Efficacy of gel electrophoresis for proteins and biotechnological products – an overview.

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DOI:

Article Info:

Received 28 June 2020
Accepted 23 Aug 2020
Published 1 Dec 2020

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Abstract:

Nucleic acids or proteins electrophoresed within a matrix or gel that immersed in a buffer provides ions needed to carry a current and for pH maintenance at a relatively constant value. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) utilizes SDS as an anionic detergent that causes protein denaturation that linearized protein molecules. Each molecule of SDS has the ability to binds to two amino acids. As a result, the ratio of charge to mass becomes constant for all denatured proteins in the mixture. The molecules of protein migrate toward the positive pole and separated in the gel depending only on their molecular weights. The chains of polyacrylamide are cross linked by N, N-methylene bisacrylamide comonomers and ammonium persulfate used as an initiator for polymerization as they act as radical source and N, N, N', N'- tetramethylethylenediamine (TEMED) used to catalyse the polymerization. Electrophoresis of proteins and nucleic acids by using agarose or polyacrylamide gels were illustrated in this review.

Key words: Electrophoresis, Agarose, SDS, Urea PAGE.

تسليط الضوء على فعالية عملية الترحيل الكهربائي على البروتينات ونواتج عمليات التقنية الحيوية

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الخلاصة:

الاحماض النووية والبروتينات تمر اثناء عملية الترحيل الكهربائي عبر وسط او قالب من مادة هلامية مغمورة في محلول منظم يوفر الايونات اللازمة لحمل التيار كما يساعد على المحافظة على الرقم الهايدروجيني عند قمية ثابتة نسبيا. في عملية الترحيل الكهربائي باستخدام مادة دوديسيل كبريتات الصوديوم- بولي أكريلاميد الهلامية فان دور مادة دوديسيل كبريتات الصوديوم هو توفير الوسط الايوني السالب الذي يعمل على تغيير شكل البروتينات الى شكل خطي. كل جزيئة من مادة دوديسيل كبريتات الصوديوم لها القدرة على الارتباط بحامضين امينيين مما يؤدي الى ثبات نسبة الشحنات الى الكتلة لكل البروتينات التي تم تغيير شكلها في المخلوط.تتحرك البروتينات باتجاه القطب الموجب وتنفصل في الوسط الهلامي اعتمادا على وزنها الجزيئي .سلاسل مادة البولي اكريلاميد تتقاطع بواسطة مادة ن،ن- ميثيلين بيس اكريلامايد كومونيمر ومادة بير سلفات الامونيوم التي تعمل كبادئ لعملية البلمرة كونه يعمل كمصدر للجذور الحرة بينما تعمل مادة رباعي ميثيل إيثيلين ثنائي أمينلتنشيط عملية التبلمر.في هذه البحث يتم استعراض وتوضيح عملية الترحيل الكهربائي للبروتينات والاحماض النووية بواسطة مادتي الاغاروز والبولي اكريلامايد الهلاميتين.

الكلمات المفتاحية: الترحيل الكهربائي، البروتينات، الاغاروز الهلامي، دوديسيل كبريتات الصوديوم ، يوريا اكريل اميد الهلامي

Introduction

Electrophoresis is defined as a separating technique for purifications of macromolecules of nucleic acids and proteins which show a difference in their size, charge, or conformation [1-4]. Its concept based on the capability of charged molecules to migrate toward either cathode or anode when subjected to an electrical field according to their charge [5, 6]. Due to the presence of phosphate backbone in nucleic acid, they exhibit a consistent negative charge, in contrary to proteins that have the capability to behave as either negatively or positively charged [4, 7].

Electrophoresis is a process that used for molecules sorting on the bases of molecular size. By subjecting the molecules (such as DNA) to an electric field they can move through agarose or polyacrylamide gel. The subjected electric field consists of two ends, one of them has the negative charge that pushes the negatively charged molecules through the gel, and the second end has a positive charge which do the opposite as it pulls these molecules throughout the gel [3-8]. The molecules to be sorted applied firstly in a well that made in the gel material which placed in a special chamber for electrophoresis that connected to a source of power. In the electrical field that generated by applying the current of electricity, the molecules with large size moved slowly through the gel whereas molecules with small size moved faster. Molecules with different sized molecules form distinct bands on the gel [8].

