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An Overview of Capillary Electrophoresis (CE) in Clinical Analysis

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Abstract
The development and general applications of capillary electrophoresis (CE) in the field of clinical chemistry are discussed. It is shown how the early development of electrophoresis was closely linked to clinical testing. The rise of gel electrophoresis in clinical chemistry is described, as well as the eventual developments that lead to the creation and the use of modern CE. The general principles of CE are reviewed and the potential advantages of this method in clinical testing are examined. Finally, an overview is presented of several areas in which CE has been developed and is currently being explored for use with clinical samples.

Keywords Capillary electrophoresis, Clinical chemistry, Clinical applications, History of capillary electrophoresis

1 Introduction

Electrophoresis has been an important tool in clinical analysis for decades [1–3]. The primary mode of separation in this method is based on the different rates of migration of analytes in an electric field. However, there are many formats for this type of separation and

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a variety of schemes by which the migration rates of chemicals in a sample can be modified [4–9]. These features, plus the fact that many biological agents hold some charge, have made electrophoresis a key method in clinical analysis for the examination of amino acids, peptides, proteins, and nucleic acids, as well as many small charged solutes [1–3].

One way of using electrophoresis is to apply small amounts of a sample to a support (usually a gel or paper) and allow the components in this sample to travel in a running buffer through the support in the presence of an electric field. These approaches are known as "gel electrophoresis" or "paper electrophoresis" and they have historically been the most common types of electrophoresis found in clinical laboratories [3, 4]. It is also possible to separate the components of a sample by using a narrow capillary that is filled with a running buffer, followed by placement of this buffer and its contents into an electric field. This second method, known as "capillary electrophoresis" or "CE" [5–8], is the focus of this text. This chapter provides an overview of CE as related to the historical development of this technique and its use in clinical analysis. A summary of the clinical applications of CE, as discussed in more detail in later chapters, is also presented.

2 Origins of Electrophoresis in Clinical Testing

It has been known for over a century that substances like proteins and enzymes will travel in an electric field [10–12]. However, the use of this phenomenon for routine chemical separations did not occur until the late 1930s, when Arne Tiselius demonstrated that electrophoresis could be utilized for the separation of serum proteins [13]. The approach used by Tiselius, as later recognized by the 1948 Nobel Prize in Chemistry, was the first practical example in which electrophoresis was used for clinical analysis. The apparatus that was used by Tiselius in these experiments consisted of a U-shaped tube into which he placed his sample and a running buffer (see **Fig. 1**). When an electric field was applied across this tube, the proteins in the sample began to separate based on their charge and size as they migrated toward the electrode of opposite charge. The result was a series of broad and only partially resolved bands that were then used to study the protein content in the sample [8, 9]. The method that was employed by

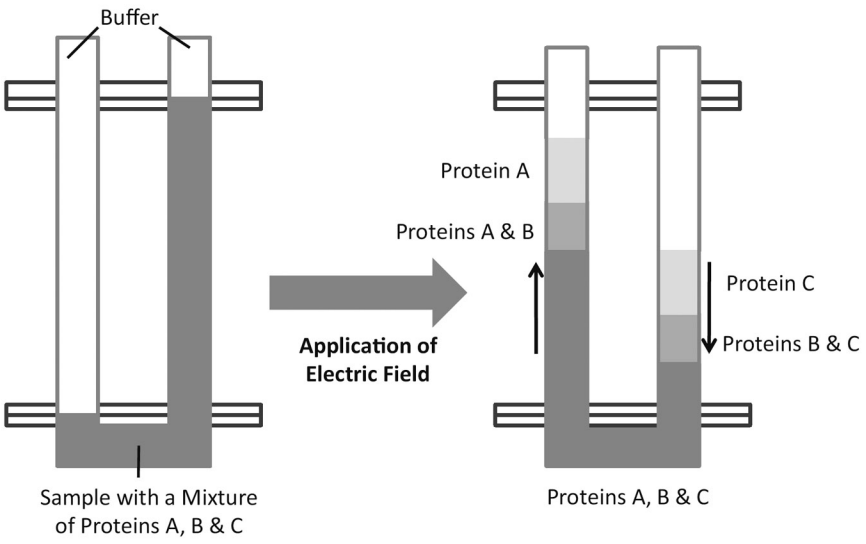

Fig. 1 General design of the apparatus that was used by Arne Tiselius in his early work with electrophoresis and the separation of serum proteins

