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Determination of melamine in different milk batches using a novel chemosensor based on the luminescence quenching of Ru(II) carbonyl complex

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ABSTRACT

A novel, simple, sensitive and precise spectrofluorimetric method was developed for measuring the melamine concentration in different milk batch samples. The method was based upon measuring the quenching of the luminescence intensity of the produced yellow colored ruthenium$^{(II)}$ carbonyl complex of the general formula $[Ru(CO)_2(L)]$ (where L = anion of tetradentate Schiff base). The $Ru^{(II)}$ complex exhibited characteristic luminescence band in the visible region. The remarkable quenching of the luminescence intensity of $[Ru(CO)_2(L)]$ complex by various concentrations of melamine was successfully used as a chemosensor for the assessment of melamine in different milk samples at λ_{ex} = 400 nm and pH 7.4 in DMSO with a linear dynamic range 1.0×10^{-6} to 3.0×10^{-9} mol L^{-1} and lower detection limit (LOD) and quantification detection limit (QOD) of 3.3×10^{-10} and 1.0×10^{-9} mol L^{-1}, respectively.

1. Introduction

Melamine is a kind of triazine analogue with three amino groups, with chemical formula $C_3H_6N_6$. Officially it is 1,3,5-triazine-2,4,6-triamine in the IUPAC nomenclature system. Melamine can also be described as a trimer of cyanamide, three cyanamide units joined in a ring.

Melamine has a molecular mass of just over 126, forms a white, crystalline powder, and is only slightly soluble in water. Like cyanamide, melamine contains 66% nitrogen by mass. Indeed, it is this high nitrogen level that gives it the analytical characteristics of protein molecules, so it was previously considered as a non-protein nitrogen (NPN) supplement. Melamine is unethically added to food products to have more protein than they actually have – likely because they have been heavily cut with water in an effort to lower production costs. Standard tests such as the Kjeldahl and Dumas tests [1], estimate protein levels by measuring the nitrogen content, so they can be misled by adding nitrogen-rich compounds such as melamine.

Melamine is a toxic compound to both animals and human beings [2]. Unfortunately, it is possible that melamine accumulates in the body and causes toxicity problems. Ingestion of melamine may lead to reproductive damage, or bladder or kidney stones, which can lead to bladder cancer [3]. Infants fed regularly with milk containing melamine will be particularly susceptible to these effects. Up to now, only a few articles have been reported for determination of melamine in milk or milk-based products. Melamine has been determined in milk and milk powder by HPLC–DAD [4], thin layer chromatography–DAD [5] or HP capillary electrophoresis–UV [6]. A high-performance micellar electrokinetic capillary chromatography with amperometric detection (MECC–AD) method for the fast determination of melamine has been developed [7]. A simple and rapid ultra-performance liquid chromatographic (UPLC) method with tandem mass spectrometry has been developed and validated for the determination of melamine in milk, milk products, bakery goods, and flour [8].

Some authors have also tried to detect melamine in milk by ultrasound-assisted extractive MS [9], using low-temperature plasma (LLP) probe combined with MS/MS [10] or by surface desorption atmospheric pressure chemical ionization (DAPCI) MS [11]. Melamine was determined in raw milk and dairy products using HILIC–electrospray MS/MS, after an off-line SPE clean-up [12]. A method based on reversed-phase LC–MS/MS using heptafluorobutyric acid (HFBA) as the ion-pair reagent for melamine determination was reported [13].

Molecularly imprinted polymers were prepared using melamine and was used as selective sorbent for the separation and extraction of melamine from dairy products and food, followed by detection of melamine using high-performance liquid [14] and GC–MS [15]. The use of fast semi-automated method

