A NOTE ON THE ASSAY OF SOME SULPHYDRYL COMPOUNDS

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An assay of thioglycollic acid has been developed using potassium iodate solution which is considered to be as accurate as the Pharmacopoeial method. The proposed method has been applied to the assay of cysteine hydrochloride, dimercaprol and glutathione.

During an investigation of the antagonism of mercurial bacteriostats by sulphydryl-containing compounds a suitable rapid and simple method of assay of these compounds was required. The requirements were that its accuracy should be of the same order as that of other methods of assay, and it should be applicable to aqueous solutions of the compounds and their sodium salts.

Thioglycollic Acid

Fresh commercial samples of thioglycollic acid are liable to contain small quantities of dithiodiglycollic acid and dithioglycollide.

Older, or improperly stored, samples may contain appreciable amounts of dithiodiglycollic acid and possibly some 2:2:5:5-tetracarboxymethylmercapto-1:4-dithiane[2].

The assay of thioglycollic acid, described in Appendix I of the British Pharmacopoeia 1958 (p. 800), is in two stages, determining both functional groups; the acidity being determined by titration with sodium hydroxide solution and, after neutralisation, the sulphydryl content by titration with iodine solution in the presence of excess sodium bicarbonate. The colour of the indicator (cresol red) remaining after neutralisation and addition of the sodium bicarbonate makes the use of starch mucilage imperative in the second part of the assay. The titration with sodium hydroxide solution will determine all material having titratable –COOH groups, i.e., thioglycollic, thiodiglycollic and dithiodiglycollic acids and the tetracarboxy acid; whereas the titration with iodine solution will determine only sulphydryl-containing substances, viz. thioglycollic acid.

In a sample of thioglycollic acid which has deteriorated the apparent $HS{\cdot}CH_2{\cdot}COOH$ content, by alkali titration, will be high whilst that indicated by iodimetric titration will be lower[2]. Since the Pharmacopoeia gives no upper limit for the $HS{\cdot}CH_2{\cdot}COOH$ content of samples, the object of the first stage of the assay is not apparent, other than as a test for the absence of salts of thioglycollic acid. A further factor not taken into account by the official method is temperature, which is reported[1] to markedly affect the iodine consumption of thiol acids, the theoretical iodine consumption occurring only at or near 0°.

To determine the $HS{\cdot}CH_2{\cdot}COOH$ content of thioglycollic acid directly and rapidly, the following method is suggested:

Dissolve about 0·3 g., accurately weighed, in 20 ml. of water, add 2 ml.

of glacial acetic acid and 0·2 g. of potassium iodide. Shake gently until solution is complete and titrate with M/60 potassium iodate solution until a faint permanent yellow colour is obtained. The addition of starch mucilage is unnecessary. Each ml. of M/60 potassium iodate is equivalent to 0·009212 g. of thioglycollic acid.

Three different samples of thioglycollic acid were assayed by both the Pharmacopoeial and the above methods, with the results shown in Table I.

TABLE I

PERCENTAGE (W/W) THIOGLYCOLLIC ACID WITH (IN PARENTHESES) STANDARD DEVIATION

Sample	B.P. method		Potassium iodate method
	(i) Alkimetric	(ii) Iodimetric	
A	90·92 (0·06)	86·46 (0·19)	86·18 (0·02)
B	90·43 (0·12)	84·77 (0·36)	84·33 (0·01)
C	93·93 (0·03)	90·40 (0·13)	89·55 (0·06)

RESULTS AND DISCUSSION

From the results in Table I it can be seen that the iodine titrations give results slightly higher than those obtained by potassium iodate titration. This is believed to be due to the formation of a small amount of thioglycollic acid or *S*-thioglycollylthioglycollic acid or both, by hydrolysis of dithioglycollide, reactions which can occur at room temperature in the presence of alkali[2].

Hydrolysis of the dithioglycollide may be expected to occur during the official assay where alkali is present, and since the extent of hydrolysis occurring will vary with the time taken to complete the assay, replicate titrations will possibly give inconsistent results.

The larger standard deviations obtained with the iodimetric titrations are taken as indicative of this hydrolysis.

With the potassium iodate method, an acid reaction prevails and no hydrolysis of dithioglycollide occurs, resulting in a greater reproducibility in replicate determinations, as shown by the smaller standard deviations.

A further advantage of the proposed method lies in the greater stability of potassium iodate solution compared with iodine solution, and in the case of assay of preparations such as ammonium or sodium thioglycollate.

The iodate titration can, if required, be used in place of iodine in the second stage of the Pharmacopoeial assay, similar results being obtained by either method. In this case the use of starch mucilage is necessary. Using the equation

$$pH = 1/2\ pKw + 1/2\ pKa + 1/2 \log C$$

it may be calculated that the neutralised thioglycollic acid at the completion of the first stage of the official assay has a pH of ca. 8·3.

No suitable indicator has been found for the neutralisation which has an exponent of this order and is colourless in acid solution. Such an

indicator would obviate the need for starch mucilage in the second part of the assay. Phenolphthalein has been shown[3] to be unsuitable for the alkimetric titration of thioglycollic acid, since ionisation of the sulphydryl group occurs about the pH value at which the indicator becomes coloured.

Thymolphthalein may be expected to behave similarly since its titration exponent is of the same order of that of phenolphthalein.

It is considered, however, that if both the alkimetric and the potassium iodate titrations are to be carried out, separate samples should be used for each determination.

Application to other Sulphydryl Compounds

Cysteine hydrochloride. This has been introduced into the B.P. 1958 (p. 956) as an inactivator for streptomycin and dihydrostreptomycin in Tests for Sterility of preparations containing these substances. The recommended assay (B.P. 1958, p. 751) for this material is by a Kjeldahl determination of nitrogen, a lengthy and complex procedure. Further, any cystine formed by oxidation will also be determined and hence the true cysteine content may be lower than indicated by the assay results.

By the use of potassium iodate titration, this is obviated and the time required for assay reduced to a minimum.

The weight of cysteine hydrochloride suggested for assay by the method described is about 0·4 g. Each ml. of M/60 potassium iodate is equivalent to 0·01576 g. of cysteine hydrochloride.

Both pH and temperature are reported[1] to affect the amounts of iodine combining with cysteine and it is believed that the presence of potassium iodide in the cysteine solution can reduce this error. Lucas and King[1] reported the quantitative determination by indirect iodine titration (excess iodine and back titration with sodium thiosulphate) at 0° and normal acidity.

Glutathione. Lucas and King[1] observed that glutathione could be determined quantitatively by iodimetric titration only at temperatures below 25° and reactions below pH 5. The potassium iodate method has however been satisfactorily applied to the determination of this material. The method may be applied to the determination of small quantities of glutathione on a semi-micro scale, using M/600 potassium iodate solution. The weight of glutathione suggested for assay is 20 to 50 mg. Each ml. of M/600 potassium iodate is equivalent to 0·003073 g. of glutathione.

Dimercaprol. Potassium iodate titration has been satisfactorily applied to dimercaprol which is at present officially assayed (B.P. 1958, p. 227) by titration with N/10 iodine solution. About 0·2 g. of sample is taken and each ml. of M/60 potassium iodate is equivalent to 0·006211 g. of dimercaprol. The insoluble disulphide of dimercaprol produced during the titration does not interfere with the visibility of the end point.

References

1. Lucas and King, *Biochem. J.*, 1932, **26**, 2076.
2. Schöberl and Krumey, *Ber.*, 1944, **77B**, 371.
3. Larsson, *Z. anal. Chem.*, 1929, **79**, 170.