Expert Opinion

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Non-invasive iontophoretic delivery of peptides and proteins across the skin

Taís Gratieri, Dhaval Kalaria & Yogeshvar N Kalia†
University of Geneva & University of Lausanne, School of Pharmaceutical Sciences, Geneva, Switzerland

Introduction: Peptides and proteins are playing an increasingly important role in modern therapy. Their potency and specificity make them excellent therapeutic agents; however, their physicochemical properties and stability requirements almost invariably necessitate their administration by subcutaneous, intramuscular or intravenous injection. Controlled non-invasive administration using more patient-friendly advanced delivery technologies may combine the precision afforded by parenteral administration with improved compliance and the potential for individualized therapy.

Areas covered: Transdermal iontophoresis enables hydrophilic charged molecules to be administered through the skin in an effective, non-invasive, patient-friendly manner. This review presents the basic concepts and an analysis of the effect of iontophoretic parameters and molecular properties on electrotransport rates across the skin along with a summary of experimental studies with peptides and proteins. The last section covers other techniques used in conjunction with iontophoresis.

Expert opinion: It has long been known that iontophoresis can administer therapeutic amounts of biologically active peptides into the body. More recent studies have shown that it is also capable of delivering structurally intact, functional proteins non-invasively into and across intact human skin. The next step is to develop cost-effective and easy-to-use iontophoretic patch systems that ensure biomolecule stability, optimize delivery efficiency and address unmet therapeutic needs.

Keywords: iontophoresis, non-invasive, peptide and protein therapeutics, skin, topical, transdermal

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1. Introduction

In recent years an increasing number of peptides and proteins have been proposed as active pharmaceutical ingredients. The average number of new candidates studied has steadily increased from 1.2 a year in the 1970s, to 4.6 a year in the 1980s, to 9.7 a year in the 1990s, to 16.8 a year so far in the 2000s [1]. Given the improved efficacy of peptide synthesis and protein production, it is likely that these potent, selective therapeutics will be the subject of further investment from the pharmaceutical industry [2]. New rational synthetic strategies could also shorten the time spent on research and development and reduce production costs [3] and together with the traditional benefits of peptides (high biological activity, high specificity and low toxicity) make them even more attractive as therapeutic candidates [4,5]. Indeed, advances in biotechnology and bioinformatics have enabled significant progress in the identification of

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Article highlights.

- The control afforded by constant current iontophoresis over transport rates means that peptide/protein delivery kinetics can mimic endogenous secretion profiles.
- Moreover, complex input kinetics can be used to optimize and individualize therapy.
- Several therapeutic peptides, including luteinizing hormone-releasing hormone and its analogues, vasopressin, somatostatin, somatorelin calcitonin, human parathyroid hormone and insulin, have been investigated in preclinical and Phase I clinical trials; importantly, these confirmed that biological activity was retained post-iontophoresis.
- More recently, it has been demonstrated that it is possible to deliver intact functional proteins across the skin non-invasively. Two-pronged approaches combining iontophoresis with the use of drug carriers or the reversible impairment of skin barrier function have been proposed in order to find potential synergies and to expand further the range of molecules that can be delivered by the transdermal route.

This box summarizes key points contained in the article.

proteins and rational drug design [6,7]. Medicinal chemistry approaches have been used to produce proteolytically stable molecules and to increase receptor selectivity [8]. In many cases chemical modifications have also been applied successfully to overcome the short half-lives of peptide and protein drug candidates [9]. However, the challenge of reaching and maintaining therapeutic levels in the target tissue remains. Poor oral bioavailability means that biopharmaceuticals are primarily administered by subcutaneous, intramuscular or intravenous injection; these invasive procedures entail varying degrees of discomfort and pain for patients and may have an impact on compliance. Furthermore, the pharmacokinetics may result in appreciable variability in blood concentrations with associated side effects [10].

In common with injection-based therapy, the more patient-friendly transdermal route avoids degradation in the gastrointestinal tract and potential first-pass metabolism; although the skin does contain metabolizing enzymes, drug molecules encounter a significantly less challenging enzymatic barrier [11]. Approved peptide and protein therapeutics vary significantly with respect to their physicochemical properties; for example, the molecular mass of romiplostim is almost 170-fold higher than that of thyrotropin-releasing hormone (60 kDa and 360 Da, respectively). Given the properties of their constituent amino acids it is to be expected that they have significant hydrophilic character and are often charged at physiological pH. These physicochemical properties do not favor their partitioning into the lipid-rich intercellular space in the stratum corneum. Nevertheless, these so-called 'undesirable' properties make peptides and proteins excellent candidates for iontophoretic delivery [12,13].

Iontophoresis is a non-invasive technique that involves application of a mild electric current to enhance the penetration of hydrosoluble, ionized molecules into and through tissues [14]. The amount of substance delivered is directly proportional to the quantity of charge passed and depends on the intensity of the applied current, the duration of current application and the area of the skin surface in contact with the active electrode compartment. In addition to precise control over delivery, other advantages include faster onset and offset times. Moreover, the current profile can be customized to enable complex drug input kinetics, for example, pulsatile delivery. This makes iontophoresis very attractive for the treatment of metabolic diseases, as natural physiological secretion profiles can be mimicked. Iontophoresis has been investigated for the delivery of peptides since the 1980s [15,16], with insulin being the most studied molecule at that time [17,18]. Progress in the last decade in microelectronics and engineering processes enabled the development of miniaturized and cost-effective delivery systems [19]; thus, compact fully integrated iontophoretic systems are now available that are far-removed from the first-generation fill-on-site devices. Transdermal iontophoresis is one of the few transdermal technologies that has resulted in the development of products approved by the regulatory authorities – LidoSite® (Vyteris Inc., Fairlawn, NJ, USA) (topical lidocaine delivery for local anesthesia) and Ionsys™ (Alza Corporation, Mountain View, CA, USA) (systemic fentanyl for acute postoperative pain). Although both lidocaine and fentanyl are low-molecular-mass therapeutics, given the excellent patient compliance for passive transdermal systems and their financial success, the appeal of delivering peptides and proteins less invasively should encourage the pharmaceutical industry to explore transdermal technologies such as iontophoresis. The first part of the review presents the basic concepts and an analysis of the effect of iontophoretic parameters and molecular properties on electrotransport rates across the skin; the second part provides an overview of studies with therapeutic peptides and proteins, and the paper concludes with a summary of combination approaches using iontophoresis with a second delivery technology.

2. Iontophoretic transport mechanisms

Molecular transport during iontophoresis can be attributed to three component mechanisms: (enhanced) passive diffusion, electromigration (EM) and convective solvent flow, also called electroosmosis (EO) (**Figure 1**). Assuming that each phenomenon is independent, the total flux of a molecule during iontophoresis can be described as the sum of the fluxes resulting from these three processes (Nernst–Planck theory) [20]:

$$J_{TOT} = J_P + J_{EM} + J_{EO} \quad (1)$$

where J_{TOT} is the total flux, J_P is the passive flux, and J_{EM} and J_{EO} are the fluxes resulting from EM and EO,

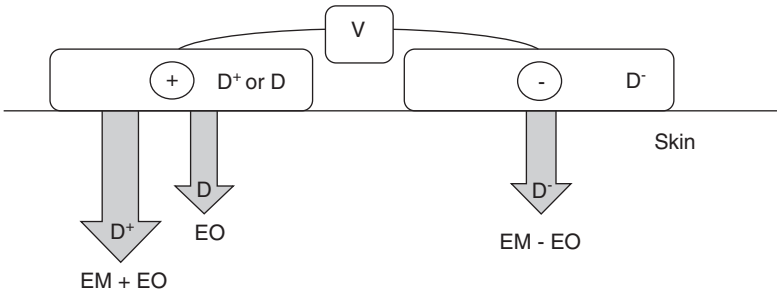

Figure 1. Electromigration (EM) and electroosmosis (EO) and their roles in the transport of charged and neutral molecules during iontophoresis under physiological conditions.

respectively. The role of passive diffusion in iontophoretic delivery is usually minor compared with the two other mechanisms [21].

