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Biomedical applications of capillary electrophoresis

L A Kartsova, E A Bessonova

Institute of Chemistry, Saint Petersburg State University
Universitetskiy prosp. 26, Peterhof, 198504 Saint Petersburg, Russian Federation

The review deals with modern analytical approaches used in capillary electrophoresis for solving medical and biological problems: search for biomarkers of various diseases and rapid diagnosis based on characteristic profiles of biologically active compounds by capillary electrophoresis with mass spectrometric detection; monitoring of the residual drugs in biological fluids for evaluating the efficiency of drug therapy; testing of the enantiomeric purity of pharmaceutical products; the use of novel materials as components of stationary and pseudo-stationary phases in capillary electrophoresis and capillary electrochromatography to increase the selectivity of separation of components of complex matrices; and identification of various on-line preconcentration techniques to reduce the detection limits of biologically active analytes. A topical trend in capillary electrophoresis required in clinical practice, *viz*., the design of microfluidic systems, is discussed.
The bibliography includes 173 references.

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I. Introduction

Electrophoresis as a method for separation of ionic analytes was proposed in the 1930s by Swedish biochemist Arne Tiselium, professor at the Uppsala University,[1–3] who separated blood serum proteins (α-, β- and γ-globulins) in a buffer solution upon application of an electric field. At the end of the 20th century, owing primarily to the studies of Hjertén,[4] Jornenson and Lukacs[5,6] and Terabe,[7] a new analytical method was developed called capillary electrophoresis (CE). The essence of the CE is the separation of components of complex mixtures present in the buffer electrolyte that fills a quartz capillary *via* voltage application and the detection of the components in a liquid flow. There exists a number of modifications of the CE technique.

Capillary zone electrophoresis (CZE) is based on the difference in the electrophoretic mobility of charged particles in aqueous or non-aqueous electrolytes.

Micellar electrokinetic chromatography (MEKC) serves for the separation of ionic and neutral compounds by using micelle-forming surfactants. So-called pseudo-stationary phase is formed and analytes are distributed between the micelles and the buffer electrolyte depending on their hydrophobicity.

Microemulsion electrokinetic chromatography (MEEKC) is based on a similar separation principle: analytes are distributed between hydrophobic negatively charged microemulsion drops and the buffer.

Capillary gel electrophoresis (CGE), which is mainly used for biopolymer separation is accomplished in a capillary filled with a gel (agarose, acrylamide and so on). The electrophoretic mobility of biopolymers is affected by the type of porosity of the gel matrix. The molecules are separated according to their sizes.

Affinity capillary electrophoresis (ACE), based on the selective interaction of proteins with particular ligands, finds increasing use for the separation of proteins. This method is utilized to study the antigen – antibody, protein – drug, cell – virus, virus – neutralizing antibody or drug interactions and other types of interaction.

L A Kartsova Doctor of Chemical Sciences, Professor of the Chair of Organic Chemistry at the Institute of Chemistry, SPbSU.
Telephone: +7(812)428 – 4044, e-mail: kartsova@gmail.com
E A Bessonova. Candidate of Chemical Sciences, Assistant Professor of the same Chair.
Telephone: +7(812)428 – 4044, e-mail: bessonova.elena.a@gmail.com
Current research interests of the authors: high performance liquid chromatography, gas chromatography, capillary electrophoresis, capillary electrochromatography, micellar electrokinetic chromatography, gas chromatography/mass spectrometry; analysis of biological objects and biologically active compounds, sample preparation, complexation and molecular recognition in chromatography and capillary electrophoresis, macrocycles for selective separation.

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Capillary electrochromatography (CEC) is a hybrid method combining the principles of capillary electrophoresis and high performance liquid chromatography (HPLC). The mobile phase migration through a capillary column is induced by electric field due to the electro-osmosis effect, the analytes being separated due to both different electrophoretic mobilities and different interactions with the stationary phase. The range of objects analyzed by this method includes inorganic and organic ions, neutral organic molecules, peptides, proteins, nucleic acids and so on.

Currently, capillary electrophoresis is among the most promising and effective methods for the separation and analysis of complex mixtures finding more and more extensive use. The method is fast and requires only microvolumes of the analyte solution. In addition, the absence of a column with a solid sorbent eliminates the problem of sorbent ageing; also, almost no organic solvents are needed.

The zone and micellar CE versions and capillary electrochromatography are used most widely.

II. Capillary electrophoresis for solving medical and biological problems

Medical and biological research predominates among CE applications. This includes rapid diagnosis of some diseases (microalbuminuria, Parkinson's and Alzheimer's diseases, pheochromocytoma, neuroblastoma, urinary stone disease, *etc.*)[8,9] based on characteristic profiles of biologically active compounds, monitoring of residual drugs in biological fluids for estimating the efficiency of drug therapy,[10,11] determination of the enantiomeric purity of pharmaceutical products[12,13] and the search for biomarkers of various diseases.[14,15] Quite a number of publications are devoted to chiral electrophoretic separation of pharmaceutical agents including non-steroidal anti-inflammatory drugs.[16–21] The chiral selectors used most often in CE include cyclodextrins (CD) and their charged derivatives,[16,22–26] crown ethers,[27,28] macrocyclic antibiotics,[29,30] copper(II) complexes with L-amino acids (L-proline, L-hydroxyproline), proteins (albumin, avidin), calixarenes, chiral surfactants and microemulsions[31–36] and molecular micelles.[37]

This is exemplified in Fig. 1, which presents electropherogram of the enantiomers of amphetamine and related compounds with neutral 2-hydroxypropyl-β-CD in the working buffer.[38] It was noted that anionic CD (sulfate, sulfonate and carboxylate derivatives) ensure higher selectivity of separation of basic and neutral analytes.[24,39–41] Successful separation of the venlafaxine antidepressant drug and its metabolites into enantiomers using sulfo-γ-CD was reported.[42]

It was shown that unique opportunities for the chiral separation are provided by using mixed selectors (Fig. 2).[43] Also, the search for new chiral agents is in progress.

Examples of natural micelle-forming chiral compounds are bile acids, which form anionic micelles.[44–46] The simultaneous use of two selectors — β-CD and chiral taurocholate micelles[47] — provided separation of enantiomers of fluorescent amino acid derivatives using a 15 mm-long quartz capillary in only 7 s.

The affinity of hydrophobic biologically active analytes to micellar pseudo-stationary phases is controlled by adding organic solvents, which change the micellization conditions. A reasonable solution to this problem is transition from micelles and vesicles formed upon self-assembly of small molecules to large macromolecules, which can also act as a pseudo-stationary phase. This approach has been implemented in drug detection.[48] The polymeric micellar selector has a minor effect on the exchange kinetics, while providing the possibility to control the overall time of analysis.[49]

New chiral microemulsions for separating the enantiomers of ephedrine derivatives having a weak psychotropic action have been developed.[50,51] The application of these emulsions reduces the assay time and increases its efficiency compared with micellar systems containing a similar selector.[52]

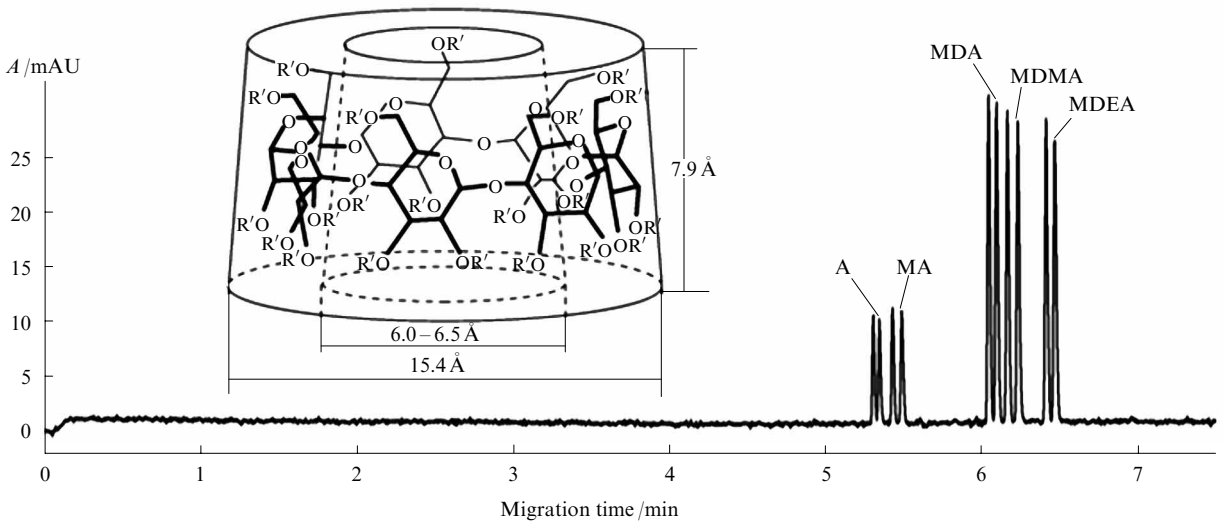


Figure 1. Electropherogram of the separated enantiomers of pharmaceutical drugs with β-CD as the chiral selector.[38]
A is amphetamine, MA is methamphetamine, MDA is 3,4-methylenedioxyamphetamine, MDMA is 3,4-methylenedioxymethamphetamine, MDEA is 3,4-methylenedioxyethylamphetamine. Here and below *A* is absorbance, AU is the absorbance unit, mAu = AU × 10^{-3}.

