Pharmacokinetics of Orally Administered Poly(Ethylene Oxide)-block-Poly(ε-Caprolactone) Micelles of Cyclosporine A in Rats: Comparison with Neoral®

Binkhathlan Z1,3, Ali R1, Qamar W2, and Lavasanifar A3,4

1 Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.  
2 Central Laboratory, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.  
3 Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.  
4 Department of Chemical and Material Engineering, University of Alberta, Edmonton, Alberta T6G 2V4, Canada.

Received, May 26, 2018; Accepted, August 20, 2018; Published, August 22, 2018.

ABSTRACT - PURPOSE: The aim of this study was to assess the pharmacokinetics of methoxy poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-b-PCL) micellar formulation of cyclosporine A (CyA) following oral administration in rats making comparisons with its commercial microemulsion formulation, Neoral®.  
METHODS: PEO-b-PCL copolymer was synthesized and used to form micelles encapsulating CyA. The release of CyA from Neoral® and PEO-b-PCL as well as PEO-b-PCL degradation were assessed in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Polymeric micellar CyA and Neoral® were administered by oral gavage to healthy Wistar rats. At predetermined intervals, rats (n=5 for each time point) were euthanized, samples of blood and plasma were collected and analyzed for CyA using an LC-MS/MS assay. Blood and plasma pharmacokinetic parameters of CyA in its polymeric micellar formulation were compared to those of Neoral®. RESULTS: Polymeric micelles of CyA showed < 15 and 10% increase in diameter in SGF and SIF, respectively, within 24 h. PEO-b-PCL showed signs of minimal degradation when incubated for > 8 h in SGF, but was stable in SIF. Drug release in both SGF and SIF was comparable between the two formulations except for significantly higher release of CyA in SIF only at 24 h time point from Neoral®. Following oral administration (10 mg/kg), the blood AUC0-∞ and tmax of CyA in the polymeric micellar formulation was comparable to that for Neoral®. However, the Cmax of CyA-loaded PEO-b-PCL micelles was significantly (p < 0.05) higher than that obtained with Neoral® (2.10 ± 0.41 versus 1.40 ± 0.25 µg/mL, respectively). CyA had higher blood-to-plasma concentration ratios in polymeric micelles compared to Neoral®, in vivo. CONCLUSION: Our results show that PEO-b-PCL micelles can serve as stable and good solubilizing carriers for oral delivery of CyA providing similar pharmacokinetic profile to that of Neoral®.

INTRODUCTION

Cyclosporine A (CyA) is a cyclic undecapeptide that is highly lipophilic with an aqueous solubility of 23 μg/mL. It is a potent immunosuppressive agent that has been approved for over 30 years by the US FDA for prophylaxis of organ rejection in kidney, liver, and heart transplants (1, 2). Initially oral Sandimmune® was introduced into European market in 1981 as a self-emulsifying drug delivery system (SEDDS) formulation containing Labrafil® M1944CS, olive oil and ethanol (3-5). When dispersed in water, this formulation forms an oil-in-water coarse macroemulsion with high polydispersity values (3). In 1994 again CyA was introduced as a self-microemulsifying drug delivery system (SMEDDS) formulation containing Cremophor RH40, corn oil glycerides, propylene glycol and ethanol under the brand name of Sandimmune Neoral® (Currently known as Neoral®) (2, 5). This formulation forms a spontaneous emulsion with a homogenous droplet size of approximately 30 nm (3). Compared with the older oral Sandimmune®, Neoral® provided more extent in oral absorption and bioavailability (1, 6). The enhanced bioavailability of cyclosporine A has been attributed to the improved dispersion characteristic of Neoral® (7).

Despite the availability of different CyA formulations currently in the market, there is still a need for improvement. Presently, the use of CyA has been limited owing to the reported side effects,
not only caused by the drug itself but also by the excipients present in the formulations (i.e. organic solvents and surfactants). Unpredictable pharmacokinetics as well as the narrow therapeutic window of CyA are still considered major concerns. In order to overcome these limitations, there have been several attempts for developing alternative oral formulations for CyA based on nano drug delivery systems. For instance, Guan et al. investigated the potential of liposomes containing sodium deoxycholate bile salt as an oral drug delivery system for CyA (8). The CyA-loaded liposomes exhibited a mean diameter size of less than 100 nm, with high drug loading, which provided an aqueous solubility of CyA reaching 2 mg/mL. Following an oral administration of CyA (15 mg/kg) to male Wistar rats, the liposomal formulation showed a comparable pharmacokinetic profile to Neoral® in terms of C_{max} and AUC_{0-\infty} in blood. However, the liposomal formulation had a significantly longer t_{max} compared to Neoral® (4.67 versus 2.67 h, respectively) (8). Zhang et al. developed polymeric micelles prepared from poly(ethylene glycol)-block-poly(D,L-lactic acid) (PEO-b-PDLLA) and used them as nanocarriers for the solubilization and oral delivery of CyA (9, 10). The optimum CyA-loaded PEO_{5000}-b-PDLLA_{5000} micelles had a mean diameter size of around 72 nm with narrow distribution. Moreover, CyA-loaded micelles showed a relatively lower cumulative release in simulated intestinal fluid up to 12 h (9). Following an oral administration of CyA (10 mg/kg) to Sprague-Dawley rats, CyA-loaded micelles showed a pharmacokinetic profile similar to Neoral® (9).

We have previously shown that methoxy poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-b-PCL) micelles can solubilize CyA and favorably alter its pharmacokinetic and biodistribution profile after a single intravenous dose in rats (11, 12). Specifically, PEO-b-PCL micelles showed a 90% decrease in blood CL and V_{Dss} of CyA in comparison to CyA in Cremophor EL/ethanol (Sandimmune®, intravenous formulation). Moreover, the blood AUC of CyA in Sandimmune® was only 12% of that obtained with the polymeric micellar formulation (11, 12).

The main objective of current study was to evaluate the potential of PEO-b-PCL micelles as a delivery system for CyA for oral administration. We assessed the stability of CyA-loaded PEO-b-PCL micelles in simulated gastric and intestinal fluids. The in vitro release of CyA from PEO-b-PCL was also evaluated and compared to Neoral® in both media. We finally performed a comparative pharmacokinetic study of CyA-loaded PEO-b-PCL micelles versus the marketed formulation of CyA (Neoral®) following a single oral administration to rats.

**MATERIALS AND METHODS**

**Materials**

Cyclosporine A was obtained from Molekula Limited (Newcastle, UK). Methoxy PEO (M₄, 5,000), stannous octoate (95%), ε-caprolactone (97%), and THF (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride injection (USP) 0.9% was obtained from Pharmaceutical Solutions Industry (Jeddah, Saudi Arabia). Neoral® oral solution (100 mg/mL; Novartis, Switzerland, MFD: 8/2014; EXP: 7/2017, LOT: H5186) was obtained from King Abdulaziz University Hospital Pharmacy (Riyadh, Saudi Arabia). Phosphoric acid was obtained from BDH Chemical Ltd (Poole, England). Zinc sulphate is purchased from LOBA Chemie Pvt. Ltd. (Mumbai, India). Simulated gastric and intestinal fluids (SGF & SIF) were purchased from Biorelevant.com Ltd (London, UK) and prepared as per the provided instructions. Dialysis bags (Spectra Por S/P 3 Dialysis Membrane Trial Kit, 3,500 Dalton 18mm) were purchased from Cole-Parmer Canada (Montreal, QC, Canada). Acetonitrile, ammonium acetate, methanol, and water were all HPLC grade and were purchased from BDH Chemical Ltd (Poole, England). All other chemicals were reagent grade. Deionized water was prepared in-house using Millipore system.