Tiselius is now known as "moving boundary electrophoresis" because it produced a series of moving boundaries between regions that contained different mixtures of proteins [3, 9]. In modern laboratories, it is more common to use more efficient separation devices and to instead use a small amount of sample that will allow analytes to be separated into narrow bands or zones. These conditions result in a general approach that is now known as "zone electrophoresis" [5–9].

It is interesting that even though Tiselius employed an open tube system, the use of gels or other supports instead of open tubes became the main format for electrophoresis that was employed in clinical laboratories for over 50 years [1–4]. The emphasis on gel or paper electrophoresis during this time was due to the smaller sample requirements, greater ease, and better reproducibility of conducting separations by this approach as opposed to using large open tubes filled with a running buffer. The popularity of gel electrophoresis in particular was further enhanced through the development of improved supports for these separations, such as polyacrylamide gels, and the introduction of new methods based on gel electrophoresis. Two examples of these methods that are still common in clinical and biomedical laboratories are sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF) [3–5, 9, 14].

Interest did continue in further pursuing the use of open tube systems for electrophoresis [14–19]. For instance, Hjerten developed a system in 1967 that utilized 1–3 mm I.D. quartz capillaries to carry out electrophoretic separations in free solution [15]. This system was used for the separation of proteins, nucleic acids, and microorganisms, among other analytes, and included an online detector. Unfortunately, the relatively large diameters of the capillaries in this system required the continuous rotation of these capillaries to minimize the effects of Joule heating and convection [14]. It was demonstrated in 1974 by Virtanen that narrower 0.2 mm I.D. capillaries could be used to eliminate the need for rotation to minimize the effects of heating convection [14, 16]. However, it was not until the commercial development of small diameter silica capillaries in the late 1970s, and the subsequent work by Jorgenson and Lukacs with 75–100 µm I.D. silica capillaries in the early 1980s, that CE became a viable alternative to gel electrophoresis for the separation of clinical and biological samples [17–19].

3 Basic Principles and Advantages of Capillary Electrophoresis

Figure 2 shows a typical system for CE, as might be found in a clinical laboratory [3, 6–8]. This system includes a power supply, which can often provide an applied potential up to 25–30 kV, and a computer for control of the system and for the collection of data. There are also two electrodes for applying an electric field across the capillary and buffer containers that supply a contact between these electrodes and the solution within the capillary. Modern CE systems include an online detector, which may be based on the use of UV-vis absorbance, laser-induced fluorescence, electrochemical detection, or mass spectrometry [3, 8]. In addition, the system has some means for injecting samples onto the capillary. Typical volumes for the injected samples are in the pL-nL range and can be applied by using methods such as electrokinetic injection or hydrodynamic injection [6–8].

The capillaries in most modern CE systems have inner diameters of 20–100 µm and lengths of 20–100 cm [6–8]. The use of these narrow bore tubes allows the heat that is generated in the presence of an electric field to be quickly dissipated to the surrounding environment. The removal of this heat helps to provide much more efficient

