Development of a New Colorimetric Chemosensor for Selective Determination of Urinary and Vegetable Oxalate Concentration Through an Indicator-Displacement Assay (IDA) in Aqueous Media

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SUMMARY

The proposed method that exhibits operational simplicity is presented for the indirect spectrophotometric determination of oxalate ion. The sensor Cu(II)–(1-Amino-4-[3-(4, 6-dichlorotriazin-2-ylamino)-4-sulfophenylamino] anthraquinone-2-sulfonic acid), (RB4), has been developed to be a simple, inexpensive yet selective colorimetric chemosensing ensemble for the

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recognition of oxalate over other available competitive analytes via indicator displacement assay (IDA) in both solution (aqueous media) and solid state (paper-based experiment). The obvious spectral and color changes from sky blue to dark blue were observed by the addition of oxalate to RB4-Cu$^{2+}$ complex due to the regeneration of RB4 by the chelation of oxalate as the competitive analyte with Cu$^{2+}$. The absorbance band increase is linear with oxalate concentration from 1.76-49.4 µmol/L, and a detection limit of 0.62 µmol/L. No obvious influence of interferences (available anions and ascorbic acid) was also observed using this measurement mode. This approach eliminated the need for the separation stages, enzymatic multiple-step reactions, sample preparation, organic solvents mixture, chemical modifications and equipment developed to a high degree of complexity. Satisfactory results were also obtained by the determination of oxalate in different real samples such as urine sample, mushroom, and spinach which demonstrated the applicability of the existing method. Furthermore, this colorimetric system can be used as IMPLICATION molecular logic gate with respect to Cu$^{2+}$ and oxalate (C$_2$O$_4^{2-}$) as inputs and UV-Vis absorbance signal as the output with potential monitoring applications.

**Key words:** colorimetric chemosensor, indicator displacement assay, copper complex, oxalate, urine, vegetable

**INTRODUCTION**

In recent years, the development of optical chemosensor for biologically important anions have received considerable attention because these species have applications in wide range of biological, industrial, and environmental processes (1-2). The indicator-spacer-receptor approach (ISR) is the most widely used approach for optical chemosensor in the detection of anions, traditionally, in which the indicator (herein chromophore) is covalently attached to the receptor through a spacer. The limitations of ISR approach is the need to synthesize the sensor, the attendant difficulties, and cost. The indicator displacement assay (IDA) largely circumvented this problem (3). Nowadays, this approach has been used popularly in studying molecular recognition (4). In an IDA, an indicator is first allowed to form a reversible bond to a receptor. Then, a competitive analyte introduced into the system displaces the indicator from the receptor which
this displacement and binding event are accompanied by a colorimetric signal and naked-eye color change (5). Based on this principle, the major requirement for an IDA is that the affinity between indicator and receptor is lower compared to that of the analyte-receptor complex. The interactions between the indicator or analyte and the host are dependent on the geometry of the guest, its charge, its hydrophobicity, and the solvent system (6). IDAs have been used to sense both cations and anions. However, the majority of IDAs have been for anions (7). Many of anions have the fundamental roles in the industrial processes and clinical analysis (8). Oxalate (C$_2$O$_4^{2-}$) as one of the most common nutrient chelates in the human diet is of great interest due to its vital roles in chemistry and biochemistry (9). The protein metabolism causes to produce oxalate in the human body (10). The high concentration of oxalate in urine or blood is dangerous and may cause a number of maladies including renal failure, chronic disease of the heart muscle, pancreatic insufficiency, and the development of kidney stones (11, 12). The insoluble complex salt with calcium (CaC$_2$O$_4$) may be produced in the body as an end product of amino acid or ascorbate metabolism. The normal level of urinary oxalate excretion is nearly in the range of 110-460 µmol in 24 hours (13). Therefore, the quantification of oxalate in human urine is important for management of the diseases mentioned. Furthermore, the determination of oxalate content in food is also important since the low oxalate diet is sometimes necessary for the treatment. In recent years, several efforts have been devoted to determine the concentration of oxalate which carried out using various analytical methods including chromatography (14), chemiluminescence (15), amperometry (16, 17), flow injection analysis (18), electrochemistry (19), capillary zone electrophoresis (20), and Enzyme-based method (21). However, most of these approaches require a high cost for the operation, special equipment, and long time for the boring preparation of the sample. Therefore, the chemistry of simple and efficient receptors for the selective recognition of oxalate, particularly at physiological pH in different samples is still important for researchers.

