Research Article

Electrochemical Investigation of N^{10}-[5'-(N-diethylamino)pentyl]-2-chlorophenoxazine (DPCP) and its Applications in Redox Titrations involving Chloramine-T (CAT)

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Accepted on: 25-05-2016; **Finalized on:** 30-06-2016.

ABSTRACT

N^{10}-[5'-(N-Diethylamino)pentyl]-2-chlorophenoxazine (DPCP) undergoes a reversible one-electron oxidation with cerium (IV) to form a pink colored radical cation [$DPCP^{+\bullet}$]. Here the DPCP and Ce (IV) are in the stoichiometric ratio of 1:1. In the presence of more than one equivalent of cerium (IV), the radical cation formed undergoes further oxidation by losing one more electron to form a brownish yellow colored dication [$DPCP^{2+}$]. Formation of radical cation and dication were characterized by UV-Vis, IR and mass spectrometry. The biological activity of phenoxazines are linked to a great extent with their ability to undergo reversible redox conversion and therefore the electrochemical behavior of DPCP is investigated by cyclic voltammetry. The cyclic voltammogram of DPCP exhibited two anodic waves at 760 mV and 1170 mV and two cathodic peaks at 688 mV and 1020 mV at a scan rate of 50 mV/sec. The peak at 760 mV corresponds to the oxidation of the DPCP to radical cation [$DPCP^{+\bullet}$] and the second anodic peak at 1170 mV corresponds to the oxidation of radical cation to dication [$DPCP^{2+}$]. Oxidation of DPCP by bromine in acid medium resulted in three products as evidenced by HPLC and the tentatively predicted structures based on mass spectral data support the formation of the brominated oxidized products. The use of DPCP as redox indicator in the volumetric estimation of bio analytically important species such as ascorbic acid, methionine and isoniazid in real samples is significant. Therefore an optimum condition for the successful use of DPCP as a redox indicator in the micro and macro estimation of these reductants using chloramine-T as an oxidant have been developed. The indicator gives sharp and stoichiometric end points. During the titration, DPCP gives a sharp color change from colorless to blue *via* pink.

Keywords: Phenoxazine derivative, redox indicator, cyclic voltammetry.

INTRODUCTION

Phenoxazine and its derivatives found fairly useful in the field of medicinal chemistry. They exert anticancer effects on a variety of carcinoma cell lines both *in vitro* and *in vivo.*[1-8] They have also been found to be antimicrobial[9-12], antibacterial[20], tranquilizers[21], anti-inflammatory[22], antimalarial[23], antipsychotropic[24], antiviral[25], antituberculor[26,27], and they have been shown to cause apoptotic cell death[6,13-15], they help in the suppression of Akt signaling[5,16-19], shown to prevent human amyloid disorders[28], and to protect neuronal cells from death by oxidative stress.[29] Thimmaiah[18,30-35] have reported the chemistry and biology of series of N^{10}-substituted phenoxazines.

Within the series, compounds having propyl-bridge were less potent than compounds with 4 carbon chain length, and those with acetyl-bridge instead of alkyl group exhibited no activity. In view of these interesting results, the authors have speculated that by increasing the alkyl chain length to $(-CH_2)_5$ and $(-CH_2)_6$ at N^{10}-position, the potency will be increased to a considerable extent due to increase in lipophilicity compared to their respective, propyl or butyl counterparts. Towards this goal, a series of 2-Chloro-N^{10}-pentyl or hexyl substituted phenoxazines and N^{10}-hexyl substituted phenoxazines have been synthesized, characterized, and evaluated for their ability to block the activation of Akt/mTOR/p70S6/S6 kinase pathway and induce apoptosis in rhabdomyosarcoma cells.

As anticipated, the potency of pentyl or hexyl derivatives to inhibit the phosphorylation of Akt has been increased to a significant extent and induced apoptosis in rhabdomyosarcoma cells at 100 nM levels, as compared to their respective propyl and butyl counterparts.

A separate manuscript delineating these interesting results will be communicated to a journal for publication shortly.

Thimmaiah[36-38] have already reported the redox chemistry of a few N^{10}-substituted phenoxazines. The pharmacological activity of phenoxazines could be due to their metabolites.

It is believed that phenoxazines undergo metabolism *in vivo* to form intermediates *via* oxidized species such as radical cations and dications.

Thus understanding the mechanism of their oxidation is of paramount importance.

