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THE AMPEROMETRIC TITRATION OF SULFHYDRYL GROUPS WITH SILVER NITRATE

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In the amperometric titration of SH groups of proteins and related compounds with $AgNO_3$, it is usually assumed that the number of SH groups in the titration mixture is equal to the number of Ag ions added up to the point where the two branches of the titration curve intersect. In the author's experience, however, this is not correct for some compounds when these are titrated in two media suitable for proteins.

EXPERIMENTAL

The electrode, containing a protruding Pt wire (length 10 mm, diameter 0.5 mm), was rotated at 1400 r.p.m. It was cleaned daily in hot nitric acid and coated with silver for 5–10 min at 10–20 μA, using the same buffer and the same potential as in the titrations (ammonia buffer −250 mV, tris buffer* −100 mV *versus* saturated calomel electrode).

* Tris(hydroxymethyl)aminomethane.

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The galvanometer, with a resistance for critical damping in series, was shunted by a resistance of 300 ohms, in order to obtain a sufficiently low resistance in the electrode circuit. In this manner a full-scale deflection corresponded to a current of 20 μA. Readings were made one minute after each addition of reagent.

The tris buffer contained 0.13 *N* tris, 0.01 *N* KCl, and 0.01% gelatin[4], and was acidified with HNO_3 to pH 7.7. During the titrations in ammonia buffer, which contained 0.1 *N* NH_3 and 0.25 *N* NH_4NO_3[1], a current of N_2 was passed through the solution. The presence of 0.01% gelatin was found to be advantageous also in the latter buffer.

Except for cysteine ester, all compounds were obtained commercially. Thioglycolic acid was freshly distilled from zinc. Stock solutions of the compounds to be investigated were prepared daily and kept under nitrogen or carbon dioxide.

To 30 ml buffer 0.5–2 ml stock solution was added and the titration carried out with 0.002 *N* $AgNO_3$, prepared daily by dilution of 0.1 *N* solution. Deionized water was used throughout.

*Cysteine ethyl ester hydrochloride**

0.2 g cysteine hydrochloride was dissolved in 6 ml 0.7 *N* HCl in dry alcohol and the solution refluxed for 6 hours. The mixture was cooled and ether added until slight turbidity developed. On standing overnight needles separated out. The crystals were centrifuged off and washed with ether.

Yield 104 mg (44%), m.p. 116–117°C (CHERBULIEZ[5] reports 115°C). N contents, calculated 7.5[4]%, found 7.5[2]%.

RESULTS

As shown in Table I, of the five compounds investigated, three gave values which were distinctly too high. These values appeared to be independent of the concentration of the compounds in the titration mixture in the range investigated ($0.3–1.2 \cdot 10^{-4}$ moles/litre). Fig. 1 shows two examples of the titration curves obtained.

TABLE I

EXPERIMENTAL SH VALUES AS PERCENTAGE OF THE THEORETICAL VALUES

Compound	*In ammonia buffer pH 9.1*	*In tris buffer pH 7.7*
tert-Dodecyl mercaptan	—	100 ± 1%
Glutathione	92 ± 2%	98 ± 1%
Cysteine hydrochloride	133 ± 3%	155 ± 3%
Cysteine ethyl ester hydrochloride	108 ± 2%	143 ± 4%
Thioglycolic acid	132 ± 3%	136 ± 3%

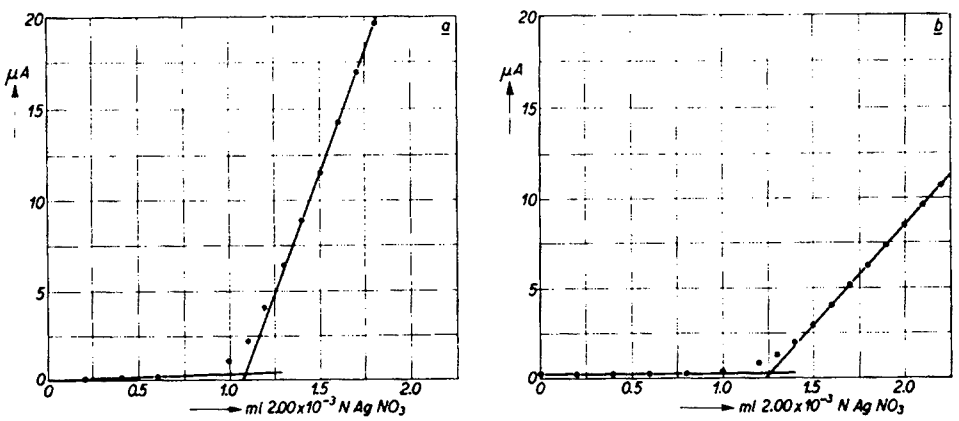

Fig. 1. Titration curves of 0.258 mg cysteine hydrochloride in: *a*. ammonia buffer, *b*. tris buffer

* Prepared by P. VAN ZONNEVELD.

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At least 10 determinations were carried out on each compound in each buffer. Scores of titrations of cysteine were made with three preparations of cysteine hydrochloride (S and Cl contents were checked) using different electrodes, both vibrating and rotating, in ammonia buffers of various compositions, etc. In all cases the values observed were too high.

The high value for cysteine in ammonia buffer does not agree with the results of KOLTHOFF AND STRICKS[1], who report a recovery of 100%. On the other hand, a recovery of 135% can be calculated from data given by STAIB AND TURBA[2]. The present result with cysteine was also confirmed by DRENTH[3].

The few data available suggest that the proximity of an amino and/or carboxyl group close to the SH group is responsible for the observed deviations. The latter may be attributed to non-stoichiometric combination of the Ag^+ or to a poisoning of the electrode by the compounds involved, leading to delayed action of the electrode and hence to high values. Some indication that the latter explanation is not correct is provided by the observation that an equimolecular mixture of cysteine and glutathione in tris buffer consumed an amount of Ag^+ equal to the sum of the amounts each compound consumes separately. Hence the titration of glutathione was not affected by the presence of cysteine. If the electrode had been poisoned by the cysteine, glutathione would also have shown too high a value.

With regard to the application of the amperometric titration with $AgNO_3$ to protein SH groups, some confidence seems to be justified, since glutathione, which is more like a protein than the other compounds hitherto investigated, yields nearly theoretical values. STAIB AND TURBA[2], in determining the SH contents of proteins, made use of a "standard curve" based upon titrations of cysteine. In view of the present data, such a procedure does not seem to be correct. It will be clear that an investigation of more compounds, especially cysteine peptides, is desirable.

SUMMARY

Three out of five sulfhydryl-containing compounds, subjected to amperometric titration with silver nitrate, appear to combine with more Ag than corresponds to their sulfhydryl content.

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