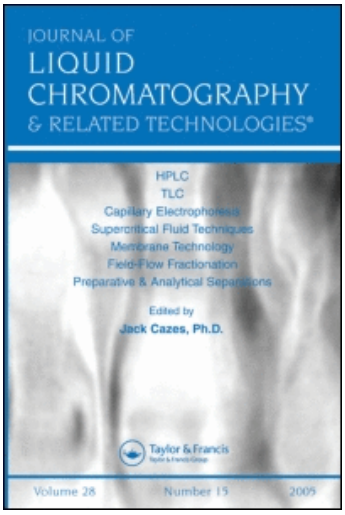

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CLINICAL APPLICATIONS OF CE

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INTRODUCTION

Capillary electrophoresis (CE) is a relatively new analytical separation technique in the clinical laboratory. The clinically relevant attributes of CE include: high resolution, rapid analysis time, nanolitre sample volume requirements, reduced cost, and full automation. In turn, the CE user is faced with the task of obtaining physiologically relevant detection limits and avoiding sample matrix interference. For this separation technique to be utilized routinely in the clinical laboratory, selectivity, reproducibility, and throughput must match, if not exceed, that of traditional slab gel and HPLC based techniques. In discussing the clinical applications of CE, it is also necessary to draw the distinction between various sample types.

Biomolecules of clinical interest have been analyzed by CE in various sample matrices. The majority of work has focused on the detection of bioanalyte standards added to different buffers and complex solutions. These numerous studies provide an important framework for the development of CE-based clinical assays, and demonstrate the immense potential for this technique in the clinical laboratory. Of greater interest to the clinical chemist, and the focus of this section, is the qualitative/quantitative determination of biomolecules *endogenous* to a real human sample matrix. CE can be used effectively in the analysis of biomolecules found in real sample matrices, such as serum, urine, cerebrospinal

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fluid, seminal plasma, saliva, cell plasma, and tissues. Presented, herein, are a variety of clinical applications of CE divided into three main categories: biofluid profile analysis, immunoassays, and clinical CE analysis using polymer sieving media. Future trends of CE in a clinical setting will be discussed.

BIOFLUID PROFILE ANALYSIS

Serum

Electrophoresis in the clinical laboratory is routinely used for the diagnosis of myeloma, a malignant disorder distinguished by the presence of monoclonal protein in the serum. Serum protein analysis has traditionally been performed by cellulose acetate electrophoresis (CAE) or agarose gel electrophoresis (AGE) under non-denaturing conditions. However, assets such as on-line UV detection, full automation, rapid analysis time, and capillary array instrumentation has made capillary zone electrophoresis (CZE) an attractive alternative to CAE and AGE .

Due to the complexity of the serum protein population, components are normally separated into 6 characteristic regions, defined as γ, β_2, β_1, α_2, α_1, and albumin. Abnormalities in a serum profile are thus, characterized by distinct peak shapes with diagnostic correlations obtained using the relative protein concentration in each region via peak areas. Since an augmentation of the diagnostic interpretation is often necessary, classification of the monoclonal protein is performed using immunosubtraction. Immunosubtraction is performed by incubating a serum sample with a solid phase having immobilized antibodies specific for a protein or a particular epitope on that protein. The recognized antigen is retained by the solid phase, allowing for the determination of its identity through a comparison of the subtracted CZE profile with that of the original CZE profile (figure 1).

Urine

Electrophoretic analysis of urine is performed primarily for the detection and quantification of Bence Jones protein, a protein associated with multiple myeloma, and to detect and distinguish between glomerular and tubular proteinurias. Diagnosis of such conditions have, in the past, involved electrophoresis of concentrated urine using CAE, AGE, or high resolution AGE. The UV detection of Bence Jones protein and proteins indicative of renal dysfunction (β_2-microglobulin, retinol binding protein, and α_1-microglobulin) from unconcentrated urine vindicates the practicality of CE in the clinical laboratory. Analysis

