

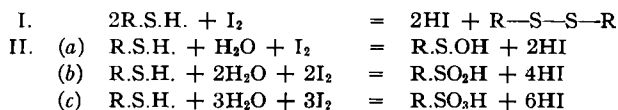
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THE ASSAY OF CYSTEINE HYDROCHLORIDE.*

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The assay of cysteine has been discussed by Okuda (1) and by Lucas and King (2). Their methods involve the use of aqueous solutions and require exact control of acidity, iodine concentration and temperature. Moreover, the factors used to calculate cysteine are more or less empirical; such factors are not as desirable to use in quantitative analysis as stoichiometrical ones derived directly from chemical equations. We have succeeded in avoiding their use.

In an aqueous acid solution, cysteine can react in more than one way. If we indicate the cysteine molecule as R.S.H., the reactions can be expressed by the equations which follow:



The oxidation of cysteine with iodine in aqueous acid solution proceeds in accordance with II as well as I. The equations under II require water as a reactant, while the equation under I does not.

We have found that the oxidation of cysteine proceeds substantially according to Equation I when carried out in such a manner that at the end of the titration the solvent medium consists of ethyl alcohol containing not more than 20% water by volume. The use of alcohol as a titrating medium and of *N*/10 aqueous iodine as oxidant permits a rapid and practically quantitative estimation of cysteine in cysteine hydrochloride monohydrate. It avoids the undesirable use of empirical factors and at the same time simplifies the determination. Another advantage in the use of alcohol as solvent is that the cysteine formed, or its salt (hydrochloride or hydroiodide) separates from the reaction mixture as a white crystalline precipitate, thus favoring the reaction in the sense given, *i. e.*, a reactant is removed from the reaction phase.

Table I gives the results obtained by the Okuda (1) method using the stoichiometrical factor derived from Equation I and an empirical factor derived from Okuda's discussion.

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TABLE I.—PURE CYSTEINE HYDROCHLORIDE MONOHYDRATE CONTAINS THEORETICALLY 68.98% CYSTEINE BASE.

	Cysteine Hydrochloride Hydrate, Grams, Sample Taken.	Potassium Iodate, Cc. <i>M</i> /300.	% Cysteine Base.	
			By Stoichiometrical* Factor.	By Empirical ¹ Factor.
(a)	0.0942	35.08	90.22	71.61
(b)	0.0818	31.15	92.26	73.23
(c)	0.0539	20.65	92.82	73.68
(d)	0.0703	25.50	87.88	69.76
(e)	0.0947	35.30	90.31	71.68

* Factor = 4.127 cc. *M*/300 potassium iodate = 0.01 Gm. cysteine.

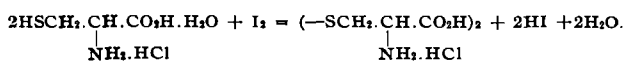
¹ Factor = 5.2 cc. *M*/300 potassium iodate = 0.01 Gm. cysteine.

Although absolute accuracy is not claimed for our method, it enables the determination to be made rapidly and the results are superior to those obtained in aqueous acid solution.

The following are the directions for the assay:

A 0.2–0.3 Gm. sample is placed in a 250-cc. glass-stoppered Erlenmeyer flask; 100 cc. of absolute ethyl alcohol are added, the flask stoppered and the flask with contents allowed to stand about thirty minutes. The alcohol solution is then titrated with accurately standardized aqueous 0.1 normal iodine until the yellow color produced by one drop of iodine solution persists for thirty seconds upon vigorous shaking; 0.05 cc. is subtracted as a correction for the excess drop added to produce the yellow color, and the percentage of cysteine calculated on basis of Equation I above.

TABLE II.



