Comparative Study of Two *In Vitro* Methods for Assessing Drug Absorption: Sartorius SM 16750 Apparatus *Versus* Everted Gut Sac

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ABSTRACT – Purpose. Oral drug administration remains the most common and most convenient way used in clinical therapy. The availability of a simple, rapid, economic and reproducible *in vitro* method to assess the rate, extent and mechanism of intestinal drug absorption is a very helpful tool. The purpose of this study was to compare the performance of Sartorius SM 16750 Absorption Simulator apparatus to Everted Gut Sac (EGS) technique in terms of predicting drug permeability. **Methods.** Permeation studies across these two *in vitro* models were performed with six drugs selected across the Biopharmaceutics Classification System (BCS) categories: tramadol (class I of BCS), doxycycline (class I of BCS), diclofenac (class II of BCS), clopidogrel (class II of BCS), metformin (class III of BCS) and chlorothiazide (class IV of BCS). **Results.** Apparent permeability coefficient (P_{app}) and diffusion profiles obtained with EGS and Sartorius SM 16750 apparatus were similar for diclofenac and metformin, whereas, we noticed significant differences ($p \leq 0.05$), for tramadol, doxycycline, clopidogrel and chlorothiazide. **Conclusion.** Compared to Everted Gut Sac model, Sartorius SM 16750 absorption simulator apparatus seems to have limited application for the assessment of intestinal drug absorption since it does not take into consideration the involvement of others processes than the passive transcellular pathway as mechanism of drug absorption.

INTRODUCTION

Oral drug administration remains the most common and the most convenient way used in clinical therapy. Solid oral drugs represent approximately 80% of the marketed dosage forms **(1)**. Compared to other oral dosage forms, they offer many advantages: they are stable, they have a smaller bulk, their dosing is accurate, and their manufacturing is relatively easy **(2)**. Generally, drugs administered orally are absorbed in the upper small intestine. The absorption capacity declines down the intestine and decreases markedly after the ileum, resulting in a small absorption window **(3)**. Consequently, intestinal absorption is one of the key factors for the bioavailability of oral dosage forms. It is a complex transfer process that takes place across the epithelial mucosa and that is influenced by a variety of factors including the physicochemical properties of the drug (e.g. molecular weight and/or size, degree of ionization pKa, solubility, oil/water partition coefficient, stereochemistry, charge distribution, chemical stability), the physiological properties of the gastrointestinal (GI) tract (e.g. gastric emptying, GI motility, area available for absorption, pH values in the various regions of the GI tract, blood flow), and the formulation aspects (e.g. particle size, crystal form and polymorphism, dissolution rate, absorption enhancers, tablets, capsules, solutions, etc.) **(4,5,6)**. There are 3 main mechanisms involved in the transfer of drug compounds across the intestinal epithelial mucosa: 1) The passive transcellular diffusion through the cell membrane which is the predominant route for hydrophobic drugs and which follows the concentration gradient meaning that the absorption rate is proportional to the drug concentration; 2) The passive paracellular transport through the tight junctions between the enterocytes which occurs with small hydrophilic compounds; 3) The transcellular transport which uses transporters that may function either passively or actively. **(5,7-9)**. Several methods have been used to assess drug absorption; they include physicochemical models, *in silico* computational models, *in situ* models, *in vitro* models and *in vivo* animal models **(9, 10)**. Ideally, the models used for the evaluation of intestinal drug absorption and permeability should be reliable, inexpensive, fast and highly predictive **(11, 12)**.

