CHAPTER 3

CHEMICAL SWITCH BASED REUSABLE DUAL OPTOELECTRONIC SENSOR FOR NITRITE
3.1. INTRODUCTION

Nitrite is a naturally occurring chemical made up of nitrogen and oxygen. Nitrogen and nitrogen compounds, such as nitrite, are found in air, soil, water and plants. In certain conditions, when oxygen is unavailable, nitrate may be converted to nitrite\(^1\). Therefore many sources of nitrate are also potential sources of nitrite. Green leafy and root vegetables, such as spinach and carrots, provide more than 85 per cent of dietary nitrate, which may be converted to nitrite by the human body during digestion. Though the majority of ingested nitrate is cleared rapidly from the body via excretion, some of it is transported to the salivary glands\(^2\) and secreted in the mouth. There it may be reduced by existing bacteria to nitrite and carried to the stomach upon swallowing. Dietary nitrate may also come from drinking water\(^3\). Though the United State Environmental Protection Agency has set a maximum limit for nitrate in drinking water, the levels of nitrate in water vary greatly and may be quite high in some locations. Nitrate content in both drinking water and vegetables is influenced by the use of nitrate fertilizers\(^4\). Sources of nitrite in natural water including chemical fertilizers, animal manure, improperly treated septic and sewage discharges, decaying plant or animal material, erosion of natural deposits. One of the main sources of ingested nitrite originates from sodium nitrite used as a food preservative in cured meats, fish and certain cheeses.

Nitrite has been used as a food preservative and anti-botulinal agent for decades\(^5,6\). It has also been a subject of controversy since the 1970s, when some of its reaction-products (i.e. nitrosamines) were associated with cancer in laboratory animals. However, following a 1981 review of all scientific data on nitrite, the National Academy of Sciences (NAS)/National Research Council (NRC) indicated that 1) nitrite does not directly act as a carcinogen in animals 2) nitrate, converted to nitrite in the human body, is neither carcinogenic nor mutagenic.
and 3) nitrite-used to preserve foods account for only a very small proportion of the human body's total exposure to nitrosamines. Today, it is clear that the benefits of nitrite in cured foods far outweigh any potential risks.

Nitrite is a salt used to preserve meat, fish and poultry. It is also a chemical substance in the human body, formed through normal physiological processes and the digestion of foods containing nitrite. Nitrite is added to certain foods to prevent the growth of the spore-forming bacterium clostridium botulinum, whose toxin causes botulism, leading to paralysis and potentially death. Botulinum toxins are the most toxic compounds known, 15,000 times greater than nerve gas and 100,000 times greater than sarin responsible for many deaths centuries ago before cured with nitrite. The word botulinum comes from the Latin word botulus, meaning sausage, which was an addition to serving as an antimicrobial, nitrite is used to produce the characteristic flavour, texture and pink colour of cured meats.

Nitrite is regulated as either a prior-sanctioned ingredient (approved for specific use before 1958) or as a food additive depending on the type of food to which it is added. For curing red meat and poultry products, nitrite is a prior-sanctioned ingredient, not requiring pre-market approval. However, for curing certain fish and use in curing mixes, nitrite is considered a food additive and requires pre-market clearance. Examples of food additive uses for nitrite include smoked or cured chub, tuna fish, sablefish, salmon, shad and home meat-curing preparations. However, nitrite use is strictly regulated in the United States. The United State Department of Agriculture (USDA) is responsible for regulating the use of nitrite in red meat and poultry products. The U.S. Food and Drug administration oversees matters concerning the safety of cured fish and products using nitrite as a food additive. Permitted uses are limited.
Accidental exposures to nitrites in chemical laboratories and ingestion in suicide attempts have been reported. Deliberate abuse of volatile nitrites (amyl, butyl and isobutyl nitrites) frequently occurs. Nitrite exposure also can occur from certain medications such as quinone derivatives (antimalarials), nitroglycerine, bismuth subnitrite (antidiarrhoeal), ammonium nitrate (diuretic), amyl and sodium nitrites (antidotes for cyanide and hydrogen sulfide poisoning) and isosorbide dinitrate/tetranitrates (vasodilators used in coronary artery disease therapy). However, the dietary intake of nitrite from cured meats is only a minor source of the body's total exposure. A significant amount of nitrite in the body is produced endogenously (internally), rather than introduced from dietary sources.

