Compartmental Modeling of Transdermal Iontophoretic Transport: I. *In Vitro* Model Derivation and Application

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Purpose. The objective of this study was to develop a family of compartmental models to describe in a strictly quantitative manner the transdermal iontophoretic transport of drugs *in vitro.*

Methods. Two structurally different compartmental models describing the *in vitro* transport during iontophoresis and one compartmental model describing the *in vitro* transport in post-iontophoretic period are proposed. These models are based on the mass transfer from the donor compartment to the acceptor compartment via the skin as an intermediate compartment. In these models, transdermal iontophoretic transport is characterized by 5 parameters: 1) kinetic lag time (t_L), 2) steady-state flux during iontophoresis (J_{ss}), 3) skin release rate constant (K_R), 4) the first-order rate constant of the iontophoretic driving force from the skin to the acceptor compartment (I_1), and 5) passive flux in the post-iontophoretic period (J_{pas}). The developed models were applied to data on the iontophoretic transport in human stratum corneum *in vitro* of R-apomorphine after pretreatment with phosphate buffered saline pH 7.4 (PBS) and after pretreatment with surfactant (SFC), as well as the iontophoretic transport of 0.5 mg ml^{-1} rotigotine at pH 5 (RTG).

Results. All of the proposed models could be fitted to the transport data of PBS, SFC, and RTG groups both during the iontophoresis and in the post-iontophoretic period. The incorporation of parameter I_1 failed to improve the fitting performance of the model. This might indicate a negligible contribution of iontophoretic driving force to the mass transfer in the direction from the skin to the acceptor compartment, although it plays an important role in loading the skin with the drug. The estimated values of J_{ss} of PBS, SFC, and RTG were identical ($p > 0.05$) to the values obtained with the diffusion lag time method. Moreover, time required to achieve steady-state flux can be estimated based on the parameter t_L and the reciprocal value of parameter K_R. In addition, accumulation of drug molecules in the skin is reflected in a reduction of the value of the K_R parameter.

Conclusions. The developed *in vitro* models demonstrated their strength and consistency to describe the drug transport during and post-iontophoresis.

KEY WORDS: iontophoretic driving force; post-iontophoretic driving force; skin release rate constant; steady-state flux; time to achieve steady-state flux.

INTRODUCTION

Transdermal iontophoresis is a method for controlled delivery of drugs via the skin by the application of a low intensity of electric current. Important features of this approach are that 1) the transport can be greatly enhanced relative to the passive diffusion, and 2) the delivery rate can be actively controlled by modulation of the current density thereby allowing individualized dosing (1).

The feasibility of drug administration by transdermal iontophoresis is often studied in *in vitro* systems in human or animal skin preparations. In these investigations, the *in vitro* transport data is commonly analyzed on the basis of diffusion lag time methods (2–5), by determination of parameters such as the steady-state flux (J_{ss}) and the diffusion lag time (T_{lag}). Briefly, in this approach, J_{ss} is estimated from the slope of the linear portion of the cumulative amount of iontophoretic transport vs. time profile. T_{lag}, which is the time required to achieve steady-state flux if the skin concentration gradient during steady-state flux is already established at the start of the permeation process, is estimated from the intercept of that linear portion to the time axis.

Unfortunately, the diffusion lag time method has several limitations. First, this method excludes several data points outside the linear portion of the cumulative flux vs. time curves. This is important, as these points contain pivotal information on the mechanism of the transport. Second, the linear portion of the cumulative flux vs. time profile does not always reflect the steady-state flux. To illustrate this, Fig. 1 shows the correlation of the flux and the cumulative flux vs. time profiles for three different conditions. In this figure, panel A shows the situation where a real steady-state is achieved, whereas the panels B and C show the conditions where steady-state has not yet been achieved and the situation where the steady-state is not achieved due to the depletion of the drug concentration in the donor phase, respectively. Interestingly, if the last five data points are analyzed with linear regression method, the correlation of the cumulative flux vs. time is linear in all cases ($R^2 > 0.999$).

Considering the limitations of the diffusion lag time method, several authors prefer to evaluate transdermal iontophoretic transport based on the maximum flux obtained, which is in most instances the flux at the end of iontophoresis period (6–8). Another approach that has been used is the analysis of the cumulative amount of drug transported during the whole period of iontophoresis (9,10). All these methods lack the important information, namely the gradual change in transport rate. This issue might be crucial especially when

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ABBREVIATIONS: IDF, iontophoretic driving force; I_0, the zero-order iontophoretic mass transfer from the donor compartment into the skin compartment; I_1, the first-order rate constant of the iontophoretic driving force in the transport from the skin into the acceptor compartment; J(t), flux at time t; J_{ss}, steady-state flux; J_{pas}, passive flux post-iontophoresis; K_R, the first-order rate constant of drug release from the skin into acceptor compartment (*in vitro*) or to the systemic circulation (*in vivo*); PIDF, post-iontophoretic driving force; P_{PI}, the zero order post-iontophoretic mass transfer due to PIDF; S, diffusion active area or patch area; T_{lag}, the diffusion lag time; t_L, the kinetic lag time of the drug molecules to enter the skin compartment; t_N, the net time of current application; t', the net time post-iontophoresis; T, time of current removal; X(t), drug amount in the skin compartment at time t; $X_A(t)$, drug amount in the acceptor compartment at time t; X_T, drug amount in the skin when the current is switched off at time T.