The "gel" term in this technique is referred to the matrix that utilized to comprise and sort targeted molecules. Generally, it is composed of a polymer that cross-linked to introduce a porosity chosen according to the weight, size and composition of the targeted molecules that subjected to the analysis [9]. For the sorting of small nucleic acids (DNA, RNA, or oligonucleotides) or proteins, the gel used consist usually of variable cross-linker and acrylamide concentrations which lead to the formation of various mesh sizes of polyacrylamide networks. On the other hand, the sorting of larger nucleic acids (more than a few hundred bases) need a matrix purified agarose. Acrylamide, in contrast to polyacrylamide, must be handled with appropriate safety precautions due to its neurotoxicity to avoid poisoning. Agarose is an uncharged carbohydrate that consists of long unbranched chains with no cross-links that cause a formation of a gel containing large pores which permit for the sorting of macromolecules and macromolecular complexes [10].

Hydrated gel networks of gel electrophoresis showed several intended characteristics for electrophoresis. They support wide range of mechanically stable experimental modes such as electrophoresis in horizontal/vertical pattern in slab gels or tubes/capillaries electrophoresis. In addition, the mechanical stability allows for manipulation after electrophoresis performance that make it possible for more techniques including blotting, electro-elution, or Mass Spectrometry.

(MS) identification /finger printing of proteins either intact of in slices of gel in digested form. The chemical inertness of the gels allows for sample content resolution according to physical rather than chemical differences between these components [11].

The aim of this study is to update and highlight on the efficacy of gel electrophoresis in the separation purification and identification of DNA, RNA, proteins and biotechnological products.

Types of gel electrophoresis and apparent gel pore size:

Agarose gels: it is copolymer that formed by alternate linking of 1, 4-linked 3, 6 anhydro-α-L-galactose and 1,3-linked β-D-galactose and, unusually replaced by pyruvate, sulphate and/or carboxylate moieties [12]. In solutions, Agarose molecules exhibit a random coil structure at elevated temperatures [13]. Agarose chains form bundles of helical fibre when subjected to cooling which bind together through non-covalent hydrogen bonds; at lower temperatures, the gelation occurs as a result of the fibre bundles cross-linking in "junction zones" by forming further hydrogen bonds [14]. Exchange of strand partner takes place in the junction zones by the rearrangement hydrogen bond [15].

Plots of Ferguson can be used to determine the agarose gels' effective size of the gel pore by plotting the mobility's log against the concentration of the gel [16] of various DNA. In accordance to the assumed distribution of Gaussian for pore sizes, the median radius of the gel's pore that causes a reduction in the mobility of DNA to half of that with zero concentration of gel that is equal to the DNA gyration radii [17]. The median of the radius of the pore of Agarose gel 1% was observed to be approximately 100 nm [18]. The pore radii of Agarose gel can be determined from DNA gel electrophoresis lattice models [19] which tend to be approximately lower by 2 folds than those estimated with the plot of Ferguson methods, whereas the estimated pore radii with nuclear magnetic resonance technique [20] or with the technique of atomic force microscopy (AFM) [21] are nearly higher by 2 folds. The determination of gel pore radii by AFM or NMR are subjected to further argument that these methods showed higher accuracy, given that the values measured by electrophoretic methods represent pores subgroup that are accessed by the migrating DNA molecules. However, the average pore size of the electrophoretic method accessible pores in a given gel matrix is possibly more relevant for the interpretation of gel electrophoresis technique [12].