**Methods**

**Synthesis and characterization of PEO-b-PCL copolymers**

Previously reported method was employed for synthesis of PEO-b-PCL block copolymer (11, 13). Briefly, methoxy PEO (MW 5,000 g/mol; 5 g), ε-caprolactone (13 g) and stannous octoate (0.2% w/w) were added to a previously flamed ampoule, nitrogen purged, then sealed under vacuum. The reaction proceeded at 140 °C for 4 h. 1H NMR spectrum of PEO-b-PCL in CDCl₃ at 500 MHz (Bruker Ultra shield 500.133 MHz spectrometer) was used to determine the number average molecular weight of the block copolymer. The
degree of polymerization of \(\varepsilon\)-caprolactone was estimated by comparing the peak intensity of PEO \((-O–CH_2–CH_2; \delta = 3.65 \text{ ppm})\) to that of PCL \((-O–CH_2; \delta = 4.075 \text{ ppm})\). The number-averaged molecular weights, weight-averaged molecular weights and polydispersity index (PDI) of the copolymer were determined by gel permeation chromatography (GPC) (Viscotek TDA 305-040 Triple Detector Array, Viscotek Corp., Houston, TX, USA). A sample of 20 µL of polymer solution in THF (15 mg/mL) was injected into a 300 × 7.5 mm PLgel 5 µm MIXED-D column (Agilent Technologies Inc., Santa Clara, CA, USA) with guard column (PLgel MIXED, 7.5 x 50 mm, 5 µm, part no. PL1110-1520). The mobile phase was THF delivered at a flow rate of 1 mL/min. The calibration curve was constructed using polystyrene standards (molecular weight range: 1,570–46,500 Da).

**Preparation and characterization of CyA-loaded PEO-b-PCL micelles**

CyA-loaded PEO-b-PCL micelles were prepared as previously reported (12, 14). Briefly, CyA and copolymer were dissolved in acetone with an initial concentration of 3 and 10 mg/mL, respectively, followed by drop-wise addition of acetone to distilled water in a ratio of 1:6. The hydrodynamic diameter of CyA-loaded PEO-b-PCL micelles was measured by dynamic light scattering (Zetasizer Nano ZS, Malvern Instrument Ltd., UK). Concentrated sucrose was added to the polymeric micellar solution in an appropriate volume to achieve a final sucrose concentration of 95.76 mg/mL to adjust the tonicity. The micellar solution was then centrifuged at 13,000 rpm for 5 min, to remove unloaded CyA.

The level of encapsulated CyA was determined in the supernatant using HPLC after destroying the micellar structure through addition of 100 times volume of mobile phase. The HPLC system (Waters® 1500 series controller, USA) is equipped with UV/Vis detector (Waters® 2489 a Dual™ Absorbance detector, USA), pump (Waters™ 1525 a Binary pump, USA), and an automated sampling system (Waters® 2707 Plus Autosampler, USA). The HPLC system was monitored by Breeze software (Waters®). CyA was analyzed by injecting 100 µL sample using a mobile phase consisted of acetonitrile: deionized water (75:25 v/v) adjusted to pH of 3 by phosphoric acid and pumped through a reversed-phase C18 column (Macherey-Nagel, 4.6 x 150 mm, 10 µm particle size) at a flow rate of 1.0 ml/min. CyA concentration was detected using UV detector adjusted at 230 nm. The column temperature was adjusted at 60 °C. CyA encapsulation efficiency was determined using the following equation:

\[
\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of loaded CyA (mg)}}{\text{Amount of CyA added (mg)}} \times 100
\]

**Stability of CyA-loaded micelles in simulated gastric and intestinal fluids**

Eight mL of micellar solution with known CyA content was placed into a dialysis bag with molecular weight cutoff of 3.5 kDa. The dialysis bag was immersed into a flask containing 240 mL of release medium (SGF or SIF) containing 30% (v/v) ethanol (to provide sink conditions), which was kept in a constant temperature shaking water bath at 37 °C and 100 rpm. At predetermined time intervals, aliquots (0.5 mL) from inside the bag was collected. For each sample, the diameter size was measured by DLS and then was freeze-dried and reconstituted in 0.5 mL THF and injected into the GPC system.

**In vitro release of CyA from PEO-b-PCL micelles in simulated gastric and intestinal fluids**

The release study was performed as previously described (9, 10). Briefly, one mL of micellar solution with known CyA content was placed into a dialysis bag with molecular weight cutoff of 14 kDa. The dialysis bag was immersed into a flask containing 60 mL of release medium (SGF or SIF) containing 30% (v/v) ethanol (to provide sink conditions), which was kept in a constant temperature shaking water bath at 37 °C and 100 rpm. At predetermined time intervals, aliquots (1 mL) of the release medium were taken and immediately replaced with the same volume of fresh release medium. Level of released CyA in each sample was measured by UPLC after drug extraction. Nimodipine at a concentration of 5
µg/mL was used as internal standard. The internal standard solution (20 µL) was added to release samples. Drug and internal standard were then extracted using 5 mL diethyl ether. After vortex mixing and centrifugation, the residue was reconstituted in 0.5 mL acetonitrile. Samples of 5 µL were injected into the UPLC system (Waters® ACQUITY™ UPLC-H Class) which was equipped with a Waters® ACQUITY™ UPLC BEH™ C18 Column (1.7 µm, 2.1 x 50 mm) with a mobile phase of acetonitrile: methanol: deionized water (75:5:20 v/v) adjusted to pH of 3 by phosphoric acid. The column temperature was set at 60 °C. CyA concentrations were determined by UV detection at 230 nm (Waters® ACQUITY™ Tunable UV detector). The calibration samples were prepared at a concentration range of 1–100 µg/mL. Each experiment was conducted in triplicate.

At a similar CyA concentration, the drug release profile from Neoral® as well as from ethanolic solution (CyA dissolved in ethanol 60% v/v) was evaluated. The percentage of cumulative released drug for ethanolic solution of CyA (control), Neoral®, and PEO-6-PCL micellar formulation was calculated and plotted versus time.

