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Short Communication

CEToolbox: Specialized calculator for capillary electrophoresis users as an android application

CE has been demonstrated to be a useful and powerful separation method for the characterization of charged and neutral molecules. Since the end of the 1980s and the development of the first commercialized CE device, the use of this separation method has continued to grow for academic and industrial research involving inexorably increasing of the number of CE users. Whatever the application domain, each CE user is daily confronted to the same problems often based on basic calculations of separation properties. In order to help the community of CE users to get quickly and easily a lot of information, and desiring to provide a tool running on mobile platforms, CEToolbox has been developed as a free Android application. Within few clicks, CEToolbox offers extensive injection information as injected volume, total capillary volume, proportion and amount of injected sample, rinsing time, and electrical field. Moreover, three additional tabs allow to obtain the calculation of the viscosity and the conductivity of BGE, and the separation flow rates. Finally, a last tab is dedicated to the calculation of electroosmotic mobility and effective mobilities for a maximum of 20 compounds. CEToolbox, which can be downloaded for free on Google and F-Droid application stores, was developed to simplify the daily of CE users regardless of the CE devices.

Keywords:
Conductivity / Flow rate / Mobility / Separation characteristic calculation / Viscosity
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CE has been commercially introduced as a separating technique during the early 1980s [1,2]. Last two decades have witnessed the emergence of CE as one of the most important tools in analytical science. CE has been demonstrated to be a useful and powerful separation method for the characterization of charged and neutral molecules [3,4]. CE mechanisms are based on analyte separation under an electrical field. This technique provides fast highly efficient separation with minimum sample volume requirement, which is essential for analytes that are only available in scarce amount. CE has, with different modifications such as CZE [5], CIEF [6], CEC [7], MEKC [8], ITP [9], and CGE [10], proved a steady growth of interest in numerous research fields such as biology [11], chemistry [12], pharmaceutical [13] and biopharmaceutical [14], environment [15], or food [16]. Due to this interest, CE is significantly established in industrial and academic laboratories, which represents a huge number of CE users.

Regardless of the research field, every CE user has in common a large number of basic problematic. Indeed, CE geometry, instrumentation, background electrolyte (BGE) properties, or mobilities determination involves a lot of fastidious calculation that CE users perform on a daily basis, such as injected volume computation, viscosity, and conductivity of the BGE or electroosmotic mobility (μ_{eof}) value.

Software, such as CE Expert (Beckman Coulter, Brea, CA, USA) introduced in 1997, were developed to obtain information on CE injection. By entering known parameters such as capillary dimensions, hydrodynamic injection pressure, and injection time, several parameters including injection volume can be automatically calculated. Nevertheless, existing software provide calculations for a limited numbers of parameters and moreover have not been updated to run on recent operating systems. However, it was noted that the interest in tools to help CE user's community is growing, and still much appreciated. For example, ECHMET (http://echmet.natur.cuni.cz/) project developed at Charles University in Prague proposes several Freewares allowing, among other things, to model and simulate electrophoretic separation.

In this project, we have developed CEToolbox application, desiring to provide a tool running on new media platforms, especially mobile platforms, and to increase the level of information in terms of separation characteristics. Within

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Color online: See article online to view Figs 2 and 3 in color.

Equation (1)
Hydrodynamic Injection (nL)

$$V_{inj} = \frac{\pi . d_c^4 . \Delta P . t}{128 . \eta . L} . 10^{12}$$

Equation (2)
Capillary Volume (nL)

$$V_{tot} = \frac{\pi . d_c^2 . L}{4} . 10^{12}$$

Equation (3)
Capillary Volume to Window (nL)

$$V_d = \frac{\pi . d_c^2 . l}{4} . 10^{12}$$

Equation (4)
Injection plug length (mm)

$$l_{inj} = \frac{4 V_{inj}}{\pi . d_c^2} . 10^{-9}$$

Equation (5)
Plug (% of total length)