Therefore, the authors have selected DPCP and studied the mechanism of oxidation of this compound by cyclic voltammetric and spectral methods. Considering the redox behavior of DPCP, the authors have proposed this reagent as a sensitive redox indicator in the titrimetric estimation of the biologically important reductants.

MATERIALS AND METHODS

Reagents

Biotin and chloramine-T were purchased from Sigma-Aldrich company. Ascorbic acid, methionine, isonicotinic acid hydrazide (INH), and phenylhydrazine hydrochloride were purchased from Ranboxy Chemicals. All other chemicals and supplies were obtained from standard commercial sources unless otherwise indicated.

Solutions

0.1 % DPCP solution was prepared and stored in amber bottle and kept in the refrigerator. Stock solutions of chloramine-T (CAT), ascorbic acid, methionine, isonicotinicacid hydrazide (INH), phenylhydrazine hydrochloride and biotin were prepared and standardized by recommended methods.

Electronic Spectra of Oxidation Products of DPCP

10^{-5} M DPCP solution was freshly prepared and treated with 0.25, 0.50, 0.75, 1.00, 2.00, 3.00 and 4.00 equivalents of cerium (IV) sulphate in 0.5 M sulphuric acid and the UV-Visible absorption spectra was recorded on a Schimatzu uv1800 spectrophotometer in the range of 200-600 nm at room temperature.

Cyclic Voltammetry

50 mL of 10^{-4} M DPCP solution in anhydrous acetonitrile containing 0.1 M with respect to tetrabutylammonium perchlorate was prepared and deoxygenated by bubbling with dry nitrogen gas for 15 min prior to all runs.

The electrode potential was scanned between 200 and 1400 mV at scan rates of 10, 25, 50, 100 mV/sec at room temperature in a one compartment cell using glossy carbon electrode as the working electrode, platinum as the auxiliary electrode (counter electrode) and saturated calomel electrode as the reference electrode using CHI Electro Chemical Analyzer Model 6039E USA.

Titration of Ascorbic acid, Methionine, Isonicotinic Acid Hydrazide, Phenylhydrazine Hydrochloride and Biotin

20 mL of 0.05 - 0.01 N ascorbic acid, phenyl hydrazine hydrochloride, methionine or isonicotinic acid hydrazide, 4 mL of 10% potassium bromide and 0.2 mL of 0.1% DPCP were mixed and diluted to 40 mL with sufficient sulfuric acid, hydrochloric acid or phosphoric acid or 10 mL of 0.01 - 0.005 N ascorbic acid or methionine or 0.01 - 0.0025 N biotin, 2 mL of 10% potassium bromide and 0.1 mL of 0.1% DPCP were mixed and diluted to 25 mL with enough sulfuric acid, hydrochloric acid or phosphoric acid and titrated with 0.05 - 0.01 M CAT or 0.01 - 0.0025 M CAT until the appearance of blue color. In the titration of methionine, INH and biotin, the indicator was added near the end point.

Determination of Ascorbic Acid or Isoniazid (INH) in Pharmaceuticals

Tablets containing ascorbic acid in the range 150 - 500 mg or INH in the range 100 - 300 mg were finely powdered and weighed accurately. Then it is transferred into a small volume of doubly distilled water and stirred well for about 15 - 20 min. The residue was filtered through a Whatman No. 42 filter paper and washed with water. The filtrate was made up to 100 ml in a volumetric flask. Different aliquots of this solution were titrated against CAT following the recommended procedure and the amount of vitamin-C or INH was calculated. In the INH titration the indicator was added near the end point.

Determination of Ascorbic Acid in Citrus Fruits

The juice of a fresh yellow lemon was extracted as quickly as possible to prevent aerial oxidation of ascorbic acid before analysis. The extracted juice was filtered through Whatman No.42 filter paper and diluted to 100 mL with doubly distilled water in a volumetric flask. Different aliquot of this sample solution were titrated against CAT following the recommended procedure and the ascorbic acid content was calculated.

Determination of Methionine in Aminodrip

A known volume of aminodrip solution was transferred into a 100 mL volumetric flask and made up to the mark using doubly distilled water. Different aliquot of this sample solution were titrated against CAT following the recommended procedure and the methionine content was calculated.

