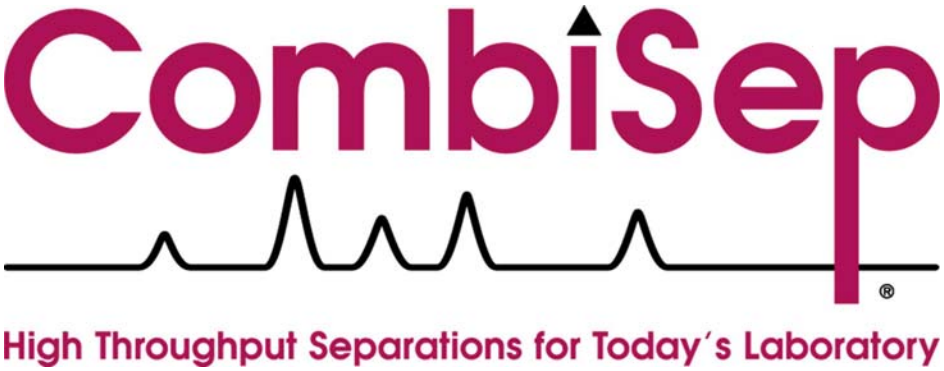
pK_a Estimator Elite™

User Manual

pK_a Analysis Software for Capillary Electrophoresis



Version 2.0.

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Document Notation

Within this document the following conventions are used:

`Courier New:`	Used to denote file path names and form names in CombiSep's *pKa Estimator Elite*™ software.
Italics:	Software names, File names and extensions are denoted with italics.
`bold, Courier New:`	Used to denote Windows™ commands and names of fields and buttons in *pKa Estimator Elite*™.

General Software Information

Throughout the software, fields with a blue background are fields that require user input. Fields with a yellow background are fields that the software will use to display computed values. For example, in Figure 1, the **`Date Created`**, **`Time Created`** and **`Path`** fields will be filled in by the software. The other fields shown require user input.

1. Introduction

1.1 Evaluation of pK_a Values by Capillary Electrophoresis

The acid dissociation constant (pK_a value) of an ionizable compound is defined as the pH value at which the dissociated and undissociated species are of equal equilibrium concentration, and is a fundamental physicochemical property that strongly influences many properties. For example, the neutral form of the compound is less water soluble, more lipophilic, and possesses higher membrane permeability than the ionized form. Experimental knowledge of compound pK_a values provides a measure of the extent of compound ionization across the pH range of pharmaceutical relevance, and is highly beneficial for predicting compound absorption, distribution, metabolism, and excretion (ADME) properties. The pK_a value also plays an important role in the development of drug delivery formulations.

Over the past decade, capillary electrophoresis (CE) has emerged as a valuable tool for the evaluation of compound pK_a values, as it possesses many favorable qualities:

- **Potential impurities and degradants can be separated from the target compound**
- **Intimate knowledge of sample concentration is not required for analysis**
- **Sparingly soluble compounds with a suitable UV chromophore can be analyzed**
- **No changes in spectral properties are required for detection of a pK_a value**
- **Minimal sample amounts are required for analysis (μg amounts)**

Numerous publications have appeared describing the use of CE for pK_a analysis including a recent review [1]. Several of these references are listed at the end of this

manual. Users are encouraged to study these articles to improve their knowledge of CE-based pK_a analysis, and best understand the strengths and limitations of the technique.

To determine the pK_a value(s) of a compound by CE, it is necessary to measure the migration times of the compound in relation to a neutral marker (usually DMSO) over a range of pH values. From the migration times of the compound (t_a) and neutral marker (t_m) in seconds, the effective mobility (μ_{eff}) can be calculated via Equation 1:

Eqn 1

$$\mu_{eff} = L_d L_c / V \left(1/t_a - 1/t_m \right)$$

Where L_d is the length of the capillary to the detector, L_c is the capillary total length (in cm), and V is the applied voltage (V). A plot of μ_{eff} vs pH yields a sigmoidally-shaped titration curve, from which the inflection point(s) define the pK_a value(s). Using non-linear regression analysis a best-fit line can be applied to the data using a set of standard fitting equations depending on the number of ionizable groups, and the pK_a value determined. Equations used for the evaluation of up to three ionizable groups have been described in the literature references available at the end of this manual [1,2].

1.2 *pKa Estimator Elite*™ Software Overview

The introduction of *pKa Estimator Elite*™ software from CombiSep provides a simple, straightforward method for calculation of pK_a values from CE data. Equations for performing non-linear regression analysis of up to three ionizable groups along with an empirical estimation of compound valency have been incorporated into the software to

simplify data analysis. All that is required of the user is to enter the migration times of the compound being evaluated and the neutral marker, compound molecular weight, and experimental information regarding capillary lengths and voltage. The *pKa Estimator Elite*™ program then generates a plot of μ_{eff} vs pH, and using a previously described empirical relationship between μ_{eff} and molecular weight [2], determines which ionization equation best fits the data. A best-fit line is then plotted and the apparent pK_a value (pK_a') is displayed, along with a goodness of fit (R^2 value). Version 2.0 of *pKa Estimator Elite*™ provides additional reporting capabilities. Comments describing the experiment can be entered and saved with the pK_a result, along with entries for the compound name and analyst name. Report generation capabilities have been added allowing the user to generate a printable Excel-based report. The single page report contains all pertinent experimental information and results and also contains a field for the user to paste in a compound structure image file if desired. The Excel format provides a means to export the numerical results to a user defined database and better archive experimental results.

By utilizing the automated capabilities of *pKa Estimator Elite*™, users can save valuable time and resources otherwise required for data analysis of pK_a values by CE. The remainder of the manual provides the user a complete description of the various functions and operations of the *pKa Estimator Elite*™ software. At the end of the manual, a generic experimental CE protocol with example results is described for collecting migration time data necessary for pK_a determination.

Chapter 2. Installation

2.1 System Requirements

This software must be installed on a computer with a Windows™ operating system to function properly. If using an Intel® Pentium® 4 computer with Hyper-Threading Technology, the Hyper-Threading feature must be disabled for the *pKa Estimator Elite*™ software to operate properly. The Hyper-Threading feature can be disabled by entering the BIOS settings of the computer on start-up and selecting **`Disable`** for the Hyper-Threading option.

To properly generate Microsoft© *Excel* reports the macro security settings must be set to trust Visual Basic Project. To change this setting, open Microsoft© *Excel* and navigate to `Tools-Macro-Security`. Select the `Trusted Publishers` tab and ensure that the "Trust access to Visual Basic Project" setting is enabled.

1. To install *pKa Estimator Elite*™ place the CD in the CD drive. Navigate to pK_a `Estimator Elite Installer →setup.exe` and double-click on `setup.exe`.

2. Follow the setup instructions provided by the installation wizard. The default installation directory is `C:\Program Files\pKa Estimator Elite`.

2.2 Running *pKa Estimator Elite*™

Using the **`Start`** button on the computer navigate to `Programs→pKa Estimator Elite→pKa Estimator` (or the directory the user installed it to if the default directory was not used) and select.

2.3 Exiting *pKa Estimator Elite*™

An **`Exit`** button is available on each tab of *pKa Estimator Elite*™ for exiting the program. To stop running the software click the **`Exit`** button and the software will close automatically.

Chapter 3. Using the **Edit Default Values** Tab

3.1 Entering Default Values

If this is the first time the user has used *pKa Estimator Elite*™, or the user has just completed a series of CE runs and experimental parameters such as the pH, ionic strength I(M), capillary length, or voltage are different than previously used, a *Default Values* file can be created in the Edit Default Values tab.

