Electrochemical Methods for Drug Characterisation and Transdermal Delivery

Capillary Zone Electrophoresis, Conductometry and Iontophoresis

BY

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ABSTRACT


This thesis concerns the development and utilisation of techniques for characterisation and transdermal delivery of various systems for pharmaceutical applications.

The degree of dissociation of drug molecules and the mobilities of the different species formed are essential factors affecting the rate of drug delivery by iontophoresis. Hence, determination of drug mobility parameters and equilibrium constants are important for the development of iontophoretic systems. With capillary zone electrophoresis using a partial filling technique and methyl-β-cyclodextrin as chiral selector, the enantiomers of orciprenaline were separated. The association constants between the enantiomers of the drug and the selector were also evaluated. Precision conductometry studies were performed for the hydrochloride salts of lidocaine and 5-aminolevulinic acid in aqueous propylene glycol and water as media, respectively.

Iontophoresis is a technique for drug delivery where charged molecules are transported into and through skin by application of a weak direct electrical current. The drugs 5-aminolevulinic acid and its methyl ester were used as model compounds and incorporated in two different drug delivery vehicles, a sponge phase and a carbopol gel. The bicontinuous structure of the sponge phase, constituted of monoolein and a mixture of propylene glycol and water, makes it interesting for use in iontophoretic delivery, since ions can move more or less freely in the aqueous as well as in the lipid domains. Furthermore, all three components are known for their penetration enhancing abilities. Hydrogels like carbopol gels are interesting media with respect to iontophoretic studies, since devices for iontophoresis often utilize hydrogels as contact interfaces between the skin and the electrodes. The results indicate that the transport achieved iontophoretically using the gel (1 % active substance) was comparable with the passive delivery of clinically used formulations (16 % - 20 % active substance).

Keywords: Capillary zone electrophoresis, precision conductometry, iontophoresis, passive diffusion, lidocaine, ALA, ALA-derivatives, sponge phase, carbopol gel

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Till mina föräldrar
List of papers

This Thesis is based on the following papers, which will be referred to by their roman numerals in the text.

I. Determination of association constants between enantiomers of orciprenaline and methyl-\(\beta\)-cyclodextrin as chiral selector by capillary zone electrophoresis using a partial filling technique

II. On iontophoretic delivery enhancement: Electrical conductance and transport properties of lidocaine hydrochloride in aqueous propylene glycol

III. Improvements of conductivity measurements of electrolyte solutions using a new conductometric cell design

IV. Transport properties and association behaviour of the zwitterionic drug 5-aminolevulinic acid in water. A precision conductometric study

V. Transdermal drug delivery by means of a lipid sponge phase - iontophoretic and passive delivery of 5-aminolevulinic acid (ALA) and its methyl ester (m-ALA)
Nadia Merclin, Johanna Bender, Emma Spar, Hans Ehrsson, Sven Lingström, manuscript

VI. Iontophoretic delivery of 5-aminolevulinic acid and its methyl ester using Carbopol gel as drug vehicle
Nadia Merclin, Tobias Bramer, Katarina Edsman, manuscript
Abbreviations

CE        capillary electrophoresis
CZE       capillary zone electrophoresis
UV detection ultraviolet detection
EOF       electroosmotic flow
CDs       cyclodextrins
β-CD       beta cyclodextrin
m-β-CD     methyl beta cyclodextrin
PFT        partial filling technique
PG         propylene glycol
LidHCl     lidocaine hydrochloride
ALAHCl     5-aminolevulinic acid hydrochloride
m-ALA      5-aminolevulinic acid methyl ester
h-ALA      5-aminolevulinic acid hexyl ester
o-ALA      5-aminolevulinic acid octyl ester
SC         stratum corneum
TEWL       transepidermal water loss
PDT        photodynamic therapy
BCC        basal cell carcinoma
ALAS       ALA synthase
PpIX       protoporphyrin IX
MO         monoolein
DMSO       dimethylsulfoxide
NMP        N-methyl-a-pyrrolidone
PEG        polyethylene glycol
The common denominator in the six papers included in this Thesis is the utilisation of techniques where charged particles migrate in an applied electrical field. The main mechanisms for transport of components by these methods are electromigration and electroosmosis (current-induced convective flow of water). Through the latter, also polar neutral molecules can be delivered. Capillary zone electrophoresis (CZE) and precision conductometry was used to characterize different systems that can be used for iontophoretic delivery.

The skin has increasingly become a route for the delivery of drugs with a range of compounds for transdermal delivery resulting in a great deal of interest in this area of research. The passive delivery of most compounds across the skin is limited due to the barrier properties of the outermost layer of the skin called the stratum corneum. Several penetration enhancement methods are being investigated and one of them is iontophoresis.

Iontophoresis is a non-invasive technique where a mild electric current is used to facilitate the delivery of charged molecules through electromigration and neutral molecules via electroosmosis. When performing iontophoresis in vivo, current is passed through a drug-containing active electrode in contact with the skin. A second passive electrode completes the circuit. The mobility and state of dissociation of the drug molecules are important factors affecting the rate of drug delivery. Hence, access to drug mobility parameters, as well as equilibrium constants is important in the development of such systems.

