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The Latest Development of Capillary Electrophoresis Technology in the Separation and Detection of Biological Macromolecules

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Abstract: The purpose of this paper is to discuss the application, advantages and disadvantages of Capillary Electrophoresis (CE) in the separation and detection of biological macromolecules, and to discuss the latest progress of this technology in the separation and detection of biological macromolecules. As a separation technology based on the migration velocity difference of ions or charged particles under the action of electric field, CE has been widely used in biochemistry, molecular biology, biomedicine and other fields. Firstly, this paper introduces the basic principle and development of CE, and emphasizes the importance of biomacromolecules research. Then, the application of CE in the separation and detection of biological macromolecules is described in detail, including the advantages of separation efficiency, analysis speed and sample consumption, as well as the limitations. Finally, the advantages and disadvantages of CE are summarized, and the future development direction and application prospect are put forward. Although CE has the advantages of high separation efficiency, rapid analysis and low sample consumption, it also has some limitations such as high requirements on sample properties. In the future, with the continuous improvement and development of technology, the application prospect of CE in the separation and detection of biological macromolecules will be broader.

1. Introduction

In the research of biochemistry, molecular biology, biomedicine and other fields, the separation and detection of biological macromolecules has always been a very key part[1]. With the rapid development of science and technology, the research on biological macromolecules is getting deeper and deeper, which requires us to have more accurate, rapid and efficient separation and detection technology[2]. In this context, CE came into being and gradually developed into an important tool in the field of biomacromolecule analysis [3]. CE is a separation technology based on the difference of migration velocity of ions or charged particles in liquid in capillary tube under the action of electric field[4]. It uses a capillary with a small diameter, thus increasing the electric field intensity and significantly improving the separation efficiency. In addition, due to the use of high-voltage electric field and small-diameter capillary, this technology realizes rapid analysis and

is very suitable for high-throughput research[5]. CE is also multifunctional, and can adapt to different types of separation requirements by adjusting electrolyte solution and operating conditions.

Biological macromolecules, such as protein, nucleic acid and polysaccharide, are the basis of life activities. They are involved in cell structure, information transmission, metabolic regulation and other links[6]. Therefore, the in-depth study of biological macromolecules is helpful to reveal the mystery of life, understand the occurrence and development mechanism of diseases, and provide ideas for the development of new drugs[7]. In this context, CE, as a powerful tool for separation and detection of biological macromolecules, is particularly important. In this paper, the principle, application, value and significance of CE in the study of biomacromolecules will be introduced in detail, and the latest progress of CE in the separation and detection of biomacromolecules will be further discussed.

2. CE overview

2.1. The basic principle of CE

CE is a separation technology based on the difference in the migration speed of ions or charged particles in liquid under the action of electric field in capillary[8]. Its basic principle is similar to the traditional electrophoresis technology, but a thinner capillary is used as the separation channel, thus improving the separation efficiency and analysis speed.

In capillary electrophoresis, a sample is injected into a capillary filled with electrolyte solution, and then a voltage is applied across the capillary to form an electric field. Charged particles begin to migrate under the action of electric field, and the migration speed depends on the charge, size and shape of particles and factors related to the properties of electrolyte solution[9]. By monitoring the migration time of particles in capillary and detecting methods such as fluorescence and ultraviolet, the separation and quantitative information of samples can be obtained.

2.2. Equipment and workflow of CE

The main equipment of CE includes: capillary, high-voltage power supply, injection system, detection system and data processing system. Its workflow is usually as follows:

Capillary preparation: select a suitable capillary, and carry out cleaning and pretreatment.

Sample preparation: the sample to be separated is properly treated for injection into the capillary.

Injection: the sample is injected into the capillary through the injection system.

Electrophoretic separation: applying a high-voltage power supply to make the charged particles in the sample undergo electrophoretic separation in the capillary.

Detection: Use appropriate detection methods (such as fluorescence and ultraviolet) to detect the separated components.

Data processing: the detection signal is analyzed and processed by the data processing system to obtain the separation and quantitative results of the sample.