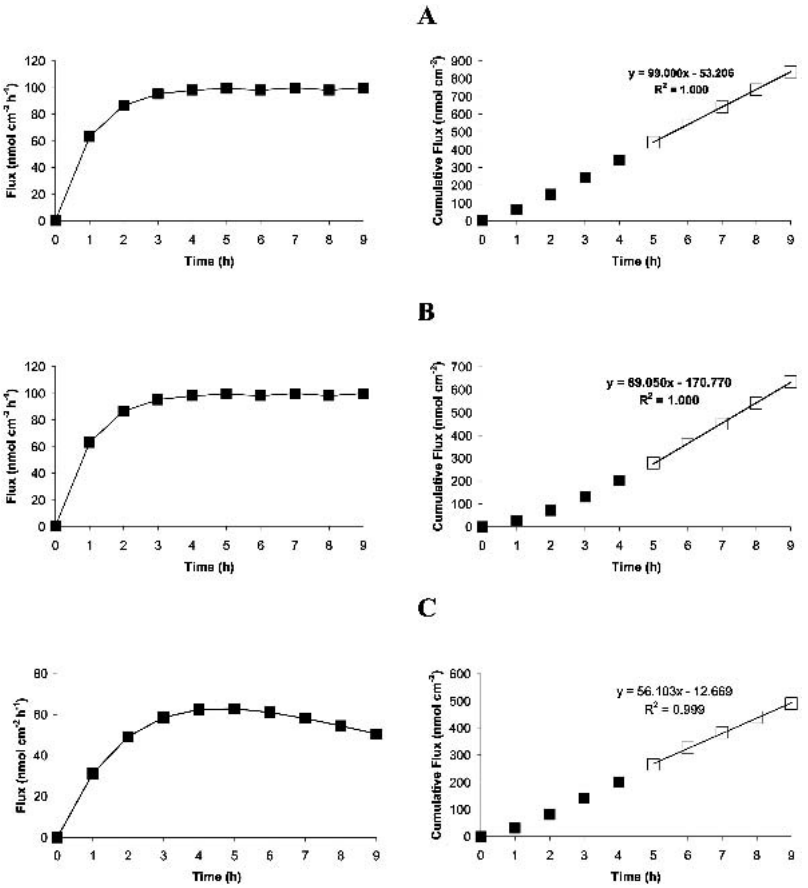

Fig. 1. The correlation of the flux vs. time profiles to the cumulative flux vs. time profiles. Although only in part A the real steady-state is achieved, the cumulative flux profiles in parts B and C also exhibit linear correlation to the time, demonstrating that linearity in cumulative flux does not always indicate a steady-state achievement.

performing an extrapolation to the *in vivo* situation. For example the method to predict the *in vivo* drug concentration in plasma based on the cumulative amount obtained during several hours of iontophoresis, only estimates the total drug input into the body over the entire period (single point analysis).

Hence, an alternative analysis method of *in vitro* data that overcomes the aforementioned disadvantages is required. An ideal method should be able to estimate both steady-state flux and time to achieve a steady-state even if the real steady-state is not achieved during the period of experimentation using all information obtained during the experiment (i.e., without excluding any data-points). Preferably this novel method should also be able to estimate *in vivo* profile based on the *in vitro* flux profile.

Therefore, the aim of this study is to develop a novel mathematical model to describe the transdermal iontophoretic transport vs. time profile, during and after iontophoresis *in vitro*. The model is subsequently applied to fit the previously published data of *in vitro* iontophoretic transport across human stratum corneum (HSC) of R-apomorphine after the pretreatment with phosphate buffered saline at pH 7.4 (PBS) and after the pretreatment with surfactant (SFC) (11) as well as the iontophoretic transport of 0.5 mg ml^{-1} of rotigotine at pH 5 (RTG) (12).

THEORY

Iontophoresis is a permeation process in which molecules are transported from the donor solution into the skin and then from the skin into the acceptor compartment (blood capillary in the *in vivo* situation). This process can be described as a drug mass transfer process from one to another compartment. Thus, we implement compartmental modeling to describe iontophoretic transport.

In contrast with passive diffusion driven by a concentration gradient, iontophoretic mass transfer is driven by a potential gradient resulting in a current flow from anode to the skin and then to the cathode. During iontophoresis the driving forces and the negative charge of the skin results in three important processes: passive diffusion, electro-osmosis, and electro-repulsion. It has been widely accepted that for small ionic drugs, electro-repulsion is the most dominant factor during iontophoresis. During iontophoresis the contribution of passive diffusion is usually very low and in most cases, even negligible. The electro-osmosis is considered to be particularly important for drugs with a relatively large molecular size, that is, peptides and proteins (13). In the models presented in this paper, we do not distinguish between those factors, and we just use the general term iontophoretic driving force (IDF).

In an *in vitro* experiment, either stratum corneum, dermatomed skin or whole skin from humans or animals such as pig, rat, or mouse is used to study the iontophoretic permeation process. In this paper, the term skin is used for either one of these preparations.

In most *in vitro* iontophoresis experiments, in addition to the iontophoretic period, the passive post-iontophoresis flux profile is also determined. Therefore, in addition to the iontophoretic period, we also include in our model the post-iontophoretic transport period, as the reduction in flux during this period provides pivotal information on the mechanisms of the iontophoretic transport.

Models for Iontophoretic Period

We propose two types of models for the iontophoretic period. Basically, due to a constant current application and assuming that no drug depletion in the donor compartment occurs, there is a constant IDF during the iontophoresis that modulates drug transport across the skin. Therefore, for both models we propose a zero-order mass transfer from the donor solution into the skin during the iontophoresis. However, according to electro-diffusion theory (14,15), the transport during iontophoresis is due to the drug ion migration caused by the flow of current between the anode and the cathode. Therefore, an important question is whether an iontophoretic driving force (IDF) not only influences the transport from the donor solution into the skin, but also the transport from the skin into the acceptor compartment. To address this issue, two models are proposed: model 1 considers IDF to influence only on the transport into the skin, while in model 2 IDF affects also the transport from the skin to the acceptor compartment in a direct manner.

Model Type I: Iontophoretic Period in Which IDF Influences the Transport into the Skin Only

There are two mass transfer steps during iontophoresis. First, the transport of the drug from the donor phase into the skin membrane driven by the IDF, and second, the passive transport (release) from the skin membrane to the acceptor compartment. As illustrated in Fig. 2A, a constant IDF drives a zero-order mass transfer I_0 from the donor phase into the skin. For the passive drug release from the skin into the acceptor phase, we propose that the transport is a first-order kinetic process. The skin release rate constant (K_R) is introduced into the model. By switching the current on, IDF starts driving the drug molecules enter the skin. However, the drug molecules may require a significant time to reach the skin compartment. To address this situation, a kinetic lag time t_L is introduced into the models. In the special situations in which the drug molecules can reach the skin compartment in a negligible time, this lag time can be constrained to zero. Furthermore, as soon as the drug reaches the skin, the release process is also started.

