SUMMARY

A comparative study of the various methods of determining exchangeable cations was made in order to assess their relative applicability. It seems that SCHOLLENBERGER's method is quite well suited for cation exchange studies as the ammonium thus adsorbed gives a fairly accurate measure of the cation exchange capacity at pH 7

RÉSUMÉ

Une étude comparative a été effectuée des différentes méthodes de détermination des cations échangeables. Il semble que la méthode de SCHOLLENBERGER convient très bien pour les études d'échange de cations: l'ammonium ainsi adsorbé peut donner une mesure assez précise de la capacité d'échange des cations au pH 7.

ZUSAMMENFASSUNG

Die vorliegende Arbeit beschreibt einen Vergleich der verschiedenen Methoden zur Bestimmung von austauschbaren Kationen Es scheint dass die SCHOLLENBERGER'sche Methode recht gut für Kationenaustausch-Untersuchungen geeignet ist; das adsorbierte Ammonium gibt ein ziemlich genaues Mass der Kationen-Austauschfähigkeit bei pH 7.

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THE PAPER CHROMATOGRAPHY OF SOME MANGANESE AND COPPER VALENCY STATES

by

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INTRODUCTION

Manganese and copper are known to undergo valency changes in biochemical systems; for example Mn in soil bacteria and Cu in the enzymes tyrosinase and ascorbic acid oxidase.

The separation of the valency states of Mn and Cu by paper chromatography was therefore undertaken as a preliminary to a study of possible valency changes of these metals during metabolism.

EXPERIMENTAL

Manganese

The solutions used were prepared as follows:

- Mn^{+2} : a saturated solution of $Mn(COOCH_3)_3 4H_2O$ in glacial acetic acid was employed.
- Mn^{+3} : Mn(COOCH₃)₂ 4H₂O (3.1 g) was dissolved in glacial acetic acid (10 ml) with warming. Pow-dered KMnO₆ (0.75 g) was added and the mixture boiled for 2 min. It was then cooled and filtered (Whatman No. 41) and the filtrate diluted with glacial acetic acid.
- Mn^{VII} : A solution of powdered KMnO₄ in glacial acetic acid was prepared with the addition of a few drops of water to increase the solubility of the salt.

References p. 438

Separation of the valencies These were performed by downward runs on acid-washed Whatman No 1 paper strips with an eluant prepared by mixing glacial acetic acid (10 ml) with acetic anhydride (30 ml) and running the wet spots as quickly as possible. The blank strips were put into the eluant, and when the liquid front approached the "start line" the mixed Mn^{+3} - Mn^{+3} or Mn^{+3} - Mn^{VII} solutions were spotted on to the paper. This ensured minimum delay before the separation began. It was important not to over-load the strips as this caused tailing to take place.

A run of only 5 cm was necessary to show whether an Mn^{+3} solution contained Mn^{VII} or Mn^{+3} . In the first instance a pink spot above the brown Mn^{+3} spot showed Mn^{VII} but the chromatograms had to be developed to show Mn^{+3} . This was done by dipping them into a solution of "tetrabase" in acetone and drying them Blue spots appeared for Mn^{+3} and Mn^{VII} at R_F values 0.94 and 0.48 respectively A blue colour was sometimes observed with Mn^{+2} ($R_F \cong 0.16$) if it had been run with Mn^{+3} . Mn^{+2} was also detected as a yellow spot by dipping the strip in a solution of oxine in ether, drying it and exposing it to ammonia vapour.

 Mn^{+2} and Mn^{VII} interacted to give Mn^{+3} (see preparation of Mn^{+3} above) and so a separation between these two valencies was not feasible. To avoid the reduction of higher Mn valencies on the paper it was essential to exclude ketones, ethers or alcohols from the cluant. Using the given cluant, the Mn^{VII} spot remains moderately stable during a short run (5-7 cm), but tends to reduce gradually to Mn^{+3} and Mn^{+2} giving short forward and backward "tails". This reduction was speeded up if either a stronger (formic) or weaker (propionic) acid was used in the cluant.

Copper

The solutions used were prepared by dissolving Cu_2Cl_3 and $CuCl_2.2H_2O$ in a minimum of 10N hydrochloric acid, and diluting the solutions with about ten times their volume of acetone If it was desired to keep the cuprous solution, it was stored under CO_4 .

