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MICRODETERMINATION OF GLUTATHIONE, THIOGLYCOLIC ACID AND ISONICOTINIC ACID HYDRAZIDE

M. SARWAR and SHAHEEN ZAIDI
PCSIR Laboratories, Lahore-16

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In our previous communications we have used N-bromosuccinimide for various selective as well as general oxidations.[1-9] The same compound was also used for certain determinations based on the addition of positive bromine or displaced iodine to organic compounds.[10-13]

In the present investigation, the analytical use of N-bromosuccinimide has been extended to a few other organic compounds, *viz.* glutathione, thioglycolic acid, and *iso*-nicotinic acid hydrazide. The widely used volumetric methods for the determination of glutathione, and thioglycolic acid are based on iodometry.[14-17] Iodine is not a primary standard. Its solutions are unstable and need repeated standardization, thus increasing the chances of error and making the method also time consuming. For *iso*-nicotinic acid hydrazide electrically generated bromine has been used for its volumetric determination.[18] Though the existing method for the determination of *iso*-nicotinic acid hydrazide[18] is reliable, it is tedious and time consuming and expensive equipment is required. In present investigation, N-bromosuccinimide has been used for redox reactions. This is a primary standard and can be shelved for indefinite period of time. Its solutions when dilute in the range of 0.01 to 0.005N can be stored for a couple of weeks if refrigerated and protected from light. N-bromosuccinimide is the only standard solution required in the procedure and its use makes the method less time consuming, less susceptible to error and economical.

Experimental

Reagents : (*i*) *N-Bromosuccinimide.* Accurately weighed 89.0 mg of recrystallized compound were dissolved in distilled water and diluted to 100 ml. the recrystallized product was 99.99% pure.

(*ii*) *Glutathione* (98% *E. Merk Product*). The solution was prepared by dissolving 300.0 mg of the reagent in distilled water and diluted to 100 ml. and standardized by the usual method.

(*iii*) *Thioglycolic acid.* (92% *B. D. H. Product*). Dissolved 300.0 mg of the accurately weighed reagent in distilled water and diluted to 100 ml and standardized by the usual method.

(*iv*) *Isonicotinic acid hydrazide.* (98% *B. D. H. Product*). Exactly 300.0 mg of analytical grade reagent were dissolved in distilled water and diluted to 100 ml.

(*v*) *Bordeaux Red, 0.05% solution in water.* All the other reagents used were of analytical grade.

Procedure. An accurately measured volume (1 ml.) of the test solution was placed in a 50-ml Erlenmeyer flask. Two drops of bordeaux red were added as indicator and the resulting solution was titrated against 0.01 N solution of N-bromosuccinimide. The reagent was added dropwise from a microburette graduated at 0.02 ml. The end point was reached when the rose red colour of Bordeaux red changed to distinct yellow by slightest excess of N-bromosuccinimide. In the case of *iso*-nicotinic acid hydrazide the test solution was made alkaline by the addition of 1 ml of 1% $NaHCO_3$ solution.

Calculations. Amount of the test compound (mg) = N. V. E.

N = normality of N-bromosuccinimide.

V = volume of N-bromosuccinimide (ml).

E = equivalent weight of the test compound.

Eq. wt. of glutathione = mol. wt/5

Eq. wt. of isonicotinic acid hydrazide = mol. wt./4

Eq. wt. of thioglycolic acid = mol. wt./5

Results and Discussion

Results in Table 1 show that the glutathione, thoiglycolic acid and *iso*-nicotinic acid hydrazide can be determined quantitatively in microamounts. The stoichiometry of the reaction can be represented by the following equations :

(i) $2R\text{-}SH + 5Br^+ + 4H_2O \longrightarrow R\text{-}\overset{\overset{O}{\|}}{S}\text{-}\overset{\overset{O}{\|}}{S}\text{-}R + 5Br^- + 10H^+$

(Glutathione and thioglycolic acid)

(*ii*) $R\text{-}CONH\text{-}NH_2 + 2Br^+ + H_2O \longrightarrow R\text{-}COOH + N_2 + 4H^+ + 2\ Br^-$ (*iso*-nicotinic acid hydrazide).

The equivalent weights of the respective substances were calculated taking these electronic changes into consideration. In equation (i) there is a five-electron change which takes place in the process of redox reaction; therefore the equivalent weights of glutathione and thioglycolic acid are 1/5 of their molecular weights. In equation (ii) there is a four-electron change which occurs and the equivalent weight of *iso*-nicotinic acid hydrazide is 1/4 of its molecular weight.

The stoichiometry of the reactions has been proved by the quantitative results which are shown in the Table. These compounds can be determined with reasonable precision and accuracy. The method is comparatively less time consuming than the existing methods.[14-18]

TABLE

Compound	Amount taken (mg)	Amount found (mg)	Relative standard deviation (%)
Glutathione	0.54	0.55	0.9
	1.0	1.0	0.6
	1.5	1.49	0.8
	2.4	2.43	0.4
	3.0	2.99	0.5
Thioglycolic	0.71	0.71	0.25
acid	1.42	1.43	0.5
	1.97	1.97	0.3
	2.84	2.85	0.06
Iso-nocotinic	0.60	0.606	0.7
acid hydrazide	1.02	1.01	0.1
	1.60	1.60	0.2
	2.00	1.99	0.5
	2.55	2.53	0.4

Limitations of the method. Cystein, cystine, methionine and ascorbic acid interfere in the procedure. The method also cannot be applied to the mixture of glutathione, thioglycolic acid and *iso*-nicotinic acid hydrazide. It is a remote possibility that glutathione, thioglycolic acid and *iso*-nicotinic acid hydrazide would be present together. This method is applicable only to individual compounds.

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