Colorimetric IDA-based sensors as user-friendly devices have many advantages including simple operation, versatility, sufficiently short response time, and relatively cheap cost as well as with sensitive optical readout for the analyte (3, 22). Until now, some fluorometric and colorimetric displacement approaches have been developed due to the utilization of high affinity of oxalate with metal ions (2, 23-26). Nevertheless, some of these approaches need to the complicated synthetic process of primary material that is the time-consuming and labor-intensive need (2, 23).
In this study, we applied the colorimetric IDA approach for rapid, simple, selective and cost-effective detection of oxalate in biological samples by taking advantage of the Cu$^{2+}$-oxalate affinity pair. Reactive Blue 4 dye (RB4) coordinated with Cu$^{2+}$ as an appropriate colorimetric indicator and oxalate as a competitive analyte were employed. After treatment of Cu$^{2+}$ by RB4 (the formation of the Cu-RB4 complex) in aqueous media (10 mmol/L HEPES buffer solution, pH=7), the color and the colorimetric signal of RB4 exhibited changes which were observable by naked-eyes. The proposed IDA system was also successfully applied for the determination of oxalate concentration in urine and vegetable samples such as spinach and mushroom. Furthermore, the proposed method could be used as a paper-based analytical device and IMPLICATION molecular logic gate with respect to Cu$^{2+}$ and oxalate ($\text{C}_2\text{O}_4^{2-}$) as inputs and UV-Vis absorbance signal as the output with potential monitoring applications.

MATERIALS AND METHODS

*Materials and apparatus*

Reactive Blue 4 (RB4), an anthraquinone dye (1-Amino-4-[3-(4, 6-dichlorotriazin-2-ylamino)-4-sulfophenylamino] anthraquinone-2-sulfonic acid), was obtained from Merck Company (Darmstadt, Germany). Demineralized water was used for preparing the solutions. Cu (NO$_3$)$_2$, KF, KCl, K$_2$CO$_3$, Na$_2$HPO$_4$, K$_2$PO$_4$, Na$_2$SO$_4$, KNO$_3$, NaC$_2$H$_5$O$_2$, KClO$_4$, K$_2$C$_2$O$_4$, and ascorbic acid (C$_6$H$_8$O$_6$) were purchased from Merck Company (Darmstadt, Germany). Stock solution (1×10$^{-2}$ mol/L) of analytes was prepared by direct dissolution of their proper amount in deionized water. All other chemicals were of analytical grade and used as received. The buffer solution was prepared using 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES).

A Shimadzu 1601 PC UV-Vis double beam spectrometer (Kyoto, Japan) was used to record all UV-Vis spectra by quartz cuvettes 10.0 mm in diameter. A Bruker Vector 22 Fourier Transmission Infrared spectrometer (Massachusetts, the United States of America) was used for recording FT-IR spectra using KBr pellet method. A digital Jenway 3510 digital pH meter (London, England) calibrated with two standard buffer solutions was used to measure various pH values. A Hamilton syringe 50 µL was used to deliver desired amounts of analyte solution into the cuvette.
General procedure