RESULTS AND DISCUSSION

Characterization of Oxidized Products of DPCP by Spectral Method

The UV-Vis spectrum of DPCP is recorded by varying the concentration of cerium (IV) sulphate. The λ_{max} and molar extinction coefficient (ε) values are recorded as shown in the **Table 1**. The UV-Vis spectrum of DPCP in the presence of 0.4 M sulfuric acid before oxidation exhibited two λ_{max} values at 241 nm (98,000 L $mol^{-1}cm^{-1}$) and 327 nm (ε =18,400 L $mol^{-1}cm^{-1}$). On adding Ce (IV) sulphate, DPCP undergoes a reversible one-electron oxidation to form a radical cation [$DPCP^{+\bullet}$] which was characterized by two λ_{max} values at 410 nm and 541 nm in the visible region. Further addition of Ce (IV) sulphate results in the increase the intensity of the pink color due to the increase in the concentration of radical cation [$DPCP^{+\bullet}$]. This results in the increase in the molar extinction coefficient value (ε) from 10,400 to 28,292 L mol^{-1} cm^{-1} at λ_{max} 541 nm, and it reaches the maximum at the stoichiometric amount 1:1 [DPCP : Ce (IV)]. The fate of the radical cation is examined by increasing the concentration of Ce (IV) further. In the presence of more than one equivalent of Ce (IV), the radical cation underwent a second one-electron oxidation

to form a dication [DPCP^{2+}] **(Scheme 1)**. Examination of the spectrum revealed that the oxidation of [DPCP$^{+\bullet}$] to [DPCP^{2+}] has resulted in drastic change in the intensity of peaks at λ_{max} 541 and 410 nm in the visible region. The 'ε' value of the dication [DPCP^{2+}] at 410 nm increased from 6,760 to 11,652 L mol^{-1}cm^{-1} and the peak at 541 nm due to radical cation almost disappeared. The disappearance of the peak at 541 nm with increasing concentration of Ce (IV) suggested that the pink colored radical cation [DPCP$^{+\bullet}$] was further oxidized to a brownish yellow colored dication [DPCP^{2+}]. Although two equivalents of Ce (IV) [DPCP : Ce (IV) = 1:2] was required theoretically for the quantitative two electron-oxidation of DPCP to [DPCP^{2+}], actually, slightly greater than two equivalents of Ce (IV) was involved in the oxidation of radical cation to dication. The IR signals of the dication at 3064, 2935, 2850, 1600, 1490, 1435, 1271, 1145 and 740 cm^{-1} **(Fig 1)** indicated the presence of characteristic functional groups of the phenoxazine type of molecule. The mass spectrum of the dication species was recorded and it displayed a protonated peak at m/z 359 (data not shown). Phenoxazines being weak bases, they usually get protonated in the mass spectral analysis. Examination of the mass spectral data revealed that the oxidized products of DPCP yield abundant molecular ion either in the monoprotonated or diprotonated form. The molecular ion peak is the base peak. The phenoxazine ring system remains stable, whereas fragmentation reactions were observed due to cleavage of bonds in the N^{10}-side chain portion. Since the molecular weight of the dication remained unchanged even after consuming more than one equivalent of Ce (IV), it can be easy to deduce that DPCP has lost only two electrons to form the dication.

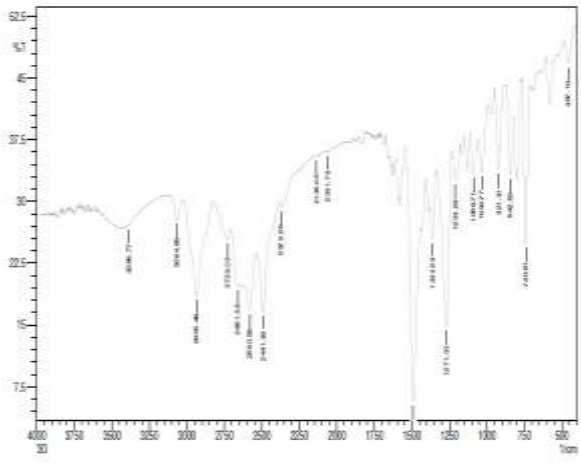
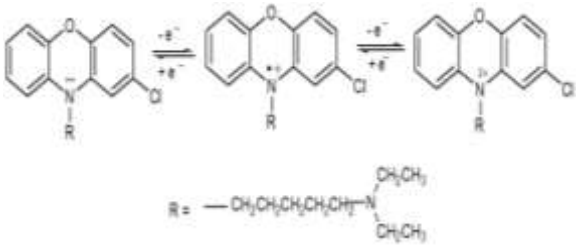


