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Utilization of azamacrocyclic ionophores as recognition elements for the potentiometric determination of gallic acid in various matrices

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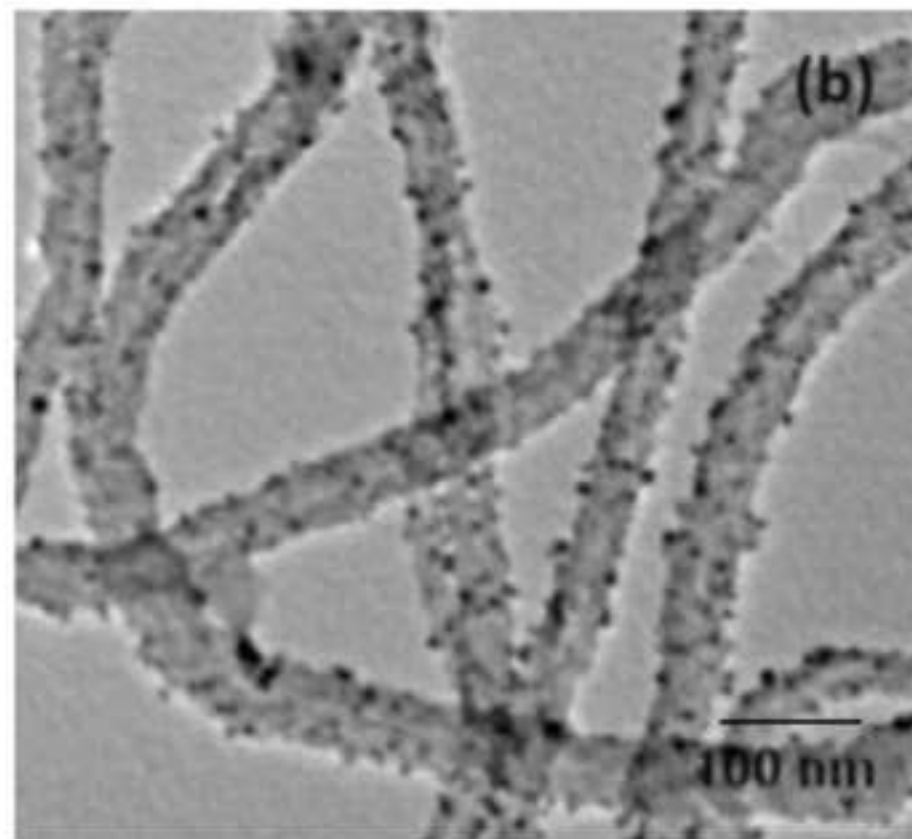
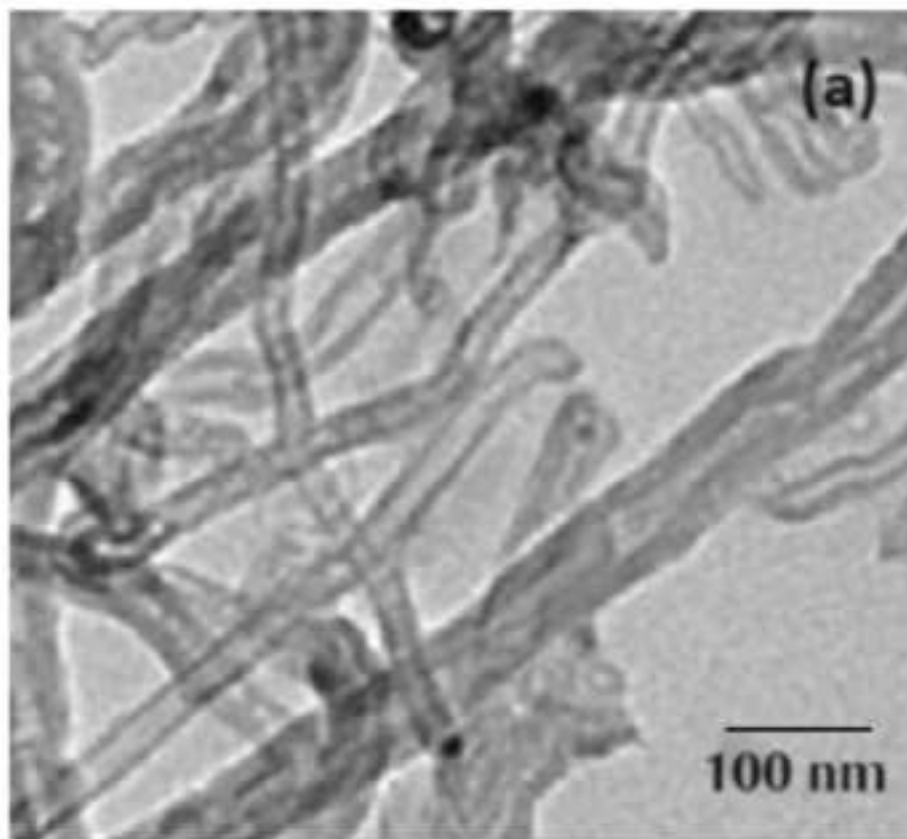
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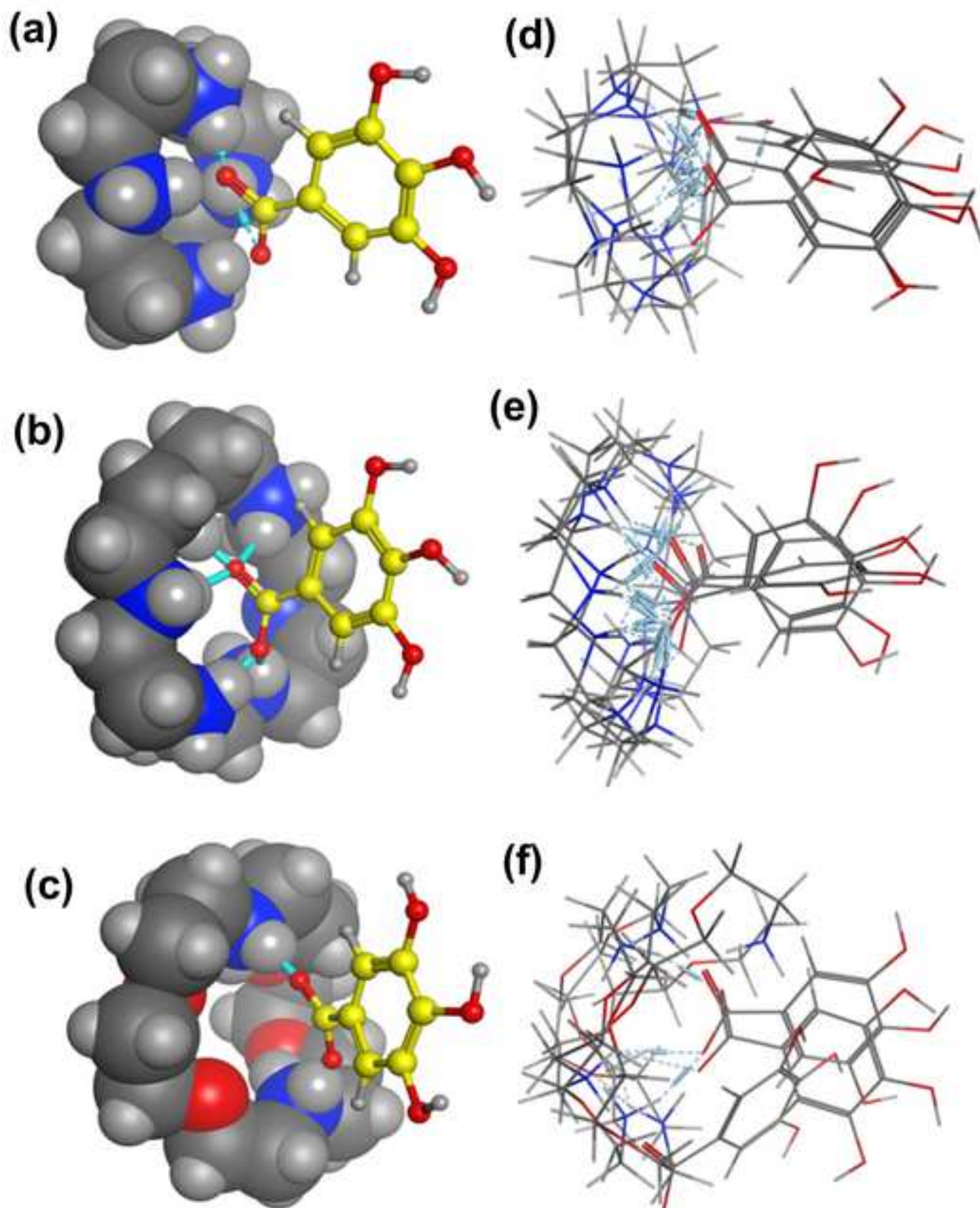
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Highlights:

- Azamacrocyclic ionophores are utilized as recognition elements in electrochemical sensors.
- A comparative study was performed between cyclen, hexacyclen, and kryptofix-22 ionophores and their sensitivity to the drug approved by molecular docking.
- MWCNTs/TiO₂ nanocomposite was utilized as an ion-to-electron transducer.
- The sensors were assessed following IUPAC guidelines.
- Feasible and time-saving application of the proposed sensors in different matrices was conducted.