$$\%_{tot} = \frac{V_{inj}}{V_{tot}} . 100$$

Equation (6)
Plug (% of length to window)

$$\%_d = \frac{V_{inj}}{V_d} . 100$$

Equation (7)
Minutes to replace 1 Volume (min)

$$t_{\min} = \frac{32 . \eta . L^2}{d_c^2 . \Delta P . 60} \times 10^{-3}$$

Equation (8)
Injection Flow rate (nL/min)

$$Q_{inj} = \frac{V_{tot}}{t_{\min}}$$

Equation (9)
Electrical Field

$$E = \frac{U}{L} . 10^{-2}$$

Equation (10)
Analyte injected (ng)

$$m = C_{(g/L)} . V_{inj}$$

Equation (11)
Analyte injected (pmol)

$$n = \frac{C_{(g/L)} . V_{inj}}{M_w} . 10^3$$

Equation (12)
Electoosmotic mobility (m²/V/s)

$$\mu_{eof} = \frac{l . L}{t_{eof} . U}$$

Equation (13)
Separation Flow Rate (nL/min)

$$Q_{sep} = \frac{\pi . d_c^2 . \mu_{eof} . E}{4} \times 60 . 10^{14}$$

Equation (14)
Viscosity (cp)

$$\eta = \frac{d_c^2 . \Delta P . t_m}{32 . l . L} \times 10^3$$

Equation (15)
Conductivity (mS/m)

$$\kappa = \frac{4 . L . I}{\pi . d_c^2 . U} \times 10^3$$

Equation (16)
Effective mobility (m²/V/s)

$$\mu_{eff} = \frac{l . L}{t_m . U} - \mu_{eof}$$

Figure 1. Theoretical equations used to develop CEToolbox. V_{inj}: Injected volume (nL); V_{tot}: total capillary volume (nL); V_{d}: volume to detection window (nL); l_{inj}: injection length (mm); $t_{\min}$: time to replace 1 volume (min); L: full length (m); l: length to detection (m); t: time of injection (s); d_{c} : inner diameter (m); η: viscosity (Pa.s); κ: conductivity (mS/m); ΔP: applied pressure (Pa); Q_{inj}: injected flow rate (nL/min); Q_{sep}: separation flow rate (nL/min); E: electrical field (V/cm); U: voltage (V) ; I: electric current (A); $C_{(\text{g/L})}$: concentration (g/L); $C_{(\text{mol/L})}$: concentration (mol/L); M_w: molecular weight (g/mol); t_{eof}: electroosmosis time; t_{m}: migration time; μ_{eof}: electroosmosis mobility; μ_{eff}: effective mobility.

few clicks on your smartphone or your tablet working on Android, CEToolbox provides extensive CE separation information as injected volume, total capillary volume, proportion and amount of injected sample, rinsing calculation, and electrical field. Moreover, using an optimized interface, four additional tabs allow to calculate viscosity and conductivity of BGE, separation flow rate, and values of μ_{eof} and effective mobilities (μ_{eff}). CEtoolbox provides CE information regardless the CE system used.

CEToolbox is a Free software running on Android platform. Developed in Java and distributed under the Apache 2 license, the code is available on GitHub and deposited at Zenodo (http://doi.org/10.5281/zenodo.4284345). The application can be downloaded freely from Google Play (https://play.google.com/store/apps/details?id = com.github.cetoolbox), F-Droid (https://f-droid.org/en/packages/com.github.cetoolbox/), or from the project web page (https://cetoolbox.github.io/).

The first releases of CEToolbox have been developed using the Eclispe software (https://www.eclipse.org/) and the Android Development Tools. This environment changed from version 2 of the software, as Eclipse was replaced by Android Studio (https://developer.android.com/studio). This integrated development environment facilitates the development, the maintenance, and the publication of CEToolbox. The implementation of the scientific code has been done in a dedicated Java class, which can be easily taken over by other projects. The software does not require any particular authorization to run on Android terminals and a particular effort has been made to keep the application lightweight, allowing support for relatively old terminals and maintaining a size of less than 100 kB. Support is provided to CEToolbox users through the GitHub ticketing system.

