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 paper proposes a method that exhibits operational simplicity for the indirect spectrophotometric determination of oxalate ion. We developed Reactive Blue 4 (RB4) as a sensor by complexation with copper ion as a simple, inexpensive yet selective colorimetric chemosensing ensemble for the recognition of oxalate over other available competitive analytes *via* indicator displacement assay (IDA) in both solution (aqueous medium) and solid state (paper-based experiment). The addition of oxalate to RB4-Cu^{2+} complex changed the colour from sky blue to dark blue 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 to 49.4 μmol/L with a detection limit of 0.62 μmol/L. This measurement mode did not show any influence of interferences (available anions and ascorbic acid). This approach eliminated the need for the separation stages, enzymatic multiple-step reactions, sample preparation, organic solvent mixture, chemical modifications and equipment developed to a high degree of complexity. The oxalate determination gave results in different real samples such as urine, mushroom and spinach, which demonstrated the applicability of the existing method. Furthermore, this colorimetric system can serve as IMPLICATION molecular logic gate using Cu^{2+} and oxalate ($C_2O_4^{2-}$) 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

INTRODUCTION

In recent years, the development of optical chemosensors for biologically important anions has been considerably attractive because they have applications in a wide range of biological and industrial processes (*1,2*). The indicator-spacer-receptor approach (ISR) is the most widely used approach in the detection of anions with optical chemosensors in which the indicator (herein chromophore) is covalently attached to the receptor through a spacer. The limitations of ISR approach are the need to synthesize the sensor, and cost. The indicator displacement assay (IDA) largely circumvented this problem (*3*). Nowadays, this approach is popular in studying molecular recognition (*4*). In an IDA, an indicator first forms a reversible bond with a receptor. Then, a competitive analyte introduced into the system displaces the indicator from the receptor. Displacement and binding reaction are accompanied by a colorimetric signal and visible colour change by the naked eye (*5*). Based on this principle, the major requirement for an IDA is that the affinity between indicator and receptor is lower than that of the analyte-receptor complex. The interactions between the indicator or analyte and the host depend 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 anions have the fundamental roles in the industrial processes and clinical analyses (*8*). Oxalate ($C_2O_4^{2-}$), as one of the most common nutrient chelates in the human diet, is of great interest due to its vital role in chemical and biochemical processes (*9*). The protein metabolism causes the production of oxalate in the human body (*10*). The high concentration of oxalate

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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_2O_4) may be an end product of amino acid or ascorbate metabolism in the body. The normal level of urinary oxalate excretion is in the range of 110–460 µmol in 24 h (*13*). Therefore, the quantification of oxalate in human urine is important. Furthermore, the determination of oxalate content in food is also important since the low oxalate diet is sometimes necessary for the treatment. Several methods developed in recent years to determine the concentration of oxalate include chromatography (*14*), chemiluminescence (*15*), amperometry (*16,17*), flow injection analysis (*18*), electrochemistry (*19*), capillary zone electrophoresis (*20*), and enzyme-based methods (*21*). However, most of these approaches require a high cost for the operation, special equipment, and long time for tedious 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 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 time-consuming and labour-intensive approaches need complicated synthetic processing of primary material (*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) in a complex with Cu^{2+}, as an appropriate colorimetric indicator, and oxalate, as a competitive analyte, were employed. After treatment of Cu^{2+} with RB4 (the formation of the RB4-Cu^{2+} complex) in aqueous medium (10 mmol/L HEPES buffer solution, pH=7), the colour and the colorimetric signal of RB4 exhibited changes that were observable by the naked eye. The proposed IDA system can also successfully determine oxalate concentration in urine, spinach and mushroom. Furthermore, the proposed method could be used as a paper-based analytical device and IMPLICATION molecular logic gate using Cu^{2+} and oxalate ($C_2O_4^{2-}$) 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-sulfophenyl-amino]anthraquinone-2-sulfonic acid), was obtained from Merck (Darmstadt, Germany). Demineralized water was used to prepare the solutions. $Cu(NO_3)_2$, KF, KCl, K_2CO_3, Na_2HPO_4, K_3PO_4, Na_2SO_4, KNO_3, $NaC_2H_3O_2$, $KClO_4$, $K_2C_2O_4$ and ascorbic acid ($C_6H_8O_6$) were purchased from Merck. Stock solution (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 in quartz cuvettes 10.0 mm in diameter. A Bruker Vector 22 Fourier transform infrared spectrometer (Billerica, MA, USA) recorded the FTIR spectra using KBr pellet method. A digital Jenway 3510 digital pH meter (London, UK) calibrated with two standard buffer solutions was used to measure various pH values. A Hamilton syringe of 50 µL was used to deliver desired amounts of analyte solution into the cuvette.

