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ORIGINAL ARTICLE

Concentration Profiles of Carvedilol: A Comparison Between In Vitro Transfer Model and Dissolution Testing

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Abstract

Purpose The study aims to investigate the transfer behavior of the weakly basic BCS class II model drug carvedilol from the stomach to the small intestine and compare the concentration profiles of carvedilol that were determined during the in vitro transfer model and dissolution testing.

Methods An in vitro transfer model, previously introduced by Kostewicz et al., was used in this study. A donor phase of simulated gastric fluid was used to predissolve Dilatrend® tablet (25 mg carvedilol). Media that simulate and cover the physiological pH and buffer capacity ranges of the intestinal fluid were used as acceptor phases. pH measurements were reported to investigate the effect of addition of donor phase containing predissolved carvedilol on lowering the pH of the acceptor media. The f_2 similarity factor was used to compare the concentration profiles of carvedilol determined during the in vitro transfer model.

Results Carvedilol was completely dissolved in all tested acceptor phases, resulted in no precipitation. The buffering capacity of the acceptor phase plays an important role in determining its pH. A discrepancy was found between the concentrations of carvedilol in all tested acceptor phases obtained using the transfer model and those reported using dissolution apparatus II in corresponding media.

Conclusions Results showed that dissolution testing using apparatus II might not be sufficient to predict its transfer from the stomach into the small intestine and that the in vitro transfer model may be more effective at mimicking the conditions in the gastrointestinal tract.

Keywords Transfer model · Weak base · BCS class II · Dissolution · Gastrointestinal fluid · Carvedilol

Introduction

Carvedilol is a weak base BCS class II drug of low solubility and high permeability [1]. Our previous study has shown that the solubility and dissolution rate of carvedilol from Dilatrend® immediate release (IR) tablets (25 mg carvedilol), using USP apparatus II, exhibited pH– and buffer capacity–dependent behavior, where solubility and hence dissolution rate of carvedilol increase at low pH and high buffering capacity and decrease at high pH and low buffering capacity.

Although the in vitro dissolution testing, using USP apparatus I and II, is an important tool in drug development and quality control procedure, it does not accurately predict the in vivo dissolution of orally administered drug products of weakly acidic or weakly basic drugs [2, 3]. In these studies, tablets are tested in dissolution media of fixed pH and buffer capacity. These experimental conditions do not mimic the transfer of drugs within the gastrointestinal (GI) tract from the stomach to the small intestine at specific gastric emptying rate [4, 5].

An in vitro transfer model used to simulate the transfer of dissolved drug out of the stomach to the small intestine was introduced by Kostewicz et al. [6, 7]. The in vitro precipitation of pure drug substances or IR tablets of weakly basic drugs upon entry into the small intestine was investigated, where the solubility of these drugs was high when dissolved in simulated gastric fluid and then decreased upon transfer into a simulated intestinal fluid [3, 5–12]. For example, Tsume et al. [3] studied the transfer behavior of the weak base anticancer drug dasatinib using the in vitro transfer model mini-

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gastrointestinal simulator (mGIS) and the USP dissolution apparatus II to predict drug supersaturation and/or precipitation when it moves from the stomach to the small intestine. Results showed that the dissolution of dasatinib using apparatus II was negligible in the simulated intestinal fluid without pancreatin SIFsp (< 1%), while dissolution using mGIS in simulated gastric fluid without pepsin (SGFsp)/SIFsp was complete (~100%). In addition, it was found that the dissolution profile of dasatinib produced using the mGIS matched the human plasma profile, whereas that produced using apparatus II did not. This indicates that mGIS might be suitable to predict the in vivo dissolution of weak base drugs. In addition, Ruff et al. [7] used the in vitro transfer model to more accurately mimic the in vivo supersaturation and precipitation behavior of the weak base ketoconazole (KTZ). The supersaturation and precipitation data from the transfer experiments were coupled to the physiologically based pharmacokinetic (PBPK) model using Stella® software. KTZ demonstrated supersaturation followed by precipitation under various in vitro conditions simulating the proximal small intestine. When the in vitro data was coupled to the PBPK model, the simulated profiles matched the observed mean plasma profiles for KTZ. Furthermore, Okumu et al. [8] investigated the dissolution behavior of the weak base etoricoxib using USP apparatus II and the in vitro transfer model when entering the small intestine. GastroPlus™ was used to simulate the drug absorption and to establish an in vivo in vitro correlation. Data showed that when using the in vitro transfer, higher solubility was achieved under such simulated intestinal conditions.

Therefore, the objective of this study was to investigate the transfer behavior of the weakly basic BCS class II model drug carvedilol from the stomach to the small intestine. Towards this aim, a donor phase of simulated gastric fluid without pepsin (SGFsp, pH 1.2) was used to predissolve the Dilatrend® IR tablet (25 mg carvedilol). In addition, acceptor phases that simulate and cover the physiological pH (4.5–7.8) and buffer capacity (0.003–0.130 M/ΔpH) ranges of the intestinal fluid were used in this study. pH measurements were reported at time intervals corresponded to those of the transfer experiments to investigate the effect of addition of donor phase containing predissolved carvedilol on lowering the pH of the acceptor phase. Furthermore, the concentrations of carvedilol determined using the in vitro transfer model ($C_{Transfer}$) were compared to those reported in our previous study [1] using the in vitro dissolution apparatus II ($C_{Dissolution}$) after 120 min to determine any discrepancy between these two in vitro methods.

