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A Potentiometric Sensor for Highly Selective and Sensitive Determination of Flurazepam in Pharmaceutical and Biological Samples Based on Molecularly Imprinted Polyaniline as the Ionophore

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ABSTRACT

In this study, a molecularly imprinted polymer (MIP) specifically designed for flurazepam (FZM) was synthesized using polyaniline as the base material. This polymer was subsequently utilized as an ionophore in the fabrication of a potentiometric sensor aimed at the precise determination of FZM concentrations. The optimal sensor composition was determined to consist of 8% ionophore, 2% sodium tetraphenylborate (NaTPB), 30% polyvinyl chloride (PVC), and 60% dioctyl phthalate (DOP). This particular formulation yielded the highest Nernstian response, with a slope of 59.6 millivolts per decade. The developed sensor exhibited a broad linear detection range, spanning from 1.0×10^{-8} to 1.0×10^{-3} mol L^{-1}, and demonstrated an impressive detection limit as low as 7.0×10^{-9} mol L^{-1}. Furthermore, the sensor's operational pH range was determined to be between 3.0 and 8.0, ensuring functionality across varying conditions. The response time of the sensor was remarkably rapid, requiring only 5 seconds to stabilize, while its lifespan extended up to two months under optimal storage and usage conditions. To validate the practical utility of the proposed sensor, its performance was rigorously tested in both pharmaceutical formulations and biological samples. The results revealed %Recovery values within the range of 97.5% to 105.0%, signifying highly accurate and reliable performance in real-world applications. These findings underscore the effectiveness and potential applicability of the developed electrode for precise and efficient FZM determination in diverse sample matrices.

1. Introduction

The design, synthesis, investigation, and development of various sensors have become one of the main focuses in modern chemistry, as these sensors play a vital role in pharmaceutical analysis and clinical diagnostics [1-3]. These sensors enable rapid, cost-effective, and real-time detection of therapeutic agents, while also supporting applications such as counterfeit drug identification and monitoring of substance abuse. Advances in molecular recognition have significantly improved sensor selectivity and sensitivity, positioning them as critical tools for drug analysis, therapeutic monitoring, and personalized medicine. As such, the innovation of sensor technologies for targeted pharmaceutical detection continues to be a dynamic and impactful research area.

The development of highly selective and sensitive analytical methodologies for the determination of pharmaceutical compounds is a cornerstone of modern analytical chemistry [4]. As pharmaceutical formulations become increasingly complex and the demand for precise

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therapeutic monitoring grows, the need for innovative sensor technologies capable of detecting trace levels of active pharmaceutical ingredients (APIs) in both pharmaceutical and biological matrices has never been more critical [5]. Among the various classes of pharmaceuticals, benzodiazepines, such as flurazepam (FZM), have garnered significant attention due to their widespread use in the treatment of insomnia and anxiety disorders [6]. However, their potential for misuse, dependency, and adverse side effects necessitates robust analytical tools for their accurate quantification in clinical and quality control settings [7-9]. FZM, a long-acting benzodiazepine, is commonly prescribed for its sedative and hypnotic effects [10]. Its pharmacological activity is primarily mediated through its interaction with gamma-aminobutyric acid-A (GABA-A) receptors in the central nervous system [11]. Despite its therapeutic benefits, FZM is associated with several challenges, including narrow therapeutic windows, risks of overdose, and potential for abuse [12]. Furthermore, its metabolism results in the formation of active metabolites that contribute to its prolonged pharmacological effects [13]. Consequently, monitoring flurazepam levels in pharmaceutical formulations, as well as in biological fluids such as plasma or urine, is crucial for ensuring patient safety, optimizing therapeutic efficacy, and preventing misuse [14].

Traditional analytical techniques for the determination of flurazepam, including high-performance liquid chromatography (HPLC) [15], gas chromatography (GC) [16], and mass spectrometry (MS) [17], have been widely employed due to their high sensitivity and accuracy. However, these methods often require extensive sample preparation, expensive instrumentation, and highly skilled personnel [18]. Additionally, the time-consuming nature of these techniques poses limitations for real-time monitoring and high-throughput applications [19]. In this context, electrochemical sensors have emerged as a promising alternative due to their inherent advantages, such as simplicity, cost-effectiveness, rapid response times, and potential for miniaturization [20]. Potentiometric sensors, a subclass of electrochemical sensors, have gained particular interest for their ability to provide selective and sensitive detection of target analytes based on ion-selective electrodes (ISEs) [21]. The performance of potentiometric sensors is largely dictated by the ionophore material incorporated within the sensor membrane. Ionophores play a critical role in selectively binding the target analyte and facilitating its detection [22]. To enhance the selectivity and sensitivity of potentiometric sensors, researchers have increasingly turned to molecular imprinting technology (MIT) as an innovative approach to designing ionophores with tailored recognition properties [23]. Molecularly imprinted polymers (MIPs) are synthetic materials that possess specific binding sites complementary to the size, shape, and functional groups of a target molecule [24]. These materials are synthesized by polymerizing functional monomers in the presence of a template molecule, followed by the removal of the template to create imprinted cavities [25]. MIPs offer several advantages, including high selectivity, chemical stability, and reusability [26]. When integrated into sensor platforms, MIPs serve as highly selective recognition elements capable of distinguishing target analytes even in complex sample matrices [27]. Polyaniline (PANI), a conducting polymer with excellent electrochemical properties, has been widely explored as a substrate for MIP synthesis due to its ease of fabrication, environmental stability, and tunable conductivity [28-30]. PANI-based MIPs combine the molecular recognition capabilities of imprinted polymers with the electroactive properties of polyaniline, resulting in hybrid materials that are particularly well-suited for potentiometric sensing applications [31]. The incorporation of molecularly imprinted polyaniline as an ionophore material in potentiometric sensors holds great promise for achieving highly selective and sensitive detection of target analytes such as flurazepam [32].

