Arabian Journal of Chemistry (2016) 9, S812–S820

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Novel coupling reactions of phytochemicals with sulfa drugs and their applications in the determination of nitrite at trace level in environmental samples

Masoumeh Moghimi [a,*], Mohammad T. Baei [b], Faramarz Rostami Charati [c], Akheel Ahmed Syed [d]

[a] *Department of Chemistry, Gonbad Kavoos Branch, Islamic Azad University, Gonbad Kavoos, Iran*
[b] *Department of Chemistry, Azadshahr Branch, Islamic Azad University, Azadshahr, Iran*
[c] *Department of Chemistry, Faculty of Science, Gonbad Kavoos University, P.O. Box 163, Gonbad, Iran*
[d] *Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, India*

Received 4 May 2011; accepted 20 August 2011
Available online 28 August 2011

KEYWORDS

Spectrophotometry;
Nitrite;
Sulfonamides;
Phytochemicals;
Environmental samples

Abstract Twelve spectrophotometric methods based on new reactions for the determination of trace amounts of nitrite in environmental samples were developed. Replacement of toxic reagents was explored to attain the standards of clean chemistry. These methods utilize two classes of compounds namely; phytochemicals and sulfonamides, in the presence of limited amounts of sodium hydroxide. The methods were based on the oxidation of sulfanilamide (SAA), sulfadoxine (SDX), sulfamethoxazole (SMX) or sulfadiazine (SDZ) by nitrite in sodium hydroxide medium and coupling with cardol, cardanol or anacardic acid which yielded yellow, orange and orange red color derivatives having an absorbance maximum in the range 430, 460 and 470 nm, respectively. The colors developed were stable for about 3 h. Beer's law was obeyed for nitrite in the concentration range 0.08–0.90, 0.16–1.04, 0.08–0.80 and 0.08–0.80 μg ml^{-1} for cardol; 0.80–4.40, 1.60–5.72, 0.52–5.20 and 0.80–4.40 μg ml^{-1} for cardanol and 0.80–5.70, 1.04–6.20, 1.30–5.20 and 0.80–4.00 μg ml^{-1} for anacardic acid, respectively. The reaction conditions and other important analytical parameters were optimized to enhance the sensitivity of the methods. Interference if any, by non-target ions was also investigated. The methods were applied determining nitrite in environmental samples. The performances of these methods were evaluated in terms of Student's

* Corresponding author. Tel.: +98 9111792262.
E-mail address: moghimi@gonbadiau.ac.ir (M. Moghimi).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2011.08.008

t-test and variance ratio *F*-test to find out the significance of the proposed methods over the reference spectrophotometric method.

1. Introduction

The monitoring of nitrite in environmental samples is being practiced by most health authorities' worldwide, with legislation being levied on its permissible levels in drinking water; at present the maximum contamination level in drinking water is 1 μg mL^{-1} (Environmental Protection Agency, 2004). Excessive concentration of nitrite in drinking water could be hazardous to health, especially for infants and pregnant women. Nitrite oxidizes iron in hemoglobin of the red blood cells to form methemoglobin, which loses its oxygen carrying ability. This creates the condition known as methemoglobinemia (Eaton et al., 1995). Nitrite also has a direct impact on the health because of its reaction with amines or amides in human body to produce different types of nitrosamines, which are very powerful carcinogens (Lijinsky, 1992). Therefore, elucidation of nitrite concentrations is desirable from the stand point of environmental analytical chemistry.

Environmental analytical chemistry contributes significantly to the growth of environmental responsibility for sustainable development through environmental monitoring. This has been possible due to the advances in analytical techniques which enable the study of toxicants and their role in environmental pollution. Nowadays, analytical methodologies are well established for environmental monitoring. However, a paradoxical situation has emerged as the majority of the analytical methods employed to investigate environmental problems generate chemical wastes, which contribute to environmental pollution (Anastas, 1999). In some situations, the chemicals employed are more toxic than the species being monitored. As a consequence, some environmental analytical chemists are focusing their work on the development of methodologies less harmful to humans and to the environment. Nowadays, in the development of a new analytical method or a procedure the amount and toxicity of the reagents used and of the wastes produced are as important as any other analytical feature.

Various techniques developed so far for the determination of nitrite in environmental samples include: chromatography (Tsikas et al., 1994; Helaleh and Korenaga, 2000; Butt et al., 2001; Yu et al., 2001), electroanalytical (Davis and Compton, 2000; Badea et al., 2001; Ensafi and Kazemzadeh, 1999) and optical techniques (Huang et al., 2000; Odake et al., 2001; Afkhami et al., 2005). Among the optical techniques, simple methods based on UV–vis spectrophotometry have become an accepted analytical tool for the determination of nitrite in environmental samples.

Visible spectrophotometric methods are convenient, sensitive and are relatively inexpensive. These methods employ different routes for the determination of chromogen produced (Revanasiddappa and Kumar, 2003; Baveja and Gupta, 1983; Sunitha and Gupta, 1984; Baveja and Gupta, 1981; Rathore and Tarafder, 1989; Kaur and Gupta, 1987; Kaveeshwar and Gupta, 1991; Kesari and Gupta, 1998; Revanasiddappa et al., 2001; Nair and Gupta, 1979; Ozmen et al., 2006; Chaube et al., 1982; Satake and Wang, 1997; Sreekumar et al., 2003; Yue et al., 2004; Horita and Satake, 1997; AOAC, 1997). Most of the spectrophotometric procedures for nitrite determination are based on diazo-coupling reaction resulting in dye formation and these are characterized by high sensitivity, but often have drawbacks of pH dependence, diazotization temperature and coupling time. Besides, these procedures often use large sample volumes of carcinogenic reagent(s), which makes it outside of the standards of clean chemistry (Allen, 1989). A comparison of a few selected procedures; their spectral characteristics and drawbacks are enumerated in Table 1.