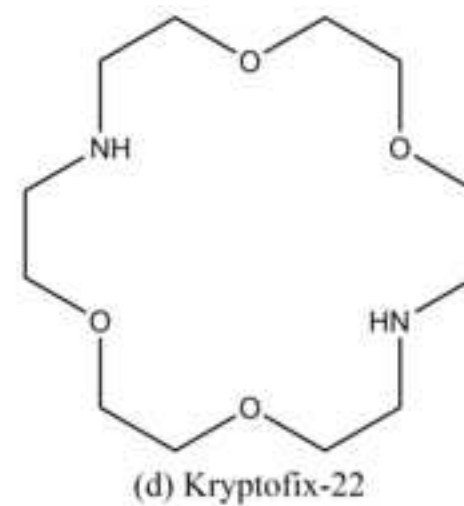
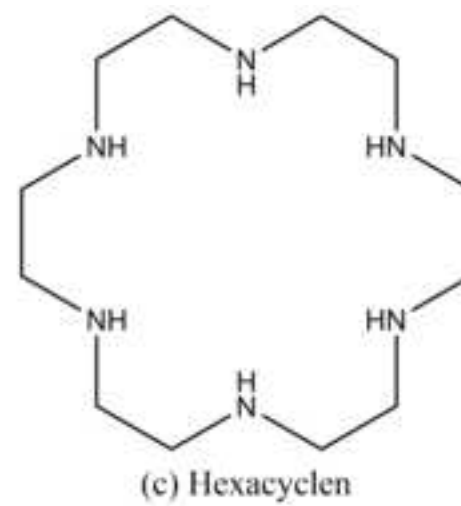
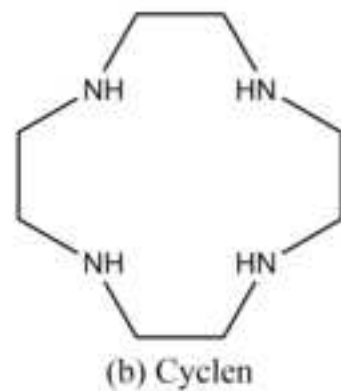
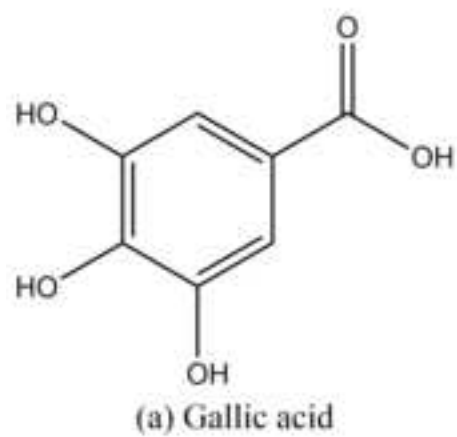


Figure 2

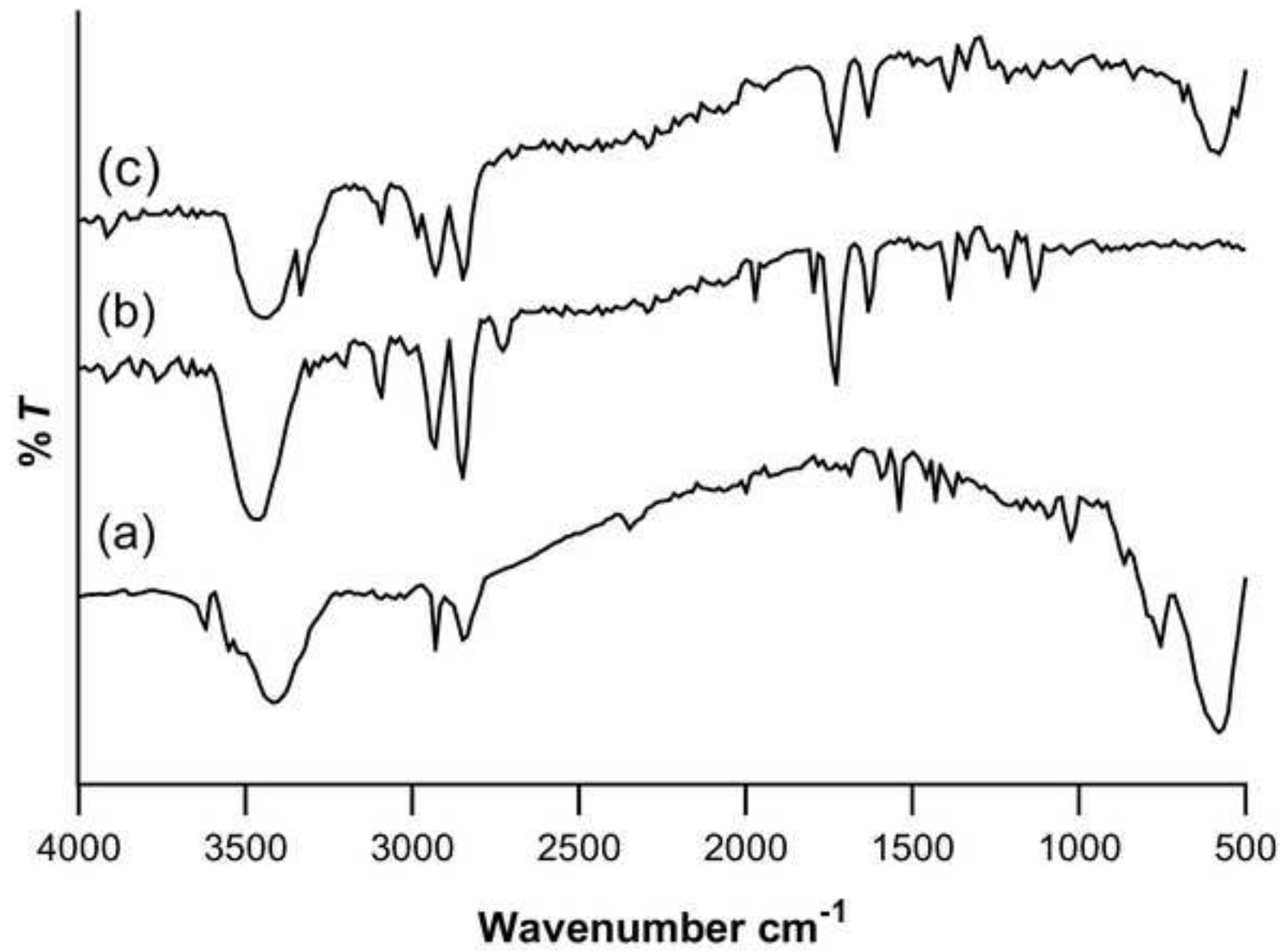
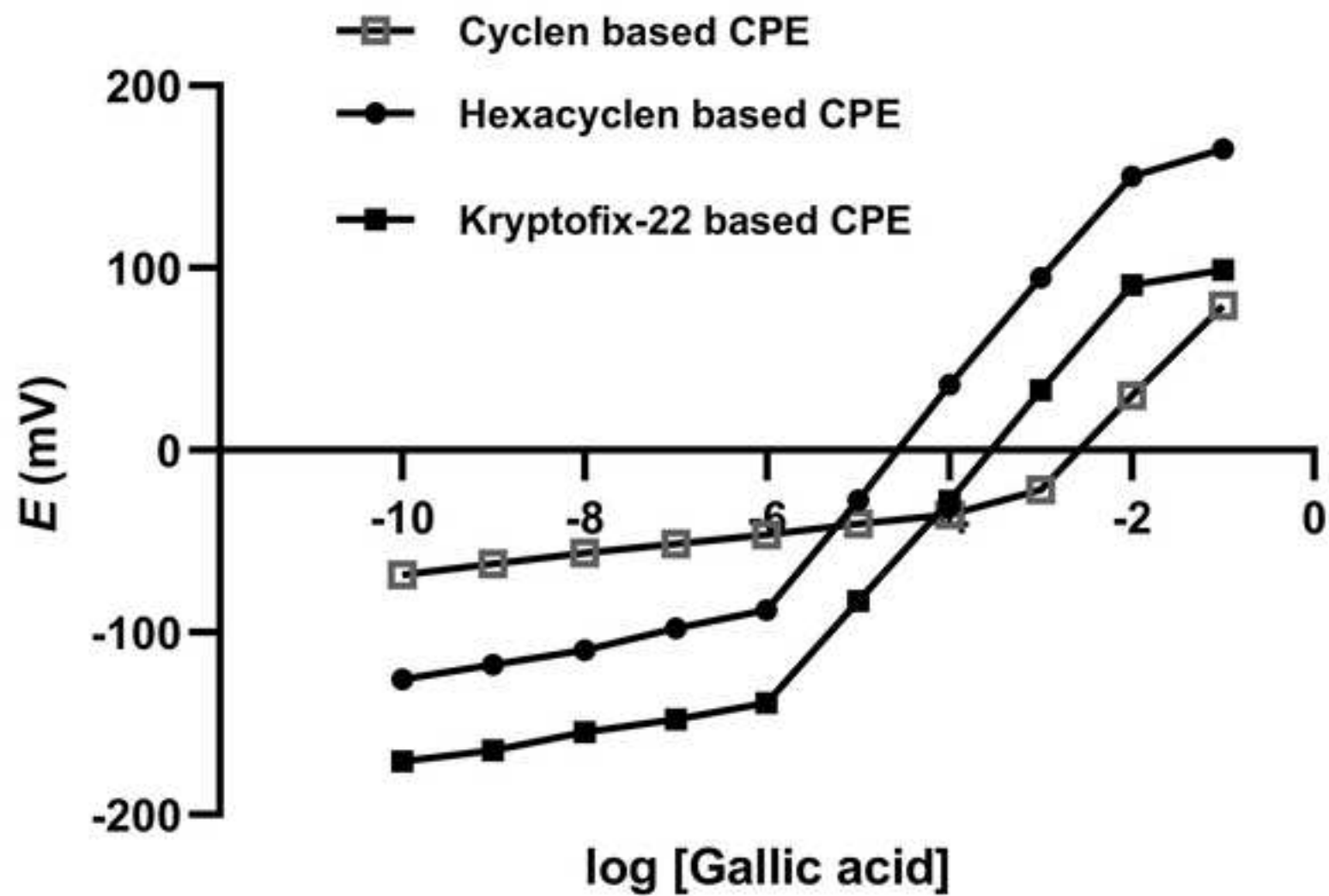


