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## Development of an innovative optical sensor to detect extremely low levels of chromium in real samples using colorimetric methods

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The investigation focused on a methodology for concentrating and analyzing Cr(vi) in aqueous samples. This objective was accomplished through the creation of a cellulose triacetate (CTA) matrix-based membrane optode. This optode was constructed by physically integrating a specific chromophore, 1,3-benzenediamine, *N,N'*-bis(2-furanylmethylene) (BDBFM), known for its selectivity towards Cr(vi), alongside the plasticizer dioctylphthalate (DOP). The effectiveness of integrating Aliquat 336, an anion exchanger, was evaluated in the process of immobilizing both BDBFM and the Cr(vi)–BDBFM complex within the optode matrix. The progressive intensification of the violet color observed on the optodes, directly correlating with the amount of loaded Cr(vi), highlights the potential of this method for colorimetric screening of Cr(vi) in aqueous samples. The developed optode was also employed for the determination of the total chromium content by converting Cr(III) to Cr(vi) *via* oxidation using 0.1 M hydrogen peroxide. The concentration of Cr(III) can be quantified by subtracting the amount of Cr(vi) from the total chromium content. This optode enabled the quantitative detection of Cr(vi), even at levels as low as 2.85 ng mL<sup>-1</sup>. The suggested sensor displayed a low detection limit, fast response time, cost effectiveness, ease of preparation and also remarkable selectivity regarding some anions and cations. Regeneration of the optode can be easily accomplished by employing 0.05 M HNO<sub>3</sub>, while demonstrating remarkable reproducibility and reversibility in its response, with a relative standard deviation (RSD) below 1.9. The suggested method was effectively utilized to measure chromium levels in a diverse range of samples, such as food, water, and environmental, and biological samples.

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### Environmental significance

The optode developed in this study offers a straightforward and efficient method for detecting Cr(vi) ions. Its utilization of cellulose triacetate as a matrix ensures favorable optical and mechanical properties. Notably, the optode exhibits a reversible color change from yellow to violet upon exposure to Cr(vi) ions, and it can be easily regenerated using a 0.05 M HNO<sub>3</sub> solution with complete reversibility. Compared to previously reported optical sensors for Cr(vi) detection, the proposed optode demonstrates a significantly faster response time. An important aspect of this study is the novel application of BDBFM in combination with the proposed optode for the determination of Cr(vi) ions. This approach has not been documented in the existing literature, making this study the first to employ BDBFM in conjunction with the proposed optode. Furthermore, the versatility of the optode sensor extends to its successful implementation in monitoring BDBFM across various sample types, including water, food, and biological, and environmental samples. This wide range of applications showcases the potential of the optode sensor beyond its primary use in Cr(vi) determination.

## 1 Introduction

Environmental pollutants such as heavy metals and organic and inorganic compounds in recent years have received a lot of attention.<sup>1–4</sup> Inadequate wastewater treatment facilities in various industries lead to the discharge of heavy metals into aquatic environments.<sup>5,6</sup> Chromium, in particular, is a significant environmental pollutant commonly associated with

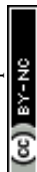
industrial activities such as steelworks, tanning factories, wood preservation, and industrial electroplating. Chromium is an element that necessitates assessment in environmental and pharmaceutical products, primarily due to the health risks it poses. Additionally, it has been found to cause various types of DNA lesions, as indicated by studies.<sup>7–9</sup> Compounds of Cr(III) are recognized for their important role in glucose and lipid metabolism and are regarded as essential trace elements that contribute to maintaining efficient protein metabolism in humans.<sup>10–15</sup>

Chromium has the ability to enter the human body through inhalation or ingestion *via* drinking water, and its presence in water, air, and biological samples is typically at low levels.

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grade HCl from Merck (Germany), were consistently used throughout the study. 2-Nitrophenyl octyl ether (NPOE) from Fluka, cellulose triacetate with a M. wt 72 000–74 000 (Alfa Biochem), bis-(2-ethylhexyl) terephthalate (DOTP) from Acros, dioctyl phthalate (DOP) (99%, Sigma-Aldrich), di-(2-ethylhexyl) phosphoric acid (D2EHPA) (97%, Sigma-Aldrich), dioctyl sebacate (DOS) from Fluka, 1,5-diphenylcarbazine (DPC) from Merck, and trioctyltrimellitate (TOT) from Aldrich were used as received. Dinonylnaphthalene sulfonic acid (DNNS) obtained from Fluka and trioctylmethyl ammonium chloride (Aliquat-336) were both utilized in their received form without further modification.

To prepare stock solutions of Cr(vi), K<sub>2</sub>CrO<sub>4</sub> was dissolved in water, resulting in a concentration of 1000 mg L<sup>-1</sup> for both Cr(vi) and Cr(III). For Cr(III) stock solutions, Cr(NO<sub>3</sub>)<sub>3</sub> was dissolved in 0.5 M HNO<sub>3</sub>. Model and standard solutions were then prepared by diluting the stock solutions accordingly. Borate, acetate, thiel, phosphate, and universal buffer solutions of pH 2.0 to pH 12 were prepared to create different pH conditions using established methods.<sup>56</sup> To generate stock solutions of interfering ions, salts from Merck were dissolved in double-distilled water to achieve a concentration of 20 000 µg mL<sup>-1</sup>.