Model independent approach, using difference factor and similarity factor, was adopted to compare the release profile of CyA from Neoral® versus CyA-loaded PEO-6-PCL micelles (15). The difference factor ($f_1$), which is the percent error between the two curves over all time points is calculated as follows:

$$f_1 = \frac{\sum_{j=1}^{n}|R_j - T_j|}{\sum_{j=1}^{n} R_j} \times 100$$

Where $n$ is the sampling number, $R_i$ and $T_j$ are the percent CyA released from Neoral® (reference) and CyA-loaded micelles (test), respectively, at time $j$. The similarity factor ($f_2$) was calculated as follows:

$$f_2 = 50 \times log \left( \left[ 1 + \frac{(1/n) \sum_{j=1}^{n} (R_j - T_j)^2}{n} \right]^{1/2} \right) \times 100$$

In general, $f_1$ values lower than 15 (0-15) and $f_2$ values higher than 50 (50-100) indicate similar dissolution (release) profile (16).

Pharmacokinetic study in rats
Animal studies were conducted based on protocols approved by the Experimental Animal Care Centre Review Board (No. C.P.R- 4525), Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The protocols were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications 8th edition, 2011). Male Wistar rats (250-350 g) were housed in temperature-controlled rooms with 12 h of light per day. The animals had free access to food and water prior to experimentation. On the day of study, rats were allowed free access to water, but food was withheld overnight. The next morning, rats were divided into two groups (45 rats/group): control group received Neoral® oral solution (100 mg/mL), after 20 times dilution with normal saline, and test group received CyA in the polymeric micellar formulation. CyA, from each formulation, was administered as single dose of 10 mg/kg orally through gavage. At 30 min, or 1, 2, 4, 6, 9, 12, 24, and 48 h after oral administration, each rat (n = 5 for each time point) was anaesthetized and exsanguinated by cervical dislocation. At each time point, samples of whole blood from each rat were collected and split into two heparinized tubes. One was for whole blood, which was directly stored at –20 °C, while the other was immediately centrifuged for 3 min to collect plasma, which was also kept at –20 °C until assessed for drug concentration. The blood and plasma concentrations of CyA were analyzed by a liquid chromatography-mass spectrometry (LC-MS/MS) method as described below and the blood or plasma concentration versus time curves were profiled.

In vitro blood-to-plasma concentration ratio of CyA
To investigate the influence of formulation on CyA distribution in blood, the in vitro blood-to-plasma concentration ratio of CyA was determined for CyA dissolved in absolute ethanol, CyA-loaded micelles, and Neoral® (after 10 times dilution with saline). Briefly, known amounts of CyA from each formulation were added to heparinized tubes containing freshly obtained rat blood to provide final concentrations of 0.5, 1, and 2.5 µg/mL.

Animals (NIH Publications 8th edition, 2011). Male
(within the range obtained from the pharmacokinetic study). The tubes were placed in a shaking water bath at 37 ºC for 1 h. At that time, the tubes were removed and 200 µL of blood was transferred to new glass tubes (n = 3) containing 100 µL of Zinc Sulphate solution (0.2 M). The remaining blood was centrifuged at 2500 × g for 10 min. A volume of 200 µL of the plasma layer was transferred to new glass tubes (n = 5). Samples were kept frozen at -20 ºC until being assayed for CyA concentrations.

**Determination of CyA levels in blood and plasma**

The concentrations of CyA in blood or plasma samples were analyzed by a previously reported LC-MS/MS method (17). Briefly, the analyses were performed using a Waters Acquity H-Class UPLC system coupled with a Waters triple quadrupole mass spectrometer with an autosampler (Milford, MA, USA). Chromatographic separation was achieved using Acquity UPLC BEH™ C18 column (1.7 µm, 2.1 × 50 mm, Waters, USA) connected to an Acquity UPLC BEH™ 1.7 µm VanGuard™ Pre-column 2.1 × 5 mm both maintained at 55º C using a column heater. The mobile phase comprised of methanol (A) and 3mM ammonium acetate buffer (B), pumped at a rate of 0.35 mL/min. The gradient program of inlet method was: 80% A and 20% B for first 0−0.49 min, changed to 100% A for 0.5−0.8 min, and then again changed to 80% A and 20% B for 0.81−3.0 min. The total analysis time was 3 min including re-equilibration of the column. The mass spectrometric detection was carried out using electrospray ionization (ESI) probe operated in positive ionization mode and single ion recording (SIR) for both the analyte (CyA) and internal standard (Cyclosporine D; CyD). Nitrogen was used as desolvation gas at 600 L/h flow rate with desolvation temperature set at 350º C. The temperature of the source was set at 150º C. The cone voltage was optimized at 92 V to maximize the signal corresponding to the sodium adduct of the selected compounds. The analysis was carried out using SIM at m/z 1225.2 for CyA and m/z 1239.2 for CyD. MassLynx software (Version 4.1, SCN 714) was used for data acquisition and processing. The assay quantification limit was 0.025 ng/mL.

The drug and the internal standard were extracted from blood and plasma samples through a simple protein precipitation method as described previously (17). Briefly, to a 300 µL of blood or plasma sample, 50 µL of CyD (5 µg/mL) was added and vortexed. Thereafter, 150 µL of Zinc Sulphate solution (0.2 M) was added and vortexed for about 30 s and then 500 µL of methanol was added. The samples were again vortexed for about 30 s, left for 5 min, and then centrifuged for 6 min at 13,000 rpm. Supernatant was removed and again centrifuged for 8 min at 13,000 rpm to clean the samples. After centrifugation, 200 µL of supernatant was transferred into HPLC vial with insert, and 10 µL of the sample was injected for analysis Concentration ranges of 0.025–5 µg/mL were employed in the calibration samples.

**Data and statistical analysis**

Non-compartmental methods were used to calculate the pharmacokinetic parameters. The elimination rate constant (λz) was estimated by linear regression of the plasma concentrations in the log-linear terminal phase and the corresponding half-life (t1/2) was calculated by dividing 0.693 by λz. The AUC0−∞ was calculated using the approach outlined by Bailer (18), which incorporates partial AUC and variability associated with each of the mean concentrations at each sampling point. Pairwise comparisons of the AUC were then undertaken at α = 0.05. The critical value of Z (Zcrit) for the two-sided test after Bonferroni adjustment was 2.24 (19), and the observed value of Z (Zobs) was calculated as previously described (20, 21). The oral clearance (CL/F) was calculated by dividing dose by AUC0−∞. The maximum plasma concentration (Cmax) and the time at which it occurred (tmax) were determined by visual examination of the data (22). The relative oral bioavailability (F) was calculated by dividing the blood AUC of CyA obtained from polymeric micellar formulation (test) by the blood AUC of Neoral® (reference formulation).

All data are reported as mean ± SD, unless otherwise indicated. Differences between the means were compared by unpaired Student’s t-test assuming unequal variance. The level of significance was set at α = 0.05.

**RESULTS**

**Characterization of CyA-loaded PEO-b-PCL micelles**

PEO-b-PCL copolymer was successfully synthesized as confirmed by 1H NMR and GPC data. The degree of polymerization of ε-
Caprolactone was found to be 117, which translates to a PCL molecular weight of 13,400 g/mol. The total molecular weight of PEO-b-PCL based on 1H NMR spectrum was 18,500 g/mol, which is close to the value obtained from GPC i.e. 20,500 (Table 1). Cyclosporine A achieved a high encapsulation efficiency (~74%) reaching an aqueous solubility of 2.22 mg/mL in PEO-b-PCL micelles (Table 1). The mean $Z_{\text{ave}}$ diameter of CyA-loaded PEO-b-PCL micelles was 83.8 nm. The values for encapsulation efficiency and diameter size of the micelles are similar to those we previously reported (12).

