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RESEARCH PAPER

Strategy for non-target ionic analysis by capillary electrophoresis with ultraviolet detection

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Abstract A strategy for non-target analysis of samples with unknown composition by capillary electrophoresis (CE) with ultraviolet (UV) detection is suggested. The strategy is based on the preliminary identification of analytes and further optimization of the conditions for their separation using the developed computational tool set ElphoSeparation. It is shown that, in order to record electrophoretic peaks with the mobilities from the maximum to minimum possible values, the positive and negative voltage polarity and hydrodynamic pressure should be used. To choose the optimal separation conditions, dynamic maps of electrophoretic separation (DMES) are suggested. DMES is a bar chart with theoretical resolutions of adjacent peaks presented in ascending order of the migration time. The resolution is calculated as the division of the difference of the effective electrophoretic mobilities of adjacent analytes by their average peak width in terms of electrophoretic mobility. The suggested strategy is tested by the example of the analysis of herbal medicine (Holosas) on the basis of rose hips. The approach should be used to analyze samples with not very complex composition, such as environmental water and precipitation samples, process liquors, some vegetable extracts, biological fluids, food, and other samples for the determination of widespread compounds capable of forming ionic species. For samples with complex composition, the approach used together with other techniques may produce advantageous information due to specificity of the method, particularly it can be useful for determination of compounds suffering from low volatility or thermal stability, and for analysis of samples with difficult matrices.

Keywords Capillary electrophoresis · Optimization of separation · Non-target analysis · Identification · Selectivity · Peak width

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Introduction

Capillary electrophoresis is successfully used for ionic and molecular analysis in many application areas due to rapidity, low cost, and high separation efficiency of analysis. Samples under study are often complex multicomponent solutions obtained from food [1, 2], process liquors [3], atmospheric aerosols [4], plant extracts and materials [5, 6], biological fluids [7], biocultivation products [8], and many others. Generally, capillary electrophoresis, if not hyphenated to mass spectrometry (MS) detection, is used for target analysis in order to determine the substances which are a priori expected to be found in the given sample. However, in such cases, it is possible that the analytes under study are concurring with nonmetering substances. For example, if plant extracts and foods are analyzed, a limited number of organic acids are determined, and the interfering effect of other acids is not taken into account [1, 6–8].

Recently, non-target analysis has widely been used in a number of application areas. Non-target analysis is the determination of analytes a priori unknown to a chemist before analyzing samples [9–11]. This analysis has a special place in metabolomics [12–15], food authentication [16], environment analysis [4, 17], etc. Generally, non-target analysis is carried out using chromatographic and electrophoretic techniques with MS detection where mass spectral libraries are required. Ultraviolet (UV) detection is insufficiently specific, but the advantage of capillary electrophoresis (CE) is an ability of changing the effective electrophoretic mobilities of analytes due to varying the background electrolyte (BGE) composition. Thus, performing the analysis by CE with UV detection (CE-UV) using two or more BGEs with different composition allows one to enhance the reliability of identification and quantification because the identification is based on matching several values of effective electrophoretic mobilities and partly UV spectra.

At present, one of the main restrictions for using CE-UV for non-target analysis is the absence of a tool for searching the substances with a certain value of effective mobility at a specified composition of BGE. The effective electrophoretic mobility of ions is the velocity of their motion at the electric field intensity of 1 V/m. For the ions of strong acids and bases, it depends on the ionic strength of BGE, temperature, viscosity, and content of complexing agents [18]. For the ions involved in acid-base and complexing equilibria, the effective mobility also depends on the mole fractions and ionic mobilities of the species being the weighted mean value over all the species [19]. There have been attempts to use the effective electrophoretic mobilities, generally obtained by CE-UV, for the identification. In 1995–1998 for a comprehensive screen for drugs of forensic interest in whole blood, Hudson et al. [20, 21] composed the libraries of electrophoretic mobilities for more than 600 drugs using four BGEs: 100 mM phosphate (pH 2.38, 2.5, 9.5) and 100 mM borate (pH 8.5). The cellular electrophoretic mobility database was presented by Slivinsky et al. [22]. Yassine et al. used the correlation of effective mobility to $Z/M^{2/3}$ for confirming the chemical known/unknown structures of compounds [4]. But the limitations of the libraries of electrophoretic mobilities for the given BGEs are obvious. There are several hundred probable BGEs, and it is impossible to measure the effective electrophoretic mobilities for each substance using every BGE.

There are two computer programs to predict the effective electrophoretic mobilities (without searching the substances with a certain value of effective mobility). The Buffer Workshop program [23] (MB Design Solutions, http://bufferworkshop.com) allows one to calculate the mobility of a substance at a specified pH, but the influence of the ionic strength on the mobility can simultaneously be taken into consideration for only 16 acids or bases, with the BGE constituents being among them. At the same time, the program consists of the data for 640 substances (507 acids and 133 bases), although for several substances only the values of acidity constants are presented. The PeakMaster 5.3 program [24] (free downloaded from http://www.natur.cuni.cz/gas) allows one to calculate the effective electrophoretic mobilities for selected substances but the program consists of the data for 516 substances which can be added to the calculation table one by one.

To use CE-UV for non-target analysis, besides the preliminary identification, it is necessary to choose the optimal conditions for separating the preliminary identified ions. For optimization of the separation of the given substances, the trial-and-error procedure, chemometric methods [25–28], and methods based on CE theory [29, 30] are used. A shortcoming of the trial-and-error procedure is that it is suitable for the separation of small number of analytes. The chemometric methods are based on a large array of experimental data. Thus, the methods based on the CE theory seem to be the most suitable approach.

