Introduction of a new nitrite-selective optical sensor for food analysis using a copper(II) Schiff base complex

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Abstract

A copper(II) Schiff base complex, [Cu(cd4OMeSalMeen)], methyl-2-{{1-methyl-2-(4-methoxy-phenolamino-ethyl)-methylidynenitrilo}ethyl}amino-1-cyclopentene dithiocarboxylate copper(II), was incorporated into triacetylcellulose membrane and applied as ionophore in order to develop an anion-selective optical sensor for the analysis of nitrite by absorption spectrophotometry. At optimum pH 3.0, a linear calibration curve was observed for nitrite in the range of 0.50 to 7.00 mgL⁻¹ with a detection limit of 0.04 mgL⁻¹. The response time of the optode (t95%) was found to be 8–10 min, depending on the nitrite ion concentration. The proposed sensor was fully recovered in nitric acid solution (0.1 M) and had acceptable reproducibility (RSD=1.9%). The optode exhibited good selectivity toward nitrite ion according to hard-soft acid base (HSAB) principle and the formation constant was calculated. The application of the sensor for determination of nitrite content in meat products was quiet successful.

Keywords: Optical sensor; copper(II) Schiff base; nitrite; spectrophotometry; formation constant.

Introduction

Nitrite has an important role in food industry. Various applications have been introduced for nitrite in the industry [1], but its efficient role as a food additive is of great interest, especially in meat products. The preservative use of nitrite is due to its ability to prevent microbial growth, gives taste and color to the meat and inhibits lipid oxidation that leads to rancidity. Despite the above mentioned advantages, it can be toxic in high concentrations, causing environmental pollution and serious health problems for human [2]. The major concern is the formation of carcinogenic nitrosamines in meat products from the reaction of the amines with nitrite and subsequently their conversion to diazonium ions under acidic conditions (such as what occurs in the human stomach). Some of these nitrosamines can lead to the cancer because they form carbocations which can react with biological nucleophiles (such as DNA or an enzyme) in the cells and disrupt their normal function [3-5]. Obviously, it is a great deal to find a simple, fast and low-cost method for determination of nitrite in food.

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Different approaches have been developed for the analysis of nitrite [6], including kinetic [7,8], chromatography [9,10], spectrophotometric [11-15], spectrofluorimetric [16-19], capillary electrophoresis [20-22], atomic absorption spectroscopy [1], pulse polarography [23] and electroanalytical methods [24-26]. However, some of these methods are expensive and time-consuming [27], and some of them suffer from poor sensitivity [17].

Among these techniques, optical sensors appeared to be more suited as they offer good selectivity, sensitivity, low detection limit and less complication [28-30]. Recently, sensors based on optical signal measurements and fabricated with Schiff bases as ionophores have been applied to determine specific ions. The selection of these bulk optodes is mainly because of their stability under different conditions and the possibility of tuning their electronic and steric features [31]. This method have been used successfully to analyze some metal cations such as Cr(III) [32], Ce(III) [33], Co(II) [34], Fe(III) [35,36], Pb(II) [37] and Ag(I) [38]. Nevertheless, only few articles have been reported the use of Schiff base ionophores for monitoring the anions [25,39].

Our research has been focused on applying [Cu(cd4OMeSalMeen)] Schiff base complex (Figure 1) as a new sensor for quantitative determination of nitrite and development of this optode for analyzing nitrite in real samples.

**Experimental**

**Reagents and solutions**

All chemicals were of the best available analytical grade and employed without any prior purification. Doubly distilled water was used throughout. Ethylenediamine was obtained from Merck. A stock solution of nitrite, 100 mg L\(^{-1}\), was prepared by dissolving sodium nitrite (Merck) in water. Sample solutions were obtained daily by appropriate dilution. Buffer solutions, containing boric acid, acetic acid and phosphoric acid (0.04 M each) were prepared and different pHs were adjusted by addition of sodium hydroxide (0.2 M). [Cu(cd4OMesalMeen)] stock solution, 1.0 × 10\(^{-4}\) M, was prepared daily by dissolving 0.0042 g of the Schiff base and diluting with 100 mL ethylenediamine.

**Apparatus**

The absorbance measurements were carried out on a Shimadzu 1601 PC UV-Visible spectrophotometer. A pH-meter, Jenway model 3510, containing a combined glass electrode was used for monitoring pH adjustments.

