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Electrokinetic Detection and Characterization of Intact Microorganisms

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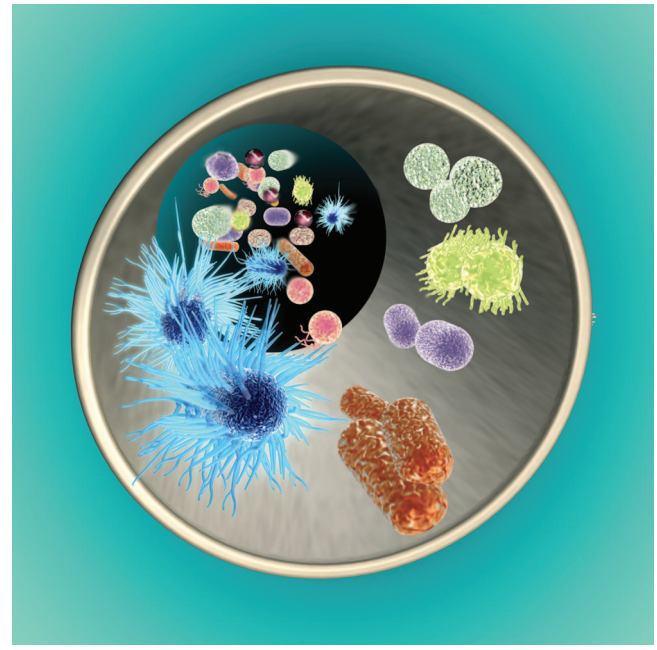
Achievements in bacteria analysis with electromigration techniques may improve medical diagnoses, detection of food contamination, and sterility testing. (To listen to a podcast about this feature, please go to the *Analytical Chemistry* website at pubs.acs.org/ac.)



An important research problem in the modern clinical laboratory is the discovery of new biomarkers, the presence of which can indicate a particular disease state; for example, the presence of an antibody may indicate an infection. The complex nature of biological samples and the low concentrations of analytes demand a system with high sensitivity and efficiency. Capillary zone electrophoresis (CZE) is such a system, and it promises to rival HPLC when applied to the separation of charged and neutral species in biochemistry, pharmaceutical science, bioscience, ion analysis, food analysis, environmental science, medical diagnosis, and clinical settings.[1,2] One area in which CZE may have an inherent advantage over HPLC is the analysis of small particles of colloidal sizes, such as cells.

The rapid and sensitive determination of pathogenic microorganisms is extremely important in biotechnology, quality control of probiotics, and bacterial analysis, but it is critical for early and effective disease management and antimicrobial therapy, especially for infant patients, whose immunological systems are not fully developed. Some microbes are active ingredients in health products, medicines, and supplements. On the other hand, some pathogenic microorganisms can be considered possible biological warfare agents and constitute a significant cause of death in many countries (*Bacillus anthracis*, *Rickettsia rickettsii*, and *Salmonella typhi*).[2] Biological weapons include bacteria, viruses, fungi, and toxins found in nature that can be used to kill or injure people. Pathogenic bacteria are the most common causes of food- and water-borne illnesses.

However, conventional microbiological techniques of bacterial identification, culture, and isolation by biochemical and serological assay are time-consuming and labor-intensive. Therefore, fast and selective analytical techniques that more rapidly and effectively determine microbial contamination or infection are required. In this article, we summarize the most important achievements in microbial separation by electromigration techniques and describe the different applications.

ORIGIN OF MICROBIAL CHARGE

Small viruses have diameters in the range of several tens of nanometers. Bacteria are generally larger, some by a factor of 100, and the increased size leads to increased complexity. Viruses exist mainly in helical and/or icosahedral forms, whereas bacteria can adopt an enormous variety of shapes and sizes, both among and within species. These physiological differences can make the characterization and identification of bacterial cells by electromigration techniques more difficult (Figure 1a). The microbial surface charge originates from the ionization of surface molecules and the adsorption of ions from solution. Bacterial cell walls and