2.3. Advantages and limitations of CE

As shown in Table 1, the main advantages and limitations of CE are listed simply and clearly, which is helpful for a more comprehensive and in-depth understanding of CE.

Table 1: Advantages and limitations of CE

Advantage	Describe	Limitations	Describe
High separation efficiency	CE adopts fine-diameter capillary, which increases the electric field intensity and makes the separation efficiency higher.	The requirements for samples are higher	Samples need to have certain charge or derivable properties in order to be effectively separated and detected. This limits its application to some specific types of samples.
Rapid analysis	Due to the adoption of high-voltage electric field and small-diameter capillary, the analysis speed is faster, which is suitable for high-throughput analysis and improves the analysis efficiency.	The equipment cost is high	The equipment required for CE is relatively sophisticated and expensive. This increases the cost of technology application and may also limit its popularization in some environments with limited resources.
Less sample consumption	CE only needs a small amount of samples for analysis, which reduces the sample consumption cost and is more suitable for precious or limited samples.	High technical requirements for operation	Operators need to have certain experience and skills. This may bring some challenges to the popularization and application of technology.
Versatility	By adjusting the electrolyte solution and operating conditions, various separation modes can be realized, such as ion exchange, size exclusion and so on, which increases the flexibility and application scope of the technology.	-	-

3. Application of CE in separation of biological macromolecules

3.1. Separation and purification of biological macromolecules

Biological macromolecules, such as protein, nucleic acids (DNA, RNA), polysaccharides, etc., are vital components in the life system. The separation and purification of these biomacromolecules are very important for the research in biochemistry, molecular biology, biomedicine and other fields. In order to study the structure, function and interaction of these biomacromolecules, it is first necessary to separate them from complex biological samples and purify them.

Traditional separation methods of biological macromolecules include chromatography and gel electrophoresis. As an efficient separation technology, CE is especially suitable for the separation and purification of biological macromolecules. CE can achieve high resolution separation on the micro scale, and has the advantages of rapidity, automation and less sample consumption.

3.2. Application of CE in protein separation

Protein is one of the most important macromolecules in organisms, and participates in various biological processes. CE played an important role in the separation of protein[10]. By choosing appropriate buffer and separation conditions, capillary electrophoresis can realize the separation of protein based on the charge, size and hydrophobicity of protein. This is of great significance for

protein omics research, protein interaction analysis, protein drug development and other fields.

In protein separation, common capillary electrophoresis modes include capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE) and affinity capillary electrophoresis (ACE). Among them, CZE is mainly separated according to the charge and size of protein, CGE is separated according to the size of protein by filling gel in capillary, and ACE is used to capture and separate protein by specific interaction (such as antigen-antibody interaction).

3.3. Application of CE in nucleic acid separation

Nucleic acid is the main carrier of biological genetic information, which is very important for genomics, transcriptomics and other fields. CE also plays an important role in nucleic acid separation. Similar to protein separation, nucleic acid separation can also be based on charge and size.

In nucleic acid separation, common capillary electrophoresis modes include capillary array electrophoresis (CAE) and single molecule capillary electrophoresis (SMCE). CAE can separate and analyze multiple nucleic acid samples in parallel in the same capillary, which improves the separation flux. However, SMCE can directly detect and analyze a single nucleic acid molecule, which has the advantages of high sensitivity and high resolution.

4. Application of CE in the detection of biological macromolecules

4.1. Overview of detection methods of biological macromolecules

The detection of biomacromolecules is an important analytical task in the fields of biomedicine, biochemistry and molecular biology. Common detection methods of biological macromolecules include spectroscopy, mass spectrometry, chromatography, electrophoresis and so on. These methods have their own characteristics, so we can choose the appropriate method according to the actual needs.

CE is an important electrophoresis method, which has unique advantages in the detection of biological macromolecules. It combines the high separation efficiency of electrophoresis with the high sensitivity of capillary, and realizes the detection of biological macromolecules with high resolution and sensitivity.