Materials

Carvedilol powder (Moehs Catalana, S. L., Barcelona, Spain) was provided as a gift from Pharma International Company (Amman, Jordan). Dilatrend® immediate-release tablets 25 mg carvedilol (ROCHE Australia, lot no. M2084B05) were purchased from the market. All chemical reagents were of analytical grade. Double-distilled water was obtained using a GFL water distilling apparatus (Germany).

Methods

Donor Phase and Acceptor Phases

Gastric fluid without pepsin (SGFsp, USP 38; pH 1.2) which simulates gastric condition [13] was used as a donor phase. Acetate buffer (USP 40–NF 35, pH 4.5) which simulates duodenal fluid, intestinal fluid without pancreatin (SIFsp, USP 26; pH 6.8), and fasted–state simulated intestinal fluid (blank FaSSIF, pH 6.5) and fed–state simulated intestinal fluid (blank FeSSIF, pH 5) which simulate the physiological conditions in the proximal intestine in the fasted and fed states [14], respectively, were used as acceptor phases. The word blank preceding FaSSIF and FeSSIF indicates that these media were prepared without the addition of surfactants or bile salts. A series of phosphate buffers (pH 6.8) of different concentrations (6.25–100 mM), which covers the physiological buffer capacity range of the intestinal fluid, were used as acceptor phases and prepared as previously described [1, 15]. Moreover, acceptor phases of phosphate buffers (pH 7.2 and 7.8) that simulate the pH during the fasted state of the proximal ileum [16, 17] and colon [18], respectively, were prepared as previously described [1, 15]. Double–distilled water (DDH_2O) of zero ionic strength and zero buffer capacity [19] was used as a reference for the acceptor phases. The chemical composition and physicochemical properties of the donor and acceptor phases are illustrated in Table 1.

Determination of Carvedilol in the Donor Phase

The amount of carvedilol predissolved in the donor phase SGFsp was determined using UV–Vis spectrophotometer before each transfer experiment. Briefly, one Dilatrend® tablet containing 25 mg carvedilol was placed in a beaker containing 100 mL SGFsp maintained at 37 ± 0.5 °C. Dilatrend® tablet was stirred in SGFsp for 30 min to simulate the gastric residence time, where gastric residence time has been estimated to be approximately 15–60 min [8, 9]. Ten milliliters of the dispersed solution was withdrawn and centrifuged at 3500 rpm for 15 min. Then, the supernatant was diluted appropriately with SGFsp. The concentration of carvedilol in SGFsp was determined by measuring carvedilol absorbance by a UV spectrophotometry (Varian, Cary 50 UV-Vis spectrophotometer, USA) at λ_{max} 285 nm using a standard calibration curve of carvedilol prepared in SGFsp. The amount of carvedilol in SGFsp was performed in at least triplicate.

Table 1 Composition and physicochemical properties of the donor and acceptor phases used in the in vitro transfer model

Donor phase	Composition	pH	Buffer capacity (M/ΔpH)
SGFsp	83.7 mM HCl (37% *w*/*v*) and 34.2 mM NaCl in 1 L DDH_2O	1.2 ± 0.1	–
Acceptor phases	Composition	pH	Buffer capacity (M/ΔpH)
Acetate buffer	28.0 mM CH_3COOH and 36.5 mM $CH_3COONa.3H_2O$ in 1 L DDH_2O	4.5 ± 0.1	0.034
Blank FaSSIF	28.4 mM Na_2HPO_4, 8.7 mM NaOH, and 105.9 mM NaCl in 1 L DDH_2O	6.5 ± 0.1	0.120
Blank FeSSIF	101.0 mM NaOH, 144.1 mM CH_3COOH, and 203.2 mM NaCl in 1 L DDH_2O	5.0 ± 0.1	0.130
SIFsp	50.0 mM KH_2PO_4 and 22.4 mM NaOH in 1 L DDH_2O	6.8 ± 0.1	0.034
Phosphate buffer 6.25 mM	3.13 mM Na_2HPO_4 and 3.13 mM NaH_2PO_4 in 1 L DDH_2O	6.8 ± 0.1	0.003
Phosphate buffer 12.5 mM	6.25 mM Na_2HPO_4 and 6.25 mM NaH_2PO_4 in 1 L DDH_2O	6.8 ± 0.1	0.006
Phosphate buffer 25 mM	12.5 mM Na_2HPO_4 and12.5 mM NaH_2PO_4 in 1 L DDH_2O	6.8 ± 0.1	0.012
Phosphate buffer 50 mM	25.0 mM Na_2HPO_4 and 25.0 mM NaH_2PO_4 in 1 L DDH_2O	6.8 ± 0.1	0.024
Phosphate buffer 100 mM	50.0 mM Na_2HPO_4 and 50.0 mM NaH_2PO_4 in 1 L DDH_2O	6.8 ± 0.1	0.047
Phosphate buffer 100 mM	28.0 mM NaH_2PO_4 and 72.0 mM Na_2HPO_4 in 1 L DDH_2O	7.2 ± 0.1	0.058
Phosphate buffer 100 mM	8.5 mM NaH_2PO_4 and 91.5 mM Na_2HPO_4 in 1 L DDH_2O	7.8 ± 0.1	0.037