According to the mass transfer scheme in Fig. 2A, an ordinary differential equation (ODE) of mass transfer into and from the skin can be written as follows:

$$\frac{dX(t)}{dt} = I_0 - K_R X(t_N) \tag{1}$$

where I_0 is a zero-order mass transfer driven by a constant IDF, K_R is a first-order skin release rate constant, $X(t)$ is the drug amount present in the skin at time t, and t_N is the net time that can be described as:

$$t_N = t - t_L \tag{2}$$

As at time zero no drug is present in the acceptor compartment, the initial condition of $X(t)$ at $t = 0$ is:

$$X(0) = 0 \tag{3}$$

Solving Eq. 1 by using this initial condition yields:

$$X(t) = \frac{I_0}{K_R}(1 - e^{-K_R t_N}) \tag{4}$$

The rate of drug release from the skin into the acceptor compartment is written as:

$$\frac{dX_A(t)}{dt} = K_R X(t) \tag{5}$$

where $X_A(t)$ is the amount of drug present in the acceptor compartment. Substitution of $X(t)$ in Eq. 4 yields:

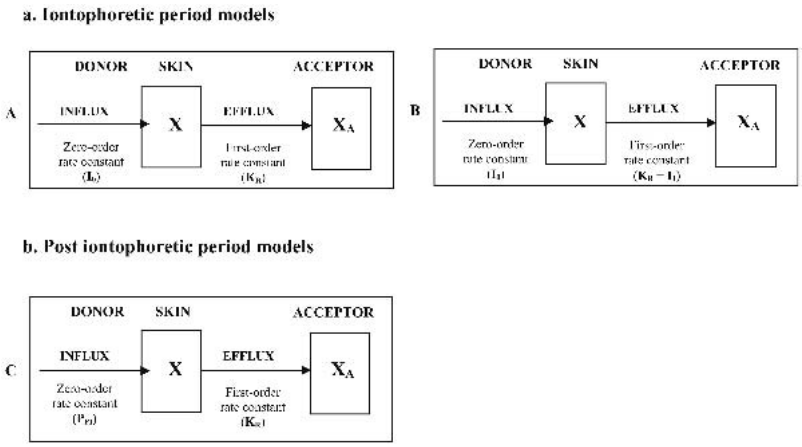

Fig. 2. Schemes of compartmental mass transfer during iontophoretic periods (A and B) and post-iontophoretic period (C). X, drug amount in the skin compartment; X_A, drug amount in the acceptor compartment.

$$\frac{dX_A(t)}{dt} = I_0(1 - e^{-K_R t_N}) \quad (6)$$

Flux is the amount of material flowing through a unit cross section of a barrier in unit time. Considering this definition we can write:

$$J(t) = \frac{dX_A(t)}{S\,dt} \quad (7)$$

where *J(t)* is the flux at time *t* and *S* is the diffusion area (patch area). Combination of Eqs. 6 and 7 yields:

$$J(t) = \frac{I_0}{S}(1 - e^{-K_R t_N}) \quad (8)$$

From this equation, the steady-state flux is obtained as:

$$J_{ss} = \frac{I_0}{S} \quad (9)$$

Model Type II: Iontophoretic Period in Which IDF Influences the Transport into and from the Skin

In this model, it is assumed that besides a constant mass transfer of drug into the skin I_0, IDF also contributes to the drug mass transfer from the skin into the acceptor compartment. However, as the drug amount in the skin is considered to change with time, we propose that second mass transfer rate constant (I_1) is a first-order rate constant rather than a zero-order rate constant. The scheme of the drug mass transfer is depicted in Fig. 2B.

According to the figure, the ODE of the mass transfer into the skin is as follows:

$$\frac{dX(t)}{dt} = I_0 - K'_R X(t) \quad (10)$$

where:

$$K'_R = K_R + I_I \quad (11)$$

Solving Eq. 9 by using the initial condition of Eq. 3 yields:

$$X(t) = \frac{I_0}{K'_R}(1 - e^{-K'_R t_N}) \quad (12)$$

The rate of drug release from the skin into the acceptor compartment is written as:

$$\frac{dX_A(t)}{dt} = K'_R X(t) \quad (13)$$

Analogous to model 1, the flux at time t (*J(t)*) is obtained from the solution of Eqs. 12 and 13 as follows:

$$J(t) = \frac{I_0}{S}(1 - e^{-K'_R t_N}) \quad (14)$$

Models for Post-Iontophoretic Period

During post-iontophoretic period, theoretically IDF is removed after switching off the current. However, it has been reported that during iontophoresis, dependent on the current density, an increase in the hydration of the stratum corneum lipid structure occurs (16,17). This may result in an enhancement of the passive flux during the post-iontophoretic period. For example, passive transport of acyclovir across nude mouse skin during the post-iontophoretic period was reported to be significantly enhanced in comparison with the pre-iontophoresis period (18). Including this aspect in our model, a post-iontophoretic driving force due to an enhanced passive diffusion after current application (PIDF) is considered to be significant. As during the iontophoretic period the level of IDF is much higher than PIDF, we assume that this driving force (PIDF) becomes significant only after the IDF removal (post-iontophoresis) and not during the iontophoresis. Furthermore, as the skin could be considered to be more permeable, at this phase the kinetic lag time (t_L) could be neglected as well. The mass transfer process is illustrated in Fig. 2C. According to this figure, the rate of mass transfer can be described by the equation below:

$$\frac{dX(t)}{dt} = P_{PI} - K_R X(t') \quad (15)$$

where P_{PI} is the post-iontophoretic drug transfer due to PIDF and t' is the net time after current removal that can be described as:

$$t' = t - T \quad (16)$$

in which T is time of current removal. To solve the ODE above for *X(t')* the initial condition $X(0) = X_T$ is used to derive the equation below:

$$X(t) = \frac{P_{PI}}{K_R}(1 - e^{-K_R t'}) + X_T e^{-K_R t'} \quad (17)$$

X_T is the amount of drug in the skin when switching off the current at time T and is calculated based on either Eq. 4 or Eq. 12. According to the aforementioned flux definition, the equation for flux *J(t)* is derived as:

$$J(t) = \frac{P_{PI}}{S}(1 - e^{-K_R t'}) + \frac{K_R}{S} X_T e^{-K_R t'} \quad (18)$$

