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Full Paper

Electromembrane Extraction of Nilotinib using Green Agarose-Based Membrane Modified with a Deep Eutectic Solvent of Choline Chloride and Methacrylic Acid

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Abstract- A novel and eco-friendly approach is introduced for the quantification of nilotinib in biological samples, employing gel-based electromembrane extraction (G-EME) coupled with fluorescence detection. In this method, a deep eutectic solvent (DES) composed of choline chloride and methacrylic acid (ChCl–MAA) was incorporated into agarose membranes (AG@DESChCl–MAA) to enhance extraction performance. The inclusion of DES significantly improved the conductivity, selectivity, and migration efficiency of nilotinib across the membrane. This system offers several advantages, including simplicity of operation, low cost, portability, and the elimination of toxic organic solvents. The developed method demonstrated good precision, with intra- and inter-day relative standard deviations (RSDs) of 6.5% and 8.6%, respectively. A linear calibration curve was obtained in the concentration range of 0.65 to 10.0 mg·L^{-1}, with a detection limit (LOD) of 0.20 mg·L^{-1}. These results confirm the potential of the AG@DESChCl–MAA-based G-EME system as a sensitive, green, and cost-effective alternative for nilotinib analysis in complex matrices.

Keywords- Agarose membrane; Deep eutectic solvent; Fluorescence detection; Gel electromembrane extraction; Nilotinib

1. INTRODUCTION

Accurate quantification of analytes in biological samples requires meticulous sample preparation to eliminate matrix interferences and concentrate the analyte into a suitable medium for instrumental analysis [1]. Traditional extraction techniques such as solid-phase

extraction (SPE) and liquid-liquid extraction (LLE) are commonly employed; however, they typically involve the use of large volumes of hazardous organic solvents and solvent evaporation steps, which may lead to analyte loss or degradation [2–4]. In contrast, membrane-based extraction techniques offer several advantages, including higher enrichment factors, improved clean-up efficiency, enhanced selectivity, and reduced solvent consumption [4].

Among emerging membrane-based techniques, membrane-protected micro-solid phase extraction (μ-SPE), hollow fiber liquid-phase microextraction (HF-LPME), and electro-membrane extraction (EME) have gained considerable attention. μ-SPE involves enclosing sorbents within a protective membrane barrier, thereby preventing direct contact with the sample and improving matrix clean-up. HF-LPME is effective for pre-concentration and clean-up but suffers from long extraction times (30–50 min). EME addresses this drawback by applying an electric potential across a supported liquid membrane (SLM), enabling rapid and selective transport of charged analytes from the donor phase (DP) to the acceptor phase (AP) [4–7].

The SLM is typically formed by trapping an immiscible organic solvent within the pores of a porous membrane [8]. The applied voltage drives the analyte migration through the membrane. Recently, gel-electromembrane extraction (G-EME) was developed as a greener modification of EME, in which the organic solvent in the SLM is replaced with a biodegradable gel, such as agarose [9–14]. G-EME is mostly solvent-free, eliminates the interface between aqueous and organic phases, and is particularly effective for polar compounds with low affinity for organic solvents. However, it suffers from lower selectivity and the adverse effects of electro-endosmotic flow (EEO), which causes liquid displacement across the gel and impacts extraction efficiency. In agarose-based G-EME systems, analyte flux is driven by electromigration and EEO. While electromigration enhances extraction, EEO can reduce efficiency by altering acceptor phase volumes [9–14]. Recent studies have highlighted the potential of agarose modifications, such as amino acid- or DES-doping, to overcome these challenges and improve extraction reproducibility [15].

To address these limitations, the incorporation of deep eutectic solvents (DESs) has been proposed. DESs are a class of green solvents, typically formed by hydrogen bond donors and acceptors, offering low toxicity, biodegradability, cost-effectiveness, and compliance with green chemistry principles [16–18]. Their unique physicochemical properties make them ideal for use as functional additives in membrane preparation, improving selectivity, conductivity, and structural homogeneity [19]. However, achieving compatibility between DESs and polymer matrices remains a key challenge. In line with recent reviews on green sample preparation methods and advances in DES-based functional polymers for pharmaceutical analysis [20, 21], the use of DESs provides an effective strategy to enhance both environmental friendliness and analytical performance.

In this study, we report for the first time the synthesis and application of agarose-based G-EME membranes modified with a novel DES composed of choline chloride and methacrylic acid.