Transfer Experiments

To simulate the drug transfer out of the stomach into the small intestine, a Dilatrend® tablet was dispersed in 90–mL SGFsp donor phase. A programmable syringe pump (Graseby 2100, Smiths Medical Instrument, China) was used to transfer the donor phase containing predissolved drug into a dissolution vessel containing 810–mL acceptor phase. A transfer rate of 2 mL/min which represents the physiological flow rate out of the stomach into the intestine was used in this study [6, 7]. The acceptor phase was maintained at 37 ± 0.5 °C. A paddle with rotational speed of 50 rpm, which simulates the rotational speed for the dissolution testing of carvedilol tablets recommended by the USP (USP 40–NF 35), was used in the acceptor phase. A calibrated USP apparatus II (Varian, VK 7000) was fitted with an auto-sampling station consisting of a VK810 peristaltic pump and a VK750 digitally controlled heater/circulator. The dissolution apparatus was connected to a UV spectrophotometer (Cary 50, UV-Vis spectrophotometer). Samples were taken automatically at 0, 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min and passed through a 45-μm polyethylene filter (SUN-SRi, Rockwood, TN, USA). The sample volume was 15 mL, sufficiently enough to flush the filter before making the UV measurement. The auto-sampling system pulls the sample into the flow cell, measures the absorbance of the sample, and then returns it to the vessel. Thus, no correction was made for the drug quantity removed during sample and no replacement of sample volume was needed. The absorbance was measured at λ_{max} 285 nm. The percent dissolved of carvedilol in the acceptor phase was calculated against standard calibration curves of carvedilol. Calibration curves were made for each transfer experiment, where carvedilol standard solutions were made from a combination of the donor phase SGFsp and each acceptor phase in a ratio of 1:9 *v*/*v*, similar to that used in the transfer experiments. The percent dissolved of carvedilol in the acceptor phase was determined as a function of time and compared against the theoretical concentration. The theoretical concentration was calculated, where the amount of carvedilol dissolved in the donor phase was considered to be 100% and the percent release shown in dissolution plots is based on that amount. The experimental setup for the in vitro transfer model is illustrated in Fig. 1.

pH Measurements During the Transfer Experiments

The final volume and pH of the acceptor phase in the dissolution vessel varied with the volume of donor phase transferred. The pH of the acceptor phases was monitored during the transfer experiments and was recorded at time intervals corresponding to those of transfer experiments (0, 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min).

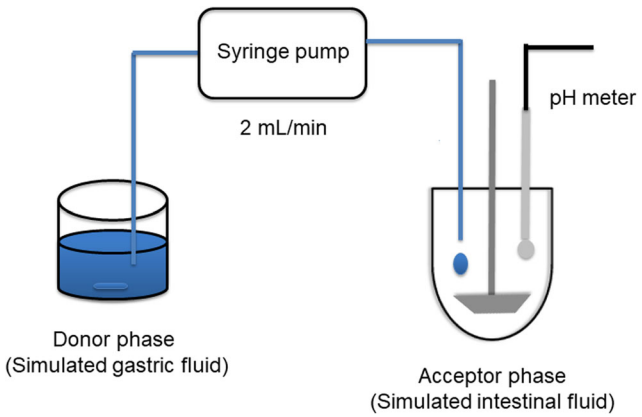

Fig. 1 Experimental setup for the in vitro transfer model of Dilatrend® IR tablets

Evaluation of the Concentration Profiles

The similarity factor (f_2) was estimated to compare the concentration profiles of carvedilol in all acceptor phases. The similarity factor (f_2) was calculated using Eq. 1 [20]:

$$f_2 = 50 \times \log\left\{\left[1 + (1/n)\sum_{j=1}^{n}\left(R_j - T_j\right)^2\right]^{-0.5} \times 100\right\} \quad (1)$$

where n is the number of sampling points and R_j and T_j are the percent of the reference and test products dissolved at time point j, respectively. The values of f_2 were estimated where only one measurement above 85% dissolution of both reference and test products was used in the calculation. [21]. If the f_2 value was less than 50, then the concentration profiles were considered dissimilar. If f_2 was between 50 and 100, then the concentration profiles were considered similar [20].

Percent Difference Between $C_{Transfer}$ and $C_{Dissolution}$

Percent difference between $C_{Transfer}$ and $C_{Dissolution}$ was calculated using Eq. 2:

$$\%\text{Difference} = \frac{\text{CTransfer} - \text{CDissolution}}{\text{CDissolution}} \times 100\% \quad (2)$$

If % difference was positive, then this is a percentage increase, indicating that the concentration of carvedilol obtained from the in vitro transfer model was higher than that obtained from the in vitro dissolution apparatus II after 120 min. However, if % difference was negative, then this is a percentage decrease, indicating that the concentration of carvedilol obtained from the in vitro transfer model was lower than that obtained from the in vitro dissolution apparatus II after 120 min.