**Synthesis of the ionophore**

The ionophore used throughout this research, [Cu(cd4OMesalMeen)] Schiff base, was obtained as follows: At first, Methyl-2-(1-methyl-2'-aminoethane) amino-1 cyclopentenedithiocarboxylate (HcdMeen) was prepared applying published methods. Then, the ligand was formed by adding the equimolar amount of appropriate salicylaldehyde derivative and other diketon to a solution of HcdMeen in methanol. The yellow precipitate was recrystallized from 1:1 solution of methanol/chloroform. The preparation of the Schiff base was performed at the next step. A solution of copper(II) acetate (0.1 mmol) in 10 mL
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A solution from copper complex (10^{-4} M) with a constant ionic strength (I) of 0.1 M sodium perchlorate was prepared. Of each solution, 2.5 mL was transferred into the thermostated cell compartment of the UV-Vis instrument, which was kept at constant temperature 10, 20, 30, and 40 (±1°C) by circulating water. Each solution was titrated by adding aliquots of the nitrite ion using a Hamilton microliter syringe. Concentrations of nitrite ion were varied in the range of 1 to 10 fold in excess. The absorption measurements were monitored at various wavelengths in 300-500 nm regions where the difference in absorption between the substrate and the product was the largest after the equilibrium attained.

Sample preparation  
Meat products (six different brands of sausage: A, B, C, D, E, F) were collected from local markets in Shiraz, Iran. Extraction of nitrite from the meat products was made using the AOAC sample preparation procedure [41,42]. 5 g of each sample was transferred to the mixer and homogenized carefully. 50 mL of hot water (80 ºC) and a pellet of sodium hydroxide (to prevent the liberation of nitrous acid) were added and the mixture was shaken about 15 min for the complete extraction of nitrite. The filtrate was then taken into a 500 mL beaker and the diluted solution was analyzed using the fabricated Schiff base sensor.

Results and discussion  
Working principles and spectral characteristics of the optode  
According to the experiments, the proposed Schiff base membrane revealed remarkable tendency toward NO_2^- ion over a wide variety of tested SCN^-, CN^-, I^-, OH^-, CH_3COO^-, PO_4^{3-}, SO_3^{2-} ions. The response mechanism of the optode is completely according to the hard-soft
acid base principle (HSAB) and is believed to be based on the complexation of nitrite ion (as borderline base) to the central copper(II) atom of the Schiff base (as borderline acid). The absorption spectra of the immobilized Schiff base and the resulting complex were recorded at the buffered solution (pH 3). As illustrated in Figure 2, the spectrum of the Schiff base includes two intense bands.

![Figure 2. Absorption spectra of the optical sensor in the presence of (0.0–8.0 mgL$^{-1}$) nitrite ion by addition of 1.0 mgL$^{-1}$ in each interval at pH=3.](image)

The absorption band at 395 nm is attributed to a metal to ligand charge transfer (MLCT) and the other band at 310 nm is assigned to a π→π* transition [43]. All absorption measurements have been performed conveniently by monitoring the decay of the MLCT band at the corresponding $\lambda_{\text{max}}$ (395 nm). The variation of the electronic spectra for [Cu(cd4OMesalMeen)] titrated with nitrite ion in ethylenediamine at $T = 283$ °K (Figure 2) is shown in which the absorption of adduct was different from the copper complex and nitrite ion. The position of the maxima is slightly changed upon the addition of nitrite which is most likely due to the subsequent interaction of nitrite with the ionophore. As the nitrite concentration is increased, the absorption bands at 395 nm and 310 nm are decreased. The isosbestic points for this system show that there are only two species in equilibrium.

**Effect of pH on the optode response**

In order to investigate the impact of pH on the response of the optode, the absorbance data were recorded at different pHs ranging from 1–11. As can be seen from Figure 3, the maximum value of $\Delta A$ (defined as the difference in absorbance value between immobilized Schiff base and the complex) was occurred at pH 3. Therefore, this pH was chosen for further experiments.
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**Figure 3.** Effect of pH on the response of the nitrite-optical membrane sensor.

**Response time**

The rate determining step in this type of reactions is considered as the diffusion of the analyte to the optical membrane and association with the ionophore. The time required to reach 95% of this action is called "response time". The time-dependent response feature of the present bulk optode was studied by recording the absorbance change from a buffer solution at pH 3 to a buffered nitrite solution of 7.50 mgL$^{-1}$. The results are plotted in Figure 4. The response time of the membrane was found to be 8–10 min depending on the concentration of nitrite.