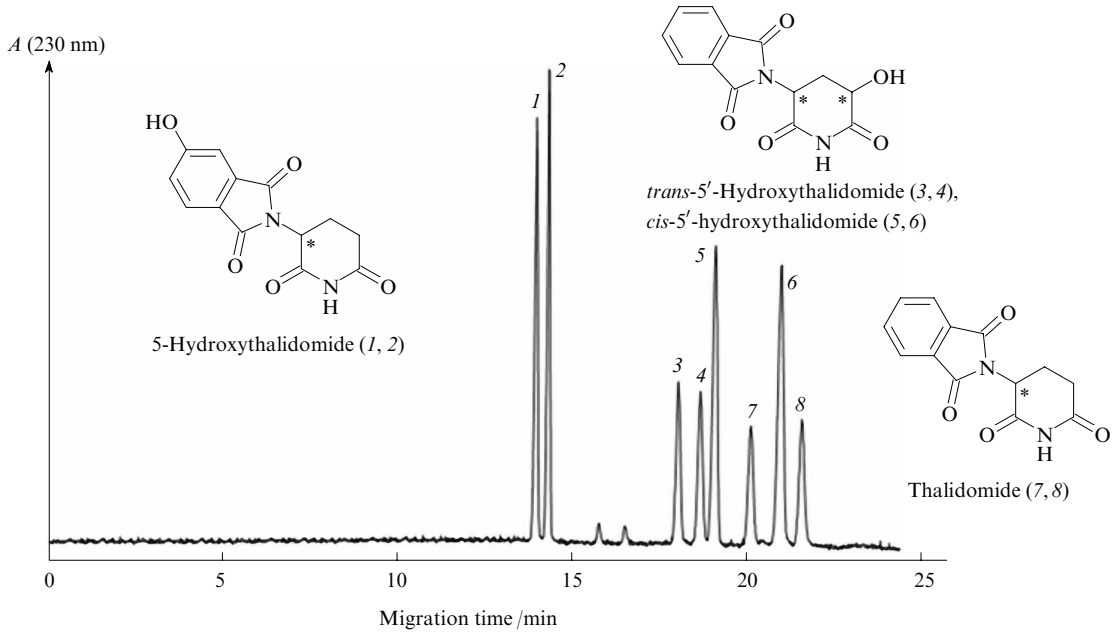
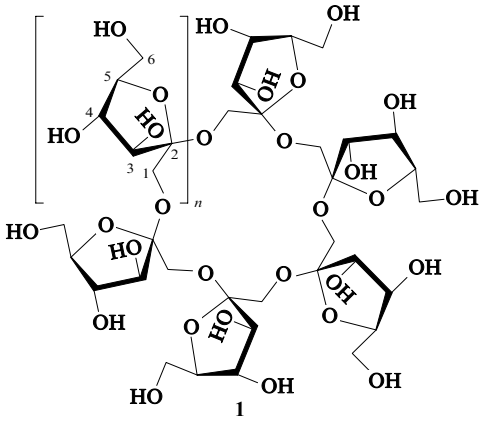


Figure 2. Separation of thalidomide and hydroxylated thalidomide enantiomers with two selectors, sulfobutyl(4.0)-β-CD and β-CD, present simultaneously in the ammonium acetate buffer solution.[43]
The capillary is coated by polyacrylamide.

Structures 1



1

Armstrong and co-workers[53] proposed a number of neutral and sulfonated cyclofructans (Structures **1**) for the CE separation of enantiomers. They are not inferior in efficiency to cyclodextrins but provide higher enantioselectivity of separation for those classes of analytes that are poorly separated by using cyclodextrins.

Figure 3 illustrates the advantage of the CEC method over capillary HPLC in the separation of enantiomers of substituted β-amino alcohol on the same chiral cation exchange sorbent (Fig. 4):[54] the CEC method is more efficient and the time of assay is, in this case, twice shorter. Polar organic solvents having high dielectric permittivity and low viscosity served as the mobile phase.

The enantioselectivity of chiral phases in organic solvents is known to substantially differ from that in aqueous eluents. In a medium with high acetonitrile content, hydrophobic interactions decrease and the role of ionic interactions is enhanced. Non-aqueous capillary

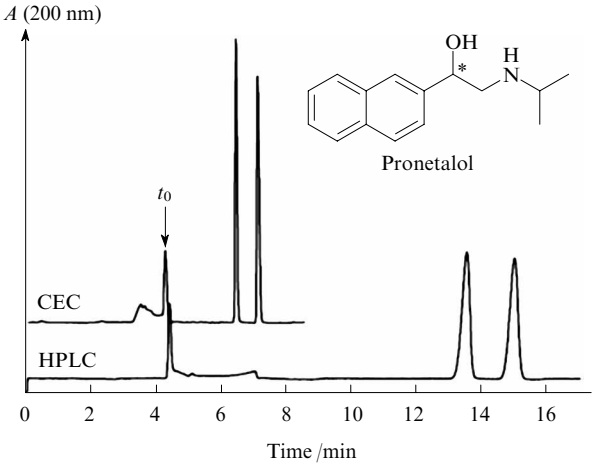


Figure 3. Separation of pronetalol enantiomers by CEC and HPLC on a chiral cation exchange sorbent.[54]
Electrolyte in CEC: acetonitrile–methanol (80 : 20 v/v), 25 mM HCO_2H and 12.5 mM 2-aminobutanol; t_0 is the time of appearance of the peak of a non-sorbed component.

electrophoresis is suitable for successful separation of highly hydrophobic samples insoluble in aqueous solutions.

New scope of analytical applications of capillary electrophoresis for solving medical and biological problems is opened up by using the ligand exchange principle (ligand exchange capillary electrophoresis, LECE). Particularly this method with a copper–ammonia buffer was used[55] for simultaneous CE determination of both glucose as an absorbing copper complex (direct detection) and physiologically significant K^+ and Na^+ cations (indirect detection) in

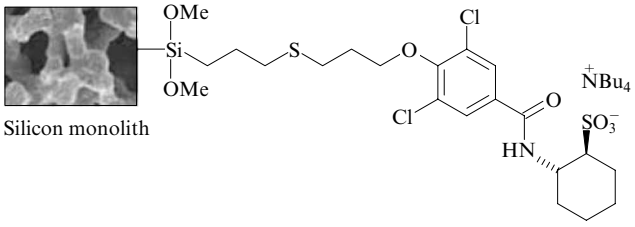


Figure 4. Chiral cation exchanger: a silicon monolith functionalized by the chiral selector, *trans*-(1*S*,2*S*)-2-(*N*-4-allyloxy-3,5-dichlorobenzoyl)aminocyclohexanesulfonic acid.[54]

blood serum of kidney failure patients before and after dialysis (Fig. 5).

Examination of survey papers published in the last decade attests to increasing interest of analytical chemists in the CE technique.[8, 56–58] Apart from the studies that make use of traditional spectrophotometric detection, a growing fraction of studies are devoted to the use of electrochemical, laser-induced fluorescence (LIF) and chemiluminescence detectors.[8, 59–63] These types of detectors provided a more than 1000-fold decrease in the limits of detection of diagnostically important analytes.

The active demand for CE for solving clinical and medical problems is due to its higher (compared to HPLC) efficiency, wide range of possible analytes, simple sample preparation and other advantages. The diversity of method

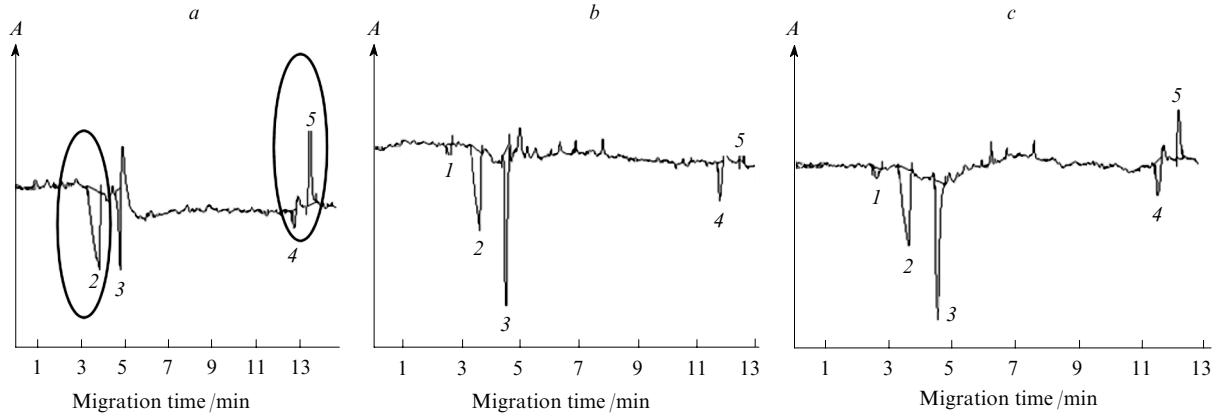


Figure 5. Simultaneous LECE-UV determination of sugars and inorganic cations.[55]
(*a*) Dialysing solution; (*b*) blood serum before dialysis; (*c*) blood serum after dialysis. Conditions: Agilent CE system; capillary: L_{eff}/L_{tot} = 50/60 cm (L_{eff} and L_{tot} are the effective and total lengths, respectively), inner diameter: 50 μm; diode matrix detector: 200–600 nm; working electrolyte: 1 mM Cu^{2+}, 175 mM NH_3; 30 mbar, 10 s; voltage 25 kV. Peaks: (*1*) K^+, (*2*) Na^+, (*3*) system peak, (*4*) DMSO as electroosmotic flow (EOF) marker, (*5*) glucose.