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The purpose of the present study is to compare the performances of the Sartorius SM 16750 Absorption Simulator with the Everted Gut Sac (EGS) technique in the evaluation of drug permeability. The EGS technique is a valuable *in vitro* procedure that can be used to assess the permeability characteristics of various drug classes **(8, 13)**. The bio-mimetic artificial membranes, such as those used with the Sartorius SM 16750 Simulator, represent an interesting alternative *in vitro* method to assess drug absorption properties. They are rapid, economic, reproducible and easy to carry out **(14)**. For the evaluation of these two *in vitro* models, 6 compounds were chosen. These are: tramadol, doxycycline, diclofenac, clopidogrel, metformin and chlorothiazide. The selected drugs belong to different classes of the Biopharmaceutic Classification System (BCS) (Table 1). Tramadol, a centrally acting opioid analgesic **(15)** and doxycycline, an antibiotic **(16)**, belong to class I of the BCS **(17, 18)**. Diclofenac, a nonsteroidal anti-inflammatory drug **(19)** and clopidogrel, a thienopyridine antiplatelet agent **(20)**, belong to class II of the BCS **(21)**. Metformin, a biguanide antidiabetic agent **(22)** and chlorothiazide, a thiazide diuretic and antihypertensive agent **(23)**, belong to class III and IV of the BCS respectively **(24, 25)**. According to this classification system **(26)**, tramadol and doxycycline are highly soluble and highly permeable drugs, diclofenac and clopidogrel are poorly soluble but highly permeable substances, metformin is a highly soluble but poorly permeable compound and chlorothiazide is a poorly soluble and poorly permeable drug.

Table 1. The Biopharmaceutic Classification System (BCS): A guiding tool for predicting the intestinal drug absorption according to solubility and permeability in aqueous medium.

Class I	**Class II**
High solubility	Low solubility
High permeability	High permeability
Class III	**Class IV**
High solubility	Low solubility
Low permeability	Low permeability

MATERIALS AND METHODS

Drugs and Chemical Reagents

Clopidogrel hydrogenosulfate and tramadol chlorhydrate were kindly provided by Medis Laboratories (Nabeul, Tunisia); metformin chlorhydrate and chlorothiazide were provided by Siphat Laboratories (Tunis, Tunisia), doxycycline was provided by the National Drug Control Laboratory (Tunis, Tunisia) and diclofenac sodium was provided by Unimed (Sousse, Tunisia). Lauric alcohol, caprylic acid, monopotassium phosphate and disodic phosphate were purchased from Sigma Aldrich Laborchemikalien GmbH (Germany). NaOH, HCl, citric acid, NaCl, KCl, HCO_3Na, and $CaCl_2$ were acquired from Chemi-Pharma Laboratories (Tunis,Tunisia). All chemicals and reagents used were of analytical grade. All drug solutions were freshly prepared before use.

Buffer Solutions

Buffer solutions were prepared according to the European Pharmacopoeia as follows:

Buffer Solution Phosphate pH 6.8

77.3 ml of disodic phosphate R (71.5 g/l) were mixed with 22.7 ml of citric acid solution R (21 g/l). The pH of the solution was adjusted using a citric acid solution R (21 g/l) when necessary.

Buffer Solution Phosphate pH 7.4

250 ml of potassium phosphate were added to 393.4 ml of NaOH 0.1 M.

Diffusion Assays

Diffusion assays were performed using a bio-mimetic artificial membrane and the EGS technique.

The Sartorius Absorption Simulator Model

Figure 1 shows a schematic representation of the Sartorius SM 16750 Absorption Simulator (Sartorius Membranfilter GmbH, Germany) **(27)**. This apparatus consists of a donor compartment **(A)** filled with a pH 6.8 buffer solution and a receiver compartment **(B)** filled with a pH 7.4 phosphate buffer solution. Both media were maintained at 37 ± 0.5 °C and circulated continuously on the two sides of the diffusion cell thanks to a peristaltic pump at a rate of 9.5 ml/min. The diffusion cell contains a nitrocellulose artificial membrane (OSMONICS Micronsep® model, Bioblock, France, diameter = 90 mm and pore size =0.45 µm). This membrane was impregnated, by immersion for 1 hour, with a lipidic mixture consisting of caprylic acid and lauric alcohol (50:50 w/w). The excess of lipidic mixture was eliminated with absorbing paper. The percent of lipidic mixture absorption, calculated by weighing the membrane before and after

impregnation, ranged between 90 and 110%. The drug tested was added to the donor compartment, samples were withdrawn from the receptor compartment at 15, 30, 45, 60, 75, 90, 105, and 120 minutes, assayed spectrophotometrically and immediately put back in the medium. The experiments were conducted 6 times for each drug. Drug absorption was expressed in percentage. No interferences were observed with the components of the membrane during the diffusion assays.

