Electrophoresis types and applications

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The inception of the concept for electrophoresis was made in 1807. After which it has been a staple tool used by biologist and chemist over the centuries. Various systems of electrophoresis have been introduced, from paper electrophoresis to today's modern automated electrophoresis system. The development of electrophoresis systems have been driven by the advancement of technology and also by the requirement for better and faster resolution of results. This paper reviews the development and progress of electrophoresis over the last two centuries and summarizes possible future trends of electrophoresis.

Keywords: Electrophoresis; History; Development; Advancement; Application

History of electrophoresis development

The concept of electrophoresis was discovered a couple of centuries ago by Ruess [1]. In 1807, he noted the migration of particles towards the anode when applying an electrical current through a suspension of clay in water. However, it was not until the year 1942 that electrophoresis became a major scientific method or procedure. Coleman and Miller [2] conducted an experiment and discovered the migration of neutral hexose toward the anode in a borax (sodium borate) solution. Subsequently, the usage and limitations of the electrophoresis for other compounds was tested. These compounds included those containing adjacent "-OH" groups and also high concentration of neutral sugars [3, 4, 5]. In 1952, Consden and Stanier [6] successfully separated sugar using electrophoresis and only then was electrophoresis started being used for DNA and RNA separation.

Majority of today's current technology in electrophoresis emerged from the advancements made in the seventies and still being used until today. The utilization of Tris-borate-EDTA (TBE) in the electrophoresis of DNA was first demonstrated in 1968 by Peacock, et. al.[7]. Next, Richards et. al. [8] introduced the use of Tris media (Tris-acetate-EDTA (TAE) and Tris-borate-EDTA (TBE)) as the buffer solution for separation of RNA. While Danna et. al. [9] has expanded the usage of electrophoresis as a tool for analysis of DNA. Upon electrophoresis, they managed to determine the relative molarity and length of fragments of SV40 DNA digested with a Hemophilus inXuenzae restriction enzyme. Ethidium bromide was recognized as a suitable DNA stain when Aaij and Borst used it in the conductive medium to show the differences between linear and circular DNA in 1972 [10].

Types and advancements of Electrophoresis

2. 1 Paper electrophoresis

Paper electrophoresis is one of the simplest methods of electrophoresis. 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 [14].

Paper electrophoresis has often been compared to paper chromatography due to their similarity in their mode of action. However, chromatography separates a sample based on its polarity while paper electrophoresis

separates the sample based on its charge by applying a running electrical charge from one terminal to the other [14].

2. 2 Agarose gel electrophoresis

Agarose is a polysaccharide that forms pores with sizes ranging from 100 to 300 nm in diameter. The size of the pore correlates with the concentration of the agarose gel. The higher the concentration of the agarose gel the smaller the pore size and vice versa. Agarose gel electrophoresis is often used to separate DNA or RNA fragments of different length. It involves the movement of negatively charged DNA or RNA molecules from the negative electrode to the positive electrode. The molecules are separated based on their molecular size. [15]

2. 3 Polyacrylamide gel electrophoresis (PAGE)

There are two types of polyacrylamide gel namely the dissociating and non-dissociating gels. A non-dissociating gel separates the proteins in their native form 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 subsequent experiment. A dissociating gel denatures the protein into its constituent polypeptides to determine the polypeptide composition of the sample [14]. Native gel electrophoresis is a non-denaturing gel that has a higher resolving power than the SDS-PAGE when used for protein separations [15].

Polyacrylamide gel is made up of chains of acrylamide monomers (CH2= CH-CO-NH2) that is cross linked with N, Nâ€²-methylenebisacrylamide units (CH2= CH-CO-NH-CH2-NH-CO-CH-CH2), also commonly known as “ bis”. The concentration of bis shall determine the pore size of the polyacrylamide gel.

Due to its higher resolving power than agarose gel electrophoresis, polyacrylamide gel is most commonly used in examining proteins and families of chain-terminated DNA molecules [16]. Additionally, native polyacrylamide gel is capable of separating DNA molecules with a size difference of one nucleotide, such as in single nucleotide polymorphism (SNP) studies.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a rapid method for quantifying and characterizing protein or small molecular weight peptides [17]. SDS-PAGE separates proteins based on their molecular weight [18]. There are two variants of the polyacrylamide gel namely the gradient and SDS-Urea gels. Gradient gels are often employed to separate proteins in just a single resolving gel without the need of stacking gel. SDS-Urea gels are used when the charge of the proteins is significantly similar to the mass of the protein, such as membrane proteins and immunoprecipitates [15]. Solubilization, denaturation and dissociation of the polypeptides chains without altering the proteins intrinsic charge is achieved with use of urea.

