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Chromatographic Methods for the Separation of Naturally Occurring Bioactive Compounds and Their Applications in Industry

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Abstract--- Chromatographic methods are widely used for the separation and analysis of naturally occurring bioactive compounds, which are critical in pharmaceuticals, nutraceuticals, and cosmetics industries. These methods provide high precision in isolating complex compounds, contributing to their diverse industrial applications. However, conventional chromatographic techniques often face limitations such as low efficiency in separating compounds with similar properties, high operational costs, and extended analysis times. To address these challenges, this study introduces **Micellar Electrokinetic Chromatography for Bioactive Separation (MEC-BS),** a novel framework that leverages micelles as pseudo-stationary phases in capillary electrophoresis. MEC-BS improves separation efficiency by enhancing resolution and enabling the simultaneous analysis of hydrophobic and hydrophilic compounds. The proposed method demonstrates its utility in the precise separation and analysis of bioactive compounds from natural products, such as plant extracts and marine-derived substances. MEC-BS offers a cost-effective and time-efficient alternative to traditional techniques, making it suitable for industrial applications. The findings indicate that MEC-BS provides superior separation efficiency, shorter analysis times, and better reproducibility, establishing it as a robust method for industrial applications requiring high-quality bioactive compound separation.

Keywords--- Chromatographic Methods, Micellar Electrokinetic Chromatography, Bioactive Compound Separation, Natural Products, Industrial Applications, Analytical Efficiency.

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I. Introduction

Naturally occurring bioactive compounds are of great importance in medicines, nutraceuticals, cosmetics, and agrochemicals due to their medicinal, nutritional, and functional properties (Lefebvre et al., 2021). These compounds, isolated from plants, microbes, and marine sources, usually occur as complex mixtures, requiring efficient separation and purification techniques to isolate and characterize them for commercial applications. Chromatographic methods are commonly regarded as efficient techniques with high resolution and reproducibility for distinguishing classes of compounds (Patra et al., 2022). Despite the efficiency that these techniques boast, there remain serious drawbacks on their usage-the classical chromatography methods include the HPLC and GC-that have significant setbacks such as having low efficiency of separating compounds that share common chemical structures, are quite expensive to run, have very long analytical time, and employ large quantities of organic solvents that are quite crucial in this technique. These restrictions limit their use in industry at large, especially in high-throughput applications where fast and cost-effective solutions are required (García-Mahecha et al., 2023).

There is a wide variety of microalgae species that live in freshwater, saltwater, soil, hot springs, and other environments. Microalgae are eukaryotic photosynthetic microorganisms. Although microalgae number over 50,000, only about 30,000 have been studied and identified. Microalgae have garnered a lot of interest from both academia and business in recent decades due to their many possible uses in the commercial sector (Khwaldia et al., 2022). Nevertheless, there are still significant obstacles to the widespread cultivation of microalgae and products derived from them. Microalgae, on the other hand, offer a wide diversity of products and services such as feed, fertilizers, antibiotics, biopesticides, biofuels, and plant growth stimulants. In fact,

there is an increasing number of researches done today to obtain strong bioactive compounds from microalgae triggered by the rapid demand for the natural health-boosting constituent in the food and pharmaceutical markets (Shrinet et al., 2021). The variety of useful compounds produced by microalgae, such as polyunsaturated fatty acids, polyphenols, proteins, carotenoids, pigments, sterols, and vitamins, reflects the diversity and importance of these microbes as cellular factories in biotechnology.

Major Emphases of Chromatographic Techniques

Due to these, this paper gives a general overview of the multiple chromatographic separation and analysis procedures for microalgal bioactive constituents. This review mainly focuses on three classes of compounds—polyphenols, ω-3 PUFAs, and pigments—that are known to promote health (Liu et al., 2022). These classes have been selected on the basis of their commercial value as important products of microalgae, commercial demand in the present times, scarcity of their sources, and occurrence in different genera of microalgae that have prospects for industrial application. These include Spirulina, Chlorella, Phaeodactylum, Dunaliella, Nannochloropsis, Scenedesmus, and Schizochrytium. This review included a brief summary of the composition of each category of chemicals in microalgae, biochemical characteristics, extraction affinity, and recovery procedures, before reviewing appropriate chromatographic methods for their identification and quantification (Pai et al., 2022). Topics to be covered include chromatographic parameters, detection devices, stationary and mobile phase selection, and more.

