Comparative evaluation of rivastigmine permeation from a transdermal system in the Franz cell using synthetic membranes and pig ear skin with in vivo-in vitro correlation

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In the present study, *in vitro* permeation experiments in a Franz diffusion cell were performed using different synthetic polymeric membranes and pig ear skin to evaluate a rivastigmine (RV) transdermal drug delivery system. *In vitro-in vivo* correlations (IVIVC) were examined to determine the best model membrane. *In vitro* permeation studies across different synthetic membranes and skin were performed for the Exelon® Patch (which contains RV), and the results were compared. Deconvolution of bioavailability data using the Wagner–Nelson method enabled the fraction of RV absorbed to be determined and a point-to-point IVIVC to be established. The synthetic membrane, Strat-M™, showed a RV permeation profile similar to that obtained with pig ear skin ($R^2 = 0.920$). Studies with Strat-M™ resulted in a good and linear IVIVC ($R^2 = 0.991$) when compared with other synthetic membranes that showed $R^2$ values less than 0.90. The $R^2$ for pig ear skin was 0.982. Strat-M™ membrane was the only synthetic membrane that adequately simulated skin barrier performance and therefore it can be considered to be a suitable alternative to human or animal skin in evaluating transdermal drug transport, potentially reducing the number of studies requiring human or animal samples.

**Keywords:** transdermal drug delivery system; permeation, synthetic membrane; Strat-M™; Franz diffusion cell; rivastigmine; point-to-point IVIVC.
1. Introduction

Transdermal drug delivery is an attractive alternative delivery route compared with more conventional routes, such as oral drug delivery, as it avoids first pass metabolism, and may overcome issues of poor patient compliance (Bartosova and Bajgar, 2012). A transdermal drug delivery system (TDDS) is a formulation or device (e.g., transdermal patch) which provides controlled and continuous drug delivery through the skin, maintaining the drug concentration in the blood within the effective therapeutic window (Kalia and Guy, 2001, Wokovich et al., 2006). Over the past two decades, TDDS development has become increasingly important in the pharmaceutical industry, leading to an increased number of TDDS being approved by regulatory authorities for commercialization (Prausnitz et al., 2004; Prausnitz and Langer, 2008; Wiedersberg and Guy, 2014). The rivastigmine Exelon® Patch is an example of a commercially available TDDS which is used for the symptomatic treatment of mild to moderately severe dementia in Alzheimer’s disease (Williams et al., 2003). Rivastigmine (RV) is a molecule with a partition coefficient (log $P_{octanol/water}$) of 2.1, exhibiting good permeability and solubility and thus falling into Class I of the Biopharmaceutics Classification System (Tannergren et al., 2009).

The therapeutic efficiency of a TDDS, such as a patch, depends on its performance, which involves two main steps: (1) drug release from the patch, and (2) permeation and diffusion through the stratum corneum, via the epidermis and dermis until it reaches the systemic circulation (Kalia and Guy, 2001; Wokovich et al., 2006). Thus, the efficacy of a TDDS must be tested by means of reproducible and reliable in vitro performance tests that are able to measure drug release and permeation for the finished dosage form (Ueda et al., 2009). A permeation test coupled with an in vitro-in vivo correlation (IVIVC) can aid the development of a new transdermal system by anticipating patch performance before clinical trials, saving time and reducing development costs. Furthermore, this type of testing can be applied to evaluate the performance of products that have undergone scale-up and post-approval changes (e.g., drug supplier, formulation and manufacturing site changes) (FDA, 2014) relieving companies of the need to repeat extensive studies. However, few reports can be found in literature on IVIVCs for TDDS (Shen and Burgess, 2015).

The flow-through or static diffusion cells are recommended by international guidelines to measure drug release and permeation rate for topical and transdermal dosage forms (EMA, 2012; FDA, 1997a; Franz, 1975; Ueda et al., 2009), and for the development of IVIVCs (Shen and Burgess, 2015). The most common diffusion cells are Franz-type (static) cells which
consist of two compartments - donor and receptor - which must be separated by a membrane. Synthetic membranes are commonly used for drug release studies and natural (human or animal) skin for permeation studies (Addicks et al., 1987; Agyralides et al., 2004; Clement et al., 2000; Davaran et al., 2005; Frum et al., 2007; Hai et al., 2008; Joshi et al., 2012; Limponsa and Umprayn, 2008; Ng et al., 2010; Santoyo et al., 1996; Uchida et al., 2015), due to the different diffusion properties offered by membrane models.