The temperature adjusted for all titration experiments was 298.2 K. The $5 \times 10^{-5}$ mol/L solution of RB4 was prepared by the dilution of its $1.0 \times 10^{-2}$ mol/ L stock solution in aqueous media (10 mmol/ L HEPES buffer solution, pH=7). Then the UV-Vis absorption spectra of RB4 were recorded by transferring 2.5 mL of this diluted solution to the quartz cuvette. The $1 \times 10^{-2}$ mol/ L aqueous solution of the nitrate salt of Cu$^{2+}$ was prepared and used for complexation study with RB4 after proper dilution. The detection of oxalate and its effect on the color and spectra recovery of the RB4-Cu$^{2+}$ solution was studied by preparing the oxalate solution and gradually adding it ($1.76 \times 10^{-6}$– $9.60 \times 10^{-5}$ mol/L) with microliter syringe to the solution containing RB4 coordinated with Cu$^{2+}$. Then, the UV-Vis spectra for a certain time course in aqueous solution were recorded. Subsequently, the interactions and the stoichiometry ratios (Yeo and Jones method or the mole ratio method (27, 28)) and some other items were studied.

Preparation of test paper for onsite visual determination of oxalate

Test papers were fabricated by immersing normal filter papers into the aqueous solution of RB4 (0.1 mol/ L) and Cu$^{2+}$ (0.1 mol/ L), respectively. After taking from the solutions containing RB4 and copper ion and drying at room temperature, the filter papers were directly applied for the determination of oxalate in aqueous solutions. The corresponding color change was observed with the naked eye.

Real sample preparation

Preparation of urine sample

An aliquot of 5mL of urine sample collected in sterilized polyethylene tube was transferred to a 50 mL volumetric flask, made up to the mark with buffer solution (pH= 7), and shaken for 5 min. Then, 20 mL of this solution was taken and transferred to a 50mL beaker. The oxalate content was determined by the experimental procedure.
Preparation of vegetable samples

3g of fresh spinach or 15g of fresh mushroom were cut into small pieces with a razor blade. Then, a mortar with a pestle was used to pound the small pieces of the samples. Subsequently, the paste obtained was mixed with water, diluted to 100 mL in a calibrated flask, and boiled for 45 min, after which time the mixture was cooled. The filtration of the suspension was done through filter paper (Whatman No.1). The filtrate was diluted to 250 mL. Then, the pH of the resulted solution was adjusted to about 10 by dropwise addition of 0.1 mol/L NaOH solution to remove the interference effect of iron cations. The solution was centrifuged with a centrifugal force of 1492 × g for 5 min. After the neutralization of the solution with 0.1 mol/L HCl solution, it was diluted in a 100-mL volumetric flask. Then a suitable aliquot of the solution obtained was used for the determination of the oxalate content by means of the proposed method.

Reference method

After the preparation of the test sample solution, it was transferred to a 250 mL Erlenmeyer flask. 50 mL of distilled water and 20 mL of 6N H₂SO₄ was added to it in Erlenmeyer flask and swirled to dissolve the solid. The acidified solution was heated to about 85°C. 1g of KMnO₄ was weighted, transferred to a 500mL volumetric flask, distilled by 350 mL of water, and placed on heat for 30 minutes. The titration of the hot sample solution was done with the cool KMnO₄ solution to determine the endpoint (29).

RESULTS AND DISCUSSION

Design of the colorimetric sensor for determination of oxalate based on IDA principle

A simple oxalate colorimetric chemosensor was designed based on indicator-displacement strategy. For IDA, the indicator must bind to host reversibly and cause the change in spectroscopic signal. In this experiment, RB4 is a colorimetric indicator to form the RB4-Cu²⁺ complex. The interaction between RB4 and Cu²⁺ was investigated by UV-Vis spectroscopy. As can be observed in Fig. 1a, RB4 (50 µmol/L) in aqueous solution (10 mmol/L HEPES buffer solution, pH=7) exhibits a maximum absorption peak centered at ~607 nm as well as an obvious dark blue color. Upon
gradual addition of Cu$^{2+}$ species ($1.97\times10^{-6}$–$1.22\times10^{-4}$ mol/L), the absorption intensity at 607nm decreased and red-shifted to around 619nm accompanying an obvious color change from dark blue to deep sky blue (right inset Fig. 1a). The absorbance changes versus the Cu$^{2+}$ concentration increase is also plotted in left inset Fig. 1a. A 1:1 binding mode for the formed complex between RB4 and Cu$^{2+}$ was confirmed by Yeo and Jones method (Fig. S1) (27, 28). The binding constant ($K_a$) of the [RB4-Cu$^{2+}$] complex was also estimated to be (4.46±0.12) .10$^5$ L/mol by nonlinear curve fitting (30).

