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Research Article

TITRIMETRIC DETERMINATION OF ASCORBIC ACID AND ISONICITONIC ACID HYDRAZIDE IN DRUG FORMULATIONS WITH CHLORAMINE-T AS OXIDANT

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ABSTRACT:

A simple and titrimetric method was developed for the determination of ascorbic acid and Isonicitonic acid hydrazide in drug formulations with chloramine-T and O-ainsidine as indicator. Inspite of the beautiful red colored oxidized product of O-anisidine, the studies on its application in analytical techniques are scanty. So, authors have taken up the investigation on the utility of O-anisidine as a new analytical reagent in the chloramine-T indicator reaction. The detailed reaction between chloramine-T and O-anisidine has enabled the authors to utilize O-anisidine as indicator in titration of ascorbic acid and isonicitonic acid hydrazide. Suitable conditions has been established with different acids viz., hydrochloric acid, sulfuric acid, phosphoric acid, and acetic acid to give sharp colour change at the equivalent point. The present method has been applied for the estimation of ascorbic acid and also isonicotonic acid hydrazide (INH) in pharmaceutical formulations and results obtained are in good agreement with the values obtained by standard methods.

Key Words: O-Anisidine, Chloromine-T, Ascorbic acid, Isonicitonic Acid Hydrazide.

INTRODUCTION:

O-Anisidine is used as a reagent for the spectrophotmetric determination of gold by Jenic Joseph.et.al[1] in biological samples and also in some salts such as silver nitrate in acid media. In this paper, authors have carried out titrimetric determination of Ascorbic Acid and Isonicitonic Acid Hydrazide, utilizing O-

Anisidine as a new redox indicator in chloramine-T (CAT) titration. CAT is the sodium salt of N-chloro-p-tolune sulphonamide, $CH_3C_6H_4SO_2NClNa$ .It is a colourless crystalline solid and exists as the trihydrate. It is soluble in water as well as in organic solvents such as acetic acid [2,3] Puttaswamy et.al[4] reported kinetics of ruthenium (III) - and osmium(VIII)-catalyzed oxidation of ornidazole with chloramine-T in acid and alkaline media. Mechanistic aspects for the oxidation of sunset yellow dye by chloramine-T in presence of perchloric acid and in sodium hydroxide medium catalyzed by Os(VIII) was reported by Vinod.et.al [5]. Saldanha et.al[6], Basavaiah[7], Jacob and Nair[8], Gowda and Mahadevappa[9] and Hoda [10] reported oxidations reactions with chloramine-T. Various indicators in chloramine-T titrations are summarized in **Table-1.**

Indicator	Analyte(s)	References
Methyl Red	Ascorbic acid	Verma[18]
Indigo Carmine	Methionine and its metal complexes	Gowda and Mahadevappa[9]
	Fe(ll)	Atanas'ev and Vral'skaya[31]
Variamine Blue	Ascorbic acid	Erdey and Kalpar[14]
Starch-Iodide	As(lII), Sb(III), Sn(Il) and Fe(II)	Macillan and Easton[32]
	I-, hydrazine, As(III), Sn(Il),	Singh et al[19]
	Hg(I), SCN~ and Fe(II)	Verma[18]
Chloroform + IC1	Ascorbic acid	Singh et al.[33]
	I-, hydrazine, As(III), Sn(II),	
Cacotheline	Hg(I), SCN~, Fe(II) and tartar	Krishna Murthy et al.[19]
1, 4-di-amino-2,3-dihydroanthraquinone	emetic. Sn(Il), Ti(III) and ascorbic	Captain et ai.[20,21]
Capri blue, Solochrome prune	Acid	Rao and Rao[24]
As, Gallamine Blue, Celestine Blue, Meldola's Blue, Cresyl	As(III) and ascorbic acid	
Fast Violet -Acetate and Resazurin	As(III), Sb(III), T1(I), hydrazine, hydroquinone	
N-substituted phenothiazines	and ascorbic acid	Gowda and Ahmed[22,23]
	Hydroquinone, metol, ascorbic acid	
Phenosafranine, methylene violet, Amethyst violet, safronine-T, Wool fast blue BL and Aposafranine	As(llI), Sb(lll), hydroquinoneT ascorbic acid, hydrazine and INH	Rao & Sastri[30]
Azine dyes		Rao and Kumari[25]