The best in vitro model reported to date for the prediction of the in vivo permeation of topical products is the use of excised human skin in the Franz Cell (Franz et al., 2009; Yang et al., 2015), and is also strongly recommended by regulatory agencies (FDA, 1997a; EMA, 2012; OECD, 2004). Nonetheless, the use of excised animal skin is also recognized as acceptable, and is frequently used as a replacement for human skin (EMA, 2012; OECD, 2004), since human skin use is subject to national and international ethical considerations and is not always accessible (OECD, 2004). A number of studies have demonstrated that pig ear skin exhibits similar structural and biochemical characteristics to human skin (Barbero and Frasch, 2009; Dick and Scott, 1992; Simon and Maibach, 2000), making it well-suited for permeation studies, providing comparable results to human skin (Dick and Scott, 1992; Godin and Touitou, 2007). In addition, it is generally recognized that natural tissue samples can demonstrate variability from one species to another and even within the same species (e.g. based on age, sex, race, degree of hydration), potentially affecting drug permeability across skin (WHO, 2006). The ready availability of skin sources is not always possible, hindering the application of in vitro performance testing for TDDS in Franz Cells.

The lack of biological skin availability recurrently leads to the use of synthetic membranes for in vitro performance testing; a fit for purpose synthetic membrane must be inert, provide high permeability and not occlude the drug penetration (EMA, 2012; FDA, 1997a; Ueda et al., 2009). The Food and Drug Administration (FDA, 1997a) has suggested the application of simple polymeric membranes, which have often been used in in vitro drug permeation studies, such as: polysulfone (Clement et al., 2000, Ng et al., 2010), polyethersulfone (Joshi et al., 2012; Ng et al., 2010; Uchida et al., 2015), cellulose (Santoyo et al., 1996; Clement et al., 2000; Ng et al., 2010; Borges et al., 2013), or polydimethylsiloxane (Addicks et al., 1987; Clement et al., 2000; Frum et al., 2007; Ng et al., 2010). However, depending on the physicochemical characteristics of the synthetic membrane, the Franz Cell test may only reflect the drug release, and may not reflect the drug permeation across the skin, providing limited information about the effectiveness of the TDDS (Borges et al., 2013).
Due to the difficulty in obtaining biological skin samples, sample to sample variability and ethical issues, there is an increasing interest in establishing an inexpensive and reproducible in vitro membrane model that simulates the skin barrier performance in terms of release and permeation. In this context, the aim of the present study was to develop an in vitro drug permeation test utilizing synthetic membranes that can potentially replace the use of human or animal skin. The study was coupled with the development of an IVIVC method for rivastigmine patch (Exelon® Patch) as the chosen model TDDS.

2. Materials and methods

2.1 Materials

All reagents used were of analytical grade. Sodium hydroxide (NaOH), ammonium hydroxide (NH₄OH), ammonium monobasic phosphate (NH₄H₂PO₄), sodium phosphate (Na₂HPO₄) sodium chloride (NaCl), potassium chloride (KCl) and potassium monobasic phosphate (KH₂PO₄) were purchased from Vetec (Rio de Janeiro, Brazil). The high performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Tedia (Rio de Janeiro, Brazil). For all filtration procedures 0.45 µm polyvinylidene fluoride filters were used (Millex Millipore, São Paulo, Brazil). Purified water was obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Rivastigmine USP Reference Standard (batch no. F0J302) was purchased from the U.S. Pharmacopeia. The TDDS containing RV, which is commercially available as Exelon® Patch10 (produced by Novartis (Brazil)), was purchased from a local pharmacy. Each transdermal patch of 10 cm² contains 18 mg of RV and is reported to release 9.5 mg RV over a period of 24 hours (Novartis, 2015).

Pig ear skin was obtained from young animals sacrificed at a local slaughterhouse (Suibom Comércio Atacadista de Carne Ltda/ME, Minas Gerais/ Brazil). Synthetic membranes with different characteristics were acquired from different suppliers and are listed in Table 1.

