



Novel oxidative electrophilic coupling reactions of phenoxazine derivatives with MBTH and their applications to spectrophotometric determination of residual chlorine in drinking water and environmental water samples

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ABSTRACT

Novel, sensitive and rapid spectrophotometric methods, using phenoxazine (PNZ), 2-chlorophenoxazine (CPN) and 2-trifluoromethylphenoxazine (TPN) as chromogenic reagents for the determination of residual chlorine are proposed. The methods are based on the reduction of chlorine by an electrophilic coupling reagent, 3-methyl-2-benzothiazoline hydrazone hydrochloride hydrate (MBTH) in mild hydrochloric acid medium and subsequent coupling with PNZ, CPN or TPN. The blue color formed in the reaction showed maximum absorbance at 680–690 nm and obeyed Beer's law over the range 0.1–2.2 $\mu\text{g ml}^{-1}$. The molar absorptivity values with PNZ, CPN and TPN were 2.80×10^4 , 2.67×10^4 and $1.91 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and Sandell's sensitivity values were 0.028, 0.027 and 0.028 $\mu\text{g cm}^{-2}$ respectively. The proposed methods were successfully applied in the determination of residual chlorine in drinking water and environmental water samples. The performance of proposed methods was evaluated in terms of Student's *t*-test and variance ratio *F*-test which indicated the significance of proposed methods over the standard spectrophotometric method.

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1. Introduction

Chlorine has been used as an oxidizing agent in waste water treatment; a control for fouling organisms in industrial cooling towers; pulp and paper effluents and as a disinfectant for potable water supplies. The presence of chlorine in natural waters can have potentially severe consequence of riverine flora and fauna [1]. Earlier, chlorine toxicity research was focused mainly on fouling organisms in industrial cooling water systems, and paper mill effluents. However, for last three decades, chlorine has received negative publicity, mainly due to the discovery in the 1970s that chlorination of water containing organic compounds could lead to the formation of trihalomethanes (THMs). THMs have diverse negative effects on environmental and human health, including toxicological, mutagenic and carcinogenic effect, as well as induction of congenital malformations and related foetal growth [2–4]. Consequently a large number of alternative disinfectants were reported in the literature yet, no disinfectant offers as many advantages as chlorine in terms of convenience, reliability, ease of use and measurement and cost effectiveness. Because of the widespread use of chlorine as chemical disinfectant and the practice to maintain a small concentration of residual chlorine in the water disinfected

by chlorination that reaches the consumer there is a continual interest in the development of analytical methods that are simple, sensitive, selective and rapid for the determination of various forms of chlorine in environmental matrices. Among the various forms of chlorine, total residual chlorine, which is the free residual chlorine plus the combined residual chlorine, is of paramount importance. Methods having different efficiency and convenience have been developed for the determination of chlorine in aqueous samples, which include electroanalytical [5–7], chromatographic [8] and optical methods [9–21]. Among optical methods simple visible spectrophotometric methods take precedence over chemiluminescence and UV-methods because of the complexity involved in the chemiluminescence procedures and lack of selectivity of UV-methods. The most widely accepted visible spectrophotometric methods are based on the oxidation reaction of aromatic amines such as *o*-tolidine [10,12] and 4-nitrophenylhydrazine [14] forming colored products. On account of the fairly high reactivity of these reagents, specification of residual chlorine is either troublesome or necessitates very careful control of the reaction conditions. *N,N*-diethyl-*p*-phenylenediamine (DPD) is evidently the most widely used spectrophotometric reagent for the determination of chlorine in water sample [9]. Ready kits are available for this and extensively used. Although expensive, these kits are stable, while normal reagents have a fairly short shelf-life [22].

Phenoxazine derivatives are isolog of phenothiazines and exist in neutral form, as monocations, as dications and even as

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trications depending on the environment [23]. Their molecular structure and luminescent properties have been studied to a great extent [24]. Phenoxazine derivatives are nervous system depressants particularly with sedative, antiepileptic, tranquillizing activity [25], antitubercular activity [26]. In recent years, phenoxazine derivatives are reported to be potential chromophoric compounds in host–guest artificial photonic antenna systems [27], which are known to exert intensive anticancer activity on malignant tumors in children [28] and are reported to be more potent and less toxic chemosensitizers [29]. Besides, they have impressive applications as biological stains [30], as laser dyes [31].