Brucine	Ascorbic acid, hydroquinone, 1NH, and hydrazine	Rambabu[34]
Methyl orange	Ascorbic acid, Cu(l), Hydrazine, As(lII), Sb(lII), Sn(II), Ti(III), Fe(II) Cu(I)	Murthy[35]

Materials and Methods

Reagents

Chloramine –T

Approximately 0.1N solution of chloramine-T is prepared by dissolving 15 g of the sample (M & B) in one litre of demonized water. The solution is standardized against standard arsenic (III) solution in sodium bicarbonate medium as per the method of Bishop and Jennings[11]. The standardization can also be carried out against standard potassium iodide solution through sodium thiosulphate using starch as indicator.

Stability

Dietzel and Taufel[12] have observed that there is a decrease in purity of CAT by 1.4% in 12 months, when the sample is stored in a brown glass bottle and by about 5% when stored in a clear glass bottle. According to Rao et al[13] solutions of CAT in 0.3 - 2.0 M perchloric acid, 0.1 - 2.0 M sulphuric acid and 2.0 M sodium hydrate, as well as solutions of pH values 7.7 and 9.18, are highly stable. Solutions of CAT in hydrochloric acid are stable only in media of 0.1 - 0.3 M and there is a decrease in stability above 0.3 M. These authors ascribe the decrease to the oxidation of chloride to chlorine by CAT. Bishop and Jennings[11] have made extensive investigations on the stabilities of aqueous chloramine-T solutions (0.05 N) in clear glass and brown glass bottles. From their observations, they have concluded as follows: 1. Solutions stored in brown glass bottles do not show any decrease in strength for quite some time, when stored in dark or day light. 2. The decrease is perceptible after five months and is about 0.5% in one year. 3. Exposure of solutions of CAT to day light resulted in a continuous and immediate drop in the strength of the solution, the drop being 0.4% in the first week and about 20% in one year.

EDTA

ETDA (0.5 g per 1000 ml) is used as stabilizer for the ascorbic acid solution which is standardized against standard chloramine-T solution using variamine blue as indicator[14].

O-Anisidine

1% (or) O-anisidine in 2% Methanol is prepared from Aldrich chemical company, INC, USA. The Solution is diluted with triply distilled water and stored in amber-coloured bottle. O-Anisidine has been standardized by Spectrophotometric[15], chromatography[16] or TLC [17].

Ascorbic Acid

0.1N ascorbic acid solution is prepared by dissolving required quantity of reagent grade sample of the substance in triply distilled water and diluting to desired volume. A small quantity of EDTA (0.5g per L) is added as stabilizer. The ascorbic acid solution thus prepared is standardized against potassium bromate using (p)–ethoxy chrosidine as Indicator.

Isonicitonic acid Hydrazide solution (INH)

0.1N solution of INH is prepared by dissolving the required amount of substance (fluka) in triply distilled water and solution is standardized as per standard method.

Titration of Ascorbic Acid

Verma[18] proposed starch-iodide and methyl red as indicators in the titration of ascorbic acid with chloramine-T. Other indicators used for this titration are: Cacotheline[19], anthraquinone derivatives[20, 21], N-substituted phenothiazines[22, 23], oxazine dyes and azine dyes [24, 25, 26].