Figure 5



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Conflict of interest statement

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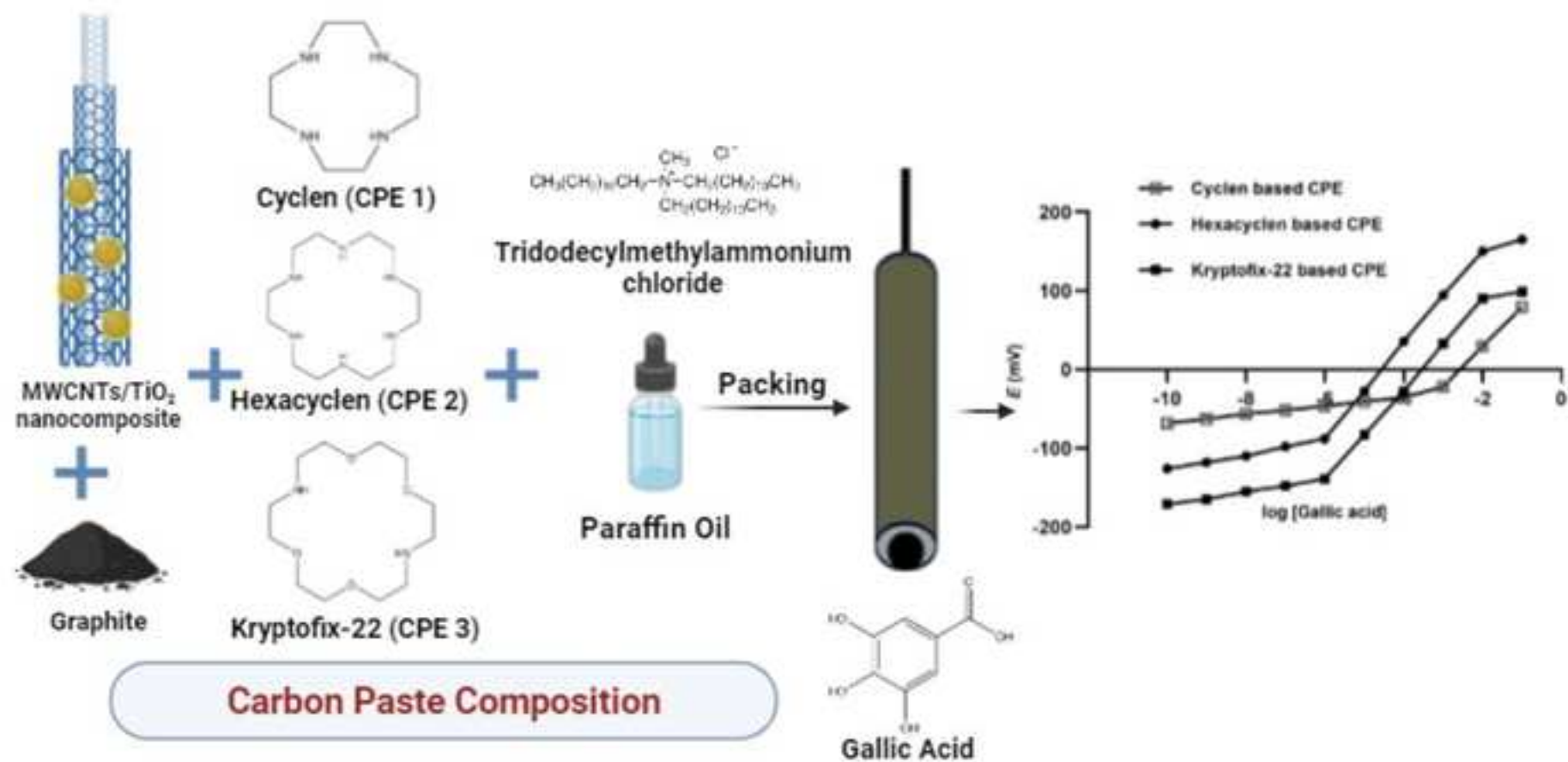
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The person who meets the authorship criteria is listed as an author, and the authors certify that they take public responsibility for the content, including the concept, design, analysis, interpretation of data, writing, or revision of the manuscript. Furthermore, the authors certify that this material has not been and will not be submitted to or published in any other publication before its appearance in Microchemical Journal.

Sincerely.

Nehad A. Abdallah



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Utilization of azamacrocyclic ionophores as recognition elements for the potentiometric determination of gallic acid in various matrices

Abstract

Gallic acid is a naturally occurring low molecular weight triphenolic molecule identified as a potent antioxidant, protecting against various types of reactive species and non-radicals. Three novel carbon paste electrodes were developed for the potentiometric determination of gallic acid. Azamacrocyclic ionophores; cyclen, hexacyclen, and kryptofix-22 were utilized as recognition ionophores separately. A multi-walled carbon nanotubes/ titanium oxide nanocomposite was employed as the ion-to-electron transducer in the designed sensors. The prepared transducer was characterized by FT-IR and TEM. Molecular docking was implemented to study the recognition mechanism between these ionophores and the gallic acid molecule. It revealed promising binding interactions between gallic acid and the three recognition ionophores, especially hexacyclen and Kryptofix-22. In studying the potentiometric response of the proposed sensors, they exhibited high stability, sensitivity, accuracy, and precision in the quantitation of gallic acid. Gallic acid can be accurately quantified over the linearity range of $1 \times 10^{-1} - 1 \times 10^{-3}$ M, $1 \times 10^{-2} - 1 \times 10^{-6}$ M, and $1 \times 10^{-2} - 1 \times 10^{-6}$ M with a Limit of detection (LOD) 4×10^{-4} M, 3×10^{-7} M, and 7×10^{-7} M, for CPE1, CPE 2 and CPE 3, respectively. The electrodes showed high efficiency in determining gallic acid in *Krameria lappacea* root alcoholic extract, black tea, green tea, and human plasma without sophisticated pretreatment steps.

Keywords: Carbon Paste Electrode; Potentiometry; Azamacrocyclic ionophores; MWCNTs; Gallic acid

Introduction

Gallic acid (GA) (3,4,5-trihydroxybenzoic acid), a naturally occurring triphenolic molecule, has been identified as a potent antioxidant. It offers significant defense against oxidative damage induced by reactive species often found in biological systems, comprising hydroxyl, superoxide, and peroxy, as well as non-radicals, hydrogen peroxide, and hypochlorous acid [1, 2]. Because of its redox properties, this phenolic naturally occurring compound appears to be a powerful antiradical agent, working as a hydrogen donor, reducing agent, and metal chelator. It possesses many biological activities, including anticancer, antifungal, antibacterial, antiviral, antiulcer, and cholesterol-lowering effects [3, 4].

Several synthetic substances can mitigate the harmful effects of oxidative stress but can also result in adverse side effects. Therefore, the emphasis has turned to discovering antioxidant compounds with therapeutic potential that have minimal or no adverse effects for prophylactic treatments. As a result, considerable attention is being devoted to naturally occurring antioxidants such as GA [5, 6]. GA has been discovered as one of the major antioxidant molecules responsible for many herbal extracts' significant antiradical, anti-inflammatory, cardioprotective, neurological, and anticancer properties. GA is considered one of the principal natural phenolic acids present in tea leaves, grapes, and many other processed herbs and drinks [7]. Rhatany (*Krameria lappacea*) is a given name to various species of *Krameria*, a hemiparasite plant found in South America, particularly in Peru, Ecuador, and Bolivia [8, 9]. Many studies reported that *Krameria* extracts are rich in GA and other phenolic constituents with hepatoprotective, antioxidant, antimicrobial, and anti-inflammatory activities [10, 11].

Regarding all of these different biological and pharmacological impacts, determining GA is extremely important. Chemiluminescence [12, 13], HPLC [14-19], LC-MS/MS [20, 21], UV-Vis spectrophotometry [22-24], potentiometry [25, 26], and voltammetry [27-33] were used for assessing the GA. Though most of these techniques are quite sensitive, some necessitate expensive tools and complex pre-treatment measures. Therefore, this work focused on the quantitative determination of GA in different herbal extracts and other matrices with high accuracy and sensitivity without requiring extensive sample preliminary treatment. Potentiometric sensors have recently acquired popularity due to their sensitivity, simplicity, quick response, and selectivity.

The literature revealed that two potentiometric electrodes were applied for GA quantitation [25, 26]. Both of them were designed as classical liquid contact ion-selective electrodes with polyvinyl chloride (PVC) membrane sandwiched between an internal filling solution and the sample to develop a liquid-contact interface. This classical electrode configuration requires additional maintenance due to the evaporation of the inner filling solution, and the leaching of some membrane components over time [34, 35]. In this work, GA was determined in *Krameria lappacea* root alcoholic extract and other matrices utilizing cutting-edge potentiometric carbon paste electrodes (CPEs).

CPE is considered one of the most important analytical techniques because of its rapid and straightforward construction, durability due to its regenerable surface capabilities, and low background current [36, 37]. To achieve high selectivity and sensitivity to the target analyte, functional components would be added to the carbon paste to boost the CPE's electrochemical performance and specificity.