The selection of methacrylic acid (MAA) as a DES component is further justified by recent evidence demonstrating its effectiveness in selective recognition and strong interactions with pharmaceutical analytes [22]. Owing to its carboxylic and vinyl functional groups, MAA plays a pivotal role in membrane performance. These groups facilitate extensive hydrogen-bonding with the agarose network and strengthen electrostatic as well as π–π interactions with drug molecules such as nilotinib. Consequently, the incorporation of MAA improves DES–polymer compatibility, enhances extraction capacity, increases selectivity, and contributes to greater membrane stability.

The proposed membrane design aims to enhance extraction performance while mitigating EEO. Nilotinib, a tyrosine kinase inhibitor used in the treatment of chronic myeloid leukemia, was selected as the target analyte due to its clinical significance and the need for trace-level determination in biological matrices. The developed DES-based agarose membranes exhibited high extraction efficiency, ease of fabrication, reusability (up to three cycles), and long-term stability (up to nine months). Notably, the presence of methacrylic acid was found to facilitate specific interactions through its carboxyl groups with nilotinib, thereby significantly improving sensitivity and selectivity. Key parameters affecting extraction efficiency were optimized using the one-variable-at-a-time (OVAT) approach.

2. EXPERIMENTAL SECTION

2.1. Chemicals and reagents

All chemicals were of analytical grade. Nilotinib (≥99% purity) was obtained from Tawfigh Daro Pharmaceutical Company (Tehran, Iran) and used without further purification. A 1000 $mg.L^{-1}$ stock solution of nilotinib was prepared in methanol, covered with aluminum foil to prevent light-induced degradation, and stored at 4 °C. Working solutions were freshly prepared by appropriate dilution with ultrapure water.

Choline chloride (ChCl) and methacrylic acid (MAA) were purchased from Merck (Darmstadt, Germany) and used to prepare the deep eutectic solvent (DES). Other reagents, including agarose and solvents, were also obtained from Merck. Ultrapure water was produced using a Milli-Q® system (Millipore, MA, USA).

2.2. Instrumentation

Electromembrane extraction was performed using a PV-300 DC power supply (Paya Pajoohesh Pars, Tehran, Iran) with voltage and current ranges of 0–300 V and 0–1.0 mA, respectively. Two platinum wire electrodes (0.25 mm diameter; Pars Platin, Tehran, Iran) were

employed as anode and cathode. The acceptor phase was placed in a cylindrical glass cell (10 mm inner diameter, 8 cm height).

A magnetic stirrer (Heidolph Instruments, Schwabach, Germany) operated at 900 rpm was used during both membrane preparation and extraction. A pH meter (pH-L220, ISTE, Iran) was used to measure pH, and an analytical balance (A220XB, Precia, France; precision: 0.0001 g) was employed for weighing.

Fluorescence measurements were conducted using a spectrofluorophotometer (RF-5301PC, Shimadzu, Japan) at room temperature. Excitation and emission wavelengths were optimized for nilotinib and set at 260 nm and 367 nm, respectively, based on fluorescence intensity measurements.

2.3. Preparation of $DES_{ChCl\text{-}MAA}$

The DES was prepared by mixing ChCl and MAA in a 1:1 molar ratio. The mixture was heated at 100°C under continuous stirring until a clear and homogeneous liquid formed, indicating successful eutectic formation. The DES was cooled to room temperature and stored in a tightly sealed container until further use.

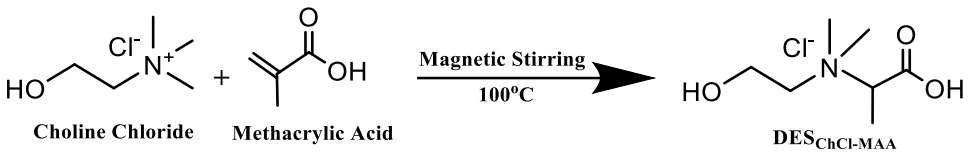

Figure 1. Schematic illustration of the $DES_{ChCl\text{-}MAA}$ synthesis based on 1:1 molar mixture of ChCl and MAA

2.4. Membrane Preparation

The agarose@$DES_{ChCl\text{-}MAA}$ membrane was prepared by uniformly dispersing a precise amount of agarose powder into a defined volume of a mixture containing water and the synthesized $DES_{ChCl\text{-}MAA}$ at 100°C, under continuous magnetic stirring. Once a clear and homogeneous solution was obtained, a specific amount of acetic acid was added to improve the mechanical durability of the membrane. The entire membrane preparation process was completed in approximately 2 minutes.