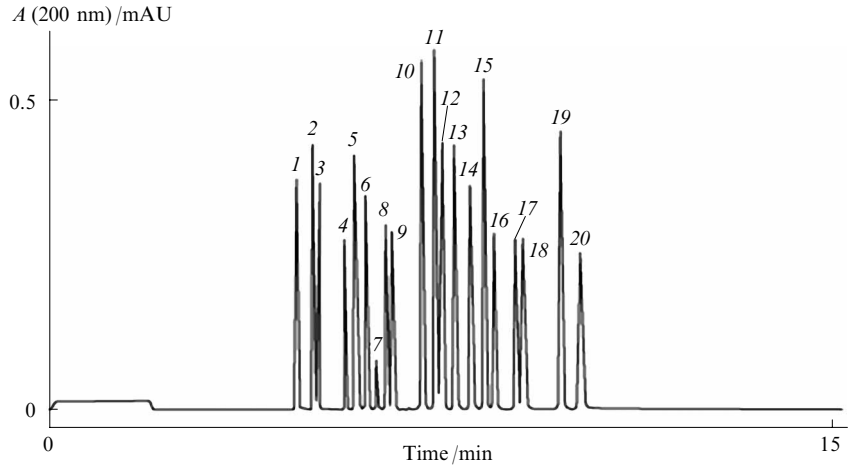


Figure 6. CZE electropherogram of a mixture of 20 important drugs.[69]
Quartz capillary: 60 cm × 50 μm; 100 mM phosphate buffer, pH 2.4; voltage 25 kV (*1*) pheniramine, (*2*) chlorpheniramine, (*3*) brompheniramine, (*4*) anileridine, (*5*) amphetamine, (*6*) methamphetamine, (*7*) trifluoperazine, (*8*) pseudoephedrine, (*9*) ephedrine, (*10*) methoxamine (internal standard), (*11*) diphenhydramine, (*12*) dextromethorphan, (*13*) codeine, (*14*) hydroxyzine, (*15*) salbutamol, (*16*) metoprolol, (*17*) trazodone, (*18*) haloperidol, (*19*) verapamil, (*20*) loperamide.

alternatives in combination with various on-line preconcentration regimes promotes successful application of CE in clinical laboratories to separate small ions, neutral molecules and biopolymers. Most of the published electrophoretic studies were performed using capillary zone electrophoresis, micellar electrokinetic chromatography and capillary electrochromatography and were largely devoted to problems of metabolomics and pharmacokinetics.[8, 10, 59, 64–68]

Noteworthy are several advantages of CE over HPLC for determination of residual drug amounts in biological fluids. High polarity of major pharmaceutical products hampers HPLC analysis for these compounds, as opposed to electrophoretic assay. Figure 6 presents the results of CE separation of 20 significant drugs (alkaloids, β-blockers, antidepressant drugs and so on).[11, 69, 70] As a working electrolyte, phosphate buffer (pH < 7) was used: under these conditions, the capillary surface had a slight negative charge and the amino groups of analytes being separated were protonated. These conditions of electrophoretic analysis made it possible to compose screening protocols for detection of pharmaceutical agents and narcotic drugs in the forensic toxicological analysis.

However, the CE method has also a number of limitations including insufficient separation selectivity and low reproducibility of the migration parameters; low concentration sensitivity of UV detection, which is inapplicable for the analysis of biological objects; and irreversible adsorption of biopolymers on the quartz capillary walls.

A priority task of the research in this field is to overcome the limitations of CE for medical diagnostic applications, while preserving its benefits. A lot has already been achieved along this route.

III. New stationary and pseudo-stationary phases for capillary electrophoresis and capillary electrochromatography

The use of polymeric surfactants,[71] micellar polymers,[72, 73] dendrimers and hyperbranched polymers[74–76] as working buffer components can serve to control the separation selectivity for MEKC determination of analytes.

The irreversible adsorption of proteins on the quartz capillary walls, resulting in irreproducibility of migration parameters and baseline instability, is eliminated by the formation of dynamic or covalent coating of the quartz capillary. Attractive prospects in this area are opened by using hyperbranched polymers as stationary and pseudo-stationary phases; this is due to specific physicochemical characteristics of these materials: stable micelle-like structure; the presence of intramolecular cavities, which account for the possible formation of host–guest complexes with the analyte; and numerous terminal functional groups.[77–83]

A similar function, that is, preventing the sorption of proteins during electrophoretic separation, can also be performed by polymeric ionic liquids (IL) introduced into the buffer electrolyte.[84–86] For instance, poly(3-butyl-1-vinylimidazolium) bromide dynamically modifies the quartz capillary walls, inducing electroosmotic flow reversal and preventing the adsorption of proteins.[87] High efficiency (up to 10^5 t.p. m^{-1}, t.p. is the theoretical plate) and reproducibility of migration parameters of biopolymers were achieved (Fig. 7).

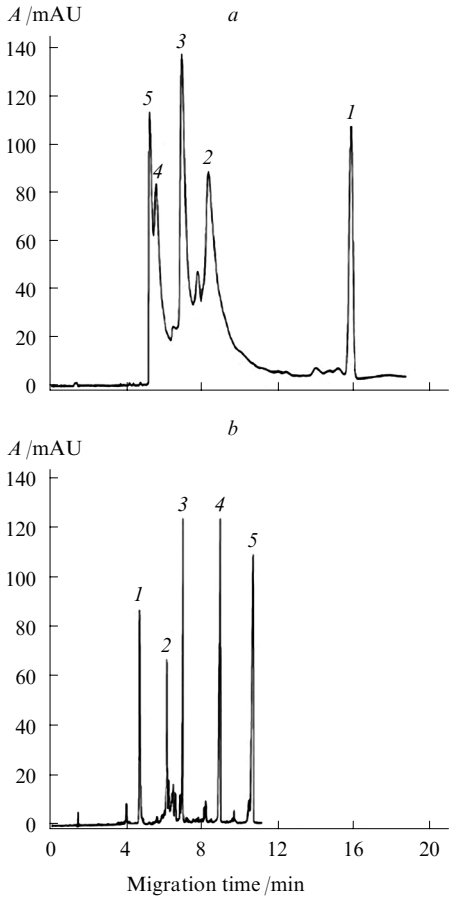


Figure 7. CZE electropherogram of a model mixture of major proteins without ionic liquid (*a*) and with 0.003 mass % poly(3-butyl-1-vinylimidazolium) bromide added to the buffer electrolyte (60 mM sodium phosphate, pH 4.0) (*b*).[87]
CE conditions: voltage 20 kV (*a*) and −20 kV (*b*).
(*1*) DMSO; (*2*) α-chemotrypsinogen A, (*3*) ribonuclease A, (*4*) cytochrome *c*, (*5*) lysozyme.

Yanes *et al.*[88] used the addition of ionic liquids containing 1-alkyl-3-methylimidazolium cation to the buffer electrolyte for the separation of polyphenol type antioxidants. It was found that IL modify the quartz capillary walls and thus reverse the EOF and increase the separation selectivity. In addition, IL interact with analytes *via* π–π-bonding to form positively charged complexes, which migrate towards the cathode (Fig. 8).

