

Review

Separation Sciences in the Czech Republic: From Historical Foundations to Recent Advances

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Abstract

Research in the analytical and preparative fields of separation science has a long-standing tradition in the Czech Republic and former Czechoslovakia. Czech and Slovak scientists recognized the advantages of the two most widely used separation techniques, i.e., chromatography and electrophoresis, soon after their discovery and broader adoption. Their contributions to the development of the fundamentals and applications of chromatography and electrophoresis are demonstrated not only by numerous publications in renowned scientific journals and books, but also by several breakthrough innovations that led to patents and sometimes also to successful commercialization. The contributions of the earlier most prominent Czech scientists to the development of separation sciences have already been summarized in the literature. In the following lines, we instead aim to highlight less well-known, yet important, contributions within the Czech chromatographic and electrophoretic community. Moreover, recent developments since the last comprehensive reviews are covered in greater depth.

Keywords: chromatography; electromigration methods; history; Czech Republic; Czechoslovakia; method development; fundamentals

1. Introduction

Separation science involving chromatographic, electromigration, and related methods is one of the most important disciplines in the field of chemical sciences because both natural and synthetic chemical substances are usually present in more or less complex mixtures, but for investigation of their properties and applications, they have to first be isolated in a pure form. In other words, they must be separated from the other substances in the usually rather complex matrices. For this purpose, various separation methods are necessary. Czech chemists were aware of this fact and had already started research, development, and application of then new separation methods, chromatography, and electrophoresis, in the 1940s [1–3]. These years correspond to the period when separation methods started to slowly gain wider scientific recognition.

The comprehensive history of Czech chromatographic achievements and the impact of Czech scientists on national and international journals and conferences can be found in many sources. Among English-written materials, the traces of Czech scientists can be found in the book published on the occasion of the 75th chromatography anniversary [4], the personal remarks of Zdeněk Deyl [5], or, in the historical overview addressed by Pavel



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Jandera, one of the pioneers of high-performance liquid chromatography in the Czech Republic [6]. Therefore, the current article focuses on a brief review of the lesser-known yet important information which may have been published only in the Czech language. Moreover, the contribution of Czech scientists to the development of chromatographic and electrophoretic methods in the last twenty years, not yet covered in previous reports [6], is briefly summarized. The description of the development and application of separation methods is divided into two sections: chromatographic methods, which mainly cover high-performance liquid chromatography, and electromigration methods.

2. Chromatographic Methods

2.1. Historical Introduction

The chromatographic technique developed by M.S. Tswett at the beginning of the twentieth century [7] penetrated only slowly into the wider scientific community in the following decades. Although Czech scientists, such as Julius Stoklasa, were already involved in early discussions following Tswett's work, their studies did not represent a systematic application of chromatography [8]. The method itself was not established in Czechoslovakia until the mid-20th century, when gas chromatography and later high-performance liquid chromatography were introduced and developed.

For Czech scientists (or Czechoslovak, as both countries were united between 1918 and 1992 with a six-year break during the Second World War), the introduction of chromatography started with two major events. The first was the series of review articles in the historical Czech journals of the 1940s written by Josef Košťř [1–3], and the second was the organization of the first national conference on chromatographic techniques in 1950, organized by František Šorm (later director of the Institute of Organic Chemistry and Biochemistry, and president of the Czechoslovak Academy of Sciences, Prague), with the aim of transferring the ideas of the Faraday Society Discussion on Chromatographic Analysis held at the University of Reading (22–24 September 1949) to the national environment [4,9].

As noted by Jandera [6], the further development of chromatographic techniques in Czechoslovakia in the following decades was strongly driven by numerous chemists, most notably Jaroslav Janák, Ivo Hais, and Karel Macek. Jaroslav Janák later developed gas chromatographs for the analysis of natural gases and gases of similar composition with volumetric detection. This was the origin of the first world patent on gas chromatographs (Figure 1) [10], patented only three years after the pioneering work by A.T. James and A.J.P. Martin [11]. In the early 1950s, gas chromatography started to be adopted across several Czechoslovak laboratories, particularly in Brno (J. Janák, with M. Rusek, K. Tesařík and others), Prague (E. Smolková-Keulemansová, L. Feltl, and V. Pacáková), and Pardubice (J. Franc, J. Churáček). Within a short period, a scientific community was established mainly around the above laboratories, and intensive development of both instrumentation and methodology mainly for industrial applications followed. Czechoslovak scientists were also very active through organization and participation in national chromatographic meetings, and, despite political constraints, more limitedly in international conferences.

In the field of liquid chromatography, the early period was characterized by the introduction of classical column liquid chromatography and, simultaneously, by the implementation of paper and later thin layer chromatography. In 1954, Hais and Macek published a comprehensive monograph on paper chromatography [12], representing one of the earliest systematic treatments of this rapidly developing technique. The work appeared only two years after one of the earliest books in this field had been published by Block, LeStrange, and Zweig in 1952 [13].



Figure 1. Janak's volumetric gas chromatograph—the instrument is displayed in the vestibule of the building of the Institute of Analytical Chemistry, Czech Academy of Sciences, in Brno.

Another strong Czechoslovak contribution to the advances of planar chromatography is attributable to the research group of J. Gasparič (Pardubice), who systematically investigated retention mechanisms and the identification of various groups of compounds. In cooperation with M. Večeřa and M. Jureček, a series of articles were published in Czechoslovak journals in the 1950s and early 1960s, the monograph including a complex laboratory manual of identification of organic compounds using chemical reactions. Thus, the methods of paper and gas chromatography were published [14].

In the 1950s and 1960s, gas chromatography underwent systematic expansion in both experimental and theoretical aspects. Advanced techniques were developed, including high-temperature and pyrolysis gas chromatography (i.e., in Janák's group in Brno, and in Pardubice by J. Franc; the in-house laboratory developed a high-temperature gas chromatograph built by J. Franc, which was later manufactured by Dioptra Turnov and sold within Eastern Bloc countries). In Brno, thermodynamic interpretation of chromatographic retention data was extensively studied by J. Novák and J. Drozd as well. During this period, the field of planar chromatography started to orient toward biochemical and pharmaceutical applications, especially for the studies of drug metabolisms [5].

Not surprisingly, in the late 1960s, the chromatographic community in Czechoslovakia was quite strong and well-established, with a good reputation abroad. Thus, the introduction of high-performance liquid chromatography by C. Horváth and S. Lipsky [15] made the research groups in Czechoslovakia well-prepared for the upcoming boom of the technique. Research groups in Prague (M. Popl, L. Vodička, J. Kříž) and Pardubice (J. Churáček, P. Jandera) contributed to the development of in-house HPLC instrumentation and column-packing technologies using fine silica-based sorbent particles since the beginning of HPLC era. The schematic picture of the instrumentation developed by P. Jandera

for HPLC analyses using in-laboratory packed columns with adoption of the in-laboratory built flow-through detection cell on a Spekol spectrophotometer (Carl Zeiss Jena) is shown in Figure 2.