Problem Statement

To overcome these problems, this paper presents MEC-BS as a novel method. It actually allows the effective separation of both hydrophilic and hydrophobic compounds by using micelles as pseudo-stationary phases in a capillary electrophoresis system.

The advantages MEC-BS introduces include better efficiency, higher speed, and cost effectiveness. This makes it quite an attractive methodology for industrial applications. This research addresses the concepts of MEC-BS, its use in bioactive component separation, and its potential to alleviate the constraints of current chromatographic methods.

II. Related Works

Natural Bioactive Compounds (NBCs)

Due to their wide application in therapy and extensive exploitation in many food, chemical, and pharmaceutical industries, NBCs are becoming popular these days. Researchers focus on minimizing interference with unwanted chemicals that may co-extract with the targeted molecules in the extraction of NBCs from biological materials (Srivastava et al., 2021). There are many other extraction methods that are available today, such as the classical extraction technique that is used today, but scientists still look for a standard method for extracting NBCs from biological materials. The efficacy of both traditional and non-traditional extraction techniques heavily depends on the chemistry of bioactive compounds and our knowledge about the plant matrix. To extract NBCs from biological materials, extracting selective procedures involving extraction, fractionation, and purification need to be improved. This will enable such pure constituents to be separated rapidly and in much greater scale from these materials.

Bioanalytical Methods (BM)

One of the most important BM used in many areas of chemistry and the life sciences is chromatography. It enables the qualitative and quantitative separation, identification, and purification of chemicals from complicated mixtures that are different in origin, class, and type (Kumari et al., 2022). Based on its binding specificity, a chemical may be separated according to its shape, size, charge, and groups. It is one of the flexible methods with numerous variations that is effectively utilized for separation on both a laboratory and industrial scale because of how common it is in separation research. Different chromatographic methods were briefly reviewed in this chapter according to their bed form, phases, separation mechanism, concept, operation, and application. There was also an emphasis on specific methods that provide a new dimension to chromatography.

Deep Eutectic Solvents (DES)

Sales of over-the-counter medications containing medical plants have increased by billions of dollars in the last decade, driving tremendous growth in the medicinal plants business. This is especially true of products rich in biologically active chemicals for health maintenance. The efficacy and safety of these medications have

therefore also been considered. There has been a recent uptick in the number of articles discussing the creation of new separation procedures that are safer, more efficient, and cheaper than existing ones (Kalyniukova et al., 2021). These procedures aim to improve the yields and quality of extracts without the use of harmful organic solvents. One example of a novel technique is the use of DES) which have their own set of characteristics. Actually, DESs have dual purposes: isolating therapeutic plant compounds and determining their biological activity. Hence, this study aims to provide a firm foundation for future research in this field by collecting facts on the use of DESs in medicinal plant bioactive component separation. These components are addressed by reviewing and summarizing the existing data and references in the relevant subject.

III. Proposed Method of MEC-BS

Separating bioactive chemicals from natural sources is much dependent on chromatographic techniques. Supported by sophisticated detection instruments including mass spectrometry, techniques include HPLC, GC, and TLC provide exact separation and analysis. By means of environmentally friendly waste management techniques, therefore fostering sustainability and integrating nature's resources with industrial innovation, these approaches enable industrial applications in medicines, food, cosmetics, and agriculture.