Xiong *et al.*[89] employed carbon nanotubes (CNT), which have unique electrical and optical properties and large surface area, as pseudo-stationary phases for MEKC and stationary phases and chiral selectors for CEC assays. Since hydrophobic CNT are poorly soluble in aqueous and polar organic media, which restricts their application for CE, various sorts of nanotube modification were proposed: the introduction of functional groups on the CNT surface and modification with cyclodextrins, ionic liquids and so on.[90–96]

Figure 9 illustrates an example of using carboxylated multiwalled nanotubes as a pseudo-stationary phase for electrophoretic determination of urinary modified purine

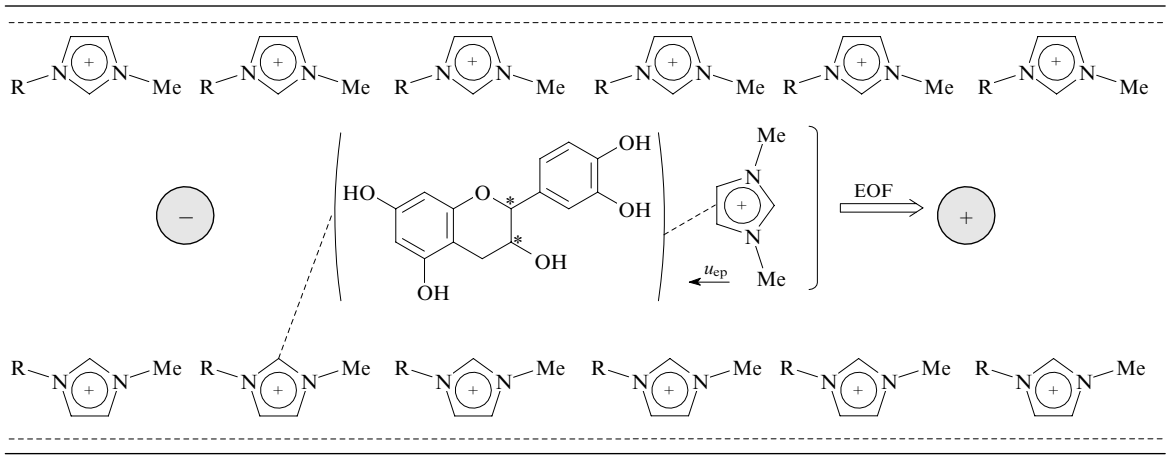


Figure 8. Illustration of the electrophoretic separation of polyphenols using ionic liquids with 1-alkyl-3-methylimidazolium cations.[88] u_{ep} is the electrophoretic velocity.

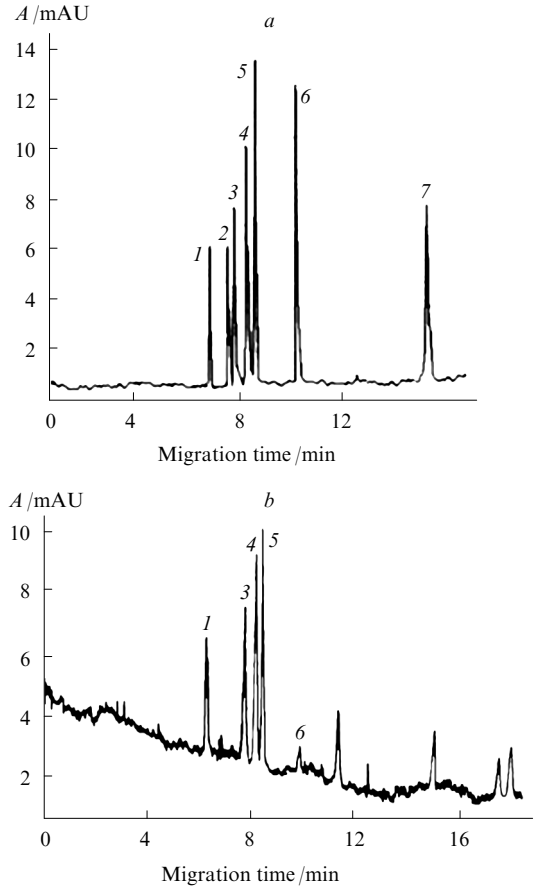


Figure 9. Electropherograms of a mixture of purine and pyrimidine base standards (*a*) and RNA hydrolysate (*b*) obtained with addition of 8.0×10^{-5} g ml^{-1} of carboxylated multiwalled nanotubes to the buffer electrolyte.[89]
Buffer electrolyte: 23 mM sodium tetraborate, pH 9.2; voltage 18 kV; absorption at 254 nm.
(*1*) Cytosine, (2) thymine, (*3*) adenine, (*4*) guanine, (*5*) uracil, (*6*) hypoxanthine, (*7*) 8-azaadenine.

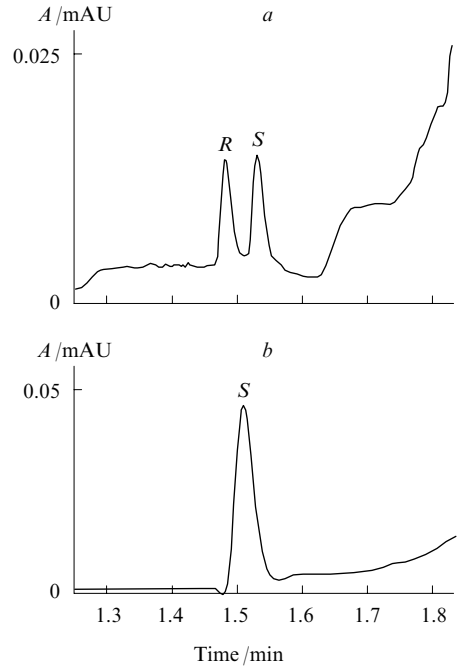


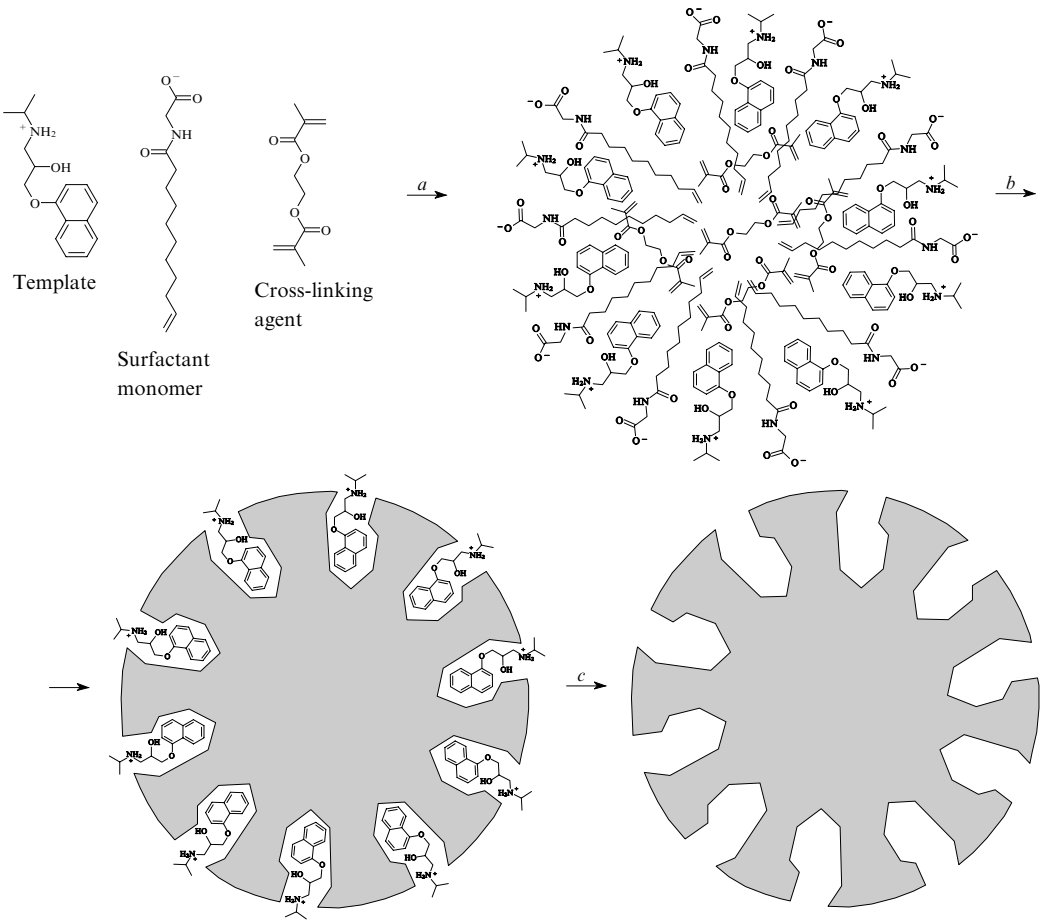
Figure 10. Electropherograms of separated *R*,*S*-enantiomers of propranolol (*a*) and *S*-propranolol (*b*) using nanoparticles obtained according to Scheme 1.[97]

and pyrimidine bases, which are biomarkers of some types of cancer.[89]

Novel materials for the CEC separation of biologically active analytes include also 'molecular imprinted' polymers. Scheme 1 illustrates the synthesis of imprinted nanoparticles, and Fig. 10 shows how they are used in CEC for the separation of enatiomers of the propranolol β-blocker.[97]

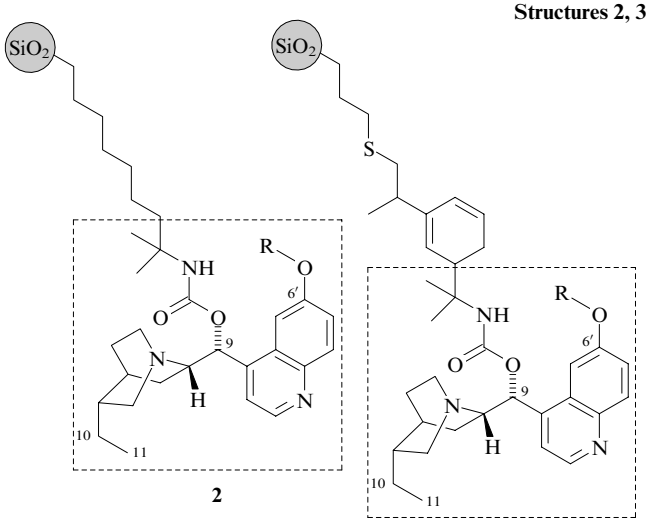
For maintaining stable EOF during capillary electrochromatography, Lindner sorbents with alkaloid bases (quinine, cinchonidine) have been proposed[98] (Structures **2** and **3**). The appropriate conformational pre-organization of chiral selector moieties — quinuclidine bicyclic structure, quinoline ring and carbamate group — is a critical param-

Scheme 1



(*a*) Formation of the pre-polymerization complex between the template and the surfactant monomer, (*b*) polymerization in the presence of the component, (*c*) template removal.

