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Historical Development, Design Features and Function of Electrophoresis Machine

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ABSTRACT

This paper tends to give insight on historical development of electrophoreses machine, its design development and function. Electrophoretic separation was first demonstrated in the year of 1807 and has since been a staple tool used by biologists and chemists for more than a century since its inception. This paper reviews the evolution of the electrophoresis technology and provides an insight into the possible future development of electrophoresis in medical facilities. It is both exciting and equally perplexing to explore the promises that this seeming simple separation technology used for the separation of genes and detection of proteins holds for the future. The development of electrophoresis from its first observation some 200 years ago via conventional gels for macromolecule separation and capillary electrophoresis (CE) to current developments centered around lab-on-chip. The most basic electrophoresis system consists of a chamber or cell, a membrane, an incubator or oven, and a power supply. The chamber contains two buffer compartments, one at each end of the chamber. A bridge between the two compartments supports a membrane, typically cellulose acetate or some type of gel.

I. INTRODUCTION

Electrophoresis combines the prefix “electro,” referring to electricity, and “phoresis,” which comes from the Greek verb “phoros” and means “to carry across.” Thus, electrophoresis means that electricity will carry something across. That “something” includes DNA, ribonucleic acid (RNA), genes, pharmaceuticals, metabolites, nucleotides, and peptides released by lysed cells. The electricity, acting on the surface charge of the substance of interest, carries it across a porous membrane at a speed proportional to the surface charge and the strength of the applied electrical field. Coupled with DNA copying techniques, this offers a powerful means of identifying a plethora of conditions and ailments. In situations where the molecules cannot be adequately detected, specific antibodies are mixed with the sample materiel. These antibodies attach themselves to the molecule—for example, a particular protein associated with HIV—and can be seen. Staining is sometimes used to increase visibility. In this paper we study electrophoresis machine not only the process. Thus electrophoresis machine is a device used in the medical laboratory to separate molecules in their liquid state based on their ability to move in an electric field in other word, is a device used in separating molecular objects in their liquid state such as separating genes, testing for the resistance of a specific kind of antibiotics. The application of electrophoresis has led to the development of a number of virtually error-free testing methods, such as Northern and Southern blotting, and the more commonly known Western blotting, used as a confirmatory test for a number of serious illnesses, including hepatitis and AIDS. Electrophoresis machine is a machine used in detecting the genotype in human which are basically AA, AS, and SS. Many parents witnessed sudden death of their children at their teen age, without knowing the cause or how to avert it.

The advent of the knowledge of electrophoresis give light on the genotypic constitution of cells.

The knowledge of electrophoresis diagnose the causes of different diseases that causes death example multiple myeloma, sickle cell, diseases at birth, chronic liver disease etc.

The knowledge did not only unravel the causes but also led to the development of authentic cure. Also intending couple’s eyes were opened to their genotype status, giving them room to decide on whether to go on with their marriage or not, as the likelihood or otherwise of having sickle cell anemia as a child is made known to them.

II. HISTORY OF ELECTROPHORESIS AND ELECTOPHORESIS MACHINE

The concept of electrophoresis was discovered a couple of centuries ago, in 1807 by Ruess (Ruess, 1809) who conducted an experiment of applying an electrical current through a suspension of clay in water. He noted the migration of particles towards the anode. However, it was not until the year 1942,