All values provided by CEToolbox were calculated from equations detailed in Fig. 1.

CE is a miniaturized technique which makes it difficult to visualize volumes of rinsing, BGE, or sample injected. The CE user is regularly confronted with several questions concerning total capillary volume, injected volume, time of rinsing, and sample concentration. The first tab of CEToolbox is dedicated to obtain injection information. With a single click on the "Injection" tab of the interface (Fig. 2), the CE user can enter all known separation parameters such as capillary length (L), length to window (l), inside diameter (d_{c}), pressure (ΔP), time (t), BGE viscosity (η), and voltage (U), and known sample parameters as concentration (C) and molecular weight (M_w). In order to be compatible with all the existing CE systems, pressure can be expressed in two different units, mbar or psi. Moreover, in order to be universal in terms of amount calculation, concentration can also be expressed in two different units, mmol/L and g/L. After clicking on the "Calculate" button, a window appears displaying all injection information (Fig. 2). About separation properties, CEToolbox provides hydrodynamic injection volume (Equation 1),

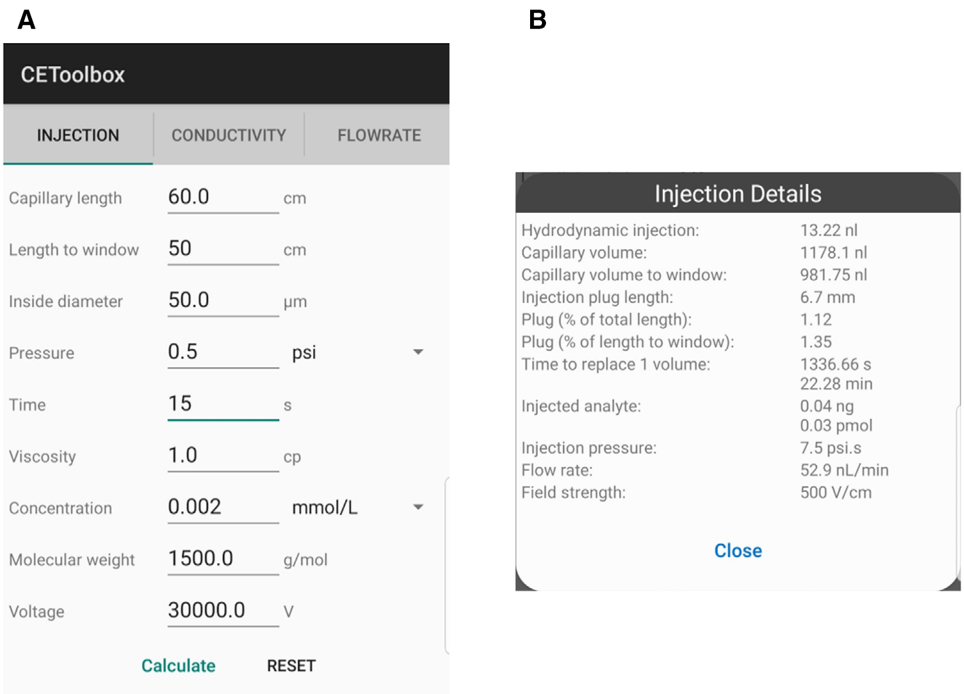

Figure 2. CEToolbox Injection interface. (A) Parameters window. (B) Results window.