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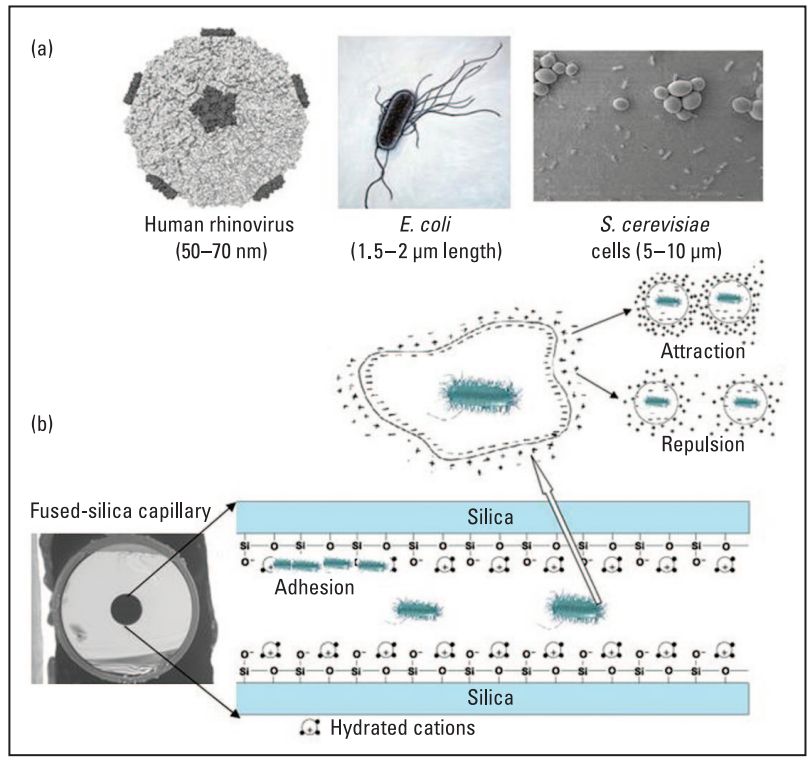

Figure 1. (a) Comparison of viral, bacterial, and fungal cell dimensions. (b) Interactions between a fused-silica capillary and a charged bacterial cell surface.

membranes contain numerous proteins, lipid molecules, teichoic acids, and lipopolysaccharides that contribute to this characteristic charge. However, at physiological pH (5–7), most bacterial strains are negatively charged because the number of carboxyl and phosphate groups exceeds the number of amino groups.

The compositions of Gram-positive and -negative bacteria differ considerably. The cell wall of Gram-positive bacteria consists of a 15–80-nm-thick rigid layer of peptidoglycan on top of the phospholipid-rich cytoplasmic membrane. The cell wall of Gram-negative bacteria consists of a much thinner peptidoglycan layer 1–2 nm thick sandwiched between two phospholipid bilayer membranes. The outer of these two membranes contains pores that are formed by aggregates of proteins and that facilitate the selective transport of molecules through the membrane. As charged particles, bacterial and viral cells undergo electrophoresis in free solution, with their mobility determined by the ionic strength and pH of the buffer solution.[1–7]

Microorganisms are the new "smart" particles that can change their surface properties on the basis of interactions with one another and the surrounding environment. This change in surface characteristics dictates how bacterial cells interact when forming biofilms and aggregates. Other considerations are the numerous types of interactions with the solvent and the solutes that occur on the fused-silica capillary surface. In the case of bacteria, strong adhesion and aggregation effects are observed. Bacterial adhesion and surface colonization are highly correlated with the bacteria's surface physicochemical properties. The various strains of microorganisms have different surface properties, resulting in different adhesion kinetics and affinity for the substrate. Interactions among microorganisms vary from weak association to strong electrostatic or covalent bonds (Figure 1b). These interactions are unstable, and bacteria can easily be removed from adsorbed surfaces by different solvents or by agitation.

Nevertheless, when bacterial cells are in close proximity to a fused-silica surface or coated capillary wall, they can form short-range, specific interactions that are very stable. Although the exact mechanisms are not completely understood, it is believed that extracellular proteins (outer membrane proteins, flagella, etc.), polysaccharides, and lipopolysaccharides play a role in strengthening bonds between a bacterium and a substrate (stationary phase) or between other microbes of the same or different species.[3,4] Aggregation may also be indicative of pathogenic properties of the microbe. These aggregates make it difficult to interpret the separation process of different microorganisms; many microbes release enzymes and proteins that produce unwanted peaks. Variations in microbial cell wall composition are caused by isolation and preparation procedures, growth conditions, and age. Appropriate selection of the experimental conditions can help minimize the excretions that cause the additional peaks. Moreover, the best experimental environment for one or a few species of microorganisms may not be right for other species, which is why bacterial determination is not easy to perform.[6–16]

SELECTIVITY AND SEPARATION PERFORMANCE

Ebersole and McCormick were the first researchers to partially separate *Enterococcus faecalis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* by CZE.[17] *S. pyogenes* and *S. pneumoniae* were resolved in nearly 70 minutes (min) with 250-cm-long, 100-μm-i.d. capillaries. Four years later, Pfetsch and Welsch applied a similar method to

separate *Pseudomonas putida*, *Pseudomonas* sp., and *Alcaligenes eutrophus*, but the bandwidths were still broad.[18] They proved that the electrophoretic mobilities of bacteria are characteristic features and can be determined easily by CZE, and they found the most appropriate ionic strengths, pH values, and buffer solutions to do so.[18]

