

## Transdermal alniditan delivery by skin electroporation

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### Abstract

The aim of this study was to evaluate the transdermal permeation of alniditan by electroporation and to compare with iontophoretic delivery. The influence of the electrical parameters of electroporation was investigated in vitro using a factorial design study. The transdermal flux of alniditan was enhanced by two orders of magnitude by application of high voltage electrical pulses. The electrical parameters of electroporation – i.e. the voltage, the duration and the number of pulses – allowed a control of drug permeation. Both transport during and after pulsing were shown to be important for alniditan transdermal delivery by electroporation. Electroporation was found more efficient in promoting alniditan permeation than an iontophoresis transferring the same amount of charges.   1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Electroporation; Iontophoresis; Alniditan; Mechanism; Comparison

### 1. Introduction

Because of the excellent barrier properties of the stratum corneum, few drugs can passively penetrate through the skin at sufficient quantities to achieve their therapeutic effects. Many efforts have been made over the years with the aim to overcome the stratum corneum barrier. Chemical or physical approaches were explored [1].

Iontophoresis is now considered as an interesting method for enhancing the transdermal permeation of drugs. It consists in applying for min or h a low current density as a driving force [1–4].

Skin electroporation – the application of short (from Ms to less than s) high voltage pulses (from

about 100 to 1000 V) – has recently been shown as a promising method to overcome the stratum corneum barrier [5,6]. The hypothetical mechanism of electroporation involves the formation of new aqueous pathways across the stratum corneum lipid bilayers [7,8]. The transdermal transport of molecules has been shown to be increased by up to four orders of magnitude [9]. However, the investigation of skin electroporation for transdermal drug delivery was restricted until now to a small number of molecules such as calcein [5] and sulphorhodamine [10], drugs like metoprolol and fentanyl [6,11], peptides (LHRH [12]), oligonucleotides [13,14] or polyanions (Heparin [15]).

Alniditan ( $pK_a$  8.3 and 11.5; molecular weight 302.4;  $P_{oct/water\ pH\ 8}$ : 1.59) is a novel chemical entity issue from Janssen Research for the treatment of migraine. It induces a selective vasoconstriction and

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acts primarily on the 5-HT<sub>1D</sub> serotonergic receptor. Because of its poor oral bioavailability, different routes of administration were evaluated. The feasibility of the transdermal delivery of alniditan by iontophoresis was demonstrated *in vitro* and confirmed in a phase I clinical trial [4].

The aim of this study was to investigate *in vitro* the transdermal delivery of alniditan by electroporation. The influence of various electroporation protocols was first studied. The mechanism of alniditan permeation enhancement was also examined. Finally, the transport of alniditan by electroporation was compared to iontophoretic transdermal permeation.

## 2. Material and methods

### 2.1. Chemicals

Alniditan and [<sup>3</sup>H] – alniditan were supplied by Janssen (Beerse, Belgium). The salts used to prepare the buffers (analytical grade) were purchased from UCB (borate; RPL, Leuven, Belgium) and from Merck–Belgolabo (acetate and phosphate; Overijse, Belgium). Glucose was obtained from UCB. All solutions were prepared in ultra pure water (Sation 9000, Sation, Barcelona, Spain).

### 2.2. Material and procedures

Full thickness abdominal skin was directly used after excision from male hairless rats (7 to 11 weeks old; Iops hairless mutant, Iffa Credo, St Germain-les-Arbresles, France). Three centimetres-squared skin separated the donor and receptor compartments of polycarbonate vertical cells (Makrolon, Obra, Liège, Belgium). The donor compartment, in contact with the stratum corneum, was filled with 1.5 ml of drug solution containing alniditan 5 mg/ml, [<sup>3</sup>H]-alniditan 1  $\mu$ Ci/ml and acetate (pH 5) or borate (pH 9.5) buffer 0.05 M. The dermis side of the skin was facing the receptor compartment (7.5 ml) filled with phosphate buffer (pH 7.4, 0.024 M) isotonised with glucose, thermostatised at 37°C and continuously stirred. Electrodes (1 cm<sup>2</sup>, pure platinum, SA Johnson Matthey, Brussels, Belgium) were inserted on both sides of the skin and separated by 1 cm. The anode was immersed in the donor compartment.