In this study, we present the design and development of a potentiometric sensor for the determination of FZM in pharmaceutical and biological samples. The sensor is based on molecularly imprinted polyaniline (MIP-PANI) as the ionophore material. By employing molecular imprinting technology, we aim to achieve superior selectivity toward FZM while minimizing interference from structurally similar compounds or endogenous matrix components. In other words, the novelty of this research is employing MIP for the first time as an electroactive sensing material for the detection of FZM. The proposed sensor is evaluated for its analytical performance characteristics, including linear range, detection limit, selectivity, reproducibility, and stability. Additionally, its applicability to real-world samples is demonstrated through the analysis of FZM in pharmaceutical formulations and biological fluids. This work represents a significant advancement in the field of potentiometric sensing by leveraging the synergistic properties of molecular imprinting and conducting polymers to develop a robust analytical tool for FZM determination. The findings of this study have important implications for pharmaceutical quality control, clinical diagnostics, and therapeutic drug monitoring. Furthermore, the methodology described herein can serve as a blueprint for the development of potentiometric sensors targeting other pharmaceutical compounds or analytes of clinical relevance.

2. Experimental

2.1. Materials and Reagents

In this study, all chemicals utilized were of analytical grade to ensure the highest level of precision and reliability in experimental outcomes. These chemicals were procured from Merck (Darmstadt, Germany), a reputable supplier known for its quality reagents. FZM, the target compound for this research, was supplied by Parsdarou Company, located in Tehran, Iran. Additionally, pharmaceutical formulations of FZM in the form of capsules from three distinct brands were obtained from a local pharmacy in Tehran, Iran, to ensure a representative sample set for analysis. Biological samples, including saliva and urine, were collected from a healthy volunteer who provided informed consent in compliance with ethical standards. Deionized water, which was rigorously purified to eliminate impurities, was used throughout the experiments for the preparation of solutions to ensure consistency and accuracy in all procedures.

2.2. Synthesis of Molecularly Imprinted Polyaniline (MIP)

The molecularly imprinted polyaniline (MIP) specific to FZM was synthesized using a chemical polymerization [33] technique designed to create a highly selective material for FZM recognition. Initially, 1.0 mmol of FZM was dissolved in 10 mL of deionized water containing 1.0 mmol of aniline monomer. Aniline was chosen for its ability to polymerize into polyaniline, a conductive polymer with good mechanical and chemical properties. To initiate the polymerization process, 2.0 mmol of ammonium persulfate (APS), an oxidizing agent, was added dropwise to the reaction mixture under constant stirring at room temperature to ensure uniform mixing and reaction progression. The polymerization reaction was allowed to proceed for 24 hours, providing ample time for the formation of the MIP and the incorporation of the FZM template molecules into the polymer matrix. Upon completion of the polymerization process, the MIP was separated from the reaction mixture through filtration. The collected material was thoroughly washed with a mixture of water and ethanol to remove any unreacted monomers and residual template molecules that might interfere with its performance. The thorough washing step ensured that the MIP retained only the imprinted cavities specific to FZM. The purified MIP was then dried under vacuum conditions at 50°C for 12 hours to remove any residual solvents and moisture, resulting in a stable and ready-to-use material. For comparison purposes, a non-imprinted polyaniline (NIP) was synthesized under identical conditions but without the addition of FZM as the template molecule. This control material allowed for the evaluation of the imprinting effect and the selectivity of the MIP.

2.3. Fabrication of the Potentiometric

To develop a potentiometric sensor capable of detecting FZM with high sensitivity and selectivity, the synthesized MIP was incorporated as the ionophore within a polyvinyl chloride (PVC)-based membrane. The membrane formulation consisted of 30% (w/w) PVC as the base polymer providing structural integrity, 60% (w/w) dioctyl phthalate (DOP) as a plasticizer to enhance flexibility and ion transport, 8% (w/w) MIP as the active recognition component, and 2% (w/w) sodium tetrakis(4-chlorophenyl)borate (NaTPB) as an additive to improve ion exchange properties. These components were dissolved in tetrahydrofuran (THF), an organic solvent that facilitated uniform mixing and dissolution of all ingredients. The resulting homogeneous membrane solution was carefully cast onto the surface of a graphite electrode, chosen for its excellent conductivity and stability. The coated electrodes were left to dry at room temperature overnight to allow solvent evaporation and membrane formation. This step resulted in a thin, flexible membrane with embedded MIP particles capable of selectively binding FZM molecules. To enhance sensor performance and ensure proper functioning, the fabricated electrodes were immersed in a 10^{-3} M FZM solution for conditioning prior to use. This conditioning step allowed the MIP cavities within the membrane to become fully activated and ready for analyte recognition. A silver/silver chloride electrode served as the reference electrode in all potentiometric measurements, providing a stable potential reference point.

