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Physicochemical and Biopharmaceutical Characterisation of Small Drug Molecules by Capillary Electrophoresis

BY

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Abstract
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Capillary Electrophoresis (CE) was explored as a means for physicochemical and biopharmaceutical characterisation of small drug molecules. Special attention was paid to the characterisation of acid-base and lipophilic properties of drug compounds by analysing their migration behaviour in different CE systems. The thesis comprises an overview of the field together with separate studies on the different topics.

The utility of CE for the determination of pK_a of labile drug compounds was investigated. A general methodology was developed comprising key steps such as the use of a stabilising sample diluent, electromigration injection, and analyte characterisation by UV-Vis spectroscopy. The methodology was successfully applied for two sets of drug compounds, labile at low and high pH, respectively.

CE was also evaluated for experimental modelling of passive intestinal membrane permeability by studying analyte migration in liposomal, microemulsion and micellar electrolytes. Good correlation is reported between CE migration and Caco-2 cell absorption estimates and for in vitro inhibition of thrombin. Interestingly, a slightly better correlation was obtained for liposomal electrolytes.

The utility of liposomes in CE was further extended by developing a novel procedure for immobilising liposomes inside fused silica capillaries. This approach enabled direct on-line coupling of liposome CE to high sensitivity mass spectrometry. The utility of liposome-coated capillaries is demonstrated for estimating drug passive intestinal membrane permeability. Its use in biopharmaceutical drug profiling is discussed.

Utilising advanced molecular descriptors, commonly applied to in silico prediction of passive intestinal membrane permeability, migration of analytes in micellar CE systems could be well predicted. The novel approach was based on hierarchical multivariate analytics and use of molecular descriptors for both analytes and micellar media surfactants. Demonstrated results propose that the CE format could be useful to validate how representative molecular descriptors are for describing molecular behaviour in complex liquid media, e.g. physiological systems.

Keywords: capillary electrophoresis (CE), micellar electrokinetic chromatography (MEKC), drug, liposome, microemulsion, coating, pKa, lipophilicity, multivariate analysis, molecular descriptor, hierarchical analysis, passiv absorption

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Till Lars

List of papers

This thesis is based on following papers that are referred to by Roman numerals (**I-VI**) in the body of this document:

I. Determination of dissociation constants of labile drug compounds by capillary electrophoresis. Eivor Örnskov, Anna Linusson, Staffan Folestad. *Journal of Pharmaceutical and Biomedical Analysis,* 33, (2003), p. 379-391.

II. Statistical molecular design, parallel synthesis and biological evaluation of a library of thrombin inhibitors. Anna Linusson, Johan Gottfries, Thomas Olsson, Eivor Örnskov, Staffan Folestad, Bengt Nordén, Svante Wold. *Journal of Medicinal Chemistry*, 44, (2001), p. 3424-3439.

III. Experimental modelling of passive drug absorption by capillary electrophoresis using liposomal, micellar and microemulsion electrolytes. Eivor Örnskov, Johan Gottfries, Magnus Erickson, Staffan Folestad.
Submitted to *Journal of Pharmacy & Pharmacology.*

IV. Method for immobilization of liposomes in capillary electrophoresis by electrostatic interaction with derivatized agarose. Eivor Örnskov, Sara Ullsten, Lennart Söderberg, Karin E. Markides, Staffan Folestad. *Electrophoresis*, 23, 19 (2002), p. 3381-3384.

V. Immobilised-liposome capillary electrophoresis – online mass spectrometry for biopharmaceutical drug profiling. Sara Ullsten, Eivor Örnskov, Per Sjöberg, Katarina Edwards, Göran Karlsson, Karin E. Markides, Staffan Folestad.
Manuscript

VI. Hierarchical multivariate modelling and prediction of MEKC migration using analyte and micellar media molecular descriptors. Eivor Örnskov, Anna Linusson, Erik Johansson, Staffan Folestad.
Submitted to *Electrophoresis.*

Contents

1 Introduction to drug characterisation by capillary electrophoresis

Development of new medicines is a time-consuming process that can take decades. Figure 1 illustrates main steps from an idea to a drug product include drug discovery, drug development and the product launch and marketing.

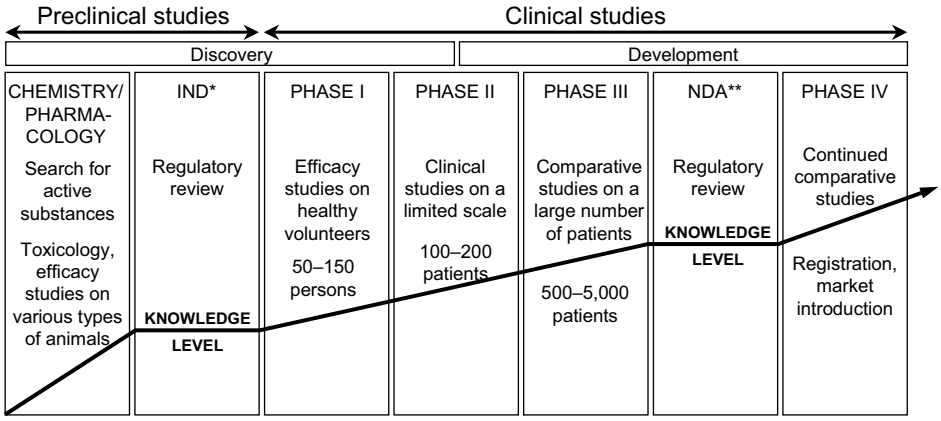


Figure 1. The R&D process of a new drug (redrawn with permission from AstraZeneca Sweden).
** Investigational new drug application for permission to administer a new drug to humans.*
*** New drug application, application for permission to market a new drug.*

The drug discovery process starts with the identification of a biological target and ends with a selection of one or a few candidate drugs that will be tested on humans. A biological target is usually a protein such as a receptor, an enzyme or an ion channel. During drug discovery, the choice of a biological target naturally depends on the possibility that modulation of the target will lead to a disease treatment. After identification of the biological

target, drug discovery comprises hit identification, lead identification and lead optimisation; see Figure 2.

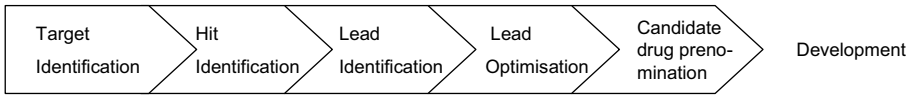


Figure 2. Drug discovery.

An identified hit is a molecule that has shown biological activity in an animal or a human or has displayed activity against a biological target in a high throughput-screening (HTS) assay. A lead is a series of hit molecules that have features that can be modified to optimise pharmacological properties of the molecule. Lead molecules can be found by investigating natural products and compounds synthesised in industrial and university laboratories. Lead molecules can also be synthesised using highly automated systems that produce many compounds but a small amount of each compound.

The lead optimisation phase is started when lead molecules demonstrate biological activity and have modifiable structural elements. Often, lead optimisation is a more time-consuming step (about 2.5 years) in contrast to lead identification and hit identification (about 18 months together). During the lead optimisation phase, many new compounds are synthesised. Compared to traditional sequential synthesis, use of highly automated synthesis systems – together with combinatorial chemistry – has enhanced the number of synthesised compounds, while it has decreased the amount of each compound that is synthesised. This phase stops with identification of a selection of one or a few candidate drugs that can be tested on humans.

Successful drug development requires optimisation of the pharmacological activity of the drug compound at the target site and the delivery to that site. Oral administration is usually the preferred administration route for a drug compound because of its ease of use and patient compliance. Opportunities to use oral administration depend on the drug compound bioavailability, that is, the fraction of a given dose that reaches the blood system. Table 1 summarises factors that influence bioavailability.

Table 1. Factors that influence bioavailability[a].

Physiological factors	**Pharmacokinetic factors**
Membrane transport	Absorption
Gastrointestinal motility	Distribution
Stomach emptying	Metabolism (gastrointestinal and liver)
Disease state	Elimination
Protein and tissue binding	
Formulation properties	**Physicochemical properties**
Crystal form	Lipophilicity
Particle size	Degree of ionisation (pK_a)
Absorption enhancers	Molecular size and shape
Dissolution rate	Hydrogen bonding
Formulation (solution, capsule, tablet, and so on)	Chirality
	Complexation
	Solid state properties (hygroscopicity, crystal form, melting point and solid form)
	Solubility
	Stability
	Surface activity

[a] Reference[1].

Delivery of drug molecules to the blood system usually includes a passive or an active transport through the intestinal membrane. The drug compound's physicochemical properties[2] govern passive trans- and paracellular membrane diffusion and also interactions involved in active membrane transport. The most important molecular physicochemical properties, for passive absorption, are summarised in the "Lipinski rule of five", which states that a compound that violates two out of four limits in H-bond-accepting properties, donor capacity, lipophilicity and size, might have absorption problems[3]. In brief, several physicochemical properties of a drug molecule govern delivery to the target site and activity at that site.

To gain access to the pharmacologically relevant physicochemical and biophysical compound properties, several non-biological and biological in vitro and in vivo methods were developed. Several methods have shortcomings: typically, they require milligram amounts of the compound, and some methods are time consuming and thus have limited throughput.

Recently, use of in silico or virtual methods for physicochemical characterisation of lead molecules became easily available thanks to great strides in computing technology[4, 5]. In silico methods are mostly based solely

on calculations that use theoretical molecular descriptors, which are mathematical representation of the molecules. The main advantage of in silico or virtual methods is that calculations can be done before the compounds have been synthesised, but these methods need experimental data to validate the theoretical models, which is a limitation[6].

So there is still a need for development of fast experimental methods for physicochemical screening that uses minute amounts of the compounds and has good capacity, that is, we need methods that are ultimately suited for high throughput screening. In this context, exploration of capillary electrophoresis (CE) is of interest because of its attractive advantages, for example, CE requires only nanogram amounts of the sample, and it yields high precision and accuracy and is a technique that is easily automated. And CE does not require high-purity substances because it enables separation of impurities and decomposition products from the main component. The characterisation mode can easily be adjusted by simply changing the separation media. Separation mechanisms in CE are relatively simple and enable use of CE data, together with theoretical molecular descriptors for in silico modelling. This accentuates CE's potential as a tool for physicochemical characterisation of small drug compounds. And the CE format enables the introduction of physiological fluids and solutions that simulate different physiological fluids. So CE can also be used as a tool for biopharmaceutical characterisation, i.e., the determination of physicochemical properties and interaction behaviour with different components at physiological conditions.

This study investigated CE as a technique for physicochemical characterisation of active pharmaceutical ingredients. The investigation was limited to small drug molecules. Special emphasis was put on testing the utility of micelles, microemulsions, liposomes and CE as experimental model systems for passive absorption of small drug molecules. For this purpose, modelling and prediction of drug passive absorption with experimental CE data and calculated molecular descriptors was studied. An additional objective was to outline a general strategy for the determination of dissociation constants by CE for labile and partially degraded drug compounds.

2 Capillary electrophoresis (CE)

This chapter briefly discusses the general theory and instrumentation of capillary electrophoresis (CE). Many textbooks[7, 8, 9] and several review articles[10, 11, 12, 13, 14] provide general information on the theory and practical aspects of electrodriven separations using capillaries. Weinberger et al[9] and Vesterberg[15, 16] describe the history of electrodriven separation techniques. Chapter 3, section 3.1 presents the specific theory for methods used in papers **I** and **II**. Chapter 4, section 4.2 presents the specific theory for the methods used in papers **II**, **III**, **IV**, **V** and **VI**.