Results

Carvedilol Assay in the Donor Phase (SGFsp)

The saturation solubility of carvedilol drug substance in SGFsp (pH 1.2) has been determined previously and found to be high of 8.3 ± 0.07 mg/mL [1]. This is due to the low pH of this medium, where carvedilol exhibited a typical weak base pH–dependent solubility profile with a high solubility at low pH and low solubility at high pH. When Dilatrend® tablet was dispersed in the donor phase SGFsp, carvedilol content was 95.9 ± 2.6% (n = 10).

In Vitro Transfer of Predissolved Carvedilol Into Simulated Intestinal Fluids

Figure 2 shows the percent dissolved of carvedilol upon transfer into acceptor phases that simulate intestinal fluid (acetate buffer, blank FaSSIF, blank FeSSIF, and SIFsp). During the first 5 min, drug was not observed in the acceptor phases, indicating a delay in drug appearance. After 5 min, the rate of carvedilol dissolved in the acceptor phases was fast and almost complete after 45 min (> 85%). When comparing the concentration profile of carvedilol in acetate buffer with those in blank FaSSIF, blank FeSSIF, and SIFsp, it was found that these profiles were similar to each other with f_2 values > 50. In addition, comparing the concentration profiles of blank FaSSIF and those of blank FeSSIF and SIFsp, concentration profiles were found to be similar, with f_2 values > 50. Furthermore, the concentration profile of blank FeSSIF and SIFsp were similar, with f_2 value > 50.

In simulated intestinal fluid of different phosphate buffer concentrations (6.25–100 mM, pH 6.8), a 5–min delay in drug appearance was observed in all acceptor phases. The rate of percent dissolved of carvedilol in phosphate buffers was fast, where > 85% were dissolved after 45 min (Fig. 3). Comparing the concentration profiles of carvedilol in phosphate buffers 6.25, 12.5, 25, 50, and 100 mM (pH 6.8), concentration profiles were found to be similar to each other, with f_2 values > 50. In water, an average carvedilol concentration of 96.2% was achieved after 45 min. Carvedilol appeared immediately in water, where no delay was observed. The concentration profile of carvedilol in water was dissimilar to those of the phosphate buffers 6.25, 12.5, 25, 50, and 100 mM (pH 6.8) with f_2 values < 50.

In phosphate buffers 100 mM (pH 7.2 and 7.8) and during the first 5 min, carvedilol was not observed in these acceptor phases, indicating a delay in drug appearance. This observation is similar to what has been found in the acceptor phases acetate buffer, blank FaSSIF, blank FeSSIF, SIFsp, and

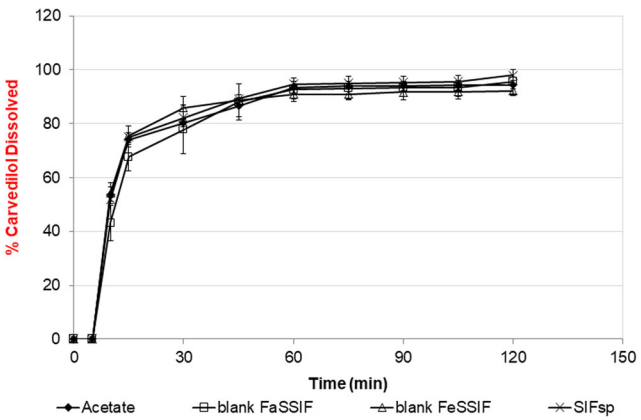

Fig. 2 Concentration profiles of carvedilol in the acceptor phases: acetate buffer, blank FeSSIF, blank FaSSIF, and SIFsp. Data are represented as the mean ± SD (n = 3)

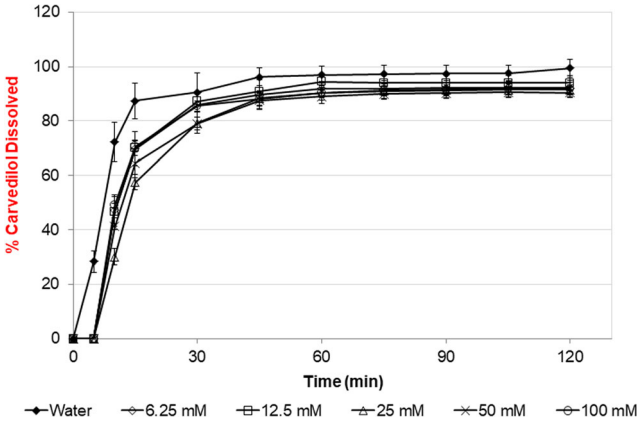

Fig. 3 Concentration profiles of carvedilol in the acceptor phases of phosphate buffers 6.25–100 mM and in double–distilled water. Data are represented as the mean ± SD ($n = 3$)

phosphate buffers 6.25–100 mM (pH 6.8). Whereas water was the only acceptor phase, where predissolved carvedilol appeared immediately after its addition to water. The percent dissolved of carvedilol was 85.4 and 85.0% in pH 7.2 and 7.8, respectively, after 45 min (Fig. 4). The concentration profile of phosphate buffer pH 7.2 was similar to that of phosphate buffer pH 7.8 with f_2 value > 50.