In this paper we report novel, sensitive and rapid spectrophotometric methods based on the use of phenoxazine (PNZ), 2-chlorophenoxazine (CPN) and 2-trifluoromethylphenoxazine (TPN) as chromogenic reagents in the presence of 3-methyl-2-benzothiazoline-hydrazone hydrochloride hydrate (MBTH) as coupling reagent for the determination of total residual chlorine (henceforth referred to as residual chlorine, RSC). The results show that the methods are simple, sensitive and rapid. Besides, the reagents offer clear advantages over most of the chromogenic reagents currently used for this purpose and the procedures show positive features over existing methods.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared from analytical grade chemicals unless specified otherwise. PNZ and MBTH were from Aldrich. The reagents were used without further purification. CPN and TPN were synthesized as per the procedures described in the literature [32].

A standard chlorine solution was prepared by passing a slow stream of chlorine from a cylinder and passed into a liter of distilled water for about 20 min and stored in an amber bottle. The concentration of chlorine solution was standardized iodometrically using standard titration method [9]. Solutions of the required strength were prepared by diluting this stock solution with distilled water just before the experiment. Stock solution of PNZ, CPN and TPN 0.05% (w/v) were prepared by dissolving 50 mg each in distilled ethyl alcohol and diluting quantitatively to 100 ml with distilled ethyl alcohol. Aqueous solution of MBTH 0.05% (w/v) was prepared and stored in amber bottle to protect from the sunlight. Solutions of diverse ions were prepared by dissolving their corresponding salts. For reference, DPD colorimetric method as described in the literature was followed [9].

2.2. Apparatus

All spectral and absorbance measurements were carried out on a Specord 50 UV–vis spectrophotometer with 1.0-cm silica quartz matched cell.

2.3. Spectrophotometric procedure

Appropriate volumes of standard chlorine solution containing known concentration were used, so that the final concentrations as mentioned in Table 1 were transferred to a series of 25-ml calibrated flasks, 1 ml of PNZ, CPN or TPN, 1 ml of 2 M HCl and 1 ml of MBTH were added. The blue color was formed almost instantaneously. The colored solutions were made up to the mark with distilled ethyl alcohol and their dilution in different solvents like water, methanol, acetic acid acetonitrile has been tested. Results showed that distilled ethyl alcohol gives maximum intensity and stability of the colored solutions. The absorbance was measured at 680, 690 and 690 nm for PNZ, CPN and TPN respectively in 1.0-cm

Table 1

Spectral data for the determination of residual chlorine using PNZ, ZPN or TPN and MBTH.

Parameters	PNZ	CPN	TPN
Color	Blue	Blue	Blue
λ_{\max} (nm)	680	690	690
Stability (h)	2	2	2
Beer's law ($\mu\text{g ml}^{-1}$)	0.10–2.20	0.20–2.00	0.20–1.90
Recommendation concentration ($\mu\text{g ml}^{-1}$)	1	1	1
Molar absorptivity ($\times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$)	2.80	2.67	1.91
Sandell's sensitivity ($\mu\text{g cm}^{-2}$)	0.028	0.027	0.028
Detection limit ($\mu\text{g ml}^{-1}$)	0.043	0.065	0.071
Regression equation ^a			
Slope (<i>a</i>)	0.278	0.287	0.232
Intercept (<i>b</i>)	0.037	0.508	0.056
Correlation coefficient	0.9990	0.9968	0.9986
R.S.D. ^b (%) (<i>n</i> = 7)	0.90	1.01	1.84

^a $y = ax + b$ where *x* is the concentration of residual chlorine in $\mu\text{g ml}^{-1}$.

^b Relative standard deviation.

quartz cell against reagent blank (colorless) which was prepared in the same manner but in the absence of chlorine.