The EGS Technique

The experiments were carried out on male Wistar rats provided by Central Pharmacy of Tunis (Tunis, Tunisia). The animals were treated according to the Canadian Council on Animal Care guidelines (1984). They were kept in an animal house at standard environmental conditions. The animals were fasted for 24 hours prior to the experiment while having free access to water. Their weight ranged between 200 and 250 g. The rats were anesthetized with ether before the experiment; the jejunum was isolated, and the animals were then sacrificed by cervical dislocation. The EGS were carefully prepared from rat jejunum as follows **(28)**: the segment was quickly excised, stripped of adhering tissue and flushed several times with a Ringer solution (9‰) containing 0.154 mM/l NaCl; 0.0034 mM/l KCl; 0.0024 mM/l; HCO_3Na and 0.0021 mM/l $CaCl_2$. The intestine was everted and immediately placed in an oxygenated medium (O_2/CO_2, 95%:5%) at 37 ± 0.5 °C. Then, it was cut into small sacs of 5cm in length which were blotted and weighed; the average weight of the sacs was 0.3498 ± 0.0264 g. For the assays, the sacs were filled with Ringer solution (9‰) (pH 7.4), hanged in a test tube containing 15 ml of the drug solution (pH 6.8) and incubated at 37 ± 0.5 °C in an oscillating water bath (OLS 200, Grant instruments, Cambridge, UK) at 60 cycles/min. Throughout the assay, the sacs were constantly oxygenated (95% O_2 - 5% CO_2). Samples were withdrawn at the same times used with the bio-mimetic artificial membrane technique and assayed spectrophotometrically. The experiments were conducted 6 times for each drug. Drug absorption was expressed in percentage.

Calculation of the Apparent Permeability Coefficients

Permeability coefficients (P_{app}) obtained with the Sartorius SM 16750 Absorption Simulator and the EGS method were calculated according to Eq. 1 **(29)**:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{AC_0} \qquad (1)$$

Where P_{app} (cm/s) is the apparent permeability coefficient, dQ/dt (µg/s) the amount of drug permeated per unit of time calculated from the regression line of time points of sampling, A (cm^2) the surface area available for permeation, and C_0 (µg/ml) the initial drug concentration in the donor compartment.

Percentage of Drug Recovery (R%) and Drug Retention (Ad%)

At the end of each experiment, the residual concentration of drug remaining in the donor medium (Sartorius SM 16750 model) or in the external medium (EGS technique) was assayed.

The percentage of drug retained (Ad%) either by the artificial lipoid barrier or by the EGS was determined by a mass balance calculation according to Eq. 2:

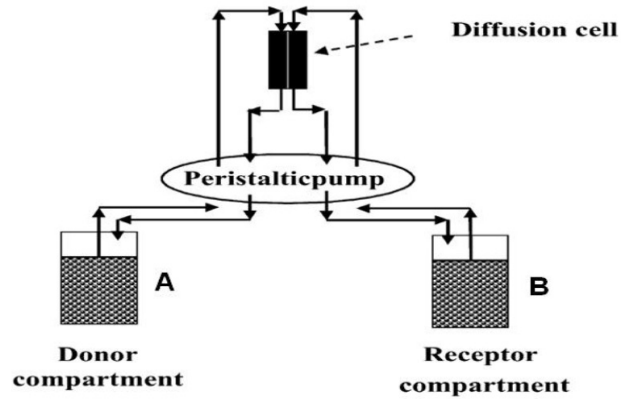

Figure 1. Schematic representation of the Sartorius SM 16750 Simulator Model

$$Ad\% = 100 - R\% = \left(1 - \frac{C_{r,end} \times V_r + C_{d,end} \times V_d}{C_0 \times V_r}\right) \times 100 \qquad (2)$$

where $C_{r,end}$ and $C_{d,end}$ are the drug concentrations measured at the end of the experiment in the receiver and donor compartments respectively, $C_{d,0}$ the initial concentration in the donor compartment, and V_r and V_d the volumes of the receptor and donor compartments respectively.

DRUG ANALYSIS

A spectrophotometric method using a UV-Visible Thermo Scientific EVO 60 spectrophotometer was developed to quantify metformin (λ_{max} = 230 nm), clopidogrel (λ_{max} = 240 nm), tramadol (λ_{max} = 271 nm), doxycycline (λ_{max} = 274 nm), diclofenac (λ_{max} = 276 nm), and chlorothiazide (λ_{max} = 294 nm).