2.2 Methods
2.2.1 High performance liquid chromatography (HPLC) analysis

RV quantification was performed using an Elite LaChrom HPLC system from Merck-Hitachi, (Darmstadt, Germany) coupled to a photodiode array detector (DAD L-2130), quaternary pump (L- 2455), column oven (L-2350), autosampler (L-2200), and Eze-Chrom software. RV in solution was quantified using a modified U.S. Pharmacopeia method (USP, 2015a). Chromatographic separation was achieved isocratically at room temperature with a Kromasil 100Å C8 column (4.6 x 150 mm; 5 μm). The mobile phase consisted of a mixture of monobasic ammonium phosphate buffer (8.6 mg/mL; pH 7.0), acetonitrile and methanol in a 50:25:25 (v/v/v) ratio and, was run at a flow rate of 1.2 mL/min (Amaro et al., 2015; Simon et al., 2016). The eluent was monitored for 6 min with ultraviolet-visible detection at 215 nm for RV quantification; the retention time was approximately 4 min.

2.2.2 Validation of the quantification method

The chromatographic method was validated by testing the parameters of specificity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) (ICH 2005; USP, 2014b). The specificity was assessed by comparing the spectral scans (200-400 nm) of the receptor medium, RV standard solution and product sample. The linearity was evaluated by linear regression analysis of three replicates obtained on different days, at 6 levels ranging from 20 to 300 µg/mL, which are equivalent to 10 – 150 % of the drug working concentration (200 µg/mL). The accuracy of the method was determined by a recovery test, in which product solutions of predetermined concentration were spiked with a known amount of RV standard solution at four levels (40, 100, 200 and 230 µg/mL) equivalent to 20, 50, 100, 115 % of working concentration. The repeatability of the method was estimated by calculating the relative standard deviation (RSD) of the quantification of six patch samples (Shabir, 2003; USP, 2015b). Each patch was submerged in 50 mL of acetonitrile and receptor medium (1:1, v/v) and placed in an ultrasonic bath for 1 hour. The solutions were diluted in receptor medium resulting in samples with a concentration of ~200 µg/mL. The LOQ and LOD were calculated based on the equations: \( \text{LOQ} = SD \times \frac{10}{S} \) and \( \text{LOD} = SD \times \frac{3}{S} \), where \( SD \) is the standard deviation of the intercept with the y-axis of three calibration curves and \( S \) is the average of angular coefficients of the respective curves (ICH, 2005). The stability of prepared solutions in receptor medium containing RV standard (10, 75 and 200 µg/mL),
was tested by storage at room temperature and evaluation at 0, 12, 24 and 48 h. The chromatographic system suitability was monitored by controlling the retention time, theoretical plates and asymmetry of the RV chromatographic signal.

2.2.3 Drug solubility in receptor medium

RV solubility was determined in phosphate-buffered saline (PBS) pH 7.4 at 37 ± 0.5 °C. PBS contains 138 mM NaCl, 2.7 mM KCl, 1.43 mM KH$_2$PO$_4$ and 8.57 mM Na$_2$HPO$_4$. The solubility studies were carried out by adding an excess of drug in a beaker containing 5 mL of receptor medium to obtain a saturated solution. The solutions were kept under constant magnetic stirring at 500 rpm for 24 h. Thereafter, the solutions were centrifuged (Eppendorf 5430R, Germany) for 15 minutes at a speed of 5000 rpm. The supernatant was filtered through a 0.45 µm Millipore membrane, and the filtrate was assayed by the HPLC method for RV quantification, according to the methodology described in section 2.2.1. The solubility test was carried out in triplicate.

2.2.4 Pig skin and synthetic membranes preparation

The young white porcine ears from different animals were initially cleaned with tap water, followed by excision of the dorsal skin from the underlying cartilage using a scalpel. The hairs and subcutaneous fat tissue were removed using surgical scissors to obtain a full-thickness skin (~400 µm), which was washed with distilled water and visually inspected to ensure its integrity (Junyaprasert et al., 2007). When not used immediately, the skin was wrapped in filter paper, moistened with saline solution and packed in aluminum foil for storage at -4°C for up to 3 days (Pupe et al., 2013). At point of use, the frozen skin samples were rehydrated by incubation in saline solution 0.9 % (w/v) at room temperature for one hour before being placed in the Franz Cell units.

No pre-treatment was performed on silicone membranes (Sil2, Sil5, Sil10) or polyethersulfone membrane (StrM). Cellulose membranes (Cup and Cel) required hydration in boiling distilled water before use.