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. Comparatively, conventional agarose gel electrophoresis only separates based on size only. This limitation makes agarose gel electrophoresis improbable for molecular fingerprinting.

Reason being some samples may have PCR fragments of similar size, thus forming only a single band or bands that are only slightly distanced apart that make it impossible to be determined [19].

As the samples pass through the gradient gel, it slowly denatures as the concentration of chemical denaturant in the polyacrylamide gel increases. When the sample reaches a threshold denaturant concentration, PCR fragments with weaker bonds will denature much quickly. This denaturation causes the PCR fragments migration along the polyacrylamide matrix to slow down dramatically. Consequently, a more diverse set of bands can be formed for easy comparison to references online [19].

The other type of gel is temperature gradient gel electrophoresis (TGGE), which operates almost similarly liked DGGE, except it uses a temperature gradient as its separating function. In TGGE, the temperature gradient is introduced perpendicularly to the sample running directions. This temperature gradient from low to high temperature allows the sample to denature accordingly based on site mutations. TGGE enables researchers to distinguish single base pair mutation in a single heterogeneous sample as the denaturing point could be either higher or lower from the original sample [20].

2. 5 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 [21, 22].

For 2D gel electrophoresis, the system combines the SDS-PAGE and isoelectric focusing techniques, thus separating the proteins based on their size and isoelectric point. Consequently, 2D gel electrophoresis gives a much better resolution of the protein. It 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 techniques have enable rapid and more accurate analysis of proteins. However, with such high sensitivity there is a higher chance of getting errors due to charge differences. Besides, the samples need to be handled with care. 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 [22].

2. 6 Zymograms

Zymogram is an electrophoresis technique which allows enzyme activity to be analysed in situ after the process of electrophoresis has been conducted on the enzyme [23]. 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 [24]. The use of zymogram allows researchers to save time by removing the need to conduct such steps for protein or enzyme characterization.

There are many ways for zymogram staining to be conducted. A paper by Coughlan [24] explains some of the methods used such as immersing the gel in a chemical reagent which highlights the bands on the gel. Another method mention 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 [24].

One of the advantages of zymogram 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 [23]. 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 [23].

The application of zymograms can vary from analysing of ribonucleases using normal electrophoresis or isoelectri focusing, two-dimensional analysis of bacteria, fungal works, and also for detection and characterization of microbial proteases [25, 26, 27, and 28]. Zymogram is also used as a way to double check on result, which enables researchers to check on suspected protein-protein or enzyme interaction [29]

2. 7 Pulsed-field electrophoresis

For very large DNA molecules around 30 to 50kb, it is not capable to be separated using normal electrophoresis process [30]. 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 [30]. 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 120o 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. Also, the larger the molecule the longer it will take to re-orient [30]

2. 8 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 SDS-PAGE for 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 [14].

There are numerous ways to detect and accumulate the data from the samples. Some of the examples of detection systems are 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 [14]. 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 [31]. An experiment conducted by Tristezza et. al [32] details protocol and usage of capillary electrophoresis for the identification of protein markers in Saccharomyces Cerevisiae. In the paper, the result from using capillary electrophoresis was compared against agarose electrophoresis to evaluate their differences in detection limit [32].

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 [33].

2. 9 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 [34].

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 [34]. 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 [35]. This is due to their extremely small size and the accuracy needed to read their results require highly accurate methods for microchip electrophoresis to work [36].

Research in developing new materials for microchip electrophoresis is widely delved into by many groups of researchers [37, 38, 39, 40]. 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 [37, 38]. The advantage of this protocol reduces the cost of production and also allows faster results development. Wang et. al. [38] 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 [39, 40].

2. 10 Fluorophore-assisted carbohydrate electrophoresis (FACE)

FACE is used to identify carbohydrates with an attached fluorescent dye by separating the carbohydrates using a polyacrylamide gel [40]. This technique is important as carbohydrates are not charged, and is the main technique used to analyze different types of carbohydrates such as glycoproteins, glycolipids, plant and bacterial polysaccharide [40]. Another

advantage to using FACE is to reduce the need for complex work such as those conducted by Gao [41]. 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 [42].