Figure 1: IR spectra of Dication

Table 1: UV-Vis spectral data for the oxidation products of DPCP

No. of equivalents of Ce (IV)	λmax (nm) (ε, L mol^{-1}cm^{-1})	λmax (nm) (ε, L mol^{-1}cm^{-1})
0.25	410 (3,000)	541 (10,400)
0.50	410 (3,800)	541 (14,900)
0.75	410 (4,760)	541 (20,935)
1.00	410 (5,400)	541 (28,292)
1.50	410 (6,760)	541 (20,100)
2.00	410 (7,600)	541 (4,200)
3.00	410 (11,652)	Disappears



Scheme1: Proposed mechanism of oxidation of N^{10}-[5'-(N-diethylamino)pentyl]-2-chlorophenoxazine (DPCP)

Electrochemical Oxidation of DPCP by Cyclic Voltammetry

The cyclic voltammetric parameters for DPCP are listed in **Table 2**. The plausible reaction mechanism for the overall redox reaction of DPCP is illustrated in **scheme 1**. The cyclic voltammogram of DPCP obtained in dry acetonitrile containing tetrabutylammonium perchlorate as supporting electrolyte has the appearance as shown in **Figure 2** and it exhibited two reversible anodic waves at 760 mV and 1170 mV and two cathodic waves at 688 mV and 1020 mV at a scan rate of 50 mV/s. In the cyclic voltammogram of DPCP, the first wave at 760 mV corresponds to the oxidation of the neutral molecule to the radical cation [DPCP$^{+\bullet}$] and the second anodic peak of 1170 mV stands for the oxidation of [DPCP$^{+\bullet}$] to a relatively stable dication [DPCP^{2+}].

The first and the second redox potentials of DPCP were found to be 724 mV and 1095 mV respectively. Of particular interest was that the second cathodic peak was found to be not significant suggesting that the dication is highly reactive.

Table 2: Cyclic Voltammetric Parameters of DPCP

Scan Rate	$(E^o_p)^1$	$(E^r_p)^1$	$(E_f)^1$	$(\Delta E_p)^1$	$(E^o_p)^2$	$(E^r_p)^2$	$(E_f)^2$	$(\Delta E_p)^2$
mV/Sec	mV	mV	mV	mV	mV	mV	mV	mV
50	760	688	724	72	1170	1020	1095	150

$(E^o_p)^1$ and $(E^o_p)^2$: anodic peak potentials; $(E^r_p)^1$ and $(E^r_p)^2$: cathodic peak potentials, $(E_f)^1$ and $(E_f)^2$: formal redox potential; $(\Delta E_p)^1 = (E^o_p)^1 - (E^r_p)^1$; and $(\Delta E_p)^2 = (E^o_p)^2 - (E^r_p)^2$: Difference between the anodic and cathodic peak potentials for the first and second electron transfer respectively.

Table 3: Estimation of bioanalytically important compounds present in the standard samples by titrating against chloramine-T using DPCP as redox indicator in different acid medium

Compound	Taken (mg)	Estimated using Present Method/mg[a]			Reference Method[b] (mg[a])
		H_2SO_4	HCl	H_3PO_4	
Ascorbic acid	88.06	87.59(0.04)	88.89(0.07)	87.59(0.05)	87.50(0.06)
	35.22	35.9(0.02)	34.3(0.06)	34.3(0.03)	34.80(0.05)
	8.806	8.639(0.03)	8.718(0.04)	8.718(0.02)	8.40(0.07)
	4.403	4.51(0.02)	4.51(0.03)	4.51(0.02)	4.55(0.04)
Methionine	74.6	75.3(0.06)	74.68(0.05)	74.46(0.04)	74.90(0.05)
	29.8	29.6(0.02)	29.6(0.07)	29.6(0.03)	30.00(0.04)
	7.46	7.38(0.03)	7.38(0.05)	7.38(0.05)	7.36(0.06)
	3.72	3.8(0.05)	3.8(0.05)	3.83(0.07)	3.82(0.05)
INH	34	33.8 (0.04)	33.8(0.05)	33.4(0.05)	33.4(0.05)
	6.84	6.4(0.04)	6.4(0.06)	6.4(0.05)	6.4(0.04)
PHH	36	36(0.04)	36(0.05)	36(0.06)	36.1(0.05)
	7.2	6.6(0.03)	6.6(0.04)	6.6(0.07)	6.8(0.07)
	1.8	1.81(0.02)	1.8(0.0)	1.83(0.02)	1.84(0.06)
	0.7	0.75(0.03)	0.76(0.04)	0.75(0.03)	0.76(0.06)
Biotin	12	12.2(0.04)	12.3(0.02)	12.09(0.06)	12.4(0.04)
	3.05	3(0.01)	3.1(0.03)	3.37(0.02)	3.2(0.05)

a= Average of five methods: Standard Deviation is given in the parenthesis; b= Potentiometric method