For the selectivity assessment, the methods based on the CE theory often use a relative difference in the effective electrophoretic mobilities [31, 32]. But the difference in mobilities and usual mobility dependences on pH do not actually reveal the separation, because they do not contain the data on the peak width which may be decisive for the separation. The studies with the theoretical consideration of the peak width are few, and a number of assumptions used are valid at infinite dilution [29, 33, 34]. Recently, a new peak broadening parameter has been suggested for characterization of the separation capability in capillary electrophoresis [35]. This is the peak width in terms of electrophoretic mobility at 5 % of the maximum peak height $w_{0.05}(\mu)$. The knowledge of the peak width in terms of electrophoretic mobility for a number of ions allows one to characterize the separation capability for a set of experimental conditions and to estimate the peak resolution R for the ions with the known electrophoretic mobilities without performing the separation:

$$R = \frac{\left|\mu_{ep,\ i+1} - \mu_{ep,\ i}\right|}{w_{0.05}(\mu_{av})} \quad (1)$$

where $\mu_{ep,\ i+1}$ and $\mu_{ep,\ i}$ are the effective electrophoretic mobilities of $i + 1$th and ith analytes, respectively, $\mu_{av} = (\mu_{ep,\ i+1} + \mu_{ep,\ i})/2$.

The purpose of this study is to suggest a strategy for non-target ionic analysis of the samples with unknown composition by CE-UV. The strategy consists of the preliminary identification of ions and further optimization of the conditions for their separation using the calculated values of the effective mobilities and peak widths in terms of electrophoretic mobility. To realize the strategy, the computational tool set has been developed. The suggested strategy is tested by means of the anionic analysis of a herbal medical product.

Materials and methods

Instrumentation

The study was carried out using a capillary electrophoresis system with a diode-array detector Agilent ^{3D}CE G1600A (Agilent Technologies, Waldbronn, Germany) of the Krasnoyarsk Regional Center of Research Equipment, Siberian Branch of the Russian Academy of Sciences. The data acquisition and processing were performed with the computer program ChemStation Rev.A.10.02. Untreated fused silica capillary with 50 μm id and the total and effective lengths of 48.5 and 40 cm (Agilent Technologies) was used. The capillary temperature was kept constant at 25.00 ± 0.04 °C. The voltage of +30 and −20 kV was used. The voltage was applied to the capillary inlet. The application of hydrodynamic pressure of 0–50 mbar to the inlet end of the capillary was used during the separation. The direct detection was made at 200, 210, 220, 245, and 254 nm with the bandwidth of 6–10 nm. For indirect detection, the signal wavelength was at 350 nm with the bandwidth of 80 nm, and five above-listed values were used as a reference. The samples were injected hydrodynamically for 2 s at a pressure of 30 mbar. The experiments were performed at least in triplicate.

A new capillary was first flushed with 1 M NaOH for 10 min, and then with ultra pure water for 10 min. At the beginning of each day, the capillary was first flushed with 0.1 M NaOH for 5 min, twice with ultra pure water for 10 min. At the beginning of the using of a certain BGE, the capillary was exposed the flushing with ultra pure water for 1 min, with BGE for 5 min, and applying the voltage of +30 or −20 kV for 10 min. The capillary was flushed with BGE for 5 min between the runs.

Chemicals

All the chemicals used were of analytical-reagent grade and were purchased from Sigma-Aldrich (Moscow, Russia). Deionized water from a water purification system Direct-Q3 (Millipore, France) was used for the solution preparation.

The herbal medicine, Holosas, on the basis of rose hips was used as a sample with unknown composition. The sample was diluted 50 times with deionized water. For indirect detection, the following BGEs were used: (1) 2.0 mM pyromellitic acid (PMA), 16 mM triethanolamine (TEA), pH 7.83; (2) 2.0 mM PMA, 9.7 mM TEA, pH 7.20; (3) 2.0 mM PMA, 6.9 mM TEA, pH 6.03; (4) 2.5 mM m-nitrobenzoic acid (m-NBA), 1.9 mM LiOH, pH 4.10; (5) 2.5 mM m-NBA, 1.7 mM LiOH, pH 3.90; and (6) 4.0 mM benzoic acid, 11.9 mM diethanolamine, pH 9.20. The concentrations of the constituents in the samples for estimating the effective mobilities using 2 mM PMA, 16 mM TEA were within 0.1–0.5 mM. For direct detection, the following BGEs were used: (1) 5.0 mM formic acid, 10.0 mM TEA, pH 7.80; (2) 5.0 mM glycolic acid, 3.1 mM LiOH, pH 4.11; and (3) 10.0 mM glycine, 7.1 mM LiOH, pH 10.10.

To deduce the regularities of the dependences of the peak width in terms of electrophoretic mobility and electroosmotic mobility on the separation conditions, the following BGEs were used: (1) 2.5 mM m-NBA, 0.9 mM LiOH, pH 3.50; (2) 2.5 mM *o*-phthalic acid, 3.8 mM LiOH, pH 5.30; (3) 2 mM PMA, 16 mM TEA, pH 7.83; and (4) 10 mM 5-sulfosalicylic acid, 29 mM LiOH, pH 11.77. The gluconic, lactic, succinic, acetic, formic, hydrochloric, chlorous acids, or their salts were used as analytes at a concentration of 0.8 mM. The samples were obtained by diluting the stock solutions with deionized water or BGE so that the sum of ion concentrations in the sample be equal to the sum of BGE ion concentrations.