Polyacrylamide gel: it is cross linked chemical gel that produced by crosslinking agent (ex. N, N′-methylenebisacrylamide; Bis) with bi-functional group that react with acrylamide to form the intended cross-linked gel [22]. The constituents of polyacrylamide gel is expressed by %T that represent the total (w/v) acrylamide plus cross-linker concentration and %C that represent the (w/w) percentage of cross linker included in %T. Polyacrylamide gels are polydisperse in their structure as a result of Bis polymerization with itself more rapidly than with acrylamide [22]. Due to that, polyacrylamide gels comprise highly cross linked, Bis-rich nodules that joined to each other by sparsely cross linked, relatively acrylamide-rich fibres [23].

As a result of the non-homogenous matrix structure of the polyacrylamide gel, the effective pore size measured by gel electrophoresis rely on the analyte size. When the analyte used is proteins, the apparent pore size consistent with the pores in the Bis-rich nodules [24]. On the other hand, when the DNA is the analyte that considered as a larger one, the small pores do not appear in the nodules and in a contrast, they will be obstructed by the movement through the acrylamide-rich fibres [24]. It was reported that, plots of Ferguson also determined DNA molecules with a size that ranged from 123 base pairs to 1600 in gels of polyacrylamide comprising 3.5% to 10.5%T and 3% Bis. Ideal examples of this plots that demonstrated for multimers of 167-bp normal fragments and curved DNA are clarified in Figure 1 [25].

Plots constructed by Ferguson that demonstrated for the multimers with curved shape showed steep slope that extrapolated to lower migration that observed in gel with zero concentration in comparison with the plots of Ferguson for matched normal samples. Plots of

Ferguson analysis demonstrate that the effective radius of the pore is ranged from 20 to 140 nm in gels comprising of 3.5% to 10.5%T and 0.5% to 10%C (25). By using scanning and transmission electron microscopy, a similar gel pore radius has been obtained [26]. However, NMR relaxation have assumed that the average radius of pore showed an average of nearly lower by 5 folds, may be due to difference in this technique that determine the average pore radii of the gel nodule and fibre [12].

Urea PAGE or denaturing urea polyacrylamide gel electrophoresis employs 6-8 M urea, which denatures secondary DNA or RNA structures and is used for their separation in a polyacrylamide gel matrix based on the molecular weight. Fragments between 2 to 500 bases, with length differences as small as a single nucleotide, can be separated using Urea PAGE. The migration of the sample is dependent on the chosen acrylamide concentration. A higher percentage of polyacrylamide resolves lower molecular weight fragments. The combination of urea and temperatures of 45-55 °C during the gel run allows for the separation of unstructured DNA or RNA molecules. In general, this method is required to analyse or purify single stranded DNA or RNA fragments, such as synthesized or labelled oligonucleotides or products from enzymatic cleavage reactions [27].

Starch: Potato starch after subjected to partial hydrolysed can be used as another medium for protein electrophoresis with non-toxic property. The opacity of these gels is slightly higher than that of acrylamide or agarose. Intact proteins (Non-denatured) can be sorted depending on their charge and size. The visualization performed by using Amido Black or Napthal Black staining. The concentrations of ideal starch gel are 5% to 10% [28].