Correlation Study between the two *in vitro* Models

The correlation study between the Sartorius SM 16750 Absorption Simulator method and the EGS model was carried out by the means of a mathematical approach using two parameters: the difference factor (f_1) and the similarity factor (f_2). This model is usually used to compare dissolution profiles **(30)**, but it can also be used to compare diffusion kinetics **(31)**.

Calculation of the Difference Factor (f_1) (32)

This factor calculates the percent difference between two curves at each time point and is a measurement of the relative error between the two curves. It's determined according to Eq. 3:

$$f_1 = \left\{ \left[\sum_{t=1}^{n} \left| R_t - T_t \right| \right] \Big/ \left[\sum_{t=1}^{n} R_t \right] \right\} \cdot 100 \qquad (3)$$

n: number of time points.
R_t: diffusion value of the reference batch at time t
T_t: diffusion value of the test batch at time t

Calculation of the Similarity Factor (f_2) (32):

This factor is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in percent diffusion between the two curves. It's calculated using Eq. 4:

$$f_2 = 50 \cdot \log\left\{ \left[1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \cdot 100 \right\} \qquad (4)$$

The curves are considered similar when f_1 value is close to zero and f_2 value is close to 100. Generally f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100) ensure equivalence of the two curves.

STATISTICAL ANALYSIS

Statistical analysis was computed with SPSS® Windows (version 14.0). The results were represented as mean ± Standard Deviation (± SD) for the six experiments. For each drug compound, the apparent permeability average values (P_{app}) obtained for the two *in vitro* models were compared using a one way analysis of variance (ANOVA) test. Student's t-test was used to compare the two data sets of drug diffusion. The difference observed was considered significant at $p \leq 0.05$.

RESULTS

Comparison of Drug Permeability Coefficients

The apparent permeability coefficients P_{app} (cm/s) of tramadol, doxycycline, diclofenac, clopidogrel, metformin and chlorothiazide obtained with the EGS method and the Sartorius SM 16750 Absorption Simulator Apparatus are shown in **table 2**. Data are expressed as mean ± S.D. The EGS permeability coefficient of tramadol was 11,843 ± 1,985 cm/s at pH 6.8, which is significantly lower than the coefficient obtained with the Sartorius SM 16750 model (28,022 ±

4,793 cm/s) ($p \leq 0.05$). Similar results were observed for doxycycline, clopidogrel and chlorothiazide with Sartorius SM 16750 permeability values higher than the EGS technique coefficients ($p \leq 0.05$). The EGS permeability coefficients were 14.351 ± 2.012 cm/s, 2.589 ± 0.426 cm/s and 0.451 ± 0.085 cm/s, whereas the Sartorius SM 16750 permeability values were 32,335 ± 4,344 cm/s, 7,693 ± 1,331 cm/s and 0,814 ± 0,077 cm/s for doxycycline, clopidogrel and chlorothiazide respectively. On the other hand, diclofenac permeability coefficients obtained by EGS and Sartorius SM 16750 absorption models did not present a significant difference ($p > 0.05$). Similar results were noticed with metformin ($p > 0.05$). The EGS permeability coefficients were 17.044 ± 2.914 cm/s and 0.737 ± 0.149 cm/s and the Sartorius SM 16750 permeability values were 19.667 ± 2.879 cm/s and 0.613 ± 0.104 cm/s for diclofenac and metformin respectively.

Percentage of Drug Recovery (R %)

Table 3 shows the percentages of drug recovery and drug retention of tramadol, doxycycline, diclofenac, clopidogrel, metformin and chlorothiazide with the Sartorius SM 16750 apparatus and the EGS model. Results are expressed as mean ± S.D. With the first model, the percentages of drug retained were 1.79 ± 1.05 %, 4.00 ± 1.36%, 2.44 ± 1.16%, 1.25 ± 1.32%, 2.79 ± 1.47% and 2.69 ± 1.47%, whereas, with the EGS technique, the percentages were 5.82 ± 1.89%, 8.08 ± 1.74%, 8.04 ± 1.70%, 5.45 ± 1.58%, 7.85 ± 1.78% and 7.95 ± 1.81% for tramadol, doxycycline, diclofenac, clopidogrel, metformin and chlorothiazide respectively. For the six drug assayed, the amount of the drug absorbed on the artificial membrane was less important than the amount retained on the EGS ($p \leq 0.05$). This may be explained by the fact that in the EGS model, drugs must cross the whole intestinal wall with a risk of accumulation in the muscular layer (9). In both cases, the percentages of drug retention were limited (below 10%).