Calculations

The effective electrophoretic mobility $\mu_{ep,i}$ was calculated using the equation:

$$\mu_{ep,\,i} = \mu_{tot,i} - \mu_{\mathrm{EOF}} \pm \upsilon_P \cdot \frac{l}{U} = \frac{l l_{\mathrm{eff}}}{U t_i} - \mu_{\mathrm{EOF}+P} \qquad (2)$$

where $\mu_{ep,i}$ and $\mu_{tot,i}$ are the effective electrophoretic and total mobilities of the *i*th analyte, respectively, μ_{EOF} is the electroosmotic mobility, υ_p is the hydrodynamic flow velocity, U is the voltage with the polarity sign ("+" or "−"), l and l_{eff} are the total and effective capillary lengths, t_i is the migration time of the *i*th analyte, and $\mu_{\mathrm{EOF\,+\,P}}$ is the electroosmotic mobility adjusted by applying hydrodynamic pressure. The values of $\mu_{\mathrm{EOF\,+\,P}}$ were calculated subject to the direction of electroosmotic flow (EOF) as follows:

$$\mu_{\mathrm{EOF}+P} = \frac{l l_{\mathrm{eff}}}{U t_{EOF}} \quad \text{or} \quad \mu_{\mathrm{EOF}+P} = -\frac{l(l - l_{\mathrm{eff}})}{U t_{\mathrm{EOF}}} \qquad (3)$$

where l_{eff} and the former equation were used when EOF was directed from the inlet to the detector, and (l-l_{eff}) and the latter equation were used when EOF was directed from the outlet to the detector, t_{EOF} is the migration time of the EOF marker or time corresponding to sp0. sp0 is the system peak forming at the capillary end in the start after the voltage application which is transferred to the detector by EOF [36, 37].

The effective electrophoretic mobility was calculated at the given pH as follows [18].

$$\mu_{ep,\ i} = \sum_{z=-6}^{+6} \mu_{i,\ z} \alpha_{i,\ z} \qquad (4)$$

where $\mu_{i,\ z}$ and $\alpha_{i,\ z}$ are the ionic mobility and molar fraction of the species with the charge z for the *i*th analyte, respectively. The details of calculation of the molar fractions are present in

the Electronic Supplementary Material (ESM) (S1). The influence of the ionic strength on the ionic mobility was taken into consideration in two ways:

1. Theory of Onsager and Fuoss (OF) for the mixtures of electrolytes [24, 38] (Eq. S4)

$$\mu_{i,\ z} = f\left(\mu^0_{i,z}, z, I, \text{BGE composition}\right) \quad (5)$$

where $\mu_i^0(z)$ is the ionic mobility of the species with the charge z for the ith analyte at $I = 0$, I is the ionic strength calculated as $I = 0.5\sum_j^s z_j^2 c_j$, z_j is the relative charge of the jth ion, c_j is the concentration of the jth ion, and s is the overall number of all the ionic species in the solution.

2. Empirical Eq. [39] Here, the equation (6) must be located where $k = 0.50$ for $|z| = 1$ and $k = 0.77$ for $|z| > 1$.

The peak width in terms of electrophoretic mobility [35] and theoretical values of electroosmotic mobility [40] were calculated using Eqs. S5 and S6, respectively (see ESM).

$$\mu_{i,\ z} = \mu^0_{i,\ z} \exp\left(-k\sqrt{|z|I}\right) \quad (6)$$

Results and discussion

The strategy for analyzing the samples with unknown composition using CE-UV is suggested. The approach consists of the following steps:

1. Sample analysis using the standard BGEs for direct and indirect detection under the conditions, allowing one to record peaks with the effective electrophoretic mobilities from the maximum to minimum possible values
2. Calculation of the effective electrophoretic mobilities and preliminary identification
3. Optimization of the separation of possible ions based on the difference in the effective electrophoretic mobilities and peak width in terms of electrophoretic mobility, and choice of the second BGE
4. Analysis using the second BGE, calculation of the effective electrophoretic mobilities, and choice of another BGE if necessary

The sample for the analysis by CE-UV must be in the liquid state, miscible with water; the tentative testing can be carried out as described in the paper of Magnuson et al. [10]. The formic and pyromellitic BGEs with pH 7.8 (containing TEA as a buffering constituent) are suitable as the standard BGEs for direct and indirect detection, correspondingly. However, it is necessary to bear in mind that some substances may be in molecular form at this pH and transform into ionic species at higher pH. Therefore, after the optimization of the separation of the preliminary identified species, it is necessary to perform the optimization of the separation of this species from those having zero mobility at pH 7.8. The pH of further used BGE should be in the range of 9–12 for the anionic analysis and in the range of 2–4 for the cationic analysis.

The number of possible ions can be reduced taking into account the following.

(a) UV absorption or UV spectrum. For example, the electrophoretic peaks of a substances with benzene ring at direct detection are greater than the peaks of these substances at indirect detection.
(b) Solubility in solutions. Possible substances can be excluded from consideration if their solubility is lower than the sensitivity of the electrophoretic determination.
(c) Other factors. For example, pharmaceutical composition is, as a rule, free from toxic substances, or a natural object without man's impact is free from artificial substances.
(d) Method of standard additions. To establish the absence of a certain substance in the analyzed sample, it is not necessary for the peak of the substance to be separate up to the baseline from the peak of another substance with close mobility. The appearance of a shoulder or an additional peak in the electropherogram after spiking the sample under study with a possible substance is to demonstrate the absence of the substance. It is recommended to use the standard additions method for both preliminary and final identifications.