The amount of nitrite formed is dependent on the nitrate reductase activity of the microbial population and the availability of nitrate. In vitro and in vivo studies have shown that nitrate can be reduced to nitrite by bacterial and mammalian metabolic pathways. The widespread occurrence of nitrate reductase activity in bacteria means that the nitrite is produced endogenously at sites populated by large numbers of bacteria, namely, the mouth, stomach (if the gastric pH is > 5), the distal small intestine and colon, the infected urinary bladder and vagina.

In humans, saliva is the major site for the formation of nitrite. About 25% of ingested nitrate is secreted in the saliva and of this about 20% is converted to nitrite in the mouth. Thus, about 5% of dietary nitrate is converted to nitrite. A direct correlation between gastric pH, bacterial colonization and gastric nitrite concentration has been observed in healthy populations with a range of pH values from 1 to 7. In individuals with gastrointestinal disorders and achlorhydria resulting from pernicious anaemia or hypogammaglobulinaemia, gastric nitrite levels can reach 6 mg/L. The situation in human neonates is not
clear. It is commonly asserted that infants younger than 3 months may be highly susceptible to gastric bacterial nitrate reduction because they have very little gastric acid production17.

During physiological and pathological hypoxia, nitrite is converted to NO via reaction with hemoglobin, myoglobin, xanthine oxidoreductase and heme-and thiol-containing enzymes and by acidic reduction18. These different reactions provide for graded nitrite reduction to NO along the entire physiological and pathological oxygen and pH spectrum. Nitrite is being recognized as a critical hypoxic buffer, potentially contributing to the regulation of hypoxic vasodilatation and hypoxic mitochondrial respiration and to the modulation of ischemia-reperfusion tissue injury and infarction. Additionally, a previously unknown function for hemoglobin as an enzymatic nitrite reductase has now been identified that converts nitrite to NO, an effect that is maximal at about 50% oxygen saturation. This newly understood role of hemoglobin as a nitrite reductase, as well other pathways for nitrite reduction to NO19, are relevant in the content of signaling, blood flow regulation, oxygen sensing and nitrite-based therapeutics. These mechanistic discoveries have now led to further identification of potential roles for nitrite in the treatment of a variety of disease characterized by ischemia and hemodynamic disregulation, including neonatal pulmonary hypertension, subarahnoid hemorrhage associated vasospam, ischemia-reperfusion injury to heart and liver, hypertension and sickle cell disease.

Moreover, when nitrite is acidified in the stomach, it stimulates antimicrobial activity. Just as nitrite protects food against clostridium botulinum, it may also protect the human stomach against other food borne pathogens. Scientific evidence indicates that foods with added nitrite and naturally containing nitrate are safe for human consumption. No restrictions of these foods
are supported by science. Although vegetables are a major source of dietary nitrate, scientists have concluded that the benefits of eating them far outweigh any potential risk of their contribution to nitrite levels in the body. In fact, the conversion of dietary nitrate to nitrite has antimicrobial benefits in the mouth and stomach. Some epidemiological studies show a reduced rate of gastric and intestinal cancer in groups with a high vegetable-based nitrate intake.

Nitrite has never been shown to cause cancer in humans or animals. The American Cancer Society concluded in its 1996 dietary guidelines that "nitrites in foods are not a significant cause of cancer". A 1996 NRC report entitled "Carcinogens and Anticarcinogens in the Human Diet" made no mention of cancer risk associated with cured meat consumption. After much debate about 1974 and 1978 rodent studies that indicated that nitrite digestive reaction-products (N-nitroso compounds) were carcinogenic, the NAS/NRC conducted a review of the safety of nitrite. In 1981, the NAS Committee on Nitrite and Alternative Curing Agents in foods issued a report that concluded that neither nitrite nor nitrate directly caused cancer in animals. Studies did not provide sufficient evidence to conclude otherwise.