2.2.5 In vitro permeation studies
The *in vitro* permeation studies were carried out in a Franz Cell system (Hanson Research, Chatsworth, LA, USA) with a diffusion area of 1.767 cm² and capacity of 7 mL for the receptor medium, PBS pH 7.4. The Franz Cell system was maintained at a constant temperature of 37 ± 0.5 °C through thermostatic bath circulation, while the receptor medium was stirred constantly at 350 rpm during the experiments. For the permeation studies, synthetic membranes with different characteristics were used (Table 1), as well as the pig ear dermis. For each membrane evaluated, an assay was performed with six diffusion cells. Each membrane was carefully placed at the interface between the donor and receptor compartments, with placement of the Exelon® Patch 10 over the membrane. Aliquots of 1 mL were collected at time 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 22, 23 and 24 hours (since the TDDS is intended for single daily application). Sink conditions were maintained with the replacement of the same volume of receptor medium held at 37 ± 0.5 °C. All collected samples were analyzed by HPLC and RV quantification was obtained by the regression equation obtained from a standard curve prepared on the same day as the analysis.

The cumulative amount of RV permeated through the membrane was calculated taking the patch area into account (mg/cm²), with results plotted as a function of time (hours). To study the kinetics of RV permeated through different membranes between 1 and 24 hours, data were treated according to zero-order (cumulative amount of drug permeated versus time), first-order (log cumulative amount of drug remaining versus time), Higuchi (cumulative amount of drug permeated versus square root of time), and Korsmeyer-Peppas (log cumulative amount of drug permeated versus log time) models. The best-fit kinetics model was selected based on the correlation coefficient values obtained by linear regression (Costa and Lobo, 2001). The significance of the differences observed from the application of different membranes was verified by a repeated measures test using one-way ANOVA (GraphPad Prism® software, La Jolla, CA, USA) and considered significant if *p* < 0.05.

2.2.6 Methodology applied in the development of IVIVC

The RV *in vitro* permeation data was retrieved from studies described in section 2.2.5, and the *in vivo* plasma concentration data (ng/mL) versus time (h) of the RV after TDDS (Exelon® Patch 10) skin application was obtained from a previously reported pharmacokinetic study (Lefèvre et al., 2008). Mean plasma concentration data of RV as a function of time was deconvoluted using the Wagner-Nelson mathematical equation (eq. 1) and thereby the RV
The fraction absorbed (%Fa) at different times was calculated (Wagner and Nelson, 1964; Yang et al., 2015).

\[ \%Fa = \frac{C_t + k_{el} AUC_{0-t}}{k_{el} AUC_{0-\infty}} \times 100\% \] (eq. 1)

Where, \( C_t \) is the plasma concentration of RV at time \( t \), \( k_{el} \) is the elimination rate constant, \( AUC_{0-t} \) is the area under the curve from time 0 to time \( t \), and \( AUC_{0-\infty} \) is the area under the curve from time 0 to infinity (Wagner and Nelson, 1964; Yang et al., 2015).

A point-to-point IVIVC for each membrane investigated was evaluated through assessment of the linear correlation between in vitro permeation and the in vivo input rate (FDA, 1997b). In vitro and in vivo fractions were plotted as independent (x) and dependent (y) variables, respectively, and a linear regression analysis established (Silva et al., 2015; Yang et al., 2015). The linear regression calculations were performed using Microsoft® Office Excel® 2007.

2.2.7 Statistical analysis

Microsoft® Office Excel® 2007 was used to determine in vitro permeation data. Difference between means was determined through statistical analysis of variance, ANOVA with Tukey's multiple comparison post-hoc test using GraphPad Prism® software (version 5.0), with a 95% confidence level.

3. Results and discussion

3.1 HPLC method validation

Accurate and reliable analytical methods for drug assay in in vitro permeation studies are mandatory to evaluate the performance of transdermal systems (USP, 2015b). A modified U.S. Pharmacopeia method was used to quantify RV hydrogen tartrate raw material. The chromatographic method demonstrated specificity, since no chromatographic signal was detected on the injection of receptor medium alone. The RV chromatographic peak obtained from the standard and sample solutions injection had a retention time of 4.07 min with a peak purity of more than 0.999. The linearity was established with a correlation coefficient of 0.999.
(Table 2). The repeatability of the method was adequate (RSD < 5%). The accuracy was satisfactory, presenting recoveries between 99.2 and 100.0 %. The solutions containing RV at concentrations of 10, 75 and 200 µg/mL remained stable after storage for 48 hours at room temperature, with RSD of 0.52, 0.73 and 0.67 %, respectively. The chromatographic system suitability to RV assay in the receptor medium exhibited satisfactory parameters, with theoretical plates higher than 4300, peak asymmetry between 1.5-1.7 and, peak purity greater than 0.99 for all determinations.

