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Characterization of Oxidation Product of N^{10}-[3'-[N-Bis-(Hydroxyethyl)Amino]Propylphenoxazine by Spectral and Cyclic Voltametric Methods and Its Applications in Redox Titrimetry

C. M. Shivaprasad[1], B. C. Channu[2]*, K. N. Thimmaiah[3] and I. Mallikarjuna[1]*

1. Department of Chemistry, Government First Grade College for Women, Vijaynagar, Mysore – 570 032, Karnataka, **INDIA**
2. Department of Chemistry, Sri. D Devaraja Urs Govt. First Grade College, Hunsur - 571 105, Karnataka, **INDIA**
3. Department of Chemistry, Northwest Mississippi Community College, Senatobia, Mississippi, **USA**
4. Department of Chemistry, Smt.VHD Central Institute of Home Science, Sheshadri Road, Bengaluru–560 001, **INDIA**

Email: drchannubc@gmail.com; mallikarjunahiremath4@gmail.com

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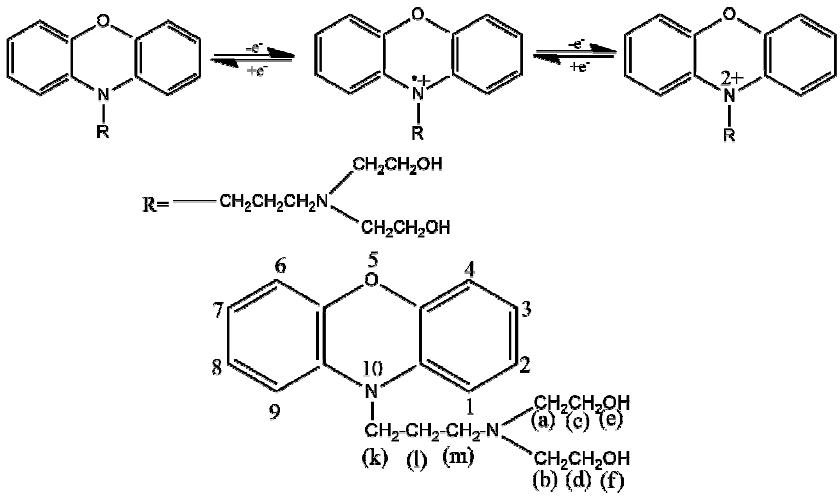
ABSTRACT

Cerium (IV) sulfate oxidizes N^{10}-[3'-[N-Bis(hydroxyethyl)amino]propylphenoxazine [BPP] to form a pink colored radical cation to undergo a reversible one-electron oxidation [$BPP^{+\cdot}$] in the presence of stoichiometric concentrations of the reactants (BPP: Ce(IV)=1:1). Ce(IV) concentration was increased and the radical cation underwent a second one-electron oxidation in the presence of more than one equivalent of Ce(IV) to form a brownish yellow colored dication [BPP^{2+}]. The dictation was established by UV-vis, IR and mass-spectral techniques. Two reversible anodic waves at 664 mV and 1122 mV and two cathodic waves at 608 mV and 968 mV were shown by the BPP cyclic voltammogram at a 24 mV/s scan rate. The peak at 664 mV corresponds to the radical cation [BPP^{+}] oxidation of BPP. The second anodic peak at 1122 mV reflects dication [BPP^{2+}] oxidation. The cyclic voltametric parameters were estimated. As demonstrated by HPLC, bromine oxidizes BPP into 4 materials. Based on mass-spectral data, the tentatively predicted structures support the development of four brominated oxidized products. The respective first and second formal BPP potentials were found to be 410-407 mV and 559-508 mV, and 762 mV in 0.5M sulphuric acid was found to be the transition potential of BPP in the titration of ascorbic acid with chloramine-T.

The optimal conditions for the effective use of BPP as a redox indicator in macro and micro estimations of ascorbic acid, methionine, isoniazid, phenylhydrazine hydrochloride and biotin using chloramine-T as an oxidant have been developed in order to explore analytical applications. It's been created. Sharp and stoichiometric endpoints are given by the indicator. BPP initially undergoes reversible one-electron oxidation during titration to form a pink colored radical cation. The radical cation is reversibly oxidized to a blue colored dictation at the endpoint with the loss of one more electron with the progression of the titration. The use of BPP as an oxidation-reduction reaction indicator for the volumetric determination of bioanalytically relevant species in real samples, such as ascorbic acid, methionine and isoniazid, was significant.

Graphical Abstract



Structure of BPP

Keywords: Phenoxazine oxidation, Characterization, Spectral, Cyclic voltammetry Redox indicator.

INTRODUCTION

Among phenoxazine derivatives, compounds of pharmacological interest have been identified and claimed to be nervous system depressants, in particular with sedative, antiepileptic, tranquilizing [1-3], spasmolytic, antituberculous and anthelmintic [4] activity. Recently, a variety of phenoxazine derivative products have been prepared by Thimmaiah *et al*., [5] and tested for their ability to reverse cancer cell resistance.The derivitives of phenoxazines pharmacological activity may be attributed to their metabolites, similar to phenothiazines [6]. For example, an oxidized species of phenothiazine cationic radical is commonly thought to be a metabolic intermediate in the formation of in vivo sulfoxide and hydroxylated products, and it has been shown that these metabolites can be formed by an aqueous buffer cation radical reaction [7].It is also hypothesized that phenoxazines could also undergo in vivo metabolism, analogous to phenothiazines, to form intermediates through oxidized species such as radical cations and dications as intermediates.

