Chapter 3

Recent Advancements in DNA Gel Electrophoresis on Pharmaceutical Sciences

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Abstract

Gel electrophoresis is a fundamental technique for the separation of charged molecules in pharmaceutical sciences, and it is widely employed in biochemistry, molecular biology, and genetics. The primary methods of electrophoresis include gel electrophoresis, zone electrophoresis, free-flow electrophoresis, and capillary electrophoresis, with gel and capillary electrophoresis being the most prevalent in biological research. In DNA electrophoresis, DNA fragments are separated based on the number of base pairs. This technique utilizes agarose gels, which facilitate the movement of DNA—characterized by a slight negative charge—toward the positive electrode at the gel's end. While the use of electrophoresis has diminished due to advancements in DNA sequencing and polymerase chain reaction (PCR) technologies, gel electrophoresis remains a valuable tool, especially for investigating the interactions of DNA with enzymes. Recent research indicates that scientists are employing agarose gel electrophoresis (AGE) to study enzyme inhibition, structure-activity relationships (SAR), binding studies, pharmaceutical formulation properties, DNA genomics and transcriptomics. Despite the prevalence of newer methodologies, gel electrophoresis continues to provide significant insights in the realm of DNA research. In this section, the recent studies of AGE will be indicated, and the role of AGE in the recent area of pharmaceutical research will be discussed to enlighten the future perspective.

1. Electrophoresis in Pharmaceutical Research

Electrophoresis is a decisive analytical technique for separating charged molecules based on their movement through a baseline in response to an applied electric field. This method is widely utilized across different preparative

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and analytical applications, making it fundamental in biochemistry, molecular biology, and genetics (Sambrook et al. 1989).

Electrophoretic techniques are broadly used in various scientific field. The main principle of this technique is the movement of sample ingredients from one charge end to another. The main requirement of this technique is the sample should be charged electronically for the reason of migration. Various approaches are used that assembled in four main types of; gel electrophoresis, zone electrophoresis, free-flow electrophoresis and capillary electrophoresis (Rana et al. 2023). Thes techniques are used different migration baseline and distinct purposes for the relevant studies. In biological term, gel electrophoresis and capillary electrophoresis are used mostly, compared between all electrophoresis types.

During electrophoresis, molecules such as nucleic acids (DNA and RNA) and proteins are subjected to an electric current, prompting them to migrate through a gel or another matrix. The separation primarily results from differences in size, charge, and conformation; for example, smaller molecules generally travel faster than their larger counterparts. Common types of electrophoresis include agarose gel electrophoresis for DNA and RNA, which allows researchers to isolate specific fragments for cloning or sequencing, and polyacrylamide gel electrophoresis (PAGE) for proteins, which helps analyse protein purity, structure, and molecular weight. Techniques such as capillary electrophoresis enable scientists to separate different substances with remarkable precision and speed (Ausubel 1988).

1.1. DNA Gel Electrophoresis

DNA electrophoresis is the technique that determine the DNA fractions by their base pair number. This technique is widely used for DNA studies including DNA damage, replication, restriction and ligation. It is possible to see the DNA material can migrate from (-) end to (+) end as their charge is slightly negative (phosphate backbone). This negativity helps to moving forward in the same column. Thus, the DNA fragmentations are aligned by their molecular weight (parallel to base pair numbers) (Figure 1.1.). This method can be employed either horizontal or vertical gel electrophoresis.

Although various gelling agents can be employed for electrophoresis, agarose remains the most suitable choice. Agarose gels are widely used because traditional methods, such as sucrose density gradient centrifugation, only provide an approximate estimation of DNA fragment size (Lee et al. 2012). In contrast, agarose allows DNA fragments to migrate freely through

the gel matrix, maximizing the distance travelled and enabling more accurate and indicative separation of nucleic acids (Lee et al. 2012).