Cashew nut (*Anacardium occidentale*) shell liquid (CNSL), a by product obtained during the processing of cashew nut, is used in the manufacture of industrially important products such as cement, specially coating, primers, etc. (Tyman, 1996). The major phenolic constituents of CNSL are 70% anacardic acid, 18% cardol, 5% cardanol and other constituents include 2-methyl cardol and small amount of polymeric material. Cardol and methylcardol (trivial names) belong to the group of natural and easily isolate amphiphilic compounds (Kozubek and Tyman, 1999). Since cardol has two OH groups it is possible that this compound will have more versatile applications than its counterpart cardanol. By far the greatest amount of work on polymeric materials derived from CNSL or cardanol has been with their use in the manufacture or modification of phenolic resins (Menon et al., 1985; George et al., 2001; Prabhakaran et al., 2001; Niimuru and Miyakoshi, 2003; Huong et al., 1996). Since cardanol is an important natural source of phenolic compounds, and thus possesses antioxidant properties (Trevisan et al., 2006) many synthetic products involving this type of additive have been developed based on CNSL derivatives (Kumar et al., 2002; Paramashivappa et al., 2001). Anacardic acids are a group of phenolic lipids, which, for convenience may be considered fatty acids with a phenolic ring instead of carboxyl at the polar group. Although anacardic acids possess several biological activities (Kubo et al., 1993; Himejima and Kubo, 1991; Muroi and Kubo, 1993; Kubo et al., 1986), they also inhibit medicinally important enzymes such as, postaglandinsynthase, tyrosinase and 5-lipoxygenase (Grazzini, 1991; Kubo et al., 1994; Shobha et al., 1994). The biological activities of anacardic acid have encouraged many researchers to develop drug analogues for different applications (Gulati and Subba Rao, 1964; Elsholy et al., 1986).

The sulfonamides are analogues of *p*-aminobenzoic acid and are known since 1932. Though a large number of sulfonamides are synthesized and reported in the literature, only about two dozen of them have been used in clinical practice (Northey, 1948). They differ only slightly in their antimicrobial activity but vary greatly in their pharmacokinetic properties, or rate of excretion. Accordingly, they are classified as short-, medium-, long- and ultra-long acting drugs (Reynolds, 1993). Sulfanilamide (SAA) belongs to short-acting, sulfamethoxazole (SMX) medium-acting and sulfadoxine (SDX) ultra-long acting sulfanomides.

Table 1 Comparison of spectrophotometric methods for the determination of nitrite.

Reagent	Range (μg ml^{-1})	$\lambda_{max\ (nm)}$	Remarks	Reference
p-Rosaniline + NEDA	0.08–0.72	565	Fe(III), Cr(IV) and s interferes	Baveja and Gupta (1983)
PNA + guaiacol	0.03–0.15	540	Extractive, NO_3^-, Al^{3+} interferes	Sunitha and Gupta (1984)
4-Nitroaniline + 1-napthol	0.02–0.14	610	Extractive, many interferences	Baveja and Gupta (1981)
p-Aminophenyl mercaptoacetic acid	0.10–1.60	495	S^- and Sb^{2+}interfere	Rathore and Tarafder (1989)
p-Aminoacetophenone + NEDA	0.10–0.80	546	Less sensitive	Kaur and Gupta (1987)
o-Nitroaniline and 1-aminonaphthalene	0.08–0.68	545	Less sensitive	Kaveeshwar and Gupta (1991)
PNA + phloroglucinol	0.004–0.04	420	Fe(III) and Cu(II) interfere	Kesari and Gupta (1998)
PNA + acethylacetone	0.50–14.00	490	Less sensitive Cu(II), Co(II), and Hg(II) interfere	Revanasiddappa et al. (2001)
PNA + 2-methyl-8-quinolinol	0.002–0.400	585	Most cations and anions interfered	Nair and Gupta (1979)
4-(1-Methyl-1-mesitylcyclobutan-3-yl)-2-aminothiazole + *n*,*n* dimethyl aniline	0.05–2.00	482	pH-dependent, time consuming and extractive	Ozmen et al. (2006)
p-Nitroaniline + 8-quinolinol	0.01–0.06	550	pH-dependent and extractive	Chaube et al. (1982)
Sulfanilic acid + 1-naphthol	0.02–0.87	418	pH-dependent and time consuming	Satake and Wang (1997)
SAA + ethylacetoacetate	0.2–3.00	356	Fe(III) interfere and less sensitive	Sreekumar et al. (2003)
Proposed methods	0.08–0.90	430	Simple, sensitive common ions do not interfere	

NEDA, *N*-(l-napthyl)ethylenediamine hydrochloride; PNA, *p*-nitroaniline; SAA, sulfanilamide.

Now a day, the toxicity of chemicals must be taken into account in the development of new analytical procedures or implementation of existing ones. However, there are many analytically reliable procedures that are not environmentally benign. Many of them are recommended as standard or reference methods.

Considering the efforts of analytical chemists to develop new methods and/or procedures, it is not realistic to hope that the replacement of all hazardous chemicals can be made within the near future. However, this should be one of the main goals of the analytical chemistries. In the present context the replacement of hazardous/toxic reagents is exemplified by the use of phytochemicals rather than synthetic compounds. The former involves multiple step (rest of the cases) synthesis and generally generates more toxic/hazardous waste. In comparison, the phytochemicals derived from natural products will be generally less hazardous/toxic.

For the first time a new class of reagents are used for the spectrophotometric determination of nitrite using phytochemicals; cardol, cardanol and anacardic acid, as chromogens and sulfa drugs which include sulfanilamide (SAA), sulfadoxine (SDX), sulfamethoxazole (SMX) or sulfadiazine (SDZ) as new coupling reagents (Fig. 1) in the presence of sodium hydroxide will be attempted, a 'soft-solvent' medium. These compounds are less toxic compare to other reagents and can lead to greener analytical methods. Impacts of varying parameters in the determination, such as effect of bases, concentration of surfactant and reagents on the absorbance and the stability of the complex will also be studied, during the course of the experimentation.