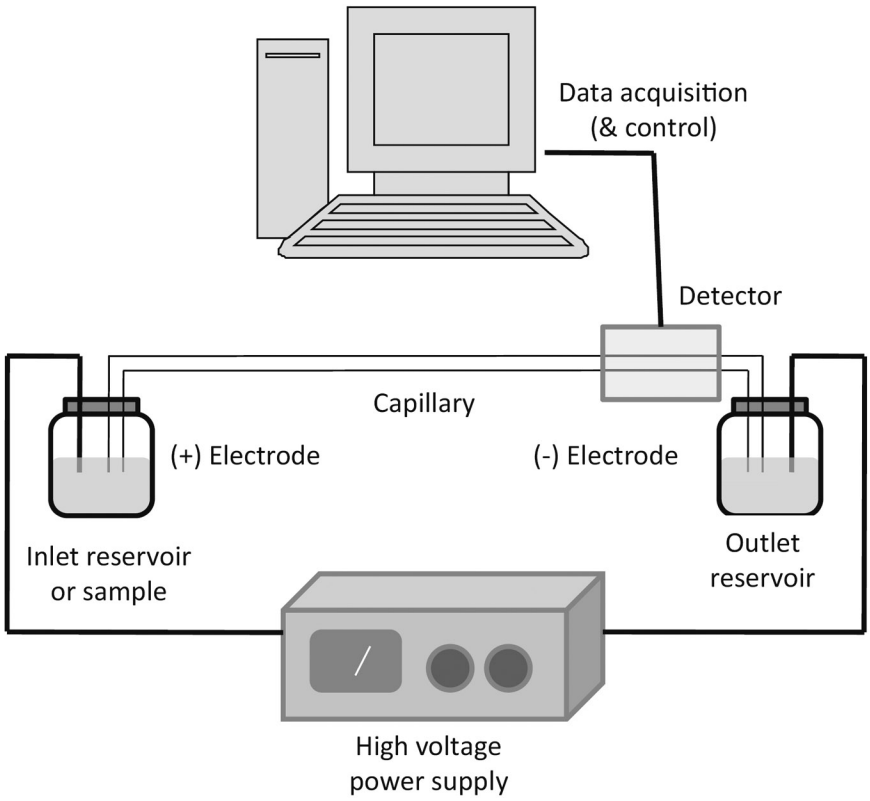

Fig. 2 General design of a modern capillary electrophoresis system. This particular configuration is based on the "normal polarity" mode, in which the sample is applied to a silica capillary at the side of the positive electrode

and faster separations than gel or paper electrophoresis. The absence of a gel or support in most types of CE also eliminates eddy diffusion and minimizes secondary interactions that may occur with the support. These conditions create a situation in which longitudinal diffusion is often the main source of band-broadening and in which more efficient separations are obtained as the voltage is increased and analytes spend less time in the capillary. The result is a fast separation with high efficiency and narrow peaks [6–8, 17–19].

There are many features of CE that make this method attractive as an alternative to gel or paper electrophoresis for clinical analysis. For instance, CE is faster and more efficient than these traditional methods. CE is also easier to perform as part of an automated system. The small sample size requirements of CE and its ability to be used with various detectors and detection formats are additional features that make this method appealing for clinical analysis [3, 20–25].

4 Applications of CE in Clinical Analysis

Following the introduction of the first commercial CE instruments in the late 1980s, there has been a steady increase in the use of CE for various samples of clinical interest. Some early reviews of clinical applications for CE can be found in references [4, 20–23].

Figures 3 and 4 illustrate how the interest in CE for clinical analysis has grown during the last three decades. This interest is indicated by both the number of publications that have appeared on this topic (Fig. 3) and citations that have been made to these papers (Fig. 4). Most of these applications have involved the use of urine, serum, plasma, or blood. In 1990, there were only two publications that dealt with such samples and that included "capillary electrophoresis" within their titles. However, between 1990 and 2017 there were over 1380 publications dealing with these samples and that included "capillary electrophoresis" in their titles. There were also over 27,000 citations to these articles over the same period of time. In addition, work appeared on more exotic specimens such as saliva, cerebrospinal fluid, and tears, with over 80 publications and almost 1760 citations to these papers appearing between 1990 and 2017.