**Figure 4.** Typical response curve of the optode at 395 nm as a function of time when the membrane is exposed to 7.5 mgL$^{-1}$ nitrite ion

**Figure 5.** Calibration curve for the determination of nitrite ion using the optode
**Dynamic range**
The absorbance data of the optode membrane sensor were obtained upon changing the concentration of nitrite from 0.50 to 8.50 mgL\(^{-1}\). As depicted in Figure 5 graph of ∆A, absorbance difference, against different concentrations of nitrite gives good straight-line between 0.50 to 7.00 mgL\(^{-1}\) fitted to the correlation equation ∆A = 0.0299 C − 0.0106 with a regression coefficient of 0.9987, where C is the nitrite ion concentration expressed in mgL\(^{-1}\). The detection limit, defined as \(3s_b/m\), where \(s_b\) is the standard deviation of the blank signal (n=14) and \(m\) is the slope of the calibration curve, was estimated to be 0.04 mgL\(^{-1}\).

**Regeneration of the optical sensor**
Since the regeneration of the optode is an important feature, different reagents such as HCl, HNO\(_3\), H\(_2\)SO\(_4\), NaOH, EDTA and KSCN were tested to find which one is capable of recovering the membrane. According to the results, the sensor showed efficient reversibility upon treatment with 0.1 M HNO\(_3\) solution. After this regeneration step, the membrane should be placed in blank buffer solution (pH 3) for 10‒15 min, and get ready for the next measurement.

**Repeatability and reproducibility**
The repeatability of the prepared membrane was evaluated by using a single membrane and performing seven replicate measurements for 4.0 mgL\(^{-1}\) nitrite buffered solution followed by recovering the membrane with 0.1 M HNO\(_3\) solution. The resulting standard deviation was found to be 2.2%.

**Effect of foreign ions**
To study the selectivity of the fabricated membrane, the absorbance signals of the optode for nitrite ion were recorded in the presence of foreign species. The analysis was carried out in the buffered solution of pH 3, containing constant amount of nitrite ion, 1.0 mgL\(^{-1}\), plus different concentrations of interfering ions (up to 1000 times of the analyte ion). The tolerance limit, defined as the concentration of added species causing less than ±5 relative error, was detected for each ion. As can be deduced from Table 1, no interference was observed and the sensor was completely selective to nitrite over the other studied foreign ions.

**Table 1. Effect of foreign ions on the determination of 1.0 mgL\(^{-1}\) nitrite ion**

| Foreign ions | Tolerated molar ratio [interference/[nitrit]| |
|-------------|-----------------------------------------------|
| I\(^-\), Cl\(^-\), NO\(_3\)^-| 300 |
| SCN\(^-\), ClO\(_3\)^-, CH\(_3\)COO\(^-\), HCO\(_3\)^-, CN\(^-\), S\(_2\)O\(_3\)^2-| 300 |
| K\(^+\), Mn\(^{2+}\), Ca\(^{2+}\), Cd\(^{2+}\), Zn\(^{2+}\), Ni\(^{2+}\), Al\(^{3+}\) | 1000 |
| NH\(_4\)^+, Fe\(^{2+}\), Sr\(^{2+}\), Cr\(^{3+}\), La\(^{3+}\), Cu\(^{2+}\), Pb\(^{2+}\), Mg\(^{2+}\), Ag\(^+\) | 500 |
| Fe\(^{3+}\) | 100 |
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| Sausage sample | Nitrite added (mgL⁻¹) | Nitrite found (mgL⁻¹) | Recovery (%) |
|----------------|-----------------------|----------------------|--------------|
| Sample A       | 0.0                   | 0                    | –            |
|                | 1.0                   | 1.1                  | 110          |
|                | 2.0                   | 2.1                  | 105          |
|                | 0.0                   | 1.5                  | –            |
| Sample B       | 1.0                   | 2.6                  | 106          |
|                | 2.0                   | 3.4                  | 93           |
|                | 0.0                   | 0.0                  | –            |
| Sample C       | 1.0                   | 1.1                  | 110          |
|                | 2.0                   | 1.9                  | 95           |
|                | 0.0                   | 0.0                  | –            |
| Sample D       | 1.0                   | 1.0                  | 100          |
|                | 2.0                   | 2.1                  | 105          |
|                | 0.0                   | 2.5                  | –            |
| Sample E       | 1.0                   | 3.6                  | 104          |
|                | 2.0                   | 4.6                  | 104          |
|                | 0.0                   | 0.0                  | –            |
| Sample F       | 1.0                   | 1.1                  | 110          |
|                | 2.0                   | 1.9                  | 95           |