Table 2 illustrates the f_2 values used to compare the concentration profiles of Dilatrend® tablets upon their transfer into all tested acceptor phases. In addition, the average percent dissolved of carvedilol in all tested acceptor phases after 120 min is summarized in Table 3.

pH Profiles of the Acceptor Phases

Figure 5a presents the pH profiles of the acceptor phases: acetate buffer, blank FeSSIF, blank FaSSIF, and SIFsp upon addition of SGFsp containing predissolved carvedilol. In acetate buffer, the pH was decreased from 4.53 to 4.01, with a maximum reduction of 0.52 pH units from the initial pH

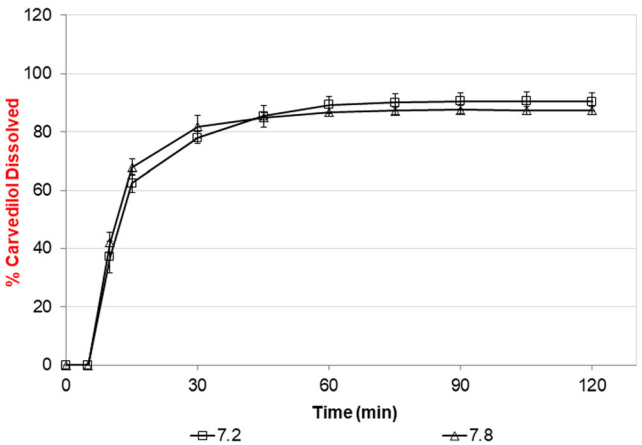

Fig. 4 Concentration profiles of dissolved carvedilol in the acceptor phases: phosphate buffers 100 mM of pH 7.2 and pH 7.8. Data are represented as the mean ± SD ($n = 3$)

value. In blank FeSSIF, blank FaSSIF, and SIFsp, a decrease in pH from 5.04 to 4.82, 6.47 to 5.45, and 6.76 to 6.40, respectively, was observed. Comparing these pH changes, blank FaSSIF showed the highest decrease in pH of 1.02 pH units from its initial pH value, throughout the transfer experiments.

To study the effect of the buffer capacity of the acceptor phase on pH decrease upon the transfer of carvedilol predissolved in SGFsp, phosphate buffers of different concentrations (6.25–100 mM, pH 6.8) were used as acceptor phases. Results showed that as the concentration of phosphate buffer increased, the pH deviation decreased in the order 100 mM (0.18 pH units), 50 mM (0.32 pH units), 25 mM (0.74 pH units), 12.5 mM (3.97 pH units), and 6.25 mM (4.37 pH units) (Fig. 5b). In phosphate buffer 100 mM, the buffer capacity was high enough (0.047 M/ΔpH) to maintain the pH of the acceptor phase during the transfer experiment. On the other hand, the buffer capacity of phosphate buffer 6.25 mM was very low (0.003 M/ΔpH), resulting in highest pH reduction. Water which was used as a reference for the acceptor phases showed a pH reduction of 3.79. Because water lacks buffering capacity [22], a high pH reduction was reported upon the addition of the donor phase.

Figure 5c illustrates the pH profiles of the acceptor phases: phosphate buffers (100 mM, pH 7.2 and 7.8) upon addition of SGFsp containing predissolved carvedilol. Due to the high concentration of phosphate buffers pH 7.2 and 7.8 (100 mM) and as a result high buffering capacity of 0.058 and 0.037 M/ΔpH, respectively, a slight pH reduction of 0.24 and 0.44 pH units was observed in pH 7.2 and 7.8, respectively.

Discussion

In our previous study [1], the saturated solubility of carvedilol drug substance and dissolution behavior of carvedilol from Dilatrend® IR tablets (25 mg carvedilol) have been determined in media that simulate gastric fluid of SGFsp and intestinal fluids of acetate buffer, SIFsp, blank FaSSIF, blank FeSSIF, phosphate buffers (6.25–100 mM, pH 6.8), and phosphate buffers (100 mM, pH 7.2 and 7.8). These media cover the physiological pH and buffer capacity range of the gastric and intestinal fluids. Results showed that carvedilol exhibited a pH–dependent solubility profile between pH 1.2 and 7.8, where carvedilol solubility was high at low pH and low at high pH [1]. Whereas, the solubility of carvedilol increased with increasing the buffer capacity of the medium. In addition, dissolution testing using USP apparatus II (paddle, 50 rpm) with dissolution volume of 900 mL revealed that the rate of carvedilol release was fast and complete in media that simulate gastric fluid with low pH. However, the rate of carvedilol release was slow and incomplete in media that simulate intestinal fluid with high pH. It is noteworthy that the saturated solubility

Table 2 The similarity factor (f_2) [20] of the concentration profiles of percent dissolved of carvedilol from Dilatrend® tablets obtained using the in vitro transfer model

