Chitosan based nano-membrane for Chromium(III) Determination in Pharmaceutical and Foodstuff Samples

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A newly fabricated chromium selective liquid electrode sensor was successfully designed according to the reaction between nano-chitosan and chromium(III) ions. The characteristics specific slope (58.5 mV/decade), linearity range of concentration from 1.0×10⁻⁶-1.0×10⁻¹ M, the limit of detection (1.32×10⁻⁸)M, selectivity behavior among some inorganic cations, the dynamic response time (10s), lifespan (four months), the potential dependence on pH and basic interesting validations and parameters were investigated. The nominated electrode was successfully utilized to detect chromium(III) ions in pharmaceutical and foodstuff samples. The data provided by the developed sensor were treated, statistically and compared with other electrodes listed in the literature.

Keywords: Membrane sensor, Nano-chitosan, Chromium ions, Pharmaceutical and Foodstuff samples

1. INTRODUCTION

Based on what was stated by the Healthy National Institutes, the effectiveness of Cr (III) in the body are not quite settled and the required values for better health are not evident. It is well known in several food sources with very small amounts, foods containing high quantities of modest sugars resort to have lower chromium quantities. Its lack in humans is tenuous, but a regimen including high quantities of normal sugars raises chromium urine secretion, contagion, squeeze, contusion, gestation, and severe practice can also exceed the chromium secreted in urine.

The standard staffing quotidian of chromium for sensible women extent from 20 to 25 micrograms, based on their age, increasing with lactation or gestation period.
In type 2 diabetics chromium supplements reduce blood sugar levels and the quotidian insulin intake. They aid with weight deprivation, lessening of body fat, and increase of penchant muscle. Other suggested benefits comprise hypertension lowering, cholesterol and afflicted signs of a representative gloominess [1-3]. Thus, it is very interesting to detect chromium in pharmaceutical and foodstuff samples.

In recent years, Chitin and chitosan(CHN) have attracted valuable attention because of their multi-potential utilizations in various ways[4-8] due to their interesting characteristics such as non-toxicity, biocompatibility, and biodegradability that make them useful to apply in the biomedical area.

Chitosan (CHN) as a prospect polysaccharide purse (Fig.1) is a linear amino polysaccharide of glucosamine and N-acetyl glucosamine units and is provided by alkaline deacetylation of chitin [9]. The prominence validations of chitosan help this polymer characteristics for several practical utilizations.

![Chemical structure of chitosan](image)

**Figure 1.** Chemical structure of chitosan

Several analytical strategies have been developed for chromium detection, atomic emission and atomic emission analysis [10, 11], spectrophotometry[12, 13], Voltammetry [14] and ion-selective electrode techniques [15-29].

In spite of the aforementioned methods have good sensitivity, several difficult troubles have been established in their utilizations. Potentiometric measurements based on the selective sensor is very simple and offers several advantages, such as easier sampling conditioned, quick restraint, strongly eclectic, very quite linearity range of concentration, simple types of equipment with an approximately low limit of detection, carried out in various types of solutions and economic [30]. However, several previously published electrodes agonized from metal ions intervention, and have a limited linearity extent of concentration.

The electrochemical technique is excessively interested by numerous researchers because of its many specifications[31]. Therefore, they have been widely utilized in biological[32-37] and environmental analysis[38-39].

Nano-chitosan can be created into fluffy films [40] and has made metal cations- sensors [41] because of the presence of a lone pair of the acetylamide, and amine groups which compose chelates with transition metal ions[42]. In this proposed work, Cr$^{3+}$ ion-selective electrode has been fabricated by adding chromium dopants into the nano-chitosan membrane at polyvinyl chloride matrix through immersion, the optimum dopant concentration and sensor validations of the designed electrode such as...
the Nernstian slope, limit of detection, the dependence of pH, dynamic response time, lifespan, and the behavior of intervention ions are studied.

