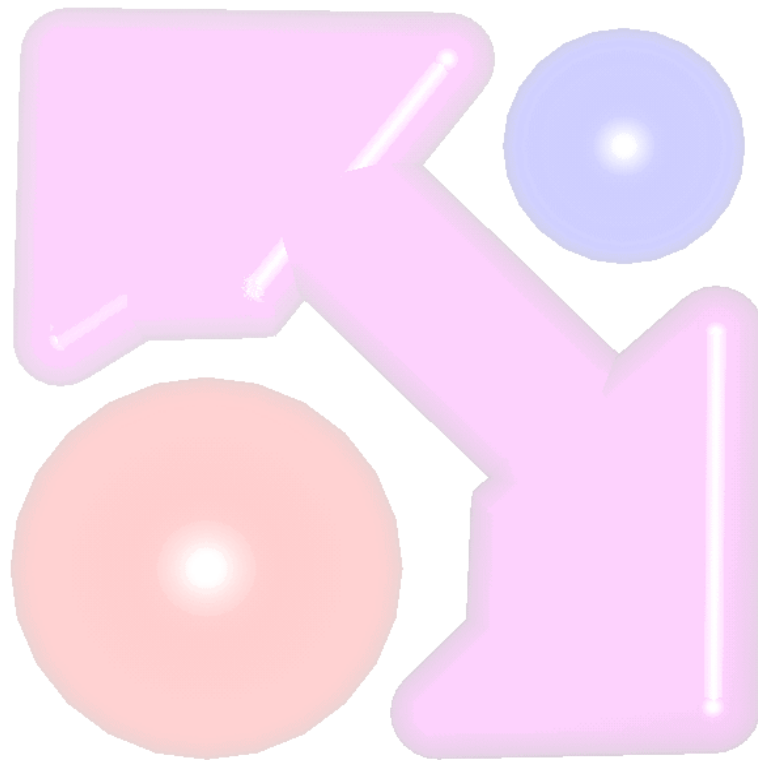


# PHoEBuS

Version 1.3

Program **H**elp for **E**lectrophoretic **B**uffer **S**tudies

## Reference Manual



By Ph. Morin, E. Vangrevelinghe and S. Mayer, Orleans (France), 1996, 1997-2001.  
Recording Number: IDDN.FR.001.270019.00.R.P.1996.000.30625.

© Copyright 2001 ANALIS sa/nv

**analisis**

Information in this document is subject to change without notice and does not represent a commitment on the part of ANALIS. The software described in this document is furnished under a license agreement or nondisclosure agreement. The software may be used or copied only in accordance with the terms of agreement. It is against the law to copy the software on any medium except as specifically allowed in the agreement. No part of this manual may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying and recording, for any purpose without the express written permission of ANALIS.

© Copyright 2001 ANALIS. All Rights Reserved.

Microsoft, Windows and Windows NT are registered trademarks of Microsoft Corporation, Pentium and Pentium II are registered trademarks of Intel Corp.

## **Analisis sa/nv**

Rue Dewez 14, B5000 Namur, Belgium

tel.: (+32) 81 25 50 50

fax: (+32) 81 23 07 79

e-mail: [ceofix@analisis.be](mailto:ceofix@analisis.be)

<http://ceofix.analisis.com>

Printed in Belgium

# Contents

---

<b>1 INTRODUCTION</b>	<b>1-1</b>
<b>1.1 GENERAL OVERVIEW</b>	<b>1-1</b>
<b>1.2 DETECTION BY <u>DIRECT</u> SPECTROMETRY</b>	<b>1-1</b>
<b>1.3 DETECTION BY <u>INDIRECT</u> SPECTROMETRY</b>	<b>1-1</b>
<b>1.4 COMPARING CALCULATED RESULTS WITH BUFFER PROPERTIES</b>	<b>1-2</b>
<b>1.5 OUTLINE OF THE MANUAL</b>	<b>1-2</b>
<b>2 SOFTWARE INSTALLATION</b>	<b>2-1</b>
<b>2.1 COMPUTER REQUIREMENTS</b>	<b>2-1</b>
2.1.1 MINIMUM CONFIGURATION	2-1
2.1.2 RECOMMENDED CONFIGURATION	2-1
<b>2.2 INSTALLATION</b>	<b>2-1</b>
2.2.1 WINDOWS NT 4.0 – WINDOWS 2000 INSTALLATION	2-1
2.2.2 WINDOWS 9X (OR MILLENIUM) INSTALLATION	2-2
<b>2.3 REGISTER YOUR COPY OF PHOEBUS</b>	<b>2-2</b>
<b>3 PHOEBUS OVERVIEW</b>	<b>3-1</b>
<b>3.1 KEY STEPS</b>	<b>3-1</b>
<b>3.2 SELECTION OF THE WORKING PROGRAM</b>	<b>3-1</b>
<b>3.3 SETTING SYSTEM PARAMETERS AND OPTIONS</b>	<b>3-2</b>
3.3.1 OVERVIEW	3-2
3.3.2 CORRECTION OF THE ACTIVITY COEFFICIENT FOR THE IONIC STRENGTH	3-2
3.3.3 VARIATION OF THE ELECTROPHORETIC MOBILITY VERSUS THE IONIC STRENGTH	3-3
3.3.4 THE EQUIPMENT TAB	3-3
3.3.5 PRINTING OPTIONS	3-4
3.3.6 THE STARTUP OPTIONS TAB	3-5
3.3.7 COMPOUND MANAGEMENT	3-6
<b>3.4 DEFINING BUFFERS AND CONVENTIONS FOR THE SELECTION OF COMPONENTS AND IONS</b>	<b>3-6</b>
<b>3.5 THE OPERATIONAL WINDOW</b>	<b>3-8</b>
<b>4 BUFFER PREPARATION - FIXED PH/IONIC STRENGTH</b>	<b>4-1</b>
<b>4.1 OVERVIEW</b>	<b>4-1</b>
<b>4.2 PREPARATION OF A PHOSPHORIC ACID/SODIUM HYDROXIDE BUFFER</b>	<b>4-1</b>
4.2.1 PREPARATION OF A BUFFER AT FIXED PH AND IONIC STRENGTH DIALOG BOX	4-2
4.2.2 ENTERING DATA	4-2
4.2.3 RESULTS	4-3
<b>4.3 ADDITIONAL EXERCISES</b>	<b>4-7</b>
<b>5 BUFFER PREPARATION - FIXED PH/CONCENTRATION</b>	<b>5-1</b>
<b>5.1 OVERVIEW</b>	<b>5-1</b>
<b>5.2 PREPARATION OF AN IMIDAZOLE/ACETIC ACID BUFFER</b>	<b>5-1</b>
5.2.1 THE PREPARATION OF A BUFFER AT FIXED PH AND CONCENTRATION DIALOG BOX	5-1

5.2.2	ENTERING DATA	5-2
5.2.3	RESULTS	5-3
<b>6 IONIC COMPOSITION OF AN ELECTROPHORETIC BUFFER</b>		<b>6-1</b>
<hr/>		
6.1	OVERVIEW	6-1
6.2	THE IONIC COMPOSITION OF AN ELECTROPHORETIC BUFFER DIALOG BOX	6-1
6.3	DETERMINING THE PROPERTIES OF A CITRIC ACID/SODIUM CITRATE BUFFER	6-2
6.3.1	DATA ENTRY	6-2
6.3.2	RESULTS	6-3
6.4	ADDITIONAL EXERCISES	6-6
<b>7 DATABASES</b>		<b>7-1</b>
<hr/>		
7.1	OVERVIEW	7-1
7.2	PHYSICO-CHEMICAL PROPERTIES OF COMMON IONS	7-1
7.2.1	VIEWING THE DATABASE	7-1
7.2.2	EDITING THE DATABASE	7-2
7.2.3	ADDING A NEW ION	7-2
7.3	CHEMICAL COMPONENT DATABASE	7-3
7.4	THE BUFFER DATABASE	7-3
<b>8 APPENDIX</b>		<b>8-1</b>
<hr/>		
8.1	SIMPLIFIED NOTATION OF IONS	8-1
8.1.1	ACIDS	8-1
8.1.2	BASES	8-1
8.1.3	ZWITTERIONS	8-1
8.1.4	CONVENTIONS	8-2
8.2	THEORETICAL BACKGROUND	8-3
8.2.1	CALCULATION OF THE ACTIVITY COEFFICIENT AT 25°C	8-3
8.2.2	VARIATION OF THE ELECTROPHORETIC MOBILITY VERSUS THE IONIC STRENGTH	8-3
8.2.3	CALCULATION OF THE CONDUCTIMETRIC CONSTANT OF THE ELECTROPHORETIC SYSTEM	8-4
8.3	REFERENCES	8-6

---

# 1 Introduction

---

## 1.1 General Overview

The PHoEBuS application program is designed to assist the scientist in creating and preparing the electrolyte to be used in a separation via capillary electrophoresis. It provides the following information for electrolytic solutions at 25°C:

- chemical characteristics (pH, ionic composition, buffer capacity)
- electrical characteristics (conductivity, estimation of the current, electrical power)
- electrophoretic characteristics (mean electrophoretic mobilities of the co-ion and the counter ion)

**In addition, the application program is also an useful tool to determine the effect of changes in the pH, the ionic strength or the concentration of one of the components when such solutions are developed for other analytical methods such as liquid chromatography, ion chromatography and titrimetry.**

There are two general modes of operation in Capillary Electrophoresis:

- Detection by Direct Spectrometry (Section 1.2)
- Detection by Indirect Spectrometry (Section 1.3)

## 1.2 Detection by Direct Spectrometry

In many cases, the electrophoretic separation is performed using an electrolyte that does not absorb at the wavelength that is used to detect the compounds of interest. When this mode is used, a direct UV spectrometric detection is possible, with the wavelength set at the absorbance maximum of the analyte(s).

The optimization of the electrophoretic separation depends on two physico-chemical parameters; the ionic strength and the pH of the electrolyte. PHoEBuS can be used to describe how to make an electrolyte with a pH and the ionic strength that is selected by the user.

## 1.3 Detection by Indirect Spectrometry

If the analyte does not absorb in the UV-visible region of the spectrum, an indirect spectrometric detection is used. In this approach, the electrolyte contains an ion that includes a chromophoric group and the spectrometric detector is set at the maximum absorbance wavelength of the ion. This mode of detection is commonly used for the analysis of inorganic cations and anions in capillary electrophoresis.

The sensitivity of detection via an indirect spectrometric method can be optimized by varying the pH and the concentration of the co-ion of the electrolyte. The application program can determine the composition that will provide the desired pH and the concentration of the co-ion of the electrolyte which are imposed by the user. In addition, the program allows for selection of the chemical form under which the co-ion will be mainly present in the electrolyte so as to optimize the transfer ratio.

In summary, the application program allows the user to select the pH and the concentration of an ion of the electrolyte (in general the co-ion). As an alternative, the user can select the pH and the concentration of one of the chemical forms of this ion (if imposed values have a chemical reality).

## 1.4 Comparing Calculated Results with Buffer Properties

PHoEBuS employs a number of theoretical models which simulate inter-ionic interactions to assist the analyst in the preparation of an electrolyte for capillary electrophoresis. It is strongly recommended that the analyst checks the properties of the buffer generated by the application program before the buffer is used since the buffer properties may differ slightly from calculations. To ensure that the buffer meets the requirements of the separation, the following points should be kept in mind:

- Calculations are based on physico-chemical parameters at 25°C (pK<sub>a</sub>, ionic product of the water, electrophoretic mobility).
- Measure the pH at 25°C using a calibrated pH meter.
- Make certain that the concentrations of stock solutions are accurate. If the stability of the stock solution is questionable, prepare a fresh solution.
- Verify that the correct value of pK<sub>a</sub> (the acidity constant) and the electrophoretic mobilities are used. This latter physico-chemical parameter is frequently poorly defined.

## 1.5 Outline of the Manual

The manual includes the following information:

- **Software Installation** (Chapter 2) - describes how to load the program on your computer.
- **PHoEBuS Overview** (Chapter 3) - explains how the desired mode of operation of the program is selected and describes how various system parameters are selected.
- **Buffer Preparation - Fixed pH/Ionic Strength** (Chapter 4) - describes how the user enters parameters to prepare a buffer with a fixed pH and ionic strength, and how the characteristics of the buffer are reported.
- **Buffer Preparation - Fixed pH/Concentration** (Chapter 5) - describes how the user enters parameters to prepare a buffer with a fixed pH and concentration of one of the components, and how the characteristics of the buffer are reported.

- **Ionic Composition of an Electrophoretic Buffer** (Chapter 6) - describes how the program can be used to describe the ionic composition of a buffer.
- **Databases** (Chapter 7) - discusses the nature of the *Ions* database and the *Buffer* database. It describes how the *Ions* database can be edited.

Chapters 4-6 describe the three operating modes of the application program and include exercises which serve to help the user become familiar with the program. Each chapter describes a specific mode of operation. The reader will note that some material is repeated in the three chapters since each mode of operation of the application program can be used on an independent basis.