1. Enter in the pH and ionic strength (I(M)) values for the experiment in the table form. If the same ionic strength was used for all pH values (recommended) simply enter the value in the first row and then click the **Fill** button (see Figures 1 and 2). The buffer pH values should be entered in increasing pH order from top to bottom in the form. If 24 pH values were used in the experiment, then click on the **24 Buffers** button. This will increase the number displayed in the **pH Count** field to 24 and will display 24 rows in the **pH** and **I(M)** tables.

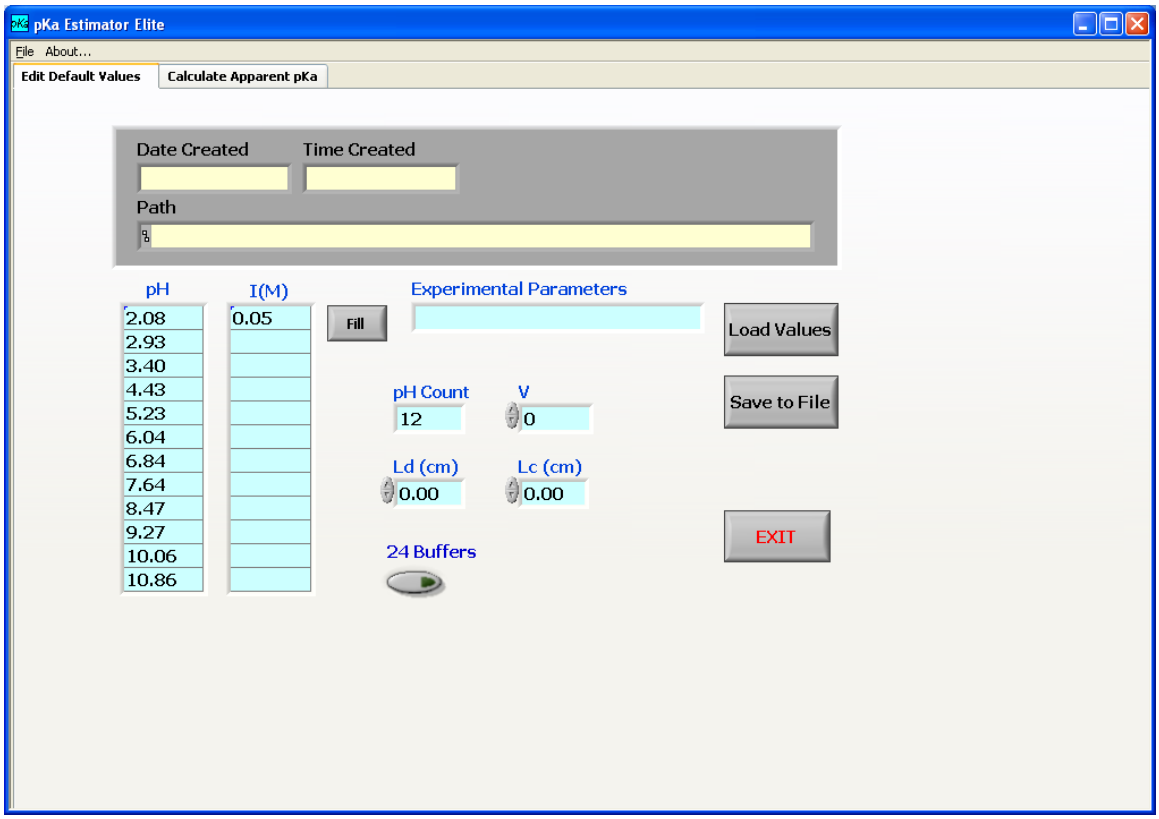

Figure 1. `Edit Default Values` form with pH values and first I(M) value entered.

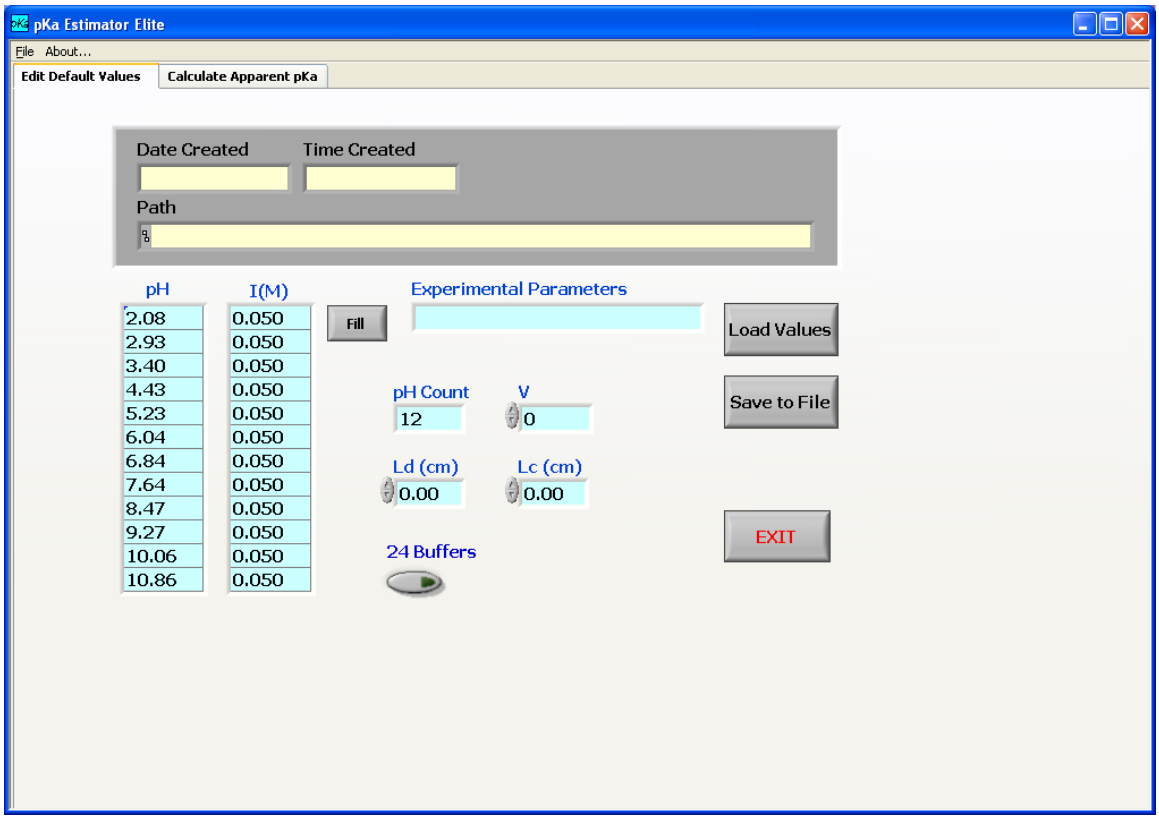

Figure 2. `Edit Default Values` form after **`Fill`** button has been pressed.

2. Enter the experimental values for **L_d(cm)**(effective capillary length to the detector in centimeters), **L_c(cm)** (total capillary length in centimeters) and **V** (voltage in volts).

3. Enter a descriptive phrase in the **`Experimental Parameters`** field for future reference (e.g., lot # of the pH buffers used).

3.2 Saving Default Values

To save the values entered into the `Edit Default Values` tab click on the **`Save to File`** button. A standard file save dialog box will appear for specifying a name to save the file to (see Figure 3). The default directory that files are saved to is `C:\Program Files\pKa Estimator Elite\data\`. If desired, the user can navigate to a different directory using the standard file save dialog box. The file that is created is saved as a comma-delimited file with a *.csv* extension. The file can be opened and edited in MicroSoft© *Excel* or text-editing software if desired. Please note that changing the format or layout of the file within *Excel* or a text-editor may make the file unreadable by the *pKa Estimator Elite*™ program. The date and time when the file is created and the path the file is saved to will be stored in the file and these values read-in and displayed in the appropriate fields the next time the file is loaded.