The proposed research displays the fabrication and estimation of chromium(III) membrane sensor. The important and very active materials in polyvinyl chloride (PVC) matrix membranes are the Cr\(^{3+}\) with the nano-chitosan chelate. The specific significant slope, extended and quick restraint for chromium ion was displayed by the proposed sensor within the linearity extent of concentration $1.0 \times 10^{-6}$-1.0$\times10^{-1}$ M, the limit of detection ($1.32\times10^{-8}$) M, the selectivity action among some cations, the lifespan (120 d), the response time (10 s), the dependence of potential on pH and the specific validations were studied. The convenient pH range was (4.0-6.0); the restraint of the suggested sensor is barely influenced by the change of pH in this period. The developed electrode is felicitously employed for the detection of chromium(III) ions in pharmaceuticals and foodstuff samples. The provided results by the developed sensor were statistically treated, and compared with the previously listed chromium sensors.

2. EXPERIMENTAL

2.1. Samples and Reagents

Chlorides of chromium, zinc, iron and aluminum, nickel and cadmium nitrates, hydrogen peroxide, ammonium, and sodium hydroxide. TBP(tributylphosphate), sulfuric, hydrochloric acids, tetrahydrofuran, methanol, and dioctyl-phenyl phosphonate (DOPP) from Merck [Germany]. Nano-chitosan, PVC, and TEHP [tris-(2-Ethylhexyl)phosphate] were provided from Sigma-Aldrich Company, (USA). Pharmaceutical formulations containing chromium; (Chelated chromium, Struvite forte and Acetazolamide) and foodstuff (Tea-Leaves, Cocoa powder and Black pepper) were purchased from the local markets in Saudi Arabia and Egypt.

2.2. Conditioned of Stock Solutions

All processes were carried out with highly pure chemicals. Stock solutions of Cr\(^{3+}\) and other metal ions of 0.1 molar solutions were conditioned, then dissolving in distilled H\(_2\)O. The $10^{-6}$ - $10^{-1}$ M solutions were conditioned by dilution. A 100 mg of nano-chitosan was conditioned by dissolving in 100 mL aqueous acetic acid 1% (v/v) to provide a stock solution; Appropriate dilutions can be made when needed and the solution should be stored at 4°C.

2.3. Sample Preparation and Cr\(^{3+}\) Assay in Pharmaceutical and Food Products.

The required potentiometric gagueing solutions were prepared as follows: a content of pharmaceutical formulations (Chelated Chromium, Struvite forte and Acetazolamide) was put into a marked conical flask, 10 ml of 30 % H\(_2\)O\(_2\) was added, and stay to stand till dissolving. Concentrated H\(_2\)SO\(_4\) 1 ml was added, heating until H\(_2\)O\(_2\) analyzed. After mineralization repeat this step five times,
adding 10 ml ammonia solution, and 25 ml water, stay an hour to settle. Hence, the solution was filtered quantitatively, then diluted with distilled water.

For the analysis of foodstuff; (Tea-Leaves, Cocoa powder and Black pepper) were chosen for estimation of Cr\(^{3+}\) ions. With respect to Black pepper and Cocoa powder samples were well rinsed, cut into bars, dried at 120 °C for 2 hs. Ten grams were weighed accurately, put into a ceramic crucible, then into a muffle furnace for 4 hs. at 600 °C for ashing, the samples were cooled to the ambient conditions after completing the ashing, diluted HCl 5 ml were added to dissolve the providing yield, transferred it to a 50 ml calibrated marked flask, diluted with bidistilled H\(_2\)O to the mark. For Tea-leaves sampling, five grams was weighed veracity, applying the same strategy as described above. A 10 ml volume of each solution was taken for the detection of chromium ions at the desired laboratory temperature of the suggested procedure and Table 3 displayed the produced results.

2.5. Fabrication of the Selective Electrode

The nano-chitosan membrane selective electrode for chromium ion determination has been fabricated. Nano-chitosan is a naturalistic multi-module including nitrogen atoms that coordinate with Cr\(^{3+}\). It was grounded to 400 mesh, 0.375 g PVC was dissolved in 20 mL of THF, stirring with a magnetic stirrer, adding 1.0 mL DOPP, then 0.750 g of it was added to the mixture progressively, and stirred for 2 h at laboratory conditions. The mixture was transferred into a glass plate and stayed till all solvents were evaporated to produce nano-chitosan membranes. It is a main active constituent, and blended with polyvinylchloride (PVC) as matrix dissolved previously to solvent tetrahydrofuran (THF) and dioctylphenylphosphonate (DOPP) is added as a plasticizer by the ratio nano-chitosan:PVC:DOPP (6:3:1). To provide the dopant desired concentration, the membrane was immersed in Cr\(^{3+}\) for 5 days. From the FT-IR spectrum the peaks of acetylamide, hydroxy, and amine groups wavelength number were noticed to assure that Cr\(^{3+}\) combined with the chitosan. The optimum concentration of Cr\(^{3+}\) dopant was developed at 1.5 M and provided a Nernstian slope of 58.50 mV/decade with the detection limit of 1.32 \(\times\) 10\(^{-8}\) M. The electrode was barely affected in the pH range 4.0 - 6.0.