The oxidation of Cr(III) to Cr(vi) was conducted utilizing a previously described method in the literature.<sup>36,57</sup> The solution's pH was adjusted to 10, and a 3.0% hydrogen peroxide solution was added, followed by heating at 80 °C for 30 minutes. Any excess hydrogen peroxide was eliminated by boiling the solution for 10 minutes. The aforementioned test procedure was subsequently employed for this solution. Subsequent to the conversion of Cr(III) to Cr(vi) *via* hydrogen peroxide oxidation under alkaline conditions, the total chromium content was determined. The concentration of Cr(III) was then calculated by subtracting the concentration of Cr(vi) from the total chromium concentration.

Upon oxidizing Cr(III) to Cr(vi) *via* the alkaline oxidation process using hydrogen peroxide, the method was applied to determine the total chromium content. Subsequently, the concentration of Cr(III) was calculated by deducting the concentration of Cr(vi) from the overall chromium concentration.

## 2.2. Apparatus

Absorbance measurements and spectral analysis were conducted using the UV-vis spectrophotometer model V-670 from JASCO (Tokyo, Japan). The acidity or alkalinity of the solutions was measured using a pH meter (Orion research model 601 A/

digital ionalyzer pH meter). Absorbance measurements were taken by placing the samples of the sensor membrane within a quartz cuvette relative to the surrounding air and blank samples of the optode. The thickness of the optode was determined using a digital microscope (Ray Vision Y 103) that was connected to a video camera (JVC TK-C 751 EG) and a digital micrometer (Mitutoyo, Japan) with a precision of ±0.001 mm.

For the analysis utilizing atomic absorption, a Shimadzu model 670 atomic absorption spectrometer equipped with flame atomization was utilized. The operational parameters were established based on the recommendations of the manufacturer. Measurements were conducted using a flame generated by a mixture of nitrous oxide and acetylene gas.

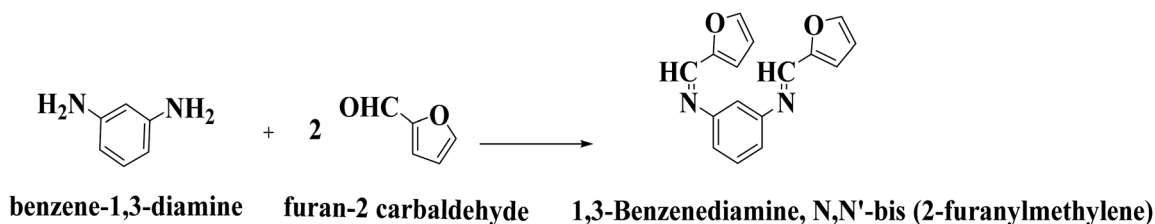
## 2.3. Preparation of the ligand

To synthesize BDBFM, a reaction was carried out by combining a solution of substituted benzene-1,3-diamine (1.08 g) (10 mmol) and furan-2-carbaldehyde (1.65 mL) (20 mmol) (density = 1.16) in 10 mL of absolute ethanol. The mixture was refluxed for 5.0 hours, followed by cooling and dilution with ice-cold water. The resulting solid was purified through recrystallization from ethanol, leading to the isolation of pure BDBFM.

The chemical structure of BDBFM was confirmed using various techniques such as FTIR, elemental analysis (C, H, and N), and <sup>1</sup>H-NMR spectroscopy. To prepare a stock solution of BDBFM, an accurately weighed quantity of the solid reagent is dissolved in the minimum required volume of ethanol, and then diluted to the desired volume in a 100 mL measuring flask (Scheme 1).

## 2.4. Preparation of optodes

For the preparation of the optode, cellulose triacetate (CTA) was dissolved in 10 mL of CHCl<sub>3</sub>. Separate solutions of chloroform were prepared, each containing a known quantity of a plasticizer, such as DOP, NPOE, T2EHP, TOT, DOS, or DOTP, and an additive (Aliquat-336 or DNNS). A solution of BDBFM with a known concentration was also prepared by dissolving the compound in ethanol. The casting solution for the optode was then created by combining appropriate volumes of the CTA solution, plasticizer solution, and BDBFM solution. If an additive was necessary, CHCl<sub>3</sub> solution of Aliquat-336 or DNNS was added to the casting solution. The resulting casting solution was thoroughly mixed through ultra-sonication for 2.0 minutes and then spread onto a flat-bottom glass Petri dish with a diameter of 9 cm. The Petri dish was placed on a leveled



Scheme 1 Synthesis of BDBFM.



surface to facilitate uniform film formation and solvent evaporation, which typically took around 24 hours. Once the solvent had completely evaporated, the optode film was carefully detached from the Petri dish surface. The resulting optode samples had dimensions of approximately 1 cm × 3 cm, providing a suitable size for subsequent analysis and testing. To ensure precise and accurate measurements, the thickness of the membrane was determined using a digital microscope (Ray Vision Y 103) in combination with a video camera (JVC TK-C 751 EG). This meticulous measurement process allowed for the reliable determination of the optode membrane's thickness, a critical parameter for evaluating and characterizing its performance.

### 2.5. Uptake of Cr(VI) and the stability of the Cr(VI)-BDBFM complex

A systematic examination was conducted to assess the influence of pH on the absorption of Cr(VI), the stability of the Cr(VI)-BDBFM complex within the optodes, and the absorbance of the corresponding blanks. To investigate this, the optode film was immersed in 2.5 mL of aqueous solutions with pH values ranging from 2.0 to 8.0, each containing 125 ng mL<sup>-1</sup> of Cr(VI), for a duration of 10 min. Under acidic conditions, acetate buffer was utilized, while borate buffer was employed under basic conditions to achieve pH adjustments. Following color development, the films were maintained at a temperature of 25 ± 2.0 °C. Cr(VI) uptake at different pH levels was evaluated by submerging the optode and measuring the absorbance when maximum sorption was achieved. The absorbance changes in the Cr(VI)-loaded optode were monitored at fixed intervals using a wavelength of 627 nm. The optode films were placed on the inner wall of a 1 cm × 1 cm × 3 cm quartz cell. To evaluate the impact of pH on the blank, the optodes were immersed in 2.5 mL solutions with pH values ranging from 2.0 to 8.0, without the presence of Cr(VI), for the same duration.