3. Results and discussion

The optical characteristics for the determination of residual chlorine with PNZ, CPN or TPN using MBTH are detailed in Table 1. Major parameters that influence the performance of the proposed methods were studied in order to establish the optimum working configurations. All the optimization steps were carried out with a chosen RSC concentration as mentioned in Table 1.

3.1. Reaction mechanism

Electrophilic coupling reaction has attracted considerable attention for quantitative analysis of many environmental toxicants [33,34]. The mechanism of the reaction between PNZ, CPN or TPN, MBTH and RSC in acidic medium to yield a blue color was postulated in Fig. 1. The oxidation of MBTH by RSC is accompanied by a simultaneous loss of one proton and two electrons forming an electrophilic intermediate, which has been postulated to be coupling species [35]. In the second step, an electrophilic reaction between the PNZ, CPN or TPN and the electrophilic intermediate takes place, with the formation of the blue colored product.

3.2. Wavelength determination

In order to get results with minimum interferences, it is necessary to find out the optimum wavelength for RSC determination by the proposed method. The wavelength must be specific for the quantitative and specific monitoring of the RSC–MBTH–PNZ, CPN or TPN methods. The wavelength of maximum absorbance was identified by scanning the product of RSC–MBTH–PNZ, CPN or TPN over the range 300–800 nm with a Specord 50 UV–vis spectrophotometer. A wavelength of 680, 690 and 690 nm respectively (Fig. 2) for the three methods was found optimum for getting the best results (Table 1).

3.3. Effect of reagents and acid concentration

The effect of PNZ, CPN or TPN reagents was studied in the range of 0.10–10.00 ml of a 0.05% (w/v) solution. To achieve the maximum color intensity a volume of 0.50–3.00 ml of the solution was necessary. Hence, 1 ml of 0.25% (w/v) solution in water in 25 ml standard flask was selected for further studies, under optimized conditions. The maximum intensity of the blue color was achieved in hydrochloric acid medium.