Table 4: Estimation of bioanalytically important compounds present in the real samples by titrating against chloramine-T using DPCP as redox indicator in different acid medium

Sample		Estimated Compound	Quantity mentioned on the tablet	Present method/mg[a]			Reference Method[b]/mg
				H_2SO_4	HCl	H_3PO_4	
Vitamin C tablets	Celin (Glaxo)	Ascorbic acid	300	298.67(0.06)	298.95(0.05)	298.57(0.04)	298.99(0.05)
	Suckcce (IDPL)	Ascorbic acid	500	497.90(0.03)	498.10(0.06)	497.80(0.04)	498.90(0.02)
	Becelac (Pfmix)	Ascorbic acid	75	74.20 (0.04)	74.30(0.02)	74.30 (0.05)	74.60 (0.04)
Lemon Juice		Ascorbic acid	-	149.50(0.08)	148.72(0.09)	-	149.97(0.03)
Orange		Ascorbic acid	-	94.52(0.06)	95.80 (0.05)	-	92.20 (0.04)
Aminodrip		Methionine	316.80	314.26(0.06)	313.78(0.03)	314.94(0.06)	314.00(0.05)
INH tablets	Isokin (Parke -Davis)	Isonicotinicacid hydrazide	300	304(0.07)	298(0.04)	-	297(0.06)
	Isonex (Pfizer)	Isonicotinicacid hydrazide	100	99.30(0.48)	99.00(0.04)	-	101.20(0.03)

a= Average of five methods : Standard Deviation is given in the parenthesis; b= potentiometric method

DPCP as Redox Indicator in Titrations with Chloramine-T

Literature survey has revealed that very few ring substituted phenoxazines have been used as indicators in the volumetric estimation of various reductants.[36-44] But most of these methods suffer from one or more limitations. For example, some of the reported methods have revealed that the titrations were carried out at higher temperature. The analytical applications of N^{10}-substituted phenoxazines as redox indicators is of great importance because these molecules possess suitable redox potentials matching the redox potentials of the analytes. Therefore, the authors have proposed DPCP as a sensitive redox indicator in the titration of methionine, isonicotinic acid hydrazide (INH), ascorbic acid, phenyl hydrazine hydrochloride or biotin against CAT.

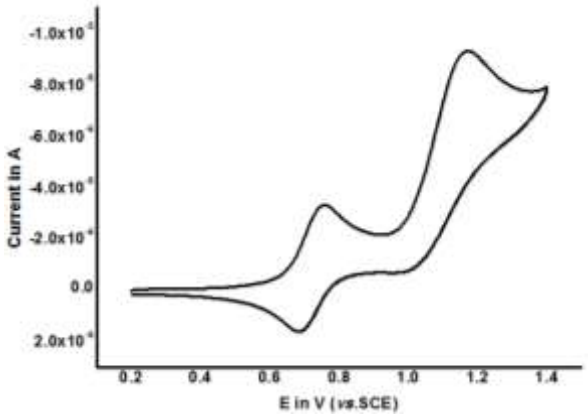
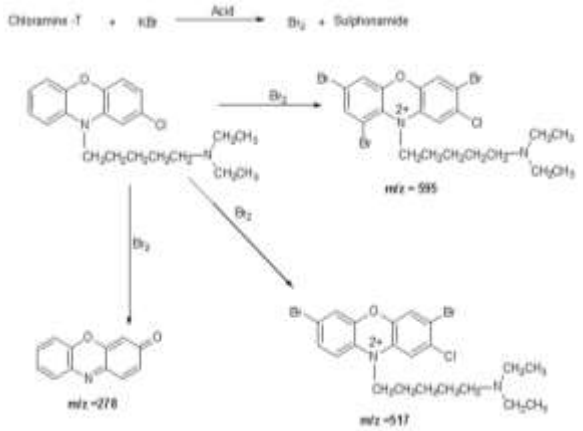


