ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2010, *55*(6), 283-288

REVIEW

CAPILLARY ZONE ELECTROPHORESIS IN DETERMINATION OF MICROORGANISMS

Ewa KŁODZIŃSKA[a], Ştefania TĂNASE [a,b], Andra Alina TOMESCU [a,b], Marius MOGA[b] and Bogusław BUSZEWSKI [a*]

[a] Chair of Environmental Chemistry and Bioanalytics, Faculty of Chemistry
Nicolas Copernicus University, Gagarin 7 Str, 87-100 Toruń, POLAND
tel. (48)(56)6114308, fax. (48)(56)6114837
[b] "Transylvania" University of Brasov, Faculty of Medicine 29 Eroilor Boulevard, 500036 - Brasov, Roumania

Received 29th July, 2009

Electromigration techniques have been recently proposed as a replacement for traditional methods of microbial identification. The results of the initial experiments indicate that diagnostics based on this technology can be extremely sensitive, inexpensive and, primarily, very fast. Capillary electrophoresis is a novel method of chemical analysis which enables separation and identification of various chemical substances. Characterized by high efficiency, repeatability of results, sensitivity toward detected substances and reliability, this technique is also extremely cost-saving as it requires small amounts of chemical substrates to be used. This study was carried out to summarized our achievements and experimental results in separation and identification of bacteria by electromigration techniques, to examine microbial behavior during separation process.

INTRODUCTION

Microorganisms are a group of organisms that are impossible to be seen by the naked human eye because of their size. They were discovered in 1675 by Anton van Leeuwenhoek, who also started studying them with the help of a microscope. Bacteria, viruses, fungi, archaea, microscopic plants (green algae), animals (plankton, the planarian and the amoeba) are all types of microorganisms.

Bacteria are a large group of unicellular microorganisms. Bacteria can be found in soil, acidic hot springs, radioactive waste, water and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals. There are 40 million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water, while the human body contains more than 150 types of bacteria (both inside and outside the body)[1]. Even if bacteria are known for their advantages, being vital for the recycling of nutrients, they are also harmful for people, being pathogenic and causing many infectious diseases, including cholera, syphilis, anthrax, leprosy etc. Almost 40% of the 50 million deaths per year are caused by infectious diseases[1].

Therefore, bacterial determination has a very important role in many branches, such as medical diagnosis, bioscience research, analysis in food industry and quality control of fermentation processes as well as in environment (water, soil)[2-5].

* Corresponding author: bbusz@chem.uni.torun.pl

WHAT IS CAPILLARY ZONE ELECTROPHORESIS?

Electrophoresis is based on the principle that electrical charged molecules migrate towards the opposite pole and separate from each other on the basis of their physical characteristics, especially on the basis of their charge to size ratio. One of the first people that worked with electrophoresis was Tiselius, who also received the Nobel Prize in 1948 for his experiments. He also proved that molecular weight of proteins is strongly related to their electrophoretic mobility[3].