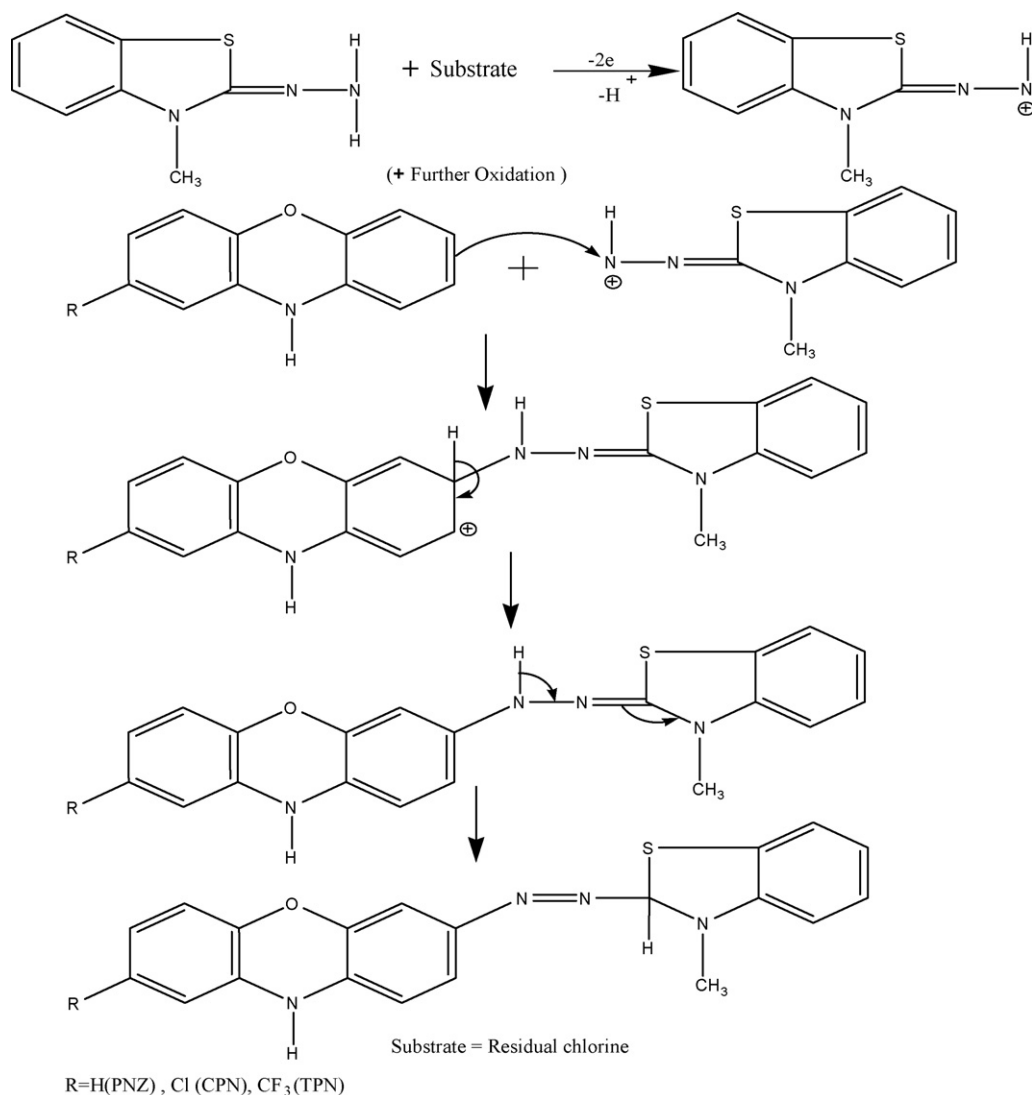


Fig. 1. Proposed reaction mechanism between MBTH and phenoxazines.

Preliminary investigations showed that hydrochloric acid was better than sulphuric, phosphoric or acetic acid. Maximum intensity of the blue color was achieved in the range of 1–6 ml of 1 M HCl. Therefore, 2 ml of 2 M HCl in 25 ml were used for getting the best results. Similarly the same procedure was adopted to know the amount of MBTH required for getting constant and maximum color

intensity. It was found that a volume of 0.50–3.00 ml of the solution is necessary. Hence, 1 ml of 0.05% (w/v) MBTH solutions were selected to get reproducible results. Experiments were carried out to optimize reaction temperature and time. It was found that the maximum color is developed within a minute at room temperature and remains almost stable for about 2 h. Increase in the temperature decreases the intensity of the blue color.

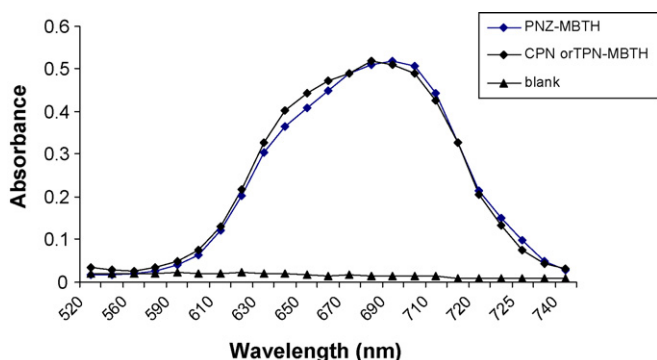


Fig. 2. Absorption spectra of PNZ-MBTH and CPN or TPN-MBTH.

3.4. Order of addition of reactants

In the course of the investigations it was observed that the sequence of addition of reactants was important as it influenced to a great extent the intensity and the stability of the colored product. The sequences (i) PNZ, CPN or TPN–acid–RSC–MBTH and (ii) RSC–acid–PNZ, CPN or TPN–MBTH gave less intense and unstable color. While, (iii) RSC–PNZ, CPN or TPN–acid–MBTH gave more intense and stable blue color. This was expected as the sequence (i) and (ii) produced radical cation. While, in (iii) electrophilic reaction was evident. It was found that the blue color formed almost instantaneously in the reaction was not affected after 1 min and remained constant up to 2 h. Therefore, 1 min was selected as the reasonable time in the absorbance study.

4. Analytical figures of merit

The proposed spectrophotometric method was evaluated under the optimum conditions with respect to linearity, accuracy, precision and interference.