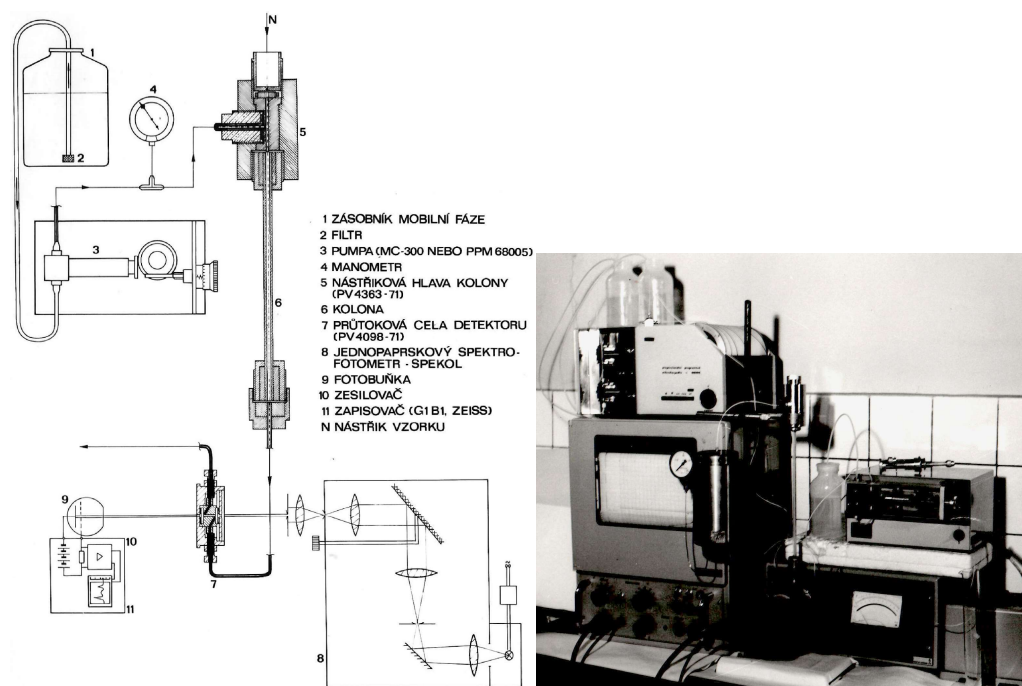


Figure 2. Poster presentation of in-laboratory built early HPLC chromatograph in the laboratory of P. Jandera (Pardubice), displayed at the exposition of technical innovations in 1970 in Brno (**left**), and photo of the real set (**right**), year ca. 1970 [16].

In parallel to the development of HPLC, microcolumn liquid chromatography was developed in Brno (M. Krejčí, K. Šlais, and later continued in the 1990s by J. Planeta, P. Karásek and coworkers), leading to improved analytical sensitivity and detection limits. The 1970s also saw the introduction of chiral stationary phases, particularly cyclodextrin-based systems, enabling the separation of enantiomers in both GC (E. Smolková-Keulemansová, L. Feltl, V. Pacáková), and more recently, in HPLC (E. Tesařová and coworkers).

In the 1980s and 1990s, attention was shifted toward the theoretical aspects of chromatography and the development of novel stationary phases. A major contribution was made by P. Jandera, who, in collaboration with J. Churáček, formulated a general theory of gradient elution in HPLC, enabling prediction of retention behavior and optimization of separation processes across various chromatographic modes, including normal-phase, reversed-phase, and ion-exchange chromatography, and later also hydrophilic interaction liquid chromatography (HILIC). A detailed review of the role of Pavel Jandera and his contribution to separation science is provided in two comprehensive articles: one written by Jandera himself [16], and another published in 2021 in memory of his sudden passing [17].

Besides the fundamental research in separation principles and mechanisms, column-packing materials were synthesized and used for the preparation of HPLC columns. Silica-based adsorbents available for both thin layer chromatography and HPLC were produced by companies like Lachema (Brno) and Kavalier (Sázava), together with ion-exchangers by Spolchemie (Ústí nad Labem). Concurrently, new packing materials and continuous separation media including polymer-based sorbents derived from hydroxyethyl methacrylate were developed. Later, at the end of the 1980s, continuous separation media in the form of organic polymer disks for the separation of biopolymers were developed in Prague by F.

Švec in cooperation with Russian scientists T. Tennikova and B. Belenkii [18], which was the initial step toward the use of organic polymer monolithic columns in HPLC [19].

From the 1990s onward, and particularly at the beginning of the 21st century, the opening of the Czech Republic to Western instrument markets in the post-communist era led to the rapid equipping of laboratories and research institutes with separation techniques coupled to mass spectrometry, i.e., GC-MS and HPLC-MS. Although both GC-MS [20] and LC-MS [21] were originally developed in as early as the 1950s and 1970s, respectively, their commercial availability followed much later. This technological advancement significantly expanded analytical capabilities, and Czech analytical scientists once again became highly active in the field. The idea of early purchase of HPLC-MS instrumentation newly available on the market was adopted by J. Churáček (at that time in his last year as the head of Department of Analytical Chemistry in Pardubice), who strongly supported the purchase of the first bench-top HPLC-MS system in the Czech Republic, which was installed in P. Jandera's laboratory at the end of 1995. Immediately, a first-year Ph.D. student, M. Holčápek, started developing his career in this field. One of the first research topics was the characterization of the transesterification reaction of triacylglycerols from plant oils in the production of biodiesel [22]. This research direction was significantly extended in the following two decades to lipidomics analyses, and later resulted in the development of the lipidomic screening method for early diagnosis of cancers [23].

At the turn of the millennium, research efforts of the Czech scientists focused on advanced chromatographic concepts such as two-dimensional liquid chromatography [24], supercritical fluid chromatography and chiral stationary phases [25], and monolithic columns based on organic polymeric compounds [26]. These approaches enable more efficient separation of complex mixtures, including biopolymers, metabolites, and natural products. The outcomes of these studies have been regularly published in international peer-reviewed journals, including world-leading journals, and presented at major scientific conferences.

The strong integration of Czech scientists within the international community of separation scientists can be illustrated by the successful organization of international conferences in recent years in the Czech Republic, including the most prestigious meetings. After two successfully organized symposia of the *International Symposium of Separation Sciences* (ISSS) series, organized under the auspices of the Central European Group of Separation Sciences (CEGSS) (2005 in Pardubice) chaired by P. Jandera, and co-chaired by P. Jandera and A. Horna, respectively (2014 in Prague), the first *International Symposium on High-Performance Liquid Phase Separations and Related Techniques* (HPLC) in the Czech Republic was chaired by M. Holčápek and F. Foret in 2017 in Prague. This meeting attracted a huge number of participants, reaching nearly 1400. In 2010 in Prague and in 2024 in Brno, *Microscale Bioseparations and Bioanalysis* (MSB) symposia, which cover the advances in microseparation techniques and innovations in bioanalytical methods, were organized by F. Foret. Yet the latest achievement is the forthcoming *International Symposium on Chromatography* (ISC), which will be held on 6–10 September 2026 in Prague, co-chaired by M. Holčápek and L. Nováková.

