

Research Article

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Bioanalytically validated potentiometric method for determination of bisphenol A: Application to baby bibs, pacifiers, and Teethers' saliva samples

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Abstract: A bioanalytically validated potentiometric method was proposed for the assay of bisphenol A (BPA) using a MWCNT-modified graphite ion selective electrode. The sensitivity of measurements was significantly enhanced by using MWCNTs as an ion-to-electron transducer layer. A precise linear analytical curve was developed to determine BPA in a specified linear range of 10,000–0.01 $\mu\text{mol}\cdot\text{L}^{-1}$, with a detection limit of 0.000104 $\mu\text{mol}\cdot\text{L}^{-1}$. After confirming that the results obtained with the proposed potentiometric sensor are not affected by the presence of possible interferents, the method was successfully applied to determine BPA concentration in different saliva samples, giving good accuracy and precision.

Keywords: bisphenol A, potentiometric sensor, multiwalled carbon nanotubes, ion-to-electron transducer, solid-contact electrode

1 Introduction

Bisphenol A (BPA) is a monomer that was first progressed in the 1890s; it has moderate water solubility, a low vapor pressure [1], and a pK_a of 10.6 [2]. BPA was introduced as an artificial estrogen and was reported to have the activity of estrone in activating the reproductive system in female rats in the 1930s.

BPA has been used in several commercial products, including thermal receipts, dental sealants, plastics, PVC, and food packaging. Humans are exposed to BPA through dermal exposure, their diet, and inhalation of household

dust. BPA is a familiar endocrine disruptor; in laboratory studies, it has been discovered to link to estrogen receptors and to have estrogenic influence [3].

Babies have been renowned as a distinguished population to be considered in risk evaluation because their health menace can vary from that of adults. This can be ascribed to physiological variations, which can be declared as immature immune system functions and lower metabolism capacities. Consequently, toxic hazard elimination is not as efficient as in adults [4]. On the other hand, due to the frequent mouthing of objects by babies, the level of exposure varies, as infants face a greater likelihood of oral exposure compared to adults [5]. Baby pacifiers, bibs, and teethers are dramatically used by infants and can be deemed as a main cause of their oral exposure to several dangerous chemicals such as monomers.

Numerous commercial baby products designed for intraoral use, such as nipples, pacifiers, and baby bottles, have been merchandised and suggested as most suitable for fitting a baby's mouth, consolidate appropriate tongue and jaw positioning, nasal breathing, and lip closure during feeding, and to facilitate a healthful oral growth. Children frequently use these items from infancy through early childhood, several times a day. BPA is used as a starting material in the industrialization of some of these products, which can be emitted into the oral environment during regular use.

Polycarbonate baby bottles have been identified as the primary cause of BPA exposure in children 6 years and below. Additionally, a survey disclosed that 21 bottle-fed babies had elevated levels of BPA in their blood serum [6].

Multiple methods have been reported in the literature for the determination of BPA in different matrices such as waste plastics [7], water [8,9], juice [10], baby bottles [11], food samples [12], saliva [13], and milk [14–16]. A very serious task is developing BPA screening methods and tools in children's saliva to prevent and minimize its negative effects on the body. For many years, electrochemical ion-selective sensors have been extensively employed as *in situ* analyzers in fields such as medicine, environmental monitoring, and various industries [17]. Hence, in this study, a simple, sensitive, and rapid potentiometric sensor for monitoring BPA

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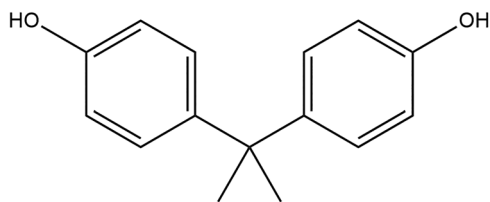


Figure 1: Bisphenol A chemical structure (driven by chemibio draw ultra).

(Figure 1) in saliva samples of babies was proposed. To the best of our knowledge, this is the first potentiometric approach for measuring BPA in saliva samples of baby bibs, pacifiers, and teethingers. It is a carbon-based ISE modified with MWCNTs for better sensitivity and more reproducible response. This study's novelty is illustrated in attempting to establish a sensitive, fast, and reliable membrane electrode to selectively detect BPA, where MWCNTs are used for the first time in the potentiometric determination of BPA.