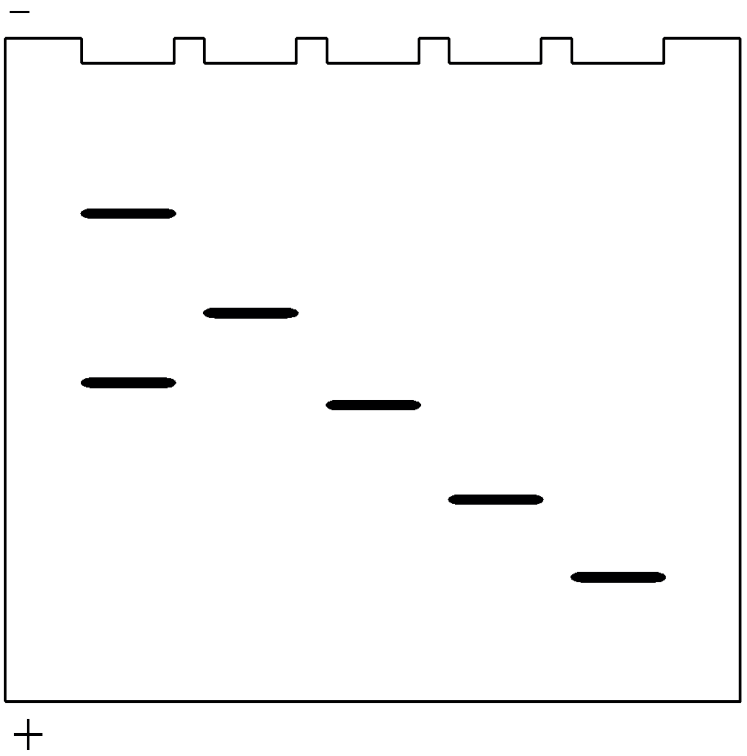

Figure 1.1. Illustration of gel electrophoresis. The DNA fragments can be shown on the gel as "bands" in separated columns.

1.2. DNA Gel Electrophoresis studies in the last decade

Although the utilization of agarose gel electrophoresis (AGE) has become increasingly limited, it remains a fundamental analytical technique in molecular biology and biochemistry. Its ability to resolve nucleic acid fragments and provide rapid, reliable results continues to make it valuable in many research settings. The electrophoretic migration of nucleic acids through agarose gels enables the separation of DNA fragments ranging from approximately 100 to 25,000 base pairs in length. Following electrophoresis, DNA bands can be visualized using UV-visible dyes that intercalate between nucleic acid bases. The widespread availability of agarose and the cost-effectiveness of its application protocol have contributed to the sustained relevance of this method, despite the advent of more sophisticated DNA-based analytical technologies (Semenov et al. 2023) .

Nevertheless, interest in electrophoretic techniques has gradually declined over the past decade, primarily due to major advances in DNA sequencing, PCR methodologies, and the integration of artificial intelligence into molecular modelling (Figure 1.2.). However, this decline reflects a methodological transition rather than a scientific retreat, as DNA-focused research-particularly within the pharmaceutical field continues to expand through the adoption of alternative analytical and computational strategies (Fonslow et al. 2009, Jaywant et al. 2024, Sowersby et al. 2024).

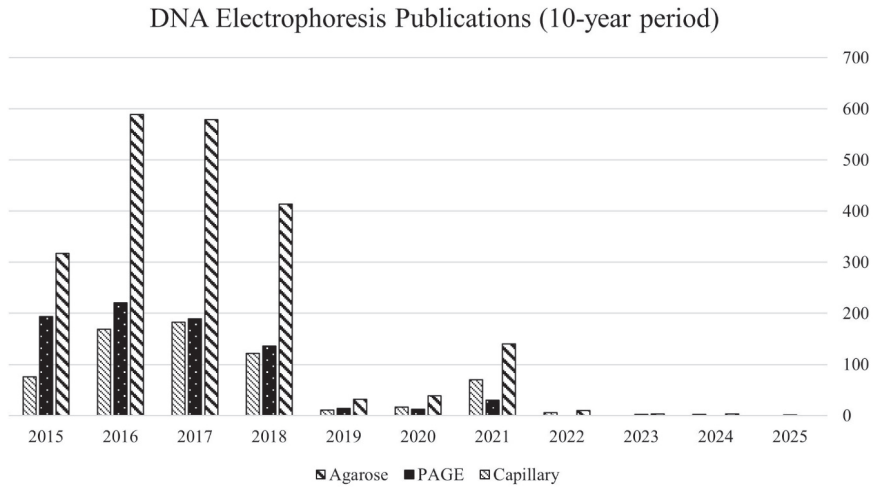

Figure 1.2. The bar chart of total publications counts of the fundamental electrophoresis techniques using with DNA-based materials (According to the PubMed Library.).

One factor contributing to the declining preference for electrophoresis is the increasing focus on short oligonucleotide studies in DNA research. Evaluating short DNA fragments with AGE presents challenges, as these sequences migrate rapidly through the gel, complicating their visualization and resolution. Nevertheless, despite these limitations, AGE continues to play a valuable role in molecular biology laboratories.

Its suitability for addressing straightforward research questions, together with its established reliability, reproducibility, and extensive troubleshooting flexibility, ensures that it remains widely used. Over the past decade, AGE has maintained relevance across several critical research areas, particularly in the analysis of nucleic acids and protein–nucleic acid interactions, where it continues to provide consistent and meaningful results (Semenov et al. 2023) (Figure 1.3.).