Separation of the valencies POLLARD, MCOMIE AND BANISTER¹ reported that Cu⁺ and Cu⁺² could be well separated by ascending paper chromatography using an eluant consisting of ether, methanol, hydrochloric acid and water (50 30:4 15 by volume) which was developed primarily for the separation of uranium valencies Repetition of these experiments by the author showed that although agood separation was obtained, some oxidation occurred with Cu⁺ causing back tailing and a weakening of the final Cu⁺ spot on the paper-strip. It was thought that the more polar constituents of the solvent (the acid and water) tended to keep the cuprous copper in ionic form thus rendering it susceptible to oxidation. When a less polar cluant was used and the run was performed in an atmosphere of CO_{g} , oxidation and tailing were eliminated

The eluant consisted of ethanol (30 ml), glacial acetic acid (5 ml) and ether (50 ml). About 100 ml of this eluant was poured into the bottom of a chromatographic jar (30 cm \times 12.5 cm internal diameter) which contained a trough mounted on a framework. A blank strip of paper was placed in the empty trough and the tank was closed with a greased ground-glass lid through which a small hole (5 mm diam.) had been bored. The tank was then filled with CO_g through a delivery tube which was inserted through the hole in the lid and allowed to reach the bottom of the vessel. The delivery tube was then withdrawn and the mixed Cu valency solution spotted onto the paper-strip by means of a capillary tube inserted through the hole in the hole in the lid. The delivery tube was re-inserted and a little more CO₂ passed; the tube was then finally withdrawn and the hole closed by a cork. The tank was allowed to stand for about 10 minutes and then the eluant was introduced into the trough (through the hole in the lid) using a filter-funnel, the stem of which had been drawn out for the purpose.

After a run of 10-15 cm, the dried strips were developed by spraying them with a solution of Na_2MoO_4 weakly acidified with 2N HCl. This showed up Cu⁺ as a blue spot ("Mo blue") The strip was re-dried and dipped into a solution of rubeanic acid in ether followed by exposure to NH_3 when both valencies were shown up as olive-green spots. If air had been effectively excluded from the jar no back-tailing of Cu⁺ was observed. Freshly-run strips were also developed by dipping them into either an aqueous solution of potassium ferrocyanide (brownish pink spots for both valencies) or an ethereal solution of oxine followed by exposure to NH_3 (yellow spots). Neither of these reagents however was as sensitive as rubeanic acid R_F values were approximately 0.2-0.3 for Cu⁺² and 0.6-0.7 for Cu⁺

Cuprous oxinale. Previous work^{3,3} upon the chromatography of Mo and Fe valencies led to the discovery of 8-hydroxyquinoline complexes of these metals in lower valence-states. In view of this finding, it was decided to investigate the possible existence of a Cu⁺-oxine complex. This was obtained as follows:

 $Cu_{a}Cl_{a}$ was prepared by boiling CuCl₂ with copper turnings in an excess of roN HCl and pouring the solution into a tenfold excess of water. The precipitate was washed by decanting it in turn with water, acetone and ether in an atmosphere of CO₂ and dried in a stream of CO₂ at 50-60°. The pure white product was preserved in CO₂ in a scaled tube Cuprous chloride (0.5g) and 8-hydroxyquinoline

(3.5 g) were shaken together dry in a stoppered tube containing CO_q . Pyridine (10 ml) was added and the mixture warmed in a slow stream of CO_g until solution was effected. Into a 350 ml flask, 150 ml of water was poured and all air expelled by a vigorous stream of CO_g . Sodium dithionite (Na₂S₂O₄) (1 g) was added, and when dissolved the mixed solution of Cu_gCl_g and oxing in pyridine was added and the flask corked and shaken vigorously.

The orange precipitate was filtered off through a Whatman No 541 filter paper at the pump in an atmosphere of CO₂ by using the simple device illustrated below in Fig. r The minimum water pressure was used to pull the filtrate through the funnel as it was important that air should not be sucked into the funnel and oxidise the cuprous complex.



Fig. 1. Simple apparatus for filtration in CO_{g} .

The contents of the funnel were washed in turn with water, accione and other (CO_g atmosphere), and transferred as quickly as possible to a boiling tube which was fitted with a bored cork carrying inlet and outlet tubes and from which air had been displaced by CO_g . The tube was placed in a water bath at 40-50° and a steady stream of dry CO_g passed until the complex was a fine, dry, orange powder. This could be preserved in CO_g in a scaled tube, but when exposed to air the powder oxidised slowly giving green cupric oxinate. The orange complex contained no Cl or S (from $S_gO_4^{-8}$) but only cuprous copper (shown by the chromatographic analysis of a solution of the powder in HCl), oxine, and pyridine (expelled by boiling with NaOH) An analogous preparation of the complex using 25 ml of ethanol instead of 10 ml pyridine as the solvent medium resulted in an orange product which could not be dried without decomposition. It appears therefore that combined pyridine is necessary for the stability of the complex.