Steady-state passive flux post-iontophoresis can be estimated as follows:

$$J_{pass} = \frac{P_{PI}}{S} \quad (19)$$

METHODS

The models were applied to analyze the intrinsic iontophoretic flux vs. time profiles of R-apomorphine across human stratum corneum (HSC). Two different groups of R-apomorphine transport were analyzed, namely the group with phosphate buffered saline at pH 7.4 pretreatment (PBS) and the group with surfactant pretreatment (SFC) (11). Moreover, one data-series of the iontophoretic transport of rotigotine, a lipophilic dopamine agonist, with the drug donor concentration of 0.5 mg ml^{-1} at pH 5 (RTG) (12) was also analyzed. In order to fit the data, the iontophoretic period and post-iontophoretic period models were combined. The term of model 1 or model 2 is given respectively for the combination of iontophoretic period type I or type II models to the post-iontophoretic period model.

Both models were applied to fit the individual data of PBS, SFC and RTG groups by using WinNonlin Professional version 4.1 (Pharsight Corporation) (19). In addition to individual data fitting, a naïve pooling approach (20) was used to

perform visual-based evaluations of the data. Diagnostics of the fitting were based on plots of the predicted and observed flux *vs.* time, the predicted vs. observed flux correlation, and the weighted residual sum of square vs. predicted flux. Nelder-Mead algorithm was used during the minimization process with values of the increment for partial derivative, number of predictive values, convergence criterion, iterations, and mode size of 0.001, 1000, 0.0001, 500, and 4, respectively. For all fittings proportional weighting to the reciprocal of the predicted value was applied.

In order to determine whether the addition of parameter I_1 (model 2) significantly improves the fitting performance, an evaluation based on F-test as described previously (20) was performed. In addition, the approximate % coefficients of variation (%CV) of the fit-parameters were also evaluated to determine the precision of the fit-parameters. When a comparison between the obtained fit-parameters was necessary, the significance of the difference of the mean values was tested using the unpaired two-tails Student's *t* test ($p < 0.05$).

RESULTS

Evaluation of the Best Model

The visual-based evaluation of model 1 to the R-apomorphine iontophoretic data of PBS and SFC groups as well as rotigotine (RTG) by a naïve pooling approach are presented in Figs. 3, 4, and 5, respectively. As clearly shown in those figures, the measured R-apomorphine and rotigotine flux from all data sets were excellently fitted by the prediction values of the model (see parts I of the figures). Moreover, the capability of the model to describe the iontophoretic transport is also demonstrated by the evaluation based on the predicted vs. observed flux correlation (parts II) and the weighted residual sum of square *vs.* predicted flux (parts III).

When the data-sets of all groups were fitted by using model 2, all of the visual evaluations demonstrated the identical situations with the fitting using model 1 (the graphs are not shown). In order to determine the best model from model 1 and model 2, F-test evaluations to the individual data-sets were performed. The results indicate that increase in the model complexity by the addition of I_1 does not significantly improve the fitting performance for PBS, SFC, and RTG data ($p > 0.05$). Furthermore, when model 2 was used, the prediction of parameter I_1 for PBS, SFC, and RTG individual data sets, in most cases yielded a negligible value with a very large %CV as an indication of the lack of precision. Based on these results, model 1 was chosen as the best model to be used for PBS, SFC, and RTG data sets. The individual fits of the data of PBS, SFC and RTG groups to model 1 as presented respectively in Figs. 6, 7, and 8, also indicate that the proposed models are able to describe the iontophoretic transport in all groups.

Parameters Estimation of J_{ss}, K_R, J_{pas}, and t_L

The best-fit results and the average %CV of parameters J_{ss}, K_R, J_{pas}, and t_L for PBS, SFC, and RTG groups by using

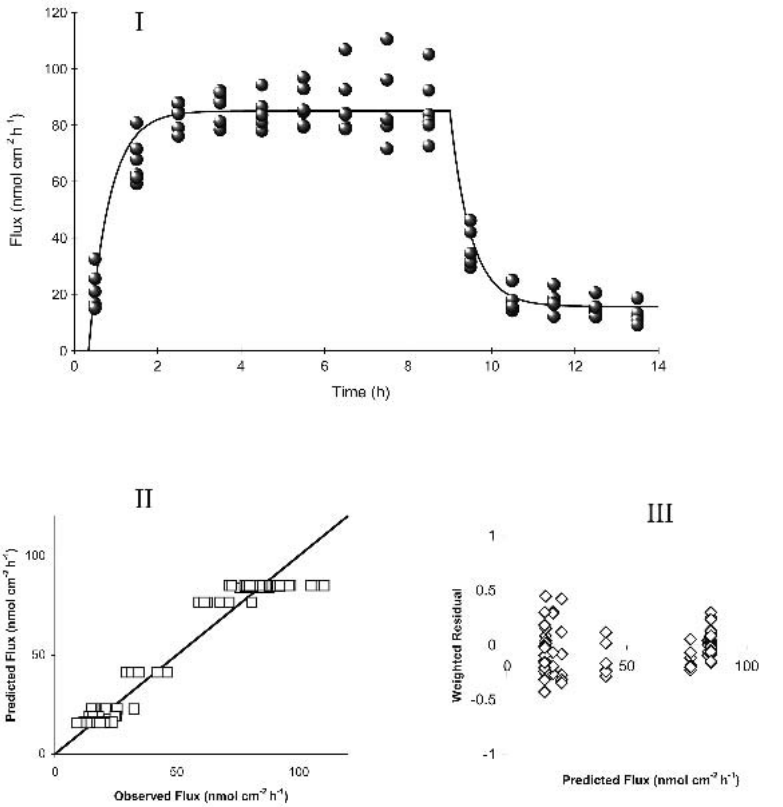

Fig. 3. The fitting result (I) and the evaluation of the predicted flux vs. observed flux (II) and the weighted residual sum of square vs. predicted flux (III) based on the naïve pooling approach of the data-set of R-apomorphine transport from PBS group during and post-iontophoresis using model 1.