During the pioneering period of chromatography, Czechoslovak and Czech scientists were highly productive in the preparation of scientific literature (some of the fundamental books were already mentioned previously). Several dozen books and book chapters in fundamental chromatographic books have also been published by renowned international publishers, most of them summarized in the review by Jandera [6]. Although the number of publications by Czech scientists has rapidly increased in recent decades, the preparation of books in English with Czech authors remains rather rare. An exception is the book *Modern HPLC in Theory and Practice* by L. Nováková, M. Douša, P. Česla, J. Urban, and co-authors,

published in June 2024, solely focused on fundamentals as well as method development and validation in HPLC [27].

2.2. Development of Chromatographic Methods in Various Research Centers in the 21st Century

In the following sections, the main achievements in the field of chromatography over the last twenty years (since the publication of the last national report by Jandera [6]) at various research centers in the Czech Republic, where the development of chromatographic methods has a long tradition, are summarized. The topics presented provide an illustrative rather than comprehensive overview of published work and may not encompass all the results achieved in this field by Czech scientists. As chromatography is currently an indispensable method of choice in most chemical analyses, the presented selection of works by Czech scientists focuses primarily on major research directions and generally omits studies of a purely applied character, which use chromatographic separations as a routine analytical tool.

2.3. Faculty of Science at Charles University in Prague

The development of chromatographic separations at the department is tightly connected with the topic of enantiomeric separations. Various chiral stationary phases were evaluated, including immobilized polysaccharides [28], cyclofructans [29], teicoplanin, vancomycin [30], and cyclodextrin phases [31]. The stationary phases were characterized by using linear free-energy relationship (LFER) models in both HPLC and supercritical fluid chromatography [32]. LFER models with classical chromatographic tests like Walters, Engelhardt, and Galushko were used for evaluation of zirconia-based stationary phases [33].

Several fundamental aspects of capillary liquid chromatography on slurry-packed columns and monolithic columns were also studied. For packed microcolumns, the influence of end frits material on separation efficiency was evaluated [34]. Developed quartz wool frits generate advantages of higher efficiency, ease of preparation, and the possibility of creation of an end-column detection window immediately after the frit. Further, coated particle-based stationary phases with polyaniline suitable for HILIC separations were developed and characterized using LFER models [35]. Organic capillary monoliths of different chemistries were prepared, mainly for the separation of small molecules [36].

In the field of gas chromatography, the methods of analysis of toxicologically relevant samples were developed by R. Čabala and coworkers [37,38]. Besides the development of separation methods for analysis, novel sample preparation microextraction procedures were developed as well [39].

2.4. Institute of Analytical Chemistry of the Czech Academy of Sciences in Brno

In the group of F. Foret, the development of organic monolithic polymers mainly for applications in glycosylation analyses was extensively studied by J. Lavická (maiden name Křenková) [40–42]. Monolithic columns for capillary chromatography were also developed by D. Moravcová, J. Planeta, P. Karásek, V. Kahle, and M. Roth [43,44]. As the research at the institute spans widely over both techniques, i.e., chromatography and electrophoresis, further discussion is also provided in Section 3.2.2.

In recognition of Jaroslav Janák, who served as the first director of the institute for more than 20 years, the Jaroslav Janák Award was established in 2014. The award is granted annually to scientists who have made a significant contribution to the development of analytical chemistry through their work. The awardees so far include the following scientists: J. Janák (2014), P. Jandera (2015), P. Boček (2016), L. Křivánková (2018), F. Švec and M. Gilar (2022), B. Gaš (2023), M. Novotný and A. Guttman (2024), and D.S. Chung (2025).

2.5. Institute of Organic Chemistry and Biochemistry at the Czech Academy of Sciences in Prague

The main analytical research at the institute is typically connected with the coupling of chromatographic methods with mass spectrometry for studying biomolecules, with applications in medicine and environmental science. J. Cvačka, V. Vrkoslav, and coworkers have contributed to the development of HPLC-MS methods for the detailed structural characterization of lipids and other bioactive compounds [45–47]. Besides HPLC, several studies have also utilized semi-preparative thin layer chromatography and gas chromatography, mainly in coupling of the techniques with mass spectrometry for analysis of lipids [48,49]. Several other research groups are using chromatography within the institute as an analytical tool supporting diverse projects; however, fundamental research specifically focused on chromatography itself remains rather limited.

2.6. Faculty of Science at the Palacký University in Olomouc

Significant contributions to the development of chromatography have been made by O. Novák and coworkers in the bioanalytical field, particularly through the development of ultra-high-performance separations coupled to tandem mass spectrometry (UHPLC-MS/MS) for analyses of plant hormones at trace levels [50–52]. The work integrates advanced chromatographic separations with miniaturized sample preparation techniques, including solid-phase and immunoaffinity chromatography [53].

Using HPLC coupled with mass spectrometry, a series of studies focused on the determination of anthocyanins was published by P. Bednář, K. Lemr, and particularly by P. Barták [54–56]. The methods developed typically utilized reversed-phase separations on conventional columns; however, microcolumn setups and UHPLC-MS/MS were used as well. In recent years, analyses of cultural heritage objects were developed using chromatographic methods as well [57,58].

Significant numbers of chromatographic methods for metabolomic analyses were developed in recent decades by D. Friedecký and coworkers [59,60]. Most of the work relies on the application of advanced chromatographic methods coupled to mass spectrometry for the precise identification and quantitation of multicomponent samples.

2.7. University of Chemistry and Technology in Prague

Significant contributions to advances in chromatographic separations, particularly enantiomeric separations, were provided by M. Kohout and coworkers. New chiral stationary phases were developed and the mechanisms of enantiorecognition were described, which enhanced the possibilities of chromatographic methods for selective separation of optical isomers [61,62].

Among the types of analyses most widely solved by chromatographic methods are the analyses of food samples, including both characterization of food composition and determination of foreign and potentially harmful species and food contaminants. A tremendous number of methods for such analyses utilizing chromatographic methods have been published by J. Hajšlová and coworkers (i.e., [63,64]). Chromatographic methods, both gas and liquid, have been systematically developed and optimized as high-performance analytical tools for the determination of trace-level contaminants in complex food matrices.