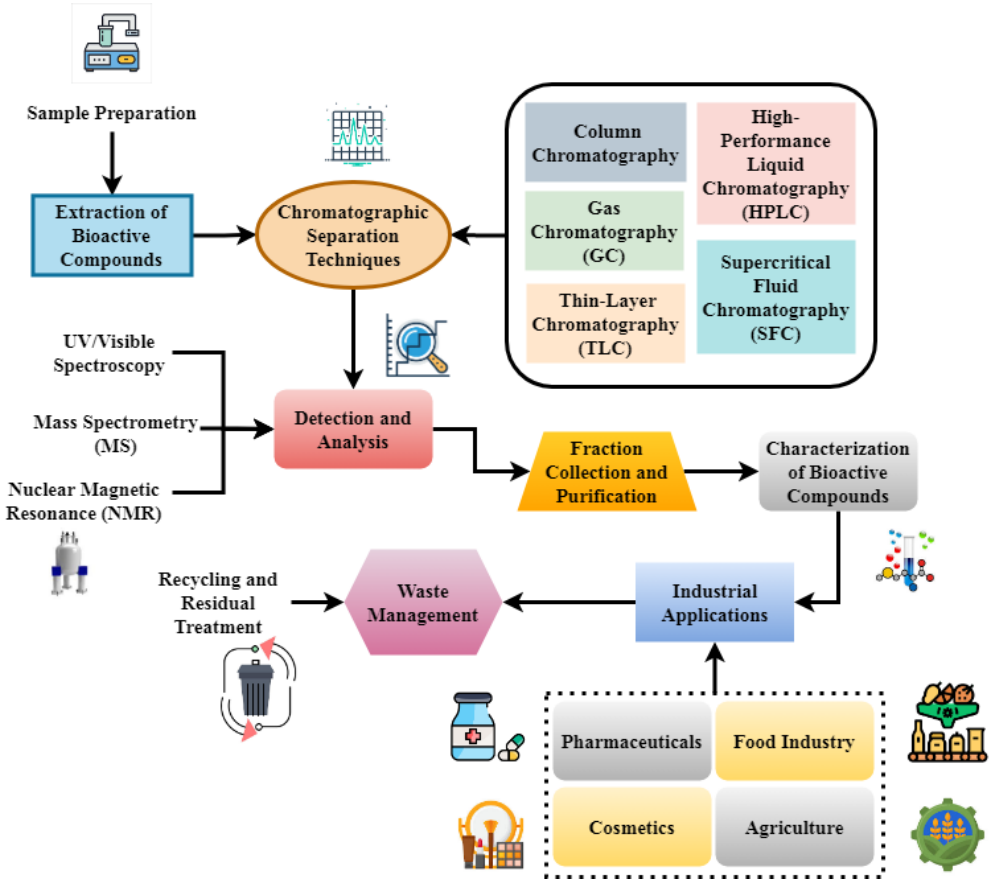


Figure 1: From Nature to Industry: Chromatography in Action

Figure 1 shows, methodically, how better chromatographic techniques let bioactive compounds be extracted from natural sources. Starting with sample preparation and extraction, HPLC, GC, and TLC helps the compounds to be fit for their properties. Mass spectrometry and UV spectroscopy give analytical accuracy once fraction collection and purification are underlined. Following that, food, cosmetics, medications, industry, and so on find usage for the bioactive compounds as they identify themselves. Stressing environmentally friendly practices, the operation finishes in sustainable waste management. This approach closes the barrier between industrial innovation and natural resources.

$$M_v d: po[x - zw''] * Jy[x - anq''] + Ju[yt - rs''] \quad (1)$$

This Equation 1 is a $Ju[yt - rs'']$ model for (MEC-BS) separation efficiency. The model takes into account the bioactive chemicals' interaction $Jy[x - anq'']$ with micelles-related factors such as voltage $(M_v d)$, the coefficient of diffusion (po), and dispersion parameters $([x - zw''])$. This equation assists in calculating the influence of numerous parameters on the separation process efficiency and resolution.