Figure 2: Cyclic Voltammogram of DPCP

Oxidation of DPCP by Chloramine-T Method



Scheme 2: Oxidation of DPCP

Chloramine-T oxidizes potassium bromide to bromine under acidic conditions. The liberated Bromine oxidizes **DPCP** to three products which were separated by HPLC and characterized by mass spectrometry. The mechanistic pathway for the oxidation DPCP by bromine is illustrated in **Scheme 2**. The mass spectral data supports the formation of the products predicted in the plausible mechanism. A signal at m/z 595 was assigned to the 3,6,8-tribromo derivative of [$DPCP^{2+}$], a signal at m/z 517 was assigned to 3,6-dibromo derivative of [$DPCP^{2+}$], and the signal at m/z 278 was assigned to 7-Bromophenoxazone. Similar oxidation products were reported for phenoxazines, N^{10}-substituted phenoxazine and 2, 10-disubstituted phenoxazines.[45,46] Bromine appears to have promoted the phenoxazine nucleus of **DPCP** to undergo electrophilic substitution reaction in positions 3, 6 and 8 along with two electron oxidation.

Determination of Formal Redox Potential and Transition Potential of DPCP

While choosing a redox indicator for a particular redox titration, it is necessary to ensure that its formal redox potential lies within those of the analytes redox potentials. The indicator must have suitable redox and transition potentials which should be within the potential break in the potentiometric titration curve. Schilt's method was used and the respective first and second formal redox potentials of DPCP were found to be 582 mV and 690 mV. Further for assessing the merit of the redox indicator, the transition potential is very helpful. Therefore, the transition potential of DPCP in the titration of ascorbic acid with CAT was determined and the value found to be 625 mV. The effect of sulfuric acid in the range of 0.5 -1.0 M on the transition potential was examined and it was found that the increase of acid concentration resulted in a slight decrease in the transition potential of DPCP.

Titration of Ascorbic Acid

In an acid medium, chloramine-T liberates bromine from potassium bromide. The liberated bromine oxidizes ascorbic acid to dehydroascorbic acid. In the titration of 0.05 - 0.005 N ascorbic acid against CAT, DPCP gives a sharp color change from colorless to blue in 1 M H_2SO_4, 1 M HCl, 2 M H_3PO_4 or 2.5 M AcOH **(Table 3).** Thus DPCP gives a sharp and reversible end point and the end point color is stable for 20 min. The effect of acidic strength on the indicator action is studied by varying the concentration of the acids and it is found that the indicator gives sharp end points in 0.4 - 1.5 M H_2SO_4, 0.5 - 2.0 M HCl,1.0 - 3.5 M H_3PO_4 or 1.0 - 4.0 M acetic acid solution containing potassium bromide. Premature end points were obtained at higher acidities and overstepping end points were obtained at lower acidities.

DPCP gives sharper end points, more accurate titre values and has less indicator corrections. Therefore it is advantageous over phenothaiazine type of indicators. Further, compared to phenothiazines (2 mL of 0.1 %) type of indicators, the amount of DPCP required for the indicator action is minimal (0.2 mL of 0.1%).

Determination of Ascorbic Acid in Real Samples

Before applying this method for the determination of the ascorbic acid in real samples, the effect of number of other substances commonly found in pharmaceuticals was first assessed. The following amounts of the tablet diluents and excipients do not interfere in the determination of 50 mg of ascorbic acid using DPCP as an indicator: citric acid (700 mg), oxalic acid (600 mg), tartaric acid (350 mg), starch (350 mg), gelatin (250 mg), talc (250 mg), dextrose (650 mg), reserpine (250 mg) and pulvisacacia (250 mg), stearic acid (250 mg), alginic acid (250 mg), sucrose (600 mg). An advantage of the proposed method is that organic compounds which are likely to be present along with ascorbic acid in commercial preparations and plant tissues do not interfere.

Determination of ascorbic acid in vitamin-C tablets [Celin (Glaxo), Suckee (IDPL) etc] by titrating against CAT using DPCP as a redox indicator is found to be most useful and accurate. The results obtained were compared with those found by the *o*-dianisidine method[47] and the official method of British pharmacopoeia[48]. It also agrees well with the claimed values on the label containing the tablets **(Table 4).**

The proposed method was also used to determine the ascorbic acid present in citrus fruit juices. The results obtained are comparable with those obtained by the published method.[49] **(Table 4).**

Titration of Methionine

In an acid medium, chloramine-T liberates bromine from potassium bromide. The liberated bromine oxidizes the sulphide group of methionine to sulphoxide. In the titration of 0.05 - 0.02 N methionine against CAT, DPCP gives a sharp color change from colorless to blue *via* pink in 1 M H_2SO_4, 1 M HCl, 2 M H_3PO_4.Thus DPCP gives a sharp and reversible end point and the end point color was stable for 15 min in H_2SO_4 or HCl and 10 min in H_3PO_4. The effect of acidic strength on the indicator action is studied by varying the concentration of the acids and it is found that the indicator gives sharp end points in 0.4 - 1.5 M H_2SO_4, 0.5 - 2.0 M HCl or 0.5 - 3.0 M H_3PO_4 containing potassium bromide. DPCP gives overstepping/late end points at lower acidities and premature end points at higher acidities. Sluggish end points were obtained in acetic acid medium.