2.4. Selectivity Study

The selectivity of the developed potentiometric sensor toward FZM was evaluated using the matched potential method (MPM) [34], a well-established approach for assessing sensor specificity. In this method, a known concentration of FZM was added to the test solution, and the corresponding potential change was recorded using the sensor. Subsequently, potential interfering species such as biomolecules or common ions present in biological fluids were introduced into the solution at increasing concentrations until an equivalent potential change was observed. This approach allowed for the determination of selectivity coefficients, which quantify the sensor's ability to distinguish FZM from other substances. The results provided valuable insights into the sensor's performance in complex matrices and its suitability for real-world applications.

2.5. Analysis of Real Samples

To demonstrate the practical applicability of the developed sensor in real-world scenarios, three types of samples were analyzed: pharmaceutical formulations (FZM capsules from three different brands), saliva samples, and urine samples. For pharmaceutical analysis, an accurately weighed amount equivalent to one capsule's labeled FZM content was dissolved in deionized water. The resulting solution was filtered to remove excipients and other insoluble components that might interfere with sensor performance [35]. The filtrate was then appropriately diluted before measurement using the potentiometric sensor. In addition to pharmaceutical formulations, biological samples were analyzed to assess the sensor's utility in clinical and diagnostic settings. Saliva and urine samples were collected from a healthy adult volunteer who had abstained from taking any medication for at least 48 hours prior to sample collection to avoid potential interference from other drugs or metabolites. The collected samples were centrifuged at 4000 rpm for 10 minutes to remove particulate matter and other debris. The resulting clear supernatants were spiked with known concentrations of FZM to perform recovery studies, which evaluated the sensor's accuracy and precision in detecting FZM in complex biological matrices. Overall, this comprehensive study highlights the successful synthesis of molecularly imprinted polyaniline specific to FZM, its incorporation into a potentiometric sensor, and its application in analyzing pharmaceutical formulations and biological samples. The results underscore the potential of molecularly imprinted materials for developing highly selective sensors tailored for specific analytes in diverse applications.

3. Results and Discussion

3.1. Membrane optimization

To enhance the functionality of an ion-selective electrode, it is imperative to conduct a comprehensive evaluation of the membrane's composition, as it directly influences the electrode's sensitivity, selectivity, and overall performance. Key components such as the ionophore, the plasticizer, and ionic additives play critical roles in shaping the membrane's behavior. The ionophore, which is responsible for selectively binding the target ion, must be carefully chosen and optimized in terms of its nature and concentration. The dielectric constant of the plasticizer, which affects the membrane's physical properties and its interaction with analytes, also requires meticulous consideration. Additionally, the inclusion of ionic additives can significantly enhance performance by reducing membrane resistance and improving ion exchange processes. Consequently, systematic testing and analysis of these elements are essential to achieve an optimal sensor design. In the development of a PVC-membrane ion-selective electrode, precise optimization of the membrane formulation is crucial, often requiring experimentation with various combinations of ionophores, plasticizers, and ionic additives to identify the most effective configuration.

Research involving 14 electrodes with distinct membrane compositions underscores the critical importance of ionophore content in determining electrode performance. Among these, seven electrodes were fabricated with varying concentrations of ionophore to assess its impact. The findings revealed that membranes lacking an ionophore (e.g., membrane 2) exhibited a limited linear range and a reduced slope, highlighting the necessity of this component for achieving adequate sensitivity and selectivity. In contrast, increasing the ionophore concentration to 8% (as in membrane 7) yielded a robust Nernstian response characterized by a slope of 56.9 mV/Decade and an expanded linear range from 1.0×10^{-8} to 1.0×10^{-3} mol L^{-1}. These results demonstrate the pivotal role of ionophore content in enhancing electrode behavior and provide valuable guidance for designing high-performance sensors. However, it is equally important to avoid excessive ionophore addition, as this can lead to membrane saturation and negatively affect performance, as observed in membrane 8, where the calibration curve slope was reduced [36].

The role of ionic additives in improving electrode performance is also significant. For instance, the use of 2% sodium tetraphenylborate (NaTPB) was shown to enhance sensitivity and extend the linear range of detection, further emphasizing the importance of optimizing additive concentrations. Similarly, the choice of plasticizer has a profound impact on electrode functionality. Among various plasticizers tested, dioctyl phthalate (DOP) emerged as the most effective due to its non-polar nature and high dielectric constant, properties that strengthen interactions between the analyte and the plasticizer. This characteristic is particularly beneficial for applications involving monovalent cations such as FZM. Membrane 13, which incorporated DOP as a plasticizer, demonstrated superior performance based on its calibration curve, underscoring DOP's suitability for enhancing membrane-based analytical systems. The favorable attributes of DOP make it a promising candidate for improving both sensitivity and selectivity in analytical techniques that depend on effective membrane-analyte interactions.