2. 11 Capacitively coupled contactless-conductivity detector (C4D)

C4D is a universal and sensitive tools, which consist of two axially placed tubular electrodes that encompass the separation capillary [43]. The technology has been around since the sixties and seventies [44], but only picked up after a study into using it for capillary electrophoresis was proposed by Zemann, et. al. [45], and Fracassi da Silva and do Lago [44, 46]. It works by gathering the signals longitudinally along the capillary, causing one of the two electrodes to be excited by an alternation current signal and the other electrode to register the signal after it has passed through. C4D is currently being designed to enable miniaturization of microchip electrophoresis and also to build a portable capillary electrophoresis system. It was first used as a detection system for isotachophoresis [44].

2. 12 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 [47]. This process can occur in either solution or immobilised to a solid support [48]. It is also used to measure the binding affinity of receptors to neutral and charged ligands [49]. 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 [50-59]. There are also different types of affinity systems. For example, Taketa and Hirai [60] explains the usage of lectin affinity for use on serum Î±-fetoprotein to determine the interaction between lectin and ligand. Smith and Kelleher [61] used concanavalin A 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 [62]. Also, its wide field of usage makes it an important technique for sampling and data collection.

2. 13 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.[63] 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.[64] 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 [65]

One of the new 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 [66, 67]. It is capable of separating different types of sample by using the appropriate gel type at high speed.

Preview of future trends

With the development of increased sensitivity in detection systems, it is possible to increase the speed and processing of electrophoresis. This also enables researchers to use less of their samples, which is the best advantage of capillary electrophoresis, as it will mean less preparation time and also less wastage of sample.

Although the technology of electrophoresis has advanced tremendously from the basic paper electrophoresis system to today’s highly advance microchip electrophoresis system, there is still a basic need for an external power supply to run the electrophoresis, more so as the system becomes more sophisticated. This serves as a bottle neck on the usage of any electrophoresis system in that the usage of any system is limited to a room with an external power supply. Without a power supply electrophoresis cannot be conducted. Active research is being carried out currently to overcome these limitations (Unpublished results).

Other disadvantages of the current electrophoresis systems and techniques were investigated by Chery, et. al. [68]. In the paper, the influence of

contaminations such as vanadium and selenium can compromise gel results, especially if the process is being left to run for a long period of time. However, the authors stressed that more testing needed to be carried out regarding the effect of contaminations on gel results viability.

With capillary electrophoresis, a lot of care into the details, such as capillary position and gas flow rates, of each experiment runs have to be taken to be able to reproduce the same results as differences in the settings will generate different results for the same experiment [69].

With the advancement in technology, both in engineering and sciences, it is now possible to produce smaller circuit boards that is light and has higher efficiencies and more efficient transformers. Portable electrophoresis system could be built on these. Currently there is preliminary research into designing such a system but the efficiency of such systems is still being tested. For example, Zhang et. al. [70] described their method to design a portable capillary electrophoresis with a conductivity detector. They discussed the use of microchip technology coupled with integrated electrodes which would act as a detector to transmit the data to be analyzed by a separate machine to generate its result. Another research project that takes advantage of the increasing technology development has developed a method in which the staining and de-staining of nucleic acid to be automated and is cheap [71]. The paper describes the method, in which the researchers use day to day materials such as a model airplane fuel pump to circulate the staining reagents via the reagent bottles to the staining chamber, which cuts cost for low budget laboratory when compared to buying and using commercial systems [71].

Also, there are many patents regarding increasing the efficiencies of current systems, such as using a different buffer system or gel type, in increasing the efficiency. There is major research being done by different groups around the world on improving currently used systems, notably the automated electrophoresis system. The main point of the research being done is to increase the detection limit and also the reduction in the amount of background noise [72] Also, the uses of different type of materials, such as coated capillaries, for running gel electrophoresis and upgrading the detection system on the current equipments are also being looked into [17, 73, 74, 75, 76, 77, 78, 79, 80]. The materials being research and developed by these groups mainly focuses on increasing the efficiencies, reproducibility and accuracy of the separation run. Work is also being done to enable retrieval of the separated sample without damaging it [77].

Conclusion

The technology of electrophoresis started in the beginning of the nineteen century and even after two centuries have passed it is still being practiced. 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.

Competing interest:

The authors declare no competing interest.