DPCP has advantages over indigocarmine[49] in that it gives sharper end points and more accurate values and functions in three acid media, while indigocarmine works only in acetic acid medium. The results obtained are comparable with the reference potentiometric method (**Table 3).**

Determination of Methionine in Aminodrip

Before applying this method for the determination of the methionie in aminodrip, the effect of number of other substances commonly found in pharmaceuticals was first assessed.

The following amounts of the tablet diluents and excipients do not interfere in the determination of 50 mg of methionine using DPCP as an indicator: L-hystidine (33.75 mg), L-arginine (495.00 mg), lysine, L-tyrosine (56.25 mg), L-cystine (129.25 mg), L-tryptophan (0.55 mg), L-threonine (129.30 mg), L-leucine (756.75 mg), L-valine (157.50 mg), L-alanine (675.00 mg), L-proline (345.00 mg), L-isoleucine (106.80 mg), L-phenylalanine (253.05 mg), L-glutamic acid (450.00 mg), L-aspartic acid (360.00 mg), L-serine (165.00 mg) and glycine (1650.00 mg), L-hydroxyproline (315.00 mg).

Determination of methionine in aminodrip by titrating against CAT using DPCP as a redox indicator is found to be most useful and accurate. The results given **Table 4** are compared with reference potentiometric method and the values agrees well with the claimed values on the label containing the tablets.

Titration of Isonicotinic Acid Hydrazide

Isonicotinic acid hydrazide commonly called as isoniazid (INH) is the most important drug in the treatment of tuberculosis. This induced the researchers to workout methods for the rapid and accurate determination of INH. Among the various methods available, titrimetric methods are recently used. Some of the indicators used till time for the determination of INH using CAT are unsatisfactory for one or other reason. For example, methyl orange & methyl red indicator works only in the phosphoric acid medium.

In acid medium, chloramine-T liberates bromine from potassium bromide. The liberated bromine oxidizes INH quantitatively to nicotinic acid and nitrogen. In the titration of 0.05 - 0.01 N INH against CAT, DPCP gives a sharp color change from colorless to blue *via* pink in 1 M H_2SO_4, 1 M HCl, 3 M H_3PO_4. Thus DPCP gives a sharp and reversible end point and the end point color was stable for 5 min in all acid media. The effect of acidic strength on the indicator action is studied by varying the concentration of the acids and it is found that the indicator gives sharp end points in 0.5 - 2 M H_2SO_4 or HCl, 2.5 - 3.5 M H_3PO_4 solution containing potassium bromide. DPCP gives overstepping/late end points at lower acidities and premature end points at higher acidities.

Determination of INH in Real Samples

Before the proposed method was applied for the determination of the INH in real samples, the effect of number of substances commonly found in pharmaceuticals was first assessed.

The following amounts of tablet diluents and excipients do not interfere in the determination of 50 mg of INH: starch (300 mg), talc (250 mg), glucose (250 mg), gelatin (75 mg), citric acid (500 mg), oxalic acid (600 mg), stearic acid (150 mg) and alcohol (10 mL).

An advantage of the present methods is that organic compounds which are likely to be present with isonicotinic acid hydrazide in commercial preparations do not interfere.

The INH content in tablet Isokin and Isonex was determined and the results were given in **Table 4.**

The results obtained are comparable with reference potentiometric method[47] and the values agrees well with the claimed values on the label containing the tablets.

Titration of Phenylhydrazine Hydrochloride

Chloramine-T liberates bromine from acidified potassium bromide which oxidizes phenylhydrazinehydrochloride to benzenediazoniumchloride. In the titration of 0.05 - 0.01 N INH against CAT, DPCP gives a sharp color change from colorless to blue *via* pink in 0.5 M H_2SO_4, 1 M HCl or 1.5 M H_3PO_4. Thus DPCP gives a sharp and reversible end point and the end point color was stable for 3 min in all acid media. The effect of acidic strength on the indicator action is studied by varying the concentration of the acids and it is found that the indicator gives sharp end points in 0.2 - 1 M H_2SO_4,0.2 - 1.5 M HCl or 1.0 - 3.0 M H_3PO_4 solution containing potassium bromide. DPCP gives late end points at lower acidities and premature end points at

higher acidities. Sluggish end points were obtained in acetic acid medium.