2.6. The Potential Layer Preparation

The selectivity and sensitivity of the ion-selective electrode are deeply affected by the composition and the nature of additives employed. Therefore, the influence of various membrane compositions on the response characteristics of the fabricated chromium-nano-chitosan membrane electrode was studied to detect the best composition of the nominated electrode. Thus, From the characteristic slope(58.50 mV/decade) of the calibration curve( Fig. 2 ) the best behavior of the membrane electrode consisting of an exactness weight of 0.03g of the compound chromium-nano-chitosan with 0.22 g PVC, and 0.75 g TEHP which were blended to provide the ion-selective sensor layer. A Teflon tube with an electrode of Ag/AgCl was filled with the recently conditioned mixture, then put into a gel by heating at a temperature of 375 K for 20 mins. After cooling, the membrane sensor was immersed for two hrs. in 10\(^{-3}\) M Cr\(^{3+}\) solution.
2.7. EMF Measurements

An Orion 90-02 reference sensor was employed with a mechanical stirrer to provide a veracity of 0.1 mV at the ambient temperature for gauging the EMF of chromium(III) membrane electrode system. An Orion 90-00-01 solution including 0.55 M potassium chloride, 1.5M potassium nitrate, 0.05 M sodium chloride, and 40 % formaldehyde 1 ml was employed to complete filling the stable reference electrode’s bridge. FT-IR spectroscopy (Prestige 21-Shimadzu) was utilized to detect the change in wavelength numbers through the complex formed by the functional groups after doping with Cr$^{3+}$.

3. RESULTS AND DISCUSSION

The interesting validations of our suggested Cr$^{3+}$ sensor were investigated to detect its prominence in practical utilization. The potential dependence on pH, characteristics specific slope, selectivity studying, response time, and detection limit were examined.

3.1. Calibration Curves

The calibration curve of the chromium sensor was presented in Figure 2. The Cr$^{3+}$ sensor’s specific slope is 58.50 mV/decade, the detection limit is 1.32x10$^{-8}$M and the recording linear concentration range is 1.0x10$^{-6}$-1.0x10$^{-1}$M. Table 1 presented the interesting validation parameters of the suggested Cr$^{3+}$ electrode.

![Figure 2](image-url)  
**Figure 2.** The proposed Cr$^{3+}$ electrode calibration curve in the linear concentration range 10$^{-6}$-10$^{-1}$M.
The specific Nernstian slope is a very interesting factor to detect ion-selective electrodes that are conveniently employed in analytical applications. It is elaborated from the slope curve of the potential gauged in (EMF/mV) versus the log concentrations of standard solution (M) as seen in Fig. 2. The optimal value of the Nernstian slope is 59.10/n (mV/decade), where ( n ) is the valency. For the proposed Cr$^{3+}$ sensor with the n value = 3 is 19.70 mV/decade. The Nernstian slope in this research was 19.50 mV/decade, this means any increment in the concentration of 10$^{-1}$ M test solutions under test, the potential shift is 19.50 mV/decade. This displays that the suggested Cr$^{3+}$ sensor is acceptable to utilize in the analytical applications of Cr$^{3+}$ because of the value allowed of the Nernestian slope is 19.70 mV/decade.

3.2. Measurement of the Functional Groups of Nano-Chitosan Membranes

Two probabilities are found for Cr$^{3+}$ ion binding to the nano-chitosan membrane, via the amine groups or amine and acetylamide groups or both [44]. Dopants with metal ion give specific validations to perform a complex with chitosan [42]. The FT-IR spectra former to membrane prior doped with Cr$^{3+}$ presented that the N-H stretch of secondary amine (acetyl amide group) at the wavelength 1652.85 cm$^{-1}$ and N-H stretch of the primary amine at the wavelength 1589.34 cm$^{-1}$.