Precision conductometry was used to acquire basic physicochemical data like the ionisation and mobility of a drug in a certain drug-solvent system of interest. By determining the concentration dependence of the molar conductivity of an electrolyte and combine such data with transport numbers, the various kinds of possible aggregates present in the solution may be identified and quantitatively established.

Evaluation of the binding affinity of drugs to various chiral selectors and especially proteins is of importance to try to understand the mechanisms in separation systems. Capillary zone electrophoresis, using a partial filling technique, was employed. The enantiomers of a compound will interact in a specific way with the chiral selector. Depending on the affinity of the solute to the chiral selector, the electrophoretic mobility will change and hence improve the enantioresolution.
2. Methods used for characterisation

2.1 Principles in capillary electrophoresis

The use of electric fields to separate molecules has been applied since the late 1800’s, but the first to demonstrate free solution electrophoresis in an open tube was Stellan Hjörten in 1967 [1]. The first paper on modern capillary electrophoresis (CE) was presented by Jorgenson and Lukacs [2]. Separation by electrophoresis occurs due to differences in the migration rates between compounds in an applied electric field. In CE, separation is carried out in narrow fused-silica capillaries usually with an inner diameter of 5-100 µm. The outside is covered with a protective layer of polyimide. The capillaries are often filled with a buffer that can support a current, but they can also be coated on the inside or packed with a stationary phase. The instrumental set-up used in CE is shown schematically in Fig. 1. The electrodes immersed into the two buffer reservoirs are connected to a high-voltage supply capable of developing a potential of 20 – 30 kV. The sample is injected into the anode end of the capillary. Under the influence of the electrical field the components migrate towards the cathode passing through a detector on the way. A point of detection is created by heat-removing the polyimide coating of the capillary. The most common source of detection is UV.

Fig. 1: Schematic representation of a CE system
2.1.1 Modes of sample injection

At one end of the capillary (usually the anode end) the sample can be introduced either by hydrodynamic or electrokinetic injection. Hydrodynamic sample injection can be accomplished by applying pressure at the injection end of the capillary. Using the Hagen-Poiseuille equation, Eq. 1, the volume of the sample loaded can be calculated:

\[
Volume = \frac{\Delta P \cdot d^4 \cdot t \cdot \pi}{128 \cdot \eta \cdot L}
\]  

(1)

The injected volume depends on capillary dimensions \((d, L)\), the viscosity \((\eta)\) of the buffer in the capillary, the applied pressure \((P)\) and the time \((t)\).

Electrokinetic injection is performed by replacing the injection-end reservoir with the sample vial and applying the voltage. In this mode of injection, the analyte enters the capillary both by migration and the pumping action of the electroosmotic flow (EOF). The amount, \(Q\), analyte injected can be calculated according to:

\[
Q = \frac{(\mu_e + \mu_{EOF}) \cdot V \cdot \pi \cdot r^2 \cdot c \cdot t}{L_c}
\]  

(2)

where \(\mu_e\) is the electrophoretic mobility of the analyte and \(\mu_{EOF}\) is the mobility of the electroosmotic flow, \(V\) is the applied voltage, \(r\) the capillary radius, \(c\) the analyte concentration, \(t\) the time and \(L_c\) the total length of the capillary.

2.1.2 Electroosmotic flow

The capillary wall in fused silica capillaries contains silanol groups. Depending on the pH, these acidic groups will dissociate giving the wall a negative charge. Cationic components in the background electrolyte will be attracted to the negatively charged wall creating an electrical double layer containing an excess of cations. The layer formed closest to the wall is called the Stern layer and is considered immobile. The second layer, the diffuse layer, consists of solvated cations. When an electric field is applied, the solvated cations in the diffuse double-layer migrate towards the cathode. The movement of the solvated ions is propagated through the solution by friction forces, causing a net movement of the bulk solution in the direction of the cathode. The potential across the layers is called the zeta potential and its magnitude is affected by the surface charge and the ionic strength of the buffer.

According to Eq. 3, the mobility of the electroosmotic flow is directly proportional to the zeta potential \((\zeta)\) and the dielectric constant \((\varepsilon)\) of the background electrolyte and inversely proportional to the viscosity of the medium \((\eta)\):
\[ \mu_{\text{EOF}} = \frac{E \cdot \zeta}{\eta} \]  

The velocity of the electroosmotic flow depends on the strength of the field \( E \) and can be calculated by Eq. 4:

\[ v_{\text{EOF}} = \mu_{\text{EOF}} \cdot E \]  

The flow profile of the electroosmotic flow in the capillary is rather flat (Fig. 2a). This is in contrast to that generated by an external pump which yields a laminar flow (Fig. 2b). In a laminar flow the difference in velocity at different distances from the capillary wall is large which in turn has a negative effect on the efficiency (band-broadening). The flat flow profile is therefore advantageous in this respect.

The electroosmotic flow will increase with pH [3-5] and decrease with increasing ionic strength [6]. In capillary zone electrophoresis (CZE) the electroosmotic flow can be advantageous, allowing faster separations as well as simultaneous separation of cations, anions and neutral solutes. However, in some modes of capillary electrophoresis, like isotachophoresis [7] and isoelectric focusing [8], the electroosmotic flow should be suppressed.