## 2 Software Installation

---

### 2.1 Computer Requirements

#### 2.1.1 Minimum Configuration

Personal Computer with Pentium 90 MHz, 16 MB RAM, 5 MB of available hard-disk space, Windows 9x operating system, graphics board with 256 color capability, quad-speed CD-ROM drive.

#### 2.1.2 Recommended Configuration

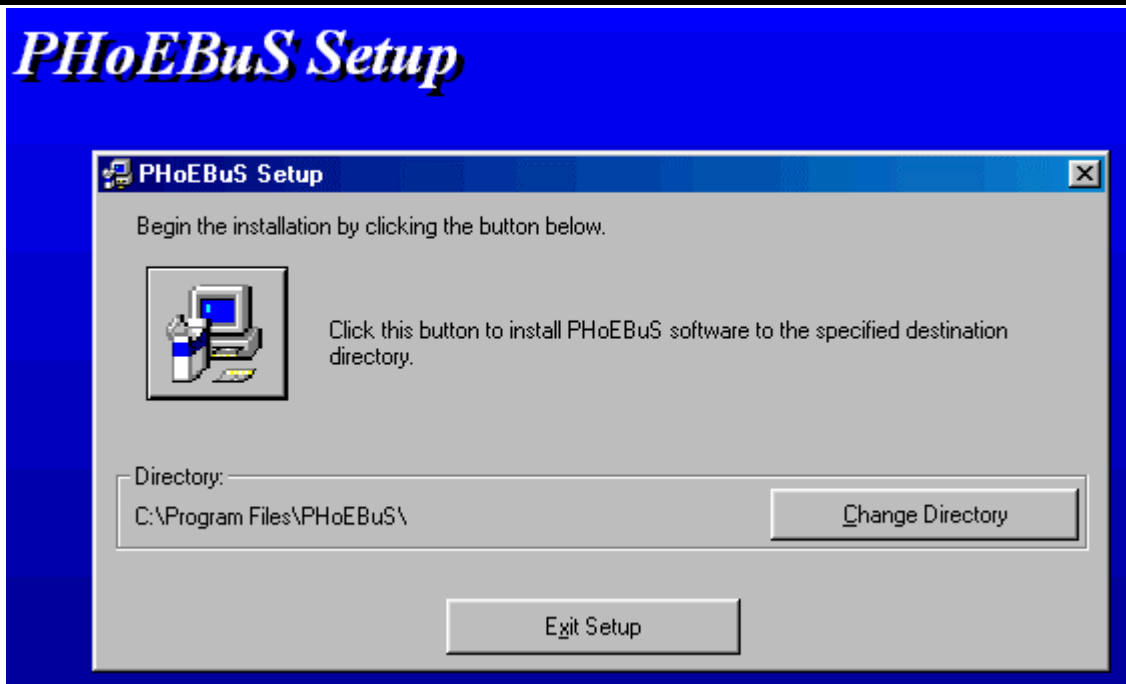
Personal Computer with Pentium II 200 MHz or higher processor, 64 MB RAM, 10 MB of available hard-disk space, Windows 9x operating system or higher, graphics board with 16 bits color capability, quad-speed CD-ROM drive or higher, printing capacity.

### 2.2 Installation

#### 2.2.1 Windows NT 4.0 – Windows 2000 Installation

To install the program on a computer with the Windows NT 4.0 – Windows 2000 Operating System:

- a) Log on to an Administrator Privileged Account.
- b) Insert the compact disc PHoEBuS into the CD-ROM drive. The Setup program should start automatically. If Setup does not start automatically, open Windows explorer, click the CD-ROM drive icon, and then double-click Setup.exe.
- c) The program will lead you through the installation. The default directory for the program is "C:\Program Files\Phoebus". If that is acceptable, click on the large button in the upper left corner of the setup window (Figure 2-1). To select another directory, click on the Change directory button and enter the desired directory.



**Figure 2-1: The Setup Window**

- d) At the conclusion of the installation process, a message will indicate that the installation was successful. Click **OK** in the installation message box to acknowledge that the program was installed. An icon for PHoEBuS will be presented on the display and may be copied as a shortcut on the desktop.

## 2.2.2 Windows 9x (or Millenium) Installation

To install the program on a computer with the Windows 9x Operating System:

- a) Insert the compact disc PHoEBuS into the CD-ROM drive. The Setup program should start automatically. If Setup does not start automatically, open Windows explorer, click the CD-ROM drive icon, and then double-click Setup.exe.
- b) The program will lead you through the installation. The default directory for the program is "C:\Program Files\Phoebus". If that is acceptable, click on the large button in the upper left corner of the setup window (Figure 2-1). To select another directory, click on the Change directory button and enter the desired directory.
- c) At the conclusion of the installation process, a message will indicate that the installation was successful. Click **OK** in the installation message box to acknowledge that the program was installed. An icon for PHoEBuS will be presented on the display and may be copied as a shortcut on the desktop.

## 2.3 Register your copy of PHoEBuS

The first time you attempt to run PHoEBuS on a particular machine you must register the installation using the process described below:

**Note: PHoEBuS registration is machine specific. You must register PHoEBuS on the machine on which it is to be used.**

In order to register you will need to :

- report the Serial Number of your copy of PHoEBuS in the Registration dialogue box (Figure 2-2). This Serial Number should be in the CD-ROM box.
- Complete the registration form named Registration\_form.pdf. This file is located in the directory where PHoEBuS has been installed (e.g. "C:\Program Files\Phoebus\"). Both the Registration and the Serial Numbers must be also reported in the form. These Numbers can be copied and pasted from the Registration dialogue box.
- Fax the completed Registration form to Analis.

**PHoEBuS - Registration Step**

You must register to be able to use PHoEBuS.

Registration Number :

Serial Number :  (supplied in the CD-ROM box)

**!!! WARNING: Registration is machine specific !!!**

To obtain your validation code, complete the registration form : [Registration\\_form.pdf](#)  
(located in the directory where PHoEBuS has been installed)  
Don't forget to report the above registration and serial numbers.

Then send the form by fax to Analis; Fax : ++ 32(0) 81 23 07 79

After your supplied information has been checked you will be given a validation code.  
In case of problem with the registration step, please contact Analis  
either by phone : ++ 32(0) 81 25 50 50 or by email : [ceofix@analis.be](mailto:ceofix@analis.be)

Validation Code :

**Figure 2-2: The Registration dialogue box**

A Validation Code will be promptly issued to you. To complete your registration you must enter this Validation Code in the space provided at the bottom of the Registration dialogue box (Figure 2-2) and click on the "Submit" button. Then if the Validation Code you have provided is correct, the main PHoEBuS program starts and the Registration dialogue box will never appear.

## 3 PHoEBuS Overview

### 3.1 Key Steps

In this chapter we describe:

- Selection of the working program (Section 3.2)
- Setting a variety of system options (Section 3.3)
- How buffers are described in the program (Section 3.4)
- The general layout of the window used in the program (Section 3.5)

### 3.2 Selection of the Working Program

The main menu of PHoEBuS indicates the three modes of operation of the application program. Select the radio button for the desired mode and click on **Execute**. The user can obtain detailed information about familiarize to the finality of each program by clicking on **Help**.

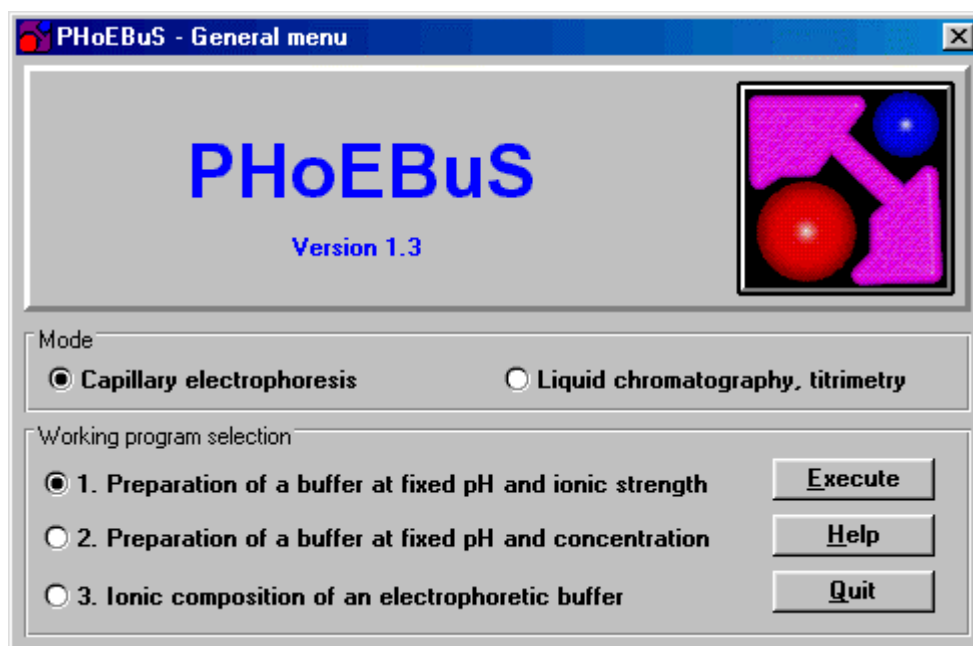



Figure 3-1: The Main Menu

**Note:** The program will not present the Main Menu when it is opened if a different startup option has been selected as described in Section 3.3.6.

### 3.3 Setting System Parameters and Options

#### 3.3.1 Overview

The application program allows for selection of a number of parameters such as the way that the activity coefficient is to be calculated and the printing options. After you have opened the desired mode of operation, click on **Parameters** in the Menu drop down menu or click on  to access the dialog box that provides access to the various pages for selection of system parameters (Figure 3-2). Many of the options described in this chapter are set when the program is installed and then are changed only on a very infrequent basis.

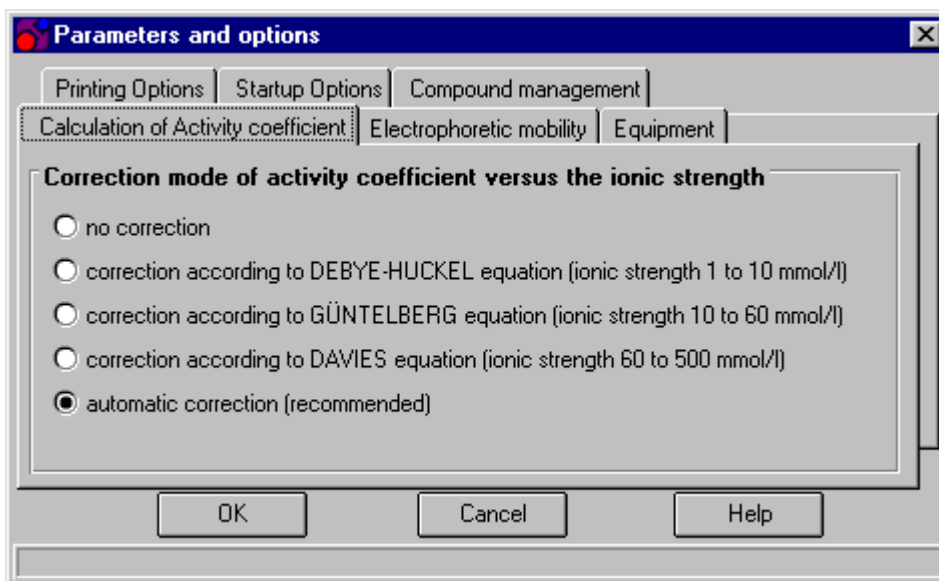


Figure 3-2: The Parameters and Options Dialog Box

Figure 3-2 presents the tab used to select the *Correction Mode of Activity Coefficient Versus the Ionic Strength* dialog box. The other dialog boxes are accessed by clicking on the appropriate tab.

#### 3.3.2 Correction of the Activity Coefficient for the Ionic Strength

The activity coefficient ( $a_i$ ) of an ion is a critical parameter in the various calculations provided by the program. It is related to its concentration ( $C_i$ ) by the expression shown in equation 3-1:

$$a_i = \gamma_i \cdot C_i \quad 3-1$$

where  $\gamma_i$  is the ionic activity coefficient of the ion  $i$ .

The program allows the user to select the mode of calculation of the ionic activity coefficient at 25° C according to the ionic strength ( $I$ ) as shown in Figure 3-2. A detailed discussion of the various approaches is presented in Appendix A2-1.

The choices provided in Figure 3-2 are:

- a) **no correction** - the calculation is made with the assumption that the ionic activity coefficient is independent of the ionic strength.
- b) **correction according to DEBYE- HÜCKEL equation (ionic strength 1-10 mmol/l)**

- c) correction according to GÜNTEMBERG equation (ionic strength 10-60 mmol/l)
- d) correction according to DAVIES equation (ionic strength 60-500 mmol/l)
- e) automatic correction (recommended).

In most instances, the automatic correction option should be selected. In this mode, the activity coefficient will be calculated via the Debye-Hückel equation if the ionic strength is less than 10 mmol/l, by the Güntelberg equation if the ionic strength is between 10 and 60 mmol/l and by the Davies equation if the ionic strength is between 60 and 500 mmol/l.

The default mode is automatic correction.

