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구리수은막 전극에을 사용한 이소니아자이드의 전위차 역적정

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Potentiometric Back Titration of Isoniazid in Pharmaceutical Dosage Forms Using Copper Based Mercury Film Electrode

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요 약. 구리수은막 전극(CBMFE)으로 전위차 역적정함으로써 이소니아자이드(INH)를 정량하는 간단하고 빠른 방법이다. 순수한 형태와 투약형태에 대해서 1.0-10.0 mg 범위에서 정량 할 수 있도록 적정조건을 설정하였다. 방법의 정밀도와 정확도는 통계적인 방법으로 평가되었으며, 정제와 시럽속에 함유된 INH 정량법은 F-시험과 t-시험을 통하여 영국약전(BP) 방법과 비교하였다.

주제어: 이소니아자이드, 구리수은막전극, 역적정

ABSTRACT. A simple, rapid potentiometric back titration of Isoniazid (INH) in the presence of Rifampicin (RIF) in tablets and syrups is described. The method is based on the oxidation of INH by a known excess of copper (II) ion and the back titration of unreacted copper (II) ion potentiometrically with ascorbic acid using a lab-made Copper Based Mercury Film Electrode (CBMFE). The titration conditions have been optimized for the determination of 1.0-10.0 mg of INH in pure and dosage forms. The precision and accuracy of the method have been assessed by the application of *lack-of-fit test* and other statistical methods. Interference was not caused by RIF and other excipients present in dosage forms. Application of the method for INH assay in tablets and syrups was validated by comparison of the results of proposed method with that of the British Pharmacopoeia (BP) method using *F*- and *t*- statistical tests of significance.

Keywords: Potentiometric back titration, Isoniazid, Pharmaceutical analysis, Copper based mercury film electrode, Statistical analysis

INTRODUCTION

The World Health Organization (WHO) has estimated that one third of the world's population is infected by Mycobacterium Tuberculosis (TB).[1-2] The treatment of TB is improved with the anti tuberculosis drugs such as Isonicotinic acid hydrazid(INH), Rifampicin (RIF) and Pyrazinamide (PZA). The determination of these drugs in vitro and in vivo has been reported.[3-5] The assay of INH has been reported in comprehensive reviews.[6-8] Zhang *et al.* have monitored spectrophotometrically the reduction of Cu (II) to Cu (I) by INH.[9] Many methods have been developed for the assay of INH based on its oxidation by many metal ions and its basicity.[9-11] Other methods include continuous-flow chemiluminescence method using reagents such as N-bromosuccinimide,[12] chromatographic techniques such as HPLC,[13] capillary gas chromatography,[14] spectrophotometry,[9,15] colorimetry,[16] cyclic voltammetry.[17]

The widely used British Pharmacopoeia (BP) method recommends visual titration of INH with bromate/bromide solution in acidic medium.[18] Visual titrimetric methods cannot be applied for the INH assay in the presence of RIF due to the intense red color of RIF. Since INH is autooxidized in solutions, direct potentiometric titration of INH is difficult.[11] Many potentiometric methods have also been reported for the assay of INH by making use of commercially available ion selective electrodes[19-23] which are either costly or not readily available in the market. Since copper based mercury film electrode (CBMFE) is cost effective, it has been applied as an indicator electrode

for the assay of INH,[24] ascorbic acid,[25-26] sulphamethoxazole.[27]

The present work deals with the potentiometric back titration of INH in the presence of RIF and vitamin B_6in tablets and syrups.The interferences of RIF and vitamin $B_6$are not significant in the proposed method. INH was treated with a known excess of copper (II) and the unreacted copper (II) was potentiometrically determined by titration against ascorbic acid making use of CBMFEas an indicator electrode.The experimental data were analyzed statistically to validate the proposed method. The *lack-of-fit test* which involves the application of analysis of variance in regression analysis was adopted for assessing the data obtained in replicate analysis of pure INH.[28-31] The results obtained in the analysis of INH in pharmaceutical dosage forms were statistically compared with that of BP method by application of F and t-test.[32]

MATERIALS AND METHODS

Materials

Tablets: Isokin tablets (Pfizer Ltd., Hyderabad, India), isocaldin tablets (Retort Laboratories, Madhavaram, Chennai), Rifa i-6 tablets (Concept pharmaceuticals Ltd., santacruz (E), Mumbai, India), R- Cinex 600 tablets (LUPIN Ltd., Mahavir Nagar, Vapi, India).