### 2.6. The influence of plasticizers on Cr(VI) in optodes

To investigate the impact of different plasticizers on the optical properties and the capacity of optodes to absorb Cr(VI), six distinct optodes were prepared, each incorporating a specific plasticizer. The plasticizers used in this study included DOP, NPOE, T2EHP, TOT, DOTP, and DOS. The optode films were immersed in stirred aqueous solutions (2.5 mL) with a pH of 4.25, containing 125 ng mL<sup>-1</sup> of Cr(VI), for a duration of 10 minutes. The color development in the optode was monitored by placing it on the inner wall of a spectrophotometric cell, and the absorbance was measured at a specific wavelength of 627 nm. This allowed for the assessment of the progress of color development in the optode films.

### 2.7. Stoichiometric determination of the Cr(VI)-BDBFM complex

The stoichiometry of the Cr(VI)-BDBFM complex was investigated using several methods, including the continuous-variation (Job's), the molar ratio, and the conductometric titration methods. These experiments were conducted under

specific conditions, with a pH of 4.25 and concentrations of 3 × 10<sup>-4</sup> M for both Cr(VI) and BDBFM. Absorbance measurements were taken at a wavelength of 627 nm, which corresponds to the maximum absorbance of the complex. These measurements were utilized to determine the precise stoichiometric ratio between Cr(VI) and BDBFM in the complex.

### 2.8. The optode tolerance to different anions in relation to Cr(VI) sorption

The tolerance of the optode to various anions, such as NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>, CH<sub>3</sub>COO<sup>-</sup>, and CO<sub>3</sub><sup>2-</sup> on the sorption of Cr(VI) was investigated. For this study, a fixed size of the optode was used along with a concentration of 125 ng mL<sup>-1</sup> of Cr(VI). Solutions containing known concentrations of the respective salts with anions or cations were mixed with the Cr(VI) solution, and the resulting solution's pH was adjusted to 4.25 using acetate buffer. It was ensured that the contribution of CH<sub>3</sub>COO<sup>-</sup> ions added for pH adjustment was taken into account. The optode samples were submerged in the solutions, and the absorption of Cr(VI) was assessed by comparing the concentration of Cr(VI) before and after the immersion of the optode samples. The volume of the solution in contact with the optode remained constant at 2.5 mL.

### 2.9. The quantification of Cr(VI) in the optode

The optode films, sized at 1 cm × 3 cm, were dried and securely affixed to the inner wall of a quartz cell with dimensions of 1 cm × 1 cm × 3 cm. In order to directly quantify the concentration of Cr(VI) in the optodes, synthetic samples were prepared by adding various concentrations of Cr(VI) (ranging from 25 to 550 ng mL<sup>-1</sup>) to 2.5 mL of acetate buffer solution at a pH of 4.25. Each sample was thoroughly stirred for 10 min to ensure consistent and uniform development of a violet color on the optode film. This procedure was repeated using fresh optode films for each concentration. Spectrophotometric analysis of the Cr(VI)-BDBFM complex within the optode samples was conducted at a specific wavelength of 627 nm ( $\lambda_{\text{max}}$ ). The resulting data were utilized to construct a calibration curve, facilitating the quantitative determination of Cr(VI) concentrations. The effectiveness of the method was further evaluated by applying it to environmental samples, where the samples were treated with known quantities of Cr(VI) ions before being exposed to the optodes.

In addition, the developed method was utilized for the determination of the total chromium content. This involved the conversion of Cr(III) to Cr(VI) through the oxidation process using hydrogen peroxide. By comparing the measured level of Cr(VI) in the sample with the overall concentration of chromium, the concentration of Cr(III) could be calculated. This approach allowed for the quantification of both Cr(III) and Cr(VI) in the sample, providing valuable information about the total chromium content.

### 2.10. The procedure for determining Cr(VI) using GFAAS

To prepare the real sample solutions, the samples were treated with a small amount of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and a 0.05%



hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) solution. The mixture was then gently heated for 5.0 min to facilitate the oxidation of  $\text{Cr(III)}$  to  $\text{Cr(VI)}$ . After cooling, a drop of hydrochloric acid (HCl) at a concentration 2.0 M was added to eliminate any excess permanganate. Once the oxidation process was completed, the samples were analyzed GFAAS, with the absorbance measured at a wavelength of 357.9 nm. The concentration of  $\text{Cr(VI)}$  in the samples was determined by referencing a calibration plot constructed using standard solutions of known  $\text{Cr(VI)}$  concentrations.

### 2.11. Statistical analysis

The experiments were conducted in six replicates, and the obtained data were presented as the mean  $\pm$  standard deviation (SD). Statistical analysis was performed using the Student's *t*-test and *F*-value. Microsoft Excel 2007 was utilized to create datasheets for data processing and analysis, including calculations of average values and recovery rates. This comprehensive statistical evaluation enabled a thorough examination of the experimental results.