4.2. Application of CE in protein detection

CE can quantitatively analyze protein by separating and detecting it. In protein detection, CE commonly used includes fluorescence capillary electrophoresis (FCE) and mass spectrometry combined electrophoresis (CE-MS). See Table 2 for details.

These methods improve the sensitivity and accuracy of protein detection, and provide a powerful tool for studying the function and disease mechanism of protein.

Table 2: Common methods of CE in protein detection

Technology	Describe	Advantage
FCE	The fluorescent labeled antibody or fluorescent dye is combined with protein, and the quantitative analysis of protein is realized by detecting the fluorescent signal.	High sensitivity: A small amount of protein can be detected by the amplification effect of fluorescence signal.
		High selectivity: The target protein can be selectively detected by specific fluorescent labeled antibody or dye.
		Suitable for complex samples: In complex biological samples, the detection signal-to-noise ratio of protein can be improved by fluorescent labeling.
CE-MS	Combining capillary electrophoresis with mass spectrometry, protein was separated by electrophoresis and identified and quantified by mass spectrometry.	High resolution: Capillary electrophoresis provides efficient protein separation, and mass spectrometry provides high resolution and high quality protein identification.
		High sensitivity: Protein with low abundance can be detected by mass spectrometry.
		Accuracy: Mass spectrometry can accurately determine the molecular weight and sequence information of protein.
		Providing structural information: Mass spectrometry can also provide structural information of protein, such as post-translation modification.

4.3. Application of CE in nucleic acid detection

Table 3: Common methods of CE in nucleic acid detection

Technology	Describe	Applied range
Sanger sequencing	The DNA fragments labeled with fluorescent dyes were separated by CE, and the fluorescent signals were detected to determine the nucleic acid sequence.	Genome sequencing
		Catastrophe analysis
		Microbiological assay
DNA fragment analysis	DNA fragments of different sizes were separated by CE and quantitatively analyzed according to migration speed.	DNA fingerprinting analysis
		Genome hybridization analysis
		Diagnosis of monogenic genetic diseases
RT-PCR	Combined with CE, the change of fluorescence signal is monitored in real time during PCR reaction, and the quantitative detection of nucleic acid is realized.	Virus nucleic acid detection
		Gene expression analysis
		Mutation screening
MSP	Methylated DNA and unmethylated DNA were separated by CE, and the specificity was detected by PCR.	Detection of tumor markers
		Epigenetic research
		Abnormal DNA methylation in diagnosis of diseases

In nucleic acid detection, CE commonly used includes Sanger sequencing, DNA fragment analysis, real-time fluorescence quantitative PCR (RT-PCR), DNA methylation specific PCR (MSP) and so on. See Table 3 for details.

5. The latest research progress of CE

5.1. New technologies and methods of CE

In recent years, CE has been bringing forth the old and bringing forth the new, and many new technologies and methods have emerged. The most representative ones include: microfluidic capillary electrophoresis: this technology combines microfluidic technology with capillary electrophoresis, and improves the separation efficiency and repeatability by accurately controlling the flow of microfluidic in capillary. Multidimensional Capillary Electrophoresis: This method integrates many different separation modes into one capillary tube, realizes efficient separation of complex samples, and improves resolution and separation effect. The appearance of these new technologies and methods has greatly enriched the application scope of CE and improved its separation and analysis ability.

5.2. Combination of CE and other technologies

The combination of CE and other technologies is also a current research hotspot. Among them, the combination with mass spectrometry (MS) is particularly eye-catching. Capillary electrophoresis-mass spectrometry (CE-MS) combines the high separation ability of capillary electrophoresis with the high sensitivity and selectivity of mass spectrometry, and can be used for the identification, quantification and structural analysis of biological macromolecules. In addition, capillary electrophoresis can also be combined with optical detection, fluorescence resonance energy transfer (FRET) and other technologies to improve the sensitivity and diversity of detection.