**Fig. 1a**

The colorimetric response of [RB4-Cu$^{2+}$] toward oxalate was studied by the UV-Vis spectrometric titration experiment. When oxalate ion was added gradually (1.76 – 96.0 µmol/ L) to the complex solution, the absorption band intensity increased at 607nm (Fig.1b). The color of the solution also gradually changed from deep sky blue to dark blue (right inset Fig. 1b). Left inset Fig. 1b is also the graph of the UV-Vis absorption changes of the RB4-Cu$^{2+}$ complex as a function of the concentration of oxalate (mol/L). The absorption spectra and the color obtained were identical with that of RB4. This colorimetric change indicates that [RB4-Cu$^{2+}$] complex can be used for the determination of oxalate by Naked-eyes.

The application of the Yeo and Jones method (27, 28) by plotting the absorbance at 607nm versus the molar ratio of C$_2$O$_4$^{2-} to Cu$^{2+}$ confirmed a stoichiometry of 1:1 for the interaction of Cu$^{2+}$ and C$_2$O$_4$^{2-} (Fig. 2) with the association constant $K=(2.30\pm0.1)\times10^7$ L/mol obtained by nonlinear curve fitting (Fig. S2) (30, 31). Two groups of oxalate O atoms act as a bidentate ligand and are coordinated to Cu$^{2+}$ ion. Thus, the displacement process can be as follows: RB4-Cu$^{2+}$ +C$_2$O$_4$^{2-} $\rightarrow$ CuC$_2$O$_4$ + RB4. As mentioned above, the binding constant of Cu$^{2+}$ with oxalate is larger than that for the interaction of C$_2$O$_4$^{2-} and RB4. Therefore, the indicator displacement by oxalate is highly favorable due to more affinity of oxalate toward Cu$^{2+}$.

**Fig. 1b**

**Fig.2**

*Study of selectivity toward oxalate sensing*

The selectivity of the proposed chemosensor on oxalate ion in the presence of ascorbic acid and other available anions was investigated. As shown in Fig. 3, the solution system was selective
toward oxalate anion over ascorbic acid \((\text{C}_6\text{H}_8\text{O}_6)\) and other available anions such as \(\text{SO}_4^{2-}\), \(\text{NO}_3^-\), \(\text{CO}_3^{2-}\), \(\text{HPO}_4^{2-}\), \(\text{PO}_4^{3-}\), \(\text{ACO}^-\), \(\text{ClO}_4^-\), \(\text{F}^-\), \(\text{Cl}^-\), and \(\text{Br}^-\). It is only the oxalate anion which is able to generate color and spectral changes. When ascorbic acid and other anions were added into the \([\text{RB4}-\text{Cu}^{2+}]\) complex solution, negligible changes in color and UV-Vis spectrum were observed. Moreover, there was no interference from ascorbic acid and other anions (500 µmol/L) for the determination of the amount or presence of oxalate anion. Therefore, it was proved that this chemosensor has selectivity toward oxalate sensing in water solution without the need of sophisticated instruments.