General procedure

The adjusted temperature in all titration experiments was 298.2 K. The $5\cdot10^{-5}$ mol/L solution of RB4 was prepared by the dilution of its 10^{-2} mol/L stock solution in aqueous medium (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. For complexation study, a 10^{-2} mol/L aqueous solution of the nitrate salt of Cu^{2+} was prepared. The detection of oxalate and its effect on the colour and absorption spectra of the RB4-Cu^{2+} solution were studied by preparing the oxalate solution and gradually adding it (1.76–96.0 µmol/L) with microlitre syringe to the solution containing RB4 complex with Cu^{2+}. Then, the UV-Vis spectra were recorded. Subsequently, the interactions and the stoichiometry ratios (Yoe and Jones method (*27*) or the mole ratio method (*28,29*)) were studied.

Preparation of test paper for onsite visual determination of oxalate

Test papers were fabricated by immersing ordinary filter papers into the aqueous solution of RB4 (0.1 mol/L) and Cu^{2+} (0.1 mol/L), respectively, dried at room temperature and then used for the determination of oxalate in aqueous solutions. The corresponding colour change was observed by the naked eye.

Real sample preparation

Preparation of urine sample

An aliquot of 5 mL of urine sample collected in sterilized polyethylene tube was transferred to a 50-mL volumetric flask, made up to the mark (pH=7), and shaken for 5 min. Then, 20 mL of this solution were taken and transferred to a 50-mL beaker. Then, the oxalate content was determined by the colorimetric method mentioned above.

Preparation of mushroom and spinach samples

A mass of 3 g fresh spinach or 15 g fresh mushroom was cut into small pieces with a razor blade, and then homogenized with a mortar and pestle. Subsequently, the obtained paste was mixed with water, diluted to 100 mL in a calibrated flask, boiled for 45 min and then cooled. The filtration of the suspension was done through Whatman no. 1 filter paper. The filtrate was diluted to 250 mL. Then, the pH of the obtained 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 (MS-3400 centrifuge; Cole-Parmer®, St. Neots, UK) at 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. A suitable aliquot of the obtained solution was used for the determination of oxalate content with the proposed method.

Reference method

After preparation of the test sample solution, it was transferred to a 250-mL Erlenmeyer flask. A volume of 50 mL distilled water and 20 mL of 3 M H_2SO_4 were added and stirred to dissolve the solid. The acidified solution was heated to about 85 °C. A mass of 1 g $KMnO_4$ was transferred to a 500-mL volumetric flask, 350 mL distilled water were added, and the solution was heated for 30 min. The hot sample solution was titrated with the cool $KMnO_4$ solution to determine the endpoint (30).

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 assay. 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-Cu^{2+} complex. The interaction between RB4 and Cu^{2+} was investigated by UV-Vis spectroscopy. **Fig. 1a** shows that RB4 (50 µmol/L) in aqueous solution (10 mmol/L HEPES buffer solution, pH=7) exhibits a maximum absorption peak at approx. 607 nm as well as an obvious dark blue colour. Upon gradual addition of Cu^{2+} (1.97–122 µmol/L), the absorption intensity at 607 nm decreased and shifted to around 619 nm accompanied with a colour change from dark blue to deep sky blue (right inset, **Fig. 1a**). The absorbance changes *versus* the Cu^{2+} concentration increase is plotted in the left inset of **Fig. 1a**. A 1:1 binding of the formed complex between RB4 and Cu^{2+} was confirmed by Yoe and Jones method (**Fig. S1**; supplementary material available at: www.ftb.com.hr) (27-29). The binding constant (K_s) of the [RB4--Cu^{2+}] complex was also estimated to be (4.46±0.12)·10^5 L/mol by nonlinear curve fitting (31).