Isotachophores (ITP) is a moving boundary electrophoretic technique. A combination of two buffer systems is used to create a state in which the separated zones all move at the same velocity. In a single ITP experiment either cations or anions can be analysed.

In isoelectric focusing (IEF) the separation is based on differences in isoelectric points and the technique has been used in separations of large biomolecules like proteins, lipoproteins and glycoproteins.
2.1.3 Electrophoretic mobility

The migration of ions in an electrical field will be affected by properties like charge and size. The electrophoretic mobility for a spherical ion is defined as:

$$\mu_e = \frac{v}{E} = \frac{\tau}{6 \pi \eta \cdot r}$$

(5)

where \(v\) is the ion velocity, \(E\) the electric field strength, \(\tau\) the net charge, \(r\) the ionic radius of the solvated species. From Eq. 5 it is evident that small, highly charged species have high mobilities whereas large, minimally charged species have low mobilities.

It is also possible to calculate the mobility from an electrophoretic experiment according to:

$$\mu = \frac{L_d}{V \cdot t_m}$$

(6)

where \(L_d\) is the distance to the detector and \(t_m\) the migration time.

The electrophoretic mobility is independent of voltage and capillary length. However, parameters like pH, ionic strength and buffer additives will affect the mobility.

In the presence of electroosmotic flow the mobility measured is called the observed mobility or the apparent mobility, which includes both the electrophoretic mobility \(\mu_e\) and the electroosmotic mobility \(\mu_{EOF}\):

$$\mu_{obs} = \mu_e + \mu_{EOF}$$

(7)

2.1.4 Efficiency and resolution

The efficiency, expressed as number of theoretical plates \((N)\) is a dimensionless quantity, which can be determined directly from an electropherogram using the following equation:

$$N = 5.54 \left( \frac{t_m}{w_{1/2}} \right)^2$$

(8)

where \(w_{1/2}\) is the width of the peak at the half peak height, expressed in time units. The resolution of two components can be expressed with respect to efficiency according to the following expression developed by Giddings [9]:

$$\text{Resolution} = \frac{N_{12} + N_{21}}{2N}$$
\[ R_e = \frac{\sqrt{N}}{4} \cdot \frac{\Delta \mu_{app}}{\mu_{app}} \]  

(9)

where \( \Delta \mu_{app} \) is the difference in electrophoretic mobility between the two components and \( \mu_{app} \) is the average electrophoretic mobility of the two components.

2.1.5 Cyclodextrins and their derivatives used as chiral selectors

Cyclodextrins (CDs) are widely used as chiral selectors. They are non-ionic cyclic oligosaccharides containing glucose units linked through \( \alpha(1 \rightarrow 4) \) linkages. The most frequently used CDs contain six, seven or eight glucose units, and are called \( \alpha \), \( \beta \) and \( \gamma \)-CDs respectively. Their shape resembles a hollow truncated cone with a cavity diameter determined by the number of glucose units. Therefore, each CD shows a different capability of complex formation with differently sized guest molecules. In Fig. 3 the chemical and cone-shaped structure is shown for \( \beta \)-CD. The interior of the molecule is relatively hydrophobic while the external surface is hydrophilic. The primary hydroxyl groups are located on the narrow side of the truncated cone while the secondary hydroxyl groups are situated on the wider edge [10]. These characteristics have made it possible to use CDs for enantioseparation. In Paper I, methyl-\( \beta \)-CD (m-\( \beta \)-CD) was used as chiral selector in the enantioseparation of orciprenaline by capillary zone electrophoresis using a partial filling technique.

Fig. 3: The chemical (a) and cone-shaped (b) structure for \( \beta \)-CD (adapted from Loftsson and Breust, 1996)
2.1.6 Partial filling technique-PFT

The partial filling technique has previously been used in the separation of several compounds and applying different selectors [Paper I, 11-14]. This technique allows for a low consumption of the selector (50-800 nL/run). Also, detection interferences between the analyte and the chiral selector are avoided.

The first step in the procedure is flushing the capillary with a buffer. The selector solution is then applied prior to the run, at the most up to the detection window, followed by introduction of the sample. During the run, both ends of the capillary are immersed in buffer. The separation of the enantiomers takes place in the selector plug. The enantiomers move out of the separation zone towards the detection window with the same velocity. The separation conditions can be chosen in such a way that the UV-absorbing separation plug

✓ migrates away from the detector
✓ is stationary in the capillary (i.e. its mobility is negligible)
✓ migrates slowly in the same direction of the enantiomers (µen) with the electroosmotic flow or its own mobility (µPL< µen)

The partial filling technique has proved to be efficient in the determination of association constants [15] and by applying varying lengths of selector the resolution and enantioselectivity can be regulated.

2.1.7 Determination of association constants

The binding affinity of drugs to different chiral selectors and especially to proteins is very important for the understanding of the mechanisms in separation systems. Examples of selectors used in CE when performing enantioseparations are proteins [16-19] and CDs [20-21].

Electrophoretic methods have previously been developed for the determination of association constants between a charged ligand and proteins [22-24]. The evaluation of association constants between different chiral selectors and enantiomers have also been performed using the partial filling technique [25].