2 Experimental

2.1 Materials and reagents

BPA (purity $\geq 97.00\%$) was purchased from Sigma-Aldrich (Cairo, Egypt); purity was confirmed by using a previously reported method [7] and found to be $99.66 \pm 1.05\%$. All chemicals and reagents used were of analytical grade, and water was bi-distilled. Polyvinyl chloride (PVC) was bought from Fluka AG (Buchs, Switzerland). Tetrahydrofuran (THF) was purchased from Millipore Sigma (Darmstadt, Germany). Dioctyl phthalate (DOP) was bought from Acros Organics (Morris Plains, NJ, USA). Multi-walled carbon nanotubes (MWCNTs) were purchased from XFNano Materials Tech Co., Ltd. (Nanjing, China). Methanol was bought from El Nasr Company (Cairo, Egypt). Acetic acid, boric acid, phosphoric acid, and potassium chloride (KCl) were purchased from Rebas International (Rotterdam, Netherlands). Human saliva samples were collected from healthy volunteers and used within 24 h. Saliva samples were collected by spitting sampling method, and sampling of whole saliva is the most common and less invasive procedure [18]. The samples were stored at -20°C when not in use.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance with the tenets of

the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

2.2 Standard solutions

2.2.1 Standard BPA stock solution

A stock solution of BPA ($1.0 \times 10^{-2} \text{ mol}\cdot\text{L}^{-1}$) was prepared by weighing 0.228 g of BPA into a 100.0 mL volumetric flask, solubilized in 0.5 mL methanol before completing to the mark using distilled water.

2.2.2 Standard BPA working solutions

Solutions of various concentrations (ranging from 1.0×10^{-3} to $1.0 \times 10^{-9} \text{ mol}\cdot\text{L}^{-1}$) were prepared by diluting BPA stock solution using Britton–Robinson buffer (BRB) [19] adjusted to pH 7.

2.3 Procedures

2.3.1 Ion-selective membrane (ISM) preparation

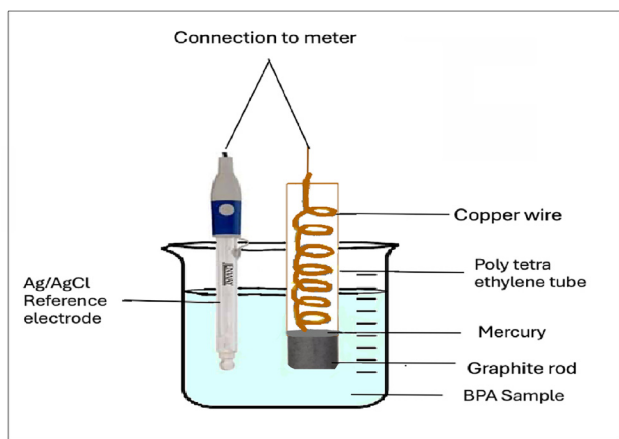
The ISM was prepared in a 5 cm Petri dish by blending 0.01 g of MWCNTs, 0.10 g of PVC, and 0.4 mL of DOP, dissolved in 6.0 mL of THF, and then sonication was applied for 5 min till complete homogeneity.

2.3.2 Electrode assembly

The potentiometric sensor was made from a 2.5 cm graphite rod (about 3 mm in diameter). One end of the rod was utilized for connection, whereas the other end, approximately 1 cm in length, was coated with a layer $>0.01 \text{ cm}$ of the ion-sensing mixture. The electrode was coated by dipping in the electroactive membrane mixture several times until the proper thickness was obtained. It was then allowed to completely dry overnight at room temperature. The uncoated graphite rod's end was plugged into a polytetraethylene tube, which was stuffed with metallic mercury. A copper wire, approximately 1 mm in diameter, was submerged in mercury. For preconditioning, the sensor was dipped in a BPA solution with a concentration of $1.0 \times 10^{-2} \text{ mol}\cdot\text{L}^{-1}$ at 25°C for 2 h before its initial use. It was stored in $1.0 \times 10^{-2} \text{ mol}\cdot\text{L}^{-1}$ KCl when not in use.