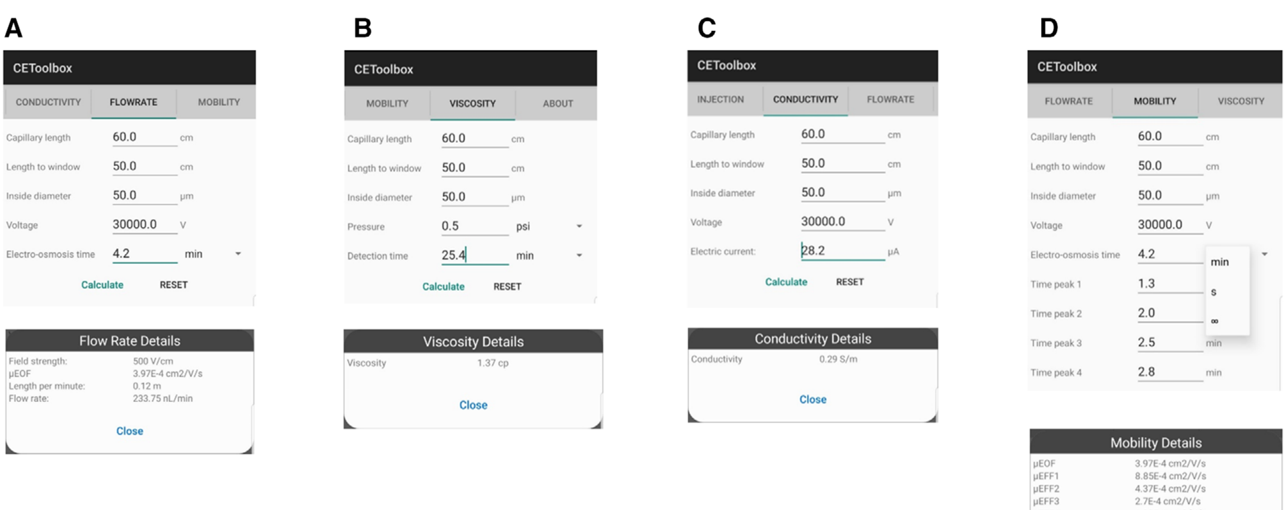

Figure 3. Interface of parameters and results windows for (A) flow rate, (B) viscosity, (C) conductivity, and (D) mobility calculation.

capillary volume (Equation 2), capillary volume to window (Equation 3), injection plug length (Equation 4), plug (% of total length) (Equation 5), plug (% of length to window) (Equation 6), time to replace 1 volume (Equation 7), injection flow rate (Equation 8), and electrical field (Equation 9). Concerning sample properties, the amount of analyte is calculated following Equations 10 and 11, and is given in the two different units (nanogram or picomole). It will be impossible for the authors to refer all the bibliographic references, which notice these sorts of information because almost all the literature using CE refer at least one of these properties. This shows the essential nature of such information to CE Users. The second tab of CEToolbox is dedicated to obtain the flow rate of the separation generated by the μ_{eof}. This is probably the least information reported in the literature, however it gives a very important idea about the speed and the behavior of the separation [17]. CE users need this information to predict and to optimize the efficiency of the separation. With a simple click on the “Flow rate” tab of the interface (Fig. 3A), CE user can enter known separation parameters such as capillary properties (L, l, d_c), tension, and electroosmosis time (t_{eof}) expressed either in minutes or in seconds. After clicking on the “Calculate” button, a window appears with the value of μ_{eof} (Equation 12) and the value of the separation flow rate expressed in nL/min (Equation 13).