In the calculations the assumption is made that an enantiomer forms a 1:1-complex with the chiral selector. Furthermore, it is also assumed that the mobility of the complex formed of CD and each enantiomer (µCD,R,S) respectively, is the same. The association constants of the two enantiomers are given by:

\[
K_R = \frac{[R-CD]}{[R][CD]} \quad \text{and} \quad K_S = \frac{[S-CD]}{[S][CD]}
\]

(10)
where $R$ and $S$ is the denotation of the two enantiomers, $CD$ is the chiral selector, in this case cyclodextrin. $K_R$ and $K_S$ are the association constants of the $R$- and $S$- enantiomer respectively.

The observed electrophoretic mobility ($\mu_{obs}$) of the solute will be the sum of the mobility of the solute within and out from the plug and the mobility of the electroosmotic flow. The mobility of the solute within the plug is in turn composed of the electrophoretic mobilities of the free and complexed solute. The observed electrophoretic mobility, $\mu_{obs}$, in Eq. 11 is valid for any of the two enantiomers, but the $R$-enantiomer is chosen as an example. The observed mobility of the $R$-enantiomer can be written as follows:

$$\mu_{obs} = X \left( \frac{[R-CD]}{[R]+[R-CD]} \right) \mu_{CD,R} + X \left( \frac{[R]}{[R]+[R-CD]} \right) \mu_R + (1-X) \cdot \mu_k + \mu_{EOF}$$

where $\mu_R$ is the electrophoretic mobility of the $R$- enantiomer in the absence of a separation plug, $\mu_{CD,R}$ and $\mu_{*R}$ stands for the mobilities of the $CD$-enantiomer complex and free enantiomer within the separation plug. The ratio between the effective plug length of the separation zone and the effective length of the capillary is denoted as $X$.

Since the viscosity is different in the selector zone and the neat electrolyte zone, a correction of the observed mobility must be made. This is achieved by measuring the current, which is inversely proportional to the viscosity [26]. The correction factor, $a$, is the ratio between the: “current in the neat electrolyte” and the “current when the capillary is filled with the separation solution at a concentration of interest”, leading to the following relationship:

$$\mu_{corr}(R) = \mu_{obs}(R) + X(a-1) \cdot \mu_{*eff}(R)$$

and

$$\mu_{*eff}(R) = \mu_{*obs}(R) \cdot \mu_{EOF}$$

$\mu_{*eff}(R)$ is the effective mobility of the $R$-enantiomer when the capillary is completely filled with the separation solution and $\mu_{*obs}(R)$ and is the observed mobility of the $R$-enantiomer. The term $[X(a-1)] \mu_{*eff}(R)$ then corrects the $\mu_{obs}$ due to the presence of a plug of separation solution. Eq. 11 can then be transferred to:

$$\mu_{corr}(R) = \mu_k + X \left( \frac{[R-CD]}{[R]+[R-CD]} \right) \cdot \mu_{CD,R} + X \left( \frac{[R]}{[R]+[R-CD]} \right) \cdot \mu_R + \mu_{EOF}$$

By combining Eq. 10 and 14 we arrive at the following expression:
\[
\mu_{\text{corr}(R)} - (1 - X)\mu_R = X\left(\frac{K_R[CD]\mu_{\text{CD},R} + \mu_R}{K_R[CD] + 1}\right) + \mu_{\text{EOF}}
\]

The association constants can be evaluated from the slope of a graph where \([\mu_{\text{corr}}(1-X)\mu_R]\) is plotted as a function of \(X\). Solving for the association constant the following relationship is obtained:

\[
K_R = \frac{\mu_R - \text{slope}}{[CD] \cdot (\text{slope} - \mu_{\text{CD},R})}
\]

The association constants between the two enantiomers of orciprenaline and CD investigated in Paper I were independent of the chiral selector concentration. Furthermore, the enantion resolution was improved by increasing the length of the separation zone.

2.2 Principles of precision conductometry

Electric conductometry is a technique where the motions of ions in a solution can be studied by applying an electric field resulting in a potential difference between two electrodes immersed in the sample. The conductivity cell is incorporated into one arm of a conductivity bridge.

In the investigations presented in this Thesis a Leeds and Northrup 4666 high-precision conductivity bridge was used in the experimental set-up.

Conductivity cells of the Daggett-Bair-Kraus type [27] fitted with bright platinum electrodes (Paper II) and platinized electrodes (Paper IV) were used. A new conductometric cell design is presented in Paper III. These two kinds of cells are depicted in Fig. 4.

\[\text{Fig. 4: Cells of the Daggett-Bair-Kraus type (a) and the newly developed cell (b)}\]
Using the newly developed cell, significant improvements over conventional conductivity technique are achieved. The time required for a run, involving determination of a complete conductivity-concentration curve (cf. figure 5), is reduced by at least a factor of ten. The amount of drug required is diminished, which is of particular importance in case of costly drugs. A high degree of reproducibility of this technique is observed.

Precision conductometry has proved to be a most powerful tool in investigating structure and transport properties of electrolytes in liquid solution. By determining the concentration dependence of the molar conductivity of an electrolyte and combine such data with transport numbers, the various kinds of aggregates present in the solution may be identified and quantitatively established.