Table 2. Apparent permeability coefficients P_{app} (cm/s) of tramadol, doxycycline, diclofenac, clopidogrel, metformin and chlorothiazide determined with the EGS and the Sartorius SM 16750 techniques.

Compound	Molecular Weight (g/mol)	Solubility	Permeability	P_{app} (EGS)(1) ($x10^{-6}$ cm/s)	P_{app} (Sartorius)(2) ($x10^{-6}$ cm/s)	Ratio (2)/(1)
Tramadol	299.84	High	High	11.843 ± 1.985	28.022 ± 4.793	2.37
Doxycycline	512.90	High	High	14.351 ± 2.012	32.335 ± 4.344	2.25
Diclofenac	318.14	Low	High	17.044 ± 2.914	19.667 ± 2.879	1.15
Clopidogrel	419.90	Low	High	2.589 ± 0.426	7.693 ± 1.331	2.97
Metformin	165.63	High	Low	0.737 ± 0.149	0.613 ± 0.104	0.83
Chlorothiazide	317.71	Low	Low	0.451 ± 0.085	0.814 ± 0.077	1.81

Table 3. Percentages of drug recovery and drug retention for tramadol, doxycycline, diclofenac, clopidogrel, metformin and chlorothiazide with the EGS and the Sartorius SM 16750 models.

In vitro absorption model	Drug	Percentage of drug recovery ± S.D (%)	Percentage of drug retention ± S.D (%)
Sartorius apparatus	Tramadol	98.21 ± 1.05	1.79 ± 1.05
	Doxycycline	96.00 ± 1.36	4.00 ± 1.36
	Diclofenac	97.56 ± 1.16	2.44 ± 1.16
	Clopidogrel	98.75 ± 1.32	1.25 ± 1.32
	Metformin	97.21 ± 1.47	2.79 ± 1.47
	Chlorothiazide	97.31 ± 1.47	2.69 ± 1.47
EGS technique	Tramadol	94.18 ± 1.89	5.82 ± 1.89
	Doxycycline	91.92 ± 1.74	8.08 ± 1.74
	Diclofenac	91.96 ± 1.70	8.04 ± 1.70
	Clopidogrel	94.55 ± 1.58	5.45 ± 1.58
	Metformin	92.15 ± 1.78	7.85 ± 1.78
	Chlorothiazide	92.05 ± 1.81	7.95 ± 1.81

Correlation Study between the EGS Model and the Sartorius SM 16750 Apparatus

Figure 2 represents the absorption rates across the nitrocellulosic membrane used with the Sartorius SM 16750 Absorption Simulator (■) and the EGS (◆) of tramadol (1000µM), doxycycline (200µM), diclofenac (50µM), clopidogrel (232µM), metformin (1000µM) and chlorothiazide (1000µM). Data are expressed as mean ± S.D (n=6).

The results for the calculation of the similarity factor (f_2) and the difference factor (f_1) are reported in **table 4.** For tramadol, (f_2 = 70.11% and f_1 = 49.79%), doxycycline (f_2 = 63.79% and f_1 = 80.66%), clopidogrel (f_2 = 69.98% and f_1 = 92.90%) and chlorothiazide (f_2 = 74.99% and f_1 = 45.01%) f_2 and f_1 were higher than 50 % and 15% respectively. However, for diclofenac (f_2 = 74.96% and f_1 = 12.60%) and metformin (f_2 = 82.52% and f_1 = 14.88%), f_2 was higher than 50 % and f_1 was less than 15%.

Table 4. f_1 and f_2 values for the diffusion profiles of tramadol, doxycycline, diclofenac, clopidogrel, metformin and chlorothiazide obtained with the EGS and the Sartorius SM 16750 models.