when Coleman and Miller (Coleman and Miller, 1942), conducted an experiment and discovered the migration of neutral hexose toward the anode with a borax (sodium borate) solution that electrophoresis became a major scientific method. Many different type of variable experiments was carried out to determine the usage and limitations for the electrophoresis of other compounds, such as those containing adjacent "–OH" groups and also high concentration of neutral sugars (Smith, 1955; Hashimoto et al., 1942;Foster, 1957). After a successful separation of sugar was conducted in 1952 by Consden and Stanier (Consden and Stanier, 1952), electrophoresis was then used for DNA and RNA separation. Around the 1970's, advancements in the process of electrophoresis in the area of DNA and RNA separation flourished, with most of today's current technology in electrophoresis emerging from then and is still being used until the present time. Some of the notable experiments conducted during the 1970's were by Richards et. al. (Richards et al., 1965) who used a Tris-based media to separate RNA, introducing the use of Tris media, Tris-acetate-EDTA (TAE) and Tris-borate-EDTA (TBE), as the buffer solution for subsequent studies. Another experiment which defined the usage of electrophoresis as a tool for analysis of DNA was conducted by Danna et. al. (Danna and Nathans, 1971) in which they introduced the usage of restriction of enzyme, produced by Hemophilus in Xuenzae, to determine the relative molarity and length of fragments of SV40 DNA. The use of ethidium bromide as a DNA stain was introduced when it was used in the conductive medium to show the differences between linear and circular DNA by Aaij and Borst in 1972 (Aaij and Borst, 1972). For the conductive buffer (running buffer), Tris emerged as the dominating buffer to be used. There are no known reasons behind Tris being the dominant buffer as there have been many alternative chemicals. Tris-borateEDTA (TBE) was introduced at 1968 by Peacock, et. al., (Peacock, and Dingman, 1968) and Tris acetate acid EDTA (TAE) was introduced at 1972 by two separate groups (Danna and Nathans, 1971;Aaij and Borst, 1972) In the past for DNA analysis work to be conducted to determine its size, a lengthy procedure must be conducted (Meyes, 1976). Electrophoresis uses the electrical field to separate and move molecules through a sieve like compound based on the molecular mass and charge ratio (Lodish, 2000). Electrophoresis is usually used for molecular biology work (ie DNA, RNA and protein separation).The process of electrophoresis can also be used for the analysis of chemistry compound such as water, soil and air quality or contamination, food quality and processing hygiene and also for possible medical forensic analysis. The fundamental principle of electrophoresis applied for these processes with only a different type of detection system being used (Kappesand Hauser, 1998). DNA, RNA, and protein works is not only limited to just separation. Electrophoresis can also be used as a purification process. This is usually done in cases where the research is limited to specific targets of interest, for example a membrane protein or a specific gene. In this situation, electrophoresis is used to separate and the protein or gene of interest is excised out from the entire sample (Kappes and Hauser, 1998).

Historical fabrication of electrophoresis defined with the work of Arne Tiselius in the 1930s, and new separation processes and chemical analysis technique based on electrophoresis continued to be developed into the 21st century. Tiselius with the support from Rockefeller foundation developed the Tiselius apparatus for moving boundary electrophoresis which was described in 1937 is the well known paper "a new apparatus for electrophoretic analysis of colloidal mixtures". The method spread slowly until the advent of effective zone electrophoresis method in the 1940s and 1950s, which used filter paper or gel as a supporting media. By the 1960s, increasingly sophisticated gel electrophoresis made it possible to separate biological based on minute physical and chemical differences, helping to drive the rise of molecular biology. Gel electrophoreses and related method became the basis for wide range of biochemical methods such as protein finger printing, southern blot, and similar blotting procedure, DNA sequencing and many more.

III. Types and advancement of Electrophoresis

Since the introduction of electrophoresis into the scientific world, many changes and improvements to the resolution, resolving, and efficiency abilities has been made to the system. From the basic paper electrophoresis to the highly advance automated microchip electrophoresis system, each different system has its own functionality and unique usage.

a. Paper electrophoresis

Paper electrophoresis is one of the simplest techniques of electrophoresis. In this technique the sample is applied onto a point of a strip of filter paper that has been moisturized with a buffer solution. Each end of the strip is then dipped into separate tanks containing the buffer solution and a different electrode (anode or cathode). An electric current is then applied and the sample will then move towards the electrode with the opposite polarity. When the process is done, the strip is then dried and viewed with a detection system (Voetand Voet, 1995). This technique has often been compared to paper chromatography due to their similarity in their mode of action. However, the difference between these two systems is that chromatography separates a sample based on its polarity while electrophoresis separates the sample based on its charge by applying a running electrical charge from one terminal to the other(Voet and Voet, 1995).

b. Agarose gel electrophoresis

Agarose is a polysaccharide that is able to form pores with sizes ranging from 100 to 300 nm in diameter, with the size of the pore correlating with the concentration of the agarose gel. The higher the concentration of the agarose gel the smaller the pore size. Agarose gel electrophoresis is often used to separate DNA or RNA fragments of different length. This technique involved the movement of charged molecule in an electric field, in which a positive and negative electrode (i.e. anode and cathode), will allow the negatively charged DNA or RNA molecules to migrate from the negative electrode to the positive electrode. The molecules are separated based on their length, size and structure(Hame and Rickwood, 1998).

c. Polyacrylamide gel electrophoresis

There are two types of gel; dissociating and non-dissociating gel. A non-dissociating gel separates the proteins in their native form, which helps to conserve the protein structures, functions and activity. It is useful when the protein of interest is wanted at the end of the procedure for experiment.A dissociating gel denatures the protein into its constituent polypeptides. It is commonly used to determine the polypeptide composition of the sample (Voet

and Voet, 1995). Native gel electrophoresis is a non-denaturing gel that has a higher resolving power than the SDS-PAGE. It is also used for protein separations. However, it does not denature the proteins, unlike SDS-PAGE which does (Hame and Rickwood, 1998).