2.1 Theory and instrumentation

Electrodriven separation is based on differences in mobilities of sample compounds, through a conducting medium, under the influence of an applied electric field. Differences in mobilities depend on the charge and radius of the sample compound and the viscosity of the conducting media.

When the electrodriven separation is performed in capillaries, the technique is called CE. The capillaries have an inner diameter of 25-150 μm and an outer diameter of 190-365 μm, with a common total length of 20-75 cm; in most cases they are made of synthetic quartz (SiO_2) of high purity (fused silica) and have an external layer of polyimide that makes them physically resistant against fractures. Use of capillaries for electrodriven separation permits use of high-field strength, up to 30 kV, which enables fast separations because of effective heat dissipation.

The electroosmotic flow (EOF) is an important phenomenon in CE, i.e., a flow of the separation media through the capillary during analysis. EOF originates from the movement of hydrated cations, that form a viscous electric double layer at the walls of an untreated fused silica capillary when applying an electric field[8]. Usually, a positive potential is applied to the electrode at the inlet end of the capillary, and the electrode at the other end of the capillary is grounded. So the direction of the EOF in untreated, fused silica capillaries is toward the cathode.

The separation medium is called the background electrolyte (BGE) and usually consists of an aqueous buffer that maintains the buffer pH and

provides conductivity. By adding modifiers to the BGE that generate different types of analyte-additive interactions, the separation of sample compounds can be enhanced.

Injection of sample compounds into the capillary is made with hydrodynamic or electrokinetic injection. All common ways of introducing the sample compounds require moving the inlet end of the capillary from the BGE reservoir to a sample solution reservoir during the injection. Hydrodynamic injection is done by applying pressure over a sample reservoir placed at the injection end of the capillary or by applying a vacuum over a sample reservoir that is placed at the other end of the capillary or by a height difference between a sample and a BGE reservoir. The Hagen-Poiseuille equation can be used to calculate the injected volume, V_s

$$V_s = \frac{\Delta P \cdot r^4 \cdot \pi \cdot t}{8 \cdot \eta \cdot L_{tot}} \qquad \text{Equation 1}$$

where ΔP is the pressure drop over the capillary, r is the internal radius of the capillary, t is the injection time, η is the BGE viscosity, and L_{tot} is the total length of the capillary. Electrokinetic injection is performed by applying a voltage with one capillary end situated in the sample reservoir for a short period.

Many detection modes are available, the most common is on-capillary detection that uses ultraviolet-visible (UV-Vis) absorption. The on-capillary detection window is produced by removing a small length of polyimide film at the outlet end of the capillary. The most common alternative detection modes include: laser-induced fluorescence, indirect absorption, mass spectrometry, electrochemical and conductivity detection.

The observed migration time of the analyte relates to the apparent mobility, μ_{app}, which in practice is a sum of the effective electrophoretic mobilities, μ_{eff}, of the analyte and the electrolyte medium, μ_{EOF}. The latter is the electroosmotic mobility. The effective electrophoretic mobility is calculated from the experimentally determined migration time for the analyte and the electroosmotic flow

$$\mu_{eff} = \mu_{app} - \mu_{EOF} = \frac{L_{tot} \cdot L_{eff}}{V} \cdot \left(\frac{1}{t_{app}} - \frac{1}{t_{EOF}} \right) \qquad \text{Equation 2}$$

where L_{tot} is the total length of the capillary, L_{eff} is the length of the capillary to the detection window, V is the applied voltage and t_{app} and t_{EOF} is the migration time of the ionic specie and the marker of the electroosmotic flow, respectively. Electroosmosis is the laminar flow that arises from the movement of hydrated cations and forms a viscous double layer at the walls

of an untreated fused silica capillary, when applying an electric field. The mobility of the EOF depends on the charge density of the silica walls and on the ionic strength, dielectric constant and viscosity of the BGE. The effective electrophoretic mobility of an analyte varies – depending on the physicochemical properties of the electrolyte. Properties, such as pH, ionic strength and viscosity, all affect electrophoretic mobility.

Good resolution of analytes is a combination of efficiency and selectivity. A special advantage with CE is that high efficiency can be obtained for small and large molecules. See the references[8, 9] that describe theoretical and practical aspects on resolution in more detail.

The basic instrumentation for all CE separation modes is the same. A CE system consists of an injection system, a separation capillary, a high voltage devise, electrodes and a detector.

Figure 3 illustrates a schematic representation of the instrumental set-up for a basic CE system.

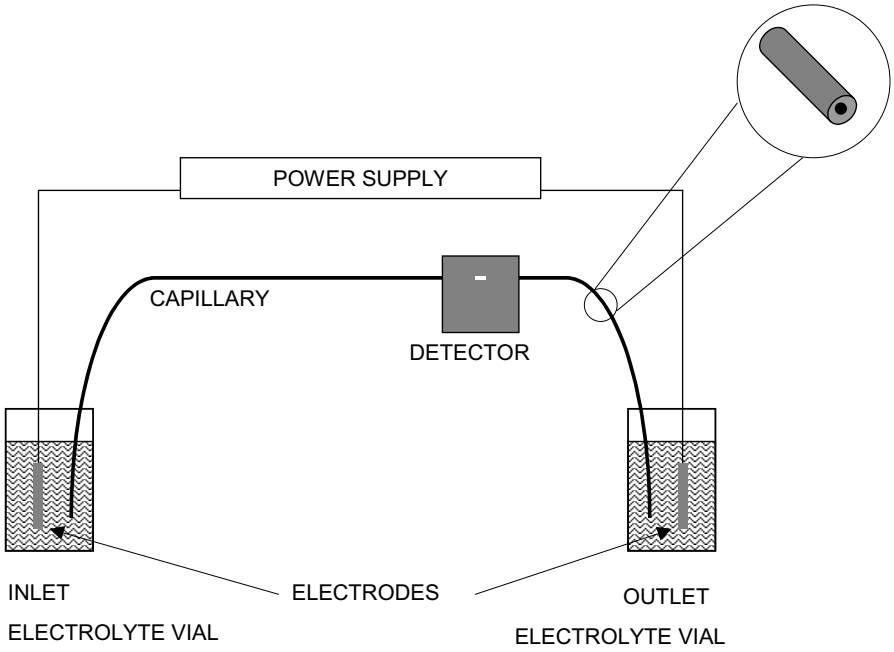


Figure 3. Schematic representation of the instrumental set-up for a basic, capillary electrophoresis system; magnified view shows a close-up of the capillary.

Electrodriven separation can also be performed using microfabricated miniaturised devices[17]. Silica and quartz have often been used as device

compartments. But more recently, disposable polymer chips have gained increased attention[18]. Applying an electric field controls the flow (from reservoirs and through the different channels of the BGE) and sample solutions on the chip. The flow of fluids on a chip device usually originates from electrokinetic phenomena and/or electroosmosis. This technique is still in its infancy; only a few commercial instruments are available. A commercial absorbance-based CE instrument with a 96-capillary array has recently been demonstrated for high throughput physicochemical characterisation of drug compounds[19]

Many different separation CE modes can be used for physicochemical characterisation of drug compounds. The most common modes and those used in papers **I-VII** are described briefly below. All modes are mainly specified by the composition of the BGE and/or the modifications of the capillary. Sections 3.1 and 4.2 present principles for capillary zone electrophoresis and electrokinetic chromatography in more detail.

Capillary zone electrophoresis

Capillary zone electrophoresis (CZE) is the most common mode; here the capillary is filled with a BGE that consists of an aqueous buffer solution. The separation is based on different effective electrophoretic mobilities of the analytes. This mode was used for determination of dissociation constants in papers **I** and **II**.

Micellar electrokinetic chromatography

Adding a surfactant to a buffer solution at a concentration over the (critical micelle concentration (CMC) causes micelles or aggregates to form. These micelles are a moving pseudostationary phase available for analyte interactions. This mode is called micellar electrokinetic chromatography (MEKC). Separation of analyte compounds, which interact with the pseudostationary phase, is performed with MEKC. This mode is commonly used for determination of retention factors, i.e., hydrophobic properties of the sample compounds; refer to papers **II**, **III** and **IV**. Holland et al[20] recently reported a new mode of MEKC that uses bilayered micelles, i.e., bicelles.

Microemulsion electrokinetic chromatography

MEKC can be extended to microemulsion electrokinetic chromatography (MEEKC) by adding modifiers, which are immiscible or partly miscible with water, to a buffer solution that contains surfactants. The general separation mechanism is the same as for MEKC. And the same type of properties, such as lipophilicity, can be characterised by using MEEKC. This mode was used in paper **III**.

Vesicle and liposome electrokinetic chromatography

It is generally recognised that the molecular geometry of the surfactant governs the shape of the aggregate that is formed. Vesicles may be formed by addition of vesicle-forming surfactant to a buffer solution. These vesicles will act as a pseudostationary phase available for analyte interactions as in MEKC. This mode is called vesicle electrokinetic chromatography. Notably, vesicles consist of bilayers that are attractive for use as model membranes. Phospholipids are surfactants that are typically found in cell membranes of living matter. When added to a buffer solution, certain phospholipids cause lipid vesicles to form. In this thesis, these lipid vesicles are called liposomes. The general separation mechanism is normally the same as for vesicle electrokinetic chromatography. Free liposomes added to a buffer solution were used in paper **III**, and liposome coated capillaries were used in papers **IV** and **V**.

3 Characterisation of drug acid-base properties by CE

A drug molecule's acid-base property is a key parameter for drug development because it governs solubility, partition, absorption, distribution, metabolism and elimination. As a measurement of the acid-base property, the dissociation constant(s) (pK_a) is usually determined in aqueous media.

pK_a can be predicted using computational methods. These methods have certain advantages, i.e., calculations can be performed on large virtual compound libraries. But erroneous data are often predicted for complex and flexible drug compounds that contain several functional groups. And these calculations are based on parameters in databases that contain experimental data from the literature. So enough data for new types of compounds are probably missing – for providing accurate predictions.

But several fast experimental techniques are available for determination of pK_a values. Potentiometric titration is in general use because it is fast and accurate, and automated commercial instruments are available; its shortcomings include the requirements to use: (1) milligram amounts of pure compounds and (2) mixtures of aqueous buffer and an organic solvent for low solubility compounds. Data obtained in mixtures require extrapolation to pure aqueous buffer, which may not always be valid.

An alternative to potentiometric titration is UV-Vis spectrophotometric titration because it can handle compounds with lower solubility and lower sample concentrations. But the compound must contain a UV active chromophore close enough to the site of the acid-base function in the molecule. And the determination is sensitive to impurities in the compounds[21].

Both potentiometric titration and UV-Vis spectrophotometric titration can be further miniaturised to consume only a few micrograms of a sample per analysis[22].

Techniques that can handle impure compounds are based on analytical separation principles: liquid chromatography (LC), ion-exchange chromatography, gas chromatography, paper chromatography and traditional isotachophoresis (ITP)[23]. LC is the most frequent technique because of minute compound consumption and because automatic LC systems can be modified to yield high capacity by using a flow injection analysis

approaches[24]. The main disadvantage is the risk of long retention times when using pure aqueous media mobile phases without organic additives for the determination of pK_a[25]. ITP may well be used because is consumes small amounts of compounds. But it has drawbacks, such as laborious calculations, and its main shortcoming is that the buffer pH choice is limited to pH 3-11[26].

The CE technique offers several advantages over traditional techniques for determination of pK_a. The experimental set-up is simple, and by using commercial instruments, the procedure is easy to automate. In addition, determinations can be conducted at different temperatures (15-60°C) and in different media. The CE technique is especially useful if the amount of compound is small because only a few nanogram or less is commonly used for analysis. And pK_a values may also be determined for compounds, which are impure or relatively unstable in aqueous solutions because impurities and decomposition products can be separated from the main component.