Remarks.	Weight of Sample.	Volume Iodine Solution.	Cysteine Hydrochloride Anhydrous.	Volatile Plus Anhydrous Cysteine Hydrochloride.	
A. Hydrate No. 1 (9.94% water*) titrated with aqueous iodine (1 cc. = 0.016367 Gm. anhydrous cysteine hydrochloride)	0.2275 Gm.	12.44 cc.	89.50%		
	0.4469	24.33	89.10		
			Average 89.3	99.24% (89.3 + 9.94)	
No. 1 dried, titrated with aqueous iodine (1 cc. = 0.016367 Gm. anhydrous cysteine hydrochloride)	0.1181	7.09	98.26		
	0.2116	12.91	99.86		
			Average 99.06		
Hydrate No. 2 (9.20% water*) titrated with aqueous iodine (1 cc. = 0.018030 Gm. anhydrous cysteine hydrochloride)	0.2489	12.48	90.40		
	0.3404	17.11	90.63		
	0.3079	15.59	91.29		
			Average 90.79	99.90% (90.79 + 9.20)	
B. Titration of hydrate in 100 cc. 95% alcohol using iodine in alcohol (1 cc. = 0.009102 Gm. anhydrous cysteine hydrochloride). At end-point, adding 10 cc. water and continuing titration	0.2917	27.43 (end-point in alcohol)	85.59		
		28.60 (end-point in water)	89.24		
		45.92 (end-point in alcohol)	84.97		
		48.13 (end-point in water)	89.06		
C. Mixture of dried cysteine hydrochloride and dried sodium borate ¹ Preparation No. 1—titrated with aqueous iodine (1 cc. = 0.018093 Gm. of anhydrous cysteine hydrochloride)	0.4289	13.02	54.92		
		0.3758	11.41	54.93	
		0.4164	12.72	55.21	

Preparation No. 2—titrated with aqueous iodine (1 cc. = 0.026968 Gm. of anhydrous cysteine hydrochloride)	0.7590	15.95	56.67
	0.7702	16.11	56.41
	0.7603	16.01	56.79
	0.7695	16.17	56.66
D. Sample consisting largely of cysteine hydrochloride titrated with iodine (1 cc. = 0.02703 Gm. anhydrous cysteine hydrochloride)	0.4114	2.32	15.24
	0.4190	2.31	14.90
	0.3900	2.23	15.46

* Loss over phosphorous pentoxide in vacuum; theory for monohydrate is 10.26% water.

¹ This mixture is made so that when dissolved in water the p_H will be 3.8–4.0. The amounts of dried materials vary slightly from batch to batch. The mixture usually contains about 58% of dried cysteine hydrochloride.

All of the data have been combined in Table II. Under "A" are given data from analyses on samples of pure cysteine hydrochloride both before drying and after drying over phosphorous pentoxide. Since cysteine hydrochloride monohydrate does not, according to our findings, have its full amount of water (theory 10.26%), use of the factor corresponding to the monohydrate yields values greater than 100%. Therefore, in assaying the hydrate it is best to calculate the titration in terms of anhydrous cysteine hydrochloride and add the value found for total volatile as determined by drying in vacuum over phosphorous pentoxide. This procedure results in values approximating 100% for pure samples of hydrated cysteine hydrochloride.

It has been stated above that the titration is conducted in alcohol so that at the end-point the solvent contains 20% water. When 95% alcohol was used, both to dissolve the sample and to make up the standard iodine solution, the reaction appeared to go rapidly and smoothly during the first part of the titration. However toward the end, the reaction became very sluggish, and a sharp end-point could not be obtained. The addition of 10 cc. water at the end of the titration resulted in a sharp end-point and then speeded up the reaction considerably. The alcohol end-points, which were quite uncertain because of reaction slowness, indicate a value of 95%, whereas those obtained after adding water indicate a value of 99%. The latter value is the more probable. These results show that aqueous iodine solution is superior to alcoholic iodine solution for this titration—see Section B in Table II.

Part "C" of Table II contains results obtained in assaying a mixture of cysteine hydrochloride and sodium borate. The assay method appeared satisfactory since consistent values were obtained.

Either due to incomplete reduction of cysteine or to partial oxidation of the cysteine the sample might occasionally be a mixture of cysteine and cystine. Assays on such a sample are reported under "D." In this case the material does not dissolve completely in alcohol. The quantity of cysteine hydrochloride could be readily found by the titration with iodine.

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