3.2 In vitro permeation studies

The use of a Franz Cell system requires the selection of a receptor medium which can maintain sink conditions or at least the capacity to solubilize the drug content in the patch (Clément et al., 2000). In order to maintain sink conditions, it was considered that, if the entire amount of RV contained in the patch was transferred to the receptor medium volume (as migration of the drug can occur to the diffusion area), the solution concentration should be no more than one third of the saturation solution concentration, as recommended by the U.S. Pharmacopeia (USP, 2015c). The RV solubility in PBS pH 7.4 was experimentally to be 18.69 ± 0.27 mg/mL (n = 3). Considering that the receptor compartment volume of each cell is 7 mL, and the declared maximum drug content in the patch is about 18 mg, the maximum concentration that may be attained in the receptor compartment is 2.57 mg/ml (which is 14% of the saturation solubility) and thus sink conditions are maintained.

The RV cumulative permeation profiles from TDDS are shown in Figure 1 where panel A shows the permeated amount from synthetic membranes plotted against time and, panel B shows the permeated amount from pig ear skin against time. The total drug amount (mg ± SD) permeated over 24 hours through synthetic membranes Cel, Cup, Sil2, Sil5, Sil10 and StrM was 18.53 ± 0.21 mg, 18.81 ± 1.32 mg, 19.23 ± 0.91 mg, 19.22 ± 1.35 mg, 19.41 ± 1.14 mg and 14.89 ± 2.23 mg respectively, and through pig ear skin was 15.85 ± 1.62 mg. The RV cumulative permeation profiles obtained over the 24 hours of experimentation were not significantly different for cellulose (Cel, Cup) and silicone membranes (Sil2, Sil5, Sil10) when evaluated by one-way ANOVA with Tukey's post-hoc test (p > 0.05). These permeation profiles were found to be characteristic of anomalous kinetics (non-Fickian mechanism) by fitting of the Korsmeyer-Peppas model, which resulted in $R^2$ values of 0.902, 0.837, 0.874, 0.881 and 0.899 for Cel, Cup, Sil2, Sil5 and Sil10 respectively, corroborating with previous literature (Costa and Lobo, 2001). The Sil10 membrane, which is thicker (0.254 mm) than
other membranes, demonstrated lower drug permeation at initial time points in comparison with the other synthetic membranes; however, after 2 hours the permeated amount was not significantly different to other membranes, suggesting that the membrane also offers the least possible diffusional resistance. Therefore, these results suggest that the cellulose and silicone membranes do not present considerable resistance to the diffusion of RV from the patch.

Conversely, the synthetic membrane, StrM, when compared to all other synthetic membranes evaluated in this study, exhibited a significantly different \((p < 0.001)\) permeation profile, in which the cumulative amount of RV diffused across the membrane showed similarity with the permeation data for the pig ear skin (Skin). These permeation profiles were best characterised by the Higuchi (square root of time) kinetic model for Skin \((R^2 = 0.987)\) and StrM \((R^2 = 0.997)\). This kinetic model is frequently used to describe the controlled release rate of a drug from a dosage form by a constant diffusion process (Siepmann and Peppas, 2011). The synthetic membrane StrM showed a RV permeation profile that was statistically similar to that obtained with the Skin, with comparable permeation-time profiles \((p > 0.05)\).

Considering the similarity between the RV permeation profiles obtained for StrtM and Skin membranes shown in Figure 1, a linear relationship was investigated between permeation across StrtM versus Skin. A significant and strong correlation \((p < 0.0001)\) was found with a correlation coefficient \((r)\) of 0.962 between the RV diffusion through StrM and Skin (Figure 2), suggesting that the synthetic membrane provides a diffusion barrier function which is similar to that of pig ear skin.