Structures 2, 3



2 **3**

R = Me, Bu^{t}CH$_2$

eter for stabilization of interactions in the chiral sorbent–enantiomer system, which may be through ion-pair and π–π interactions or hydrogen bonds.[99] In non-aqueous media, these chiral cation exchangers were efficiently used to separate the enantiomers of several dozens of biologically active compounds.[54, 100]

IV. On-line preconcentration methods in capillary electrophoresis for decreasing the detection limits of analytes

The contents of biologically active compounds in real objects is usually at the level of a few ng ml^{-1}. Development of electrophoretic on-line preconcentration techniques can help to decrease the detection limits of analytes,[101–109] which is of prime importance for practical implementation of CE in clinical medicine. Apart from the traditional ways of in-capillary preconcentration used for CE (stacking, sweeping, dynamic pH junction, isotachophoresis), which are related to difference between the electrolyte properties in the sample and working buffer zones, novel strategies combining several preconcentration mechanisms were developed and implemented: the electrical conductivity difference between the sample solution and the buffer electrolyte, distribution between the micelles, complexation with working buffer components, pH gradient.

One example is the combination of transient isotachophoresis (t-ITP) and electrostacking mechanisms proposed for the preconcentration of ionogenic analytes.[110] In a quartz capillary filled with a supporting electrolyte, a leading electrolyte was added. Then electrokinetic injection of a large volume of the sample dissolved in a low-conducting matrix was performed followed by hydrodynamic injec-

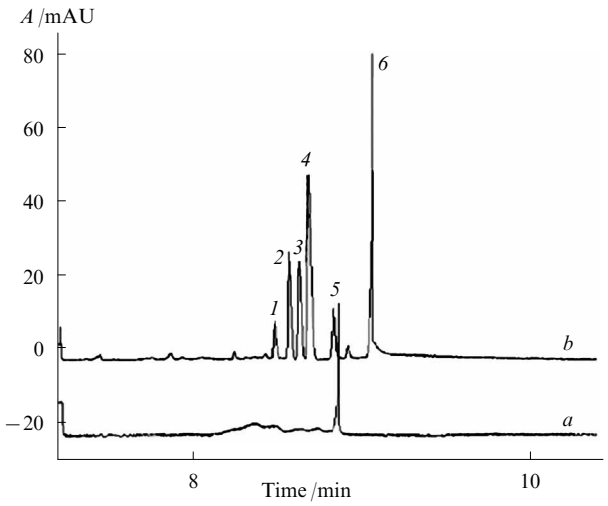


Figure 11. Electropherograms of non-steroidal anti-inflammatory drugs without preconcentration (*a*) and after on-line preconcentration (*b*).[111]
(*1*) Ibuprofen, (*2*) fenoprofen, (*3*) ketoprofen, (*4*) naproxen, (*5*) diclofenac, (*6*) indomethacin, (*7*) diflunisal.

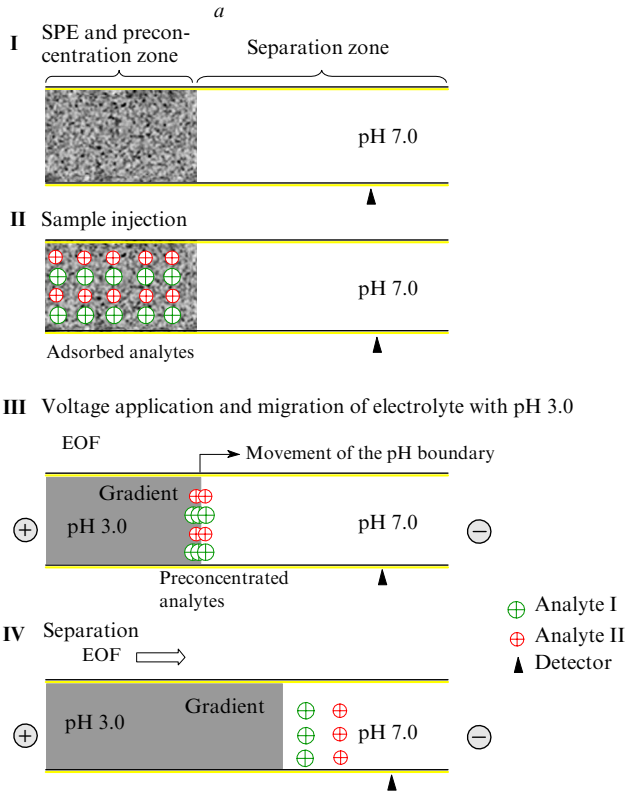
tion of the terminating buffer. In the initial instant of analysis, so-called transient isotachophoresis occurs. The application of this approach for determination of non-steroidal anti-inflammatory drugs provided preconcentration factors of up to 10^5 (Fig. 11).[111]

Of interest is yet another version of on-line preconcentration, called analyte focusing by micelle collapse, has been proposed for preconcentration of neutral and charged analytes.[112–118]

Classical sweeping is based on preconcentration of analytes by the pseudo-stationary phase formed by addition of a cationic or anionic surfactant (most often, sodium dodecyl sulfate) into the buffer electrolyte in a concentration exceeding the critical micelle concentration. Micelles penetrate into the micelle-free sample zone.[119, 120] Preconcentration occurs due to the distribution of analyte components between the working buffer and the pseudo-stationary micellar phase and various complexation processes between the analyte and buffer system components. The preconcentration factors achievable in this case range from 80 to $5 \times 10^3 - 10^4$.

The anlyte focusing by micelle collapse differs considerably from the traditional in-capillary preconcentration protocols: as opposed to sweeping, in this case, the sample solution contains micelles, while the working buffer is devoid of micelles; as opposed to stacking, in this case, the conductivity of the buffer electrolyte is lower than the conductivity of the sample solution. Micelles transfer neutral analytes into a quartz capillary. At the buffer electrolyte boundary, the concentration drops below the critical micelle concentration, which leads to destruction of the micelles and liberation of analytes. The repeated transfer and collapse of micelles results in analyte preconcentration. The attained preconcentration factors are $> 10^4$ (Ref. 121).

For dopamine, sample preparation (sorption preconcentration on a monolithic cation exchanger) followed by electrophoretic quantification of dopamine (30 ng ml^{-1}) in a biological fluid was described.[122] For this purpose, the capillary inlet was filled with a monolithic sorbent [poly(methacrylic acid-co-ethylene glycol dimethacrylate)]; upon elution from the sorbent, catecholamines were concentrated in the starting zone of the monolith due to electrostatic interactions and the pH gradient formed (Figs 12, 13).



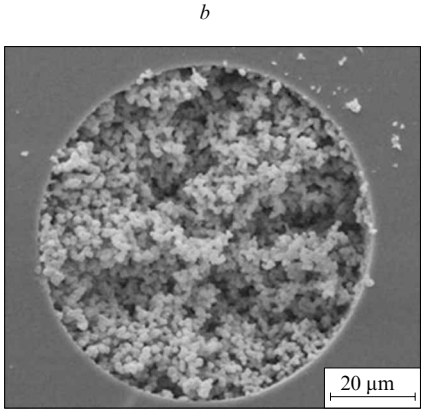


Figure 12. Scheme of solid-phase extraction (in-line-SPE) of dopamine using a monolithic cation exchanger under conditions of capillary electrophoresis (*a*) and SEM image of the cross-section of the capillary inlet (75 μm internal diameter) filled with a monolithic sorbent, poly(methacrylic acid-co-ethylene glycol dimethacrylate) (2700 magnification).[123]

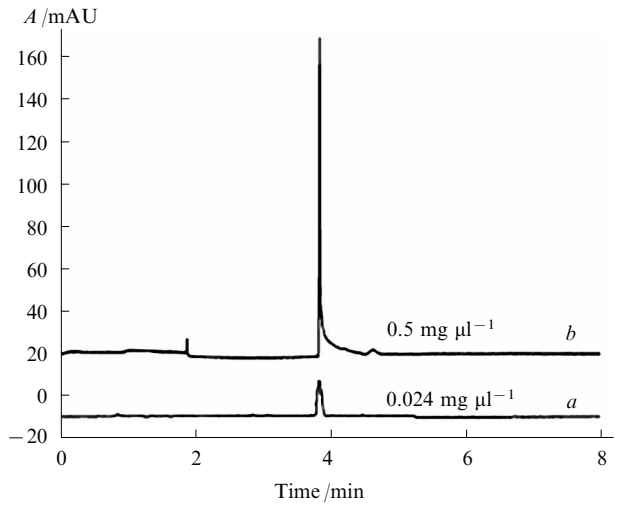


Figure 13. CZE electropherograms of a solution of dopamine standard without preconcentration (*a*) and with preconcentration (in-line-SPE-CE) (*b*).[122]
CE: 36.5 cm long quartz capillary (inner diameter 75 μm); 8 cm of the capillary is filled by the monolithic sorbent; working electrolytes: 6 mM phosphate buffer (pH 7); 12 mM phosphate buffer (pH 3); voltage 25 kV; UV detector at 200 nm.