Electrodes were connected to Easyject Plus<sup>®</sup> (Equibio, Seraing, Belgium), a high voltage pulser delivering exponential-decay pulses [6]. The electrical parameters defining the electroporation protocols are the voltage applied (V), the number of pulses (N) and the time constant ( $\tau$ ). The time constant is the time interval between the beginning of the pulse (maximum voltage) and the moment at which the voltage reaches 37% of its maximal value. The amount of transferred charges (Q) was calculated by the sum of the integral of  $I(t) dt$  of each pulse. The electrical energy applied by the pulses to the electrodes can be approximated by:  $E = N\tau(V_i^2 - V_f^2) / 2R_{\text{cell}}$ , where  $R_{\text{cell}}$  is the total resistance of the diffusion cell [20]. A few samples were freeze-dried to remove the tritium label which might have exchanged with water. No tritium label was released from alniditan after electroporation (data not shown), demonstrating the stability of [<sup>3</sup>H]-alniditan. Iontophoresis was performed as previously described [4].

An experimental design of three factors at two levels was used to study the effects of the electrical parameters on alniditan transdermal permeation. The choice of the protocols was based on previous data [9,11,20]. Influence of the voltage (100 and 200 V), time constant (about 100 and 500 ms) and the number of pulses (5 and 10, with an interpulse time of 1 min) was investigated. After logarithm transformation, the results (log cumulative quantities and log fluxes) were analysed by General Linear Model (Systat program) [11]. The logarithm transformation was required to obtain homoscedasticity of the variances (Bartlett test).

In order to check whether alniditan permeation occurred during or after the pulses, the donor compartment was filled i) with the alniditan solution during and after the pulses ii) with the alniditan solution during the pulses and replaced thereafter by a drug-free buffer iii) with a drug-free buffer during electroporation and replaced thereafter by the alniditan solution. The electroporation protocol used to study the mechanism of alniditan permeation involved application of  $5 \times (200 \text{ V} - 500 \text{ ms})$ .

The permeation studies were followed for 6 h. Samples of receptor solution (0.4 ml) were withdrawn at regular intervals and replaced by an equal volume of drug free buffer. Drug concentration was

determined by measuring the radioactivity with a liquid scintillation counter (Ready Safe Beckman, Wallac 1410 LKB). The ratio of the cumulative quantities detected in the receptor compartment to the membrane area was plotted as a function of time. The flux (per surface area) was deduced from the slope of the linear portion of each curve. Results are expressed as means  $\pm$  standard errors of the means (SEM). Statistical analysis was performed on fluxes using a one way analysis of variance (Tukey HSD test,  $P < 0.05$ ). The normal distribution was obtained after log transformation (Kolmogorov – Smirnov test).

### 3. Results

#### 3.1. Influence of electroporation on alniditan permeation

Five pulses of 100 V were applied with mean time constants of 603 ms (pH 5) and 548 ms (pH 9.5). Electroporation experiments (at pH 5 and 9.5) were compared to passive diffusion performed at both pH.

Cumulative quantities of alniditan are shown in Fig. 1. Under passive conditions, transdermal flux of alniditan was very low ( $0.12 \pm 0.02 \mu\text{g}/\text{cm}^2$ ). Application of five high voltage pulses caused a 100-fold increase in alniditan permeation as compared to passive diffusion. The permeation flux remained

elevated for at least 6 h. This elevated post-pulse flux might come from alniditan reservoir created within the skin during pulsing and/or from an increased permeability induced by electroporation.

As reported in the literature, a dramatic drop in skin resistance was observed during the first electrical pulse [5,6,16,17]. The impedance values approximated by the Easyject plus<sup>®</sup> electroporation device fell from about  $100 \text{ k}\Omega \text{ cm}^{-2}$  to about  $600 \Omega \text{ cm}^{-2}$  during the pulse.

#### 3.2. Influence of pH on alniditan transdermal permeation

Alniditan permeation by electroporation was studied at two ionisation states of the drug. At pH 5 (acetate buffer 0.05 M) and pH 9.5 (borate buffer 0.05 M), alniditan carries one and two positive charges respectively (Fig. 1).