2. Experimental

2.1. Reagents

Cardol, cardanol and anacardic acid from Vital Mallya Scientific Research Foundation, India from Ipca Laboratories Ltd. (India) and sulfanilamide (SAA), sulfadoxine (SDX), sulfamethaxazole (SMX) and sulfadiazine (SDZ) samples from SmithKline Beecham, India were received as gift samples. Sodium nitrite and sodium hydroxide (BDH, India), isopropyl alcohol were received from Ranbaxy, India. All reagents were used of analytical grade chemicals unless specified otherwise.

Stock sodium nitrite solution, 2.176×10^{-2} M (1000 μg nitrite mL^{-1}) in 1000 mL volumetric flask was prepared by dissolving 1.50 g sodium nitrite (predried at 110 °C for 4 h) in water. A small amount of sodium hydroxide to prevent nitrite decomposition and a few drops of chloroform to prevent bacterial growth were also added. Solutions of the required strength were prepared by diluting this stock solution with distilled water.

Stock solution (0.25% w/v) of Cardol, cardanol and anacardic was prepared by dissolving 250 mg each in isopropyl alcohol and diluting quantitatively to 100 ml with isopropyl alcohol.

Solutions (0.1% w/v) of SAA, SDX, SMX and SDZ were prepared by dissolving 100 mg each of them and diluting quantitatively to 100 ml with distilled water, 10 ml of 2 N hydrochloric acid was added during the preparation of sulfadoxine, sulfamethaxazole and sulfadiazine to improve its solubility.

2.2. Apparatus

Specord 50 UV–vis spectrophotometer with 1.0-cm silica quartz matched cell (Jasco, Tokyo, Japan) was employed for measuring the absorbance.

2.3. Procedure

To a series of 25-ml calibrated flasks; 1.5 ml of (0.1% w/v) SAA, SDX, SMX or SDZ reagent, an aliquot of standard solution of nitrite, 2.0 ml of (0.25% w/v) cardol, cardanol and anacardic acid and 1.0 ml of 1 M NaOH were added and the mixture

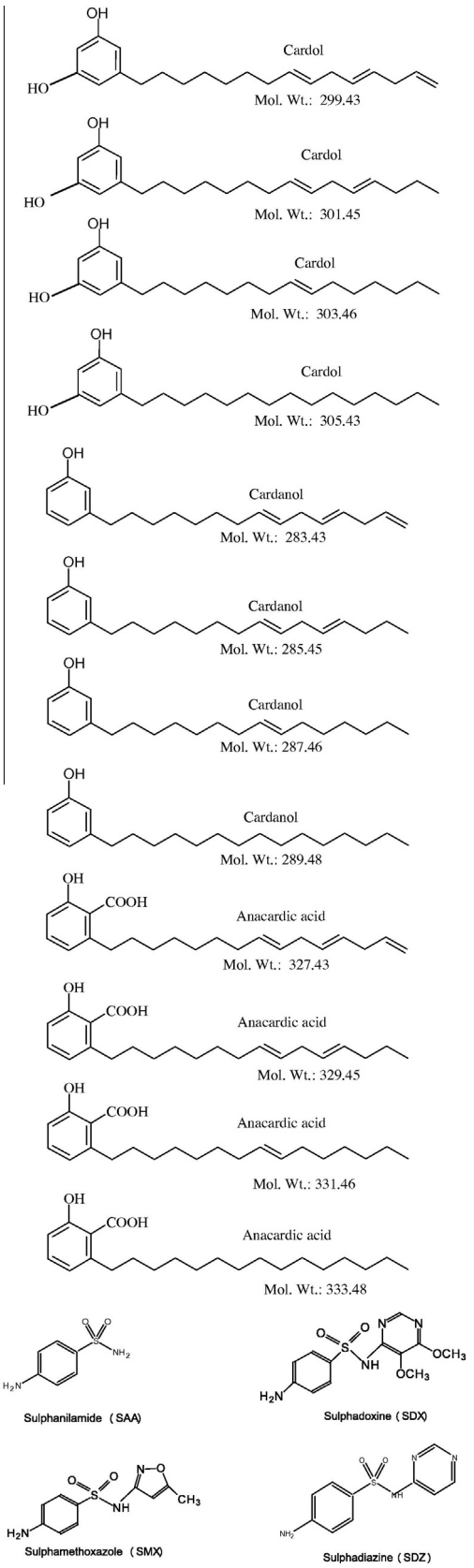

Figure 1 Structure of cardol, cardanol, anacardic acid and sulfonamides.

was shaken thoroughly and allowed to stand for 15 min for cardol and 10 min for cardanol and anacardic acid at room temperature. The yellow, orange and orange red colored products obtained from each was made up to the volume with distilled water and the absorbance were measured at 430, 460 and 470 nm for cardol, cardanol and anacardic acid against the corresponding reagent blank, respectively. Concentration of nitrite in test solution was calculated from the regression equation computed from the Beer's law data as a reference (Table 2).

3. Result and discussion

The spectral characteristics for the determination of nitrite with cardol, cardanol and anacardic acid using SAA, SDX, SMX or SDZ and are detailed in Table 2.

3.1. Wavelength determination

In order to have minimum interferences, it was necessary to identify optimum wavelength for nitrite determination in the method. This wavelength must be specific for the quantitative and specific monitoring of the nitrite SAA, SDX, SMX or SDZ-cardol, cardanol or anacardic acid. The wavelength of maximum absorbance was identified by scanning the product of SAA, SDX, SMX or SDZ–nitrite–cardol, SAA, SDX, SMX or SDZ–nitrite–cardanol and SAA, SDX, SMX or SDZ–nitrite–anacardic acid over the range 300–800 nm with a specord 50 UV–vis spectrophotometer. A wavelength of 430, 460 and 470 nm, respectively, was found optimum for getting best results (Figs. 2–4).

