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Design and mechanistic evaluation of novel pore-regulated polymer matrices for transmucosal drug delivery

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2012-02-14
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CHAPTER FOUR

Quantitative And Qualitative Assessments Of The *In Vivo* Drug Plasma Levels Subsequent To The Administration OF The Optimized Pore-Regulated Polymer Matrix Formulations And The Pharmacokinetic And Pharmacodynamic Modeling Of The Generated Data

4.1. INTRODUCTION

In Chapter three, the newly constructed optimized P-RPM formulation was characterized and its potential to function as a versatile transbuccal drug delivery system capable of effectively loading and modulating the *in vitro* dissolution and release of the selected model drugs (PS and CBZ) with differing aqueous solubility and permeability levels was investigated. Furthermore, the *in vitro* drug release performance of the optimized P-RPM was compared with that of equivalent leading commercially available oral tablet products. In view of the promising outcomes of the *in vitro* and *ex vivo* evaluations presented in Chapter three, it becomes essential to assess the *in vivo* performance of the formulation with respect to the changes in plasma levels over time and attempt to further evaluate the generated data through pharmacokinetic and pharmacodynamic modeling approaches. The development of suitable *In vitro-In vivo* correlation data will be included in the modeling process.

Generally, researchers use animals as models of humans in carrying out both long and short term experimental investigations. The major aim is to predict the response of humans to such treatment under examination and to evaluate the correlation between *in vitro* and *in vivo* data. These animal models are employed because research cannot always be carried out on humans for practical and ethical reasons. Besides, human studies are usually more expensive and riskier than animal bioavailability studies. In contrast to *in vitro* studies, animal models provide a dynamic interaction of multiple factors relating to the host, drug and possibly delivery system (Craig, 1993). In addition, animal models allow for frequent sampling of tissues, blood and other body fluids which may not be possible in humans. This approach allows investigators to characterize the effects of a much wider range of drug doses or types than would be ethical in human trials. Also, a reduction of inter-individual variations inherent in human studies and maximization of experimental reproducibility can be attained with animal models (Craig, 1993).

Pharmacokinetics is a term which describes the quantitative relationship between the dose of drug administered/regimens and plasma/tissue levels which may be measured as absorption, distribution, binding, excretion while pharmacodynamics depicts the association between plasma/tissue levels and the magnitude of the observed pharmacologic effects (Pérez-Urizar *et al*., 2000). Pharmacokinetic and pharmacodynamic (PK-PD) modeling involves the description, estimation and prediction of pharmacokinetic and pharmacodynamic parameters as well as their

relationships utilizing computerized mathematical simulations (Pérez-Urizar *et al*., 2000; Olsen *et al*., 2008). The information provided by PK-PD models can be valuable in optimizing dose, dosing regimens or the dosage form as a whole. Therefore, PK-PD modeling approaches can form an essential part in the development and evaluation of drugs or drug delivery systems displaying optimal physiological and therapeutic benefits with minimized undesired toxic effects (Grabnar *et al*., 1998; Pan and Hedaya, 1998; Pérez-Urizar *et al*., 2000; Frederique *et al*., 2009).

Recently, the concept and application of the *In vitro-In vivo* correlation (IVIVC) approach for evaluating pharmaceutical dosage forms have been a major center of attention for the pharmaceutical industry, academia and regulatory sectors. Two definitions of IVIVC have been proposed by the United States Pharmacopoeia (USP) and Food and Drug Administration (FDA). The USP defines IVIVC as an establishment of a rational relationship between a biological property, or a parameter derived from a biological property produced by a dosage form and a physicochemical property or characteristic of the same dosage form. The FDA on the other hand defines IVIVC as a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and a relevant *in vivo* response. Generally, the *in vitro* property is the rate or extent of drug dissolution or release while the *in vivo* response is the plasma drug concentration or amount of drug absorbed (Emami, 2006). The processes of pharmaceutical formulation development and optimization is an essential part of manufacturing and marketing of any therapeutic dosage form which may be time consuming and expensive (Emami, 2006; Sirisuth and Eddington, 2009). The optimization of such pharmaceutical formulations may require altering formulation constituents, manufacturing technologies, equipments and batch sizes. In the past, when these kinds of modifications were applied to a formulation, *in vivo* bioavailability studies would be required to be carried out so as to ensure that the new formulation is bioequivalent with the older one. Consequently, without doubt, the implementation of this type of requirements can impede marketing of the new formulation as well as elevate the cost and duration of production (Emami, 2006; Sirisuth and Eddington, 2009). Therefore, IVIVC, a regulatory approach developed by the FDA was developed to minimize the need for such additional bioavailability studies (especially in human beings) as part of the formulation design and optimization. IVIVC enables the development of *in vitro* tests that reflects the bioavailability data (Emami, 2006; Cardot *et al*., 2007). Concisely, an IVIVC can be employed to support biowaivers as well as be used to establish dissolution specifications,

support and/or validate the use of dissolution methods (Emami, 2006). Hence, an IVIVC may reduce amount of bioequivalence studies needed for approval as well as during scale-up and post-approval changes. However, the application of IVIVCs is limited to a certain product and cannot be used across drug products especially those with differing release mechanisms. For IVIVC determinations, five correlation levels namely levels A, B, C, D and multiple-level C correlations are commonly employed. Levels A, B and C are referred to as the main levels of correlation (Sirisuth and Eddington, 2009).

The goal of this Chapter was to evaluate the changes in the plasma concentration levels of PS and CBZ with time following the administration of the optimized P-RPM formulation using quantitative and qualitative approaches as well as to generate suitable PK-PD models for the evaluation and prediction of the *in* vivo performance of the P-RPM formulation for transbuccal drug delivery. In addition to these objectives, the *in vivo* performance of the drug loaded P-RPM formulation was compared to that of the commercially available oral tablets of PS and CBZ. These investigations were carried out utilizing large white pigs as animal models. To accomplish the set targets, a suitable solid phase extraction procedure for the isolation of drug from plasma samples and Ultra Performance Liquid Chromatographic methods for the quantification of PS and CBZ was developed. Also, Fourier Transform Infrared (FTIR) Spectroscopy was used quantitatively to assess the presence of PS and CBZ in the plasma. PK-PD modeling of the generated data was performed using computerized simulations/models. As part of the modeling, mathematically and statistically viable processes were used to generate IVIVC models for both *in vitro* drug dissolution and *in vivo* bioavailability data.

4.2. EXPERIMENTAL SECTION

4.2.1. Materials

The optimized drug loaded P-RPM formulation explored in Chapters two and three were employed for this experimental phase. Amitriptyline hydrochloride, anhydrous theophylline, potassium dihydrogenphosphate monobasic buffer salt, phosphoric acid (85%), acetonitirle (HPLC grade) and methanol (HPLC grade) were purchased from Sigma Chemical Company (St. Louis, USA). The leading commercially available (in South Africa) oral formulations of PS (Epanutin®) and CBZ (Tegretol®CR) were obtained from Pfizer Limited, Johannesburg, South Africa and Novartis Pharmaceuticals Corporation, Isando, South Africa respectively. Nylon 3/0

non-absorbable surgical suture was procured from Shandong Sinorgmed Co., Ltd., China. Formalin was obtained from Sizachem, Durban, South Africa. Haematoxylin and eosin stains were acquired from Brisben Chemicals, Maharashtra, India and GFS Chemicals, Inc., Powell, OH, USA respectively. Midazolam was purchased from Roche Products (Pty) Limited, Isando, South Africa and ketamine was obtained from Bedford Laboratories, Bedford, OH, USA.

4.2.2. Quantitative evaluation of *in vivo* drug absorption employing the optimized P-RPM formulations

4.2.2.1. Grouping of animals and procedure for *in vivo* studies

Animal studies were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand, Johannesburg, South Africa (Clearance Certificate Number: AESC 2007/42/05) under the supervision of the Central Animal Service Unit of the same institution. For this experimental phase, the drug loaded optimized P-RPM formulations were employed in replicates. In total, 30 healthy, female large white pigs with an average weight of 36±3kg were utilized. For experimental purposes, these animals were divided into five main groups of six pigs each.

The experimental treatments performed on each group made up of five pigs included:

Group 1: Pigs in this group were employed to test the PS (50mg) loaded optimized P-RPM formulation.
Group 2: Pigs in this group were employed to test the CBZ (50mg) loaded optimized P-RPM formulation.
Groups 3 and 4: The commercially available PS (100mg) (Epanutin®) and CBZ (200mg) (Tegretol®CR) oral tablet formulations were tested respectively using these groups of animals. This set of formulations was tested to provide data that can be used for comparison with the newly constructed P-RPM formulation to assess its efficiency in terms of attaining therapeutic plasma concentrations.
Group 5: This faction was employed to assess the drug-free or placebo formulation.

Administration of the respective drug-loaded formulations

For the newly constructed P-RPM formulation, pigs in groups 1, 2 and 5 were employed. Each formulation was applied to the non-keratinized region of the pig's buccal mucosa and an enclosed mucosal pouch sutured around it using sterile, non-absorbable suture, nylon 3/0 that was removed at the end of the study. Despite that the formulation was bioadhesive in nature, this procedure was devised to prevent the pig from chewing or swallowing the formulation and was conducted under anaesthesia within 45 minutes.

On the other hand, the commercially available tablet formulation separately containing PS or CBZ was administered orally to each pig in an upright position by gastric dosing using a specialized, large bore stomach tubing device (length 70cm, diameter 1.4cm) which was introduced through the mouth and oesophaus into the stomach. Each tablet was separately administered via the tubing device by flushing it with about 20mL of potable tap water. This process was employed to facilitate the swallowing of the respective tablet through the gastrointestinal tract for absorption into the blood stream to take place. This process was done under anaesthesia within 30 minutes. All animals were fasted but allowed to have access to drinking water during the first 8 hours of the study period.

Anaesthesia

Each pig was anaesthetized using a combination of ketamine (11mg/kg) and midazolam (0.3mg/kg) given by intramuscular injection and maintained by an inhalation anaesthetic dose of 2% isoflurane and 100% medical oxygen gas for the duration required for the administration of the respective formulations.

Blood Sampling

For the five groups of animals, blank blood sampling (10mL) was done before administering any formulation (t_{0hour}). Thereafter, blood was sampled after the pigs had regained consciousness over 24 hours at t_{1hour}, t_{2hour}, t_{4hour}, t_{6hour}, t_{8hour}, t_{10hour} and t_{24hour} through the marginal ear vein using an intravenous cannula set. A week before commencing each set of experiments, each group of pigs was habituated to the process of blood sampling through the marginal ear vein by feeding it while touching the ear. A solution of heparin in normal saline was used to flush out the cannula to keep it unblocked from blood clotting throughout the study period. Blood sampling at t_{10hour} and t_{24hour} were included to assess drug plasma levels post the targeted duration of action of the

P-RPM formulation (i.e. 8 hours). Blood samples were collected into heparinized vacutainers (Weihai Hongyu Medical Devices Co., Ltd., Shandong, China). Blood samples were centrifuged at 3000rpm for 15 minutes (Bench top Centrifuge, TD5A-WS, ShagaiHai LU Xiang Yi, China), the supernatant plasma pipetted off and stored away immediately in Eppendorf tubes at -70°C until analysis. Figures 4.1, 4.2, 4.3 and 4.4 presents photographs showing the different phases involved in the administration of the drug loaded tablet and P-RPM formulations.