2.3.3 Potentiometric measurements

Potentiometric measurements were conducted using a Jenway pH meter 3310 Orion, equipped with a double-junction Ag|AgCl reference electrode. A schematic diagram of the electrode assembly is demonstrated in Scheme 1. For pH adjustment, a Bandelin Sonorox Rx 510S magnetic stirrer (Budapest, Hungary) and a Jenway pH glass electrode (UK) were employed. The calibration curve was constructed by immersion of the developed electrode, in conjunction with the reference electrode, in BPA solutions of concentrations ranging from 1.0×10^{-2} to $1.0 \times 10^{-9} \text{ mol}\cdot\text{L}^{-1}$. The electrode was left immersed in solutions till a constant reading was obtained. The spotted emf from the proposed sensor was plotted in relation to $-\log [\text{BPA}]$ concentrations. A regression equation was calculated for the suggested sensor. IUPAC recommendations were followed for the performance validation of the sensor [20].



Scheme 1: Schematic diagram of electrode assembly.

2.3.4 Sensor selectivity

The selectivity of BPA sensing electrode was investigated by observing the potential of $1.0 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ BPA with the same concentration of interfering ions such as phthalates (organic leaching chemicals termed as endocrine-disrupting compounds), inorganic leaching compounds (PbCl_2 and ZnO), and saliva components (sodium chloride, magnesium bicarbonate, calcium bicarbonate, sodium phosphate, urea, and ammonium oxide). Selectivity coefficient values ($K_{A,B}^{\text{pot}}$) were obtained using the separate solution method (SSM) [21] in which the potentials were obtained separately for $1.0 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ BPA and then for $1.0 \times$

$10^{-4} \text{ mol}\cdot\text{L}^{-1}$ interferent solution. The selectivity coefficients were obtained using the following equation:

$$\log K_{A,B}^{\text{pot}} = (E_B - E_A)/S + (1 - Z_A)/Z_B \log a_A \quad (1)$$

where $K_{A,B}^{\text{pot}}$ is the selectivity coefficient, E_A and E_B are the potentials of the drug and interferent solutions, respectively, S is the slope of the calibration plot, a_A is the activity of the drug, and Z_A and Z_B are charges of the drug and interfering ions, respectively.

2.3.5 pH effect on sensor performance

Over a pH range of 2.0–11.0 regulated by using Britton–Robinson buffer, the influence of pH on the readings of the investigated sensor was investigated for 1.0×10^{-4} and $1.0 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ BPA solutions. Potential values were attained and registered at each pH level.

2.3.6 Application to saliva in contact with baby bibs, pacifiers, and teethers

Simulation of the real status of babies chewing bibs, teethers, or pacifiers was done by leaving each one separately in contact with human saliva for 3 h at 37°C in a stirring water bath. Into a set of volumetric flasks (10.0 mL), 1 mL of each of the resultant saliva was added and then accurately spiked with different volumes of the prepared stock standard solution and filled to the mark with BRB (pH 7) to obtain $0.01\text{--}8.0 \mu\text{g}\cdot\text{mL}^{-1}$ BPA. All biological samples were stored at -20°C until use. The measurements were completed in agreement with the “Potentiometric measurements” section. The corresponding regression equation was used to obtain the recovery percentages.

3 Results and discussion

Potentiometric methods offer several benefits compared to other sample analysis techniques. These include a wide linear range, quick response time, low cost and energy consumption, ease of preparation, and excellent sensitivity and selectivity for a variety of samples. Additionally, potentiometric methods do not require pre-treatment or the use of organic solvents, making them more environmentally friendly. In contrast, other analytical methods are often unsuitable for large-scale monitoring due to their complexity, high cost, energy, and time demands, and need for sample pre-treatment. As a result, potentiometric assays are a more advantageous choice for sample analysis [22]. A big challenge that has always been facing analysts is

Table 1: Performance characteristics of BPA by the suggested sensor

Parameter	Sensor
Slope (mV/decade) ^a	62.48
Intercept (mV) ^a	642.95
Correlation coefficient (<i>r</i>)	0.9995
Response time	10 s
Working pH range	6–8
Concentration range (mol·L ⁻¹)	1.0×10^{-2} – 1.0×10^{-8}
Stability (days)	30
Accuracy ^a (mean ± SD)	100.62 ± 1.171
Precision (SD)	
Inter-day precision ^b	1.061
LOD (mol·L ⁻¹) ^c	1.04×10^{-10}

^aAverage of three determinations. ^bInter-day precision was evaluated with a sample size of 9, involving the average of three concentrations repeated over three consecutive days. ^cLimit of detection, is determined by the intersection of the extrapolated arms of the Nernstian and non-responsive calibration plot parts.

building a solid contact ion selective electrode (SC-ISE) with harmonious and repeatable potentials. Thus, the main goal of this work is the synthesis of a portable, economical, solid-contact sensor with high sensitivity, reproducibility, and harmonious readings for selective determination of BPA.