Formation constant and thermodynamic free energy calculations for copper(II) Schiff base complex with nitrite ion in ethylenediamine solvent

The formation constant of the copper(II) Schiff base complex were calculated by using Ketelaar’s equation (1).[44]

\[
P = \frac{1}{(\varepsilon_c - \varepsilon_A - \varepsilon_D)} \left[ \frac{1}{K} + \left( \frac{C_A + C_D}{C_A C_D} \right) \right]
\]  

where \( P = C_A^c C_D^c / (A - A_A^c - A_D^c) \) \( A \) and \( C_A^0 \) and \( C_D^0 \) are the initial concentrations (mol. dm⁻³) of the acceptor and the donor, respectively. \( A \) is the optical density of the solution including the acceptor and the donor, \( A_A^c \) and \( A_D^c \) are the optical densities of the pure acceptor and the pure donor in the solution of concentration \( C_A^0 \) and \( C_D^0 \). \( \varepsilon_c \), \( \varepsilon_A \) and \( \varepsilon_D \) are the molar extinction coefficients (dm³. mol⁻¹. cm⁻¹) of the complex, the acceptor and the donor, respectively. \( K_{eq} \) is the equilibrium constant of the formed adduct and the cell optical path length is 1 cm. The linear plots of \( P \) against \( (C_A^0 + C_D^0) \) for [Cu(cd4OMesalMeen)] titrated with nitrite ion at various temperatures in ethylenediamine are shown in Figure 6. It signifies that only a 1:1 adduct is formed, since the mixture of 1:1 and 1:2 or only 1:2 complex in a system would lead to a curve. The formation constant of the studied complex was calculated from the ratio of the slope to the intercept by least-square method. The \( K \) measurements were repeated at least twice and were reproducible.
The thermodynamic parameters were calculated by using the well-known van’t Hoff equation (2).

\[ \ln K_{eq} = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \]  

(2)

where \( K \) is the equilibrium constant, \( R \) is the gas constant and \( T \) is the temperature in Kelvin. Thermodynamic parameters of the studied adduct were obtained from the linear plots of \( \ln K_{eq} \) against \( 1/T \). The values of \( \Delta H^\circ \) and \( \Delta S^\circ \) were obtained from the slope and the intercept, respectively. The formation constant and the thermodynamic parameters data are collected in Table 3.

The linear plots of \( \ln K \) against \( 1/T \) for the interaction of \([\text{Cu(cd4OMesalMeen)}]\) with nitrite ion at various temperatures in ethylenediamine is shown in Figure 7.

![Figure 6. Plots \( P \) against \( C \) for \([\text{Cu(cd4OMesalMeen)}]\) with nitrite ion in ethylenediamine, where \( P = C_A \times C_n/(A - A_i - A_n) \) and \( C = (C_A + C_n) \) at 293 K.](image1)

![Figure 7. Plot of \( \ln K \) against \( 1/T \) for \([\text{Cu(cd4OMesalMeen)}]\) with nitrite ion, in ethylenediamine](image2)

| \( K_{eq} \times 10^2 \) (K)/(dm\(^3\).mol\(^{-1}\)) | \( \Delta H^\circ \) (kJ mol\(^{-1}\)) | \( \Delta S^\circ \) (J K\(^{-1}\).mol\(^{-1}\)) | \( \Delta G^\circ \) (kJ mol\(^{-1}\)) | 
|----------------|------------------|------------------|------------------|
| 14.19±1.31 (T=273) | -16.16±3.91 | -42.47±9.20 | -29.03±2.73 |
| 8.98±0.37 (T=283) | -16.16±3.91 | -42.47±9.20 | -29.03±2.73 |
| 5.55±0.08 (T=293) | -16.16±3.91 | -42.47±9.20 | -29.03±2.73 |

\( \Delta G^\circ \) values are calculated from \( \Delta G^\circ = \Delta H^\circ - T \Delta S^\circ \) at \( T = 303^\circ K \).
Conclusion
A copper(II) Schiff base complex, [Cu(cd4OMeSalMeen)], was successfully immobilized on triacetylcellulose membrane and a new optical sensor was resulted for the analysis of nitrite ion. The proposed optode has provided a useful means for the determination of nitrite content in meat products as it possesses the advantages of simple preparation and handling, fast and easy regeneration, and low cost and good selectivity. The formation constant and thermodynamic parameters were calculated for the interaction of the Schiff base with nitrite ion and showed that the reaction was exothermic.

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