The column is equilibrated by a phosphate buffer solution (pH 7) (**I**). Then hydrodynamic injection of the sample dissolved in the same buffer electrolyte (**II**) is performed. Catecholamines are adsorbed on the sorbent surface due to electrostatic interactions with the negatively charged methacrylate anions. Then the capillary ends are immersed into a buffer electrolyte with pH 3, which is lower than the methacrylic acid pK_a. This suppresses dissociation of methacrylic acid (**III**) and decreases the retention of the analytes. As the voltage is applied, a step pH gradient is formed, the analytes are desorbed and migrate to the buffer with higher pH, where they are again adsorbed on the monolithic sorbent *via* electrostatic forces. This cycle is repeated until the preconcentrated analytes are completely eluted from the monolithic sorbent. Then CZE separation of the preconcentrated zones is carried out (**IV**).

V. Capillary electrophoresis with mass spectrometric detection for identification of drugs and disease markers

Considerable prospects are opened up by employing mass spectrometry in capillary electrophoresis (CE-MS) for detection and identification of biologically active substances and drugs in natural objects.[123–127] Figure 14 illustrates the on-line coupling of a capillary electrophoresis system with a mass spectrometer. This combination gives a complex set of ~1000 mass spectra. The treated CE-MS data are represented as a 3D graph where the coordinates are time, molecular mass and analytical signal intensity.[128, 129]

Currently, two key technical solutions have been proposed for coupling a CE system with an electrospray ionization mass spectrometer, one involving and one not involving the sheath liquid, which markedly extended the analytical potential of the electrophoretic determination.[123, 124, 129, 130] In proteomics, the former version is more demanded.[126, 131–137] In this case, the electrical contact between CE and the ion source is provided by coaxial supply of a sheath liquid flow, which is mixed with the buffer electrolyte. However, the sheath liquid can reduce the efficiency, which results in sample smearing. This effect is mitigated by using counter-pressure at the capillary inlet. In the sheathless interfaces, the voltage needed for CE separation is applied directly to the capillary outlet. For this purpose, quartz capillaries with a metal deposited at the capillary end or metallic microelectrodes are used.[138] Although most of these interfaces provide a very high sensitivity, there are some limitations concerned with obtaining stable spraying, destruction of the metal coating with time, clogging of the capillary tip at the outlet. A new method has been proposed: the use of a capillary with a porous tip as the source of nanospraying;[128, 139] this was implemented for identification of

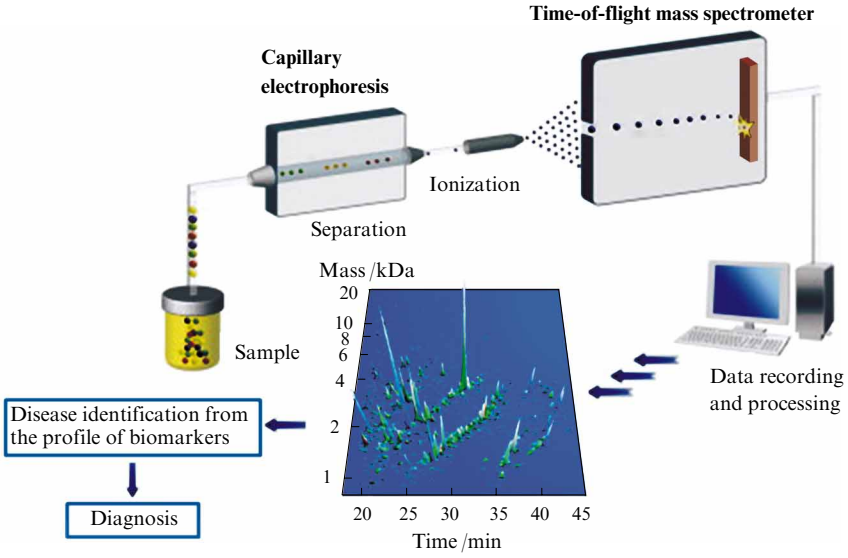


Figure 14. Diagram of on-line coupling of a capillary electrophoresis setup and a mass spectrometer.[128, 129]

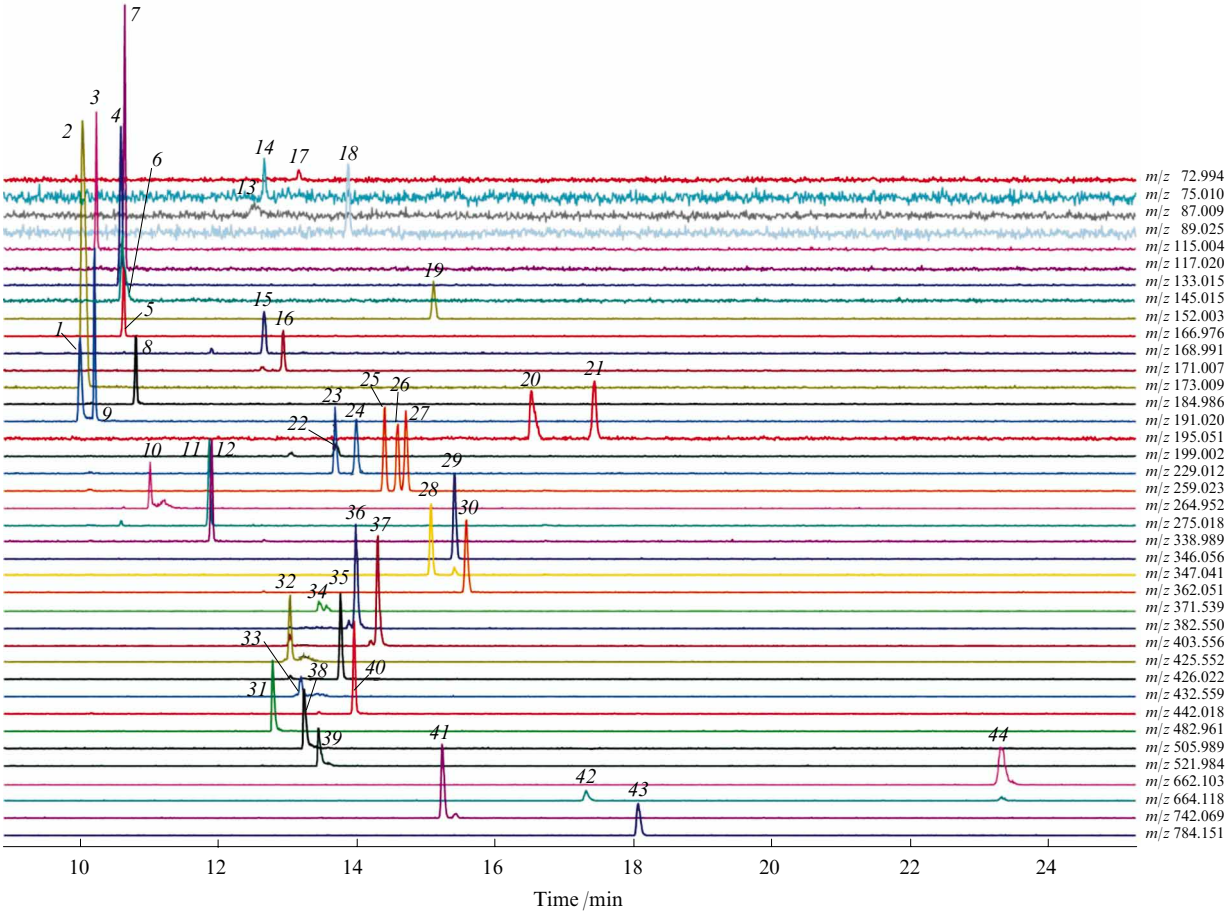


Figure 15. CE-MS electropherogram of a mixture of anionic metabolites (selected ion monitoring mode) with a time-of-flight analyzer and with the use of a platinum spray needle: phosphorylated saccharides, carboxylic acids, nucleotides, nicotinamide adenine dinucleotides and so on (10 μmol litre^{-1}).[143]
(*1*) Isocitrate, (*2*) *cis*-aconitate, (*3*) fumarate, (*4*) malate, (*5*) phosphoenolpyruvate, (*6*) 2-oxoglutarate, (*7*) succinate, (*8*) 3-phosphoglycerate, (*9*) citrate, (*10*) 2,3-diphosphoglycerate, (*11*) 6-phosphogluconate, (*12*) fructose 1,6-diphosphate, (*13*) pyruvate, (*14*) glycolate, (*15*) dihydroxyacetone phosphate, (*16*) glycerophosphate, (*17*) glyoxylate, (*18*) lactate, (*19*) cysteinesulfinate, (*20*) ^{13}C-MES (MES is 2-morpholinoethanesulfonic acid, internal standard), (*21*) gluconate, (*22*) erythrose 4-phosphate, (*23*) ribulose 5-phosphate, (*24*) ribose 5-phosphate, (*25*) glucose 1-phosphate, (*26*) fructose 6-phosphate, (*27*) glucose 6-phosphate, (*28*) IMP, (*29*) AMP, (*30*) GMP, (*31*) UTP, (*32*) malonyl-CoA, (*33*) succinyl-CoA, (*34*) NADPH, (*35*) ADP, (*36*) CoA, (*37*) acetyl-CoA, (*38*) ATP, (*39*) GTP, (*40*) GDP, (*41*) NADP, (*42*) NADH, (*43*) flavin adenine dinucleotide, (*44*) NAD.

proteins in complex mixtures and for separation of 'protein–protein' and 'protein–erythrocyte metal ion' complexes,[140, 141] for the analysis of the tryptic digest of albumin and peptide mixtures.[142] Higher stability of electrospraying, high sensitivity and good reproducibility of the results were noted.