Approximately 350 µL of the resulting solution was pipetted into microtubes and kept at 4 °C for at least 3 h with open caps to facilitate gelation. After gelation, the tubes were sealed and stored at 4 °C. The resulting membranes remained stable and functional for several months. Prior to use, the tips of the microtubes were trimmed to expose the gel section, ensuring uniform membrane length in all experiments.

2.5. Agarose@$DES_{ChCl\text{-}MAA}$ based EME procedure

The prepared agarose@$DES_{ChCl–MAA}$ membrane was employed as the extraction medium in the EME process. Prior to each experiment, the tips of the microtubes containing the gel

membranes were precisely trimmed using a sharp blade to ensure consistent membrane length and optimal performance.

A 7.0 mL aqueous sample solution containing nilotinib (pH 7.9) was used as the donor phase (DP) and placed in a 10 mL macro-glass vial. The acceptor phase (AP), consisting of 150 µL of an acidic aqueous solution (pH 3.8), was gently introduced into a micro-vial containing the agarose@$DES_{ChCl–MAA}$ membrane positioned at its bottom. The membrane served as a physical barrier separating the DP and AP compartments.

Subsequently, the micro-vial holding the gel membrane and AP was immersed into the macro-vial containing the DP. Two platinum wire electrodes were used: the anode (positive) was inserted into the DP, and the cathode (negative) was placed in the AP. Both electrodes were connected to a DC power supply, and their tips were shaped into small loops to enhance the local electric field and maintain a stable inter-electrode distance during extraction.

The macro-vial was placed on a magnetic stirrer set to 900 rpm to ensure continuous agitation throughout the extraction period. A constant voltage of 50 V was applied for 20 minutes.

Upon completion of the extraction, the acceptor phase—now enriched with extracted nilotinib—was carefully collected and subjected to quantitative analysis using fluorescence spectrophotometry (RF-5301PC, Shimadzu, Japan).

3. RESULTS AND DISCUSSION

3.1. FTIR confirmation of $DES_{ChCl–MAA}$

The successful formation of the DES composed of ChCl and MAA was confirmed by Fourier-transform infrared (FTIR) spectroscopy (Fig. 2). The FTIR spectrum displayed a broad and intense absorption band in the range of 3200–3400 cm^{-1}, corresponding to the O–H stretching vibrations. This broadening is indicative of extensive hydrogen bonding between the hydroxyl group of ChCl and the carboxylic acid group of MAA—an essential feature of $DES_{ChCl–MAA}$ formation.

Additionally, a distinct band near 1710 cm^{-1} was attributed to the C=O stretching vibration of the carboxylic acid in MAA. The observed shift and reduction in intensity of this band, compared to pure MAA, further support the occurrence of strong hydrogen bonding interactions with ChCl.

Absorption bands observed at 2950–2850 cm^{-1} were assigned to C–H stretching vibrations of methyl groups. A band around 1630 cm^{-1} was related to C=C stretching vibrations in the vinyl group of MAA. Medium-intensity peaks between 1400–1500 cm^{-1} were attributed to $–CH_3$ bending modes and possibly to C–N stretching. Finally, characteristic peaks in the region of 1050–950 cm^{-1} were assigned to C–N stretching vibrations originating from the quaternary ammonium group in ChCl.

These spectral features collectively confirm the formation of a hydrogen-bonded eutectic mixture, validating the successful synthesis of $DES_{ChCl–MAA}$.

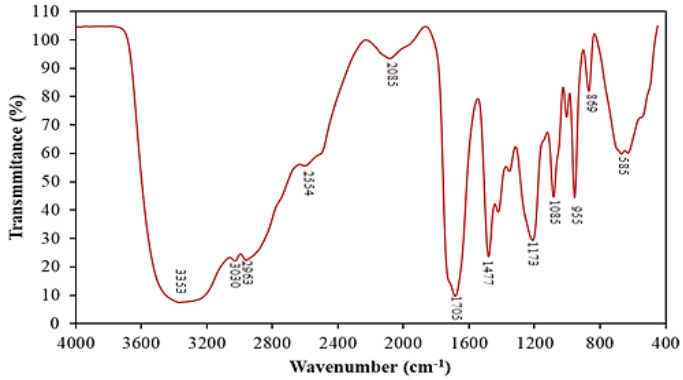

Figure 2. The FTIR spectrum of the prepared $DES_{ChCl-MAA}$

3.2. Effect of DES incorporation on membrane extraction performance

Unmodified agarose membranes were prepared by dissolving 3% (w/v) agarose powder in distilled water brought to boiling temperature. Upon reaching the first boil, the solution was removed from heat, and 0.075 µL of acetic acid was added under stirring to enhance membrane durability. Approximately 350–400 µL of the resulting hot solution was transferred into microtubes and stored at 4 °C for at least 2 hours to allow the formation of uniform, bubble-free gel membranes.