4.1. Linearity (Beer's law application), accuracy, precision

The linearity of the spectrophotometric method for the determination of RSC was evaluated under optimum conditions. The regression calibration equation obtained under optimum conditions for RSC with PNZ was: $Y = 0.037 + 0.278X$; $r = 0.999$ and $n = 5$, where Y is the absorbance and X is the RSC concentration as $\mu\text{g ml}^{-1}$, r is the correlation coefficient. The calibration curve was linear over the range 0.10–2.20 $\mu\text{g ml}^{-1}$. The detection limit gives an indication of the lowest concentration of RSC that can be distinguished from the background absorbance with 99% certainty. The detection limit was calculated as follows ($D_L = 3.3 \delta/m$), where " δ " is the standard deviation of the blank, " m " is the slope of the calibration curve. Keeping PNZ as an example, the calculated detection limit was 0.043 $\mu\text{g ml}^{-1}$ of RSC. The accuracy of the proposed system was evaluated by comparing the results obtained with drinking water and environmental water samples (from sewage water, lake water and well water) using the proposed spectrophotometric method as well as the standard spectrophotometric method [9]. The results from the proposed spectrophotometric method compared very well with those from the standard method. The % R.S.D. was found to be < 1.4 ($n = 5$). The proposed method was found as accu-

rate and precise as that of standard method. To further establish the validity and accuracy of the proposed method, recovery tests by standard addition technique were performed. Known amounts of standard solutions at two different levels were added (standard addition method) to a fixed amount of real sample and the mixtures were analysed by the proposed procedure; each test was repeated 5 times. The results presented in Tables 2–4 show good recoveries and non-interference from commonly encountered constituents normally in the environmental samples.

4.2. Interferences

Interference of foreign species was investigated by analysing a standard solution of 1.0 $\mu\text{g ml}^{-1}$ chlorine to which increasing amounts of interfering species were added. The tolerable limit of a foreign species was taken as a relative error not greater than 3%. It was found that 600-fold excess of Cl^- , Br^- , NO_3^- , SO_4^{2-} , 500-fold of $\text{C}_2\text{O}_4^{2-}$, HPO_4^{2-} , H_2PO_4^- , EDTA and 450-fold of K^+ , Na^+ , Mg^{2+} , Ba^{2+} , Ca^{2+} and tartarate did not interfere with the proposed method

4.3. Method validation

To validate the proposed spectrophotometric method, Student's t -test was performed on the results of 5 real samples (Tables 2–4). Comparison was made between the proposed spectrophotometric method and the standard method to find 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 meth-

Table 2
Determination of residual chlorine using PNZ and MBTH.

Sample	Chlorine added ($\mu\text{g/ml}$)	Proposed method		Reported method [9]		t -Value ^b	F -value ^c
		Found (μg)	Recovery %, \pm R.S.D. ^a	Found (μg)	Recovery %, \pm R.S.D. ^a		
Drinking water	–	0.510	–	0.520	–	1.60	3.80
	0.500	1.020	101.00 \pm 0.46	1.030	101.90 \pm 0.31	0.67	1.29
	1.500	2.030	100.90 \pm 0.62	2.020	100.50 \pm 0.81	1.70	4.52
Sewage water	–	0.360	–	0.350	–	1.90	5.20
	0.500	0.860	100.90 \pm 0.81	0.865	100.60 \pm 1.01	1.25	2.37
	1.500	1.860	100.10 \pm 0.16	1.870	100.50 \pm 0.62	0.89	3.76
Lake water	0.500	0.490	99.00 \pm 0.81	0.498	99.60 \pm 0.67	1.98	3.98
	1.500	1.460	98.10 \pm 0.42	1.498	99.90 \pm 1.03	1.98	3.98
Well water	0.500	0.510	102.20 \pm 1.10	0.492	98.40 \pm 0.52	0.38	5.31
	1.500	1.470	98.60 \pm 0.71	1.492	99.50 \pm 0.37	0.61	4.98

^a Average of 5 determinations \pm 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 3
Determination of residual chlorine using CPN and MBTH.