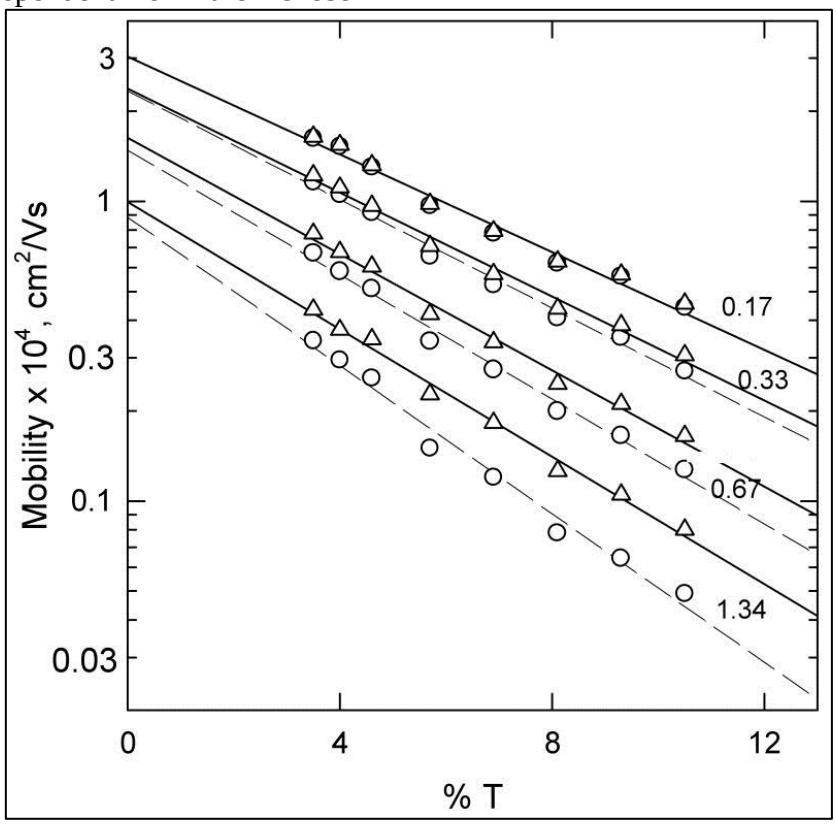

Figure (1): Ferguson plots observed for normal (Δ) and anomalous (○) DNA fragments in polyacrylamide gels. The logarithm of the mobility is plotted as a function of polyacrylamide concentration, %T, in gels containing 3%C. The normal and anomalous fragments contained

one, two, four or eight monomers, from top to bottom; the size of each pair of fragments, in kilo base pairs, is indicated beside each pair of lines. All lines were drawn by linear regression (25)

The interactions of DNA with agarose and polyacrylamide gel matrices

DNA electrophoretic mobility is highly dependent on the nature of the matrices in which the separation takes place. In the following, the interactions of DNA with agarose and polyacrylamide gel matrices are presented

Interaction of DNA with agarose gels: Based on the theory of gel electrophoresis that established by Ogston-Rodbard-Chrambach [29], polyelectrolyte movement through the gel matrix is estimated by the gel's fractional volume that accessed for macromolecules migration of. Hence, plots of Ferguson are supposed to estimate the mobility of free solution of analyte when the gel concentration is zero [30]. Plots of Ferguson that constructed for DNA should extrapolate with major intercept at gel concentration equal to zero, because the mobility of DNA molecules free solution of greater than ~400 bp showed an independency from the molecular mass [31]. Nevertheless, similar to the gels formed of agarose as illustrated in Figure 2, the interception at zero gel concentration reduced in a concomitant with an increment in the size of DNA. Hence, the migration of molecules of DNA in gels of agarose showed to be hindered by a mechanism that is based on the molecular mass in beside their sieving effect. The effect of molecular mass showed to be a transient interaction between DNA molecules and the gel fibres of agarose gel throughout electrophoresis. This interaction is not surprise given that DNA is a polyelectrolyte with highly negative charge and agarose molecules have the capability to bind anions [32]. If the mobility obtained at agarose gel concentration equal to zero are linearly extrapolated in case of zero DNA molecular mass, the consequence movement showed values of (3.0 ± 0.1) × 10-4 cm2/Vs in 40 mM Tris-acetate-EDTA buffer [33], which showed a mobility with nearly equal to that obtained by using similar electrolyte background [31].