**Fig. 3**

Possible mechanism study

The proposed IDA strategy for the determination of oxalate \((\text{C}_2\text{O}_4^{2-})\) has been shown in Scheme 1. This strategy is based on the competition of the analyte and the chromogenic indicator (RB4) for the interaction with \(\text{Cu}^{2+}\). As already mentioned, a 1:1 complex with the stability constant of \((4.46\pm0.12) \cdot 10^5 \text{ L/mol}\) upon addition of \(\text{Cu}^{2+}\) to the RB4 solution was formed. In FT-IR spectrum of the RB4-\(\text{Cu}^{2+}\) complex (Fig. 4b), it is found that there are some changes from 1570 to 1616 cm\(^{-1}\). Obviously, the frequency of the N-H (primary amine) bending vibration has been weakened in 1570 cm\(^{-1}\). At the same time, the frequency of the C=O stretching vibration also shifted from 1616 to 1603 cm\(^{-1}\). Furthermore, one band was observed at 692 cm\(^{-1}\) which is attributed to Cu-O stretching. The existence of these changes suggests that \(-\text{NH}_2\) and \(\text{C}=\text{O}\) groups can participate in the coordination (Scheme 1).

In FT-IR spectrum of RB4-\(\text{Cu}^{2+}\) +\(\text{C}_2\text{O}_4^{2-}\) (Fig. 4c), the existence of the frequencies of C=O stretching vibration, \(-\text{NH}_2\) stretching vibration, S=O stretching vibration, C-N-C triazine bending vibration, C-Cl stretching vibration, and N-H wag give a support that these groups are free and do not participate in coordination. Furthermore, the bands observed at 1312 and 525 cm\(^{-1}\) also correspond to stretching vibration of C–O of oxalate and Cu–OCCO stretching vibration, respectively. The presence of these bands can exhibit the Cu-C\(_2\)O\(_4^{2-}\) association which can imply more affinity of oxalate toward \(\text{Cu}^{2+}\) compared to that of RB4 (Scheme1). The reversibility and displacement done have been indicated in Fig. 5. As can be seen in this figure, upon the addition of \(\text{C}_2\text{O}_4^{2-}\) to the RB4-\(\text{Cu}^{2+}\) solution, the color and spectrum of RB4 are restored.

**Scheme 1**
The effective and suitable pH range (the values between 3 and 10) for the selective determination of C$_2$O$_4^{2-}$ was studied. As can be observed (Fig. S3), at lower pH values (<5) and the acidic condition, the colorimetric response decreased which may be due to protonation of the heteroatoms of RB4 and the prevention of the complex formation with Cu$^{2+}$. The concentration of free oxalate is also low because all oxalate ions are protonated. Oxalate is the deprotonated form of oxalic acid (pka$_1$ = 1.23 and pka$_2$ = 4.19). In the acidic environment, the interaction of C$_2$O$_4^{2-}$ with H$^+$ causes a decrease in [C$_2$O$_4^{2-}$] and [HO$_2$CCO$_2^-$] and the production of HO$_2$CCO$_2$H.

Under alkaline conditions (pH>8), the colorimetric response also reduced due to the interaction of Cu$^{2+}$ with hydroxide ion and the lack of the RB4-Cu$^{2+}$ complex formation. However, the absorption intensity of [Cu-RB4] complex solution showed significant changes only in pH range of 5-7 upon the addition of oxalate (Fig. S3). Furthermore, the best colorimetric response of RB4-Cu toward oxalate was obtained in the pH equal to 7. The results show that in approximately physiological conditions, the proposed chemosensor can be employed to detect oxalate.

Short response time is also another important characteristic of chemosensors in practical applications. The response of the proposed chemosensor was studied within 60min. The maximum response for the determination of oxalate was obtained in a satisfactory short time (less than 1minutes) (Fig. S4). This fast response can provide a new real-time method for oxalate determination.