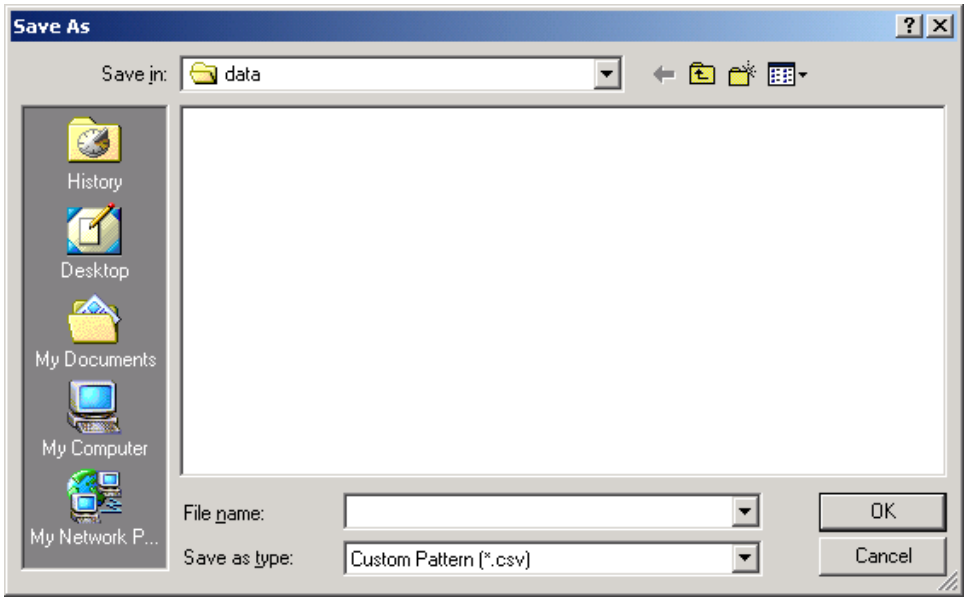

Figure 3. Standard Save Dialog Box.

3.3 Loading Default Values

To load experimental parameters from an existing *.csv* file click on the **Load Values** button and use the standard file open dialog box to navigate to the folder the file is in and select it. The default directory that files are read from is `C:\Program Files\pKa Estimator Elite\data\`. The software will automatically determine from the *.csv* file that is loaded whether 12 or 24 pH buffers were used, thus the user does not need to click the **24 Buffers** button prior to loading a file that contains 24 buffers. The **24 Buffers** button will be set to True automatically when a file containing 24 buffers is loaded (see Figure 4). ***Note***: The user must use the **Load Values** button to open a saved default values file for the information on the `Edit Default Values` tab to be transferred to the `Calculate Apparent` pK_a tab. Alternatively, the user can enter this information directly in the `Calculate Apparent` pK_a tab without creating a default values file.

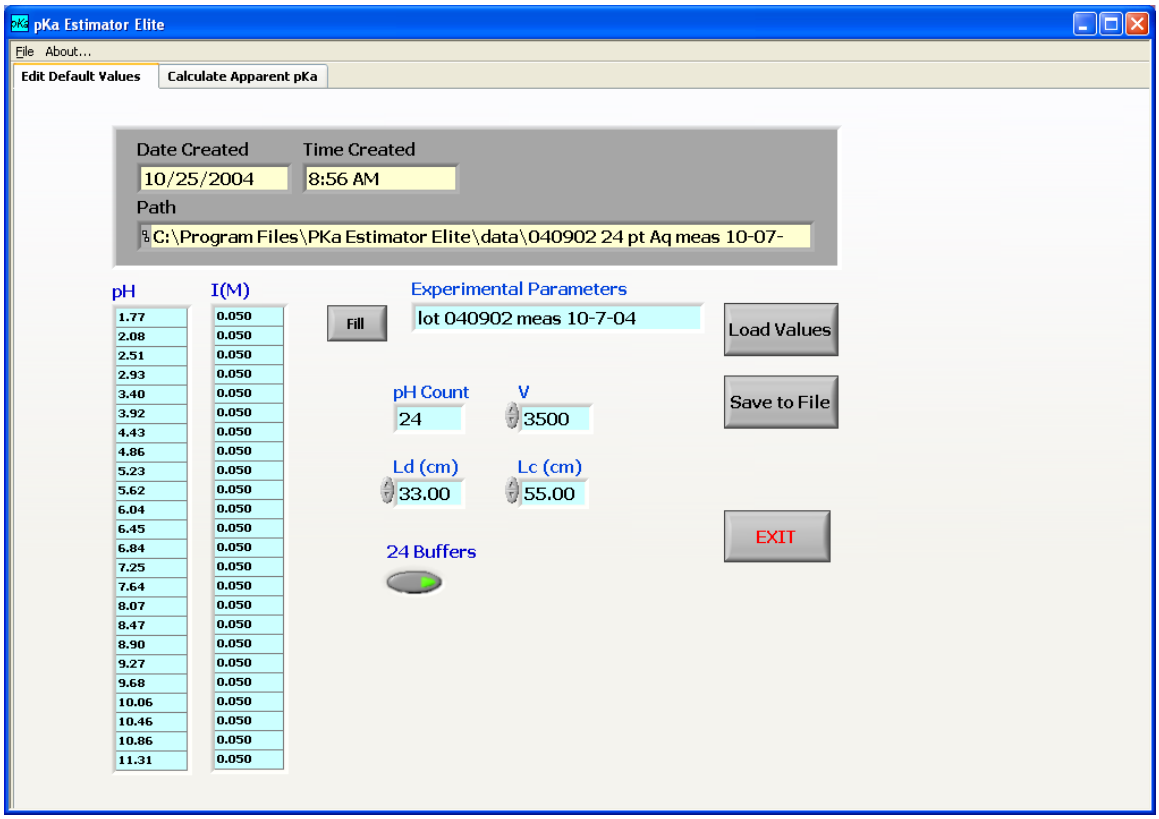

Figure 4. `Edit Default Values` form after loading a file that contains 24 buffers.

Chapter 4. Determining the Apparent pK_a Value

Click on the `Calculate Apparent pK`$_a$ tab to move to that screen. The L_d, L_c and V values from the first tab will automatically be displayed in the appropriate fields on this form (see Note in Section 3.3 above). Alternatively, experimental information can be entered directly into this form without using the `Edit Default Values` tab. If data has been previously entered on the `Edit Default Values` tab the user can modify the L_d, L_c and V values on this form. Enter in the migration times for the analyte (t_a) and EOF marker (t_m) in seconds for each pH value and then press the **`Refresh`** button. If the user wants to exclude the migration time data from a particular pH value in the table from the pK_a calculation, click on its corresponding **`Use`** light to turn off the light and then press the **`Refresh`** button. At this point, the graph will display a scatter plot of pH vs. calculated effective mobility (μ_{eff}) for the data (see Figures 5 and 6).