The colorimetric response of [RB4-Cu^{2+}] complex towards 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 607 nm (**Fig.1b**). The colour of the solution also

gradually changed from deep sky blue to dark blue (right inset, **Fig. 1b**). Left inset in **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 obtained colour were identical with that of RB4. This colorimetric change indicates that [RB4-Cu^{2+}] complex can be used for the determination of oxalate by the naked eye.
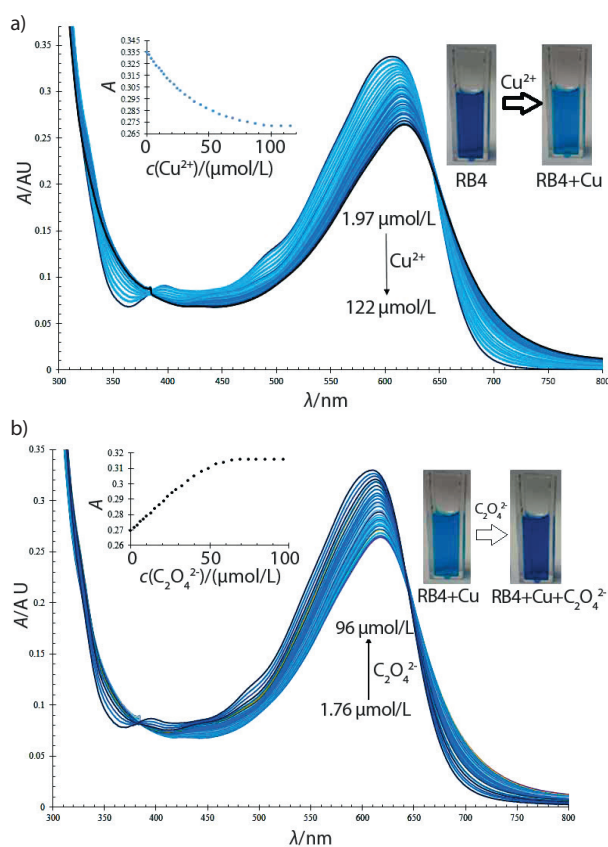

Fig. 1. The UV-Vis spectrum of: a) Reactive Blue 4 dye (RB_4; 50 µmol/L) with different concentrations of copper ions. Left inset is the graph of the UV-Vis absorption changes of RB4 at 607 nm as a function of the concentration of Cu^{2+} (µmol/L). Right inset is the photograph of the corresponding solution (the colour change from dark blue to deep sky blue); b) RB4-Cu^{2+} complex (equimolar concentration of 50 µmol/L RB4 with 50 µmol/L Cu^{2+}) after the addition of different oxalate concentrations. 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. Right inset is the photograph of the solution colour change from deep sky blue to dark blue

The application of the Yoe and Jones method (27-29) by plotting the absorbance at 607 nm *versus* the molar ratio of $C_2O_4^{2-}$ to Cu^{2+} confirmed a stoichiometry of 1:1 for the interaction of Cu^{2+} and $C_2O_4^{2-}$ (**Fig. 2**) with the association constant K_s=(2.3±0.1)·10^7 L/mol obtained by nonlinear curve fitting (**Fig. S2**) (31,32). Two oxygen atoms from oxalate act as a bidentate ligand. Thus, the displacement process can be as follows:

$$RB4\text{-}Cu^{2+} + C_2O_4^{2-} \rightarrow CuC_2O_4 + RB4 \qquad /1/$$
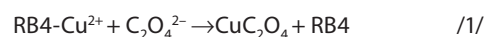
As mentioned above, the binding constant of Cu^{2+} with oxalate is larger than that of Cu^{2+} with RB4. Therefore, the indicator displacement by oxalate is highly favourable due to higher affinity of oxalate towards Cu^{2+}.

a)

b)