Recording peaks with mobilities from the maximum to minimum possible values

Generally, experimental conditions suitable for the separation of the given analytes are such that in the electropherograms, the peaks appear with effective electrophoretic mobilities within the limited range. For example, let us consider a usual BGE for the inorganic anion analysis, containing 2.25 mM PMA, 1.6 mM TEA, 6.5 mM NaOH, 0.75 mM hexamethonium hydroxide, pH 7.7 [36]. For the electroosmotic mobility of $25.8 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, voltage of −30 kV, and capillary lengths of 80.5/72 cm, within 30 min (t_{rec}), the peaks appear with the effective electrophoretic mobilities from $-\infty$ to $-36.5 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} in the electropherograms (see Eq. 2). When the voltage is +30 kV and other conditions are the same, the peaks with the mobilities from $+\infty$ to $-15.1 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} appear in the electropherograms (Fig. 1a). Thus, the ions with the mobilities from −15.2 to $-36.4 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} are not recorded under these conditions. With decreasing the electroosmotic mobility, this gap shifts toward zero (Fig. 1a) but the value of the gap does not change since it is equal to $(2ll_{eff})/(|U|t_{rec}) > 0$. It slightly changes with changing the capillary length (Fig. 1a, b).

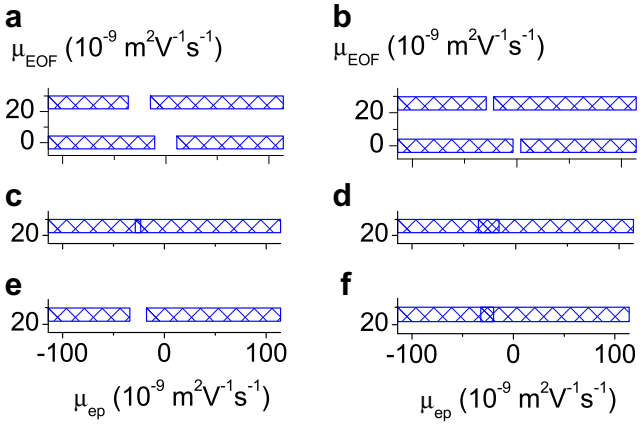

Fig. 1 Ranges of analyte effective electrophoretic mobilities which can be recorded under different conditions. Hydrodynamic pressure (mbar) were **a**, **b** 0, **c**–**f** 50. Total capillary length (cm): **a**, **c**, **e** 80.5 ($\upsilon_p = 5.0 \cdot 10^{-4}$ m s^{-1}); **b**, **d**, **f** 48.5 ($\upsilon_p = 8.4 \cdot 10^{-4}$ m s^{-1}). t_{rec} (min): **a**–**d** 30, **e**–**f** 15. The voltage values of −30 and +30 kV are the left and right of the gap or crossing, respectively

All the peaks can be recorded by the pressure-assisted CE (PACE) which allows widely varying the electrolyte flow velocity in the capillary due to the application of hydrodynamic pressure during the separation [36, 37, 41]. Figure 1c, d shows the mobility ranges with hydrodynamic pressure application (50 mbar) and capillary lengths of 80.5 and 48.5 cm. In such cases, all the ions can be recorded. For a smaller capillary, the time for the electropherogram recording can be decreased from 30 to 15 min (Fig. 1e, f). Thus, to record peaks with the mobilities from the maximum to minimum possible values, it is convenient to use the capillary length of 48.5 cm and recording time within 15–30 min with the positive and negative voltage polarity and hydrodynamic pressure of 50 mbar. Another way to record all the peaks is to use two BGEs at positive and negative voltage polarity, with BGE for the negative polarity containing an EOF modifier in a greater amount as compared to BGE for the positive polarity.

When the indirect detection and EOF directed from the capillary outlet to inlet are used, the system peaks can appear in the electropherograms with the mean or high analyte concentrations. Their migration times can be estimated and the conditions can be chosen when the system peaks do not concur with the analyte peaks as described by Sursyakova et al. [36, 37].

The ElphoSeparation tool set

A special tool set named as ElphoSeparation was developed in MS Excel. There are two modules of ElphoSeparation for preliminary identification and two module for optimization of the separation, performing the calculations with the OF or empirical equations (Eqs. 5, 6). The ElphoSeparation contains the database of the ionic mobilities and acidity and basicity constants for 516 substances from the literature data (Hirokawa's and PeakMaster 5.3 data) [24]. The tool set database allows one to add other substances up to 1000 ones. The mobility calculation is carried out using Eqs. S1–S6 (see ESM).

The modules for the preliminary identification operate as follows (ESM Fig. S1). It is necessary to indicate the following: (1) for OF Eq., the pH, concentrations, charges, and ionic mobilities of the BGE constituents (up to four cations and anions including H^+ and OH^-) and for the empirical equation, only pH and ionic strength of BGE; (2) the effective mobility with the admissible deviation (%). The ElphoSeparation instantly shows all the analytes with these mobilities. In contiguous cells, the ionic mobilities and acidity and basicity constants are displayed. Having copied these properties into the optimization module of ElphoSeparation with the blank database, one can trace the mobility change among the analytes under study. Here, the percentage of the admissible deviation is of decisive importance. The reproducibility of the experimentally obtained effective mobilities does not, as a rule, exceed 1 % [42]. To determine how the applied theory describes the experiment, the effective mobilities of a number of anions were measured using pyromellitic BGE (pH 7.83). Table S1 shows the comparison of the experimental effective mobilities $\mu_{ep,\,i}$ with the calculated mobilities in terms of percent of the mobility deviation. As follows from the data of Table S1, it is better to use the module of ElphoSeparation with OF Eq. and 3 % of the admissible deviation for preliminary identification. But for the mobilities $|\mu_{ep,\,i}| < 10$, it is preferable to use the absolute value of 0.5–1 (Table S2).