## 3 Results and discussion

### 3.1. Determining the stoichiometric ratio of the $\text{Cr(VI)}$ -BDBFM complex

The stoichiometry of the  $\text{Cr(VI)}$ -BDBFM complex was investigated using two methods: Job's method and the molar ratio procedure. The absorption spectra of BDBFM and the  $\text{Cr(VI)}$ -BDBFM complex were initially recorded and are illustrated in Fig. 1. The maximum absorption wavelength for BDBFM was found to be 493 nm, while the complex with  $\text{Cr(VI)}$  exhibited its

peak absorption at 627 nm. Both methods were subsequently carried out at 627 nm, the wavelength at which the complex demonstrated the highest absorbance.

In Job's method, absorbance was plotted against the mole fraction of  $\text{Cr(VI)}$ , with varying concentrations of BDBFM and  $\text{Cr(VI)}$ . The plot exhibited an inflection point at 0.35, indicating the presence of two BDBFM molecules in the formed complex. Furthermore, the molar ratio method yielded a BDBFM to  $\text{Cr(VI)}$  ratio of 2.0, providing additional evidence for the stoichiometric ratio of (2 : 1) for BDBFM to  $\text{Cr(VI)}$ . To further validate these findings, conductometric titration was employed.

$\log K$ , which is the conditional formation constant, was determined by employing the Harvey and Manning equation with the data obtained from the aforementioned methods. The calculated  $\log K$  value was determined to be 4.46, while the true constant was found to be 4.30. The interaction between  $\text{Cr(VI)}$  and BDBFM leads to the formation of a distinctive violet-colored complex known as the cationic complex  $[\text{Cr(VI)}-(\text{BDBFM})_2]^{n+}$ . This complex formation is highly specific to  $\text{Cr(VI)}$  and is widely utilized for the colorimetric determination of  $\text{Cr(VI)}$  in aqueous samples.

For the specific detection of  $\text{Cr(VI)}$ , a membrane optode was developed by incorporating BDBFM into a CTA matrix that was plasticized with DOP. Upon exposure of this optode to an aqueous solution containing  $\text{Cr(VI)}$  at pH 4.25, a vibrant violet color develops within both the membrane and the surrounding solution. This color change serves as a visual indicator for the presence of  $\text{Cr(VI)}$  and allows for its sensitive and selective detection.

To ensure the optimal performance of the optode in extracting  $\text{Cr(VI)}$  from aqueous solutions, it is crucial for the membrane to possess certain characteristics such as homogeneity, flexibility, thinness, and mechanical strength. These criteria served as the foundation for the optimization of the membrane composition. Various combinations of supporting polymers, extractants, and reagents were initially tested to identify the most suitable combination for sensing  $\text{Cr(VI)}$  ions. The concentrations of Aliquat 336, DOP, and CTA, which served as the optimal carrier, plasticizer, and polymer for the chromophore BDBFM, were adjusted to enhance the sensitivity, selectivity, and stability of the optode. Among the critical components, the base polymer and the plasticizer play a significant role in determining the strength and flexibility of the membrane. Thus, different concentrations of these components were tested at three levels, while maintaining the Aliquat 336 and BDBFM concentrations at 40% and 1.0%, respectively, in all three setups. A combination of 6.0% DOP and 53% CTA resulted in minimal chromophore leakage, but the membrane exhibited rigidity due to the closely packed CTA strands caused by the low amount of plasticizer. On the other hand, the membrane containing 16% DOP was flexible but overly oily due to the excessive amount of plasticizer. The issue of leaching of the chromophore was addressed during the optimization process. It was observed that the optode composition consisting of 10% DOP and 49% CTA exhibited the best stability and flexibility while minimizing leaching. The ratios of DOP and CTA, along with the corresponding characteristics of

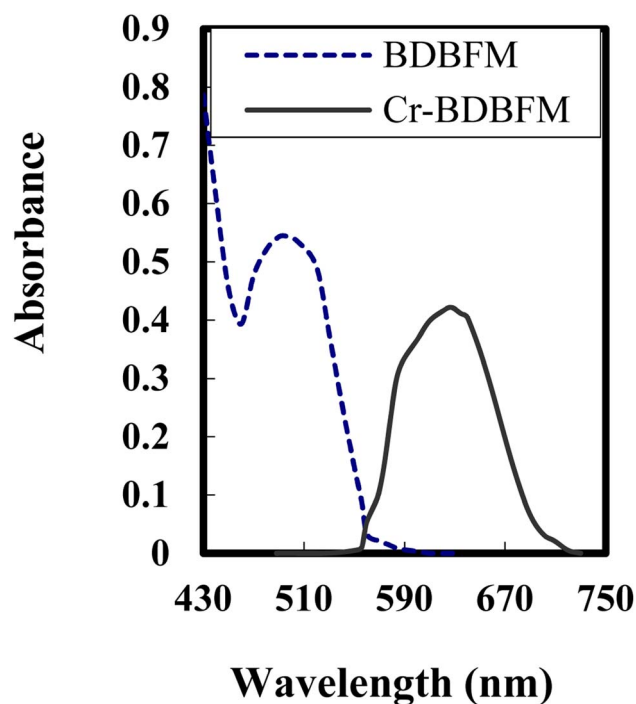


Fig. 1 Absorption spectra of  $3 \times 10^{-4}$  mol  $\text{L}^{-1}$  of BDBFM and its complex with 125 ng per mL  $\text{Cr(VI)}$  ions at pH 4.25.