2.8. Faculty of Science at Masaryk University in Brno

Coupling separation techniques, particularly chromatography, with mass spectrometry using laser desorption/ionization techniques is not as straightforward as in the case of continuous ionization techniques, such as electrospray ionization. This topic has been systematically investigated by J. Preisler and coworkers, who developed several methods with off-line coupling of liquid chromatography and electromigration techniques

with matrix-assisted laser desorption/ionization (MALDI) or surface-assisted laser desorption/ionization (SALDI) techniques [65,66]. The approach is beneficial, especially in proteomics and analyses of complex biomolecular systems.

Microcolumn monolithic chromatography has been extensively studied by J. Urban, who joined the Faculty of Science at Masaryk University in Brno in 2017 (formerly at the Faculty of Chemical Technology in Pardubice). In Brno, continuous research in organic monoliths consisted of various fundamental studies into the retention of small molecules on polymethacrylates [67], preparation of several innovative chromatographic devices [68,69], and recently on the characterization of morphologic properties of hybrid organo-silica monoliths [70].

2.9. Faculty of Chemical Technology at the University of Pardubice

Research in the field of chromatographic separations in the last two decades in Pardubice has focused mainly on two topics. The first was the systematic development of two-dimensional liquid chromatography, mainly in comprehensive mode, which also utilized preparation and application of tailored monolithic stationary phases; the second comprised the continuous development of optimization approaches for gradient elution and characterization of retention in various separation modes. Both topics were covered by P. Jandera and coworkers [71–73]. As the scientific contribution of P. Jandera has already been comprehensively reviewed, including also in this period, readers are directed to this source [17]. In recent years, the group of P. Česla continues with research work into the field of two-dimensional liquid chromatography [74] and the development of new optimization approaches for gradient elution [75,76].

Further development of chromatographic methods in Pardubice, especially for the analysis of lipids, has been carried out by M. Holčápek's group [23]. Chromatographic methods using argentation chromatography [77], two-dimensional liquid chromatography [78], and HILIC for lipidomic analyses were developed [79].

HPLC methods coupled with mass spectrometry, especially for analysis of naturally occurring compounds, were also developed in Pardubice by L. Česlová and J. Fischer [80].

2.10. Faculty of Pharmacy at Charles University in Hradec Králové

In Hradec Králové, chromatographic methods intended mainly for pharmaceutical analyses were developed using several approaches, including ultra-high-performance liquid chromatography [81], HILIC [82], subcritical [83], and supercritical fluid chromatography [84] by L. Nováková, P. Solich, and coworkers. In 2017, F. Švec joined the permanent staff of the faculty, which resulted in strengthening of the research in several directions, including sample preparation for chromatographic analyses using polymeric materials [85], novel separation media for supercritical fluid chromatography [86], and description of retention interactions in this technique [87], particularly in cooperation with D. Šatínský, L. Nováková, H. Sklenářová, J. Lenčo, P. Chocholouš, and other coworkers.

3. Electromigration Methods

3.1. Historical Introduction

Electrophoresis is an excellent separation method based on the different migration velocities of electrically charged chemical species and (bio)particles in liquid media in electric fields. It can be applied for analytical and preparative separations of various compounds and particles, including: small ions of inorganic and organic acids and bases via medium-sized oligopeptides and oligonucleotides, large biomacromolecules of proteins and nucleic acids, and (bio)particles such as viruses, cells, organelles, and synthetic nano- and microparticles. Electrokinetic phenomena, electrophoresis, and electroosmosis were first

observed at the beginning of the 19th century [88]. The theoretical bases of electromigration were established by F. Kohlrausch at the end of the same century [89], but the first analytical application of electromigration was presented by A. Tiselius only in 1930 [90].

Later, he developed a special electrophoretic apparatus with optical refractive index detection [91] and used it for the separation of blood serum proteins into several fractions (albumins and alpha-, beta- and gamma-globulins), using a method called moving boundary electrophoresis. For these achievements, he was awarded the Nobel Prize in 1948.

A good base for the development of electromigration separation methods in Czechoslovakia was the basic research in the area of general electrochemistry performed already in the 1920s by J. Heyrovský [92]—Nobel Prize laureate in 1959 for the invention of polarography. To the best of our knowledge, the first electrophoretic experiments in Czechoslovakia were performed by Gordon et al. in 1949 [93] at the Central Institute of Chemistry in Prague (the predecessor of the later Institute of Organic Chemistry and Biochemistry of the Czechoslovak/Czech Academy of Sciences) headed by F. Šorm, who (together with J. Košťál at the Faculty of Science at Charles University in Prague) strongly supported the introduction of chromatographic and electrophoretic methods into chemical and biochemical laboratories. He organized the first national conference on chromatographic and electrophoretic methods in 1950 [6]. The Czech pioneers of electrophoresis were aware of the disturbing effects (thermoconvection and sedimentation) of moving boundary electrophoresis performed by Tiselius in a free solution in a vertical U tube [91]. Therefore, they carried out the electrophoretic separation of proteins (ferritin, hemoglobin, egg white proteins) in agar gel placed on a cooled glass plate in a home-made apparatus. In this anticonvective and partially sieving medium, it was possible to semipreparatively separate ferritin from hemoglobin and partially fractionate egg white proteins.

In the 1950s, different types of paper electrophoresis were developed. Non-cooled low-voltage so-called descending paper electrophoresis was constructed by O. Mikeš [94] at the Institute of Organic Chemistry and Biochemistry (IOCB) at the Czechoslovak Academy of Sciences (CAS). The sample (hydrolyzed proteins and peptides) was applied in the middle of the vertically oriented paper filled with buffer and situated in two electrode compartments with platinum electrodes. The resulting movement of the sample components consisted of a combination of electrophoretic migration and electroosmotic flow, and suction flows of the buffer solution inside the paper. As a result, O. Mikeš and V. Holeyšovský were the first in the world to have applied diagonal paper electrophoresis for the 2-D separation of amino acids and peptides, especially useful for the determination of disulfide bridges in proteins and peptides [95]. Improved and faster separations were achieved by the cooled high-voltage paper electrophoresis with fixed heat exchanger designed by Z. Prusík and B. Keil [96]. Both types of these paper electrophoresis apparatuses have been widely applied for analytical and semipreparative separations of amino acids and peptides in the elucidation of the primary structure (amino acid sequences including determination of disulfide bridge positions) of proteins and polypeptides.

Development of the instrumentation and theory of paper electrophoresis continued into the 1960s and early 1970s. V. Jokl from the Pharmaceutical Faculty of Charles University in Hradec Králové studied the separation of metal ions by paper electrophoresis in ligand-containing buffers. The ligands, e.g., EDTA, formed complexes with metal cations and thanks to different strengths of complexation, even the metal ions with the same or close mobilities, such as cations of rare earth elements, especially several lanthanides, could be separated. From these measurements, the empirical equations for the estimation of the ionic mobilities of these complexes were derived [97]. J. Vacík at the Faculty of Science at the Charles University in Prague investigated paper electrophoresis both from a theoretical and experimental point of view. He and Z. Fidler solved the continuity equation for nonlinear

distribution functions in paper electrophoresis with the counterflow of the background electrolyte using an analog computer [98].