Syrups: Isocaldin Retort syrup (Retort Laboratories, Madhavaram, Chennai), Docina syrup (Ashok Pharmaceuticals, Trustpuram, Chennai, India). Pure INH was bought from Sigma Aldrichchemicals. All other chemicals used were of analytical grade and were prepared as given below.

INH solution (0.025 M): INH solution was prepared by dissolving 0.3429 g in 100 mL graduated flask and made up to the mark by using deaerated water, and standardized by the BP method.[18]

Ascorbic acid solution (0.05 M): Ascorbic acid solutionwas prepared by dissolving 0.8807 g in 100 mL graduated flask and made up to the mark by using deaerated water and standardized potentiometrically by titration with potassium hexacyanoferrate (III).[33]

Copper sulphate solution (0.1 M): Copper sulphate solution was prepared by dissolving 6.242 g of copper (II) sulphate pentahydrate in 250 mL graduated flask and made up to the mark.

Mercury (II) nitrate solution (0.02 M): Mercury (II) nitrate solutionwas prepared by dissolving 0.34 g of mercury (II) nitrate monohydrate in 80 mL of distilled water containing 2 mL of nitric acid (2 M), and diluted to 100 mL in a graduated flask.[34]

Ammonium thiocyanate solution (0.5 M): Ammonium thiocyanate solutionwas prepared by dissolving 9.515 g in 250 mL graduated flask and made up to the mark by using deaerated water.

Instrumentation

Potentiometric titrations were carried out using CBMFE as an indicator electrode and saturated calomel electrode as a reference electrode using a pH meter (Elico, LI-120, India).

Fabrication of CBMFE

CBMFE was prepared as reported in our earlier publications.[24-26] A plastic sleeved copper wire (99% purity) of 10 cm length and 1 mm thickness was taken and plastic sleeve was removed at both ends to expose about 1 cm copper wire. Epoxy seal was applied at the junction of copper wire and plastic sleeve to impede entry of solution in to the sleeve. The exposed 1 cm portion of one end of the wire was polished by abrasion with a fine emery paper and washed with water followed by treatment with concentrated HNO_3 for a few seconds and finally rinsed with water. When the polished wire was dipped in acidified mercury (II) nitrate (0.02 M) solution for 10 min, a thin layer of mercury got deposited over the polished wire due to reduction. Finally, the electrode surface was gently wiped with a filter paper and rinsed with water.

Back titration of INH in pure form

An aliquot containing 1.0-10.0 mg of INH was transferred in to a titration cell followed by the addition of 5.0 mL of acetate buffer solution (pH 5.0) and 2 mL of ammonium thiocyanate (0.5 M) solution. A known excess of copper (II) sulphate(1.0-3.5 mL; 0.1 M) was added to the contents of the titration cell while a light yellow colored precipitate was formed. The contents were stirred gently and the excess copper sulphate was titrated against ascorbic acid solution (0.0125 M-0.05 M) using CBMFE as an indicator electrode, and double junction saturated calomel electrode as a reference electrode.

INH assay in pharmaceutical dosage forms

The following pharmaceutical samples were prepared with deaerated water and kept protected from light.

Tablets: Twenty tablets containing INH were weighed and powdered. An appropriate amount of powdered sample equivalent to 300 mg of INH was dissolved gently in about 30 mL of deaerated water and the residue was filtered using Whatmann-41 filter paper and washed well.

The combined filtrate and washings were made up to 100 mL graduated flask and 2.0 mL of the solution was taken for titration.

Syrups: A certain volume of the syrup solution equivalent to about 300 mg of INH was transferred into a 100 mL graduated flask and made up to the mark and 2.0 mL of this solution was taken for the titration.