2.1 Electromigration

Electromigration refers to the ordered movement of the ions in the presence of the applied electric field. This process may be described by Faraday's law [14,20,22-24]:

$$J_{EM} = \frac{It_D}{AFz_D} \quad (2)$$

where I is the applied current (amperes), t_D is the transport number of the drug, A is the cross-sectional area through which transport occurs, F is the Faraday constant (coulombs/mole) and z_D is the drug charge.

The rate of drug delivery is proportional to the applied current (I) and the ability of the drug to function as a charge carrier, which is expressed by the transport number ($0 < t_D < 1$). This parameter describes the fraction of the total charge carried by each species. The transport number of a drug depends on its mobility and concentration and how they compare with the corresponding properties of the other charge carriers present in the system, and is a measure of their relative efficiency as charge carriers [25-27]. Specifically,

$$J_{EM} = \left(\frac{1}{Z_D F}\right)\frac{z_D u_D c_D}{\sum_{n=0}^{i} z_i u_i c_i} I_d \quad (3)$$

where I_d is the applied current density (equal to I/A), and z_D, u_D and c_D refer to the charge, mobility and concentration of the drug in the membrane, respectively; the denominator is the sum of the products of these parameters for each ion in the system contributing to charge transfer across the membrane [14,28]. As peptides and proteins have a high molecular mass, they tend to have lower mobilities than other ions often present in the system, such as Na^+ or Cl^-. Therefore, to increase delivery efficiency, the system needs to be designed to avoid competition with these highly mobile ions. In theory, other approaches would be to increase drug ionization by modulating the pH to increase z_D, or increasing drug concentration in the donor, c_D; however, in practice these changes in the formulation are not always possible and other factors must be taken into account, such as drug stability or skin tolerance to a specific pH.

2.2 Electroosmosis

The skin has an isoelectric point (pI) of ~ 4 – 4.5 [29] and at physiological pH it is negatively charged and acts as a cation-selective ion-exchange membrane. As a consequence, under the influence of an electric field, a convective solvent flow is generated in the anode-to-cathode direction [30-33].

The practical consequence of EO is that it contributes to the permeation of cations but opposes the movement of anions. Furthermore, under physiological conditions, neutral molecules can also be transported from the anode into the body [34]. The relative importance of EM and EO to the total flux of proteins and peptides has been the subject of much investigation [12,21,35,36].

In these studies, a neutral polar molecule with negligible passive transdermal transport (e.g., acetaminophen or mannitol) was used as an EO 'marker' and its flux used to report on EO solvent flow from anode to cathode and hence estimate the EO contribution to the total transport of the molecule of interest. During iontophoresis, the linear velocity (V_w) of the current-induced water flow (centimeters per hour) across the skin can be estimated using [33]:

$$V_W = \frac{J_M}{C_M} \quad (4)$$

where J_M and c_M are the flux and donor concentration of the marker, respectively. It follows that a measurement of J_M at known c_M allows V_w to be determined. It is then possible to calculate the EO contribution to the flux of the peptide drug by multiplying V_w by its concentration in the donor solution (c_D) [29]:

$$J_{EO} = V_W \cdot c_D \quad (5)$$

Three assumptions are implicit in this analysis: i) that drug and marker are transported in a similar fashion by convective solvent flow; ii) that transport of drug and marker is independent and there is no interaction between the two species; and iii) that electroosmotic transport of the marker molecule is proportional to its concentration in the solvent [37].

Using EO markers, it was observed that certain cationic peptides and proteins are able to decrease or even abolish EO flow; surface hydrophobic regions participate in van der Waals-type interactions with structures in the transport pathway, allowing exposed cationic amino acid side chains to form electrostatic interactions with fixed negative charges in the skin [38]. The neutralization of the fixed negative charges results in reduced convective flow. The magnitude and significance of electroosmotic flow inhibition can be expressed by calculating the inhibition factor (*IF*) [36]:

$$IF = \frac{[Q_M, \text{control}]}{[Q_M, \text{peptide}]} \quad (6)$$

where Q_M, control is the amount of marker transported after an iontophoretic experiment when no peptide is present in the donor solution and Q_M, peptide is the corresponding quantity in the presence of the peptide (or protein).

The EO inhibition caused by peptides and proteins can be influenced by their physicochemical properties and the applied current density. As this is increased, more charge has to be transported across the skin; this is partly carried by the peptide, which is driven into the membrane in greater amounts, leading to a more extensive neutralization of the skin's negative charge and a more pronounced EO inhibition.

As, in principle, electric mobility tends to decrease with molecular mass, it was putatively suggested that electroosmosis would become increasingly important for larger molecules and might even be the sole electrotransport mechanism for molecules approaching ~ 1000 Da [39]. However, studies using EO markers have shown that EM can be the dominant transport mechanism for peptides and proteins; for example, for triptorelin (a decapeptide, molecular mass 1311 Da) EM accounted for ~ 80% of overall transport [12]. In such cases the impact of molecule–skin interactions and the reduction of EO flow on total flux can be negligible – as observed during the iontophoretic delivery of cytochrome *c*, a 12.4 kDa protein [35]. When a current density of 0.5 mA/cm^2 was applied, although skin permselectivity decreased, there was no significant impact on the total flux, as EM accounted for ~ 90% of total protein delivery [35]. Similarly, in the case of Ribonuclease A, an RNA cleaving enzyme with a molecular mass of 13.6 kDa, EM was the major driving force, accounting for > 80% of the total flux. Increasing current density from 0.1 to 0.3 mA/cm^2 led to a near fourfold increase in J_{EM} and a twofold increase in J_{EO}. However, a further increase in current density to 0.5 mA/cm^2 produced a decrease in J_{EO} ($IF \sim 5$), indicating either interactions between the permeant and the transport pathway or a more efficient concentration-dependent screening of membrane charge that decreased convective solvent flow. **Figure 2** shows similar marker transport at either 0.1 or 0.3 mA/cm^2 in the presence and absence of the protein; however, there was a significant decrease of marker transport at 0.5 mA/cm^2 in the presence of protein, indicating EO inhibition [40].

3. System parameters affecting delivery

3.1 System design

Iontophoretic systems comprise three main components: the active and return electrode compartments and a microprocessor-controlled power supply (**Figure 1**). Charged drugs are normally placed in the electrode compartment with the same polarity; under physiological pH, neutral molecules are placed in the anode compartment. According to Equation 3 the optimal situation for iontophoresis would be the so-called 'single-ion' case where competing co-ions are absent from the formulation [28]. However, in practice it is difficult to create a 'single-ion system'; competing ions may be present in the pharmaceutical formulation (buffering agents, viscosity modifiers and preservatives), or highly mobile inorganic ions necessary for (or generated by) electrode reactions [25].

Although there are many different types of electrode, the one most suited to iontophoresis is the Ag/AgCl couple, which is reversible at low potential, chemically stable and does not elicit pH changes [14,20,24]. Ag/AgCl electrodes need chloride ions for anodal electrochemistry; thus, the anodal compartment must contain a supply of such ions derived either from the active agent (e.g., present as a hydrochloride salt) or from an external source (e.g., NaCl), in which case the concentration of competing cations in the anodal formulation is greatly increased and the delivery efficiency of the positively charged drug decreased. Strategies to reduce this competition can involve physical (but not electrical) separation of the drug and electrode compartment, such as that based on the well-known 'salt-bridge' concept in electrochemistry (**Figure 3**).

Several recent studies on the iontophoretic delivery of peptides and proteins have been done using salt-bridge assemblies normally composed of agarose and NaCl [35-37,40]. In addition to increasing the proportion of charge carried by the peptide/protein, salt bridges allow its isolation from the electrode compartment. However, it is important to verify whether the protein adsorbs onto the salt bridge [41]. It was demonstrated that reducing the number of competing ions in the formulation significantly increased the transport of a series of tripeptides across porcine skin *in vitro* (**Figure 4**) [37]. The use of a salt bridge increased the EM contribution (which accounted for 77 – 93% of the overall transport); however, as more peptide was carrying the charge, it also increased the inhibition factor of molecules with a propensity to interact with the membrane [37].