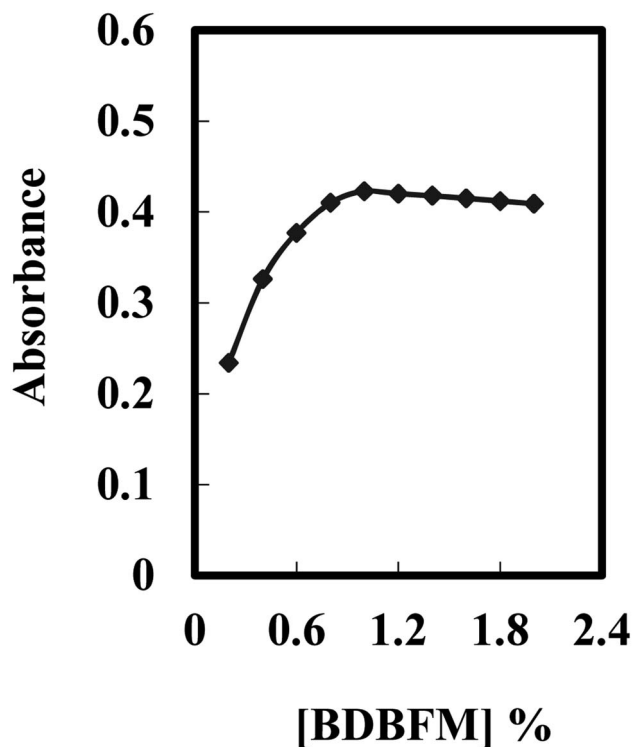
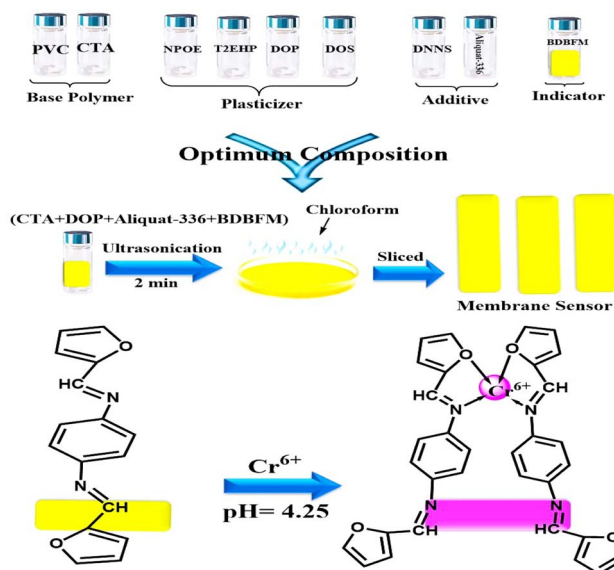


Fig. 3 Effect of the BDBFM percentage on the optode membrane immersed in  $125 \text{ ng mL}^{-1}$  of  $\text{Cr}(\text{vi})$  under optimum conditions.



Scheme 2 Schematic representation for the preparation and complexation of the formed optical sensor. The images are real photos of the sensor.

a more favorable method for determining the concentration of  $\text{Cr}(\text{vi})$  ions. By measuring the absorbance after 10 min of reaction time, the time required to generate a significant signal from the initial reaction between BDBFM and  $\text{Cr}(\text{vi})$  was significantly reduced. The stirring of the  $\text{Cr}(\text{vi})$  solution had

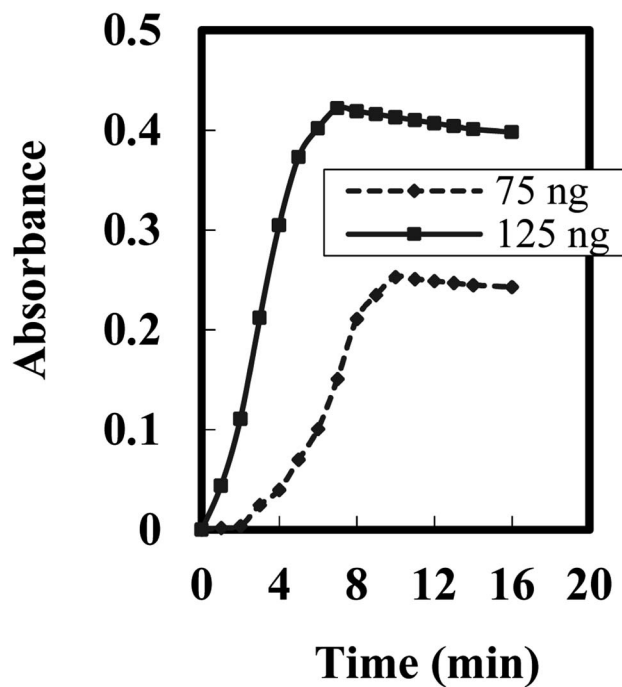


Fig. 4 Steady state response time of the optode towards different  $[\text{Cr}(\text{vi})]$  –  $75 \text{ ng mL}^{-1}$  and –  $125 \text{ ng mL}^{-1}$ .

a significant impact on the response of the formed optode. Stirring the solution resulted in an eight-fold enhancement compared to the non-stirred solution. This enhancement can be attributed to the movement of  $\text{Cr}(\text{vi})$  ions towards the immobilized BDBFM. Stirring facilitates the diffusion of  $\text{Cr}(\text{vi})$  ions across the membrane, allowing them to reach BDBFM more quickly and promoting the reaction between  $\text{Cr}(\text{vi})$  ions and BDBFM. In contrast, in the non-stirred process, the diffusion of  $\text{Cr}(\text{vi})$  ions across the membrane relies solely on the concentration gradient.<sup>61</sup>

The proposed optode exhibits a notable feature of regenerability, allowing for multiple reuses of a single optode while minimizing reagent consumption. Various regenerating reagents, including EDTA,  $\text{HNO}_3$ , SCN, and thiourea, were investigated. Regeneration with EDTA proved to be time-consuming, while regeneration with SCN and thiourea did not yield consistent results. However, 0.05 M nitric acid demonstrated complete regeneration within a short duration (less than 40 seconds) and exhibited high reproducibility. The membrane could be regenerated over 12 times without any loss of its characteristics, leading to the selection of 0.05 M nitric acid as the preferred regenerating reagent.