Furthermore, the imprinting process of the ionophore within the membrane plays a crucial role in determining

electrode response. To investigate this effect, a membrane containing a non-imprinted polymer (NIP) in place of a molecularly imprinted polymer (MIP) was constructed (membrane 3). The resulting low slope and non-Nernstian behavior confirmed that the imprinting process significantly influences the electrode's potential response [37]. These findings collectively highlight the intricate interplay between membrane composition and electrode performance, offering valuable insights for optimizing sensor design and advancing analytical methodologies.

Table 1. Membrane composition optimization

No	PVC (%wt)	Ionophore (%wt)	NaTPB (%wt)	Plasticizer (%60)	Linear Range (mol L^{-1})	Slope (mV/Decade)	LOD (mol L^{-1})
1	40	0	0	DBP	1.0×10^{-4} to 1.0×10^{-3}	8.1±0.3	9.8×10^{-5}
2	38	0	2	DBP	1.0×10^{-4} to 1.0×10^{-3}	11.9±0.4	9.5×10^{-5}
3	30	8 (NIP)	2	DOP	1.0×10^{-4} to 1.0×10^{-3}	14.8±0.2	9.1×10^{-5}
4	37	1	2	DBP	1.0×10^{-5} to 1.0×10^{-3}	24.5±0.3	8.9×10^{-6}
5	36	2	2	DBP	1.0×10^{-5} to 1.0×10^{-3}	29.6±0.2	8.6×10^{-6}
6	34	4	2	DBP	8.0×10^{-6} to 1.0×10^{-3}	40.8±0.3	7.3×10^{-6}
7	30	8	2	DBP	1.0×10^{-8} to 1.0×10^{-3}	56.9±0.1	9.7×10^{-9}
8	29	9	2	DBP	1.0×10^{-8} to 1.0×10^{-3}	55.2±0.3	9.3×10^{-9}
9	33	8	0	DBP	5.0×10^{-7} to 1.0×10^{-3}	51.2±0.2	4.1×10^{-7}
10	31	8	1	DBP	1.0×10^{-7} to 1.0×10^{-3}	52.5±0.3	3.5×10^{-7}
11	29	8	3	DBP	1.0×10^{-8} to 1.0×10^{-3}	57.1±0.2	9.2×10^{-9}
12	30	8	2	OA	3.0×10^{-8} to 1.0×10^{-3}	58.1±0.3	1.5×10^{-8}
13	30	8	2	DOP	1.0×10^{-8} to 1.0×10^{-3}	59.6±0.1	7.0×10^{-9}
14	30	8	2	NB	5.0×10^{-8} to 1.0×10^{-3}	58.1±0.3	3.0×10^{-8}

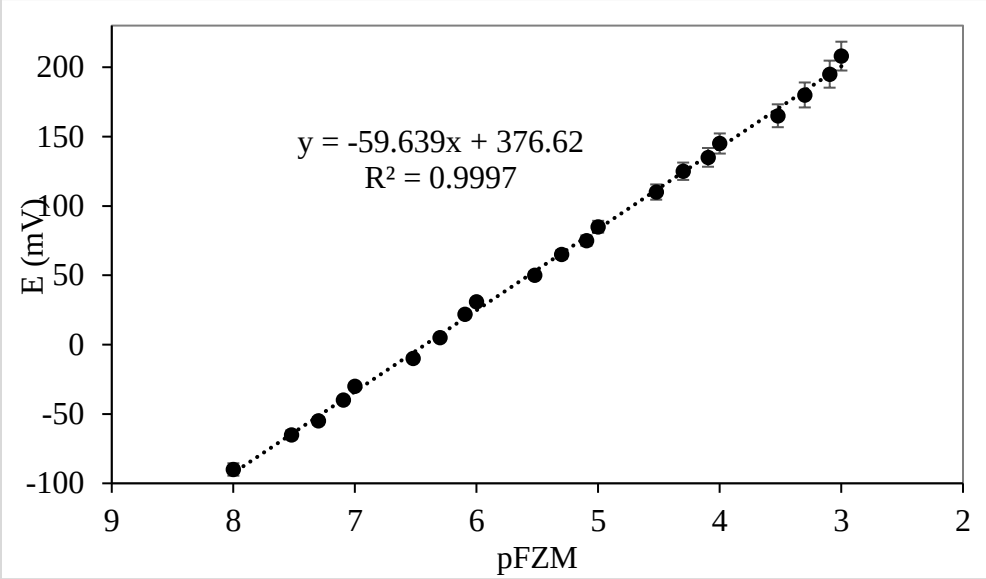

Fig. 1. The resulting calibration curve from the 13th membrane.