Reference acceptor phase	Test acceptor phase	f_2 value
Acetate buffer	Blank FaSSIF	63.7
Acetate buffer	Blank FeSSIF	78.0
Acetate buffer	SIFsp	86.3
Blank FaSSIF	Blank FeSSIF	59.1
Blank FaSSIF	SIFsp	64.7
Blank FeSSIF	SIFsp	83.6
Water	Phosphate buffer 6.25 mM	36.3
Water	Phosphate buffer 12.5 mM	36.3
Water	Phosphate buffer 25 mM	30.4
Water	Phosphate buffer 50 mM	34.5
Water	Phosphate buffer 100 mM	38.6
Phosphate buffer 6.25 mM	Phosphate buffer 12.5 mM	96.8
Phosphate buffer 6.25 mM	Phosphate buffer 25 mM	52.5
Phosphate buffer 6.25 mM	Phosphate buffer 50 mM	67.1
Phosphate buffer 6.25 mM	Phosphate buffer 100 mM	91.3
Phosphate buffer 12.5 mM	Phosphate buffer 25 mM	51.8
Phosphate buffer 12.5 mM	Phosphate buffer 50 mM	65.2
Phosphate buffer 12.5 mM	Phosphate buffer 100 mM	85.1
Phosphate buffer 25 mM	Phosphate buffer 50 mM	63.3
Phosphate buffer 25 mM	Phosphate buffer 100 mM	50.4
Phosphate buffer 50 mM	Phosphate buffer 100 mM	65.0
Phosphate buffer 100 mM, pH 6.8	Phosphate buffer 100 mM, pH 7.2	59.8
Phosphate buffer 100 mM, pH 6.8	Phosphate buffer 100 mM, pH 7.8	71.1
Phosphate buffer 100 mM, pH 7.2	Phosphate buffer 100 mM, pH 7.8	73.2

and dissolution studies were conducted under constant pH without considering the pH gradient in the GI fluid upon transition from the stomach to the intestine as present in vivo.

Therefore, carvedilol is a weak base (pK_a 7.8) which exhibited high solubility and fast dissolution in the acidic environment of the stomach due to its high ionization, and low solubility and slow dissolution in the higher pH environment of the intestine due to the high unionized fraction [9, 23]. Thus, it is expected that as carvedilol moves down the GI tract, pH rises and as a result its solubility and dissolution rate decrease and it may precipitate out [24, 25].

The in vitro transfer model was validated. For instance, Dilatrend® tablet was predissolved in the donor phase SGFsp for a constant time (30 min) and at a constant stirring rate in all transfer experiments. The transfer rate (2 mL/min) of the syringe pump was validated before starting the transfer

Table 3 $C_{Transfer}$, $C_{Dissolution}$, and percent difference between $C_{Transfer}$ and $C_{Dissolution}$ of carvedilol from Dilatrend® tablets in all tested media after 120 min

Media	$C_{Transfer}$ (%)	$C_{Dissolution}$ (%)	Percent difference
Acetate	94.5	98.2	− 3.9
Blank FaSSIF	95.6	77.0	+ 19.5
Blank FeSSIF	92.2	85.0	+ 7.8
SIFsp	98.0	62.3	+ 36.4
Water	99.4	27.6	+ 72.2
Phosphate buffer 6.25 mM	92.2	52.9	+ 42.6
Phosphate buffer 12.5 mM	94.0	73.1	+ 22.2
Phosphate buffer 25 mM	91.5	68.4	+ 25.2
Phosphate buffer 50 mM	90.4	65.2	+ 27.9
Phosphate buffer 100 mM	91.7	64.4	+ 29.8
Phosphate buffer pH 7.2	90.3	37.9	+ 58.0
Phosphate buffer pH 7.8	87.5	14.1	+ 83.9