3.1 Theory for determination of pK_a from migration data

The mobility of an ionisable analyte is a direct function of the pH of the separation medium, the electrolyte. So the pK_a value(s) of the analyte can be obtained through a series of CE experiments with buffers that have different pHs. By plotting the analyte mobility versus buffer pH, a curve analogous to a potentiometric titration curve is obtained from which the pK_a value(s) can be determined. In practice, the mobility is calculated from the migration time for the analyte and for the electroosmotic flow. Beckers et al[26], Cai et al[27], Cleveland et al[28] and Ishihama et al[29] introduced CE as a technique for determination of dissociation constants. Khaledi's *High Performance Capillary Electrophoresis*[8] reviews the technique, and section 2.1 briefly summarises the theoretical basis for CE. Basic theories for determination of pK_a using CE are also found in the literature[27, 28, 30].

Here, a description of the general theory of pK_a determination by CE, used in papers **I** and **II,** is given.

The basis for determination of dissociation constants by CE is the measurement of the migration time for an analyte, which is separated in a capillary that contains an electrolyte with a specified pH. A charged analyte in a pure water medium migrates with an intrinsic ionic mobility, μ_{ion}, when an electric field is applied. The ionic mobility depends on the charge, form and size of the ion and the attached solvation shell. If the water contains ionic additives, the ionised analyte migrates at an effective electrophoretic mobility, $\mu_{eff,}$ also referred to as the electrophoretic mobility.

Ions of the opposite sign always surround an ionised analyte in a solution. The electrostatic interaction between the analyte and the ion sphere, which migrates in the opposite direction, will affect the analyte's mobility, so analyte mobility decreases with increasing ionic strength[10].

In this way, the vector sum of the effective electrophoretic mobility of an analyte and the electroosmotic mobility gives the apparent mobility, μ_{app}, also defined by Smith et al[31] and Knox[32] as the overall mobility.

A weak basic or acidic analyte consists of several ionic or neutral species that are in fast, dynamic equilibrium together. So the analyte migrates as a single analyte. The effective electrophoretic mobility of a partially dissociated analyte is a function of the ionic mobility of the respective specie

$$\mu_{eff} = \sum_i x_i \cdot \mu_{ion} \qquad \text{Equation 3}$$

$$x_i = \frac{c_i}{\sum_j c_i} \qquad \text{Equation 4}$$

and c_i is the concentration of the individual ionic form of the analyte, *j* present in the solution[33]. The neutral parts do not contribute to the effective electrophoretic mobility, but the actual magnitude of the molar fraction of the ionic components is affected by a change in pH in the electrolyte. The dependence of the effective electrophoretic mobility of weak acids and bases on pH has a characteristic shape that corresponds to the dissociation curve of weak acids and the protonation curve of weak bases.

The effective electrophoretic mobility is assumed to mainly depend on the effective charge and pH. So the BGEs used in papers **I** and **II** were prepared to have the same ionic strength.

The thermodynamic dissociation K_a^{th} constant for the equilibrium

$$HA \leftrightarrow H^+ + A^- \qquad \text{Equation 5}$$

is defined as

$$K_a^{th} = \frac{\gamma_{A^-} \cdot \gamma_{H^+}}{\gamma_{HA}} \cdot \frac{[H^+][A^-]}{[HA]} \qquad \text{Equation 6}$$

where γ is the activity coefficients derived from $[A^-] \cdot \gamma_{A^-} = [A^-]$, γ_{A^-} is the activity coefficient of the anionic specie and γ_{H^+} is the activity coefficient of the hydrogen ion. So by assuming that the activity coefficient of the undissociated acid γ_{HA} is 1, Eq.6 can be rewritten as

$$pK_a^{th} = pH - \log \gamma_{A^-} - \log \frac{[A^-]}{[HA]} \qquad \text{Equation 7}$$

The pH value of the experiment is known, and the value of the activity coefficient for the dissociated acid ($-\log \gamma_{A^-}$) can be calculated with the Debye-Hückel equation. At a temperature of 25 °C and at an ionic strength below 0.1, the activity can then be calculated from

$$-\log \gamma = \frac{0.5085 \cdot z^2 \cdot \sqrt{I}}{1 + 0.3281 \cdot a \cdot \sqrt{I}} \qquad \text{Equation 8}$$

where a is the hydrated ion size parameter (which is set to 5 Å as in reference [28] because it is unknown) and z is the charge of the ion and I is the ionic strength of the electrolyte. Substituting Eq.8 into Eq.7 gives

$$pK_a^{th} = pH - \log \frac{[A^-]}{[HA]} + \frac{0.5085 \cdot z^2 \cdot \sqrt{I}}{1 + 0.3281 \cdot a \cdot \sqrt{I}} \qquad \text{Equation 9}$$

This correction gives the thermodynamic pK_a value as opposed to a concentration-dependent value or mixed constants. The activity of the ions compensates for (1) the attraction that ions can exert on one another and (2) the incomplete hydration of ions in solutions that are too concentrated. The lower the concentration, the smaller this interaction becomes. At infinite dilution, the pK_a concentration becomes numerically equal to the thermodynamic pK_a. Thus the main difference between a thermodynamic pK_a and the pK_a concentration is that the ions' activities must be accounted for when calculating the thermodynamic pK_a [34]. To relate pK_a to mobility, the $[A^-]/[HA]$ part of the equation is expressed in terms of electrophoretic mobility. The electrophoretic mobility is calculated from the experimentally determined migration time for the analyte and the electroosmotic flow. Eq.2 then gives the effective electrophoretic mobility of any ionic specie, μ_{eff}. The effective electrophoretic mobility of an anionic specie at different pH is given by

$$\mu_{eff} = \alpha \cdot \mu_a \qquad \text{Equation 10}$$

where α is the fraction of the solute ionised and μ_a is the electrophoretic mobility of the fully deprotonated specie (A^-). The formula for a cationic specie is analogous, except that μ_b is used as the electrophoretic mobility of

the fully protonated species (BH^+). The ratio of deprotonated and protonated anionic species can be written as

$$\frac{[A^-]}{[HA]} = \frac{\alpha}{1-\alpha} = \frac{\mu_{eff}}{\mu_a - \mu_{eff}} \qquad \text{Equation 11}$$

Then Eq.9, after substitutions, attains the form that is valid for monoprotic acids, i.e.,

$$pK_a^{th} = pH - \log \frac{\mu_{eff}}{\mu_a - \mu_{eff}} + \frac{A \cdot z^2 \cdot \sqrt{I}}{1 + B \cdot a \cdot \sqrt{I}} \qquad \text{Equation 12}$$

the formula for a base is analogous. A and B are constants that vary with temperature and the electrical properties of the solvent. At 25°C, the constants are A = 0.5085 and B = 0.3281[28].

The pK_a^{th} constant is designated pK_a from here. For determination of the thermodynamic pK_a^{th} values, pH and μ_{app} must be measured under full temperature control, so the entire length of the capillary and the electrolyte reservoirs must be precisely thermostatted, something that is not fully implemented in currently available commercial CE instruments.

Note: pK_a does not correspond to a stoichiometric constant since $\gamma_{A^-} \neq 1$. The thermodynamic constant corresponds to the stoichiometric constant only when all activity coefficients are equal to unity. The final equations for calculation of pK_a values that relate analyte mobility to electrolyte pH, valid for a weak monoprotic acid (Eq.13) and a weak monoprotic base (Eq.14), then attains the form

$$\mu_{eff} = \frac{10^{(-pKa+pH+Q)} \cdot \mu_a}{1 + 10^{(-pKa+pH+Q)}} \qquad \text{Equation 13}$$

$$\mu_{eff} = \frac{10^{(pKa-pH+Q)} \cdot \mu_b}{1 + 10^{(pKa-pH+Q)}} \qquad \text{Equation 14}$$

Here Q is set to $(0.5085 \cdot z^2 \cdot \sqrt{I})/(1 + 0.3281 \cdot a \cdot \sqrt{I})$. In this way, pK_a values can be obtained from experimental data through a simple non-linear curve-fitting procedure.

Ishihama et al[29] and by Gluck et al[30] have presented equations for determination of pK_a values of multivalent ionic solutes.

3.2 Determination of pK_a for labile drug compounds

Limited attention has been paid to the use of CE for determination of pK_a for labile compounds. To the best of our knowledge, so far, there are only four reports on determination of pK_a of labile compounds by CE,[35, 36, 37, 38] besides papers **I** and **II**. The action taken by Sheng et al[35] was preparation of new sample solutions every three hours. But this introduces limitations for automated analyses. Takayanagi et al[37] determined acid dissociation constants of phenolphthalein, and Örnskov and Folestad[36] determined dissociation constants of omeprazole analogues. Recently, Ishihama et al[38] presented pK_a values for the labile rabeprazole. The acidic pK_a, reported by Ishihama et al, agreed with the values in paper **I**; the basic pK_a was 0.6 pH units higher. Ishihama et al applied pressure during the CE analysis, which allowed completion of one CE analysis in less than one minute. These studies took advantage of the CE separation to resolve the analyte of interest from related compounds, such as degradation products. Despite these reports, a general strategy for labile compounds is only presented in paper **I**.

The study in paper **I**: (1) investigated different aspects of the developed methodology, with emphasis on injection and conditioning procedures and the effective length of the separation capillary (2) extended the preliminary study by Örnskov and Folestad[36] to a wider range of drug compounds that are labile in aqueous solutions and (3) outlined a general methodology for determination of pK_a by CE.

A **B**

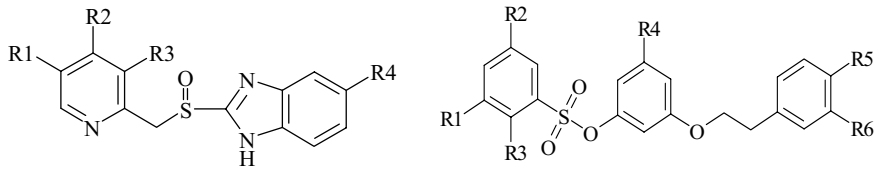


*Figure 4 **A**. General structure of pyridinyl-methyl-sulfinyl-benzimidazoles (PMSBs). **B**. General structure of benzenesulfonic acid phenethyloxy-phenyl esters (BSAPs). The substituents at the R5 position consist of a primary amine (-CH2NH2) or an amidine (-CHNHNH2), the substituent at R6 consists of a hydroxyl group or a hydrogen.*

Pyridinyl-methyl-sulfinyl-benzimidazoles (PMSBs) and benzenesulfonic acid phenethyloxy-phenyl esters (BSAPs) were used as model compounds; see Figure 4. The PMSBs constitute a group of drug compounds that have an acidic pK_a at 8-9 and a basic pK_a at 3-5 and that are known to degrade under neutral and acidic conditions[39]. Note that for the PMSBs, the pyridine nitrogen atom can accept a proton and the benzimidazole ring NH group can

release a proton – thus acting as an acid[40]. BSAPs represent compounds that are labile at the other end of the pH range, i.e., at high pH; refer to paper **II**.

Several key steps were evaluated to optimise the overall methodology. These experiments comprised sample matrix and injection, separation time and peak characterisation. PMSBs were selected as a first set of model compounds.

Figure 5 shows typical CE separations at different pH for the rabeprazole compound. From these electropherograms, it is obvious that degradation during CE separation increases at lower pH. And at pH 3.5, the electroosmotic flow is about 4 times lower at pH 10.6. The holding time thus became inevitably longer, which enhances degradation of the labile compounds.