Drug	Difference factor (f_1) (%)	Similarity factor (f_2) (%)
Tramadol	49.79	70.11
Doxycycline	80.66	63.79
Diclofenac	12.60	74.96
Clopidogrel	92.90	69.98
Metformin	14.88	82.52
Chlorothiazide	45.01	74.99

DISCUSSION

Gastro-intestinal absorption is one of the key factors involved in the bioavailability of orally administered drug compounds. A great variety of *in vitro*, *in situ* and *in vivo* methods have been developed to assess the rate, extent and mechanism of intestinal absorption **(8, 27, 33)**. Several diffusion studies have been conducted using either the Sartorius SM 16750 Apparatus with a bio-mimetic artificial membrane or the EGS model.

The Sartorius SM 16750 Absorption Simulator is an *in vitro* model which simulates passive diffusion using an artificial lipoid membrane. The use of bio-mimetic artificial membrane techniques, such as used with the Sartorius SM 16750 Absorption Simulator method, offers several advantages. These techniques allow a rapid screening of a large number of compounds; they are simple to carry out; they are not expensive, and they avoid the use of animals or organs. Since the majority of drugs are mainly absorbed through passive transfer, the Sartorius SM 16750 Apparatus provides a suitable method for a variety of drugs and offers an effective approach for the assessment of drug absorption **(34)**. Several diffusion studies were performed using this model with suitable and valuable results **(35-37)**. The Sartorius SM 16750 Absorption Simulator used to perform the diffusion experiments presented the following characteristics: a donor and a receiver compartment adequately thermostated for a constant experimental temperature (37 ± 0.5°C); a peristaltic pump which allowed a regular, uniform and continuous circulation of both donor and receiver media assuring the dynamic conditions of the assay and avoiding the formation of undesired unstirred water layers at the level of the diffusion membrane; and an artificial lipoid membrane which was the essential part of this apparatus and which had comparable permeability with the natural gastro-intestinal barrier for passively transported substances. The nitrocellulose membrane was impregnated with a mixture of caprylic acid and lauryl alcohol (50%-50%) to mimic the intestinal barrier **(34)**. Cellulose nitrate provided rigidity to the membrane whereas caprylic acid provided the hydrophilic character that exists in biological membranes, and lauryl alcohol was used to give the membrane a lipophilic character in order to simulate the intestinal barrier. It is important to notice that the variation in the composition of the lipidic phase used to soak the artificial membrane may affect significantly drug permeability **(27)**. In our study, the lipidic phase proportions allowed to simulate the intestinal barrier and to give suitable results **(34)**. The 0.45µm membrane pores were filled with the lipidic phase, so that lipid-soluble drugs could dissolve in the membrane, and then diffuse thanks to a concentration gradient across the lipidic pores whose sizes were significantly greater than the molecules.