The ion-to-electron transducer is the first vital element in constructing solid-contact ion-selective electrodes. The interaction between the analyte-sensing paste and the electron conductor is facilitated, and the transducer confidently converts the concentration of ions into electron signals, effectively stabilizing the potential at the substrate/ion-selective paste interface [38-42]. In the presented study, a nanocomposite of multi-walled carbon nanotubes (MWCNTs) and titanium oxide nanoparticles (TiO_2) was synthesized and used as an ion-to-electron transducer for the designed CPEs. MWCNTs are distinguished by their mechanical and electrical properties, high charge transfer capacity, large surface area, and electronic transport properties, in addition to their mechanical and thermal stability [43, 44]. The hydrophobic characteristic of MWCNTs avoids the development of a water layer at the metal conductor/carbon paste interface, boosting the CPE's stability. Incorporating metal oxide nanoparticles onto the surface of MWCNTs enhances their electrical, magnetic, catalytic, and optical properties [45-47]. TiO_2 nanoparticles exhibit remarkable electrical properties, significantly improving the transfer of electrons, electrode conductivity, and analytical response [38].

The ion recognition element is the second critical component of the proposed CPEs. It is vital to select an effective ligand/ ionophore upon the fabrication of a new chemical sensor. The ionophore is responsible for specific analyte binding as well as ensuring sensor stability, repeatability, and durability. In this study, three CPEs were designed utilizing three different azamacrocyclic ionophores: cyclen, hexacyclen, and kryptofix-22, separately. The protonated amines of azamacrocyclic ionophores can bind a wide range of anions, depending on their size through the formation of ionic hydrogen bonding [48].

The effective application of these ionophores as recognition elements for determining the GA is dependent on the pH of the medium and the pKa of the GA. The protonation of the GA reduces its anionic charge and negatively influences its binding/coordination with the cationic center in the ionophore. At the same time, the anion recognition sites of the azamacrocyclic ionophores require protonation to bind an anion. In light of these two opposing variables, the anion recognition process often occurs at an optimal pH level [48]. Cyclen with pKa values of 10.7 and 9.7, hexacyclen with pKa values of 10.2, 9.2, and 8.7, kryptofix-22 with pKa values of 9.3 and 8.2 are protonated at pH values below their pKa values [49-51]. GA is a weak acid with a pKa value of 4.4. It exists in molecular form at low pH ($\text{pH} < 4.4$) and becomes ionized as pH increases [52, 53]. Hence, it should be considered that the recognition of the gallic anion by the protonated azamacrocyclic ionophores is pH-dependent. **Fig. 1** represents the chemical structure of the gallic acid and the three azamacrocyclic ionophores.

This study is considered unique in using azamacrocyclic compounds as recognition elements for GA potentiometric assessment. The generated CPEs were successfully employed to selectively detect GA in pure form, *Krameria lappacea* root alcoholic extract, tea extracts, and spiked human plasma.

2. Experimental

2.1. Apparatus

Potentiometric measurements were conducted using a CLEAN digital ion analyzer PH 600, model 007747 (China). The potentiometer was linked to the fabricated indicator electrode and the reference electrode (Ag/AgCl -Thermo-Orion, model 900201). A magnetic stirrer (Heidolph MR Hei, model 100818877) was used for stirring GA samples during the measurement.

2.2. Materials and chemicals

Analytical-grade chemicals were used. Titanium oxide TiO_2 , multi-walled carbon nanotubes (MWCNTs), *N, N*-dimethylformamide (DMF), and graphite powder were acquired from Sigma Aldrich, USA. Tridodecyl(methyl)ammonium chloride (TDMAC) was purchased from Acros Organics. Cyclen, hexacyclen, and kryptofix 22 were obtained from Merck, USA. The standard material of GA monohydrate (HPLC > 98%) was supplied from Alpha Chemika, Mumbai-400002 (India). Salicylic acid and vanillic acid were obtained from Fluka. The Britton-Robinson aqueous buffer solution was prepared by mixing the appropriate volumes of phosphoric acid (0.04 M), boric acid (0.04 M), and acetic acid (0.04 M). Sodium hydroxide solution (0.2 M) was used for pH adjustment.

2.3. Preparation of standard solutions

The GA standard solution (1×10^{-1} M) was prepared by dissolving 1.7 g of the standard material in bi-distilled water. Concentrations ranging from 1×10^{-10} M to 1×10^{-2} M were obtained by dilution of the stock solution with pH 6 Britton-Robinson buffer.

2.4. Procedure

2.4.1. Preparation of a nanocomposite of MWCNTs and TiO_2

The preparation of MWCNTs/ TiO_2 nanocomposite was conducted as described in our prior work [45-47]. Briefly, the carboxylation of pristine MWCNTs was carried out using a blend of HNO_3 and H_2SO_4 (1:3 v/v). Deionized water was used to wash the produced MWCNTs-COOH which was subsequently vacuum-dried and ultrasonically mixed with TiO_2 nanoparticles in DMF solvent for 12 h at room temperature before being magnetically stirred at 700 rpm. The MWCNTs/ TiO_2 nanocomposite was washed and vacuum dried at 70°C for 24 h.

2.4.2. Fabrication of the proposed CPEs

The optimal potentiometric response was obtained through the preparation of a carbon paste using 50:10:7:3: 30 % w/w graphite, MWCNTs/ TiO_2 , ionophore, TDMAC, and paraffin oil. Three different ionophores (cyclen, hexacyclen, and kryptofix-22) were utilized for the fabrication of CPE1, CPE2, and CPE3; respectively. To eliminate any air gaps, the paste components were thoroughly mixed and carefully placed in the Teflon chamber of the CPE's metallic spacer. The paste surface was polished with clean filter paper. Before measurement, each CPE was conditioned in 1×10^{-4} M GA aqueous solution for 1 hour. Following multiple measurements, a new paste surface would be obtained by scraping off 2 to 3 mm of the old surface to regenerate the electrode surface.

2.5. Study the selectivity of the proposed CPE

The CPE's selectivity in the presence of structurally similar molecules and interferents was assessed by the matched potential approach. The selectivity coefficient ($K^{\text{pot}}_{\text{GA, interferent}}$) was calculated as the exact proportions of the primary ion (GA) and the interfering ion that produce an equal change in a reference solution potential value under precisely the same conditions. The selectivity coefficient is calculated following the IUPAC criteria using Equation (1) [54]:

$$K^{\text{pot}}_{(A, B)} = \frac{a'_A - a_A}{a_B} \quad (1)$$

The symbols A and B represent GA and interfering ions, respectively. The symbol a_A indicates the activity of the reference GA solution, while a'_A denotes the activity of the additional GA that changes the potential reading. a_B is the interfering ion's activity that is added to the reference solution, causing an equivalent potential shift.

2.6. Molecular Docking Study

A combined flexible alignment and molecular docking study for GA complexes with cyclen, hexacyclen, and kryptofix-22 (ionophores) was performed using Molecular Operating Environment (MOE) 2022 [55] (Chemical Computing Group, Montreal, QC, Canada). The three-dimensional structure of cyclen was obtained from the crystal structure (pdb code: 3I8Y) [56] after removing the protein of human chromobox homolog 2 (CBX2). The three-dimensional structure of hexacyclen and kryptofix-22 were used after the modification of 18-crown-6 which was extracted from the crystal structure of the DMP19 complex (pdb code: 3WUR) [57]. The modification was achieved by using a builder in which all oxygens in 18-crown-6 were changed to nitrogen in the case of hexacyclen while only two oxygens were changed for kryptofix-22. GA was built in MOE using the builder. To prepare the three ionophore structures for the flexible alignment process, partial charges were added (protonation at pH = 6). Potential energy was minimized by applying the force field MMFF94x and refinement at gradient RMS of 0.1 Kcal/mol/Å². Finally, join chains were applied to generate the ligand complexes with complete flexibility. Molecular dynamic simulation (MDS) was performed on the optimal flexible alignment pose using the Nosé-Poincaré-Andersen (NPA) algorithm for creating the trajectory from 0 picoseconds (ps) to 1500 ps [58].

2.7. Determination of gallic acid in different matrices.

2.7.1. Quantitation of gallic acid in the alcoholic extract of *Krameria Lappacea*

Krameria lappacea roots were purchased from a local herb store in Al-Madinah Al-Munawarah, Saudi Arabia. The identity of the plant was identified by a taxonomist at the College of Science, Taibah University, Al-Madinah Al-Munawarah. The roots were cut into small pieces and pulverized with a grinder. 1 g of the powder was extracted with 200 mL of ethanol at room temperature for 48 h. The extract was filtered with Whatman filter paper, and the ethanol solvent was evaporated at 50 °C. The produced residue was redissolved in a minimum amount of ethanol and completed with 100 mL of 0.1 M Britton-Robinson buffer (pH 6). Standard aliquots of different concentrations of GA were added to 10 mL of the plant extract and the electromotive force (*e.m.f*) was recorded by immersing each of the CPE2 and CPE3, separately in conjunction with the Ag/AgCl reference electrode.

2.7.2. Quantitation of gallic acid in tea samples

The concentration of GA in tea samples was determined by taking 1 g of dried powdered green and black tea leaves bought from the local market and mixing it with 25 mL of double distilled water. The samples were then heated to 80 °C for 10 minutes and cooled to room temperature. After that, the tea extracts were filtered and diluted to 100 mL in pH 6 Britton-Robinson buffer. Potentiometric measurements are carried out by spiking 10 mL of tea extract with known concentrations of standard GA and recording the *e.m.f*. using CPE2 and CPE3.

2.7.3. Quantitation of gallic acid in spiked human plasma

The concentration of GA in plasma samples was measured directly with no need for extensive extraction processes. 1 mL of plain plasma samples (provided by Experiments and Advanced Research Unit, Cairo, Egypt) was spiked with different aliquots of standard GA, followed by 1 mL of acetonitrile and centrifuged at 5000 rpm for 10 minutes. In 10 mL volumetric flasks, 0.5 mL of the clear supernatants were diluted with Britton-Robinson buffer and their *e.m.f* was recorded by using CPE2 and CPE3. The corresponding regression equation was applied for the quantitation of GA.