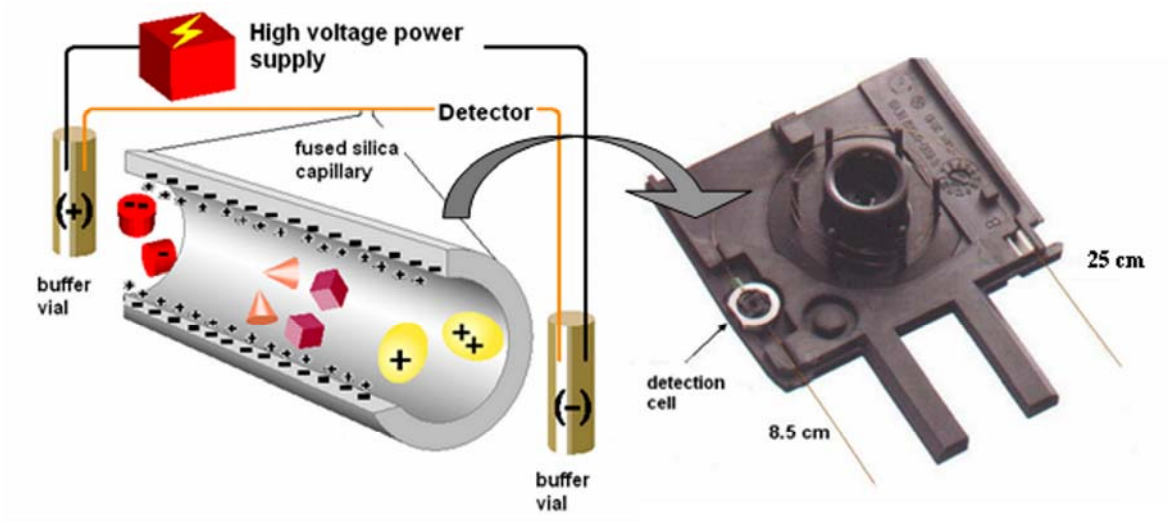


Fig. 1 – A schematic diagram of the capillary electrophoresis system and the picture of cassette with detection cell for electrophoretic measurements [6].

However, electrophoresis had two limiting factors: (i) the fact that only low voltage could be used in order to prevent over-heat and (ii) the detection of molecules after electrophoresis. Capillary electrophoresis (CE), which was first introduced in 1960s, solved those two problems.

The basic components of capillary electrophoresis system are: a high voltage power supply, a fused silica capillary with an internal diameter ≤ 200 μm, electrodes, two buffer containers that hold both the electrode and the capillary and a detector (Fig. 1).

In performing CE, the capillary is filled with an appropriate separation buffer at the desired pH and the sample is introduced at the inlet. Both ends of the capillary and the electrodes are placed into buffer containers and up to 30 kV applied to the system. Determined by their charge and mass, the ionic species in the sample plug migrate (in a certain direction and with a certain speed) and, eventually, they pass a detector where information is collected and stored by the software[4]. Electrophoretic mobility (μ_{ef}) is the force that applied to ions helps cationic substances pass the detector, while neutral compounds remain static and anionic compound are moved into a different direction; hence it is not the only force that appears in CE. The electrophoresis mobility is given by:

$$\mu_{ef} = \frac{q}{6\pi\eta r} \tag{1}$$

where: q is the force of molecule, r – diameter of molecule and η is the buffer viscosity.

Electroosmotic flow (EOF) is the other force that under an applied field takes all the components towards the detector[5], and in buffer is given by

$$\mu_{EOF} = \frac{\varepsilon\zeta}{4\pi\eta} \tag{2}$$

where: ε is the dielectric constant of the buffer, ξ is the zeta potential which is given by

$$\xi = 4\pi\delta q \tag{3}$$

where: δ is thickness of the diffuse of the double layer, q_e is the charge per unit surface area.

The thickness of the diffuse of the double layer is inversely proportional to the concentration of the buffer. The electroosmotic flow is proportional to the zeta potential. The data (the separated chemical compounds) appear as a function of time of the detector response, mainly peaks with

different migration times being shown in the electropherogram.

There are many types of CE which are distinct between themselves depending on the type of capillary and electrolytes used.

Capillary zone electrophoresis (CZE), also known as free-solution CE (FSCE) is the simplest form of CE. Differences in the charge-to-mass ratio of the compounds are the basis of the mechanism of separation. Homogeneity of the buffer solution and constant field strength all over capillary's length are the fundamental points in performing CZE.

DETERMINATION OF BACTERIA FOR MEDICAL DIAGNOSIS

The search for and the identification of an etiological factor of an infectious disease is known as "microbiological diagnosis". In order to diagnose an infectious disease and to give the proper treatment to a patient it is necessary first to identify the microorganism that caused the infection and finally test its sensitivity to different drugs[6].

Nowadays, in medicine there are different ways of identifying bacteria and diagnosing infectious diseases, such as (1) microscopic examination of exudates, body fluid or tissues after staining (Gram staining, acid-fast), or by dark-field examination or immunofluorescent-labeled antibody tests, or by the newer techniques for antigen identification such as counterimmunoelectrophoresis and latex agglutination, as used in respiratory infections, (2) appropriate bacteriological culture techniques, (3) a variety of new techniques to identify the antigen and the particular antigenic strain, many based on molecular methods, (4) serological tests[7] and (5) polymerase chain reaction (PCR). The PCR technique is becoming more utilized by many laboratories, despite being very costly. The reason is that it represents a relatively fast and accurate manner of identifying pathogenic bacteria through their genetic material. It also allows antibiotic resistance to be efficiently and precisely assessed. On the other hand, PCR has a limited screening potential, this being its main disadvantage[6].