No statistical difference was found between drug fluxes by electroporation at pH 5 or 9.5. However, since the drug is less stable at pH 9.5, pH 5 was chosen for the following experiments.

#### 3.3. Influence of electrical factors on alniditan transdermal permeation

The experimental protocol was selected because it was previously shown to provide a dramatic enhancement in transdermal transport of other drugs [6,9,11,18]: i) Exponential-decay pulses were more efficient in increasing drug permeation than square wave pulses. ii) A small number of long duration pulses seemed at least as efficient as a large number of short duration pulses.

In order to evaluate the influence of the voltage, time constant and number of pulses on alniditan permeation, a factorial design study with three factors at two levels was performed [11]. The low (–1) and high (+1) levels were respectively 100 V and 200 V for the voltage applied to the electrodes, 100 ms and 500 ms for the duration and 5 and 10 for the number of pulses. Fluxes are shown in Fig. 2 and corresponding cumulative quantities are given in Table 1.

A significant enhancement in drug flux was induced by electroporation versus passive diffusion (Tukey HSD test,  $P < 0.05$ ). The influence of each

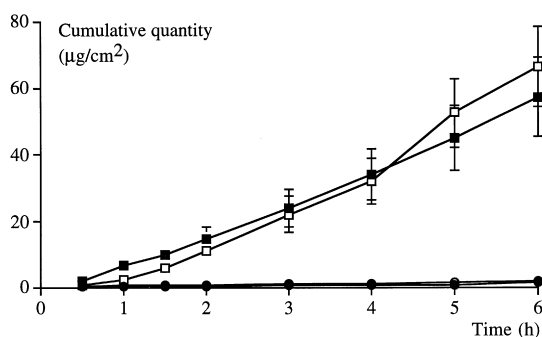


Fig. 1. Alniditan cumulative quantity ( $\pm$ SEM) versus time detected in the receptor compartment after electroporation performed at pH 5 ( $5 \times (100 \text{ V} - 603 \text{ ms})$ ; acetate buffer, 0.05 M; closed square) or pH 9.5 ( $5 \times (100 \text{ V} - 548 \text{ ms})$ ; borate buffer, 0.05 M; open square) or after passive diffusion performed at pH 5 (closed circle) and 9.5 (open circle).

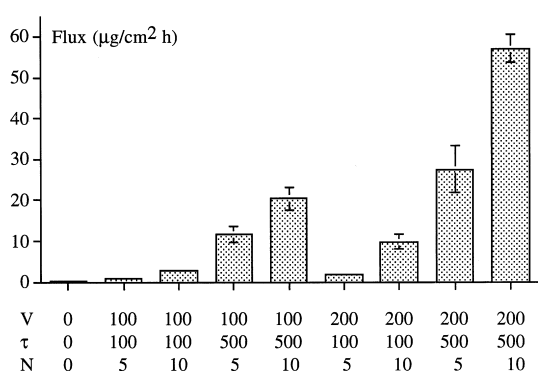


Fig. 2. Alniditan transdermal permeation fluxes by electroporation as a function of the protocol ( $V$ : 100 and 200 V, time constant  $\tau$ : 100 and 500 ms and number of pulses  $N$ : 5 and 20). The donor solution contained alniditan (5 mg/ml) in an acetate buffer pH 5, 0.05 M.

electrical factor is listed below and the results of the factorial analysis of variances are detailed in Table 2.

### 3.4. Voltage

The influence of the voltage (applied to the electrodes) on cumulative quantities of drug detected in the receptor compartment is shown in Fig. 2. Increasing the voltage significantly enhanced the drug flux ( $P < 0.05$ , Tukey test), except when five pulses were applied with a time constant of 100 ms. However, the overall effect of voltage was shown to be not significant in the factorial design (Table 2).