However, the NRC called for further studies on the metabolism of nitrite in humans, sources of human exposure to N-nitroso compounds (NOCs) in order to develop ways to minimize them. The NRC also recommended that the food industry 1) reduce nitrite in products to the greatest extent possible without compromising protection against botulism and 2) eliminate the use of nitrate in most cured meat and poultry products. In its 1982 follow-up report, the NRC examined alternatives to nitrites but found none with antibotulinal effects. In the late 1980s, the U.S. government's Interagency Working Group on nitrite research evaluated all data on nitrite and suggested protocols for new rodent studies. The National Toxicology Program (NTP) recently conducted studies for two years.
based on these protocols. The food industry responded to concerns about NOCs in the 1970s and 1980s by virtually eliminating the addition of nitrate to foods and reducing residual nitrite (analytically detectable) levels in cured meat products five-fold without compromising antibotulinal effects. The industry also began using agents to block or inhibit the formation of NOCs from nitrite. Such agents include ascorbate (vitamin C), erythorbate (chemically similar to vitamin C) and tocopherol (vitamin E). Most cured meats produced in the United States contain ascorbate or erythorbate. For bacon, adding one of these inhibitors is mandatory.

NOCs, including nitrosamines and nitrosamides, are formed by a process called N-nitrosation. With the presence of nitrite, this process can take place in the human stomach (because nitrosamines are much more stable after food processing than nitrosamides, they are of greater potential concern). Nitrite can be introduced in the stomach by consuming foods that contain it and from endogenous conversion of nitrate to nitrite. Nitrate can be derived from dietary sources or from the body's normal nitrogen metabolism. The contributors of nitrosamines are tobacco products, foods are minor contributors to overall exposure to preformed nitrosamines. The use of nitrite in bacon results in very low levels of nitrosamines, which at higher levels, have been shown to be carcinogenic in laboratory animals. As a result, the USDA established a surveillance program for preformed nitrosamines in bacon more than a decade ago. Beer, whiskey and other malt-brewed alcoholic beverages have also been shown to contain very low levels of preformed nitrosamines.

Nitrosamines and nitrosamides are recognized animal carcinogens and some, e.g. the tobacco-specific nitrosamines N-nitrosornornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNN and NNK), are human carcinogens. Nitrosamines need to be activated metabolically by cytochrome
P450 enzymes to electrophilic intermediates in order to exert a carcinogenic effect, while nitrosamides are direct-acting carcinogens. Nitrosation of amines produces electrophiles that can alkylate nucleophilic sites on biological molecules. Nitrosation of primary exocyclic amino groups on DNA, followed by deamination, may lead directly to mutations.

Nitrosating agents—e.g. nitrous acid (HNO₂) and nitrogen oxide (N₂O₅)—that arise from nitrite under acidic gastric conditions react with amines or amides to form nitrosamines or nitrosamides and the induction of tumours in animals via endogenous synthesis of N-nitroso compounds has been demonstrated²⁴. In animal or human studies, N-nitroso compounds have been associated with 15 different types of cancers, including tumours in the bladder, stomach, brain, esophagus, bone and skin, kidney, liver, lung, oral and nasal cavities, pancreas, peripheral nervous system, thyroid, trachea, acute myelocytic leukaemia and T and B cell lymphoma²⁵-²⁷. More than one hundred of these N-nitroso compounds have been tested for carcinogenicity in animals and 75-80% of them have been found to be carcinogens²⁵. IARC²⁶ concluded that 11 N-nitroso compounds were carcinogenic in man. In humans, the organs thought to be more at risk from cancer are the stomach, esophagus, nasopharynx and bladder. Ascorbic acid (vitamin C), a known inhibitor of nitrosation reactions, lowers the incidence of tumours in these experiments. The effect of ascorbic acid in the reduction of the risk for cancers that is associated with ingested nitrite has also been shown in epidemiological studies. These observations support the role of endogenous nitrosation in tumorigenesis.