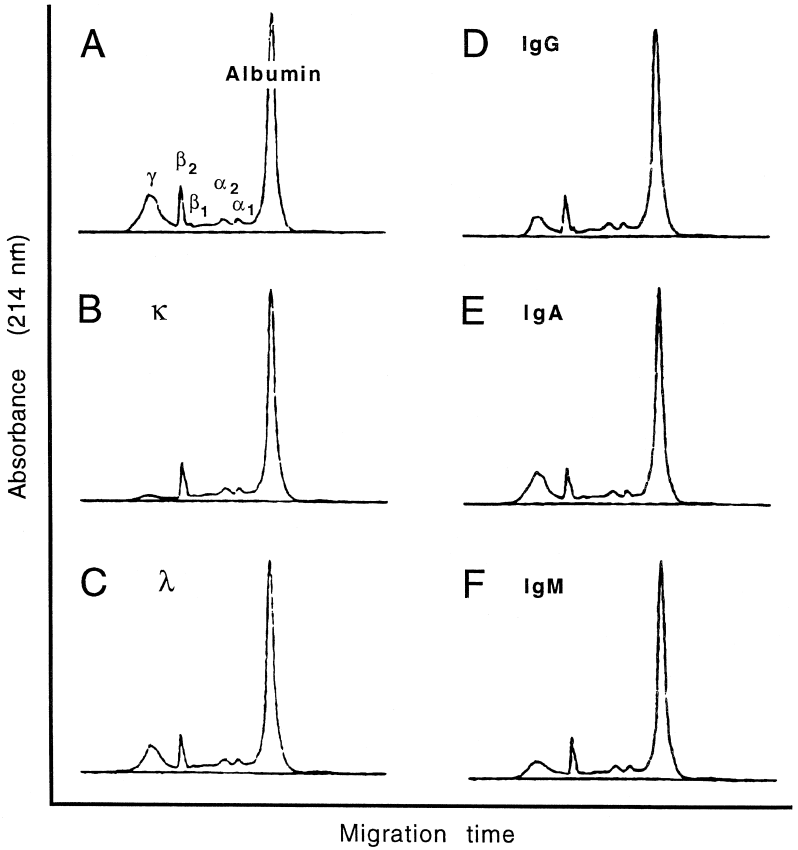

Figure 1. Capillary zone electrophoresis analysis of human serum showing the 6 characteristic protein zones (A). Identification of IgMκ monoclonal protein in the serum profile using sequential immunosubtraction procedures (B-F). (Modified figure from R. P. Oda, R. Clark, J. A. Katzmann, J. P. Landers, Electrophoresis, 18, 1715, 1997.)

of various endogenous compounds in urine, including amino acids, peptides, small ions, and organic anions, without sample pretreatment can also be successfully performed using the various modes of CE and a host of detection schemes.

Seminal Plasma

Seminal plasma profiles are useful in the assessment of accessory sex gland function. The seminal plasma levels of various proteins, such as transfer-

rin, prostate specific antigen (PSA), and albumin are of clinical importance. CE has been performed for the determination of small molecular weight compounds and the identification of transferrin, alpha-1-antitrypsin, albumin, and PSA zones in seminal plasma. Though few examples of CE seminal plasma profiles exist, improved resolution and the use of immunosubtraction is expected to make these profiles clinically useful.

Cerebrospinal Fluid (CSF)

CE is ideal for CSF analysis due to its many advantages over the traditional separation of CSF components using AGE. Staining and destaining procedures are unnecessary, profiles are obtained on the order of minutes versus hours, and little CSF (microlitre volume) is required. CSF protein electrophoresis is clinically important in the detection of oligoclonal bands associated with multiple sclerosis. CE is effectively used in the diagnosis of multiple sclerosis by providing high resolution of these oligoclonal bands in the gamma globulin region of a CSF profile. This separation technique has also been demonstrated to be useful in rapid screening of multiple sclerosis without the need for sample preconcentration.

Cell Plasma

Analysis of the contents of single human cells is yet another example of the clinical utility of CE. Since the inner diameter of a capillary is typically less than 100 μm, one can position a single cell in the end of a capillary where it is lysed, its contents derivatized, and separated electrophoretically. The low concentrations of intracellular species necessitates the use of detection schemes such as laser-induced fluorescence (LIF). Quantitation of proteins, enzymatic activity, ions, and small biomolecules, within single human erythrocytes, can be achieved using CE. Perhaps the most common use of CE in a clinical setting for the analysis of cellular contents is in screening for hemoglobin variants (figure 2).

The hemoglobin profiles of patients can be obtained in under 20 minutes, providing important information on hemoglobinopathies, diabetes, and thalassemia syndromes. Furthermore, the use of CE for obtaining cell profiles is attracting considerable attention in the realm of functional genomics and the early diagnosis of disease.