4.2.2.2. Quantitative analysis: Instrumentation and chromatographic conditions

Quantitative analyses for changes in plasma levels of PS and CBZ over time were performed using the Waters Acquity Ultra Performance Liquid Chromatographic (UPLC) system equipped with the Acquity PDA and ELSD detectors and with the Acquity UPLC BEH C18, 1.7µm, 2.1×50mm column (Waters Corporation, Milford, Mass, USA).

Phenytoin sodium

The mobile phase was made up of 54% phosphate buffer, pH 5.2 and 46% of a mixture of acetonitrile (18%) and methanol (82%). For the UPLC analysis, settings were: isocratic flow rate at 0.5mL/minute, column temperature at 25°C, total run time for analysis 4 minutes, injection volume of 1µL and UV detection at a wavelength of maximum absorbance at 214nm. Amitriptyline hydrochloride was employed as an internal standard.

Carbamazepine

The mobile phase was made up of 50% methanol and 50% of phosphate buffer, pH 2.5. For the UPLC analysis, settings were: isocratic flow rate at 0.3mL/minute, column temperature at 25°C, total run time for analysis 3 minutes, injection volume of 5µL and UV detection at a wavelength of maximum absorbance at 285nm. Theophylline (anhydrous) was employed as an internal standard.

The wash solutions used for the separate analysis of both model drugs were the strong, weak and seal washes and they were composed of 90/10; acetonitrile/water, 10/90; acetonitrile/water and 100% double deionized water (Millipore water purification system, Millipore, Molsheim, France) respectively. All prepared solutions were filtered using the 0.22µm membranes (Sterile Milipore filter, Millipore Corporation, Bedford, MA, USA) under vacuum and degassed before their usage for UPLC analysis.

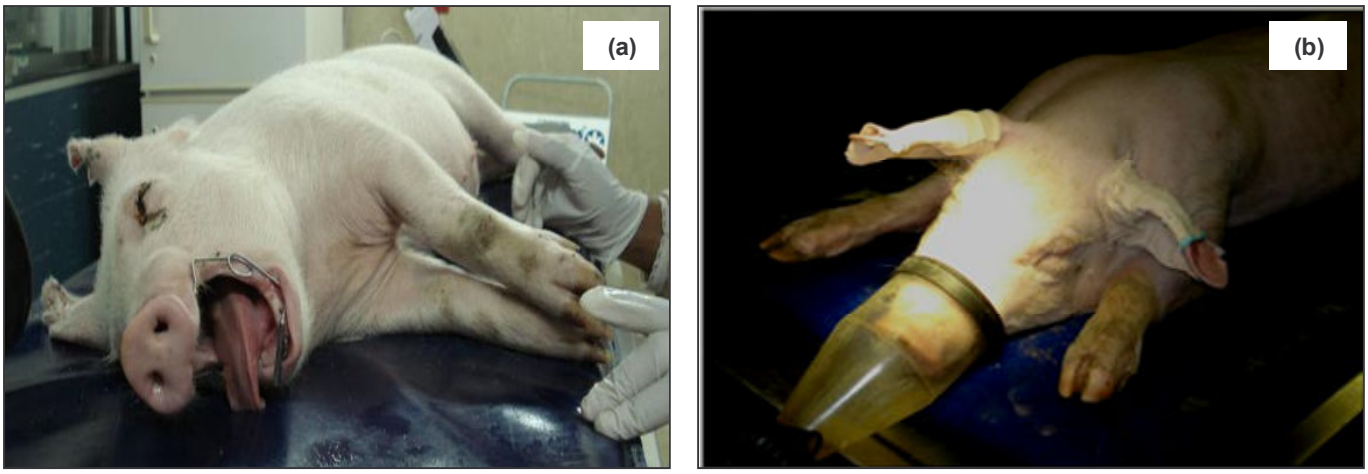

Figure 4.1: Digital photographs showing: (a) pig after intramuscular anaesthesia using midazolam and ketamine; (b) maintenance of anaesthesia employing isoflurane and oxygen by inhalation.

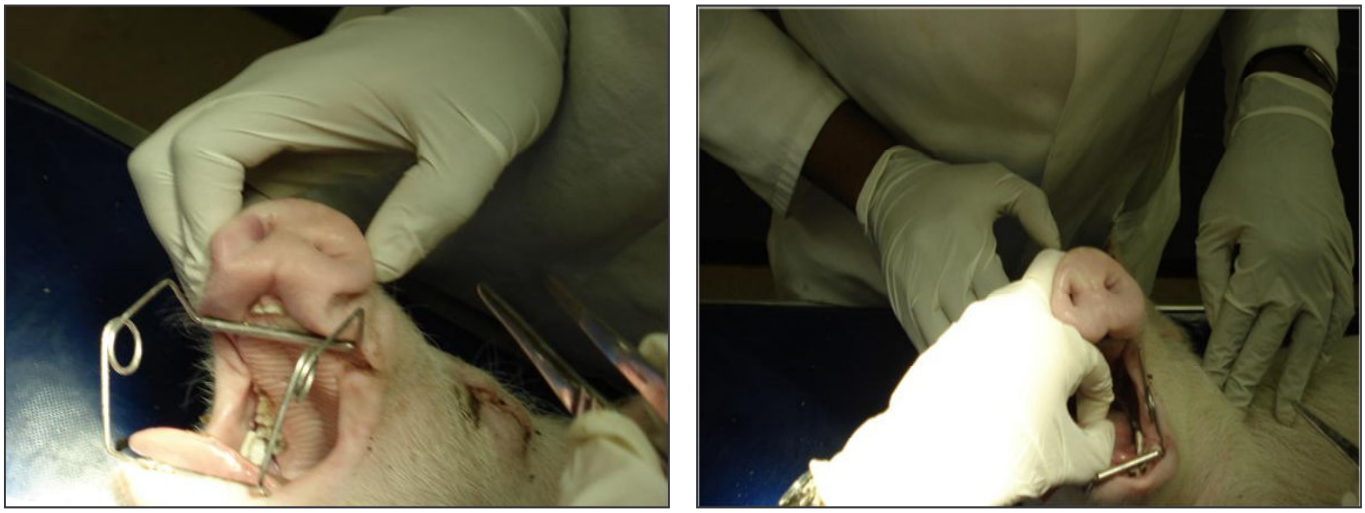

Figure 4.2: Digital photographs taken from different angles showing the placement of the P-RPM formulation in the non-keratinized region of the pig's oral cavity by veterinary experts.

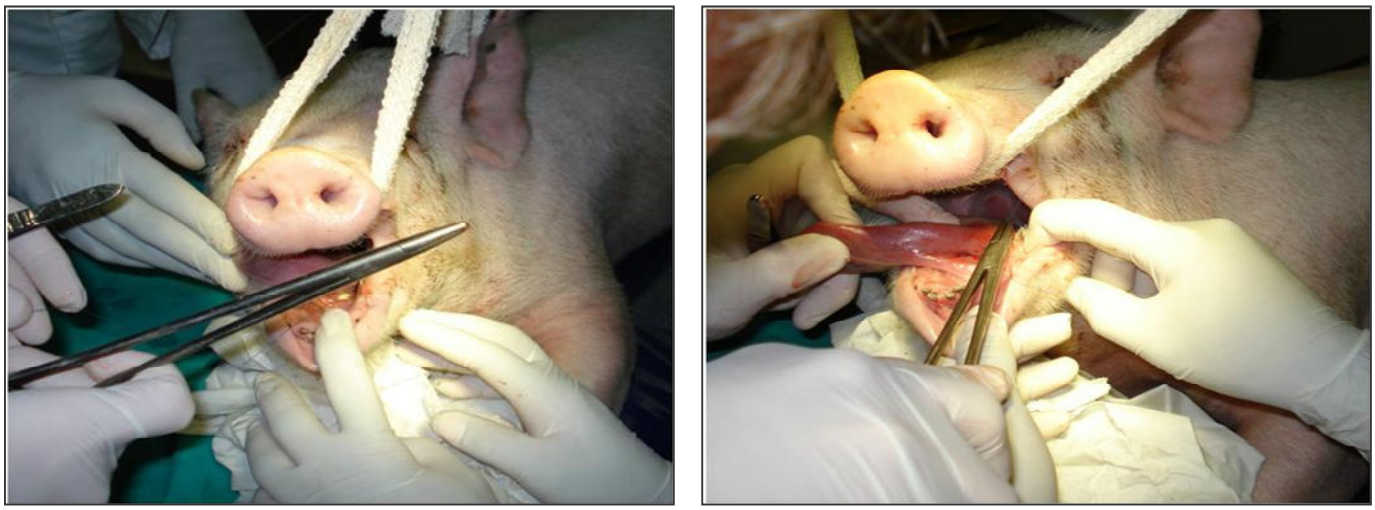

Figure 4.3: Digital photographs showing the suturing process which resulted in the formation of a mucosal pouch which held the P-RPM formulation in place to prevent the pig from chewing or swallowing it.



Figure 4.4: Digital photograph demonstrating the insertion of gastric tubing for passing the commercially available tablet oral formulations of CBZ (Tegretol®CR) and PS (Epanutin®) into the stomach.

4.2.2.3. Preparation of standard solutions and calibration curves for PS and CBZ

The internal standards employed for PS and CBZ were amitriptyline hydrochloride and theophylline respectively because their extraction properties are similar to those of the test drugs, their stability in the solvents employed for the extraction process and their good response at the detection wavelengths.

The standard stock solutions of PS and amitriptyline hydrochloride (internal standard) were separately prepared by dissolving specific quantities in methanol to yield a concentration of 10mg/100mL. The standard solutions employed in preparing the calibration curve of the test drug and internal standard were obtained by further dissolving appropriate quantities of the stock solutions in methanol. The seven different concentrations obtained were 10, 50, 250, 500, 1,000, 2,500 and 5,000 ng/mL. The drug-free plasma sample was spiked separately using the above-listed solutions. The internal standard working solution was prepared at a concentration of 5µg/mL and was added to all the plasma samples prepared for analysis using the UPLC. The calibration curve was prepared as a plot of ratio of area under the curve (AUC) of PS to that of the amitriptyline hydrochloride against the corresponding known concentration of PS.

The same procedure as described for preparing the calibration curve for PS was followed for CBZ except that theophylline was utilized as internal standard at a concentration of 2µg/mL. The calibration curve was prepared as a plot of ratio of area under the curve (AUC) of CBZ to that of theophylline against the corresponding known concentration of CBZ.