The main problems in electrophoretic analysis of bacteria are separation performance and selectivity. In electrophoresis of intact bacterial cells, separation can be degraded by adhesion of the bacteria to the fused-silica surface, resulting in a nonreproducible electroosmotic flow (EOF). In particular, cationic components from the bacterial envelope have a tendency to interact with anionic silanolate groups, resulting in band broadening and distortion, which decrease separation efficiency and selectivity. In addition, the migration of bacteria in clusters or aggregates decreases the magnitude of electrophoretic mobilities, leading to poor reproducibility of migration times and suppression of the EOF.[12,13]

On the other hand, bacteria−wall interactions can be reduced or eliminated by dynamic or chemical modification of the active sites on the capillary surface.[6−16] In the case of chemical modification, the modifier is introduced onto the capillary wall by the formation of covalent bonds; for example, a reactive bifunctional silane precursor such as γ-methacryloxypropyltrimethoxysilane can be used. The silane groups may be attached and polymerized by monomers, forming the outer layer on the capillary surface. By using acrylamide-, trimethylchlorosilane-, and divinylbenzene (DVB)-modified capillary columns, Buszewski and co-workers were able to separate five species of bacteria in 8.5 cm in 10 min.[13] Because the bacteria were separated in the same buffer in modified capillaries (trimethylchlorosilane and DVB), weak adhesion to the capillary wall likely had some effect on the separation, which resulted in slightly greater mobility values in the DVB-coated capillary.

These observations can be confirmed by the results of capillary rise measurements. In the DVB-modified capillary, no rise was observed, which is indicative of the high hydrophobicity of the inner capillary wall.[13] The contact angle θ was calculated from the capillary rise of water h_r (cm) by

$$\cos\theta = \frac{\frac{1}{4}h_r d(\rho - \rho_v)g}{\gamma}$$

in which d is the i.d. of the capillary (cm), ρ is the density of the liquid used (g/mL), ρ_v is the density of air saturated with vapor from the liquid (g/mL), g is the acceleration due to gravity (cm/s^2), and γ is the surface tension of the liquid (dynes/cm).[13]

Armstrong et al. published a series of papers on the mechanistic aspects of the separation process, separating bacterial aggregates, on-line monitoring of bacterial migration, identifying the causative pathogens of urinary tract infections, determining bacterial viability, quantitation of bacteria, and determining live bacterial cells in consumer products.[6,8−11,19] The researchers used primarily two electromigration techniques for the separation of several species of microorganisms. The first approach involved CZE of bacteria with a dissolved poly(ethylene oxide) (PEO) solution and UV absorbance detection at $\lambda = 214$ nm. The second approach used capillary isoelectric focusing (CIEF) of three similarly sized bacteria on the basis of their surface charges.[6] Successful CIEF separation of yeast cells (4 μm diam) in 100-μm-i.d. capillaries coated with hydroxypropyl methylcellulose has been obtained. Yeast cells cultured to different cell densities exhibited pI differences of up to 1.2 units.[19]

Most research groups have used a method that provided separations of bacterial cells with very short analysis times and extremely sharp peaks.[6−16,20−22] These "apparent efficiencies" were attributed to a very small addition of modifying agents to the running buffer. Most frequently, a very dilute (0.0125−0.025 w/w) solution of PEO was used to decrease the EOF, prevent adsorption of bacteria to the fused-silica surface, and promote consolidation of the cells into a narrow band. Without PEO, the EOF was too fast, and all species of bacteria migrated with EOF as a broad band. Finally, it was possible to transform broad peaks near the EOF (a series of spikes from scattered light from the microbial and colloidal particles) into sharp and symmetrical peaks.[20]

Another method involves immunofluorescent staining and adding *N*-hydroxyethylacrylamide or identification of *S.aureus* in bacterial mixtures by means of specific mAb-coated latex coupled with CZE.[23,24] Completely different approaches use chemical modification of capillary walls and monolithic stationary phases.[12−16] All of this research that focused on many different microorganisms indicated the possibility of using CZE and/or a microfluidic approach for a variety of important microbiological analysis applications.