The phenoxazine derivatives have characteristic property of donating electrons. The 2-electron oxidation of phenoxazines can be attributed to the transition of color from colourless to pink due to the formation of radical cations and then to brownish yellow due to the formation of dications. The property associated with the development due to oxidation of different coloured species has made them ideal for use in titrimetry as redox indicators. The understanding of their mechanism of oxidation is of vital importance [8]. In view of the formation of the radical cations and dications from phenoxazines during oxidation and even in vivo systems as metabolic intermediates. The authors therefore selected one of the phenoxazine derivatives, namely 10-[3'-[N-Bis-(hydroxyethyl)amino]propyl]phenoxazine [BPP]. This compound was analysed by spectral and cyclic voltammetric methods and thereby studied the mechanism of oxidation of this compound. In addition, the authors have suggested this reagent as a sensitive redox indicator in order to find its application in analytical chemistry, based on the fact that this compound imparts different colours during oxidation.

MATERIALS AND METHODS

Apparatus: UV-Visible spectra with l cm matched cells were recorded in methanol using the JASCO UV-Vis spectrophotometer. It has been recorded by a Perkin-Elmer Model 1320 spectrophotometer and

the infrared spectrum of BPP as KBr pellets and its oxidized product in nujol in the range 4000-400 cm^{-1}. ^{1}H (270 MHz) and ^{13}C-NMR (67.8 MHz) spectra were recorded in $CDC1_3$ solution in a 5 mm tube on a JEOL CPF-270 Fourier transform spectrometer with TMS as internal standard. The mass-spectral data was gathered using the hybrid tandem mass spectrometer E_1BE_2-qQ geometry spectrometer of (where E, an electric sector; and Q, a quadrupole mass analyser). A one-compartment cell (platinum anode and cathode and saturated calomel electrode were used as the reference electrode) was used for Cyclic voltammetric measurements [**8**].

Substances: The required chemical substance such as phenaxazine-1-brorno-3-chloropropane, N,N,diethanolainine,tetrabutylammonium-bromide and folic acid procured from Aldrich Chemical Co. (Milwaukee, Wisconsin, USA) purchased.

Solutions: Recommended methods have been adopted for the standardization for stock solutions of chloramine-T, ascorbic acid, methionine, isonicotinic acid hydrazide, phenylhydrazine hydrochloride and folicacid. A 0.1 % ethanol/water solution of BPP was prepared and stored in an amber container.

Procedure

Synthesis 10-(3'-Chloropropyl)phenoxazine: In 40 mL of benzene 7.0 g of phenoxazine (0.04 mol) was dissolved and 175 mL of 6N potassium hydroxide and 6.44 g tetrabutylammonium bromide (0.02 mol) were added to it. The contents of the reaction were stirred for 60 min at laboratory temperature. 1-bromo-3-chloropropane (0.1 mol) is slowly added to the contents of the reaction were stirred for a whole day(24 h) at room temperature. Benzene was evaporated and ether was used to separate from the aqueous layer. The ether layer was washed with water and the organic layer was dried and rotavaporated over anhydrous sodium sulphate.On silica gel, the residue was chromatographed. Petroleum ether-ethyl acetate(3:1) pure 10-(3'-chloropropyl)phenoxazine eluted as white crystals(7.94 g 80 %, mp, 53°C); UV,IR, ^{1}H and ^{13}C NMR, mass spectral and elemental analysis characterized the substance.

10-[3'-[N-Bis(hydroxyethyl)amino]propyl]phenoxazine: One gram (3.86 mmol) of 10-(3'-chloro-propyl) phenoxazine was dissolved and 1.5g of KI, 2.1g of K_2CO_3 and 1.62 g (15.4 mmol, 1.5mL) of diethanolamine were added to 150 mL of anhydrous acetonitrile. The mixture was overnight refluxed until a significant amount of product was produced. The reaction mixture was water-diluted and ether-extracted (3 x 100mL). The ether layer was washed and dried with water over anhydrous sodium sulphate and rotavaporated. Solid recrystallisation in ethyl acetate and petroleum ether resulted in pure 10-[3'-[N-Bis(hydroxyethyl)amino]propyl]phenoxazine(1.4g, 90%, mp 83-84 $^{\circ}C$); elemental analysis, UV,IR, ^{1}H and ^{13}C NMR, mass spectral methods characterized the sample.

UV-Visible spectra of oxidation product of BPP: A freshly prepared 2 x 10^{-5} M BPP solution was treated with 0, 0.25, 0.5, 0.75, 1.0, 2.0 or 3.0 cerium(IV) sulfate equivalents in 0.5M sulfuric acid and electronic absorption spectra were registered in the 200-600nm range at room temperature.

Cyclic voltammetry: In an anhydrous acetonitrile containing 0.1M with respect to tetrabutylammonium perchlorate, a 50 mL solution of 6.0 x 10^{-4} M phenoxazine or BPP was prepared and previous to all these processes for about 15 minutes the dry N_2 was passed through it for removal of oxygen. At a scan rate of 12, 24, 48 or 96 mV per second at room temperature, the electrode potential was scanned between +100 and +1200 mV [**8**].

Titration of 0.05-0.005N ascorbic acid or phenylhydrazine hydrochloride: 20 mL of 0.05-0.01N ascorbic acid or phenylhydrazine hydrochloride, 4 mL of 10% potassium bromide and 0.2 mL of 0.1% BPP or 10 mL of 0.01-0.005N ascorbic acid, 2 mL of 10% potassium bromide and 0.1 mL of 0.1% BPP were combined and diluted to 40 mL or 25 mL of adequate sulphuric acid, hydrochloric acid, phosphoric acid or acetic acid and titrated [**8**] with 0.05-0.01N or 0.01-005N CAT solution to the appearance of blue colour.

Titration of 0.05-0.005N methionine, isonicotinic acid hydrazide or 0.01-0.0025 N folic acid:20 mL of 0.05-0.01N methionine or isonicotinic acid hydrazide solution, 4 mL of 10% potassium bromide or 10 mL of 0.01-0.005N methionine or 0.01-0.0025N folic acid and 2 mL of 10% potassium bromide were mixed and diluted to 40 mL or 25 mL with sufficient sulphuric acid, hydrochloric acid or phosphoric acid and titrated to 0.05-0.01N, 0.01-0.005N or 0.01-0.0025N CAT solution to the appearance of blue colour adding 0.2 mL or 0.1 mL of 0.1%BPP indicator near the end point.