### 3.5. Time constant

The influence of two time constants was investi-

Table 1

Alniditan transdermal permeation fluxes and cumulative quantities as a function of electroporation protocol

| Treatment | V<br>(V) | $\tau$<br>(ms) | N  | Cumulative<br>quantities at 6 h<br>( $\mu\text{g}/\text{cm}^2$ ) | Flux<br>( $\mu\text{g}/\text{cm}^2 \cdot \text{h}$ ) | n |
|-----------|----------|----------------|----|------------------------------------------------------------------|------------------------------------------------------|---|
| Diffusion | 0        | 0              | 0  | 1.5 ± 0.5                                                        | 0.1 ± 0.0                                            | 4 |
| 1         | 100      | 100            | 5  | 8.8 ± 2.3                                                        | 0.9 ± 0.1                                            | 5 |
| 2         | 100      | 100            | 10 | 20.8 ± 5.5                                                       | 2.8 ± 0.5                                            | 4 |
| 3         | 100      | 500            | 5  | 57.2 ± 11.8                                                      | 11.7 ± 1.9                                           | 6 |
| 4         | 100      | 500            | 10 | 108.7 ± 18.8                                                     | 20.3 ± 2.9                                           | 4 |
| 5         | 200      | 100            | 5  | 7.1 ± 0.9                                                        | 1.7 ± 0.7                                            | 4 |
| 6         | 200      | 100            | 10 | 49.7 ± 11.6                                                      | 9.8 ± 1.8                                            | 5 |
| 7         | 200      | 500            | 5  | 155.8 ± 32.6                                                     | 27.4 ± 5.7                                           | 6 |
| 8         | 200      | 500            | 10 | 387.1 ± 34.4                                                     | 56.8 ± 3.5                                           | 6 |

Table 2

General linear model analysis.

| Variable        | log fluxes  |         | log cumulative quantities |         |
|-----------------|-------------|---------|---------------------------|---------|
|                 | Coefficient | P value | Coefficient               | P value |
| Voltage (V)     | 0.002       | 0.690   | -0.002                    | 0.727   |
| Number (N)      | 0.192*      | 0.050   | 0.085                     | 0.449   |
| Time ( $\tau$ ) | 0.008*      | 0.000   | 0.005*                    | 0.004   |
| Interactions:   |             |         |                           |         |
| V*N             | 0.001       | -0.098  | 0.001                     | 0.102   |
| V* $\tau$       | 0.000       | 0.775   | 0.000                     | 0.188   |
| $\tau$ *N       | -0.000*     | 0.007   | -0.000                    | 0.296   |

The analysis was based on log fluxes data or log cumulative quantities (detected at 5 h). The coefficient and significance of the electrical factors (voltage (V), number of pulses (N) and time constant ( $\tau$ )) or interaction terms are given (\* indicates a significant difference:  $P < 0.05$  is significant;  $P < 0.01$  is highly significant)

gated. As shown in Fig. 2 and in Table 1, the increase in time constant from  $98 \pm 5$  to  $519 \pm 22$  ms induced a significant enhancement in alniditan permeation. The influence of the time constant on alniditan transport by electroporation was significant (Table 2).

### 3.6. Number of pulses

Alniditan permeation was studied after application of five and ten single electrical pulses. The inter-pulse time was 1 min. Multiplying the number of pulses significantly enhanced the drug permeation (Table 1; Tukey HSD test,  $P < 0.05$ ), except when 100 V – 500 ms pulses were applied. The factorial analysis (Table 2) revealed a significant effect of the number of pulses on alniditan flux. Moreover, a significant interaction appeared between the time constant and the number of pulses.

### 3.7. Mechanisms of transdermal delivery of alniditan by electroporation

Alniditan transport could occur during pulsing by electrophoresis and diffusion through new aqueous pathways and/or after pulsing by diffusion through the electropermeabilised skin [6,18].

In order to understand the mechanism of alniditan permeation by electroporation, alniditan was placed in the donor compartment during and/or after high voltage pulses. For this set of experiments,  $5 \times (200 \text{ V} - 500 \text{ ms})$  were applied.

When alniditan was in the donor compartment during and after pulses application, the quantity measured at 6 h was  $128.5 \pm 19.7 \mu\text{g}/\text{cm}^2$  (Fig. 3).

When the drug was present only during pulsing (i.e. the donor compartment was emptied and replaced by a drug-free buffer after the last pulse), the cumulative quantity at 6 h decreased to  $49.3 \pm 4.1 \mu\text{g}/\text{cm}^2$ . These results indicate that about half of alniditan is transported into or through the skin during the 5 min of electrical treatment.