Patch-based iontophoretic systems using the same principles have been developed in which the electrode compartment contains an ion-exchange resin to trap Ag^+ ions released from the anode and is separated from the drug reservoir by a size exclusion membrane [42,43].

3.2 Current

Many studies have demonstrated that the iontophoretic flux of a peptide can be enhanced by increasing the

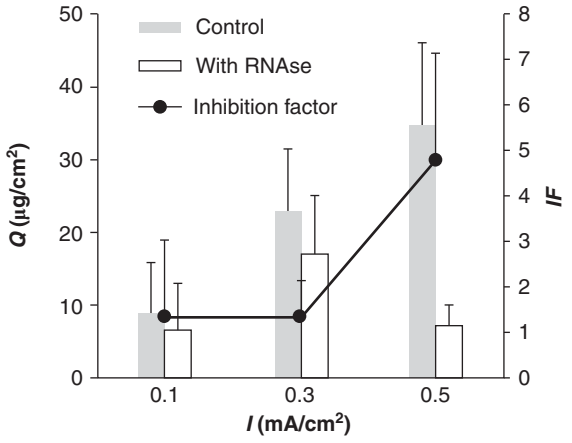

Figure 2. Cumulative acetaminophen permeation (*Q*) across the skin after 8 h of transdermal iontophoresis at 0.1, 0.3 and 0.5 mA/cm^2 in the presence and in the absence of Ribonuclease A [40].

applied current [21,44,45]; linear correlations between flux and current density have been reported for thyrotropin (362 Da) [15] and triptorelin (1311.5 Da) [12].

Although an increased current density usually results in increased permeation, straightforward linear correlations are not always observed. For example, a poor correlation was found between DGAVP (9-desglycinamide, 8-arginine-vasopressin) flux and applied current; a more than sixfold increment in current density did not even double the flux [46]. When working with cationic peptides and proteins one point to be considered with respect to the effect of increasing the current is the potential inhibition of the electroosmotic flow caused by neutralization of the skin's negative charge [36]. This may reduce the response to increases in current. However, lack of response to increases in current can also be a result of concentration polarization of the molecule in the transport pathways; this results in a plateauing of the current–response profile. This behavior was observed with cytochrome *c* [35] and in the iontophoretic delivery of Ribonuclease A [40]. In this study, a threefold increase in current density from 0.1 to 0.3 mA/cm^2 resulted in a corresponding increase in protein permeation; however, a further increase in applied current density to 0.5 mA/cm^2 did not produce a statistically significant increase in transport (**Figure 5**).

Linear relationships between steady-state flux and current intensity provide flexibility in controlling drug input kinetics. In terms of patient compliance and current tolerability, the upper limit for the current density applied *in vivo* is considered to be ~ 0.5 mA/cm^2 [47]. Although tingling and itching sensations as well as erythema (which resolves without sequelae) are frequent and well-tolerated side effects, higher current densities can provoke pain and discomfort [47]. Another approach to reduce patient discomfort and skin irritation is the use of a pulsed current profile (direct current with a varying on/off ratio) instead of a continuous direct current [48-51]. The hypothesis is that pulsed waveforms allow time for the skin to depolarize and return to its initial state before the onset of the next pulse, provided that the depolarization period is sufficiently long to discharge the membrane capacitance. In this way, charge does not accumulate in the stratum corneum and skin irritation resulting from polarization is presumably avoided [52,53]. It has been shown that a pulsed direct current profile using 0.5 mA/cm^2 is more efficient at transporting luteinizing hormone-releasing hormone (LHRH) and nafarelin than direct current profiles across the human epidermis *in vitro* [54]. Although the use of a pulsed current profile seems to be promising for the delivery of peptides and proteins, few groups have worked with these current profiles. More mechanistic studies on the efficiency of such systems are needed.

3.3 Drug concentration

A priori, Equations 2 and 3 suggest that increasing the drug concentration in the formulation will result in an increased transdermal flux via an increase in t_D, and indeed this has been observed in some cases. The steady-state flux of H-Tyr-D-Arg-OH (molecular mass 337 Da, charge = +1) increased linearly with donor peptide levels over the concentration range examined (**Figure 6**). EM was the dominant transport mechanism, accounting for > 70% of iontophoretic delivery. Both EM and EO contributions displayed a linear dependence on peptide concentration, suggesting that peptide–peptide and peptide–skin interactions were of an insufficient level to have an impact on electrotransport. Moreover, the relative contribution of EO to peptide electrotransport was not significantly different at each concentration, confirming the absence of an inhibition effect [21].

For certain molecules, however, the flux–concentration profiles reach a plateau above which further increases in concentration have only a limited or negligible effect on flux. One fact to be considered is the concentration and mobilities of competing ions. When the donor formulation contains a source of competing ions, the initial linear dependence that is observed between flux and drug concentration may fade as concentration increases: once the product of the drug concentration and mobility is in sufficient excess of the corresponding values for the competing ions, the flux may become independent of drug concentration. A second factor to be considered is the ability of the molecule to bind with skin structures along the iontophoretic transport pathway. The transport of some positively charged peptides that interact with skin and affect the EO, such as nafarelin and leuprolide, shows a nonlinear dependence on concentration [38,55-62]. In these cases, the impact on drug delivery can depend on the relative contribution of EO to the iontophoretic transport of the molecule in question [59,63].

A third factor is that simply increasing the amount of drug in the formulation may not increase the number of molecules

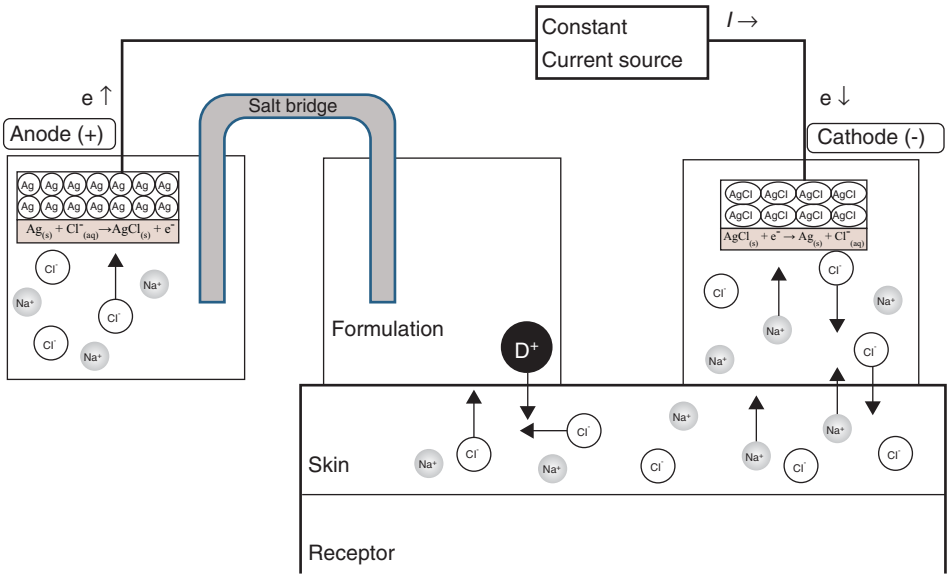

Figure 3. Iontophoresis using a Ag/AgCl electrode system. The anodal compartment is connected to the drug-containing compartment by a salt bridge. Application of an electric potential causes a current to flow through the circuit. At the anode–solution interface, the Ag^+ and Cl^- react to form insoluble AgCl, which is deposited on the electrode surface. Electromigration transports the cations, including the drug molecule, from the anodal compartment into the skin. At the same time, endogenous anions, primarily Cl^-, move into the anodal compartment. In the cathodal chamber, Cl^- ions are released from the electrode and electroneutrality requires that either an anion is lost from the cathodal chamber or that a cation enters the chamber from the skin.
Adapted from [13].