Traditional methods (inoculating into a medium, culturing, staining and analyzing with a microscope) are usually enough for the proper diagnosis of an infectious disease, sophisticated methods being useless. Problems appear in case of more severe diseases, when time is crucial and the diagnosing has to be fast and efficient. Therefore, when we are handling with infections that have a rapid progress and that can have serious damages in short periods of time (for example septic shock, meningitis etc.) the correct treatment is crucial and has to be applied almost immediately[6].

Recently, much work has been done in order to improve traditional methods of diagnosing and to search for novel techniques that could ease medical diagnosis. Electromigration techniques have been recently proposed for the identification of microorganisms. Bacteria have a surface charge that originates from the ionization of surface molecules and of the adsorption of ions from solution. The characteristic charge of bacterial cell wall and membranes is given by their components: proteins, lipid molecules, teichoic acids, lipopolisaccharides (Table1).

The presence of negatively charged ions of carboxyl and phosphate groups on the bacterial cell wall allows bacteria to enter the mechanism of electrophoresis. Thus, bacterial cells can be submitted to electromigration techniques in a free solution, their mobility being depended of ionic strength and pH of buffer solution[2,7,8].

Table 1

Relation between interaction groups localized on the surface of bacteria and protonisation reaction

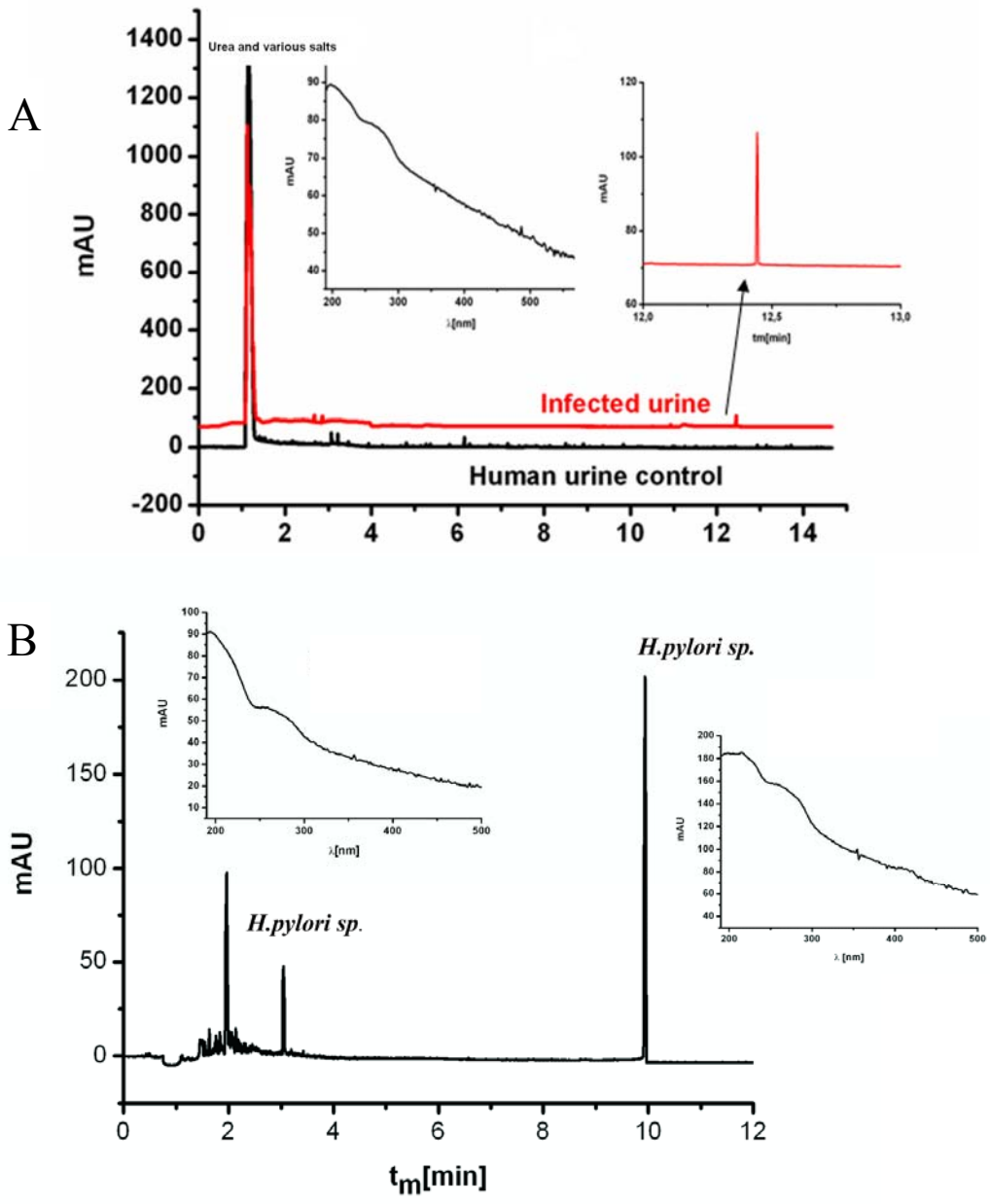
Groups localized on the bacteria surface	Reaction
polysaccharide, protein, peptidoglycan	$-COOH \Leftrightarrow -COO^- + H^+$
protein, peptidoglycan	$-NH_3^+ \Leftrightarrow -NH_2 + H^+$
teichoic acids	$-HPO_4 \Leftrightarrow -PO_4^- + H^+$
phospholipids	$H_2PO_4 \Leftrightarrow HPO_4^- + H^+$
phospholipids	$-HPO_4^- \Leftrightarrow -PO_4^{2-} + H^+$