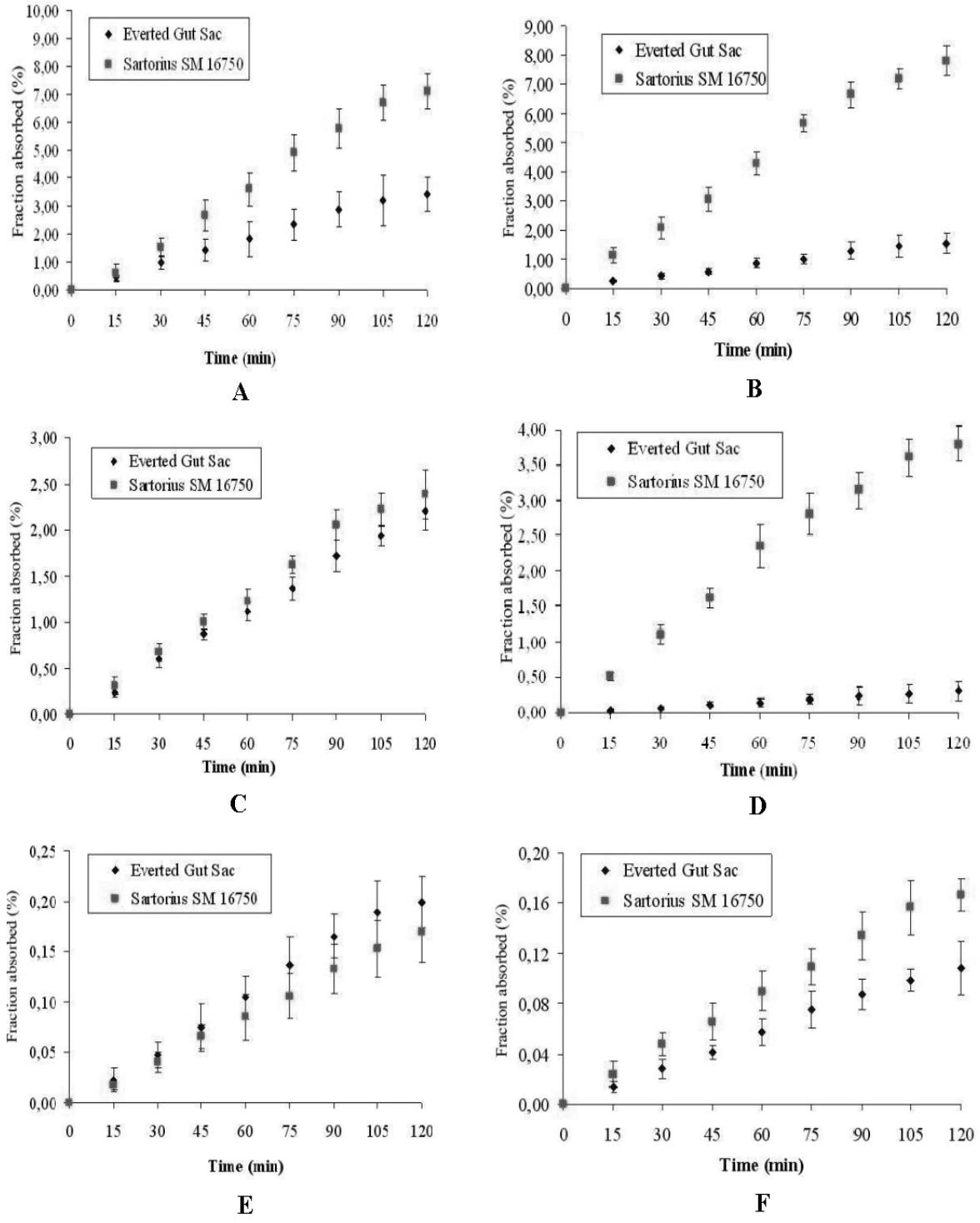

Figure 2. Absorption rates of drug compounds across the Sartorius SM 16750 (■) and the EGS (◆) models: (A) Tramadol (1mM), (B) Doxycycline (200µM), (C) Diclofenac (50µM), (D) Clopidogrel (232 µM), (E) Metformin (1mM) and (F) Chlorothiazide (1mM). Data are expressed as mean ± S.D (n=6).

The second *in vitro* model tested was the EGS of the rat small intestine. This technique has been used first to study the transport of macromolecules **(38)**. Afterward, it was improved

and used mainly to quantify the paracellular transport of hydrophilic molecules and to estimate the effects of potential enhancers on their absorption **(39)**. This model was also used to study drug transport across the intestine **(40)**, to determine kinetic parameters with high reliability and reproducibility **(8, 39)**, to study the mechanism of drug absorption, the uptake of liposomes and the effect of pharmaceutical excipients on drug absorption **(9, 41)**. The EGS method is considered as a valuable *in vitro* procedure for predicting the permeability characteristics of various drug classes **(13)** and studying the effect of efflux transporters, such as P-glycoprotein (P-gp), on xenobiotic transport through the intestinal barrier **(42)**. EGS is a relatively fast and inexpensive method **(9)**. During permeation experiments carried out with the EGS technique, the use of Ringer solution (9‰) associated with permanent oxygenation of the medium (O_2/CO_2, 95%:5%) ensured tissue viability for up to 120 minutes. Under these conditions, histological studies have shown the validity of the everted intestinal segments after 2 hours of experiment **(43)**.

Compared to the EGS model, the artificial membrane methods have some drawbacks. They do not take into consideration the potential role of enzymes, carrier-mediation (influx and efflux transporters), and pores which all reflect the active and the paracellular route in intestinal drug absorption **(34, 44, 45)**. In the EGS, all cell types and mucous layers are maintained **(9)**; therefore, influx transporters such as oligopeptide transporters and bile acid transporters **(46)**, efflux transporters like P-glycoprotein and multidrug resistance-associated proteins (MDR1 and MDR2) and the paracellular pathway still exist **(47-49)**.