For sensitivity and reproducibility determinations, the lowest standard concentration in the calibration curve was considered as the lower limit of quantification (LOQ). Samples at a concentration of 10ng/mL were utilized as the LOQ for both PS and CBZ.

4.2.2.4. The drug extraction procedure

For both PS and CBZ, the standard and test samples were extracted by the liquid-liquid coupled with solid-liquid phase extraction procedure. For the standard solutions, 500µL of each drug solution (different concentrations dissolved in methanol) was added separately to 500µL of drug-free plasma. Each sample was vortex mixed for 60 seconds (Vortex-2 Gennie, Scientific Industries Inc., Bohemia, NY, USA) and centrifuged at 3,000rpm for 15 minutes. The clear supernatant solution was then pipetted directly unto the single use HLB 3cc (Waters

Corporation, Milford, Mass, USA) for solid phase extraction. 0.5mL methanol was employed for elution of respective drugs. The methanol extracts were transferred to the UPLC machine for analysis. In addition to methanol acting as an extracting liquid, it functioned as deproteinating agent for drug molecules bound to plasma proteins and eluting solvent.

4.2.3. Qualitative evaluation of plasma samples using the Fourier Transform Infra Red Spectroscopy (FTIR)

With the qualitative analysis, certain portions of plasma samples stored away at -70°C were utilized. The samples assessed were those of 2, 4 and 8 hours for visualizing and further substantiating the capability of the optimized P-RPM to systemically release via the transbuccal route of drug delivery. They were assessed by the Perkin Elmer 100 Series FTIR Spectrophotometer, (Beaconsfield, UK) equipped with the Spectrum V 6.2.0 software. The frozen plasma samples at time 0, 2, 4, 8 and 24 hours were lyophilized (-55 ± 2°C; 0.42 mBar) over 48 hours. 10mg of the lyophilized plasma sample for the respective formulations was placed on the sample holder on the machine stage. The instrument stage was cleaned with methanol before each sample was placed upon it for analysis and blank background scans were taken before placing the sample for analysis. Samples were analyzed at wavenumbers ranging from 4000-600cm^{-1}, scan time = 32 scans and resolution of 4cm^{-1}. All recorded scans were an average of five repeated scans per sample.

4.2.4. PK-PD modeling of the *in* vivo drug absorption data employing non-compartmental algorithms

WinNonLin® software (V5.2.1 with built-in IVIVC toolkit, Pharsight Software, Statistical Consultants Inc., Apex, NC, USA) was employed as a tool for the PK-PD modeling and the computation and evaluation of all relevant pharmacokinetic and pharmacodynamic parameters. The input data used for the modeling process comprised of the *in vivo* drug (PS and CBZ) absorption values which were obtained for both the P-RPM and conventional formulations described in this Chapter (Section 4.3.1) over a period of 24 hours utilizing the large white pig model.

4.2.4.1. Model selection and configuration

With regards to this phase, successful PK-PD modeling which is described by a single bolus dose of drug released over time was dependent upon the thorough selection of an applicable

pharmacokinetic model that is based on the dose and duration of absorption of PS and CBZ available from the *in vivo* plasma concentration-time profiles obtained during the *in vivo* studies.

A non-compartmental pharmacokinetic model was selected since it was able to reduce any assumptions in the modeling of the plasma concentration-time data particularly for potentially variable study settings as with the relatively prolonged administration of model drugs via the transbuccal route directly into the systemic circulation. Non-compartmental analysis required fewer assumptions compared to compartmental analysis and thus avoided a few pertinent challenges observed with typical compartmental analysis such as the ability to accurately describe the kinetics of drug distribution within a multitude of compartments. However, it is also important to note that the non-compartmental analysis approach employed in this study was not model-independent and a few assumptions were still made, but the number and implication of these assumptions were less than with compartmental analysis. Non-compartmental analysis is preferred over compartmental analysis in bioequivalence evaluation since non-compartmental analysis is undertaken with minimum of intervention by the investigator and the rules of calculus are defined prior to the analysis. Two steps are crucial in the process such as the computation of the area under the curve (AUC) from zero to the last time point with quantifiable concentration and computation of the terminal elimination constant. These quantities are used key parameters in the evaluation of bioequivalence (Pan and Hedaya, 1998; Pérez-Urizar *et al*., 2000; Frederique *et al*., 2009). Prior to the model fitting of the *in vivo* drug absorption data, WinNonlin® was used to accurately derive the initial parameter estimates in order to avoid data convergence to a local minimum. The *in vivo* drug absorption data obtained from the animal studies was initially fitted into the selected non-compartmental model in order to establish the need for necessary modifications to this selected model. Once this was established, the model was fitted to the *in vivo* drug absorption data utilizing non-linear regression analysis in order to estimate the salient pharmacokinetic parameters and their accuracy. Consequent to this step, the modeling process outputs were diagnostically evaluated via pertinent statistical descriptors such as the goodness of fit, correlation, residual analysis and parameter accuracy. This formed the basis of assessing the suitability of the employed model for fitting the *in vivo* drug absorption data from the P-RPM formulation and explicated the data in comparison to benchmarked products for the PS and CBZ loaded P-RPM formulations respectively. Representative pharmacokinetic and pharmacodynamic model-generated *in vivo* drug absorption profile and drug effect schematic are depicted with Figures 4.5 and 4.6.

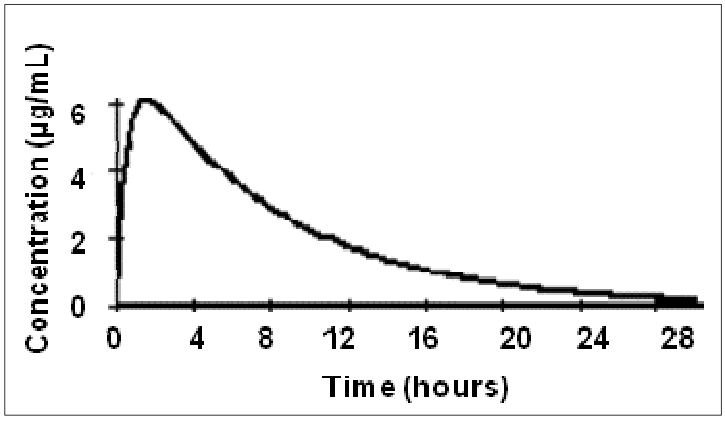

Figure 4.5: A schematic of WinNonlin® compiled non-compartmental pharmacokinetic model of plasma concentration of drug versus time via extravascular administration from the drug loaded P-RPM formulation.

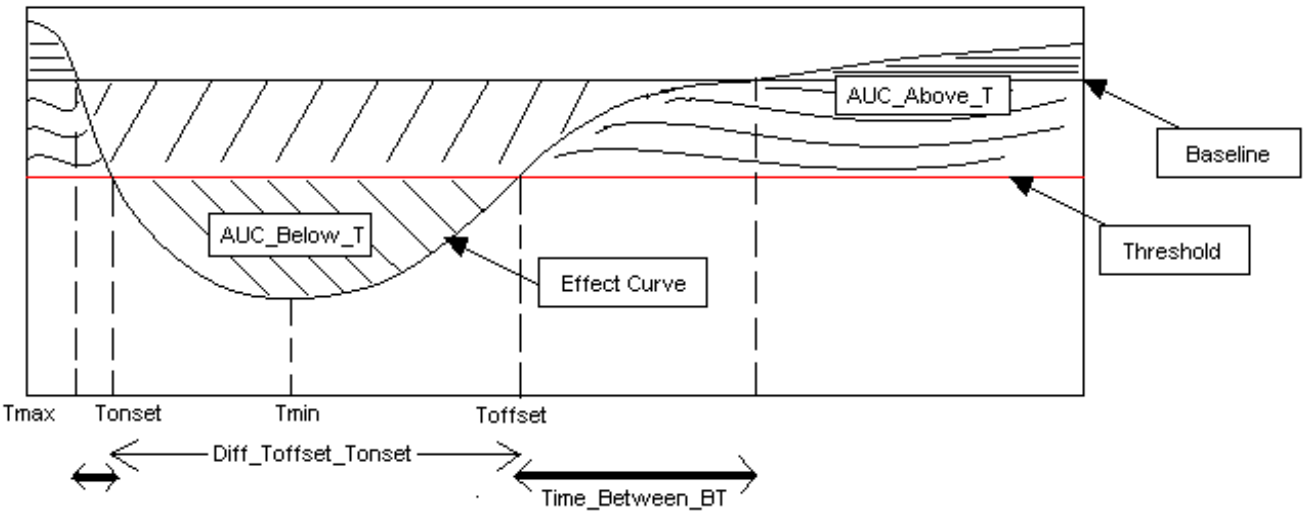

Figure 4.6: A schematic of WinNonlin® compiled pharmacodynamic model for drug effect data estimation of the P-RPM formulation.

4.2.4.2. Algorithm selection for PK-PD modeling

WinNonLin® software was also used to establish a "best fit" model to the *in vivo* drug absorption data via estimation of the variance-inflation factors for the pharmacokinetic and pharmacodynamic parameters with the "goodness of fit" as a pertinent statistical criterion for accurate parametrical estimation.

WinNonLin® provided the “least squares” estimates of the various parameters for the PK-PD model employing a linear approximated Levenberg and Hartley modified Gauss-Newton regression algorithm for rapidly minimizing the sum of squared residuals to achieve convergence (Hartley, 1961; Davies and Whitting, 1972; Beck and Arnold 1977). The Levenberg-Hartley (Gauss-Newton) regression algorithm is known to perform well on a wide class of PK-PD modeling processes including ill-conditioned data sets whereby certain parameters are absent such as in this case. Although the Gauss-Newton algorithm conventionally requires the estimated variance-covariance matrix of the parameters, the Levenberg-Hartley modification suppressed the magnitude of the change in parameter values between iterations, thus enabling the algorithm to perform well on ill-conditioned data sets. Convergence criteria of 0.0001 were used during the minimization process and a maximum of 1000 iterations were allowed. Convergence was achieved with a relative change in the weighted sum of squares (<0.0001). With the Levenberg-Hartley modified Gauss-Newton regression algorithm in WinNonLin®, two successive transformations were used to affect bounding of the parameter space.

4.2.4.3. Computation of salient PK-PD parameters

The apparent first-order rate constant associated with the terminal (log-linear) portion of the plasma concentration-time profiles was computed. This was estimated via linear regression of time versus logarithm of concentration and referred to as lamda z (λ_z). The apparent volume of distribution (V_z) was also fitted. Individual estimates of these parameters were obtained and the fits were evaluated by the assessment of the residuals. The maximal plasma concentration (C_{max}) and time required to attain the C_{max} (T_{max}) were calculated from the fitted curves. The systemic drug clearance (Cl) and the area under the plasma concentration-time curve ($AUCt_{0-\infty}$) were computed by the trapezoidal rule and extrapolated to infinity. In an attempt to comply with the theory of statistical moments, the average total time which the drug molecules spent in the body after administration was also computed as the Mean Residence Time (MRT) and convoluted as the Mean Absorption Time (MAT) to determine the average time for drug to enter the systemic circulation. An assumption that the drug molecules were completely absorbed from the site of administration of the P-RPM formulation was made.