OBSERVING BACTERIAL MIGRATION BEHAVIOR

Migrating bacterial zones can be measured several ways. The majority of commercial systems use UV, UV−vis absorbance, or fluorescence as their primary mode of detection. Laser-induced fluorescence (LIF) has been used in CZE systems with very low detection limits (10^{-18}−10^{-21} mol). The sensitivity of the technique is attributed to the high intensity of the light and the ability to accurately focus it on the capillary. However, in the case of bacterial separation, it is very important to understand the unusual sample focusing of the microorganisms and to prove the presence of undesired phenomena such as self-aggregation, migration in clusters, and irreversible adhesion to the fused-silica surface. For this reason, specialized devices such as the CCD camera or different types of microscopes or microfluidic devices have been used.[25−27]

In 1996, Preisler and Yeung first examined changes in the EOF in a fused-silica capillary during electrophoresis by using PEO dissolved in the running buffer.[28] An imaging CCD camera was used to follow the motion of a fluorescent neutral marker zone. In 2002, Armstrong and co-workers used a CCD camera coupled with LIF to monitor bacterial migration behavior and record moving pictures of the microbial electrophoretic process.[26] They showed that under certain experimental conditions, a band compaction (focusing) process occurred inside the capillary as bacterial cells migrated in a dc field. Many different parameters, such as ionic strength of the buffer solution, pH values, and different types of polymers with various molecular weights, were studied.

Three possible mechanisms were outlined that could account for the solution aggregation of bacterial cells in the presence of PEO and an electric field. The first involved the field-induced

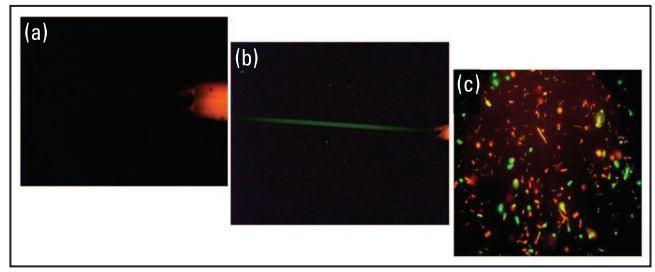

Figure 2. Data from CZE/stereomicroscopy. (a) Fluorescence from the capillary only. (b) Green (live) fluorescent bacteria adhered to the capillary. (c) Total map of red (dead) and green fluorescent bacterial cells.

aggregation model, in which the particle (bacterial cell) is attracted to (positive dielectrophoresis) or repelled from (negative dielectrophoresis) the high-electric field regions, depending on the particle's polarizability and the frequency of an applied ac field.[20] The second model involved the presence of a "hairy" layer (the dilute adsorbed polymer) near the bacterial surface. It was proved that the electrokinetic movement of small ions is slowed within the hairy layer, creating a localized region of lower conductivity; if a concurrent, local enhancement of the electric field results, focusing can occur. The third mechanism is the shape-induced differential mobility model, which illustrates differential migration depending on the orientation of the cells in the electric field. For rod-shaped microorganisms such as *E. coli* and *Bacillus infantis*, the orientations are randomly distributed; when an electric field is applied, these bacteria migrate at different velocities. This unsystematic movement allows for the possibility that bacterial cells will collide and aggregate, depending on their surface properties. It is apparent that the electrophoretic mobility of the clusters is different from that of the individual cells, and the rates at which different cells aggregate also vary.[20]

Buszewski et al. successfully visualized bacterial adhesion to the capillary wall and migration in clusters without adding PEO to the running buffer.[27] In this case, the acridine-orange-stained bacterial cells were monitored with a CZE apparatus coupled with a fluorescent stereomicroscope equipped with a CCD camera and specialized software. This novel construction allowed for on-line observation of the electrophoretic movement. Moreover, the adhesion of the bacterial cells could be observed as a slight green fluorescence of the inner capillary wall, a signal that was not observable with a diode array detector (Figure 2).

Microfluidic devices recently used for CZE and on-chip reactions typically have cross-sectional dimensions of 10−50 μm, which are similar to those of biological cells.[29] Li and Harrison reported the initial steps for on-chip cell transport and manipulation of *E. coli*, yeast, and erythrocytes.[29] They used applied potentials in a network of channels to control the direction of cell flow and also demonstrated a simple lysing reaction by on-chip mixing of cells and reagents. In this case, the observations were performed with a microscope equipped with a video camera. Shintani and co-workers developed and optimized a system coupling microcapillary electrophoresis (MCE) and LIF detection for the analysis of bacteria.[25] They successfully separated pure cultures of lactic bacteria and *Saccharomyces cerevisiae* within 200 seconds by adding 0.05% of sodium alginate and 0.1% NaCl.[25]

CE OF VIRAL CELLS

Early work involving microbes and CZE was performed in 1987 by Hjerten et al.[30] By using a fused-silica capillary dynamically coated with methylcellulose, they analyzed *Lactobacillus casei* and tobacco mosaic virus. However, there was no separation of the different species of microorganisms because all of them moved together with the EOF. Later, a series of investigations reported CE of human rhinoviruses (HRVs), which are best known for causing the common cold, bronchitis, and, often, asthma.[31−34] HRVs have a diameter of ~35 nm and a molecular mass of 8×10^6 Da. They are composed of 60 copies each of four viral capsid proteins (VP1−VP4) that form an icosahedral shell that encloses an RNA genome with positive (messenger-sense) polarity. These highly ordered structures easily undergo conformational modifications during infection upon exposure to low pH or to elevated temperature.[31−34]