Procedure

5.0 ml of 0.1030N ascorbic acid is taken in the titration vessel, required amount of acetic, hydrochloric, sulphuric or phosphoric acid to give desired concentration were added and 0.1 ml of 1% o-anisidine indicator and distilled water added to total volume of 50ml. The titration is carried out with 0.1079N chloramine-T to a colour change from colourless to yellow. By the experimental observations it is found that the indicator is not functioning well for any concentrations of the acetic and phosphoric acid. No sharp colour change is observed even after the addition of excess of oxidant. The colour change of indicator at higher titer values are observed, but in case of hydrochloric acid medium between 0.5-6.0N the indicator functioned satisfactorily. The colour change

of the indicator is from colourless to yellow. In case of sulphuric acid medium between 0.5-6.0N the indicator also functions satisfactorily. Stiochiometric results are observed and the colour change of the indicator is from colourless to yellow. Some typical results are given in Table-2.

Table-2: 5.0ml 0.1030N ascorbic acid = 4.80 ml of 0.1079N CAT.

Overall strength of acid, N	Volume of CAT consumed, ml	Observations
Hydrochloric acid		
0.5	4.80	Colourless to yellow and is sharp.
2.0	4.80	The colour change is very sharp
4.0	4.80	Same as above
6.0	4.80	Same as above
Sulphuric acid		
0.5	4.80	Indicator transition is sharp.
2.0	4.80	Colourless to yellow and is very sharp
4.0	4.80	Same as above
6.0	4.80	Same as above
Acetic acid		
0.5	4.70	Colourless to red and is at higher value.
2.0	4.70	Same as above
4.0	4.70	Same as above
6.0	4.70	Indicator transition is not sharp.
8.0	4.70	Same as above
Phorphoric acid		
4.0	4.70	The indicator colour change is at higher value.
6.0	4.70	Same as above
8.0	4.70	Same as above
10.0	4.70	Colour transition is not found
12.0	4.70	Same as above

Effect of Indicator concentration:

The effect of concentration of O-anisidine is also studied using different volumes of the indicator at 2.0N hydrochloric and 2.0N sulphuric acid concentrations, in titrating ascorbic acid with chloramine-T. The colour change of the indicator is sharp from colourless to yellow in 0.05-0.3 ml of 1% O-anisidine, therefore 0.1 ml of indicator is prescribed for the titration of ascorbic acid with CAT. Some typical results are given in the Table-3.

Table-3: 5 ml 0.1036N ascorbic acid = 4.8 ml of 0.1079N CAT.

Volume of Indicator, ml	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
Volume of CAT, (ml)	4.8	4.8	4.8	4.8	4.7	4.7
In hydrochloric acid, 2N						
In sulphiric acid, 2N	4.8	4.8	4.8	4.8	4.7	4.7

Recommended procedure:

An aliquot of 0.1030N ascorbic acid is taken in the titration vessel, an overall acidity of 2.0N hydrochloric or 2.0N sulphuric acid is maintained in a total volume of 50 ml, 0.1 ml of indicator is added and the titrations carried out with 0.1046N chloramine-T to a colour change from colourless to yellow and is found to be very sharp. Some typical results of the estimation of ascorbic acid are given in **Table-4.**

Estimation of ascorbic acid with CAT

Amount of ascorbic acid, mg		
Taken	**Found**	**Relative error (%)**
Sulphuric acid, 2N		
5.032	5.045	- 0.258
7.504	7.504	Nil
10.026	10.026	Nil
20.082	20.060	0.109
30.051	30.042	0.029
40.062	40.048	0.034

Hydrochloric acid, 2N		
5.032	5.027	0.009
7.504	7.504	Nil
10.026	10.012	0.013
20.082	20.060	0.010
30.051	30.040	10.036
40.062	40.062	Nil

Reverse titration:

Reverse titration is carried out for the estimation of chloramine-T with ascorbic acid by adopting same procedure in different hydrochloric, sulphuric, acetic and phosphoric acid concentrations. It is found that the indicator is not functioning well at any acid concentrations. So reverse titration is not recommended for the titration of chloramine-T with ascorbic acid.