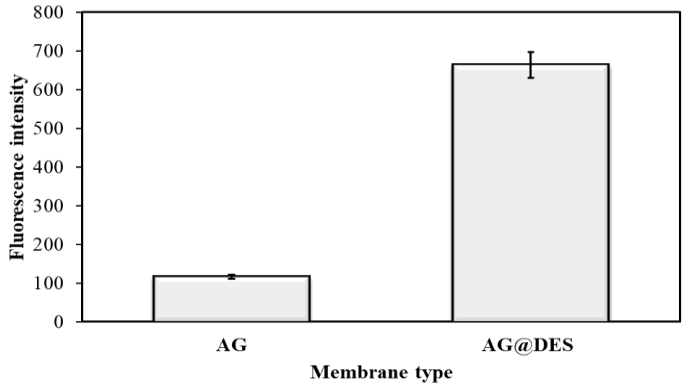

Figure 3. Comparison of fluorescence intensity of nilotinib extracted using unmodified agarose (AG) and DES-modified agarose (AG@DES) membranes. Experimental conditions: nilotinib concentration, 5.0 mg·L^{-1}; donor phase pH = 7.0; acceptor phase pH = 3.0; extraction voltage = 50 V; extraction time = 5 min; sample volume = 8 mL; acceptor phase volume = 150.0 µL; stirring rate = 900 rpm. Error bars represent standard deviations (n = 3)

In contrast, DES-modified agarose membranes (AG@DES) were synthesized by dispersing a precise amount of agarose powder into a preheated (100 °C) mixture of water and the

prepared ChCl–MAA deep eutectic solvent. Once a homogeneous and transparent solution was obtained, acetic acid was added to improve mechanical strength. The entire preparation process was completed within 2 minutes. About 350 µL of the hot mixture was transferred into microtubes and refrigerated at 4 °C for a minimum of 3 hours with the caps left open. After gelation, the microtubes were sealed and the membranes stored for long-term use. Prior to extraction, the microtube tips were uniformly trimmed with a blade to expose the gel and ensure consistent membrane length across all experiments.

The preconcentration of nilotinib was carried out using the G-EME method, and fluorescence measurements were subsequently performed (Figure 3).

3.3. Optimization of EME parameters

To improve the efficiency of detecting and quantifying trace amounts of nilotinib, several key parameters influencing the extraction process were systematically optimized. These parameters included agarose concentration, DES content, acetic acid concentration, applied voltage, extraction time, and the pH values of both the donor phase (pH_{DP}) and acceptor phase (pH_{AP}).

Initially, membrane-related factors—such as agarose gel concentration, DES content, and acetic acid amount—were optimized to achieve desirable membrane integrity and performance. Subsequently, the influence of operational parameters, including donor phase pH, acceptor phase pH, applied voltage, and extraction duration, was investigated. All optimizations were performed using the one-variable-at-a-time (OVAT) approach.

3.4. Effect of agarose concentration in membrane formulation

The influence of agarose concentration on the extraction efficiency was investigated in the range of 1.0–5.0% (w/v). As shown in Figure 4, the recovery of nilotinib increased with increasing agarose content, reaching a maximum at 4.0% (w/v), and then decreased slightly at 5.0%.

At lower agarose concentrations (e.g., 1.0%), the membrane structure lacked mechanical stability, leading to membrane disruption and partial dissolution into the donor phase during extraction. Additionally, at such low gel concentrations, electroosmotic flow (EOF) was more pronounced, resulting in water migration from the donor phase to the acceptor phase and causing an undesirable increase in AP volume. EOF refers to the movement of liquid under the influence of an applied electric field through a porous membrane or capillary, and it becomes more significant in small-diameter systems.

Conversely, increasing the agarose concentration beyond 4.0% led to a more rigid gel structure, which hindered the migration of charged analytes across the membrane, thereby reducing extraction efficiency.