Measure of viscosity and conductivity of BGE is highly significant for the CE user especially for CGE [18] and CIEF [19] applications and for separation using BGE with high ionic strength [20]. Indeed, viscosity is involved in several separation parameters as equation of the hydrodynamic injection volume (Equation 1) and time to replace one volume of the capillary (Equation 7). Equation 1 shows that viscosity is a very significant factor in fluid flow. Because volume delivered varies linearly with the viscosity value, a small change in BGE viscosity can cause a large change in fluid delivery. Concerning conductivity, in CE, analytes are separated under an electrical field. One of the most important issues can be caused by the Joule effect due to high current generated by the electrical field applied. In that case, BGE conductivity value can help the CE user to prepare experimental protocols and to avoid some troubles such as analyte degradations. François et al. promote CE instrumentation as an integrated apparatus for measurement of viscosity and conductivity of BGE [21]. For viscosity measurements, a short plug of a flow marker in the BGE was injected under a pressure of 50 mbar for a few seconds, according to the estimated viscosity. The marker zone was displaced by introducing the BGE (free of the marker) under a known pressure until the marker was detected. The measurement of the detection time of the marker (t_d) was used to calculate viscosity according to Hagen–Poiseuille law (Equation 14). For conductivity determinations, a range of voltages was applied to the capillary filled by the BGE. The voltages applied were chosen so that the linearity of Ohm's law was perfectly verified. Conductivity was obtained from the measured current intensity according to Ohm's law after rearrangement described in Equation 15. To obtain these two information, two different tabs are dedicated on CEToolbox. With a single click on the "Viscosity" tab of the interface (Fig. 3B), the CE user can enter known separation parameters as capillary properties (L, l, d_c), pressure, and detection time (t_d). After clicking on the "Calculate" button, a window appears with the value of the viscosity expressed in centipoise (cP). Concerning "Conductivity" tab (Fig. 3C), separation parameters are capillary properties (L, l, d_c), voltage, and electric current (I). Window of results gives the value of conductivity expressed in mS/m. These four tabs allow CE users to address simple questions for the preparation of experimental setup.

The fifth tab of CEToolbox is dedicated to mobility calculation. In that case, information are not supplied for preliminary study but when the separation is ended. Indeed, the electropherogram provides lots of information about the quality of the separation. One of the most important data is migration time (t_m) of each compound. However, due to the presence of an electroosmotic flow, CZE is often criticized for a lack of robustness in terms of t_m. Every CE user is aware that in CZE, a significant parameter for analyte characterization is the effective mobility (μ_{eff}). μ_{eff} represents the mobility of the compound without taking into consideration the electroosmotic flow (Equation 16). Experimental determination of mobility can be achieved by the ratio of the speed and the electrical field. Despite the simplicity of these equations, CE users need a calculator to obtain mobility information. The general approach consists of using the software provided by the CE constructor, which obviously gives this information, especially for complex separation. However, for separation not exceeding 20 compounds, no calculation tool is available and CE users tend to set up a homemade calculation file. With the availability of CEToolbox, CE users are not required any more to develop their own solutions to calculate μ_{eff}. CEToolbox permits to determine μ_{eff} using t_m for a separation not exceeding 20 compounds. With a single click on the "Mobility" tab of the interface (Fig. 3D), CE users can enter all the known separation parameters as capillary properties (L, l, d_c), tension, electroosmosis time, and analyte migration time. After clicking on the "Calculate" button, a window appears with the calculated value of μ_{eof} and the different values of μ_{eff}. It is important to note that if μ_{eof} is negligible, the box reserved for t_{eof} value must be replaced by the infinity symbol.

Finally, CEToolbox remains a theoretical calculator and all the theoretical principles used to develop this application have been exposed in this article. In consequence, the results presented should be taken as approximations and not as absolutes.

We have developed CEToolbox application, desiring to provide a useful calculator for CE users in the aim to facilitate their daily experiments in the laboratory. This application is anchored in the use of new media platform that have already integrated the life of the laboratory. Today, CEToolbox appears to be the most comprehensive specialized calculator for the CE user. Whatever CE system is used, CEToolbox provides extensive CE separation information as injection, viscosity, conductivity, flow rate, and mobility properties. CEToolbox is a software running on Android platform and is available freely on Google and F-Droid application store, and also from the project web page: https://cetoolbox.github.io. Even if it is easy to use the application on other platform such as classic computers using an Android emulator, in the near future and in order to facilitate the diffusion and the use of CEToolbox, it is planned to port it to other computing systems (iOS, Mac OS X, Microsoft Windows, etc.).

The authors declare no conflict of interest.

Data availability statement

The main data of this article are the source code of the CEToolbox application. It is openly available and the version used for this article has the following DOI: https://doi.org/10.5281/zenodo.4643845

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