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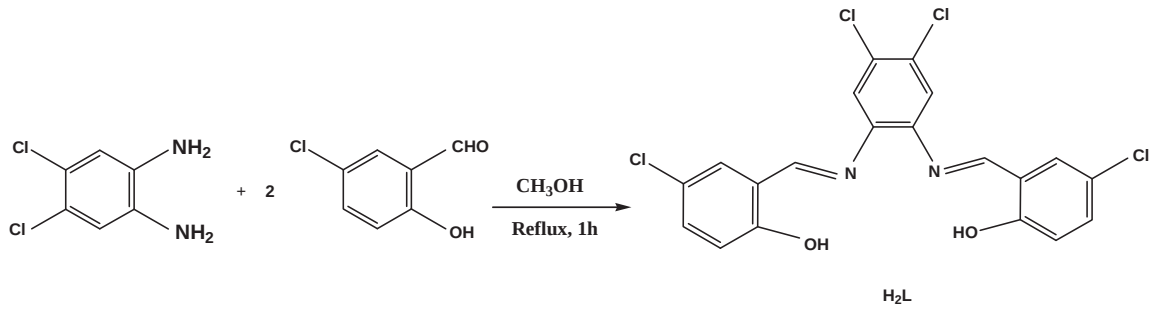
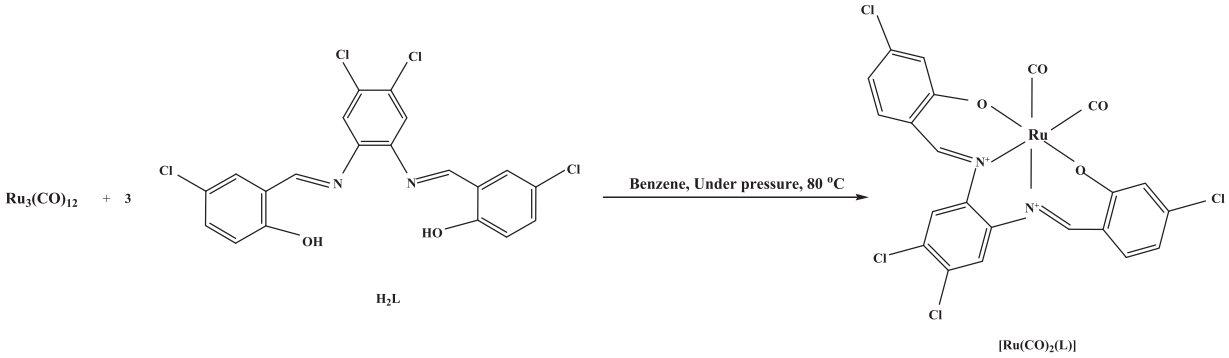


Scheme 1. Synthetic route for the Schiff base ligand H_2L.



Scheme 2. Synthetic route for Ru(II) carbonyl complex $[Ru(CO)_2(L)]$.

employing direct analysis in real time (DART) ion source coupled to time-of-flight mass spectrometry (TOFMS) for analysis of melamine (MEL) and cyanuric acid (CYA) in milk powder and milk based products has been demonstrated [16]. Melamine was determined in feed, egg and liquid milk by capillary electrophoresis (CZE) was reported [17]. Multimode reader has been generally applied in immunoassay, the 96-well micro-plate was modified with molecularly imprinted melamine sol–gel film, which the highly selective and high throughput detection of melamine was achieved [18].

However, complicated pre-concentration, time-consuming steps and high-cost instruments limit the application of the existing methods. Hence, there have been increasing demands for new, fast, simple, convenient and sensitive methods for the determination of melamine. Unfortunately, so far few of papers have been reported concerned about optical methods to determine melamine in milk. Near infrared reflectance spectroscopy (NIRS) method for detecting of melamine in milk powder [19]. Melamine was screened in milk products with a low-cost disposable micro fluidic device coupled with ultraviolet (UV) detection [20]. A spectrophotometric method (Mannich reaction) for determination of melamine [21]. Compared to those existing methods, much importance has been attached to the chemosensors due to their simplicity, low-cost, accurateness, sensitivity and high stability. Fluorometric determination of melamine in tainted milk using cucurbit[7]uril (CB7) sensor [22].

Visual detection of melamine in raw milk using gold nanoparticles (Au NPs) as colorimetric probe was reported [23,24]. Preparation of Monoclonal Antibody for Melamine and development of an indirect competitive ELISA for melamine detection in raw milk, milk powder and animal feeds was also reported [25].