4.2.4.4. Bioequivalence analysis

In accordance with the FDA bioequivalence guidelines, bioequivalence testing was undertaken by setting a 90% confidence interval for the difference of the means of the logarithmic transformed values of $AUCt_{0-\infty}$, AUC_{last} and C_{max}. An acceptance criterion between the intervals 0.8-1.25 was set for bioequivalency. The anti-logarithm of the confidence limits obtained constitute the 90% confidence interval for the ratio of the geometric means between the values for the PS and CBZ loaded P-RPM formulations (test products 1 and 2) and the two comparator commercial products, Epanutin® and Tegretol®CR respectively (reference products 1 and 2).

4.2.4.5. Determination of *in vitro-in vivo* correlation

The existence of an IVIVC relationship was evaluated using the WinNonLin® software (V5.2.1 with built-in IVIVC toolkit, Pharsight Software, Statistical Consultants Inc., Apex, NC, USA). The output of the *in vitro* PS and CBZ dissolution profiles (Section 3.3.1.4 of Chapter three) and their corresponding *in vivo* bioavailability data (Section 4.3.1.2 of Chapter four) were employed to compute the fraction of drug released *in vitro* and the fraction of drug absorbed *in vivo* respectively which were then used to develop a linear, point-to-point level A IVIVC relationship (Emami, 2006; Cardot *et al*., 2007).

4.3. RESULTS AND DISCUSSION

4.3.1. *In vivo* plasma levels of PS and CBZ and comparison with the conventional formulations

4.3.1.1. Calibration curves of PS and CBZ

Based on the previously described UPLC procedure employed for these investigations, the retention times (RT) of 1.02, 2.26, 0.89 and 0.25 minutes were obtained for PS, amitriptyline hydrochloride, CBZ and theophylline respectively. Figures 4.7 (a) and (b) separately show the typical three-dimensional chromatograms of both PS and CBZ with their respective internal standards. The calibration curves for PS and CBZ together with their respective internal standards in plasma was subsequently constructed with the Sigma Plot software employing a linear polynomial approach. These are illustrated with Figures 4.8 (a) and (b).

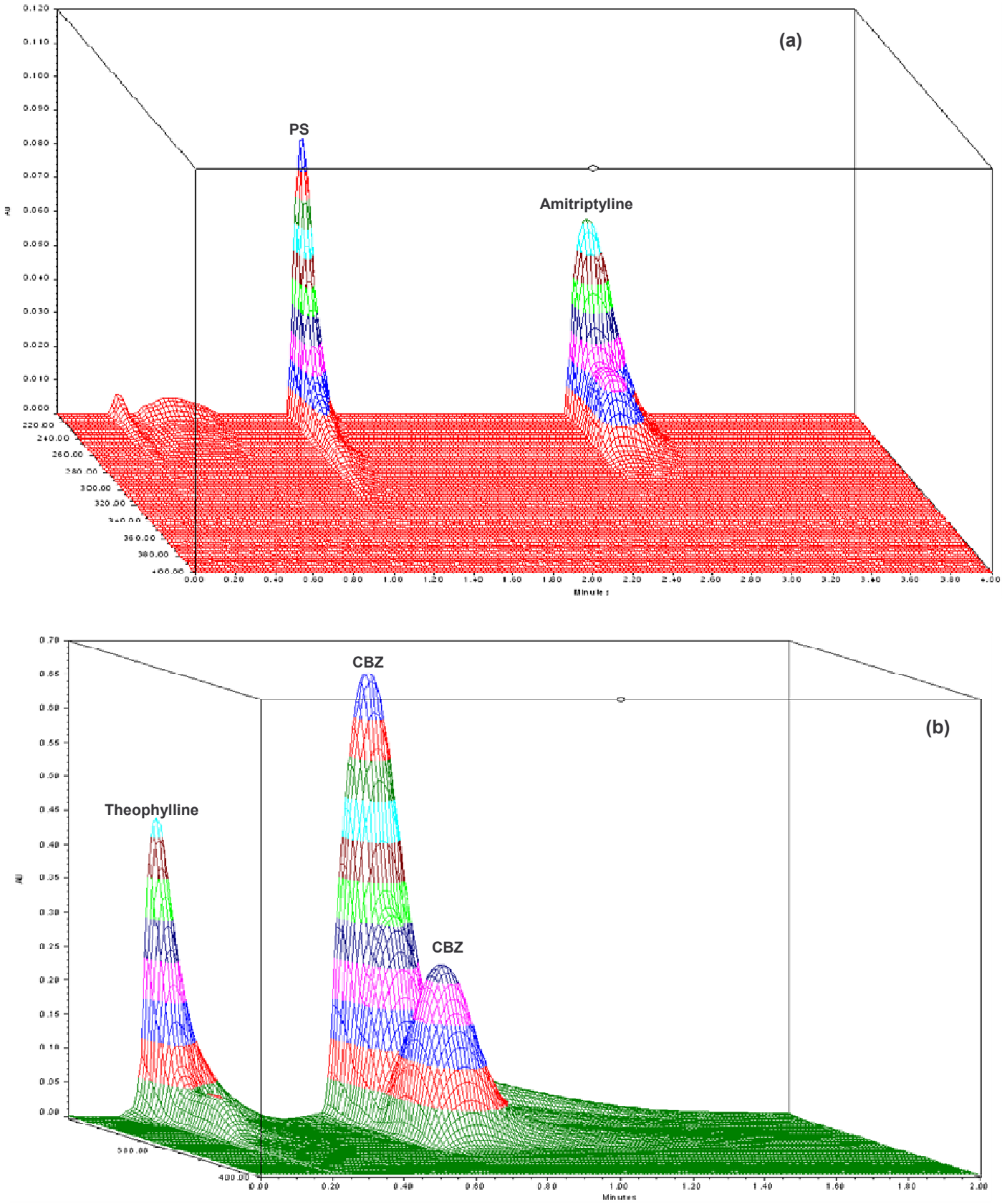

Figure 4.7: Three-dimensional UPLC chromatogram of (a) PS with its internal standard, amitriptyline hydrochloride and (b) CBZ with its internal standards, theophylline.

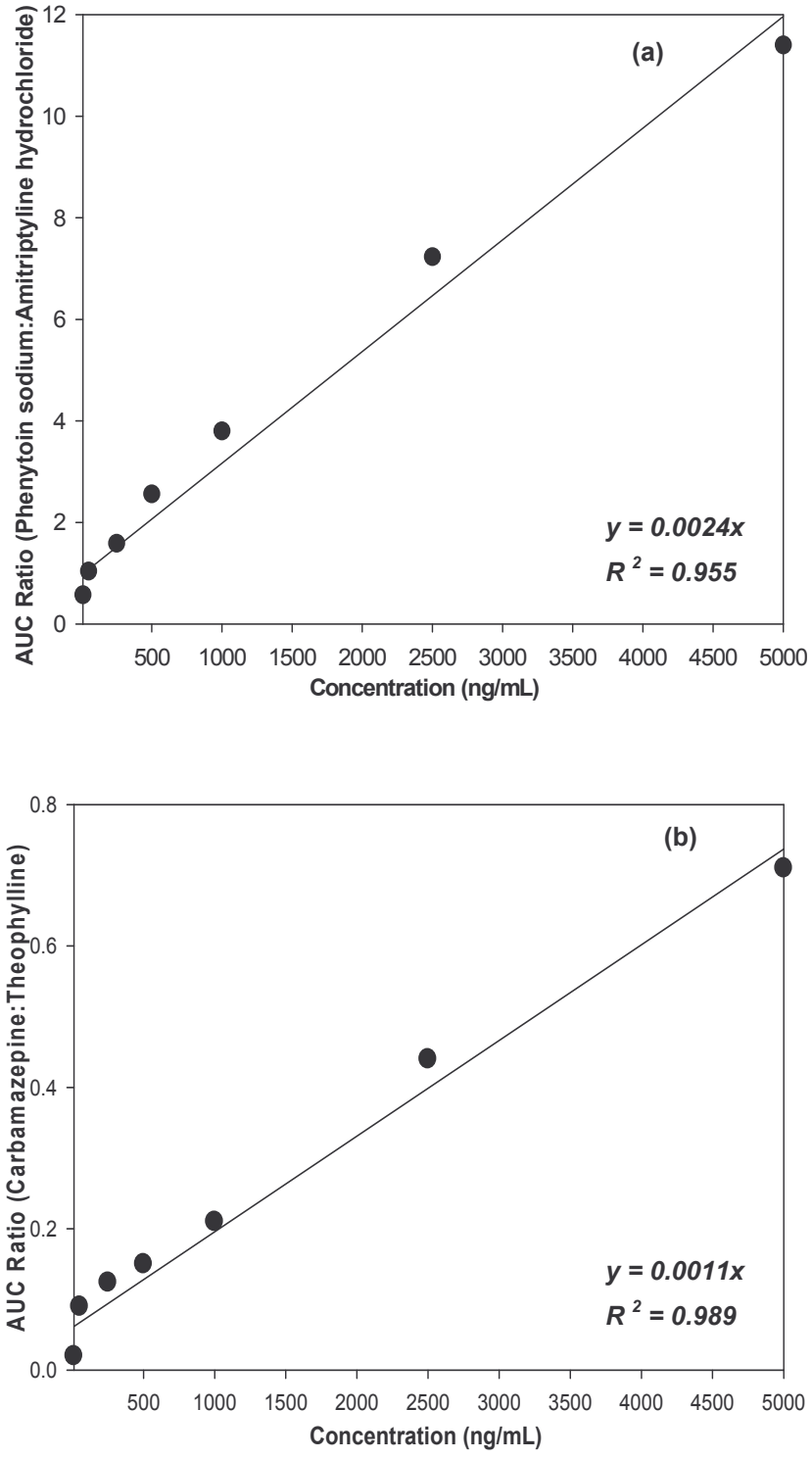

Figure 4.8: Calibration curves of (a) PS in plasma using amitriptyline hydrochloride as an internal standard at 214nm and (b) CBZ in plasma using theophylline as an internal standard at 285nm (N=3 and Standard Deviation ≤ 0.35 in all cases).

4.3.1.2. Plasma-concentration time curves for the different formulations

The tested formulations separately loaded with PS or CBZ were well tolerated by the pigs as no obvious side effects were observed during the test duration and over four weeks post experimentation. Also, animals gained weight normally and progressively over this specified period of time showing that the formulation may be potentially non-toxic. Generally, it was also observed that the P-RPM formulation applied to the pig's buccal mucosa as described earlier had dissolved significantly (with visible minute remnants) at the site of application by the end of the eighth hour duration of study which indicates that this formulation may not need to be removed after some time post administration.

Plasma concentration - time curves for both PS and CBZ loaded P-RPM formulations and their corresponding conventional formulations, Epanutin® and Tegretol®CR respectively, are depicted in Figures 4.9 and 4.10 respectively.