ANALYSIS OF THE COMPLEX

Wet-ashing with nitric and perchloric acid resulted in the formation of blue crystals which did not give the reactions of ionic copper. The crystals were decomposed by hot NaOH giving colloidal cupric oxide which could not be filtered. Calcination of the complex caused volatilization and loss of copper. In view of these difficulties, the method of BERG⁶ was used as follows:

0.5-0.7 g of the Cu⁺ complex was accurately weighed and transforred to a 400 ml beaker with distilled water (30-50 ml), 5 ml of 10 vol. H_gO_g and 2 ml of a strong solution of NaOH were then added. The beaker was covered with a watch glass and the mixture bolled until the smoll of pyridine had disappeared. 10N HCl was added drop by drop until the precipitate had just dissolved and the solution, after cooling, was transferred quantitatively to a conical flask. Four times its volume of distilled water was added, followed by an excess of potassium iodide. The liberated I_g was titrated with $N/_{s0}$ Na₃S₂O₈ using starch as indicator. A mean value of 16.3% Cu was obtained. The method was checked using cupric oxinate Found Cu: 18.0 and 18.3. Calc. for Cu(CH_ON)_: Cu. 18.1.

References p. 438

Nanalyses were done by the micro-method of Dumas. The N content varied according to the method by which the complex was dried.

At 60-70° in CO₂, found N: 7.5. At 40° in CO₂, found N: 7.9. At 40° in pyridine-saturated CO₂, found N: 8.3. Thus it seemed that some N (presumably as pyridine) was lost during the drying process. A check analysis on cupric oxinate gave N:8.1. Calc. for Cu(C₉H₆ON)₂: N, 8.0.

The complete composition of the somewhat unstable cuprous complex was: Cu: 16.3, C: 59.6, H: 3.9, N: 8.3. $(CuC_9H_6ON\cdot C_9H_7ON)_2\cdot C_5H_5N$ (cf. Ag⁺ compound⁵) requires Cu: 16.2, C: 57.0, H: 3.6, N: 8.9, and this is suggested by the author as a possible formula for the complex.

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SUMMARY

Chromatographic separations upon paper strips are described for Mn^{+2} and Mn^{+3} , Mn^{+3} and Mn^{VII} , and $Cu^{+}and Cu^{+2}$. The last separation was performed in an atmosphere of CO_2 to prevent the oxidation of Cu^{+} . An unstable cuprous-8-hydroxyquinoline complex was prepared from cuprous chloride and oxine in pyridine medium. Analysis of the complex suggested the composition $(CuC_9H_6ON\cdot C_9H_7ON)_{g} \cdot C_8H_8N$.

RÉSUMÉ

Des séparations chromatographiques sur bandes de papier sont décrites pour Mn^{+2} et Mn^{+3} , Mn^{+3} et Mn^{VII} , Cu^+ et Cu^{+2} . Cette dernière séparation a été effectuée dans une atmosphère d'anhydride carbonique pour empêcher l'oxydation de Cu⁺. Un complexe instable Cu(I)-8-hydroxyquinoléine a été préparé à partir de chlorure de cuivre(I) et d'oxine, dans la pyridine. L'analyse de ce complexe donne la composition suivante (CuC₉H₆ON·C₉H₇ON)₂·C₅H₅N.

ZUSAMMENFASSUNG

Die Trennung, durch Chromatographie auf Papierstreifen, von Mn^{+3} und Mn^{+3} , Mn^{+3} und MnVIIund von Cu⁺ und Cu⁺² wurde beschrieben. Die letztere Trennung wurde in Kohlendioxyd-Atmosphäre ausgeführt um Oxvdation des Cu⁺ zu verhindern. Ein unbeständiger Cupro-8-Hydroxychinolin-Komplex wurde aus Kupfer[1]-chlorid und Oxin in Pyridin hergestellt. Die Analyse diese Komplexes hess auf die folgende Zusammensetzung schliessen: $(CuC_9H_8ON \cdot C_9H_7ON)_3 \cdot C_5H_8N$.

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438