Many applications for CE in clinical chemistry have been explored and developed, as is illustrated in **Fig. 5** [1, 3, 23–25]. One set of these applications has involved the use of CE in the analysis of clinical biomarkers. These biomarkers may be serum proteins, enzymes, peptides, carbohydrates, lipids, and small organic or inorganic compounds [21, 24]. For instance, some chapters in this text will examine the use of CE to analyze glucose and lactose or serum transthyretin in clinical samples [1, 2]. The use of CE for metabolomics will also be considered in this text. In this latter field, a group of analytes that are involved in one or more metabolic pathways are monitored to determine how their composition changes in the presence or absence of a given clinical condition [24].

Several additional applications for CE in clinical chemistry will be examined in this text [20–29]. One area in which CE has been employed is hematology, or the study of diseases that are related to blood and blood-forming components [1, 27–29]. Other uses that have reported for CE are in the areas of bacteriology and virology, for the detection or characterization of bacteria or viruses, and in the field of endocrinology, for the analysis and study of hormones within the

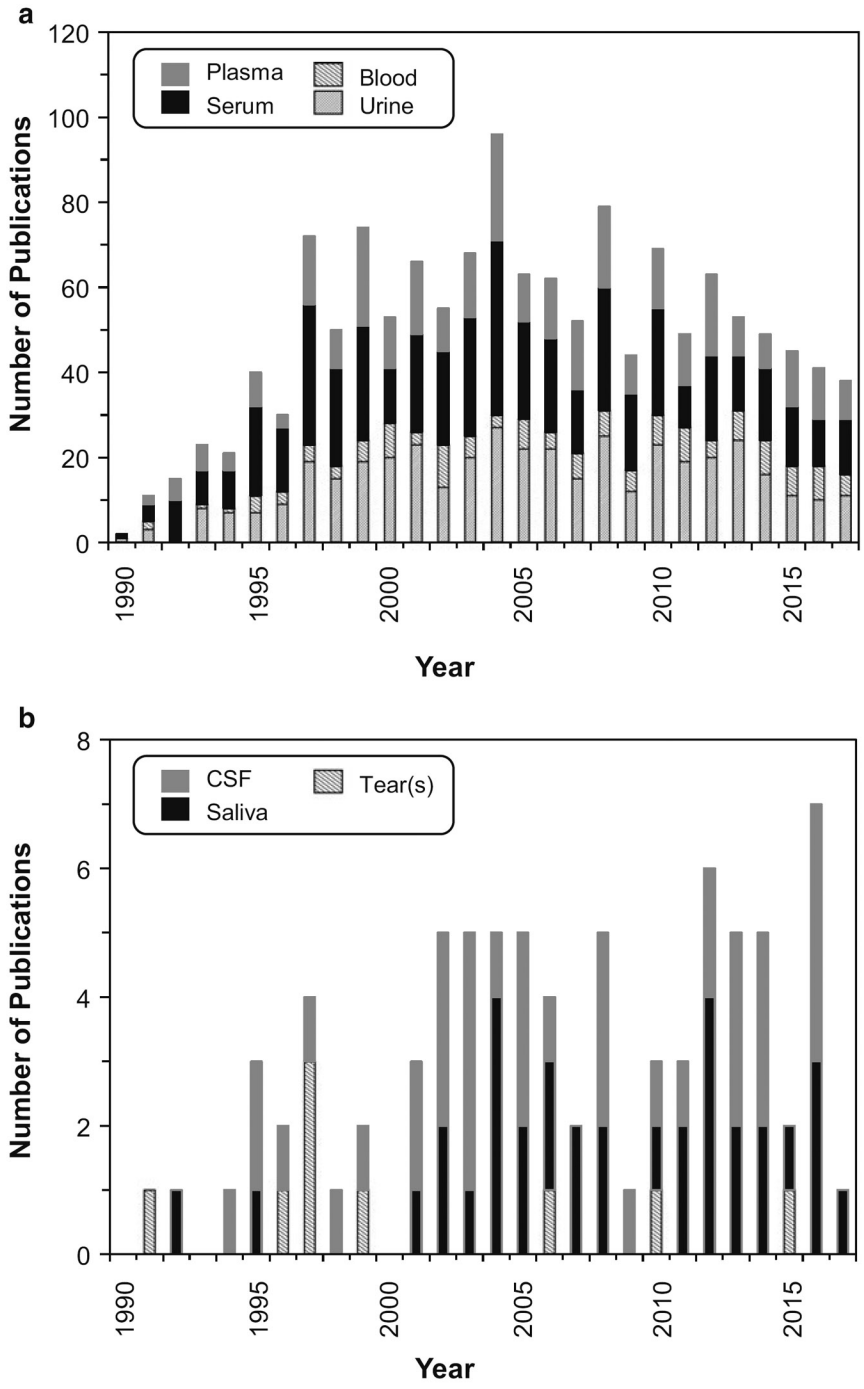

Fig. 3 Number of publications appearing between 1990 and 2017 that included the term "capillary electrophoresis" in their titles and that involved typical clinical samples. The results in (**a**) are for papers that contained "urine," "serum," "plasma," or "blood" in the title of the paper. The results in (**b**) are for papers that contained "tear(s)," "saliva," or "cerebrospinal fluid" (CSF) in the title. The results were generated on March 23, 2018, using the Web of Science. The phrases "inductively coupled plasma" and "inductively-coupled plasma" were excluded during the search for papers that included the term "plasma" in their titles

