APPENDIX

Indirect Polarographic Method for the Estimation of Thiol Groups and Disulphide Bonds

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The reaction of methylmercuric iodide with thiol groups has been developed for the estimation of thiol groups and of disulphide bonds (Leach, 1960).

The method consists of amperometric measurement of excess of methylmercuric iodide by using the dropping-mercury electrode. Fig. 1(a) shows the usual method of following the current carried at -0.7v when serial additions of the mercurial are made. Extrapolation of lines passing through the experimental points gives the equivalence point (X) at the intersection. As each addition of the mercurial plus purging to remove oxygen takes time the thiol groups being estimated are exposed to pH conditions on the alkaline side and in some systems there is a loss of thiol. In crude systems such as flour suspensions there are also oxidative and other competing reactions removing thiols after the addition of the suspending medium and before deoxygenation. It has been necessary therefore to devise a system that permits both the addition of excess of methylmercuric iodide to prevent these reactions and the subsequent determination of the equivalence point.

Fig. 1(b) shows that it is possible to do this by removing the excess of methylmercuric iodide and so dropping the current to a base level by allowing the mercurial to react with GSH. In the system described a sample containing $1.1\mu\text{mole}$ of thiol has been added to an aqueous phase containing $1.3\mu\text{moles}$ (i.e. excess) of the mercurial along with the carrier electrolyte etc. After purging with nitrogen to remove oxygen the current at -0.7v was measured (point A) and two further increments of $0.4\mu\text{mole}$ added, the current again being measured (points B and C). At point C $4\mu\text{moles}$ of GSH were added, the system was purged and the current was measured again (point D): this has fallen owing to the removal of the methylmercuric iodide as the mercaptide. The mercaptide so formed is associated with a small amount of current carried and this must be considered. The slope of the line associated with the glutathione-methylmercuric mercaptide, however, is easily established by the addition of a known amount of the mercurial (up to $1\mu\text{mole}$, to give point E). Extrapolation of the appropriate

Fig. 1. Direct titration (a) and indirect titration (b) of excess of methylmercuric iodide in the estimation of thiol groups and disulphide bonds. Experimental details are given in the text.

experimental points gives the equivalence point at the intersection (*X*). Each experimental point must be corrected for a dilution factor accompanying the addition of reagents. These are, however, prepared as concentrated as possible to keep the volumes down.

The general system used was as follows. The amperometric titrations were carried out in conjunction with a dropping-mercury electrode and a Metrohm Polarecord (type E 261) assembly. The titrations were usually carried out with approx. 0.5–1 μ mole of thiol or disulphide at pH 9 (0.2M-ammonia-ammonium chloride or 0.2M-tris-hydro-

chloric acid buffer) at 40° in a volume of 7–20ml. For the estimation of disulphide bonds the solution was made 0.27M with respect to sodium sulphite. Methylmercuric iodide was dissolved in 25% (v/v) dimethylformamide; in this way solutions of satisfactory concentration (2mM) can be obtained (Leach, 1960). For protein thiols and disulphides the estimation was done in the presence of 8M-urea.

REFERENCE

Leach, S. J. (1960). *Aust. J. Chem.* **13**, 520.