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Virtually specific UV-molecular probe for nitrite sensing

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A highly sensitive and selective UV-molecular probe has been developed for detection of low concentrations of nitrite in aqueous solution based on monotonous increase in absorbance of rhodamine 6G at 385 nm. Addition of nitrite also results in a bathochromic shift in UV absorption maximum of rhodamine 6G from 355 to 385 nm. The optimal conditions for parameters like concentration of H_2SO_4 and rhodamine 6G, response time and stability is reported. Under optimised conditions, the developed UV-probe enables the determination of 0 to 0.5 mgL^{-1} of nitrite. On the other hand, the addition of other anions like I^-, SCN^-, ClO_4^-, $[HgI_4]^{2-}$ and $[Zn(SCN)_4]^{2-}$ shows a bathochromic shift from 525 (the visible range absorption maximum) to 575 nm with no perceptible absorption at 385 nm. This enabled a virtually specific UV-molecular probe for rapid, precise and reliable monitoring of traces of nitrite in environmental samples and food materials with no interference from other anionic or cationic species. Studies with pyronine G also exhibit similar spectral characteristics on addition of nitrite.

Keywords: UV-probe, nitrite, rhodamine 6G, colorimetry

Nitrite is one of the widest known toxic inorganic pollutants. Nitrite occurs extensively in natural waters, soil, fertilizers, food materials and physiological systems. It is produced as an important intermediate in the biological nitrogen cycle. In water, nitrite can react with secondary amines to form carcinogenic as well as mutagenic N-nitrosoamines[1] and thus nitrite content could be used as an important indicator for water pollution[2]. Excess uptake of nitrite could cause gastric cancer[3] and its reaction with haemoglobin interferes with the physiological function of the oxygen carrier, which results in methaemoglobinaemia (blue baby syndrome), a rare disease predominantly affecting infants[4]. The World Health Organization (WHO) and European Community have recommended that the nitrite level in drinking water should not exceed 0.2 mgL^{-1} and 0.1 mgL^{-1} respectively[5,6]. These environmental and health hazards of nitrite have prompted researchers to develop efficient methods for the sensitive and selective determination of nitrite, in order to understand its distribution and pollution potential.

Among various techniques *viz* spectroscopic, electrochemical and chromatographic, spectral techniques are most widely used for nitrite determination due to excellent limits of detection and facile assay type protocols. A broad range of techniques have been evaluated, which include UV-Vis, chemiluminescence, fluorimetric, IR, Raman and molecular cavity emission protocols[7]. The most common approach to the determination of nitrite is the Griess assay which utilizes diazotization reaction wherein sulphanilamide and N-(1-naphthyl) ethylene-diamine are used as target amine and coupler respectively[8]. The other approach is the kinetic spectroscopy protocols wherein nitrite acts as catalyst for oxidation of gallocyanine by bromate[9]. Karthikeyan *et al.*[10] described a spectrophotometric procedure by measuring the decrease in absorbance of rhodamine 6G at 525 nm, the absorption maximum of the dye. Vishnuvardhan *et al.*[11] have recently described chemical switch based reusable dual optoelectronic sensor for nitrite. Rhodamine 110 has been used for determination of nitrite based on

fluorescence quenching[12,13]. A spectrofluorimetric procedure for nitrite based on unsymmetric rhodamine was reported by Liu *et al*.[14]

Although a number of methods are available for determining the presence of toxins, there is a growing need for rapid on-site analysis with optical probes for detecting these toxic ions and molecules. The design and development of molecular probes that are able to determine the presence of anions and cations in a straightforward manner will be of interest, since these probes offers the sensitive detection of trace amounts of analytes. Rhodamine 6G and simpler model of such dye pyronine G are metallo-chromic dyes and have been employed for aqueous spectrophotometric determination of several inorganics. The probes which employ these dyes are interesting as they have broad absorption band in the UV and visible spectra with quite large extinction coefficient, and high fluorescence quantum yields. To our knowledge, no UV-molecular probe for sensing of trace amounts of nitrite is hitherto reported. It is now proposed to have a UV-molecular probe for sensing of nitrite based on the increase in absorbance at 385 nm.