Application of the Developed Method

The indicator method now developed have been applied for the determination of the ascorbic acid contents of commercial pharmaceutical preparations of vitamin 'C' tablets and the results obtained are in excellent agreement with those obtained by other standard methods. The following procedure is adopted for preparing solutions of ascorbic acid from the tablets. One tablet is ground to fine powder, dissolved in deionised water, filtered through G-4 sintered glass funnel and made upto 100 ml. A known volume of this solution is treated with two drops of 0.1% O-anisidine, the mixture is diluted to 50 ml and titrated with 0.IN chloramine-T solution. A similar aliquot is titrated with the CAT solution using starch as indicator. The above procedure is repeated with different commercial samples of vitamin 'C' and typical results obtained in these two methods are incorporated in

Table-5.

Trade name and manufacture	Indicator and amount of ascorbic acid found in g.	
	Starch	0-anisidine
Celin, Glaxo	0.05 ± 0.01	0.05 ±0.01
Sorvocin, E1PW	0.49 ± 0.01	0.50 ±0.01
Suckcee, IDPL	0.49 ± 0.01	0.49 ±0.01
Chewcee, Lederle	0.50 ± 0.01	0.50 ±0.01

Titration of Isonicotinic Acid Hydrazine

Naer et al.[27] proposed an indirect iodometric method for the titration of Isonicotinic acid hydrazide (INH) with chloramine-T. Berka and Zyka as well as Pinzauti et al.[28] have reported direct potentiometric titrations of INH with chloramine-T. Rao and Kumari[29] and Rao and Sastri[30] used azine dyes as visual indicators in the titration of chloramine-T with INH in hydrochloric acid medium. They have extended their studies for the determination of INH in commercial pharmaceutical samples. The authors of this paper have undertaken a study of the feasibility of the INH - chloramine-T titration using O-anisidine as indicator. The results of these studies are given in this section.

Procedure

In order to establish the optimum conditions for the determination of isonicotinic acid hydrazide (INH), the author has carried out the following experiments in different acid media. 5.0 ml of 0.1079N INH is taken in the titration vessel, required amount of hydrochloric, sulphuric, acetic or phosphoric acid are added to give the desired concentration and 0.1 ml of 1% O-anisidine indicator and distilled water added to a total volume of 50 ml. The titration is carried out with 0.1079N chloramine-T to a colour change from colourless to pinkish red. Some typical results are given in Table-6.

Table-6: 5.0ml 0.1040N INH = 4.81 ml of 0.1079N CAT

Overall strength of acid, N	Volume of CAT consumed, ml	Observations
Hydrochloric acid		
0.5	5.0	Colour transition is not found.
2.0	5.0	Same as above
3.0	5.0	Red colour developed slowly near the end point, not sharp.
4.0	5.0	Same as above
5.0	5.0	Same as above
Sulphuric acid		
0.5	5.00	The indicator colour change at higher value.
2.0	4.90	Same as above
4.0	4.90	Same as above
6.0	4.90	Indicator transition is not sharp.
Acetic acid		
0.5	4.81	Colourless to red and waiting for 15 sec. is necessary near the endpoint.
2.0	4.81	The colour change is very sharp
4.0	4.81	Same as above
6.0	4.81	Same as above
Phosphoric acid		
4.0	5.00	Turbid solution is obtained, not sharp.
6.0	5.00	Same as above
8.0	4.81	Indicator colour change is colourless to red on heating
10.0	4.81	Same as above
12.0	4.81	Sharp colour change from colourless to red
14.0	4.81	Same as above.

From the experimental observations it is found that the indicator is not functioning well at any concentrations of hydrochloric and sulphuric acid, no sharp colour change is observed. Red colour is developed at the higher volumes of the titrant, but in case of acetic acid medium between 0.5-6.0N the indicator functioned satisfactorily. The colour change of the indicator is from colourless to pink. In case of phosphoric acid medium between 8.0-14.0N the indicator is functioning satisfactorily at 40°C by heating and the colour change of the indicator is from colourless to red.