After the tentative identification to preliminarily determine pH and ionic strength of next BGE, it is convenient to use the optimization module of ElphoSeparation with the empirical equation because of no need to specify the BGE composition and the mobility dependences on pH can be obtained with pH ranging from 2 to 12. Considering these dependences and varying the ionic strength if necessary, the pH range can be found with non-concurrence of the curves. To find the exact BGE composition, the optimization module of ElphoSeparation with the OF Eq. should be used. It is worth noting that for sufficient buffer capacity, the pH of BGE should be in the range of $pK_a \pm 1$ (pK_a of a buffering constituent in BGE).

For a high number of substances, none of BGEs using which all the analytes can be baseline separated, and the mobility dependences on pH is not informative enough. In this case, the differences in the effective mobilities of the adjacent ions $\mu_{ep,\,i+1} - \mu_{ep,\,i}$ and resolution are more useful for the optimization. The differences in the

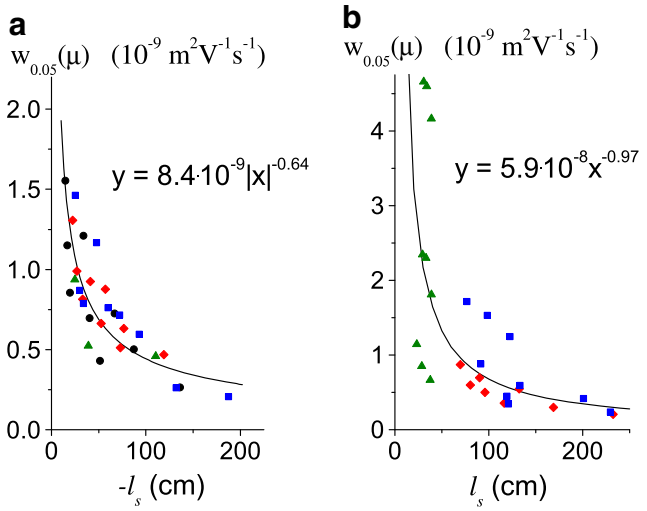

Fig. 2 Dependences of the peak width in terms of electrophoretic mobility on the virtual distance of separative migration for **a** the positive (+30 kV) and **b** negative (−20 kV) voltage polarity. BGEs for indirect detection with the pH range of 3.5–11.8, contained benzene carboxylate probes: *black circle* is the sulfosalicylic BGE, pH 11.8, *black diamond* is the pyromellitic BGE, pH 7.8, *black square* is the phthalic BGE, pH 5.3, *black triangle* is the nitrobenzoic BGE, pH 3.5. The detection was made at 350 nm with the bandwidth 80 nm as a signal wavelength and 220 nm as a reference with the bandwidth 6 nm

effective mobilities can be used for finding the absence of substances in the sample analyzed. As shown above, the mobilities μ_i obtained from ElphoSeparation with OF Eq. agree with the experiment within 3 %. It follows that if there is no peak in the electropherogram with the specified mobility ±3 %, the possible substance is absent in the sample. However, in order not to confuse the concerned *i*th analyte with the adjacent analytes, the relative mobility differences $\Delta\mu_i = 2(\mu_{ep,\,i+1} - \mu_{ep,\,i}) / (\mu_{ep,\,i+1} + \mu_{ep,\,i})$ and $\Delta\mu_{i-1}$ should be no lower than 3–4 %. If a few peaks with a sufficiently distant distribution appear in the electropherograms, then the absence of substances can be found using the standard BGE with a slightly different pH as compared to the initial BGE. The change of $\Delta\mu_i$ with varying separation conditions can be conveniently traced by means of the histogram dependences of $\Delta\mu_i$ on *i*.

The resolution is calculated using Eq. 1 based on the mobility differences of the adjacent analytes $\mu_{ep,\,i+1} - \mu_{ep,\,i}$ and peak width in terms of electrophoretic mobility $w_{0.05}(\mu)$. For a high number of substances, the dynamic maps of electrophoretic separation (DMES) are suggested, which are for the first time presented in the paper. DMES is a bar chart with the theoretical resolutions of the adjacent peaks given in the ascending order of the migration time in the electropherograms. Varying the electroosmotic mobility or pH of BGE within the obtained preliminary range, the resolution change can be traced and the optimal separation condition can be found.

It was found that the peak widths in terms of time strongly differ from the theoretical peak widths, including the effect of longitudinal diffusion, contribution to variance caused by a finite injection volume, length of the detector window, and hydrodynamic flow [29, 34]. At the same time, the experimentally obtained peak width in terms of electrophoretic mobility is found to be well approximated by the power function of the virtual distance of the separative migration $l_{s,\,i}$:

$$w_{0.05}(\mu_i) = a_i l_{s,i}^{b_i} \tag{7}$$

$$l_{s,\,i} = \left(\mu_{ep,i}/\mu_{tot,i}\right) l_{eff} = f_{m,\,i} l_{eff} \tag{8}$$

where $l_{s,\,i}$ is the distance covered by the *i*th analyte without taking into account EOF in the capillary during the migration time t_i [35], a_i and b_i are the coefficients depending on the injected volume of the sample, effective electrophoretic mobility of the analyte and BGE ions, molar extinction coefficient of the BGE probe, temperature, capillary length, etc., and $f_{m,\,i}$ is the electromigration factor. The approximate values of the peak width can be used for estimation. For a number of anions using the BGEs for indirect detection with the pH