Polyacrylamide gel electrophoresis consist of polyacrylamide gel, which is made up of chains of acrylamide monomers (CH2=CH-CO-NH2) that is cross linked with N, N′-methylenebisacrylamide units (CH2=CHCO-NH-CH2-NH-CO-CH-CH2), also commonly known as a “bis”. The pore size of the polyacrylamide gel is also determined by the concentration of “bis”. Polyacrylamide gel is used to separate strands of DNA molecules whose size is very close to each other, often by one nucleotide such as in SNP, and also proteins. This is because the polyacrylamide gel has a much higher resolving power than agarose gel electrophoresis (Brown, 2002).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a low cost, reproducible and rapid method for quantifying, comparing and also to characterize protein, peptides and small molecular weight nucleic acids (Judd, 2002). This technique of electrophoresis separates proteins by their molecular weight (Laemmli, 1970).

There are two variant of the SDS-PAGE technique, the gradient and SDS-Urea gels.Both are based on the SDS-PAGE electrophoresis but with a higher resolving power based on the needs of the sample. Gradient gels are often used to separate small and large proteins in a single gel. SDS-Urea gels are used when the charge of the proteins is significantly relative to the mass of the protein, such as membrane proteins and immunoprecipitatesasSDS-urea gel helps to solubilizes, denatures and dissociates the polypeptides chains without altering the proteins intrinsic charge, allowing the proteins to be separated base on their net charge (Hame and Rickwood, 1998).

d. **Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE)**

Denaturing Gradient Gel Electrophoresis (DGGE)is a method that is employed to separate PCR-generated DNA products for molecular fingerprinting. DGGE is different from conventional agarose gel electrophoresis as it separates the PCR products based on its sequence size differences and also its rate of denaturing. This specialty makes it important as conventional agarose gel electrophoresis only separates based on size only, which makes it improbable for molecular fingerprinting as some samples may have PCR fragments of similar size, thus forming only a single band or bands that are only slightly distanced apart it is impossible to determine (Zijnge et al., 2006). As the samples pass through the gradient denaturing gel, it slowly denatures as the concentration of chemical denaturant in the polyacrylamide gel. When the sample reaches a threshold denaturant concentration, PCR samples with weaker bonds will denature much quickly, thus its migration along the polyacrylamide matrix will slow down dramatically, forming a more diverse set of bands which can then be easily compared to references online (Zijnge et al., 2006). The other type of gel is TGGE,which functions principally similar to those of DGGE, except it uses a temperature gradient as its separating function. In TGGE, the gradient temperature is introduced perpendicularly from the sample running directions. This allows the sample to migrate from the low temperature to the high temperature, which allows the sample to denature accordingly based on site mutations. TGGE enables researchers to distinguish single base pair mutation in a single sample as the denaturing point could be either higher or lower from the original sample (Henco et al., 1994).

e. Isoelectric focusing and 2-dimension (2D)gel electrophoresis

Isoelectric focusing is a technique of separating the proteins based on their net charge, or also known as the isoelectric point of the protein. This is done by placing the sample of proteins in a pH gradient slab that is generated by an electric field. This will cause the proteins to migrate in the pH gradient field until it reaches a pH in which its isoelectric point is zero (O’Farrells, 1975;Bollag and Edelstein, 1999). For 2D gel electrophoresis, the system utilizes the SDS-PAGE and isoelectric focusing techniques. The technique of 2D gel electrophoresis separates the proteins based on their size and also their isoelectric point, thus giving a much better resolution of the protein and can also be used to separate a protein if the charge and size of the protein is known. The advantage of the higher resolving power of the isoelectric focusing and 2D electrophoresis techniquesallowsforrapid and accurate analysis of proteins.However, with such high sensitivity there is a higher chance of getting errors. These techniques are highly sensitive to charge differences, and as such the samples needed to be handled with care, as any interactions of the sample with other lipids or other proteins may cause a change in the charge of the protein of interest, thus getting a negative result (Bollag and Edelstein, 1999).