Procedure for the assay of ascorbic acid and isoniazid in pharmaceutical preparations: For approximately 15 minutes, an appropriately weighed quantity of well-powdered tablets containing 150-500mg of vitamin C or 100-300mg of INH was agitated in double distilled water. The residue was filtered and washed with water using Whatman No.42 filter paper. The filtrate was made up to 100 mL and, following the prescribed protocol, different aliquots of this solution were titrated and the amount of vitamin C or INH was measured (in INH titration, indicator added near end-point).

Determination of ascorbic acid in citrus fruits: In order to avoid aerial oxidation of ascorbic acid, fresh yellow lemon, orange or red tomatoes were taken and juice extracted as quickly as possible before examination. Then, through Whatman No.42 filter paper, the juice was filtered and diluted to 100mL with double distilled water. Different aliquots were titrated in accordance with the recommended protocol and measured ascorbic acid content.

Determination of methionine in aminodrip: In a 100 mL volumetric flask, a known volume of amino drip solution was transferred and updated to the label. Subsequent to the prescribed protocol, different aliquots of this solution were titrated and the sum of methionine content was calculated.

RESULTS AND DISCUSSION

N-Alkylation of phenoxazine via phase transfer catalysis: Compared to the previously mentioned preparatory method, phenoxazine undergoes N-alkylation more easily in the presence of a phase transfer catalyst (PTC) [**9**]. In the existence of tetrabutylammonium bromide [$(n\text{-}C_4H_9)_4N^+Br^-$], stirring of the parent phenoxazine at room temperature with 1-bromo-3-chloropropane in a bi-phase system consisting of an solvent benzene and 6.0 N aqueous KOH solution results in the generation of 10-(3.-chloropropyl) phenoxazine at good yield. Here, from the water phase to the benzene phase in which actual reaction takes place, ammonium salt transfers hydroxide ions. These findings are interpreted as phenoxazine deprotonation by [OH^-], which is transferred into the benzene layer by the catalyst. The anion produced may be considered as a stabilized phenolate anion, which is afterwards alkylated to form the aromatized system [**10**]. 10-[3'-[N-Bis-(hydroxyethyl)amino] propyl]phenoxazine (BPP) (Scheme 1) was prepared, dried under high vacuum, separated by column chromatography, identified by UV, IR-, ^1H and ^{13}C-NMR and mass spectral studies. At 218, 239 and 322 nm, the UV-spectrum of BPP in methanol showed three λ_{max} values which can be assigned to π-π^*, π-π^*, and n-π^* transitions. The IR band may be assigned to the O-H stretching frequency at the 3300 cm^{-1} zone. Eight aromatic protons were seen in the ^1H-NMR spectrum and the data is in line with the assigned structure. The proton assignment is completely accompanied by the curves of integration. Six signals representing 12 aromatic carbons were exhibited in the ^{13}C-NMR spectrum. At m/z 328, the GC-mass spectrum demonstrated an extreme molecular ion [$M^{+\cdot}$] peak. The spectral data is consistent with the structure allocated.

Identification of the oxidized product of BPP by spectroscopic techniques: Phenoxazine derivatives, which are much more possible to occur in vivo as metabolic entity, are excellent electron donors that readily form radical cations. The production of radical cations in vivo as metabolic entities necessitates a thorough understanding of the oxidation mechanism [**11**]. The electrochemical and homogeneous oxidations to its extremely persistent radical cation [$POZ^{+\cdot}$] and dication [POZ^{2+}] of the parent phenoxazine (POZ) were studied. Even up to 150°C, the radical cation[$POZ^{+\cdot}$] and dication[POZ^{2+}] have been found to be persistent (no nitrogenic proton loss). ^1H-NMR measurements [9] presented clear proof that the N-H bond (Scheme 1) when the radical cation was oxidized, it stayed in place. A great deal of

attention has been given to the action of POZ towards oxidizing agents and the association that this behavior brings with the chemistry of phenoxazine oxidized products.

Scheme 1

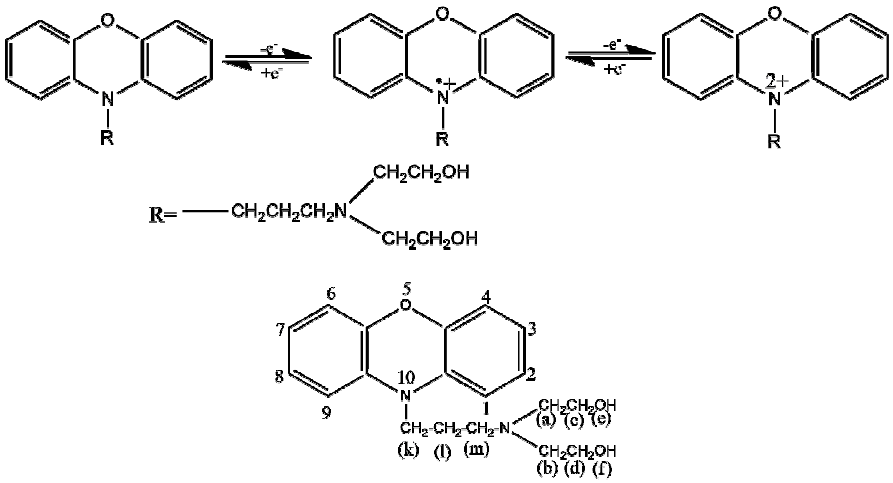


Figure 1. Structure of BPP.

Before and after oxidation of BPP by Ce(IV), the absorption spectrum of BPP was reported and the values of λ_{max} and molar extinction coefficient(ε) were calculated. The BPP before oxidation exhibited three λ_{max} values at 218 nm (ε = 48,795 L $mol^{-1}cm^{-1}$) due to pi-pi* transition and 239 nm (ε = 62,385 L $mol^{-1}cm^{-1}$) due to pi-pi* transition and other peak at 322 nm (53,125 L mol^{-1} cm^{-1}) due to n-pi* transitions were observed in the presence of 0.5M sulphuric acid (Figure 1).