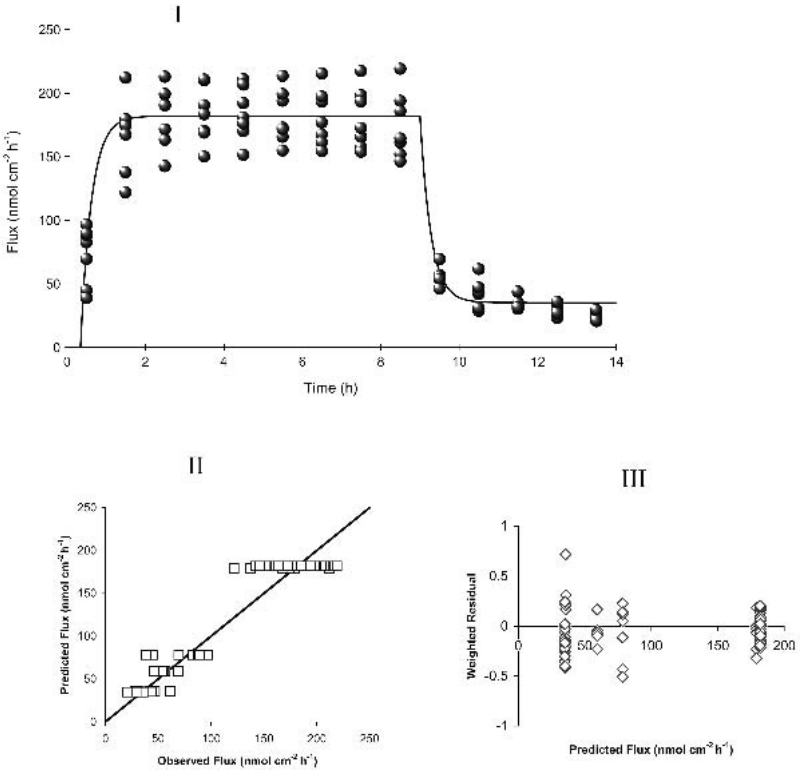

Fig. 4. The fitting result (I) and the evaluation of the predicted flux vs. observed flux (II) and the weighted residual sum of square vs. predicted flux (III) based on the naïve pooling approach of the data-set of R-apomorphine transport from SFC group during and post-iontophoresis using model 1.

model 1 as the best model are presented in Table I. The average estimation of J_{ss} was 84.8, 180.3, and 25.3 nmol cm^{-2} h^{-1}, respectively for PBS, SFC, and RTG. The %CVs were in the range from 3 to 6. The average parameter J_{pas} of PBS and SFC were 15.1 and 30.6 nmol cm^{-2} h^{-1} with the %CVs of 6 and 9 for PBS and SFC, respectively. For RTG, the average J_{pas} was approximately 5 nmol cm^{-2} h^{-1}, however as the average %CV was very large (>1000), this value was neglected. The average parameter K_R was estimated as 2.4, 2.7, and 0.5 h^{-1} for PBS, SFC, and RTG respectively, with the %CVs were in the range from 13 to 17. The last parameter t_L was estimated as approximately 0.4, 0.3, and 0.3 h for PBS, SFC, and RTG, respectively, with the %CVs were in the range from 6 to 32. Based on the unpaired Student's *t* test performed, surfactant pretreatment significantly increased J_{ss} and J_{pas} of R-apomorphine in comparison to the values of PBS (p values <0.05). The values of K_R in both groups were very similar ($p = 0.675$). In addition, there was a trend of reduction in t_L of R-apomorphine due to surfactant pretreatment although this reduction was slightly above the threshold border of the level of confidence ($p = 0.07$). Moreover, the value of K_R of RTG was significantly less than PBS or SFC ($p < 0.001$) while the value of t_L was similar to both PBS and SFC ($p > 0.05$).

Comparison of the Model Prediction Results to the Value Obtained with the Diffusion Lag Time Method

The comparison between the parameter values obtained with model 1 and the previous published value of J_{ss} and J_{pas} of R-apomorphine and rotigotine is presented in Table I. The unpaired Student's *t* test revealed that the values of J_{ss} predicted with model 1 in all groups were not significantly different to the previously published values obtained using diffusion lag time method ($p > 0.05$). Furthermore, the values of J_{pas} in both PBS and SFC groups predicted with model 1, were slightly higher ($p < 0.05$) than the values reported in the literature.

DISCUSSION

Despite its simplicity, the proposed model demonstrates the ability to describe the iontophoretic transport of R-apomorphine and rotigotine. First of all, based on the naïve pooling approach (see part I of Figs. 3, 4, and 5) and the individual fit approach (see Figs. 6, 7, and 8), the predicted values were very close to the observed value. This situation is also demonstrated in the visual evaluations based on the predicted flux *vs.* the observed flux and the weighted residual sum of square vs. the predicted flux as presented in part II and part III in Figs. 3, 4, and 5.

Moreover, the model predicted parameter of J_{ss} in all groups were statistically identical to the values obtained with the diffusion lag time method to estimate steady-state fluxes (11,12). Although our estimation of J_{pas} for PBS and SFC groups were slightly higher than those reported in the literature, this issue might be related on which data points of the post-iontophoretic period was chosen to estimate the steady-state passive flux with the diffusion lag time method. Nevertheless, the low %CV of J_{pas} obtained might be an indication that the model estimation might be better than that with the