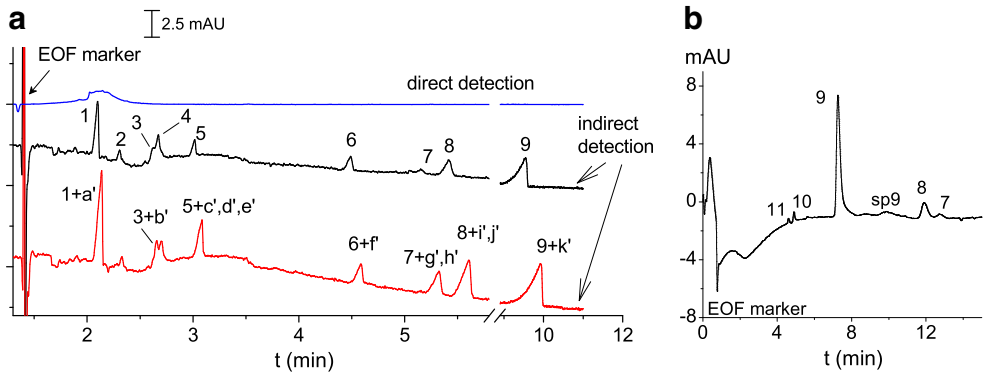

Fig. 3 Electropherograms of the herbal medicine diluted 50 times. BGEs with pH 7.8 for direct and indirect detection were used. The voltage was **a** +30 kV and **b** −20 kV. Hydrodynamic pressure was 50 mbar. The following acid standards were added: *a*'—gluconic, *b*'—lactic, *c*'—pyruvic, *d*'—acetic, *e*'—glycolic, *f*'—phosphoric, *g*'—formic, *h*'—succinic, *i*'—malic, *j*'—hydrofluoric, *k*'—citric. The detection wavelength was as in Fig. 2

Table 1 The effective mobilities (95 % confidence interval, $n = 3$) of the recorded electrophoretic peaks using the pyromellitic BGE (pH 7.83) and possible acids corresponding to these values

№	$\mu_{ep,\,i}$ (10^{-9} m^2 V^{-1} s^{-1})	Acid
1	−24.1 (±0.1)	Glucuronic, gluconic, glutamic
2	−28.5 (±0.2)	Hydroxytrimethylacetic, valeric, aspartic, diethylacetic, isocaproic, trimethylacetic, 2-ethylbutyric, caproic
3	−34.0 (±0.2)	Methoxyacetic, lactic, glyceric, propionic, Gly-Glu, carboxymethyl cysteine
4	−34.7 (±0.2)	
5	−39.2 (±0.2)	Pyruvic, acetic, glycolic
6	−50.5 (±0.3)	Oxaloacetic, mesaconic, glutaconic, itaconic, citraconic, glutaric, phosphoric
7	−53.5 (±0.1)	Formic, 2-ketoglutaric, malic, hydrofluoric, succinic, methylmalonic
8	−54.5 (±0.1)	
9	−62.9 (±0.3)	Citric, tartronic
10	−74.0 (±0.2)	Sulfuric
11	−75.8 (±0.4)	Hydrochloric

range of 3.5–11.8, containing benzene carboxylate probes, the coefficients in Eq. 7 were found (Fig. 2). For $l_s > 200$–250 cm, $w_{0.05}(\mu) \approx 0.4 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. With the concentration decreasing by three times, the peak width changes by 0.8–1.5 times. Therefore, for the estimation, Eq. 7 with obtained coefficients can be used.