3.2. Reaction mechanism

Aromatic diazonium ions couple with active substrates such as amines and phenols (Schirmer, 1982; Norwitz and Keliher, 1981; Baiocchi et al., 1982). Because of the size of the attacking species, substitution is effected mostly para to the activating group, unless that position is already occupied, in which case ortho substitution takes place. In case of cardol, the substituent being in the *meta* position, the substitution is preferably in the para position. The media pH is of paramount importance for the activation of substrates. Alkaline medium is recommended for phenols because phenols themselves are not active enough for the diazotisation reaction. Nevertheless, there is a risk of unstable derivatives and large values of blank due to the process called hydroxy-de-diazonization (March, 1992), which reacts with excess of the reagent in basic medium. The SAA, SDX, SMX and SDZ sulfa drugs containing aryl primary amino groups undergo diazotization reaction using sodium nitrite solution to produce diazonium groups, which react with cardol, cardanol and anacardic acid in sodium hydroxide medium to produce yellow, orange and orange red colored dyes, respectively.

3.3. Effect of reagents and base concentration

The effect of SAA, SDX, SMX or SDZ was required for getting constant and maximum color intensity. It was found that 0.50–2.00 ml of the solution was needed to get good results. Hence, 1.5 ml of (0.1% w/v) SAA, SDX, SMX or SDZ solutions is sufficient to get reproducible results.

Table 2 Spectral data for determination of nitrite using sulfonamides as coupling agents and cardol, cardanol and anacardic acid as chromogens.

Parameters	Cardol				Cardanol				Anacardic acid			
	SAA	SDX	SMX	SDZ	SAA	SDX	SMX	SDZ	SAA	SDX	SMX	SDZ
Color	Yellow	Yellow	Yellow	Yellow	Orange	Orange	Orange	Orange	Orange red	Orange red	Orange red	Orange red
λ_{max} (nm)	430	430	430	430	460	460	460	460	470	470	470	470
Stability (h)	3	3	3	3	3	3	3	3	3	3	3	3
Beer's law (μg ml^{-1})	0.08–0.90	0.16–1.04	0.08–0.80	0.08–0.80	0.80–4.40	1.60–5.72	0.52–5.20	0.80–4.40	0.80–5.70	1.04–6.20	1.30–5.20	0.80–4.07
Recommended ion concentration (μg ml^{-1})	0.32	0.56	0.32	0.40	2.00	2.60	2.10	2.80	2.10	2.60	2.60	2.00
Molar absorptivity (L mol^{-1} cm^{-1})	3.99 x10^4	2.55×10^4	4.41×10^4	3.72×10^4	6.82×10^3	5.07×10^3	6.03×10^3	5.91×10^3	6.17×10^3	4.63×10^3	4.85×10^3	6.81×10^3
Sand ell's sensitivity (μg cm^{-2}) $\times 10^{-3}$	1.15	1.80	1.04	1.23	6.75	9.06	7.62	7.77	7.45	9.94	9.50	6.76
Regression equation[a]												
Slope (a)	0.795	0.677	0.762	0.843	0.170	0.127	0.140	0.161	0.124	0.113	0.153	0.180
Intercept (b)	0.025	−0.056	0.058	−0.018	−0.033	−0.037	−0.011	−0.064	0.027	−0.026	−0.100	−0.051
Correlation coefficient	0.9944	0.9972	0.9889	0.9897	0.9892	0.9967	0.9959	0.9975	0.9882	0.9909	0.9897	0.9912
Reaction time (min)	15	15	15	15	10	10	10	10	10	10	10	10
R.S.D.% (n = 7)	0.22	0.27	0.22	0.34	0.23	0.85	0.42	0.98	0.68	0.72	0.54	0.46

[a] Regression curve: $y = ax + b$ where x is the concentration of nitrite in μg ml^{-1} and y is absorbance.

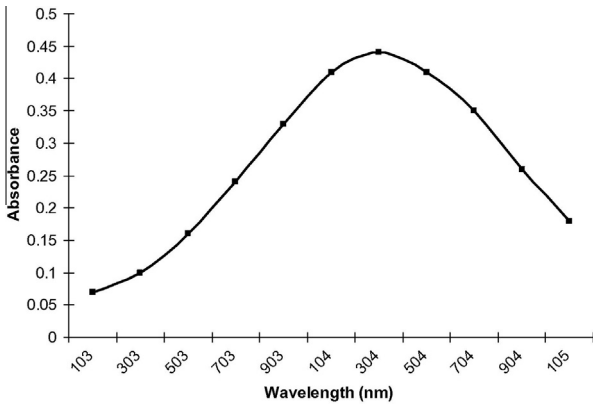

Figure 2 Absorption spectrum of SAA–nitrite–cardol.

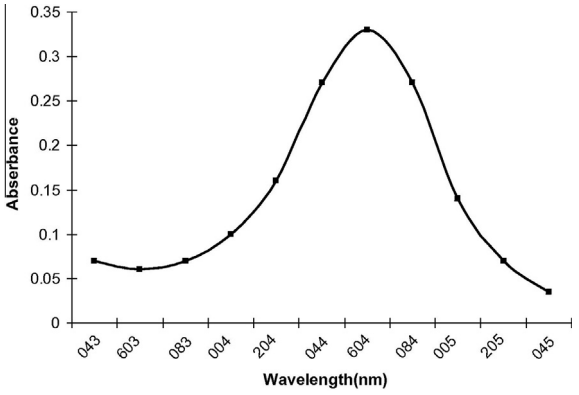

Figure 3 Absorption spectra of the reaction product of SAA–nitrite–cardanol.

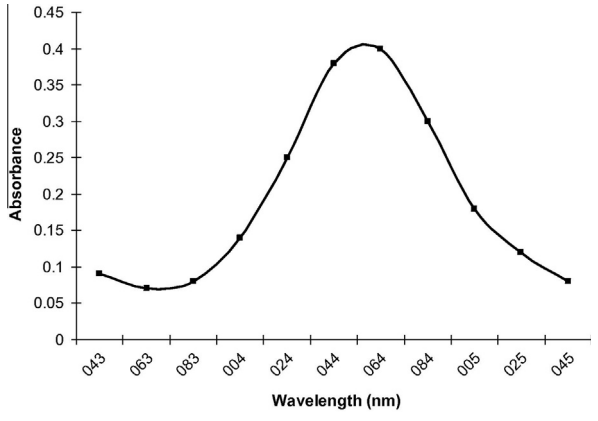

Figure 4 Absorption spectrum of the reaction product of SAA–nitrite–anacardic acid.