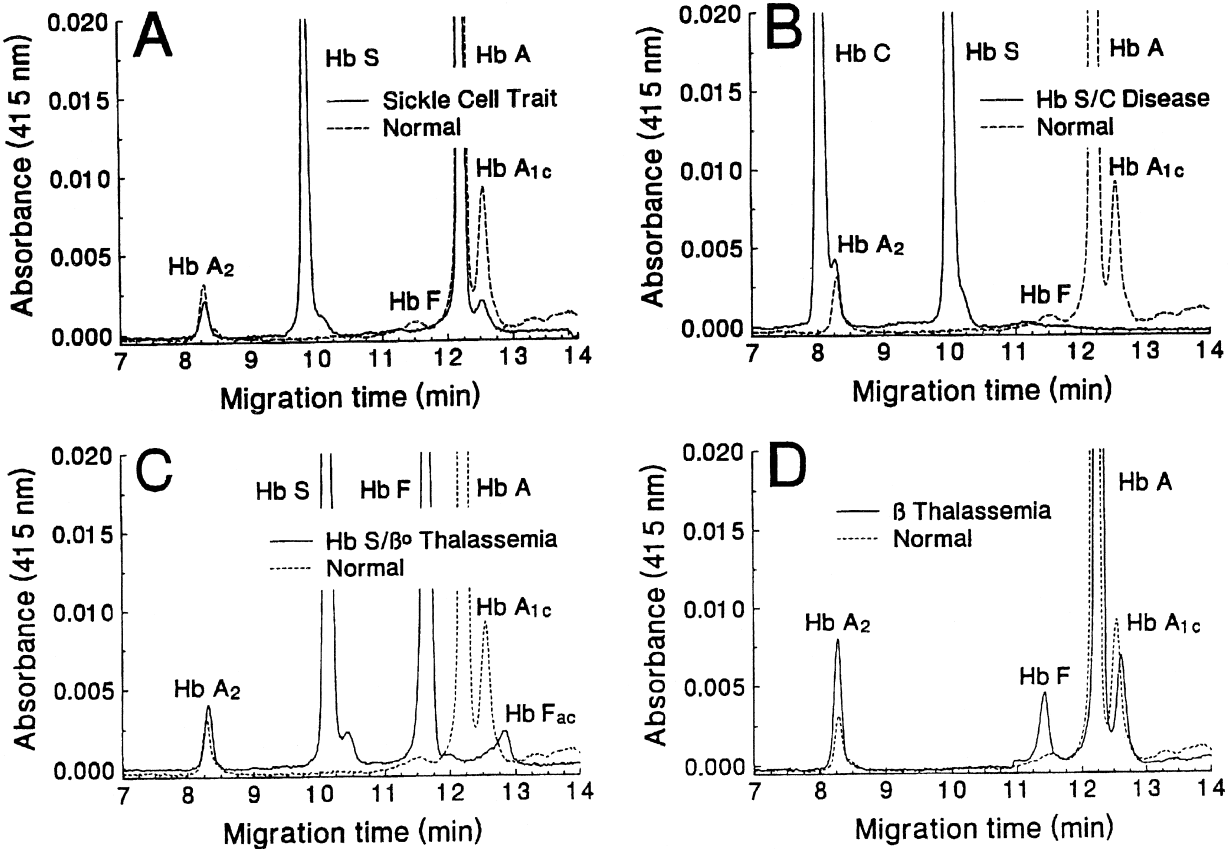

Figure 2. Comparison hemoglobin variants in normal blood and those from blood from subjects with sickle cell traits (A), Hb S/C disease (B), Hb S/β^{o}-thalassemia (C), or β-thalassemia (D). (Figure and caption from J. M. Hempe and R. D. Craver, Clin. Chem., 40, 2288, 1994.)

IMMUNOASSAYS

Immunoassays provide a means for clinical laboratories to specifically and quantitatively measure minute amounts of analyte (antigen) in complex sample mixtures. Immunoassays center on the use of antibodies raised against a clinically relevant molecule. Such immunoglobulins inherently exhibit high specific binding affinity for their antigen. The high sensitivity of immunoassays stem from conjugation of antibodies or antigens with labels based on fluorescence, chemiluminescence, radioactivity, or enzymatic amplification.

Two modes of immunoassays exist for CE: competitive and direct. Competitive immunoassays typically involve the use of an antigen conjugated to a highly fluorescent dye and an antibody, or a fragment of an antibody, which recognizes that antigen. Briefly, a set amount of antigen-dye conjugate is added to the sample of interest, followed by the addition of a limited quantity of antibody. Incubation of the resulting solution allows for endogenous antigens to compete with labeled antigens for a limited number of antibody binding sites. CE-LIF analysis of a nanolitre-size sample aliquot provides separation and detection of the free labeled antigen and the fluorescent antigen-antibody complex.

A calibration curve using the signal intensities of either the free labeled antigen, the fluorescent complex, or their ratio, can then be constructed. Quantitation of the steroid hormone cortisol in human serum, which can indicate adrenal malfunction, is an example of a real sample competitive CE immunoassay.

Direct CE immunoassays rely on the use of fluorescently labeled antibodies or antibody fragments (called affinity probes). In this approach, an excess quantity of affinity probe relative to the antigen is incubated in the sample solution to ensure complete antigen capture. Following the injection of a small sample aliquot into the capillary, an electrophoretic separation of the excess affinity probe and fluorescent complex is performed. LIF detection of the fluorescent complex provides a means to determine antigen concentration via peak intensity and a pre-constructed calibration curve. The measurement of human serum immunoglobulin A concentration has been described using this method.