Mann et al. demonstrated a CZE method for the analysis of an intact recombinant adenovirus.[35] They used a capillary coated with polyvinyl alcohol, which eliminates the need for detergent additives in the running buffer. This system allows for the observation of new viral peaks that appear after a freeze−thaw cycle, which indicates discrete modifications to the viral surface. In the same year, Okun et al. used affinity electrophoresis to investigate the formation of complexes of HRV2 and HRV14 with nonaggregating, neutralizing monoclonal antibodies.[36] The migration behavior is modified upon binding of specific antibodies; this allows identification of affinity complexes and their separation from unbound reagents.[36]

In 2001, on the basis of the thermodynamic formation of complexes between an HRV and neutralizing ligands, Okun et al. quantified the extent of the affinity reaction by relating the equilibrium concentration of one reactant to the initial concentration of the other reactant added to the incubation mixture.[37] They proposed an equation that describes the reaction between one molecule of the receptor and the equivalent binding sites, assuming that the interaction is independent of the presence of the other sites. In addition, they determined the stoichiometric composition for the complex of a nonaggregating antibody (mAb 8F5) with HRV2 and the soluble receptor fragment of MBP-$VLDLR_{2-3}$ with HRV2. The dissociation constant was determined to be several hundred nanomoles per liter.[37]

In 2004, Kenndler et al. published a series of papers concerning selective CE detection of HRV by labeling the capsid proteins and genomic RNA of HRV with different fluorescent dyes.[38−40] The

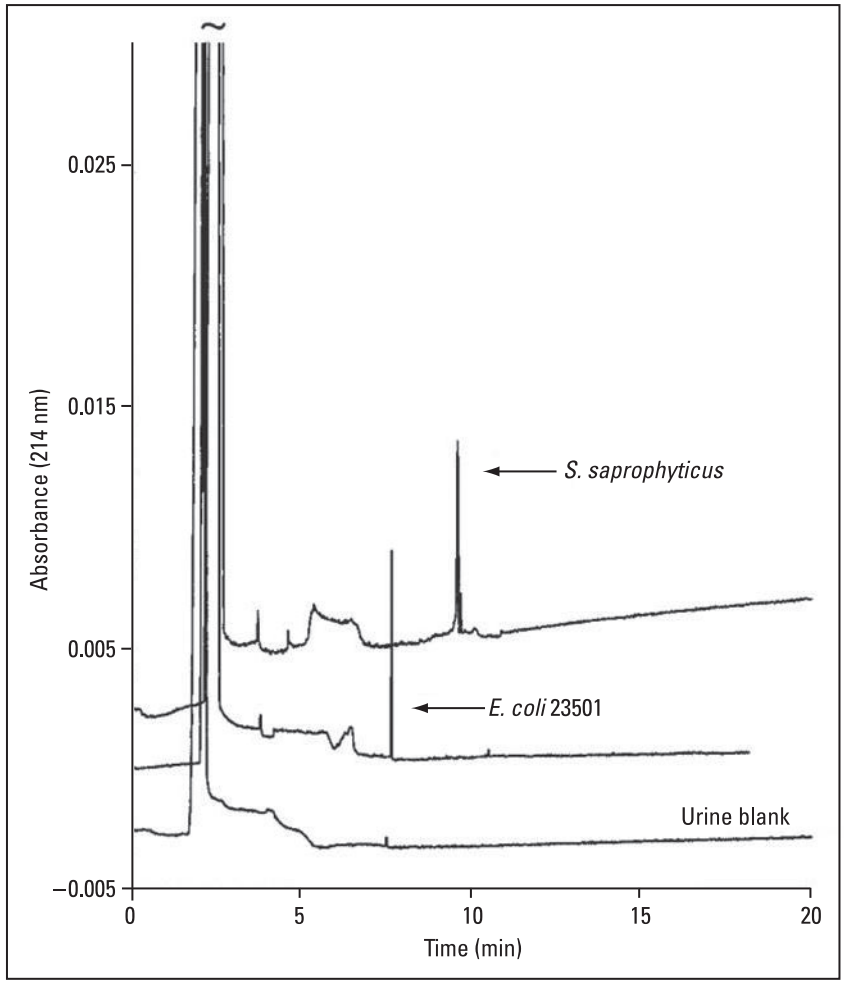

Figure 3. *S. saprophyticus* and *E. coli* in identical urine matrixes. (Adapted from Ref. 9.)