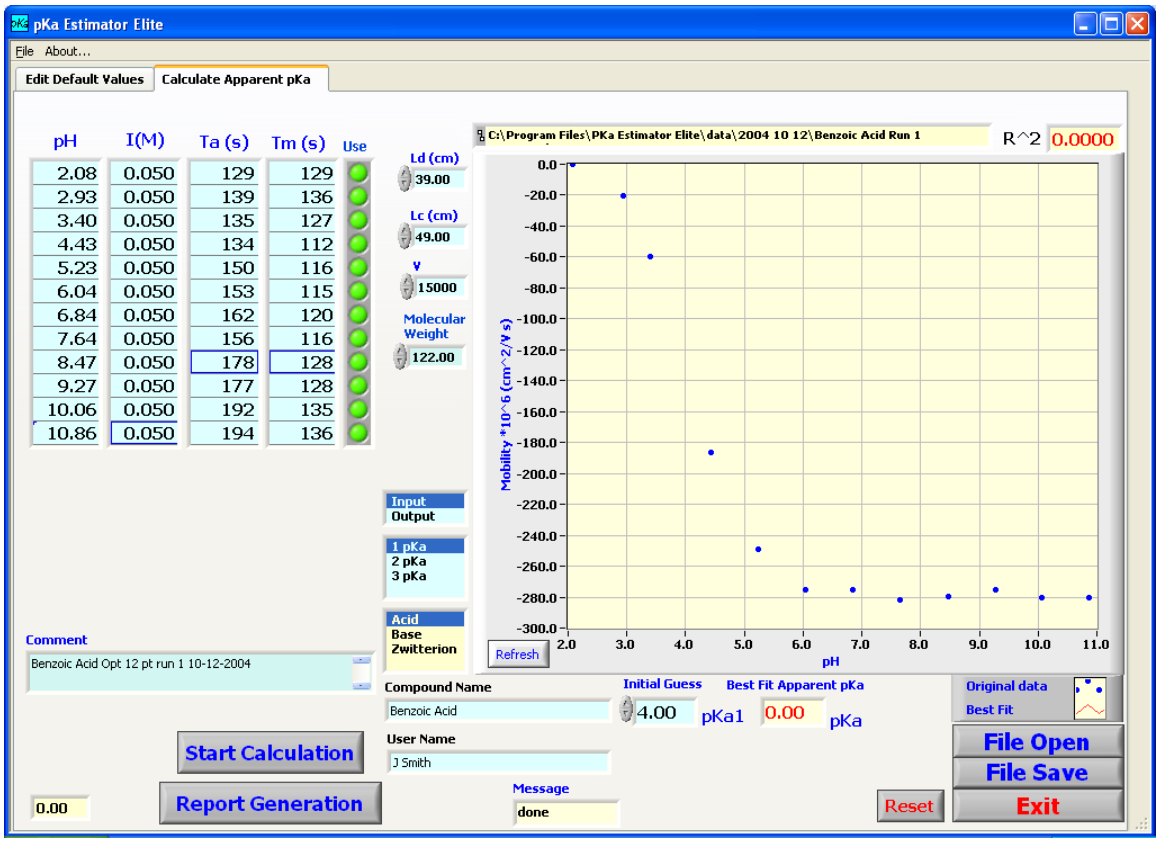

Figure 5. `Calculate Apparent` pK_a form prior to pK_a determination (12 buffers).

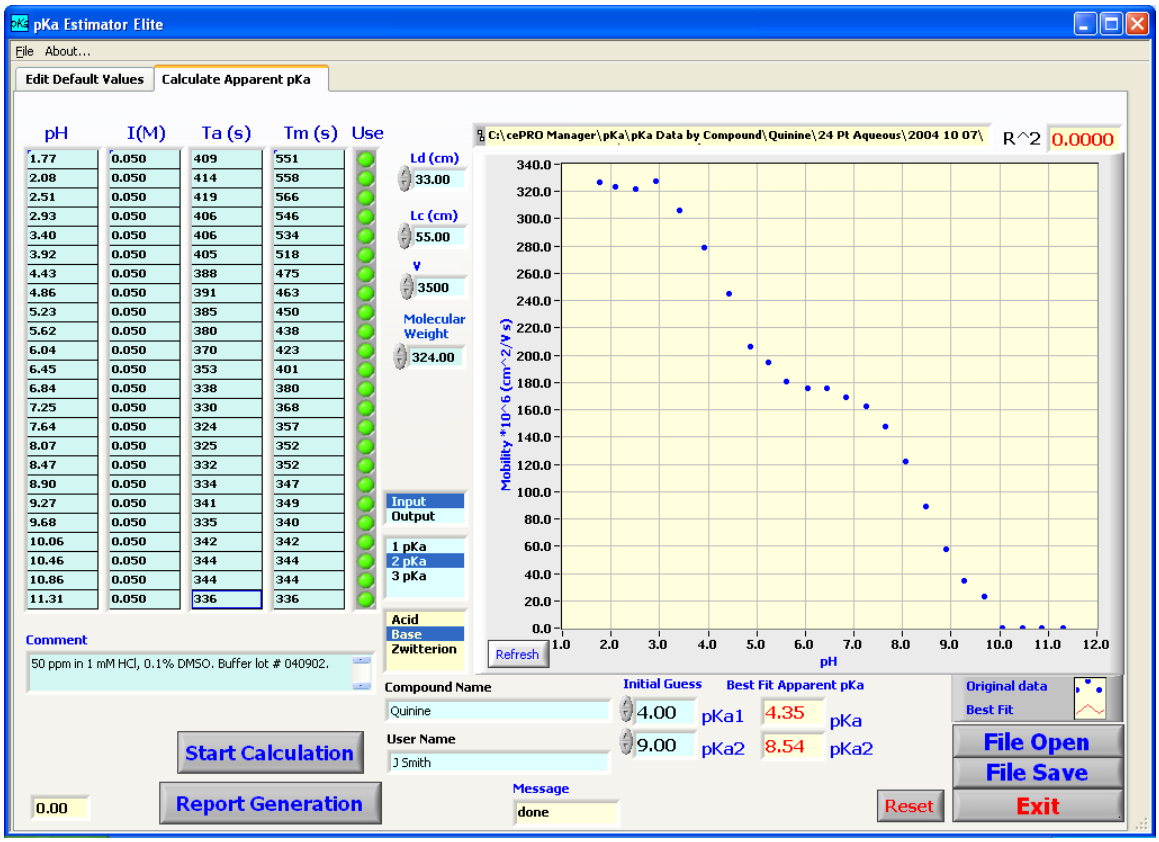

Figure 6. `Calculate Apparent` pK_a form prior to pK_a determination (24 buffers).

4.1 Entering the Compound Molecular Weight

The *pKa Estimator Elite*™ software selects from nine different model equations for determining a best-fit line to the data. The software applies the best fitting equation to the migration data based on the maximum positive and/or negative effective mobility of the sample compound along with its molecular weight. Therefore, for the calculation to determine which model equation to use (monobase, dibase, etc.), the molecular weight of the compound should be entered into the **`Molecular Weight`** field. Contributions from counter ions should be subtracted from the total molecular weight if a salt form is being

analyzed. If the user attempts to calculate the pK_a value without first entering a molecular weight a prompt will appear reminding the user to input a value (see Figure 7).

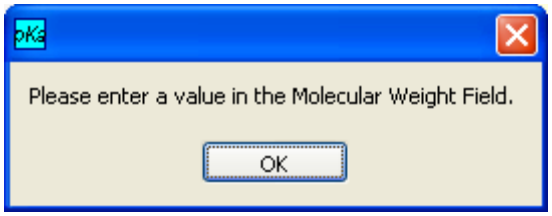

Figure 7. Prompt to remind user to enter a molecular weight value.

4.2 `Initial Guess` Values

Below the scatter plot is a column of one or more fields labeled **`Initial Guess`**. These values are used as initial seed values for the non-linear regression analysis. The use of initial guess values may aid the software in situations where a best-fit solution is not easily calculated (see below). To change the values in the **`Initial Guess`** field(s) before calculation select the number of fields (pK_a values) to display by selecting **`1` pK_a**, **`2` pK_a** or **`3` pK_a** in the **pK_a** control to the left of the plot (see Figure 8) and enter new values in the **`Initial Guess`** fields (see Figure 9). If a pK_a calculation has already occurred, simply press the **`Start Calculation`** button again after changing the initial guess values to re-determine the best-fit line.