When alniditan was placed in the donor compartment immediately after pulsing, the quantity at 6 h increased from  $1.5 \pm 0.5 \mu\text{g}/\text{cm}^2$  (passive diffusion control) to  $48.1 \pm 5.2 \mu\text{g}/\text{cm}^2$  (post-pulsing passive diffusion). This elevated transdermal post pulse flux was due to electrically-induced changes in skin

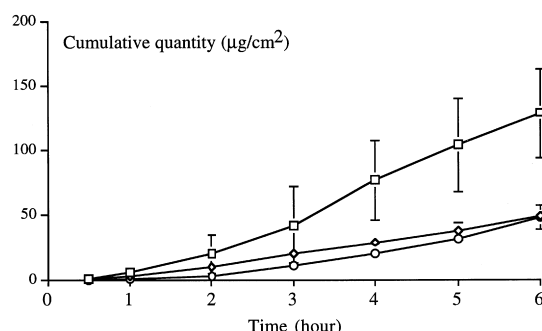


Fig. 3. Cumulative quantity of alniditan (mean  $\pm$  SEM) versus time.  $5 \times (200 \text{ V} - 500 \text{ ms})$  were applied. The donor compartment was filled i) with the alniditan solution (5 mg/ml; acetate buffer pH 5, 0.05 M) during and after the pulses (square) ii) with the alniditan solution during the pulses and replaced thereafter by the drug-free buffer (rhomb) iii) with the drug-free buffer during electroporation and replaced thereafter by the alniditan solution (circle).

permeability. Moreover, the fact that the flux remained high for 6 h (Fig. 3) suggests a weak reversibility in skin permeability enhancement induced by these electrical conditions. The drug fluxes measured between 1–2 h or 5–6 h after pulsing were  $2.43 \pm 1.2$  and  $16.37 \pm 4.79 \mu\text{g}/\text{cm}^2$ .

### 3.8. Comparison with iontophoresis

In order to check whether skin electroporation could be more potent than iontophoresis to promote alniditan transdermal permeation, the alniditan transport by electroporation was compared to iontophoretic permeation. Iontophoresis conditions were similar to the clinical study [1] and compared to a low number of exponential-decay pulses of high voltage – long duration [6,9,11,18].

The application of  $5 \times (100 \text{ V} - 500 \text{ ms})$ ,  $10 \times (200 \text{ V} - 100 \text{ ms})$  pulses induced the same permeation enhancement as an iontophoresis applied 30 min with a mean current density of  $0.4 \text{ mA}/\text{cm}^2$  (borate buffer, 0.1M). 1 h iontophoresis at a mean current density of  $0.4 \text{ mA}/\text{cm}^2$  induced the same drug transport as the application of  $5 \times (200 \text{ V} - 500 \text{ ms})$  (Fig. 4 and Table 3). At the same quantities of charges transferred, alniditan transport by electroporation was enhanced by a factor of three as compared to iontophoresis.

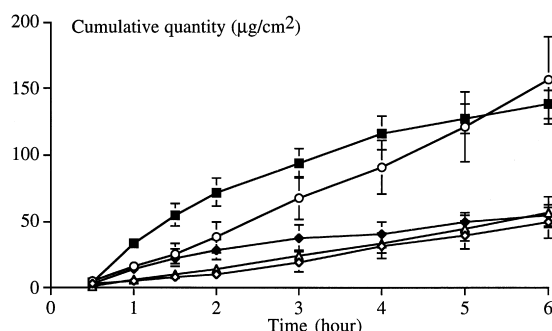


Fig. 4. Cumulative quantity of alniditan versus time detected in the receptor compartment after iontophoresis or electroporation. Various electroporation protocols were applied:  $10 \times (200 \text{ V} - 100 \text{ ms})$  (open rhomb),  $5 \times (200 \text{ V} - 500 \text{ ms})$  (open circle),  $5 \times (100 \text{ V} - 500 \text{ ms})$  (open triangle) and compared to iontophoresis performed at a current density of  $0.4 \text{ mA/cm}^2$  during 30 min (closed rhomb) or 1 h (closed square).

#### 4. Discussion

Alniditan, an antimigraine drug, has a poor oral bioavailability. Because of its therapeutic indications, a rapid delivery is desirable. The feasibility of transdermal delivery of alniditan by iontophoresis was previously shown [4]. The aim of this study was to investigate the transdermal delivery of alniditan by electroporation. The enhancement factor versus passive diffusion and iontophoresis, the influence of the electrical parameters and the mechanism of alniditan permeation by electroporation were investigated.