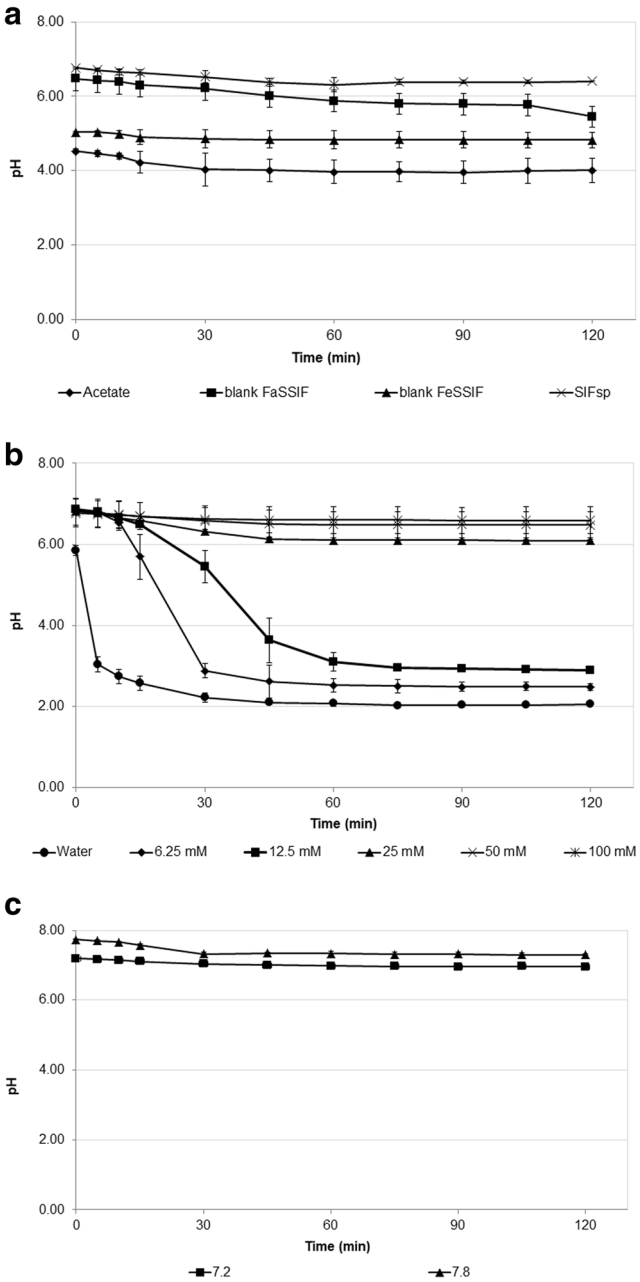

Fig. 5 **a** pH profiles upon addition of the predissolved carvedilol in SGFsp into the acceptor phases: acetate buffer, blank FeSSIF, blank FaSSIF, and SIFsp. Data are represented as the mean ± SD ($n = 3$). **b** pH profiles upon addition of the predissolved carvedilol in SGFsp into the acceptor phases: phosphate buffers 6.25–100 mM and in double-distilled water. Data are represented as the mean ± SD ($n = 3$). **c** pH profiles upon addition of the predissolved carvedilol in SGFsp into the acceptor phases: phosphate buffers 100 mM of pH 7.2 and 7.8. Data are represented as the mean ± SD ($n = 3$)

experiments to ensure a constant transfer rate of the donor phase to the acceptor phase during experiments. The initial concentration of carvedilol in the SGFsp was determined for many replicates with low standard deviation (95.9 ± 2.6%, $n = 10$). The temperature of the donor phase containing Dilatrend® table in 100–mL beaker was maintained at 37 ± 0.5 °C by placing the beaker on a hot plate adjusted at 37°C. In addition, the temperature of the acceptor phase was set at 37 ± 0.5 °C in the dissolution apparatus. SGFsp containing predissolved carvedilol was continuously stirred inside the syringe pump during the transfer experiments to prevent undissolved particles of tablet excipients from settling inside the syringe. Furthermore, the standard deviation of the percent dissolved of carvedilol and pH of the acceptor phase was less than 5.0% and 0.5 pH units, respectively, for all transfer experiments which were performed in triplicate. Lastly and for comparison purposes, volume of the donor and acceptor phases (90 and 810 mL, respectively) used in the in vitro transfer model were chosen to achieve a total volume of 900 mL and match that of the dissolution medium used in dissolution apparatus II.

Although Kostewicz et al. initially used the in vitro transfer model to study the precipitation and dissolution kinetic of plain weakly basic drug powders in the small intestine [6, 7], many research groups used this model to investigate the transfer behavior of weakly basic drugs in their solid oral drug products (i.e., tablets). For instance, Tsume et al. have used solid tablets to study the precipitation behavior of the weakly basic drugs pioglitazone and dasatinib in the intestine [3, 9].

The precipitation phenomenon of several weakly basic BCS class II drugs has been previously studied using the in vitro transfer model [3, 5–9] that was originally developed by Kostewicz et al. [6, 7]. It has been reported that although these drugs are subjected to precipitation upon entry into the small intestine, pharmacokinetic studies have shown that some drugs may exhibit high oral bioavailability (> 80%) [8, 9].

In this study, the in vitro transfer model (Fig. 1) was used to study the percent dissolved of carvedilol upon entry into the intestine. The simulated gastric fluid SGFsp was used as a donor phase, whereas simulated intestinal fluids (SIFsp, FaSSIF, FeSSIF, and phosphate buffers) of different pH values and buffer capacities were used as acceptor phases (Table 1). SGFsp, and SIFsp, FaSSIF, and FeSSIF have been used as donor and acceptor phases, respectively, in many previous studies [3, 5–10, 26, 27].

Concentration profiles of all acceptor phases exhibited a 5–min delay in the appearance of the drug. Water was the only acceptor phase where no delay was observed. This might be due to the variation in the chemical composition and physicochemical properties (pH, buffer capacity, and ionic strength) between water and other acceptor phases, where water has zero buffer capacity and zero ionic strength [19]. For example, although the concentration profiles of carvedilol in phosphate buffers 6.25–100 mM (pH 6.8) were similar to each other with $f_2 > 50$. The concentration profiles of these buffers were dissimilar to that of water with $f_2 < 50$ (Table 2). This is because the chemical composition and physicochemical properties of the acceptor phase have an impact on the dissolution rate of poorly soluble drugs like carvedilol [1]. Water has zero buffer

capacity, whereas phosphate buffers 6.25–100 mM (pH 6.8) have a buffer capacity range of 0.003–0.047 M/ΔpH. This variation resulted in dissimilar concentration profiles.

Carvedilol exhibited a high percent dissolved when it is transferred from the donor phase and emptied into acceptor phases, resulted in no precipitation within 120 min. Average carvedilol concentration ($C_{Transfer}$) achieved after 120 min in all tested acceptor phases was between 87.5 and 99.4% (Table 3). This was consistent with Okumu et al. who used a transfer model to investigate the solubility of the weak base BCS class II drug etoricoxib when entering the small intestine [8]. Okumu et al. found that no precipitation was observed for etoricoxib due to the high solubility of this drug in simulated intestinal fluids (FaSSIF and SIF) after its complete dissolution in the simulated gastric fluid (SGF) [8].