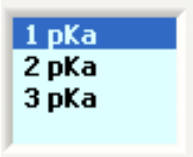

Figure 8. **pK_a** control with **`1` pK_a** highlighted.

Figure 9. **`Initial Guess`** and **`Best Fit Apparent pK`$_a$** when **`3 pK`$_a$** is selected.

Occasionally the *pKa Estimator Elite*™ software is unable to find a best-fit line for the data. If this occurs a pop-up window will be displayed to inform the user of the situation and will prompt the user for new initial guess values (Figure 10). Look closely at the scatter plot of pH vs. effective mobility to determine which initial guess values need to be modified to more closely match the location of the inflection points. Often one of the initial guess values needs to be modified by as little as ±0.5 pH units to enable the software to find a best-fit curve. After modifying the values in the **`Initial Guess`** fields click **`Start Calculation`** to find the best-fit line.

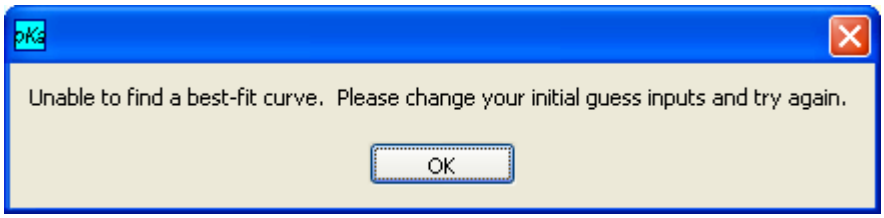

Figure 10. Pop-up when the software is unable to find a best-fit curve.

4.3 Determining the Apparent pK$_a$ Value

When ready, click on the **`Start Calculation`** button to calculate the apparent pK$_a$ value(s). A dialog box will appear to inform the user which model equation has been chosen and to ask if the user would like to choose a different model equation (see Figure 11). To choose a different model equation, click **Yes**. Clicking yes will open a pop-up

form where the user can select a different model equation (see Figure 12). The pop-up form will close automatically shortly after a selection is made. The software will then proceed to find a best-fit line to the data points using non-linear regression. A progress bar will appear in the upper center of the scatter plot to inform the user of the status of the best-fit calculation. When the calculation is complete, the progress bar will disappear.

After the best-fit line has been determined the R^2 value for the fit will be displayed in the **R^2** field that is located in the upper right corner of the `Calculate Apparent pKa` form. This value gives the user a statistical measurement of how closely the best-fit line matches the measured data points. The best-fit line is plotted on the existing graph and the apparent pK_a value(s) appear in the **`Best Fit Apparent pKa`** field(s) (see Figures 13 and 14). ***Note:*** The apparent pK_a value (often written as pK_a') is the value displayed by the software. Under typical experimental conditions, the ionic strength is set to level ionic strength of I = 50 mM. In order to convert the apparent pK_a' value to a thermodynamic value at I = 0 mM (or to a different ionic strength), a correction factor should be employed. Correction factors for acids and bases are listed in Appendix 1. Correction factors for zwitterionic compounds are less straightforward, and as a result apparent pK_a' values are most commonly listed in the literature.

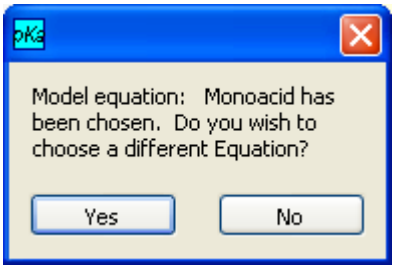

Figure 11. Prompt to select new model equation (Yes) or accept calculated model equation (No).

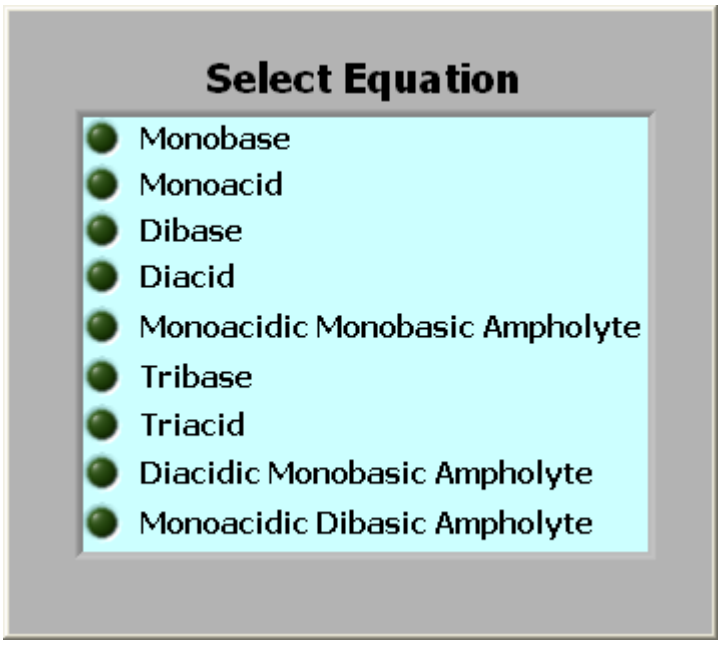

Figure 12. `Equation Selection` form for user to select a different model equation.

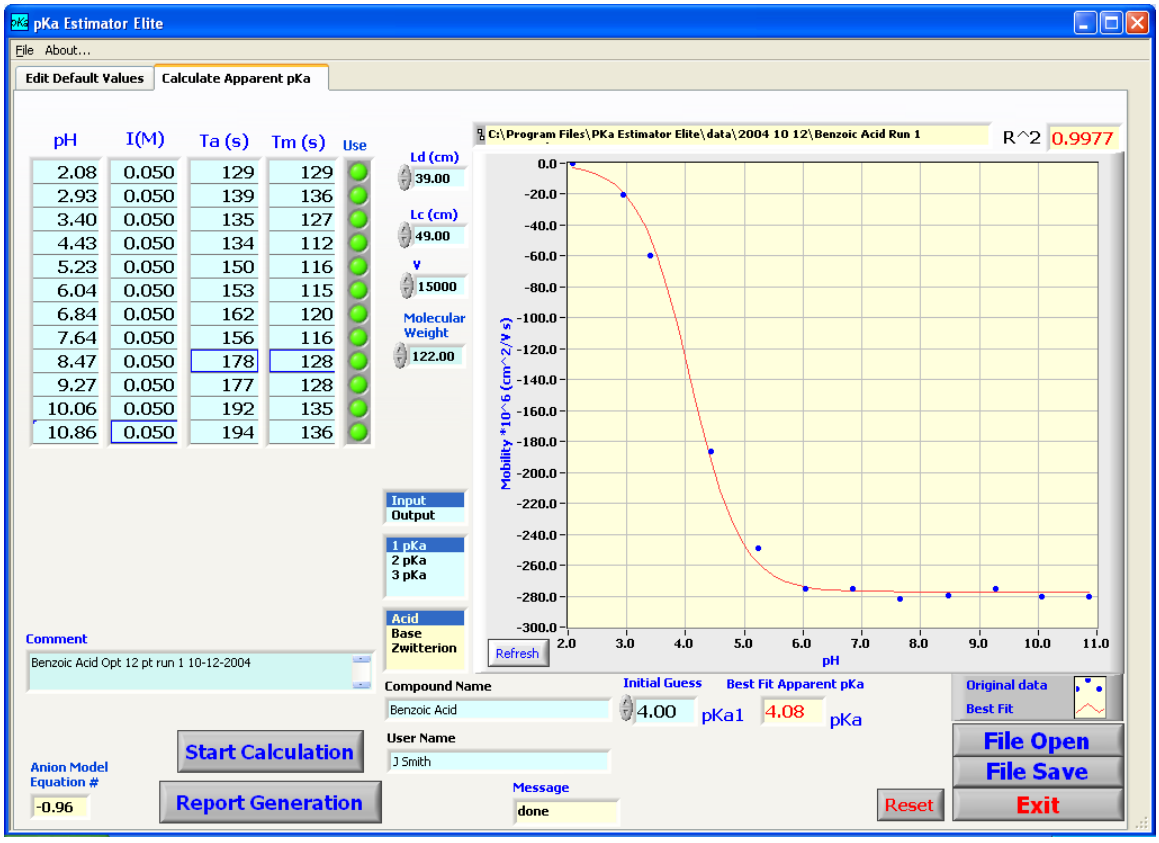

Figure 13. `Calculate Apparent pK`$_a$ form after pK$_a$ calculation (12 buffers).