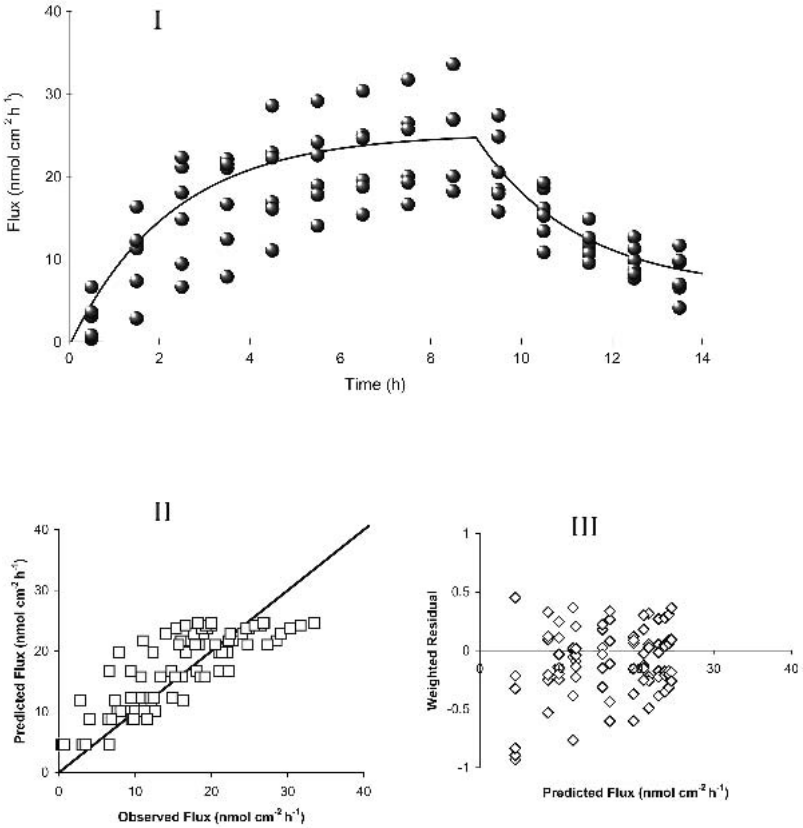

Fig. 5. The fitting result (I) and the evaluation of the predicted flux vs. observed flux (II) and the weighted residual sum of square vs. predicted flux (III) based on the naïve pooling approach of the data-set of rotigotine transport during and post-iontophoresis using model 1.

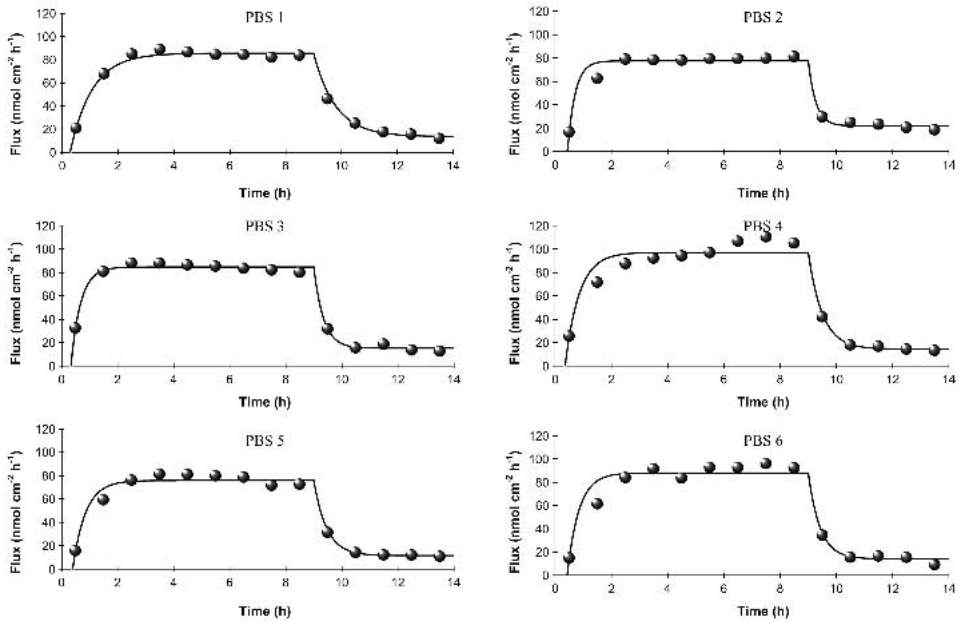

Fig. 6. The individual R-apomorphine (PBS group) transport during 9 h iontophoresis and 6 h post-iontophoresis (n = 6) and the individual model prediction curves based on model 1.