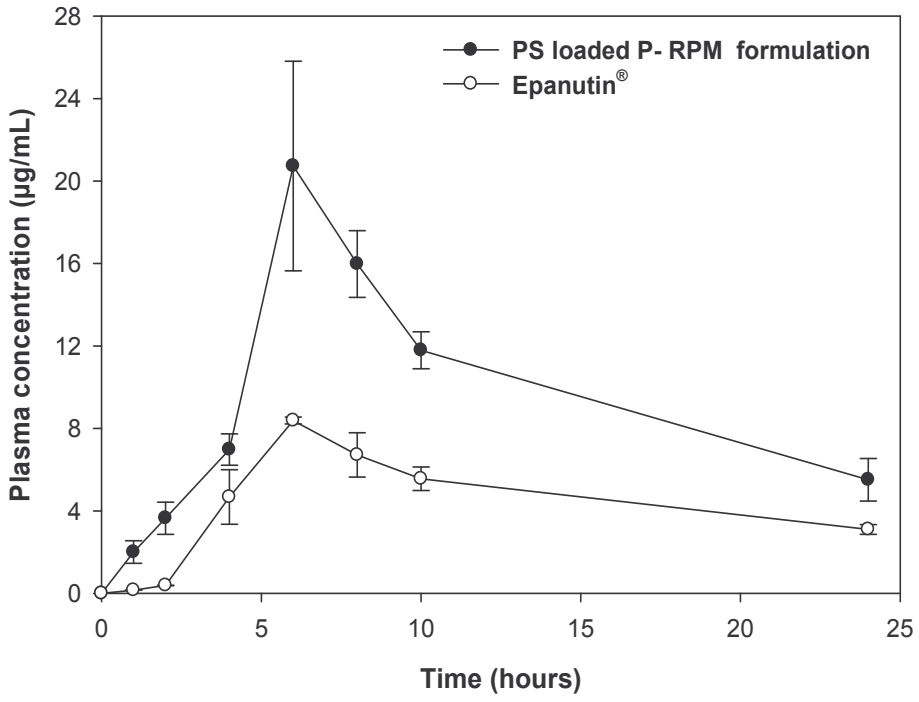

Figure 4.9: Plasma concentration versus time profile for the PS (50mg) loaded P-RPM formulation and Epanutin® (100mg) (N=6).

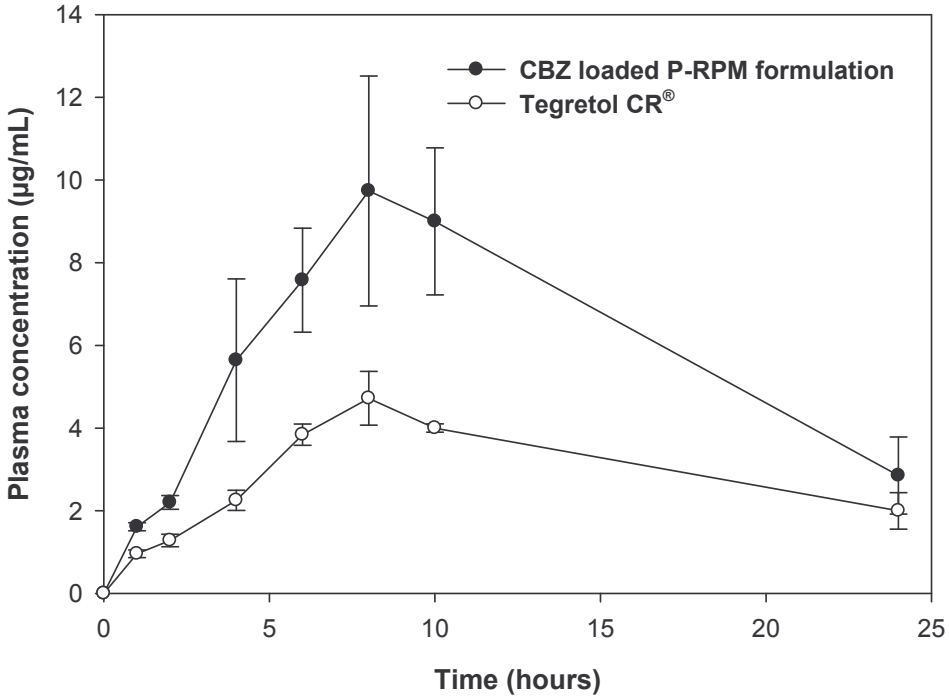

Figure 4.10: Plasma concentration versus time profile for the CBZ (50mg) loaded P-RPM formulation and Tegretol®CR (200mg) (N=6).

The changes in plasma levels of both the conventional and newly fabricated P-RPM formulations of PS and CBZ over time can be described as being biphasic (Figures 4.9 and 4.10). Post administration, gradual increases in plasma levels up until certain time points when subsequent decreases occurred were observed. The observed pattern may be attributable to the degrees of solubility of the individual drugs in plasma, permeability levels at mucosal sites as well as the differences in the nature and excipient compositions of the drug delivery formulations under study. This also may be associated with the varying plasma protein binding capability of both PS and CBZ. Furthermore, the differences in the degrees of physicochemical solubility and permeability at the mucosal site of PS and CBZ appeared to have significantly influenced their plasma levels with formulations containing PS displaying higher peak plasma concentrations (Figure 4.9) than those loaded with CBZ (Figure 4.10). For each set of formulation being compared, the newly fabricated P-RPM formulation attained higher plasma drug levels than those of the commercially available formulations, Epanutin® and Tegretol®CR, at each time point (Figures 4.9 and 4.10) despite the fact that the dose administered with the P-RPM formulations were less (PS = 50mg and CBZ = 50mg) than that given with the commercially available formulations (PS; Epanutin® = 100mg and CBZ; Tegretol®CR = 200mg).

This reveals the potential of the newly fabricated matrix to perform as a suitable drug delivery system for transbuccal drug delivery. The capabilities of the newly fabricated P-RPM drug delivery systems to potentially deliver higher plasma drug levels over time than the commercially available tablet formulations despite that drug loading levels were much lower. However, drug dosages included in the P-RPMs should be well regulated to prevent toxic plasma concentration. In addition, the potential of these newly fabricated P-RPM drug delivery systems to perform (by attaining higher plasma concentrations) despite lower drug loading levels may also serve as an advantage in minimizing side effects and consequently enhancing patient compliance. Also, this can be of economic value as the cost of production may be less because a lower quantity of active drug is required.

4.3.2. Elucidation of the performed PK-PD modeling

4.3.2.1. Evaluation of model validity and summarized comparative assessment of non-compartmental PK-PD model parameters for the tested formulations

During analysis, it was observed that a thousand iterations were adequate to assemble drug release data to a minimum mean square error with a convergence criteria setting of 0.0001. The accuracy of the model was further analyzed by the parametrical support from salient statistical descriptors such as the coefficient of regression (R^2) which is a measure of the model validity and stability. The R^2 value was computed with respect to the model parameters and an R^2 value of >0.90 was set in all instances for this particular set of data. The goodness-of-fit measures and a summary of the formulations analyzed are provided in Table 4.1. These generated values showed the superior accuracy and precision of the employed PK-PD model.

Table 4.1: Diagnostic numerical criteria employed for the assessment of the PK-PD model goodness-of-fit

Formulation	R^2	Correlation XY
PS loaded P-RPM	0.9728	0.9863
CBZ loaded P-RPM	0.9967	0.9983
Epanutin®	0.9853	0.9926
Tegretol®CR	0.9908	0.9977

In order to further visualize the validity and accuracy of the employed non-compartmental modeling approach, the relationship between the observed/experimental and fitted/predicted plasma concentration-time data were visualized. A close relationship, illustrated with Figure 4.11, was observed for these set of parameters for all the formulations tested.

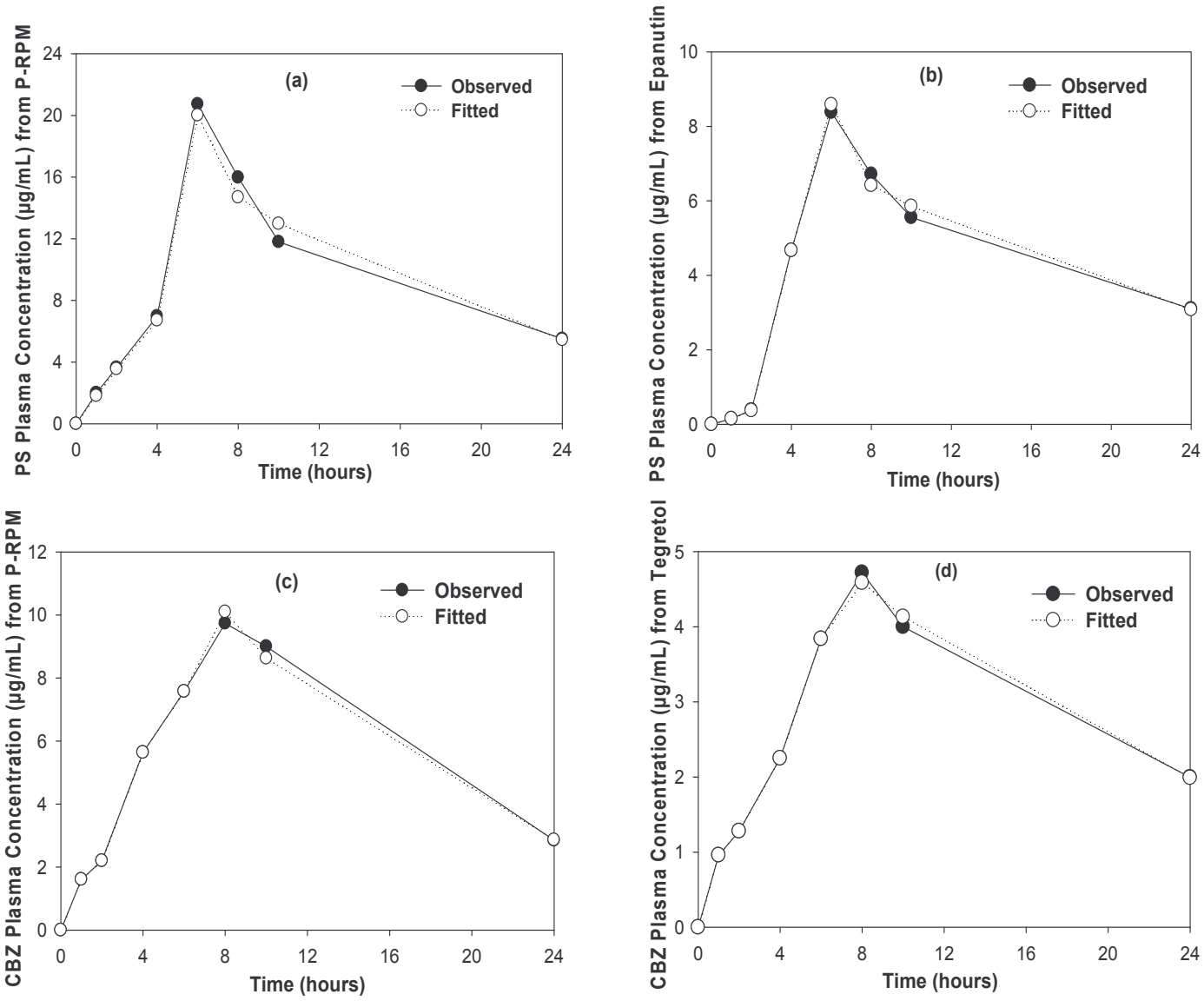

Figure 4.11: Profiles showing the close relationships between the observed and fitted plasma concentration-time data of: (a) PS released from the P-RPM, (b) PS released from the Epanutin®, (c) CBZ released from the P-RPM and (d) CBZ released from the Tegretol®CR (N=5 and Standard Deviation ≤ 0.10 in all cases).