5.3. Challenges and future development direction of CE in the separation and detection of biological macromolecules

Although CE has made remarkable progress in the separation and detection of biological macromolecules, it still faces some challenges. For example, improving the separation efficiency and resolution: for more complex and larger-scale biomacromolecule samples, it is still an urgent need to further improve the separation efficiency and resolution. Enhance detection sensitivity: especially for the detection of low-abundance biological macromolecules, improving detection sensitivity is the key. Automation and high-throughput quantification: In order to meet the requirements of high-throughput analysis in biomedicine, clinical medicine and other fields, automation and high-throughput quantification of CE are inevitable trends.

The future development direction includes: developing new capillary and coating materials to improve the separation effect and prolong the service life of capillary. Researchers should further optimize the combination of capillary electrophoresis and other technologies to achieve more efficient and sensitive biomacromolecule analysis. They can use artificial intelligence, machine learning, and other technologies to improve the speed and accuracy of capillary electrophoresis data analysis and promote the in-depth development of related research.

6. Conclusions

CE, as a separation technology based on the difference of migration speed of ions or charged

particles in liquid under the action of electric field, has made remarkable progress in the past decades. In this paper, the application of CE in the separation and detection of biological macromolecules is studied in detail, and the advantages of this technology such as high separation efficiency, rapid analysis and low sample consumption are analyzed, and its latest research progress is discussed. The results of this study can provide valuable reference and enlightenment for researchers in related fields.

CE provides strong support for the research in the fields of biochemistry, molecular biology and biomedicine. This technology has the advantages of high resolution, high speed and low sample consumption in the separation of biological macromolecules, and plays an important role in promoting the research in biochemistry, molecular biology, biomedicine and other fields. However, CE also has some limitations, such as high requirements for samples, high equipment cost and high operating technology. However, with the continuous progress and innovation of science and technology, it is believed that CE will play an increasingly important role in the separation and detection of biological macromolecules, and provide strong support for the research and application in life sciences, medicine and other fields.

References

[1] Liang Yu, Zhang Lihua, Zhang Yukui. Capillary electrophoresis mass spectrometry and its application in proteomics [J]. Chromatography, 2020, 38 (10): 8

[2] Peng Le, Cheng Jiawei, Li Shunan, et al. Progress in the Application of Capillary Electrophoresis in Biomacromolecular Analysis [J]. Analytical Testing Technology and Instruments, 2022, 28 (4): 384-391

[3] Sun Miao, Li Linsen, Zhu Chao, et al. 2017 Annual Review of Capillary Electrophoresis Technology [J]. Chromatography, 2018 (4): 327-333

[4] Yu Fangzhi, Zhang Dapeng, Yuan Zheng. Application of affinity capillary electrophoresis technology in the study of protein-DNA interactions [J]. Chromatography, 2020, 38 (10): 1133-1142

[5] Wang Binbin, Pan Lingling, Wu Xiaoliang, et al. Analysis of the Development Trend of Capillary Electrophoresis and New Research Progress in the Biological Field [J]. Chinese Journal of Science and Technology Database (Citation Edition) Medical and Health, 2022 (4): 6

[6] Yu Fangzhi, Zhang Dapeng, Yuan Zheng, Zhao Qiang, Wang Hailin. Application of affinity capillary electrophoresis technology in the study of protein-DNA interactions [J]. Chromatography, 2020, 38 (10): 10

[7] Li Yujie, Li Shengfang. Application of capillary electrophoresis technology [J]. Chemical Management, 2019 (13): 2

[8] Di Shengmiao, He Ziwei. Application of capillary electrophoresis technology in the analysis of monoclonal antibody drugs [J]. Chinese Journal of Science and Technology Database (Abstract Edition) Medical and Health, 2022 (2): 161-163

[9] Zheng Xiyan, Liu Yu, Jia Xirong, et al. Application of capillary electrophoresis technology in drug analysis [J]. Pharmaceutical Information, 2020, 9 (4): 5

[10] Guo Dongliang, Wang Yanfen, Li Yu, et al. Overview of Biomacromolecule Visualization Technology [J]. Journal of Computer Aided Design and Graphics, 2021, 33 (12): 9