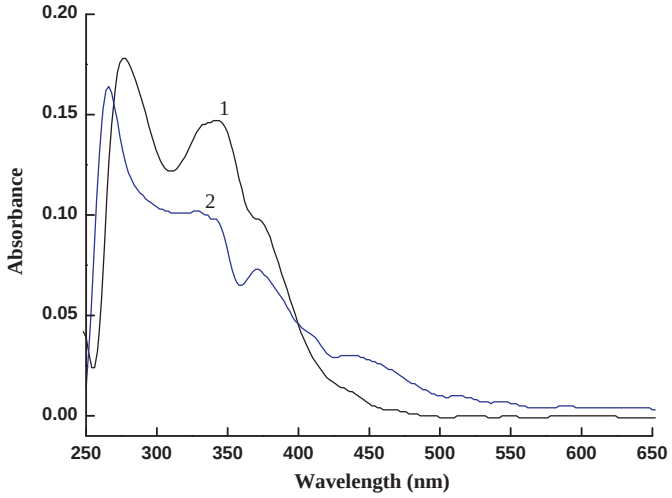


Fig. 1. Absorption spectra of 1×10^{-5} mol L^{-1} of Schiff base ligand (1) and Ru(II) complex (2).

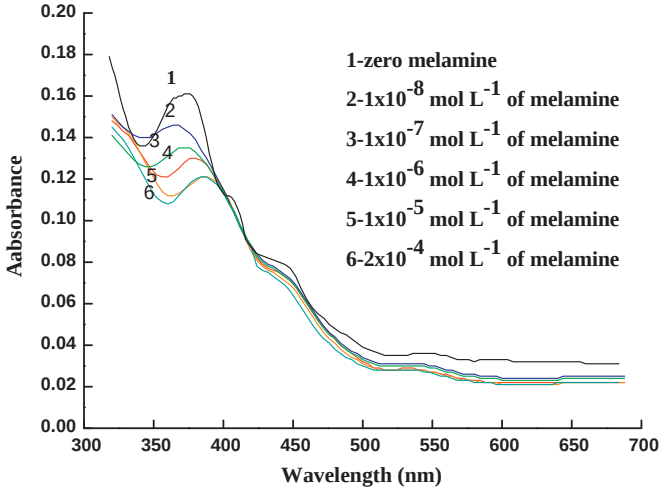


Fig. 2. Absorption spectra of 1×10^{-5} mol L^{-1} of $Ru^{(II)}$ complex in presence of different melamine concentrations.

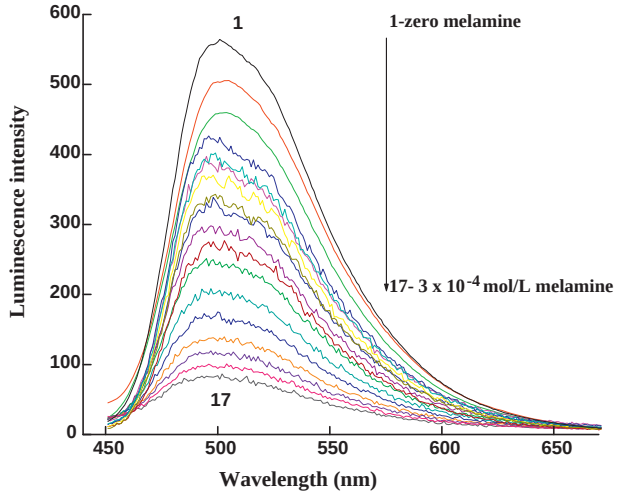


Fig. 3. Luminescence spectra of 1×10^{-5} mol L^{-1} of $Ru^{(II)}$ complex in presence of different melamine concentrations at $\lambda_{ex.}$ = 400 nm.

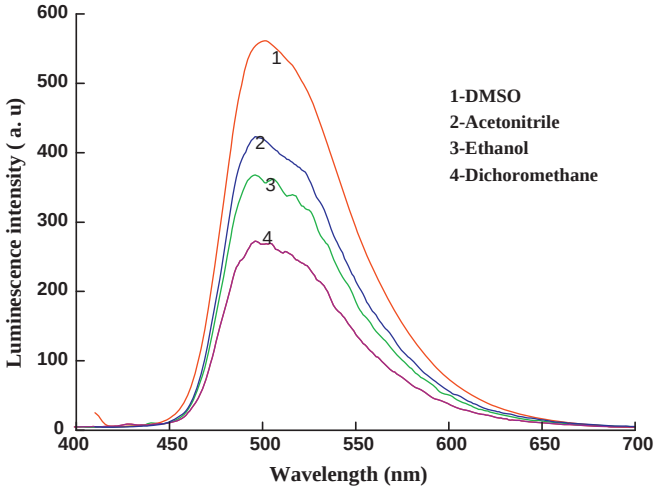


Fig. 4. Luminescence spectra of 1×10^{-5} mol L^{-1} of $Ru^{(II)}$ complex in different solvents at $\lambda_{ex.}$ = 400 nm.