To investigate whether electroporation could significantly increase alniditan permeation, electroporation was compared to passive diffusion. As expected,

passive permeation of alniditan was very low at both pH studied ( $0.12 \pm 0.02 \text{ µg/cm}^2$ ) [4]. Application of high voltage pulses significantly increased the permeation by more than two orders of magnitude. Similar enhancement factors were found for other small positively charged organic drugs in the studies using hairless rat skin [6,9,11].

Previous studies showed that the different electrical factors (voltage, time constant and number of pulses) allow control of the quantity of drug delivered by electroporation [5,6,11,20]. To check whether the electrical parameters also allow control of the quantity of alniditan delivered, different protocols were applied and a factorial analysis was performed. The factorial analysis revealed that the time constant and the number of pulses significantly influenced the alniditan permeation flux. Even though the Anova test demonstrated in most cases a significant effect no significant influence was found for the voltage in the factorial analysis (Table 2). Furthermore, using the coefficient, the effects of each factor on increasing alniditan permeation can be classified: the higher the coefficient, the greater the effect. The greatest influence was found for the number of pulses, followed by the time constant. The non significant effect and weak influence of the voltage contrast with previous studies which found a significant influence and a great effect of the voltage [9,11].

The reason for the discrepancy with the previous studies can probably be found in the range of voltage and the variable donor solutions used in the different studies. Application of 100 or 200 V corresponded to

Table 3

Alniditan cumulative quantities detected at 0.5, 1 or 6 h as a function of electrical treatment, energy applied to the electrodes and amount of charges transferred during the electrical treatment. (mean  $\pm$  SEM)

| Protocol                                           | Cumulative quantity ( $\text{µg/cm}^2$ ) |                |                  | Q(C)  | E(J)  |
|----------------------------------------------------|------------------------------------------|----------------|------------------|-------|-------|
|                                                    | 0.5 h                                    | 1 h            | 6 h              |       |       |
| $5 \times (100 \text{ V} - 500 \text{ ms})$        | $2.0 \pm 0.7$                            | $6.7 \pm 1.3$  | $57.2 \pm 11.8$  | 1.0 C | 111.8 |
| $10 \times (200 \text{ V} - 100 \text{ ms})$       | $3.6 \pm 3.1$                            | $5.3 \pm 4.1$  | $49.7 \pm 11.6$  | 0.9 C | 89.9  |
| $5 \times (200 \text{ V} - 500 \text{ ms})$        | $5.5 \pm 2.1$                            | $16.7 \pm 4.6$ | $155.8 \pm 32.6$ | 2.2 C | 224.6 |
| $0.5 \text{ h} - 0.4 \text{ mA/cm}^2$ <sup>a</sup> | $4.0 \pm 1.1$                            | $14.4 \pm 4.4$ | $54.8 \pm 8.0$   | 2.2 C | 7.8   |
| $0.5 \text{ h} - 0.4 \text{ mA/cm}^2$ <sup>b</sup> | $5.2 \pm 1.3$                            | $14.0 \pm 3.5$ | $75.9 \pm 6.4$   | 2.2 C | 7.8   |
| $1 \text{ h} - 0.4 \text{ mA/cm}^2$ <sup>a</sup>   | $4.5 \pm 1.3$                            | $33.2 \pm 4.6$ | $138.0 \pm 10.7$ | 4.3 C | 15.6  |

Experiment performed with:

<sup>a</sup> Pt electrodes and 0.1 M borate buffer,

<sup>b</sup> Ag/AgCl with 0.05 M ethanolamine buffer.

a transdermal voltage ranging from about 15 up to 25 V. In contrast, in the factorial analysis of fentanyl transport by electroporation, the voltage ranged between 50 V to 250 V which led to a significant increase in transmembrane voltage from about 20 up to 60 V [11].

The mechanism of alniditan transport by electroporation has been investigated. A dramatic decrease in skin resistance has been measured during pulsing, consistent with the hypothesis that new aqueous pathways are created by high voltage pulses [10].

