A Potentiometric Procedure for the Assay of Isonicotinic Acid Hydrazide (Isoniazid)

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A potentiometric procedure for the assay of isonicotinic acid hydrazide (isoniazid) with vanadium(V) at room temperature is described. The reduction of vanadium(V) to vanadium(IV) by isoniazid in an acidic medium is catalysed by osmium tetroxide, and the application of this method to the assay of isoniazid in pharmaceutical preparations is considered. Oxalic acid interferes in the determination, although commonly used excipients such as starch, dextrin, sucrose, glucose, lactose and gum acacia do not interfere.

Several colorimetric[1,2,3,4] and titrimetric[5 to 17] procedures have been reported for the assay of isoniazid in pharmaceutical preparations. The hydrazino group in the compound is susceptible to oxidation and many of the cited titrimetric procedures depend on this property. The potentiometric titration of isoniazid with potassium bromate[7] in an 8 to 12 per cent. hydrochloric acid medium and in the presence of potassium bromide is considered to be the best method, although Kühni, Jacob and Grossglauser[13] state that the titration of isoniazid with 0·05 N potassium bromate gave results that were 0·5 per cent. too high.

The redox reaction between isoniazid and quinquivalent vanadium does not appear to have been considered as the basis of a quantitative titrimetric method. Recently Krych and Lipiec[18] have reported a spectrophotometric method for the determination of vanadium(V) with isoniazid that involves measuring at a wavelength of 420 nm the absorbance of the orange - red complex formed between the reactants at a pH of 1·98. These authors state that the complex is not stable for longer than 10 to 15 minutes and that the results are reproducible only to within 4 per cent. Gowda and Gopala Rao[19] proposed a method for the assay of isoniazid that involves treating an aliquot of an aqueous solution of the compound with an excess of 0·05 M sodium vanadate solution in a medium of 4 M sulphuric acid, allowing the reaction mixture to stand for one minute and titrating the unreacted vanadate with a standard solution of ammonium iron(II) sulphate. *N*-Phenylanthranilic acid is the redox indicator used in this case. These authors claimed definite advantages for their method over iodimetric and bromate titration procedures because the common excipients such as lactose, glucose and starch did not interfere in the determination. We have undertaken a detailed study of the reaction and have succeeded in developing an accurate potentiometric method for the assay of the compound in pharmaceutical preparations.

Experimental

Reagents—

Sodium vanadate solution, 0·1 N—A standard vanadium(V) solution was prepared (by dissolving sodium orthovanadate in water) and standardised against a standard solution of potassium dichromate, which was in turn standardised against a solution of ammonium iron(II) sulphate, the end-points in both the titrations being located potentiometrically.

Isonicotinic acid hydrazide—The isoniazid used in this investigation was of U.S.P. grade. A 0·05 M solution in water was prepared from a sample that had been twice recrystallised from aqueous ethanol and dried at 110 °C for 2 hours. The aqueous solution thus prepared was standardised against a standard solution of potassium bromate following the potentiometric method described by Vulterin and Zyka.[7]

Osmium tetroxide—A 0·1 per cent. solution in 0·1 N sulphuric acid was prepared and stored in an amber glass bottle (sample supplied by Johnson Matthey Chemicals Ltd., London).

This reagent (in 0·2-ml amounts) was used as a catalyst in all the experiments described in this paper.

Orthophosphoric acid—E. Merck's "Pro Analysi" grade orthophosphoric acid (85 per cent.) was used throughout this investigation (any analytical-reagent grade acid can be used).

Sulphuric acid—Analytical-reagent grade nitrogen-free sulphuric acid was used without further purification.

All other reagents and chemicals used were of analytical-reagent grade.

Nydrazid and Isonex tablets, manufactured by Squibb Pharmaceuticals and Dumex Pharmaceuticals (India), respectively, were extracted with water and the aqueous extracts analysed to determine the active constituent (isoniazid).