capsid of HRV2, consisting of four viral proteins, was labeled with fluorescein isothiocyanate (FITC) and analyzed by CE with UV and LIF detection.[39,40] To confirm the identity of the peak, the researchers used heat denaturation, proteolytic digestion, and receptor binding. The results clearly demonstrated that fluorescence labeling of the viral capsid might lead to a valuable probe for studying infection processes in living cells.[40] The authors proved that viral capsid can be stained with AMCA-S and that it is possible to selectively label the genomic RNA and the capsid with two different stains. They also showed that bioaffinity is preserved after capsid derivatization (the virus still binds soluble receptor fragments) and, finally, that the double-labeled virions are still infective and dangerous.[40]

In the same year, Shiha and co-workers identified hepatitis C virus in human urine samples.[41] The characteristic peak at migration time 2.72 min was collected by using CZE and was subjected to nested PCR. The amplified product of the fractionally collected peak was detected by agarose gel electrophoresis. In 2006, Kremser et al. described the influence of detergent additives on the mobility of native and subviral rhinovirus particles by CE.[42,43] Analysis was performed in borate buffer at pH 8.3 with an electrolyte and three detergents as additives. Moreover, the influence of capsid modification with the amine reactive fluorescent dye Cy3.5 on migration behavior was discussed. The researchers found that reproducibility strongly depended on the presence of the detergents above their critical micelle concentration.[42,43]

In their next paper, Kremser et al. demonstrated that the nonionic detergent dodecylpoly(ethyleneglycol ether) (D-PEG) was suitable for analysis of subviral particles because it preserves their integrity, in contrast to commonly used SDS.[43] They obtained a well-defined peak for 80S particles in borate buffer containing 10 mM D-PEG. The identity of the peak was confirmed by complexation with mAb 2G2, which recognized a structural epitope exclusively present on subviral particles but not on the native virus.[43] Upon incubation of the 80S particles with mAb 2G2, the peak disappeared. Incubation at 40 °C and then 65 °C allowed for the transformation of intact HRV2 virus into 80S particles. The researchers also demonstrated that subviral particles labeled with FITC or Cy3.5 were stable under the experimental conditions in which D-PEG was used. These particles were still recognized by mAb 2G2, suggesting that the exposed lysines that are derivatized by the reagent do not form part of the epitope of the antibody.[43]

Most recently, Bilek et al. demonstrated CE-LIF of the attachment of HRV2 to an artificial cell membrane. This membrane was mimicked by liposomes containing a lipid with a nitrilotriacetic

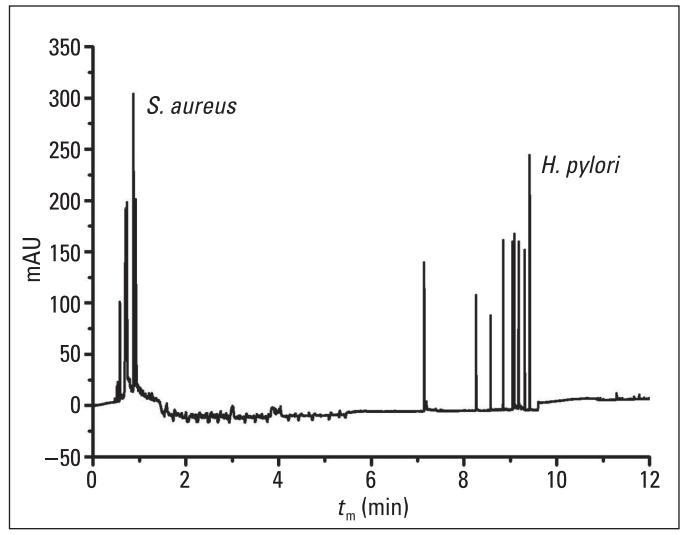

Figure 4. Separation of *S. aureus* (Gram-positive) and *H. pylori* (Gram-negative).[47]

acid group. Detection was accomplished when the minor receptor group HRV2 bound specifically to the receptor-decorated vesicles, and the major receptor group HRV14, which uses a different type of receptors for cell binding, did not attach to the liposomes.[44]

ELECTROPHORESIS OF BACTERIA FOR MEDICAL DIAGNOSIS

Thousands of serious cases of bacterial infections are diagnosed every year. Immediate treatment is required, but doctors have always faced difficulty in obtaining the correct diagnosis. Conventional bacterial identification tests are time-consuming and laborious. Therefore, CZE for the determination of intact bacterial cells represents not only a new frontier for separation sciences but also a completely new approach for medical diagnosis, quality control, and analysis of food contamination.