For the PMSB model compounds, an analyte cannot be dissolved in the BGE, as is common in conventional CE, particularly not in separations that use low pH electrolytes. The PMSBs were thus dissolved in a high pH buffer (pH=11) to minimise any analyte degradation in the sample vial during the automated analysis sequence. Typically, a CE complete measurement sequence (conditioning, injections and analysis) could last 30-90 minutes, which is why it was necessary to dissolve the PMSBs in a stabilising diluent. Still, a potential risk using high pH solvent is that a sample diluent may alter the separation conditions by introducing a too-large zone of a different buffer into the separation electrolyte (inside the capillary). No significant differences could be observed, provided that the injection volume is kept small (i.e.< 0.03% of capillary volume). When large injection volumes were tested (i.e. 0.6% of capillary volume), the peak broadening increased with larger injection volumes. The influence on pK_a determination from an increase in injection volume (about a factor of 20) using hydrodynamic injection was investigated. A shift is observed toward lower pH in the mobility versus pH plot; refer to paper **I**.

The next step was to investigate the effect of injection method on determination of pK_a. The main advantage with electromigration is that a smaller volume of sample diluent is introduced into the capillary – to avoid altering the electrolyte conditions, i.e., the BGE pH. Although hydrodynamic injection is advantageous in that the sample is injected as a plug, so the analyte is maintained in a stabilising sample matrix until the effective separation starts. A potential advantage with electromigration over hydrodynamic injection is the built-in segregation between analyte and related substances. But in this study, no such advantages could be observed because the major degradation product had a similar mobility as the main drug compound. The effect of injection method, electromigration or hydrodynamic injection, was further investigated during determination of the dissociation constants of lansoprazole, pantoprazole and rabeprazole.

Generally, the lower pyridinyl dissociation constants were about 0.1 pK_a units higher when hydrodynamic injection was used. This implies that the pH 11 sample diluent increases the local pH in the capillary and therefore yields a slightly higher dissociation constant. The upper dissociation constant of the PMSBs were the same for hydrodynamic and electromigration injection.

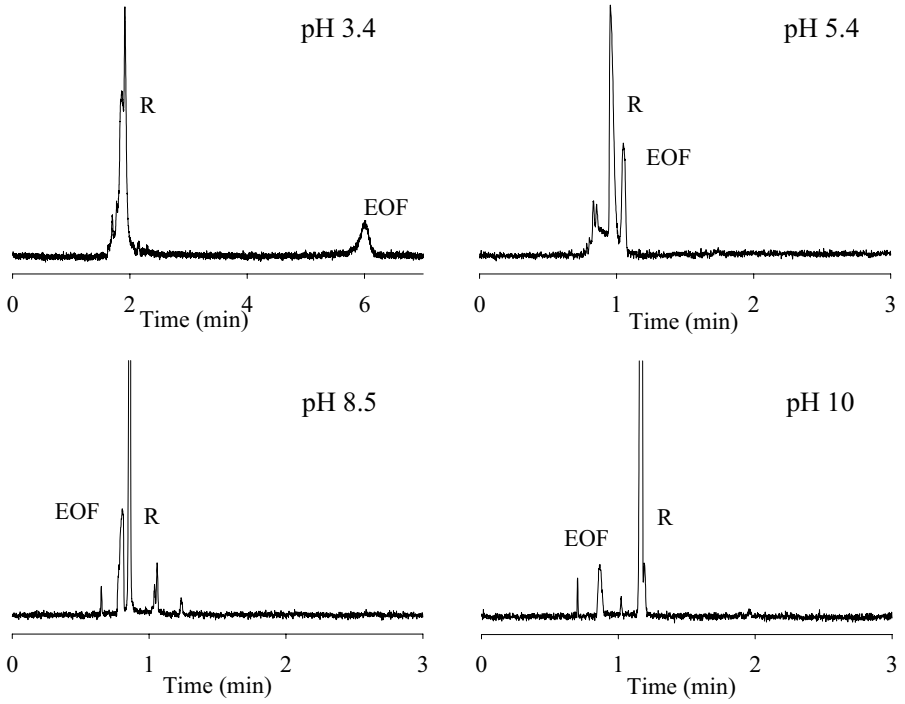


Figure 5. CE separation of rabeprazole that degrade at neutral and acidic pH. Electropherograms from CE separations in electrolytes of different pH. R = rabeprazole, EOF = marker of electroosmotic flow (benzyl alcohol).

To minimise analyte degradation during CE separations, short analysis times were tested through use of a short separation capillary – effective length 8.5 cm compared with 26.5 cm. But no significant effect regarding less degradation of PMSBs was observed – mainly because the sample diluent was the most critical step in the analytical procedure for the PMSBs.

A general problem with severe degradation of analytes during separation is the need for correct identification of peaks in the electropherogram. Typically, peaks from degradation products may overlap with the main

analyte of interest, which is why peak characterisation then is needed. Figure 4 in paper **I** illustrates CE separation of rabeprazole; here, the main component is highly degraded. Using the UV-VIS spectra acquired online during separation, it was possible to distinguish between the main component and two of its degradation products.

Besides the PMSBs, a second set of model drug compounds, BSAPs, was also investigated. Figure 4 displays the general structure of these BSAPs. These compounds are ionisable and highly lipophilic and were found to be stable in pure DMSO and in mixtures of water and DMSO. But in buffer at pH > 6, these compounds rapidly degrade as demonstrated in paper **I**.

The strategy used here was to dilute the compounds dissolved in DMSO with water and use this solution as the stabilising sample matrix during injection. In addition, a short analysis time was found to be crucial to minimise degradation during separation in buffers at pH > 6. The short analysis time was obtained by using an effective 8.5-cm capillary length. In this way, the analyte yields a sufficient peak height to be distinguished from degradation products and the EOF marker. When using 26.5 cm, a longer effective length, the main analyte peak could not be distinguished from other peaks. Identification of the correct migration time through visual inspection of the electropherogram and the peak shape was important for the BSAPs because of more severe degradation compared with the PMSBs.

Several aspects must be considered in the course of modifying CE for determination of labile compounds. Paper **I** proposed that a general strategy should follow these three steps:

General strategy for determination of pK_a of labile compounds using CE

1. Dissolve compounds in a stabilising medium to minimise analyte degradation in the sample container during the CE separation sequence.
2. Keep analysis times short to minimise analyte degradation in the capillary during the CE separation.
3. Use any means that can enable peak characterisation of the main component, for example, acquisition of UV-Vis absorption spectra or use of mass spectrometry detection.

The general methodology outlined in paper **I** and used in paper **II** together with results from application to the PMSBs and BSAPs model drug compounds demonstrate the power of CE as a general format for physicochemical profiling of drug compounds. In particular, the nanotechnological features of CE also make it interesting for use in the early stage of drug discovery when compounds are often less pure and only available in minute amounts. Thus it is shown here that pK_a can be determined with high precision and accuracy also for labile drug compounds.

Use of short-end injections to minimise the analysis time, first suggested by Örnskov and Folestad[36] and then in paper **I**, was combined with pressure-assisted CE by Wan et al, which allowed rapid pK_a determinations using CE[41]. And use of mass spectrometry has recently been demonstrated for high throughput determination of pK_a using CE[42].

4 Characterisation of drug lipophilicity properties by CE

Knowing the lipophilicity of a drug compound is especially important when predicting efficient delivery to the active site and when predicting the activity at the target site, metabolism, elimination and many other processes in a living organism.

Traditionally, the lipophilicity of a compound is expressed as the partition coefficient of neutral compounds in 1-octanol/water (log P_{ow}) or as the distribution coefficient log D_{ow} for ionised compounds at a specified pH. Log P_{ow} values can be found in databases for many compounds. Most of these data are determined by the classic shake-flask technique, which is based on measurements in a biphasic system that consists of 1-octanol and water. But determination using this classic technique requires a relatively large amount of pure compounds and is a rather tedious process.

Many theoretical, in silico, and experimental methods were developed for determination of lipophilicity[43, 44, 45]. Though giving reliable data for uncomplicated compounds, the theoretical methods often fail to account for intramolecular effects of more complex compounds[46], as discussed in paper **III**.

Today, potentiometric techniques are generally used for log P_{ow} determinations but have the same general limitations as for determination of pK_a values, as mentioned in Chapter 3. In addition, this technique cannot always handle non-ionic or too hydrophilic compounds with log P_{ow} smaller than ca –0.5[43]. The determination of lipophilicity parameters that use separation-based techniques has the same advantages as for determination of pK_a for example: ease of automatisation, only milligram sample requirements and the ability to handle impure and labile compounds. Use of LC is mostly widespread, but the mechanisms that govern analyte-separation media interactions are complicated, and the correlation to lipophilicity (log P_{ow}) is not straightforward. An alternative separation technique is the analytical micro-volume liquid-liquid flow extraction system, which can easily be automated and requires small amounts of sample[47].

During recent years, MEKC[48, 49] has become an alternative method for characterisation of lipophilic properties. MEKC is attractive for compounds that span a wide range of hydrophilic to hydrophobic properties and offers

high separation efficiency. This was demonstrated in paper **III** for a set of β-blockers, which span a wide range of lipophilic properties; see Figure 8. Notably, there are more pure analyte-separation media interactions in CE than in LC. That means that MEKC is a more straightforward format for modelling lipophilicity. But for highly lipophilic compounds, accurate determination of retention factors may be connected with some difficulties.

Good correlation was demonstrated between MEKC data (log *k*') and log P_{ow} first by Ong et al[50], and later by others[8]. Furthermore, microemulsions and liposomes as the pseudostationary phase in CE have been shown to generate log *k*' that correlates with log P_{ow}, refer to papers **III** and **IV**. Other modes of CE, such as vesicle electrokinetic chromatography – bilayer aggregates formed by surfactants – have also been shown to yield data that correlate to log P_{ow}.

The rationale for using CE with liposomal, micellar and microemulsion electrolytes as tools for physicochemical characterisation of drug compounds is that separations are mainly due to solute differences in lipophilicity. And it is possible to adjust the properties of the pseudostationary phase in the electrolyte simply by adjusting the composition of the electrolyte, for example, by changing type of surfactant or lipid.

4.1 Stationary and pseudostationary phases

All aggregates used as the pseudostationary phase in papers **II-V**, consisted of various surface-active molecules added to the buffer solution. For example, liposomes are prepared by using liposome-forming molecules such as water-insoluble phospholipids. Phospholipids (such as 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine, a common component of human membranes) added to buffer solutions at certain concentrations, form stable spherical vesicles composed of curved bilayers with a diameter of typically about 120 nm. Use of liposomes in CE enables detailed studies of physical and physicochemical properties of the bilayer and the structure and dynamics on the molecular level. Figure 7 illustrates the large size difference of liposomes and micelles.

Although dynamic systems show high performance, use of liposomes as a free-flowing pseudostationary phase is incompatible with direct coupling to MS. Immobilisation of liposomes onto the fused-silica surface (in paper **V)** enables the capillary electrophoresis system to be coupled online to MS (paper **IV**).

Micelles are prepared by adding surface-active micelle-forming molecules, such as SDS, to a buffer solution at a concentration over the critical micellar concentration. Many different surfactants are used as the

micelles in MEKC; bile acids are among the most frequently used; see Figure 6. In contrast to liposomes, micelles are small (about 3 nm for SDS micelles) and dynamic aggregates with a relatively short lifetime, about 20 milliseconds [51]. The number of molecules that form a micelle is higher for micelles made of SDS than for the bile acids, which gives a lower charge density for the latter micelle aggregates.