Nitrosation of proline to form the non-carcinogenic nitrosamine N-nitrosopropylene²⁸ has been used as a test of endogenous nitrosation and a large number of studies have now demonstrated the nitrosation of proline in humans. In a typical experiment, proline and nitrate are administered and the nitrite that
arises from oral nitrate reduction can then nitrosate proline in the stomach. Gastric nitrosation of proline in humans can be inhibited by vitamin C, as seen in animal experiments. Examples of endogenously formed carcinogenic nitrosamines in humans are $N$-nitrosodimethylamine and $N$-nitrosopyrrolidine. Nitrite-cured meat contains substantial amounts of nitrosatable compounds ($N$-nitroso compound precursors). Other pathways for endogenous nitrosation include bacterially mediated and nitric oxide-related nitrosation.

Thus, there is an active endogenous nitrogen cycle in humans that involves nitrate and nitrite. In the presence of amines or amides, endogenous nitrosation\textsuperscript{29} takes place in the acidic environment of the human stomach. The nitrosating reactions are enhanced following ingestion of additional nitrate, nitrite or nitrosatable compounds. Some of the $N$-nitroso compounds that are formed in humans under these conditions are known carcinogens.

A two days symposium was held at the United State National Institute of Health (NIH) in Bethesda, Maryland, to address recent advances in our understanding of the role of nitrite in physiology, pathophysiology and therapeutics. The topics covered a wide breadth, including mechanisms of nitrite formation, transport and enzymatic reduction to NO, nitrite as a subtraction for nitration, nitrosation and nitrosylation reactions, the existence of a symbiotic nitrogen cycle in humans and the emerging role for nitrite in blood flow homeostasis hypoxic vasodilation and therapeutics\textsuperscript{18}.

Chemical switches find wide interest in science and technology due to their ability to undergo structural changes in response to a respective stimulation\textsuperscript{30,35}. However, a search for a chromophore, whose changes in colour can be used to sense nitrite both qualitatively and quantitatively in presence of several other coexisting anions, is highly desirable. Such reagents would be especially valuable if they are commercially marketed without resorting to
dedicated synthetic protocols. Also extraction of these reagents into toxic organic solvents has to be avoided. Here, focus has been made on Rhodamine 6G (Rh 6G) as a potential off-the-shelf chromoionophore that can sense nitrite. Rh 6G, a xanthene dye is an important analytical reagent which is stable, pH independent and has been applied to resonance scattering, spectrophotometric, fluorimetric and polarographic analysis.

Optical nitrite sensor on chemical modification of a polymer film with gallo cyanine and Safranine O has been reported. However, chemical switch behaviour is not attempted in these works. Jiang et al. have reported a resonance-scattering method for the determination of traces of nitrite in water with Rh 6G in HCl-KI medium. This is an indirect method for the determination of nitrite where nitrite ion reacts with I· to form Iγ in Dil. HCl and Iγ combines with Rh 6G+ to form (Rh 6G.Iγ)n association particles with resonance scattering at 320, 400 and 595 nm. Similarly, Jiao et al. have reported a reversible chemosensor for nitrite based on the fluorescence quenching of a carbazole derivative, which is also based on the reaction between nitrite ions and excess I· to form Iγ. These reports have a drawback that the selectivity of nitrite sensing could be influenced by the presence of several other species coexisting with nitrite which will also react with I· to form Iγ. Now it is proposed Rh 6G based system for the sensing of nitrite capable of switching absorbance 'ON' and 'OFF' in aqueous solutions itself.