The first who tried to perform bacterial determination using CE was Hjerten et al. in 1987, showing that tobacco mosaic virus and *Lactobacillus casei* would migrate through a capillary and that the orientation of the tobacco mosaic virus affected its electrophoretic mobility[9]. However, because all the

species moved with the EOF, no separation has been achieved. The first successful separation was achieved by Ebersole and McCormick, who partially isolated *Enterococcus faecalis*, *Streptococcus pyogenes, Streptococcus agalacticae, Streptococcus pneumoniae* and *Streptococcus aureus*[10]. The same method was applied by Pfetsch and Welsch[11] for *Pseudomonas putida, Pseudomonas species* and *Alcaligenes euthropus*. An effective separation of *Micrococcus luteus, E. aerogenes, Pseudomonas fluorescens* and *S. cerevisiae* was obtained by Armstrong and co-workers[12,13].

Determination of bacteria is not easy to perform, since they are living organisms. Some of them easily adhere to various surfaces or to other microorganisms or they can form clusters, as natural behavior. Moreover, there can appear many peaks because of bacterial secretions of enzymes or proteins[2]. Therefore, when working with microbial cells the use of different compounds is needed in order to prevent those unwanted results.

A few years ago we also conducted a promising research about the possibility of performing bacterial separation and identification with CZE. Their experiments have shown that this method can offer a sensitive, inexpensive and fast manner of identifying microorganisms[6]. Buszewski and co-workers utilized capillary surfaces with chemical modification, using different monomers (for example: divinylbenzene, trimethylchlorosilane, acrylamide) or by using monolithic columns for the bacterial identification. Thus, they succeeded in suppressing EOF and reducing the adsorption of the cells to the capillary wall (Fig. 2)[1,6, 14-17].