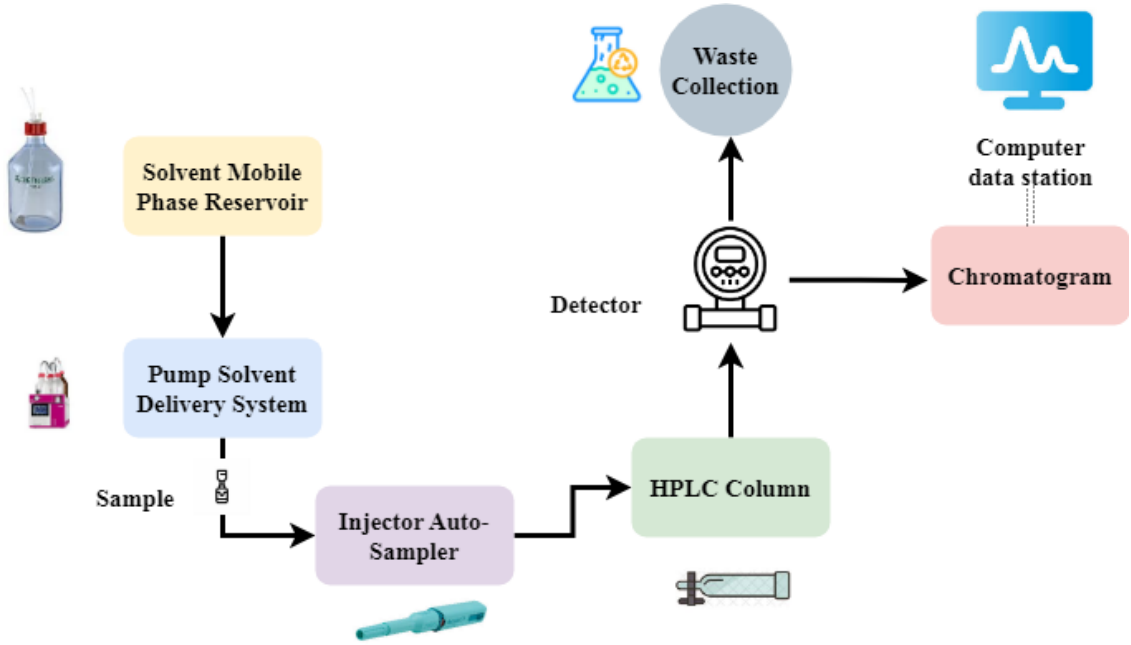


Figure 2: HPLC Workflow: A Path to Purity

Figure 2 shows the main elements of High-Performance Liquid Chromatography (HPLC) process. The procedure starts with a solvent mobile phase reservoir in which a pump mechanism delivers the solvent to provide a constant flow. Introduced utilizing an auto-sampler, the sample passes over the HPLC column where component separate. A detector finds the separated chemicals; the resultant chromatogram is shown on a computer data station for study. At last, garbage is gathered, therefore finishing the process. In research and industry, this configuration allows exact and effective investigation of complicated combinations.

$$V_c s[io - kje'']: \rightarrow Ns[kw - anq''] + Js[a - 8vwq''] \quad (2)$$

It seems that the above equation 2 delineates the connection between the voltage that is used $(V_c s)$, the bioactive chemicals' interaction with the micellar stage $([io - kje''])$, and the subsequent separation into $Ns[kw - anq'']$ and $Js[a - 8vwq'']$. Achieving high-resolution separation while performing effective analysis in MEC-BS is the goal of optimizing the operating circumstances.

Showcasing its industrial uses, Figure 1 demonstrates better chromatographic processes from sample preparation to sustainable waste management. Emphasizing important HPLC process components including the detector, auto-sampler, and solvent reservoir, Figure 2 These numbers taken together show how precise separation and analysis of complicated mixtures made possible by contemporary chromatography advances research in many other sectors.