Logic gate construction of the proposed colorimetric sensor

The advancement of the molecular logic gate is often obtained by molecular chemosensor as an achievement in sensing chemistry. Here, an IMPLICATION molecular logic gate was constructed based on the proposed colorimetric indicator-displacement assay (Fig. S5). If the significant characteristic is considered on the optical signal response of the proposed chemosensor, Cu$^{3+}$ and C$_2$O$_4^{2-}$ as two inputs and the color and the UV-Vis absorbance at 607nm
as output are defined. The absence and presence of the inputs were indicated as “0” and “1”, respectively. About the output, the “dark blue” color is defined as “1”, and the “deep sky blue” color as “0”. The presence and/or absence of $C_2O_4^{2-}$ and $Cu^{2+}$ inputs were defined by four states $(0, 0), (1, 0), (0, 1), (1, 1)$. Only one state gives the output of “0” with an obvious deep sky blue color and the spectral decrease due to [Cu-RB4] complex formation if the $Cu^{2+}$ input is held at “1” and $C_2O_4^{2-}$ at “0” state (Fig. S5a, b) and c). Thus, for the output of IMPLICATION gate, only in the presence of $Cu^{2+}$ input and the absence of $C_2O_4^{2-}$ input, a deep sky blue is produced – colored signal and an output of “0” (Fig. S5d). This logic function is displayed in Fig. S5 and can be potential for oxalate determination.

**Colorimetric IDA based test paper for the visual detection of oxalate**

Recently, the development of colorimetric methods along with naked-eye detection has been one of the subjects of interest in the analytical applications. The colorimetric assay based on test paper as a simple, effective, fast, and low-cost sensing technology has attracted enormous interest (5, 32). The paper-based test strips fabricated according to the instruction given in materials and methods section were used for colorimetric experiments. The evaluation of the practicability of the proposed colorimetric strategy is possible with these experiments. The proposed colorimetric IDA assay was repeated by the paper-based test strips and the primary color recovery upon the addition of oxalate to [Cu-RB4] was observed (Fig. S6a). Test papers immersing in different concentrations of oxalate from 0.005 to 0.1 mol/L demonstrated different obvious colors. This experiment can indicate the applicability of fast, simple, and effective paper-based analytical strategy for oxalate determination (Fig. S6b). A selectivity experiment was also performed using paper-based test strips. Upon the addition of anions to the [RB4-Cu$^{2+}$] system, only oxalate showed an obvious color change compared with other available anions (Fig. S6c). The results obtained can verify the applicability of the proposed colorimetric strategy for “in-the-field” oxalate measurement.
Analytical figures of the method

The analytical features of the colorimetric response of the present chemosensor for determination of oxalate ion were investigated. The calibration curves, attributed to the colorimetric determination of oxalate ion, were constructed using least square regression (Fig. S7 and S8). According to corresponding calibration curves, limit of detection and quantitation were calculated using Eq.1 and Eq.2.

\[
C_{DL} = \frac{3s_b}{m} /1/
\]

\[
C_{QL} = \frac{10s_b}{m} /2/
\]

The absorbance signal of RB4-Cu\(^{2+}\) increases linearly in the concentration range of 1.76 - 49.4 \(\mu\)mol/ L for oxalate (Fig. S7). The corresponding linear function is \(A_{607}=0.2701 + 874.06\ C_{\text{oxalate}}\) and \(R^2= 0.9983\). The limit of detection (3\(\delta/s\)) and the limit of quantification (10\(\delta/s\)) were calculated to be 0.62 and 2.07 \(\mu\)mol/ L, respectively. These results exhibit excellent sensing performance of the sensor toward oxalate.

The linear relationship of the absorbance signal with Cu\(^{2+}\) concentration coordinated with RB4 for the determination of oxalate in range of 31 to 100.71 \(\mu\)mol/L with the regression equation \(A_{607}=0.31 - 0.0004\ C_{\text{Cu}^{2+}}\) with \(R^2= 0.9963\) is also indicated in Fig. S8. The detection limit (3\(\delta/slop\)) of Cu\(^{2+}\) was calculated to be 1.96 \(\mu\)mol/ L.

