

Determination of Thiols with *o*-Iodosobenzoate

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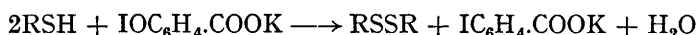
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Thiol groups are titrated directly with *o*-iodosobenzoate reagent at pH 7 employing leuco-2,6-dichlorophenolindophenol with potassium iodide as indicator. This modification avoids errors that result from side-reactions of iodine, which take place if the excess of iodosobenzoate is made to react with acidified potassium iodide.

Thiol groups react with potassium *o*-iodosobenzoate at pH 7 according to the reaction^{1,2}



Hellerman *et al.*¹ determined cysteine and glutathione by treating the sample with the minimal suitable excess of the reagent, as ascertained by a series of preliminary tests, and determining the residual amount of reagent iodimetrically. This method of determining thiols has been simplified by us as follows: a solution of the sample is titrated directly with a standard solution of *o*-iodosobenzoate at neutral pH, using leuco-2,6-dichlorophenolindophenol with potassium iodide as indicator; the end-point, which is indicated by the formation of a blue colour, is sharp, reproducible and stable.

The reaction of *o*-iodosobenzoate with -SH groups at pH 7 is fast but that with the iodide is slow so that it is necessary to titrate the sample solution slowly in order to locate the correct end-point. The titration can, however, be repeated by first adding quickly all but about 1 ml of the previously required volume of titrant and then titrating slowly to the end-point.

Experimental

Reagents

Potassium *o*-iodosobenzoate solution, 0.05 N. This solution was prepared by adding to the free acid a slight excess of 1 M potassium hydroxide solution and diluting appropriately; the solution was standardised iodimetrically.² A 0.01 N solution was made by suitably diluting the concentrated reagent with water.

Phosphate buffer, pH 7. The buffer was prepared by dissolving 117.7 g of dipotassium hydrogen orthophosphate plus 44.1 g of potassium dihydrogen orthophosphate in 1 l of water.

Leuco-2,6-dichlorophenolindophenol indicator solution. This solution was prepared by the following method and was found to serve the purpose satisfactorily. To 25 ml of a 0.05 per cent. solution of the sodium salt of the indicator about 0.5 g of potassium acetate was added and the blue colour was bleached by the dropwise addition of a 0.05 per cent. solution of ascorbic acid. The reduced indicator oxidises when exposed to the air and it is necessary to decolorise the solution before use.

Procedure

Dissolve an accurately weighed amount of sample containing 0.05–1.0 mequiv of -SH group in 10 ml of water and add 5 ml of phosphate buffer and 5 ml of water. Add 1 ml of leuco-2,6-dichlorophenolindophenol indicator solution together with about 50 mg of potassium iodide. Titrate the solution with the 0.01 N *o*-iodosobenzoate reagent when the sample solution contains up to 0.25 mequiv of an -SH group and with 0.05 N titrant for larger amounts. The sample solution must be titrated slowly (10 ml of titrant are added during a period of 2 min) and near the end-point the addition of each drop of titrant is followed by swirling of the solution for 10 s; the end-point is shown by the appearance of the vivid blue colour of the oxidised form of the indicator.

Results

In Table I, the results given by the proposed method for some water-soluble thiols are compared with those obtained by other methods. In comparison with the original procedure,¹ the present method is not only more precise but requires less time in obtaining accurate results. Solutions of ascorbic acid and sodium thiosulphate were titrated by the proposed procedure and the determination of these compounds provided a useful means of standardising the *o*-iodosobenzoate reagent.

TABLE I
DETERMINATION OF THIOLS

Compound	Purity, per cent., by—	
	proposed method*	other method
Cysteine	98.5	98.3 (<i>o</i> -Hydroxymercuribenzoate ³)
Glutathione	97.6	97.8 (Iodimetry ⁴)
2-Mercaptoethylammonium chloride	97.9	98.0 (Iodimetry ⁵)
2-Mercaptoethanol	98.8	98.9 (Pb ⁴⁺ titration ⁶)
Mercaptoacetic acid	80.0	80.2 (Iodimetry ⁵)
2-Diethylaminoethanethiol hydrochloride	98.2	98.4 (Pb ⁴⁺ titration ⁶)
2-Mercaptopropionic acid	97.3	97.2 (Iodimetry ⁵)

* Mean of eight determinations; relative standard deviation in the range 0.1–0.2 per cent.

Starch with potassium iodide can also be used as the indicator but leuco-2,6-dichlorophenolindophenol is recommended for the following reasons. Firstly, the tendency of the liberated iodine to react with disulphides at pH 7 gives rise to a vanishing end-point. Secondly, when a dilute solution of *o*-iodosobenzoate (0.01 N) is employed the blue colour given by starch-iodine is less readily discernible; the colour at the end-point with 2,6-dichlorophenolindophenol is more intense. Thirdly, if over-titration occurs, the end-point is indicated by a dirty green colour rather than the vivid blue colour when leuco-2,6-dichlorophenolindophenol is used.

The results obtained for mercaptosuccinic and 3-mercaptopropionic acids were high and their determination by this method is therefore not recommended.

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