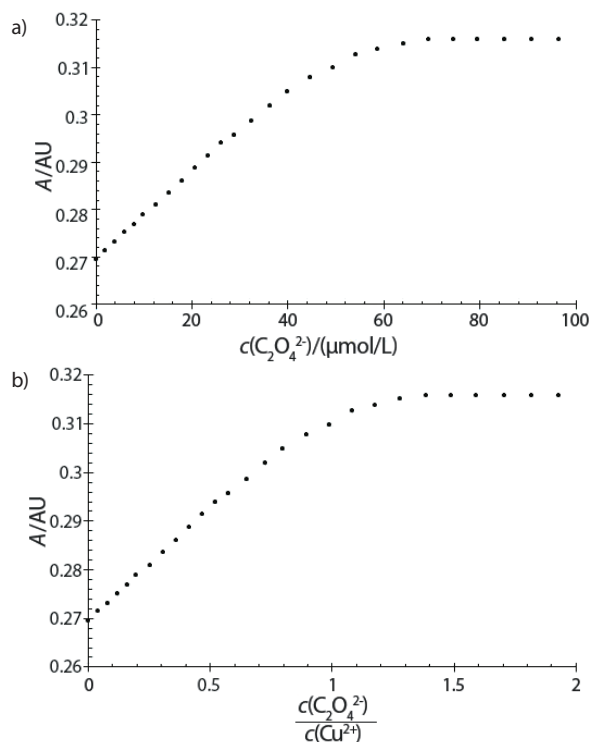**Fig. 2.** The graphs show: a) the increased absorbance at 607 nm as a function of the concentration of oxalate (1.76–96.0 μmol/L) added to Reactive Blue 4-Cu^{2+} complex (50 μmol/L RB4 with 50 μmol/L Cu^{2+}) in 10 mmol/L HEPES buffer solution, pH=7, and b) the absorbance at 607 nm as a function of the molar ratio of oxalate to 50 μmol/L Cu^{2+} in 10 mmol/L HEPES buffer solution, pH=7

Study of selectivity towards oxalate

The selectivity of the proposed chemosensor towards oxalate ion in the presence of ascorbic acid and other anions was investigated. The solution system was selective towards oxalate anion over ascorbic acid ($C_6H_8O_6$), SO_4^{2-}, NO_3^-, CO_3^{2-}, HPO_4^{2-}, PO_4^{3-}, AcO^-, ClO_4^-, F^-, Cl^- and Br^- (**Fig. 3**). Only the oxalate anion is able to generate colour and spectral changes. When ascorbic acid and other anions (500 μmol/L) were added into the [RB4-Cu^{2+}] complex solution, negligible changes in colour and UV-Vis spectrum were observed, showing that they do not interfere with the detection of the presence or amount of oxalate anion. Therefore, it was proven that this chemosensor has selectivity towards oxalate in water solution without the need for sophisticated instruments.

Possible mechanism study

Scheme 1 shows the proposed IDA strategy for the determination of oxalate ($C_2O_4^{2-}$). This strategy is based on the competition of the analyte and the chromogenic indicator (RB4) for the interaction with Cu^{2+}. As already mentioned, a 1:1 complex with the stability constant of $(4.46\pm0.12)\cdot10^5$ L/mol formed after the addition of Cu^{2+} to the RB4 solution. There are some changes in the FTIR spectrum of the RB4-Cu^{2+} complex from 1570 to 1616 cm^{-1} (B in **Fig. 4**). Obviously, the frequency of the N-H (primary amine) bending vibration weakened at 1570 cm^{-1}. At the same time, the frequency of the
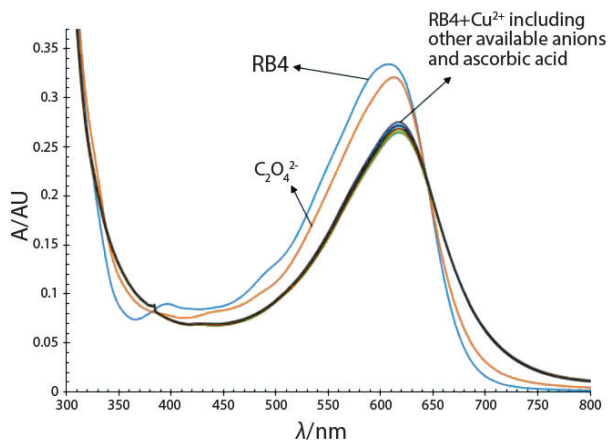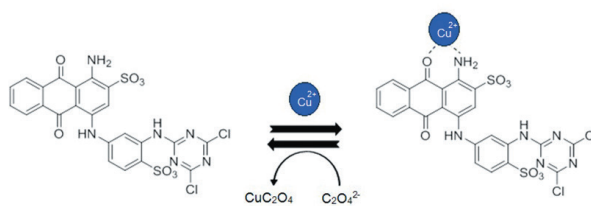