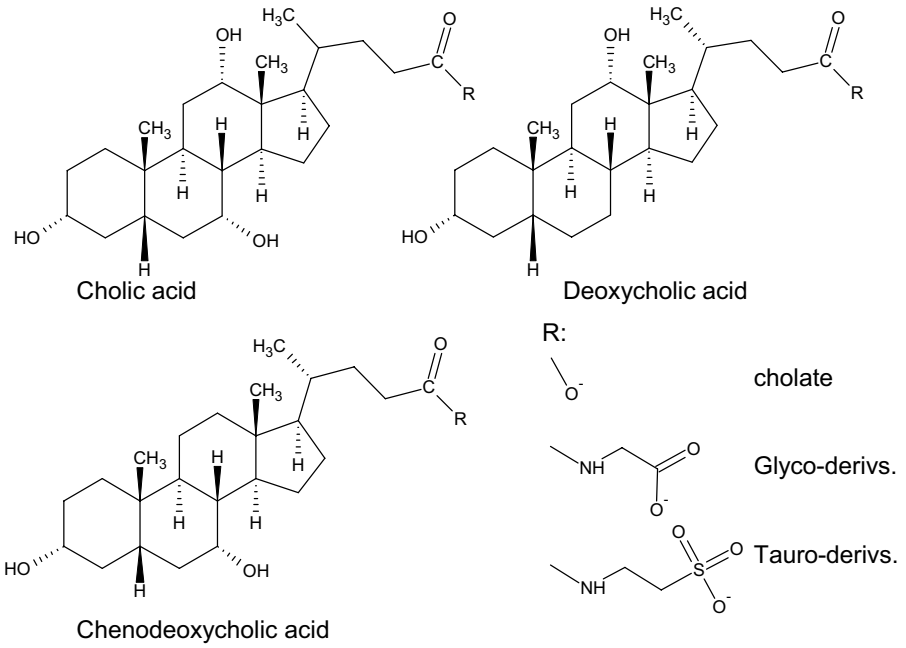


Figure 6. Chemical structures of the most common bile acids in humans and their glyco and tauro derivates; bile acids are commonly used as micellar aggregates in CE.

Electrolytes that contain microemulsions will form by adding an immiscible or a partially miscible liquid, such as an organic solvent to a micelle solution, and the electrolytes are stabilised by an interfacial film of surfactant. The microemulsions usually have a diameter of 0.01-0.1 μm [52]. Until now, microemulsions as the pseudostationary phase in CE (MEEKC) [53] is a relatively seldom-used mode of CE. This is surprising because microemulsion media for CE separations are easy to prepare and are stable for several weeks.

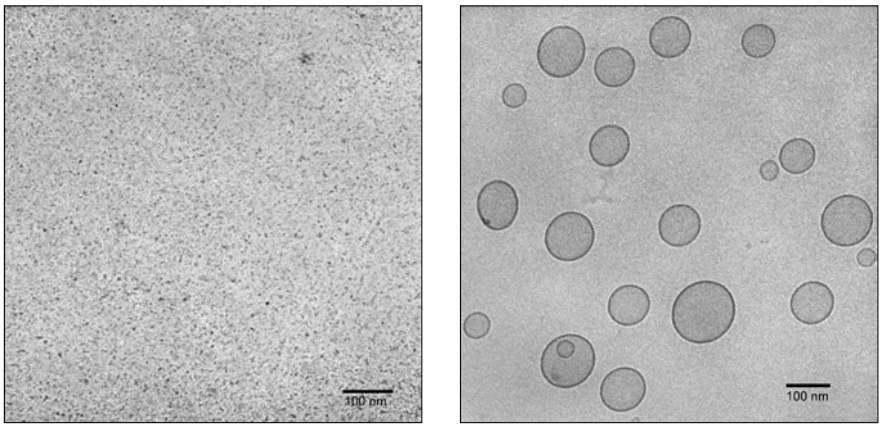


Figure 7. Cryo-transmission electron images of sodium dodecyl (SDS) micelles (left) and (POCP/PS) liposomes (right). Liposomes consisted of 3 mM 80/20 v/v% phosphatidylcholine (POPC) and phosphatidylserine (PS) in sodium phosphate buffer pH = 7.4, I = 0.02. Images prepared by Göran Karlsson at the dept. of Physical Chemistry, Uppsala University, Sweden.

4.2 Theory for determination of lipophilicity from migration data

The basic principle is described below in a condensed form for determination of lipophilicity properties by means of MEKC. Khaledi and co-workers presented a thorough description of these theories[54, 55]. The distribution of an analyte between the aqueous, i.e., the bulk buffer solution, and the pseudostationary (micellar) phase is governed by K, the distribution coefficient, and the ratio between the volumes of V_{ps}, the pseudostationary phase, and V_{aq}, the aqueous phase, according to

$$k = K \cdot \frac{V_{ps}}{V_{aq}} \qquad \text{Equation 15}$$

$$k = K \cdot [(n \cdot (C_{surf} - CMC))/(1-(n \cdot (C_{surf} - CMC)))] = \frac{n_{ps}}{n_{aq}} \qquad \text{Equation 16}$$

where k is the retention factor, n is the partial molar volume of micellar phase, C_{surf} is the total surfactant concentration, and CMC is the critical micelle concentration. As long as V_{ps} is smaller than V_{aq}, the denominator $(1-(n \cdot (C_{surf}-CMC)))$ is about equal to 1. In this way, k directly relates to the number of moles of analyte distributed in the pseudostationary phase and the aqueous phase, n_{ps} and n_{aq}, respectively. For neutral analytes, k values can be determined in a straightforward way by measuring the migration time for the analyte, t_s, the aqueous phase, t_0, and pseudostationary phase, t_{ps}. But for ionised analytes, the observed migration time is also affected by their electrophoretic mobility in the aqueous phase and by any interaction with surfactant monomers. So the analyte migration time must be corrected for these contributions to obtain a measure of the distribution between the aqueous and pseudostationary phase.

To simplify the theoretical treatment of the electrophoretic separation of ionised analytes, when using various electrolytes that contain a pseudostationary phase, it is more direct to describe this in terms of mobilities. For cationic analytes, and anionic analytes, the corrected retention factor (k') is calculated from

$$k' = \frac{\mu_{eff} - \mu_0}{\mu_{ps} - \mu_{eff}} \qquad \text{Equation 17}$$

where μ_{eff} is the electrophoretic mobility of the analyte in the electrolyte that contains pseudostationary phases aggregates, μ_0 is the electrophoretic mobility of the analyte in the electrolyte in absence of pseudostationary phase aggregates, and μ_{ps} is the mobility of the pseudostationary phase.

μ_0 is simplest to determine using an electrolyte without any surfactant additives. But several assumptions are made in this case, i.e., the negligible influence of micelles on ionic strength, dielectric constant and viscosity. And insignificant binding between the free surfactant or lipid monomers and charge analyte is assumed. If complete ion-pair binding between the charged analytes and surfactants monomers occurs, then $\mu_0 = 0$.

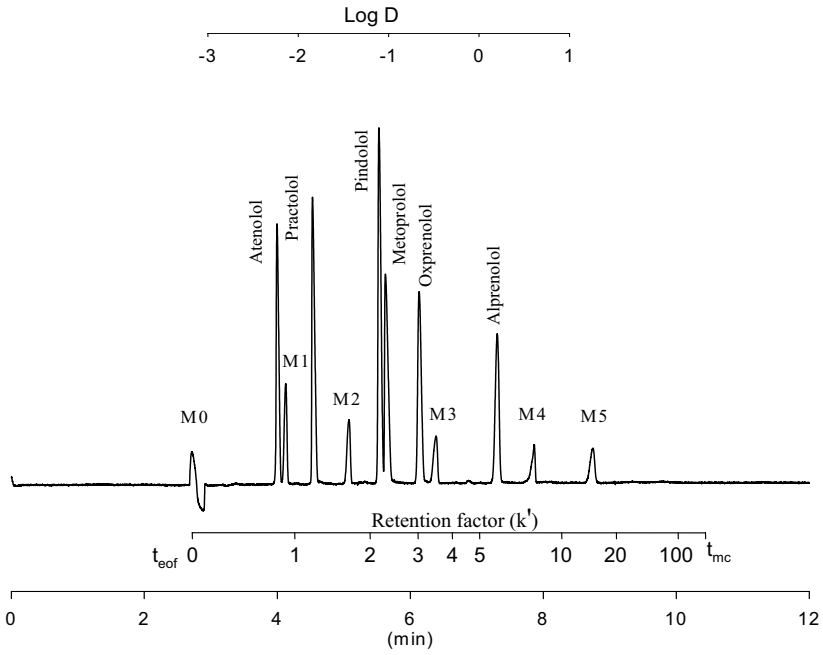


Figure 8. Separation of model compounds in micellar electrokinetic chromatography. The retention factor scale is given in the electropherogram, with the normal time scale. The upper scale represents the logarithmic distribution ratio (log D) scale. Six β-adrenoceptor antagonists (0.4 mg/ml) were injected together with five alkyl phenyl ketones (0.05 mg/ml). M0 = a compound migrating with the EOF (acetonitrile and methanol). M1 = acetophenone, M2 = propiophenone, M3 = butyrophenone, M4 = valerophenone, M5 = hexanophenone. Conditions: Separation media consisted of sodium phosphate buffer pH = 7.4, I = 0.05 and 100 mM sodium deoxy cholate. Untreated fused silica capillary, 35.0 cm (26.5 cm to detector) of 50 µm I.D., 375 µm O.D. UV-Vis detection at 230 nm. Hydrodynamic injection 20 mbar (5 s). Separation at 37 °C and 300 V/cm (about 62µA).

The mobilities needed to calculate *k*' values can be calculated using experimental migration data. For determination of t_{EOF}, the migration time of the electroosmotic flow, the signal from the analyte diluent, can be used. The migration time of the pseudostationary phase, t_{ps}, can be calculated by an iterative procedure[56, 57, 58] using the migration data for a homologous series of five alkyl phenyl ketones (alkylphenone), acetophenone, propiophenone, butyrophenone, valerophenone and hexanophenone (HP); see Figure 8.

The calculation procedure of μ_{ps} starts with assuming that HP, one of the most lipophilic alkylphenone, migrates at the same mobility as the pseudostationary phase aggregates. By using the mobility of HP as a

substitution of μ_{ps}, log k' for all five alkylphenones were calculated according to Eq.17. The equation for the linear relationship between log k' and the carbon number of the alkyl group was then calculated. So a temporary log k' for HP was obtained from this equation. Thereafter, a new μ_{ps} was obtained from Eq.17 using the calculated k' for HP. Then the log k' values of all alkylphenones were recalculated with the new μ_{ps} and a new equation for the linear relationship between log k' and the carbon number was determined. This procedure was repeated until successive iterations showed no substantial differences in calculated μ_{ps}.

For CE separations that use stationary lipophilic phases, such as immobilized liposomes (in papers **IV** and **V)**, the corrected retention factor (k') is calculated from

$$k' = \frac{\left(\frac{t_s - t_{eof}}{t_{eof}}\right) + \frac{\mu_{eff}}{\mu_{eff} + \mu_{eof}}}{1 - \frac{\mu_{eff}}{\mu_{eff} + \mu_{eof}}} \qquad \text{Equation 18}$$

where t_s is the elution time of a specific solute, t_{eof} is the elution time of an unretained and uncharged solute, μ_{eff} is the electrophoretic mobility of a specific solute, and μ_{eof} is the mobility of the electroosmotic flow[59].