**Stability of CyA-loaded micelles in SGF and SIF**

The diameter size of micelles incubated in SGF and SIF at 37 °C was measured at predetermined time intervals. As shown in Figure 1, the mean $Z_{\text{ave}}$ diameter slightly increased in SGF from 83 nm at time 0 (in distilled water) to around 93 nm in the first hour and remained constant throughout the entire period of the experiment (24 h). Upon incubation in SIF, the mean $Z_{\text{ave}}$ diameter of the micelles gradually increased by 5–10% after the first hour of incubation. The micelles diameter ranged from 81.1 to 92.7 nm throughout the experiment (Figure 1).

In order to assess the stability of the block copolymer at these media, aliquots were taken from inside the dialysis bags (0.5 mL), freeze-dried, reconstituted in THF, and then injected into the GPC system. The chromatograms of PEG-b-PCL copolymer at different time points recovered from CyA-loaded micelles in SGF (Figure 2). The shape of PEO-b-PCL peak and consequently the PDI ($M_w/M_n$) calculated did not change until 8 h incubation, where the peak shape started to slightly change (small shoulder was observed). These changes were more noticeable at 12 and 24 h incubations at SGF. These changes were reflected at the calculated PDI of the copolymer, which increased from 1.54 at 4 h to 1.60, 1.63, and 1.65 at 8, 12, and 24 h, respectively.

The chromatograms of PEG-b-PCL copolymer at different time points obtained from CyA-loaded micelles incubated in SIF. In contrast to SGF, both the shape of the peaks and PDI of the copolymer did not change in all samples (Figure 2).

### Table 1. Characteristics of the polymeric micellar formulation of CyA

| Block copolymer | $M_n$ (g/mol)$^a$ | $M_n$ (g/mol)$^b$ | PDI$^c$ | initial drug concentration (mg/mL) | Average diameter (nm)$^d$ | PDI$^e$ | Encapsulation Efficiency ± SD (%)$^f$ |
|-----------------|------------------|------------------|---------|-----------------------------------|--------------------------|---------|-------------------------------------|
| PEO-b-PCL       | 18500            | 20500            | 1.55    | 3                                 | 83.8 ± 1.6               | 0.17 ± 0.09 | 73.77 ± 1.18                       |

$^a$ Number-average molecular weight measured by 1H NMR. $^b$ Number-average molecular weight measured by GPC using PS standards. $^c$ Polydispersity index ($M_w/M_n$) determined by GPC. $^d$ Mean diameter size estimated by DLS technique. $^e$ Polydispersity index measured by DLS technique. $^f$ Measured by HPLC. Values are recorded as mean ± SD (n = 3).

**Figure 1.** Variation in diameter size of CyA-loaded PEO-b-PCL with incubation time in SGF and SIF at 37 °C and 100 rpm. Each data point represents the mean ± SD (n = 3).
| SGF  | 0 h | 1 h | 2 h | 4 h | 8 h | 12 h | 24 h |
|------|-----|-----|-----|-----|-----|------|------|
| ![Detector response (mV)](image1) | ![Retention volume (mL)](image2) | ![Detector response (mV)](image3) | ![Retention volume (mL)](image4) | ![Detector response (mV)](image5) | ![Retention volume (mL)](image6) | ![Detector response (mV)](image7) | ![Retention volume (mL)](image8) |
| SIF  | CyA in THF | 1 h | 2 h | 4 h | 8 h | 12 h | 24 h |
| ![Detector response (mV)](image9) | ![Retention volume (mL)](image10) | ![Detector response (mV)](image11) | ![Retention volume (mL)](image12) | ![Detector response (mV)](image13) | ![Retention volume (mL)](image14) | ![Detector response (mV)](image15) | ![Retention volume (mL)](image16) |

**Figure 2.** GPC chromatograms of CyA-loaded PEO-b-PCL at different incubation times in SGF or SIF. Samples were taken from inside the dialysis bag, lyophilized, and then reconstituted in THF prior to their injection into the GPC system. The chromatogram at 0 h is for a sample of CyA-loaded PEO-b-PCL that was lyophilized directly following micelle preparation.
In vitro release of CyA from PEO-b-PCL micelles in SGF and SIF

Figure 3 shows the release profile of CyA from the different formulation in SGF and SIF. For PEO-b-PCL micelles, CyA had a similar release profile in both media with around 68% and 61% released within 24 h in SGF and SIF, respectively. Neoral® showed a drug release profile comparable to PEO-b-PCL micelles in SGF with around 65% of the drug released within 24 h. In SIF, Neoral® showed a CyA release profile which was comparable to that for PEO-b-PCL micelles up to 12 h (~ 50%), but it showed a significantly higher drug release in the 24 h reaching 85% compared to 61% obtained with PEO-b-PCL micelles (p < 0.5, unpaired Student’s t-test). The control ethanolic solution of CyA showed a similar release profile in both media with around 50% drug release within the first two hours, and 80-100% drug was released within 10 h.

The calculated $f_1$ and $f_2$ for CyA-loaded micelles in SGF were 5.5 and 51.6, respectively. In SIF, the $f_2$ and $f_3$ were 13.9 and 51.1, respectively. Therefore, the release profile of CyA-loaded micelles is considered similar to that of Neoral®.

Pharmacokinetics of CyA in blood

Figure 4 shows the concentration-time profile of CyA for control formulation (Neoral®) and the polymeric micelles formulation in blood following an oral dose of 10 mg/kg in rats. The 48 h profile shows a rapid absorption phase for CyA reaching a mean $C_{\text{max}}$ of 1.40 mg/L and 2.10 mg/L for Neoral® and the polymeric micellar formulation, respectively. The difference in $C_{\text{max}}$ between the two formulations was statistically significant (p < 0.05, Student’s unpaired t-test). The $t_{\text{max}}$ was 2 h in both formulations. The relative bioavailability of CyA, where the AUC of Neoral® served as the reference formulation, was around 96%. The mean blood CL/F for Neoral® and polymeric micellar formulations were 0.419 L/h/kg and 0.435 L/h/kg, respectively. The pharmacokinetic parameters of CyA in blood are listed in Table 2.
to Neoral®, the difference was not statistically significant ($p > 0.05$). The $t_{\text{max}}$ was 2 h in both formulations. The relative bioavailability of CyA in the polymeric micelles, based on plasma AUC, was approximately 81%. The mean plasma CL/F for Neoral® and polymeric micellar formulations were 1.307 and 1.614 L/h/kg, respectively. The pharmacokinetic parameters of CyA in plasma are listed in Table 3.