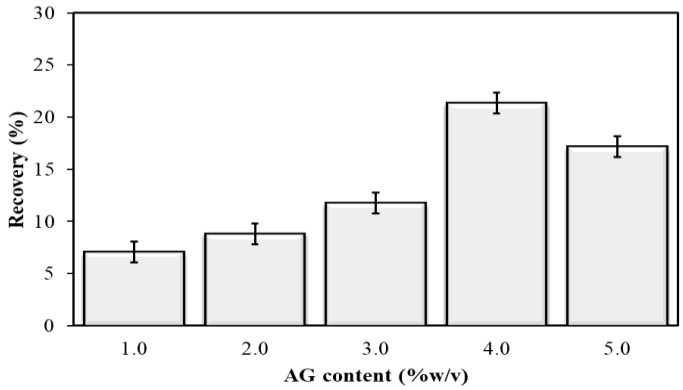

Figure 4. Influence of agarose concentration (% w/v) in the AG@DES membrane on the extraction recovery of nilotinib. Conditions: pH_{DP} = 7.9, pH_{AP} = 3.8, extraction voltage = 50 V, time = 5.0 min, sample volume = 8 mL; acceptor phase volume = 150.0 µL; stirring rate = 900 rpm, DES content = 25% (v/v). Error bars represent standard deviations (n = 3)

These observations suggest that 4.0% (w/v) agarose provides an optimal balance between membrane integrity and analyte transport rate for effective G-EME performance.

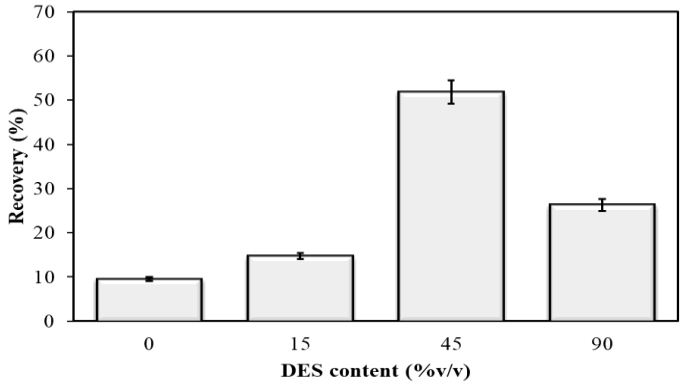

Figure 5. Effect of DES content (% v/v) in the AG@DES membrane on the extraction recovery of nilotinib. Experimental conditions were the same as in Figure 2, except that the DES content was varied while agarose concentration was fixed at 4% (w/v). Error bars represent standard deviations (n = 3)

3.5. Effect of DES content in membrane formulation

Following the optimization of agarose concentration (4% w/v), the influence of DES content in the membrane was examined by varying the volume percentage of DES from 0% to 90% (v/v) with deionized water. After preparing each mixture, the solution was heated to boiling, and 4% (w/v) agarose was added to form the gel. The extraction performance of each membrane formulation was then evaluated, and the results are presented in Figure 5.

As shown, the recovery of nilotinib increased with DES content, reaching a maximum at 45% (v/v). This enhancement can be attributed to the improved conductivity and increased polarity of the membrane, which facilitates analyte migration and interaction during the EME process. However, at 90% (v/v) DES, a decline in recovery was observed, possibly due to excessive viscosity or reduced gel stability, which may hinder analyte transport and compromise membrane homogeneity.

These results indicate that 45% (v/v) DES in the membrane composition offers the most favorable conditions for extraction performance.

3.6. Effect of acceptor phase pH

The pH of the acceptor phase plays a critical role in the efficiency of analyte extraction, particularly for ionizable compounds such as nilotinib. To evaluate this effect, the pH of the acceptor phase was varied from 3.0 to 7.0, while all other conditions remained constant. As illustrated in Figure 6, the highest recovery was achieved at pH 4.0, followed closely by pH 3.0. At acidic pH values, the analyte is more likely to be in its protonated, positively charged form, which favors its electrophoretic migration through the AG@DES membrane toward the cathodic acceptor phase. Additionally, low pH values suppress electroosmotic flow (EOF), thereby stabilizing the volume of the acceptor phase and improving analyte enrichment. However, as the pH increased beyond 4.0, a sharp decline in recovery was observed. This decrease can be attributed to reduced analyte charge, weakening of the electric field-driven migration, and possibly increased analyte solubility in the donor phase, resulting in decreased partitioning toward the acceptor compartment.