Yet another way for increasing the sensitivity of CE-MS has been proposed.[143] The CE-MS determination of anions is considerably affected by the needle material. Usually, the spray needle is made of stainless steel; however, it is oxidized and corroded at the anode due to electrolysis processes. The resulting iron oxides plug the capillary tip, which shortens its service life. The formation of complexes between anionic analytes and iron oxides or nickel ions is also possible, which markedly decreases the sensitivity of detection of anions. The use of platinum spray needle prevents corrosion and metal oxidation. The sensitivity of determination of anionic metabolites was found to increase 63-fold; this is most pronounced for analytes with high chelating properties: carboxylic acids, nucleotides or coenzyme A (Fig. 15).† The limits of detection were 0.8–24 fmol.

Figure 16 shows the general block diagram of CE-MS applied for drug screening. In the clinical and forensic toxicology, drugs present in biological objects are determined in two stages. The first stage includes fast and selective drug screening in a sample using CE with electrospray ionization time-of-flight mass-spectrometric detection and on-line preconcentration. This reduces the detection limit down to ~2 ng ml^{-1}. The second stage comprises

† In Fig. 15: CoA is coenzyme A; AMP, ADP, ATP are adenosine mono-, adenosine di- and adenosine triphosphates; GMP, GDP, GTP are guanosine mono-, guanosine di- and guanosine triphosphate; IMP is inosine monophosphate; UTP is uridine triphosphate; NAD and NADH are nicotinamide adenine dinucleotide and its reduced form; NADP and NADPH are NAD phosphate and and its reduced form.

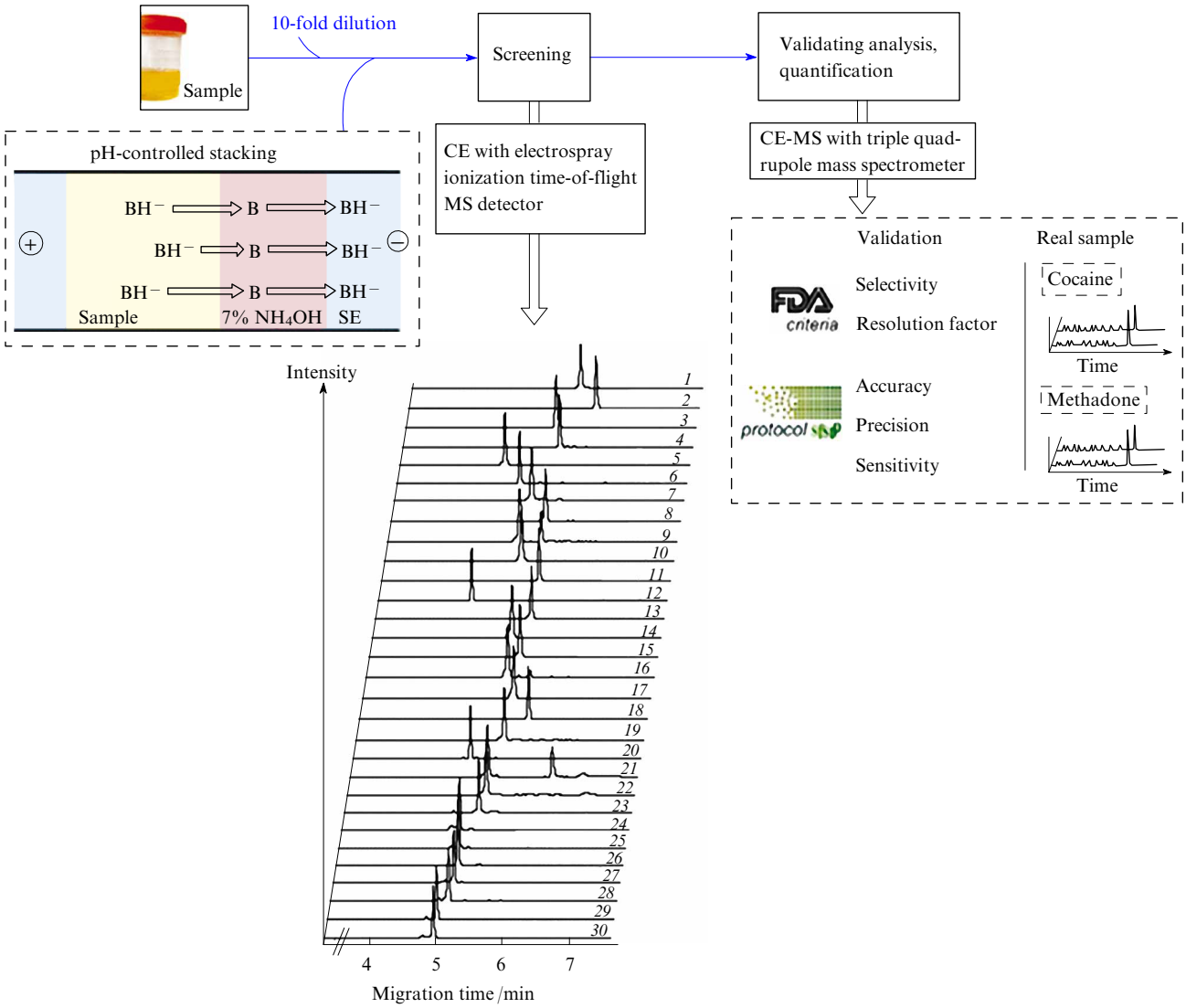


Figure 16. Diagram of two-stage CE-MS screening of drugs.[144]
SE is a supporting electrolyte; (*1*) D-propoxyphene, (*2*) fentanyl, (*3*) trimipramine, (*4*) metoprolol, (*5*) procaine, (*6*) ketamine, (*7*) 1,5-dimethyl-3,3-diphenyl-2-ethylidenepyrrolidine, (*8*) methadone, (*9*) pethidine, (*10*) dextromethorphan, (*11*) nalbuphine, (*12*) buprenorphine, (*13*) pholcodine, (*14*) codeine, (*15*) ethylmorphine, (*16*) morphine, (*17*) 6-monoacetylmorphine, (*18*) heroin, (*19*) cocaethylene, (*20*) anhydroecgonine methyl ester, (*21*) norcocaine and benzoylecgonine, (*22*) cocaine, (*23*) methylphenidate, (*24*) norephedrine, (*25*) ephedrine and pseudoephedrine, (*26*) 3,4-methylenedioxyethylamphetamine and *N*-methyl-1-(3,4-methylenedihydroxyphenyl)-2-butanamine, (*27*) 3,4-methylenedioxymethamphetamine, (*28*) 3,4-methylenedioxyamphetamine, (*29*) methamphetamine, (*30*) amphetamine.

independent validation of the results obtained and quantitative analysis by CE with a triple quadrupole mass spectrometer.[144]

In recent years, a key trend in practical clinical medicine is rapid diagnosis of various diseases based on characteristic electrophoretic profiles of biologically active compounds (metabolic and biochemical profiles; proteomic profile; urine profile; basic profile; and electrophoretic and chromatographic profiles). These studies form the research area called metabolomics — recording and examination of low-molecular metabolic profiles specific to processes occurring in living cells; treatment on these profiles provides an additional diagnostic criterion.[145] The CE-MS method is the subject of most publications devoted to the search for biomarkers of cardiovascular diseases,[146] diabetes mellitus (diabetic nephropathy),[147,148] kidney diseases,[86,149–151] Alzheimer's disease,[152] cancer, *etc.*,[153–158] using metabolic profiles.

The human urine and blood serum contain several hundred polypeptides — specific biomarkers of many diseases; these compounds are actively determined by CE-MS.[159–163] Figure 17 depicts an example of separation of a polypeptide mixture and the corresponding 3D electropherogram in the time–molecular mass–analytical signal intensity coordinates.