APPARATUS—

The potentiometric titration assembly used consists of a Pye potentiometer graduated in millivolts, a galvanometer (Cambridge Instrument Co., London), a saturated calomel reference electrode, a bright-platinum rod (0·2 mm in diameter) as indicator electrode and a porous-plate salt-bridge filled with a saturated solution of potassium chloride. The titration mixture is stirred with an electromagnetic stirrer.

Preliminary experiments showed that a direct titration of isoniazid with sodium vanadate in an acidic medium containing any mineral acid would not be possible because the red complex formed on the addition of sodium vanadate decomposed slowly and the potentials were not stable. The use of several catalysts such as orthophosphoric acid, iodine monochloride, osmium tetroxide and copper(II) sulphate did not improve the situation. The reverse titration, *i.e.*, that of vanadate with isoniazid, is also slow at both room and elevated temperatures in media containing various concentrations of sulphuric, perchloric and phosphoric acids (ranging from 0·25 to 4·0 M). However, the reaction was markedly catalysed by osmium tetroxide, although in a 0·5 to 2·0 M sulphuric acid medium the potentials did not attain stable values. Nevertheless, further experiments showed that the addition of 1·0 ml of orthophosphoric acid gave stable potentials. We have therefore carried out experiments to ascertain the optimum concentrations of the catalyst and phosphoric or sulphuric acid that will give a satisfactory potentiometric titration, each time keeping one of the parameters constant.

Effect of varying the catalyst concentration—Experiments on the variation of the osmium tetroxide catalyst concentration in the range 0·05 to 2·0 ml of a 0·1 per cent. solution in 50 ml of the titration mixture (containing the optimum concentration of either phosphoric acid or sulphuric acid for this reaction) have shown no significant deviations in the accuracy of the method. However, we noticed that when the catalyst solution was present in volumes below 0·2 ml, the potentials were not stable near the equivalence point, thus causing considerable delays with each determination.

Effect of varying phosphoric or sulphuric acid concentration—In these experiments 0·2 ml of osmium tetroxide was added to the titration mixture and the acid concentration was varied. Experiments showed that variation of the phosphoric acid volume from 0·5 ml to 20·0 ml (in a total volume of 50 ml of titration mixture) did not affect either the accuracy or the speed of the titration, while greater concentrations of phosphoric acid resulted in a higher consumption of isoniazid and an abnormal drift in the potentials near the equivalence point. Moreover, the potential break near the equivalence point was not sharp but evenly distributed between successive additions of the titrant, thus leading to substantial errors.

In the case of titrations in media 0·25 to 4·0 M in sulphuric acid, we found that without the addition of at least 1·0 ml of phosphoric acid the potentials were not stable and that the break in potential at the equivalence point could not be located accurately. At higher concentrations of sulphuric acid the results were always several per cent. lower, even if phosphoric acid and osmium tetroxide were added. Because of these findings, we recommend the following procedure for the potentiometric assay of isoniazid with sodium vanadate at room temperature.

RECOMMENDED PROCEDURE—

A suitable volume of the standard vanadium(V) solution is transferred to a 150-ml titration vessel and 1 to 20 ml of 85 per cent. orthophosphoric acid (or a mixture of 2·5 to 10·0 ml of sulphuric acid (1 + 1) and 1·0 ml of 85 per cent. orthophosphoric acid) *plus* 0·2 ml

of 0·1 per cent. osmium tetroxide solution are added. The resulting mixture is diluted to 50 ml and titrated potentiometrically with 0·05 M isoniazid solution, the potentials being noted 1 minute after the addition of each portion of the titrant. A typical potential *versus* volume graph is given in Fig. 1.