These findings highlight the importance of acidic conditions in maximizing the extraction efficiency of nilotinib in the G-EME system.

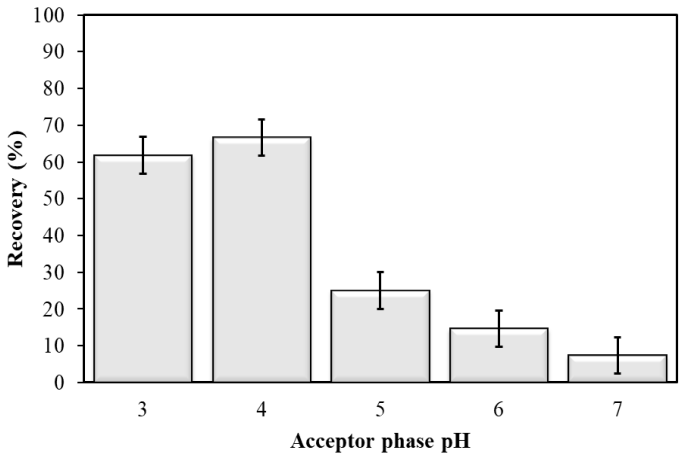

Figure 6. Effect of acceptor phase pH on the recovery of nilotinib. Experimental conditions were the same as in Figure 2, except that the pH of the acceptor solution was varied from 3.0 to 7.0. Error bars represent standard deviations (n = 3)

3.7. Effect of donor phase pH

The effect of donor phase pH on the extraction recovery of nilotinib was studied over the pH range of 5.0 to 9.0, while other experimental parameters were kept constant. As shown in Figure 7, the highest recovery was obtained at pH 7.0, with a significant decrease observed at both lower and higher pH values. At acidic pH levels (pH = 5–6), the analyte likely exists in a protonated form with limited migration under the applied electric field, and may also exhibit stronger interactions with the donor matrix, reducing its transfer across the membrane. In contrast, at alkaline conditions (pH 8–9), the reduced extraction efficiency can be attributed to deprotonation of the analyte, leading to weaker electrostatic interactions with the negatively charged AG@DES membrane and possible repulsion effects.

Optimal extraction at pH 7.0 suggests a balanced state for the ionization of nilotinib, where it exists in a sufficiently charged form and maintains favorable interactions with the membrane while preserving migration potential under the electric field. This pH was therefore selected as the optimal condition for further experiments.

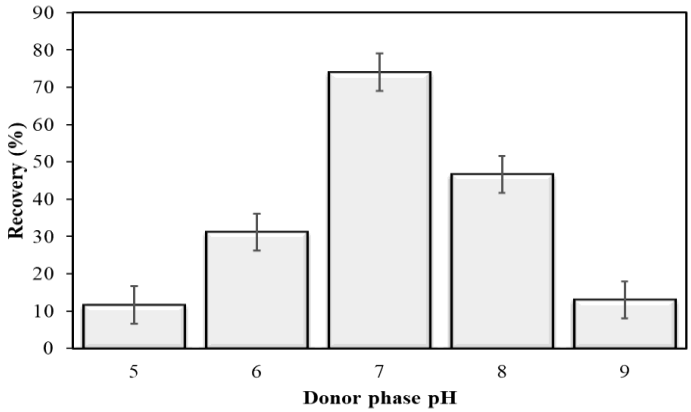

Figure 7. Effect of donor phase pH on the recovery of nilotinib. Experimental conditions were the same as in Figure 2, except that the pH of the donor solution was varied between 5.0 and 9.0. Error bars represent standard deviations (n = 3)

3.8. Effect of EME time

The influence of extraction time on the recovery of nilotinib was studied in the range of 0 to 30 minutes, under constant conditions for other parameters. As shown in Figure 8, the extraction efficiency increased steadily until 20 minutes, and then reached a plateau thereafter. At shorter durations (e.g., 5–15 minutes), the migration of the analyte across the AG@DES membrane was still in progress, resulting in moderate recovery. The sharp increase in recovery up to 20 minutes indicates efficient analyte transport driven by the applied electric field, facilitated by the optimized membrane properties.

Beyond 20 minutes, no significant improvement in recovery was observed, suggesting that the system had reached equilibrium and the extraction process had achieved its maximum

efficiency. Thus, 20 minutes was selected as the optimal extraction time for subsequent experiments, balancing both performance and practicality.