### 3.3.3 Variation of the Electrophoretic Mobility Versus the Ionic Strength

Two models are commonly used to describe the dependence of the electrophoretic mobility  $m_{ep}(i)$  of the ion (i) with the ionic strength  $I$  of the electrolyte at 25 °C, the Friedl model and the Debye-Hückel model.

The default selection is the Friedl model. If desired, the user can select the Debye-Hückel model or no correction via the *Correction mode of Electrophoretic mobility versus the ionic strength* tab (Figure 3-3). This dialog box is accessed by clicking on the *Electrophoretic Mobility* tab when the Correction mode of activity coefficient versus the ionic strength dialog box (Figure 3-2) is presented. A detailed discussion of the theoretical background of the models is presented in Appendix A2.2.

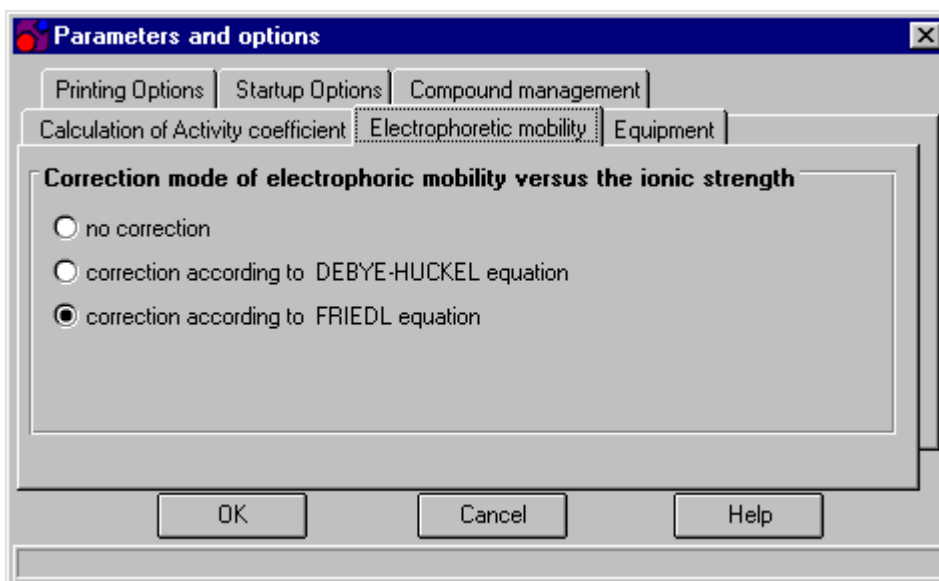
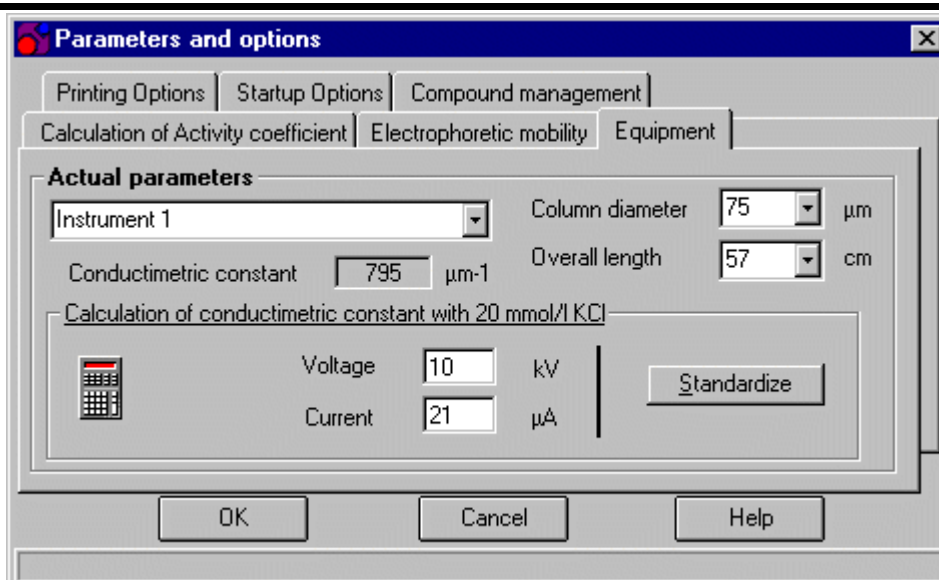


Figure 3-3: The Electrophoretic Mobility Tab

### 3.3.4 The Equipment Tab

PHoEBuS provides two general approaches for estimation of the conductimetric constant, automated determination and standardization of the system. These approaches are presented on the *Equipment* tab of the Parameters and options dialog box (Figure 3-4) which is accessed by clicking on the Equipment tab on the Parameters and options dialog box.



**Figure 3-4: The Equipment Tab**

a) Automated Determination of the Conductimetric Constant

If you choose this option, click on the down arrow by the instrument field and select the system that you are using (up to five can be entered), enter the column diameter (in  $\mu\text{m}$ ) and the capillary length (in cm), then press . The conductimetric constant of the system (in  $\mu\text{m}^{-1}$ ) will be automatically calculated and can be viewed by opening the dialog box again.

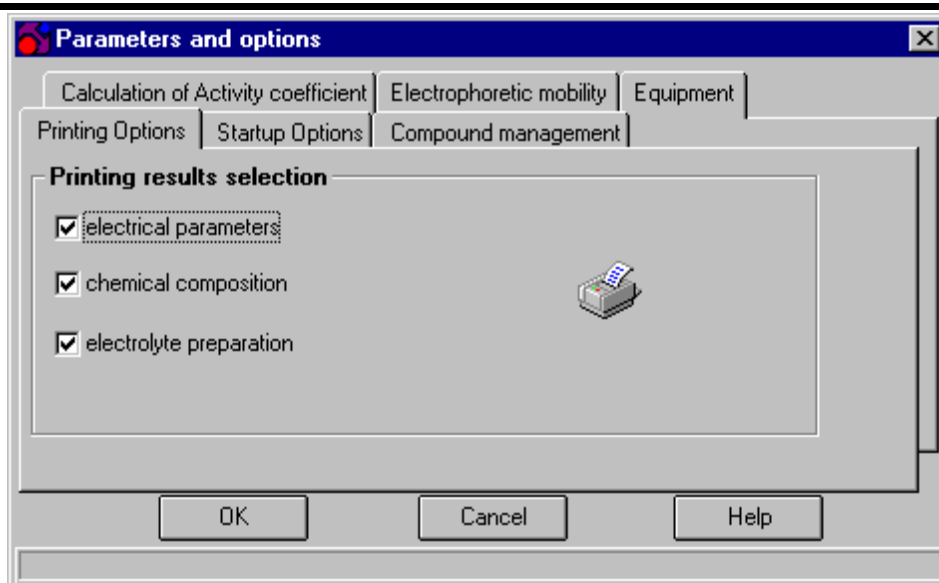
b) Experimental Determination of the Conductimetric Constant

If the dimensions of the capillary column are unknown or badly defined, the program allows to determine the conductimetric constant of the electrophoretic system. To determine the conductimetric constant of the system:

- 1) Fill the capillary with a 20 mmol/l KCl solution and apply a moderate voltage (e.g. 10 kV).
- 2) Read the value of the current and enter the observed values of the voltage (kV) and the current ( $\mu\text{A}$ ) in the appropriate fields in Figure 3-4.
- 3) Click on  to calculate the conductimetric constant (in  $\mu\text{m}^{-1}$ ) of the electrophoretic system, then click on  to store the value.

### 3.3.5 Printing Options

The program allows the user to select the parameters that should be included in the printed report. To access the *Printing options* tab (Figure 3-5), click on the *Printing Options* tab on the Parameters and Options dialog box.

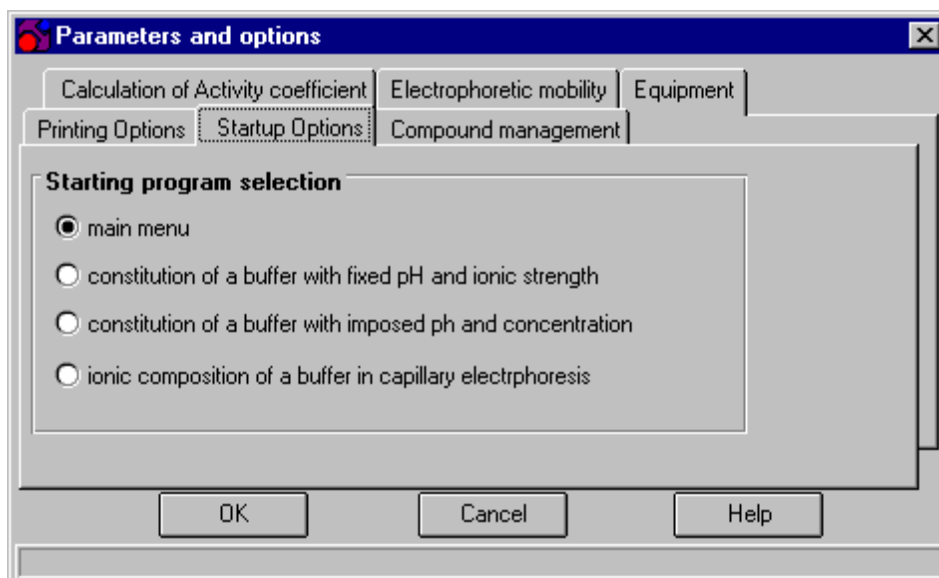


**Figure 3-5: The Printing Results Tab**

The desired selection(s) can be made by clicking in the check box adjacent to each entry.

### 3.3.6 The Startup Options Tab

The *Startup Options* tab is provided to allow you to select the mode of operation that should be presented when the program is powered up (Figure 3-6). To select the desired option, select the appropriate radio button.



**Figure 3-6: The Startup Options Selection Tab**

**Note:** The General Menu, which is used to select other program options can be accessed via the *Return to general menu* option on the Menu drop down menu.

### 3.3.7 Compound Management

The *Compound Management* tab is provided for editing the buffers used in the program (Figure 3-7).

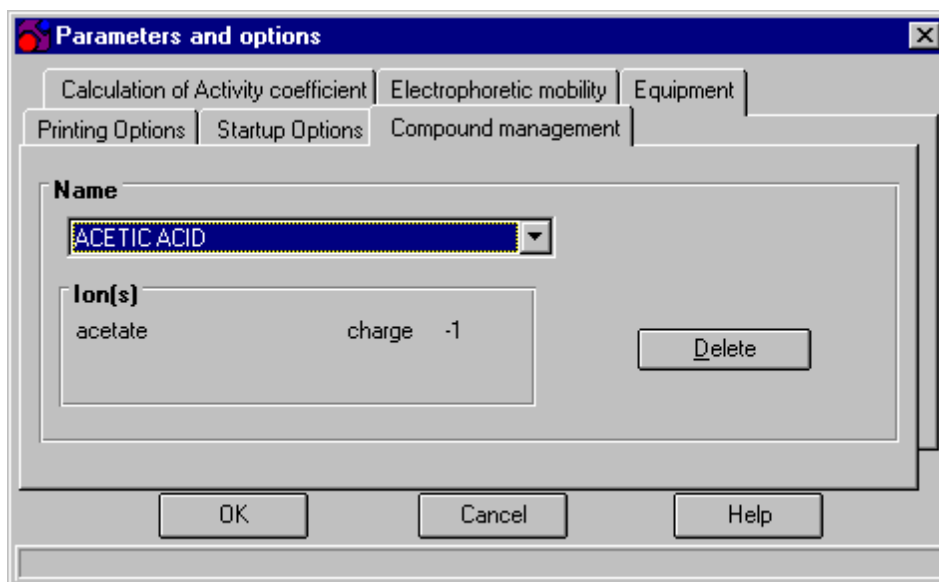


Figure 3-7: The Compound Management Tab

To delete a compound, highlight it and press the **Delete** button. The program will present a dialog box for the user to verify the deleting of the entry.

## 3.4 Defining Buffers and Conventions for the Selection of Components and Ions

The user selects the buffer system in each of the three modes of operation of the program. This section describe how a constituent of a buffer is selected and provides information about a few of the acids, bases, salts and buffers that are commonly used in capillary electrophoresis.

Selection of a component of a buffer is performed via the *components* field (Figure 3-8) of each of the working windows (e.g. the Ionic Composition of an electrophoretic buffer at 25° C, which is described in Chapter 4).

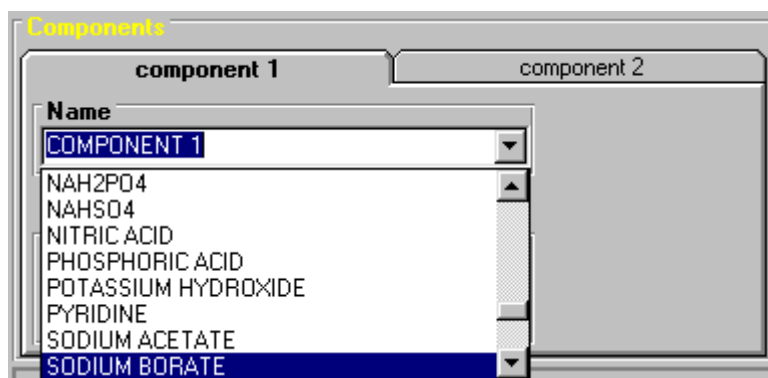


Figure 3-8: The Component Field

To select the component to be used, use the scroll bar to view the component name, and click on it. The field will present the number of ions, the names of the ions and their charge as shown in Figure 3-9.