Armstrong and Schneiderheinze applied CE to the rapid identification of the bacterial pathogens *E. coli* and *Staphylococcus saprophyticus*, which are responsible for urinary tract infections.[9] Because urine is a water-based matrix with few macromolecular or colloidal contaminants, this seems to be an ideal method for microbial identification. They showed that the two pathogens can be separated by using a polymer-based CE approach (Figure 3). A similar method was used by Kłodzińska et al. to identify different *E. coli* strains in human urine.[14] The urine that was found to contain pathogenic bacteria by microscopic examination was directly injected into the CE system. However, to check the selectivity and sensitivity of the proposed method, the researchers attempted to resolve different strains of the same species of *E. coli* in a single run. The urine was inoculated with a different, pure culture of *E. coli* (PCM 2561) and then directly injected into the CE system. Several signals at different migration times originating from PCM 2561 were obtained. This constitutes proof of the technique's high selectivity and ability to differentiate among several pathogenic strains. Another approach for the analysis of *E. coli* in human urine involves the addition of calcium and myoinositol hexakisphosphate to the running buffer, but this method suffers from broad bandwidths.[14]

Under very similar experimental conditions, the same group was able to separate two clinical strains of *Helicobacter pylori* cultured from cancer tissue.[15] *H. pylori* causes >80% of gastric ulcers and is directly associated with the development of gastric cancer. Noninvasive methods to monitor infection would help in rapid identification of *H. pylori* even when different strains are present. This approach could be very useful for optimizing antibiotic treatments.[15]

To increase selectivity and sensitivity, Buszewski et al. used an acrylamide-modified capillary column to analyze strains of *S. aureus* from wounds and bedsores and used monolithic capillary columns based on styrene divinylbenzene to identify *E. coli* in human urine.[14] By using an open tubular capillary column made from three acrylamide layers, they successfully separated two clinical species of *H. pylori* and *S. aureus* (Figure 4).[15] Results were finally confirmed by molecular analysis of a part of the *coag* gene from analyzed strains of *S. aureus*. To perform coagulase gene typing, the repeated units encoding hypervariable regions of the *S. aureus* gene were amplified by PCR, followed by restriction enzyme digestion and analysis of restriction fragment length polymorphism patterns.[37]

ACTIVE BACTERIA IN PHARMACEUTICAL AND CONSUMER PRODUCTS

Specific strains of microbes can be beneficial to human health. Some excellent examples of these products are pills and syrups for patients who are lactose intolerant. Other examples are commonly used dietary supplements (probiotics) that contain potentially beneficial bacteria or yeasts. Lactic acid bacteria have been used in the food industry for many years because they convert sugars and other carbohydrates into lactic acid. Viability and the nature (strain or species) of the microbes determine whether they produce any beneficial effects. Arm-