A new strong impulse for the advancement of electromigration methods in Czechoslovakia was the invention of a new technique—displacement electrophoresis, by A.J.P. Martin and F.M. Everaerts in 1967 [99]. This method, later called isotachopheresis (ITP) [100], was performed in a free solution with increased viscosity by addition of hydroxyethylcellulose in narrow bore Pyrex glass tubes/capillaries of 0.5/0.8 mm ID/OD. Due to the concentrating and self-sharpening effect of the discontinuous electrolyte system governed by the Kohlrausch regulation function [89], highly efficient and sensitive separations of cationic or anionic species were achieved, and capillary ITP (CITP) was considered as a very strong analytical and preparative separation method.

Soon after the introduction of lab-made CITP devices, the first commercially available instrument for CITP, Tachophor, started to be produced by the Swedish company LKB in Bromma-Stockholm in 1970. It was equipped with a narrow bore polytetrafluoroethylene (PTFE) tube/capillary of 0.5/0.8 mm ID/OD and 230–800 mm length. Separation was monitored first by temperature (thermocouple) detector and later by more sensitive universal electric conductivity detectors and specific UV-absorption detectors at fixed wavelengths of 206 or 254 nm. However, due to economic limitations, this apparatus was unavailable for researchers in Czechoslovakia. For that reason, before starting the research and development of this method, they were first forced to construct their own apparatuses. In the beginning, it was a handicap, but finally it turned out to be an advantage because Czech and Slovak researchers were then able to become leading specialists in the field of new instrumentation and methodology development of CITP, and later, other capillary electromigration (CE) methods. They constructed several unique apparatuses with parameters comparable to and competitive with the LKB instrument. The high sensitivity and separation power made CITP an attractive analytical method. It started to be investigated and developed in several research institutions in Czechoslovakia. In the next sections, in approximately chronological order, the development of electromigration methods in various Czech research centers is presented.

3.2. Development of Electromigration Methods in Various Research Centers

3.2.1. Faculty of Science at Charles University in Prague

The first center, where the instrumentation and methodology of CITP were developed, is the Department of Physical Chemistry, located in the Faculty of Science at the Charles University in Prague, and linked to the group of J. Vacík. He and his skillful electrotechnical assistant, J. Zuska, began collaborating with the “father of CITP”, F. Everaerts, and his technical assistant, T. Verheggen, from the Technical University of Eindhoven in 1969. Within this cooperation, a new lab-made CITP apparatus was constructed, and some papers were published. In the first of them, the application of counterflow in CITP was described in 1970 [101]. It is worth mentioning that the major components of the lab-made CITP device, including the hydrodynamic system, dosing valve, high-voltage power supply providing constant electric current, and thermocouple-based temperature detector, were also lab-made from home and imported components. This device was later improved by the construction of the universal contact conductivity detector and then by the capacitively coupled contactless conductivity detector (C4D) developed by then Ph.D. student, B. Gaš [102]. This detector was later improved and adapted for application in capillary zone electrophoresis (CZE) [103] and produced by J. Zuska’s company ADMET. It is still frequently used as a universal detector in commercial CE devices. In the 1980s, J. Vacík continued in his theoretical study of electrophoresis. Together with V. Fidler and Z. Fidler, they published the results of the solution to the continuity equation in ITP using the hybrid computer [104]. Although the described system was composed of only four strong

electrolytes, it demonstrated well the dynamics of the separation–adjustment of analyte concentration in the ITP steady state following the Kohlrausch regulation function.

A very successful successor of J. Vacík was B. Gaš, later Dean of the Faculty and Vice-Rector of Charles University. He was engaged mainly in the study of theoretical principles of electromigration processes. Together with V. Hruška, they have in detail described the role of the Kohlrausch regulation function and other conservation laws in electrophoresis and isotachophoresis [105]. He created new theoretical models of electrophoresis based on a series of nonlinear or linearized partial differential equations and, found their numerical solutions [106]. Together with his Ph.D. students (M. Jaroš, V. Hruška, P. Dubský) and coworkers (E. Tesařová, K. Včeláková-Ušelová, I. Zusková, L. Mullerová, M. Riesová, and J. Svobodová), he developed and experimentally verified the advanced programs for the simulation of electromigration processes, PeakMaster [107] and Simul [108]. The latest versions of these programs, PeakMaster 6 and Simul 6, are freely available at <https://echmet.natur.cuni.cz/#portfolio> (accessed on 10 April 2026).

These programs represent powerful tools for the simulation and optimization of experimental conditions of all major CE methods: CZE, CITP, capillary isoelectric focusing (CIEF), affinity capillary electrophoresis (ACE), and capillary electrokinetic chromatography (CEKC). PeakMaster was originally based on linearized, and later, on nonlinear models of CZE and CEKC. It enables the calculation of positions and shapes of analyte and system peaks without restricting the number of analytes, selectors, the complexation stoichiometry, or simultaneous acid–base equilibria. It performs all calculations and shows the properties of the background electrolyte (BGE) and the expected electropherograms or electrokinetic chromatograms within a few seconds.

The program Simul 6 is based on a model consisting of continuity equations for the movement of electrolytes in a separation channel, acid-based equilibria of weak electrolytes, and the electro-neutrality condition. It accounts for any number of multivalent electrolytes or ampholytes and provides a complete picture of the dynamics of electromigration and diffusion in the separation channel. Simul 6 is a very powerful tool for the simulation of CZE, CITP, and CIEF. It allows monitoring of the dynamics of electroseparation processes, including the transient states, testing of the properties of electrolyte systems, and optimization of experimental conditions of CE methods *in silico*. Hence, it significantly improves the efficiency of the development and optimization of experimental conditions for practical CE separations.

Using these models and programs, several special phenomena in CE methods were discovered and experimentally verified, such as prediction of the position and shape of the system peaks [109] and oscillating electrolytes [110]. Recently, B. Gaš and his coworkers published a new program for the determination of thermodynamic acidity constants and ionic mobilities of weak electrolytes from CE data [111] and a new mathematical model of electromigration when electroneutrality conditions are not met [112].

In the Faculty of Science, CITP and other CE methods were also investigated within the Department of Analytical Chemistry. I. Jelínek, J. Snopek, and E. Smolková-Keulemansová introduced the application of cyclodextrins (CDs) as stereoselective additives to the leading electrolyte in CITP in the 1980s. Using this approach, they succeeded in separating positional isomers and enantiomers of various non-chiral and chiral compounds and drugs [113–115]. CZE and affinity CE methods have been developed by P. Coufal, V. Pacáková, K. Štulík, and M. Křížek and have been applied in the analysis of various inorganic and organic compounds, as well as for investigation into their noncovalent interactions [116–118].