### Table 2. Pharmacokinetics of CyA in blood after oral administration of Neoral® or CyA-loaded micelles to rats at a dose of 10 mg/kg

| Parameter          | Neoral® | CyA micelles |
|--------------------|---------|--------------|
| AUC$_{0-24h}$ (mg.h/L) | 16.17 ± 0.25 | 18.76 ± 0.36* |
| AUC$_{0-48h}$ (mg.h/L) | 21.64 ± 0.28 | 21.65 ± 0.38 |
| AUC$_{0-\infty}$ (mg.h/L) | 23.87 | 22.97 |
| $t_{1/2}$ (h) | 14.78 | 11.80 |
| CL/F (L/kg/h) | 0.419 | 0.435 |
| C$_{\text{max}}$ (mg/L) | 1.40 ± 0.25 | 2.10 ± 0.41* |
| $T_{\text{max}}$ (h) | 2 | 2 |

* Statistically significant from Neoral® ($p < 0.05$).

### Table 3. Pharmacokinetics of CyA in plasma after oral administration of Neoral® or CyA-loaded micelles to rats at a dose of 10 mg/kg

| Parameter         | Neoral® | CyA micelles |
|-------------------|---------|--------------|
| AUC$_{0-24h}$ (mg.h/L) | 6.72 ± 0.17 | 5.93 ± 0.18* |
| AUC$_{0-\infty}$ (mg.h/L) | 7.65 | 6.19 |
| $t_{1/2}$ (h) | 8.85 | 5.90 |
| CL/F (L/kg/h) | 1.307 | 1.614 |
| C$_{\text{max}}$ (mg/L) | 1.03 ± 0.49 | 1.16 ± 0.33 |
| $T_{\text{max}}$ (h) | 2 | 2 |

* Statistically significant from Neoral® ($p < 0.05$).

The in vivo blood-to-plasma concentration ratio versus time profile for CyA in each formulation. In all data points, the blood-to-plasma ratio of CyA was above 1 in both formulations, which indicates binding of CyA to blood cells (mainly red blood cells). Nonetheless, the values were different between the two formulations, and the differences were statistically significant at three time points ($p < 0.05$). The values of blood-to-plasma ratios ranged from 1.9−6.3 for CyA in the polymeric micellar formulation and from 1.5−4.4 for Neoral®. The in vitro blood-to-plasma concentration ratios of CyA ranged from 0.54 to 0.89 for Neoral® and from 0.49 to 0.61 for CyA-loaded PEO-b-PCL micelles (Figure 7). The range for CyA in ethanol, on the other hand, was from 1.40 to 1.45 (Figure 7).

### DISCUSSION

Although polymeric micelles are recognized to be good solubilizing agents for poorly soluble drugs, studies on their use as solubilizing agents for oral drug delivery is limited. One potential concern hindering oral use could be the perceived instability of micelles in the gastrointestinal (GI) environment. Despite the perception, several studies have shown the stability of polymeric micelles in the challenging environment of the GI tract. For instance, the stability of griseofulvin-loaded PEO$_{5000}$-b-PDLLA$_{4000}$ micelles was evaluated in SGF and SIF at 37 ºC for 11 days (23). The specific viscosity of the copolymer decreased very slowly with time from day 1 until day 11. Moreover, the drug release profile in SGF and SIF was biphasic with no burst release, and only 10% release within the first two days reaching ~ 50% release at day 6. This was in contrast to the free drug, that was entirely released within 24 h. In another study, on CyA-loaded PEO$_{5000}$-b-PDLLA$_{5000}$ micelles, micellar diameter and amount of encapsulated drug have not been changed throughout the incubation of micelles in SGF or SIF for 12 hours (at 37 ºC) (10). The optimum micellar formulation showed a sustained release of CyA in SIF with only 40% drug release within 6 hours and up to ~80% release after 24 hours. The free drug, on the other hand, showed more than 80% release within 6 h and around 100% release at 24 h.

Permeability of the micellar formulation from the GI epithelium is another issue that has not been investigated adequately. For instance, Préat and coworkers have investigated the potential of polymeric micelles prepared from methoxypoly(ethylene glycol)-poly(ε-caprolactone/trimethylene carbonate) [PEG-p(CL-co-TMC)] (Molecular weight ~ 5,000 g/mol) for oral administration utilizing risperidone as a model drug (24, 25).
Figure 5. Plasma concentration versus time profile of CyA following an oral dose (10 mg/kg) of Neoral® and PEO-b-PCL micellar formulation to rats. Each data point represents the mean ± SD (n = 5 rats/data point).

Figure 6. In vivo Blood-to-plasma concentration ratios versus time profile in rat following an oral dose (10 mg/kg) of Neoral® and PEO-b-PCL micellar formulation. Each data point represents the mean ± SD (n = 5 rats/data point). * Indicates statistical significance (p < 0.05).

Figure 7. In vitro Blood-to-plasma concentration ratios at three different concentrations of CyA in ethanol, Neoral®, and PEO-b-PCL micellar formulation. Each data point represents the mean ± SD (n = 3). * Significantly different from CyA in ethanol (p < 0.05). # Significantly different from Neoral® (p < 0.05).
They used an in vitro model of the intestine (Caco-2 cells) to assess the intestinal permeability of $[^{14}C]$-radiolabeled PEG$_{750}$-P(CL-co-TMC) micelles. The mechanistic studies suggest that the drug-loaded micelles were absorbed by pinocytosis, whereas the polymeric unimers diffused passively across the membrane concomitantly with micellar endocytosis (26). Moreover, based on the total radioactivity in plasma after single intravenous and oral dose to rats, the oral bioavailability of $[^{14}C]$-radiolabeled PEG$_{750}$-P(CL-co-TMC) micelles were estimated at 40%, indicating that polymers may be orally bioavailable to some extent, although it was not clear if the polymer is absorbed in a micellar form.

The main objective of this study was to evaluate the potential of PEO-b-PCL micelles as an oral delivery system for CyA making comparisons with its commercial oral formulation Neoral®. We have investigated the use of PEO-b-PCL micelles as an oral delivery system for valspodar, which is a non-immunosuppressive derivative of CyA, before (27). Following a single oral administration of valspodar-loaded micelles (10 mg/kg), the median $t_{\text{max}}$ of valspodar was ~ 2 h for both the micellar formulation as well as control formulation (valspodar dissolved in Cremophor El/ethanol). Likewise, the $C_{\text{max}}$ did not differ significantly between the two formulations, with a relative bioavailability of valspodar in the polymeric micellar formulation of around 120% (27).

The pharmacokinetic profile of PEO$_{5000}$-b-PDLLA$_{5000}$ micellar formulations of CyA has already been evaluated and compared to that of Neoral® before (9). Following a single oral dose (10 mg/kg) of CyA-loaded micelles to rats, the pharmacokinetic parameters of $C_{\text{max}}$, $T_{\text{max}}$, and AUC$_{0-\text{t}}$ were comparable to those obtained from the marketed oral CyA formulation (Neoral®) (9).