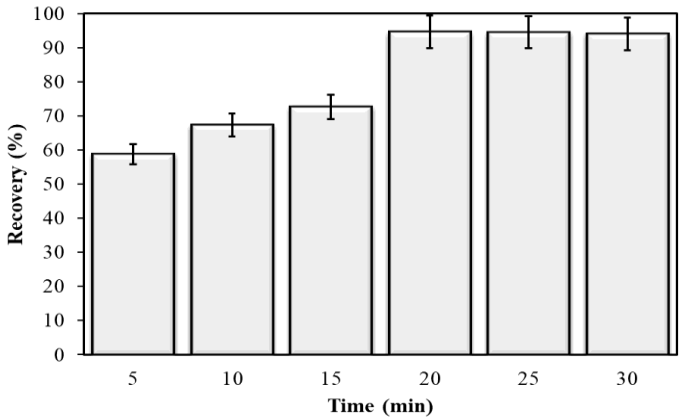

Figure 8. Effect of extraction time on the recovery of nilotinib. All experimental conditions were kept constant as optimized previously, except for the extraction time, which was varied between 5 and 30 minutes. Error bars represent standard deviations (n = 3)

3.9. Effect of applied voltage

The applied voltage plays a critical role in electro-membrane extraction (EME) as it directly influences the migration rate of charged analytes across the membrane. To evaluate this effect, the voltage was varied between 25 and 75 V while keeping all other parameters constant.

As shown in Figure 9, the recovery of nilotinib increased significantly from 62.3% at 25 V to a maximum of 99.6% at 50 V, indicating enhanced electrophoretic transport of the analyte under a moderate electric field. However, a further increase in voltage to 75 V led to a noticeable decline in recovery.

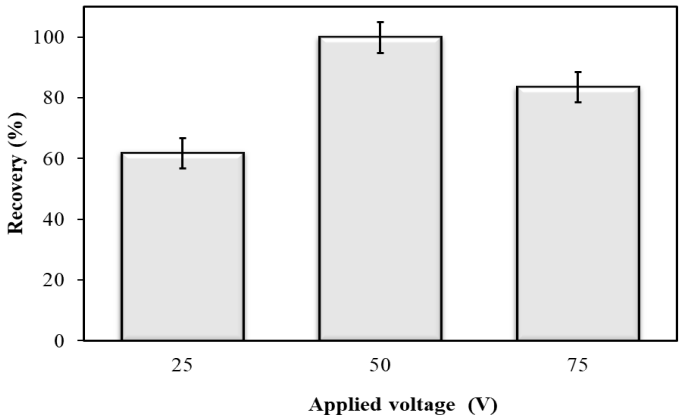

Figure 9. Effect of applied voltage on the extraction efficiency of nilotinib. Conditions: agarose 4% w/v, DES 45% v/v, donor phase pH 7.9, acceptor phase pH 3.8, extraction time 20 min, stirring rate 900 rpm. Error bars represent standard deviations (n = 3)

This decrease at higher voltage may be attributed to possible disturbances such as membrane instability, bubble formation due to electrolysis, or excessive Joule heating, which can negatively affect the analyte transport and the structural integrity of the membrane. Therefore, 50 V was selected as the optimal voltage for subsequent experiments.

3.10. The analytical figure of merit

It was essential to evaluate the analytical performance of the proposed method under optimized extraction conditions, as summarized in Table 1. The assessed parameters included the limit of detection (LOD), limit of quantification (LOQ), linearity, correlation coefficient (r), enrichment factor (EF), relative standard deviation (RSD%), and extraction recovery (ER%).

Based on the calculations, the LOD and LOQ, determined as 3σ/S and 10σ/S respectively (where σ is the standard deviation of the blank and S is the slope of the calibration curve), were found to be 0.20 and 0.66 mg.mL^{-1}. For nilotinib, the method exhibited excellent linearity with a correlation coefficient (r) of 0.9912. The enrichment factor was calculated to be 9.20 ± 0.45. The intra-day precision was assessed by performing five replicate measurements within a single day, while inter-day precision was evaluated by conducting three replicate measurements per day over five consecutive days. The RSD values for spiked samples were found to be below 8.8%, confirming the satisfactory repeatability and reproducibility of the method.