Table 1
Sensitivity and regression parameters for chemosensor.

Parameter	Method
λ_{em}, nm	505
Linear range, mol L^{-1}	1.0×10^{-6}–3.0×10^{-9}
Limit of detection (LOD), mol L^{-1}	3.3×10^{-10}
Limit of quantification (LOQ), mol L^{-1}	1.0×10^{-9}
Intercept (a)	0.01
Slope (b)	1.0×10^{7}
Standard deviation	0.01
Variance (Sa^2)	6.8×10^{-4}
Regression coefficient (r)	0.99
C_O, mol L^{-1}	9.1×10^{-7}
R_O, Å	0.75

At present, luminescent complexes are attracting more and more interest from researchers due to their important applications in the field of light-emitting diode devices [26], fluorescent sensor [27] and biological probes [28]. The chemiluminescent activities of the ruthenium complexes form the basis of their uses for the development of sensitive and selective methods for determination of a number of analytes [29]. The continuing quest for new complexes of ruthenium is primarily due to their efficacy in such a broad range of applications. However literature survey reveals that little work has been done on tetradentate Schiff base complexes of Ru(II).

The present investigation aims to synthesize a novel reliable, sensitive, low cost-effective luminescent sensor based on $Ru^{(II)}$ carbonyl tetradentate Schiff base complex to determine melamine at trace levels to identify potential health risks involved with consumption of contaminated milk products.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were analytical-reagent of the highest grade. 5-Chloro-salicylaldehyde, 2,3-diaminonaphtalene and $Ru_3(CO)_{12}$ were purchased from Sigma–Aldrich (Saint Louis, USA). Pure standards of melamine, distilled water and pure grade solvents were

Table 2
Evaluation of intra-day and inter-day accuracy and precision.

Sample	Melamine taken[a]	Intra-day accuracy and precision (n = 3)			Inter-day accuracy and precision (n = 3)		
		Melamine Average found ± CL[b]	%RE[c]	%RSD[d]	Melamine average found ± CL	%RE	%RSD
Beyti	2.0	2.03 ± 3.20	1.50	1.29	2.06 ± 2.81	3.00	1.13
	5.0	4.95 ± 3.05	1.00	1.23	5.05 ± 2.78	1.00	1.12
	8.0	8.09 ± 2.95	1.125	1.19	8.12 ± 2.76	1.50	1.11
Labanita	2.0	2.51 ± 3.13	2.00	1.26	1.99 ± 2.76	2.00	1.11
	5.0	5.02 ± 3.00	0.40	1.21	5.04 ± 2.63	0.80	1.06
	8.0	7.99 ± 2.86	0.13	1.15	8.03 ± 2.53	0.38	1.02
Bekhero	10.0	10.07 ± 5.86	0.70	2.36	9.89 ± 5.74	1.10	2.31
	15.0	15.12 ± 5.49	0.80	2.21	15.14 ± 5.62	0.93	2.26
	20.0	19.79 ± 5.09	1.05	2.05	20.33 ± 5.27	1.65	2.12
Juhayna	10.0	10.31 ± 3.38	3.10	1.36	9.79 ± 4.25	2.10	1.71
	20.0	20.22 ± 3.00	1.10	1.21	20.24 ± 3.63	1.20	1.46
	30.0	30.19 ± 2.81	0.63	1.13	30.23 ± 3.03	0.76	1.22
Joy	5.0	5.11 ± 7.88	2.20	3.17	5.19 ± 7.98	3.80	3.21
	10.0	10.12 ± 6.06	1.20	2.44	10.14 ± 6.11	1.40	2.46
	15.0	15.09 ± 4.80	0.60	1.93	15.13 ± 4.82	0.86	1.94
Enjoy	5.0	5.11 ± 8.25	2.20	3.32	5.19 ± 8.26	3.80	3.33
	10.0	10.12 ± 7.98	1.20	3.21	10.14 ± 8.10	1.40	3.26
	15.0	15.09 ± 7.71	0.60	3.10	15.13 ± 8.00	0.86	3.22

[a] The values are multiplied by 10^{-7} mol L^{-1} for method.
[b] CL, confidence limits were calculated from: $CL = \pm tS/(n)^{1/2}$. The tabulated value of t is 4.303, at the 95% confidence level; S = standard deviation and n = number of measurements.
[c] %RE, percent relative error.
[d] %RSD, relative standard deviation.