Skin is constituted by a multilayer structure, and the outmost layer, the stratum corneum, is an effective barrier against drug permeation across skin due to the presence of lipid components (Prausnitz and Langer, 2008). Previously, Clément and co-workers (Clément et al., 2000) evaluated synthetic membranes impregnated with synthetic hydrophobic material and demonstrated the importance of the lipophilic characteristics to act as skin lipid-like materials, simulating the stratum corneum barrier. Uchida and co-workers (Uchida et al., 2015) observed, by microscopic analysis, the presence of synthetic lipids in two of the three polymeric layers which form the StrM membrane, imparting additional hydrophobic skin-like properties to this synthetic membrane. It was also reported that polymeric layers create a porous structure with a total thickness of approximately 325 \(\mu m\), which is divided into three layers of different densities, providing a permeation gradient in terms of pore size and diffusivity (Merck, 2012; Uchida et al., 2015).

Consequently, this similar RV diffusion behaviour is thought to be related to the hydrophobic characteristics and irregular polymeric structure of the StrM membrane, which
provided for the correlation between *in vitro* drug permeation profiles obtained using the synthetic StrM membrane and pig ear skin. The results presented here indicate that the synthetic membrane may be successfully used as a substitute for biological skin for *in vitro* permeation studies, taking into consideration that pig ear skin exhibits structural and biochemical characteristics similar to those of human skin (Barbero and Frasch, 2009; Dick and Scott, 1992; Simon and Maibach, 2000).

3.3 *IVIVC* studies

The collected *in vitro* permeation data was mathematically correlated with the bioavailability data of RV from TDDS, Exelon® Patch 10, as the same principles of *IVIVC* utilized for extended release oral dosage forms (FDA, 1997b) can be applied to non-oral dosage forms, such as parenteral depot formulations and new drug delivery systems (Nandy et al., 2011; Shen and Burgess, 2015).

Based on the RV plasma concentration data versus time obtained from the bioavailability curve (Lefèvre et al., 2008), the RV fraction absorbed (%Fa) as a function of time was calculated using the Wagner-Nelson mathematical equation. RV pharmacokinetics from a transdermal patch has been previously described by a one compartment model (Mercier et al., 2007; Lefevre et al., 2008). Figure 3 presents the curve of the RV fraction absorbed versus time after dermal application of the TDDS until $C_{\text{max}}$ was reached.

According to Yang and co-workers, only Level A correlations are applicable to *IVIVC* of TDDS, due to the complex mechanism of drug permeation (Yang et al., 2015). Therefore, the point-to-point *IVIVC* approach was used in this study for the RV patch (TDDS), i.e. a point-to-point relationship between *in vitro* dissolution/permeation and the *in vivo* absorption of drug from the dosage form (FDA, 1997b). The correlation was performed by plotting the data obtained during the time interval from 0 to 8 hours after dermal application, to reflect the time required for RV plasma concentration to reach a maximum ($t_{\text{max}}$) before beginning to decrease as a function of elimination processes. The coefficient of correlation ($R^2$) and the slope were obtained by plotting the *in vivo* fraction absorbed (%Fa) against the *in vitro* fraction permeated, assuming the *in vivo* time was the same as *in vitro* time (point-to-point) without any change in the time scale. *IVIVC* data is shown in Table 3.

The validity of the regression curves was represented by the $F_{\text{cal }}> F_{\text{tab}}$, indicating that the slope is significantly different from zero with $p$ values no more than 0.0007 ($p < 0.05$). The best-fit values ($R^2 > 0.90$) for linear correlation were found for Skin and StrM
membranes. For these membranes all points evaluated fitted close to the linear plot with minimum scatter (Figure 4), resulting in a low residual standard deviation ($S_yx$). On the other hand, the Sil2, Sil5, Sil10, Cup and Cel membranes showed a high residual standard deviation, reflecting a less linear distribution, that is, the regressed data was less homogeneously distributed and more scattered around the regression line of the model, resulting in a poorer correlation, with $R^2 < 0.90$, and reduced predictability of the model.

It is interesting to note that the StrM synthetic membrane presented a strong and significant linear correlation with the $in vivo$ data, with $R^2 = 0.991$, in the same way as the pig ear skin (Skin), with $R^2 = 0.982$. These good and similar correlations may be justified by the similar properties of the pig ear skin and StrM synthetic membrane and human skin. The good linear correlations obtained by in vitro RV permeation across StrM and Skin with $in vivo$ RV absorption indicates that the methodology used in the in vitro permeation studies reflects the $in vivo$ conditions, which is the correlation level which is most desirable for TDDS (Shen and Burgess, 2015; Yang et al., 2015).

