decreases considerably with precipitation of brown hydroxide. It seems that the solution remains more constant when sodium bicarbonate is present in excess (solid salt on the bottom of the container).

Oxidation of cerous ion. A comparison of the reduction potential of Co(III)/Co(II), which is about 1.8 volts, with that of Ce(IV)/Ce(III), which is about 1.5 volts, shows that it is theoretically just possible to oxidize cerous ion quantitatively by means of cobaltic ion in the usual analytical concentration range. The following experiments confirm this.

An about 0.05 M cerous nitrate solution was prepared and analyzed. Evaporation of a sample and ignition to CeO₂ gave the molarity 0.0515 (mean of 3 determinations). The colour of the CeO₂ showed that it was not quite pure, but with a slight content of other rare earths. The true content of cerous ion in the solution was determined by oxidation with sodium bismuthate^{5,6} and titration with hydrogen peroxide of the Ce(IV) formed. Two determinations gave 0.0511 M and 0.0511 M.

The cerium content in 20 ml of the 0.0511 M solution was now oxidized with cobaltic ion as follows: 30 ml conc. sulfuric acid were diluted with water to 100 ml. After cooling 20 ml cerous nitrate solution were added. 150 ml ca. 0.01 M cobaltic carbonate solution were now gradually added. The Ce(III) was oxidized nearly immediately, and the colour of the solution showed excess of cobaltic ion. The solution was now heated to decompose the cobaltic ion, and boiled for one minute, to expel the oxygen formed. The ceric ion formed was now titrated with hydrogen peroxide. Two determinations gave 0.0509 M and 0.0510 M.

Cobaltic ion may be used for many other quantitative oxidations, for example oxidation of chromic chromium to chromate chromium.

1. Field, F. J. *Chem. Soc.* **14** (1862) 51.
2. Job, A. *Ann. chim. et phys.* **20** (1900) 214.
3. Willard, H. H. and Hall, D. J. *Am. Chem. Soc.* **44** (1922) 2237.
4. Metzl, A. *Z. anal. Chem.* **53** (1914) 537.
5. Metzger, F. J. *J. Am. Chem. Soc.* **31** (1909) 523.
6. Someya, Kin'ichi *Z. anorg. Chem.* **168** (1928) 56.

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Separation of Saturated Straight Chain Fatty Acids II. Qualitative Paper Ionophoresis

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When analysing the homologous series of saturated straight chain fatty acids the separation can be achieved by paper ionophoresis. Barnett^{1,2} describes the separation of long chain fatty acids — from capric to stearic acid — using 3 N aqueous ammonia as solvent. The analysis is, however, difficult to carry out because of the high volatility of ammonia. In the procedures described below this difficulty is avoided by substituting ammonia with sodium hydroxide.

The acids from acetic to pelargonic acid can be separated from each other ionophoretically by means of aqueous sodium hydroxide buffered with boric acid to pH 9.0. The mobilities of these acids at +25° C and at a voltage of 5 V/cm were as follows: acetic 31, propionic 27, butyric 25, valeric 23, caproic (hexoic) 21.5, α -nonylic (heptoic) 20, caprylic (octoic) 19, and pelargonic (nonoic) acid 18 mm/h.

The acids from acetic to palmitic acid can be separated from each other ionophoretically by using a 0.2 N solution of sodium hydroxide in glycerol and a temperature of +90° C. The short chain acids are separable with the method described below but their location on the glycerolic paper is rather cumbersome because of the slow absorption of the indicator solution and because of the disturbing effect of the atmospheric carbon dioxide. Therefore the description will be limited to the separation of the acids from caproic acid upwards. As an interesting detail may be mentioned that it is possible to follow the movement of all these acids throughout the analysis by dissolving a suitable indicator, e. g. phenolphthalein, in the glycerol solution and removing the carbon dioxide from the air-tight reaction chamber equipped with a window. The mobilities of different acids at a voltage of 20 V/cm were as follows: caproic 7.7, α -nonylic 6.6, caprylic 5.8, pelargonic 5.3, capric (decoic) 4.9,

undecic 4.6, lauric (dodecic) 4.4, myristic (tetradecic) 4.1, and palmitic (hexadecic) acid 3.9 mm/h.

Experimental: Separation of the acids from acetic to pelargonic acid. The paper was used as 10 × 50 cm strips, each for the investigation of three samples. The distance between electrode vessels was 40 cm from liquid to liquid. The acids were placed on the paper as free acids in ethanol as close to the buffer solution in the cathode vessel as possible. In order to avoid the volatilization of the acids they were neutralized on the paper with ammonia vapour. After the solvent had evaporated the whole paper was sprayed with buffer solution the excess of which was removed by two dry filter papers. The paper was then immediately placed between the electrode vessels and kept there horizontally by means of glass rods fastened to the ends of the paper. The whole apparatus was surrounded by saturated water vapour. After ionophoresis the paper was dried. The acids were located with methyl red. The acids from valeric to pelargonic acid can also be located ³ with a solution of rhodamin B.

Separation of the acids from caproic to palmitic acid. The paper strips were of the same size as above, but in some cases it was advantageous to use shorter strips than 50 cm in order to speed up the analysis without increasing the electric potential. The paper was immersed in 0.2 N glycerolic sodium hydroxide. The excess of glycerol was removed with the edge of a glass plate. The ethanol solutions of the acids were placed on the paper and the paper laid horizontally between the electrode vessels. After the paper had reached the temperature of the chamber (90°C) the current was put on. The acids were located by spraying the paper first with 2% aqueous copper acetate, removing the glycerol after 10 min. with water, drying the paper, and spraying it finally with a 0.5% solution of rhodamin B in water or in 0.05 N hydrochloric acid. On the dried paper the acids were located conveniently in ultraviolet light.

1. Barnett, A. J. G., Lees, H. and Smith, D. K. *Biochem. J. (London)* **53** (1953) xxxiii.
2. Barnett, A. J. G. and Smith, D. K. *Nature* **174** (1954) 659; *J. Sci. Food Agr.* **6** (1955) 53.
3. Kaufmann, H. P. and Budwig, J. *Fette u. Seifen* **53** (1951) 390.

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The Effect of Nitrous Oxide on Nitrate Utilization by *Azotobacter vinelandii*

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Nitrous oxide competitively inhibits nitrogen fixation by *Azotobacter vinelandii* but exerts no effect on ammonia utilization ¹⁻³. Virtanen and Lundbom ⁴ have found in their long time growth experiments that N₂O inhibits not only the fixation process but also nitrate reduction by this organism. Because of the importance of this finding for the understanding of the mechanism of biological nitrogen fixation, the effect of N₂O on nitrate utilization by *A. vinelandii* was reinvestigated in short time respiration experiments.

Fig. 1. Lack of inhibition by N₂O of growth of *A. vinelandii* (strain O) supplied nitrate. Growth was measured by following manometrically the increasing rate of oxygen uptake by the culture.

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