Sample	Chlorine added ($\mu\text{g/ml}$)	Proposed method		Reported method [9]		t -Value ^a	F -value ^b
		Found (μg)	Recovery %, \pm R.S.D. ^c	Found (μg)	Recovery %, \pm R.S.D. ^c		
Drinking water	–	0.510	–	0.520	–	1.20	2.02
	0.500	0.991	98.00 \pm 0.67	1.030	101.90 \pm 0.31	1.50	5.10
	1.500	2.015	100.30 \pm 0.94	2.020	100.50 \pm 0.81	2.10	3.40
Sewage water	–	0.360	–	0.350	–	0.90	1.60
	0.500	1.020	101.00 \pm 0.31	0.865	100.60 \pm 1.01	1.20	2.50
	1.500	2.021	100.50 \pm 0.56	1.870	100.50 \pm 0.62	1.40	4.20
Lake water	0.500	0.489	98.00 \pm 0.92	0.498	99.60 \pm 0.67	1.31	3.10
	1.500	1.501	100.10 \pm 0.52	1.498	99.86 \pm 1.03	1.90	2.90
Well water	0.500	0.503	100.60 \pm 0.72	0.492	98.40 \pm 0.52	0.68	4.23
	1.500	1.494	99.60 \pm 0.86	1.492	99.50 \pm 0.37	2.07	2.57

^a Tabulated t -value at 95% confidence level is 2.78.

^b Tabulated F -value at 95% confidence level is 6.39.

^c Average of 5 determinations \pm relative standard deviation.

Table 4
Determination of residual chlorine using TPN and MBTH.

Sample	Chlorine added ($\mu\text{g/ml}$)	Proposed method		Reported method [9]		<i>t</i> -Value ^a	<i>F</i> -value ^b
		Found (μg)	Recovery %, \pm R.S.D. ^c	Found (μg)	Recovery %, \pm R.S.D. ^c		
Drinking water	–	0.510	–	0.520	–	1.70	3.40
	0.500	1.020	101.00 \pm 0.85	1.030	101.90 \pm 0.31	0.80	5.10
	1.500	1.995	99.20 \pm 0.31	2.020	100.50 \pm 0.81	0.90	4.60
Sewage water	–	0.360	–	0.350	–	1.10	2.80
	0.500	0.997	98.70 \pm 0.51	0.865	100.60 \pm 1.01	1.30	5.10
	1.500	2.035	101.20 \pm 0.63	1.870	100.50 \pm 0.62	1.80	4.90
Lake water	0.500	0.489	98.00 \pm 0.25	0.498	99.60 \pm 0.67	1.30	4.80
	1.500	1.502	100.10 \pm 1.40	1.498	99.90 \pm 1.03	0.79	5.10
Well water	0.500	0.491	98.20 \pm 0.92	0.492	98.40 \pm 0.52	2.10	1.80
	1.500	1.496	99.70 \pm 0.60	1.492	99.50 \pm 0.37	1.56	2.70

^a Tabulated *t*-value at 95% confidence level is 2.78.

^b Tabulated *F*-value at 95% confidence level is 6.39.

^c Average of 5 determinations \pm relative standard deviation.

ods for RSC determination differ significantly at the 95% confidence level. The calculated *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 [9].

5. Conclusion

A first-ever use of phenoxazine, 2-chlorophenoxazine and 2-trifluoromethylphenoxazine belonging to the class of tricyclics involving electrophilic coupling reaction with 3-methyl-2-benzothiazoline hydrazono hydrochloride hydrate (MBTH) is proposed for the spectrophotometric determination of residual chlorine in drinking water and environmental water samples. Although a variety of methods are available for the determination of residual chlorine, the proposed methods, besides being simple, sensitive, reliable and cost-effective also have the advantage of determination without the need for heat extraction. The methods do not involve troublesome reaction conditions and can be compared in sensitivity and reliability with standard method. Application of the method in the determination of residual chlorine in a variety of real samples has demonstrated its practical utility. A value-addition to this method can be achieved if the procedure is combined with on-line or at-line system and this is currently under investigation.

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