The utility of CE-based immunoassays in a clinical setting is, to date, limited. The use of labeled antigens for competitive immunoassays has been confined to assays involving small antigenic molecules, which contain few derivatizable sites. Furthermore, the complex chemistry associated with the homogenous labeling of antibodies and antibody fragments and the difficulty in separating bound from unbound affinity probes, has made direct assays nontrivial. Despite these drawbacks, CE in the realm of clinical immunoassays remains attractive. This is due, in part, to advantages over conventional immunoassays that include the dispensing of sample clean-up and washing protocols, low reagent consumption, reduced incubation period, rapid analysis, and the potential for simultaneous assay of multiple analytes.

CLINICAL CE ANALYSIS USING POLYMER SIEVING MEDIA

Polyacrylamide and/or agarose slab gel electrophoresis is commonly used in the clinical laboratory for the separation of proteins and nucleic acids. Both these techniques bear significant time and labour costs and are, at best, semiautomated. CE is proving to be a viable high throughput alternative, particularly in the realm of genetic analysis. The use of polymer solutions (non-gel) in CE allows for the separation of bioanalytes according to differences in mass. The major advantage of polymer sieving media over chemical gels is that they can be easily replaced after every analysis. In addition, several sieving media have been developed, and the separation conditions optimized for the rapid, high resolution (single-base substitution) separation of DNA restriction fragments and polymerase chain reaction (PCR) products. Detection schemes using LIF and fluorescent labeling reagents provide an alternative to time consuming staining/destaining procedures and isotopic labeling.

Typical methods for the detection of polymorphisms in the human genome such as restriction fragment length polymorphism (RFLP), variable number of tandem repeats (VNTR), microsatellite analysis, and single strand conformation polymorphism (SSCP), can be readily performed using CE. In this section, emphasis is placed on the analysis of clinically relevant diagnostic nucleic acids obtained from the cells of human patients.

CE has been used effectively in the analysis of RFLP products for the DNA diagnosis of diseases such as cancer, cystic fibrosis, Alzheimer's, and Duchenne muscular dystrophy. CE-based SSCP analysis of the p53 tumor suppressor gene from white blood cells of normal and multiple myeloma patients can be used in the detection of p53 gene mutations and in genetic screening for cancer. Differences in the number of base-pair repeats can be determined by VNTR analysis using CE. This has been applied to the quantitation of repeats in human apolipoprotein B alleles for assessing the risk of coronary heart disease.

Genomic instability of specific short tandem repeats (microsatellites) at specific loci in the human genome can be determined by CE. For example, the CE profile of PCR products from tumor and normal DNA of a colorectal cancer patient can be obtained in less than 15 minutes. Other diseases to which microsatellite CE analysis has been successfully applied include Kennedy disease, cystic fibrosis, and the pre-natal diagnosis of Down's syndrome.

As a final note, CE is also applicable to the analysis of antisense DNA in biofluids such as serum and urine, and reverse-transcriptase PCR products for the detection/quantitation of various viruses such as Hepatitis C and HIV-1. Of particular interest, is the adaptation of such genetic analyses to high-throughput CE instrumentation, as has been done successfully in the case of DNA sequencing.

THE FUTURE OF CE IN THE CLINICAL LABORATORY

Although CE has proven to be competitive with conventional instrumentation methodologies, it remains to be seen whether CE-based assays will displace existing clinical assays. An increase in acceptance for CE in the clinical laboratory will require the development of assays having high accuracy, precision, robustness, and appropriate detection limits. From an instrumental point of view, foreseeable developments in CE technology that will have a dramatic impact on its promotion to the clinical laboratory include improved limits of detection, ultra-rapid analysis time, and high sample throughput. The issue of detecting dilute analytes in biological fluids has been addressed by several research groups. On-line solid-phase preconcentration (spPC) through the use of bead containing microcartridges or membranes having non-specific or specific affinity coatings have been shown to enhance sensitivity by up to 1000 fold. A unique feature of spPC is that sample clean-up/desalting allows for additional information on the analyte when coupled to mass spectrometric, nuclear magnetic resonance, or circular dichroism detection schemes.

The advent of microchip CE holds great promise for ultra-rapid analysis in the clinical laboratory. The fabrication of narrow channels within glass or fused silica substrates is made possible through the application of photolithography and chemical etching techniques. Due to the open geometry of the microchip, very short separation distances can be used resulting in exceptionally fast sample component separations. For example, analysis of human serum samples subjected to a competitive immunoassay for cortisol was shown to be complete in under 30 seconds. Microchip technology also allows for the integration of dense channel arrays within a single chip, thereby providing a means for high throughput sample analysis.

The concept of arrays in the capillary format, catering to high throughput in a clinical setting, has been realized by the recent introduction of a commercial 7 capillary CE instrument specifically designed for serum protein electrophoresis analysis. The routine use of CE in the clinical laboratory will, therefore, largely depend on the development of such high throughput technologies.

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