This study investigated the correlation between the Sartorius SM 16750 Absorption Simulator method and the EGS technique in assessing the *in vitro* permeability of six drug compounds. The drugs, which were selected across the BCS, were: tramadol and doxycycline (two highly soluble and highly permeable drugs), diclofenac and clopidogrel (two poorly soluble but highly permeable molecules), metformin (a highly soluble but poorly permeable drug), and chlorothiazide (a poorly soluble and poorly permeable compound).

By comparing the drug permeability coefficients obtained with the two *in vitro* models, it was found that the EGS apparent permeability coefficients (P_{app}) were lower than the Sartorius SM 16750 Apparatus coefficients for 4 compounds: tramadol, doxycycline, clopidogrel and chlorothiazide ($p<0.05$), whereas no significant differences in P_{app} were observed with diclofenac and metformin ($p>0.05$). The comparison of diffusion profiles obtained with the two *in vitro* models showed that, for tramadol, doxycycline, clopidogrel and chlorothiazide, diffusion curves (% of drug absorbed per unit time) were not comparables since f_1 values were higher than 15%. These results suggest passive transcellular diffusion is not the only mechanism for the passage of these molecules across the gastro-intestinal barrier and that other processes may be involved in their transport. For tramadol, the significant difference between the results can be explained by the possible involvement of uptake transporters in the intestinal transport and by the presence of transporters other than P-gP such as proton based efflux pumps implicated in limiting the transepithelial passage of this compound **(50, 51)**. Doxycycline's passage across the intestinal barrier occurs by a passive transcellular pathway mainly **(22)**; however, the transepithelial transport of the drug is decreased by P-glycoprotein efflux pump **(52, 53)**. Previous studies about clopidogrel showed that its absorption decreases by the intestinal efflux transporter P-glycoprotein **(54, 55)**. Finally, for chlorothiazide, previous studies reported that its main transport route was paracellular permeation **(22)**. Besides, chlorothiazide's absorption seemed to be decreased by a non P-glycoprotein intestinal efflux transporter **(25)**.

Diclofenac and metformin diffusion studies undertaken with the Sartorius SM 16750 Simulator apparatus and EGS method showed similar results in terms of permeability values ($p>0.05$) and diffusion profiles ($f_1<15\%$ and $f_2>50\%$ for both compounds). These results were expected for diclofenac since its passage across the intestinal epithelial cell layer occurs by passive diffusion **(56)**. For metformin, a similarity in the results found with the two *in vitro* methods was noticed although metformin's passage through the intestinal barrier occurs mainly thanks to a paracellular mechanism **(57, 58)**. Actually, metformin permeability is concentration-dependant, and the permeability tends to decrease when metformin concentration increases. In a previous study undertaken with Caco-2 cells model **(58)**, it was reported that the predicted permeability of metformin showed a decrease by approximately 70% when drug concentration increased from 0.05 to 10mM. This change was

attributed to a decrease in the paracellular permeability **(58)**.

During permeation experiments undertaken with the EGS and the Sartorius SM 16750 models, it was important to compare the percentage of drug recovery. Results showed that these percentages were high with the two models (> 90%), indicating that the amount of drug retained by the artificial membrane or the EGS was very limited. This phenomenon was reported previously **(59)**.

CONCLUSION

In conclusion, the comparison between the performances of the Sartorius SM 16750 Absorption Simulator and the Everted Gut Sac technique in terms of predicting drug permeability showed a good correlation only for diclofenac and metformin. Whereas, the Sartorius SM 16750 gave an over-estimated apparent permeability (P_{app}) for tramadol, doxycycline, clopidogrel and chlorothiazide compared to EGS model. This over-estimation can be explain by the fact that passive transcellular diffusion was not the only mechanism for the passage of these four molecules across the gastro-intestinal barrier and that other processes were involved in their transport (carrier-mediated transport and paracellular route). It could be concluded that the absorption simulator method, less invasive and easier to carry out, gives comparable results with the EGS technique only when drug passage across the intestinal barrier occurs by passive transcellular diffusion and when no influx or efflux systems are implicated in the transepithelial passage. The results of the study suggest that the Sartorius SM 16750 has limited application for the assessment of drug intestinal absorption compared to the EGS model.

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