In this Faculty, original electrophoretic methods were also developed within the Department of Biochemistry. Here, V. Hořejší and M. Tichá introduced the application of affinity electrophoresis in polyacrylamide and agarose gels for the investigation and quan-

titative evaluation of glycoprotein interaction with lectins and other ligands immobilized in the gel [119,120].

3.2.2. Institute of Analytical Chemistry of the Czech Academy of Sciences in Brno

Chronologically, the second strong center for the development of CITEP and other CE methods was the Institute of Analytical Chemistry (IAC) at the Czechoslovak/Czech Academy of Sciences (CAS) in Brno. Here, research was first conducted by P. Boček, later director of the Institute. He and his coworker, M. Deml, constructed their first special lab-made CITEP device in 1975 [121]. The separation channel with the rectangular cross section was milled in a poly(methylmethacrylate) block placed on an aluminum heat exchanger cooled by circulating liquid medium. It was equipped with a home-made universal electric potential gradient detector [122]. This apparatus was used for investigations into ITP separation process. Regarding instrumentation and methodology, the papers on the following topics represent valuable contributions to the development of CITEP: continuous sample injection [123], application of concentration cascade of the leading electrolyte [124], large volume sample pre-separation for trace analysis [125], utilization of complex equilibria for influencing the effective mobility and separability of analytes [126], and separation of polystyrene particles and circular DNA in liquid agarose gel [127]. Theoretically important papers described: (i) employment of solvent ions (hydrogen cation or hydroxide anion in the case of water) as terminating ions of the cationic and anionic ITP electrolyte systems [128], (ii) factors controlling the migration order of the zones [129], (iii) conditions for occurrence of migrating system peaks depending on the number of co-ions and pH of the BGE [130], and (iv) conditions for correct ITP migration and separability of analytes [131].

The members of Boček's group significantly contributed first to the development of CITEP and later to other CE methods. In CITEP, P. Gebauer introduced the concept of the zone existence diagram [129] and studied the behavior of kinetically labile complexes in ITP [132]. He introduced the so-called peak shape diagram, which allowed identification of the conditions for symmetrical peaks in CZE [133], and he explained the schizophrenic behavior of some analytes [134].

L. Křivánková, director of the Institute after P. Boček, and her coworkers classified cationic and anionic CITEP electrolyte systems into stable buffered systems, stable non-buffered systems, and non-stable non-buffered systems [135]. She investigated possibilities to decrease the limits of detection (LODs) in CITEP analyses by combining two capillaries with different diameters, and applied this 2D separation method for practical analyses of diluted analytes. In collaboration with other group members, she contributed especially to the development of methodology based on the combination of CITEP with CZE [136,137] and transient ITP in CZE [138].

F. Foret, current director of the Institute, significantly contributed to the development of both instrumentation and methodology of CE methods. Already in 1986, he had constructed a combined UV-absorption and conductivity detection cell for CE methods [139]. In the 1990s, during his stay at the Barnett Institute at the Northeastern University in Boston, he introduced online coupling of CZE and CITEP-CZE and microchip CE separations of proteins with electrospray ionization mass spectrometry (ESI-MS) detection [140,141]. After returning to Brno in 2002, he established his own group of "Bioanalytical Instrumentation" and continued in the development of interfaces for the hyphenation of CE and microchip CE devices with ESI-MS detection [142,143] and their applications in bioanalysis. Recently, he developed a new original electromigration method—epitachophoresis [144]. It is based on the electromigration of ionogenic species in discontinuous electrolyte systems in a circular separation compartment, with the anode in the center and cathode at the circumference

of the circle. It can be applied for the isolation of nucleic acids and other biopolymers at a relatively large scale.

V. Dolník et al. introduced a charge-based transient-state ITP model for optimization of ITP separation [145]. J. Pospíchal et al. demonstrated the possibility to modify the pH of the separation system by addition of another electrolyte from the other electrode vessel by changing the ratio of electric currents from the two electrode vessels [146]. K. Klepárník et al. developed CE separation of ssDNA fragments in short fused-silica capillaries at highly alkaline BGEs at pH > 12 [147], and he significantly contributed to the development of interfaces for CE coupling with ESI-MS detection [148,149]. R. Vespaľec was engaged mainly in the development of CE methods for the enantioseparation of chiral compounds [150] and analysis of cluster boron anions [151].

Pavel Kubáň, current head of Boček's former group, and his coworkers introduced a new method for sample preparation—liquid membrane electroextraction. They have applied it to the extraction of heavy metal cations and other ionogenic compounds from complex matrices, with subsequent analysis of extracted ions by CE with contactless conductivity detection [152]. Recently, this group succeeded in the coupling of hollow fiber liquid phase microextraction and CE analysis of human biofluids [153], and combined dried blood spot self-sampling with automated CE processing for clinical analysis [154]. His brother, Petr Kubáň, is engaged in the development of miniaturized portable CE devices with contactless conductivity or fluorescence detection and their application for the on-site determination of small organic and inorganic ions in various human biological fluids [155,156].

The significant contributions of Boček's and Foret's groups, the so-called "Brno electrophoretic school", to the development and application of CE methods is also confirmed by the fact that they have published two books comprehensively describing theoretical bases, instrumentation and methodology developments, and applications of both CITP [157] and CZE [158] methods. Last but not least, it is worth mentioning that P. Boček served and F. Foret is still serving as senior deputy editor of *Electrophoresis*—the major international journal dedicated to electromigration methods. In addition, F. Foret and his coworkers organize a series of symposia called International Interdisciplinary Meeting, CECE YYYY (<https://www.ce-ce.org/>), where CECE comes from the original name of the meeting, Central Europe Capillary Electrophoresis.

At the IAC of the CAS, in addition to P. Boček's and F. Foret's group, CE methods were also investigated and developed in the groups of K. Šlais and M. Roth. K. Šlais synthesized low-molecular-mass pI markers for the calibration of the pH gradient in capillary and slab gel IEF [159], and introduced the concepts of electrofocusing in ITP-generated pH gradients in tapered capillaries [160,161] and the preparative separation of amphoteric analytes by divergent flow IEF [162]. M. Roth et al. contributed to the development of supercritical water-treated fused silica capillaries with two sections of different IDs and inner wall roughness [163,164]. These capillaries were used for the preconcentration and separation of bacteria [165] and for the identification of milk protein allergens [166].

3.2.3. Institute of Organic Chemistry and Biochemistry at the Czech Academy of Sciences in Prague

In addition to the aforementioned high-voltage paper electrophoresis [96], Z. Prusík and coworkers developed a unique device for continuous carrierless free-flow electrophoresis (Figure 3) [167].



Figure 3. Apparatus for continuous free-flow electrophoresis.