Similarly, the same procedure was adopted to ascertain the amount of cardol, cardanol or anacardic acid required for getting constant and maximum color intensity. It was found that 0.1–10.0 ml (0.25% w/v) solution to achieve the maximum color intensity; volume of 1.0–3.0 ml of the solution gave good results. Hence, 2.0 ml of (0.25% w/v) cardol, cardanol or

Table 3 Determination of nitrite in different environmental sample using cardol and SAA.

Sample	Nitrite added ($\mu g\ ml^{-1}$)	Proposed method		Reference method (AOAC, 1997)			
		Nitrite recovered ($\mu g\ ml^{-1}$)	Recovery (%) ± R.S.D.[a]	Nitrite recovered ($\mu g\ ml^{-1}$)	Recovery (%) ± R.S.D.[a]	*t*-Value[b]	*F*-Value[c]
Well water	0.100	0.098	98.0 ± 0.73	0.101	101.0 ± 0.41	1.43	5.60
	0.300	0.302	100.6 ± 0.52	0.306	102.0 ± 0.58	2.20	2.70
	0.600	0.598	99.6 ± 0.57	0.599	99.8 ± 0.33	1.90	5.52
Lake water	0.100	0.099	99.0 ± 0.48	0.099	99.0 ± 0.25	1.34	2.71
	0.300	0.310	103.0 ± 0.64	0.304	101.3 ± 0.68	1.10	4.80
	0.600	0.607	101.0 ± 0.32	0.598	99.6 ± 0.61	2.50	5.32
Tap water	0.100	0.102	102.0 ± 0.37	0.098	98.0 ± 0.91	1.64	5.22
	0.300	0.298	99.3 ± 0.65	0.305	101.6 ± 0.76	1.80	4.86
	0.600	0.601	100.1 ± 0.22	0.590	98.3 ± 0.82	2.00	5.53
Mineral water	0.100	0.101	101.0 ± 0.61	0.103	103.0 ± 0.50	1.56	4.24
	0.300	0.307	102.3 ± 0.37	0.297	99.0 ± 0.67	1.60	1.90
	0.600	0.597	99.5 ± 0.76	0.601	100.1± 0.82	2.34	3.86
Road side soil	0.100	0.097	97.0 ± 0.81	0.097	97.0 ± 0.68	2.65	1.48
	0.300	0.310	103.0 ± 0.41	0.303	101.0 ± 0.23	1.34	1.55
	0.600	0.605	100.8 ± 0.91	0.598	99.6 ± 0.72	0.96	5.33

[a] Average of five-determination. RSD-relative standard deviation.
[b] Tabulated *t*-value at 95% confidence level is 2.78.
[c] Tabulated *F*-value at 95% confidence level is 6.39.

Table 4 Determination of Nitrite in different environmental samples using cardol and SDX.

Sample	Nitrite added ($\mu g\ ml^{-1}$)	Proposed method		Reference method (AOAC, 1997)			
		Nitrite recovered ($\mu g\ ml^{-1}$)	Recovery (%) ± R.S.D.[a]	Nitrite recovered ($\mu g\ ml^{-1}$)	Recovery (%) ± R.S.D.[a]	*t*-Value[b]	*F*-Value[c]
Well water	0.300	0.305	101.6 ± 0.91	0.308	102.6 ± 0.31	1.10	5.64
	0.600	0.589	98.2 ± 0.62	0.603	100.5 ± 0.53	1.36	5.32
	0.800	0.801	100.1 ± 0.57	0.806	100.7 ± 0.82	1.54	4.98
Lake water	0.300	0.298	99.3 ± 0.50	0.294	98.0 ± 0.50	2.10	4.18
	0.600	0.608	101.3 ± 0.32	0.590	98.3 ± 0.67	1.95	3.65
	0.800	0.803	100.3 ± 0.76	0.810	101.2 ± 0.71	1.70	5.70
Tap water	0.300	0.297	99.0 ± 0.62	0.310	103.3 ± 0.65	2.00	3.41
	0.600	0.607	101.2 ± 0.65	0.610	101.6 ± 0.58	2.20	2.31
	0.800	0.798	99.7 ± 0.75	0.795	99.4 ± 0.73	1.90	1.65
Mineral water	0.300	0.301	100.3 ± 0.84	0.303	101.0 ± 0.23	1.56	1.58
	0.600	0.603	100.5 ± 0.90	0.608	101.3 ± 0.34	1.62	4.90
	0.800	0.795	99.4 ± 0.50	0.807	100.8 ± 0.45	2.10	2.00
Road side soil	0.300	0.308	102.6 ± 0.41	0.297	99.0 ± 0.52	1.80	3.62
	0.600	0.591	98.5 ± 0.37	0.589	99.6 ± 0.68	1.67	4.10
	0.800	0.810	101.2 ± 0.25	0.807	100.8 ± 0.63	1.46	3.25

[a] Average of five-determination. R.S.D.-relative standard deviation.
[b] Tabulated *t*-Value at 95% confidence level is 2.78.
[c] Tabulated *F*-value at 95% confidence level is 6.39.

anacardic acid solution in 25-ml standard flask was selected for further studies, under optimized conditions.

Maximum intensity of the yellow, orange and orange red color was achieved in the range of 1–6 ml of 1 M NaOH. Therefore, 1.0 ml of 1 M NaOH in 25 ml was used for getting the best results.

3.4. Order of addition

During the course of the study it was observed that the sequence of addition of reactants is also important as it influences to great extent intensity and the stability of the color product. The sequences (i) cardol, cardanol or anacardic acid–NaOH–

Table 5 Determination of Nitrite in different environmental samples using cardol and SMX.