3. Results and discussion

3.1. Characterization of the ion-to-electron transducer element

The FT-IR spectrum of TiO₂ nanoparticles in **Fig. 2(a)** shows broadband at 578 cm⁻¹, which is attributed to Ti-O-Ti stretching and bending modes. In **Fig. 2(b)**, a stretching vibration of the C=O group is observed at 1726 cm⁻¹. The band at 3482 cm⁻¹ indicates an O-H stretching vibration. Additionally, the peaks ranging from 2850 to 2920 cm⁻¹ suggest stretching vibrations of C-H. These observations indicate the successful introduction of carboxyl groups onto the surface of MWCNTs after the reaction with nitric acid. Furthermore, the FT-IR spectrum of MWCNTs@TiO₂ in **Fig. 2(c)** reveals a wide band centered at 584 cm⁻¹, demonstrating the adhesion of TiO₂ nanoparticles to the surface of MWCNTs. Decreasing the intensity of C=O stretching at 1718 cm⁻¹ demonstrates the consumption of COOH of MWCNTs by co-precipitation with TiO₂ nanoparticles.

In the TEM image, **Fig. 3(a)**, the MWCNTs displayed characteristic tubular structures with predominantly smooth surfaces. The clarity of the MWCNTs sidewalls was notable. After the reaction of TiO₂ with MWCNTs-COOH, the metal oxide spherical nanoparticles effectively coated the surface of MWCNTs, increasing the thickness of their sidewalls, as shown in **Fig. 3(b)**.

3.2. Molecular dynamic simulation (MDS)

The molecular dynamic studies showed promising binding interactions between GA and all three electron recognition elements, individually, with a preference for hexacyclen and kryptofix. As shown in **Fig. 4**, binding interactions were observed between the carboxylate moiety of GA and the protonated N of the ionophores. H-bonding was obtained between the carbonyl O of GA and NH²⁺, in addition to strong electrostatic interactions between carboxylate anion O⁻ of GA and the positively charged nitrogen in the three ionophores. Owing to the presence of six nitrogens in hexacyclen (**Fig. 4b**), multiple electrostatic interactions were observed compared with cyclen (**Fig. 4a**) and Kryptofix-22 (**Fig. 4c**). Nevertheless, based on the size-fit, the size of kryptofix-22 is slightly larger than that of hexacyclen, which may allow gallic acid to interact with kryptofix-22 cavity more easily compared to hexacyclen. Size differences were measured according to the average distance of three diameters for the two amino groups and four oxygens in kryptofix-22 (5.85 Å), and six amino groups in hexacyclen (5.70 Å) mainly after energy minimizing by Moe. Additionally, the electrostatic repulsion between adjacent NH²⁺ groups in hexacyclen may play a crucial role in delaying the interaction between gallic acid and hexacyclen experimentally, providing an advantage for kryptofix-22 to interact faster with gallic acid. Thus, the charge distribution on hexacyclen could affect its stability and the rate of binding interaction with gallic acid.

Figures 4d, 4e, and 4f illustrate the conformational stability of GA with cyclen, hexacyclen, and Kryptofix-22 during the 1500 ps simulation time. The trajectories showed that there was no deviation as well as the consistency during the simulation.

3.3. Potentiometric response characteristics of the proposed CPE

3.3.1. Optimum composition of the designed CPEs

The crucial step in constructing the CPE is the determination of the optimal structure of the electrode paste. Several experiments were conducted utilizing CPEs having different component ratios as shown in **Table 1**. The hexacyclen ionophore was used as a model recognition element to establish the best electrode composition. Sensor 1 was created by uniformly mixing 70% w/w of graphite powder with 30% w/w of paraffin oil. A homogenous and durable paste is produced. Sensors 2, 3, and 4 were developed by mixing different proportions of hexacyclen and TDMAC. The presence of TDMAC as a lipophilic cationic additive demonstrated an appropriate anionic Nernstian slope and optimal potentiometric response. Sensor 2 demonstrated a better Nernstian response than sensor 1 owing to the presence of 7% hexacyclen. The influence of transducer composition was investigated by the design of three sensors (sensors 5, 6, 7) with MWCNTs, TiO₂, and MWCNTs/TiO₂ composite; respectively. Overall, the influence of MWCNTs/TiO₂ on the CPE response was remarkable. It resulted in a better Nernstian slope, a shorter response time, and higher sensitivity. The optimal response was obtained by combining graphite, paraffin oil, MWCNTs/TiO₂, ionophore, and TDMAC at a ratio of 50:30:10:7:3% w/w. This ratio was taken into account when fabricating the Other CPEs using cyclen and kryptofix-22 ionophores.

3.3.2. Potentiometric response characteristics of the recognition elements

As previously stated, using polyazamacrocycles as recognition elements is extremely promising. Polyazamacrocycles are protonated at lower pH to produce cationic sites -NH⁺ (pK_a = 9.8), which bind anionic guests via electrostatic and H-bonding interactions. In this investigation, three CPEs with three distinct polyazamacrocycle ionophores, cyclen (CPE 1), hexacyclen (CPE 2), and kryptofix-22 (CPE 3) were developed and used to selectively recognize the GA at pH 6. The potentiometric responses of the CPEs were studied following IUPAC criteria [54] and summarized in **Table 2**.

Figure 5 shows that CPE 2 and CPE 3 had linear potentiometric responses with perfect Nernstian slopes of 59.8 ± 0.74 and 57.478 ± 0.82 mV/decade, respectively. CPE 3 demonstrated greater stability and sensitivity at LOD 1×10^{-7} M. Two aspects may contribute to GA's efficient identification with hexacyclen and kryptofix-22. First, there is an electrostatic and hydrogen bonding between the protonated versions of the ionophores and the GA. Second, the size-fit of the ionophores and the guest GA molecule. However, by using the larger spacer groups in kryptofix-22, the protonated forms of kryptofix-22 can act as anion receptors that sense the anionic form of GA faster than hexacyclen-based CPE.

Three quality-control standards of concentrations 1×10^{-3} M, 1×10^{-4} M, and 1×10^{-5} M were utilized for testing the precision of CPE 2 and 3. While 1×10^{-1} M, 1×10^{-2} M, and 1×10^{-3} M were used in the case of CPE 1. The RSD % was below 2%, particularly in CPE 2 and 3. The reproducibility was evaluated using three separately prepared sensors for each of the studied CPEs under the same conditions and the RSD % was less than 2%. It proved the agreement of the results from replicate analysis of the same sample upon using different electrodes of the same composition.

The dynamic response time is the time needed for the electrode to attain ± 1.5 mV of the original equilibrium potential values after a 10-fold increase in the analyte concentration. CPE 3 was found to have the fastest response time within 10 seconds. The time required to reach equilibrium values following a 10-fold decrease in GA concentration was considered and found to be longer. It took approximately 160 s, 100 s, and 40 s for CPE 1, CPE 2, and CPE 3, respectively as shown in **Fig. S1**. It ensures the reversibility and the fast response of the designed CPEs.

The stability of the proposed CPEs was detected by continuously monitoring their calibration slope, linearity range, and response time without surface renewal of the electrodes' paste. For CPE 1, CPE 2, and CPE 3, electrode responses remained within $\pm 2\%$ of their initial values after 41, 63, and 80 days, respectively.

3.4. pH effect on the response of the CPEs

The efficient alignment of GA with the recognition element directly affects the response of the studied sensors. GA is a phenolic compound with a pKa value of 4.4 [52, 53]. It is represented as a weak acid and existed in the molecular form at pH values lower than 4.4 and became ionized as the pH of the solution increased. In addition, the anion recognition sites very often need protonation for anion binding. Because of these two opposing factors, the pH value is considered a key factor for the anion recognition that generally occurs at an optimum pH value.

A standard concentration of 1×10^{-3} M was prepared at different pH values ranging from 2 to 10 and applied to study the impact of pH. CPE 2, and CPE 3 showed stable, constant readings over the range of 4.5 to 6.5 and 4.5 to 6, respectively, as shown in **Fig. S2**. In the case of CPE 1, it showed a stable potential response over the pH range of 5 to 7. Thus, pH 6 was chosen as the recommended pH value for the suggested sensors. The observed CPE's stable response over these ranges can be related to the protonation of the ionophores and the presence of GA in anionic form. It promotes the binding of anionic guests via electrostatic and H-bonding interactions. Above pH 9, potential measurements were reduced due to the presence of non-protonated ionophores, which resulted in restricted interaction with GA. Nonetheless, below pH 3, the ionophores are fully protonated, whereas GA is non-ionized, reducing the potentiometric reading of the sensors.

3.5. Selectivity Study

The selectivity of the studied CPEs was assessed by the matched potential method. The method involved the addition of a known amount of GA solution (a_A') to a reference solution of 1×10^{-3} M GA for CPE1 and 1×10^{-4} M GA for CPE 2 and CPE 3. The subsequent potential change (ΔE) was calculated. This is followed by the addition of the interfering ion solution with an activity of (a_B) to the reference solution to generate an equal change in the CPE potential (ΔE). The selectivity coefficient ($\log K_{GA, Interferent}^{pot}$) was calculated according to IUPAC guidelines [54]. **Table 3** displays the values of the selectivity coefficient, demonstrating the great selectivity of CPE 2 and CPE 3 towards GA in the presence of other interferents.

Table S1 demonstrates a short comparison of the response characteristics between the proposed CPEs and the previously published ones. All the literature-stated sensors were designed as classical liquid contact ISEs with PVC membrane sandwiched between an internal filling solution and the sample to develop a liquid-contact interface. The PVC membrane in one of the reported electrodes is based on the application of molecularly imprinted polymer for GA as a recognition element [25]. The other reported one utilized calix-6-arene and 2-hydroxy propyl β -cyclodextrin separately as ionophores in a polymeric PVC membrane [26]. The biggest obstacle for the classical liquid contact ISEs is the difficulty of the miniaturization, fragility, the need for additional maintenance due to evaporation of the inner filling solution, and the leaching of some membrane components over time [34, 35]. All these factors passively affect the stability and the sensitivity of the ISE.