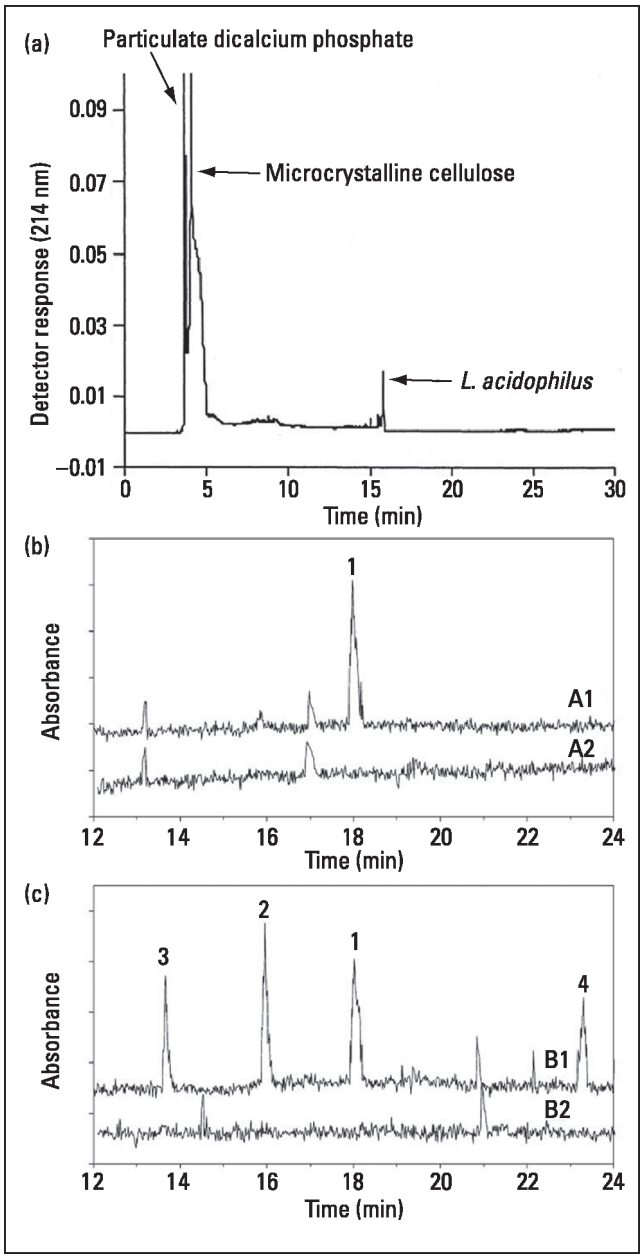

Figure 5. (a) Direct injection analysis of dissolved Schiff tablets. Electropherograms for (b) cornflakes and (c) juice. A1 and B1 are spiked samples; A2 and B2 are blanks. 1, *E. coli*; 2, *S. enteriditis*; 3, *Listeria monocytogenes*; 4, *Enterococcus faecium*. [(b) and (c) (adapted from Ref. 45.)

strong and co-workers developed the first high-efficiency microbial assays of tablets and powder-based commercial products (Figure 5a).[10] Moreover, they evaluated cell viability with CE-LIF by penetrating the cells with a mixture of SYTO 9 green fluorescent nucleic acid stain and red fluorescent propidium iodide stain. Viable cells produce an enhanced green fluorescence, whereas dead cells produce a red fluorescence.

Rapid developments in genomics and proteomics led to the introduction of capillaries filled with monoliths. Buszewski et al. were the first to use lauryl methacrylate to determine *Lactobacillus rhamnosus* in pharmaceutical products by capillary electrochromatography.[1,16]

STERILITY TESTS AND MONITORING BACTERIAL CONTAMINATION IN FOOD

Some of the tests for waterborne salmonellosis, a major public-health problem, are not sensitive enough and have a lengthy analysis time. In 2004, Palenzuela et al. proposed a CE-based method with UV detection for the identification and quantitation of bacterial contamination in cornflakes, juice, milk, baby food, and frankfurters (Figures 5b and 5c).[45] They took advantage of the interactions of calcium and myoinositol hexakisphosphate ions with the bacterial cell surface, which change its electrical properties and thus its electrophoretic mobilities. The technique was successfully adapted to identify contaminated food. The precision of real spiked samples is 3.3−7.0%, and the overall analysis time is 7 hours (h), much shorter than the 24−48 h required for plate counts. The validity of the method was established by comparison with the standard plate-counting method.[45]

Large-scale testing of pharmaceutical and medicinal products is very daunting. Especially in the health care industry, it is necessary to be sure that tissues, blood, and plasma are not infected with pathogenic bacteria. Hospitals must have the ability to diagnose bacterial infections as soon as possible for faster patient recovery. Until now, single-cell detection was the last significant hurdle to the development of a rapid, microfluidic sterility test for use by public organizations and private industries.[46] Armstrong et al. proposed a new procedure that gives a yes/no answer to the presence of any microorganisms in a single sample.[46] By using the synthetic compound SB3-40 in place of the nutrient broth blocking agent, the researchers decreased background noise by a factor of 40, which allowed for the identification of a single cell in the sample. Future experiments will focus on applying this test to real samples for diagnosis.[46]

CONCLUSION

The importance of electromigration techniques in molecular biology and medicine is increasing rapidly, especially in systematic studies of proteomes and metabolomes. Current research suggests that CZE allows for the identification of individual bacteria even in the range of different strains of the same species. The main benefit is the speed of analysis: final results can be available in 6−40 min.

Recent research has demonstrated practical uses for electrophoretic techniques, especially in medical diagnosis. For the environment, the largest application is monitoring bacteria in drinking water, wastewater, rivers, and reservoirs. The speed of these methods has the potential to provide revolutionary solutions because conventional microbiological detection techniques often require several days to produce results. Microscopes equipped with CCD cameras allow viability control and visualization of separation processes and can help elucidate problems with the separation. On the other hand, miniature devices could significantly enhance the throughput of cell analysis by integrating automated sample preparation steps with reagent mixing and by reducing separation times. The future holds the real possibility of simple devices that can rapidly perform a single type of analysis such as sterility tests, blood or lymph analysis, or wastewater analysis and give results in real time.

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Ewa Kłodzińska is an assistant chair and Bogusław Buszewski is chair of environmental chemistry and bioanalytics at Nicolaus Copernicus University (Poland). Kłodzińska's main research focuses on application of electromigration techniques, separation of pathogenic bacteria, and 2D PAGE and molecular biology methods for bacterial identification. Buszewski's research covers analytical chemistry, physicochemistry of surface processes, development of new stationary phases and columns, separation mechanisms (HPLC, TLC, GC, and CZE), hyphenated techniques, sample preparation, adsorption, and chemometry. Address correspondence about this article to Kłodzińska at 7 Gagarin St., 87-100 Torun, Poland (eklo@chem.uni.torun.pl).

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