Table 1. Quantitative performance for the determination of nilotinib using the developed AG@DES based EME-fluorescence method under optimized conditions

LOD[a]	LOQ[a]	DLR[a]	r	%RSD[b]		EF[c]
				Intra-day	Inter-day	
0.20	0.66	0.65 – 10.0	0.9912	6.5	8.6	9.20 ± 0.45

[a] Concentration is based on mg.mL^{-1}
[b] %RSDs were obtained by five replicate measurements

3.11. Comparison with Previously Reported Methods

To further evaluate the efficiency and applicability of the proposed AG@DES-EME-Flu method, a comparison was made with other previously reported analytical techniques used for NIL determination, as summarized in Table 2. These methods include cyclic voltammetry (CV), HPLC-UV, fluorescence spectrometry, UPLC-MS/MS, resonance Rayleigh scattering (RRS), and SPE-LC-MS.

The proposed method exhibited a linear dynamic range (LDR) of 0.65–10.0 mg·L^{-1}, which is comparable to or broader than several conventional methods such as CV (0.01–1.06 mg·L^{-1}),

HPLC-UV (0.01–5.0 mg·L^{-1}), and RRS (0.1–1.0 mg·L^{-1}). While some techniques like SPE-LC-MS and UPLC-MS/MS achieved lower LODs (0.0015 mg·L^{-1} and 0.006 mg·L^{-1}, respectively), they typically require complex instrumentation and longer sample preparation steps.

In contrast, the current method offers a good balance between sensitivity and operational simplicity. Moreover, it benefits from a low-cost setup, eco-friendly membrane composition, and a fast extraction process under mild conditions. The combination of these advantages positions the AG@DES-EME-Flu system as a promising alternative for the trace-level analysis of nilotinib in aqueous samples.

Table 2. Comparison of the proposed method with the other reported methods for NIL drug

Method	LDR (mg.L^{-1})	LOD & LOQ (mg.L^{-1})	Ref.
CV[a]	0.01 – 1.06	0.003 & 0.01	[23]
HPLC-UV[b]	0.125 – 7.0	0.38 & 0.125	[24]
Fluorescence	0.01 – 10.0	0.003 & 0.01	[25]
UPLC-MS/MS[c]	0.02 – 4.0	0.006 & 0.02	[26]
RRS[d]	0.1 – 1.0	0.03 & 0.1	[27]
SPE-LC-MS[e]	0.005 – 5.0	0.0015 & 0.005	[28]
Fluorescence	1.0 – 9.0	0.3 & 1.0	[29]
HPLC-UV	0.01 – 5.0	0.003 & 0.01	[30]
AG@DES-EME-Flu[f]	0.65 – 10.0	0.20 & 0.66	This work

[a] Cyclic Voltammetry
[b] High performance liquid chromatography coupled with UV detection
[c] Ultra high-performance liquid chromatography - tandem mass spectrometry
[d] Resonance Rayleigh Scattering
[e] Solid phase extraction - liquid chromatography - tandem mass spectrometry
[f] AG@DES based EME coupled with fluorescence

4. CONCLUSION

In this study, a novel and green electromembrane extraction (EME) method was developed using a hydrogel membrane composed of agarose and a deep eutectic solvent (DES) based on choline chloride and methacrylic acid (ChCl-MAA). The inclusion of methacrylic acid (MAA) is particularly significant, as its carboxylic and vinyl groups provide strong hydrogen-bonding and electrostatic interactions with the agarose matrix, thereby improving membrane homogeneity, stability, and affinity toward nilotinib. These interactions not only enhance the compatibility of the DES within the agarose structure but also play a decisive role in boosting extraction selectivity and efficiency. The prepared AG@DES membrane offered excellent stability and compatibility with fluorescence detection, enabling efficient preconcentration and determination of nilotinib in aqueous solutions.

Critical parameters influencing extraction efficiency—including agarose and DES content, applied voltage, extraction time, and donor/acceptor phase pH—were thoroughly optimized.

Under optimal conditions, the method exhibited good linearity, acceptable precision, and enrichment factor. Although the LOD and LOQ were slightly higher compared to instrumental methods such as LC-MS/MS, the simplicity, cost-effectiveness, and environmental friendliness of this approach make it a valuable alternative for trace analysis of nilotinib.

Overall, the proposed AG@DES-EME-Flu platform demonstrates high potential for analytical applications involving polar pharmaceutical compounds, especially in resource-limited or field-based settings.

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Declarations of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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