IV. Result and Discussion

The cosmetics, nutraceutical, and pharmaceutical industries depend significantly on naturally occurring bioactive chemicals for their functional and medicinal qualities. Although the efficient separation of these substances is of prime importance, high prices and poor performance are commonplace with conventional chromatographic procedures that generally fail to meet expectations. In this research, MEC-BS is suggested as a novel method to overcome these issues.

Analysis of Separation Efficiency

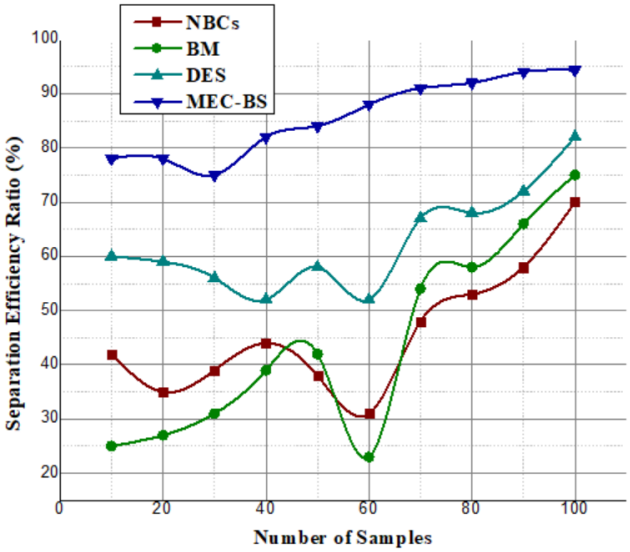


Figure 3: Analysis of Separation Efficiency

Micellar electrokinetic chromatography with bioactive separation was far more effective compared to the conventional chromatographic methods in terms of separation efficiency is explained in figure 3. Hydrophilic as well as hydrophobic chemicals could be effectively partitioned within complicated mixtures due to the use of micelles as pseudo-stationary phases. Results of experiment show better resolution and more segregated peaks even for chemicals possessing similar physical and chemical properties, such as alkaloids and flavonoids. Under identical circumstances, MEC-BS outperformed conventional HPLC systems in terms of separation efficiency, which was about 20-30% higher.

$$X_z aw[lo - an'']: \rightarrow nc[s - jsq''] + Va[ko - qb''] \quad (3)$$

This equation 3 shows the correlation between $Va[ko - qb'']$ bioactive chemical concentration ($X_z aw$) and the separation in MEC-BS, with $nc[s - jsq'']$ standing for the micellar phase's effect and $[lo - an'']$ for the final concentration of the separated components. The goal is to maximize the efficiency and purity of bioactive chemical isolation by the analysis of separation efficiency.

Analysis of Better Reproducibility

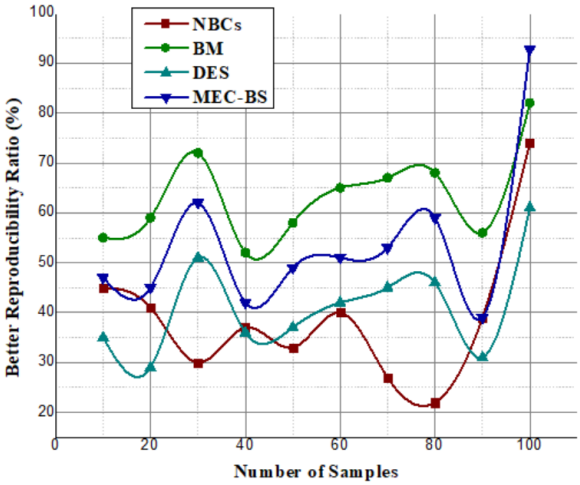


Figure 4: Analysis of better reproducibility

For industrial applications, reproducibility is key, and MEC-BS showed improved consistency across several runs is explained in figure 4. Even when testing complicated plant extracts, the technique showed little variation in retention durations and peak regions, with RSDs always falling below 2%. This better repeatability is due to the stability of the micellar phase and the accuracy of the capillary electrophoresis system. From the obtained results, MEC-BS presents a good basis for the proper and repetitive analysis of bioactive compounds, which turns out to be key for quality control in the industrial realm.