3.1 Performance of the investigated sensors

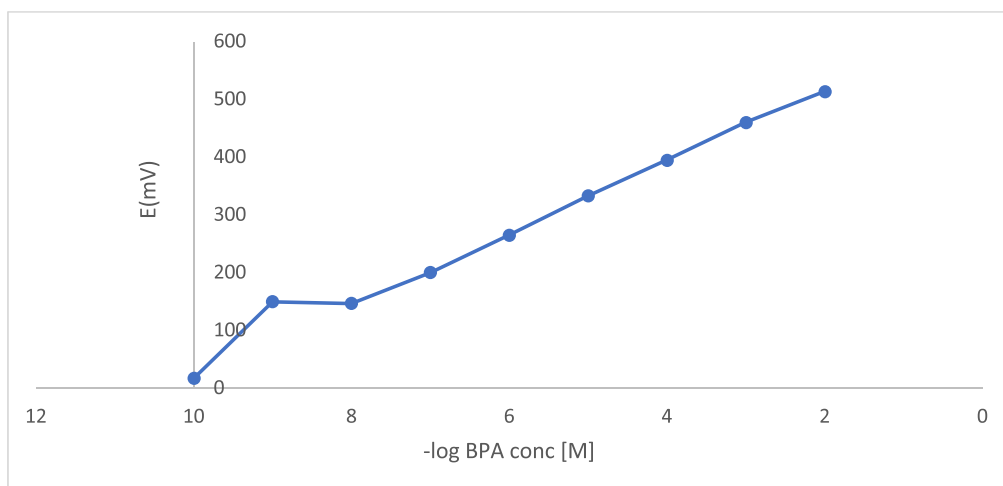
The calibration plot slope for the developed MWCNT-SCE exhibited a cationic response and was found to be 62.48 mV/concentration decade. Polyvinyl chloride (PVC) served as the polymer matrix in creating the ion-sensing mixture, and DOP was used as a plasticizer, along with

THF. Additionally, MWCNTs were added and proven to be utilized as a transducer layer to enhance sensitivity and increase stability and reproducibility [23].

In this study, the performance of a sensor based on MWCNTs was assessed. As per IUPAC guidelines, the performance characteristics of the proposed sensor were evaluated [24], as shown in Table 1. The suggested sensor covered a wide dynamic linear concentration range of 1.0×10^{-2} – 1.0×10^{-8} M, as illustrated in Figure 2. The point of intersection of the linear extrapolated portions of the two curves is used for determining the limit of detection (LOD) and was found to be 1.04×10^{-10} M [24]. Fast response time is a major factor for the rapid analysis of a large number of samples and, therefore, participates in the real application of the developed sensor [25]. A short response time of 10 s was observed for the developed sensor. The short response time is because of the use of MWCNTs characterized by causing a high double-layer capacitance. As opposed to conducting polymers, the transduction mechanism is found in redox reactions and ion-exchange mechanisms, and MWCNTs are sturdier against any redox side reactions that might occur in the sensor [26].

3.2 pH effect on sensor performance

To optimize experimental conditions, the impact of pH on the efficacy of the proposed sensor was examined. No significant variation in the sensor's measurements was observed within the pH range of 6.0–8.0. Above pH 8, *E* decreases dramatically, and below pH 6, BPA begins to precipitate; therefore, *E* is not stable anymore, as illustrated in Figure 3. This could be attributed to the fact