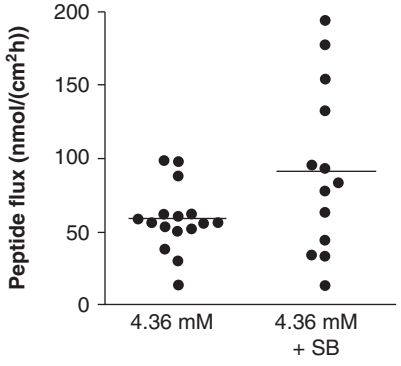

Figure 4. The influence of the salt bridge (SB) on the transport of a series of tripeptides across porcine skin *in vitro*.
Adapted from [37].

in the membrane. The formation of aggregates may hinder peptide delivery, reducing drug mobility and affecting EM. This was reported as being responsible for the anomalous iontophoretic behavior observed with triptorelin, where a twofold increase of peptide in the formulation produced a twofold decrease in delivery [12].

3.4 pH

In principle, drug permeation can be optimized by controlling the ionization state of the drug and/or that of the skin through manipulation of formulation pH. The degree of drug ionization will affect the mobility and hence EM, whereas the ionization state of the skin determines EO solvent flow. As peptides generally possess a mixture of weakly acidic and basic groups, then, depending on the specific pH value, they can be predominantly anionic, cationic or neutral. With respect to the skin, its charge should be such that it favors EO flow in the direction of drug movement. For example, for basic peptides with pI > 8, it will usually be more appropriate to keep the pH at least one unit below the pI (~ 7) so that the molecule will be positively charged and the skin negatively charged, facilitating both EM in the anode and EO, whereas in the case of acidic peptides with pI < 4, it may be useful to use pH 5 or 6, providing EM in the cathode and reducing the effect of EO [14]. The effect of pH on the iontophoretic delivery of two amino acids, histidine and lysine, was compared at pH 4 and 7.4 [64]. In the case of lysine (pK_a 9.59), increased delivery was observed at pH 7.4 because then EO also contributed to the transport of the charged amino acid. By contrast, for histidine (pK_a 6.5), higher transport was observed at pH 4 than at pH 7.4 because histidine is > 90% uncharged at pH 7.4 and is no longer subject to EM and, as a result, its iontophoretic transport depends exclusively on EO [64]. Conversely, the transport of thyrotropin-releasing hormone (TRH) at pH 8 with 98% uncharged peptide was twice that at pH 4 where TRH is ~ 99% protonated [15].

Although modulation of formulation pH can certainly be used to improve transport, in practice the formulator has

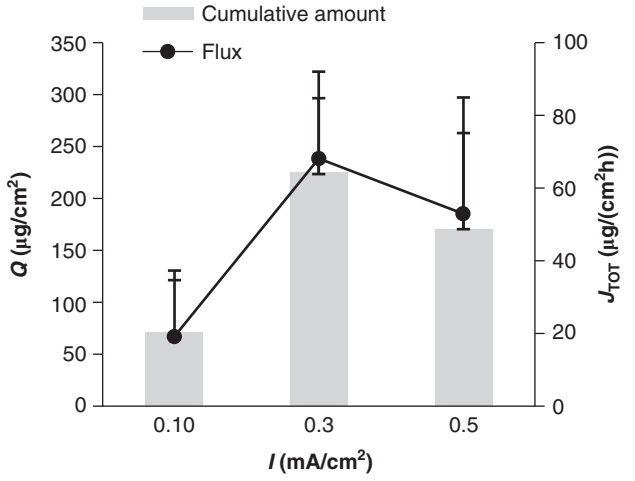

Figure 5. Effect of current density on cumulative permeation (Q) and steady-state flux (J_{TOT}) of Ribonuclease A across porcine skin [40].

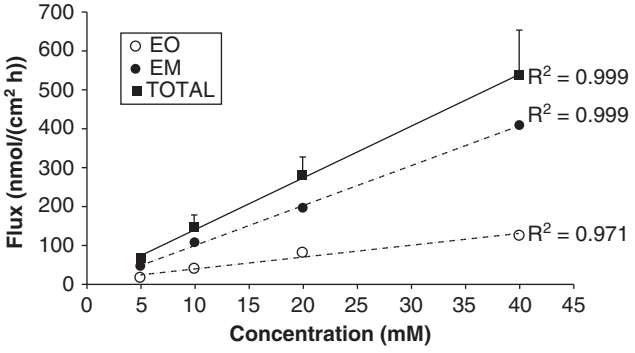

Figure 6. Electroosmosis (EO) and electromigration (EM) contributions to the iontophoretic delivery of tyrosine-D-arginine dipeptides (YdR). Data show the mean steady-state flux (± s.d.) as a function of YdR concentration in the formulation [21].

only limited options. Realistically, for a formulation to be acceptable for skin application it will probably have a mildly acidic to neutral pH (5 – 7.4) – extreme pH values can irritate the skin or even cause chemical burns. In addition, a key issue will be the stability of the biopharmaceutical as a function of pH, and a compromise is required that ensures both applicability and stability. Thus, although low pH (3.6) has been used during anodal iontophoresis of cationic insulin to decrease peptide degradation, this is not an option for application *in vivo* [65]; similarly, cathodal iontophoresis of anionic insulin has been studied at pH 10 in order to decrease aggregate formation – at pH > 9 aggregation is reduced and monomers and dimers are present in higher percentages. Indeed, the results showed much higher plasma levels in rats following iontophoresis at pH 10 as compared with a formulation at pH 7 [66]. However, such a basic formulation cannot be envisaged for use in patients.

In addition, at extremes of low and high pH, there will, by definition, be substantial concentrations of hydroxonium and hydroxide ions, respectively. These ions have high electrical mobilities and at elevated concentrations can cause significant reduction in the iontophoretic delivery of larger drug molecules, which have lower mobilities and may be present at low concentrations in the formulation. With respect to skin pH, although this can be changed for *in vitro* experiments by modifying the pH of the bathing solution in contact with the membrane, this is rather more difficult to achieve *in vivo* owing to the skin's intrinsic buffering capacity [67].

3.5 Formulation

The most obvious formulation factors affecting iontophoretic delivery are the formulation pH (discussed previously) and the presence of competing ions. As discussed in Section 3.1, in cases where the electrodes can be separated from the drug reservoir the so-called 'single-ion' situation can be applied, where the drug is the only ionic species that can contribute to charge transport, that is, all other competing cations have been eliminated from the formulation. Under these conditions, the drug competes only with endogenous counter-ions, for example a cationic drug will compete with subdermal chloride. However, in some cases addition of excipients such as stabilizers and preservatives may be necessary and the efficiency of drug delivery can be lowered [68]. In such cases, the drug concentration relative to that of competing co-ions, not the nominal drug concentration *per se*, should be considered; in fact, the use of molar fraction has also been suggested [23,26,63,69,70].

Another important point to consider is the ease of application of the formulation in a clinical situation. Although *in vitro* experiments are usually performed using aqueous solutions, pre-filled iontophoretic patch drug reservoirs will most probably use hydrogels, and these have attracted increasing attention in recent years in view of their adhesiveness and biocompatibility [71,72]. Hydrogels are three-dimensional networks of hydrophilic polymers capable of retaining large amounts of water or biological fluids within their structure; because of the high water content they possess good electrical conductivity and can be used for transdermal iontophoretic delivery [73-78]. Furthermore, network structure and composition can be manipulated to influence the permeation and diffusion characteristics of a drug within the hydrogel [79]; these changes must take into account the charge of both the polymer and the drug. Normally, drug release is reduced when the molecule has the opposite charge to the polymer and is favored when they have the same charge [80].