About half of alniditan was transported into or through the skin during the 5 min of pulses application. The transport during electroporation could occur by electrophoresis and increased diffusion through the permeabilised membrane. A good correlation was found between the amount of transferred charges and the cumulative quantities detected at 6 h (Table 1, correlation coefficient  $r^2=0.967$ ). This correlation puts forward the importance of electrophoretic movement in alniditan permeation by electroporation. Furthermore, the sustained release of alniditan in the receptor compartment suggests the creation of a drug reservoir formed during pulsing. Indeed, a reservoir has often been demonstrated in transdermal transport studies of other compounds [6,15,18]. Due to the skin model used in this study, the drug must diffuse or could partly be trapped in the dermis.

In addition, significant permeation occurred after pulsing when alniditan was added after electroporation (about half of alniditan diffused after pulse application). This increase in passive diffusion due to electroporation (enhancement factor of about 30) and the dramatic decrease in skin impedance after electroporation (data not shown) demonstrate that the stratum corneum is permeabilised by high voltage pulses. Furthermore, since instant fluxes remained high, the skin permeability enhancement was not totally reversible within 6 h *in vitro*.

The influence of the drug charge on the transdermal transport by electroporation has not been clearly established yet. An equivalent transdermal permeation for a single or double charged molecule (Fig. 1) was found in this study. This result might be surprising in regard with the statement: the higher the charge, the higher the electrophoretic drift.

However, according to the physico-chemical properties and the concentration of the molecule transported, the mechanisms of molecular transport can vary [10,18]. Pliquett and Weaver comparing calcein (−4 charged polar molecule, 623 Da) and sulforhodamine transport (−1 charged polar molecule, 607 Da) showed that the contribution of electrophoresis was important for calcein while diffusion through electroporated skin dominated for sulforhodamine [10]. The electrophoretic movement could be more important in the case of the double charged molecule although passive diffusion could dominate for the +1 charged alniditan. Moreover, iontophoretic delivery of the single charged alniditan was higher than +2 alniditan suggesting that alniditan could interact with the skin [4].

The final objective of this study was to compare the alniditan delivery by electroporation and iontophoresis. It has been argued that although both electroporation and iontophoresis involve electric fields, the two approaches are fundamentally different. Iontophoresis is known to act primarily on the drug by an electrophoretic drift, while the contributions of electroosmotic flow and increased skin permeability are mainly involved as secondary events. On the other hand, electro-induced increased permeability and electrophoretic movement through both previously existing and newly-created transport pathways have been proposed as mechanisms of drug permeation enhancement by electroporation [18]. Further, an electroloading can occur [5,6]. As for metoprolol and fentanyl, similar cumulative quantities were obtained with both methods *in vitro* in the experimental conditions used [6,11]. Nevertheless, at equivalent charges transferred, application of electroporation has always been shown more efficient in transdermal permeation enhancement for small organic molecules and macromolecules than iontophoresis [5,9,15].

Different observations indicate in the present study a difference in the mechanisms of action of both approaches. i) First, the higher cumulative quantities induced by electroporation and iontophoresis transferring the same amount of charges suggests an increase in drug transport number. While a low transport number indicates hindered transport, larger transport numbers indicate less hindrance and in this case suggest the creation of large transport pathways

during electroporation [19]. ii) The difference in permeation kinetics underscores also the difference in mechanism of action between the two electrically enhanced transdermal drug delivery. Although iontophoretic fluxes progressively decreased, constant fluxes could easily be deduced from the electroporation experiments. The difference in permeation profiles between iontophoresis and electroporation could be explained by long-lived changes in skin permeability and/or by a drug loading induced by electroporation.

## 5. Conclusion

Application of high voltage electrical pulses significantly increased the transdermal permeation of alniditan. The flux of alniditan across the skin was dependent on the protocol applied, i.e. the voltage, the number and the time constant. Both transport during and after pulsing were shown to be important for alniditan transdermal delivery by electroporation. Further, the creation of a drug reservoir within the skin during pulsing and the increase in skin permeability contributed to elevated drug flux *in vitro*. Electroporation was found more efficient in promoting alniditan permeation than iontophoresis transferring the same amount of charges, suggesting the creation of larger pathways during high voltage pulses.

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