The concentration of carvedilol in all tested acceptor phases obtained using the in vitro transfer model ($C_{Transfer}$) after 120 min was compared to that reported previously [1] using dissolution apparatus II in corresponding dissolution media ($C_{Dissolution}$). The comparison between $C_{Transfer}$ and $C_{Dissolution}$ after 120 min is illustrated in Table 3. It is clear that $C_{Transfer}$ was higher than $C_{Dissolution}$ in all tested media with percent increase between 7.8 and 83.9%, except in acetate buffer. The high $C_{Transfer}$ values might be attributed to the high solubility of carvedilol in the donor phase SGFsp, where drug remained solubilized upon addition into the acceptor phases. The high $C_{Dissolution}$ (98.2%) in acetate buffer was due to the formation of the water–soluble acetate salt of carvedilol as reported by Loftsson et al. [28]. This can be explained by the high solubility of the carvedilol acetate salt formed from the protonated carvedilol base and the anionic form of the acetic acid. T. Incecayir has reported that the solubility of carvedilol in acetate buffer (pH 4.5) was 75–fold higher than that in hydrochloric acid of very low pH (pH 1.2) [29]. In addition, Hamed et al. have shown that the solubility of carvedilol was the highest in acetate buffer (pH 4.5) when compared to its solubility in media that simulate the gastric and intestinal fluids and cover the physiological pH range of the GI fluid from 1.2 to 7.8 [1]. Therefore, a slight percent decrease of 3.9 was found between $C_{Transfer}$ and $C_{Dissolution}$ (94.5 vs 98.2%, respectively). The $C_{Transfer}$ and $C_{Dissolution}$ profiles are illustrated in Figs. 1S–12S, Supplement Information.

The discrepancy between $C_{Transfer}$ and $C_{Dissolution}$ profiles in corresponding media suggests that the in vitro dissolution testing using apparatus II for the orally administered solid dosage forms of weakly basic drugs might not be sufficient to predict their in vivo behavior [9]. Therefore, it is suggested that the dynamic in vitro transfer model might predict the in vivo behavior of carvedilol more accurately than the in vitro dissolution testing using apparatus II [9]. This is because this model simulates the transfer of drug from the stomach into the intestine and the pH gradient of the GI fluid [3, 5–10, 26].

Therefore, to design a suitable in vitro dissolution method for carvedilol, data revealed that the composition and physicochemical of the dissolution media (pH and buffer capacity) play an important role in determining the dissolution rate of carvedilol when using dissolution apparatus II. In these studies, complete dissolution was only found in acetate buffer; however, no complete dissolution was found in water and dissolution media that simulate the intestinal fluid. Whereas, in the in vitro transfer model, complete dissolution was found in all acceptor phases, indicating that the composition and physicochemical properties of the acceptor phases have no influence on the dissolution rate of carvedilol. This is because carvedilol exhibited high solubility in the donor phase SGFSp, where drug remained solubilized upon addition into the acceptor phases.

During the transfer experiments, the pH of the acceptor phases in the dissolution vessel was monitored at time intervals corresponded to those of the transfer experiments. Monitoring the pH of the acceptor phases was necessary to investigate the effect of the addition of donor phase containing predissolved carvedilol on lowering the pH of the acceptor phase. This is because lowering the pH of the acceptor phase may enhance the solubility of carvedilol and hence its dissolution [9].

The pH of the acceptor phase depends on the volume of donor phase transferred into the dissolution vessel and on the buffering capacity of the acceptor phase [6]. Since the transferred volume was constant in all acceptor phases during the transfer experiments, the buffering capacity is the only factor that determines the pH of the acceptor phase. In acceptor phases of low buffering capacities (0.000–0.006 M/ΔpH), the pH was lowered to ≥ 4 pH units as observed in water, and 6.25 and 12.5 mM phosphate buffers. However, in acceptor phases of high buffering capacity (0.034–0.130 M/ΔpH), pH was deceased to ≤ 1 pH unit as observed in 25–100 mM phosphate buffers. Therefore, pH reduction was high (≥ 4 pH units) in buffers of low phosphate concentration with low buffering capacity. Whereas, pH reduction of ≤ 1 pH unit was found in buffers of high phosphate concentration, where the buffering capacity of the acceptor phase was high enough to maintain the pH, independent of how much of the weakly basic drug carvedilol dissolved in the acceptor phase [30].

Conclusions

The present study demonstrated the importance of the in vitro transfer model to investigate the transfer behaviour of the weakly basic model drug carvedilol from the stomach into the intestine. The use of acceptor phases with pH and buffer capacity that simulate the physiological range of the intestinal fluid results in high carvedilol concentration after 120 min, indicating no precipitation. This in vitro transfer model highlights the need to consider the transfer of weakly basic drugs from the stomach into the intestine for better in vivo prediction

of bioavailability. Given the concentration profiles determined during the in vitro dissolution testing using USP apparatus II (paddles), the concentration profiles determined during the transfer experiments may provide a more physiologically–relevant in vitro model.

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