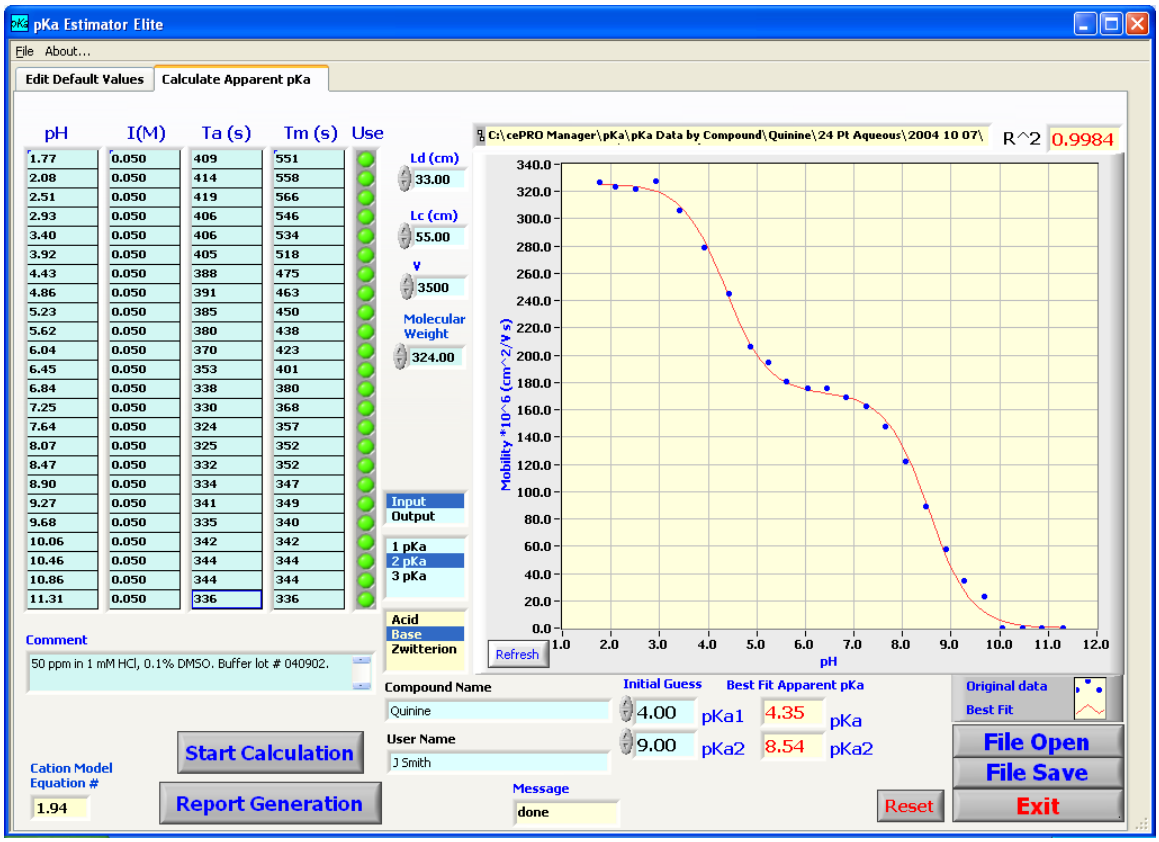

Figure 14. `Calculate Apparent` pK_a form after pK_a calculation (24 buffers).

4.4 Model Equation Number

The value reflecting the maximum fractional charge of the compound, used to determine which model equation to use, will be displayed in the **`Model Equation`** field. This value is rounded to the nearest whole number to guess the charge valency of the compound (1 = Mono-, 2 = Di-, 3 = Tri-). **`Acid`**, **`Base`** or **`Zwitterion`** will be highlighted in the indicator to the left of the graph (see Figure 15) to indicate if the compound was treated as an acid, base or a zwitterion.

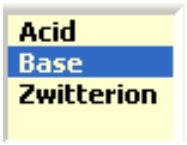

Figure 15. Indicator showing how compound was fitted - as an acid, base or zwitterion.

4.5 `Comment` Field

Information about the pK_a experimental results (e.g., sample diluent, pH points used, buffer lot #) can be entered and edited using the **`Comment`** field. This information will be saved with the *.pka* file and will be displayed whenever the file is reopened.

4.6 `Compound Name` Field

The compound name or identifier can be entered into the **`Compound Name`** field. This information will be saved with the *.pka* file and will be displayed whenever the file is reopened.

4.7 `User Name` Field

If desired, the name of the analyst performing the pKa experiment can be entered into the **`User Name`** field. This information will be saved with the *.pka* file and will be displayed whenever the file is reopened.

4.8 `Reset`

The **`Reset`** button will clear the table and graph on the `Calculate Apparent` pK_a tab. Likewise, if the user loads a new *.pka* file (see Section 4.12) the values will be cleared and replaced with the new values.

4.9 Refresh

The **Refresh** button on the graph clears the best-fit line from the graph, leaving only the scatter plot of pH vs. effective mobility.

4.10 Output

To view the calculated effective mobilities and the best-fit effective mobilities in a table format click on the **Output** option in the **Input/Output** control to the left of the graph (see Figure 16).