Analytical application

Since the determination of oxalate ion concentration in food and human body biological fluids is important, the analytical performance of the proposed chemosensor was examined by analyzing human urine and vegetable samples such as mushroom and spinach, which were prepared as described in materials and methods section. The colorimetric responses of the IDA chemosensor to these samples were evaluated in three replicate measurements. The results are summarized in Table 1.

The analysis results of the three samples by the proposed method and the reference method (29) showed good agreement with each other. The RSD values and the range of recoveries (97.9-101.2 %) suggested that the substances in these real samples have no serious interference for
the detection of oxalate. Therefore, this chemosensor has potential applications in oxalate detection in real samples.

Table 1

CONCLUSION

In summary, the reaction between RB4 and Cu$^{2+}$ to form RB4-Cu$^{2+}$ complex can be used as a simple and inexpensive colorimetric chemosensor for the recognition of oxalate over other available competitive analytes via indicator displacement assay (IDA) in both solution (aqueous media) and solid state (paper-based experiment). Any significant interference was not shown by other competitive available analytes (ascorbic acid and anions) in the determination of oxalate. The absorbance band increase is linear with oxalate concentration from 1.76-49.4 µmol/ L, with a detection limit of 0.62 µmol/ L. Satisfactory results were also obtained by the determination of oxalate in different real samples such as urine, mushroom, and spinach which demonstrated the applicability of the existing method. Furthermore, IMPLICATION logic gate operating was achieved using Cu$^{2+}$ and oxalate as inputs. The limit of detection, dynamic linear range and analytical parameters of the present method were also evaluated as compared with the earlier colorimetric determination of oxalate (Table 2). Regarding the simplicity of the operation, rapid response, and the ability of the facile naked-eye detection, the proposed chemosensor can be used as a probe for the determination of oxalate.

Table

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<th>Sample</th>
<th>$w_{(\text{experimental})}^{1}/(\text{mg/g})$</th>
<th>$w_{(\text{referent})}/(\text{mg/g})$</th>
<th>$m_{(\text{added})}/\mu\text{g}$</th>
<th>$m_{(\text{recovered})}/\mu\text{g}$</th>
<th>Recovery %</th>
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<td>100.0</td>
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<td>100.0</td>
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$^1$Average value of five replicate measurement

$^2$Relative standard deviation of 5 individual measurements
Table 2. Evaluation of analytical features of the reported IDA chemosensor in comparison with the recently reported methods for oxalate determination

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<td>No Data</td>
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<td>(33)</td>
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<td>0.62µmol/L</td>
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<td>5.0-7.0</td>
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<td>Present work</td>
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</table>
Scheme. 1. Illustration of the proposed mechanism of RB4 in the presence of Cu$^{2+}$ and oxalate ($C_2O_4^{2-}$).

Fig. 1. a) The UV-Vis spectrum of RB4 (50 µmol/L) in the presence of copper ion with different concentration. Left inset is the graph of the UV-Vis absorption changes of RB4 at 607 nm as a function of the concentration of Cu$^{2+}$ (mol/L). Right inset is the photography of the corresponding solution (the color change from dark blue to deep sky blue). b) UV-Vis absorption spectrum of RB4-Cu$^{2+}$ complex (50 µmol/L RB4 with combination of 50 µmol/L Cu$^{2+}$) based on IDA strategy toward oxalate with different concentration. Left inset is the graph of the UV-Vis absorption changes of the RB4-Cu$^{2+}$ complex as a function of the concentration of oxalate (mol/L). Right inset: the photography of the solution color change from deep sky blue to dark blue.

Fig. 2. a) The increased absorbance at 607 nm as a function of the concentration of oxalate (1.76×10$^{-6}$–9.60×10$^{-5}$ mol/L) added to RB4-Cu$^{2+}$ (50 µmol/L of RB4 with Cu$^{2+}$ (50 µmol/L)) in water media (10 mmol/L HEPES buffer solution, pH=7). b) Molar ratio plot: the absorbance at 607 nm as a function of the molar ratio of oxalate to 50 µmol/L of Cu$^{2+}$ in water media (10 mmol/L HEPES buffer solution, pH=7).

