SHORT COMMUNICATIONS

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

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A simple and rapid potentiometric method for the estimation of Isoniazid in dosage forms was developed. The method was based on treating isoniazid with iodine and titration of the iodide produced equivalent to isoniazid with silver nitrate using a copper based mercury film electrode (CBMFE) as an indicator electrode. No interference was caused by other excipients present in dosage forms. The results of the proposed method and British Pharmacopoeia method were compared using F and t statistical tests of significance.

POTENTIOMETRIC methods of isoniazid (INZ) determination which have been reported in comprehensive reviews[1-3] and other literature makes use of a variety of electrodes including Cu(II)[4], Ag(I)[5], iodide[6] and flouride[7] selective electrodes and graphite membrane electrodes[8]. Although potentiometric methods of INZ determination offer many advantages over other methods reported, such as spectrophotometry, fluorimetry, voltammetry and visual titrimetry, they require expensive ion-selective electrodes, which are not commonly available in the market.

In this work, a copper based mercury film electrode (CBMFE) which shows characteristic potentiometric response towards iodide ions was fabricated easily using a commercially available inexpensive copper wire and the electrode was applied without any further pretreatment for INZ determination. The proposed method was based on the treatment of INZ with alcoholic iodine and titration of iodide produced equivalent to INZ against silver nitrate. The results of replicate analyses of pure INZ was assessed statistically and the results of INZ determination in dosage forms by the proposed method and the B.P. method were compared by F and t tests of significance.

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All chemicals used were of analytical grade. INZ was recrystallised from ethanol before use. The titrand solution was stirred with a mini magnetic stirrer and potentials were measured using a digital pH/mV meter (precision ± 1 mV) at ambient temperature (29 ± 1°).

All solutions were prepared using deionised and distilled water. A stock solution of INZ containing 1 mg per ml was prepared. Silver nitrate (0.1 M) was prepared and standardised potentiometrically against sodium chloride. Iodine in ethanol (0.1 M) was freshly prepared and passed through the anion exchange resins (Dowex 1-X8, chloride form, 100-200 mesh) packed in a small glass tube to remove traces of iodide. Potassium hydrogen phthalate-nitrate buffer (pH 3.0) was prepared by adjusting the pH of 0.1 M potassium hydrogen phthalate solution to 3.0 with 0.5 M nitric acid. Mercuric nitrate (0.02 M) and sodium bicarboate (0.1 M) solutions were also prepared.

A polished copper wire (1 cm of the plastic sleeve removed and epoxy seal applied at the junction) was coated with a thin film of mercury by dipping the wire in acidified mercuric nitrate (0.02 M) solution for 10 min. The electrode surface was gently wiped with a filter paper and rinsed with water.

To an aliquot containing (1.0-14.0 mg) of INZ, 10.0 ml of 0.1 M sodium bicarbonate (pH 8.8) was added followed by dropwise addition of 0.1 M ethanolic iodine until yellow colour of iodine persisted for one min. The pH of the solution was reduced to 3.0 with 0.1 M nitric acid followed by addition of 10.0 ml of potassium hydrogen phthalate-nitrate buffer (pH 3.0) and diluted to 50 ml. The iodide produced was titrated against 0.01 - 0.1 M $AgNO_3$ using CBMFE as an indicator electrode and a double junction calomel electrode as a reference electrode.

Twenty tablets containing INZ were weighed and powered. An appropriate amount of powdered sample equivalent to about 250 mg of INZ was dissolved with 50 ml of water and the residue, if any, was filtered using Whatmann No. 41 filter paper and washed 5-6 times with water. The combined filtrate and washings was diluted to 250 ml. 5.0 ml of this solution was taken for titration.

In experiments where INZ present in liquid formulations was to be determined, a certain volume of the preparation equivalent to about 250 mg of INZ was transferred to a 250 ml volumetric flask and diluted upto the mark with water. 5.0 ml of this solution was taken for titration.