4.3 Determination of lipophilicity properties by CE

In many studies, correlation has been successfully demonstrated between CE migration data and log P. This was also demonstrated in papers **II**, **III**, **V** and **VI**. Table 2 lists studies that report correlation to log P using MEKC, and Table 3 lists studies that correlate MEEKC, liposomes and vesicles data to log P. All this studies elegantly demonstrate the ease with which the experimental CE technique can be varied and used for lipophilicity characterisations.

It should be noted that the most commonly used micellar phases consisted of SDS, which have been shown to give different regression results for various groups of solutes as shown by Khaledi and co-workers [60]. The micellar systems studied in paper **III** included various bile acids, SDS and CTAB. The same regression results were observed for the different model analytes in the SDS micellar systems, which likely emanates from that the model compounds comprised a narrow set of β-blockers.

The majority of the reports in Tables 2 and 3 have investigated the direct correlation between CE data and log P data solely. If the objective for

determination of lipophilicity indeed is to model cell permeability, the partitioning in the in vitro/CE system and biological systems should be based on the similar analyte-media mechanisms. Dorsey and co-workers demonstrated that water-micelle partitioning in an MEKC system is a better model of biopartitioning with respect to enthalpic and entropic contributions to the free energy transfer than the octanol-water partition model[61]. Thus because the thermodynamic signature of micelle-water partitioning is similar to biological partitioning, as opposed to octanol-water, it has been proposed that better correlation with in vitro biological parameters might be expected with micellar-based methods[62].

Thus, in the future an increasing number of reports demonstrating and discussing the potential of CE for this purpose should be expected[63]. This implies that use of CE generated lipophilicity data for quantitative structure-activity relationships (QSAR) studies or biopharmaceutical characterisations, is still in its infancy.

Table 2. Overview of lipophilicity properties, as correlations to log P_{ow}, determined by CE – using micelles as the pseudostationary phase.

Aggregate	Compounds (n)	Reference
Micelles		
	First; Phenols neutral and ionic (11)	Ong et al[50]
	Phtalate esters (7)	Takeda et al[64]
	Various neutrals (9)	Chen et al[65]
	Neutral alkylbenzenes and other neutral compounds (14)	Muijselaar et al[58]
	Drugs, neutrals and ionic (26)	Schmutz et al[66]
	Various neutrals (32)	Greenaway et al[67]
	PAHs (20)	Jinno et al[68]
	Various neutrals and ionic (100)	Herbert et al[69]
	Various neutrals (53)	Ishihama et al[70]
	Various neutrals (11), drugs (3)	Smith et al[71]
	Various drugs (16), various neutral and ionic (18)	Adlard et al[72]
	PAHs (20)	Jinno et al[73]
	Drugs, Dihydropyridines (26)	Benito et al[74]
	Various aromatic (60), drugs (9)	Yang et al[75]
	Steroids (6)	Wiedmer et al[76]
	Drugs, β-blockers (10)	Lin et al[77]
	Various neutrals and ionic (16)	Dinelli et al[78]
	Various neutrals and ionic (67)	Woodrow et al[61]
	Review	Khaledi et al[79]
	Various, neutrals and ionic (14)	Ferguson et al[80]
	Ionic tetracyclines (6)	Chen et al[81]
	Neutral, ionic drugs (53)	Hanna et al[82]
	Pesticides (neutral) (43)	Wu et al[83]
	Drugs, cephalosporins (7)	Mrestani et al[84]
	Triazines, neutrals and ionic (13)	Lin et al[85]

Table 2, continued.

Aggregate	Compounds (n)	Reference
Micelles	Triazines, neutrals and ionic (14)	Freitag et al[86]
	Phenols (11), diverse neutrals (23)	Wall et al[87]
	Various neutral (53)	Ishihama et al[88]
	Various neutrals (14), peptides (7)	Idei et al[89]
	Benzopyrenes (13)	Kodama et al[90]
	Drugs, cephalosporins (8)	Mrestani et al[91]
	Drugs, β-amino alcohols (15)	Pascoe et al[92]
	Drugs, cephalosporins (9)	Mrestani et al[93]
	Drugs, cephalosporins (8)	Mrestani et al[91]
	Various neutrals (30)	Zhao et al[94]
	Drugs, thrombin inhibitors (18)	Paper **II**
	Various aromatics, neutrals and ionic (48)	Baily et al[95]
	Various neutral (14), peptides (7)	Idei et al[96]
	Drugs (9)	Mrestani et al[97]
	Drugs, ionic (6)	Mrestani et al[98]
	Bile Acids (16)	Lucangioli et al[99]
	Drugs (18)	Gavenda et al[100]
	Drugs, neutrals and ionic (10), various neutral (36)	Taillardat-Bertschinger et al[101]
	Drugs charged (21)	Detroyer et al [102]
	Drugs, β-blockers (10), various drugs (12)	Paper **III**

Table 3. Overview of lipophilicity properties as correlations to log P_{ow}, determined by CE– using microemulsions, liposomes and vesicles as the pseudostationary phases.

Aggregate	Compounds (n)	Reference
Microemulsion		
	First; Various neutrals and ionic (9)	Watarai et al[103]
	Various neutrals (53)	Ishihama et al[70]
	Various neutrals and ionic (28), a few drugs	Gluck et al[104]
	Drugs, anionic (10) and cationic (15)	Ishihama et al[105], [106]
	Various neutrals (53)	Abraham et al[107]
	Drugs, cephalosporins (7)	Mrestani et al[84]
	Various ketones, neutral and ionic (8)	Watarai et al[108]
	Various neutrals (53)	Ishihama et al[88]
	Neutral phenones (8)	Altria et al[109]
	Drugs, cephalosporins (8)	Mrestani et al[91]
	Drugs, and various neutrals and ionic (86)	Poole et al[110]
	Drugs, bases and nucleosides (10)	Furumoto et al[111]
	Pesticides (> 80)	Klotz et al[112]
	Drugs, β-blockers (10), various drugs (12)	Paper **III**
Liposomes		
	First; Zwitterions (4)	Nakamura et al[113]
	Various aromatic neutrals (55)	Burns et al[114]
	Various neutrals (29)	Burns et al[115]
	Drugs, β-blockers (10), various drugs (12)	Papers **III** and **V**
Vesicles		
	First; various neutrals (20)	Hong et al[116]
	Drugs, β-amino alcohols (15)	Pascoe et al[92]
	Various aromatic neutrals (41)	Agbodjan et al[117]
	Drugs, and various neutrals and ionic (29)	Razak et al[118]
	Various neutrals, neutral and ionic pesticides (115)	Klotz et al[119]

5 Modelling of drug compound passive intestinal absorption using CE data

The biopharmaceutics discipline within pharmaceutical science focuses on delivery of a drug to its pharmacological target. Typically, biopharmaceutical profiling of a drug includes in vitro and in vivo studies on human dissolution and absorption. Today, vitro methods commonly use artificial or biological membrane systems or constitute assays based on biological cell layers, such as Caco-2 cells. Alternative in vitro artificial methods are separation based[63, 120]. Several modes of LC, such as immobilized artificial membranes[121] and immobilized liposome chromatography[122], have been demonstrated.

The rationale for exploring the separation-based CE technique for modelling drug absorption is this: only nanogram amounts of a drug compound are needed for the CE analysis – in contrast to the milligram amounts needed for LC methods. And because the CE technique is solution based, the composition of the electrolytes can be easily adjusted to emulate various interactions encountered in physiological medias.

5.1 Drug absorption

If the drug compound is dissolved in the intestinal content, the next barrier is the intestinal cell membrane; see Figure 9.

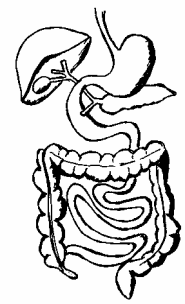


Figure 9. Overview of the human gastrointestinal tract. The small intestine tract segments are called duodenum (25 cm), jejunum and ileum (260 cm). Ileum empties into the colon (110 cm)[123]; drawing by Erika Fredriksson, Uppsala, Sweden.

Several different mechanisms are involved in drug intestinal absorption[124, 125]; the most common is passive diffusion that occurs through the epithelial cell plasma membrane or through the intercellular spaces (trans- and paracellularly, respectively); see Figure 10. Note that drug compounds may also cross the intestinal tract cell membranes by mixed transport routes[1]. Right now, an increasing number of active transport routes are being discovered in the intestinal membranes. Nevertheless, several of the drug compounds must penetrate several membranes to reach the target site, and the drug compound may not have access to the same active transport routes at the target cell or the other cell membranes that must be passed on the way to the target. So at all times, characterisation of the passive, drug-compound, membrane-permeability properties is highly relevant during drug discovery and development. Table 4 and Table 5 show some of the most frequently used in vitro techniques for assessment of human intestinal absorption during drug discovery and development.

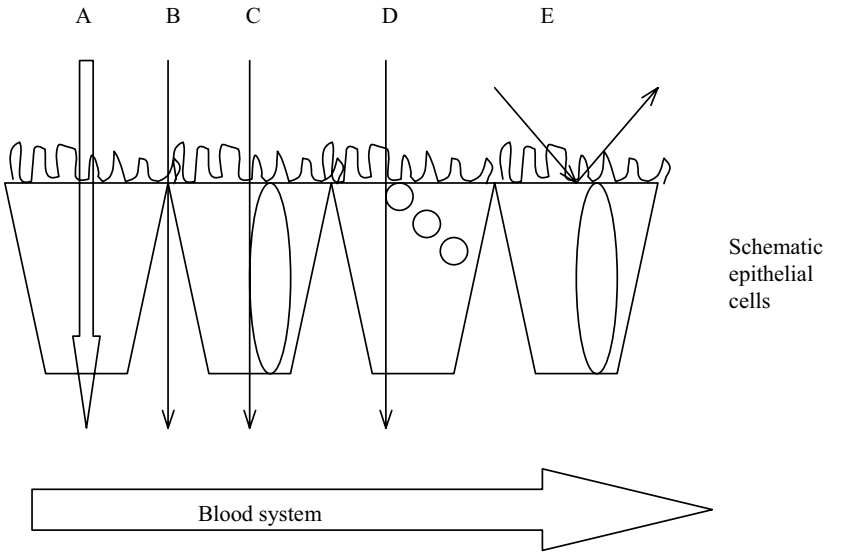


*Figure 10. Schematic representation of the main routes across the intestinal cell membrane for a drug molecule. **A**: Passive diffusion via transcellular route, either through the cytoplasmic aqueous phase or along the lipid membranes of the cell[124]. **B**: Passive diffusion via paracellular route, in the intestine a potential route for polar molecules with a molecular weight <500 . **C**: Facilitated and active (energy consuming) transport via carrier systems in the cells. **D**: Transcytosis, transcellular transport via membrane vesicles. **E**: Efflux by facilitated and active transport via carrier systems in the cell membranes.*

Table 4. Overview of common biological in vitro techniques for modelling human intestinal absorption of small drug molecules.

Technique	**Remark/application**	**References**
Biological membranes		
Using chamber, excised intestinal segments	Excised intestinal segments	Ungell[126 b]
Everted intestinal ring, slices or sacs	Excised intestinal segments	Tukker[127 b]
Cell culture models		
Caco-2 cell monolayers, human intestinal mucosal cells	Cultured cells, 3 weeks growing	Artursson et al[128 b]
Madin-Darby canine kidney cell monolayers, (MDCK)	Cultured cells, 3 days growing	Irvine et al[129 b]
Interaction with cells and cell derived vesicles		
Isolated intestinal cells	Intestinal mucosal cells	Tukker [127 b]
Brush-border-membrane vesicles	Prepared from cell homogenates or intestinal scrapings	Tukker [127 b]
Immobilized liposome chromatography	Vesicles from red blood cells, red blood cells/ghosts	Beigi et al[130]

[a] First publication
[b] Recent review

Table 5. Overview of common in vitro techniques for modelling human intestinal absorption of small drug molecules.