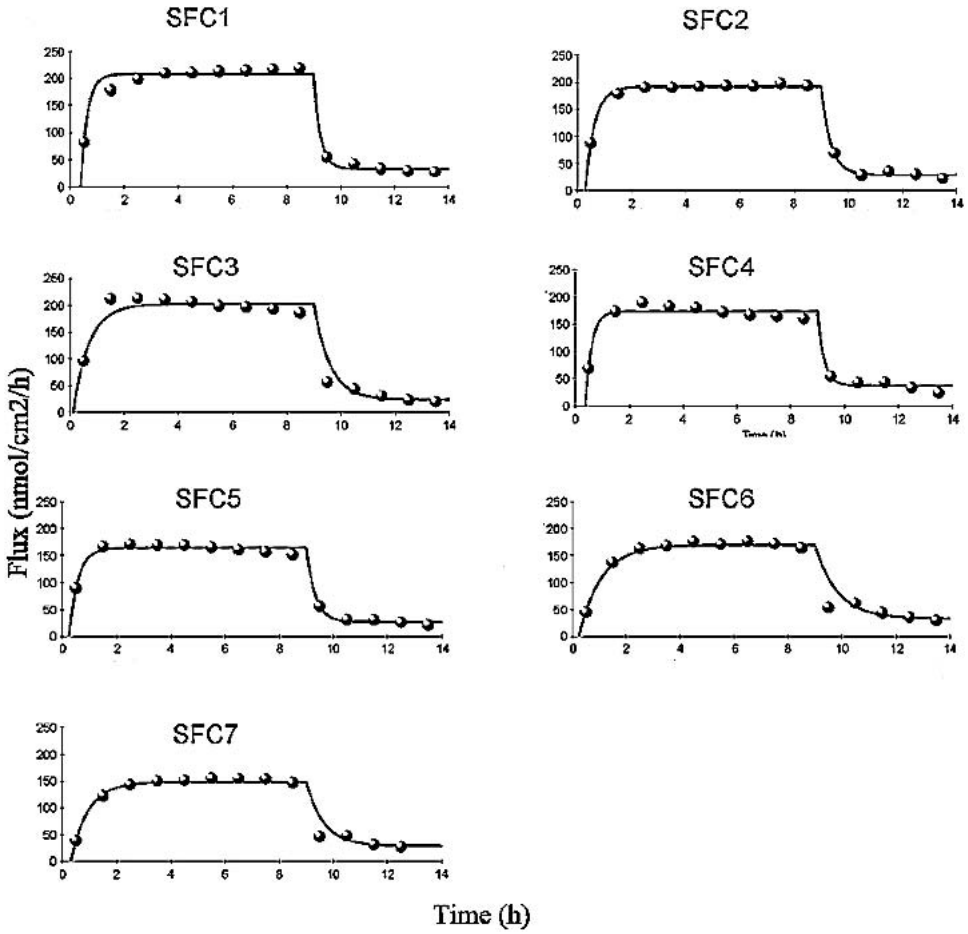

Fig. 7. The individual R-apomorphine (SFC group) transport during 9 h iontophoresis and 6 h post iontophoresis (n = 7) and the individual model prediction curves based on model 1.

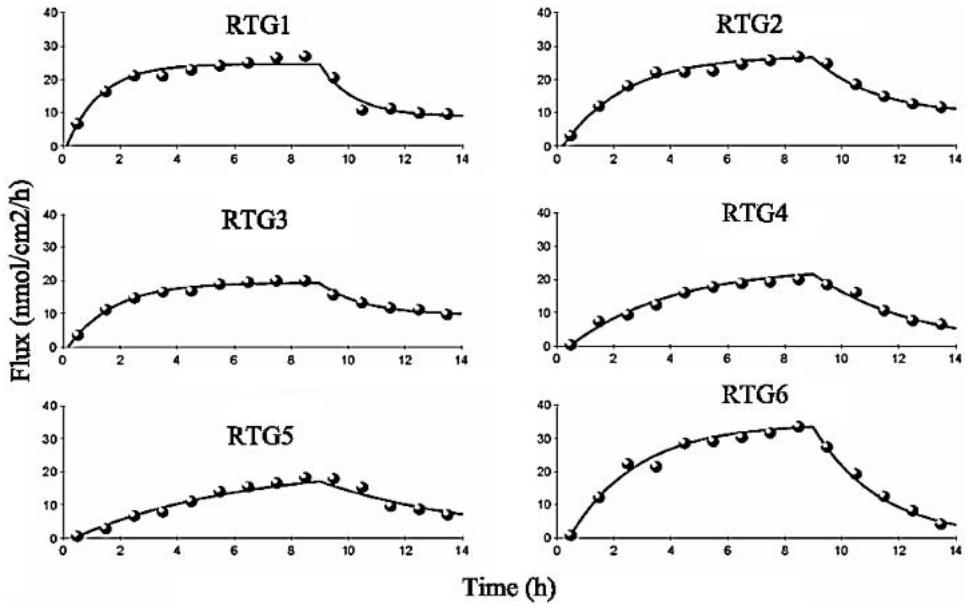

Fig. 8. The individual rotigotine transport during 9 h iontophoresis and 6 h post-iontophoresis (n = 6) and the individual model prediction curves based on model 1.

Table I. The Best-Fit Parameters and Average %CV of Iontophoretic Data R-apomorphine from PBS and SFC Groups and Iontophoretic Data of Rotigotine (RTG) Obtained with the Compartment Model and Comparison to the Published Values Obtained with the Diffusion Lag Time Method

Group	Parameter	Unit	Model prediction		Published value
			Best-fit value	%CV	
PBS	J_{ss}	nmol · cm^{-2} · h^{-1}	84.8 ± 7.7	3	92 ± 14^{NS}
	J_{pas}	nmol · cm^{-2} · h^{-1}	15.1 ± 3.5	6	10 ± 4^{S}
	K_R	h^{-1}	2.4 ± 0.8	13	—
	t_L	h	0.4 ± 0.1	6	—
SFC	J_{ss}	nmol · cm^{-2} · h^{-1}	180.3 ± 21.9	5	181 ± 23^{NS}
	J_{pas}	nmol · cm^{-2} · h^{-1}	30.6 ± 4.6	9	24 ± 3^{S}
	K_R	h^{-1}	2.7 ± 1.2	17	—
	t_L	h	0.3 ± 0.1	32	—
RTG	J_{SS}	nmol · cm^{-2} · h^{-1}	25.3 ± 5.2	6	22.7 ± 5.5^{NS}
	J_{pas}	nmol · cm^{-2} · h^{-1}	Negligible	>1000	—
	K_R	h^{-1}	0.5 ± 0.2	13	—
	t_L	h	0.3 ± 0.1	14	—

^{S}: Significant difference with the model prediction ($p < 0.05$); ^{NS}: not significant difference with the model prediction ($p > 0.05$).

diffusion lag time method. From all of these considerations we are convinced that this modeling approach might be an alternative manner to handle the *in vitro* iontophoretic diffusion data in the future.

Furthermore, the modeling approach has several benefits that are not present in the diffusion lag time method. First, the data can be analyzed directly from the original (flux) data without any requirement to transform the data, which may distort the error distribution of the data (20). Second, the entire data-set is analyzed. Specifically, there is no need to exclude some data points, as is the case in the diffusion lag time method. Thirdly, the proposed compartment model describes the flux as a function of time. This equation is useful to estimate the steady-state flux, even if steady state is not achieved during the duration of experiment.