Effect of indicator concentration:

The effect of concentration of O-anisidine is also studied using the different volumes of the indicator at 2.0N acetic and 10.0N phosphoric acid concentrations, in titrating INH with CAT. The colour change of the indicator is sharp from colourless to pink in 0.05-0.5 ml of 1% O-anisidine. So 0.2 ml of indicator is recommended for the titration of INH with CAT. Some typical results are given in Table-7.

Table-7: 5 ml 0.1040N INH = 4.81 ml of 0.1079N CAT.

Volume of Indicator, ml	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
Volume of CAT, ml	4.81	4.81	4.81	4.81	4.81	4.81
In Acetic acid, 2N						
In phosphoric acid, ION	4.81	4.81	4.81	4.81	4.81	4.81

Recommended procedure:

An aliquot of 0.1040N INH is taken in the titration vessel, an overall acidity of 2.0N acetic or 10.0N phosphoric acid is maintained in a total volume of 50ml, 0.2ml of indicator is added and the titration is carried out with 0.1079N CAT to a colour change from colourless to red. Some typical results of estimation of INH are given in Table-8.

Table-8: Estimation of INH with CAT.

Amount of INH, mg		
Taken	**Found**	**Relative error (%)**
Acetic acid, 2N		
1.090	1.090	0.642
2.693	2.693	Nil
10.801	10.801	Nil
15.604	15.592	0.076
20.806	20.708	Nil
Phosphoric acid, 12N		
1.090	1.090	Nil
2.693	2.682	0.408
10.801	10.812	-0.101
15.604	15.604	Nil
20.806	20.813	- 0.033

Reverse titration:

Reverse titration is carried out for the estimation of CAT with INH by adopting same procedure in different hydrochloric, sulphuric, acetic and phosphoric acid media. It is found that the indicator is not functioning well at any acid concentrations. So reverse titration is not recommended for the titration of CAT with INH.

Application of the developed Method

The indicator method now developed can be successfully adopted for the determination of INH contents of commercial tablets as per the following procedure. One of the tablet is ground to a fine powder, dissolved in demonized water, the solution is filtered through a G-4 sintered glass funnel and diluted to a suitable known

volume. Aliquots of this solution are titrated using O-anisidine as indicator in l.0N phosphoric acid medium, with 0.1N CAT solution. A similar aliquot is also titrated with chloramine-T solution using safranine-T as indicator[30]. The values are in excellent mutual agreement as can be seen from the results given in **Table-9**.

Trade name and manufacture	Indicator and amount of INH, g.	
	Safranine-T	**o-anisidine**
Isonex, Dumox pharmaceuticals	0.30 ±0.01	0.30 ±0.01
Nydrazid, Squibb	0.29 ±0.01	0.20 ±0.01
Isokin, Park-Davis		
Wanner-Lambent, USA	0.29 ±0.01	0.29 ±0.01
Docina-306	0.30 ±0.01	0.30 ±0.01

SUMMARY

The salient features of the studies on the application of o-anisidine as indicator in titrations of various reductants with chloramine-T are as follows:

1. A very sharp colour change from light yellow to red was observed for 0.1 ml of the indicator for a fraction of 0.1N chloramine-T solution exactly at the equivalence point in titrations with different reductants.
2. The titrations were feasible in sulphuric acid, phosphoric acid, hydrochloric acid and acetic acid media.
3. The analytes chosen in this investigation are Ascorbic acid, Isonicotinic acid hydrazide, highly satisfactory results obtained with a relative error of 0.1% with respect to the standard method. From these results the author used O-anisidine as indicator for the estimation of Ascorbic acid and Isonicotinic acid hydrazide in pharmaceutical formulations.
4. In view of the sharp colour change obtained with small amount of indicator solution and its applicability in titrations of different reductants, the advantage of its low cost and easy availability the author feels that o-anisidine can be claimed as one of the best indicators in these titrations.

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