f. Zymography

Zymography is an electrophoresis technique which allows enzyme activity to be analysed after the process of electrophoresis has been conducted on the enzyme (Scadden, and Naaby-Hansen, 2001). This technique is important as it allows the researcher to characterize the protein or enzyme on the gel without the need for the researcher to purify the protein or enzyme of interest via excising the band from the gel and “cleaning” or extracting the band from the gel before any test could be conducted on it (Coughland, 1985). The use of zymography allows researchers to save time by removing the need to conduct such steps for protein or enzyme characterization. There are many ways for zymography staining to be conducted. A paper by Coughlan (Coughland, 1985) explains some of the methods used such as immersing the gel in a chemical reagent which highlights the bands on the gel. Another method mentioned is the use of second gel which is run concurrently with the gel containing the samples, in which the second gel contains auxiliary enzymes and chromogenic reagents which allow the bands of interest to be detected on the second gel and allows for immunological detection (Coughland, 1985). One of the advantages of zymography over conventional assays is that it allows the enzyme activity to be studied based on their physical characteristics such as molecular weight or isoelectric point (Scadden, and NaabyHansen, 2001). As the examination of the sample is conducted on the gel, many other factors can be studied such as posttranslational modification of a particular enzyme, heterogeneity of enzyme isoforms and studying enzyme activitiy in their native state, in which such studies cannot be conducted in standard conventional assays (Scadden, and Naaby-Hansen, 2001). The application of zymography can vary from analyzing of ribonucleases using normal electrophoresis or isoelectric focusing, two-dimensional analysis of bacteria, fungal works, and also for detection and characterization of microbial proteases (Peterson et al., 2009;Yasuda et al., 1992;Lantz, And

Ciborowski, 1994; and Coughlin, and Green, 1983). Zymography is also used as a way to double check on result, which enables researchers to check on suspected protein-protein or enzyme interaction (Karumbaiah et al., 2007)

g. Pulsed-field electrophoresis

For very large DNA molecules around 30 to 50kb, it is not capable to be separated using normal electrophoresis process (Watson et al., 2004). This is because as the large DNA molecule migrates along the gel using normal electrophoresis, one end of the molecule will penetrate the matrix while the rest of the molecule trails along, forming a "snake" like smear (Watson et al., 2004). Pulsed-field electrophoresis was therefore created to counter this situation. In this process, the electrical voltage periodically switches between three directions, one that runs through the central axis of the gel and two that run at an angle of 120 degree on each side of the gel. With this, the large DNA molecule is allowed to re-orientate, thus preventing the formation of the "snake" like smear. However, the larger the molecule the longer it will take to re-orient (Watson et al., 2004).

h. Capillary electrophoresis

Capillary electrophoresis is carried out in very thin capillary tubes, with about 1 to 10 µm inner diameter, that is usually made out of glass, quartz or plastic. The tube is then filled with any of the required buffer such as Laemmlifor proteins or TAE buffer for agarose related work. A capillary electrophoresis run is very short, thus it is very useful for analytical work. Also, each reaction only takes a small amount of materials due to the small size of the capillary tubes. Capillary electrophoresis is suitable for use in genetic analysis, pharmaceuticals with enantiomers, counter-ion analysis in drug discovery, and protein characterization (Voet and Voet, 1995). Many types of capillary electrophoresis systems exist. Some examples of are the micellarelectrokinetic capillary chromatography, capillary electrochromatography, and the capillary array electrophoresis. Capillary array electrophoresis is an automated system that is often used for large scale projects, such as the Human Genome Project, as it allows massive screening of samples simultaneously (Mathies and Huang, 1992).Micellarelectrokinetic capillary chromatography (MEKC) is used when the sample being analyzed contains a mixture of natural organic substance with similar chemical structure (Carretero et al., 2004). An ionic surfactant is mixed into the background electrolyte with a concentration higher than the critical micelle concentration. This allows the formation of charged micelles, which allows for the sample to migrate at a pseudostationary phase in the aqueous phase causing the separation to separate differently in the run (Carretero et al., 2004). Capillary electrochromatography (CEC) is alike to liquid chromatography, except it uses an electrical field to propel the mobile phase through the stationary phase (Norman, 1999;Iain H. Grant, 1995). This allows it to have a higher efficiency capillary zone electrophoresis and high performance liquid chromatography. The application uses a capillary with a very small diameter that is packed with a solid stationary phase, in which it allows for the electrical field to move the mobile phase through it, reduced plate heights as a result of the plug flow profile and the ability to use smaller particles leading to higher peak efficiency than is possible in pressure driven systems (Norman, 1999; Iain H. Grant, 1995). There are numerous ways to detect and accumulate the data from the samples.One example of detection systems used for capillary electrophoresisis laser induced fluorescent; the detection limit is increase, as it is able to detect smaller concentration of sample but it is a much harder and more expensive detection system to use (Voet and Voet, 1995).Another method used is by the electrically floating conductivity detection system. This system is designed to be separated from the main capillary electrophoresis body. The detection system detects the conductivity by measuring the electrodes. The electrical signal that is generated by the electrodes will pass through the floating electronics and transmitted via infrared to a computer as data (Zare et al., 1998). An experiment conducted by Tristezzaet al. (Triztezza et al., 2009) details protocol and usage of capillary electrophoresis for the identification of protein markers in Saccharomycescerevisiae. In the paper, the result from using capillary electrophoresis was compared against agarose electrophoresis to evaluate their differences in detection limit (Triztezza et al., 2009). Another advantage of using capillary electrophoresis is that due to its high precision and reading capabilities, it is used for estimation of unknown DNA or RNA fragment size (Akbari et al., 2008).