Figure 3-9: The Buffer Description

The following tables provide information for a few acids, bases and buffers that are used with the program. To determine the information for others, simply click on the desired component in the component field.

**Note: It is not necessary to take  $\text{H}_3\text{O}^+$  or  $\text{OH}^-$  into account when entering the reagents in the program.**

Acid	Number of Ions	Chemical Name of the Ion	Nature of the Ion*	Ionic Charge in the Compound
HCOOH	1	formate	A	-1
H <sub>2</sub> SO <sub>4</sub>	1	sulfate	A	-2
H <sub>3</sub> PO <sub>4</sub>	1	phosphate	A	-3

Base	Number of Ions	Chemical Name of the Ion	Nature of the Ion*	Ionic Charge in the Compound
NaOH	1	sodium	C	+1
Ba(OH) <sub>2</sub>	1	barium	C	+2
Imidazole	1	imidazolium	C	+1
TRIS	1	Tris	C	+1

Salt	Number of Ions	Chemical Name of the Ion	Nature of the Ion*	Ionic Charge in the Compound
Na <sub>2</sub> HPO <sub>4</sub>	2	sodium phosphate	C A	+1 -2
NaH <sub>2</sub> PO <sub>4</sub>	2	sodium phosphate	C A	+1 -1
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub>	2	sodium tetraborate	C A	+1 -2
TRIS-HCl	2	Tris chloride	C A	+1 -1

Biological Buffer	Number of Ions	Number of Acid Groups	Number of Basic Groups	Nature of the Ion*	Ionic Charge in the Compound
MES	1	1	1	Z	-
Aspartic Acid	1	2	1	Z	-
Histidine	1	1	2	Z	-
ADA	1	2	2	Z	-

\* A: anion; C: cation; Z: zwitterion.

### 3.5 The Operational Window

There are three modes of operation in the program which are selected from the main window (Figure 3-1). When you select a working program, the monitor will present a window like that shown in Figure 3-10.

The screenshot shows the 'PHoEBuS - Preparation of a buffer at fixed pH and ionic strength (25°C)' dialog box. It features a menu bar with 'Menu', 'Buffer', and 'Help'. Below the menu bar is a toolbar with icons for file operations and a help icon. The main area is divided into two sections: 'Components' and 'Results'.

**Components Section:**

- Two tabs: 'component 1' (selected) and 'component 2'.
- 'Name' dropdown menu: 'COMPONENT 1'.
- 'number of ion(s) in this component': '1'.
- 'Ion(s)' section: Radio buttons for '1 charge' (selected) and '2 charge'.
- 'pH' and 'ionic strength' input fields (mmol/l).
- 'Ion selection' buttons: 'anion', 'cation', 'zwitterion'.
- 'acetate' text input field.
- 'charge in the component': '-1'.
- 'ION' button highlighted in blue, with navigation arrows and a 'Select' button.

**Results Section:**

- Four tabs: 'Parameters', 'Chemical composition', 'Preparation', 'Save'.
- 'Parameters' tab: 'Ionic strength' (mmol/l), 'Buffer capacity' (mmol/l,pH).
- 'Chemical composition' tab: 'Buffer mobility' (- cationic, - anionic), 'Conductivity' (mS/cm).
- 'Preparation' tab: 'Voltage' (15 kV), 'Column diameter' (50 μm), 'Overall length' (57 cm), 'Estimate' button.
- 'Save' tab: 'Instrument 1' (μA, mW).

**Figure 3-10: The Preparation of a Buffer at Fixed pH and Ionic Strength Dialog Box**

The dialog box consists of two regions; a Components region in which the components of the buffer are defined and a Results region which presents the data.

The button bar on top of the window contains the tools used to execute a command, as follows:



Return to the main menu



Open the database



Access a list of buffers for spectrometric detection



Access the parameters and options tab



Reset and erase all data entries to perform a new calculation



Calculate



Print

Commands on the drop down menus on the menu bar are directly equivalent to the buttons on the button bar. As an example, the **Menu** drop down menu includes:

**Back to main menu**

**Database of ions**

**List of buffers**

**Parameters**

**Quit (shuts the program down)**

## 4 Buffer Preparation - Fixed pH/Ionic Strength

### 4.1 Overview

The optimization of an electrophoretic separation depends on two physico-chemical parameters, the ionic strength and the pH of the buffer. The PHoEBuS application program is designed to aid in the preparation of a buffer containing two components for which the user has selected the pH and ionic strength. In addition, the program can be used to predict the chemical, electrical and electrophoretic parameters of the buffer.

The optimization of the separation involves:

- the variation of the resolution versus the pH **at constant ionic strength**
- the variation of the resolution versus the ionic strength **at constant pH**.

In this chapter, we will describe the preparation of a buffer using at a fixed pH and ionic strength. The general approach will be to lead the reader through each step in the process.

### 4.2 Preparation of a Phosphoric Acid/Sodium Hydroxide Buffer

Figure 4-1: The Preparation of a Buffer at Fixed pH and Ionic Strength Dialog Box

### 4.2.1 Preparation of a Buffer at Fixed pH and Ionic Strength Dialog Box

The dialog box that is presented when the *Preparation of a buffer at fixed pH and ionic strength* selection is made on the main window is shown in Figure 4-1.

This exercise will generate a Phosphoric Acid/Sodium Hydroxide buffer, which is commonly used for the chiral separation of a basic drug via capillary electrophoresis with detection by a direct UV absorption measurement.

### 4.2.2 Entering Data

The following steps should be used to enter the data:

- Click on the down arrow adjacent to the Name field, use the scroll bar to access Phosphoric Acid and click on it. The component 1 region will indicate 1 for the number of ions in this component and the ion 1 radio button will be activated with the legend phosphate and a charge of -3 will be indicated.
- If phosphoric acid is not included in the list of components, enter the name and click on the  button in the *Ion selection* region of the dialog box.

Use the arrow keys by      to access phosphate in the field. (The > button selects the next anion in the list, the < button selects the previous anion in the list, << button accesses the first anion in the list, while the >> selects the last anion in the list). After you have selected phosphate, the field should appear as shown in Figure 4-2. Click on .

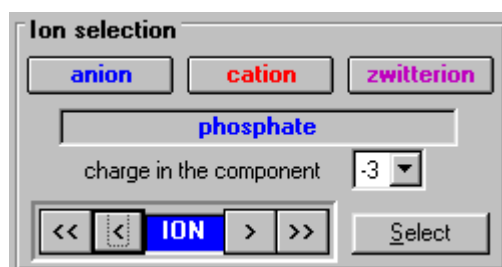



Figure 4-2: The Ion Selection Field

- Repeat steps a-b for the second component in the buffer, Sodium Hydroxide. The cation to be selected is Sodium and the charge is + 1.
- Indicate the pH of the buffer. For this example, we will use 2.5.

**Note: The useful range of the pH in capillary electrophoresis is between 1.0 and 13.0. The application program will not accept values outside these limits.**

- Indicate the ionic strength of the buffer (in mmol/l). For this example, we will use 50.

**Note:** The useful range of ionic strength in capillary electrophoresis is between 1 and 150 mmol/l. If the ionic strength is less than 1 mmol/l, it is likely that noisy electropherograms will be observed. If the value is greater than 150 mmol/l the required power dissipation is greater than the thermoregulation capacity of the instrument. The application program will not accept values outside these limits.

- f) Verify that the calculation mode for the ionic activity coefficient is correct (Section 3.3.1). For this exercise, either GÜNTEMBERG correction or automatic correction is acceptable.
- g) Click on the  icon on the menu bar to perform the calculation. The results are presented in the lower half of the screen, as discussed in Section 4.3.3.

**Note:** If the message "This electrolyte cannot be made !" is presented, the application program cannot generate a buffer with the indicated pH and ionic strength (for example, a Phosphoric Acid/Sodium Hydroxide buffer cannot be created at pH = 2 and an ionic strength of 10 mmol/l).

### 4.2.3 Results

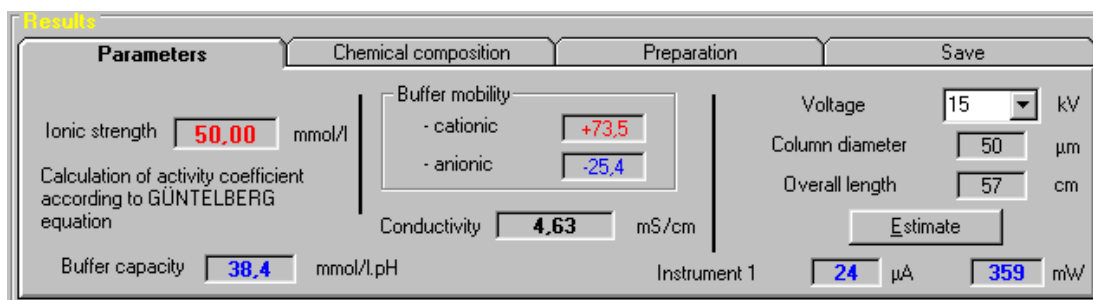
The results part of the dialog box (Figure 4-3) includes four tabs which describe:

- the electrophoretic characteristics of the buffer (Section 4.2.3.1)
- the chemical composition of the buffer (Section 4.2.3.2)
- how to prepare the buffer (Section 4.2.3.3)

a field to save data in the database. This topic is discussed in Chapter 7.

#### 4.2.3.1 The Parameter Tab

The *Parameter* tab (Figure 4-3) presents a variety of electrophoretic and electrical parameters of the buffer.



Parameter	Value	Unit
Ionic strength	50.00	mmol/l
Calculation of activity coefficient according to GÜNTEMBERG equation		
Buffer capacity	38.4	mmol/l.pH
Conductivity	4.63	mS/cm
Buffer mobility - cationic	+73.5	
Buffer mobility - anionic	-25.4	
Voltage	15	kV
Column diameter	50	μm
Overall length	57	cm
Instrument 1	24	μA
	359	mW

**Figure 4-3: The Results Field**

The parameter tab includes:

- the buffer capacity (mmol/l.pH) indicates the ability of the buffer to counteract the change in the pH. If the buffer capacity of the indicated buffer is small (<5 mmol/l.pH), the reported value will be indicated in red. This would suggest that the user might consider using a different buffer to maintain sufficient pH control to provide better repeatability and reproducibility in capillary electrophoresis.
- the electrical conductivity of the buffer (mS/cm).
- the mean electrophoretic mobilities of the anion and the cation of the buffer are expressed in  $10^{-5} \text{ cm}^2/\text{V.s}$ .
- the electrical current ( $\mu\text{A}$ ). The maximum current threshold has been fixed arbitrarily to 100  $\mu\text{A}$ . If the current is greater than 100  $\mu\text{A}$ , a warning message will be presented on the screen to alert the user to select a buffer with a lower conductivity.
- the generated electrical power (mW).

The column length and diameter are set via the Parameters and Options window (Section 3.3.4). The user can change the voltage (kV) and click on  to determine the power dissipation at various voltages.

#### 4.2.3.2 The Chemical Composition Tab

The *Chemical Composition* tab (Figure 4-4) presents a detailed description of the chemical composition of the buffer and indicates the concentration, apparent charge and activity coefficient of each of the various ionic forms (e.g.  $\text{H}_3\text{A}$ ,  $\text{H}_2\text{A}^-$  etc.). As an example, the apparent charge of phosphate is equal to -0.75 on the example in Figure 4-4.

Results				
Parameters	Chemical composition	Preparation	Save	
Component	Chemical compound	Charge	Activity coefficient	Concentration (mmol/l)
PHOSPHORIC ACID	- phosphate	-0,75		67,039
	H3A	0	1,000	17,043
	H2A(-)	-1	0,810	49,994
	HA(2-)	-2	0,431	1,88E-3
	A(3-)	-3	0,151	8,16E-13
SODIUM HYDROXIDE	- sodium	1,00		46,095
	Na(+)	1	0,810	46,095

Figure 4-4: The Chemical Composition Tab

#### 4.2.3.3 The Preparation Tab

The *Preparation* Tab presents information about how the buffer is prepared (Figure 4-5). The user can select the concentration (mmol/l) of each of the stock solution and the desired volume (ml) of the buffer via the appropriate fields.

**Results**

Parameters      Chemical composition      **Preparation**      Save

volume  ml            stock solution  mmol/l


COMPONENT	mole number (mmol)	concentration in buffer (mmol/l)	concentration in stock solution (mmol/l)	volume of stock solution (ml)
PHOSPHORIC ACID	6.70	67.04	1000	6.70
SODIUM HYDROXIDE	4.61	46.10	1000	4.61


**Figure 4-5: The Preparation Tab**


As an example, if one adds 6.70 ml of phosphoric acid stock solution and 4.61 ml of 1 M NaOH stock solution and sufficient water to dilute to 100 ml (at 25 °C), the desired phosphoric acid/sodium hydroxide buffer will be generated (with a pH of 2.5 and ionic strength of 50 mmol/l).