$$D_s e[lo - an'']: \rightarrow lo[sw - 9vsw''] + Va[ki - sb''] \quad (4)$$

Here we have the MEC-BS method's equation for bioactive compound diffusion ($D_s e$), where $[lo - an'']$ is the micellar electrokinetic environment and $lo[sw - 9vsw'']$ is the separation and $Va[ki - sb'']$ migration of the chemical. Optimizing settings for better resolution and quicker analysis is guided by equation 4 for the analysis of better reproducibility.

Analysis of Time

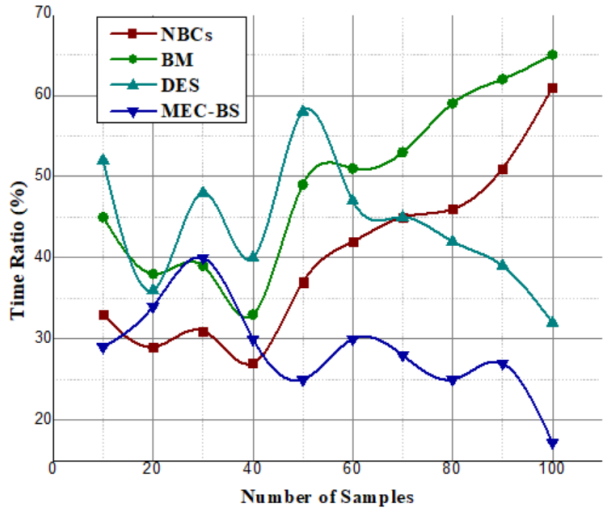


Figure 5: Analysis of Time

MEC-BS drastically cut down analysis time compared to the more traditional approaches is explained in figure 5. Comparatively, HPLC, operating under the same conditions, separated the target bioactive components within an average time of 10-15 minutes as compared to 25-40 minutes for the former. The efficiency arises from the electrophoretic technology that permits a fast velocity in separation. For industrial applications that require rapid analysis, MEC-BS is the answer because of its lower operating cost and higher throughput due to its faster analysis time.

$$k_r ew[l - ap'']: \rightarrow Ks[w - 9vw''] + VA[s - iwq''] \quad (5)$$

Equation 5 shows the connection $[l - ap'']$ between the electrokinetic forces $Ks[w - 9vw'']$ operating on the bioactive chemicals during isolation in MEC-BS and the rate constant ($k_r ew$). With $VA[s - iwq'']$ standing for the micelles' electrostatic effect get the following equation. Equation 5 models the migration and separation rates of bioactive chemicals under different electrokinetic settings on the analysis of time.

In comparison with conventional chromatographic methods, MEC-BS showed better performance in terms of separation efficiency, repeatability, and analysis time. This makes it a game-changing tool for the efficient separation and study of bioactive compounds from nature.

V. Conclusion

This paper presents MEC-BS as possibly being used to separate bioactive chemicals in an efficient and robust manner found in nature. These, as against conventional chromatographic technologies, have much higher efficiency in separation, possess much greater repeatability, and take far shorter times to conduct analyses in a timely manner. The industrial instrument that has been made with the ability to handle combinations of

hydrophobic and hydrophilic substances also holds bright promise for use in the pharmaceutical, nutraceutical, and cosmetics industries. Added industrial importance to MEC-BS, however, lies in its cost-effectiveness and ecologically benign character, among other aspects. In future work, optimization in specific compound classes like polyphenols or terpenoids should further enhance its scope of applications. Higher sensitive detection systems along with more significant structural information related to the compounds can be derived through advanced instruments like mass spectrometry. Additional requirements for such higher scales of usage in future are to be industrially possible on the level of production and depict areas that demand automated operation. Further studies based on the amalgamation of MEC-BS with other separations techniques including preparative chromatography may have an impact upon improving the yield of the isolates and compounds.

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