As part of the assessment of the accuracy of the selected PK-PD model, the weighting schemes and residuals were fitted and functionally analyzed for their randomness. The model showed a random scatter (relative to the zero point or baseline) in the residuals (Figure 4.12) and the iterations in the residuals were large. The absolute magnitude or amplitude of the residuals was relatively reduced. Thus, this indicates that the selected non-compartmental model employed for this analysis was very suitable for the evaluation of the obtained *in vivo* experimental data.

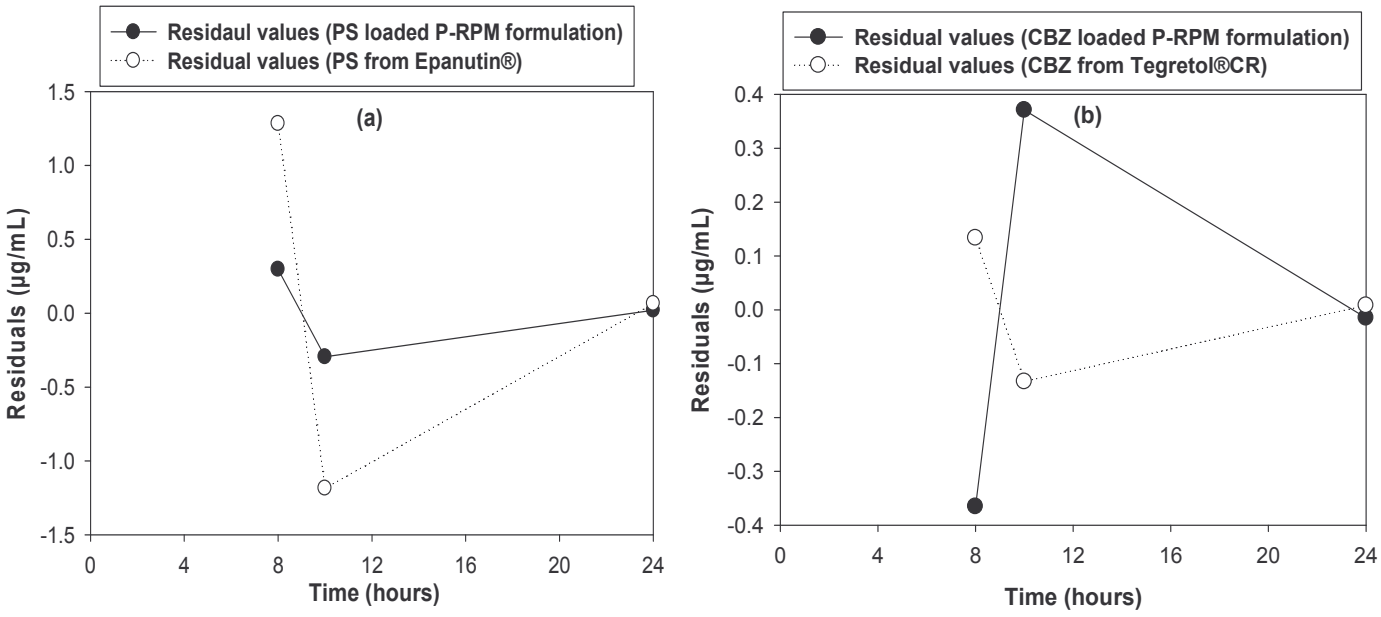

Figure 4.12: Relative residual plots obtained for fitted plasma drug concentration-time data employing the non-compartmental PK-PD model for the (a) PS loaded P-RPM and Epanutin® and (b) CBZ loaded P-RPM and Tegretol®CR formulations based on the first order rate constant associated with the terminal portion of the curve (N=5 in all cases).

A summary of the estimated pharmacokinetic and pharmacodynamic parameters for each tested formulation obtained from the non-compartmental modelling procedure are listed in Tables 4.2 and 4.3 respectively.

Table 4.2: Comparative summary of estimated pharmacokinetic parameters for the PS and CBZ loaded P-RPM formulations and their conventional formulations (N=5)

Parameter	Unit	PS-P-RPM	Epanutin®	CBZ-P-RPM	Tegretol®CR
λ_z	hour $^{-1}$	0.062	0.046	0.079	0.052
$T_{1/2}$	hour	11.170	15.104	8.803	13.272
T_{max}	hour	6.000	6.000	8.000	8.000
C_{max}	µg/mL	20.727	8.380	9.734	4.720
$C_{max/D}$	µg/mL/mg	0.415	0.084	0.195	0.024
C_0	µg/mL	1.998	0.150	1.611	0.959
T_{last}	hour	24.000	24.000	24.000	24.000
C_{last}	µg/mL	5.507	3.098	2.850	1.997
AUC_{last}	hour.µg/mL	226.672	106.309	141.947	69.986
AUC_{all}	hour.µg/mL	226.672	106.309	141.947	69.986
AUC_{inf}	hour.µg/mL	315.424	173.817	178.145	108.224
$AUC_{inf/D}$	hour.µg/mL/mg	6.309	1.738	3.563	0.541
V_z	mL	2554.432	12536.709	3564.554	35385.402
Cl	mL/hour	158.517	575.318	280.670	1848.019
$AUMC_{last}$	hour^2.µg/mL	2213.720	1105.213	1355.969	729.317
$AUMC_{inf}$	hour^2.µg/mL	5685.250	4128.968	2648.242	2340.966
MRT_{last}	hour	9.766	10.396	9.553	10.421
MRT_{inf}	hour	18.024	23.755	14.866	21.631
V_{ss}	mL	2857.123	13666.516	4172.345	39974.016
$Time_{low}$	hour	13.453	23.000	4.665	19.367
AUC_{low}	hour.µg/mL	190.277	106.309	87.459	68.678
$AUC_{inf\ low}$	hour.µg/mL	279.030	173.817	123.656	106.916
AUC_{high}	hour.µg/mL	0.150	0.000	5.262	0.000
$AUC_{inf\ high}$	hour.µg/mL	0.150	0.000	5.262	0.000
$Time_{high}$	hour	0.412	0.000	5.881	0.000

λ_z: first order rate constant of the terminal portion of the log-linear curve; $T_{1/2}$: half life; T_{max}: time of maximum plasma concentration; C_{max}: maximum concentration at T_{max}; C_0: initial plasma concentration; AUC: area under the curve; V_z: terminal phase volume of distribution; Cl: total clearance; MRT: mean residence time; V_{ss}: total volume of distribution AUMC: mean cumulative drug absorption.

Table 4.3: Comparative summary of estimated pharmacodynamic parameters for the effect of the PS and CBZ loaded P-RPM formulations and their conventional formulations (N=5)

Parameter	Unit	PS-P-RPM	Epanutin®	CBZ-P-RPM	Tegretol®CR
T_{max}	hour	6.000	6.000	8.000	8.000
T_{onset}	hour	5.894	-	6.393	-
R_{min}	ug/mL	1.998	0.149	1.611	0.959
R_{max}	ug/mL	20.727	8.380	9.734	4.720
T_{min}	hour	1.000	1.000	1.000	1.000
T_{offset}	hour	6.306	-	12.274	-
$AUC_{below\ T}$	hr*ug/mL	233.478	353.691	47.315	114.014
$AUC_{above\ T}$	hr*ug/mL	0.150	0.000	5.262	0.000
Baseline	ug/mL	10.000	10.000	4.000	4.000
Threshold	ug/mL	20.000	20.000	8.000	8.000
$Diff_T_{offset}_T_{onset}$	hour	0.412	-	5.881	-
$Time_{between\ BT}$	hour	9.136	0.000	12.454	3.633

4.3.2.2. Relative bioavailability analysis

The *in vivo* bioavailability of PS and CBZ from the P-RPM formulations relative to that of the respective conventional products was assessed using plots depicting the changes in area under the absorption-time curve (AUC) versus time as well as the mean cumulative drug absorption (AUMC) versus time (Figures 4.13 and 4.14). The AUC and AUMC were computed from the generated plasma concentration-time profiles fitted in the PK-PD model.

The AUC versus time and AUMC versus time profiles showed that the PS and CBZ loaded P-RPM formulations had higher bioavailability over 24 hours than their respective conventional formulation. Furthermore, the AUC-time and AUMC-time profiles indicated that PS and CBZ were released in a zero-order manner from the newly fabricated P-RPM formulation. However, the rate of release was marginally gradual during the first 3 hours post transbuccal administration of the P-RPM and maintained a zero-order release rate over the remaining 20-21 hours (Figures 4.13 and 4.14).

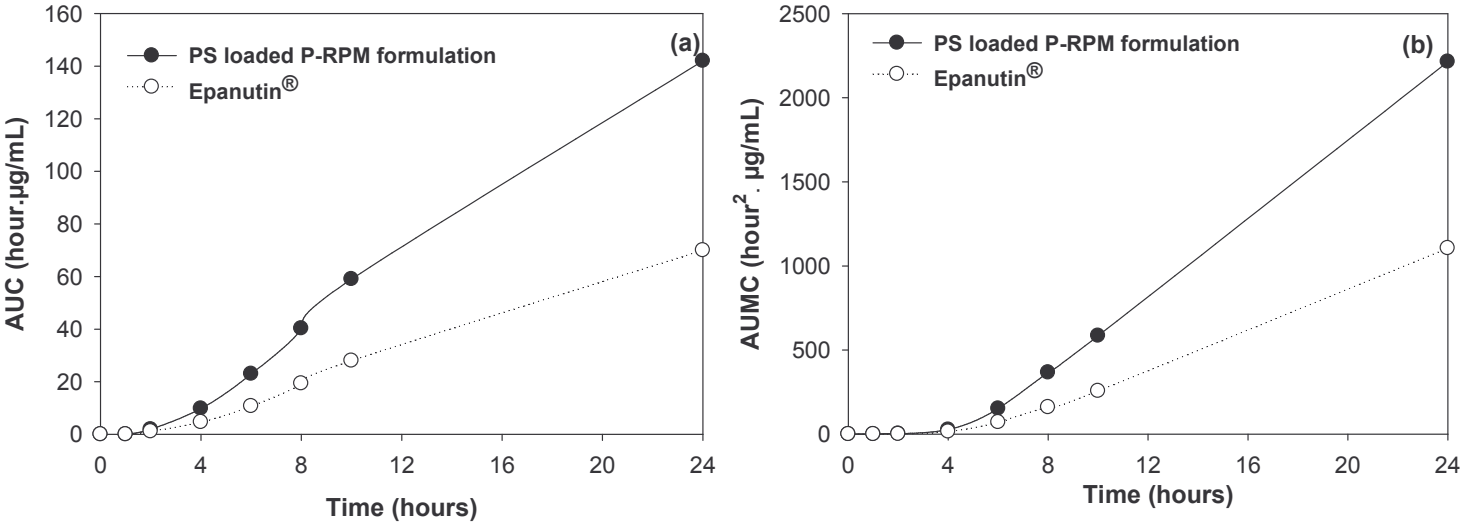

Figure 4.13: An illustration of the cumulative increase in PS concentration over a period of 24 hours computed from: (a) AUC and (b) AUMC of the mean *in vivo* concentration-time profiles of the PS loaded P-RPM formulation and the comparator product, Epanutin®.