The combined use of drug carriers and iontophoresis has also been reported. Liposomes [81-85], microemulsions [86,87] and solid lipid nanoparticles [88] have been used as vehicles for iontophoretic drug delivery. Some reports claim that charges could be imparted to neutral drugs by encapsulating

them in charged drug carriers, which could be delivered by iontophoresis [88]. Higher accumulation of drug carriers in the hair follicle has also been shown [83]. Furthermore, enhanced transport and drug accumulation in the skin have been observed on incorporation into nanoparticles [89-91]. However, information on whether these carriers can penetrate the skin is still controversial. Some reports show that the drug but not the carrier is delivered [92,93]. Therefore, with respect to the iontophoretic delivery of peptides, the concomitant use of a drug carrier system would probably be justifiable only if it represented a significant increase in molecular solubility and/or stability. Indeed, it has been suggested that encapsulation could protect a peptide from degradation [84]. Iontophoretic delivery of ciclosporin A across human cadaver epidermis has been investigated using colloidal systems (lecithin vesicles and microemulsion) [94]; the liposomes enhanced aqueous solubility ~ 100-fold. Of the formulations tested, highest drug permeation was observed from lecithin vesicles under anodal iontophoresis, followed by anodal iontophoresis of a microemulsion. Although both the microemulsion and lecithin vesicles were negatively charged, cathodal iontophoresis resulted in the lowest permeation of ciclosporin A, suggesting that the carriers were not penetrating the skin themselves. However, by improving drug solubility, they increased the concentration gradient and hence the flux. So far very few studies have focused on the use of formulation conditions to improve the iontophoretic delivery of peptides. However, that should change because peptides and proteins are comparatively high-cost molecules and an increase in drug solubility and/or stability could make possible therapy using lower more cost-effective drug loads in patch systems.

4. Molecular factors affecting delivery

For a long time the molecular mass was considered to be the main factor affecting transdermal iontophoretic transport. However, it has recently been shown that molecular mass or size is not a sufficiently discriminating parameter to explain subtle differences in transport. An investigation into the effect of charge and molecular mass on the electrotransport of a series of dipeptides showed that an increase in charge could compensate for an increase in molecular mass [95]. For example, a comparison of lysine and H-Lys-Lys-OH transport revealed that the twofold increase in molecular mass was compensated by doubling the charge; by contrast, H-His-Lys-OH, with approximately the same molecular mass as H-Lys-Lys-OH but unit charge (at pH 7.4), had a significantly lower flux. The ratio of charge to molecular mass (and hence molecular volume) will influence the electric mobility of a molecule.

Even though the charge/molecular mass ratio explains the different delivery profiles of small peptides, the effective mobility of a peptide or a protein will also depend on volume, shape and charge distribution within the molecule. It is therefore likely that secondary, tertiary and quaternary structure of a protein will be important in determining iontophoretic transport. Indeed, different sequences of the same three amino acids within a tripeptide have been shown to affect transport behavior significantly [96]. Capillary zone electrophoresis (CZE) may be a promising tool to determine the effective mobility, providing an estimation of the EM contribution to transdermal iontophoretic flux [97,98]. However, if the molecule of interest interacts with the skin transport pathway, then CZE will not provide an accurate prediction of transport [35].

5. Iontophoretic delivery of therapeutic peptides and proteins through the skin

Several studies have been performed on the iontophoretic delivery of therapeutic peptides, and these are briefly described below (see also **Table 1**).

5.1 Diabetes and insulin delivery

One of the greatest challenges for non-invasive peptide and protein delivery concerns insulin. Iontophoretic delivery of insulin has been studied extensively both *in vitro* [99-106] and *in vivo* in small animals [105,107-109]. However, the physicochemical properties of insulin hinder its iontophoretic delivery: the insulin monomer is a ~ 6000 Da negatively charged peptide (51 amino acids) with pI ~ 5.4. This means that when insulin is delivered as a cation from the anode it will tend to become 'neutral' on contact with the skin's outermost layers (pH ~ 5) before becoming predominantly negatively charged within the skin (pH > 5.4 in the inner layers) – hindering its anodal transport [24]. Conversely, when it is delivered as an anion from the cathode, not only will cathodal delivery be opposed by EO, but also insulin may decrease its anionic character in the upper layers of the skin. Hence, its pI plays an important role in its iontophoretic transport, explaining its poor permeation by both anodal and cathodal deliveries [110]. Moreover, the formation of dimers and hexamers at relatively low concentration complicates delivery further. Thus, the iontophoretic delivery of insulin analogues may be more favorable; the highest iontophoretic flux was observed for a sulfate analogue of monomeric porcine insulin, which had a pI ~ 2.5 and a net -8 charge at pH 7.4 [100]. The iontophoresis of a monomeric insulin analogue in diabetic rats (with intact skin) was also reported to reduce plasma glucose levels [107].

Although early studies showed that iontophoretic delivery of insulin resulted in a reduction in blood glucose levels in small animals, the challenge is to extrapolate these results to humans, where significantly greater quantities of the hormone are required for pharmacologic effect. A normal healthy individual produces 18 – 40 IU (where 1 IU ~ 0.04 mg) of insulin a day, which corresponds to 0.2 – 0.5 IU/(kg day). Approximately half of this is secreted in the basal state and the rest is secreted in response to meals. Therefore, the basal secretion rate is ~ 0.5 – 1.0 IU/h; an iontophoretic system would have to provide a drug input rate of 0.02 – 0.04 mg/h to match the

Table 1. List of relevant studies applying iontophoresis for delivery of therapeutic peptides and proteins *in vitro* and *in vivo*.

Therapeutic agent	Approximate molecular mass (Da)	Model	Observations
Insulin	6000	Diabetic rats	Monomeric human analogue (intact skin) and bovine insulin (impaired barrier) induced decrease in blood glucose level [107]
		Porcine epidermis *in vitro* and diabetic rats	Combination approach with permeation enhancers increased transport [105]
Human calcitonin	3500	Rats	Hypocalcemia comparable to intravenous [120]
Salmon calcitonin	3430	Rabbits	Therapeutic effect [121]
		Shaved rats	Therapeutic effect [118]
		Rats	Comparable to subcutaneous injection [10]
hPTH	4117	Rats, hairless rats, beagle dogs	Absorption via hair follicles [122]
		Ovariectomized rats	Similar results to subcutaneous injection [123]
LHRH	1182	Yorkshire pigs	Pharmacologically active LHRH delivered [131]
Nafarelin	1322	Human skin *in vitro*	75% pulsed DC current was most efficient in delivery [54]
Leuprolide	1210	Healthy males	Pharmacologically effect [61]
		Healthy males	Comparable to subcutaneous injection [134]
Triptorelin	1311	Porcine skin *in vitro*	Therapeutic amounts delivered [36]
Vasopressin	1084	Human and rat skin *in vitro*	Therapeutic dose delivered [137]
9-desglycinamide vasopressin	1028	Human skin *in vitro*	Transport achieved mainly by electroosmosis [46]
Desmopressin	1183	Diabetic rats	More effective than oral and nasal route [138,139]
Octreotide	1019	Rabbits	Increased flux as function of current and concentration [44]
Vapreotide	1131	Porcine skin *in vitro*	Peptide irreversibly binds to skin but therapeutic concentrations achieved [36]
Somatorelin	3929	Hairless porcine skin *in vitro*	Linear increase in flux with current density but independent of type of current and frequency [50]
		Hairless guinea-pig	Steady-state levels similar to subcutaneous [141]
GHRP	817	Rats	Therapeutic levels achieved [142]
Botulinum toxin	150,000	Humans with hyperhidrosis	Amelioration of symptoms [147,148]
		Rats	Toxin found in hair roots, sebaceous glands and arrector pili muscle fibers [150]

GHRP: Growth hormone-releasing peptide; hPTH: Human parathyroid hormone; LHRH: Luteinizing hormone-releasing hormone.

physiological rate of insulin secretion, and for a conveniently sized 4 cm^2 patch, this equates to drug fluxes of 5 – 10 μg/(cm^2 h) [14]. Furthermore, it is always important to bear in mind the clinical feasibility of the treatment; given the requirements for basal insulin, amounts of ~ 1 mg may need to be given and the duration of current application may become an issue for patients receiving chronic therapy. In addition, in most of the animal studies, significant barrier impairment was necessary to deliver sufficient insulin to decrease blood glucose levels [107,108]. These problems may explain why despite the considerable amount of work so far, there are no reports of successful clinical investigations into the transdermal iontophoretic delivery of insulin in humans.