To form a radical cation, BPP undergoes a reversible oxidation of one electron [$BPP^{+\cdot}$] which in the presence of stoichiometric quantities of [BPP:Ce(IV) = 1:0.50, 1:0.75, and 1:1] reactants is defined by two λ_{max} values at 410 nm and 533 nm in the visible region. As indicated by a significant increase in the molar extinction coefficient value, that is, from 18650 to 24800 L mol^{-1} cm^{-1}[9]. The intensity of the pink colour due to the formation of radical cation [$BPP^{+\cdot}$] at λ_{max} 533 nm reached the limit at the stoichiometric quantity [BPP:Ce(IV) = 1:1] of the oxidant. In order to investigate the fate of the radical cation, cerium(IV) concentration was further increased [BPP:Ce(IV) = 1:2 and 1:3]. The radical cation underwent a second one-electron oxidation to form a dication [BPP^{2+}] in the presence of more than one cerium(IV) equivalent (Scheme). Analysis of the spectrum showed that the oxidation of [$BPP^{+\cdot}$] to [BPP^{2+}] resulted in a dramatic shift in the intensity of peaks in the visible area at 533nm and 410nm. The 'ε' value of the dictation [BPP^{2+}] increased to 7900 L mol^{-1} cm^{-1}at 410 nm [**8**]. Whereas, it decreased to 7000 L mol^{-1} cm^{-1} at 533 nm. With increasing cerium(IV) concentration, the dramatic decrease in ε value at 533nm indicated that the pink colored cation radical[$BPP^{+\cdot}$] was further oxidized to a brownish yellow colored dication[BPP^{2+}]. It was noteworthy that the stoichiometric quantities [BPP:Ce(IV)=1:1]resulted in the first quantitative one-electron neutral BPP oxidation to form the radical cation [$BPP^{+\cdot}$] (Scheme). Radical cation and dictation species were also obtained in the presence of 0.5 M sulphuric acid when BPP was oxidized by hydrogen peroxide. Although two cerium (IV) equivalents [BPP:Ce(IV) = 1:2] were theoretically needed for the quantitative two-electron-oxidation of BPP to BPP^{2+}, 3 equivalents of cerium (IV) were actually involved, indicating that the kinetically slow process of oxidation of the radical cation to diction. In addition, the quantitative oxidation of BPP to a dication was demonstrated by the complete disappearance of a peak with a retention period of 16 minutes corresponding to the neutral form in the HPLC profile, followed by the emergence of a new peak with a retention time of 8 minutes less for the dictation. The IR signals at 3260, 2960, 2860, 1580, 1460, 1420, 1400, 1260, 1150 and 740 cm^{-1} suggested the existence of phenoxazine molecule-type having characteristic functional groups. The mass spectrum of dictation was reported and the protonated peak at m/z 329 was shown (Figure 2). In mass-spectral analysis, phenoxazines are typically protonated as weak bases. Analysis of mass spectral data

revealed that abundant molecular ions are formed either in the monoprotonated or diprotonated form by the oxidized product of BPP. The base peak is the molecular ion peak. The phenoxazine ring system remains intact, although fragmentation reactions have been observed in the N^{10} side chain section due to cleavage of bonds. Because even after absorbing more than one equivalent of Ce (IV), the molecular weight of the dication remained unchanged, it can be easily deduced that BPP lost only two electrons to form the dication.

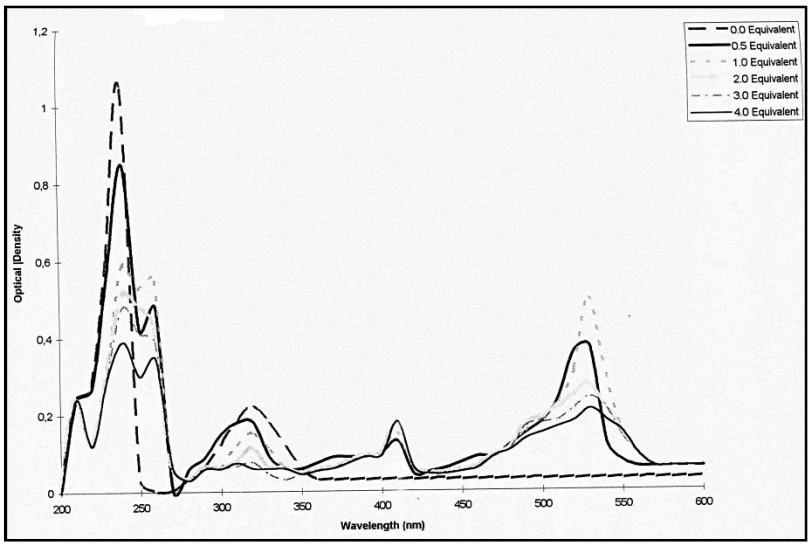


Figure 2. UV-Visible Spectra for the oxidation of BPP with Ce(IV).

Electrochemical oxidation of BPP by cyclic voltammetry: To a large degree, the biochemical functions of phenoxazines are related to their capacity to undergo reversible redox transformation and, thus, a great deal of interest has been generated in investigating their electrochemical properties. Therefore, in the present analysis, an attempt is made to study by cyclic voltammetry the electrochemical activity of the parent phenoxazine (POZ) and its derivative BPP. Table 1 list POZ and BPP cyclic voltammetric parameters [**11**].