Table 1. Interesting parameters of the nominated chromium(III) selective electrode.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nernstian slope/mV/decade</td>
<td>58.50</td>
</tr>
<tr>
<td>Intercept/mV</td>
<td>-52.10 + 0.30</td>
</tr>
<tr>
<td>Detection limit /mol dm$^{-3}$</td>
<td>1.32 x 10$^{-8}$</td>
</tr>
<tr>
<td>Linearity range/mol dm$^{-3}$</td>
<td>1.0 x 10$^{-6}$-1.0 x 10$^{-1}$</td>
</tr>
<tr>
<td>Response time/s</td>
<td>10</td>
</tr>
<tr>
<td>Lifespan/d</td>
<td>120</td>
</tr>
<tr>
<td>Working pH range</td>
<td>4.0-6.0</td>
</tr>
</tbody>
</table>

After doping the membrane with Cr$^{3+}$ presented peaks point out the existence of N-H acetylamide and N-H extension of primary amines group does not show. Whilst the peak of OH- group at wavelength number ~3400 cm$^{-1}$ decrease in strength. This is made possible that the N and O atoms are the amine ligands and hydroxide will form a chelate bond with Cr$^{3+}$ ions which are electropositive, by donating its lone pairs of the electron, the N-H bond becomes weakness and its peak did not reappear[43]. From spectral data of FT-IR mentioned above is displayed that Cr$^{3+}$ ions form a chelate complex with nano-chitosan.

3.3. Interference Study

The selectivity behavior of the Cr$^{3+}$ electrode with respect to interfering foreign ions were tested by the separate solution or by the matched potential modes reported before [45] employing the following equations:
log $K_{ij}^{pot} = E_j - E_i / S - (Z_i/Z_j - 1) \log a_i$, \quad $K_{Cr/M}^{pot} = \frac{a_i}{a_i^{z_i/z_j}}$

where $a_i$ is the $\text{Cr}^{3+}$ activity, $E_i$ is the potential of $\text{Cr}^{3+}$, $E_j$ is the potential of the interfering ion, $z_i$ and $z_j$ are the charges of $\text{Cr}^{3+}$ and interfering ions, respectively and $S$ is the slope of the electrode calibration plot.

By utilizing the separate solution mode, at the magnitude of EMF, the potential $-160 \text{ mV}$ and 0.001 M $\text{Cr}^{3+}$. For the matched potential mode, the equation is:

$$K_{Cr/M}^{pot} = \frac{a_i}{a_i^{z_i/z_j}}$$

The produced results are tabulated in Table 2.

3.4. Dynamic Response Time of the Designed $\text{Cr}^{3+}$ Sensor

The fabricated membrane ion-selective electrode response time is very interesting for practical analytical utilities. After injecting the known concentrated solution adding water (1:1) for dilution. To detect response time for the investigated sensor the utilized solutions have these specifications: $c_1 : c_2 = 1:100$, $v_1 : v_2 = 1:20$, where $c_1$ is the concentration of the sample, $c_2$, the standard concentration, $v_1$ is the volume of the sample, and $v_2$ is the volume of standard. The results provided are summarized in Fig. 3. The response of the sensor is reproducible after 10 seconds of adding chromium. The timer is started at the injection moment, the fast and stabilized potential records reflect the whole titration time. As depicted from Fig. 3, the sensor attains its equilibrium restraint in an instant time (10 s) over the working concentration range.