Table 3
Method robustness and ruggedness expressed as intermediate precision (%RSD).

Sample	Melamine taken[a]	Robustness		Ruggedness
		Parameter altered		Inter-analysts, (%RSD) ($n = 3$)
		Chemosensor conc. (%RSD)	pH[b] (%RSD)	
Beyti	5.0	1.28	1.22	1.45
Labanita	5.0	1.12	0.97	1.89
Bekhero	10.0	0.78	0.71	1.54
Juhayna	10.0	0.88	0.75	1.35
Joy	15.0	0.45	0.38	1.21
Enjoy	15.0	0.43	0.39	1.22

[a] The values are multiplied by 10^{-7} mol L^{-1}.
[b] Concentrations of Ru(II) complex were 2, 5 and 6×10^{-5} mol L^{-1}; and the values of pH were 8, 8.4 and 8.8.

purchased from Sigma–Aldrich (Saint Louis, USA). Stock solutions of melamine (1.5×10^{-3} mol L^{-1}) were prepared and dissolved in ethanol. The working standard solution of (2.0×10^{-4} mol L^{-1}) was prepared by appropriate dilution with DMSO. A chemosensor stock solution (1.0×10^{-3} mol L^{-1}) was prepared by dissolving appropriate weight of chemosensor in a small amount of DMSO in 100 mL measuring flask, then diluting to the mark with DMSO. The pH was adjusted by using 0.1 mol L^{-1} of HCl/NH_4OH.

2.2. Apparatus

All luminescent measurements were carried out on Shimadzu RF5301 Spectrofluorophotometer in the range of 290–750 nm. The absorption spectra were recorded with a Unicam UV–visible double-beam spectrophotometer from Helios Company. It employs a Tungsten filament light source and a Deuterium lamp, which have a continuous spectrum in the ultraviolet region. The spectrophotometer is equipped with a temperature-controller cell holder. (All measurements were measured at Photo energy Center, Faculty of Science, Ain Shams University).

2.3. General procedure

2.3.1. Synthesis of the Schiff base ligand (H_2L)

To a hot methanolic solution containing 10 m mol of the amine, 20 m mol of aldehyde was added drop wise. The yellow solution obtained was refluxed for 2 h.

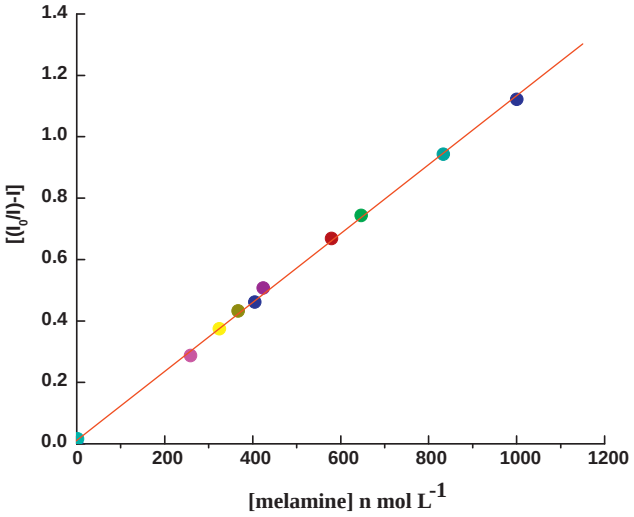


Fig. 5. Linear relation between melamine concentrations and $[(I_0/I) - 1]$.

After cooling, the precipitated Schiff base was collected by filtration and recrystallized from methanol. The ligand was dried in a desiccator over anhydrous $CaCl_2$ under vacuum. The resultant product was washed with ethanol (Scheme 1).