Experimental Section

Reagents

Analytical reagent grade chemicals were used in this study. Sulphuric acid (E Merck, Mumbai, India), rhodamine 6G and pyronine G (Aldrich, Milwaukee, WI, USA) were used as received. Stock solution of sodium nitrite was prepared by dissolving 0.1500 g of sodium nitrite (E Merck, Mumbai, India) in 100 mL of de-ionized water. A small amount of chloroform was added as stabilizer[10]. Working standards were prepared by appropriate dilution of stock solution.

Apparatus

A Shimadzu computer controlled UV-Vis spectrophotometer UV-2401 PC (Shimadzu, Kyoto, Japan), Edinburg computer controlled spectrofluorimeter, FLSP920 (Edinburgh, Livingston, UK) and Digital *p*H meter LI-120 (ELICO, Hyderabad, India) were used for absorbance, fluorescence and *p*H measurements respectively.

Procedure for determination of nitrite

An aliquot of sample solution (upto 15 mL) containing 0-4 mgL^{-1} of nitrite was taken in a 25 mL standard volumetric flask. 1.5 mL of 12.5 M sulphuric acid was added to above solution with mixing followed by the addition of 4 mL of 0.005% rhodamine 6G solution. This was then diluted to mark with de-ionized water and the absorbance or fluorescence measurements were taken at 385 and 555 nm (λ_{ex}= 355 nm) respectively against a reagent blank. The concentration of nitrite was established by reference to a calibration graph.

Analysis of natural waters

Appropriate aliquots of tap, well and sea water samples were taken for quantification *via* direct and standard addition methods by following the above mentioned procedure.

Analysis of food materials

About 1 g of sugar was dissolved in water and appropriate aliquots of this solution were taken for quantification *via* both direct and standard addition methods by following the above mentioned procedure. Milk powder was analyzed according to a reported procedure[10].

Results and Discussion

Absorption spectra

Figure 1 shows the absorbance spectra of rhodamine 6G with 0, 0.1, 0.2, 0.4 and 0.5 mgL^{-1} of nitrite (curves a to e) acidified with 0.75 M in sulphuric acid. Curves f, g and h show the absorption spectra of 0, 0.4 and 4 mgL^{-1} of nitrite in 0.75 M sulphuric acid medium. As seen from curve 'a' rhodamine 6G shows two absorption maxima – one in UV region (355 nm) and another in visible region (525 nm). On addition of nitrite to rhodamine 6G a new peak appears at 385 nm, the intensity of which increases with increase of nitrite and there is decrease in intensity for absorbance of rhodamine 6G at 525 nm. On the other hand, the addition of 0.4 or 4 mgL^{-1} of nitrite to 0.75 M sulphuric acid solution did not show any perceptible absorption at 385 nm. **Figure 2** shows similar features with pyronine G instead of rhodamine 6G. In contrast, addition of anions like ClO_4^-, SCN^- and I^- (**Figure 3**) and $[HgI_4]^{2-}$ and $[Zn(SCN)_4]^{2-}$ (Figure not shown) results in an increase in absorbance at 355 nm (hyperchromic shift), lowering of absorbance at 525 nm and an additional peak at 578 nm (a sort of red shift).