The precision and reliability of the optode are critical considerations in the development of chemical sensors. Precision refers to the ability of the optode to produce consistent results when used repeatedly, while reliability is related to the consistency of results obtained from different sets of optodes.<sup>62</sup> Precision was evaluated by independently constructing eight optodes under similar conditions and measuring the absorbance at  $\lambda_{\text{max}}$  627 nm (with three repeated determinations)









Table 5 Determination of total chromium in spiked test solutions ( $N = 5$ )

Added ( $\mu\text{g}$ )		Found <sup>a</sup> ( $\mu\text{g}$ )			Recovery <sup>a</sup> (%)			Total Cr, FAAS
Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Total Cr	Cr(III)	Cr(VI)	Total Cr	
0.00	2.00	0.00	1.98	1.98	0.00	99.00	99.00	2.05
0.50	1.50	0.49	1.52	2.01	98.00	101.33	100.50	1.97
1.00	1.00	1.01	1.01	2.02	101.00	101.00	101.00	1.95
1.50	0.50	1.48	0.49	1.97	98.67	98.00	98.50	2.04
2.00	0.00	2.01	0.00	2.01	100.50	—	100.50	1.97
2.25	0.75	2.27	0.76	3.03	100.89	101.33	101.00	2.95
3.00	0.00	2.99	0.00	2.99	99.67	—	99.67	3.05

<sup>a</sup> Average of six determinations.

Cr(VI). This means that, even in solutions containing both Cr(VI) and Cr(III) ions, the optode can accurately determine the concentration of Cr(VI). Furthermore, by oxidizing Cr(III) to Cr(VI) using H<sub>2</sub>O<sub>2</sub> as an oxidizing agent, the optode can also be used to determine the total chromium concentration in the same solution. The concentration of Cr(III) ions can then be calculated by subtracting the amount of Cr(VI) from the total chromium concentration.

In order to oxidize Cr(III) to Cr(VI), a mixture of Cr(VI) and Cr(III) ions is subjected to a treatment involving 2.0 mL of 0.1 M H<sub>2</sub>O<sub>2</sub> and 0.5 mL of 0.1 M HNO<sub>3</sub> in a beaker. The beaker is heated at approximately 80 °C for 30 minutes, while being covered with a watch glass during the oxidation process. After the solution has cooled down, its pH is adjusted to pH 4.25 using acetate buffer. Subsequently, the solution is transferred to a calibrated 10 mL flask and diluted with water up to the mark. The recommended procedure for Cr(VI) determination using the optode is then followed. Additionally, the total chromium content is determined using flame atomic absorption spectroscopy (FAAS) for comparison purposes. The results of chromium speciation at various Cr(III)/Cr(VI) ratios are summarized in Table 5.

The data presented in Table 5 reveal satisfactory agreement between the total chromium measurements obtained using the proposed optode membrane and those obtained using the GFAAS method, considering the reported experimental uncertainties. Furthermore, accurate measurements of both Cr(III) and Cr(VI) ions present in the initial solutions can be achieved using the proposed optode for all tested Cr(VI)/Cr(III) mixtures.

### 3.6. Analytical applications

The developed method was successfully utilized to perform the speciation analysis of Cr(III) and Cr(VI) in various natural water samples, including tap water, Nile river water from Benha City, and a sea water sample from the Mediterranean Sea at Port Said City. To evaluate the reliability of the method, chromium species were deliberately spiked into these samples.

Meticulous precautions were taken during the collection and preparation of the water samples to ensure precise and reliable analysis. Specially pre-washed polyethylene bottles were utilized to prevent any potential contamination. To eliminate any particulate matter, the samples underwent filtration through

a Millipore cellulose membrane that had a pore size of 0.45  $\mu\text{m}$ . To maintain sample integrity and minimize chemical changes, the samples were stored in acidified polyethylene bottles at a temperature of 4.0 °C. Nitric acid was added to achieve a concentration of 1.0%, thereby stabilizing the samples and preventing degradation or alteration of the analytes during storage. The acidification step is vital for sample preservation.

For tap water samples, an additional step was incorporated to remove chlorine. Activated charcoal was introduced into the samples and allowed to interact for a duration of 5.0 minutes. Subsequently, the samples were subjected to a secondary filtration process to eliminate any residual charcoal particles.

Before conducting the analysis, the pH of the samples was carefully adjusted to 4.25. This specific pH value was chosen to ensure optimal conditions for the subsequent analysis of Cr(VI) using the developed method. By precisely controlling the pH, the accuracy and reliability of the analysis were significantly enhanced.

The results obtained, carefully documented in Table 6, exhibit excellent agreement between the measured amounts of the analytes and the amounts added, thereby confirming the accuracy and validity of the proposed optode for detecting trace levels of chromium in both spiked and natural water samples. The relative standard deviations for Cr(III) and Cr(VI) in the water samples demonstrate a commendable range of 1.15% to 2.48% and 1.56% to 2.84%, respectively. Furthermore, the proposed procedure was successfully extended to solid samples, which underwent microwave digestion, yielding relative standard deviations ranging from 3.5% to 8.0%.