2.3.2. Synthesis of Ru^{II} complex

A mixture of $Ru_3(CO)_{12}$ (0.16 m mol) and Schiff base (0.48 m mol) in 20 ml benzene was heated at 80 °C under reduced pressure in sealed tube for 1 h, until the mixture gradually changed to deep red color. The reaction mixture was cooled and the solvent was removed on a vacuum line. The red residue formed was washed for several times by hot petroleum ether 60–80 and then recrystallized from hot methanol then the isolated complex was dried in vacuum. (Scheme 2).

2.3.3. Preparation of melamine solutions and construction of calibration curve

The pure standard solutions of melamine were prepared by different dilutions of 1.5×10^{-3} mol L^{-1} melamine stock solution to give the following concentrations of melamine, 2.0×10^{-4} to 1.0×10^{-9} mol L^{-1}. The different concentrations of malamine were added to 10 mL clean and sterilized measuring flasks containing (1.0×10^{-5} mol L^{-1}) of the chemosensor. The solutions were diluted to the mark with DMSO and the pH was adjusted to 7.4 by HCl/NH_4OH at room temperature. The above solutions were used for subsequent measurements of absorption and emission spectra

Table 4
Results of recovery study using standard addition method.

Proposed method Sample studied	Pure melamine added, mg	melamine found, mg	Pure melamine recovered (Percent ± RSD)
Beyti	2.5	2.51	100.4 ± 0.27
	4.0	4.05	101.3 ± 0.37
	5.5	5.47	99.40 ± 0.27
Labanita	2.5	2.47	98.88 ± 0.37
	4.0	4.05	101.3 ± 0.37
	5.5	5.51	100.2 ± 0.17
Bekhero	2.5	2.52	100.8 ± 0.27
	4.0	4.05	101.3 ± 0.37
	5.5	5.51	100.2 ± 0.17
Juhayna	3.0	3.05	101.6 ± 0.45
	4.5	4.55	101.1 ± 0.45
	6.0	6.01	100.2 ± 0.10
Joy	2.5	2.53	101.2 ± 0.47
	4.0	4.05	101.3 ± 0.57
	5.5	5.48	99.63 ± 0.17
Enjoy	2.5	2.48	99.20 ± 0.27
	4.0	4.05	101.3 ± 0.45
	5.5	5.52	100.4 ± 0.15

Table 5
Comparison of spectrofluorimetric technique with some existing methods for the determination of melamine.

Method	Linear range	Detection limit	Recovery %	Reference
Liquid chromatography tandem mass spectrometry	10–5000 (n mol L^{-1})	1 (n mol L^{-1})	93.9–102%	8
Molecularly imprinted sol–gel film	0.1–50 (μ mol L^{-1})	0.02 (μ mol L^{-1})	95%	18
Spectrophotometric method	1–100 (μg mL^{-1})	0.23 (μg mL^{-1})	86–89%	20
Fluorometric method	1–60 (μg mL^{-1})	0.20 (μg mL^{-1})	92–95%	22
Electrochemical sensor	1.0×10^{-8}–5.0×10^{-6} (mol L^{-1})	3.0×10^{-9} (mol L^{-1})	96%	41
Chemosensor method	3×10^{-9}–1.0×10^{-6} (mol L^{-1})	3.3×10^{-10} (mol L^{-1})	98.88–101.3%	Present work

as well as the effect of solvents. The luminescence intensities were measured at λ_{ex} = 400 nm.

2.3.4. Determination of melamine in different milk batches

A 50 mL of 1% trichloroacetic acid solution and 2 mL of 2.2% lead acetate solution were added to 5 mL of liquid milk sample in order to eliminate protein and extract the analyte. A 30 mL of the mixture was placed in ultrasonic cleaner for 20 min to mix well, standing for 2 min. Then the mixed solution was centrifuged for 40 min at 4000 rpm. Then 0.1 mL of the separated milk solution was added to 10 mL clean and sterilized measuring flasks containing (1×10^{-5} mol L^{-1}) of the chemosensor [$Ru(CO)_2(L)$] and the solution was completed to the mark by DMSO and the pH was adjusted at 7.4.