Table 1. UV-Visible spectral data of 10-[3'-[N- Bis(hydroxyethyl)amino]propyl phenoxazine (BPP)

Number of equivalents	λ_{max}(nm) (ε)	λ_{max}(nm) (ε)	λ_{max}(nm) (ε)	λ_{max}(nm) (ε)	λ_{max}(nm) (ε)
0.0	240(52,800)	-	320(12,500)	-	-
0.5	240(42,200)	256(sh)	320(9,600)	410(6,600)	533(18,650)
1.0	240(29,000)	256(27,000)	320(4,500)	410(7050)	533(24,800)
2.0	240(25,500)	256(21,000)	320(3,000)	410(7,400)	533(11,350)

The anodic peak potentials are denoted by $E_p^{o1}$and E_p^{o2}, while E_p^{r1} and E_p^{r2} are the cathodic peak potentials corresponding to E_p^{o1} and E_p^{o2} respectively. Anodic peak currents i_p^{o1} and i_p^{o2} and their corresponding cathodic peak currents i_p^{r1} and i_p^{r2} are given with E_{f1} and E_{f2} representing formal redox potential. $D_1^{1/2}$ and $D_2^{1/2}$ denote the diffusion coefficient values for the first and second anodic peaks, respectively. $\Delta E_{p1}(E_p^{o1}\text{-}E_p^{r1})$ and $\Delta E_{p2}((E_p^{o2}\text{-}E_p^{r2})$ signify the variations between the anodic and cathodic peak potential.

Although these values for a reversible one-electron transfer method are very different from the ideal value of 59 mV [**11**], it can be believed that the electrode reactions involve two nearly reversible one-electron oxidations.

Figure 3 and Table 2 displays the POZ cyclic voltammogram. Two reversible anodic waves at 605 mV and 825 mV and two cathodic waves at 537 mV and 750 mV at a scan rate of 24 mV sec^{-1} are shown.

The first anodic peak of POZ neutral molecule is corresponding to the oxidation to the radical cation of the ($POZ^{+\cdot}$) and the second anodic peak is the oxidation to the dication of the radical cation (POZ^{2+}) (Scheme).

The electrolytic analysis was conducted as a supporting electrolyte in the presence of tetrabutylammonium perchlorate. At a scan rate of 24 $mVsec^{-1}$, the cyclic voltammogram revealed two reversible anodic waves at 664 mV and 1122 mV and two cathodic waves at 608 mV and 968 mV. The peak at 664 mV corresponds to the radical cation [$BPP^{+\cdot}$] through oxidation of the neutral molecule [BPP] and the second anodic peak at 1122 mV reflects the radical cation oxidation [BPP^{2+}] [**11**]. The redox potential of the first and second of BPP was found to be 636 mV and 1045 mV respectively. Of note, it was found that the second cathodic peak was not crucial indicating that the cation is kinetically active species.This may possibly be due to the presence of water traces in the solvent,acetonitrile.A significant finding was that BPP's anodic and cathodic peak potential was found to be greater than that of the parent compound's corresponding POZ values.

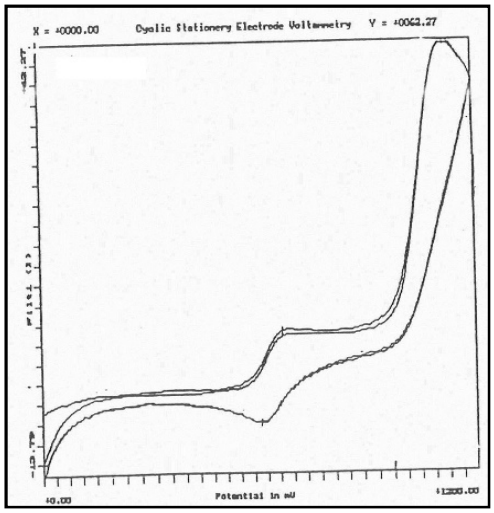


Figure 3. Cyclic voltammogram of 10-[3'-[N-Bis(hydroxyethyl)amino]propyl]phenoxazine.

Table 2. Cyclic voltammetric parameters of 10-[3'-[N-Bis(hydroxyethyl)amino]propyl phenoxazine (BPP)

Scan rate mV/s	$E_p^{o^1}$	$E_p^{r^1}$	E_{f^1}	ΔE_{p^1}	$E_p^{o^2}$	$E_p^{r^2}$	E_{f^2}	ΔE_{p^2}	$i_p^{o^1}$	$i_p^{r^1}$	$\frac{i_p^{r^1}}{i_p^{o^1}}$	$i_p^{o^2}$	$i_p^{r^2}$	$\frac{i_p^{r^2}}{i_p^{o^2}}$
12	608	563	586	45	1060	960	1010	100	8	4	0.5	53	1.8	0.03
24	664	608	636	56	1122	968	1045	154	11.4	5	0.4	62	8.1	0.13
48	636	566	601	70	1062	968	1015	94	17	8	0.5	83	7.0	0.08
96	622	565	594	57	1003	929	966	74	18	9	0.5	72	8.0	0.11

The measured potential values were almost unchanged, irrespective of the different scan rates, suggesting that the electrode mechanism is regulated by diffusion. For POZ ($\sqrt{D_1}$ = 3.96 x 10^{-5}; $\sqrt{D_2}$ = 6.68 x 10^{-5}) and for BPP (($\sqrt{D}$ = 2.82 x 10^{-5}; $\sqrt{D_2}$ = 1.53 x 10^{-4}), values are similar to that of the reference compound benzil (1.1 x 10^{-5} cm^2 s^{-1}) [**11**].

Therefore, in favor of a reversible electron mechanism, strong evidence exists. In the voltammogram, the second cathodic peak potential of BPP was found to be negligible, implying that the dictation is kinetically active species, and electrochemical processes require electronic oxidation only in POZ and BPP.

BPP as a redox indicator in titrations with chloramine-T: The transition in color from different shades of red to yellow, which follows the reduction of substituted 3H-phenoxazin-3-ones, has made them suitable as bromometric and stannometric redox indicators. A chemical literature survey reported that only a few ring-substituted phenoxazines were used as titrimetric indicators in the determination of different reductants. Most of these approaches suffer from one or more constraints. Some of the methods mentioned showed, for example, that the titrations were conducted at higher temperatures. In addition, it is also noted in the literature that, as redox markers, no N^{10}-substituted phenoxazines have been proposed to date.