To further demonstrate the practical applicability of the proposed system, a variety of food samples were carefully analyzed. Beverage samples, including orange juice, soda, cola, apple juice, and Sprite drink, were carefully collected from a local market in Port Said, Egypt. To prepare these samples for analysis, a degassing step was performed by vortexing them for 2.0 minutes. Subsequently, the samples were diluted five times with high purity water. After filtration using a 0.45  $\mu\text{m}$  membrane filter, the solutions were refrigerated at 5 °C to guarantee their preservation and stability for future utilization. Solid samples such as coffee, tea, and tobacco were obtained from supermarkets in Benha City for analysis. Before subjecting these solid samples to the proposed optode testing, a digestion



Table 6 Determination of Cr(III), Cr(VI) and total chromium in some natural water samples (N = 5)

Samples	Added ( $\mu\text{g}$ )		Found <sup>a</sup> ( $\mu\text{g}$ )			Recovery (%)		
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Total	Cr(III)	Cr(VI)	Total
Tap water from Benha City, Egypt	—	—	0.220	0.400	0.620	—	—	—
	0.3	0.5	0.522	0.910	1.432	100.38	101.11	100.85
	0.5	0.30	0.720	0.695	1.415	100.00	99.29	99.65
	0.4	0.4	0.616	0.81	1.426	99.35	101.25	100.42
River water from Shobra, Egypt	—	—	0.430	0.880	1.310	—	—	—
	0.5	0.75	0.925	1.627	2.552	99.46	99.82	99.69
	0.75	0.50	1.170	1.370	2.540	99.15	99.28	99.22
	0.60	0.60	1.020	1.480	2.500	99.03	100.00	99.60
Sea water from Port Said, Egypt	—	—	0.510	1.130	1.640	—	—	—
	0.30	0.20	0.818	1.333	2.151	100.99	100.23	100.51
	0.25	0.25	0.755	1.370	2.125	99.34	99.28	99.30
	0.20	0.30	0.717	1.433	2.15	100.99	100.21	100.47

<sup>a</sup> Average of six determinations.

process was carried out utilizing a microwave system. The digestion procedure involved specific time intervals and power levels. The samples were microwaved for 2.0 minutes at 250 W, followed by 2.0 minutes at 0 W, 6.0 minutes at 250 W, 5.0 minutes at 400 W, and 8.0 minutes at 550 W. Venting was then conducted for an additional 8.0 min. During the digestion process, 1.0 gram of the tobacco, coffee, or tea samples was combined with 6.0 mL of 65% HNO<sub>3</sub> and 2.0 mL of 30% H<sub>2</sub>O<sub>2</sub> in the microwave system. The resulting mixture was subsequently diluted to a total volume of 25 mL using bi-distilled water. A blank sample was prepared following a similar procedure, excluding the addition of the solid sample. By adhering to these meticulous preparation procedures, both the beverage and solid samples were properly treated and made ready for subsequent analysis using the proposed optode method.

Food products, particularly those of wheat, play a significant role in the human diet. It is crucial to detect the presence of toxic Cr in the form of Cr(VI). In this study, a total of 50 commercially available bread samples were collected from a local market in Benha, Egypt. The extraction of Cr(VI) from these samples was performed using a 0.01 M NaOH solution at room temperature for a duration of 17 hours.<sup>77</sup> Following the extraction process, the supernatant was obtained by centrifugation, and its composition was examined using the graphite furnace atomic absorption spectrometry (GFAAS) technique. The aforementioned procedure was applied to the resulting solutions, with the pH carefully adjusted to 4.25, allowing for the determination of chromium content.

For the analysis of a soil sample collected from Benha, Egypt, a precise procedure was followed. Initially, 2.0 grams of the soil sample were carefully transferred to a beaker. To initiate the digestion process, 20 mL of aqua regia was added to the beaker. The solution obtained was evaporated in a fume hood until dryness. This evaporation process was repeated twice to ensure thorough digestion of the sample. Next, 10 mL of bi-distilled water was added to the remaining residue in the beaker. The mixture was mixed well and then filtered using a high-quality blue band filter paper from Macherey-Nagel, a reputable company based in Düren, Germany. To remove any remaining

impurities, the insoluble portion was meticulously washed with bi-distilled water.

The final solutions obtained from the filtration process were subjected to the proposed procedure, as previously described, to determine the chromium (Cr) content. Additionally, the Cr content was assessed using a sensitive technique utilized for analyzing trace elements which is GFAAS. During the analysis of real soil samples, the total chromium content was assessed as Cr(III) by converting any present Cr(VI) to Cr(III) utilizing the prescribed procedure as recommended.<sup>78,79</sup>

By utilizing the optimized parameters as described earlier, the system provided robust results for the food samples, as presented in Table 7. The accuracy of the method was evaluated by comparing these results with those obtained using GFAAS. Rigorous statistical tests, including the calculation of *F*-values and *t*-tests at a 95% confidence level, were performed, revealing no significant deviations between the two methods.<sup>80</sup>

The analytical performance of the developed optode was thoroughly assessed for the detection of Cr(VI) in human blood (serum) and urine samples. Human serum and urine samples were collected from the Benha University Hospital to perform analytical investigations. In the case of the serum samples, the protein components were precipitated by subjecting 5.0 mL of the serum to centrifugation at 4000 rpm for 5.0 minutes. Trichloroacetic acid was used in a small volume during the centrifugation process to aid in protein precipitation. Following centrifugation, the serum portion was carefully decanted, and then diluted with deionized water at a ratio of 1 : 10. For calibration purposes, a spiked sample was prepared by adding precise amounts of a Cr(III) standard solution to 0.1 mL of the human serum sample. The samples, including the spiked sample, were thoroughly mixed, and additional dilutions were made as required. The chromium concentrations in these samples were directly analyzed using the proposed optode technique, enabling accurate determination of the chromium levels.