In the current study, CyA was encapsulated in PEO-b-PCL micelles effectively through a co-solvent evaporation method. A high level of drug loading was achieved, which resulted in an aqueous solubility of nearly 2.2 mg/mL (Table 1). Although there was around 15% increase in the mean diameter of micelles upon incubation in SGF ($p < 0.05$), the size was constantly less than 100 nm throughout the whole period of the experiment (24 h) (Figure 1). Moreover, despite the fact that core-forming block in these micelles is a polyester (PCL), which is prone to hydrolysis especially in an acidic aqueous media such as SGF, the GPC data showed that the copolymer forming polymeric micelles was 100% stable for at least 8 hours (Figure 2). This implies the protection of the poly(ester) structure within the micellar core resulting in stability of PEO-b-PCL in acidic conditions of SGF.

In SIF, the mean diameter of micelles increased by 5–10% ($p < 0.05$). Several factors are known to influence the size of micelles. The differences in pH as well as ionic strength between the two media might have been the cause of micelle size difference. A similar variation in size was obtained with CyA-loaded PEO-b-PDLLA micelles reported in Zhang et al. work (10). However, it is not clear whether the change in average diameter of micellar population observed here (which is only 12% difference) has made a significant contribution to pharmacokinetics of CyA micellar formulation. In contrast to the profile in SGF, the GPC data showed stability of PEO-b-PCL copolymer in the micellar form in SIF for the entire period of the experiment (24 h). This is not surprising since the pH of SIF is close to neutral pH, while SGF has an acidic pH (~ 2.0), which is known to facilitate the hydrolysis of poly(esters). Taken together, PEO-b-PCL micelles used in this CyA formulation have an excellent stability profile in SGF and SIF at 37 °C.

We then investigated the capability of PEO-b-PCL micelles to control the release of the encapsulated drug under conditions relevant to the GI conditions, i.e. fasting SGF and SIF media. As shown in Figure 3, both Neoral® and CyA-loaded PEO-b-PCL micelles were able to sustain the release of CyA in both media. In SGF, the 24 h cumulative release of CyA in Neoral® and PEO-b-PCL micelles were 65% and 68%, respectively (Figure 3A). In SIF, 81% of CyA was released from Neoral® after 24 h incubation, whereas PEO-b-PCL had a significantly lower CyA release (only 61%, $p < 0.05$) (Figure 3B). In studies by Zhang et al., the cumulative release of CyA from the optimum PEO-b-PDLLA formulation was around 85% at 24 h in both SGF and SIF (with 30% ethanol) (9, 10). PEO-b-PCL micelles seem to have a better control over the release of CyA compared to PEO-b-PDLLA in the same media. It should be noted though that cyclosporine A is a cyclic peptide and belongs to BCS Class IV (i.e. low solubility & low permeability) (28). Therefore, the likelihood of obtaining an in vitro-in vivo correlation (IVIVC) for a cyclosporine A formulation is not that high (28). That said, the significant increase in drug release obtained with Neoral® in SIF at 24 h was associated...
with a significantly higher CyA concentration ($p<0.05$) in blood compared to CyA-loaded micelles (361 versus 175 ng/mL, respectively).

The clinical use of CyA has been associated with potentially severe toxic side effects on kidneys (29). Moreover, CyA is known to induce renal failure and increase the synthesis of reactive oxygen species, thromboxane, and lipid peroxidation products in kidneys (30). Micelles of PEO-$b$-PCL were successful carriers efficiently changing the pharmacokinetic and biodistribution profile of CyA by reducing CyA accumulation in normal tissues (including kidneys) and increasing its levels in blood after a single intravenous dose (11, 12, 31). These micelles have also been shown to control the release of CyA over a prolonged time (in 4% w/v bovine serum albumin solution, 37° C). While the control formulation (Sandimmune® for intravenous injection) almost completely released the incorporated CyA within 24 h, only less than 6% of the drug released within the same period from PEO-$b$-PCL micelles (12). Additionally, PEO-$b$-PCL copolymers have been shown to be compatible with several body tissues, and safe to use in animals through different routes of administration (32-34). The positive impact of PEO-$b$-PCL micelles on the pharmacokinetics and tissue distribution of CyA after intravenous administration could be seen following oral administration should all, or at least certain proportion, of CyA-loaded micelles get absorbed as intact micelles.

Our in vitro data showed the PEO-$b$-PCL micellar formulation of CyA to have the sufficient stability and release profile to act as a carrier for CyA in the GI tract and via oral administration. The question was whether these micellar formulations can provide similar or even improved pharmacokinetic profile to that of commercial CyA formulation, the same way they did when their intravenous administration was used (11, 12, 31). To answer this question, we investigated the pharmacokinetics of CyA following oral administration of each formulation both in blood and plasma. Following a single oral dose of 10 mg/kg to rats, PEO-$b$-PCL micelles provided approximately 50% higher $C_{\text{max}}$ compared to Neoral® when blood samples were assessed ($p < 0.05$). In fact, the $\text{AUC}_{0-6}$ for the PEO-$b$-PCL micellar formulation of CyA was also significantly higher than that for Neoral®, perhaps pointing to better absorption of the former formulation. Interestingly, the pharmacokinetic profile of CyA following oral administration of its PEO-$b$-PCL formulation showed a slight sign of enterohepatic circulation, which was not observed for the Neoral® formulation, which again implies an improvement in the extent of absorption/reabsorption by an unknown mechanism only for PEO-$b$-PCL formulation of CyA. The $t_{\text{max}}$, $\text{AUC}_{0-48h}$, and $\text{AUC}_{0-\infty}$ were similar for both formulations. The mean elimination $t_{1/2}$ of CyA was about 3 h longer in Neoral®. However, owing to the destructive nature of the sampling procedure, it is not known whether or not the difference in $t_{1/2}$ between the two formulations is statistically significant since the $t_{1/2}$ for individual rats could not obtained. Nonetheless, by comparing the drug concentrations for each formulation in the last three data points (12, 24, and 48 h), which were used to estimate the elimination $t_{1/2}$, the difference in drug concentration at 24 h was the only one significantly lower for the polymeric micellar formulation ($p < 0.05$). For comparison, in studies by Zhang et al. on the pharmacokinetics of CyA-loaded PEO5000-$b$-PDLLA5000 using Neoral® as a reference formulation (9), at a single oral dose of 10 mg/kg in rats, the CyA blood concentration versus time profile for PEO-$b$-PDLLA was comparable to that of Neoral®, CyA-loaded PEO-$b$-PDLLA micelles had a slightly lower $C_{\text{max}}$ compared to Neoral® though the difference was not statistically significant. On the other hand, CyA-loaded PEO-$b$-PDLLA micelles tended to have a higher $\text{AUC}_{0-72h}$ compared to Neoral®, but the difference was not statistically significant (9).

In contrast to what we have seen in blood pharmacokinetics, the $C_{\text{max}}$ of CyA-loaded micelles was only 10% higher than that for Neoral®, when plasma samples were assessed, but the difference was not statistically significant ($p > 0.05$). The calculated $\text{AUC}_{0-24h}$ for CyA-loaded micelles was around 12% lower than that for Neoral® ($p < 0.05$). This was reflected in the higher plasma CL/F value for CyA-loaded micelles compared to Neoral®. Nonetheless, the relative bioavailability of CyA-loaded micelles was higher than 80%. Overall, the results of pharmacokinetic assessments in blood and plasma, did not support a notable change in the profile of CyA by its PEO-$b$-PCL micellar formulation compared to Neoral® following oral administration, except for a significant enhancement in $C_{\text{max}}$ only in blood.