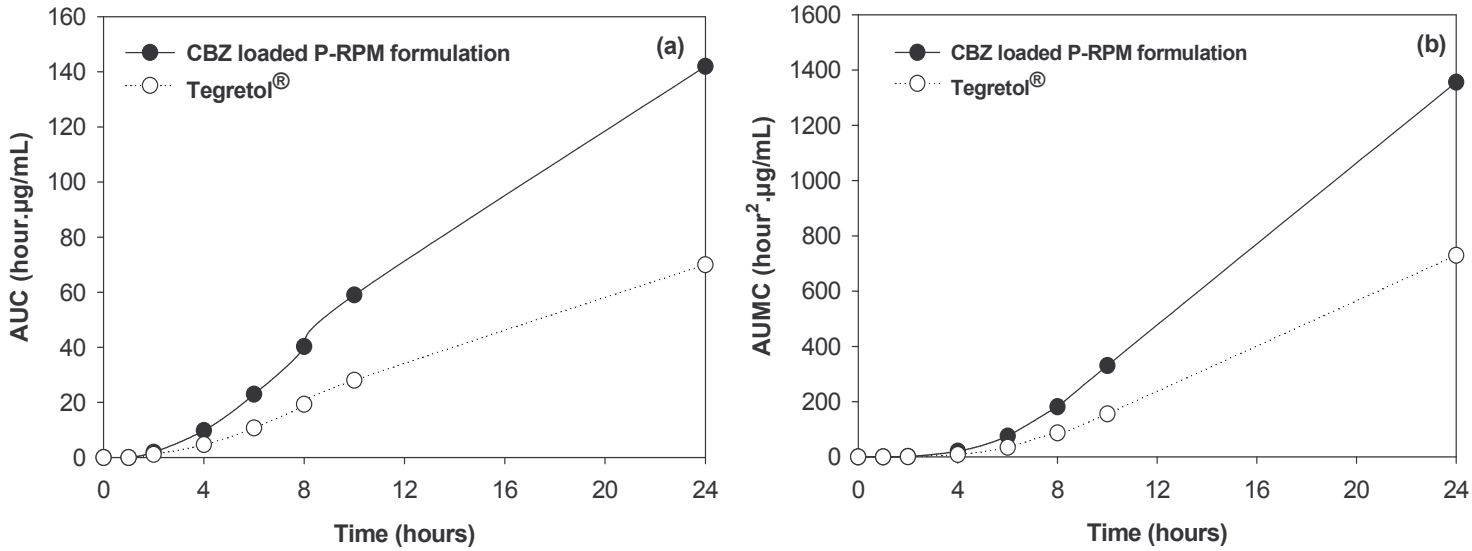

Figure 4.14: An illustration of the cumulative increase in CBZ concentration over a period of 24 hours computed from: (a) AUC and (b) AUMC of the mean *in vivo* concentration-time profiles of the CBZ loaded P-RPM formulation and the comparator product, Tegretol®CR.

In addition, relative bioavailability was evaluated by comparing the numerical values for the maximum plasma concentrations (C_{max}) and time (T_{max}) for the PS and CBZ loaded P-RPM and their commercially available counterparts (Table 4.2). Overall, both the PS and CBZ loaded P-RPM formulations attained higher peak plasma concentrations (PS: 20.727µg/mL; CBZ: 9.734µg/mL) when compared with their respective conventional formulations (Epanutin®: 8.380µg/mL; Tegretol®CR: 4.720µg/mL) at maximum time. The outcome shows that the respective drug loaded P-RPM formulation has higher bioavailability than their comparator products.

4.3.2.3. *In vitro-In vivo* correlation

Figures 4.15 and 4.16 presents the point-to-point level A IVIVC plots relating the fraction released (*in vitro*) against fraction absorbed (*in vivo*) for the PS and CBZ loaded P-RPM formulations. A linear correlation existed for both sets of data with the R^2 values close to unity (PS: 0.931 and CBZ: 0.909) serving as confirmation. Based on this outcome, it can be proposed that for the P-RPM formulations developed in this study, *in vitro* dissolution data may be representative of an *in vivo* pharmacokinetic data and serve as a suitable surrogate.

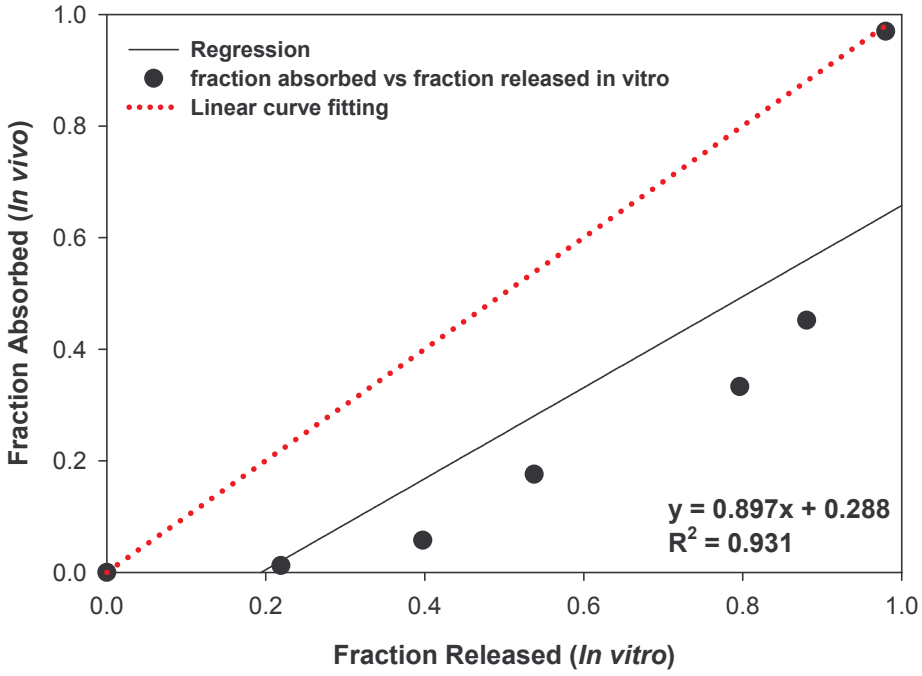

Figure 4.15: A point-to-point level A IVIVC model plot for the PS loaded P-RPM formulation.

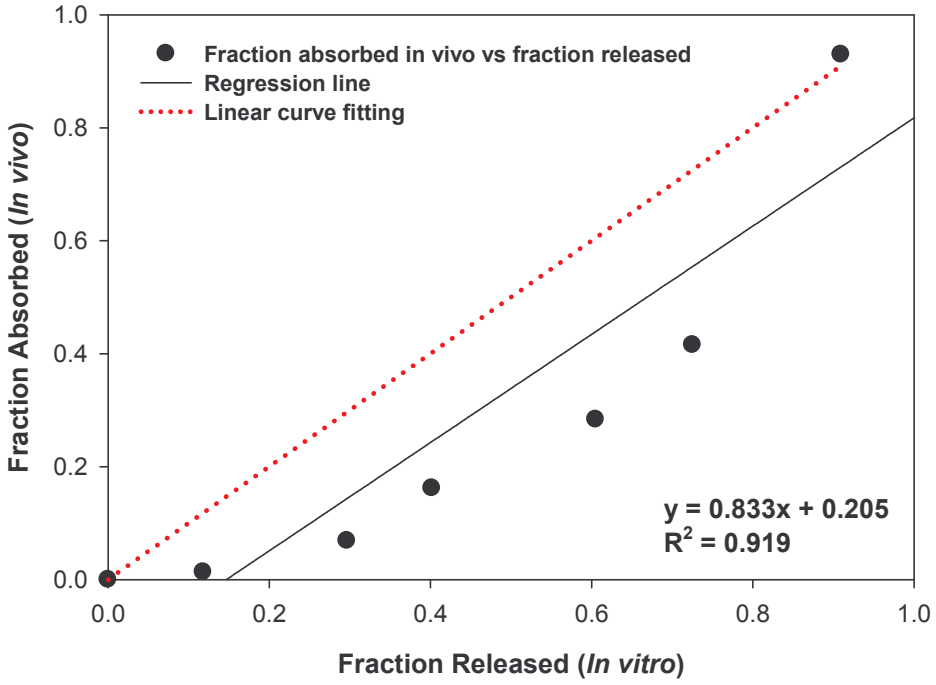

Figure 4.16: A point-to-point level A IVIVC model plot for the CBZ loaded P-RPM formulation.

4.3.3. Qualitative analysis of i*n vivo* drug plasma levels of PS and CBZ from the P-RPM formulations

Qualitative analysis of the collected plasma samples conducted employing the FTIR spectrophotometric analysis further substantiated the outcome of the quantitative analyses of PS and CBZ plasma concentrations over time.

4.3.3.1. Phenytoin sodium

In summary, the FTIR spectra showed the presence of PS in plasma when compared to the baseline at t_{0hour} before drug administration and pure drug spectra. The centres of activity that spanned over N-H (3050 - 2925cm^{-1}), C=N (1607 - 1623cm^{-1}), C=C (1501 - 1515cm^{-1}) and C-ONa (1384 - 1367cm^{-1}) bond vibrations that are characteristic of PS chemical structure, were identified in the plasma samples at t_{2hours}, t_{4hours}, and t_{8hours}. The FTIR spectra are presented in Figure 4.17.

The band at 3279 cm^{-1} (t_{0hour}), 3236cm^{-1} (t_{2hours}), 3244cm^{-1} (t_{4hours}) and 3236cm^{-1} (t_{8hours}) are due to N—H stretching frequency of the PS. The changes in intensity are indications that this bond is being perturbed during administration into the body fluids as well as changes in the level of

drug within the plasma. New stepwise bands were observed at 3050 - 2925cm^{-1} showing that there is hydrogen bonding on the administration of the drug. Thus the perturbation of the N—H bond may be due to hydrogen bonding, presumably between the drug and the plasma protein molecule or body fluids.