Sample	Nitrite added ($\mu g\ ml^{-1}$)	Proposed method		Reference method (AOAC, 1997)			
		Nitrite recovered ($\mu g\ ml^{-1}$)	Recovery (%) ± R.S.D.[a]	Nitrite recovered ($\mu g\ ml^{-1}$)	Recovery (%) ± R.S.D.[a]	*t*-Value[b]	*F*-Value[c]
Well water	0.100	0.097	97.0 ± 0.52	0.101	101.0 ± 0.48	1.83	3.40
	0.300	0.298	97.6 ± 0.28	0.298	99.3 ± 0.50	1.63	5.70
	0.600	0.590	98.3 ± 0.68	0.603	100.5 ± 0.78	2.00	4.90
Lake water	0.100	0.103	103.0 ± 0.60	0.098	98.0 ± 0.91	2.50	4.98
	0.300	0.308	102.6 ± 0.31	0.295	98.3 ± 0.87	0.98	5.40
	0.600	0.605	100.8 ± 0.48	0.597	99.5 ± 0.63	1.34	5.83
Tap water	0.100	0.098	98.0 ± 0.23	0.101	101.0 ± 0.41	1.23	5.32
	0.300	0.302	100.6 ± 0.76	0.302	100.6 ± 0.22	2.15	2.31
	0.600	0.607	101.2 ± 0.71	0.599	99.8 ± 0.51	2.32	1.58
Mineral water	0.100	0.101	101.0 ± 0.50	0.099	99.0 ± 0.74	1.80	4.62
	0.300	0.297	99.0 ± 0.67	0.301	100.3 ± 0.28	1.58	2.77
	0.600	0.598	99.6 ± 0.31	0.598	99.6 ± 0.37	1.78	3.80
Road side soil	0.100	0.102	102.0 ± 0.37	0.097	97.0 ± 0.45	1.34	1.41
	0.300	0.298	99.3 ± 0.91	0.309	103.0 ± 0.62	1.56	5.62
	0.600	0.592	98.9 ± 0.74	0.607	101.2 ± 0.81	1.76	2.80

[a] Average of five-determination. R.S.D.-relative standard deviation.
[b] Tabulated *t*-value at 95% confidence level is 2.78.
[c] Tabulated *F*-Value at 95% confidence level is 6.39.

nitrite–SAA, SDX, SMX and SDZ; (ii) nitrite–NaOH–cardol, cardanol or anacardic acid–SAA, SDX, SMX and SDZ gave less intense and unstable yellow, orange and orange red color (iii) SAA, SDX, SMX and SDZ–nitrite–cardol, cardanol or anacardic acid–NaOH gave more intense and stable yellow and orange red color hence it was selected for further work.

4. Analytical figures of merit

The spectrophotometric methods were evaluated under the optimum conditions with respect to linearity, accuracy, precision, molar absorptivity and Sandell's sensitivity.

The linearity of the spectrophotometric method for the determination of nitrite was evaluated under optimum conditions. The regression calibration equation obtained under optimum conditions for nitrite, cardol and SAA was: $Y = 0.025 + 0.795X$; $r = 0.9944$ and $n = 7$, where Y is the absorbance and X is the nitrite concentration as mg/L. The calibration curve was linear over the range 0.08–0.90 mg/L. The detection limit (D_l) gives an indication of the lowest concentration of nitrite that can be distinguished from the blank absorbance with 99% certainty. The D_l was calculated as: $D_l = 3.3\delta/m$. Where δ is the standard deviation of the blank absorbance $n = 10$, m is the slope of the graph. The calculated D_l for keeping cardol as an example was 0.032 mg/L of nitrite. The molar absorptivity was 3.99×10^4 L mol^{-1} cm^{-1} for cardol-SAA complex and Sandell's sensitivity 0.0011 $\mu g\ cm^{-2}$. Sandell's sensitivity (S) represents the number of micrograms of the determinant per milliliter of a solution having an absorbance (A) of 0.001 for a path length (l) of 1-cm. Thus, $S = 10^{-3}/a = \mu g\ cm^{-2}$ where a is the specific absorptivity and its value (in mL g^{-1} cm^{-1}) corresponds to the determinant in a cuvette with an optical length of 1-cm. Also, a = (b/molecular weight of nitrite ion) × 1000, where b = molar absorptivity = A/C l, where C is the molar concentration of the determinant and l = 1-cm path length. The accuracy of the method was evaluated by comparing the results obtained for real environmental samples (obtained from lake, well and tap water and soil) with the proposed spectrophotometric methods and with the result of standard spectrophotometric method. The results obtained in the proposed spectrophotometric methods compared very well with those from the standard method. The %RSD was found to be < 0.8 ($n = 5$). The proposed method was found to be as accurate and precise as that of the official method (Tables 3–6).

To further confirm the validity and accuracy of the proposed method recovery tests were performed by standard addition method. Each test was repeated five times. The results presented in Tables 3–6 indicate very good recoveries and non-interference from commonly encountered constituents normally present in the real sample.

4.1. Interferences

To study the selectivity of the proposed method, the effect of various ions on the determination of 0.32 $\mu g\ mL^{-1}$ nitrite using cardol-SAA was tested under the optimum conditions. The tolerance limit was defined as the concentration of added ion causing less than ±3% relative error. The result show that the ions Na^+, Mg^{2+}, Zn^{2+} and K^+ did not interfere even when present in 1000-fold excess over nitrite. Fe^{3+}, MnO_4^{2-}, Cr^{6+} caused negative interference (Table 7).

4.2. Method validation

To validate the proposed spectrophotometric method, Student's *t*-test was performed on the results of five real samples (Tables 3–6). Comparison was made between the proposed spectrophotometric method and the standard method to find

Table 6 Determination of nitrite in different environmental samples using cardol and SDZ.