The optode demonstrated effective detection of Cr(VI) in these samples, as well as in dietary supplements and artificially spiked human serum and urine samples. The samples were



Table 7 The level of total chromium in some real samples after application of the presented and GF-AAS procedures ( $N = 5$ )

Sample	Added ( $\mu\text{g g}^{-1}$ )	Found <sup>a</sup> ( $\mu\text{g g}^{-1}$ )		RSD (%)	$t$ -Test <sup>b</sup>	$F$ -Value <sup>b</sup>
		Optode	GF-AAS			
Tobacco	—	1.48	1.45			
	0.50	2.00	1.85	2.74	1.34	
	1.00	2.45	2.60	2.22		2.87
Coffee	—	0.27	0.26			
	0.75	1.00	0.98	3.15	1.58	
	1.50	1.75	1.80	2.76		3.21
Red tea	—	0.18	0.18			
	0.25	0.38	0.36	2.48	1.21	
	0.50	0.70	0.65	2.25		2.58
Green tea	—	0.15	0.16			
	0.40	0.56	0.53	3.27	1.77	
	0.80	0.93	1.00	2.57		3.43
Soil from Benha City	—	24.7	24.5			
	1.50	26.35	25.75	1.98	0.84	
	3.00	27.50	27.80	2.32		2.46
Orange juice <sup>c</sup>	—	0.25	0.25			
	6.0	6.40	6.15	2.53	1.54	
	12.0	12.05	12.50	1.97		3.16
Soda <sup>c</sup>	—	0.65	0.70			
	8.0	8.75	8.60	2.16	1.37	
	16.0	16.90	16.40	2.44		2.95
Cola <sup>c</sup>	—	0.80	0.75			
	3.0	3.70	3.90	2.65	1.85	
	6.0	6.95	6.60	2.33		3.48
Apple juice <sup>c</sup>	—	0.25	0.25			
	9.0	9.40	9.10	1.87	1.62	
	18.0	18.00	18.55	2.05		3.23
Sprite drink <sup>c</sup>	—	0.45	0.50			
	7.0	7.30	7.70	1.77	1.73	
	14.0	14.70	14.25	2.11		3.37

<sup>a</sup> Average of six determinations. <sup>b</sup> The theoretical values of  $t$ - and  $F$ - at  $P = 0.05$  are 2.57 and 5.05, respectively. <sup>c</sup> [ $\text{ng mL}^{-1}$ ].

carefully prepared following the experimental procedures outlined in the study and analyzed using the standard addition method. The results, presented in a systematic manner in Table 8, exhibited excellent recoveries ranging from 97.67% to 102.67% and low relative standard deviations (RSDs) of less than 2.12%.

Table 8 Validation of chromium speciation based on the proposed and GFAAS methods with standard addition in human serum and blood samples

Sample	Added ( $\text{ng mL}^{-1}$ )		Found <sup>a</sup> ( $\text{ng mL}^{-1}$ )			Recovery (%)	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Total	Cr(III)	Cr(VI)
Whole blood	—	—	$0.655 \pm 0.026$	$0.090 \pm 0.003$	$0.745 \pm 0.028$	—	—
	0.3	—	$0.960 \pm 0.048$	$0.095 \pm 0.004$	$1.055 \pm 0.056$	100.52	—
	—	0.2	$0.660 \pm 0.028$	$0.285 \pm 0.013$	$0.945 \pm 0.045$	—	98.28
Whole blood	—	—	$0.960 \pm 0.055$	$0.205 \pm 0.021$	$1.165 \pm 0.064$	—	—
	0.4	—	$1.350 \pm 0.073$	$0.210 \pm 0.011$	$1.560 \pm 0.078$	99.26	—
	—	0.3	$0.975 \pm 0.041$	$0.520 \pm 0.018$	$1.495 \pm 0.065$	—	101.96
Whole blood <sup>b</sup>	—	—	$0.215 \pm 0.009$	$1.075 \pm 0.057$	$1.290 \pm 0.069$	—	—
	0.5	—	$0.725 \pm 0.031$	$1.060 \pm 0.047$	$1.785 \pm 0.084$	101.40	—
	—	0.4	$0.210 \pm 0.011$	$1.485 \pm 0.075$	$1.695 \pm 0.092$	—	101.71
Serum	—	—	$0.725 \pm 0.033$	$0.130 \pm 0.006$	$0.855 \pm 0.043$	—	—
	0.6	—	$1.310 \pm 0.062$	$0.135 \pm 0.005$	$1.445 \pm 0.071$	98.87	—
	—	0.5	$0.735 \pm 0.036$	$0.645 \pm 0.016$	$1.380 \pm 0.048$	—	101.57
Serum	—	—	$1.250 \pm 0.058$	$0.080 \pm 0.002$	$1.330 \pm 0.064$	—	—
	0.7	—	$1.925 \pm 0.077$	$0.075 \pm 0.003$	$2.000 \pm 0.082$	98.72	—
	—	0.6	$1.235 \pm 0.061$	$0.690 \pm 0.012$	$1.925 \pm 0.078$	—	102.22

<sup>a</sup> Mean of three determinations  $\pm$  confidence interval ( $P = 0.95$ ,  $n = 5$ ). <sup>b</sup> Whole blood (WB) diluted with DW (1 : 5), and the concentration is out of linear range, final Cr conc. = found Cr conc.  $\times$  5 (dilution factor).





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