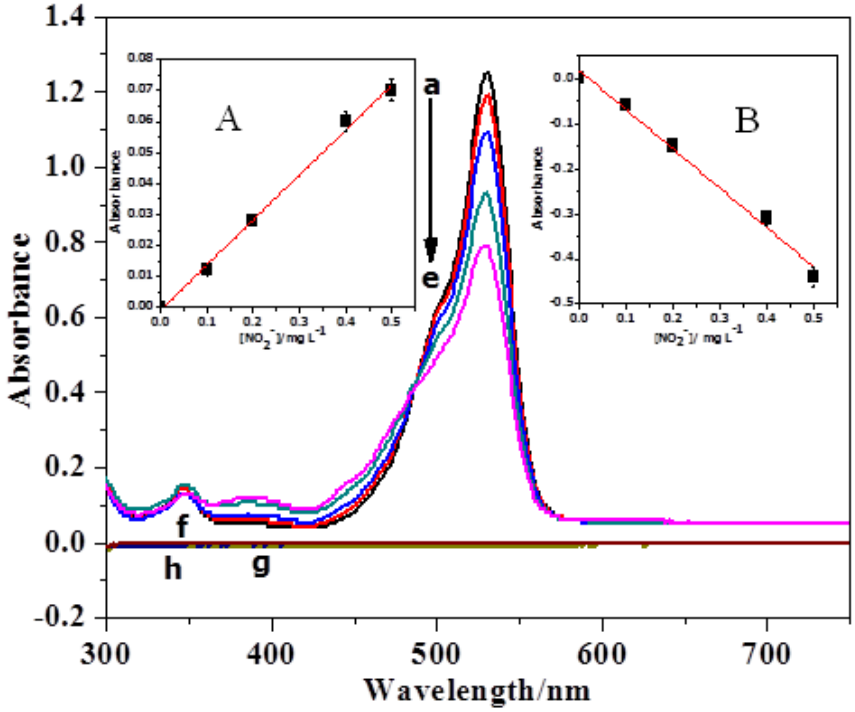

Figure 1 — Absorbance spectra of rhodamine 6G with 0, 0.1, 0.2, 0.4 and 0.5 mgL^{-1} of nitrite in 0.75 M H_2SO_4 (a to e) and absorbance spectra of 0, 0.4 and 4 mgL^{-1} of nitrite in 0.75 M H_2SO_4 (f, g, h) and calibration graph of nitrite at 385 nm [inset (A)] and at 525 nm [inset (B)].

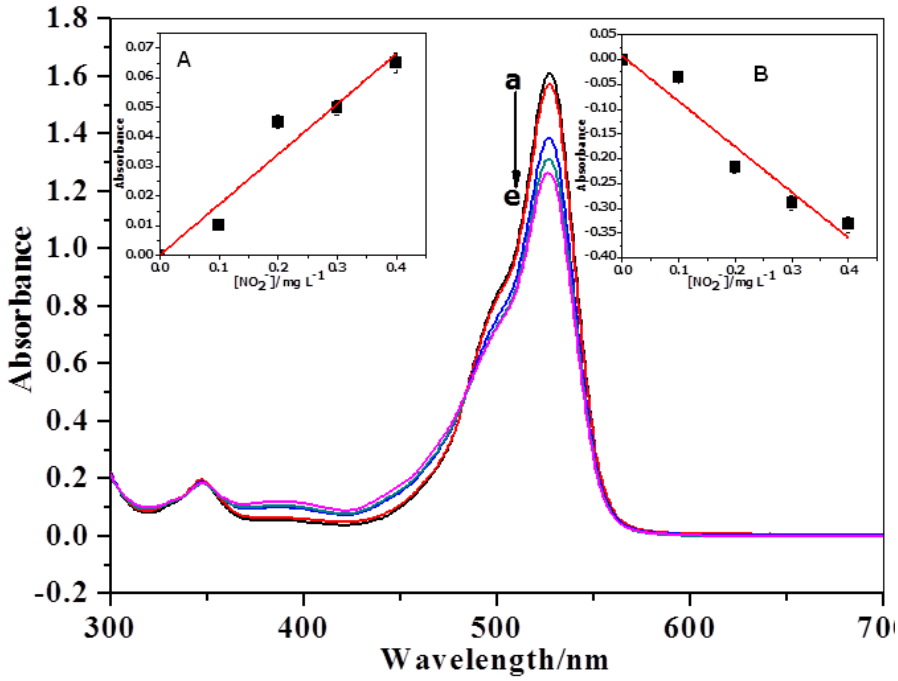

Figure 2 — Absorbance spectra of pyronine G with 0, 0.1, 0.2, 0.3 and 0.4 mgL^{-1} of nitrite in 0.75 M H_2SO_4 (a to e) and calibration graph of nitrite at 385 nm [inset (A)] and at 525 nm [inset (B)].