![Figure 3](image_url)

**Figure 3.** Time of response for $\text{Cr}^{3+}$ sensor cations concentration, (A) $10^{-6}$ M, (B) $10^{-5}$ M, (C) $10^{-4}$ M, (D) $10^{-3}$ M, (E) $10^{-2}$ M and (F) $10^{-1}$ M.
Table 2. The selectivity behavior (K) of Cr\textsuperscript{3+} sensor (reference electrode Ag/AgCl).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Separate Solution Mode (SSM)</th>
<th>Matched Potential Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td></td>
<td>(E_i = E_j)</td>
<td>(a_i = a_j)</td>
</tr>
<tr>
<td>Ni\textsuperscript{3+}</td>
<td>0.241 + 0.0023</td>
<td>0.272 + 0.030</td>
<td>0.236 + 0.0130</td>
</tr>
<tr>
<td>Zn\textsuperscript{2+}</td>
<td>0.188 + 0.0133</td>
<td>0.186 + 0.022</td>
<td>0.185 + 0.0230</td>
</tr>
<tr>
<td>Fe\textsuperscript{3+}</td>
<td>0.171 + 0.0111</td>
<td>0.172 + 0.002</td>
<td>0.174 + 0.0032</td>
</tr>
<tr>
<td>Al\textsuperscript{3+}</td>
<td>0.083 + 0.0032</td>
<td>0.088 + 0.012</td>
<td>0.084 + 0.0021</td>
</tr>
<tr>
<td>Cd\textsuperscript{2+}</td>
<td>0.076 + 0.0022</td>
<td>0.074 + 0.013</td>
<td>0.071 + 0.0013</td>
</tr>
</tbody>
</table>

3.5. Dependence of The Developed Sensor Potential on the pH

The influence of the potential sensor on the pH was examined by gauging the potential according to the chemical specification of chromium(III) cations. Sodium hydroxide, and/or hydrochloric acid drops were added to the understudying sample of the 0.001 M Cr\textsuperscript{3+} cations. Afterward the pH was registered, the electromotive force; EMF was reported after the sensor’s stabilization. The dependence of the EMF on pH is displayed in Fig.4. At lower and higher than this working pH range (4.0-6.0) an obvious lowering in the potentials owing to the hydrolysis of Cr\textsuperscript{3+} cations or the non-completing complexation reaction.

Figure 4. Dependence of the electrode potential on pH in 10\textsuperscript{-3} M Cr\textsuperscript{3+} cations concentration.

3.6. Lifetime of the Cr\textsuperscript{3+} Selective Electrode

The lifespan of the studied Cr\textsuperscript{3+} electrode was examined by gauging the slope of the sensor stayed at 4 \textdegree C. The lifespan of the electrode is four months based on the produced data. In recently prepared conditioned solutions systematic studies were completed once a week in a regular strategy.
3.7. Detection of Cr\textsuperscript{3+} cations in Pharmaceutical and Foodstuff Samples.

The estimation of Cr\textsuperscript{3+} cations in foodstuff and pharmaceutical samples was examined applying the conditioned sensor to test its practical utilities. The modes of the calibration curve and standard additions were utilized. The quantities of Cr\textsuperscript{3+} in the samples were computed from detected calibration plots and their statistical analysis validations are tabulated (Table 3).

Table 3. Chromium(III) estimation in pharmaceuticals and foodstuff samples applying the suggested electrode.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Calibration Curve Mode</th>
<th>Standard addition Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample Data mg / Kg</td>
<td>Cr\textsuperscript{3+} Found mg / Kg</td>
</tr>
<tr>
<td>Chelated chromium</td>
<td>380</td>
<td>380.65</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>170</td>
<td>171.45</td>
</tr>
<tr>
<td>Strovite forte</td>
<td>250</td>
<td>251.55</td>
</tr>
<tr>
<td>Tea-Leaves</td>
<td>350</td>
<td>350.65</td>
</tr>
<tr>
<td>Black pepper</td>
<td>530</td>
<td>531.86</td>
</tr>
<tr>
<td>Cocoa powder</td>
<td>450</td>
<td>451.85</td>
</tr>
</tbody>
</table>

- The averages of (five) estimations.