If a buffer cannot be prepared from the available stock solutions (e.g. a 1 mmol/l stock solution of sodium hydroxide was used), the legend **Too Dilute** will be indicated in the volume of stock solution field for the base.

If you want to change the concentration of the stock solution for the generation of the buffer, select the component via the drop down menu (in the center of the Results area), then enter the desired value in the stock solution field. As an example, if you wanted to use an NaOH stock solution containing 500 mmol/l, click on the down arrow adjacent to Phosphoric Acid to access a list of all species, click on Sodium Hydroxide and then enter 500 in the stock solution field.

Click on  to print results. The display will present a dialog box which is used to enter the buffer name. Enter the desired name and press OK (if a name is not desired, simply press OK). A typical report is shown in Figure 4-6. The operator can enter the measured pH, the estimated current, the date of buffer preparation on the report in the appropriate locations.

For the preparation of an other phosphoric acid-sodium hydroxide buffer having a similar ionic strength (50 mmol/l) but a different pH value, modify the value of the pH then click on .


For the preparation of an other phosphoric acid-sodium hydroxide buffer having a similar pH (2.5) but a different ionic strength, modify the value of the ionic strength then click on .

PHoEBus Software - OPTION 1		10/30/97 at 7:38:16 AM	
<b>PREPARATION OF SODIUM HYDROXIDE / PHOSPHORIC ACID BUFFER</b>			
at pH 2.50 and 50.00 mmol/l Ionic strength - 25°C			
- Estimated pH	2.500	(measured pH:	)
- Buffer capacity	38.4 mmol/l. pH		
- Average cationic mobility	+73.5 .1E-5 cm <sup>2</sup> /V.s		
- Average anionic mobility	-25.4 .1E-5 cm <sup>2</sup> /V.s		
<b><u>CHEMICAL COMPOSITION OF BUFFER</u></b>			
<b>Component</b>	<b>Charge</b>	<b>Activity coefficient*</b>	<b>Concentration (mmol/l)</b>
- SODIUM HYDROXIDE <sub>..</sub>			46.095
sodium ion	1.00		46.095
Na(+)	1	0.810	46.095
- PHOSPHORIC ACID <sub>..</sub>			67.039
phosphate ion	-0.75 <sup>**</sup>		67.039
H3A	0	1.000	17.043
H2A(-)	-1	0.810	49.994
HA(2-)	-2	0.431	1.88E-3
A(3-)	-3	0.151	8.16E-13
<b><u>BUFFER PREPARATION</u></b> - Total volume: 100 ml			
- SODIUM HYDROXIDE			
Concentration of stock solution:			1000 mmol/l
Volume of stock solution:		4.61 ml	
Mole number :		4.61 mmol	
- PHOSPHORIC ACID			
Concentration of stock solution:			1000 mmol/l
Volume of stock solution:		6.70 ml	
Mole number:		6.70 mmol	
<b><u>ELECTRICAL PARAMETERS</u></b> (Instrument 1)			
- Column length	57	cm	
- Column diameter	50	µm	
- Applied voltage	15	kV	
- Conductivity	4.63	mS/cm	
- Estimated current	24	µA	(measured current: )
- Estimated power	359	mW	
*..Calculation of activity coefficient according to GÜNTEMBERG equation.			
** Apparent charge of ion			
<b>Date of buffer preparation:</b>		<b>by:</b>	

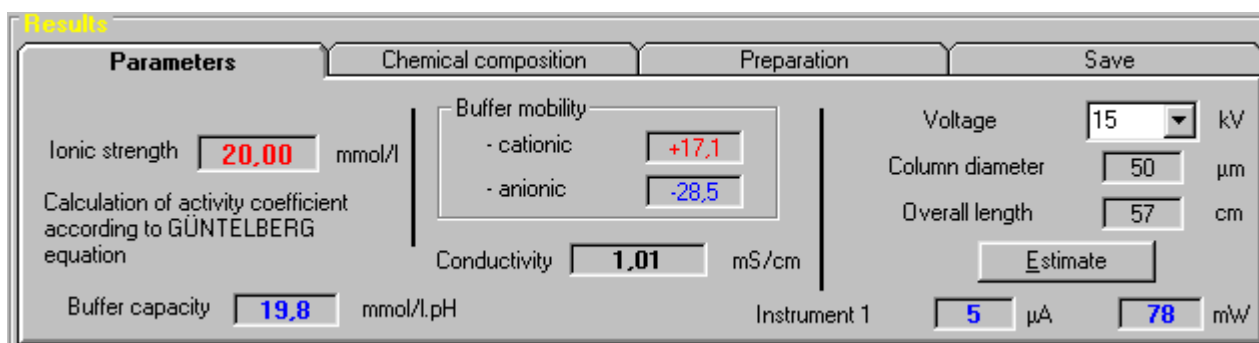
Figure 4-6: A Typical Report

### 4.3 Additional Exercises

In this section we will generate a buffer containing TRIS (Tris (hydroxymethyl)aminomethane or 2-amino-2-(hydroxymethyl)-1,3-propanediol) and the MES (4-morpholino-ethanesulfonic acid). This buffer is commonly used in the 5-9 pH range. The general approach is identical to that described above. For the purpose of this exercise:

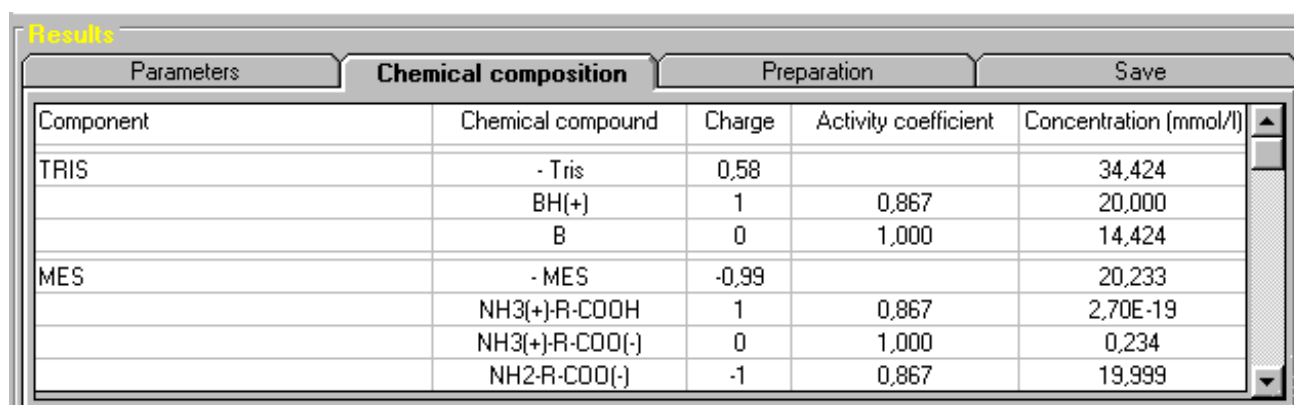
- Select TRIS as component 1.
- The number of ions field for TRIS should be set to 1 and the charge is +1.
- Select MES as component 2 (MES is a zwitterion, so the user should click on **zwitterion** and search with **<< < ION > >>** until MES is presented).
- The number of ions field for MES should be set to 1 and the charge is 0.
- Enter pH = 8.
- Enter Ionic Strength = 20 mmol/l.
- Click  to obtain the results.

The *parameters*, *chemical composition* and *preparation* tabs are shown in Figures 4-7 through Figure 4-9, respectively.



The screenshot shows the 'Parameters' tab of the software interface. It displays various input fields and calculated values for a MES/TRIS buffer. The ionic strength is set to 20.00 mmol/l, and the buffer capacity is 19.8 mmol/l.pH. The calculation of activity coefficients is based on the GÜNTEMBERG equation. The buffer mobility is shown as +17.1 for cationic and -28.5 for anionic, resulting in a conductivity of 1.01 mS/cm. The voltage is set to 15 kV, the column diameter is 50 µm, and the overall length is 57 cm. The instrument settings are 5 µA and 78 mW.

Figure 4-7: The Parameters Tab - MES/TRIS Buffer



The screenshot shows the 'Chemical composition' tab of the software interface. It displays a table with the following data:

Component	Chemical compound	Charge	Activity coefficient	Concentration (mmol/l)
TRIS	- Tris	0,58		34,424
	BH(+)	1	0,867	20,000
	B	0	1,000	14,424
MES	- MES	-0,99		20,233
	NH3(+)-R-COOH	1	0,867	2,70E-19
	NH3(+)-R-COO(-)	0	1,000	0,234
	NH2-R-COO(-)	-1	0,867	19,999

Figure 4-8: The Chemical Composition Tab - MES/TRIS Buffer

**Results**


Parameters      Chemical composition      **Preparation**      Save

volume  ml            stock solution  mmol/l

COMPONENT	mole number (mmol)	concentration in buffer (mmol/l)	concentration in stock solution (mmol/l)	volume of stock solution (ml)
TRIS	34.56	34.56	1000	34.56
MES	20.23	20.23	1000	20.23

**Figure 4-9: The Parameters Tab - MES/TRIS Buffer**

The data in Figures 4-7 to 4-9 are interpreted in the same fashion as discussed above. As an example, if we want to prepare a pH = 8 MES/TRIS buffer with an ionic strength of 20 mmol/l, using stock solutions containing 1000 mmol/l, we would add 20.23 ml of MES and 34.42 ml of TRIS and dilute to 1000 ml.

Click on  to print results.

## 5 Buffer Preparation - Fixed pH/Concentration

---

### 5.1 Overview

The PHoEBuS application program is designed to aid in the preparation of a buffer containing two components with a user selected pH and a fixed concentration for one of the components. The program can be used to describe the electrophoretic properties of the buffer, how to prepare the buffer and the composition of the buffer.

The optimization of the separation and the sensitivity of detection depends primarily on:

- the concentration of the chromophore co-ion at **constant pH**
- the buffer pH at **constant co-ion concentration**

In this chapter, we will describe the preparation of a buffer at a fixed pH and with a fixed concentration of one of the components. This mode of calculation is commonly used when indirect detection is employed (i.e. the compound of interest does not have a chromophoric group); typical application is the analysis of inorganic ions via capillary electrophoresis. In this case, the spectrometric detector is fixed at the maximum absorbance of the co-ion. The sensitivity of the analysis is a function of the pH and the concentration of the co-ion.

The application program is designed to create a buffer containing two or three components in which the pH and the concentration of the buffer co-ion (or the concentration of one of the chemical forms of the co-ion) are fixed by the user. The general approach will be to lead the reader through each step in the process.

### 5.2 Preparation of an Imidazole/Acetic Acid Buffer

#### 5.2.1 The Preparation of a Buffer at Fixed pH and Concentration Dialog Box

The dialog box that is presented when the *Preparation of a buffer at fixed pH and concentration* selection is made on the main window is shown in Figure 5-1.

**Figure 5-1: The Preparation of a Buffer at Fixed pH and Concentration Dialog Box**

An Imidazole/Acetic Acid Buffer is commonly used for the separation of a mixture of inorganic cations with indirect UV detection. The imidazolium ion absorbs strongly at 214 nm and has an electrophoretic mobility that is similar to that of the inorganic cations that are commonly separated. This buffer contains both the imidazolium ion and the acetate ion.

### 5.2.2 Entering Data

The following steps should be used to enter the data:

- Click on the down arrow adjacent to the Name field, use the scroll bar to access *Imidazole* and click on it. The component 1 field will indicate 1 for the number of ions in this component and the ion 1 radio button will be activated with the legend imidazolium and a charge of +1.
- If the imidazole ion is not included in the list of components, enter the name and click on the **cation** button in the *Ion selection* region of the dialog box.
- Use the arrow keys by **<< < ION > >>** to access imidazolium in the field. (The > button selects the next cation in the list, the < button selects the previous cation in the list, the << button accesses the first anion in the list, while the >> button selects the last cation in the list). The component field should appear as shown in Figure 5-2. Click on **Select**. The component field should appear as shown in Figure 5-2.

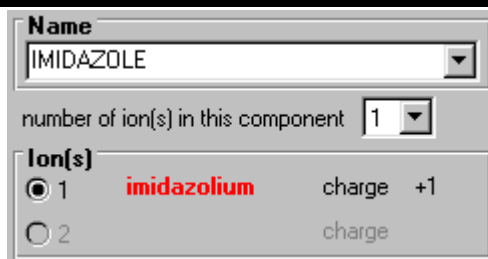



Figure 5-2: The Ion Selection Field

**Note:** The user can select either the total concentration of imidazole or the concentration of the imidazolium cation. When indirect detection is employed, the concentration of the chromophore (the co-ion) has to be taken into account in the process of optimizing the transfer ratio.