We then investigated CyA concentrations in plasma, to further investigate the potential effect of the two formulations in changing blood distribution
of CyA. We found the concentrations of CyA in plasma samples to be lower than those obtained in blood for both formulations. This was not surprising since, CyA is known to bind to red blood cells, which results in higher concentration in whole blood compared to plasma (i.e. blood-to-plasma ratio > 1). When comparing the two formulations, it appeared that the blood to plasma ratio for Neoral® to be significantly lower than that of polymeric micellar formulation at three time points, namely 4, 6, and 9 h post dose ($p < 0.05$).

To shed further light on the behind these in vivo findings, we determined the blood-to-plasma concentration ratio of CyA for Neoral® and PEO-b-PCL formulations in vitro at three different concentrations, within the range obtained in the in vivo pharmacokinetic studies. And the results were also compared to that for drug solution in ethanol (Figure 7). Indeed, Neoral® showed significant reductions in the blood-to-plasma concentration ratio of CyA at all the three drug concentrations studied when compared to CyA in ethanol solution ($p<0.05$). Surprisingly, and in contrast to the in vivo results, PEO-b-PCL micelles have shown even lower blood-to-plasma concentration ratios of CyA compared to Neoral® and CyA in ethanol, in vitro. The significantly lower in vitro blood-to-plasma concentration ratios of CyA in Neoral® and PEO-b-PCL micelles can be attributed to the protective effect of micellar formulations in Neoral® and PEO-b-PCL on CyA preventing its binding to the red blood cells (35, 36). This will lead to higher plasma concentrations and ultimately a lower blood-to-plasma concentration ratio. The PEO-b-PCL micellar formulation, however, might have released CyA during or before absorption and appearance in blood; leading to higher in vivo blood to plasma ratios compared to Neoral®. However, this has not been the case in vivo, where higher CyA blood:plasma ratios were observed for PEO-PCL micellar formulation compared to Neoral®.

Neoral® is an oral solution that immediately forms a microemulsion in an aqueous environment. Cremophor RH40 (PEG-40 Hydrogenated Castor Oil) is one of the major components of Neoral® solution. It is a non-ionic surfactant similar to Cremophor EL (PEG-35 Hydrogenated Castor Oil), a major component of Sandimmune® injection. They both were reported to be toxic (37). Additionally, there are several reports showing that Cremophor EL influences the pharmacokinetics of various drugs including CyA (35, 38-40).

Furthermore, Jin et al have reported that administration of Cremophor EL at different concentrations significantly altered the values of blood-to-plasma concentration ratio and the apparent tissue to plasma concentration ratio of CyA in rats (35). Moreover, Cremophor EL has been shown to inhibit the uptake of CyA by red blood cells in vitro (35). It has also been found that Cremophor EL releases CyA that is adsorbed on the interior surface of blood vessels (35). Although these effects were observed for Cremophor EL after intravenous administration, it is possible that the lower blood to plasma ratios obtained with Neoral® in the current study was caused by Cremophor RH40.

The higher in vivo blood-to-plasma ratio of CyA delivered by the PEO-b-PCL micellar formulation compared to Neoral® may reflect the more inert nature of PEO-b-PCL compared to Neoral® vehicle in terms of CyA interaction with red blood cells following absorption to blood stream. It may also point to the absorption of released CyA from the PEO-b-PCL formulation over vehicle associated CyA from Neoral® to the blood stream following oral administration. These explanations are speculations at this point and more investigations are needed to clarify the real reason behind this observation. Nevertheless, this should not detract from the importance and value of PEO- b-PCL micelles as vehicles that can enhance the solubility and oral delivery of CyA even when compared to its optimum oral formulation, i.e., Neoral®. This is besides the fact that PEO-b-PCL micellar formulation offers the advantage of simplicity of composition and preparation.

CONCLUSIONS

Our results show that PEO-b-PCL micelles can act as effective solubilizing agents and serve as good alternatives to commercially available excipients used in the oral formulations of a poorly soluble drug, i.e. CyA. In addition, our results imply the more inert nature of PEO-b-PCL formulations in affecting the distribution of CyA in blood components after absorption to the systemic circulation. The latter can set the PEO-b-PCL micelles apart and distinguished from solubilizing agents used in Neoral®, the oral CyA delivery system used in clinic.
ACKNOWLEDGMENTS

The authors are grateful to the College of Pharmacy Research Center and the Deanship of Scientific Research at King Saud University, Riyadh, Saudi Arabia for technical and financial support.

REFERENCES

1. Flechner SM. Cyclosporine: a new and promising immunosuppressive agent. The Urologic clinics of North America. 1983;10(2):263-75.

2. Guada M, Beloqui A, Preat V, Dios-Vieitez Mdel C, Blanco-Prieto MJ. Reforunulating cyclosporine A (CsA): More than just a life cycle management strategy. Journal of controlled release : official journal of the Controlled Release Society. 2016;225:269-82.

3. Andrysek T. Impact of physical properties of formulations on bioavailability of active substance: current and novel drugs with cyclosporine. Molecular Immunology. 2003;39(17):1061-5.

4. Yee GC. Dosage forms of cyclosporine. Pharmacotherapy. 1991;11(6):1498-525.

5. Novartis. Package insert: Neoral; 2009 [Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/050715s027,050716s028lbl.pdf.

6. Mueller EA, Kovarik JM, van Bree JB, Tetzloff W, Grevel J, Kutz K. Improved dose linearity of cyclosporine pharmacokinetics from a microemulsion formulation. Pharm Res. 1994;11(2):301-4.

7. Colombo D, Egan CG. Bioavailability of Sandimmun® versus Sandimmun Neoral®: A Meta-Analysis of Published Studies. International Journal of Immunopathology and Pharmacology. 2010;23(4):1177-83.

8. Guan P, Lu Y, Qi J, Niu M, Lian R, Hu F, et al. Enhanced oral bioavailability of cyclosporine A by liposomes containing a bile salt. International journal of nanomedicine. 2011;6:965-74.

9. Zhang Y, Li X, Zhou Y, Fan Y, Wang X, Huang Y, et al. Cyclosporin A-loaded poly(ethylene glycol)-b-poly(d,l-lactic acid) micelles: preparation, in vitro and in vivo characterization and transport mechanism across the intestinal barrier. Mol Pharm. 2010;7(4):1169-82.

10. Zhang Y, Li X, Zhou Y, Wang X, Fan Y, Huang Y, et al. Preparation and evaluation of poly(ethylene glycol)-poly(lactide) micelles as nanocarriers for oral delivery of cyclosporine a. Nanoscale Res Lett. 2010;5(6):917-25.