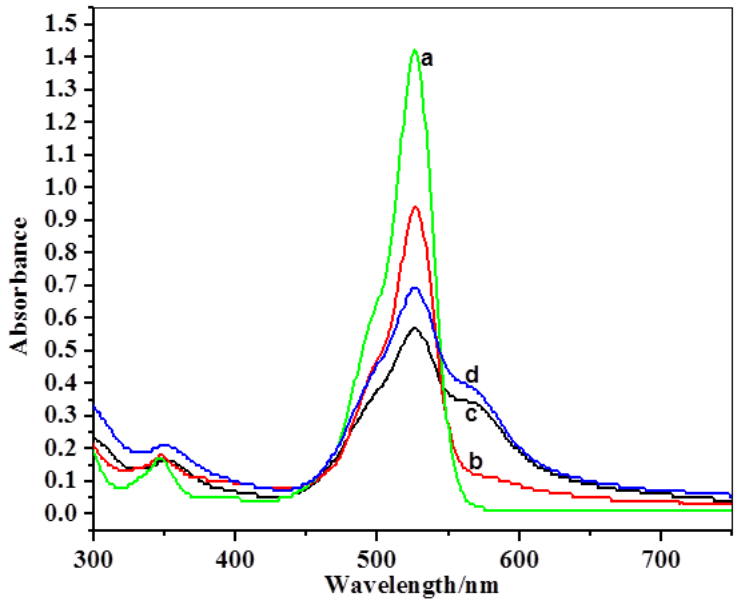

Figure 3 — Absorption spectra of rhodamine 6G (a) with 12 mM of I^- (b) 41.5 mM of SCN^- (c) and 44.5 mM of ClO_4^- (d).

Therefore nitrite sensing based on absorbance measurements at 385 nm is more specific compared to absorbance decrease at 525 nm reported in our previous works[10,11]. Similar features and conclusions were arrived in case of pyronine G dye based probe also.

Analytical optimization studies

The study on influence of acidity with sulphuric acid indicates that constant and maximum absorbance was obtained in the range 0.5 to 1 M. The effect of rhodamine 6G on the colour system indicates that a minimum of 4 mL of 0.005% rhodamine 6G in a total volume of 25 mL is sufficient to obtain maximum absorbance. The response time of UV-molecular probe is less than 30 sec and remains stable up to 30 min. Under these conditions, the determination of nitrite is possible in the concentration range 0 to 0.5 mgL^{-1} with a detection limit of 0.06 mgL^{-1}. Similar calibration range was noticed with fluoroprobe also. The developed analytical procedure based on UV-molecular probe is more precise with RSD of 2.9% compared to fluoroprobe having RSD of 5% for five replicate determinations of 0.4 mgL^{-1} of nitrite.

Selectivity studies

The tolerance ratio of various cationic, anionic and neutral electrolyte species on the determination of 0.4 mgL^{-1} of nitrite with UV-molecular probe and colorimetric probes (by measuring increase in absorbance at 385 nm or decrease in absorbance at 525 nm) and fluoroprobe (measuring decrease in fluorescence intensity at 555 nm on exciting at 355 nm) are summarized in **Table I**. The deviation of more than 3% of nitrite absorbance or 5% of fluorescence is taken as interference. As seen from the **Table I**, for all other tested species except iodide, UV-molecular probe with measurement at 385 nm offers higher tolerance ratios compared to that with measurement at 525 nm or fluoroprobe. Thus, the UV-probe with measurement at 385 nm is virtually specific as none of the tested species interfere even at levels much higher than nitrite.

Switching phenomenon

The addition of 24.74 µM of sulphamic acid results in annulling of absorbance change observed on addition of nitrite at 385 nm in ~30 minutes analogous to visible absorption probe[11] as described elsewhere. However, the UV-molecular probe switches back to nitrite absorbance on addition of further 0.4 mgL^{-1} of nitrite almost instantaneously as in case of visible probe.

Application to real samples

The applicability of the designed UV-molecular probe was tested for the analysis of natural waters and selected food materials. The analysis was carried by both direct and standard addition methods. The results obtained for various real samples are shown in

Table I — Tolerance ratio of various interferents ([Nitrite] = 0.4 mgL^{-1}, Total volume = 25 mL)