3.2. EXPERIMENTAL

3.2.1. Procedure for Determination of Nitrite

An aliquot of sample solution (up to 20 ml) containing 0-10 μg of nitrite was taken in to a 25 ml standard volumetric flask. 0.75 ml of 12.5 mol l⁻¹ sulphuric acid was added to the above solution with mixing followed by 1 ml of
0.01% Rh-6G solution. This was then diluted to the mark with deionized water and the absorbance was measured at 525 nm against a reagent blank. Establish the concentration of nitrite by reference to a calibration graph.

3.2.2. Analysis of Natural Waters

Appropriate aliquots of tap, river and pond water samples were taken for quantification by following the above mentioned procedure.

3.2.3. Analysis of Food Materials

About 1 g amount of table salt and sugar were dissolved in water and appropriate aliquots of this solution were taken for quantification by following the above mentioned procedure.

3.3. RESULTS AND DISCUSSION

3.3.1. Chemical Switching-Absorbance

A systematic study of addition of 5 μmol l⁻¹ nitrite to various 1 ml of 0.01% water soluble triphenylmethane cationic dyes was undertaken by both naked eye detection and spectrophotometric measurements after adjusting the overall acidity to pH 5.0 and 0.1 and 0.75 mol l⁻¹ H₂SO₄. The cationic dyes chosen for this study are Rh 6G, Rh B, methylene blue, crystal violet, brilliant green, xylenol orange, catechol violet, ethyl violet and malachite green. Such studies indicated that Rh 6G alone gives a decrease in visual colour intensity and consequent lowering of absorbance on addition of nitrite at acidities higher than 0.1 mol l⁻¹ in H₂SO₄. On the other hand in case of other cationic dyes, there is no visual colour change or change in absorbance spectra. The addition of nitrite to Rh 6G acidified to 0.75 mol l⁻¹ (overall) sulphuric acid results in a decrease in absorbance at λ_{max} of Rh 6G i.e. 525 nm, which had formed the basis of colorimetric determination
of nitrite described briefly in earlier report. However, it is clear from Curve b of Fig. 3.1 that the addition of nitrite to Rh 6G results in an additional peak at 385 nm: a blue shift of Rh 6G dye. The absorbances at this $\lambda_{\text{max}}$ with different concentrations of nitrite obey Beer-Lambert's law with a molar extinction coefficient ($e$) of $3.5 \times 10^3$ $\text{mol}^{-1} \text{cm}^{-1}$. The colour change is vividly seen by the naked eye on addition of NO$_2$ to Rh 6G solution in presence of H$^+$ ions, as the orange red colour of Rh 6G changes to yellow (See inset of Fig. 3.1).

Fig. 3.1: UV-Visible spectrum of the switch 'on-off' absorbance (a) Rh 6G (b) Rh 6G + nitrite (4.64 $\mu$mol l$^{-1}$) (c) Rh 6G + nitrite (4.64 $\mu$mol l$^{-1}$) + sulphamic acid (24.74 $\mu$mol l$^{-1}$) (The visual colour change of Rh 6G (a), Rh 6G + nitrite (b) and Rh 6G + nitrite + sulphamic acid (c) shown in the inset)

Fig. 3.2 shows the changes in the UV-Visible spectrum of Rh 6G with increasing concentration of nitrite. In the absence of nitrite, the spectrum of Rh 6G is characterized by a peak at $\lambda_{\text{max}}=525$ nm, the intensity of which goes on decreasing with an increase in the concentration of nitrite and another peak
appears at 385 nm, whose colour intensity increases. The absorbance change on incremental addition of nitrite to Rh 6G solution (Inset a) and calibration plot at 525 nm (Inset b) with a linear range of 0.58-8.12 μmol l⁻¹ of nitrite are shown in Fig. 3.2. The addition of 24.74 μmol l⁻¹ of sulphamic acid to a solution of Rh 6G containing nitrite (4.64 μmol l⁻¹) reverts the decrease in absorbance of the dye noticed on addition of nitrite (See inset of Fig. 3.1).