More recently, effort has been directed at combining other methods with iontophoresis to increase its efficiency. For example, chemical penetration enhancers have been used in combination with iontophoresis in an attempt to increase the permeability of the skin without severe irritation or damage to its structure [105,106,111]. In these studies, a pretreatment with penetration enhancers was usually done before iontophoresis. However, it is important to evaluate the practicality of the different protocols because the duration of pretreatment may be too long; for example, a 2 h pretreatment with saturated and unsaturated fatty acids before iontophoresis was used to produce synergistic enhancement of insulin flux through rat skin *in vitro* [103]. Although an insulin flux of nearly 7 IU/(cm^2 h) was observed from a hydrogel patch using 2 min pretreatment with different classes of permeation enhancers (5% 1,8 cineole, oleic acid and sodium deoxycholate in propylene glycol:ethanol (7:3)) used synergistically followed by 1 h of anodal iontophoresis with a sinusoidal waveform (0.5 mA/cm^2 with 1 kHz frequency), the experiment was performed *in vitro* at pH 3.6 across porcine epidermis prepared by trypsin digestion [105]. Electroporation [66], encapsulation into liposomes [112] and nanovesicles as well as microneedles [113] have been used in combination with iontophoresis to enhance penetration ability of insulin through skin. These techniques are treated in more detail in Section 6.

5.2 Osteoporosis and Paget's disease

5.2.1 Calcitonin

Calcitonin is a 32-amino acid peptide secreted by the thyroid gland. Its major physiological role is to control calcium concentration and metabolism in the body in conjunction with parathyroid hormone. Clinically, it is indicated in the treatment of Paget's disease, in the therapy of postmenopausal osteoporosis, and in malignant hypercalcemia. It is generally given as a subcutaneous or intramuscular injection [114]. However, owing to a short half-life multiple injections are required for optimal pharmacological effect, thus patient compliance is low. The other marketed alternative is a nasal spray, which suffers from low bioavailability [115]. Other disadvantages include irritation of nasal mucosa and variable absorption in the case of nasal disease conditions.

Several studies, both *in vitro* and *in vivo*, have investigated the iontophoretic delivery of this peptide, which carries a positive charge at physiological pH [10,79,109,115-120]. A pulsed current iontophoretic protocol (30 kHz, 30% duty cycle applied for 45 min) was used to deliver salmon calcitonin to shaved rats [118]; in this study, no significant difference in the hypocalcemic effect was observed on increasing the dose, suggesting that a dose–response plateau had been reached [118]. This was also observed by Santi *et al.*, who demonstrated that increasing the intravenous dose of salmon calcitonin from 10 to 25 IU/kg did not produce a significant increase in the hypocalcemic effect [121]. A cutaneous first-pass effect during salmon calcitonin delivery has been proposed [117,118], and the enzymatic inhibitors aprotinin and camostat mesilate were shown to enhance the hypocalcemic effect of salmon calcitonin in rats [117], although aprotinin was not found to modify human calcitonin delivery kinetics across hairless rat skin *in vitro* [120]. More recently, salmon calcitonin was iontophoresed to hairless rats using a wearable and disposable device (WEDD, Travanti Pharma, Inc., (Mendota Heights, MN, USA)); the decrease in calcium levels was similar to that after subcutaneous injection [10]. This device uses Zn and AgCl as the anode and cathode, respectively; when the circuit is closed oxidation of Zn and reduction of AgCl occur spontaneously because of the difference in potential between the two metals. However, such a device imposes a constant voltage (1 V) across the skin, and not a constant current, therefore drug fluxes may vary with skin resistance. Further, this voltage is insufficient for certain applications, including the above study, which required an external power source providing an extra 9 V.

5.2.2 Human parathyroid hormone

Human parathyroid hormone (hPTH) is an 84-amino acid residue peptide that is used in the treatment of osteoporosis because it promotes osteoblast growth. It can have either an anabolic or a catabolic effect on bones, depending on its input kinetics – pulsatile delivery favors its anabolic and antiosteoporotic effects. Suzuki *et al.* conducted a detailed *in vivo* investigation into the pulsatile anodal iontophoretic delivery of hPTH (1 – 34), a pharmacologically active fragment, in Sprague-Dawley rats, hairless rats and in beagles [122]. It was found that an increase in peptide concentration or current density resulted in increased plasma levels of the peptide. The study also demonstrated a linear relationship between the absorption rates and the ratio of hair follicles to epidermal thickness. Based on these results, the main transport route for hPTH (1 – 34) during iontophoresis was suggested to be via the hair follicles, implying that absorption in man might be intermediate between that in hairless rats and beagle dogs. In a subsequent study the anabolic effect of hPTH as measured by changes in bone mineral density in an ovariectomized rat model following pulsatile iontophoresis was compared with that after subcutaneous injection [123]. Their results suggested that a thrice-weekly three pulse protocol (3 × 30 min application of 0.1 mA/cm^2 using a 120 µg patch loading) was equivalent to daily subcutaneous injections of 5 µg/kg.

5.3 Luteinizing hormone-releasing hormone and its analogues

Luteinizing hormone-releasing hormone (LHRH, gonadorelin, molecular mass ~ 1182 Da) is a decapeptide that is secreted by the hypothalamus in a pulsatile mode to activate pituitary release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Pulsatile administration of LHRH is used in the therapy of hypogonadotropic hypogonadism. Its positive charge at physiological pH along with its short half-life of ~ 5 min makes it an ideal candidate for transdermal iontophoresis. The iontophoretic delivery of LHRH has been investigated both *in vitro* [54,124-130] and *in vivo* [131]. Iontophoresis of LHRH using the IPPSF model was performed at pH 6 for 2 h; LHRH delivery was monitored for a further 3 h after terminating current application [131]. The flux gradually increased during iontophoresis and decreased rapidly on terminating current flow. It was also shown that the iontophoretically delivered hormone retained its biologic activity.

Nafarelin is a LHRH superagonist with increased efficacy that can be attributed to its high binding affinity to the LHRH receptor and its relatively long biological half-life. Several studies have reported the transdermal iontophoresis of nafarelin *in vitro* [54,57,58,132]. A recent study compared constant/pulsed iontophoresis of nafarelin across human skin [54]; five different current profiles – 100% DC, 75% on/25% off or 50% on/50% off pulsed DC, 75%+/25%- or 50%+/50%-AC – were used in the iontophoretic experiments (current density 0.5 mA/cm^2, pulsed current frequency 500 Hz). The results showed that the 75% on/25% off pulsed DC was the most efficient current profile followed by the 75%+/25%- AC current profile (**Figure 7**). Transdermal administration of lipophilic peptides such as nafarelin with adjacently located positively charged and lipophilic moieties has been difficult owing to adsorption in the negatively charged transport pathways in the skin [38,58].

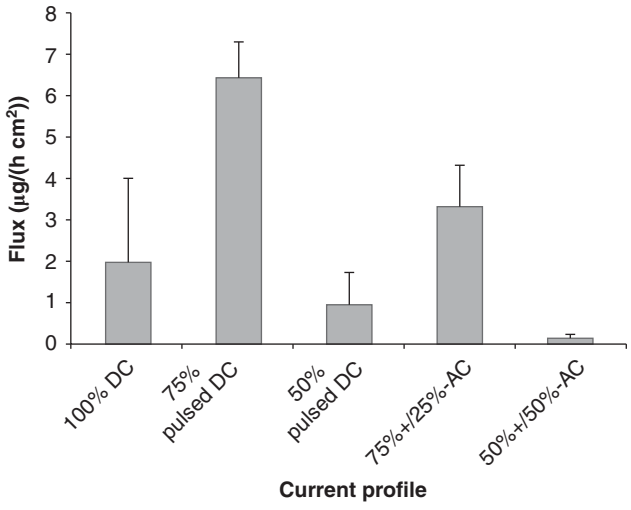

Figure 7. Iontophoretic flux of nafarelin as a function of various current profiles.