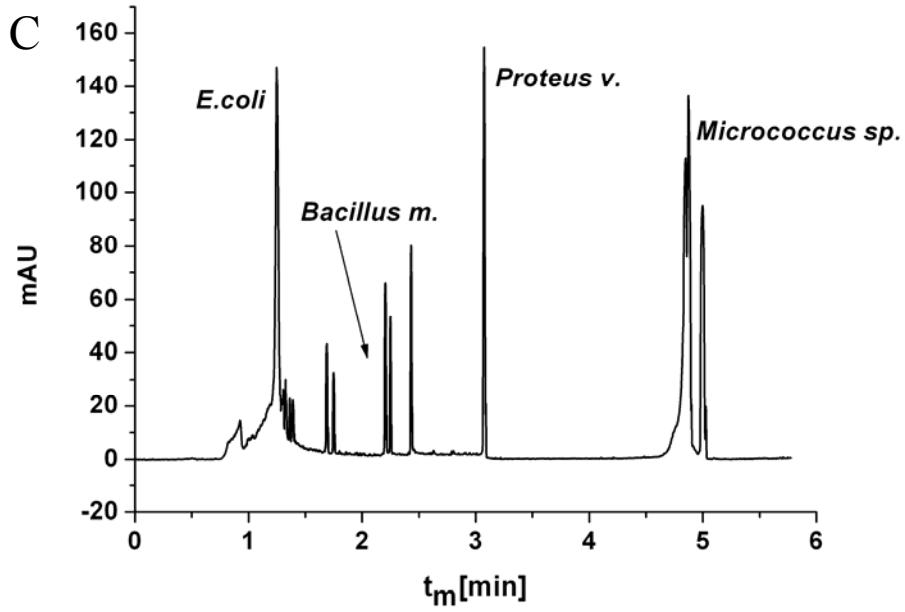


Fig. 2 – Direct injection of urine sample into the CE system; Capillary: bare fused silica (L_{eff} = 25 cm).The buffer was 0.0125% PEO dissolved in TBE buffer (pH = 8.53), detection at 214 nm, U = 20kV, injection 200 mbar·s (A), Separation of two bacterial strains. Capillary: bare fused silica (L_{eff} = 25 cm). The buffer was 0.0125% PEO dissolved in MES buffer (pH = 6.1) (B) and Separation of four species of bacteria using divinylbenzene modified capillary on 8.5 cm distance, Buffer TBE (pH = 8.53), detection at 210 nm, $V = -15$ kV, injection 100 mbar s [6,8].

DETECTION

Several types of signal transfer devices can be applied as detector of analytes in CZE separation. In many cases UV-Vis or diode-array photometer, electrochemical detectors (amperometric or coulometric, conductivity), fluorescence or mass spectrometer are used.[3,13,16] However, in literature very selective and specific detectors such as laser inducted fluorescence detector were described,[18] NMR,[19] ICP-MS[20] as well as stereoscopic microscope working in fluorescence modus coupled with CZE.[17]

UV or UV-Vis absorbance is used as the main mode of detection by many systems. Those are used partially as a capillary's detection cell and the other must be optically transparent. Because capillaries used in CZE are coated with a polymer in order to increase stability, for UV detection, capillaries are coated with transparent coatings to prevent breaking. When performing UV detection the most important step is choosing the proper detection wavelength. The spectra of bacterial suspensions were recorded in the wavelength range of 190 nm to 350 nm. When using a wavelength of 214 nm, the detection has a low sensitivity and quantification is very hard to be made on its basis.[15]

Another method of detection is fluorescence. It can be used only for samples that are naturally fluorescent or that are modified with chemical compounds in order to become fluorescent. This method is very sensitive, as the incident light has a high intensity and it can be focused on the capillary.[16] There can be applied a wide range of dyes to bacteria and thus they can be detected through fluorescence, especially laser induced fluorescence. This additional step could be the only disadvantage of this method. The application of fluorescent dyes makes bacteria visible, so it is also possible to view the process of separation.[17]

The capillary electrophoresis system can be also directly connected with a mass spectrometer (MS), which allows us to identify the sample components.

APPLICATIONS

As mentioned before, the determination of microorganisms is very important and it is finding many applications in people's lives. Although its role in medicine, in the diagnosis of infectious diseases, seems to be its greatest importance, its role in environmental issues or in the quality control of food should also be recognized.