RESULTS AND DISCUSSION

CBMFE as a potentiometric sensor

CBMFE can be prepared readily within ten minutes by the proposed inexpensive method using a commercially available copper wire. The electrode showed potentiometric response towards Cu (II) in solution. The potentiometric response characteristics of CBMFE such as stability, reproducibility etc. were exhaustively studied by the corresponding author and reported earlier.[26] The freshly prepared CBMFE had a uniform, adhesive and stable film of metallic mercury of thickness 1.5 μm over the surface of copper. The mercury layer deposited on copper wire consists of stable mercury compound with the base metal and uniform metallic layer of mercury on it. The ability to form a film of uniform thickness results from the interaction of mercury with copper.[35] The mercury film deposited contained about 0.003% w/w dissolved copper at 20 °C. The presence of copper in the mercury film may be responsible for CBMFE's response towards Cu(II) in solutions.

Reaction involved in pretreatment and titration

When INH is treated with a known excess of Cu (II), INH is oxidized by copper (II) ion to isonicotinic acid in acidic medium.[9] The redox reaction involving four electron transfers can be written as

$$C_5H_4NCONHNH_2 + 4\ Cu^{2+} + H_2O \rightarrow 4\ Cu^{+} + C_5H_4NCOOH + N_2\uparrow + 4\ H^{+} \quad (1)$$

This reaction is facilitated by the pale yellow colored complex formation due to the reaction of Cu^{+} with isonicotinic acid(INA) formed in the reaction-1 in the presence of SCN^{-} ion.[36]

The unreacted copper (II) was back titrated against ascorbic acid after the treatment of INH with a known excess of copper (II). Copper (II) gets quantitatively reduced by ascorbic acid to copper (I) which is stabilized by thiocyanate ion as cuprous thiocyanate[37] ($K_{sp}=4.8\times10^{-15}$).

$$C_6H_8O_6 + 2\ Cu^{2+} \rightleftarrows 2\ Cu^{+} + C_6H_6O_6 + 2\ H^{+}\ E^0 = 0.376\ V \quad (2)$$

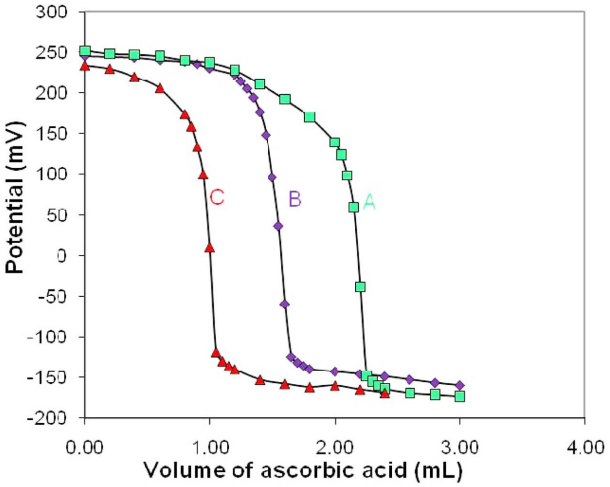

***Fig.* 1.** Potentiometric back titration of INH.

Calculation of equilibrium constant ($K_{eq}=12.66$) based on the formal redox potential in acidic medium indicates the feasibility of oxidation of ascorbic acid to dehydroascorbic acid by Cu (II) ion.

Back titration of INH

The experimental conditions were optimized for the back titration of 1.0-10.0 mg of INH. During the potentiometric titration, stable equilibrium potential was instantly established after each addition of the titrant. However, near the end point, a wait of one minute was necessary. The titration curve was well developed and a potential break of 300 mV was observed for the addition of 0.05 mL of ascorbic acid (0.03M) (*curve*B in *Fig.* 1).The detection limit of the proposed method was 1.0 mg. Below 1.0 mg, results were not precise and accurate.