Interferent	Tolerance ratios		
	UV-molecular probe (385 nm)	Colorimetric probe[11] (525 nm)	Fluoroprobe (555 nm)
I^-	5	20	2.5
NO^{3-}	2.5×10^4	10^4	2.5
Br^-	7.5×10^4	10^4	0.1
SCN^-	1.4×10^2	40	1.4×10^{-2}
ClO_4^-	2×10^3	10^2	20
PO_4^{3-}	10^5	10^3	1
Cd^{2+}	2×10^4	1	2
Hg^{2+}	5×10^2	5×10^3	10^{-7}
Pb^{2+}	10	10^{-3}	10
Zn^{2+}	10^4	10^4	10^{-3}
Cu^{2+}	4×10^2	0.1	10^{-4}
NaCl	3.5×10^4	1	3.5×10^4
KCl	2.5×10^4	10	10^4
$MgCl_2$	5×10^4	1	5×10^4

Table II — Analysis of environmental samples and food materials

Sl.No.	Sample	Nitrite concentration (0.4 mgL^{-1})	
		Direct method[a]	Standard addition method
1	Tap water	0.20	0.23
2	Well water	0.05	0.07
3	Sea water	0.03	0.05
4	Sugar	0.18	0.18
5	Milk powder	0.33	0.25

[a]Singular determinations

Table II. All these studies show that the proposed method possesses good accuracy and is well comparable with standard addition method which indicates the utility of developed reusable probe for rapid, reliable and routine monitoring of nitrite in various environmental samples and food materials.

Mechanism of nitrite sensing by UV-molecular probe

An explanation as given elsewhere[11] for nitrite sensing is the formation of electron deficient nitrosyl cation which can attack the electron rich secondary amine group of rhodamine 6G resulting in the formation of N-nitrosamine derivative of rhodamine 6G which reverses on addition of sulfamic acid. It was postulated that the π conjugation of dye is lost on formation of N-nitrosamine derivative and this results in a blue shift to 385 nm. The fluorescence measurements also indicate similar structural changes in rhodamine 6G on addition of nitrite and subsequent reversal by sulfamic acid. We could not isolate the N-nitrosamine derivative of rhodamine 6G under analytical conditions to get clinching spectral evidence of this mechanism.

Table III — Effect of addition of miscible solvents and temperature on nitrite-rhodamine 6G system ([Nitrite] = 0.4 mgL^{-1}, Total volume = 25 mL)

Parameters	Absorbance (A_{385})
Acetone (mL)	
0	0.105
0.5	0.101
1.0	0.095
2.0	0.090
Methanol (mL)	
0	0.105
0.5	0.032
1.0	0.021
2.0	0.015
Temperature (°C)	
30	0.105
50	0.096
70	0.079

Alternately, Ramkrishna[16,17], Prasada Rao[18-20] and other groups have employed visible aqueous molecular probes for various inorganics based on the formation of negatively charged binary complexes $[HgI_4]^{2-}$, $[Zn(SCN)_4]^{2-}$, $[PdI_4]^{2-}$, *etc*.[15] which then associate with cationic dyes like rhodamine 6G or pyronine G. This has resulted in appearance of new peak at 578 nm a sort of red shift due to the formation of dye aggregates which are stabilized by gelatin preventing further aggregation to form particles. In addition to red shift there is a concomitant hypochromic shift at 525 nm. Similar spectral features were also noticed with I^-, SCN^- and ClO_4^-, but at high concentrations (**Figure 3**). On the other hand, the addition of nitrite results in hypochromic shift at 525 nm and red shift of UV absorption peak of 355 nm to 385 nm. All other anions mentioned above show hyperchromic shift at 355 nm. The decrease in absorbance of nitrite-rhodamine 6G colour system at 385 nm by the addition of acetone or methanol and on increase of temperature is given in **Table III**. From the absorbance values, it is clear that as the concentration of acetone or methanol increases, the absorbance decreases. This indicates the dissociation of rhodamine 6G aggregates in solution. The decrease in absorbance with increase in temperature is a result

Table IV — Effect of addition of surfactant on nitrite-rhodamine 6G system ([Nitrite] = 0.4 mgL^{-1}, Total volume = 25 mL)