3. Results and discussion

3.1. Absorption and luminescence spectra

The electronic absorption spectra of the Schiff base ligand and the $Ru^{(II)}$ complex were recorded in DMSO solution in the region 200–700 nm (Fig. 1). The electronic absorption spectrum of H_2L ligand displayed two bands at 272 and 338 nm, which are assigned for the π–π* and *n*–π* transitions, respectively. The electronic absorption spectrum of the Ru(II) precursor in DMSO showed four bands at 264, 342, and 372 and 434 nm, respectively. The high intensity bands at round 264 nm and 339 nm have been designated as n–π* and π–π* transitions, respectively, for the electrons localized on the azomethine group of the Schiff base [30]. The absorption spectrum of $Ru^{(II)}$ complex show another types of transitions different from the free ligand. The first one was at 372 nm which can be assigned to ligand to metal charge transfer [31,32]. The other one appeared at 442 nm which can be attributed to the spin allowed $^1A_{1g} - {}^1T_{1g}$ transition [33]. The pattern of the electronic absorption spectrum of the complex indicated the presence of an octahedral environment around $Ru^{(II)}$ ion, similar to that of other ruthenium(II) octahedral complexes [34,35]. The luminescence emission spectrum of $Ru^{(II)}$ complex shows a characteristic emission band of the Ru complex at 505 nm and a shoulder at 522 nm. Upon addition of different concentrations of melamine to the solution of Ru(II) complex in DMSO a blue shift in absorption band was observed and the intensity of the absorption bands were decreased (Fig. 2). Also, it can be seen that the characteristic peak of $Ru^{(II)}$ complex at 505 nm has been remarkably quenched after the addition of different concentrations of melamine (Fig. 3), which indicates that melamine quenches the energy of the $Ru^{(II)}$ complex in both ground and excited states.

3.2. Effect of experimental variables

3.2.1. pH effect

The pH variation of the solution containing $Ru^{(II)}$ complex chemosensor and melamine will affect the complexation between the chemosensor and melamine as a quencher. Melamine has a pK_a of 5.10 [36], with equilibrium between two forms: one is molecular and the other is positively charged. Two pHs were tested, pH 7.4 where the melamine is in its molecular form, and pH 3 where the melamine is quantitatively protonated. Using optimal conditions at pH 7.4, melamine makes a quenching to the luminescence intensity of the chemosensor.

3.2.2. Effect of solvent

The influence of the solvent on the luminescence intensity of 1.0×10^{-5} mol L^{-1} of the chemosensor $Ru^{(II)}$ complex was studied under the conditions established above.

The results show that there is no quenching in the emission intensity of Ru(II) complex in the presence of DMSO [37] (Fig. 4).

3.2.3. Effect of the amount of chemosensor and melamine

The influence of the amount of $Ru^{(II)}$ complex on the luminescence intensities of Ru(II) complex in DMSO was studied under the conditions established above. The luminescence intensity of $Ru^{(II)}$ complex at 505 nm increased upon increasing the concentration of $Ru^{(II)}$ complex up to 1×10^{-5} mol L^{-1} then becomes constant. Thus, 1.0×10^{-5} mol L^{-1} $Ru^{(II)}$ complex concentration was used for further analysis in DMSO.

Also, the luminescence intensity of $Ru^{(II)}$ complex at 505 nm decreased upon increasing the concentration of melamine up to 3×10^{-4} mol L^{-1} then becomes constant. Thus, 3.0×10^{-4} mol L^{-1} melamine concentration was used for complete quenching in DMSO.

3.3. Analytical performance

3.3.1. Analytical parameters of chemosensor method

A linear correlation was found between luminescence intensity of $Ru^{(II)}$ complex at λ_{em} = 505 nm and concentration of melamine in the range given in 1×10^{-6}–3.0×10^{-9} mol L^{-1}. Fig. 5 represents the calibration curve, which was obtained by applying the Stern–Völmer plot at λ_{em} = 505 nm [38]:

$$\left[\left(\frac{I_0}{I}\right) - 1\right] = \kappa_{sv} \quad [Q] \tag{1}$$

where, I_0 and I are the luminescence intensities of $Ru^{(II)}$ complex in absence and in presence of the quencher (melamine), respectively, [*Q*] is the concentration of the melamine, and κ_{sv} is called Stern–Völmer constant. When the luminescence intensity of the chemosensor was plotted against the concentration of the quencher, the slope of the fitted data is the Stern–Völmer constant. From κ_{sv}, the critical concentration, C_o can be calculated from the relation