11. Aliabadi HM, Brocks DR, Lavasanifar A. Polymeric micelles for the solubilization and delivery of cyclosporine A: pharmacokinetics and biodistribution. Biomaterials. 2005;26(35):7251-9.

12. Aliabadi HM, Brocks DR, Mahdipoor P, Lavasanifar A. A novel use of an in vitro method to predict the in vivo stability of block copolymer based nano-containers. Journal of controlled release : official journal of the Controlled Release Society. 2007;122(1):63-70.

13. Aliabadi HM, Mahmud A, Sharifabadi AD, Lavasanifar A. Micelles of methoxy poly(ethylene oxide)-b-poly(epsilon-caprolactone) as vehicles for the solubilization and controlled delivery of cyclosporine A. Journal of controlled release : official journal of the Controlled Release Society. 2005;104(2):301-11.

14. Aliabadi HM, Elhasi S, Mahmud A, Gulamhusein R, Mahdipoor P, Lavasanifar A. Encapsulation of hydrophobic drugs in polymeric micelles through co-solvent evaporation: The effect of solvent composition on micellar properties and drug loading. International Journal of Pharmaceutics. 2007;329(1–2):158-65.

15. Moore JW. Mathematical Comparison of Dissolution Profiles. Pharmaceutical Technology. 1996;20:64-75.

16. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. European Journal of Pharmaceutical Sciences. 2001;13(2):123-33.

17. Mohd Aftab A, Fahad Ibrahim A-J, Abdallah MA-M, Abdul A, Mohammad R. Validated UPLC-MS Method for Pharmacokinetic Investigations of Cyclosporine-A in Blood. Current Pharmaceutical Analysis. 2015;11(3):210-5.

18. Bailer AJ. Testing for the equality of area under the curves when using destructive measurement techniques. J Pharmacokinet Biopharm. 1988;16(3):303-9.

19. Miller RGJ. Simultaneous Statistical Inference. 2 ed: Springer-Verlag New York; 1981. XVI, 299 p.

20. Yuan J. Estimation of variance for AUC in animal studies. Journal of pharmaceutical sciences. 1993;82(7):761-3.

21. Mehvar R, Robinson MA, Reynolds JM. Molecular weight dependent tissue accumulation of dextrans: in vivo studies in rats. Journal of pharmaceutical sciences. 1994;83(10):1495-9.

22. Penugonda S, Parang K, Mehvar R. Plasma pharmacokinetics and tissue disposition of novel dextran-methylprednisolone conjugates with peptide linkers in rats. Journal of pharmaceutical sciences. 1996;83(10):1299-304.

23. Pierr E, Avgoustakis K. Poly(lactide)-poly(ethylene glycol) micelles as a carrier for griseofulvin. J Biomed Mater Res A. 2005;75(3):639-47.

24. Mathot F, van Beijsterveldt L, Pret V, Brewster M, Arien A. Intestinal uptake and biodistribution of novel polymeric micelles after oral administration. Journal of controlled release : official journal of the Controlled Release Society. 2006;111(1-2):47-55.
25. Ould-Ouali L, Noppe M, Langlois X, Willems B, Te Riele P, Timmerman P, et al. Self-assembling PEG-p(CL-co-TMC) copolymers for oral delivery of poorly water-soluble drugs: a case study with risperidone. Journal of controlled release: official journal of the Controlled Release Society. 2005;102(3):657-68.

26. Mathot F, des Rieux A, Arien A, Schneider YJ, Brewster M, Preat V. Transport mechanisms of mmePEG750P(CL-co-TMC) polymeric micelles across the intestinal barrier. Journal of controlled release: official journal of the Controlled Release Society. 2007;124(3):134-43.

27. Binkhathlan Z, Hamdy DA, Brocks DR, Lavasanifar A. Development of a polymeric micellar formulation for valspodar and assessment of its pharmacokinetics in rat. Eur J Pharm Biopharm. 2010;75(2):90-5.

28. Ghadi R, Dand N. BCS class IV drugs: Highly notorious candidates for formulation development. Journal of Controlled Release. 2017;248:71-95.

29. Calne RY, White DJ, Thiru S, Evans DB, McMaster P, Dunn DC, et al. Cyclosporin A in patients receiving renal allografts from cadaver donors. Lancet. 1978;2(8104-5):1323-7.

30. Parra T, De Arriba G, Arribas I, Perez de Lema G, Rodriguez-Puyol D, Rodriguez-Puyol M. Cyclosporine a nephrotoxicity: Role of thromboxane and reactive oxygen species. The Journal of Laboratory and Clinical Medicine. 1998;131(1):63-70.

31. Aliabadi HM, Elhasi S, Brocks DR, Lavasanifar A. Polymeric micellar delivery reduces kidney distribution and nephrotoxic effects of Cyclosporine A after multiple dosing. Journal of pharmaceutical sciences. 2008;97(5):1916-26.

32. Binkhathlan Z, Qamar W, Ali R, Kfoury H, Alghonaim M. Toxicity evaluation of methoxy poly(ethylene oxide)-block-poly(epsilon-caprolactone) polymeric micelles following multiple oral and intraperitoneal administration to rats. Saudi Pharm J. 2017;25(6):944-53.

33. Xu L, Xu X, Chen H, Li X. Ocular biocompatibility and tolerance study of biodegradable polymeric micelles in the rabbit eye. Colloids Surf B Biointerfaces. 2013;112:30-4.

34. Gou M, Wei X, Men K, Wang B, Luo F, Zhao X, et al. PCL/PEG copolymeric nanoparticles: potential nanoplatforms for anticancer agent delivery. Curr Drug Targets. 2011;12(8):1131-50.

35. Jin M, Shimada T, Yokogawa K, Nomura M, Mizuhara Y, Furukawa H, et al. Cremophor EL releases cyclosporin A adsorbed on blood cells and blood vessels, and increases apparent plasma concentration of cyclosporin A. Int J Pharm. 2005;293(1-2):137-44.

36. Liu B, Gordon WP, Richmond W, Groessl T, Tuntralad T. Use of solubilizers in preclinical formulations: Effect of Cremophor EL on the pharmacokinetic properties on early discovery compounds. European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences. 2016;87:52-7.

37. Kiss L, Walter FR, Bocsik A, Veszelka S, Özsávári B, Puskás LG, et al. Kinetic Analysis of the Toxicity of Pharmaceutical Excipients Cremophor EL and RH40 on Endothelial and Epithelial Cells. Journal of pharmaceutical sciences. 2013;102(4):1173-81.

38. Gelderblom H, Verweij J, van Zomeren DM, Buijs D, Ouwens L, Nooter K, et al. Influence of Cremophor EL on the bioavailability of intraperitoneal paclitaxel. Clinical cancer research: an official journal of the American Association for Cancer Research. 2002;8(4):1237-41.

39. Sparreboom A, van Zuylen L, Brouwer E, Loos WJ, de Bruijn P, Gelderblom H, et al. Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. Cancer research. 1999;59(7):1454-7.

40. Gelderblom H, Verweij J, Nooter K, Sparreboom A. Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. European Journal of Cancer. 2001;37(13):1590-8.