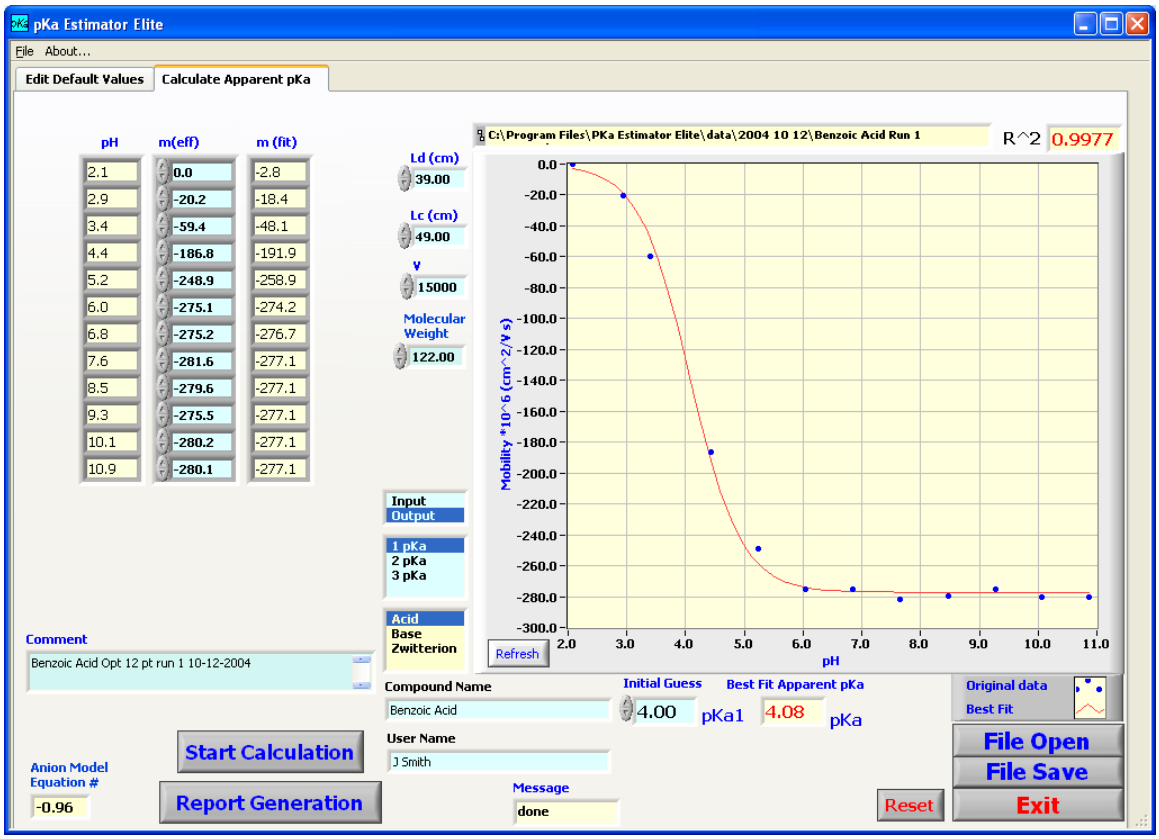

Figure 16. Calculate Apparent pK_a screen showing output fields.

4.11 Saving a File

To save all of the experimental information (migration times, pH values, L_d, L_c, V, molecular weight value, comments, compound name, user name and pK_a results) to a file click the **`File Save`** button. A standard file save dialog box will appear for specifying the name to save the file to. As with the *Default Values* files, the default directory is `C:\Program Files\pKa Estimator Elite\data\`. The file that is created is a comma-delimited file, however, it is given a *.pka* extension by the software.

4.12 Opening a File

To open an existing *.pka* file click on the **`File Open`** button. A standard file open dialog box will appear for selecting the file to open. The default directory is `C:\Program Files\pKa Estimator Elite\data\`. All of the experimental information will be loaded along with the pK_a results. To work with an existing *.pka* file the user does *not* need to go through the step of entering the default values.

4.13 `Report Generation`

Version 2.0 of the *pKa Estimator Elite*™ software introduces the capability to create a report of the pK_a results in a Microsoft© *Excel* format. To generate a report, press the **`Report Generation`** button. A standard file save dialog box will appear for specifying the name to save the file to. As with the *Default Values* files, the default

directory is `C:\Program Files\pKa Estimator Elite\data\`. The file that is created is given an *.xls* extension by the software.

The *Excel* report contains all of the experimental information, a plot of effective mobility vs. pH value, and a space at the bottom to paste in an image file of the compound structure if available (Figure 17).

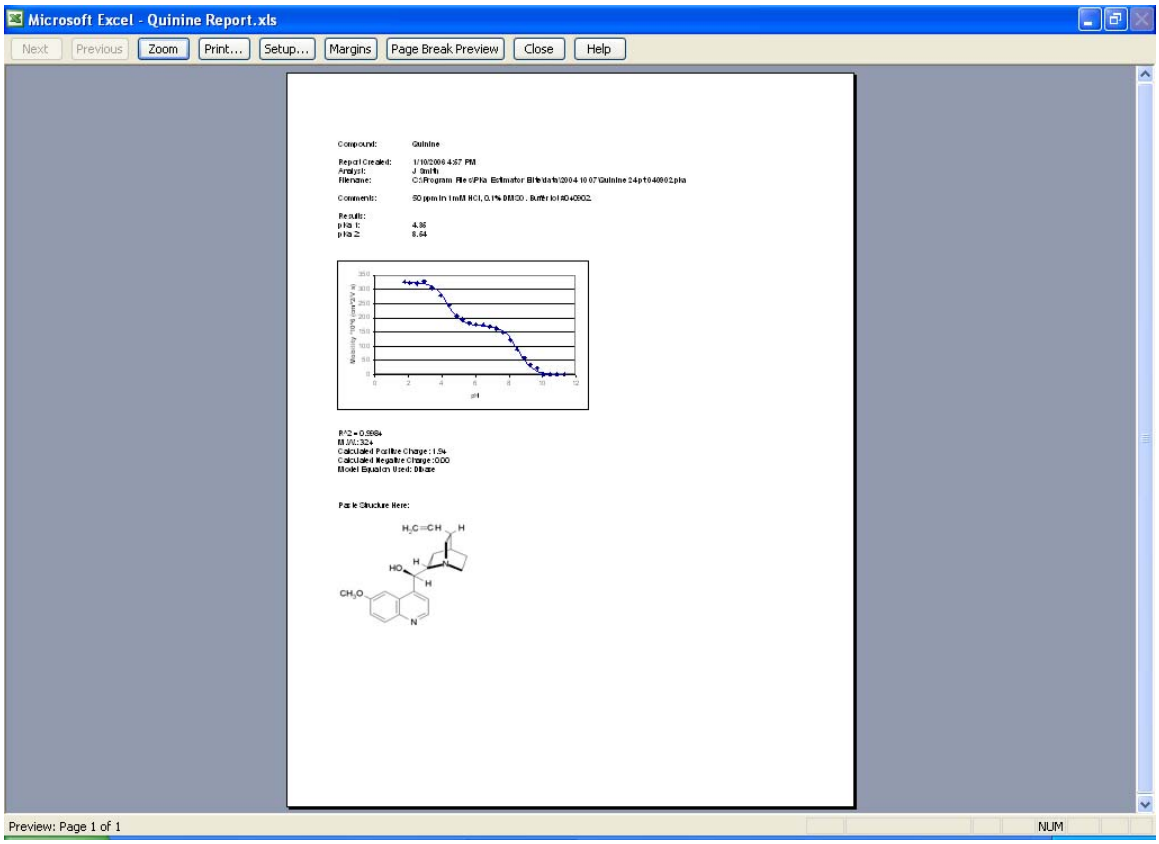

Figure 17. Microsoft© *Excel* report generated from the pK_a results.

Chapter 5. Summary

This manual walks the user through how to use CombiSep's *pKa Estimator Elite*™ software. This software enables the user to easily consolidate experimental conditions with CE migration time data. The consolidated data is then used to find a non-linear, best-fit line to the pH vs. effective mobility plot from which a compound's apparent pK_a value can be obtained. Microsoft© *Excel* reports can be generated and saved to better archive experimental results. Appendix 1 provides a generic CE protocol to help the user get started generating data and become familiar with pK_a determination by CE. For further questions regarding the use of *pKa Estimator Elite*™ software, please contact CombiSep technical support at 1-888-822-7949 (toll free) or by email at tech-support@combisep.com.

Appendix 1. Example Procedure for pK_a Measurement by CE

The following is a generic procedure for pK_a measurement by CE. It was adapted from several previously published literature protocols [1-3]. This method is intended to serve as a starting point for users gaining experience in using CE for pK_a determination, and can be readily adapted to different instruments, capillary lengths, and pH buffer systems. It is assumed the user has a working knowledge of CE.

1.1 CE Instrument Configuration

This method was developed using a Beckman MDQ single capillary CE system, but is readily translatable to any single capillary CE instrument. The instrument configuration is summarized in Table 1.

