolution in best correlating media.

The medium assigned by the Office of Generic Drugs (OGD) for the marketed formulation is 1–2% SLS in water. Most of the time compendial/OGD media and methods fail to correlate with in vivo behavior of BCS Class II drugs because they are designed for quality control rather than for formulation development. Small changes in in vitro dissolution media would be reflected in the in vivo absorption profile (*21*). Based on the IVIVC, FaSSIF was the most promising dissolution medium for classical formulations like suspensions, and media containing 0.5% SLS for cubosome formulations.

Prediction of plasma profiles from in vitro data can be explained as follows. Superposition calculates the in vivo curves based on absorption (*17*). Table 2 presents the percentage error with respect to C_{max} and *AUC* based on IVIVC. Predicted and observed plasma profiles are as shown in Figure 5. A maximum 12% of variation was observed between the predicted and observed values.

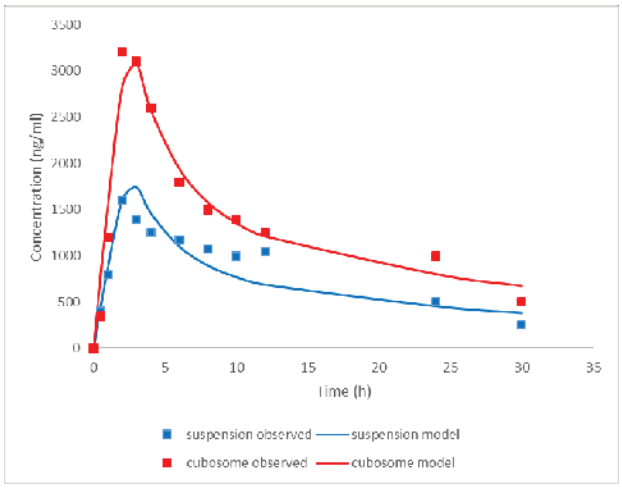

Figure 5. IVIVC predictability for cubosomes and suspension. Superposition of observed in vivo data and in vivo data predicted from in vitro data by convolution..

Between cubosome and suspension, the rate of absorption is not dramatically different; the main difference is in the extent of absorbed drug. The results seem promising to start the development of the formulations. This experiment allows selection of the best dissolution test for both formulations. Based on the selected dissolution methods, the next step is to optimize the formulation for humans.

CONCLUSION

The dissolution behavior of the BCS Class II drug EFV from IR and modified-release (SR cubosomes) in various classical and biorelevant dissolution media was studied. Based on the IVIVC, the optimal dissolution media selected were 0.5% SLS in water for cubosomes and FaSSIF for suspensions. Lipase from lipolytic media caused hydrolysis of monoolein that was used as the lipid in the formulation of cubosomes, which resulted in faster dissolution in lipolytic media. The predicted plasma profile based on IVIVC was obtained. Predicted profiles presented the right shape, and the predicted parameters were satisfied even if not within regulatory limits. These findings allowed selection of the best in vitro tool for both formulations before any human data could be obtained and thus helped in developing a better formulation. A simple approach was used to develop an IVIVC that can be used as an alternative in early development and to select the best dissolution method and formulation candidate.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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