It consisted of two parallel glass plates with dimensions of $500 \times 500 \times 4$ mm with a narrow (0.5 mm) gap between them. Separation voltage (up to 3 kV) was oriented perpendicularly to the laminar flow of the background electrolyte in the gap between the glass plates that were maintained at 4°C by fast circulating cold air. The sample was introduced as a narrow stream in the center of the inlet side of the flow-through chamber. During the flow-through inside the chamber, positively charged sample components migrated to the cathode and negatively charged components to the anode, whereas non-charged components moved in a straight direction. At the outlet side of the chamber, the sample components were collected in 48 fractions. In this apparatus, different electrophoretic methods could be performed: zone electrophoresis, affinity electrophoresis, ITP, and IEF in a continuous flow-through arrangement. This apparatus has been applied for purification of peptides and proteins (hormones, enzymes, and enzyme inhibitors) with a preparative capacity of 50–100 mg per hour.

In the late 1970s and early 1980s, Z. Prusík and his then Ph.D. student, V. Kašíčka, developed a lab-made apparatus for CITP. It was equipped with a PTFE capillary (0.45/0.70 mm ID/OD, 25–40 cm length) and a universal electric potential gradient detector and specific UV-absorption detector operating at fixed wavelengths, 206 or 254 nm. The apparatus was widely used for the determination of the CITP purity degree of synthetic peptides purified by chromatographic methods [168]. This apparatus was modified by online coupling with a sorption element containing immobilized transferrin. This setup represented the first online coupling of solid-phase extraction with a CE method. It was used for the capture and concentration of monoclonal antibodies (mAbs) against transferrin from the ascitic fluid, with subsequent mild electrodesorption, reconcentration, and analysis of the released mAbs by CITP [169].

In 1987, a new lab-made CE device with UV-absorption detection was developed [170]. Utilizing the advantage of possessing apparatuses for both analytical CZE and preparative free-flow zone electrophoresis (FFZE), a model of correlation between CZE and FFZE was developed and applied for the conversion of analytical capillary separations of peptides into a preparative process with a capacity of several tens of milligrams of peptides per hour [171].

In the area of instrumentation, the advanced version of the home-made CE device was equipped with an external electric field control of electroosmotic flow, which allowed optimization of the separation conditions of CE analyses of peptides [172]. Recently,

two types of interfaces for online coupling of the home-made CE device with ESI-MS detection were constructed [173].

In the last 25 years, V. Kašička and coworkers (D. Koval, P. Sázelová, V. Šolínová, S. Ehala-Štěpánová, and R. Konášová) have developed several CE methods for the separation, analysis, and physicochemical characterization of biomolecules and functional organic molecules. For example, CZE was used for the determination of acidity constants, actual and limiting ionic mobilities and ionic radii of nucleotides [174], and peptides [175]. ACE was employed for investigation into the noncovalent interactions of biomolecules, and for the separation of enantiomers of various organic molecules and drugs [176,177]. In addition, during this period, V. Kašička chaired the Chromatography and Electrophoresis Group of the Czech Chemical Society, and served as the Associate Editor of the *Journal of Separation Science* and *Separation Science Plus*. Since 2026, he is a member of the Editorial Advisory Board of the *Journal of Chromatography A*.

3.2.4. Faculty of Science and Faculty of Medicine and Dentistry at Palacký University in Olomouc

In the Department of Analytical Chemistry within the Faculty of Science, Z. Stránský established chronologically the fourth center for the development of CITP in Czechoslovakia. He developed several CITP methods; for example, the determination of cationic herbicides and insecticides in waters and soils [178]. His main contribution consists of the construction of a lab-made CITP device, which has become the basis for the production of a simple CITP apparatus called Agrofor. It has been produced since 1986 in the agricultural cooperative Odra in Krmelín. As the apparatus name suggests, it was used mainly in agricultural and food analytical laboratories for the determination of low molecular mass cations and anions in various matrices, e.g., organic and inorganic acids in silages. Since 1990, the production of Agrofor was transferred to the company Recman (<http://www.recman.cz/> accessed on 10 April 2026), and the apparatus was renamed to Ionosep. Z. Stránský also supported several scholars who also significantly contributed to the development of CE methods. J. Ševčík, K. Lemr, P. Bednář, P. Barták, J. Petr, and V. Maier elaborated upon several important CE methods with UV-absorption and MS detection and applied them to the separation, analysis, and characterization of various compounds and particles, such as organic compounds and drugs, including their enantiomers [179–183], natural compounds [184], proteins [185], and microorganisms [186].

In the 1980s, in addition to the Faculty of Science, the CITP method was applied for the analysis of small ions at the Faculty of Medicine and Dentistry at Palacký University. I. Válka, D. Walterová, and V. Šimánek used CITP for the analysis of papaverin and other alkaloids in medicinal preparations [187,188].

3.2.5. University of Chemistry and Technology in Prague

At the Faculty of Food and Biochemical Technology, Department of Food Conservation, F. Kvasnička elaborated upon several CITP [189] and combined CITP-CZE methods [190]. He used them for the analysis of organic and inorganic acids and bases [191], amino acids [192], biogenic amines [193], saccharides [194], and sweeteners [189] in complex matrices of food, food supplement and nutrient, feed, and beverage samples [195,196].

At the Faculty of Chemical Engineering, V. Král and his coworkers, D. Sýkora, P. Řezanka, and K. Záruba, were and still are employed in the development of CE methods. They are focused mainly on the synthesis of special uni- and multimodal stationary and pseudostationary phases based on porphyrins, cyclodextrins, bare, and cyclodextrin-modified gold nanoparticles and cysteine-modified silver nanoparticles for the techniques of open-tubular capillary electrochromatography and capillary electrokinetic chromatography [197,198]. The developed phases and methods were applied for the separation

and analysis of various compounds, e.g., amino acids, peptides, polyaromatic hydrocarbons [199], and enantiomers of Troger's base derivatives [200].

3.2.6. Institute of Physiology at the Czech Academy of Sciences in Prague

Z. Deyl and coworkers constructed a lab-made CE device in the late 1980s [201]. Z. Deyl and I. Mikšík applied this device, and later, commercial CE apparatuses, for the analysis of various biologically active compounds, such as amino acids, peptides, proteins, nucleotides, and steroids. They separated different types of collagen polymers by capillary sieving electrophoresis using entangled polymer networks of non-crosslinked linear polyacrylamide [202]. They investigated both enzymatic and non-enzymatic posttranslational modifications, glycation and glycosylation, respectively, of blood serum proteins by means of CE separation of tryptic hydrolysates of these proteins using different capillary coatings and additives, such as Pluronic copolymer liquid crystals [203] and heptansulfonic acid [204]. They have prepared different types of stationary and pseudostationary phases for the separation of peptides and proteins by open-tubular capillary electrochromatography (OT-CEC) and CEKC [205]. In addition to research activities, Z. Deyl chaired the Chromatography and Electrophoresis Group of the Czech Chemicals Society from 1991 to 1997, and served as the editor of several books on separation methods [206–209] and of many special thematic and symposium issues of the Journal of Chromatography A and Journal of Chromatography B. I. Mikšík was the co-editor of one of the above books [208], and was also the editor of the Bibliographic Section of the Journal of Chromatography A.