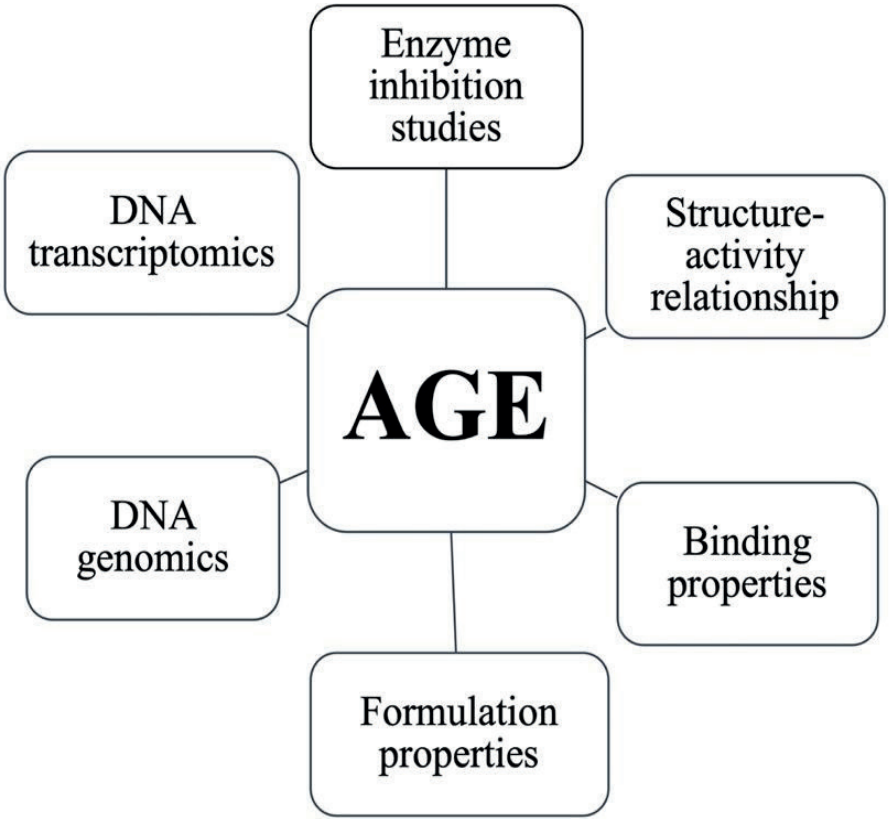

Figure 1.3. Research areas where active pharmaceutical studies have been conducted in the last 10 years.

2. Current Agarose Gel Electrophoresis methods on DNA fragment measurement

2.1. Enzyme inhibition studies

In the recent years, DNA material has been used as the model for enzyme inhibition that selected DNA-targeting enzymes using gel mobility and fraction assays. The DNA fragments clearly showed that the blockage of the functionality can be made by ligands, and it generates data about the mechanism of action for these compounds. Hence, the candidate compounds were investigated both DNA binding and functional interactions.

DNA topoisomerases are considered critical targets in the development of novel anticancer therapies. Compounds targeting these enzymes can act either by directly interacting with the DNA or by modulating the enzyme

itself. Protocols designed to evaluate the activities of topoisomerases and their inhibitors are collectively referred to as topoisomerase enzyme assays. These assays include a topoisomerase I activity test, which monitors the unwinding of supercoiled DNA; a topoisomerase II assay, which evaluates the decatenation of double-stranded DNA; and assays that measure DNA–protein covalent complexes, which represent essential intermediates in the reactions of both type I and type II topoisomerases with DNA (Osheroff et al. 1999, Nitiss et al. 2012).

As an example, tacrine–coumarin derivatives have been reported to exhibit activity against topoisomerase enzymes in lung carcinoma cells (Konkoľová et al. 2021). In that study, agarose gel electrophoresis was employed as a comprehensive assay to evaluate seven different compounds across a range of concentrations. The resulting data provided mechanistic insight into enzyme inhibition and were further supported by corresponding cell viability results. Similarly, agarose gel electrophoresis has been applied to investigate DNA–phytochemical interactions (Hsieh et al. 2020). In the present study, topoisomerase activity was assessed, and the DNA-unwinding capabilities of phytochemicals were evaluated using the different coiling forms of the pBR322 plasmid. Agarose gel electrophoresis enabled visualization of supercoiled DNA relaxation, demonstrating how ligand addition modulated topoisomerase-mediated DNA unwinding and highlighting the continued utility of AGE in functional nucleic acid research. In addition to investigating supercoiling mechanisms, other DNA-binding assays can be employed to evaluate compound–DNA interactions, such as those involving restriction endonucleases (Okumuş et al. 2022) and telomerases (Zhu et al. 2021). In these studies, electrophoretograms were used to visualize the effects of enzymatic reactions on DNA before and after treatment with the compounds. The DNA substrates varied depending on the enzyme under investigation, ranging from plasmid DNA to whole genomic DNA.