DPCP has advantages over indigocarmine in that, it is used at laboratory temperature and gives sharper end points and more accurate values and functions in three acid media, while indigocarmine works at elevated temperature and only in hydrochloric acid medium. The results obtained are comparable with the reference potentiometric method (**Table 3).**

Determination of Biotin

Biotin also called as vitamin H is necessary for the growth of animals. Literature survey has been revealed that few analytical methods have been reported for the estimation of biotin[36-38], but they have not been widely accepted. For example, titrimetric procedure using iodine trichloride as an oxidant involves extraction step using organic solvent, which is tedious. Therefore the author believed that it is worthwhile to develop simple, inexpensive and accurate method for the estimation of biotin. Therefore DPCP has been proposed as redox indicator for the estimation biotin using chloramine-T. Chloramine-T liberates bromine from acidified potassium bromide which oxidizes sulphur atom of biotin to sulphoxide.[50]

In the titration of 0.01-0.0025 N biotin against CAT, DPCP gives a sharp color change from colorless to blue *via* pink in 1M H_2SO_4, 1 M HCl or 1.5 M H_3PO_4. Thus DPCP gives a sharp and reversible end point and the end point color was stable for 15 min in all acid media. The effect of acidic strength on the indicator action is studied by varying the concentration of the acids and it is found that the indicator gives sharp end points in 0.3 - 1.5 M H_2SO_4, 0.3 - 2.3 M HCl or 1.0 - 3.5 M H_3PO_4 solution containing potassium bromide. DPCP gives late end points at lower acidities and premature end points at higher acidities. Sluggish end points were obtained in acetic acid medium.

The results obtained are comparable with the reference potentiometric method (**Table 3)** and agreed well with the claimed values.

Effect of Bromide Concentration

The effect of bromide concentration on the estimation of ascorbic acid, methionine, isonicotinic acid hydrazide, phenylhydrazine hydrochloride and biotin was studied and it was found that the minimum amount of KBr required in the titrations involving 0.05 - 0.01 N reductant is 0.3 - 0.5 g in a total volume of 60 mL or in the titrations involving 0.01 - 0.005 N reductant is 0.20 - 0.40 g in a total volume of 35 mL. Higher concentrations (up to 3%) do not affect it but lower concentrations results in sluggish end points.

Effect of Indicator Concentration

At least 0.2 mL of 0.1 % DPCP in a total volume 60 mL or 0.05 mL of 0.1 % DPCP in a total volume of 35 mL was necessary for proper indicator action. Higher concentration of indicator greater than 0.4 mL or greater than 0.1 mL give higher titre values and lower concentrations give sluggish end points. The average indicator correction was found to be 0.05 mL of 0.05 N CAT for 0.2 mL of 0.1 % DPCP or 0.2 mL of 0.005 N CAT for 0.1 mL of 0.1 % DPCP indictor.

CONCLUSION

DPCP has advantages over Indigocarmine, phenothiazine and other indicators in that it gives sharper end points and more accurate values and functions in more than one acid media. It is used at laboratory temperature and has less indicator correction.

During the titration of ascorbic acid, methionine, isonicotinic acid hydrazide, phenyl hydrazine hydrochloride and biotin, it was found that DPCP undergoes one electron oxidation to give a radical cation which is pink in color. The radical cation undergoes further one more electron oxidation to give a blue colored dication at the equivalence point. The redox and the transition potentials for DPCP have been determined and the values lie within the potential break in the potentiometric titration of the reductants with CAT in the presence of H_2SO_4, HCl and H_3PO_4.

Further, the formal and transition potentials indicates that DPCP serves as a good indicator in the titration of the reductants with CAT.

In the titration of the Methionine, INH and Biotin, if the indicator is added at the beginning of the titration, no clear end point was obtained because of the partial destruction of the indicator.

When titrating unknown samples, an approximate titre value was found by adding 1 - 2 mL of 0.1 % DPCP at the beginning of the titration and then correct titre value was found by adding the indicator near the end point.

Acknowledgement: The authors are grateful to University Grants Commission (UGC), Government of India, for financial support.

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Source of Support: Nil, **Conflict of Interest:** None.