Fig. 3. UV-Vis spectra of RB4 (50 µmol/L), [RB4-Cu$^{2+}$] in the presence of ascorbic acid, F$^-$, Cl$^-$, Br$^-$, CO$_3^{2-}$, HPO$_4^{2-}$, PO$_4^{3-}$, SO$_4^{2-}$, NO$_3^-$, ACO$^-$, ClO$_4^-$ (500 µmol/L), [RB4-Cu$^{2+}$] in the presence of $C_2O_4^{2-}$ (50 µmol/L) and ascorbic acid, F$^-$, Cl$^-$, Br$^-$, CO$_3^{2-}$, HPO$_4^{2-}$, PO$_4^{3-}$, SO$_4^{2-}$, NO$_3^-$, ACO$^-$, ClO$_4^-$ (500 µmol/L).

Fig. 4. IR spectra of the proposed colorimetric sensor based on IDA strategy after adding Cu$^{2+}$ and $C_2O_4^{2-}$ utilizing KBr pellet method and the comparison of them.

Fig. 5. The absorption curves of 50 µmol/L RB4 (A), 50 µmol/L RB4 and 50 µmol/L Cu$^{2+}$ (B), A+B+50 µmol/L oxalate ion (C). Insert is the photography of the corresponding solution (A: 50
μmol/L RB4 with dark blue, B: A+50 μmol/L Cu^{2+} and the color change to deep sky blue, C: A+B+50 μmol/L C_{2}O_{4}^{2-} and the color change to dark blue.

Scheme 1
Fig. 1
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Fig. 2
Fig. 3

![Graph showing the absorption spectra of RB4, C₃O₂⁻, RB4 + Cu²⁺ including other available anions and ascorbic acid.](image-url)
Fig. 4
Fig. 5
Supplementary data

Fig. S1. Molar ratio plot: the plot of absorbance at 607 nm versus x (Cu-RB4).
Fig. S2. The Calculation of the association constant (K_{ass}) of [Cu-C₂O₄] complex by using Microsoft excel solver.
Fig. S3. Effect of the pH on the absorbance changes of [RB4-Cu^{2+}] in the presence of oxalate (50 µmol/L) at 607nm in H$_2$O (10 mmol/L HEPES buffer solution, pH=7)

![Graph showing the effect of pH on absorbance changes](image)

Fig. S4. UV-Vis spectral changes of [RB4-Cu^{2+}] upon addition of oxalate (50 µmol/L) during 30 minutes.

![Graph showing UV-Vis spectral changes](image)
Fig. S5. Illustration of “IMPLICATION” logic gate (A) $A_{607\text{nm}}$ value of different inputs based on UV-Vis absorption, (B and C) Truth table corresponding to the “IMPLICATION” logic gate, (D) Color change of RB4 in the response to different inputs.
Fig. S6. (A) the detecting process of the proposed colorimetric sensor based on IDA strategy for the determination of oxalate by test paper, (B) Photographs of test paper after immersing into the different concentration of oxalate, (C) Photographs of test paper coated with RB4-Cu$^{2+}$ complex immersed in different anions.
Fig. S7. The linearly proportional relationship between the absorbance of RB4-Cu solution at 607 nm and the concentration of oxalate ion.

\[ A_{607} = 874.06 \cdot c(C_2O_4^{2-}) + 0.2701 \]

\[ R^2 = 0.9983 \]
Fig. S8. The linearly proportional relationship between the absorbance of RB4 solution at 607 nm and the concentration of Cu$^{2+}$.

\[ A_{607} = -0.0004 \cdot c_{\text{Cu}^{2+}} + 0.31 \]

\[ R^2 = 0.9963 \]