3.3.2. Applicable pH range

Nernstian behavior is a fundamental principle for the optimal performance of ion-selective electrodes, as it ensures accurate and reliable measurements of ion concentrations in various solutions. This behavior, however, is only observable within a specific pH range, making it essential to identify and define this range to design and utilize dependable and efficient ion-selective electrodes. By determining the pH range where Nernstian behavior is maintained, researchers can ensure that these electrodes provide consistent and precise readings across diverse samples and experimental conditions. In a detailed

experimental study, an FZM solution with a concentration of $1.0\times10^{-}5$ mol L^{-1} was analyzed across a broad pH spectrum ranging from 1.0 to 12.0. The pH of the solution was carefully adjusted using concentrated hydrochloric acid and sodium hydroxide to explore its behavior under varying acidic and alkaline conditions. Potential measurements were systematically recorded and plotted against the pH values to investigate the solution's electrochemical response and its stability across different pH levels. The primary objective of this experiment was to evaluate the FZM solution's sensitivity and response to pH changes, thereby generating reliable data to understand its behavior in different environments. The results, as depicted in Fig. 2, revealed that the sensor exhibited stable and unaffected responses within the pH range of 3.0 to 8.0. This stability indicates that the electrode is highly effective in detecting FZM ions within this specific range, making it suitable for applications requiring precise ion detection under these conditions. However, under highly acidic conditions, particularly at pH levels below 3.0, the ionophore in the electrode becomes protonated. This protonation leads to a competitive interaction between hydronium ions and the target analyte for binding with the ionophore [38]. Such competition generates a dynamic interplay where both species compete for complex formation with the ionophore, thereby reducing its binding efficiency and selectivity. These interactions are critical for understanding how the ionophore behaves in low pH environments and their broader implications for analytical applications. The protonation of the ionophore at low pH levels significantly shifts the equilibrium of complex formation, which can negatively impact the overall analytical process by reducing accuracy and reliability. On the other hand, in alkaline conditions with a pH above 8.0, cations in the solution tend to form complexes with hydroxyl ions. This competing interaction further disrupts the functionality of the ionophore, compromising its ability to selectively bind with the target analyte. Based on these observations, it was concluded that the ion-selective electrode operates effectively only within the defined pH range of 3.0-8.0, ensuring optimal performance and accurate measurements under these specific conditions. This understanding is vital for advancing the development of ion-selective electrodes tailored for precise analytical applications in various scientific fields.

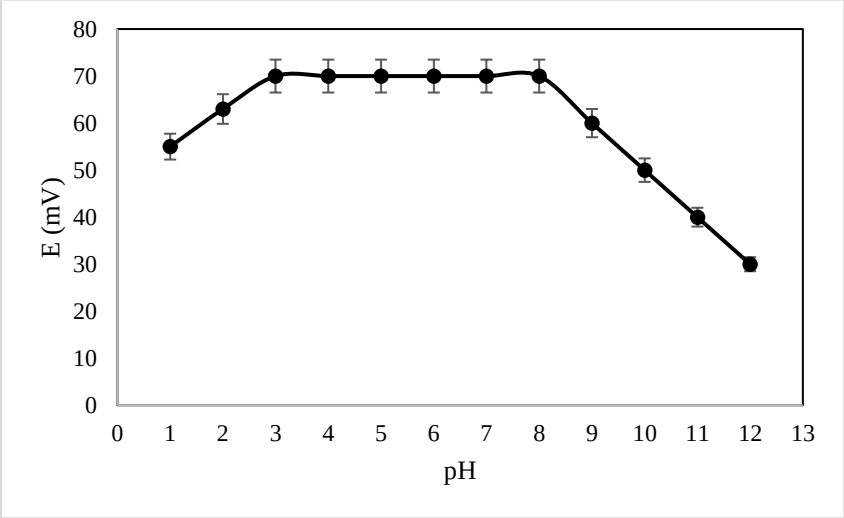

Fig. 2. The influence of pH on the potential response of the suggested sensor in a 1×10^{-5} mol L^{-1} solution of FZM at ambient temperature.

3.3.3. Response time, lifespan, repeatability, and reproducibility

The precise detection and quantification of ion concentrations through potentiometric sensing systems are significantly impacted by the response time, which is primarily governed by the kinetics of complexation and decomplexation of analyte ions in the presence of an ionophore at the membrane interface. To investigate this phenomenon, a study was conducted wherein both reference and indicator electrodes were immersed in FZM solutions with activities differing by a factor of ten. The performance of the sensor was assessed by continuously recording the electromotive force (EMF) during the experiment. The findings revealed that the sensor demonstrated an impressively rapid response time of