$$C_o = \frac{1}{\kappa_{sv}} \tag{2}$$

and the critical radius R_o can be calculated from

$$R_o = 7.35 C_o^{-1/3} \tag{3}$$

where R_o was defined as the *D*–*A* distance at which the transfer and the spontaneous deactivation of the donor-excited expresses the transport efficiency state is equally probable [39]. The regression analysis of luminescence intensity data using the Stern–Völmer

equation was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values were presented in Table 1. The limit of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [40] using the formulae: LOD = 3.3S/b and LOQ = 10S/b mol L^{-1} (where S is the standard deviation of blank luminescence intensity values, and b is the slope of the calibration plot) are also presented in Table 1.

3.3.2. Accuracy and precision of the method

To compute the accuracy and precision, the assays described under "general procedures" were repeated three times within the day to determine the repeatability (intra-day precision) and three times on different days to determine the intermediate precision (inter-day precision) of the method. These assays were performed for three levels of analyte. The results of this study are summarized in Table 2. The average percentage relative standard deviation (%RSD) values were ≤1.93% (intra-day) and ≤1.94% (inter-day) indicating high precision of the method. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and the taken concentrations of melamine. Bias{bias % = [(concentration found − known concentration) × 100/known concentration]} was calculated at each concentration and these results are also presented in Table 2. Percent relative error (%RE) values of ≤3.8% demonstrates the high accuracy of the proposed method.

3.3.3. Selectivity

Interference studies were carried out prior to the application of the proposed method for the assay of melamine in spiked milk products. The influences of several substances possibly existing in the real samples on the determination of 1.0×10^{-7} M melamine were investigated. The results show that 500-fold concentration of vitamin B_1 (0.08 mg), vitamin B_2 (0.34 mg), starch (50 mg), lactose (10 mg), calcium salt (240 mg), calcium dihydrogen orthophosphate (180 mg), sodium salt (70 mg) and magnesium salt (100 mg) have no influence on luminescence intensity of the chemosensor suggesting the proposed method has a good selectivity for melamine.

3.3.4. Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the concentration of $Ru^{(II)}$ complex and pH. The effect of the changes was studied on luminescence intensity of the chemosensor. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as %RSD (≤1.28%). Method ruggedness was expressed as the RSD of the same procedure applied by three different analysts. The inter-analysts RSD were within 1.44% for the same melamine concentrations ranged from 1.4–1.89% suggesting that the developed method was rugged. The results are shown in Table 3.

3.3.5. Application to samples

In order to evaluate the performance of the proposed method for real samples analysis, the method was applied to the analysis of melamine in the liquid milk bought from a local market. The liquid milk was pretreated according to the general procedure.

The recovery experiment for melamine was carried out by adding the melamine standard in real milk sample and the luminescence intensity of the chemosensor was measured. The contents of melamine in different milk samples and recovery as well as RSD are given in Table 4. The recovery of melamine from different milk samples was in the range of 98.88–101.6% with the RSDs of 0.1–0.57%.

To further investigate the performance of this proposed chemosensor, we compared the results obtained above with other methods [8,18,20,22,41]. As shown in Table 5, this chemosensor exhibits remarkable advantages, such as the higher sensitivity, a wider linear range and a lower detection limit. It is noticed that the recovery obtained in this method meets the requirements for determination of melamine in real sample.

4. Conclusion

A novel reliable, sensitive, low cost-effective method for the determination of melamine residue in different milk batches was developed. The proposed method depends on the luminescence quenching of a novel $Ru^{(II)}$ complex synthesized by direct reaction between $Ru_3(CO)_{12}$ and a novel synthesized quadridentate N_2O_2 Schiff base.

The newly synthesized luminescent ruthenium can permit the determination of the melamine in different milk batches at λ_{ex} = 400 nm and pH 7.4 in DMSO with a linear dynamic range 1.0×10^{-6} to 3.0×10^{-9} mol L^{-1} and lower detection limit (LOD) and quantification detection limit (QOD) are 3.3×10^{-10} and 1.0×10^{-9}, respectively.

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