Attempts to titrate INH directlywith copper (II) did not yield reproducible quantitative recoveries of INH, and the low end point value was obtained without much change in potential break which could probably be due to auto oxidation of INH partly.[11]

Effect of pH in the potentiometric back titration of INH

Fig. 2 shows the potentiometric curves of the back titration of INH at various pH levels. The end point corresponding to the quantitative recovery was reproducible in the pH range 4.0 to 6.5 and end point volume varied out of the pH range. Above the pH6.5, the variation in end point could be due to hydrolysis of Cu (II) and aerial oxidation or extensive degradation of ascorbic acid in basic medium.[38]

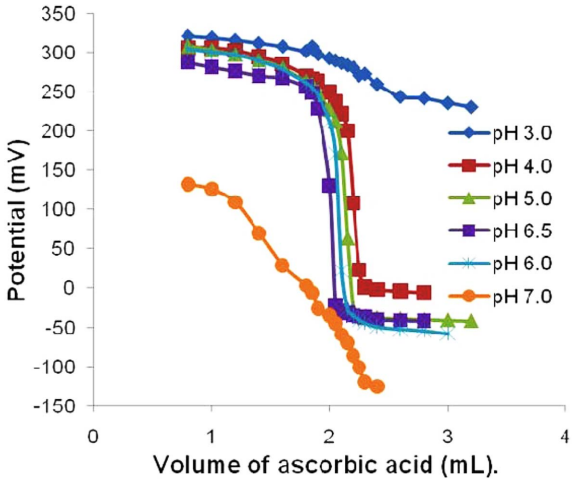

***Fig.* 2.** Effect of pH in the potentiometric back titration of INH.

Below pH 4.0, the oxidation of ascorbic acid could be slow causing variation in end point.[39]

Precision and accuracy

Five standard solutions of INH of different concentrations were prepared. Sixreplicate analyses were carried out on each of these in order to assess the precision and accuracy of the proposed method. The results obtained are presented in *Table* 1. The overall percentage relative deviation (co-efficient of variations) for thirty determinations was 0.523%. It indicates that the proposed method is precise and free from random errors. Over all standard analytical error for thirty determinations was 0.0076%. The overall mean recovery was 99.78 % which indicates that the proposed method was accurate.

Student's t-test to detect systematic error

In order to detect any systematic error associated with the analysis, two tailed t-test was applied on the experimental data given in *Table* 1. The amount taken for analysis (μ) at each concentration level was compared with the amount found ($\bar{x}$) by Student's t-test. The Student's t-value was calculated at each concentration level using equation $[|\mu-\bar{x}|/(s/\sqrt{n})]$. The hypothesis considered for the testing was that the amount found ($\bar{x}$) by six replications of analysis did not differ significantly from the amount (μ) taken. The mean value of Student's t-value calculated for five concentrations was 1.01 which was less than the critical value of 2.57 at 5% level of significance and five degrees of freedom. Thus the hypothesis was retained to make decision that amount found by the analysis did not differ from that taken. It also indicated that the proposed method is free from any systematic error.

Lack-of-fit test to detect systematic error

A regression analysis of the amount taken for analysis Vs the amount found by six replications at each concentration level was performed for the data given in the *Table* 1 and a significant test namely *lack-of-fit test* was performed to check whether the data fit a linear model indicating the agreement between the amount taken and found in the analysis. In the test, a hypothesis stating the data to fit a linear model is considered and an F-value is calculated.[28,29] The calculated value of F was 0.53 which was less than the critical value of 2.99 at 5% level of significance and 3, 25 degrees of freedom.It also indicated that the proposed method is free from any systematic error.

Study of possible sources of interference

In order to apply the proposed method for the assay of INH in pharmaceutical preparations, six replicated determinations of 5.0 mg of INH in the presence of two fold amount of vitamin B_6, three fold amount of RIF were carried out. The results of interference analysis showed that error did not exceed 1-2% and the method can be applied for the INH assay in pharmaceutical preparations.