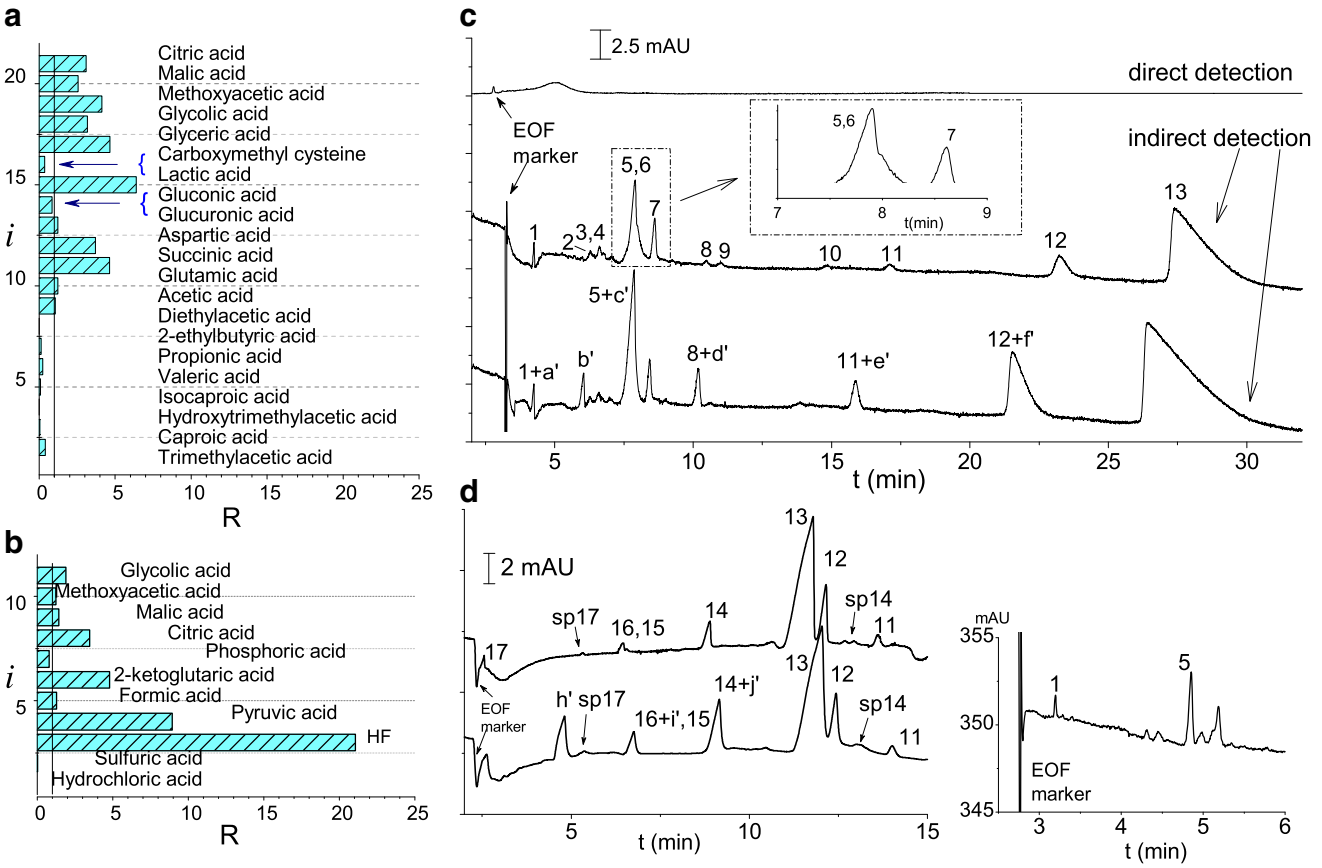

Fig. 4 **a**, **b** Dynamic maps of electrophoretic separation for the acids preliminary identified in the herbal medicine. **a** +30 kV, μ_{EOF+P} = $32 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, **b** −20 kV, $\mu_{EOF+P} = 14 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}. Benzoic BGE with pH 4.1. **c**–**e** Electropherograms of the herbal medicine diluted 50 times. The voltage was **c**, **e** +30 kV and **d** −20 kV. Hydrodynamic pressure was **c** 50 mbar, **d** 10 mbar, and **e** 0. For direct detection, BGE was glycolic with pH 4.1. For indirect detection, BGE was m-nitrobenzoic with **c**, **d** pH 4.1 and **e** pH 3.9. The following acid standards were added: *a*'—acetic, *b*'—succinic, *c*'—gluconic, *d*'—lactic, *e*'—glycolic, *f*'—malic, *g*'—citric, *h*'—hydrofluoric, *i*'—phosphoric, *j*'—formic. The detection wavelength was as in Fig. 2

Table 2 The effective mobilities (95 % confidence interval, $n = 3$) of the recorded electrophoretic peaks using the m-nitrobenzoic BGE (pH 4.10) and possible acids corresponding to these values

№	$\mu_{ep,\,i}$ (10^{-9} m^2 V^{-1} s^{-1})	Acid
1	−7.7 (±0.1)	Acetic
2[a]	−15.7 (±0.1)	Unknown
3[a]	−16.5 (±0.1)	Aspartic acid or unknown
4[a]	−16.8 (±0.1)	
5	−19.1 (±0.1)	Gluconic
6	−19.2 (±0.1)	Glucuronic acid or unknown
7	−20.2 (±0.1)	
8[a]	−22.3 (±0.1)	Lactic
9[a]	−22.8 (±0.1)	Carboxymethyl cysteine or unknown
10[a]	−25.3 (±0.2)	Methylmalonic
11[a]	−26.0 (±0.1)	Glycolic
12	−27.4 (±0.2)	Malic
13	−28.0 (±0.2)	Citric
14	−31.8 (±0.1)	Phosphoric
15[a]	−38.2 (±0.1)	Formic
16	−38.5 (±0.1)	Pyruvic
17	−75.5 (±0.4)	Sulfuric + hydrochloric

[a] Trace amount

Example of using the suggested strategy

The suggested approach was applied in the anionic analysis of a sample with complex composition, herbal medicine (Holosas) based on rose hips. Figure 3a, b shows the electropherograms of the medicine, obtained using the formic and pyromellitic BGEs with pH 7.8 (direct and indirect detection, respectively) at positive and negative voltage polarity and hydrodynamic pressure of 50 mbar. With the direct detection at −20 kV, no peak is observed; with the direct detection at +30 kV, a wide peak appears (Fig. 3a). This is probably a group of polymeric substances with close molecular weights and charges such as tannins or pectins. This wide peak is likely to appear within 2–3 min under the indirect detection as a little stretched dip.

In the case of the indirect detection, 11 peaks appear, with 2 of them being not baseline separated. Their effective mobilities were calculated using Eqs. 2–3. The potential substances with these mobilities ±3 % were found using ElphoSeparation with OF Eq. (Table 1). The following substances were not taken into consideration: (1) substances with benzene ring since the direct detection did not give any intensive narrow peaks; (2) poorly soluble substances such as pelargonic, caprylic, sebacic, isocitric, and isocaproic acids; (3) toxic and artificial substances such as cacodylic, acrylic, sulfonic, and halogen-containing acids, with the medicine being analyzed; and (4) substances which were not confirmed by standard additions method, namely ascorbic, maleic, fumaric, tartaric, and muconic acids. Standard additions method showed the potential presence of a number of substances (Fig. 3a).