**Figure 2:** Profile of the proposed sensor's potential (mV) vs $-\log$ BPA [M] concentrations.

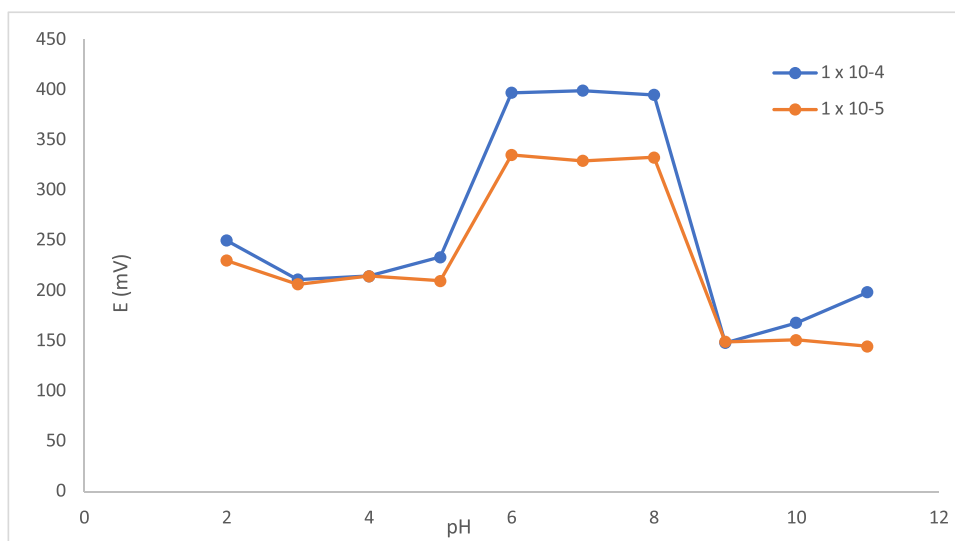


Figure 3: Effect of pH on the sensor response (2–11).

Table 2: Potentiometric selectivity coefficients of the suggested sensors ($K_{\text{pot}}^{\text{BPA, interferents}}$)

Interfering species (I)	($K_{\text{pot}}^{\text{BPA, interferent}}$)
Dibutyl phthalate	1.5×10^{-3}
Diethyl phthalate	2.2×10^{-4}
PbCl_2	7.8×10^{-2}
ZnO	3.5×10^{-2}
NaCl	2.0×10^{-4}
$\text{Mg}(\text{HCO}_3)_2$	3.8×10^{-5}
$\text{Ca}(\text{HCO}_3)_2$	3.2×10^{-5}
Sodium phosphate	4.2×10^{-4}
Urea	6.0×10^{-5}
Ammonium oxide	6.2×10^{-4}

that BPA, at $\text{p}K_a = 10.6$ [2], in aqueous solutions, and at pH values <10.6 , exists in its molecular form, while at pH values >10.6 , it is present as ionized forms (HBPA^- or as BPA^{2-}). Inspired by previous findings in the literature [27], the application of the nonclassical response in BPA detection was explored. In order to guarantee that BPA exists

mostly in its neutral form, BRB with a pH of 7 was used as the background solution to achieve sensitive detection of neutral BPA.

3.3 Determination of the sensor's selectivity coefficients

Selectivity of the ion-selective electrode is one of its most fateful characteristics. It is frequently utilized to determine the potential accuracy of measuring the sample of interest. Evaluation of selectivity coefficients was performed utilizing a separate solution technique [21]. Selectivity coefficients were assessed for organic leaching chemicals, termed endocrine-disrupting compounds, such as phthalates, inorganic leaching compounds (PbCl_2 and ZnO), and saliva components (sodium chloride, magnesium bicarbonate, calcium bicarbonate, sodium phosphate, urea, and ammonium oxide). Table 2 lists the selectivity coefficient values. The results indicate that MWCNT-SCE exhibited perfect selectivity toward BPA as opposed to other examined interferents. The sensor showed the highest

Table 3: Analysis of BPA in spiked saliva samples

	Plastic bibs			Teethers			Pacifiers		
	Spiked ($\mu\text{g}\cdot\text{mL}^{-1}$)	Found ($\mu\text{g}\cdot\text{mL}^{-1}$)	$R\%^a$	Spiked ($\mu\text{g}\cdot\text{mL}^{-1}$)	Found ($\mu\text{g}\cdot\text{mL}^{-1}$)	$R\%^a$	Spiked ($\mu\text{g}\cdot\text{mL}^{-1}$)	Found ($\mu\text{g}\cdot\text{mL}^{-1}$)	$R\%^a$
Blank	0	ND	ND	0	ND	ND	0	ND	ND
Sample 1	0.5	0.51	102	0.5	0.5	100	0.5	0.53	106
Sample 2	1	1.01	101	1	1.05	105	1	1	100
Sample 3	3	3.1	103.33	3	2.8	93.33	3	3.02	100.66
Mean $\pm$ SD	102.11 ± 1.1717			99.44 ± 5.853			102.22 ± 3.289		

^aAverage of three determinations.