- c) To fix the concentration of the imidazolium ion; click on the drop down arrow on the concentration field and select  $BH^+$ , then indicate the desired concentration. For the sake of this exercise, enter 10 (mmol/l).
- d) Repeat steps a-c for the second component in the buffer, Acetic Acid. Instead of selecting a concentration for this reagent, click on the check box adjacent to *to be determined* in the concentration region of the Components field.
- e) Indicate the pH of the buffer. For this example, we will use 4.5.

**Note:** The useful pH range for capillary electrophoresis is between 1.0 and 13.0 and the application program will not accept values outside these limits.

When the user fixes the concentration of a chemical form of the ion instead of the total concentration, the suggested pH range of the buffer is indicated. In this example the advisable pH range is between 1 and 8.15 for the imidazolium cation.

- f) Verify that the calculation mode for the ionic activity coefficient is correct (Section 3.3.2). For this exercise, either GÜNTEMBERG correction or automatic correction is acceptable.
- g) Click on the  icon on the menu bar to perform the calculation. The results are presented in the lower half of the screen, as discussed in Section 4.3.3.

**Note:** If the message "This electrolyte cannot be made !" is presented, the application program cannot generate a buffer with the indicated pH and concentration.

### 5.2.3 Results

The Results part of the dialog box (Figure 5-3) includes four tabs which describe:

- the electrophoretic characteristics of the buffer (Section 5.2.3.1)
- the chemical composition of the buffer (Section 5.2.3.2)

- how to prepare the buffer (Section 5.2.3.3)
- a field to save data in the database (Chapter 7)

### 5.2.3.1 The Parameter Tab

The Parameter presents variety of electrophoretic and electrical parameters of the buffer.

Parameters	Chemical composition	Preparation	Save
Ionic strength: <b>10.04</b> mmol/l	Buffer mobility: - cationic: <b>+52.9</b> - anionic: <b>-16.1</b>	Voltage: 15 kV	
Calculation of activity coefficient according to GÜNTEMBERG equation	Conductivity: <b>0.86</b> mS/cm	Column diameter: 50 μm	
Buffer capacity: <b>15.0</b> mmol/l.pH		Overall length: 57 cm	
		Instrument 1: 4 μA	66 mW

**Figure 5-3: The Results Field**

The parameter tab includes the following information about the buffer:

- the ionic strength of the solution (mmol/l)
- the buffer capacity (mmol/l.pH) indicates the ability of the buffer to counteract the change in the pH. If the buffer capacity of the indicated buffer is small (<5 mmol/l.pH), the reported value will be indicated in red. This would suggest that the user might consider using a different buffer to maintain sufficient pH control to provide better repeatability and reproducibility in capillary electrophoresis.
- the electrical conductivity of the buffer (mS/cm).
- the mean electrophoretic mobilities of the anion and the cation of the buffer are expressed in  $10^{-5} \text{ cm}^2/\text{V.s}$ .
- the electrical current (μA). The maximum current threshold has been fixed arbitrarily to 100 μA. If the current is greater than 100 μA, a warning message will be presented on the display to alert the user to select another buffer that has a lower conductivity.
- the generated electrical power (mW).

The column length and diameter are set via the Parameters and Options window (Section 3.3.4). The user can change the voltage and click on  to determine the power dissipation at various voltages.

### 5.2.3.2 The Chemical Composition Tab

The *Chemical Composition* tab (Figure 5-4) presents a detailed description of the chemical composition of the buffer and indicates the concentration, apparent charge and activity coefficient of each of the various ionic forms ( $\text{BH}^+$ , B, HA,  $\text{A}^-$  etc.).

Results

Parameters		Chemical composition		Preparation		Save	
Component	Chemical compound	Charge	Activity coefficient	Concentration (mmol/l)			
IMIDAZOLE	- imidazolium	1,00		10,020			
	BH(+)	1	0,900	10,000			
	B	0	1,000	0,020			
ACETIC ACID	- acetate	-0,38		26,479			
	HA	0	1,000	16,444			
	A(-)	-1	0,900	10,035			

Figure 5-4: The Chemical Composition Tab

### 5.2.3.3 The Preparation Tab

The *Preparation Tab* presents information about how the buffer is prepared (Figure 5-5). The user can select the concentration of each of the stock solution and the desired volume of the buffer via the appropriate fields.

Results


Parameters		Chemical composition		Preparation		Save	
volume	100	ml	ACETIC ACID	stock solution	200	mmol/l	
COMPONENT	mole number (mmol)	concentration in buffer (mmol/l)	concentration in stock solution (mmol/l)	volume of stock solution (ml)			
IMIDAZOLE	1.00	10.02	100	10.02			
ACETIC ACID	2.63	26.33	200	13.16			

Figure 5-5: The Preparation Tab

As an example, at 25 °C, if one adds 10.02 ml of 0.1 M imidazole stock solution, 26.48 ml of 0.2 M Acetic Acid stock solution and sufficient water to dilute to 100 ml, the desired buffer will be generated with a pH of 4.5 and a final concentration of imidazolium cation equal to 10 mmol/l.

If a buffer cannot be prepared from the available stock solutions (e.g. a 1 mmol/l stock solution of Imidazole was used, the legend *too dilute* will be presented in the volume of stock solution field for the base).

If you want to change the concentration of the stock solution for the generation of the buffer, select the component via the drop down menu (in the center of the Results area), then enter the desired value in the stock solution field. As an example, if you wanted to use an Acetic Acid stock solution containing 500 mmol/l, click on the down arrow adjacent to Imidazole (on the preparation tab) to access a list of all species, click on Acetic Acid and then enter 500 in the stock solution field.

Click on  to print results. The display will present a dialog box which is used to enter the buffer name. Enter the desired name and press OK (if a name is not desired, simply press OK). A typical report is shown in Figure 5-6. The operator can enter the measured pH, the estimated current, the date of buffer preparation on the report in the appropriate locations.



## 6 Ionic Composition of an Electrophoretic Buffer

### 6.1 Overview

The ionic composition of an electrolyte routine of *PHoEBuS* is used to determine the following information about a buffer when the chemical composition of the buffer is provided:

- chemical parameters such as pH, ionic composition, buffer capacity
- electrical parameters such as electrical conductivity, electrical current, generated electrical power.
- electrophoretic parameters such as mean mobilities of the cation and the anion of the buffer.

### 6.2 The Ionic Composition of an Electrophoretic Buffer Dialog Box

When the ionic composition of an electrophoretic buffer option is selected on the main window, the dialog box shown in Figure 6-1 is presented:

The dialog box is titled "PHoEBuS - Ionic composition of an electrophoretic buffer at 25°C". It contains the following elements:

- Component(s) section:**
  - Tabs for component 1, component 2, component 3, and component 4.
  - Name:** COMPONENT 1
  - Concentration:**
    - dissolution (mmol/l)
    - dilution (ml)
    - mmol/l
  - number of ion(s) of this component:** 1
  - Ion(s):**
    - 1 charge
    - 2 charge
- number of component(s):** 2
- final volume:** [ ] ml
- Ion selection:**
  - Buttons: anion, cation, zwitterion
  - Text: acetate
  - charge in the component: -1
  - Navigation: <<, <, ION, >, >>
  - Button: Select
- Results section:**
  - Parameters:** pH, ionic strength (mmol/l), Buffer capacity (mmol/l.pH)
  - Chemical composition:** Buffer mobility (- cationic, - anionic), Conductivity (mS/cm)
  - Save:** Voltage (15 kV), Column diameter (50 μm), Overall length (57 cm), Instrument 1 (μA, mW)
  - Button: Estimate
- Footer:** select the number of component(s) in the buffer

Figure 6-1: The Ionic Composition of an Electrophoretic Buffer Dialog Box

The upper portion of the dialog box is used to enter information about the buffer and the lower portion presents the results of the calculations.

## 6.3 Determining the Properties of a Citric Acid/Sodium Citrate Buffer

### 6.3.1 Data Entry

The following steps should be used to enter the data:

- Click on the down arrow adjacent to the name field, use the scroll bar to access Citric Acid and click on it. The number of ions in this component field will indicate 1, the radio button labeled 1 below Ions will be selected and the adjacent line will indicate citrate, with a charge of -3.
- If citric acid is not present in the list of compounds, enter the name and click on  in the Ion Selection region of the dialog box.
- Use the arrow keys by  to access citrate in the field. (The > button selects the next anion in the list, the < button selects the previous anion in the list, << button accesses the first anion in the list, while the >> selects the last anion in the list).
- Click on .
- If the solution containing citric acid has been prepared by weighing, indicate its concentration in the buffer. For this example, we will use 4 mmol/l.
- The left side of the component window the component field should appear as shown in Figure 6-2.

The screenshot shows a dialog box titled "Component(s)" with four tabs: "component 1", "component 2", "component 3", and "component 4". The "component 1" tab is active. It contains a "Name" dropdown menu with "CITRIC ACID" selected. Below it is a "number of ion(s) of this component" dropdown menu set to "1". Under "Ion(s)", radio button "1" is selected, showing "citrate" and "charge -3". Radio button "2" is unselected, showing "charge". The "Concentration" section has "dissolution" selected with a value of "4" mmol/l. "dilution" is unselected, with empty fields for "ml" and "mmol/l".


**Figure 6-2: The Citric Acid Component Field**

As an alternative, the solution could be prepared by dilution of a stock solution. In this instance, you would click to select the dilution radio button and indicate the concentration of the stock solution (mmol/l), the appropriate volume (ml) and the final volume of the buffer (ml) on the right side of the field. This approach is described in detail below.

- Click on the tab for the second component and select **sodium citrate**. There are two ions to be considered with this reagent.

- h) For the sodium cation, click on **cation** and search with **ION** until the sodium is presented. Make certain that the electrical charge of this cation is set to +1, then click on **Select**.
- i) Click on the Figure 2 relative to the ion n°2 (**citrate** anion).
- j) For the citrate anion, click on **anion** and search with **ION** until the citrate ion is presented. Then indicate its electrical charge in the component (-3 in our example) then click on **Select**.
- k) For this case, we will dilute a 60 ml of a stock solution containing (100 mmol/l for sodium citrate) to a final volume of 1000 ml (Figure 6-3).

**Figure 6-3: The Citric Acid Window**

- l) Verify that the calculation mode for the ionic activity coefficient is correct (Section 3.3.2). For this exercise, either GÜNTEMBERG correction or automatic correction is acceptable.
- m) Click on the  icon on the menu bar to perform the calculation. The results are presented in the lower half of the screen.

## 6.3.2 Results

The results part of the dialog box includes three tabs which describe:

- a variety of electrophoretic parameters (Section 6.3.2.1)
- the chemical parameters in the buffer (Section 6.3.2.2)
- a field to save data in the database. This topic is discussed in Chapter 7.

### 6.3.2.1 The Parameter Tab

The Parameter presents variety of electrophoretic and electrical parameters of the buffer.

Parameters		Chemical composition		Save	
pH	4.984	Buffer mobility		Voltage	15 kV
Ionic strength	26.65 mmol/l	- cationic	+50.3	Column diameter	50 μm
Calculation of activity coefficient according to GÜNTEMBERG equation		- anionic	-29.3	Overall length	57 cm
Buffer capacity	7.0 mmol/l.pH	Conductivity	1.53 mS/cm	<input type="button" value="Estimate"/>	
		Instrument 1		8 μA	118 mW

**Figure 6-4: The Parameters Field**

The parameter tab includes the following information about the buffer:

- the ionic strength of the solution (mmol/l)
- the buffer capacity (mmol/l.pH) indicates the ability of the buffer to counteract the change in the pH. If the buffer capacity of the indicated buffer is small (<5 mmol/l.pH), the reported value will be indicated in red. This would suggest that the user might consider using a different buffer to maintain sufficient pH control to provide better repeatability and reproducibility in capillary electrophoresis.
- the electrical conductivity of the buffer (mS/cm).
- the mean electrophoretic mobilities of the anion and the cation of the buffer are expressed in  $10^{-5}\text{cm}^2/\text{V.s}$ .
- the electrical current ( $\mu\text{A}$ ). The maximum current threshold has been fixed arbitrarily to  $100\ \mu\text{A}$ . If the current is greater than  $100\ \mu\text{A}$ , a warning message will be presented on the display to alert the user to select another buffer that has a lower conductivity.
- the generated electrical power (mW).



The column length and diameter are set via the Parameters and Options window (Section 3.3.4). The user can change the voltage and click on  to determine the power dissipation at various voltages.


### 6.3.2.2 The Chemical Composition Tab

The *Chemical Composition* tab (Figure 6-5) presents a detailed description of the chemical composition of the buffer and indicates the concentration, apparent charge and activity coefficient of each of the various ionic forms (e.g.  $\text{H}_3\text{A}$ ,  $\text{H}_2\text{A}^-$ , etc.). As an example, the apparent charge of the citrate anion is -1.80.