Thus, 37 acids remained after excluding. There is no pH which allows one to separate all the analytes (ESM Fig. S2). Having plotted in ElphoSeparation the histogram dependences of $\Delta\mu_i$ on i and varying the pH of the pyromellitic BGE within the range of 6–8 in increments of 0.1, it is found that a number of analyte can be excluded by separation of the sample at pH 7.2 and 6 (ESM Figs. S3–S5). After this excluding, 28 acids remained and so, since some standards of 28 acids were not available, a pH was found at which 13 available acids were separated among themselves and from the other acids. From the plots of the effective mobilities vs. pH, the pH range of 3.8–4.8 was found at which the 13 available acids were separated among themselves (ESM Fig. S6). For indirect detection, this pH value can be obtained using benzoic BGE ($pK_a^0 = 4.203$). Using DMES and varying the pH of this BGE within the range of 3.8–4.8 in increments of 0.1, the pH values (3.8, 4.0, 4.1 4.2, and 4.6) were found at which the 13 available acids were separated from other acids with the resolution exceeding 1. Then, using DMES and these pH values, the pH value of 4.1 was found at which the 28 acids were best separated. As shown in Fig. 4a, b, most analytes were separated with $R > 1$ except for the glucuronic and gluconic acids (the mobilities were 18.5 and $19.0 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}, respectively) and carboxymethyl cysteine and lactic acid (the mobilities were about $23 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}). A well-known rule states that the peaks will be the narrowest when the electrophoretic mobility of the BGE co-ions is close to the electrophoretic mobility of the analyte. Therefore, the optimal BGE is the m-nitrobenzoic buffer solution ($pK_a^0 = 3.493$) with pH 4.1 because the effective mobility of nitrobenzoate at this pH is $25 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} while the mobility of benzoate is $14 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} ($I = 2$ mM).

Figure 4c, d shows the electropherograms of the herbal medicine obtained using the m-nitrobenzoic and glycolic BGEs with pH 4.1 (indirect and direct detection, respectively). With the direct detection at −20 kV, no peak is observed; while with the direct detection at +30 kV, a wide peak appears being the same as that described for the formic BGE, pH 7.8. With the indirect detection, 17 peaks are observed. Table 2 shows their effective mobilities and potential analytes corresponding to these mobilities.

As shown in Fig. 4c, the peak of gluconic acid interferes with the peak of an unidentified compound. To separate the peaks, the electropherograms were recorded using m-nitrobenzoic BGEs at pH within the range of 3.8–4.2 in increments of 0.1. As illustrated in Fig. 4e, the peak of gluconic acid is separated from others peaks at pH of 3.9. Table S5 shows the concentrations of the identified acids.

Then, optimization of separation of the identified acids and possible compounds was carried out, whose electrophoretic mobilities with the pH 7.8 close to zero value (−1

$\pm 1\ m^2\ V^{-1}\ s^{-1}$), but at higher pH values it is >>0. In this case, it is convenient to find the conditions under which the unidentified acids appear in the electropherograms before the identified acids. The direct and indirect detection showed that the compounds were not present in the herbal medicine in a noticeable amount, apart from carbohydrates whose separation can be optimized at the pH of about 12 if necessary (see ESM Tables S6, S7 and Figs. S7 and S8).

Thus, using the strategy allows us to carry out the non-target anionic analysis of the sample with unknown composition by CE-UV. In contrast to usual analysis by CE based on a single BGE for the determination of a limited number of anions, this approach uses a number of BGEs allowing one to separate and quantify all the preliminarily identified analytes.

Conclusions

The strategy for non-target ionic analysis using CE-UV has been suggested and tested. The strategy is based on preliminary identification of analytes and further optimization of the conditions for their separation using the calculated values of the effective mobilities and peak widths in terms of electrophoretic mobility. This strategy is unique for the CE technique due to ability to readily change the effective electrophoretic mobilities, which is simply implemented by changing the BGE composition. The approach is more suited to the samples with not very complex composition, such as environmental water and precipitation samples, process liquors, somewhat vegetable extracts, biological fluids, food, etc. for the determination of widespread compounds capable of forming ionic species.

It is obvious that the conventional techniques for non-target analysis cannot entirely be replaced by CE-UV. Nevertheless, this technique allows one to obtain advantageous information because the analysis is carried out without sample heating, which is important for the determination of compounds suffering from low volatility or low thermal stability such as bacterial endotoxins [43], many pesticides [44], inorganic compounds [45], etc. The other advantage of the suggested approach is that, in CE-UV, the choice of the detection mode (direct or indirect) and optimization of separation conditions can allow the interfering effect of non-separated substances and sample matrix to be avoided. With the MS detection, this interfering effect is a serious problem, specifically in high-performance liquid chromatography with electrospray ionization MS detection [46]. The others modes of MS detection have limitations too. The hybrid Orbitrap has low detection sensitivity and suffers from false negatives; triple-quadrupole and hybrid linear ion-trap triple-quadrupole MS–MS suffer from many false negatives and very high time consumption [47]. CE–MS is still insufficient robust [48].

For the samples with complex composition, the suggested approach can be used together with other techniques. It was shown that in order to characterize the complex samples consisting of components with different volatility and polarity, it is necessary to use the combination of several techniques [49], and results of CE-UV can be useful because of specificity of the method. For example, Hurtado-Fernández et al., when analyzing avocado fruit by CE-UV, carried out the identification and quantification of some more phenolic acids unidentified by ultra-high performance liquid chromatography with MS detection [50]. The CE-UV can be useful as an additional method to CE–MS because in many cases the latter does not allow the determination of the ions with high mobility due to the negative influence of EOF [51]. In addition, this approach allows the use of buffer solutions close to physiological conditions that it is important to determine native (active) protein forms in pharmaceutical products [52]. The ElphoSeparation tool set developed can be also used for optimization of separation of known analytes by CE with any detection method and finding what widespread substances can interfere with the analytes under study at a specified BGE composition.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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