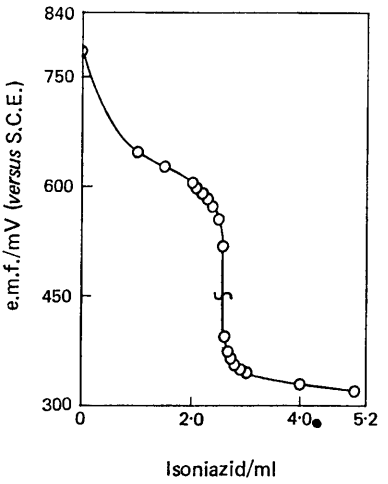

Fig. 1. Potentiometric titration of vanadium(V) with isoniazid

The potential break at the equivalence point is about 100 to 120 mV per drop (approximately 0·04 ml) of 0·05 M isoniazid. A large number of determinations of isoniazid have been carried out according to the recommended procedure and the results compared with those obtained by the B.P. method.[20] Some typical results are given in Table I and show that the proposed method yields results that agree with those by the standard B.P. method with an average deviation of 0·45 per cent.

TABLE I

COMPARATIVE ASSAYS OF ISONIAZID

	Isoniazid/mg							
By B.P. method ..	1·04	3·19	6·04	9·34	12·2	16·3	22·4	25·9
By proposed method ..	1·05	3·21	6·03	9·29	12·1	16·4	22·4	26·1
Deviation, per cent. ..	0·96	0·63	0·17	0·54	0·82	0·61	0·00	0·71

Mean deviation = 0·56 per cent.

APPLICATION OF THE PROPOSED METHOD TO THE ASSAY OF ISONIAZID IN PHARMACEUTICAL PREPARATIONS—

Two tablets are dissolved in 50 ml of water, the resulting mixture is filtered through an IG4 sintered-glass crucible and the filtrate is made up to 100 ml. This solution is then transferred to a microburette and the isoniazid content ascertained by potentiometric titration against a standard solution of vanadium(V) according to the recommended procedure. The results thus obtained were compared with those obtained by using the standard B.P. method,[20] and are given in Table II. The relative deviation for each method is also shown.

Interferences—Sucrose, glucose, lactose, starch, dextrin and gum acacia, which are usually used as excipients in pharmaceutical preparations, do not interfere in this determination. However, oxalic acid interferes at all concentrations and uranium(VI) and chromium(III) also interfere. In all the interference studies the excipients were added in amounts up to a 50-fold excess relative to the calculated amount of isoniazid at the end of each titration.

TABLE II

ASSAY OF ISONIAZID IN TABLETS

Tablet assayed	Amount of isoniazid found with the B.P. method/mg	Deviations from average	Amount of isoniazid found with the proposed method/mg	Deviations from average
Nydrazid	94·9	−0·20	95·4	+0·20
	94·7	−0·40	94·9	−0·30
	94·9	−0·20	95·4	+0·20
	95·3	+0·20	94·9	−0·30
	95·4	+0·30	95·0	−0·20
	95·4	+0·30	95·5	+0·30
	Mean 95·1	s.d. 0·30	Mean 95·2	s.d 0·28
Isonex	101·2	+0·10	101·4	+0·30
	101·1	0·00	101·0	−0·10
	101·1	0·00	100·9	−0·20
	101·2	+0·10	101·0	−0·10
	100·9	−0·20	101·1	+0·00
	101·0	−0·10	101·3	+0·20
	Mean 101·1	s.d. 0·12	Mean 101·1	s.d. 0·20

DISCUSSION

Under the experimental conditions prescribed, 4 moles of vanadium(V) are reduced per mole of isoniazid oxidised, according to the reaction—

$$C_5H_4NCONH.NH_2 + 4\ V(V) + H_2O \rightarrow C_5H_4NCOOH + 4\ V(IV) + N_2 + 4H^+$$

Whereas an orange-red 1:1 complex formed between vanadium(V) and isoniazid at a pH of 1·98 has been reported by Krych and Lipiec,[18] under our experimental conditions isoniazid is quantitatively oxidised to nitrogen and isonicotinic acid. (When a drop of osmium tetroxide solution is added to the vanadium(V) - isoniazid complex at pH 2, copious evolution of nitrogen occurs and the solution turns blue, thus showing that the complex undergoes internal oxidation - reduction under the catalytic influence of octavalent osmium.) Further work on the elucidation of the reaction mechanism is in progress and will be reported separately.

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