Fig. 3. UV-Vis spectra of Reactive Blue 4 (RB4; 50 μmol/L), [RB4-Cu^{2+}] complex in the presence of (c=500 μmol/L): ascorbic acid, F^-, Cl^-, Br^-, CO_3^{2-}, HPO_4^{2-}, PO_4^{3-}, SO_4^{2-}, NO_3^-, AcO^-, ClO_4^-, and [RB4-Cu^{2+}] complex with $c(C_2O_4^{2-})$=50 μmol/L in the presence of (c=500 μmol/L): ascorbic acid, F^-, Cl^-, Br^-, CO_3^{2-}, HPO_4^{2-}, PO_4^{3-}, SO_4^{2-}, NO_3^-, AcO^- and ClO_4^-

Scheme 1. Illustration of the proposed reaction mechanism of Reactive Blue 4 (RB4) with Cu^{2+} and oxalate ($C_2O_4^{2-}$) ions

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 $–NH_2$ and C=O groups can participate in the coordination (**Scheme 1**).

In FTIR spectrum of RB4-Cu^{2+}+$C_2O_4^{2-}$ (C in **Fig. 4**), the existence of the frequencies of C=O, $–NH_2$, S=O, C–Cl stretching vibrations, C–N–C triazine bending vibration and N–H wag
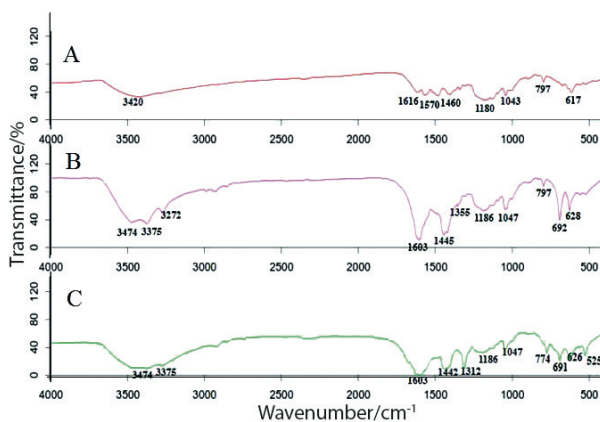

Fig. 4. Comparison of the IR spectra of the proposed colorimetric sensor based on indicator displacement assay (IDA) strategy after adding Cu^{2+} and $C_2O_4^{2-}$ utilizing KBr pellet method

shows that these groups are free and do not participate in coordination. Furthermore, the bands observed at 1312 and 525 cm^{-1} correspond to the stretching vibrations of C–O of oxalate and Cu–OCCO, respectively. The presence of these bands indicates the Cu–C$_2$O$_4^{2-}$ association, which can imply better affinity of oxalate towards Cu^{2+} than that of RB4 (**Scheme 1**). The addition of C$_2$O$_4^{2-}$ to the RB4-Cu^{2+} complex solution restored the colour and spectrum of RB4 (**Fig. 5**). C$_2$O$_4^{2-}$ has a stronger binding capability with Cu^{2+} ion than with RB4 and can form a more stable CuC$_2$O$_4$, so after the addition of C$_2$O$_4$, the UV-Vis absorption spectrum and colour of RB4 were recovered and the colour and absorbance changes were reversible with the addition of Cu^{2+} and C$_2$O$_4^{2-}$.
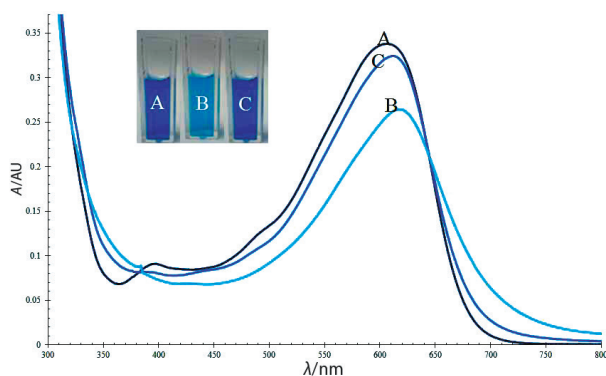