Results				
Parameters	Chemical composition			Save
Component	Chemical compound	Charge	Activity coefficient	Concentration (mmol/l)
CITRIC ACID	- citrate	-1,80		4,000
	H3A	0	1,000	0,012
	H2A(-)	-1	0,851	1,009
	HA(2-)	-2	0,524	2,740
	A(3-)	-3	0,234	0,238
SODIUM CITRATE	- sodium	1,00		18,000
	Na(+)	1	0,851	18,000
	- citrate	-1,80		6,000
	H3A	0	1,000	0,018
	H2A(-)	-1	0,851	1,514
	HA(2-)	-2	0,524	4,111
	A(3-)	-3	0,234	0,357

**Figure 6-5: The Chemical Composition Tab**

To determine the properties of a citric acid-sodium citrate buffer having different concentrations, simply modify the component concentrations, then click on . As an example, if you wanted to prepare a solution from 75 ml of the stock solution rather than 60, access the sodium citrate field in the components region, edit the ml field and click  again.

Click on  to print results. The display will present a dialog box which is used to enter the buffer name. Enter the desired name and press **OK** (if a name is not desired, simply press **OK**). A typical report is shown in Figure 6-6.

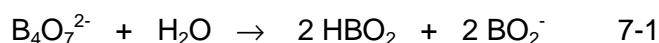
PHoEBus Software - OPTION 3			
10/30/97 at 7:38:16 AM			
<b>PREPARATION OF CITRIC ACID / SODIUM CITRATE BUFFER</b>			
- 4.00 mmol/l CITRIC ACID - 6.00 mmol/l SODIUM CITRATE			
- Estimated pH	4.984	(measured pH:	)
- Ionic strength	26.65 mmol/l		
- Buffer capacity	7.0 mmol/l. pH		
- Average cationic mobility	+50.3 .1E-5 cm <sup>2</sup> /V.s		
- Average anionic mobility	-29.3 .1E-5 cm <sup>2</sup> /V.s		
<b><u>CHEMICAL COMPOSITION OF BUFFER</u></b>			
Component	Charge	Activity coefficient*	Concentration (mmol/l)
- CITRIC ACID			
citrate ion	-1.80**		4.000
H3A	0	1.000	0.012
H2A(-)	-1	0.851	1.009
HA(2-)	-2	0.524	2.740
A(3-)	-3	0.234	0.238
- SODIUM CITRATE			
sodium ion	1.00**		6.000
Na(+)	1	0.851	18.000
citrate ion	-1.80**		6.000
H3A	0	1.000	0.018
H2A(-)	-1	0.851	1.514
HA(2-)	-2	0.524	4.111
A(3-)	-3	0.234	0.357
<b><u>ELECTRICAL PARAMETERS</u></b> (Instrument 1)			
- Column length	57	cm	
- Column diameter	50	µm	
- Applied voltage	15	kV	
- Conductivity	1.53	mS/cm	
- Estimated current	8	µA	(measured current: )
- Estimated power	119	mW	
* Calculation of activity coefficient according to GÜNTELBERG equation.			
** Assumed charge of ion			

Figure 6-6: A Typical Report

## 6.4 Additional Exercises

In this section, we will describe a buffer that is generated from sodium tetraborate. This buffer has only one component and we will weigh sufficient buffer to prepare a solution of 20 mmol/l.

In aqueous solution, the tetraborate anion hydrolyzes as shown in equation 7-1.



This anion behaves as if it was an equimolar mixture of boric acid and borate anion and the PHoEBuS software takes into account the hydrolysis of the tetraborate anion.

The components window is shown in Figure 6-7, while the two results tabs are shown in Figure 6-8 and 6-9.

Figure 6-7: The Components Field

Figure 6-8: The Parameters Tab

Component	Chemical compound	Charge	Activity coefficient	Concentration (mmol/l)
SODIUM TETRABORATE	- borate	-0,50		80,000
	HA	0	1,000	40,019
	A(-)	-1	0,825	39,981
	- sodium	1,00		40,000
	Na(+)	1	0,825	40,000

Figure 6-9: The Composition Tab

## 7 Databases

### 7.1 Overview


The PHoEBuS application program includes three databases:

- a database that describes the physico-chemical parameters of a wide number of ions (Section 7.2)
- the chemical component database (Section 7.3)
- a database that describes buffers for spectrometric detection (Section 7.4)

### 7.2 Physico-chemical Properties of Common Ions

#### 7.2.1 Viewing the Database

The physico-chemical properties of common ions database contains a variety of information about ions that are commonly used in capillary electrophoresis, including pKa and the electrophoretic mobility at infinite dilution at 25 °C. The data comes from references 5-9 in appendix 3.

To open the database dialog box (Figure 7-1), click on  from any of the three applications or select **Database of ions** from **Menu** drop down menu.

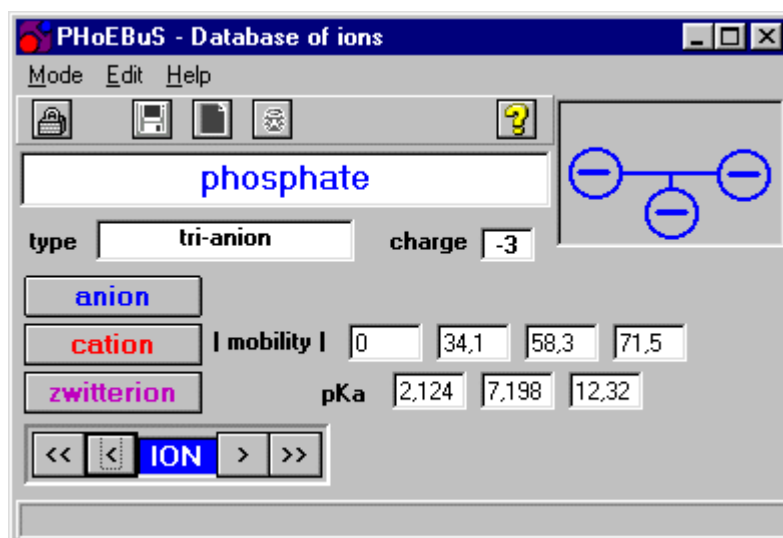









Figure 7-1: The Database Dialog Box

In Figure 7-1, we present data for phosphate. The ion mobilities are indicated for all four species ( $H_3A$ ,  $H_2A^-$ ,  $HA^{2-}$  and  $A^{3-}$ ) and the pKa values are presented for each of the three equilibria. The maximum charge on the ion is presented in the charge field and via the icon in the upper right corner.

To view data for a given anion, cation or zwitterion, click on the appropriate button ,  or , then access the desired ion using the cursor .

### 7.2.2 Editing the Database







To edit the data for a ion:

- Click on . A dialog box will be presented to verify that modifications are desired. Select OK from this dialog box.
- Select the ion for which data is to be edited, and enter the desired data.
- Click . A dialog box will be presented to verify that modifications should be saved. Select OK from this dialog box.
- Click  to re-lock the data base.

**Note: The electrophoretic mobility at infinite dilution of an ion must be indicated as its absolute value ( $10^{-5} \text{ cm}^2/\text{V.s}$ ).**



### 7.2.3 Adding a New Ion


If desired, you can add a new ion. To add a new ion:

- Click on . A dialog box will be presented to verify that modifications are desired. Select OK from this dialog box.
- Click on .
- Indicate the type of ion to be added by pressing ,  or .
- Type in the desired name (e.g. p-chlorobenzoate), then enter the appropriate electrophoretic mobility(ies) and pKa value(s).
- Save the new data by clicking on . A dialog box will be presented to verify that modifications should be saved. Select OK from this dialog box.


### 7.2.4 Deleting a Ion

To delete an ion:

- Click on . A dialog box will be presented to verify that modifications are desired. Select OK from this dialog box.
- Select the ion that is to be suppressed from the list of ions.
- Click on  to delete the ion. A dialog box will be presented to confirm that you want to delete this ion.

d) Click  to re-lock the data base.

### 7.3 Chemical Component Database

If desired, you can save all the ions of a component in the program database. This is done by clicking on the **Save** tab of the Results region of the dialog box (e.g. Figure 7-3) and then pressing . This lets you save the information about the ions in memory so that they can be used in future calculations.

A typical save tab is shown in Figure 7-3.

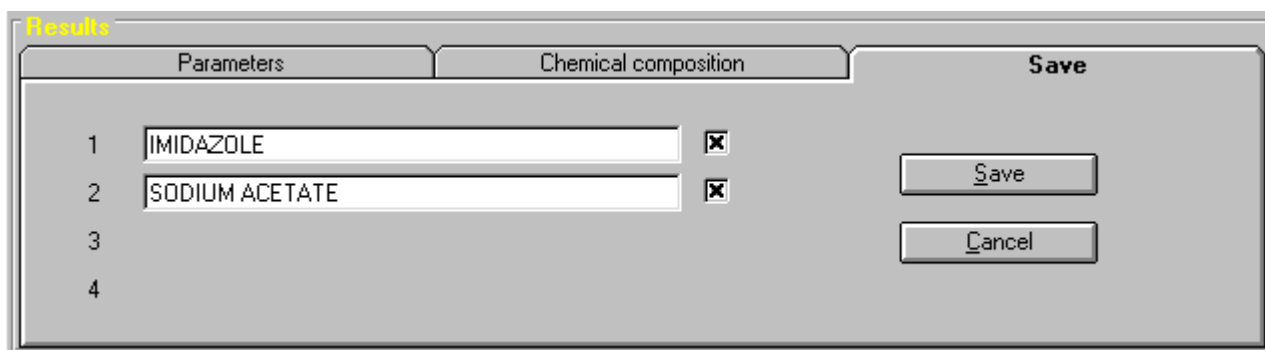



Figure 7-3: The Save Tab

If you want to delete a component, click on , to present the *Parameters and Options* dialog box, then select the *Compound management* tab (Figure 7-4).

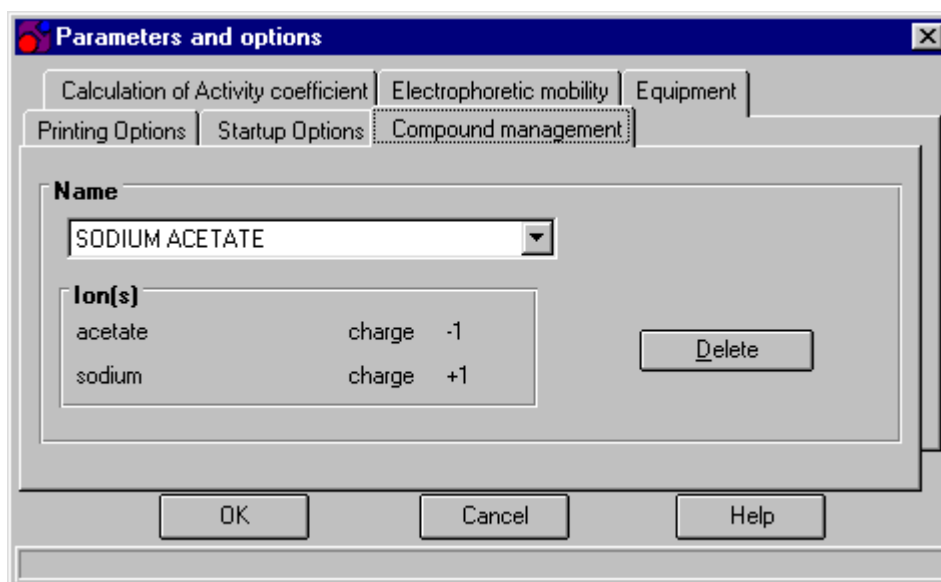


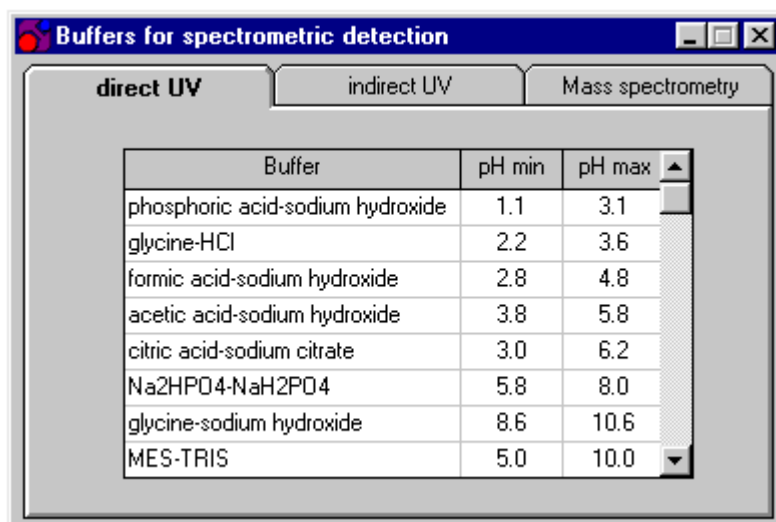


Figure 7-4: The Compound Management Tab

Select the component to delete in the list, then click .

### 7.4 The Buffer Database

The buffer database provides the information about buffers that are commonly used for direct and for indirect UV spectrometric detection. To access the buffer database (Figure 7-5), click on .