The freshly prepared CBMFE has smooth, uniform, ahdesive and thin layer of mercury. The electrode showed stable, rapid and reproducible response towards iodide ions in aqueous solution. Electrode displayed linear Nernstian response in the range 10^{-5} - 10^{-1} M (constant ionic strength of 0.1 M KNO_3; pH 4 with acetate buffer) with slope of 54 mV per decade of concentration and detection limit of 2.8 x 10^{-6} M. The response time was instantaneous for 10^{-1} - 10^{-2} M and it raised to 90 seconds for 10^{-5} M solution. The potential remained constant in the pH range 2.5 - 10.0 for 10^{-3} - 10^{-4} M and also in the pH range 3.5 - 11.5 for 10^{-2} - 10^{-1} M.

Titration of 1.0 - 14.0 mg of INZ investigated was based on the instantaneous oxidation of INZ to isonicotinic acid in sodium bicarbonate solution (pH 6.5 to 9.0) by addition of a known excess of ethanolic iodine[9] and the titration of resultant iodide with silver nitrate in acidic medium. Since CBMFE responded towards iodide ion, the titration of iodide produced was monitored with CBMFE. During the titration, the equilibrium potential was rapidly established after each addition of titrant. For the titration of 10.0 mg of INZ, an end point inflexion of 40 mV was observed for addition of 0.02 ml of 0.099 M $AgNO_3$. The end point was located from the first derivative curve. Titration of 10.0 mg of INZ was carried out by treating it with different amounts of excess of iodine and also at various pH values in acidic range in order to study the effect of iodine and to fix optimal pH for the titration. The presence of 1.5 times the equivalent amount of iodine required did not affect quantitative titration of iodide in the pH range 2-5. The pH of 10.0 mg of INZ in the presence of excess iodine in 0.1 M $NaHCO_3$ was 8.5. Potassium hydrogen phthalate-nitrate buffer was used to maintain pH in the range 2-4 for titration of idoide produced.

Iodine oxidises INZ instantanously in bicarbonate medium. On the other hand, iodine has been shown to react with ethanol very slowly, in basic medium[10]. Moreover, a very little excess of iodine remains in solution after the quantitative oxidation of INZ in solution. Besides, it was also experimentally ascertained that no blank correction is necessary for this procedure.

In order to assess precision and accuracy of the procedure, seven replicate titrations of standard solutions of INZ at eight concentration levels (1-14 mg) were carried out and overall mean recovery, mean relative standard deviation (RSD%) and mean standard analytical error of the results obtained were 99.77%, 1.077 and 0.0362 respectively. The amount taken for analysis was compared with the mean of the amount found by seven replications at each concentration by calculating Student's t- value at 5% level of significance. The results indicated the absence of any systematic error in the analysis[11].

The proposed method was checked for the possible interference of usual excipients present along with INZ in dosage formulations. The error did not exceed 1-3% when 5.0 mg of INZ was determined by five replications in the presence of 10 mg of vitamin B6 and 25 mg of each of starch, lactose, gum acacia, magnesium stearate and sucrose.

The proposed method was applied for INZ determination in four tablet formulations and two syrup formulations. The result of seven replicate determinations of INZ by the proposed method was compared with the British Pharmacopoeia method using in situ generated bromine as

Table I: Results of seven replicate determinations of INZ in pharmaceutical preparations and statistical analysis of the data

Brand name*	Stated amount (mg)	Found by proposed method (mg) Mean ± Std. dev.	Found by B.P. method (mg) Mean ± Std. dev	F	t
Tablets					
Isonex (Pfizer)	300/tab	298.28 ± 1.80	300.00 ± 2.24	1.54	1.58
Isokin (Parke-Davis)	300/tab	298.71 ± 2.06	301.00 ± 1.63	1.59	2.30
Dosina* (Asok Pharmaceutical)	300/tab	297.28 ± 2.56	295.85 ± 1.95	1.72	1.18
Isokaldin* (Retard Lab)	300/tab	306.57 ± 3.21	306.29 ± 2.69	1.42	0.177
Syrups*					
Isokaldin (Retard Lab)	300/10 ml	293.43 ± 1.40	318.71 ± 1.25	1.25	35.6
Dosina (Asok Pharmaceutical)	300/10 ml	305.00 ± 2.45	317.86 ± 2.19	1.25	10.35