Sample	Nitrite added ($\mu g\ ml^{-1}$)	Proposed method		Reference method (AOAC, 1997)			
		Nitrite recovered ($\mu g\ ml^{-1}$)	Recovery (%) ± R.S.D.[a]	Nitrite recovered ($\mu g\ ml^{-1}$)	Recovery (%) ± R.S.D.[a]	*t*-Value[b]	*F*-Value[c]
Well water	0.200	0.198	99.0 ± 0.41	0.201	100.5 ± 0.50	1.65	3.40
	0.400	0.390	97.5 ± 0.32	0.407	101.7 ± 0.74	1.40	5.60
	0.600	0.599	99.8 ± 0.82	0.603	100.5 ± 0.53	1.30	5.70
Lake water	0.200	0.202	101.0 ± 0.89	0.198	99.0± 0.31	2.21	4.86
	0.400	0.403	100.7 ± 0.56	0.401	100.2 ± 0.85	2.00	5.32
	0.600	0.597	99.5 ± 0.21	0.602	100.3 ± 0.58	1.90	2.64
Tap water	0.200	0.205	102.5 ± 0.33	0.203	101.5 ± 0.91	1.80	4.51
	0.400	0.401	100.2 ± 0.62	0.390	97.5 ± 0.65	1.76	5.10
	0.600	0.610	101.6 ± 0.57	0.607	101.2 ± 0.38	0.59	4.70
Mineral water	0.200	0.201	100.5 ± 0.48	0.199	99.5 ± 0.62	0.98	3.56
	0.400	0.398	99.5 ± 0.71	0.407	100.2 ± 0.73	2.00	3.25
	0.600	0.601	100.1 ± 0.57	0.598	99.6 ± 0.34	1.72	4.90
Road side soil	0.200	0.197	98.5 ± 0.39	0.202	101.0 ± 0.81	1.90	1.56
	0.400	0.404	101.0 ± 0.62	0.397	99.3 ± 0.74	2.60	3.40
	0.600	0.596	99.3 ± 0.65	0.603	100.5 ± 0.60	1.40	2.86

[a] Average of five-determination. R.S.D.-relative standard deviation.
[b] Tabulated *t*-value at 95% confidence level is 2.78.
[c] Tabulated *F*-Value at 95% confidence level is 6.39.

Table 7 Effect of diverse species of salts on the determination of nitrite (0.32 $\mu g\ ml^{-1}$) using cardol-sulfonamides.

Foreign ions	Tolerance limit ($\mu g\ ml^{-1}$)
Na^+, Al^{3+}, K^+, Ca^{2+}, Ti^{4+}, EDTA, Pb^{2+}, Mg^{2+}, SO_4^{2-}, F^-, Br^-, CO_3^{2-}	10000
Bi^{3+}, Ba^{2+},	1000
Ni^{+2}	500
Sr^{2+}, Zn^{2+}	100

out whether the two methods give the same results at the 95% confidence level. The *t*-test with multiple samples was applied to examine whether the two methods for nitrite determination differ significantly at the 95% confidence level. The calculated Student's *t*-value and *F*-value did not exceed the tabulated value indicating that the proposed method is as accurate and precise as the official method (AOAC, 1997).

5. Applications to polluted water and soil

Samples of potable water were collected in wide-mouthed plastic vessels from different taps and different packaged water bottles. The samples were frozen at 0 °C within 1 h of collection. Samples were filtered through Whatman No. 41 paper before analysis.

Samples of manured garden soil, farmland soil and road side soil were collected. Each sample was broken into lumps, and 5-g portion was dried at 55 °C in an oven for 12–16 h. The dried sample was ground, passed through a 2-mm mesh sieve and transferred to Whatman No. 50 filter paper on a Buchner funnel. Sufficient water (containing one or two drops of concentrated sulfuric acid) was poured on to soak the soil completely. After a few minutes, gentle suction was applied and the soil was washed with double distilled water until about 250 mL of filtrate was collected. The filtrate was made up to a standard volume and aliquots were analyzed (Chaube et al., 1984).

6. Conclusion

The proposed methods, besides being simple, inexpensive, sensitive and precise as compared to the existing methods also claim the advantage of determination without the need for extraction or heating. The method does not involve complicated reaction conditions. The proposed diazotization method has got significant advantages over other existing methods in terms of simplicity and was free from most of the interfering substances. Statistical analysis of the results revealed that the proposed method yield as accurate and reproducible values as that standard method in the determination of nitrite in various soil and water matrices. Applications of the method in the determination of nitrite in a variety of real natural samples have demonstrated its practical utility.

An attempt has been made to bring the two major fields of organic molecules, namely phytochemicals and pharmaceuticals to evolve compounds with less toxicity and thus making the methods more greener. Pharmaceuticals and phytochemicals are the major class of organic molecules with varied phytochemicals and different functional groups. It is envisaged that synthetic molecules reaction will generate more toxic waste than synthetic and extracting molecular reaction.

Acknowledgments

The authors thank Vital Mallya Scientific Research Foundation, India and to SmithKline Beecham, India for gift samples. One of the authors (MM) thanks the University of Mysore for granting permission to carry out the research work.