The analytical aspects of N^{10}-substituted phenoxazines as redox indicators are of great importance because some of the 2,10-substituted phenoxazines have sufficient redox potential and there is a clear need for the production of highly sensitive redox indicators in chloramine-T titration (CAT). The authors therefore performed a thorough study of the indicator properties of BPP in the present communication and suggested it as a sensitive redox indicator in the titrations of ascorbic acid, methionine, isonicotinic acid hydrazide, phenylhydrazine hydrochloride and folic acid with CAT [**8**].

Oxidation of BPP by chloramine-T method: In the acid medium, CAT oxidizes potassium bromide into bromine, and the released bromine oxidizes BPP into four products. The colour switches from colorless to pink and then to blue during oxidation. After separation by HPLC, the spectral data of the products indicated the formation of four oxidized products. for example, a band at m/z 565, corresponds to the formation of the 3,7,9-tribromo derivative of [BPP^{2+}]. During oxidation, the main signal at m/z 486 was tentatively assigned to the formation of a 3,7-dibromo derivative [BPP^{2+}]. Another important signal at 276 m/z could be due to the deprotonated 7-bromophenoxazone form. A great deal of interest was generated by careful analysis of the mass-spectral data of the oxidized products of POZ or BPP. The bromine produced by CAT oxidation of potassium bromide under acidic conditions appears to have stimulated the electrophilic replacement reaction of the phenoxazine nucleus at positions 3, 7 and 9, along with 2-electron oxidation. The predicted mass-spectral data-based structures such as 3,7-dibromo and 3,7,9-tribromo derivatives were consistent with the published data. The color changed from colorless to pink due to one-electron oxidation when CAT was initially added to the acidic solution of POZ or BPP containing potassium bromide, and subsequent addition probably resulted in the appearance of a blue colored brominated dication due to second one-electron oxidation. Under these conditions, it is thought that the compound undergoes an electrophilic bromine substitution reaction followed by oxidation to produce brominated dication. The stability of the blue colored dictation was tested and for 2 hours it was found to be stable. In addition to a reducing agent such as ferrousammonium sulphate or ascorbic acid, the instantaneous absence of the pink color of the radical cation or blue color of the brominated diction implied that the two-electron oxidation mechanism was reversible in nature. Additional spectral data includes confirmation of the structure of these oxidized materials. Experiments are, however, underway to isolate the individual oxidized POZ and BPP products by HPLC and to characterize them by spectral techniques.

Determination of formal redox potentials and transition potentials of BPP: Knowledge of formal and transition potential was needed for the effective application of BPP as a redox indicator. Potentiometric and Schilt methods were used, and 410 mV and 559 mVwere found to be the respective first and second formal potential of BPP.

The formal potential values determined by Schilt's method were contrasted with those determined by cyclic voltammetry of the corresponding potential values. Data comparison showed that the first formal possibilities defined by both techniques are comparable. However, it was found that the second formal potential calculated by cyclic voltammetry was significantly higher than those determined by the system of Schilt. No adequate explanation was given for this discrepancy except that cyclic voltammetric measurements were performed in anhydrous acetonitrile, while the 0.5 M H_2SO_4 solution of BPP was used for Schilt method calculation.

In addition, the transfer potential is very helpful for determining the merits of a redox predictor. The transition potential of BPP in ascorbic acid titration with CAT was therefore calculated and the value was found to be 762 mV. The effect on the transition potential of sulphuric acid in the range 0.5 - 1.0 M was investigated and it was found that the rise in acid concentration resulted in a small decrease in BPP's transition potential.

Titration of ascorbic acid: In an acid medium which oxidizes ascorbic acid to dehydroascorbic acid, CAT releases bromine from potassium bromide.

BPP produces sharp and reversible endpoints with a dazzling color transition from colorless to blue in 0.5-1.5 M H_2SO_4 or HC1, 2.0-3.5M H_3PO_4 or 1.0-4.0M HOAc during the titration of 0.05-0.005 N ascorbic acid. For 20 minutes, the end point color is constant. Overstepping endpoints were obtained at higher acidity [**8**].

Sluggish endpoints were obtained for the 0.01-0.005N ascorbic acid titration in the acetic acid medium. The effect of the bromide concentration was investigated and the minimum amount of KBr needed for the 0.05-0.01N ascorbic acid titration was 0.3-0.4g for a total volume of 60 mL and 0.15-0.20g for the 0.01-0.005N ascorbic acid titration for a total volume of 35mL. Higher concentrations (up to 3%) have little effect and lower concentrations have led to slow endpoints. For proper indicator action, at least 0.2 mL of 0.1 percent BPP at a total volume of 60 mL or 0.05 mL of 0.1 percent BPP at a total volume of 35 mL was required for proper indicator actions. The higher concentrations of the indicators > 0.4 mL or > 0.1 mL give higher titration values and lower lower concentrations give stagnat endpoints. 0.1mL of 0.05 N CAT for 0.2 mL of 0.1 percent BPP or 0.2 mL of 0.005 N CAT for 0.1 mL of 0.1 percent BPP indicator was found to be the average indicator correction. In that it provides finer endpoints and more precise title values and has less indicator correction, BPP has advantages over phenothiazine indicators. In addition, the amount of BPP required for the indicator action is very small (0.2 mL of 0.1%) relative to phenothiazines (2 mL of 0.1%).

The effect of the number of substances commonly present in pharmaceuticals was first evaluated before the proposed method was applied to the determination of ascorbic acid in real samples.

For the calculation of 50 mg of ascorbic acid, the following quantities of tablet diluents and excipients do not interfere: starch (300 mg), gelatin (250 mg), talc (250 mg), stearic acid (250 mg), alginic acid (250 mg), citric acid (650 mg), oxalic acid (500 mg), sucrose (600 mg), dextrose (700 mg), reserpine (250 mg) and pulvisacacia (300 mg), respectively.