3.2.7. Faculty of Environmental Studies at J. E. Purkyně University in Ústí nad Labem

P. Janoš developed CZE and CITP methods for metal ion analysis in inorganic chemistry, e.g., CITP determination of cations of rare earth elements in various technical materials [210], and a CZE method for the separation and analysis of lanthanides and actinides [211].

3.2.8. Faculty of Pharmacy at Charles University in Hradec Králové

M. Polášek, M. Pospíšilová, V. Jokl, and P. Jách elaborated upon several CE methods for the qualitative and quantitative analysis of various pharmaceuticals, e.g., CITP determination of non-steroidal anti-inflammatory drugs [212], anionic counter-ions in basic drugs [213], and polyol (sorbitol and xylitol) additives in multi-component pharmaceutical formulations [214]. CZE was used for a fast assay of glucosamine in pharmaceuticals and nutraceuticals [215] and MEKC was employed for the determination of impurities in indomethacin [216].

3.2.9. Faculty of Science at Masaryk University in Brno

Z. Glatz and his coworkers (M. Zeisbergerová-Pelcová, L. Michalcová, R. Řemínek, H. Nevídalová, and J. Schejbal) at the Department of Biochemistry significantly contributed to the application of CE methods for the characterization of enzymes and their substrates and inhibitors [217–219] using in-capillary immobilized enzymes or the in-capillary mixing of enzymes, substrates, and inhibitors by means of electrophoretically mediated microanalysis (EMMA) [220]. The second area of their research activities included the development of different modes of affinity capillary electrophoresis (ACE), mobility shift ACE (ms-ACE), and frontal analysis ACE (FACE), and their application to investigations into the noncovalent molecular interactions of proteins with low-molecular-mass compounds and drugs [221,222].

J. Havel and his coworkers (P. Jandík, M. Farková, M. Spanilá-Bitová, and J. Pazourek) at the Department of Chemistry first used CZE for the analysis of metal ions and for the determination of stability constants of their complexes with sulfate anions [223]. Later, they introduced the application of artificial neural networks for the optimization of solid-phase extraction and other experimental conditions of CE methods [224,225].

J. Preisler and his colleagues (T. Rejtar, O. Pes, and V. Kanický) at the same department also significantly contributed to the development of CE and MS methods. In particular, through their advanced instrumentation via online coupling with matrix-assisted laser desorption ionization (MALDI)-MS and inductively coupled plasma (ICP)-MS detection, and through their application in the field of metalloproteomics [65,226,227].

3.2.10. Faculty of Chemical Technology at the University of Pardubice

P. Jandera and his coworkers at the Department of Analytical Chemistry dealt mainly with the development and application of liquid chromatographic methods, but they were also partially engaged in the development of related CE methods. P. Česla constructed a new interface for the online coupling of LC and CE methods [74], and together with P. Jandera and J. Fischer, applied 1D CE and 2D LC-CE (MEKC) methods [228,229] for the separation of both achiral and chiral low-molecular-mass compounds, such as aromatic sulfonic acids [230], phenolic acids and flavonoids [229], anacardic acids [231], and benzothiazole derivatives of amino acids [232].

3.2.11. Third Faculty of Medicine at Charles University in Prague

P. Tůma and E. Samcová significantly contributed to the development of new instrumentation, methodology, and applications of CE methods. In collaboration with F. Opekar from the Faculty of Science, Charles University, they constructed various contactless conductivity detectors [233,234]. With regard to methodology, they concentrated large sample volumes introduced into the capillary into short zones, using an organic solvent (acetonitrile) as the terminating electrolyte of the ITP system [235]. In addition, they accelerated CE separations by combining two capillaries with different inner diameters [236]. Moreover, they combined electromembrane extraction [237] or microdialysis [238] with CE for analyses of biomolecules and drugs. The developed instrumentation and methods were applied for analyses of many types of low-molecular substances (organic and inorganic acids and bases, amino acids, carbohydrates, and drugs) in complex mixtures of clinical samples of blood serum, urine and saliva [239], and in foods and beverages [240].

3.2.12. Faculty of AgriSciences at Mendel University in Brno

R. Kizek, M. Vaculovičová, A. Vojtěch, M. Macka, and their colleagues used CE methods for the separation and analysis of quantum dots [241] and other nanomaterials [242] and for the study of their conjugates and complexes with peptides [243], DNA [244], and metalloproteins [245]. After transfer from the above IAC, J. Pospíchal and E. Glovinová also worked at Mendel University. They explored, for example, the development of CIEF without carrier ampholytes [246] and CITEP analysis of heavy metals after their preconcentration by the gradient ligand focusing method [247].

3.2.13. Czech Separation Scientists Abroad

Finally, yet importantly, it is necessary to present here the important contributions to separation science of Czech specialists acting in research centers abroad. In particular, (i) the fundamental contribution of F. Švec (University of Berkeley, CA, USA), Editor-in-Chief of the Journal of Separation Science and Separation Science Plus, to the development of organic polymeric monolithic stationary phases for capillary liquid chromatography and capillary electrochromatography [19,209,248,249], (ii) the relevant contribution of M. Novotný (University of Bloomington, IN, USA) to the development of several CE methods for the separation of peptides, proteins, and glycoproteins [250,251], and (iii) the significant methodology and instrumentation developments of CE methods by M. Macka (University of Tasmania, Hobart, Australia) [252,253].

4. Conclusions

Considering their relatively small population, we believe we can conclude that Czech scientists significantly contributed to the theory of chromatographic and electromigration methods as well as to instrumentation and methodology developments. They applied developed methods for highly efficient separation, highly sensitive analysis, isolation, purification, and physicochemical and biochemical characterization of various low- and high-molecular-mass compounds and (bio)particles.

LC and CE methods developed by Czech scientists have significantly contributed to the knowledge of the chemical and physical properties and biological functions of (bio)molecules and (bio)particles, and to the better understanding of processes occurring in living organisms and non-living systems. Overall, research by Czech scientists has significantly advanced LC and CE methods toward faster separations, higher separation efficiencies and sensitivities, and wider use of these methods for analytical and preparative purposes in many areas of research, as well as in industrial and agricultural practice.

The successful development and application of these methods in the Czech Republic can also be assumed in the coming years since, in most of the aforementioned institutions, many clever and skillful young scientists are ready to continue the top-quality research and development of LC and CE methods. Progress can be expected especially in the development of multidimensional LC and CE methods and their coupling with high-resolution MS detection. Moreover, new microfluidic and lab-on-a-chip micro-total analytical systems (μ TAS) will be designed to achieve higher separation efficiency, higher sensitivity, and higher speed analyses of a wide spectrum of (bio)molecules and (bio)particles in complex (bio)matrices. In all these developments, undoubtedly, artificial intelligence will be utilized.

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