2.2.1 Experimental procedure; calibration and measurements

The cell constant, \( k \), of the conductivity cells used is determined by measuring the resistance of aqueous potassium chloride according to Lind et al. [28]. To eliminate the frequency dependence of the relaxation effect, resistance measurements are performed at several frequencies, \( \nu \). The resistance, \( R \), is plotted against the inverse of the frequency, and extrapolated to \( 1/\nu = 0 \). This procedure yields the resistance value, \( R_0 \), at infinite frequency. The cell constant is then calculated using the following equation:

\[
k = \kappa_{KCl} \cdot R_0
\]

(17)

where \( \kappa_{KCl} \) is the conductivity of potassium chloride.

Stock solutions of the drugs in the different solvent media investigated were prepared on weight basis. Weights were corrected to \( \text{v}ac\nu \). A DMA O2 C digital precision density meter was used to determine the densities of the stock solutions and solvents. When cells of the Daggett-Bair-Kraus type were employed portions of a stock solution were successively added, by means of a calibrated precision burette, directly into the conductivity cell containing a predetermined weight of solvent. The burette with stock solution and the cell were both kept at a temperature of 25.00 ± 0.02 °C.

When using the new cell design, it is possible to reverse the procedure for the measurements, i.e. to start with a small volume of stock solution in the cell, to which portions of solvent is added. Furthermore, there is no need to keep the temperature constant (formerly at 25.00 °C), since it is measured directly in the stirred sample by means of a thermistor, followed by re-evaluation of the cell resistance to the desired standard temperature.

The \( R_0 \)-value for each concentration of the drug was determined by the same procedure as in calibrating the cell. Finally, the molar conductivity of the drug solution, \( (\Lambda_{\text{drug}}) \), is calculated according to:

\[
\Lambda_{\text{drug}} = \frac{\kappa_{\text{drug}}}{c} = \frac{k}{R_0 \cdot c}
\]

(18)
The concentration dependence of the molar conductivity is, besides ion aggregation, ascribed to the relaxation and the electrophoretic effect, respectively.

2.2.2 Electrophoretic effect

An ion in an applied electric field will move through a viscous medium and drag along the solvent in its vicinity. Neighbouring ions will move against or together with a stream of solvent molecules, either in the same or opposite direction as the first ion. Onsager and Fuoss [29] treated the electrophoretic effect by using the Boltzmann distribution law.

2.2.3 Relaxation effect

The ionic atmosphere around ions in a solution will be symmetrically distributed if the solution is in equilibrium. No net force is therefore exerted on the ions. This spherical symmetry will be disturbed when an electric field is applied and will most likely lead to a decreased velocity of the ions. This phenomenon is called the relaxation effect [29] and conductivity data are corrected for this effect by extrapolation of the resistance to infinite frequency.

2.2.4 Remarks on the choice of systems investigated

The system lidocaine in aqueous propylene glycol (PG) was investigated (Paper II). This is an interesting system as PG is a well-known transdermal penetration enhancer and one of the most common solvents used pharmaceutically. It has also been shown, that permeation of lidocaine increases significantly when an aqueous propylene glycol (20% PG) is used as base in transdermal formulations [30].

Aqueous lidocaine hydrochloride was used as model system when a new conductometric cell design for conductance measurements was developed and tested (paper III). The experimental data were fitted to a derived conductance equation (see section 2.2.5) and to the data previously reported by Sjöberg et al. [31].

In iontophoretic delivery it is important to have a maximum fraction of the drug in the reservoir in a charged state. One of the main factors affecting the ionic state of a drug is the character of the solvent used. Solvent media often used are water or water based gels. Therefore, the zwitterionic drug 5-aminolevulinic acid (ALA) was investigated in water (Paper IV).

2.2.5 Interpretation of conductance data using the Fuoss, Hsia, Fernandez-Prini (FHFP) conductance equation

Assumptions concerning existing equilibria in the medium used is a starting point in deriving a conductance equation for the system investigated. Lidocaine hydrochloride is
considered to be completely dissociated into LidH⁺ and Cl⁻ ions in water. The LidH⁺ can dissociate into Lid and H⁺. Any pairing between LidH⁺ and Cl⁻ is assumed to be negligible. The only equilibrium necessary to be considered is that between LidH⁺ ions, electrically neutral Lid molecules and protons, i.e.

\[
\text{LidH}^+ \leftrightarrow \text{Lid} + \text{H}^+
\]  

(19)

The thermodynamic dissociation constant, \( K_a \), of LidH⁺, defined in terms of activities of the species involved results in the following equation:

\[
K_a = \frac{a(\text{Lid}) \cdot a(\text{H}^+)}{a(\text{LidH}^+)}
\]  

(20)

Assuming activity coefficients of these univalent ions to be equal and that of the electrically neutral Lid molecule to be equal to unity, the dissociation constant can be expressed in terms of concentrations:

\[
K_a = \frac{c(\text{Lid}) \cdot c(\text{H}^+)}{c(\text{LidH}^+)} = \frac{c \cdot \alpha^2}{1 - \alpha}
\]  

(21)

where \( c \) is the analytical LidHCl concentration and \( \alpha \) the degree of dissociation of LidH⁺. The dissociation constant is related to the molar conductivity expressed by the following equation:

\[
\Lambda = m \left[ \lambda_0 \left( \text{H}^+ \right) \alpha + \lambda_0 \left( \text{LidH}^+ \right) \left( 1 - \alpha \right) + \lambda_0 \left( \text{Cl}^- \right) \right]
\]  

(22)

where \( \lambda_0 \) is the limiting molar conductivity for the ions.