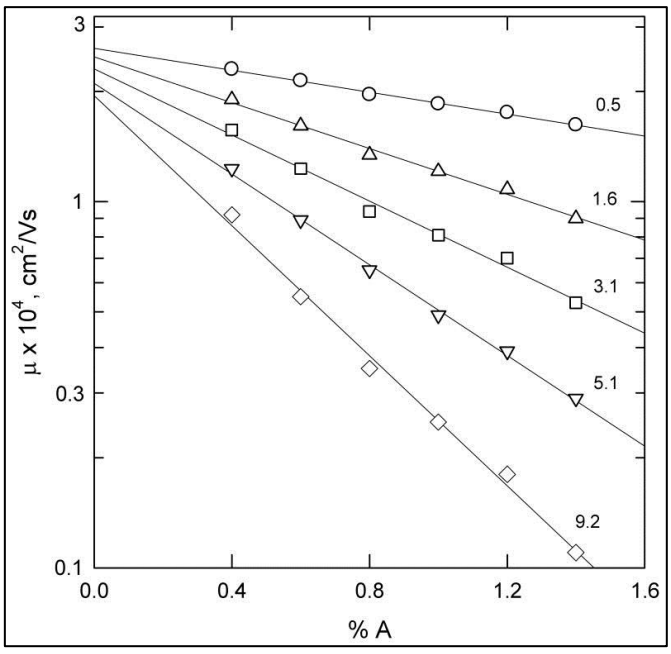

Figure (2): Ferguson plots observed for normal DNA molecules in agarose gels. The logarithm of the mobility, extrapolated to zero electric field strength at each gel concentration, is plotted as a

function of agarose concentration, %A. The lines were drawn by linear regression; the size of each DNA, in kilo base pairs, is given beside each line (33).

Interaction of DNA with polyacrylamide gels: The plots of Ferguson that showed for molecules of DNA in gels of polyacrylamide extrapolate to various mobility at concentration of gel that equal to zero, as illustrated in Figure 1. If the motilities observed at zero gel concentration are linearly extrapolated with zero DNA molecular mass, the calculation of the free migration of solution of DNA revealed to be $(3.1 \pm 0.1) \times 10^{-4}$ cm^2/ Vs in 40 mM Tris-acetate-EDTA buffer, equal within experimental error to value get from the extrapolation of plots of Ferguson obtained in gels formed of agarose [33]. Hence, DNA molecules subjected to an electrophoresis with gels of polyacrylamide are obstructed in such manner that depends on the molecular mass that occurs besides sieving, mainly due to transient interactions between the gel matrix and the migrating DNA molecules [34]. This mechanism showed to retard curved DNA molecules more than DNA molecules with normal shape that have an equal base pair number, due to plots of Ferguson for curved DNAs have slopes with steeper fashion and extrapolate to mobility nearly at lower concentration of gel equal zero [35].

The particular essential role regarding to the slow anomalous migration that results from sieving and from the tendency of the DNA molecules of a curved shape to interact with a gel matrix formed of polyacrylamide that can be estimated by determining both normal and curved DNA molecules mobility in gels in which the size of pore is changed by altering % T at constant % C (the common method of altering the size of the pore in gel matrix) and by altering % C at constant % T. in the case of anomalies mobility that owned mainly to sieving effects, it must be non-dependant on the method utilized to change the size of the pore in the gel. However, if the favourable interactions between the fibres of polyacrylamide gel with curved molecules of DNA are cause for the slow anomalous migration, the anomalies mobility must be related to the acrylamide concentration gel, and independent from the apparent pore size of the gel. If the pore size of polyacrylamide gel is reduced by elevating % T with % C kept constant, the slow anomalous migration of the molecules of DNA that possess curved shape will elevate with a reduction in the radius of pores of the gel [35], as assumed, the effect of sieving considered as the major contributor in mobility anomalies [36]. However, if the size of the pore of gel changed with altering % C whereas % T still constant, the slow anomalous migration is non-depending on the radius of gel pore given that the apparent radii of gel pores is greater than the gyration DNA radius. If the gyration radius of DNA is greater than the apparent radii of gel pores, the slow anomalous mobility of the DNAs with a curved shape reduced with the increment in the radius of pore within the gel till the radius of the pore showed a similar value to that of the gyration radius of DNA, beyond this point, the anomalies mobility behave as constant and not affected by the size of the pore. Sieving effects are considered as a secondary importance reason for the slow anomalous migration obtained for curved DNAs after the factor that include the interaction of DNA with curved molecular shape with some gel rich with acrylamide fibres that considered as the primary cause in polyacrylamide gels [35].