*Name of manufacturers are given in parenthesis

* Also contains 6 mg of vitamin B_6 per 300 mg of isoniazid

titrant[12-13]. All sets of results (Table 1) were compared statistically by calculating F-ratio and Student's t-value[11]. Calculated values of F-ratios did not exceed the two tailed critical value of 4.28 at 5% level of significance and (6,6) degree of freedom indicating that variances of two methods did not differ significantly. Calculated t-values for four tablets did not exceed two tailed critical value of 2.18 at 5% level of significance of 12 degrees of freedom indicating that mean values of seven replicate determinations of INZ by two methods did not differ significantly. However, for syrups analysed, recoveries by the B.P. method was higher than proposed method.

CBMFE has been demonstrated as an inexpensive alternative to expensive ion-selective electrodes for pharmaceutical analysis. The proposed method is simple, precise and accurate, and can be used with advantage for the analysis of INZ in coloured formulation solutions.

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Spectrophotometric Determination of Novalgin in Pharmaceutical Preparations by the Molybdenum Blue Method

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A colour reaction has been developed for the determination of novalgin in pharmaceutical preparations. The method is simple and sensitive with molar absorptivity 3.8 x 10^4l $mole^{-1}$ cm^{-1}. Novalgin is determined Spectrophotometrically by molybdenum blue method. Beer's law is obeyed in the concentration range of 1-10 µg/ml of novalgin.

NOVALGIN (Analgin or dipyrone) is the sodium salt of [(2,3-dihydro-1, 5-dimethyl -3-oxo-2-Phenyl-1 H-Pyrazol-4-yl) methylamino] methane Sulphonic acid. It is a commonly used analgesic drug. Its determination in pharmaceutical preparations is, therefore important. An indirect spectrophotometric method for the determination of novalgin in tablets by use of potassium iodate has been developed.[1] Another spectrophotometric method for the determination of novalgin has been studied by Qureshi and coworkers.[2] The method is based on reduction of iron (III) with novalgin and subsequent complexation of iron (II) with 1,10-phenanthroline. N-bromosuccinimide in acetic acid medium has been used as an analytical reagent for spectrophotometric determination.[3] In another reaction hydrolysed novalgin reacts with phenol and potassium ferrocyanide giving an orange red colour[4] that absorbs maximally at 525 nm. Buhl and Hachula[5] have reported an indirect spectrophotometric method based on reduction of Ce (IV) to Ce(III) by novalgin and its subsequent determination with arsenazo III. Similarly in few other methods the reducing interaction of a number compounds, such as tetracycline, cystein, and ascorbic acid[6-8] have been utilized for their determination. The methods are based on their interaction with ammonium molybdate and phosphoric acid to produce the blue colour which is regarded as molybdenum blue. In our studies, novalgin has also been found to inteact with ammonium molybdate and phosphoric acid to produce the blue colour solution. This colour reaction has been studied for spectrophotometric determination of drug.

An ECIL GS 866 D spectrophotometer (manufacturer) with 10 mm matched quarz cells was used.

All reagents used were of analytical grade. Solutions of 0.005 M ammonium molybdate and 1.0 M phosphoric acid were prepared in distilled water.

Onen hundred mg of novalgin was dissolved in 100 ml of distilled water. A 10 ml was diluted portion accurately to 100 ml with distilled water to obtain a working standard of 100 µf/ml solution for the preparation of calibration graph.

To an aliquot volume of 0.1 ml to 1.0 ml containing 10 µg/ to 100 µg of novalgin 2ml of ammonium molybdate, 2 ml of phosphoric acid and 4 ml of distilled water was added