approximately five seconds, enabling accurate measurement of FZM activity. This swift response underscores the sensor's capability for real-time analytical applications [39]. In addition to evaluating the response time, the research also focused on assessing the durability and long-term performance of the electrodes over an extended period of 12 weeks. This was aimed at determining their reliability in delivering consistent and dependable measurements over time. The results showed that the sensor exhibited a stable and steady incline in performance over the first eight weeks of monitoring, as illustrated in Fig. 4, maintaining consistent Nernstian behavior without any observable deviations. This stability was attributed to the limited solubility of the ionophore in inorganic solvents, combined with its remarkable chemical stability. However, beginning in the eighth week, a gradual increase in the sensor's limit of detection (LOD) was observed, reaching a value of 8.0×10^{-7} mol L^{-1}. This change in LOD consequently led to a reduction in the linear detection range, as highlighted in prior studies [38]. To further evaluate the precision of the fabricated ion-selective electrode, 10 FZM solutions were prepared with activities of 1.0×10^{-6} mol L^{-1} and 1.0×10^{-4} mol L^{-1}, and their potential responses were measured. The resulting relative standard deviation (RSD%) values were found to be 3.66% and 2.01%, respectively, indicating a high degree of precision in measuring FZM concentrations at different levels of activity. The relatively low RSD% values reflect excellent repeatability, showcasing the sensor's capability to consistently deliver accurate and reliable results under varying conditions. Furthermore, to examine reproducibility, 10 separate electrodes were utilized to measure the potential response of two FZM solutions with differing activity levels. The RSD% values for these measurements were determined to be 4.98% and 3.71%, respectively, further demonstrating the sensor's reliability and consistency in determining potential responses across multiple electrodes. Overall, this study highlights the impressive performance characteristics of the ion-selective electrode, including its rapid response time for real-time measurements, long-term stability, and precision in detecting varying concentrations of FZM. The findings emphasize its potential utility in practical applications requiring accurate and reliable ion concentration measurements over extended periods [40]. However, the observed increase in LOD after eight weeks suggests areas for further optimization to enhance the sensor's longevity and maintain an extended linear detection range.

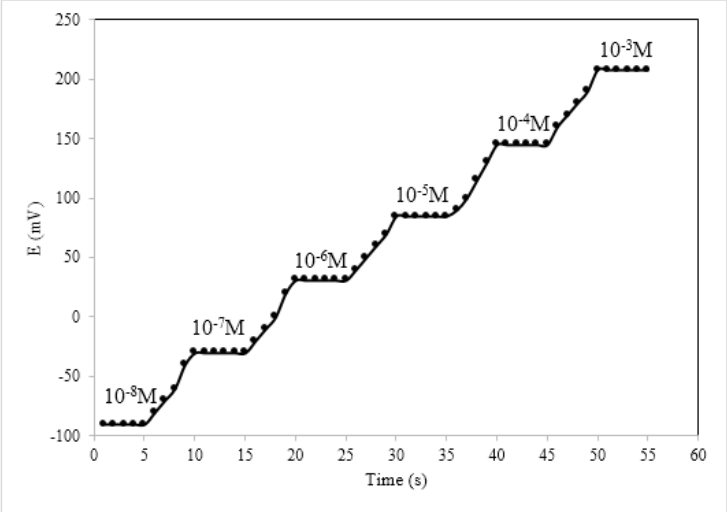

Fig. 3. The dynamic response time of the suggested electrode.

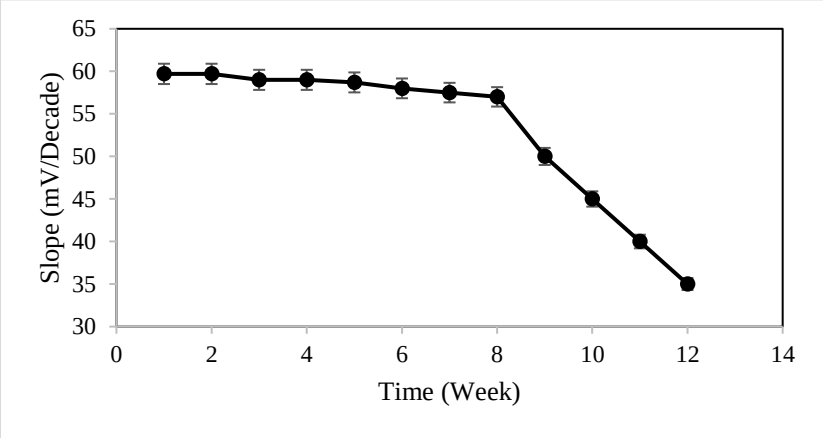

Fig. 4. The lifetime of the sensor.