Fig. 3.2: Absorbance spectra of Rh 6G with increasing amounts of 0, 0.58, 1.16, 2.32, 4.06, 5.8 and 8.12 μmol l⁻¹ of nitrite (a to g) and corresponding plot showing incremental addition of 0, 1.45, 2.9, 4.35 and 5.8 μmol l⁻¹ of nitrite (i to v) (inset 'a') and calibration graph (inset 'b') respectively.

Successive cyclic addition of 1/4/8 μg of nitrite and 60 μg of sulphamic acid in each case to Rh 6G in 0.75 mol l⁻¹ H₂SO₄ solution resulted in absorbance changes as shown in Fig. 3.3. It is clear from the figure that successive addition of
nitrite and sulphamic acid results in "CHEMICAL SWITCHING" of Rh 6G cationic dye in absorbance mode allowing the possibility of reusability of sensor. The absorbance of the Rh 6G dye was switched 'off' on addition of nitrite ion in the presence of H⁺ ions. However, chemical switching to 'ON' absorbance occurs upon addition of sulphamic acid. The switching 'off' process is irreversible and it does not return to 'on' mode unless sulphamic acid is added.

Fig. 3.3: Absorbance change during the cyclic addition of 1, 4 and 8 μg of nitrite and 24.74 μmol l⁻¹ (60 μg) of sulphamic acid and corresponding calibration plots for I and II cycle addition of nitrite (see inset)

3.3.2. Chemical Switching – Fluorescence

The emission spectrum of 1 ml of 0.01% Rh 6G present in 25 ml adjusted to an overall acidity of 0.75 mol l⁻¹ in H₂SO₄ with excitation at 355 nm is shown in Curve a of Fig. 3.4. The addition of 4.64 μmol l⁻¹ nitrite results in significant
QUENCHING as seen from the Curve b of Fig. 3.4 at emission maximum of Rh 6G i.e. 565 nm. Again, as in case of absorbance studies, the addition of 24.74 μmol l⁻¹ sulphamic acid results in retrieving the original fluorescence intensity of Rh 6G. This cycle is repeated once again on subsequent sequential addition of nitrite and sulphamic acid. Thus, a CHEMICAL SWITCHING behaviour is once again noticed in case of fluorescence studies also as in case of absorbance studies described above.

Fig. 3.4: Fluorescence spectrum of the switch 'on-off' fluorescence (a) Rh 6G (b) Rh 6G + nitrite (4.64 μmol l⁻¹) (c) Rh 6G + nitrite (4.64 μmol l⁻¹) + sulphamic acid (24.74 μmol l⁻¹)

3.3.3. Analytical Optimization Studies

The influence of acidity on the change in absorbance during the reaction of Rh 6G with nitrite was studied. The decrease in absorbance was found to be constant and maximum in the range 0.50-1.25 mol l⁻¹ H₂SO₄ (overall). The effect of the concentration of Rh 6G on the maximum change in absorbance was obtained 1 ml of 0.01% Rh 6G in a total volume of 25 ml. The minimum time
required for the complete reaction of nitrite with Rh 6G was 5 min. and remained stable up to 30 min. indicating that the species formed by the reaction of nitrite with dye was relatively stable. The time required for the reaction of sulphamic acid with the species formed by the reaction between nitrite and Rh 6G is greater than 30 min. i.e. the time required to regain the colour intensity nearly equal to that of the free dye is greater than 30 min. The variation in absorbance with change in concentration of sulphamic acid added to the reaction mixture containing 5.8 \( \mu \text{mol} \ l^{-1} \) of nitrite and Rh 6G indicate that a minimum of 24.74 \( \mu \text{mol} \ l^{-1} \) of sulphamic acid is needed to switch the colour system.