Moreover, prediction of the *in vivo* plasma concentration vs. time profile is in most cases based on a zero-order input model as proposed by Gibaldi and Perrier (21) as was used by Singh *et al.* to analyze *in vivo* iontophoretic data from several studies (22). However the application of that model is justified only if the steady-state flux is instantaneously achieved. This requirement might be not the case in many iontophoretic *in vivo* studies, in which the drug input rate into the systemic circulation changes as a function of time. The proposed compartmental model describes this input profile and constitutes therefore a suitable basis for prediction of the plasma concentration *vs.* time profile *in vivo* upon administration by transdermal iontophoresis

The addition of parameter I_1 (model 2) does not improve the fit parameter of the experimental data, as in most cases its values were negligible with a large SD. This observation might indicate that, at least in case of R-apomorphine and rotigotine, IDF does not significantly contribute to the drug transport from the skin to the acceptor phase in a direct manner. Interestingly, based on the theory of ionic mobility (14,15), the drug transport during iontophoresis is due to ionized drug migration across the skin as a result of the potential gradient. This implies that the drug migration is a continuous flow of drug ions across the tissue. Although our model analysis seems to be in contradiction with this theory, a consideration about the physicochemical properties of the drug ions must also be taken into account. In fact, the ionic drugs might behave differently from a small ion such as Na^+ that can freely flow as a mobile ion following the current flow. In contrast, drug ions, such as R-apomorphine and rotigotine, are relatively large and have more chance to interact and accumulate in the skin than the small ions. As a result the drug ions mobility due to IDF are reduced and the transport from the skin to the acceptor phase is dominated by a "passive" partitioning into the acceptor phase, which can be indicated by the value of K_R parameter.

Moreover, our approach to use a single parameter K_R as the first-order skin release rate constant is different from the previous approach proposed by Guy *et al.* (23) and Guy and Hadgraft (24) to model passive diffusion across the skin in the *in vivo* situation. In their approach, besides a first-order kinetic rate constant of the drug release from the stratum corneum to the blood capillary or acceptor phase in an *in vitro* situation (the so called k_2), a second first-order rate constant in the opposite direction (from the blood circulatory to the skin), the so called k_3, was also proposed. The latter was included in the model to address the possibility of the back-transfer of the drug from the viable epidermis to the stratum corneum. The proportion of k_2/k_3 was found to be directly related to the octanol-water partition coefficient of the drug (23).

There are at least four reasons to propose a single rate constant of the transport between skin and the acceptor phase rather than also involving k_3. First, most drugs applied by iontophoresis are rather hydrophilic, which reduces the contribution of back diffusion. Second, the addition of this parameter increases the complexity of the model, whereas our aim was to develop a useful model with a relatively simple approach and equation. Third, a complex model makes the prediction of the fit-parameters for a limited number of data points with an acceptable and justified precision more difficult. Fourth, it has been reported elsewhere that when using the previous model for *in vitro* passive permeation, parameter k_3 values were always negligible with the very large SD values even for rather lipophilic drugs. On the basis of these consid-

erations it was concluded that removing this parameter from the model does not reduce its value for describing the *in vitro* transport (25).

Rotigotine, which is relatively lipophilic [log p = 4.03 (12)], had a slower rate of drug release from HSC to the acceptor phase as indicated by a lower K_R than R-apomorphine [log p = 2.15 (26)]. This might indicate that rotigotine has more retention and accumulation in HSC in contrast to R-apomorphine. Moreover, the slow of rotigotine partition into the acceptor phase might also be due to its low solubility in the acceptor phase (PBS pH 7.4) as a consequence of its lipophilic nature. Interestingly for R-apomorphine, the values of K_R were relatively similar in both PBS and SFC groups. This might indicate that surfactant pretreatment does not significantly change the partition rate of R-apomorphine into the acceptor phase.

Introduction of a kinetic lag time parameter (t_L) into the model is aimed to address the required time for the drug molecules to enter the skin compartment. Interestingly, there was a trend that the surfactant pretreatment resulted in a shorter t_L in comparison to the values of PBS group. Although the reduction in t_L was not significant, as the p value was almost at the threshold border (p = 0.07), it might indicate that surfactant pretreatment facilitates the partitioning of the drug from the donor to the stratum corneum due to an increase in stratum corneum permeability. Further studies are required to confirm this analysis.

In our model, we proposed that the time to reach a steady-state flux is dependent on both t_L as a parameter representing how fast the drug enters the skin and K_R as a representative of the rate of release of the drug from the skin to the acceptor phase. An approximation of the time to reach a steady state flux can be estimated according to the following equation:

$$99\%T_{ss} = t_L - \frac{ln(0.01)}{K_R} \tag{20}$$

in which $99\%T_{ss}$ refers to the time to achieve 99% of the steady-state flux.

With this model, it can be deduced that the slow profile declination post-iontophoresis is not only due to the significant passive diffusion post-iontophoresis due to skin barrier perturbation during current application as always addressed before (16,17), but also dependent on K_R. As demonstrated in case of rotigotine, even with a very low J_{pas}, due to the low of K_R, the reduction in flux post-iontophoresis will also be slow. As a result, the passive flux post-iontophoresis was much higher than the passive flux prior to iontophoresis.

Moreover, as aforementioned in the previous section, in this model we do not distinguish between the type of skin membrane. Although it might be too simplistic to the *in vivo* skin situation, the value of fit parameter might address the difference between each type of membrane. If a dermatomed skin is used, K_R might be lower as a result of a slower drug release from the skin due to increase in membrane thickness and also the present of a stagnant tissue fluid at pH 7.4 that might inhibit the drug movement. Furthermore, if an *in vivo* model has also been developed using this modeling approach, comparing the fit parameters from *in vitro* to the *in vivo* situation might reveal to a selection of the best *in vitro* system that really mimics to the *in vivo* situation.

In summary, we have developed mathematical models describing the *in vitro* transport during iontophoresis as well as the transport in post-iontophoretic period. All of the proposed models properly converge to the transport data of PBS, SFC, and RTG groups both for the iontophoretic and the post-iontophoretic periods. However, based on the statistical analysis, the incorporation of parameter I_I does not improve the fitting performance of the model, thereby suggesting a negligible iontophoretic driving force contribution in the mass transfer in the direction from the skin to the acceptor compartment. The excellence of the proposed models is also demonstrated from the estimated values of parameters of J_{ss} from both groups that are statistically identical ($p > 0.05$) to the published values obtained with the diffusion lag time method. Moreover, time to achieve steady-state flux can be estimated based on the parameter t_L and the reciprocal value of parameter K_R. In addition, whether the drug molecules accumulate in the skin might also be deduced based on K_R parameter.

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