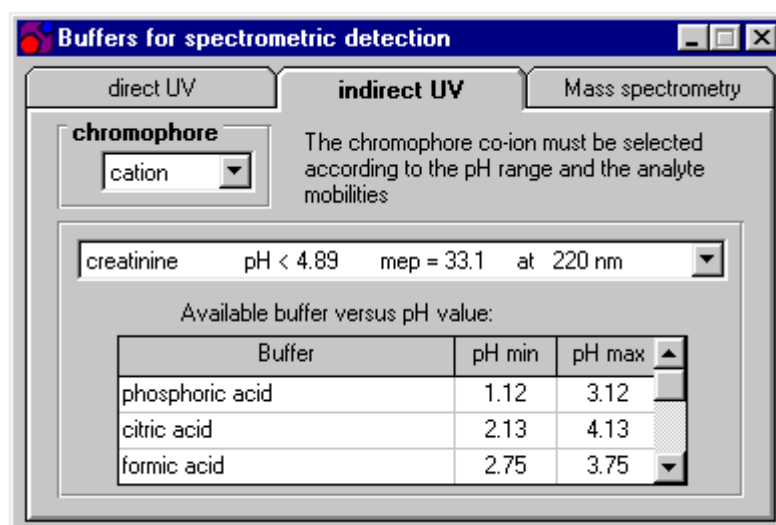


Buffer	pH min	pH max
phosphoric acid-sodium hydroxide	1.1	3.1
glycine-HCl	2.2	3.6
formic acid-sodium hydroxide	2.8	4.8
acetic acid-sodium hydroxide	3.8	5.8
citric acid-sodium citrate	3.0	6.2
Na <sub>2</sub> HPO <sub>4</sub> -NaH <sub>2</sub> PO <sub>4</sub>	5.8	8.0
glycine-sodium hydroxide	8.6	10.6
MES-TRIS	5.0	10.0

**Figure 7-5: Buffers for Direct UV Detection**

The *direct UV* tab (Figure 7-5) describes the pH range for each buffer.

The *indirect UV* tab (Figure 7-6) presents the maximum pH, electrophoretic mobility and wavelength of maximum absorbance for each chromophoric co-ion as well as the buffer range for various buffers.



The chromophore co-ion must be selected according to the pH range and the analyte mobilities

creatinine pH < 4.89 mep = 33.1 at 220 nm

Available buffer versus pH value:

Buffer	pH min	pH max
phosphoric acid	1.12	3.12
citric acid	2.13	4.13
formic acid	2.75	3.75

**Figure 7-6: Buffers for Indirect UV Detection**

The top portion of Figure 7-6 presents information about various co-ions for the separation. As an example, if you want to analyze cations, select **cation**. A list of the co-cations that could be used will be presented (creatinine, 2-aminopyridine, imidazole, benzylamine and ephedrine).

The bottom part of the window presents the pH range for various buffers that could be used for the separation.

To analyze anions, a similar procedure is used (the co-anions are sorbate, chromate and anisate).

## 8 Appendix

### 8.1 Simplified Notation of Ions

The following nomenclature is used to describe acids, bases and zwitterions in the program and on printed reports

#### 8.1.1 Acids

monoacidic	HA	A <sup>-</sup>			
diacidic	H <sub>2</sub> A	HA <sup>-</sup>	A <sup>2-</sup>		
triacidic	H <sub>3</sub> A	H <sub>2</sub> A <sup>-</sup>	HA <sup>2-</sup>	A <sup>3-</sup>	
tetraacidic	H <sub>4</sub> A	H <sub>3</sub> A <sup>-</sup>	H <sub>2</sub> A <sup>2-</sup>	HA <sup>3-</sup>	A <sup>4-</sup>

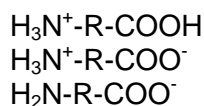
#### 8.1.2 Bases

monobasic	BH <sup>+</sup>	B			
dibasic	BH <sub>2</sub> <sup>+</sup>	BH <sup>+</sup>	B		
tribasic	BH <sub>3</sub> <sup>+</sup>	BH <sub>2</sub> <sup>+</sup>	BH <sup>+</sup>	B	

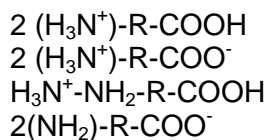
Alkaline and alkaline-earth cations are written with the appropriate chemical symbol (Na<sup>+</sup>, Ca<sup>2+</sup>, Li<sup>+</sup>, Ba<sup>2+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, etc.).

#### 8.1.3 Zwitterions

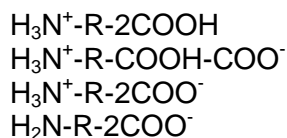
Zwitterions with an acidic group (COOH) and a basic group (typically NH<sub>3</sub><sup>+</sup>/NH<sub>2</sub>):



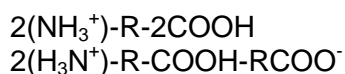
Zwitterions with an acidic group (COOH) and two basic groups (typically NH<sub>3</sub><sup>+</sup>/NH<sub>2</sub>):

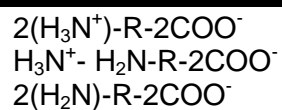


Zwitterions with two acidic groups (COOH) and a basic group (typically NH<sub>3</sub><sup>+</sup>/NH<sub>2</sub>):



Zwitterions with two acidic groups (COOH) and two basic groups (typically H<sub>3</sub>N<sup>+</sup>/NH<sub>2</sub>):





#### 8.1.4 Conventions

For a strong base, ( $\text{pK}_a > 20$ ), the concentration of its conjugated acid will be only mentioned in the table of results.

The ionic dissociation product of the water is  $13.9965 \text{ mol}^2/\text{l}^2$  at  $25^\circ\text{C}$ .

## 8.2 Theoretical Background

### 8.2.1 Calculation of the Activity Coefficient at 25°C

The equation used to calculate the activity coefficient  $\gamma_i$  is dependent on the ionic strength ( $I$ ) of the buffer, as described in Section 3.3.2. The ionic strength ( $I$ ) is given by:

$$I = \frac{1}{2} \left( \sum_i C_i Z_i^2 \right)$$

where  $C_i$  is the concentration of the ion (mol/l),  $Z_i$  is the charge on the ion.

In most cases, the user should select the automatic correction option, which selects the appropriate equation.

- a) **Debye-Hückel limit equation** (reference 1, appendix 3) :

$$\log \gamma_i = -0.51 Z_i^2 \sqrt{I} \quad (I < 1 \text{ mmol/l})$$

- b) **Debye-Hückel equation** (reference 2, appendix 3) :

$$\log \gamma_i = -0.51 Z_i^2 \frac{\sqrt{I}}{1 + B a \sqrt{I}} \quad (I < 10 \text{ mmol/l})$$

where  $a$  is a dimensional parameter of the ion corresponding to the minimal approach distance of an other ion,  $B$  is a coefficient that is equal to  $0.33 \times 10^{10} \text{ m}^{-1}$  in aqueous solution at 25°C.

- c) **Güntelberg equation** (reference 2, appendix 3).

$$\log \gamma_i = -0.51 Z_i^2 \frac{\sqrt{I}}{1 + \sqrt{I}} \quad (I < 60 \text{ mmol/l})$$

This equation is obtained when  $a$  is approximately 0.3 nm (so that the value of  $Ba$  is unity).

- d) **Davies equation** (reference 3, appendix 3) :

$$\log \gamma_i = -0.51 Z_i^2 \left( \frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3 I \right) \quad (I < 500 \text{ mmol/l})$$

This equation is used for solutions with a greater ionic strength.

### 8.2.2 Variation of the Electrophoretic Mobility Versus the Ionic Strength

Two models are commonly used to describe the dependence of the electrophoretic mobility  $m_{ep}(i)$  of the ion ( $i$ ) with the ionic strength  $I$  of the electrolyte at 25 °C. The user can select the desired method (or no correction) as described in Section 3.3.3.

**Friedl model** (reference 4, appendix 3) :

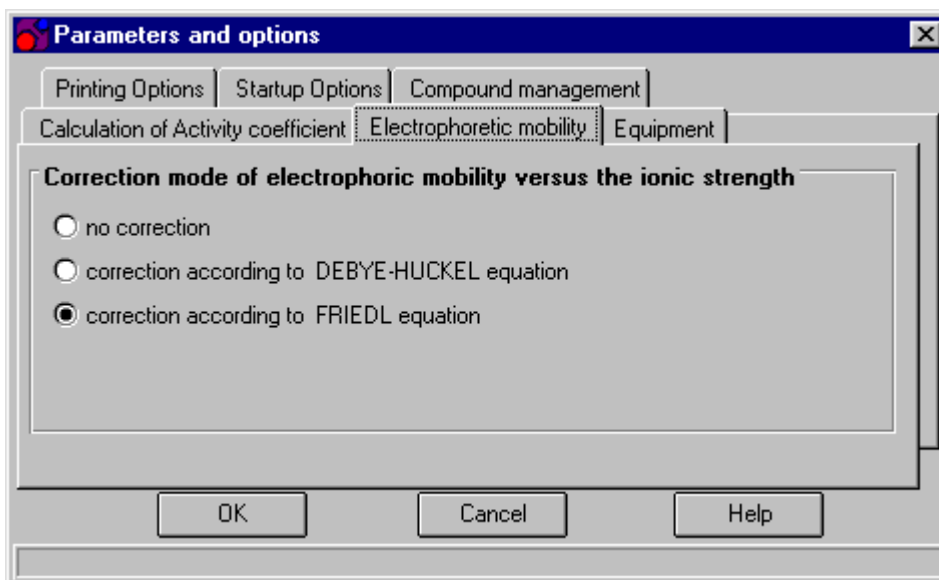
$$m_{ep}(i) = m_{ep}^{\infty}(i) \exp\left(-0.77 \sqrt{|Z_i| I}\right)$$

**Debye-Hückel model** (reference 5, appendix 3) :

$$m_{ep}(i) = m_{ep}^{\infty}(i) - \left(0.23 Z_i^2 m_{ep}^{\infty} + 31.4 \times 10^{-5} Z_i\right) \sqrt{I}$$

where  $m_{ep}^{\infty}(i)$  is the electrophoretic mobility of the ion  $i$  to infinite dilution ( $\text{cm}^2 \cdot \text{V}^{-1} \cdot \text{cm}^{-1}$ ),  $Z_i$  is the charge of the ion  $i$ ,  $I$  is the ionic strength of the electrolyte ( $\text{mol/l}$ ).

The default selection is the Friedl model. If desired, the user can select the Debye-Hückel model or no correction via the Correction mode of Electrophoretic mobility versus the ionic strength dialog box (Figure 8-1). This dialog box is accessed by clicking on the *Electrophoretic Mobility* tab when the Correction mode of activity coefficient versus the ionic strength dialog box is presented.



**Figure 8-1: Correction Mode of Electrophoretic Mobility versus the Ionic Strength Dialog Box**

Click on the mode of desired correction, then click on . The window Parameter and options closes. The selected mode of calculation is memorized and is taken into account for next calculations.

### 8.2.3 Calculation of the Conductimetric Constant of the Electrophoretic System

The current that is established in a capillary filled with an electrolyte submitted to a high voltage expressed by the equation 3-4:

$$I_c = V \cdot \kappa / K \quad 3-4$$

where  $I_c$  is the electrical current (A),  $V$  is the applied tension (V),  $\kappa$  is the conductivity of the electrolyte ( $\text{S} \cdot \text{cm}^{-1}$ ),  $K$  is the conductimetric constant of the electrophoretic system ( $\text{cm}^{-1}$ ).

The conductivity  $\kappa$  of the electrolyte is expressed by equation 3-5:

$$\kappa = \sum_i \lambda_i C_i |Z_i| = F \sum_i m_{ep}(i) C_i |Z_i| \quad 3-5$$

where  $\lambda_i$  is the ionic conductivity of the ion  $i$  ( $\text{S} \cdot \text{cm}^2 \cdot \text{mol}^{-1}$ ),  $Z_i$  is the charge of the ion  $i$ ,  $C_i$  the concentration of the ion  $i$  ( $\text{mol/ml}$ ),  $F$  is Faraday's constant.

The reader should recall that

$$\lambda_i = F m_{ep} (i) \quad 3-6$$

thus the conductimetric constant  $K$  depends on the overall length  $L_T$  and the inner diameter  $\phi$  of the capillary according to:

$$K = 4 L_T / \pi \phi^2. \quad 3-7$$

### 8.3 References

- 1) Debye P, Hückel E, *Phys. Z.*, **24** (1923) 185.
- 2) Güntelberg E, *Z. Phy. Chem.*, **123** (1926) 199.
- 3) Davies C, *J. Chem. Soc.*, (1938) 2093.
- 4) W. Friedl, J. Reijenga, E. Kenndler, *J. Chromatogr.*, **709** (1995) 163.
- 5) Debye P, Hückel E, *Phys. Z.*, **23** (1923) 305.
- 6) Handbook of Chemistry and Physics, 62<sup>nd</sup> edition (1981-1982), CRC Press.
- 7) T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, J. Yagi, J. Akiyama, *J. Chromatogr.*, **271** (1983) D1-D106.
- 8) T. Hirokawa, M. Nishino, Y. Kiso, *J. Chromatogr.*, **252** (1982) 49.
- 9) T. Hirokawa, Y. Kiso, B. Gas, I. Zuskova, J. Vacik, *J. Chromatogr.*, **628** (1993) 283.