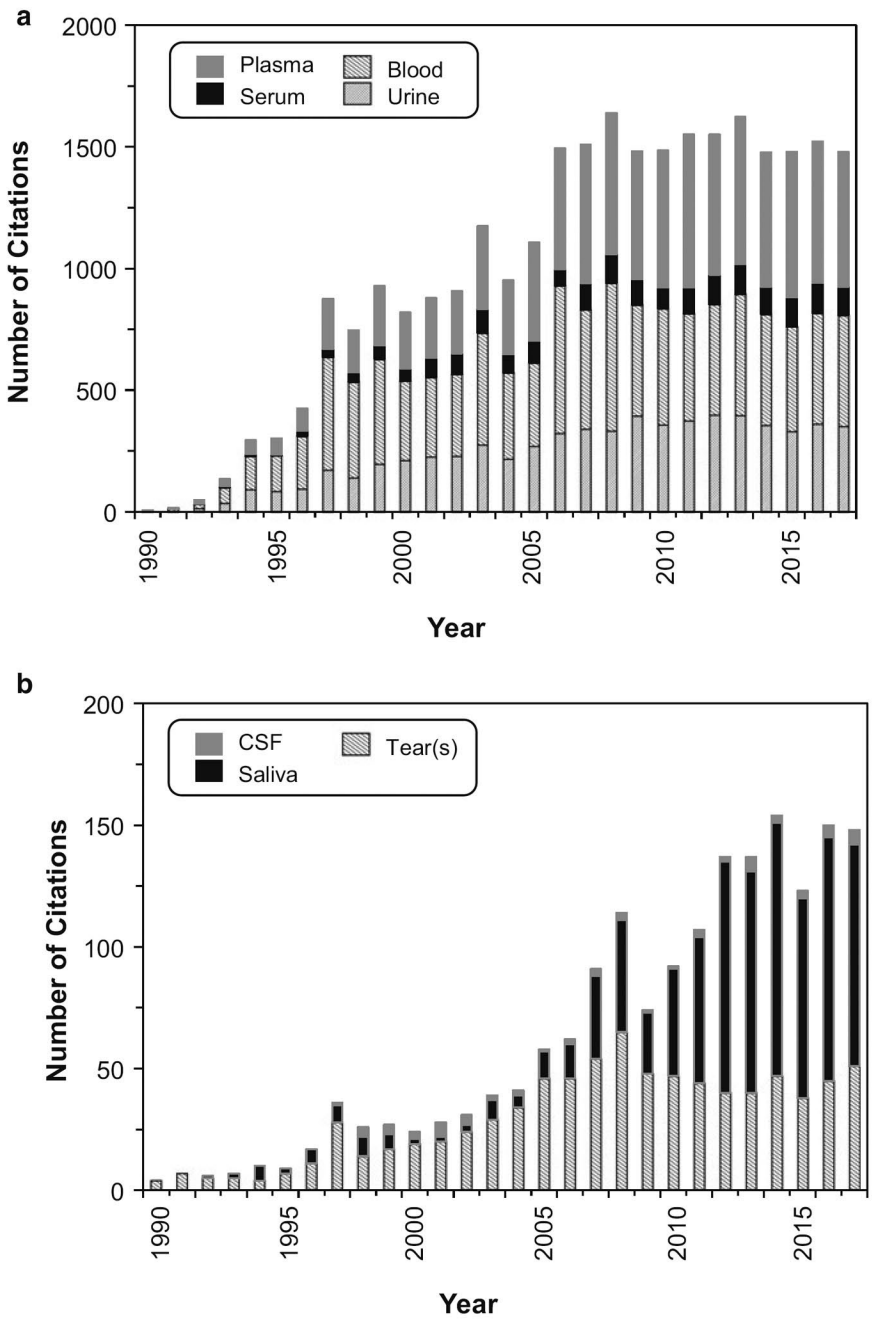

Fig. 4 Number of citations to publications that involved the use of capillary electrophoresis and typical clinical samples. The search parameters and procedures were the same as used in Fig. 3. The results in (**a**) are for papers that contained "urine," "serum," "plasma," or "blood" in the title of the paper. The results in (**b**) are for papers with titles that contained the terms "tear(s)," "saliva," or "cerebrospinal fluid" (CSF)

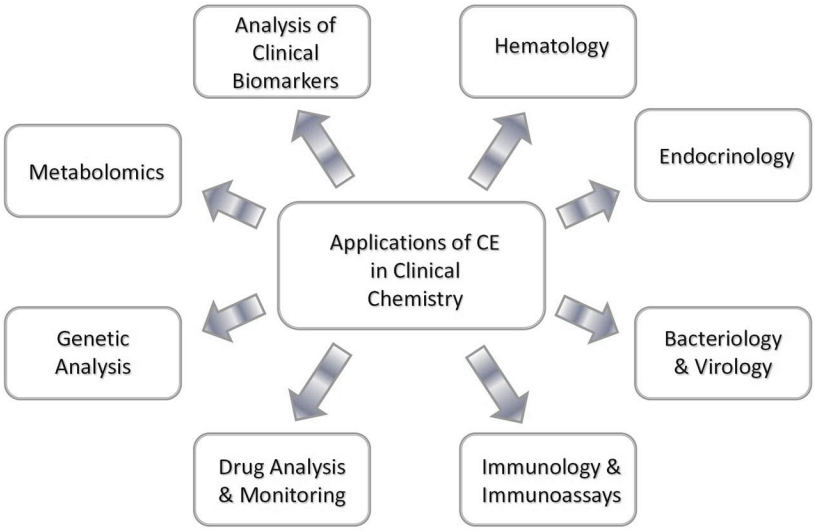

Fig. 5 Applications for CE in the field of clinical chemistry

body [1, 2, 24]. Another important set of applications for CE is in the field of immunology, which deals with diseases of the immune system [1, 2, 24]. A closely related topic is that of CE-based immunoassays, in which CE is combined with the use of antibodies or antibody-related agents for the selective binding and recognition of particular analytes in a sample [26]. CE has also been utilized for the analysis of drugs in clinical samples [21, 24, 25], as might be used for therapeutic drug monitoring or for the detection of drugs of abuse [1, 2]. Finally, CE can be used as a tool for genetic analysis and the identification of inherited disorders [24]. Many of these areas will be explored in more detail in the following chapters.

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