Technique	**Remark/application**	**References**
Interaction with liposomes, vesicles, micelles and artificial membranes		
Nuclear magnetic resonance spectroscopy	Liposomes	Fruttero [131b]
Surface plasmon resonance	Liposomes	Danelian et al[132 a]
Solid-supported lipid membranes (TRANSIL)	Unilamellar liposomal membranes	Loidl-Stahlhofen et al[133 b]
Spectroscopic techniques	Liposomes	Santos et al[134] de Castro et al[135]
Equilibrium dialysis	Liposomes (standard method)	Krämer[136]
Potentiometric titrations	Liposomes and micelles	Krämer[136 b]
Ultrafiltration/ultracentrifugation methods	Liposomes	Krämer[136 b] Austin et al[137]
Fluorosomes/fluorescence spectrophotometer	Liposomes	Melchior[138]
Microcalorimetry	Bile acid aggregates	Grosvenor et al[139]
Parallel artificial membrane permeability assay	Mixture of lecithin, organic solvents	Kansy et al[140a]
Immobilised artificial membrane chromatography	Phospholipid bilayer	Pidgeon et al[121 a]
Immobilised liposome chromatography	Liposomes	Beigi et al[122 a]
Micellar liquid chromatography	Micelles	Molero-Monfort et al[141]
CE-based methods	Vesicles, liposomes, microemulsions, micelles	Table 3 and 4, papers **II-V**.

[a] First publication
[b] Recent review

5.2 Modelling and prediction by CE using liposomes, vesicles, micelles and microemulsions

The ultimate goal of in vitro and in silico methods is to model and predict human drug absorption. Consequently, a complete validation of new in vitro and in silico methods should include direct correlation between in vitro data and human drug absorption data. However, although most in vitro methods and in silico techniques provides high precision data, human absorption data are often significantly more varying. Human drug absorption is a physiological process comprising several mechanisms; see section 5.1. Human drug absorption data reported in the literature are characterised by high variability and low quality and are frequently derived for marketed drugs, which are generally highly absorbed and reasonably well behaved in vivo. It should be realized that new methods may be skewed toward well-behaving drugs. Sigmoidal correlation with human drug absorption data has thus been observed, which complicates prediction of absorption for compounds with very high or low permeability.

Caco-2 cells permeability data are frequently used as a substitute for human drug absorption data during development of in vitro and/or in silico methods[142]. But most drugs are absorbed in the higher gut, so it is questionable as to how well colon-derived cell lines, such as Caco-2, can model human drug absorption[143]. Moreover, the junctions of Caco-2 cells are relatively dense, why absorption rates are much slower than in the intestine [144].

A benefit from separation techniques, such as CE, is that they model passive transport – without contribution from active transporters or from degrading enzymes that may be found within in vitro cell systems and animals. In addition, the analytical techniques also offer biomimetic conditions such as human body temperature and pH. Moreover, separation techniques are robust, i.e., they generate data with good precision and have high capacity. Comparisons of separation techniques indicate that they generate similar data. For example, it has been reported that the surfactant, used as the lipophilic phase, seem to be more important than the type of the separation technique used to generate the partitioning data[102].

The major advantage with the CE technique is that only nanogram amounts of a drug compound are needed, and it is simple to adjust the properties of the electrolyte, for example, by changing the type of surfactant or lipid (papers **II**, **III** and **IV**). Papers **IV** and **V** presented a novel method for immobilising liposomes on capillary walls, and immobilised liposome

capillaries were used for studies of drug-liposome interactions that use CE-MS (paper **V**). To further mimic physiological conditions, the applicability of using simulated human intestinal fluid as the electrolyte in CE was investigated, Paper **III**. In this study, the interactions between components of simulated intestinal fluid, lecithin-cholic acid mixed micelles, and drug molecules were characterized.

There are so far a limited number of studies investigating direct correlation between CE migration data and drug absorption data. Khaledi and co-workers showed high correlation between MEKC log *k*' for uncharged corticosteroides and intestinal absorption in rat, and between MEKC log *k*' and protein binding to human serum albumin[145]. Roses et al have shown correlation between MEKC log *k*' and narcotic activity towards the tadpole[146]. However, they reported that MEKC log *k*' data could not model the rate of skin permeation from water and blood-brain distribution.

Linusson et al showed for eighteen thrombin inhibitors good correlation between MEKC log *k*' and inhibition of thrombin. In addition, MEKC log *k*' data for the thrombin inhibitors compounds was also used as an estimate of passive absorption, paper **II.** Örnskov and co-workers showed good correlation between MEKC log *k*' for six β-blockers to Caco-2 cell permeability, and permeation through intestinal segment of rat ileum and rat colon data[147]. Recently, Detroyer et al[102] also reported good correlation between MEKC log *k*' and Caco-2 cell permeability data for five β-blockers.

Woodrow et al reported for chlorobenzenes that MEEKC data correlated better with water-fish lipid partitioning data than log P_{oct} data[61]. In addition, Ishihama et al have shown good correlation between MEEKC log *k*' and toxicity for phenols[70], and between MEEKC log *k*' and human skin permeability for acidic non-steroidal anti-inflammatory drugs[105].

In paper **III** CE migration data for nine β-blockers and a set of various drug compounds, obtained at 37 °C using micelles, microemulsions and liposomes, were shown to give high correlations to both Caco-2 cell permeability, permeation through intestinal segment of rat ileum, rat colon data and human absorption data. Interestingly, slightly better correlation to intestinal absorption was found for the tested liposomal systems. In paper **V** CE migration data for β-blockers and a set of various drug compounds, using immobilized liposomes, were shown to correlate to both Caco-2 cell permeability and human absorption data.

In all, there is no doubt that CE is a promising tool for experimental modeling of passive absorption and this application of the CE technique is likely to be further developed.

6 Modelling drug-electrolyte interactions in CE using molecular descriptors

Computer-generated models of CE separations may be used in different contexts: fundamental research, method development, and education. For method development, an ultimate goal of computer-generated models is to provide the analytical chemist with a means to simulate and quantitatively predict the migration of new compounds.

The MEKC mode of CE enables fundamental mechanism studies of analyte/drug-electrolyte/micelle interactions because MEKC is a solution-based technique[148]. Several computer- generated MEKC modelling strategies have been proposed, as recently reviewed by Sentellas et al[149].

Many diverse molecular descriptors have been developed for predicting drug molecular permeability, for example, in the human intestinal tract and through the blood brain barrier. But note that molecular properties, such as hydrophobicity, hydrogen bonding, molecular size and dipolar properties, govern passive absorption of drug molecules in the intestinal tract[2]. Normally, these properties also govern migration of compounds in MEKC separations. So these descriptors could also provide a basis for modelling and predicting interactions between analytes and micellar media in MEKC.

6.1 Molecular descriptors

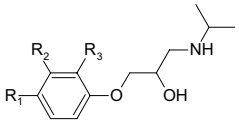
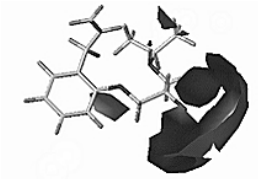
A molecular descriptor is the final result of a mathematical procedure that transforms chemical information encoded within a symbolic representation of a molecule into a useful number[150]; see Table 6. Constitutional descriptors can be calculated from a 1-dimensional representation of the molecule. Typically, they describe the molecular weight and number of atoms. From a 2-dimensional representation of the molecule, the atom types and functional groups can be used to calculate physicochemical properties, such as hydrogen-bonding capacity, lipophilicity and charge. And topological indexes can be calculated from the 2-dimensional representation of the molecule. The calculations for some descriptors, such as log P, use group contribution that is based on experimentally determined data for certain reference compounds. By using the 3-dimensional structure of a molecule,

different spatial arrangement can be famed, and the 3-dimensional structure of a molecule depends on the environment of the molecule. Descriptors generated from the 3-dimensional structure are unique for a particular molecular conformation and are considered to better reflect intramolecular interactions. A single 3-dimensional conformation is usually calculated in a fraction of a second on a computer. But a full search of the entire conformational space for one molecule can require up to several weeks of computer time when using molecular mechanics or molecular dynamics simulations. An accurate description of the electron distribution and the valence properties of the molecule can be obtained by representing the molecule by a wave function. Wave functions are generated by quantum mechanics calculations, which are very time consuming even if only one conformation of the molecular structure is considered. Typical quantum-chemical descriptors are energy-based descriptors, molecular orbital energies and polarisabilities[150].

Sophisticated molecular descriptors account for dynamic behaviour and electronic properties of the structure, which may better facilitate understanding of the interaction mechanism. But the lower dimension molecular descriptors are simpler to interpret from a chemical viewpoint. A more extensive review of descriptors and calculation methods can be found elsewhere[44, 151, 152, 153].

Four diverse software packages were used to calculate molecular descriptors based on simple and more sophisticated structure representations (paper **VI**). All these generated descriptors are commonly used for quantitative structure-activity relationships (QSAR) studies. Molecular descriptors based on simple structure representations were also used in papers **II** and **III** for QSAR studies.

Table 6. A schematic overview of molecular descriptors

Representation	Example of molecular descriptors	Generated by calculation procedure / software	References
1D structure $C_{14}H_{22}N_2O_3$	Molecular weight Number of atoms Number of bonds	Counts SaSA	154
2D structure  	Flexibility Rigidity Hydrogen bond acceptors Hydrogen bond donators Log P	Connectivity, element and fragment parameters SaSA	154
3D structure 	Surface properties Molecular shape Lipophilicity Integy moment	Force field and semi empirical Volsurf	155
	Polarizability Log P Hardness Orbital energies	Semi empirical Spartan	156
	Surface properties Polarizability Hydrogen bond properties Acid-base properties Orbital energies	*Ab initio* Molsurf	157

[a]Semi empirical molecular orbital calculations

6.2 Chemometric modelling

Chemometrics involves experimental design and multivariate analysis methods, i.e., mathematical and statistical methods for modelling and data evaluation.

The data structure can be represented in 1- and 2-dimensional plots by using multivariate analysis methods, such as principal component analysis (PCA)[158], partial least-squares projections to latent structures (PLS)[159], and hierarchical analysis[160]. A formal description of these methods can be found elsewhere. For hierarchical analysis, see Eriksson et al and references therein[161] and for PCA and PLS, see Martens[162] and Brereton[163].

Statistical design of experiments, developed by Fisher in 1926[164], is a methodology that provides efficient experimentation. In paper **II**, a set (library) of thrombin inhibitor compounds was synthesised according to a design based on three building blocks connected to the same scaffold. These compounds were then analysed using QSAR with respect to inhibition of thrombin and bioavailability.

6.3 Prediction of migration from molecular descriptors

In paper **VI**, analyte migration in MEKC was predicted using molecular descriptors for both analyte model compounds and separation media. Investigated micellar media comprised sodium dodecyl sulphate, bile acids and the positively charged surfactant cetyltrimethylammonium bromide. Five uncharged alkyl phenyl ketones and nineteen charged β-blockers were used as model analytes. Various molecular descriptors for the analytes and the media surfactants, based on simple and more sophisticated structure representations, were calculated. PCA and PLS were used for multivariate analysis modelling and prediction of analyte migration.