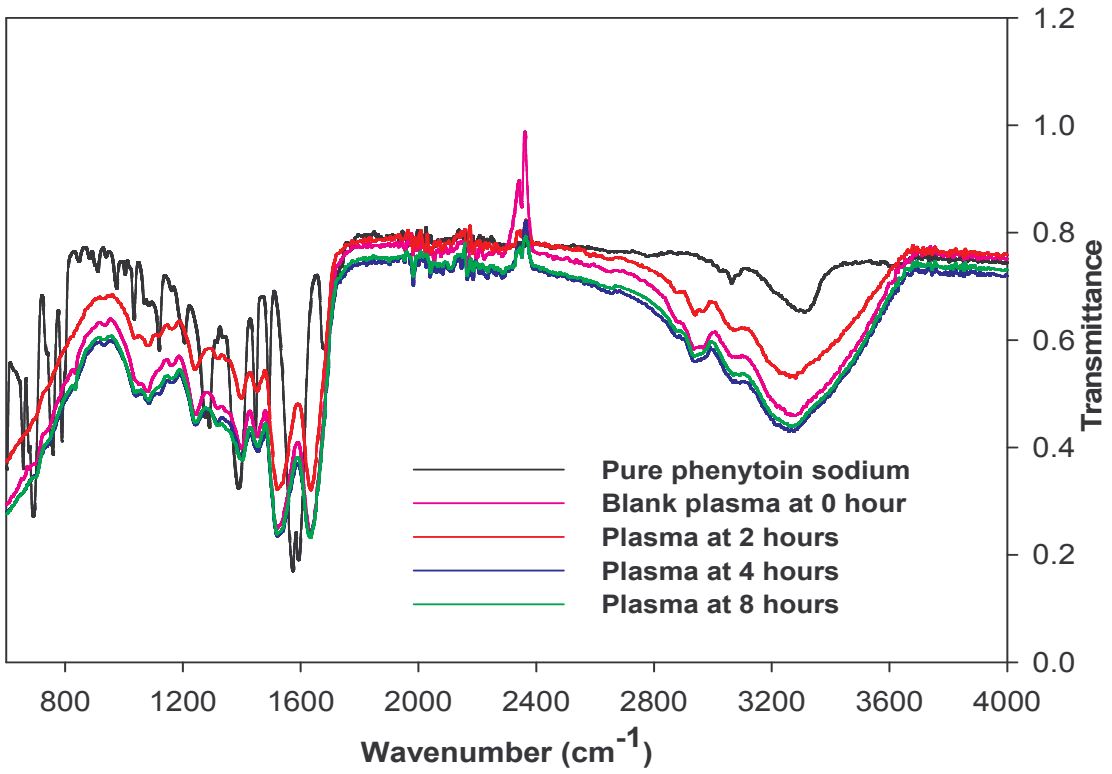

Figure 4.17: FTIR spectra showing the qualitative analysis of PS plasma levels over time in comparison with blank plasma and the pure drug.

The band at 1695cm^{-1} (t_{0hour}) is due to C=O stretch. The band appears to have disappeared when PS is administered (t_{2hours}, t_{4hours} and t_{8hours}). This thus suggests that the C=O probably undergoes some enolization in the plasma (i.e. C=O ↔C-O-H) thus indicating some electron re-arrangement within the molecule, presumably as a result of hydrogen bonding due to drug dissolution or absorption within the blood stream.

The band between 1567cm^{-1} and 1569cm^{-1} showed that the stretching frequency due to C=C and C=N in the plasma (t_{0hour}) is coupled; the band at 1567cm^{-1} is due to C=N and the one at 1567cm^{-1} is due to C=C. At t_{2hours}, t_{4hours} and t_{8nours} after drug administration, these coupled bands separate with the C=N stretch shifting to higher wavenumbers at 1607cm^{-1} (t_{4hours}) and 1623cm^{-1} (t_{2hours} and t_{8nours}) whereas the C=C stretch shifts to lower wavenumbers, 1501cm^{-1} (t_{2hours}), 1515cm^{-1} (t_{4hours}), and 1512cm^{-1} (t_{8nours}) possibly due to the aromatic rings present in PS.

The band at 1384cm^{-1} was due to C-O stretch from C-ONa bond of PS. A reduction in its intensity and shifts to 1386 - 1372cm^{-1} indicates some level of ionization due to hydrolysis of the carbonyl and sodium (C-ONa + $H_2O \leftrightarrow C\text{-}O^- + Na^+$) and possible displacement by hydrogen atom, again via hydrogen bonding with body water or plasma proteins.

Furthermore, a range of spectral transformations such as the disappearance of C=O, stretching of N-H, C=N, C=C, C-ONa bonds and peak changes below 1300cm^{-1} were observed. These alterations can also be attributed to the formation of H-bonding between drug and plasma protein or body fluids. Changes in the spectra below 1300cm^{-1} are consequences of the perturbations on N-H, C=O, C=O and C-ONa, the four centres of activity when PS interacts with proteins in the plasma.

4.3.2.2. Carbamazepine

The FTIR spectra generated displayed relevant peaks representing CBZ functional groups in the analyzed plasma samples when compared with the blank plasma examined at t_{0hours} before drug administration and pure drug spectra. The centres of activity noted spanned over aromatic C-H stretching (3090 - 3112cm^{-1}), C=C (1542 - 1547cm^{-1}), R-NH_2 (3276 - 3301cm^{-1}), C=O (1639 - 1645cm^{-1}), C-C (965 -1097cm^{-1}), solvated R-NH_2 due to the presence of blood or other body fluids (3302 - 3308cm^{-1}) and O-C-NH_2 (1638 – 1642cm^{-1}) bond vibrations/frequencies that are characteristic of the CBZ chemical structure were identified in the plasma samples at t_{2hours}, t_{4hours}, and t_{8hours}. The variations in the intensities of these characteristic bands may be as a result of bond re-orientations, stretching or bending, solvation/dissolutions/hydrogen bond formation, cyclicization of aromatic C=C bonds attributable to dissolution or absorption of CBZ molecules into the blood stream via the buccal mucosa and plasma protein binding . The FTIR spectra are presented in Figure 4.18.

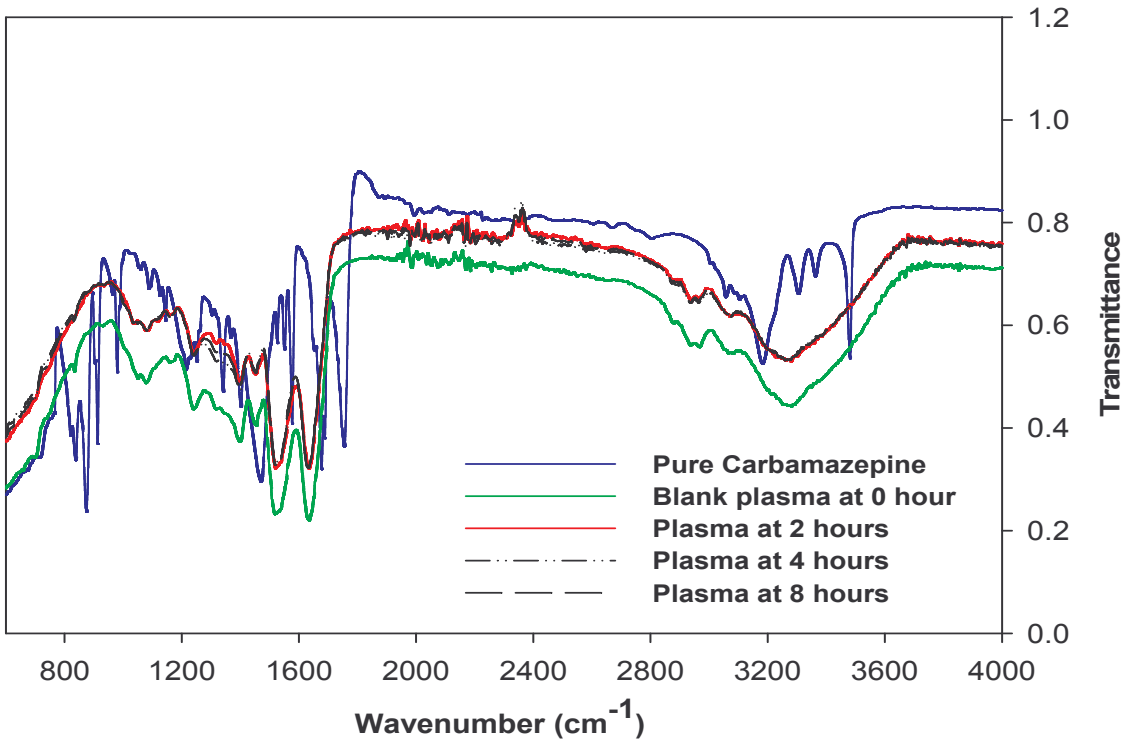

Figure 4.18: FTIR spectra showing the qualitative analysis of CBZ plasma levels over time in comparison with blank plasma and the pure drug.

4.4. CONCLUDING REMARKS

This Chapter discussed the qualitative and quantitative evaluation of the newly constructed optimized P-RPM formulation separately loaded with PS and CBZ under *in* vivo physiological condition employing the large white pig as an animal model. The P-RPM formulation demonstrated the potential to effectively regulate the systemic release of both PS and CBZ via the transbuccal route of drug delivery as visualized by outcomes from the quantitative and qualitative assessments of collected plasma samples. Drug plasma levels versus time profiles were generated for the P-RPM formulations and the commercially available formulations (for PS and CBZ). Comparisons of both sets of profiles showed the potential of the P-RPM formulations to attain higher, controlled plasma concentrations at lower drug loading levels (PS: 50mg and CBZ: 50mg) than the commercially available oral tablet formulation (100mg PS in Epanutin® and 200mg CBZ in Tegretol®CR) without any dose dumping. The selected non-compartmental PK-PD model employed displayed a desirable fit for the investigated *in vivo* data set. This

implied that most of the loaded drug molecules were preferentially released directly into the systemic circulation comparable to an intravenous bolus injection. Hence, this further confirmed the capability of the P-RPM formulation to initiate and sustain transbuccal permeation and to significantly achieve higher plasma concentrations of drug after administration as a mechanism of boosting bioavailability. The generated PK-PD parameters further revealed the differences in the performances of the PS or CBZ loaded P-RPM formulations in comparison to their respective comparator products. The developed point-to-point level A IVIVC model for both PS and CBZ loaded P-RPM formulations displayed a linear trend ($R^2 > 0.9$) indicating the possibility of applying respective *in vitro* dissolution profiles as substitutes for bioequivalency testing for this set of formulations. In the ensuing chapter, the assessment of the potential toxicity and biocompatibility of the newly designed drug loaded P-RPM formulation utilizing cytological and histological assays will be discussed.