3.3.4. Effect of Other Anionic Species

The addition of other anions like \( \Gamma \), SCN\(^-\), ClO\(_4\)\(^-\), [Hgl\(_4\)]\(^-\) and [Zn(SCN\(_4\))\(^-\)] to Rh 6G solution, the intensity of the peak at 525 nm decreases as in case of nitrite anion while a new peak appears at 578 nm a sort of red shift of Rh 6G dye. The changes in absorbance at 525 nm was less dramatic and required greater amounts of anions (unlike nitrite) in case of \( \Gamma \), SCN\(^-\) and ClO\(_4\)\(^-\) compared to [Hgl\(_4\)]\(^-\) and [Zn(SCN\(_4\))\(^-\)] to effect a commensurate change. Here, the observed red shift in the absorbance peak could be possibly due to the exchange of anions with Cl\(^-\) of the quaternary ammonium part of the dye which is well documented in literature\(^41,42\). However, the above mentioned spectral changes on addition of anions either individually or in admixtures are completely annulled by addition of nitrite, thus enabling selective sensing of nitrite. The qualitative changes of absorbance with other anions are reflected in more quantifiable terms also. About 2.5x10\(^5\) fold amounts of NO\(_3\), Br\(^-\) and PO\(_4\)\(^3\)\(^-\) and 2.5x10\(^2\), 62.5 and 1.25x10\(^3\) fold amounts of SCN\(^-\), I\(^-\) and ClO\(_4\) ions with respect to nitrite did not interfere during nitrite sensing. In order to check the applicability of the system for sensing nitrite in complex real samples, studies were carried out in terms of selectivity of the sensor for other anions, cations and neutral salts also. Table 3.1
shows the tolerance limits of various coexisting anions, cations and neutral salts with respect to 4.64 μmol l⁻¹ nitrite. The determination of 4.64 μmol l⁻¹ of nitrite was unaffected even in the presence of a multi-component mixture of PO₄³⁻/NO₃⁻/Br⁻ (1x10⁻³ %), SCN⁻ (4x10⁻³%), ClO₄⁻ (2x10⁻³%) and I⁻ (1x10⁻⁶%). Moreover, the colour reaction of nitrite with Rh 6G is highly specific as none of the anions, cations and their admixtures at 10⁵ times to that of nitrite have any deleterious effect.

Table 3.1: Interference studies (4.64 μmol l⁻¹ of nitrite)

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Tolerance limit</th>
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<tbody>
<tr>
<td>Br⁻</td>
<td>2.5x10⁵</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>2.5x10⁵</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>2.5x10⁵</td>
</tr>
<tr>
<td>SCN⁻</td>
<td>2.5x10²</td>
</tr>
<tr>
<td>I⁻</td>
<td>62.5</td>
</tr>
<tr>
<td>ClO₄⁻</td>
<td>1.25x10³</td>
</tr>
<tr>
<td>Cd²⁺</td>
<td>2.5x10⁵</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td>1.25x10²</td>
</tr>
<tr>
<td>Hg²⁺</td>
<td>6.25x10³</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>6.25x10⁵</td>
</tr>
<tr>
<td>Cu²⁺</td>
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</tr>
<tr>
<td>MgCl₂</td>
<td>1.25x10⁵</td>
</tr>
</tbody>
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3.3.5. Application to Real Samples

The applicability of the developed reusable sensor was tested for analysis of natural waters and selected food materials. The analysis was carried out by "Standard addition method" and the results obtained for various real samples are shown in Table 3.2. Furthermore, the results obtained by present method are compared with standard UV-Visible spectrophotometric method based on "p-nitroaniline-Quinoline-8-ol" as chromogenic reagent. The analytical values obtained by the newly developed sensor compares favorably with standard spectrophotometric method indicating the efficacy of developed reusable sensor for reliable and routine monitoring of nitrite in various natural waters and food materials.