3.3.4. Selectivity

The selectivity of the meticulously designed sensor was comprehensively assessed using the matched potential method (MPM), a well-established analytical technique for evaluating sensor performance in the presence of competing ionic species. This method involved rigorous testing against a total of 20 distinct ionic species to determine the sensor's ability to differentiate between the target analyte and potential interfering ions. Following this evaluation, selectivity coefficients, denoted as KMPM, were calculated to quantify the sensor's sensitivity and specificity toward various ions. These coefficients serve as a critical metric for understanding the extent to which the sensor can distinguish the target analyte from other ions that may coexist in the sample matrix. As detailed in Table 2, the KMPM values provide invaluable insights into the sensor's capacity to selectively interact with the analyte of interest while minimizing interactions with interfering species. A KMPM value approaching 1 indicates that the sensor exhibits a comparable potential response to both the target analyte and an interfering ion, suggesting limited selectivity. Conversely, a KMPM value closer to 0 signifies negligible interaction with the interfering ion, thereby reflecting a high degree of selectivity. The data presented in Table 2 unequivocally demonstrate that the selectivity coefficients for this sensor are significantly lower than 1, with values reduced by factors ranging from 100 to an impressive 10,000. This strikingly low range of KMPM values underscores the extraordinary selectivity of the sensor, which has been meticulously engineered for the precise detection of FZM. Such results strongly validate the sensor's remarkable ability to specifically identify FZM even in complex environments containing a multitude of other ionic species. This exceptional performance highlights its suitability for practical applications where high selectivity is paramount, further cementing its potential as a reliable tool in analytical and diagnostic settings.

Table 2. MPM selectivity Coefficients

Interfering species	K_{MPM}	Interfering species	K_{MPM}
Ca^{2+}	4.9×10^{-3}	Dopamine	4.8×10^{-4}
Mn^{2+}	7.8×10^{-4}	Uric acid	5.1×10^{-4}
Mg^{2+}	1.3×10^{-3}	Vitamin C	1.8×10^{-3}
Fe^{2+}	7.2×10^{-3}	Vitamin B_6	3.5×10^{-3}
Fe^{3+}	9.9×10^{-3}	K^{+}	2.1×10^{-3}
Maltose	1.0×10^{-4}	Na^{+}	1.9×10^{-4}
Glucose	3.0×10^{-4}	Ni^{2+}	2.3×10^{-3}
L-Cysteine	2.1×10^{-4}	Carbamazepine	8.7×10^{-4}
Phenylalanine	6.6×10^{-3}	Oxazepam	7.7×10^{-3}
Fructose	4.2×10^{-4}	Zn^{2+}	1.6×10^{-3}

3.3.5. The influence of partially non-aqueous media on the potential response of the electrode

To thoroughly assess the operational effectiveness of the proposed sensor in environments that are partially non-aqueous, it is essential to conduct a series of experiments

using solutions with varying proportions of organic solvents. This study aimed to address this requirement by preparing and testing three distinct sets of FZM solutions, each containing different concentrations of ethanol, acetone, and a combination of both solvents. The primary goal of these experiments was to evaluate how the sensor performs when exposed to such organic solvents, which is a crucial consideration for its potential application in analyzing real-world samples that may include these compounds. The results, as detailed in Table 3, demonstrate that the newly designed electrode retains its sensitivity and maintains a consistent linear response range even when subjected to solutions containing up to 20% organic solvent content. However, it was observed that the addition of organic solvents led to a slight decline in the slope of the electrode's response curve and an increase in the limit of detection (LOD). This behavior can be attributed to the possible leakage of ionophore and other membrane components into the test solution, a phenomenon that becomes more significant as the concentration of organic solvents increases. This observation aligns with findings reported in earlier research studies [41], which suggest that higher levels of organic content can exacerbate such effects. These insights underline the importance of optimizing the sensor's design and composition to minimize such leakage and ensure reliable performance in diverse chemical environments.

Table 3. The performance of designed sensor in non-aqueous media

Non aqueous content (%v/v)	Slope (mV.Decade^{-1})	Dynamic Range (Mol L^{-1})
0	59.6±0.1	1.0×10^{-8} to 1.0×10^{-3}
Ethanol		
5	59.5±0.2	1.0×10^{-8} to 1.0×10^{-3}
10	59.1±0.2	1.0×10^{-8} to 1.0×10^{-3}
15	58.9±0.3	1.0×10^{-8} to 1.0×10^{-3}
20	57.1±0.4	1.0×10^{-8} to 1.0×10^{-3}
25	40.8±0.5	2.0×10^{-7} to 1.0×10^{-3}
Acetone		
5	59.6±0.1	1.0×10^{-8} to 1.0×10^{-3}
10	59.4±0.3	1.0×10^{-8} to 1.0×10^{-3}
15	59.1±0.2	1.0×10^{-8} to 1.0×10^{-3}
20	58.2±0.3	1.0×10^{-8} to 1.0×10^{-3}
25	50.3±0.4	3.0×10^{-7} to 1.0×10^{-3}
Mixed 1:1 (Ethanol:Acetone)		
5	59.6±0.2	1.0×10^{-8} to 1.0×10^{-3}
10	59.3±0.3	1.0×10^{-8} to 1.0×10^{-3}
15	58.8±0.2	1.0×10^{-8} to 1.0×10^{-3}
20	57.6±0.2	1.0×10^{-8} to 1.0×10^{-3}
25	50.1±0.2	5.0×10^{-7} to 1.0×10^{-3}