Table 4: Statistical comparison between the proposed sensor and the reported reference method for the determination of BPA

Parameter	Reported method**	Proposed sensor
Mean	99.66	100.62
SD	1.05	1.171
Variance	1.103	1.371
<i>n</i>	5	5
<i>F</i> (6.39)*	—	1.243

*Tabulated *F* value at *p* = 0.5. **Reported method [7]: Voltammetric determination of bisphenol A using a carbon paste electrode based on the enhancement of cetyltrimethylammonium bromide (CTAB).

selectivity for BPA in the presence of calcium bicarbonate, while the least selectivity was recorded in the presence of lead chloride. As $K_{\text{BPA, Interferent}}^{\text{pot}}$ value reduces, it indicates that the electrode is more selective to the studied analyte (BPA) [28].

3.4 Analysis of BPA in spiked saliva samples

The primary goal of this study was to devise a sensitive and quick method for detecting BPA in saliva that comes in contact with baby bibs, pacifiers, and teethingers. Thus, the ability of the introduced SC-ISE to detect BPA in biological fluids was evaluated by direct testing of spiked saliva samples without any prior extraction steps. The proposed sensor was felicitous in obtaining accurate and precise recoveries, as shown in Table 3.

3.5 Statistical analysis

The variance ratio *F* test was used to examine the validity of the proposed approach. A statistical comparison of the suggested sensor and the reported method [7] for BPA is presented in Table 4. The results show that there is no statistically significant difference between the reported method and the proposed sensor; the calculated *F* values were less than the theoretical one at *p* = 0.05.

3.6 Comparison of the proposed sensor to other reported potentiometric methods for determination of BPA

The proposed sensor was compared with other reported potentiometric sensors for the determination of BPA, as summarized in Table 5. The developed electrode has a wider linear range and lower LOD and is considered the

Table 5: Comparison of the proposed sensor to other reported potentiometric methods for determination of BPA in different matrices

Technique	Matrix	Linear range (mol·L ⁻¹)	LOD (μmol·L ⁻¹)	Reference
Potentiometric immunosensor	Real spiked water sample	2.2×10^{-7} to 6.85×10^{-6}	0.14	[29]
MIP-based potentiometric sensor	Real plastic samples	1.0×10^{-7} to 1.0×10^{-6}	0.02	[30]
MIP paper-based potentiometric sensor	Real plastic samples	5.0×10^{-7} to 1.3×10^{-5}	0.15	[27]
Multifunctional MIP receptor-based potentiometric sensor	Spiked lake and river water samples	5.0×10^{-7} to 2.0×10^{-5}	0.23	[31]
MWCNT-modified graphite ion-selective electrode	Real spiked saliva samples in contact with baby bibs, teethingers, and pacifiers	1.0×10^{-8} to 1.0×10^{-2}	0.000104	This work

first potentiometric sensor for measuring BPA in saliva samples in baby bibs, pacifiers, and teethers.

4 Conclusion

In this research, the developed sensor was utilized for directly analyzing BPA in saliva samples in contact with baby bibs, pacifiers, and teethers. It experienced several advantages over previously developed instrumental methods, including being economic, portable, environmentally friendly, of real-time analysis, energy saving, and easy to miniaturize. Our proposed sensor exhibited accurate, selective, and precise potentiometric readings in the presence of various potential interferents. Thus, it could be applied for routine analysis without tedious extraction procedures. The addition of carbon nanotubes as ion to electron transducer led to a short response time and low signal drift.

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Author contributions: Eman Ali Al-Harbi: conceptualization, methodology, validation, investigation, and visualization. Amira Mabrouk El-Kosasy: conceptualization, methodology, validation, investigation, and visualization. Yossra Ahmed Trabik: validation, investigation, writing – original draft, and visualization.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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