The results demonstrated that the suggested CPE 2 and CPE 3 exhibited remarkable responses to GA and demonstrated a wider linearity and sensitivity than the other reported ones. The proposed sensors exhibited higher stability and longer lifetimes for 63 and 80 days of CPE 2 and CPE 3, respectively, without any surface renewal steps. The lifetime of the proposed sensors could be extended by surface renewal 3 to 4 times for the same paste.

3.6. Water layer test

The water layer test focuses on the detection of any potential drift caused by the water layer between the carbon paste and the conducting copper element [59]. In this experiment, the potential reading of 1×10^{-3} M of the GA standard solution was examined for 3 hours. This was followed by

exposing the electrode to 1×10^{-3} M of acetylsalicylic acid as an interfering ion for another 3 hours and then returning to 1×10^{-3} M of GA standard solution for 3 hours. **Fig. S3** indicates that the response of the three sensors remained constant after 3 h of exposure to the interfering ion, showing the non-existence of a water layer. This could be ascribed to the hydrophobic character of the produced paste, which prevents the development of a water layer at the interface between the paste and the CPE body's conducting surface.

3.7. Analytical applications

As previously mentioned, CPE 2 and CPE 3 are characterized by high sensitivity, stability, and a wider linearity range. It makes them successful candidates for the quantitation of GA in various matrices. 10 mL of *Krameria Lappacea* extract, black tea, and green tea extracts were spiked separately with known concentrations of standard GA. The *emf* of the solutions was measured and the GA concentration was estimated using the standard addition method. In the case of plasma samples, the developed sensors were applied for the determination of GA concentrations using the corresponding regression equation in plasma. The results in **Table 4** displayed the efficiency and accuracy of the CPEs in the determination of GA in various matrices.

A statistical comparison between the results of the studied CPEs and the reported method [15] for the determination of GA in standard solution was applied using the F-test and the student's t-test at p 0.05. As represented in **Table S2**, no significant differences have been recorded.

4. Conclusion

GA is among the most significant polyphenols, with biological characteristics that make it popular in the food, cosmetic, and pharmaceutical industries. Thus, simple, rapid, and sensitive CPEs were used to determine GA in various matrices. Aza-macrocyclic ionophores were used as GA recognition elements at pH 6. Hexacyclen and kryptofix-22 demonstrated great selectivity and sensitivity in the presence of MWCNTs/TiO₂ nanocomposite transducer. The examined sensors provided a challenging response for quantifying GA in various matrices including bulk, *Krameria Lappacea* extract, black and green tea extracts, and human plasma, with no requirement for any pretreatment measures. The LOD values for CPE 1, CPE 2, and CPE 3 were 4×10^{-4} M, 3×10^{-7} M, and 7×10^{-7} M, indicating great sensitivity and selectivity to GA. The proposed sensors would accurately determine GA in the routine operations of quality control laboratories and different healthcare activities.

Conflicts of Interest:

There are no conflicts of interest to declare.

References

- [1] A.A. Zanwar, S.L. Badole, P.S. Shende, M.V. Hegde, S.L. Bodhankar, Chapter 80 - Role of Gallic Acid in Cardiovascular Disorders, in: R.R. Watson, V.R. Preedy, S. Zibadi (Eds.) Polyphenols in Human Health and Disease, Academic Press, San Diego, 2014, pp. 1045-1047.
- [2] R. Nowak, M. Olech, N. Nowacka, Plant Polyphenols as Chemopreventive Agents, in: R.R. Watson, V.R. Preedy, S. Zibadi (Eds.) Polyphenols in Human Health and Disease, Academic Press, San Diego, 2014, pp. 1289-1307.
- [3] B. Badhani, N. Sharma, R. Kakkar, Gallic acid: a versatile antioxidant with promising therapeutic and industrial applications, RSC Advances, 5 (2015) 27540-27557. <https://doi.org/10.1039/C5RA01911G>
- [4] B.U. Islam, M. Suhail, M.K. Khan, T.A. Zughaibi, R.F. Alserihi, S.K. Zaidi, S. Tabrez, Polyphenols as anticancer agents: Toxicological concern to healthy cells, Phytother Res, 35 (2021) 6063-6079. <https://doi.org/10.1002/ptr.7216>

- [5] B. Uttara, A.V. Singh, P. Zamboni, R.T. Mahajan, Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options, *Curr Neuropharmacol*, 7 (2009) 65-74. DOI: 10.2174/157015909787602823
- [6] R. Purgatorio, A. Boccarelli, L. Pisani, M. de Candia, M. Catto, C.D. Altomare, A Critical Appraisal of the Protective Activity of Polyphenolic Antioxidants against Iatrogenic Effects of Anticancer Chemotherapeutics, *Antioxidants (Basel)*, 13 (2024) 133. <https://doi.org/10.3390/antiox13010133>
- [7] M. Govea-Salas, A.M. Rivas-Estilla, J. Ascacio-Valdés, A. Zugasti-Cruz, R. Rodríguez-Herrera, R. Belmares-Cerda, J. Morlett-Chávez, Gallic Acid as a Putative Antioxidant in Usage Against Liver Disease, in: V.B. Patel, R. Rajendram, V.R. Preedy (Eds.) *The Liver*, Academic Press, Boston, 2018, pp. 317-322.
- [8] E. Scholz, H. Rimpler, Proanthocyanidins from *Krameria triandra* root, *Planta Med*, 55 (1989) 379-384. DOI: 10.1055/s-2006-962032
- [9] M.M. Al-Oqail, Anticancer efficacies of *Krameria lappacea* extracts against human breast cancer cell line (MCF-7): Role of oxidative stress and ROS generation, *Saudi Pharmaceutical Journal*, 29 (2021) 244-251. <https://doi.org/10.1016/j.jsps.2021.01.008>
- [10] M. Onofre, C. Rocha, F. Fimbres-López, E. Rosas-Burgos, A. Burgos-Hernández, M. Plascencia-Jatomea, L. María, M. Aldana, O. Cota-Arriola, E. Quintana-Obregón, M. Cortez-Rocha, Antifungal activity of methanolic extracts of *Jacquinia macrocarpa* and *Krameria erecta* on the growth of *Fusarium verticillioides* and effect on fumonisin production, *Revista Mexicana de Micología*, 43 (2016) 1-9.
- [11] M.Á. Ramírez-Cisneros, M.Y. Rios, M. Déciga-Campos, A.B. Aguilar-Guadarrama, Phytochemical Study and Anti-inflammatory, Antidiabetic and Free Radical Scavenger Evaluations of *Krameria pauciflora* Methanol Extract, *Molecules*, 17 (2012) 861-872. <https://doi.org/10.3390/molecules17010861>
- [12] X. Wang, J. Wang, N. Yang, Flow injection chemiluminescent detection of gallic acid in olive fruits, *Food Chemistry*, 105 (2007) 340-345. <https://doi.org/10.1016/j.foodchem.2006.11.061>
- [13] S. Li, H. Sun, D. Wang, L. Qian, Y. Zhu, S. Tao, Determination of Gallic Acid by Flow Injection Analysis Based on Luminol-AgNO₃-Ag NPs Chemiluminescence System, *Chinese Journal of Chemistry*, 30 (2012) 837-841. <https://doi.org/10.1002/cjoc.201100160>
- [14] N. Denderz, J. Lehotay, Using of molecularly imprinted polymers for determination of gallic and protocatechuic acids in red wines by high performance liquid chromatography, *Journal of Chromatography A*, 1372 (2014) 72-80. <https://doi.org/10.1016/j.chroma.2014.10.070>
- [15] F.H.A. Fernandes, R.S.d.A. Batista, F.D. de Medeiros, F.S. Santos, A.C.D. Medeiros, Development of a rapid and simple HPLC-UV method for determination of gallic acid in *Schinopsis brasiliensis*, *Revista Brasileira de Farmacognosia*, 25 (2015) 208-211. <https://doi.org/10.1016/j.bjp.2015.05.006>
- [16] C. Richard-Dazeur, P. Jacolot, C. Niquet-Léridon, L. Goethals, N. Barbezier, P.M. Anton, HPLC-DAD optimization of quantification of vescalagin, gallic and ellagic acid in chestnut tannins, *Heliyon*, 9 (2023) e18993. <https://doi.org/10.1016/j.heliyon.2023.e18993>
- [17] S. Shahrzad, I. Bitsch, Determination of gallic acid and its metabolites in human plasma and urine by high-performance liquid chromatography, *J Chromatogr B Biomed Sci Appl*, 705 (1998) 87-95. [https://doi.org/10.1016/S0378-4347\(97\)00487-8](https://doi.org/10.1016/S0378-4347(97)00487-8)
- [18] Y.-X. Sheng, L. Li, Q. Wang, H.-Z. Guo, D.-A. Guo, Simultaneous determination of gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid in Si-Wu decoction by high-performance liquid chromatography DAD method, *Journal of Pharmaceutical and Biomedical Analysis*, 37 (2005) 805-810. <https://doi.org/10.1016/j.jpba.2004.11.002>
- [19] H. Wang, K. Helliwell, X. You, Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC, *Food Chemistry*, 68 (2000) 115-121. [https://doi.org/10.1016/S0308-8146\(99\)00179-X](https://doi.org/10.1016/S0308-8146(99)00179-X)