Polyacrylamide gel electrophoresis (SDS-PAGE)

Using polyacrylamide matrix for gel electrophoresis of proteins is generally named polyacrylamide gel electrophoresis (PAGE) which is considered as the most commonly applied techniques for the resolution of complex mixtures of protein sorting. It is characterized by a

convenience, short time for resolution in addition to its low cost because only micrograms quantities of protein are required in this technique [37]. The proteins charge depends on the pH of the medium which is differ from its isoelectric point that lead to the movement of proteins when an electric field is subjected. The velocity of protein migration is directly proportion to the ratio between the charges and mass of the subjected protein. Proteins have no predictable structure as in the case of nucleic acids that make the rate of their migration differ from one to another. Proteins cannot move when they are at their isoelectric point. For these situations, it is advised to denature proteins by the addition of detergent such as sodium dodecyl sulphate (SDS) to sort them exclusively in accordance to their molecular weight. SDS used due to its ability to breaks disulphide bonds which is owned to its reducing activity leading to the sorting of protein into its sub-units with the generation of negative net charge that considered as the driving force for protein movement through the gel in a rate directly proportion to their size [38].

Furthermore, denaturation that caused by SDS that lead to loses the tertiary structure of protein which makes the velocity of migration related only to the size and not to tertiary structure. So, in summary, polyacrylamide gel electrophoresis is:

- Gels prevent the convection of heat generated by the utilization of electric field, and can also act as a sieving medium, preventing molecules passage; gels can be used to keep the final separation, which facilitate the application of the stain after the electrophoresis performance [39].
- Polymerization of acrylamide is performed by using cross-linkers, the bis-acrylamide, with the addition of catalyst and initiator for the formation of poly acrylamide gel. Persulfate ion ($S_2O_8^-$), that is added as ammonium persulfate (APS) is act as an initiator for the solidification of gel and also as free radicals source, whereas TEMED (N, N, N', N'- tetra methyl ethylenediamine) act as a catalyst for the reaction of polymerization by stabilizing these free radicals. In some cases, as in the isoelectric focusing the persulfate existence can cause an interference with electrophoresis, and for this reason they replaced by riboflavin and TEMED [40].
- Polymerization of acrylamide supressed by the presence oxygen and the solution should be degassed to eliminate this effect. Furthermore, bubbles can be formed within the gel as consequences of the heat liberated during polymerization [39].
- The concentration of catalyst and the initiator (persulfate and TEMED; respectively) play role in the determination of the polymerization rate [41].
- Acrylamide/bis acrylamide ratio as well as their total concentration affects the size of the pore size and also influence the rigidity of the final gel matrix [38].

In order to that, the protein sizes range of resolution will be affected. The pore size that formed in the gel is inversely proportional with the used amount of acrylamide. For example, a gel with 7% polyacrylamide showed bigger pores than that with gel of 12% polyacrylamide concentration. Low percentage acrylamide gels used typically to resolve large proteins, whereas gels with elevated percentage utilized to resolve proteins with small size. "Gradient gels" prepared specially with high percent-acrylamide at the bottom whereas low percent-acrylamide at the top that allow for the resolution of wide range of protein molecules with a different size. The electrophoresis systems of acrylamide gel can be conducted by applying one or more buffers which are either continuous phosphate buffer system [37] or discontinuous buffer systems [42]. It was adopted that the discontinuous electrophoretic technique and what called as "Laemmli buffer" is usually used to depict the tris-glycine buffer system that is applied during SDS-PAGE [43].