i. Microchip electrophoresis

A further advancement to the capillary electrophoresis system, the microchip electrophoresis system boasts a more efficient system. One of the main benefits is an increase in throughput by many folds over the capillary electrophoresis system as the microchip systems contains numerous microchannels which allow high throughput experiments to be conducted quickly and efficiently(Hammond, 2001). Another benefit is the low fabrication cost as the intricate enclose microchannels is comprised of glass or fused silica substrates. These materials enable ultra-fast DNA separations as the sample loading formats is unique, coupled with short separation distances and optimal thermal characteristics of the glass or fused silica substrates. The microchip electrophoresis system is also fully automated, from sample handling to data analysis, which allows minimal human handling and possible error (Hammond, 2001).Example of this system is the "lab on a chip" device. Microchip electrophoresis uses laser-induced fluorescence and electrochemical detection method as their detection method due to the size of the microchip and as well as the required accuracy needed to read their results require highly accurate methods for microchip electrophoresis to work (Osbourn and Lunte, 2003;Huang et al., 2006). Research in developing new materials for microchip electrophoresis is widely delved into by many groups of researchers (Liu et al., 2006;Wang et al., 2008;Prest et al., 2001;AlvesBrito-Neto et al., 2005). Many different type of materials have been use to fabricate the microchip, from the most common material such as silica and glass substrates, to poly(dimethylsiloxane), or PDMS, and poly(methylmethacrylate), or PMMA, as a material to fabricate microchip electrophoresis via thin-casting method (Liu et al., 2006;Wang et al., 2008;). The advantage of this protocol reduces the cost of production and also allows faster results development. Wang et. al. (Wang et al., 2008) also proposed new materials such as silver ink to be used as electrode for microchip electrophoresis. The materials that are normally used as electrodes are platinum or platinum-iridium alloy, gold, copper and aluminium (Prest et al., 2001;AlvesBrito-Neto et al., 2005).

j. Fluorophore-assisted carbohydrate electrophoresis (FACE)

FACE is used to identify carbohydrates with an attached fluorescent dye by separating the carbohydrates using a polyacrylamide gel (AlvesBrito-Neto et al., 2005). This technique is important as carbohydrates are not charged, and is the main technique used to analyze different types of carbohydrates such

as plant and bacterial polysaccharide (AlvesBrito-Neto et al., 2005). Another advantage to using FACE is to reduce the need for complex work such as those conducted by Gao (Gao, 2005). For that project, FACE enables the researchers to simplify a process to detect lipid-linked oligosaccharides, which would require the samples to be metabolically labeled with radioactive sugar precursors before the molecule of interest can be detected (Starr et al., 1996).