- [20] Z. Sun, L. Zhao, L. Zuo, C. Qi, P. Zhao, X. Hou, A UHPLC–MS/MS method for simultaneous determination of six flavonoids, gallic acid and 5,8-dihydroxy-1,4-naphthoquinone in rat plasma and its application to a pharmacokinetic study of Cortex Juglandis Mandshuricae extract, *Journal of Chromatography B*, 958 (2014) 55-62. <https://doi.org/10.1016/j.jchromb.2014.03.013>
- [21] L. Wang, M.S. Halquist, D.H. Sweet, Simultaneous determination of gallic acid and gentisic acid in organic anion transporter expressing cells by liquid chromatography-tandem mass spectrometry, *J Chromatogr B Analyt Technol Biomed Life Sci*, 937 (2013) 91-96. <https://doi.org/10.1016/j.jchromb.2013.08.024>
- [22] M.S. Masoud, A.E. Ali, S.S. Haggag, N.M. Nasr, Spectroscopic studies on gallic acid and its azo derivatives and their iron(III) complexes, *Spectrochim Acta A Mol Biomol Spectrosc*, 120 (2014) 505-511. <https://doi.org/10.1016/j.saa.2013.10.054>
- [23] J. Pooralhossini, M. Ghaedi, M.A. Zanjanchi, A. Asfaram, The choice of ultrasound assisted extraction coupled with spectrophotometric for rapid determination of gallic acid in water samples: Central composite design for optimization of process variables, *Ultrasonics Sonochemistry*, 34 (2017) 692-699. <https://doi.org/10.1016/j.ultsonch.2016.07.003>
- [24] M. Zaripour, V. Zare-Shahabadi, H.J. Jahromi, Application of ultrasonic-assisted inclusion complex formation with α -cyclodextrin for simultaneous spectrophotometric determination of gallic acid and vanillic acids in fruit samples, *Spectrochim Acta A Mol Biomol Spectrosc*, 222 (2019) 117197. <https://doi.org/10.1016/j.saa.2019.117197>
- [25] T. Yang, Q. Zhang, T. Chen, W. Wu, X. Tang, G. Wang, J. Feng, W. Zhang, Facile potentiometric sensing of gallic acid in edible plants based on molecularly imprinted polymer, *J Food Sci*, 85 (2020) 2622-2628. <https://doi.org/10.1111/1750-3841.15346>
- [26] M.F.A. Ghany, L. A.Hussein, H. Z.Yamani, Comparative study of calix-6-arene and 2-hydroxy propyl α -cyclodextrin as ionophores in potentiometric ion-selective electrodes for determination of gallic acid in Acacia arabica bark extract, *Analytical Chemistry An Indian Journal*, 14 (2014) 378-383.
- [27] Z. Bitew, A. Kassa, B. Misgan, Poly(diphenylamine-4-sulfonic acid) modified glassy carbon electrode for voltammetric determination of gallic acid in honey and peanut samples, *Arabian Journal of Chemistry*, 15 (2022) 103853. <https://doi.org/10.1016/j.arabjc.2022.103853>
- [28] C.O. Chikere, E. Hobben, N.H. Faisal, P. Kong-Thoo-Lin, C. Fernandez, Electroanalytical determination of gallic acid in red and white wine samples using cobalt oxide nanoparticles-modified carbon-paste electrodes, *Microchemical Journal*, 160 (2021) 105668. <https://doi.org/10.1016/j.microc.2020.105668>
- [29] S. Feng, X. Zhou, X. Chen, G. Zhang, G. Liu, N. Wu, W. Liu, Au@Ag core-shell Nanomaterials Embedded in N-doped graphene: A Novel Electrochemical Sensor for Determination of Gallic Acid, *International Journal of Electrochemical Science*, 15 (2020) 6908-6919. <https://doi.org/10.20964/2020.07.28>
- [30] S. Lisnund, V. Blay, K. Chansaenpak, P. Pinyou, Voltammetric Determination of Gallic Acid with a Glassy Carbon Electrode modified with Reduced Graphene Oxide, *International Journal of Electrochemical Science*, 15 (2020) 7214-7227. <https://doi.org/10.20964/2020.08.06>
- [31] Z. Mahmoudi, J. Tashkhourian, B. Hemmateenejad, A disposable paper-based microfluidic electrochemical cell equipped with graphite-supported gold nanoparticles modified electrode for gallic acid determination, *Journal of Electroanalytical Chemistry*, 920 (2022) 116626. <https://doi.org/10.1016/j.jelechem.2022.116626>
- [32] F. Nazari, S.M. Ghoreishi, A. Khoobi, Bio-based Fe₃O₄/chitosan nanocomposite sensor for response surface methodology and sensitive determination of gallic acid, *International Journal of Biological Macromolecules*, 160 (2020) 456-469. <https://doi.org/10.1016/j.ijbiomac.2020.05.205>
- [33] M. Sisay, A. Kassa, A. Tesfaye, Highly selective square wave voltammetric determination of gallic acid in groundnut and tea samples using glycine(2-aminoethanoic acid) modified carbon paste electrode, *Sensors International*, 4 (2023) 100227. <https://doi.org/10.1016/j.sintl.2023.100227>

- [34] E. Bakker, P. Bühlmann, E. Pretsch, Carrier-Based Ion-Selective Electrodes and Bulk Optodes. 1. General Characteristics, *Chem Rev*, 97 (1997) 3083-3132. <https://doi.org/10.1021/cr940394a>
- [35] Y. Lyu, S. Gan, Y. Bao, L. Zhong, J. Xu, W. Wang, Z. Liu, Y. Ma, G. Yang, L. Niu, Solid-Contact Ion-Selective Electrodes: Response Mechanisms, Transducer Materials and Wearable Sensors, *Membranes* (Basel), 10 (2020) 128. <https://doi.org/10.3390/membranes10060128>
- [36] D.S. Silvester, Recent advances in the use of ionic liquids for electrochemical sensing, *Analyst*, 136 (2011) 4871-4882. <https://doi.org/10.1039/C1AN15699C>
- [37] I. Švancara, K. Vytrás, K. Kalcher, A. Walcarius, J. Wang, Carbon Paste Electrodes in Facts, Numbers, and Notes: A Review on the Occasion of the 50-Years Jubilee of Carbon Paste in Electrochemistry and Electroanalysis, *Electroanalysis*, 21 (2009) 7-28. <https://doi.org/10.1002/elan.200804340>
- [38] Y. Liu, Y. Liu, R. Yan, Y. Gao, P. Wang, Bimetallic AuCu nanoparticles coupled with multi-walled carbon nanotubes as ion-to-electron transducers in solid-contact potentiometric sensors, *Electrochimica Acta*, 331 (2020) 135370. <https://doi.org/10.1016/j.electacta.2019.135370>
- [39] Y. Shao, Y. Ying, J. Ping, Recent advances in solid-contact ion-selective electrodes: functional materials, transduction mechanisms, and development trends, *Chemical Society Reviews*, 49 (2020) 4405-4465. <https://doi.org/10.1039/C9CS00587K>
- [40] J. Ping, Y. Wang, J. Wu, Y. Ying, Development of an all-solid-state potassium ion-selective electrode using graphene as the solid-contact transducer, *Electrochemistry Communications*, 13 (2011) 1529-1532. <https://doi.org/10.1016/j.elecom.2011.10.018>
- [41] J. Ping, J. Wu, Y. Wang, Y. Ying, Simultaneous determination of ascorbic acid, dopamine and uric acid using high-performance screen-printed graphene electrode, *Biosensors and Bioelectronics*, 34 (2012) 70-76. <https://doi.org/10.1016/j.bios.2012.01.016>
- [42] Y. Shao, Y. Yao, C. Jiang, F. Zhao, X. Liu, Y. Ying, J. Ping, Two-dimensional MXene nanosheets (types Ti₃C₂T_x and Ti₂CT_x) as new ion-to-electron transducers in solid-contact calcium ion-selective electrodes, *Microchimica Acta*, 186 (2019) 750. <https://doi.org/10.1007/s00604-019-3878-7>
- [43] A.J.S. Ahammad, J.-J. Lee, M.A. Rahman, Electrochemical Sensors Based on Carbon Nanotubes, *Sensors*, 9 (2009) 2289-2319. <https://doi.org/10.3390/s90402289>
- [44] C. Hu, H. Shengshui, Carbon Nanotube-Based Electrochemical Sensors: Principles and Applications in Biomedical Systems, *Journal of Sensors*, 2009 (2009) 187615. <https://doi.org/10.1155/2009/187615>
- [45] N.A. Abdallah, S. Ahmed, Evaluation of MWCNTs/TiO₂/Chitosan composite as a carbon paste electrode for the determination of pazufloxacin, *Journal of The Electrochemical Society*, 165 (2018) H756. DOI 10.1149/2.1151811jes
- [46] N.A. Abdallah, Application of Titanium Oxide Decorated Multi-walled Carbon Nanotubes/Polyaniline as a Transducer Polymer for the Potentiometric Determination of Mirtazapine, *ChemistrySelect*, 7 (2022) e202202985. <https://doi.org/10.1002/slct.202202985>
- [47] N.A. Abdallah, Solid-Contact ISE for the Potentiometric Determination of Melitracen Hydrochloride in Pharmaceutical Tablets and Human Plasma, *Journal of The Electrochemical Society*, 167 (2020) 047504. DOI 10.1149/1945-7111/ab7182
- [48] A.K. Das, M. Das, Anion Binding Hosts, Anion Coordination and Anion Recognition, *An Introduction to Supramolecular Chemistry*, CBS Publishers & Distributors Pvt Ltd, India, 2019.
- [49] E. Kimura, A. Sakonaka, T. Yatsunami, M. Kodama, Macromonocyclic Poly amines as Specific Receptors for Tricarboxylate-Cycle Anions, *Journal of American Chemical Society*, 103 (1981) 3041-3045. <https://doi.org/10.1021/ja00401a022>