Mapping of peptides is universally used for protein identification and for determining protein characteristics. This is the first stage of quality control in the design of pharmaceutical products, which include treatment of the protein with an enzyme to obtain peptide fragments for the subsequent separation and detection by MS techniques. The CE-MS method (together with HPLC-MS) provides inde-

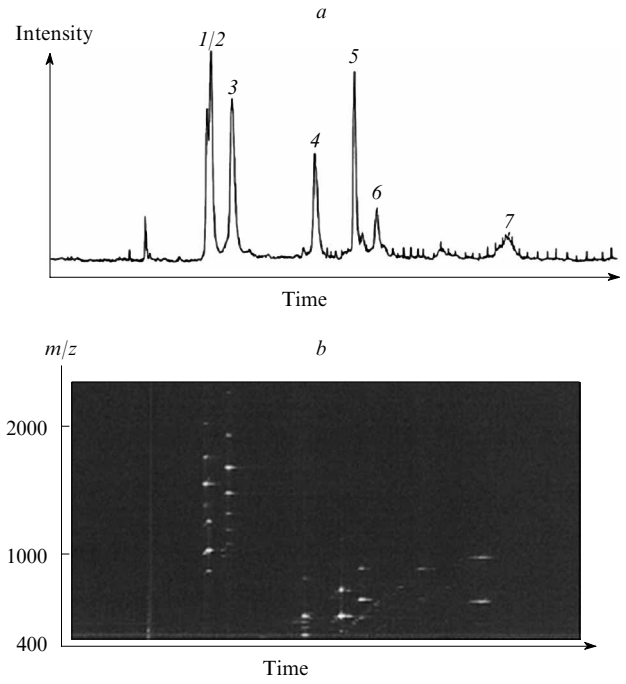


Figure 17. CE-MS separation of polypeptide standards (500 fmol).[163]
(*a*) Electropherogram of a mixture of polypeptide standards (total ion current); (*b*) the same electropherogram in the 3D format. (*1*) Aprotinin, (*2*) lysozyme, (*3*) ribonuclease, (*4*) REVQSKIGYGRQIIS (1732.96Da), (*5*) TGSLPYSHIGSRDQIIFMVGR (2333.19 Da), (*6*) ELMTGELPYSHINNRDQIIFMVGR (2832.41 Da), (*7*) GIVLYELMTGELPYSHIN (2048.03 Da).

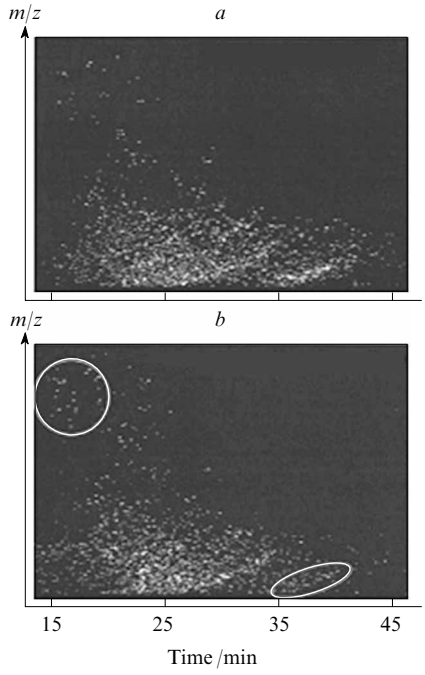


Figure 18. Electrophoretic polypeptide profile of urine in the health (*a*) and with diabetic nephropathy in which no polypeptide signals are present in the bottom right-hand corner of the electropherogram, while the polypeptide signals in the top left-hand corner increase (the areas are marked by an oval and a circle, respectively) (*b*).[163]

pendent additional information on the contents of diagnostic markers.

Determination of more than 100 polypeptides in biological samples in one assay cycle has been reported.[163]

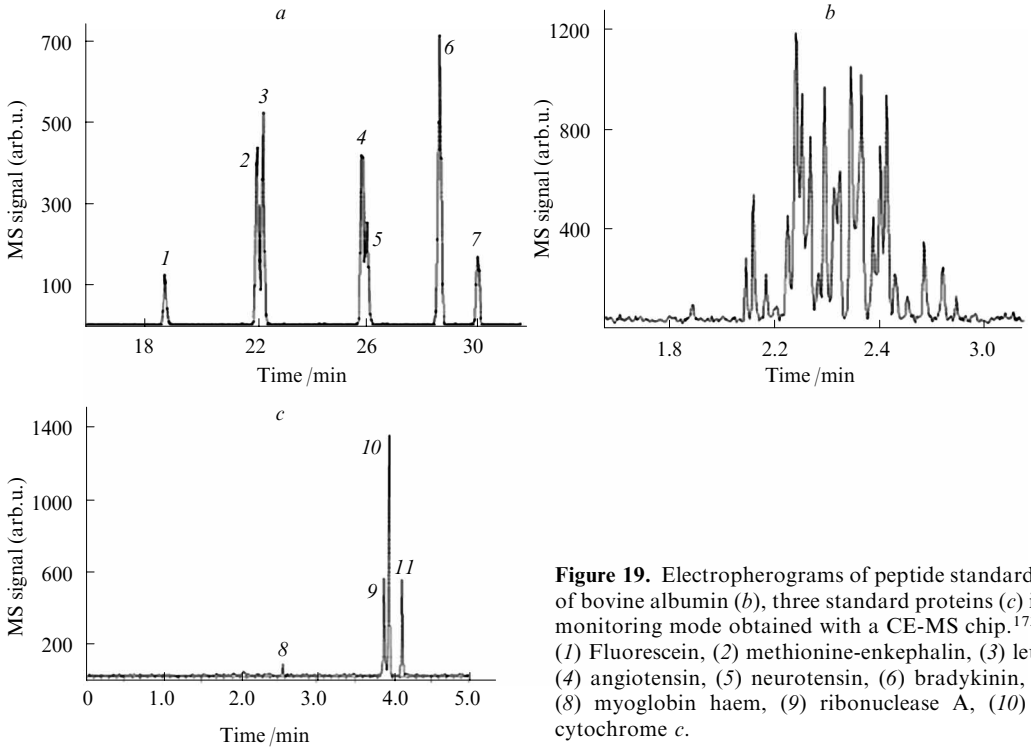


Figure 19. Electropherograms of peptide standards (*a*), hydrolysate of bovine albumin (*b*), three standard proteins (*c*) in the selected ion monitoring mode obtained with a CE-MS chip.[173]
(*1*) Fluorescein, (*2*) methionine-enkephalin, (*3*) leucine-enkephalin, (*4*) angiotensin, (*5*) neurotensin, (*6*) bradykinin, (*7*) thymopentin, (*8*) myoglobin haem, (*9*) ribonuclease A, (*10*) myoglobin, (*11*) cytochrome *c*.

Characteristic polypeptide profiles for 'health' and 'pathology' are being formed. It was noted that the ratio of particular polypeptides in urine and/or blood serum has a diagnostic value, indicating not only a definite disease but also the disease stage (Fig. 18).

The use of MEKC to obtain the profiles of 15 nucleosides in urine of thyroid cancer patients was reported.[164] Clinical investigations on the identification of nucleosides by CE with time-of-flight mass spectrometry in children diagnosed with leukemia and females diagnosed with breast cancer were carried out.[165] Since polypeptides regulate most of processes in the body, it was suggested that specific polypeptide biomarkers present in biological fluids (blood, urine, saliva, cerebrospinal fluid) can be used to diagnose various diseases.[166–168] Indeed, upon CE-MS analysis of polypeptides in urine, a database was composed[167] comprising 5000 biomarkers for diagnosis of ~40 diseases.

A topical trend in the development of the CE method demanded in clinical practice is related to miniaturization of equipment — the design of 'lab-on-chip' micro systems in which all stages of the analysis (sample injection, preconcentration, separation and detection) are collected on the same chip.[169, 170] The key advantages of these systems include high performance, rapidity and the possibility to deliver the instrument to the analytical object.

Microfluidic CE-MS systems proved to be beneficial.[171, 172] Optimization of parameters such as the length of the separating channel of the microchip and the size of the connecting capillary enhanced the efficiency of analysis. Microchips of this type were used to analyze drugs, peptides, tryptic digests and proteins.[172] The microfluidic CE-MS chip proposed[173] for analysis of mixtures of peptides, proteins and protein hydrolysates surpassed in efficiency (> 10 mln t.p.) the earlier version (Fig. 19).

* * *

The active implementation of capillary electrophoresis for solving a broad range of bioanalytical problems has substantially extended the analytical potential of separation methods. Capillary electrophoresis is several orders of magnitude more efficient than HPLC but is inferior in the separation selectivity and sensitivity, which hampers the application of the method in clinical medicine. By using various modifications of CE in combination with highly sensitive detectors, on-line preconcentration procedures, selective stationary (CEC) and pseudo-stationary phases, it becomes possible to determine biologically active compounds of various chemical nature in complex natural matrices and to solve important problems such as the control of the enantiomeric purity of pharmaceutical products, the search for markers of various diseases, diagnosis on characteristic profiles of biologically active analytes and so on.

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