Titrimetric Determination of Nitrates with an Equivalent Ratio of 1 : 8

MANY procedures for titrimetric nitrate determinations are based on reduction by ferrous salts. Ferrous iron reduces nitrates boiled in acid medium to nitrous oxide. In alkaline medium the reduction proceeds at a concentration of 28 per cent sodium hydroxide, and the product is ammonia. Cotte and Kahane[1] have found that silver sulphate catalyses the reduction at a suitably lower concentration of hydroxide. The ammonia formed was distilled into acid, the excess of which was measured. Our method is based on the same reduction; but the reduced nitrate is measured in another manner. The reduction is effected in a boiling 3 per cent sodium hydroxide solution, containing ammoniacal silver sulphate, by the action of ferrous hydroxide precipitated from a measured sample of ferrous ammonium sulphate. When the reaction is completed, the mixture is acidified with sulphuric acid and the excess of the ferrous iron is titrated with potassium permanganate. A blank titration must also be carried out. The reduction—without regard to the catalysis—proceeds by the following reactions:

(1) $KNO_3 + 2\ Fe(OH)_2 + H_2O = KNO_2 + 2\ Fe(OH)_3$;

(2) $KNO_2 + 6\ Fe(OH)_2 + 5\ H_2O = 6\ Fe(OH)_3 + NH_3 + KOH$.

As can be seen from the equations, 8 moles of $Fe(OH)_2$ are necessary for the reduction of 1 mole of nitrate. 1 ml. of 0·1 *N* potassium permanganate is equivalent to 0·7751 mgm. of NO_3.

Blank: 45·96 ml. 0·1 *N* potassium permanganate

Nitrate taken (mgm.)	0·1 *N* permanganate (ml.)	Nitrate found (mgm.)	(%)	Difference (mgm.)
1·882	43·48	1·922	102·14	+0·040
4·662	39·93	4·674	100·26	+0·012
9·335	33·94	9·317	99·81	−0·018
13·998	27·94	13·967	99·78	−0·031
18·652	21·94	18·618	99·82	−0·034

The largest quantity of nitrate which it was possible to determine amounted to 20 mgm., assuming the use of a 50-ml. burette. This is only equivalent to about 26 ml. of permanganate; but the ferrous ammonium sulphate must be employed for the reduction in large excess owing to the formation of ferrous – ferric oxide. The fact that the errors of the different nitrate concentrations are quite constant indicates the introduction of correction values.

The ions Al^{3+}, Zn^{2+}, Cd^{2+}, As^{5+}, Sn^{4+}, Mo^{6+}, U^{6+}, W^{6+}, Cl^-, PO_4^{3-}, $B_4O_7^{2-}$, CH_3COO^-, ClO_4^- do not interfere with the method. The interference of the ions: V, As^{3+}, Sn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Br^-, ClO_3^-, CO_3^-, HCO_3^-, $S_2O_8^{2-}$ can be easily eliminated or compensated.

A detailed communication on this subject will be published in the *Hungarica Chimica Acta*.

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[1] Cotte, J., and Kahane, E., *Bull. Soc. Chim.*, 542 (1946).

Production of Staphylocoagulase in a Chemically Defined Medium

IN the course of work on the purification of staphylocoagulase, a possible approach appeared to be the production of the enzyme in a protein-free 'synthetic' medium. While growth of *Staphylococcus aureus* occurs according to Fildes *et al.*[1], Gladstone[2] and Gale[3] in media simpler than that reported here, for our purpose, which is concerned exclusively with the production of coagulase, a medium containing a wide range of amino-acids and vitamins seemed advisable. This medium has yielded good growth of a number of freshly isolated strains of coagulase-positive staphylococci, without prior 'training'.

The composition of the medium per hundred c.c. of fluid is as follows: 0·025 gm. each of L-cystine, L-histidine, L-leucine, L-phenylalanine, L-proline, L-tryptophane and L-tyrosine; 0·05 gm. each of L-arginine, glycine, DL-*iso*leucine, L-lysine, DL-methionine, DL-serine, DL-threonine and DL-valine; 0·125 gm. of both DL-aspartic acid and L-glutamic acid; 0·05 μgm. of biotin; 0·125 gm. each of $MgSO_4.7H_2O$, dipotassium hydrogen phosphate, sodium chloride and ferrous sulphate (crys.); nicotinamide, pyridoxine and thiamine each at a concentration of 0·0005 *M*. All constituents were dissolved separately as stock solutions: the amino-acids with the exception of cystine and tyrosine in Sörensen's buffer *p*H 7·3; cystine and tyrosine in 3 *N* hydrochloric acid afterwards diluted with distilled water; salts and vitamins in distilled water. All solutions were sterilized by autoclaving except the ferrous sulphate, which was always freshly prepared and sterilized by filtration through a Seitz pad. The medium, made up by mixing the stock solutions, had a final *p*H of 7. 10 c.c. amounts were distributed with aseptic precautions into universal containers.

The original inoculum consisted of a saline suspension of cells, obtained by repeated spinning and washing of a 24-hours broth culture; serial subcultures were then obtained by transferring one loopful of growth in synthetic medium. Cultures were incubated at 37° C., samples withdrawn every twenty-four hours and sterilized by heating at 65° C. for 45 min. To 0·5 c.c. of such a killed culture or to a dilution of it, 0·1 c.c. of heparin (containing 10 international units) and 0·2 c.c. of human citrated plasma were added. The mixtures were incubated for twenty-four hours and the sterility of the clots tested by adding 4 c.c. of broth and incubating further.

Coagulase was demonstrated both in the primary cultures and in all sub-cultures. The amount of coagulase was small—not exceeding a titre of 1 : 16; this was generally reached after three to five days incubation of the cultures. It has been recently reported[4], however, that purified coagulase shows increased thermolability, and results obtained by one of us (A. J. O'H.) appear to support this. The evidence points to coagulase being present in the medium in a state of relatively high purity and, therefore, heat-sensitive. Thus, the titre of the enzyme demonstrated probably represents only a fraction of that actually produced in the medium. Despite the low titres so far attained, the preparation of staphylocoagulase in a protein-free medium of known chemical composition was thought interesting by itself as an instance of production of a bacterial enzyme under controllable conditions.

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