Leuprolide, another LHRH superagonist, has also been investigated [61,133,134]. A double-blind, randomized, crossover study in 13 healthy men (5 mg; 0.2 mA, 70 cm^2) showed that the patches, though large, were well tolerated and LH concentration was increased from a baseline of 11.3 ± 3.1 to 56.4 ± 49.6 mIU/ml at 4 h [133]. The iontophoresis of triptorelin (molecular mass ~ 1311 Da), another LHRH analogue, used for the treatment of sex hormone-dependent tumors and other benign gynecologic disorders, has also been studied across porcine skin *in vitro* [12]. Based on the results, application of a total iontophoretic current of 0.6 – 0.8 mA, over a 4 cm^2 contact area, would be sufficient to provide therapeutic delivery rates (27 – 36 µg/h).

Miller *et al.* conducted iontophoretic transport of LHRH, a poorly water-soluble antagonist and a superagonist (D-Trp6,Pro9-NHEt)LHRH across hairless mouse skin; the latter was delivered successfully at 0.1, 0.3 and 0.5 mA/cm [135].

5.4 Vasopressin and analogues

Vasopressin, a nonapeptide with a molecular mass of 1084 Da, is an anti-diuretic used to control polyuria and used in the management of variceal bleeding. Transdermal iontophoretic delivery of vasopressin was first investigated across rat skin [136]. Subsequent iontophoresis across human cadaver skin at a current density of 0.5 mA/cm^2 showed that the cumulative amount of intact vasopressin permeated during 8 h of iontophoretic transport was 15.37 ± 5.31 µg/cm^2, corresponding to only 1% permeation of the applied dose [137]. Nevertheless, given that the therapeutic dose of vasopressin is 25 µg, this could easily be delivered using an optimized formulation and a reasonably sized iontophoretic patch (2 – 4 cm^2). No intact vasopressin was found to permeate under passive conditions.

A vasopressin analogue, 9-desglycinamide vasopressin (molecular mass 1028 Da), which is more potent and more resistant to metabolism than vasopressin, has also been iontophoresed across dermatomed human skin [46].

Iontophoretic delivery of desmopressin acetate has been investigated *in vivo* with a diabetes insipidus model in rats [138,139]. Repeated short duration iontophoretic treatments with low current density were found to be best at maintaining a constant response. In another study, prolongation of the anti-diuretic response to desmopressin acetate in diabetic rats was compared with other routes of administration. Delivery by iontophoresis was comparable to that via the nasal route and was two to three times more effective than oral administration [140].

5.5 Somatostatin analogues

Octreotide (molecular mass 1019 Da), a synthetic octapeptide, is a somatostatin analogue available for the treatment of acromegaly and carcinoid tumors that carries a net positive charge under physiological conditions. Iontophoretic delivery of octreotide acetate has been investigated in rabbits [44]. Plasma levels of octreotide were negligible in the absence of current application, but increased in proportion to current density within the range 0.05 – 0.15 mA/cm^2 and declined rapidly after removal of the device. Similar proportional increases in plasma levels were observed when peptide concentration was increased from 2.5 to 5 mg/ml but not beyond this concentration.

Vapreotide (molecular mass ~ 1131 Da), another long-acting synthetic analogue of somatostatin used in the treatment of acromegaly and gastroenteropancreatic tumors, was iontophoresed across porcine skin *in vitro* [36]. Despite the susceptibility of vapreotide to enzymatic degradation, a flux of 1.7 µg/(cm^2 h) was achieved after 7 h of constant current iontophoresis (0.15 mA/cm^2). Post-iontophoretic extraction revealed that, depending on the experimental conditions, 80 – 300 µg of peptide were bound to the skin. Based on the clinical pharmacokinetics and observed transport rates of vapreotide across porcine skin, therapeutic concentrations might be achieved with a patch area of 15 cm^2

5.6 Somatorelin and analogues

Somatorelin, or growth hormone-releasing hormone (GRF), is a 44-residue endogenous peptide (molecular mass 5039 Da) secreted by the hypothalamus and is used to treat children with growth hormone deficiency. The iontophoretic delivery of a shortened GRF analogue, Ro 23–7861 (molecular mass 3929 Da), was studied across hairless guinea-pig skin *in vitro* [50]. A subsequent *in vivo* study in hairless guinea-pigs compared the serum GRF concentration profiles following iontophoresis with those obtained after subcutaneous and intravenous administration (10 µg/kg). Iontophoresis yielded a steady-state plasma concentration of 0.2 ng/ml that was similar to the peak plasma level achieved after a subcutaneous dose [141].

Ellens *et al.* investigated iontophoretic delivery of a growth hormone-releasing peptide (GHRP) (elimination half-life of 30 min and 71 min after intravenous and subcutaneous injection) at a current density of 0.15 – 0.2 mA/cm^2 for 2 h from a patch system specially designed for iontophoresis [142]. Blood levels of peptides persisted for at least 2 h after the current was turned off, indicative of depot formation in the skin, and flux of 0.8 – 1.2 μg/(cm^2 h) was achieved with peak plasma concentration of 20 ng/ml. The results suggested that a skin reservoir was formed during current application that released the peptide slowly into the systemic circulation after iontophoresis. Given the flux obtained with iontophoresis and the dose required to elicit a pharmacological response (21 μg/h in 70 kg adults), delivery of therapeutically relevant amounts of peptide would require use of a 15 – 20 cm^2 patch.

5.7 Botulinum toxin

Botulinum toxin A was first described for use in axillary hyperhidrosis in 1996 [143]. The safety and efficacy of this treatment have been documented [144-146]; the first case report on the successful iontophoresis of this protein to treat two patients appeared in 2004 [147], and this was followed by a pilot study performed on eight patients who were refractory to conventional therapy [148]. It is believed that the toxin works by inhibiting the release of acetylcholine at the neuromuscular junction and affecting the postganglionic sympathetic innervation of sweat glands [149]. Botulinum toxin A is a two-chain protein with a 100 kDa heavy chain joined by a disulfide bond to a 50 kDa light chain and has a pI of 6.06. An *in vivo* study found the toxin in hair roots, sebaceous glands and arrector pili muscle fibers of Wistar rats after only 10 min of iontophoresis [150]. More studies would be helpful to understand the degree of permeation and the duration of the pharmacological effect of this toxin after iontophoretic application.

6. Combination strategies to improve transdermal iontophoretic delivery

These strategies can involve either a modification of the formulation or the use of a complementary strategy to increase membrane permeability. The concomitant use of a drug carrier system and iontophoresis may result in a significant increase in molecular solubility and/or stability, for example, protect a peptide from degradation and thereby increase delivery [84]. Alternatively, other strategies involving skin barrier impairment have been proposed. As iontophoresis acts principally on the permeant, barrier impairment may offer some degree of synergy.

6.1 Iontophoresis in conjunction with electroporation

Transdermal electroporation involves the application of short (< 1 s), high voltage (50 – 500 V) pulses to the skin that disorganize the stratum corneum lipids and thereby increase drug transport. Electroporation (6 pulses of 120 V, 10 ms each) increased the transdermal iontophoretic delivery of salmon calcitonin across human epidermis fourfold; however, pulsing at lower voltages (60 and 100 V) before iontophoresis was not superior to iontophoresis alone [151]. Similarly, electroporation increased iontophoretic flux of parathyroid hormone 17-fold over 24 h [151]. Another study showed 10- and fivefold enhancements in human parathyroid hormone delivery when using electroporation pulses of 100 and 300 V followed by iontophoresis at 0.2 mA/cm^2 in comparison with the flux with electroporation alone [152]. The application of a single pulse (500 V, 5 ms) to initiate the experiment resulted in a nearly twofold increase in LHRH concentration at the end of 30 min of iontophoresis (0.4 mA/cm^2) [153]. In contrast to iontophoresis alone (0.4 mA/cm^2), electroporation of insulin (150 or 300 V, 10 ms and 10 pulses) resulted in high plasma levels of the hormone, and the combined use of electroporation and iontophoresis led to a further increase in insulin delivery in rats [66]. A single electroporation pulse before iontophoresis was found to double the iontophoretic transport of dextran sulfate as compared with iontophoresis alone (144.5 ± 10.35 and 276 ± 45.2 μg/cm^2, respectively), supporting the hypothesis that the structural rearrangement of the lipid bilayers can lead to enhanced permeability and improved transport [154].