In vitamin C tablets [Celin (Gloxo), Becozym C Forte (Roche), Chewcee (Lederle) and Sukcee (IDPL)], titration with CAT is useful for evaluating ascorbic acid. The findings of the determination of ascorbic acid in vitamin C tablets were compared with those contained in the o-dianisidine methodand the official British Pharmacopoeia methodand were also well agreed with the claimed label values for all tablets (Table 3). Ascorbic acid has been determined to be present in citrus fruit juices and the results given in Table 3 are comparable to those obtained by the N-bromosuccinimide method.

Titration of methionine: The bromine produced by oxidation of potassium bromide by CAT, oxidizes the sulphide group of methionine-to-sulphoxide. BPP offers a sharp and permanent end point for the titration of 0.05-0.005N methionine. A color shift of the indicator from colorless to blue through pink in 0.5-1.0 M H_2SO_4, 0.5-2.0 M HCl or 2.0-3.5 M H_3PO_4 solution containing potassium bromide solution occurs during titration. In a phosphoric acid medium, the end-point color is stable for 20 minutes. At lower acidity, BPP provides overstepping endpoints and at higher acidity, premature end-points. Sluggish endpoints in the acetic acid medium were obtained.

Table 3. Typical results for the substances titrated in the presence of BPP as redox indicator in sulphuric acid medium

Reductant	Taken (mg)	Found* (mg)	Relataive error (%)	Standard deviation (mg)
Ascorbic acid	88.06	88.00	-0.06	0.042
	2.20	2.18	-0.90	0.027
Methionine	74.60	74.30	-0.40	0.057
	1.86	1.87	+0.53	0.027
Isonicotinic acid	34.33	34.21	-0.34	0.022
hydrazide	1.71	1.70	-0.58	0.042
Phenyl hydrazine	36.15	36.00	-0.41	0.083
hydrochloride	1.80	1.79	-0.55	0.027
Biotin	12.21	12.24	0.24	0.027
	3.05	3.07	0.65	0.027

* Average of five determinations

The minimum quantity of potassium bromide needed for 0.05-0.01N methionine titration is 0.3-0.4g for 60 mL and 0.1-0.2 g for 0.01-0.005N methionine titration for a total volume of 35 mL.

For proper indicator action, an optimal volume of 0.2 mL of 0.1 percent BPP for macro titrations or 0.05 mL of 0.1 percent BPP for micro titration was needed. For 0.2 mL of 0.1 percent BPP and 0.2 mL of 0.005 N CAT for 0.1 mL of 0.1 percent BPP, the mean correction for indicator was found to be 0.1 mL of 0.05N CAT. The advantages of BPP over indigocarmine are that it offers i) finer endpoints and more precise values, and (ii) works in three acid media, while indigocarmine only works in the acetic acid medium.

Determination of methionine Present in Aminodrip: The effect of some of the amino acids accompanying methionine in aminodrip has been studied to determine the potential analytical application of the proposed method and does not interfere with 1M H_2SO_4, HCl or 2M H_3PO_4 at their indicated levels. The values are given Table 3. These are compared with manufacturer's specification [Compositon/100 mL of aminodrip: L-arginine (495.00 mg), L-histidine (33.75 mg), L-cystine (1229.25 mg), L-tyrosine (56.25 mg), L-tryptophan (0.55 mg), L-cysteine (5.60 mg), L-methionine (316.80 mg), glycine (1650.00mg), L-threonine (129.30 mg), L-leucine (756.75 mg), L-leucine (756.75 mg), L-methionine (316.80 mg), L-methionine (1650.00 mg), L-threonine (129.30 mg), L-leucine (756.75 mg).

Titration of isonicotinic acid hydrazide: In the treatment of tuberculosis, isoniazid (INH) is the most important medication that has induced many investigators to establish methods for its rapid and precise determination. Among them, titrimetric methods that are quantitatively based on the oxidation of INH to nicotinic acid and nitrogen involving a shift of four electrons are commonly used. For one or other explanation, some of the measures used so far for evaluating INH using CAT are unsatisfactory. Methyl red and methyl orange, for instance, function only in the phosphoric acid medium. BPP gives a sharp and permanent color change in titrations of 0.05-0.01N INH from colorless to blue through pink in 0.5-1.5 M H_2SO_4, or HCl or 2.0-3.5M H_3PO_4 solution containing potassium bromide. In all acidic media, the colour is stable for 3 minutes. Premature and overstepping endpoints were obtained respectively at lower and higher acidities.

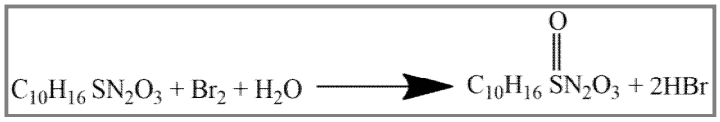
In the titration for correct end points, 0.3-0.4g of potassium bromide in a total volume of 60 mL was needed. The title values are not influenced by higher concentrations of up to 3 percent and lower concentrations result in slow endpoints. The influence of the concentration of indicators has been examined. Atleast 0,2 mL of 0,1 percent BPP was needed in a total volume of 60 mL for proper indicator function. Higher concentration (>0.4 mL) gives higher titre values and sluggish endpoints are given by lower concentration. The average correction of the indicator was found to be 0.1 mL of 0.05 N CAT for 0.2 mL of 0.1% BPP. For the calculation of 50 mg of INH, the following quantities of tablet diluents and excipients do not interfere: citric acid (500 mg), gelatin (75 mg), starch (300 mg), oxalic acid (600 mg),

glucose (250 mg), talc (250 mg), stearic acid (150 mg), alcohol (10mL). The INH content was measured for tablets such as Isokin (Parke-Davis) and Isonex (Pfizer).