Introduction of micellar media molecular descriptors enables calculation of one single global model that is valid for several micellar media. Prediction of MEKC migration by the global model was equal to or even better than by any local model. So the approach of using a combination of analyte and micellar media descriptors better models MEKC migration. But note that molecular descriptors for separation media, in paper **VI**, were delimited to representation of the surfactant monomers and not the entire micellar aggregates.

Use of hierarchical multivariate PLS modelling highly simplified interpretation of parameters that govern the separation. The analysis of the models revealed that media descriptors, such as critical packing, were important in the models based on β-blocker analytes. The descriptors generated by the Volsurf calculation procedure were found to best model MEKC separation. And the hierarchical multivariate analysis enabled simple comparison of many molecular descriptors.

The molecular descriptors used in paper **VI** are commonly used for prediction of gastrointestinal absorption properties of small drug compounds

but were also shown to be highly useful for modelling and prediction of MEKC migration. Paper **VI** proposed that MEKC separations could also be used to validate the representativeness of such molecular descriptors for molecular behaviour in complex liquid media, e.g., physiological systems. Further exploration of the presented approach should comprise experimentally designed sets of extended analyte diversity and a wider range of electrolyte conditions.

7 Concluding remarks and future work

The studies conducted within this thesis emphasise the potential of CE for physicochemical and biopharmaceutical characterisation of drug molecules. A main feature of CE is that only nanogram amount of drug compound is required for analysis. Determination of acid-base properties by CE is today in routine use. A general CE methodology was here developed that enabled determination of pK_a for labile compounds, which was missing. The CE methodology for characterisation of lipophilic properties of drug compounds is also well developed today. Still, new applications may be further explored such as enantioselective lipophilic characterisation of drug molecules.

In spite of this, the use of CE for biopharmaceutical characterisation is still in its infancy. The utility of CE for experimental modelling of passive intestinal absorption was evaluated in this thesis - for microemulsion, micellar and liposomal electrolytes. Interestingly, slightly better correlation to intestinal absorption was found for liposomal systems. This should be further investigated by testing various liposome compositions and wider sets of model drug compounds.

Immobilization of liposomes extends the utility of CE for estimating passive intestinal absorption. This novel approach developed here enabled direct on-line coupling of liposome CE to high sensitivity mass spectrometry. Further exploration of liposome CE for biopharmaceutical profiling of drug compounds should include extending the immobilized liposomes to incorporate cell membrane proteins. This would enable characterisation of interactions between drug molecules and proteins in the CE-MS format.

Utilising molecular descriptors, commonly applied to in silico prediction of passive intestinal membrane permeability, migration of analytes in MEKC could be well predicted. This novel approach was based on hierarchical multivariate analytics and use of molecular descriptors for both analytes and micellar media surfactants. Future work may include using analyte and media molecular descriptors also for more complex systems such as liposome CE. Besides, modelling separation in CE systems should be based on using experimentally designed sets of model compounds and separation conditions.

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9 Swedish summary

Fysikalkemisk och biofarmaceutisk karakterisering av läkemedelsmolekyler med kapillärelektrofores

Att utveckla nya läkemedel har historiskt sett tagit lång tid. Det finns exempel på att det kan ta tio till tjugo år från idé till dess att den färdiga produkten blir tillgänglig för patienten. En av utmaningarna vid läkemedelsutveckling är att kunna designa den aktiva substansen så att den utöver god effekt även absorberas väl genom tarmväggen. Detta för att läkemedlet ska ge optimal terapeutisk effekt. De sista tio åren har ett antal experimentella och teoretiska tekniker utvecklats med syftet att bättre kunna karakterisera de aktiva substansernas absorptionsegenskaper på ett tidigt stadium. Utvecklingen av de nya experimentella teknikerna har i hög grad styrts av att nya tekniker för syntetisering av aktiv substans genererar ett mycket stort antal substanser men samtidigt små mängder av varje substans. En fördel med teoretiska metoder är att prediktion av absorptionsegenskaper kan utföras utan att ens molekylen behöver syntetiseras. Traditionellt behöver de teoretiska metoderna fortfarande stöd av experimentella data för att prediktionerna av absorptionsegenskaper ska bli tillräckligt bra. Sålunda finns det behov av nya experimentella metoder som använder så små mängder substans som möjligt och dessutom har god analyskapacitet (stort antal prover per tidsenhet). Här erbjuder tekniken kapillärelektrofores (CE) flera fördelar: mycket små mängder substans behövs för analys (nanogram) samt att tekniken ger data med hög precision och riktighet. Dessutom är CE lätt att automatisera och det finns idag kommersiella CE-instrument med hög analyskapacitet.

Arbetet i denna avhandling har innefattat en granskning av fördelar och nackdelar med att använda analystekniken CE för fysikalkemisk och biofarmaceutisk karakterisering av läkemedelsmolekyler. Karakteriseringen baserades på CE-studier av läkemedelmolekylernas migrationsbeteende i olika elektrolytsystem. Här studerades elektrolyter innehållande enbart buffert, samt elektrolyter innehållande buffert och mikroemulsioner, miceller eller liposomer.

En första delstudie innefattade hur pK_a (syrakonstaten) kan bestämmas med hjälp av CE även för instabila och delvis nedbrutna substanser. Detta

arbete resulterade i en generell metodik som baseras på; stabil provmatris, injektion med elektromigration, minimerad analystid genom att använda en mycket kort del av separationskapillärens totala längd, samt identifiering av molekylerna med spektrofotometrisk detektion. Den föreslagna metodiken tillämpades med framgång för två set av läkemedelssubstanser som var instabila vid lågt respektive högt pH.

Nästa delstudie innefattade modellering och prediktering av passiv läkemedelsabsorption från experimentella CE-data. Migrationsbeteendet för läkemedelsmolekyler studerades i elektrolyter innehållande miceller, mikroemulsioner och liposomer. Migrationsdata korrelerades mot humana absorptionsdata, och estimat av human absorption såsom Caco-2-cellsdata. Caco-2-data genereras genom att mäta hur väl läkemedelsmolekyler penetrerar Caco-2-celler. Dessa celler kommer ursprungligen från människans tarmvägg. Korrelationsstudierna visade att CE-data korrelerar bra till humana absorptionsdata och Caco-2-cellsdata.

De migrationsdata som var baserade på användandet av liposomer i CE korrelerade något bättre till Caco-2-cellsdata. Detta indikerar att molekylers fördelning till liposomer kan vara en extra intressant modell för humana cellmembran. Genom att belägga CE-kapillärens innersida med liposomer utökas möjligheterna att använda CE för att prediktera absorptions egenskaper. Metoden för att belägga CE kapillärens innersida med liposomer är baserat på ett helt nytt koncept som här visas för första gången. Metoden är baserad på elektrostatisk immobilisering av liposomer på en underliggande laddad agarosyta. Kapillärer belagda med liposomer möjliggör direktkoppling av CE till masspektrometrisk detektion, vilket ger högre känslighet och ökad selektivitet.

Det yttersta målet med utveckling av nya in vitro- och in silico-metoder är att kunna förutsäga absorptionsegenskaper hos nya läkemedelsmolekyler. En komplett validering av nya metoder bör därför utföras mot humana absorptionsdata. Ett problem är emellertid att humana absorptionsdata ofta har stor spridning, medan flertalet in vitro tekniker ger hög precision i data. Därför används ofta så kallade Caco-2-data som ett substitut för humana absorptionsdata.

En avslutande delstudie bestod av kemometrisk modellering av CE-migrering i micellelektrolyt med hjälp av molekylära deskriptorer både för analyterna och för surfaktanterna i micelllektrolyten. Användning av molekylära deskriptorer även för micellelektrolyten vid modellering av analysseparationer visades här för första gången. Generellt styrs human absorption av molekylens lipofilicitet, vätebindningsförmåga, storlek och polaritet vilket även är de parametrar som styr fördelning till miceller i en CE-separation. För att kunna nyttja och utvärdera ett mycket stort antal molekylära deskriptorer, användes multivariat statistisk hierarkisk

modellering. De molekylära deskriptorerna som användes är vanliga vid teoretiska modelleringar och predikteringar av human absorption. Studien innefattade här prediktering av CE-migrationsdata för laddade och oladdade substanser i olika micellelektrolyter. Därför föreslås i avhandlingen att CE skulle kunna användas för att utvärdera hur representativa avancerade molekylära deskriptorer är för att beskriva en molekyls beteende i mer komplexa media, såsom fysiologiska system.

10 Abbreviations, acronyms and terms

AP	acetophenone
BSAP	benzenesulfonic acid phenethyloxy-phenyl esters
BP	butyrophenone
CE	capillary electrophoresis
CZE	capillary zone electrophoresis
EOF	electroosmotic flow
HP	hexanophenone
HTS	high throughput screening
in silico	techniques using computer (virtual) chemistry
in vitro	techniques using biological-derived material
in vivo	techniques using intact organisms or parts of the organisms
log D	logarithm of distribution coefficient
log *k′*	logarithm of corrected retention factor
log P_{ow}	logarithm of partition coefficient (octanol/water)
MEEKC	microemulsion electrokinetic chromatography
MEKC	micellar electrokinetic chromatography
pH	negative logarithm of hydrogen concentration
PMSB	pyridinyl-methyl-sulfinyl-benzimidazoles
PP	propiophenone
UV-Vis	ultraviolet-visible absorption
VP	valerophenone

11 Symbols

A	hydrated ion size parameter
α	fraction of the solute ionised
A	Debye-Hückel constant
$\mathbf{A^-}$	fully deprotonated acid specie
B	neutral base specie
B	Debye-Hückel constant
$\mathbf{BH^+}$	fully protonated base specie
°C	degree of Celsius
$\mathbf{C_{surf}}$	total surfactant concentration
CMC	critical micelle concentration
$\mathbf{c_i}$	concentration
HA	protonated acid specie
H	hydrogen ion activity
$\mathbf{H^+}$	hydrogen
I	ionic strength
j	analyte present in the solution
K	distribution coefficient
k′	corrected retention factor
$\mathbf{K_a^{th}}$	thermodynamic dissociation constant
γ	activity coefficients
$\mathbf{\gamma_{HA}}$	activity coefficient of the undissociated acid
$\mathbf{\gamma_{A^-}}$	activity coefficient of the anionic specie
$\mathbf{\gamma_{H^+}}$	activity coefficient of the hydrogen ion
$\mathbf{L_{tot}}$	total length of the capillary
$\mathbf{L_{eff}}$	length of the capillary to the detection window

η	background electrolyte viscosity
ΔP	pressure drop over the capillary
pK_a^{th}	negative logarithm of thermodynamic dissociation constant
pK_a	negative logarithm of concentration dissociation constant
Q	constant corresponding to $(0.5085 \cdot z^2 \cdot \sqrt{I})/(1 + 0.3281 \cdot a \cdot \sqrt{I})$
r	internal radius of the capillary
t	injection time
t_{app}	migration time of the ionic specie
t_{EOF}	migration time of a compound migrating with the electroosmotic flow
μ_{app}	apparent mobility
μ_{eff}	effective electrophoretic mobility
μ_{EOF}	effective electrophoretic mobility of electrolyte medium/electroosmotic mobility
μ_{mc}	mobility of the micellar phase
μ_{ion}	ionic mobility
μ_b	electrophoretic mobility of the fully protonated species BH^+
μ_a	electrophoretic mobility of the fully deprotonated species A^-
ν	partial molar volume of micellar phase
V	applied voltage
V_{mc}	volumes of the micellar phase
V_{aq}	volumes of aqueous phase
Vs	injected volume
x_i	molar fraction
Z	charge of the ion

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