References

U.S. Environmental Protection Agency, Drinking Water Standards and Health Advisories, Washington, DC, 2004 (EPA 822-R-04–005).
Eaton, A.D., Clesceri, L.S., Greenberg, A.E., 1995. StandardMethods for the Environmental of Water and waste Water, 19th ed. American Public Health Association, Washington, DC, pp. 475–484.
Lijinsky, W., 1992. Chemistry and Biology of N-Nitroso Compounds. Cambridge Monographs on Cancer Research, Cambridge.
Anastas, P.T., 1999. Crit. Rev. Anal. Chem. 29, 167.
Tsikas, D., B̈ oger, R.H., B-Boger, S.M., Gutzki, F.-M., Fr̈ olich, J.C., 1994. J. Chromatogr. B 661, 185.
Helaleh, M.I.H., Korenaga, T., 2000. J. Chromatogr. B 744, 433.
Butt, S.B., Riaz, M., Iqbal, M.Z., 2001. Talanta 55, 789.
Yu, B.S., Chen, P., Nie, L.-H., Yao, S.-Z., 2001. Anal. Sci. 17, 495.
Davis, J., Compton, R.G., 2000. Anal. Chim. Acta 404, 241.
Badea, M., Amine, A., Palleschi, G., Moscone, D., Volpe, G., Curulli, A., 2001. J. Electroanal. Chem. 509, 66.
Ensafi, A.A., Kazemzadeh, A., 1999. Anal. Chim. Acta 382, 15.
Huang, Z., Korenaga, T., Helaleh, M.I.H., 2000. Microchim. Acta 134, 179.
Odake, T., Tabuchi, M., Sato, T., Susaki, H., Korenaga, T., 2001. Anal. Sci. 17, 535.
Afkhami, A., Bahram, M., Gholami, S., Zand, Z., 2005. Anal. Biochem. 336, 295.
Revanasiddappa, H.D., Kumar, T.N.K., 2003. Chem. Anal. (Warsaw) 48, 759.
Baveja, A.K., Gupta, V.K., 1983. Chem. Anal. 28, 6.
Sunitha, S., Gupta, V.K., 1984. Int. J. Environ. Anal. Chem. 19, 11.
Baveja, A.K., Gupta, V.K., 1981. Analyst 106, 955.
Rathore, D.P.S., Tarafder, P.K., 1989. J. Ind. Chem. Soc. 26, 185.
Kaur, P., Gupta, V.K., 1987. J. Ind. Chem. Soc. 64, 428.
Kaveeshwar, R., Gupta, V.K., 1991. Analyst 116, 667.
Kesari, R., Gupta, V.K., 1998. J. Ind. Chem. Soc. 75, 416.
Revanasiddappa, H.D., Kumar, K., Bilwa, M., 2001. Mikrochim. Acta 137, 249.
Nair, J., Gupta, V.K., 1979. Anal. Chim. Acta 111, 311.
Ozmen, H., Polat, F., Cukurovali, A., 2006. Anal. Lett. 39, 823.
Chaube, A., Baveja, A.K., Gupta, V.K., 1982. Anal. Chim. Acta 143, 273.
Satake, M., Wang, G.-F., Fresenius, J., 1997. Anal. Chem. 357, 433.
Sreekumar, N.V., Narayana, B., Hegde, P., Manjunatha, B.R., Sarojini, B.K., 2003. J. Microchem. 74, 27.
Yue, X.-F., Zhang, Z.-Q., Yan, H.-T., 2004. Talanta 62, 97.
Horita, K., Satake, M., 1997. Analyst 122, 1569.
AOAC, Official Methods of Analysis of the Association of Official Analytical chemists, 16th ed., AOAC, Gaithersburg, 1997 (Method 36.1.21).
Allen, S.E., 1989. Chemical Analysis of EcologicalMaterials, second ed. Black-well, Oxford, pp. 132–134.
Tyman, J.H.P., 1996. Synthetic and Natural Phenols, Vol. 52. Elsevier, Amesterdam.
Kozubek, A., Tyman, J.H.P., 1999. Chem. Rev. 99, 1.
Menon, A.R.R., Pillai, C.K.S., Sudha, J.D., Mathew, A.G., 1985. J. Sci. Ind. Res. 44, 324.
George, J., Mitsutashi, M., Yusyase, O., Shimizi, K., 2001. Adv. Mater. 13, 715.
Prabhakaran, K., Narayan, A., Pvithram, C.J., 2001. Eur. Ceram. Soc. 21, 2873.
Niimuru, N., Miyakoshi, T., 2003. Int. J. Polym. Anal. Char. 8, 47.
Huong, N.L., Nieu, N.H., Tan, T.T.M., Griesser, U.J., 1996. Angew. Makromol. Chem. 243, 77.
Trevisan, M.T., Pfundstein, B., Haubner, R., Wü rtele, G., Spiegelhalder, B., Bartsch, H., Owen, R.W., 2006. Food Chem Toxicol. 44, 188.
Kumar, P.P., Paramashivappa, R., Vithayathil, P.J., Rao, P.V.S., Rao, A.S., 2002. J. Agri. Food Chem. 50, 4705.
Paramashivappa, R., Kumar, P.P., Vithayathil, P.J., Rao, A.S., 2001. J. Agri. Food Chem. 49, 2548.
Kubo, I., Ochi, M., Vieira, P.C., Komatsu, S., 1993. J. Agric. Food Chem. 41, 1021.
Himejima, M., kubo, I., 1991. J. Agric. Food Chem. 39, 418.
Muroi, H., Kubo, I., 1993. J. Agric. Food Chem. 41, 1780.
Kubo, I., Komatsu, S., Ochi, M., 1986. J. Agric. Food Chem. 34, 970.
Grazzini, R. Hesk, D. Heiminger, E. Mumma, R. O. Hildenbrandt, G. Reddy, C. C. Cox-Foster and D. Medlford, J. Craig, R. Biochem.Biophys. Res.Commun., 176 (1991) 775.
Kubo, I., Hori, I.K., Yokokawa, Y., 1994. J. Nat. Prod. 57, 545.
Shobha, S., Ramadoss, V., Ravindranath, C.S., 1994. B. J. Nat. Prod. 57, 1755.
Gulati, A.S., Subba Rao, B.C., 1964. Indian J.Chem. 2, 337.
Elsholy, M.A., Adawadkar, P.D., Benigni, D.A., Watson, E.S., Little, T.L., 1986. J. Med. Chem. 29, 606.
Northey, E.H., 1948. The Sulfonamides and Allied Compounds, ACS Monogr. Ser. American Chemical Society, Washington, DC.
Reynolds, J.E.F., 1993. The Extra Pharmacopoeia, 30th ed. The Pharmaceutical Press, London, p. 112.
Schirmer, R.E., 1982. Modern Methods of Pharmaceutical Analysis. CRC Press, Boca Raton, FL, 1 p.83.
Norwitz, G., Keliher, P.N., 1981. Anal. Chem. 53, 56.
Baiocchi, C., Gennaro, M.C., Campi, E., Mentasti, E., Aruga, R., 1982. Anal. Lett. 15, 1539.
March, J., 1992. Advanced Organic Chemistry. Wiley, New York, p.669.
Chaube, A., Baveja, A.K., Gupta, V.K., 1984. Talanta 31, 391.