Determination of INH in the presence of vitamin B_6 in pharmaceutical preparations

The proposed method was successfully applied for the assay of INH in pharmaceutical preparations. Six replicate determinations were performed by the proposed method

***Table* 1.** Results of six replicate determinations of INH by proposed method and statistical analyses of the data

S. No.	Amount taken (mg), (μ)	Amount found (mg)	Mean ($\bar{x}$) ± Std.dev	% Mean recovery	Std. anal. error (s/√n)	Student's t-test $[|\mu-\bar{x}|/(s/\sqrt{n})]$
1.	1.47	1.47, 1.47, 1.46, 1.46, 1.48, 1.46	1.466 ± 0.0082	99.72	0.0033	1.20
2.	3.41	3.40, 3.39, 3.39, 3.40, 3.42, 3.42	1.405 ± 0.0138	99.64	0.0056	0.88
3.	5.12	5.10, 5.90, 5.14, 5.11, 5.10, 5.13	5.115 ± 0.0187	99.90	0.0076	0.65
4.	6.72	6.70, 6.73, 6.69, 6.69, 6.75, 6.71	6.711 ± 0.0240	99.86	0.0097	0.92
5.	8.30	8.26, 8.27, 8.26, 8.27, 8.33, 8.31	8.283 ± 0.0294	99.79	0.0120	1.41
			Mean	**99.78**	**0.0076**	**1.01**

The calculated value of F in lack-of-fit test was 0.53

***Table* 2.** Results of six replicate determinations of INH by proposed method and BP method in pharmaceutical preparations and statistical analyses of the data

S. No.	Brand name & stated amount	Amount found by proposed method (mg) Mean ± Std. dev.	Amount found by BP method (mg) Mean ± Std. dev.	F*	t*
	Tablets				
1.	Isocaldin (300 mg)[a]	293.60 ± 4.5887	293.98 ± 2.2043	4.33	0.18
2.	Isokin (300 mg)[b]	301.25 ± 2.9840	297.56 ± 2.0584	2.10	2.49
	Syrups				
3.	Isocaldin (300 mg)[a]	299.83 ± 2.9146	297.10 ± 2.4731	1.38	1.74
4.	Docina (300 mg)[a]	299.25 ± 4.9757	296.54 ± 3.1433	2.50	1.12

*Calculated F and t-values for (5,5) and 10 degrees of freedom respectively at 5% level of significance.
[a]Contains 3 mg of vitamin B_6; [b]Contains 10 mg of vitamin B_6.

as well as the BP method on two types of tablets and two types of syrups which contained INH along with vitamin B_6. The BP method involved the direct titration with (bromate/bromide) mixture solutionusing methyl red as an indicator.[18] The results obtained are given in *Table* 2.

All sets of results were compared statistically by calculating F-ratios and Student's t-values.[32] The calculated values of F-ratio for comparing variances of two methods for each sample was less than the two tailed critical F-value of 7.15 at 5% level of significance and 5,5 degrees of freedom. The calculated value of Student's t-value for all the samples were also less than two-tailed critical values of 2.23 at 5% level of significance and ten degrees of freedom indicating that results of the two methods did not differ significantly.

Determination of INH in the presence of RIF in pharmaceutical preparations

Since the proposed method was validated by using the analytical tests as described above, this method was successfully applied for the assay of INH in the presence of RIF also as an active ingredient in pharmaceutical preparations. Six replicate determinations were carried out on two types of tablets containing 450 mg, 600 mg of RIF respectively and 300 mg of INH. The results obtained are given in *Table* 3. Since this tablet solutions are highly intense red colored, the detection of end point by the BP method[18] was difficult.

***Table* 3.** Results of six replicate analyses of tablets containing INH and RIF bythe proposed method

Brand name	Stated amount of INH (mg)	Amount of INH found by proposed method (mg) Mean ± Std. dev.	% Mean recovery
Rifa-i6*	300	297.20 ± 2.6784	99.06
R-Cinex600**	300	299.51 ± 1.0249	99.83

*Contains 450 mg of RIF; **Contains 600 mg of RIF.

CONCLUSION

In this proposed method, a large potential break observed facilitated the sharp detection of end point. Thus, the method is simple and accurate. Since this proposed method does not require any sophisticated instrument, it is cost effective and can be applied to monitor the purity of INH in a quality control laboratory of a pharmaceutical industry. Since this proposed method was not interfered by the RIF and vitamin B_6, it can be applied to the determination of INH in the presenceof RIF and vitamin B_6 in pharmaceutical dosage forms.

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