- [50] M. Kodama, A Thermodynamic and Kinetic Interpretation of the Macrocyclic Effect. Polarographic Studies on Copper(II) 1,4,7,10 -Tetraazacyclododecane Complexation, *Journal of the Chemical Society, Chemical Communication*, 9 (1975) 326-327. <https://doi.org/10.1039/C39750000326>
- [51] E. Luboch, A. Cygan, J.F. Biernat, Macrocyclic Polyfunctional Lewis Bases. VII. Size and Structure Controlled Stabilities of Polyaza-crown Ether Complexes with Co^{2+} - Zn^{2+} Ions, *Inorganica Chimica Acta*, 68 (1983) 201-204. [https://doi.org/10.1016/S0020-1693\(00\)88961-6](https://doi.org/10.1016/S0020-1693(00)88961-6)
- [52] F.H.A. Fernandes, H.R.N. Salgado, Gallic Acid: Review of the Methods of Determination and Quantification, *Critical Reviews in Analytical Chemistry*, 46 (2016) 257-265. <https://doi.org/10.1080/10408347.2015.1095064>
- [53] M. Jabbari, Solvent dependence of protonation equilibria for gallic acid in water and different acetonitrile–water cosolvent systems, *Journal of Molecular Liquids*, 208 (2105) 5-10. <https://doi.org/10.1016/j.molliq.2015.03.055>
- [54] L. Ernő, U. Yoshio, Performance evaluation criteria for preparation and measurement of macro- and microfabricated ion-selective electrodes (IUPAC Technical Report), *Pure and Applied Chemistry*, 80 (2008) 85-104. <https://doi.org/10.1351/pac200880010085>
- [55] Molecular Operating Environment (MOE 2022), Chemical Computing Group Inc.: Montreal, QC, Canada, 2022.
- [56] M.F. Amaya, M. Ravichandran, P. Loppnau, I. Koziarzdzki, A.M. Edwards, C.H. Arrowsmith, J. Weigelt, C. Bountra, A. Bochkarev, J. Min, H. Ouyang, Crystal structure of human chromobox homolog 2 (CBX2) in complex with H3K27(me)3 peptide, *Protein Data Bank*, 2009.
- [57] S. Mukherjee, A. Roychowdhury, D. Dutta, A.K. Das, Crystal structures of triosephosphate isomerase from methicillin resistant *Staphylococcus aureus* MRSA252 provide structural insights into novel modes of ligand binding and unique conformations of catalytic loop, *Biochimie*, 94 (2012) 2532-2544. <https://doi.org/10.1016/j.biochi.2012.07.001>
- [58] H. AlRabiah, A. Homoda, A. Bakheit, G.A. Mostafa, Cyclodextrin potentiometric sensors based on selective recognition sites for procainamide: Comparative and theoretical study, *Open Chemistry*, 17 (2019) 1222-1234. <https://doi.org/10.1515/chem-2019-0131>
- [59] M. Fibbioli, W.E. Morf, M. Badertscher, N.F. de Rooij, E. Pretsch, Potential Drifts of Solid-Contacted Ion-Selective Electrodes Due to Zero-Current Ion Fluxes Through the Sensor Membrane, *Electroanalysis*, 12 (2000) 1286-1292. [https://doi.org/10.1002/1521-4109\(200011\)12:16<1286::AID-ELAN1286>3.0.CO;2-Q](https://doi.org/10.1002/1521-4109(200011)12:16<1286::AID-ELAN1286>3.0.CO;2-Q)

Figure Captions

Fig.1. Chemical structures of (a) gallic acid, (b) Cyclen, (c) Hexacyclen, and (d) Kryptofix-22.

Fig. 2. FT-IR spectra of (a) TiO₂ nanoparticles, (b) MWCNTs, and (c) MWCNTs/TiO₂ nanocomposite.

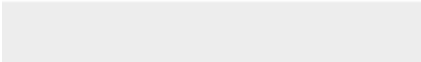
Fig. 3. TEM images of (a) MWCNTs and (b) MWCNTs/TiO₂ nanocomposite.

Figure 4: A three-dimensional representation of gallic acid (yellow) forming Hydrogen-bonding and electrostatic interactions (light blue bold lines) with (a) cyclen, (b) hexacyclen, and (c) kryptofix-22. Overlapping several trajectories of (d) cyclen, (e) hexacyclen, and (f) kryptofix-22 show conformational stability during 1500 ps simulation time.

Fig. 5. The potentiometric response of the carbon paste electrodes over gallic acid concentration range of 1×10^{-10} M to 1×10^{-2} M.



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Tables

Table 1. The impact of the electrode composition on its potentiometric performance

	Graphite (% w/w)	MWCNTs (% w/w)	TiO ₂ (% w/w)	MWCNTs/TiO ₂ composite (% w/w)	Hexacyclen (%w/w)	TDMAC (% w/w)	Paraffin Oil (%w/w)	Linearity range (M)	LOD (M)	(-) Slope (mV/decade)	Response time (s)
1	70	-	-	-	-	-	30	9 ×10 ⁻¹ - 1 ×10 ⁻¹	2 × 10 ⁻²	22.64	140
2	60	-	-	-	7	3	30	1 ×10 ⁻³ - 1×10 ⁻¹	5 ×1 0 ⁻⁴	50.76	100
3	60	-	-	-	8	2	30	1 ×10 ⁻² - 1×10 ⁻¹	9 ×1 0 ⁻³	48.69	100
4	60	-	-	-	5	5	30	1 ×10 ⁻² - 1×10 ⁻¹	6 × 10 ⁻³	49.05	110
5	50	10	-	-	7	3	30	1 ×10 ⁻⁴ - 1×10 ⁻²	6 × 10 ⁻⁵	51.47	60
6	50	-	10	-	7	3	30	1 ×10 ⁻⁵ - 1×10 ⁻²	8 × 10 ⁻⁶	53.04	50
7	50	-	-	10	7	3	30	1 ×10 ⁻⁶ - 1×10 ⁻²	3 × 10 ⁻⁷	59.44	25
8	52	-	-	8	7	3	30	1 ×10 ⁻⁵ - 1×10 ⁻²	7 × 10 ⁻⁶	53.71	35
9	48	-	-	12	7	3	30	1 ×10 ⁻⁶ - 1×10 ⁻²	8 × 10 ⁻⁷	55.04	30

Table 2. The potentiometric response characteristics of the studied CPEs.

Parameter	Cyclen based CPE (CPE 1)	Hexacyclen-based CPE (CPE 2)	Kryptofix 22-based CPE (CPE 3)
Concentration range (M)	$1 \times 10^{-1} - 1 \times 10^{-3}$	$1 \times 10^{-2} - 1 \times 10^{-6}$	$1 \times 10^{-2} - 1 \times 10^{-6}$
Slope (mV/decade)	50.7 ± 1.14	59.8 ± 0.74	57.5 ± 0.82
Intercept	131.23	272.13	204.87
Correlation coefficient (r)	0.9993	0.9995	0.9997
LOD (M)	4×10^{-4}	3×10^{-7}	7×10^{-7}
Response time (s)	42 ± 3	25 ± 2	10 ± 3
Stability (days)	41	63	80
Working pH range	5 - 7	4.5 - 6.5	4.5 - 6
Average recovery ¹	98.77 ± 0.78	99.68 ± 0.94	98.01 ± 0.92
Intraday precision ² (RSD %)	1.87	0.75	1.04
Interday precision ² (RSD %)	1.94	0.87	1.15
Reproducibility ³ (RSD %)	1.76	1.32	1.59

¹ The average of five measurements of five different concentration levels.

² The average of five determinations of three quality-control samples.

³ The mean of five determinations of three quality-control samples using three independently fabricated sensors.

Table. 3. Potentiometric selectivity coefficient ($\log K_{\text{gallic acid, Interferent}}^{\text{pot}}$) for the studied CPEs

Interfering ions	$\log K_{\text{GA, Interferent}}^{\text{pot}}$		
	Cyclen based CPE (CPE 1)	Hexacyclen-based CPE (CPE 2)	Kryptofix-22-based CPE (CPE 3)
Acetyl Salicylic acid	-3.78	-4.33	-4.65
Salicylic acid	-2.76	-4.17	-3.96
Ascorbic acid	-3.54	-3.22	-3.88
Vanillic acid	-2.63	-4.32	-4.18
Caffeic acid	-3.61	-4.01	-4.45
MgCl ₂	-2.88	-4.66	-4.18
ZnCl ₂	-3.45	-4.98	-3.93
Sucrose	-3.07	-3.96	-4.29
Rutin	-2.81	-3.99	-4.33
Glucose	-3.15	-4.32	-4.87
Lactose	-2.66	-4.76	-4.28

Table 4. Application of CPE 2 and CPE 3 for the quantitation of gallic acid in different matrices.

Sample	Hexacyclen-based CPE (CPE 2)			Kryptofix-22-based CPE (CPE 3)	
	Spiked concentration (µM)	Total found concentration* (µM)	Recovery %	Total found concentration* (µM)	Recovery %
<i>Krameria Lappacea</i> extract	0	54.5	-	56.3	-
	100	156	100.97	155	99.17
	200	253	99.41	258	100.66
Green tea extract	0	30.6	-	28.4	-
	50	83.4	103.47	80.2	102.56
	100	128.3	98.24	125.7	97.89
Black tea extract	0	43.6	-	45.0	-
	50	92.1	98.39	93.2	98.11
	100	144.4	100.56	142.6	98.34
Human plasma	20	18.8	94.00	19.1	95.50
	50	48.7	97.40	47.7	95.40
	100	98.6	98.60	97.6	97.60

* Average of three determinations

Declaration of interests

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.