Food products are composed of a large variety of molecules with different chemical properties. Although, chromatographic methods are currently being used in food analysis, bacterial contamination of food needs new and rapid methods of identification and quantification.[21] CZE represents a method through which both of those can be achieved. The advantages of this technique have made possible the analysis of different categories of food, such as water, beverages, fruits, vegetables, milk, meat, cereals, wine etc. Besides the control of microbial contamination, CZE also offers the possibility of controlling food changes (oxidation, amino acid racemization etc.).[22,23]

For the environment, CZE can be used as a method of monitoring bacteria in drinking water, wastewater, rivers and reservoirs.[16]

CONCLUSIONS

The importance of electromigration techniques in molecular biology, medicine, as well as in other branches of environmental and food analysis is increasing rapidly, as the determination and separation of microorganisms can be easily achieved through CZE.

When using CZE for bacterial determination you can find some advantages of this method, like the use of small amounts of sample, a high separation efficiency and resolution, a rapid and quantitative separation, automated instrumentation, various modes to vary selectivity and a simple separation mechanism.[24]

Acknowledgements: This work was financially supported by the CEEPUS project (CII-RO-0313-01-0809) and the Foundation for Polish Science (FNP).

REFERENCES

1. B. Buszewski and E. Kłodzińska, *Electrophoresis,* **2008**, *29*, 4177.
2. Szumski, M.; Kłodzińska, E.; Buszewski, B, J. Chromatogr. A .**1084**, 2005, 186.
3. P. Camilleri, *Capillary electrophoresis – theory and practice*, 2nd edition, CRC Press: Boca Raton, New York, 1998.
4. J.P Landers, *Handbook of capillary and microchip electrophoresis and associated microtechniques*, 3rd edition, CRC Press: Taylor & Francis Group, Boca Raton, New York, 2008.
5. J.P Landers, *Handbook of capillary electrophoresis*, 2nd edition, CRC Press: Taylor & Francis Group, Boca Raton, New York, 1997.
6. M. Jackowski.; J. Szeliga , E. Kłodzińska, B. Buszewski, Anal. Bioanal. Chem. **391,** 2006, 2160.
7. A.S. Evans: P.S. Brachman, *Bacterial Infections Of Humans* – Third Edition, 1998.
8. S. Hjerten, K. Elenbring, F. Kilar , J. Liao, A.J.C. Chen, J. Siebert, M. Zhu, J. Chromatogr. **403,** 1987, 47.
9. R.C. Ebersole, R.M. Mccormick, Biotechnology **11**, 1993, 1278.
10. A. Pfetsch, T. Welsch, Fresenius' J. Anal. Chem.; **359**, 1997, 198.
11. J.M. Schneiderheinze, D.W. Armstrong, G. Schulte, D.J. Westenberg, Microbiol Lett. **189**, 2000, 39.
12. M.J. Desai, D.W. Armstrong, Microbiol. Mol. Biol. Rev., March 2003, 38.
13. K. Hrynkiewicz, E. Kłodzińska, H. Dahm, J. Szeliga, M. Jackowski, B. Buszewski, Fems Microbiol. Lett. **286**, 2008, 1.
14. E. Kłodzińska, H. Dahm, H. Różycki, J. Szeliga, M. Jackowski, B. Buszewski, J. Sep. Sci. **29**, 2006, 1180.
15. E. Kłodzińska, B. Buszewski, Anal. Chem. **81**, 2009, 8.
16. M. Szumski, E. Kłodzińska, B. Buszewski, Microchim. Acta, **164**, 2009, 287.
17. S. Wu, N.J. Dovichi, J. Chromatogr., **480**, 1989, 141.
18. K. Albert (Ed), On-Line Lc-*Nmr* And Related Techniques: Wiley, Chichester, **2008.**
19. R. Łobinski, J. Szpunar, "Hyphenated Techniques In Speciation Analysis" Royal Society Of Chemistry (Rsc), Cambridge, 2005.
20. Y. T. Dong, Food Sci. & Tech. **10,** 1999, 87.
21. A. Cifuentes, Electrophoresis **27**, 2006, 283.
22. M. Ligor, R. Jarmalaviciene, M. Szumski, M. Maruska, B. Buszewski, J. Sep. Sci., **31**, 2008, 2707.
23. B. Buszewski, E. Kłodzińska, H. Dahm, H. Różycki, J. Szeliga, M. Jackowski, Biomed. Chromatogr. **21,** 2007, P116.