The factor, \( m \), is introduced to correct for the change in ionic mobility with the charge density of the solution. According to the FHFP equation [32-34] the dependence of this
correction factor on the concentration, \( c_i \), of free ions of a univalent electrolyte can be expressed as:

\[
m = \left( \frac{\Lambda_0 - S \cdot c_i^{1/2} + E \cdot c_i \cdot \log c_i + J_1 \cdot c_i - J_2 \cdot c_i^{3/2}}{\Lambda_0} \right)
\]  

(23)

The coefficients \( S \) and \( E \) depend on the limiting molar conductivity, \( \Lambda_0 \), the temperature, and solvent properties (dielectric constant, \( \varepsilon \), and viscosity, \( \eta \)). In addition, the coefficients \( J_1 \) and \( J_2 \) depend on the maximum distance between the charges of paired ions. This parameter was set equal to the Bjerrum radius, which for univalent electrolytes in aqueous solution at 25 °C is equal to 0.357 nm [29].

Using a computer program, developed by Beronius, the values of \( K_0 \) and \( \lambda_0(\text{LidH}^+) \) resulting in the best fit of Eq. 15 to the experimental data can be calculated. A graph showing the dependence of the molar conductivity of \( \text{LidHCl} \) in water is presented in Fig. 5.

![Graph showing the dependence of molar conductivity of \( \text{LidHCl} \) in water.](image)

Fig. 5: Dependence of molar conductivity of \( \text{LidHCl} \) in water.
The full drawn curve represents Eq. 15 fitted to the experimental points.

The data from the investigation of \( \text{ALAHCl} \) in water (paper IV) were analysed in the same way as outlined above for \( \text{LidHCl} \) in water. For \( \text{LidHCl} \) in aqueous propylene glycol (paper II) the conductivity data were analyzed in a similar way.
3. Transdermal drug delivery

3.1 Principles of iontophoresis

Iontophoresis is a technique for drug delivery where charged molecules are transported into and through biological membranes by application of a small direct electrical current (no more than 0.5 mA/cm²). The current is passed through a drug-containing electrode that is in contact with the skin. A grounding electrode is placed in the vicinity in order to complete the circuit (Fig. 6).

![Fig. 6: Schematic representation of iontophoresis in vivo](image)

Iontophoretic delivery of drugs has proved to be useful for increasing the skin permeation of several compounds. This technique also offers a high degree of control over the drug delivery rate compared to passive diffusion. Transdermal delivery of drugs is also advantageous as the drug is directed into the blood system, avoiding the first-pass hepatic circulation and systemic toxicity is virtually eliminated because of the minute amounts of drug delivered. Fear of administration, especially in comparison with administration by using syringe and needle, is eliminated.

Several drugs have been investigated and used iontophoretically, for example peptides [35-37], hormones [38-40] and antihistamines [41].
3.2 Transport mechanisms in iontophoresis

Iontophoresis enhances drug delivery across the skin by mainly two mechanisms, viz. electrorepulsion and electroosmosis. Electrorepulsion is the primary mechanism in the delivery of charged species. Cations will be driven from the anode and anions from the cathode. However, polar neutral molecules can be delivered by electroosmosis (current-induced solvent flow). Passive delivery also contributes to the total flux in iontophoretic delivery, but in almost all cases of practical importance, this flux is considered negligible.

At physiological pH, the skin is negatively charged and therefore cation-permselective [42]. Application of a current will induce electroosmotic flow in the anode-to-cathode direction [43-46], facilitating the transport of cations, inhibiting that of anions, and enabling the enhanced transport of polar, neutral solutes. However, the electroosmotic flow can be altered by hydrophobic positively charged drugs [47-49].

In principle, iontophoresis is very similar to electrophoresis, both systems require electrodes and a power supply and the transport mechanisms in capillary electrophoresis (CE) are the same. In iontophoresis the amount of electricity required to drive the molecules is small, and the skin acts as a drug transport barrier between the two electrodes. For CE, high voltage is applied in order to drive the charged species, and the length of the medium through which they travel under the electrical influence is much greater than the thickness of the skin.

3.3 Transport pathways in the skin

During iontophoresis, most ions are expected to follow the path of least resistance and to penetrate through damaged regions of the skin and down the shunts of the hair follicles and sweat glands. The current, if high enough, may form artificial shunts as the lipids of the stratum corneum (SC) temporarily disrupt to form pores [50-53]. Passive diffusion of most solutes on the other hand, occurs predominantly through SC rather than through the pores [54].