k. Affinity electrophoresis

Affinity electrophoresis systems is a technique in which the resolving capability of capillary electrophoresis is used to separate samples that undergoes specific or non-specific affinity interactions during the process of electrophoresis (Heegard et al., 1998). This process can occur in either solution or immobilised to a solid support (Heegard et al., 2003). It is also used to measure the binding affinity of receptors to neutral and charged ligands (Colton et al., 1998). The use of affinity electrophoresis is extensive in that it is able to detect affinity interactions in either free or immobilised form. Some of its uses include detection for peptides and proteins, drugs development, detection of small molecules and also for immuno-affinity works (Heegaard et al., 2001; Progent et al., 2002; Rochu et al., 2002; Mito et al., 2000; Villareal et al., 2003; Kuroda et al., 2003; Iki et al., 2000; Germanand Kennedy, 2000; Ou et al., 1999; Shimura et al., 2002). There are also different types of affinity systems. For example, Taketa and Hirai (60) explain the usage of lectin affinity for use on serum α-fetoprotein to determine the interaction between lectin and ligand. Smith and Kelleher (Smithand Kelleher, 1973) used concanavalinA affinity chromatography as an alternative detector module. Some of the advantages of using affinity electrophoresis is that the sensitivity of the technique allows for more precise detection and discrimination of normal and carcinogenic proteins from the same sample (Breborowicz, 1981). Also, its wide field of usage makes it an important technique for sampling and data collection.

l. Automated electrophoresis system

With the advancement of technology, it is now possible to conduct electrophoresis by using computerized robotics and programming that enables electrophoresis protocols to be conducted automatically. Automated systems come in many different types and form. With today's technological advances, various types of electrophoresis systems can be automated. For example,Michels et al.(Michels et al., 2002) describe the works of an automated 2D capillary electrophoresis system which they used for high throughput protein analysis. Another work conducted by Kristensen et al.(Kristensen et al., 2001) shows the usage of various automated systems, including an automated electrophoresis system, for the Human Genome Project to detect variation in the human gene. Automated electrophoresis systems are also highly accurate and precise and can even detect single-strand conformation polymorphism in genetic samples (Larsen et al., 1999). One of the advance automated system is the automated buffer-less electrophoresis system which uses pre-casted gel, either SDS or agarose, that does not require any buffer to run, which is one it biggest advantage. The system boast the possibility to view the progress of the gel run by attaching the machine to a ultra-violet light or blue light viewing add-on (GederaandHerzelia, 1996;Gedera and Herzelia, 1999). It is capable of separating different types of sample by using the appropriate gel type at high speed.

IV. DESIGN FEATURES OF ELECTROPHORESIS MACHINE

a. POWER SUPPLY UNIT

Almost all the electronic circuit from simple transistor and operational amplifier circuit up to digital and microprocessor systems requires one source of stable voltage. A regulated power supply can be constructed by using negative feedback to compare the dc output voltage with a stable reference voltage. The transformer normally, changes the mains supply voltage to a value which is safe to work with and somewhat more than the wanted dc voltage but which is not more than that the regulator can handle. But because a voltage of not less than 200v is needed for this project, a two-winding transformer which is same as auto-transformer in function is adopted for the transformer stage.

The transformer output is rectified using a bridge rectifier producing fluctuating d.c voltage which is then passed through a filter capacitor. The filter is required to remove the ripple voltage of frequency equal to twice mains frequency for bridge rectification. The filter is basically a shunt electrolytic capacitor that charged up the peak value of the input voltage with a large discharge time to prevent it been completely discharged when the fluctuating d.c voltage is going low. Hence, there is always a voltage output across any load connected. The output voltage from the filter is then fed into a linear regulator whose rating is compatible to handle this voltage.