6.2 Iontophoresis in conjunction with chemical enhancers

The use of chemical penetration enhancers is one of the more widely studied techniques for increasing transdermal drug permeation [103,105,106,111,124-128,155]. Various mechanisms have been postulated to explain how they increase permeation – including acting as solvents to dissolve skin lipids/denature skin proteins, affecting drug partitioning from the applied formulation or modifying drug solubility in the skin.

Pillai *et al.* investigated the effect of pretreatment with commonly used vehicles such as EtOH, Propylene Glycol (PG), water and their binary combinations, dimethyl acetamide (DMA), 10% dimethyl acetamide in water, ethyl acetate (EtAc) and isopropyl myristate (IPM) on insulin iontophoresis [103]. The skin barrier was severely compromised with DMA, less so by EtOH and EtAc, whereas IPM and PG had relatively minimal skin barrier-altering potential. All the solvents produced synergistic enhancement with iontophoresis. Fourier transform infrared studies showed that EtOH and EtAC caused lipid extraction, whereas IPM caused an increase in lipid fluidity. Thermogravimetric studies showed that EtOH and PG caused dehydration of skin.

6.3 Iontophoresis in conjunction with microporation

Microneedles, as the name suggests, are micrometer-scale needles (typically ~ 500 μm in length) that are used for transdermal delivery of drugs. In principle, they are sufficiently long to penetrate the stratum corneum, but

short enough not to stimulate nerves and hence pain receptors in the deeper tissues.

A study performed to evaluate the potential of combining microneedles and iontophoresis on skin permeation of D_2O and FITC-dextrans of variable molecular mass ranging from 3.8 to 400 kDa showed that convective solvent flow (electroosmosis) was unaffected by microneedle pretreatment [156]. The combination strategy significantly enhanced FITC-dextran flux compared with microneedle pretreatment alone or iontophoresis alone, whereas no synergistic effect was found on the flux of D_2O. A similar study was conducted to assess the skin transport behavior of daniplestin (molecular mass 12.7 kDa, pI 6.2) across hairless rat skin [157]. The combination approach gave higher flux values compared with iontophoresis alone. The authors followed up with an *in vivo* study in rats using a current of 0.2 mA/cm^2 for 6 h along with microneedles. Individual approaches were unable to produce detectable levels of the protein in the plasma [158]. By contrast, the combination of microneedles and iontophoresis resulted in controlled delivery over a period of 10 h.

Chen *et al.* investigated transdermal delivery of insulin from nanovesicles iontophoresed through microchannels in diabetic rats [113], which resulted in a 713-fold increase in insulin flux as compared with passive diffusion *in vitro*. *In vivo* studies in diabetic rats showed that iontophoresis of the cationic vesicles through microporated skin produced 33.3 and 28.3% reductions in blood glucose levels at 4 and 6 h; these were comparable to decreases induced by subcutaneous injection of insulin.

It has also been shown that iontophoresis across microporated skin in hairless rats results in a twofold increase in the dose of IFN-α_{2b} delivered [159].

7. Conclusion

Transdermal iontophoresis enables the controlled non-invasive delivery of therapeutics into and across the skin; numerous studies have demonstrated its ability to deliver peptides and now we are beginning to see evidence that it can also be used for proteins. Crucially, the use of activity assays and the quantification of downstream biological markers have confirmed that biological activity is retained post-iontophoresis. Despite earlier hypotheses that electrotransport of high-molecular-mass cations would be governed exclusively by electroosmosis (thus limiting potential applications), it is now clear that electromigration may play a major, if not pivotal, role in peptide and protein iontophoresis – the dominant transport mechanism being dependent on the properties of the permeant. Hence, much larger molecules can be considered as legitimate candidates for iontophoretic delivery. Transport rates are governed by: i) intrinsic physicochemical properties, although the exact relationship between delivery rates and the complex three-dimensional structures and concomitant spatial distribution of different molecular properties remains to be elucidated; and ii) iontophoretic conditions – most importantly, formulation composition and current density. So far, pre-filled iontophoretic systems for low-molecular-mass therapeutics have been approved by regulators; the challenge now is to develop commercial iontophoretic systems for the non-invasive administration of peptides and proteins that could dramatically change the delivery options of these potent and increasingly used therapeutics.

8. Expert opinion

Iontophoresis is one of the few transdermal delivery technologies that has succeeded in producing FDA-approved products – although both systems (LidoSite and Ionsys) contain low-molecular-mass therapeutics for local anesthesia and postoperative pain relief, respectively. Physicochemical properties – including good aqueous solubility and the presence of charged groups – that can render peptides and proteins 'difficult to deliver' by other approaches are ideal for iontophoresis. It offers the key advantage of enabling the controlled delivery of these therapeutics using complex input kinetics that can mimic endogenous secretion profiles – a so-called 'non-invasive infusion pump'. Until recently, it was thought that protein delivery was beyond the scope of iontophoresis; however, it has now been shown that functional proteins can indeed be iontophoresed across intact human skin. Thus, there are now published reports describing the successful non-invasive iontophoretic delivery of biomolecules ranging from thyrotropin-releasing hormone (359.5 Da) to Ribonuclease A (13.6 kDa) across intact skin; these include Phase I/II trials evaluating peptide pharmacokinetics and demonstrating pharmacological activity post-delivery. The next step is to build on these preclinical data and early stage clinical results and to develop effective iontophoretic patch systems for routine use by patients. Given the stability requirements of peptides and proteins and their susceptibility to degradation, aggregation and precipitation from solution, it may not be possible to use simple hydrogel systems as for small molecule therapeutics; dry patches where the biomolecule is hydrated immediately before use may need to be developed. In addition to the stability of the drug formulation, another major challenge will be the integrity of the other patch components, including the electronics; this will be especially critical in fully integrated patch designs. Physicochemical properties and drug pharmacokinetics/pharmacodynamics obviously put a limit on the number of peptide and protein candidates that can be delivered by transdermal iontophoresis from a realistically sized patch. For molecules with low potency, in theory there are several parameters that can be increased, including current density, duration of current application, drug loading, patch area and the use of pretreatments to impair skin barrier function, and there are many preclinical studies where these parameters have been investigated. However, for successful development of an iontophoretic product all of these 'options' need to be economically and clinically feasible and the product solutions must be

acceptable for patients – even more so for chronic therapy. For example, peptides and proteins are often expensive to produce – thus, increasing drug loading is undesirable because it might result in a product with an unacceptable final cost. The current density is normally limited to a maximum of 0.5 mA/cm^2; however, lower current densities are preferable for longer application periods and as the application area increases so as to minimize the risk of skin irritation. Moreover, lower currents are also preferred in terms of the power requirements of the device. Skin pretreatment may be feasible in preclinical studies but the increased risk of skin irritation means that it is seldom a viable option *in vivo*. In addition, to improve patient compliance the patch area should be as small as possible – preferably no more than 5 – 10 cm^{-2} if it is for prolonged use; a patch is undoubtedly better accepted if it is discrete. Other factors to be considered are ease of use and the ability of patients to manipulate the device. This is especially important for certain patients, for example, the geriatric population. Careful selection of drug candidates must be combined with well-thought-through product design and development programs that target unmet patient and therapeutic needs in order to realize the full potential of transdermal iontophoresis as a means finally to deliver biotechnology derived therapeutics non-invasively into the body.

Declaration of interest

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Affiliation

Taís Gratieri, Dhaval Kalaria & Yogeshvar N Kalia[†]
[†]Author for correspondence
University of Geneva,
School of Pharmaceutical Sciences,
30 Quai Ernest Ansermet,
1211 Geneva 4, Switzerland
Tel: +41 22 379 3355; Fax: +41 22 379 3360;
E-mail: Yogi.Kalia@unige.ch