Table 3.2: Analysis of natural waters and food samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nitrite sensor (µg/l)</th>
<th>p-nitroaniline-Quinoline-8-ol based spectrophotometric method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water (µg/l)</td>
<td>9.1 ± 2.4</td>
<td>9.2 ± 1.7</td>
</tr>
<tr>
<td>River water (µg/l)</td>
<td>21.0 ± 1.4</td>
<td>19.8 ± 1.7</td>
</tr>
<tr>
<td>Pond water (µg/l)</td>
<td>56.8 ± 3.2</td>
<td>64.1 ± 5.8</td>
</tr>
<tr>
<td>Table salt (µg/g)</td>
<td>1.20 ± 0.02</td>
<td>1.20 ± 0.20</td>
</tr>
<tr>
<td>Sugar (µg/g)</td>
<td>1.05 ± 0.13</td>
<td>0.83 ± 0.15</td>
</tr>
</tbody>
</table>

*By standard addition method
3.3.6. Discussion

The formation of ternary ion associate by interaction of charged binary anionic complexes of various metal cations like Hg, Cd, Pb, Zn etc. with cationic dye Rh 6G in presence of iodide or thiocyanate results in bathochromic shift from 525 to 578 nm. This has been advantageously utilized by various research groups for sensitive determination of metal cations directly in aqueous phase itself thus precluding the use of toxic organic solvents. In all these cases, as the concentration of metal cation increases the formation of ternary associate of [HgI4]2- and [Zn(SCN)4]2- with Rh 6G results in increase in absorbance at 578 nm with concomitant decrease in absorbance at λ max of the dye i.e. at 525 nm. On the other hand, the addition of nitrite in to Rh 6G in 0.75 mol l⁻¹ H2SO4 solutions results in hypsochromic shift to 385 nm (See Fig. 3.1) wherein the increase in absorbance at this λ max is proportional to nitrite. Again, as seen from Fig. 3.2, the decrease in absorbance at λ max of the Rh 6G is also proportional to nitrite. The magnitude of absorbance change in latter case is high. Other anions show a similar decrease at 525 nm but at much larger amounts i.e. 10²-10⁵ amounts to that of nitrite and that too in absence of nitrite (See Table 3.1). The observed bathochromic shift noticed in presence of anions other than nitrite and binary anionic complexes of metal ions could possibly be due to the exchange of anions with Cl⁻ of the quaternary ammonium part of the dye.

The addition of traces of nitrite results in alteration in Π conjugation of Rh 6G (See Scheme 3.1) thus upsetting the bathochromic shift observed in case of other anions (even at higher amounts). This enables the sensing of nitrite in presence of large excess of extraneous anions or binary anionic complexes of metal cations or their admixtures. In addition, several cations do not have any deleterious effect as they do not interact with Rh 6G in the absence of suitable anionic ligand.
In order to explore the cause of the decrease in absorbance of Rh 6G, the experiment was carried out at pH 5.0. No change was noticed in the absorbance of Rh 6G on addition of NO₂⁻. On the other hand, in solutions of pH 2.0 or lower, nitrite can significantly reduce the absorbance of Rh 6G at its $\lambda_{\text{max}}$. Based on this observation, one could envisage a plausible mechanism as shown in Scheme 3.1. Nitrite ions in the presence of H⁺ ions produce nitrosyl cation, which is electron deficient and can attack the secondary amine group of Rh 6G (1), resulting in the formation of N-nitrosamine derivative (2). In the second step, sulphamic acid reacts with N-nitrosamine derivative to give back the original dye. As seen from Scheme 3.1, the extent of conjugation in the dye is lost when the N-nitrosamine derivative is formed and this result in a blue shift to 385 nm.

A similar "on-off" switch behaviour observed in case of fluorescence measurements can also be explained by the structural changes in Rh 6G on addition of nitrite and subsequent reversal by the addition of sulphamic acid.

Scheme 3.1: Mechanism involved in the chemical switch based nitrite sensor
3.4. CONCLUSION

A chemical switch based reusable optical sensor for nitrite has been designed using the off-the-shelf readily available chromoionophore, i.e., Rh 6G. Such a chemical switch system is the first of its kind, which can discriminate nitrite from very large amounts of several anions either individually or in admixtures due to blue and red shifts of the absorbance maximum of Rh 6G with nitrite and other anions respectively. The developed sensor has been successfully demonstrated for its applicability to analysis of natural waters and food materials.
REFERENCES


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