Fig. 5. The absorption curves of 50 µmol/L Reactive Blue 4 (RB4) dye (A), 50 µmol/L RB4 and 50 µmol/L Cu^{2+} (B), A+B+50 µmol/L oxalate ion (C). Insert is the photograph of the corresponding solution (A: 50 µmol/L RB4 with dark blue, B: A+50 µmol/L Cu^{2+} and the colour change to deep sky blue, C: A+B+50 µmol/L C$_2$O$_4^{2-}$ and the colour change to dark blue

pH effect and response time

The effective and suitable pH range (values between 3 and 10) for the selective determination of C$_2$O4^{2-} was studied. At lower pH values (<5) and acidic conditions, the colorimetric response decreased (**Fig. S3**), 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 (pK_{a1}=1.23 and pK_{a2}=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 HC$_2$O$_4^-$ and the production of H$_2$C$_2$O$_4$.

Under alkaline conditions (pH>8), the colorimetric response was also weak 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 [RB4-Cu^{2+}] complex solution showed significant changes only in the pH range of 5–7 upon the addition of oxalate (**Fig. S3**). Furthermore, the best colorimetric response of RB4-Cu^{2+} to oxalate was obtained at pH=7. The results show that under 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. We tested the response of the proposed chemosensor within 60 min. The maximum response for the determination of oxalate was obtained in a satisfactorily short time (less than 1 min) (**Fig. S4**). This fast response can provide a new real-time method for oxalate determination.

Logic gate construction of the proposed colorimetric sensor

Molecular chemosensors represent an advancement of the molecular logic gate in sensing chemistry. Here, we constructed an IMPLICATION molecular logic gate based on the proposed colorimetric indicator displacement assay (**Fig. S5**). Since the significant characteristic of the proposed chemosensor was its optical signal response, Cu^{2+} and C$_2$O$_4^{2-}$ as two inputs, and the colour and the UV-Vis absorbance at 607 nm as outputs were defined. Numbers 0 and 1 indicate the absence and presence of the inputs, respectively. In the output, 1 and 0 define the dark blue and deep sky blue colours, respectively. The presence and/or absence of C$_2$O$_4^{2-}$ and Cu^{2+} inputs were defined by four pairs (0/0, 1/0, 0/1 and 1/1). Only one pair gives the output of 0 with an obvious deep sky blue colour and the spectral decrease due to [RB4-Cu^{2+}] complex formation if the Cu^{2+} input is 1 and C$_2$O$_4^{2-}$ 0 (**Fig. S5a-5c**). Thus, only with Cu^{2+} and without C$_2$O$_4^{2-}$ input, the output of IMPLICATION is a deep sky blue (**Fig. S5d**). This logic function (**Fig. S5**) 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 the detection by the naked eye has been one of the subjects of interest in the analytical applications. There is an enormous interest in the colorimetric assay based on test paper as a simple, effective, fast, and low-cost sensing technology (*5,33*). 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 with the paper-based test strips and the primary colour recovery after the addition of oxalate to [RB4-Cu^{2+}] was observed (A in **Fig. S6**). Test papers immersed in different concentrations of oxalate from 0.005 to 0.1 mol/L demonstrated different colours. This experiment can indicate the applicability of fast, simple and effective paper-based analytical strategy for oxalate determination (B in **Fig. S6**). A selectivity experiment was also performed using paper-based test strips. Upon the addition of anions to the [RB4-Cu^{2+}] complex, only oxalate showed an obvious colour change compared with other available anions (C in **Fig. S6c**). The obtained results can verify the applicability of the proposed colorimetric strategy for 'in-the-field' oxalate measurement.