TDDS development is gaining increasing interest by the pharmaceutical industries, and also represents one of the most successful and innovative areas of research in drug delivery (Bartosova and Bajgar, 2012). However, during the development of new formulations, the number of animal and human studies should be kept to the minimum necessary to achieve the aims of the research, thus, the use of predictive in vitro studies of $in vivo$ performance are increasingly needed. Despite the recognized importance of IVIVCs for oral dosage forms, the development of IVIVCs for TDDS has not yet been given proper importance, to date, and there are only a few literature reports on IVIVCs for TDDS (Chaturvedula et al., 2005; Mateus et al., 2014; Mohammed et al. 2014; Shen and Burgess, 2015, Yang et al., 2015). In view of this, the data presented in this section suggests, once more, StrM synthetic membrane as a viable alternative to human and animal skin models for transdermal permeation studies, since the results showed an in vitro permeation test coupled with IVIVC, under controlled conditions, for prediction of $in vivo$ RV transdermal absorption from a TDDS.

Furthermore, the permeation rate specifications for a transdermal dosage form are essential for the quality control analysis in order to assure a reproducible performance of TDDS, and consequently its therapeutic efficacy. If an in vitro permeation test can be related to the $in vivo$ performance of a product, it is of significant interest and importance to the pharmaceutical industry, since a generic sponsor would have an efficient tool to evaluate different formulations and select the optimal formulation for use in the pivotal bioequivalence
The use of artificial membranes in *in vitro* methods with potential to mimic the biological skin and predict product performance and drug permeability is a key step in the development phase of a new transdermal product containing RV, as well as screening of drug candidates for transdermal delivery, in order to ensure the drug permeation rate and safety in clinical studies.

### 4. Conclusion

In the current study, a point-to-point *IVIVC* was developed using the Franz Cell with a synthetic membrane for evaluation of RV delivery from a rivastigmine TDDS. This *in vitro* method may be used for quality control, stability verification, to test a product after post-approval changes and/or for equivalence studies of a new generic TDDS. Permeation of RV across the StrM synthetic membrane in Franz diffusion cells correlated well with results of permeation studies using pig ear skin.

The results demonstrated that the use of the StrM synthetic membrane in Franz diffusion cells could predict *in vivo* RV absorption, making this methodology promising to assess other drugs. This synthetic membrane can be seen as an alternative to human or animal skin when evaluating drug transdermal diffusion during the TDDS development, reducing cost and time.

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### Conflict of interest

The author(s) confirm that this study content has no conflicts of interest.
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Figure 1. *In vitro* permeation profile of RV from Exelon® Patch 10 obtained from studies in Franz Cell using different membranes. Panel (A) shows the cumulative RV permeation profiles obtained for artificial membranes: dialysis tubing cellulose (Cel), Cuprophan® (Cup), silicone of different thicknesses (Sil2, Sil5, Sil10) and Strat-M™ (StrM); and panel (B) shows the cumulative RV permeation profile obtained with pig ear skin (Skin). Values represent the mean ± standard deviation of six replicate cells.

Figure 2. Relationship between RV amount permeated (mg/cm²) through pig ear skin (Skin) versus the RV amount permeated (mg/cm²) through synthetic membrane (StrM).

Figure 3. RV fraction absorbed *in vivo* from TDDS (Exelon® Patch 10) obtained by deconvolution using the Wagner-Nelson model.

Figure 4. *In vivo-in vitro* correlation established for *in vitro* permeated data using synthetic membranes (Sil2, Sil5, Sil10, Cel, Cup, StrM) and pig ear skin (Skin) as a function of the *in vivo* fraction absorbed from TDDS containing RV (Exelon® Patch 10). Panel (A) shows the linear regression obtained from Sil 2, Sil5, Sil10, Cup and Cel membranes; and Panel (B) shows the linear regression obtained from StrtM membrane and pig ear skin (Skin).
### LIST OF ABBREVIATIONS

<table>
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>Cel</td>
<td>Cellulose dialysis tubing</td>
</tr>
<tr>
<td>Cup</td>
<td>Cellulose membrane - Cuprophan®</td>
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<tr>
<td>Fa</td>
<td>Fraction absorbed</td>
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<td>F_cal</td>
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