Circuit diagram

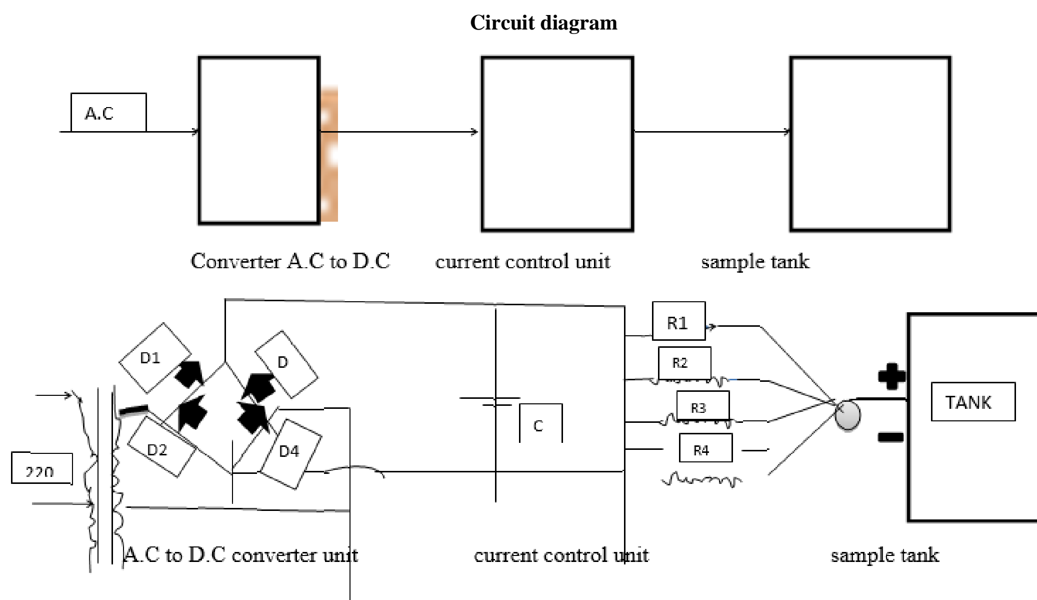

Figure 1 Circuit diagram of electrophoresis machine

b. THEORY OF OPERATION

The basic principle of electrophoresis is simple, as is the equipment needed to comprise a system. The most basic electrophoresis system consists of a chamber or cell, a membrane, an incubator or oven, and a power supply. The chamber contains two buffer compartments, one at each end of the chamber. A bridge between the two compartments supports a membrane, typically cellulose acetate or some type of gel.

The membrane is prepared by using a pipette or microsyringe to inject a small amount of marker material, unknown sample material, and normal sample material in a series of "lanes" at one end of the gel. The preparation of the unknown sample material and the method of preparation of these samples vary at the discretion of the lab technician. The lab tech places the membrane over the bridge so that each end of the membrane contacts the buffer in its respective chamber and then connects the power supply with the negative terminal connected to the end containing the samples. The tech sets the power supply for a particular voltage (or current) and time, and allows the separation to occur.

During electrophoresis, protein, DNA, or RNA segments in the samples at the bottom of each lane in the membrane migrate from negative to positive until equilibrium is reached and the segments remain in position. The process typically takes 20 minutes to an hour. There are dozens of variables that affect the outcome, including: the membrane (whether it is cellulose acetate, polyacrylamide, or some other gel material), the samples, the size and shape of the molecule of interest, the ionic strength of the buffer, the temperature, and of course, the voltage and duration of the electrical power application. Since these variables are unique to each test, specific electrophoresis tests use designated buffers, at particular temperatures, and various materials for the membrane. For example, in performing electrophoresis on cerebral spinal fluid (CSF) to diagnose multiple sclerosis, there are 15 distinct protein bands, a thin-layer agarose gel, and a buffer with a pH of 8.6. However, separations of large proteins and nucleic acids in hemoglobin most commonly use a standard agarose gel membrane and a buffer with a pH of 9.8.

After the set time, the tech disconnects the power supply and removes the membrane from the chamber. In most cases—but only when dictated by the test's protocol—the tech will dry or stain the membrane prior to examination. The tech then studies the lanes and compares the patterns in the unknown sample with the patterns in the marker materiel and normal sample materiel. Depending on the specifics of the test, there may be up to 20 "bands" or spots in each lane, and each band may be of a different size. This comparison of the bands' size and placement determines the test interpretation and the result. Alternatively, the tech may use a dedicated densitometer to determine the amount of light transmitted or reflected by each band.

V. How to Manage the Device

The biomed manager has choices in device management. Maintenance of electrophoresis systems that are visually interpreted by the lab tech can be in-house since only the incubator and power supply require attention. The remainder of the components—the chamber and membrane—require no maintenance and are simply replaced when broken. If the tech uses a densitometer, the options are different. Some densitometers are quite advanced, utilizing computer technology to remember and compare many different band patterns, which automates analysis. These densitometers may be best maintained through an annual service contract that includes periodic software and pattern upgrades. Most of the time, the original equipment manufacturer (OEM) contractor will want to cover the entire system for one price, while other contractors are willing to cover just the densitometer and leave maintenance of the incubator and power supply to the in-house biomed staff. Contract negotiations should cover these options, and ideally, contract proposals should offer coverage on an "a la carte" basis.

i. Risk Management Issues: As noted, electrical shock to the operator is a possibility. The risk of inaccurate results, such as indeterminate or false negatives, also exists since so many variables depend on the lab tech. Likewise, the mitigation of these risks rests primarily with the tech.

ii. **Troubleshooting:** It's up to lab tech to spot and correct the most common problems with electrophoresis. Of all the tests found in the laboratory today, this one probably requires the most technical skill on the part of technicians since they are responsible for the sample preparation, selection of buffers and membranes, voltage and current settings, and sample staining**.**

The three items of interest to the biomed are the incubator, power supply, and densitometer— all of which are all quite reliable. The incubator and power supply, in particular, should be no challenge to the trained biomed.