Table 1. Results of the determination of oxalate ion in real samples of mushroom, spinach and urine

Sample	$w_{experimental}$*/(mg/g)	$w_{referent}$/(mg/g)	m_{added}/μg	$m_{recovered}$/μg	Recovery/%	RSD
Mushroom	4.82	4.65	100.0	101.2	101.2	2.7
Spinach	6.31	6.42	100.0	98.8	98.8	0.8
Urine	2.35	2.37	100.0	97.9	97.9	3.5

*Average value of five replicate measurements. RSD=relative standard deviation of 5 individual measurements

Table 2. Comparison of the analytical features of the indicator displacement assay (IDA) chemosensor with the recently reported methods for oxalate determination

No	Probe sensing	LOD/(μmol/L)	c(linear range)/(μmol/L)	t(response)/s	w(water)/%	pH	Solid-state determination limit	Reference
1	Cu_2PV	n.d.	n.d.	n.d.	HEPES buffer	5.5–6.5	–	(34)
2	Cu_2L-eosin	0.079	0.0–5.0	n.d.	100	7.0	–	(35)
3	Zn_2L_2-CAS	18.7	n.d.	n.d.	100	7.4	–	(2)
4	GNPs/c-MWCNT/Au electrode	1.0	1.0–800	7	sodium succinate buffer	5.0	–	(36)
5	CuRB4	0.62	1.76–49.4	<60	100	5.0–7.0	paper test strip	present work

n.d.=not determined

Analytical features 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 **Fig. S8**). According to corresponding calibration curves, limit of detection (LOD) and quantitation (LOQ) were calculated using Eq. 1 and Eq. 2, respectively:

$$LOD = \frac{3\delta}{S} \qquad /2/$$

and

$$LOQ = \frac{10\delta}{S} \qquad /3/$$

where δ is standard deviation, and S is slope.

The absorbance signal of RB4-Cu^{2+} increases linearly in the oxalate concentration range of 1.76–49.4 μmol/L (**Fig. S7**). The corresponding linear function is:

$$A_{607\,nm} = 0.2701 + 874.06\ c_{oxalate}; \quad R^2 = 0.9983 \qquad /4/$$

The limit of detection (3 δ/S) and the limit of quantification (10 δ/S) were calculated to be 0.62 and 2.07 μmol/L, respectively. These results exhibit excellent sensing performance of the sensor towards oxalate.

Fig. S8 shows the linear relationship of the absorbance signal with Cu^{2+} concentration coordinated with RB4 for the determination of oxalate in the range of 31 to 100.71 μmol/L with the regression equation:

$$A_{607\,nm} = 0.31 - 0.0004\ c_{Cu^{2+}}; \quad R^2 = 0.9963 \qquad /5/$$

The detection limit (3 δ/S) of Cu^{2+} was calculated to be 1.96 μmol/L.

Analytical application

Since the determination of oxalate ion concentration in food and human body fluids is important, the analytical performance of the proposed chemosensor was examined by analyzing urine, mushroom and spinach samples, which were prepared as described in the 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 of the 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 2 compares the limit of detection, dynamic linear range and analytical parameters of our method with the colorimetric determination of oxalate (*2,34-36*). Regarding the simplicity of the operation, rapid response and the ability of easy detection, the proposed chemosensor can serve as a probe for the determination of oxalate.

CONCLUSIONS

In summary, the reaction between RB4 and Cu^{2+} to form RB4-Cu^{2+} complex can serve as a simple and inexpensive colorimetric chemosensor for the recognition of oxalate over other available competitive analytes *via* indicator displacement assay (IDA) in both, the solution (aqueous medium) and solid state (paper-based experiment). There were no significant interferences with other analytes (ascorbic acid and anions) in the determination of oxalate. The absorbance band increase is linear with oxalate concentration from 1.76 to 49.4 μmol/L, with a detection limit of 0.62 μmol/L. IMPLICATION logic gate operated using Cu^{2+} and oxalate as inputs. Determination of oxalate in different real samples such as urine, mushroom and spinach also gave satisfactory results, confirming the applicability of the existing method.

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