Surfactant	Concentration (M)	Absorbance (A_{385})
Triton-X 100	0	0.105
	10^{-6}	0.105
	10^{-5}	0.105
	10^{-4}	0.106
	3×10^{-4}	0.105
	5×10^{-4}	0.093
	10^{-3}	0.081
SDS	0	0.105
	5×10^{-7}	0.093
	10^{-6}	0.105
	10^{-5}	0.093
	10^{-4}	0.106
	5×10^{-4}	0.047
	10^{-3}	0.047
CTAB	0	0.105
	5×10^{-7}	0.105
	10^{-6}	0.095
	10^{-5}	0.105
	10^{-4}	0.084
	5×10^{-4}	0.095
	10^{-3}	0.084

of deaggregation. This proposition is further supported by the fact that (i) nitrite- rhodamine 6G colour system absorbance at 385 nm is reverted back on cooling after first heating to 70°C (thermal switch) and (ii) there is a decrease in nitrite absorbance at 385 nm on addition of sodium dodecyl sulphate and the absorbance remains unaffected on addition of Triton X-100 or cetyltrimethyl ammonium bromide **Table IV**. This may be due to the fact that the formation of aggregates of cationic dye rhodamine 6G is least promoted in anionic surfactant sodium dodecyl sulphate due to electrostatic attractions compared to cationic (cetyltrimethyl ammonium bromide) and non ionic(Triton X-100) surfactants[21].

Conclusions

A reusable UV-molecular probe has been designed for the first time using off-the shelf readily available chromoionophoric dyes *viz*, rhodamine 6G and pyronine G. The sensing by the designed UV-molecular probe is virtually specific as it can tolerate very large amounts of several anionic, cationic and neutral electrolyte species. As a matter of fact, the designed UV-molecular probe is much superior to colorimetric probe (reported in our previous work) and fluoroprobe in terms of selectivity and precision. The developed probe has been successfully demonstrated for the analysis of natural waters and food materials. A plausible mechanism for such highly specific UV-molecular probe is also described.

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References

1 Masuda M, Mower H F, Pignatelli B, Celan I, Friesen M D, Nishino H & Ohshima H, *Chem ResToxicol*, 13, **2000**, 301.
2 Li J, Li Q, Lu C & Zhao L, *Analyst*, 136, **2011**, 2379.
3 Konstantinou I K, Hela D G & Albanis T A, *Environ Pollut*, 141, **2006**, 555.
4 Fan A M & Steinberg V E, *Regul Toxicol Pharmacol*, 23, **1996**, 35.
5 World Health Organization Guidelines for drinking-water quality. In Chemical aspects, *3rd version*, **2006**, p.191.http://www.who.int/water_sanitation_health/dwq/GDW8rev1and2.pdf
6 Reeve R N, *Environmental Analysis* (Wiley, Chichester), **1994**, Chapter 6.
7 Matthew J, Davis J & Compton R G, *Talanta*, 54, **2001**, 785.
8 Jannasch H W, Johnson K S & Sakamoto C M, *Anal Chem*, 66, **1994**, 3352.
9 Ensafi A A & Kazemzadeh A, *Anal Chim Acta*, 382, **1999**, 15.
10 Kartikeyan S, Prasada Rao T, Iyer C S P & Damodaran A D, *Mikrochim Acta*, 111, **1993**, 193.
11 Vishnuvardhan V, Kala R & Prasada Rao T, *Anal Chim Acta*, 623, **2008**, 53.
12 Leytus S P, *Biochem J*, 209, **1983**, 299.
13 Zhang X, Wang H, Fu N N & Zhang H S, *Spectrochim Acta Part A*, 59, **2003**, 1667.
14 Liu Q H, Yan X L, Guo J C, Wang D H, Li L, Yan F Y & Chen L G, *Spectrochim Acta Part A*, 73, **2009**, 789.
15 Babko A K, *Talanta,* 15, **1968**, 721.
16 Ramakrishna T V, Aravamudan G & Vijayakumar M, *Anal Chim Acta*, 84, **1976**, 369.
17 Prasada Rao T & Ramakrishna T V, *Analyst,* 107, **1982**, 704.
18 Prasada Rao T, Reddy M L P & Pillai A R, *Talanta*, 46, **1998**, 765.
19 Mathew S, Sukumar R, Prasada Rao T & Damodaran A D, *Anal Lett,* 25, **1992**, 1941.
20 Prathish K P, James D, Jaisy J & Prasada Rao T, *Anal Chim Acta,* 647, **2009**, 84.
21 Lee B G, Jung R S & Kim K J, *Bull Korean Chem Soc*, 10, **1989**, 158.