3.4 The structure of the skin

The skin is one of the largest organs of the body, having a surface area of approximately 2 m² (for an adult). It has many functions, the most important of which is as a barrier to protect the body from hazardous external factors. Furthermore, the skin barrier prevents water from diffusing out of the body (homeostasis). However, a loss of 150-250 mL water per day per square meter of skin surface, called the transepidermal water loss (TEWL), indicates that this barrier is not water tight.

Skin is comprised of three layers: the epidermis, the dermis and the subcutis (Fig. 7).
The subcutaneous layer consists of loose connective tissue and fat. The dermis is mostly connective tissue and contains appendages as hair follicles and sweat glands, which extend upward to the surface. The dermis and epidermis (except the stratum corneum) have high water permeability. The dermis is vascularized while the dermis is avascular and extends about 100 µm below the skin’s surface.

The epidermis is defined as a stratified squamous epithelium, about 0.1 mm thick. Its main function is to act as a protective barrier.

The outermost layer of the skin, stratum corneum (SC) or the horny layer, is only about 10-20 µm thick and it constitutes the main barrier to the transport of ionized molecules [55-58]. It is comprised of sheets of cornified cells with no nuclei, called corneocytes. These cells are in turn surrounded by lipid lamellae in which the main lipid components are cholesterol, free fatty acids and ceramides [59] (Fig. 8).

Fig. 8: Molecular structure of (a) ceramide III (C16cerIII), (b) cholesterol and (c) fatty acid (PA, C16:0)
Considerable work has been focused towards the organization of these lipids within SC in order to increase the understanding of the barrier properties of the skin [60-63].

### 3.5 Investigated drugs

ALA is used clinically in combination with photodynamic therapy (PDT) in the treatment of basal cell carcinoma (BCC), a common type of non-melanoma skin cancer. By irradiation of the tissue, at an appropriate wavelength, a photochemical reaction occurs resulting in the production of toxic substances, mainly singlet oxygen, which in turn destroys the tumour cells.

The drug used in the iontophoretic experiments was 5-aminolevulinic acid (ALA). ALA is formed in the first step of the heme biosynthetic pathway (Fig. 9), from glycine and succinyl coenzyme A (CoA) by the enzyme ALA synthase (ALAS). This reaction takes place partly in the mitochondria and partly in the cytosol. The last step in the heme pathway is the incorporation of iron (Fe²⁺) into protoporphyrin IX (PpIX), (Fig. 9) under the action of ferrochelatase.

![Fig. 9: The heme synthesis](image)
When ALA is added exogenously, PpIX may accumulate because of the limited capacity of ferrochelatase, leading to a photosensitising effect of the tissue. This higher degree of PpIX accumulation has been shown in several investigations [64-68].

The penetration depth of ALA is the main factor limiting the efficacy of topical ALA-PDT. Since ALA is a hydrophilic molecule its permeation through biological barriers like cellular membranes or the stratum corneum of the skin is limited. Finding alternative strategies to improve the skin penetration have been investigated in many laboratories [69-71]. One alternative is to use the more lipophilic ALA ester derivatives [72-74] since they penetrate deeper into tumours than ALA itself. Another option is to incorporate penetration enhancers into the drug delivery vehicle [75-77] and/or to use iontophoresis [78-79].

### 3.6 Vehicles investigated in the iontophoretic experiments

Two potential drug delivery vehicles for iontophoretic delivery of ALA and its methyl ester (m-ALA) have been investigated. The first one is the sponge phase (L3) (Paper V) and besides ALA and m-ALA, hexyl- and octyl-ALA (h-ALA, o-ALA) were incorporated in this vehicle. The sponge phase consists of monoolein (glyceryl monooleate, MO), propylene glycol (PG) and water, which are all known for their penetration enhancing capabilities [80-81].

The structure of the sponge phase is shown in Fig. 10.

![Fig. 10: The sponge (L3) phase redrawn from Snabre et al., 1990.](image)

It is also possible to form the monoolein sponge phase using solvents as DMSO, NMP and PEG 400. In the phase diagram of this system (Paper V), the sponge phase is a narrow region at constant water content. It is in equilibrium with a lamellar phase at low water content and a cubic phase at low solvent content.

This phase is a thermodynamically stable isotropic liquid, which forms spontaneously, making the preparation procedure very easy. The structure is bicontinuous, i.e. it is possible
to dissolve both water- and oil soluble substances. The incorporation of poorly soluble substances was shown by Alfons et al. [82] and Ridell et al. [83].

When investigating the iontophoretic delivery of ALA from L₃, three compositions were employed. The content of MO and PG was varied while the water content was kept constant. The results achieved for the delivery of ALA from L₃ indicated that there might be an optimal composition of the phase in order to obtain the highest flux. The reason for this optimum is not clear, but it has been shown that the molar conductivity of a PG/water mixture decreases slightly with increasing PG content [84]. The viscosity of a medium will affect the mobility of ions and their transport numbers. In addition, PG is more viscous than water. This might explain the decrease in ALA flux when using the sponge phase with the highest PG content (50%). The sponge phase with the lowest content of PG (20%) resulted in low fluxes, they are comparable to the physiological buffer system used. When inspecting the vehicle after an iontophoretic run it was observed that the composition had been affected, ending up with possibly a mixture of a liquid and a cubic phase. This change might have influenced the flux.