Titration of phenylhydrazine hydrochloride: CAT releases bromine from acidified potassium bromide, which oxidizes phenylhydrazine hydrochloride to benzene diazonium chloride with four electron shifts. In 0.05-0.01N phenylhydrazine hydrochloride titration, BPP gives sharp and reversible endpoints. In 0.2-0.5 M H_2SO_4 0.2-1.0 M HCl or 1.0-3.5 M H_3PO_4, a solution containing potassium bromide, there is a dazzling color change from colorless to blue. For 3 minutes, the endpoint colour is constant. At lower and higher acidities, BPP gives late and early endpoints respectively. In the acetic acid medium, premature end points were obtained.

The effect of the concentration of bromide was investigated and the minimum required amount of potassium bromide was 0.3-0.4 g for a total volume of 60 mL. The effect of the indicator concentration was analyzed and the right indicator behavior demanded at least 0.2mL of 0.1 percent BPP. For 0.2 mL of 0.1 percent BPP, the average indicator correction was found to be 0.1 mL of 0.05N CAT. BPP has advantages over indigocarmine in that it (i) offers sharper endpoints and more precise values, (ii) works in three acid media, whereas indigocarmine works only in HCl medium, and (iii) BPP is used as a laboratory temperature indicator, while indigocarmine works only at elevated temperatures.

Determination of folic acid: For the growth of animals, folic acid (vitamin H) is essential. Some physico-chemical folic acid assays have been reported for the important role of biotin as the prothetic group of certain corboxylating, transcarboxylating or decarboxylating enzymes, but they have not been commonly accepted for routine folic acid assays. A literature survey revealed that for the determination of the folic acid using iodine trichloride as an oxidant involves a tedious extraction stage using an organic solvent. For this only a few titrimetric procedures are available. While more methods for biotin estimation are published, most of them require the availability of expensive instrumental set-up, and the authors therefore considered it worthwhile to develop easier, quicker, more sensitive, more precise and less expensive methods for folic acid determination. Therefore, as a redox indicator for the oxidimetric estimation of folic acid using CAT, the authors have suggested BPP. In an acid medium which oxidizes the sulphide group of folic acid to sulphoxide, CAT releases bromine from potassium bromide.



BPP offers sharp and permanent endpoints in the titration of 0.01-0.0025 N folic acid, followed by a dazzling color shift from colorless to blue through pink in 0.3-1.5M H_2SO_4, 0.3-2.0M HCl or 1.0-3.5M H_3PO_4 contiaing in a potassium bromide solution. In all acidic media, the end-point color is stable for 25 minutes. Specifically, late and premature end points were obtained at lower and higher acidity levels. For this titration, acetic acid was not appropriate [**11**].

The effect of the concentration of bromide was investigated and the minimum quantity of potassium bromide needed for titration was 0.2g for a total volume of 35mL. The title value was not affected by higher concentrations of up to 3% and lower concentrations resulted in sluggish endpoints. The effect of the indicator concentration was analyzed and a minimum of 0.1 mL of 0.1 percent BPP was required for the correct operation of the indicator at a total volume of 35 mL. Indicator volume of 0.15mL of 0.1. percent in all acid media gave higher title values and lower concentrations gave slow endpoints. Direct titration of the indicator solution with 0.0025N CAT under similar conditions was found to be the indicator correction. For 0.1 mL of 0.1 percent BPP, the average indicator correction was found to be 0.2mL of 0.0025 N CAT.

Potentiometric titration of folic acid: The presence of 1-3 percent potassium bromide in the potentiometric titration of 0.01-0.0025 N folic acid with CAT is advantageous since it stabilizes the

potentials and boosts the potential break. With 0.1 mL of 0.01 N CAT solutions, it takes around 2 minutes to reach the equilibrium potential, and the potential break is 307 mV. Titration in a medium containing 0.4-1.0 M H_2SO_4, 0.5-1.5 M HCl, or 2.5-3.0 M H_3PO_4 yields accurate and repeatable results. Increases in acid concentration resulted in increased titers. The potentials of the systems were not stabilized in the absence of potassium bromide.

The method proposed is simple, speedy and reliable. To date, except for starch, no studies are available on the use of internal redox indicators in folic acid titration against CAT. The authors therefore suggest that BPP could become the first of its kind to be used as a redox indicator for CAT biotin titration.

The BPP solution is stable at room temperature for a few days and then slowly oxidizes atmospherically and photochemically to give a very light pink color that does not interfere with the action of the indicator. During the titration of ascorbic acid, methionine, INH, phenylhydrazine hydrochloride, or folic acid, the predicted mechanism of oxidation of BPP was found to undergo reversible one-electron oxidation to give a pink-colored radical cation. The radical cation undergoes further one-more electron oxidation at the point of equivalence to give a blue-colored brominated diction. In the presence of H_2SO_4, HCl or H_3PO_4, the redox and transition potentials of BPP have been calculated and the values lie within the potential break inthe potentiometric titrations of CAT reductants. In addition, the formal and transition potentials suggest that BPP serves as a strong predictor for CAT reductant titrations.

No definite endpoint was obtained in the titration of methionine, INH and biotin, if the indicator was applied at the beginning of the titration due to the partial destruction of the indicator. An approximate title was found while titrating unknown samples by adding 1-2 mL of 0.1 percent BPP at the beginning of the titration, and then the correct titer was found by adding the indicator at the end point. For various reductants examined, the titration results given in are typical and are considered to compare well with the results obtained by other titrimetric methods available.

APPLICATION

This reagent as a sensitive redox indicator finds application in analytical chemistry because this compound imparts different colors during oxidation.

CONCLUSION

In summary, the cation radical [$BPP^{+\cdot}$], which undergoes a reversible one-electron oxidation in the presence of stoichiometric concentrations of the reactants (BPP: Ce(IV)=1:1). The studies further evidence that the increased concentration of Ce(IV), the radical cation underwent a second one-electron oxidation in the presence of more than one equivalent of Ce(IV) to form a brownish yellow coloured dication [BPP^{2+}]. Furthermore. two reversible anodic waves at 664 mV and 1122 mV and two cathodic waves at 608 mV and 968 mV proved by BPP cyclic voltammogram at a 24 mV/s scan rate.

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