Incubator and power supply failures are quite rare. When they happen, they are usually hard failures, such as incubators that will either not heat or overheat, and power supplies that provide no output or indication of output on the display device, timer failure, and open leads from the power supply to the membrane. If not detected during the electrophoresis setup, power supply problems manifest themselves by a lack of pattern migration during electrophoresis. A schematic is not required if the biomed is familiar with the general design of the incubators and power supplies.

Maintaining the densitometer is less straightforward. As mentioned earlier, maintenance of the device is often contracted since it contains proprietary software, and software upgrades can be included in the service contract. If maintenance is performed in-house, good service literature—including functional descriptions, block diagrams, schematic diagrams, and circuit-board layouts—is critical.

VI. Future Development of Electrophoresis Systems

The forecast here is on three fronts. First, pulsed field electrophoresis (PFE) uses a switch connected to the output of the power supply to change polarity of the power applied to the membrane on a preprogrammed basis. The DNA molecules then uncoil and orient in one direction. When the polarity is reversed, the molecules reorient themselves to the new polarity. The large DNA molecules take longer to realign than the smaller ones, so there's a separation by size. This separation could not be accomplished with conventional electrophoresis. Applications based upon this variant are under development.

Second, a new electrophoresis technique—called "two-dimensional electrophoresis" or "2 DE"— is proving to be useful. This technique first requires the membrane to be exposed to isoelectric-focusing electrophoresis in one direction, then the electrical field is changed by 90 degrees, and gel electrophoresis occurs perpendicularly to the first. The resulting bands then have a two-dimensional character to them. This technique requires computerized scanning to interpret the information, and it may wind up in the laboratory of the future.

Finally, refinements in both optical technology and computer programming are expected to further improve membrane analysis. Integrated systems using higher-speed and more feature-packed computers will integrate densitometry and provide data analysis capabilities that we only now dream of.

In 1807, Ferdinand Friedrich Reuss, a German doctor, recorded the first-known observation of clay particles, dispersed in water, moving under the influence of a spatially uniform electric field provided by a voltaic pile (a crude battery). Through these experiments, Reuss determined the existence of "barriers" in forms of sand and clay between the poles of the battery. Although Reuss was a doctor of both medicine and surgery he was more of a general scientist, and he conducted more sporadic experiments and published several papers on this and related observations until 1821. But Reuss was relatively obscure in the western European scientific community and a disastrous fire destroyed most of his research papers, so his work got little notice. (There is also some historic speculation that, although this was an interesting phenomenon, Reuss lacked a practical application for it, so he turned his attention elsewhere.)

Reuss' observations lay virtually dormant, except for a few experiments by other scientists of the era, such as Johann Wilhelm Hittorf and Walther Nernst, who measured the behavior of small ions moving through liquid solutions when influenced by an electric field.

In 1930, Arne Tiselius rediscovered this occurrence and wrote about it in his doctorate thesis, "The Moving-Boundary Method of Studying the Electrophoresis of Proteins." Tiselius continued his experiments on the application of physics and physical methods to general biochemical problems, winning the 1948 Nobel Prize in Chemistry. A major result of his experiments was the development in 1937 of a "Tiselius apparatus," a U-shaped assemblage of glass tubes constituting the first true electrophoresis device. This device not only enabled new applications of this developing technology in analyzing chemical mixtures, it prompted some major chemical research centers to acquire their own Tiselius apparatuses and conduct their own experiments.

Tiselius and his apparatus made a profound impact on both biology and biochemistry. But he was not alone. From the 1940s to the 1960s, other scientists developed techniques using filter paper and various gels as supporting media for electrophoresis. These more sophisticated gels allowed the separation of biological matter based on small chemical and physical differences, giving rise to the field of molecular biology and forming the basis of sophisticated electrophoresis tests, including protein fingerprinting, Southern, Western, and similar blotting procedures, and DNA sequencing.

VII. CONCLUSION

The technology of electrophoresis started in the beginning of the nineteen century and even after two centuries have passed it still remains as relevant as it has been in the separation science. Although present electrophoresis is being done in many different ways and method that the equipment and style is so different from the original design, yet the core principle remains the same. By following the trends in changes to the technology of electrophoresis, the next step of development would be miniaturization and portability of systems.

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