No detectable amounts of h-ALA and o-ALA were observed. It is possible for the more lipophilic esters, h-ALA and o-ALA, respectively, to interact not only with the lipid bilayer in the sponge phase but also with the skin itself [72]. The delivery of m-ALA was comparable to that of ALA most likely because of the difference in pH in the two vehicles. Adjusting the pH for the vehicles where ALA was included was not possible, the final pH was ~ 4-5 while the vehicles containing the esters were adjusted to pH ~7.

The loading capacity can be substantial in the sponge phase, which is demonstrated (Paper V) by the incorporation of up to 16 % m-ALA, which corresponds to the concentration of the active substance (m-ALA) in the clinically used formulation, Metvix®. Unguentum M (20% ALA) is another common formulation used in PDT-treatment. The two ointments were studied in vitro and the results were compared to the ones attained with the high drug-containing L₃. The fluxes attained after passive delivery of m-ALA from L₃ showed comparable fluxes to the formulations used in clinical practice.

Gels have been used for the delivery of drugs for both systemic and local action (see for example the review by Peppas, et al. [85]). They are also considered to be suitable as a delivery vehicle for iontophoresis [86].

Water based gels have been used as iontophoretic electrodes [87] and are also good electroconductive media. Gel formulations have the additional advantage of ease of application. Different types of gels have been used for the iontophoretic delivery of several drugs [88-90].

A carbopol gel was investigated as a potential drug delivery vehicle for iontophoresis with ALA and m-ALA as model compounds (Paper VI). Carbopol gels are high-molecular weight polymers of acrylic acids with a high content of carboxyl groups. In contact with an aqueous solution at neutral pH the polymer particles swell and form a gel.

The results obtained for ALA are approximately 4 times higher as compared to a physiological buffer system. The gel contains 1 % (60 mM) drug while the concentration of drug in the buffer corresponds to ~ 0.25 % (15 mM). These results are comparable, even though the concentration of ALA is higher in the gel since ALA transport increases linearly with the drug concentration over the interval 1 mM-100 mM [91].
The transport of m-ALA from the gel resulted in a 6-fold enhancement over ALA delivery.

The skin uptake was evaluated for both drugs after the iontophoretic experiments were terminated. The uptake in stratum corneum is 9 times higher for m-ALA than for ALA, corresponding well with previously published data by Lopez et al. [72]. In this study m-ALA was delivered from water with the pH adjusted to ~ 7. Despite the fact that a much higher iontophoretic flux of m-ALA is reached using water as a vehicle, the uptake in the stratum corneum is larger when using the carbopol gel. The skin uptake of ALA was also higher when delivered from the gel even though the iontophoretic fluxes are comparable to literature data [72].

Compared with passive delivery from Metvix® and Unguentum M it seems that iontophoretic delivery from the gel formulation is better than or comparable to the passive delivery in spite of 10-20 times lower concentration of ALA or m-ALA in the gel formulation.

In Fig. 11 the iontophoretic fluxes are shown for the two investigated vehicles, L3 and carbopol gel. For comparison, data from experimental runs using physiological buffer and water are also included.

Fig. 11 a: Anodal iontophoretic fluxes of ALA from L3 (○), carbopol gel (◊) and buffer (△).
The ALA delivery from L₃ resulted in somewhat higher fluxes than when it was delivered from the gel. As mentioned previously the final pH in the L₃ vehicle was approximately 4-5 but the pH in the gel was ~ 7. The pH will of course affect the degree of dissociation of the drug and the electroosmotic flow. The iontophoretic delivery of ALA at pH 7 occurs primarily through electroosmosis. However, the skin’s cation permselectivity [42] can be taken advantage of if the pH is lowered, since the drug will become positively ionized. Lopez et al. [91] showed a pH independence between pH 4 and 7.4. The investigated vehicles gave much better results compared to the buffer system. It should be mentioned though, that results obtained for the delivery of ALA from buffer by Lopez et al. were comparable to the results presented here.

The transport of m-ALA from the gel was comparable to the delivery from L₃. High fluxes of m-ALA through skin is observed when using water with the pH adjusted to ~7. The physiological buffer contains salt (133 mM) and using water will remove competing ions (i.e. Na⁺) from the formulation. Removal of salt from L₃ and the gel did not affect the flux of m-ALA.

The highest reproducibility in the delivery of ALA and m-ALA, was achieved using carbopol gel. Both L₃ and carbopol gel gave comparable or even better results than the ones achieved by in vitro passive delivery from the formulations in clinical use.
4. Populärvetenskaplig sammanfattning

Huden är kroppens största organ och har flera viktiga funktioner. Hudens huvuduppgift är att skydda och isolera de underliggande vävnaderna från yttre påverkan. Huden skyddar de inre organen från mekaniska och kemiska skador samt värmeskador. Den bildar en vattentät barriär som bevarar och upprätthåller nivåerna av kroppsvätskor, vilket är av avgörande betydelse för vår överlevnad. Dessutom upprätthåller den kroppstemperaturen (± 37 °C) genom att bevara eller avge värme, beroende på kroppens behov.


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6. References


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