3.3.6. Analytical applications

The specially designed electrode was utilized to evaluate the levels of FZM in three distinct pharmaceutical samples, and the findings from these analyses are comprehensively documented in Table 4. Upon careful examination of the results, it becomes evident that the data obtained from the analytical procedures align closely and consistently with the labeled amounts of the drug as indicated on the respective pharmaceutical products. This strong correlation underscores the reliability and accuracy of the employed analytical method. Subsequently, the concentration of FZM was also determined in two biological specimens, namely saliva and urine, with the corresponding results meticulously detailed in Table 5. A thorough review of these results reveals that the measured values are in excellent concordance with the theoretical values that were initially spiked into the samples. Furthermore, the recovery percentages achieved during these analyses ranged between 97.50% and 105.00%, demonstrating a high level of precision and accuracy in the quantification process. Additionally, the relative standard deviation (%RSD) values recorded were all below 4.14%, further confirming the robustness and reproducibility of the analytical method employed. These findings collectively highlight the effectiveness and reliability of the designed electrode for both pharmaceutical and biological sample analysis [42].

3. 3. 7. Comparison with Former Reports

The most important analytical figures of merit are compared to the ones of formerly reports and the results are shown in Table 6. As can be seen, the linear range and detection limit of all former reports are worse than the proposed method except LC-MS-MS technique which requires very expensive instrumentation and the analysis procedure is too complicated. The other reported potentiometric sensor not only is worse than this method because of its limited dynamic range and high detection limit but also suffers from poor selectivity because flurazepam-tetraphenyl borate ion pair was employed as the ionophore in the developed electrode which can also adsorbs other benzodiazepines with similar structures. As the main limitations of potentiometric sensors is their dependency on the ionic strength of the test solution which should be solved in future reseaches.

Table 4. Analysis of FZM in pharmaceutical samples

Sample	Found (mg)	Labeled (mg)	%Recovery	%RSD
Capsule 1	14.8	15	98.66	2.09
Capsule 2	15.1	15	100.66	3.11
Capsule 3	14.9	15	99.33	3.99

Table 5. Analysis of FZM in biological samples

Sample	Added (M)	Found (M)	%Recovery	%RSD
Saliva	0	N. D	---	---
	4×10^{-7}	3.89×10^{-7}	97.50	3.51
	6×10^{-5}	6.03×10^{-5}	105.00	2.88
	8×10^{-4}	7.97×10^{-4}	99.63	2.07
Urine	0	N. D	---	---
	4×10^{-7}	4.01×10^{-7}	102.50	4.14
	6×10^{-5}	5.98×10^{-5}	99.67	3.85
	8×10^{-4}	8.01×10^{-4}	101.25	2.99

Table 6. Comparison the analytical figures of merit of the proposed method with former reports

Technique	Linear Range (M)	Detection Limit (M)	Reference
HPLC (UV detector)	5.1×10^{-5}-5.1×10^{-3}	1.3×10^{-7}	[43]
LC-MS-MS	1.3×10^{-9}-1.3×10^{-8}	1.28×10^{-9}	[44]
GC-MS	2.5×10^{-5}-1.1×10^{-3}	1.2×10^{-6}	[45]

Potentiometric Sensor based on Flurazepam-tetraphenylborate	1.0×10^{-6}-1.0×10^{-3}	9.0×10^{-7}	[46]
Potentiometric Sensor based on PANI-MIP	1.0×10^{-8} to 1.0×10^{-3}	7.0×10^{-9}	This work

4. Conclusion

In this research, a molecularly imprinted polymer (MIP) specifically tailored for the detection of flurazepam (FZM) was successfully synthesized, employing polyaniline as the fundamental base material. This innovative polymer was subsequently incorporated as an ionophore in the development of a potentiometric sensor designed to achieve precise and reliable quantification of FZM concentrations. The composition of the sensor was meticulously optimized to include 8% ionophore, 2% sodium tetraphenylborate (NaTPB), 30% polyvinyl chloride (PVC), and 60% dioctyl phthalate (DOP). This carefully formulated combination was found to deliver the most responsive Nernstian behavior, achieving a slope of 59.6 millivolts per decade. The sensor demonstrated an extensive linear detection range, effectively measuring FZM concentrations from as low as 1.0×10^{-8} mol L^{-1} to as high as 1.0×10^{-3} mol L^{-1}, with an exceptional detection limit reaching 7.0×10^{-9} mol L^{-1}. Additionally, the sensor exhibited robust performance across a pH range of 3.0 to 8.0, ensuring its functionality under diverse environmental and experimental conditions. One of the standout features of this sensor was its remarkably swift response time, requiring only 5 seconds to achieve stabilization, which highlights its efficiency in real-time applications. Furthermore, the sensor demonstrated impressive longevity, maintaining optimal performance for up to two months when stored and handled under appropriate conditions. To evaluate its practical applicability, the sensor underwent rigorous testing in both pharmaceutical formulations and biological samples. The results from these tests indicated %Recovery values ranging between 97.5% and 105.0%, underscoring the sensor's high accuracy and reliability in real-world scenarios. These findings highlight the effectiveness of the developed electrode and its significant potential for precise and efficient determination of FZM concentrations across a wide array of sample matrices, making it a valuable tool for both pharmaceutical analysis and broader scientific applications.

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