In discontinuous systems the buffer used firstly improve the mobility of all proteins in the front of migration that lead to entire sample accumulation into the well after loading. The real sorting starts at the point of migration front attain to the second buffer boundary. The first gel, "stacking", has greater pore (lower percentage of acrylamide/bisacrylamide) and showed a more acidic pH than the second gel which is the actual protein separating gel. This system is mainly suitable for analysing samples that kept their resolution even at diluted concentrations [44]. For peptides with a molecular weight of less than 14 kDa, the resolution is not enough by using conventional tris-glycine systems. The solution of this problem is the development of Schägger and Von Jagow new system, in this technique the introduction of an additional spacer gel is conducted and the buffer molarity is increased and glycine is replaced by tricine as terminated ion instead of. This method yields linear resolution from 100 to 1 kDa [45].

Different between SDS and urea PAGE

SDS and urea are both protein denaturing reagents which act in different ways on protein molecules. SDS is a strong, negatively charged detergent which binds the proteins molecules so that they all have approximately the same charge to mass ratio and can be separated according to their mass by the molecular sieving effect of the PAGE gel. In a urea gel, typically 6M for proteins, the charge to mass ration is determined, not only by its size but also by the intrinsic charge on each protein molecule at the pH of the PAGE gel buffer [38]. These two methods therefore provide for two different ways of testing for protein purity. Proteins that have the same mobility in SDS-PAGE are likely to have electrophoretic moblilties that are different from each other in urea- PAGE (non-SDS PAGE). The urea-PAGE method may be easier that isoelectric focusing. When purifying proteins, especially from complex protein mixtures for which there is little prior information, it is advisable not to rely on SDS-PAGE as the sole test of purity. Another method, in addition, such one that depends on the intrinsic charge of the proteins will give a more rigorous test. Non-ionic detergents or neutral-charge detergents can be added to the urea to improve solubility if that is a problem [39, 46].

Applications of gel electrophoresis

- It is widely after digesting DNA molecules by restricted enzymes to estimate the size of the resulting fragments as in the cloned DNA restriction mapping of cloned DNA [45].
- Polymerase chain reaction (PCR) products analyzation, e.g. in molecular genetic diagnosis or genetic fingerprinting [47].
- Sorting and separating genomic DNA fragments that result after restriction before subjected to Southern blot technique, or of RNA before subjected to Northern blot technique [48].

Gel electrophoresis used in several applications that include molecular biology, microbiology, forensics, genetics and biochemistry. The gel is visualized by UV light and a gel imaging device for the analysis of the results. The image is captured and recorded by using a camera operated by a computer and the band or spot intensity of the intended sample is determined and compared with that of markers or standard applied on the same gel. The analysis and estimation process are performed mainly by specialized software designed for this purpose. Relaying on the analysis type that conducted, other techniques are often applied in concomitant with gel electrophoresis results that provide a vast versatility of applications with field-specificity [49].

Nanoparticles**:** A newly emerged gel electrophoresis application for the sorting or characterization of metal or metal oxide nanoparticles (ex. Ag, Au, SiO_2, ZnO) according to their shape, size, or even the chemistry of the surface. The goal of this

application is to achieve sample with more homogeneity in its particle size content (e.g. narrowing the distribution of particle size), that is capable to utilized in more products/processes (e.g. self-assembly processes). For the nanoparticles sorting within a gel, the size of a particle which is about the size of the mesh is considered as the main factor, whereby two mechanisms of migration were identified: the first one is the unrestricted mechanism which occur when the particle size is smaller than the mesh size whereas the second mechanism is the restricted mechanism which exist when the size of the particle is nearly similar to mesh size [50].

Conclusion:

gel electrophoresis has become a general technique that used globally in molecular biology discipline for nucleic acids and proteins separation. The use of gel electrophoresis exceed the traditional uses as preparative method that performed in parallel with common molecular biology techniques such as cloning and PCR, but additionally it also plays an crucial role in the separation of nucleic acid and applied in the analysis techniques in newly applied technologies such as editing of genome and next-generation sequencing. Recently, electrophoretic methods showed a great participation in promoting advances in various fields such as molecular biology, organic chemistry and biochemistry and will continue to be an essential technique for several other genetic applications and technology, proteins and nucleic acids sequencing, studies of malfunctions and diseases such as cancer, and in the species and individual's determination, e.g., in forensic medicine.

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