3.8. Comparison with the Previously Published Sensors

The produced results by the suggested and fabricated Cr\textsuperscript{3+} sensor were treated statistically and compared to the other various recently published sensors. Some validation parameters of the Cr\textsuperscript{3+} sensors listed previously are compared with the developed electrode in Table 4 to confirm if the suggested electrode provides good results and be accepted for Cr\textsuperscript{3+} estimation foodstuff, and pharmaceutical samples. From the data introduced in Table 4 linear range (1.0 x 10\textsuperscript{-6} - 1.0 x 10\textsuperscript{-1} M), Nernestian slope (58.50 mV/decade), the lowest limit of detection is that reported in this research (1.32 x 10\textsuperscript{-8} M) which is better than the other described Cr\textsuperscript{3+} sensors [15-21], it has a long lifespan, (120 days) compared to the previously reported sensors. Furthermore, the developed electrode is designed easily, economically. Thus, it can be confident one to state that our suggested electrode is practically employed with others for the detection of Cr\textsuperscript{3+} cations.
Table 4. Comparison between interesting validation parameters of the designed Chromium(III) sensor with others reported in the literature.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Linearity Range of Concentration (M)</th>
<th>Time of Response (S)</th>
<th>Detection Limit (M)</th>
<th>Nernstian Slope mV/decade</th>
<th>Applying pH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>This research data</td>
<td>1.0 x 10^{-6} - 1.0 x 10^{-1}</td>
<td>10</td>
<td>1.32 x 10^{-8}</td>
<td>58.50</td>
<td>4.0 - 6.0</td>
</tr>
<tr>
<td>[15]</td>
<td>1.66 x 10^{-6} - 1.0 x 10^{-2}</td>
<td>10</td>
<td>8.0 x 10^{-7}</td>
<td>19.5</td>
<td>3.0 - 5.5</td>
</tr>
<tr>
<td>[16]</td>
<td>1.0 x 10^{-6} - 1.0 x 10^{-1}</td>
<td>8</td>
<td>5.8 x 10^{-7}</td>
<td>19.7</td>
<td>2.7 - 6.6</td>
</tr>
<tr>
<td>[17]</td>
<td>1.0 x 10^{-6} - 1.0 x 10^{-1}</td>
<td>15</td>
<td>7.0 x 10^{-7}</td>
<td>19.9</td>
<td>3.0 - 6.6</td>
</tr>
<tr>
<td>[18]</td>
<td>3.0 x 10^{-6} - 1.0 x 10^{-2}</td>
<td>20</td>
<td>6.3 x 10^{-7}</td>
<td>19.8</td>
<td>2.7 - 6.5</td>
</tr>
<tr>
<td>[19]</td>
<td>4.0 x 10^{-6} - 1.0 x 10^{-1}</td>
<td>15</td>
<td>2.0 x 10^{-7}</td>
<td>20.0</td>
<td>2.8 - 5.1</td>
</tr>
<tr>
<td>[20]</td>
<td>7.0 x 10^{-6} - 1.0 x 10^{-1}</td>
<td>10</td>
<td>--------------</td>
<td>19.8</td>
<td>3.5 - 6.5</td>
</tr>
<tr>
<td>[21]</td>
<td>1.0 x 10^{-5} - 1.0 x 10^{-2}</td>
<td>10</td>
<td>8.6 x 10^{-6}</td>
<td>--------------</td>
<td>3.4 - 5.2</td>
</tr>
</tbody>
</table>

The calibration curves displayed an excellent linearity response on an expanding concentration domain. No considerable intervention was noticed from the important excipients included in the sample media under investigation. Several techniques provide a precious revival with respect to the known real values and no considerable alteration was presented for either exactitude or veracity.

4. CONCLUSION

In this research, novel chromium(III) sensor was developed. The suggested sensor is specified by superior validation parameters: for the significant Nernstian factor, long duration-time and relatively short response time. The analytical characteristics of the examined electrode are summarized in Tables 1 and 2.

The suggested sensor was applied for chromium(III) ions detection in pharmaceutical and foodstuff samples that employed in widespread. The modes of standard additions and calibration curves were utilized. The analysis of results displayed that the calibration curve technique is prioritized in the detection of chromium(III) ions. Thus, the fault is not exceeded 2% owing to the repeatability and reproducibility of the process. The mode of sensor fabrication was set as veracity and accurate in comparison to the other published methods which are utilized in common in their estimation in pharmaceutical and foodstuff samples as introduced in (Table 4).

In General, the fineness of the data was excellent because of the luck of the selected practical applications applying the proposed sensor. The time consumed is examined with no effect on the precision and reproducibility of the results.
CONFLICT OF INTEREST
The authors have no conflicts of interest to declare that are relevant to the content of this article.

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