Table 1. Instrument Configuration for pK_a Measurement by CE

Capillary Dimensions	75 μm i.d., 360 μm o.d.
Capillary Total Length (cm)	39 cm
Capillary Effective Length (cm)	49 cm
Temperature	25 C
UV Wavelength	Monitor at 214 nm, 229 nm, 240 nm (collect spectrum from 200 nm - 300 nm if desired)
Applied Voltage	15 kV (~ 300 V/cm)
Sample Injection	0.5 psi, 5 sec
Capillary Pre-Conditioning	15 min 0.1 N NaOH, 10 min water at 50 psi

1.2 Sample Preparation

Weigh out 0.1 mg/ml of compound into a tared vial. Dissolve basic compounds in 1 mM HCl and acidic compounds in 1 mM NaOH, and add 0.1% (v/v) DMSO (neutral marker). Sonicate and filter if necessary for compounds that are not fully dissolved. If the sample is presented as a 10 mM stock solution in DMSO, dilute to 0.2 mM (20x), 2% DMSO sample concentration with 1 mM HCl (bases) or 1 mM NaOH (acids) and monitor at 229 nm and/or 240 nm.

1.3 pH Buffer Preparation

Add 1.25 ml each of 12 different pH buffers of equal ionic strength (kits available from Microsolv or CombiSep) to a series of inlet and outlet vials (2 vials per pH, 24 vials total). Load the different pH buffer vials into the instrument autosampler such that the highest pH value buffer is analyzed first and subsequent pH buffers are analyzed in order to the lowest pH value. Analysis of the highest pH value first will serve to minimize changes in pH due to absorbed CO_2 from the environment throughout the time interval of the experiment.

1.4 CE Run Sequence Parameters

It has been demonstrated that the use of pressure-assisted CE can significantly reduce migration times while preserving data quality for pK_a analysis [1-5]. Table 2 lists example pressure settings as function of pH under the above instrument configuration, along with approximate migration times for a neutral marker (EOF) and current levels for a buffer series of I = 50 mM.

Table 2. Approximate CE Run Parameters for Generic CE pK_a Method

pH Value	Pressure Level (psi)	Migration Time for EOF (min)	Total Current (μA)
2.1	1.0	2.2	105
2.9	0.9	2.3	85
3.4	0.9	2.2	80
4.4	0.8	1.9	50
5.2	0.7	2.0	55
6.0	0.7	2.0	50
6.8	0.6	2.0	50
7.6	0.6	2.0	50
8.4	0.6	1.9	75
9.2	0.6	1.9	60
10.0	0.6	1.9	50
10.8	0.6	1.9	50

Using the above parameters, the analysis of basic compounds can be completed in approximately 2.5 min per CE run/pH value, and acidic compounds can be analyzed in approximately 3 min per CE run/pH value. The resulting total time to complete an unattended analysis at 12 different pH points (using a 3 min CE run time) was ~80 min. The above method was found to provide a reasonable compromise between speed and separation resolution. An increase in the pressures used in conjunction with CE can slightly decrease the total analysis time, at the expense of reduced resolution between the analyte and EOF marker.

1.5 Example Results

Table 3 lists experimental results for several test compounds achieved using the above method with a comparison to some literature results [2-6]. These results can serve as a good measure of CE system and buffer performance when users are attempting to validate their method.

Table 3. Experimental Results for pK_a Determination by CE

Compound	MW	n	pK_a' Value (I = 50 mM)	pK_a Value (I = 0 mM)	Literature Value (I = 0 mM)	References
Benzoic Acid	122	3	4.06 ± 0.03	4.14	4.20;4.26;4.22;4.10	2;3;6
Metoprolol	267	3	9.64 ± 0.02	9.56	9.51;9.44	4;6
Quinine	324	5	4.29 ± 0.12	4.04	3.93;4.13;4.14;3.88	2;3;5;6
			8.55 ± 0.05	8.47	8.27;8.39; 8.39;8.43	2;3;5;6
Warfarin	308	3	4.90 ± 0.03	4.98	5.15;4.98;5.06;4.94	3;4;5;6

Note: All values were converted to I = 0 mM for comparative purposes. References 2-5 are by CE; reference 6 is by potentiometry.

Appendix 2. Ionic Strength Correction Factors

To correct for the effects of ionic strength on compound pK_a values, equations 2 and 3 can be applied for acids or bases, respectively[1]:

Eqn 2 (Acids)
$$pK_a = pK_a' + \frac{0.5085 z^2 \sqrt{I}}{1 + 0.3281 \alpha \sqrt{I}}$$

Eqn 3 (Bases)
$$pK_a = pK_a' - \frac{0.5085 z^2 \sqrt{I}}{1 + 0.3281 \alpha \sqrt{I}}$$

In each equation, z is the valency of the ion, I is the ionic strength of the buffer (M), and α is the ion size parameter, usually assumed to be 5 (angstroms). Table 4 displays the ionic strength corrections for acids and bases for up to three ionizable groups. Note that the correction factor for the second ionization of a diprotic compound takes into account the monoprotic species it is in equilibrium with (i.e., for a diacid the correction is 0.33 – 0.08 = 0.025). The same applies to triprotic compounds. The correction factor for zwitterionic compounds is more complex, and in general apparent pK_a values are reported in the literature. Ideally, literature pK_a values should be clearly stated as apparent or thermodynamic values and the ionic strength of the buffer medium provided, although this is not always the case.

Table 4. Ionic Strength Corrections (Apparent pK_a to Thermodynamic pK_a)

Ionization Type	Correction Factor (10 mM)	Correction Factor (50 mM)	Correction Factor (150 mM)
Monoacid (-1 → 0)	0.04	0.08	0.12
Diacid (-2 → -1)	0.13	0.25	0.36
Triacid (-3 → -2)	0.22	0.42	0.60
Monobase (+1 → 0)	-0.04	-0.08	-0.12
Dibase (+2 → +1)	-0.13	-0.25	-0.36
Tribase (+3 → +2)	-0.22	-0.42	-0.60

Literature References

Listed below are several selected references describing the use of CE for pK_a analysis. This list is not comprehensive; rather it is intended to give the user a general idea of how CE has been applied for the determination of compound pK_a values.

Review Paper on pK_a Determination by CE

1. Poole SK, Patel S, Dehring K, Workman H, Poole CF: **Determination of acid dissociation constants by capillary electrophoresis**. *J. Chromatogr., A* 2004, **1037**:445-454.

Additional References

2. Miller J, Blackburn AC, Shi Y, Melzak AJ, Ando HY: **Semi-empirical relationships between effective mobility, charge, and molecular weight of pharmaceuticals by pressure-assisted capillary electrophoresis: Applications in drug discovery**. *Electrophoresis* 2002, **23**:2833-2841.

3. Jia Z, Ramstad T, Zhong M: **Medium-throughput pKa screening of pharmaceuticals by pressure-assisted capillary electrophoresis**. *Electrophoresis* 2001, **22**:1112-1118.

4. Ishihama Y, Nakamura M, Miwa T, Kajima T, Asakawa N: **A Rapid Method for pKa Determination of Drugs Using Pressure-Assisted Capillary Electrophoresis with Photodiode Array Detection in Drug Discovery**. *J. Pharm. Sci.* 2002, **91**:933-942.

5. Wan H, Holmen A, Nagard M, Lindberg W: **Rapid Screening of pKa values of pharmaceuticals by pressure-assisted capillary electrophoresis combined with short-end injection**. *J. Chromatogr., A* 2002, **979**:369-377.

6. Avdeef A: In *Absorption and Drug Development*. Edited by: John Wiley & Sons, Inc.; 2003:22-41.