2.2. Structure-activity relationships and binding properties

Structure-activity relationships (SAR) studies have one of the greatest importance nowadays, due to identify the drug mechanism on various media. DNA-targeting treatment strategies need to be revealed the DNA binding mechanism of compounds and how resulted this binding on cellular metabolism. On the other hand, Electrophoretic Mobility Shift Assay (EMSA) studies is used to identify the binding effect of compounds on selected DNA targets. For example, the DNA stress studies can be assayed with EMSA, and revealing the immuno-modular response of DNA-binding compounds (Khan et al. 2022). Thus, compound binding induces

shifts in DNA fragments on the gel in a concentration-dependent manner. These observations are consistent with other DNA-related studies, further supporting the reliability of agarose gel electrophoresis. This demonstrates that AGE can continue to serve as a valuable tool for analyzing and explaining activity-related changes in DNA, both in current research and future applications.

There are new protocols using agarose gel electrophoresis and structural analysis were also published into a protocol book entitled "Bacterial Chromatin" (Dame 2024), which shows protocols including AGE and EMSA in current studies, focused on bacterial DNA. The application of AGE has become increasingly prominent in structure–activity relationship (SAR) studies of ligand molecules, while EMSA assays remain highly adaptable for evaluating new classes of compounds targeting diverse types of DNA. EMSA combined with AGE has also been employed for the in vitro assessment of temperature-dependent DNA binding (Hutin et al. 2024). Thus, the effect of temperature on DNA binding was revealed, and the binding kinetics were quantified using AGE. Both complexed and free DNA were visualized on the same gel under varying temperature conditions. These data provide insights not only into binding kinetics but also into the physicochemical properties of the DNA fragments, yielding results that are comparable to those obtained using other DNA-related techniques, such as PCR.

DNA binding dyes are used in multiple purposes in pharmaceutical research. In order to show the DNA fragments under the UV light, dyes are playing crucial roles. For different purposes, various DNA dyes were commercially available. To indicate the mechanism of binding of these dyes, a comprehensive EMSA assay was applied to DNA (Bawane et al. 2024). According to this assay, various dyes have been identified their identical retention profiles according to the DNA-binding capacity. The relative binding levels of dyes are differed from each other, and this will result a signature pattern in the AGE-EMSA.

2.3. Formulation properties

Drug formulation systems are used for better drug activity and lower cytotoxicity. DNA-based AGE strategies were used to test the plasmid DNA interactions of new liposomal formulations (Manturthi et al. 2022). The application involved both the free and complexed forms of the therapeutic carrier interacting with DNA, resulting in a characteristic shift on AGE that reflected complex formation. Not only drug formulation, but also vaccine

formulations have been standardized with AGE interaction studies. For deciding the best composition of viral vaccine formulation, viral capsid and nucleic acid EMSA assay were done simultaneously (Sacherl et al. 2023).

New formulation strategies are used for different purposes that targeting different biological mechanisms. Photosensitization is one of them, and this strategy promotes to reduce the cellular integrity by the light exposure. AGE study was applied in a study that developing a new formulation strategy for vitamin E photooxidation and potential DNA damage (Teychené et al. 2020). The DNA damage was profiled with AGE-EMSA in this study; hence the radical oxygen species were identified. T4 Endonuclease enzyme was treated before and after irradiation step, and the fragment response was distinct. After quantifying the fragments, the capacity of photosensitization of vitamin E was determined.

Recombinant plasmid DNAs are novel strategies for vaccines and gene therapy. Their formulations are usually in colder temperatures to keep their stability maximize. Defining the stability characteristics and formulation integrity of components, AGE used to show the potential plasmid damage (Kieu Doan et al. 2023). Consequently, optimized conditions were established to enhance the stability of these plasmid DNAs. EMSA was successfully applied, with results depending on both incubation time and the concentration of the protective polymer.

2.4. DNA Genomics and transcriptomics

Gel electrophoresis continues to contribute to genomics and metabolomics studies, such as DNA footprinting assays in diverse contexts. Although PCR has largely supplanted this method, several confirmatory gel-based assays have still been reported in recent studies. The Electrophoretic Mobility Shift Assay (EMSA) on agarose gel provides the data about the modifications on promoter regions and gene expression profiling. Anti-sense oligonucleotides were used to inhibit these transcriptional regulator and resulted the promoter region inactivity of gene expression (Numata et al. 2024). Additionally, gene mutations can be monitored using EMSA with various DNA fragments together. The mutant genes were found identical shift distance according to their genomic variations, especially on promoter regions which responsible with the transcriptomic functionality (Ausubel 1988, Gurevich et al. 2010).

Overall, these findings demonstrate that EMSA and gel electrophoresis remain versatile and reliable tools for investigating DNA-ligand interactions, assessing enzymatic activity, and monitoring genetic variations, providing valuable mechanistic and functional insights that complement modern molecular techniques.

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