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SUMMARIES OF PAPERS PUBLISHED IN ANALYTICA CHIMICA ACTA

Vol. 29, No. 2, August 1963

A KINETIC STUDY OF THE REDUCTION OF VANADIUM(V) CUPFERRATE BY CHLOROFORM

The authors have carried out a kinetic study of the reduction of vanadium(V) cupferrate by chloroform. The reaction is accompanied by a colour change and can thus be followed spectrophotometrically. A theoretical interpretation of the results is given.

P. CROWTHER AND D. M. KEMP, *Anal. Chim. Acta*, 29 (1963) 97–102

SUBSTOICHIOMETRIC DETERMINATION OF TRACES OF MOLYBDENUM BY NEUTRON ACTIVATION ANALYSIS

A rapid, simple activation method for the selective determination of traces of molybdenum is proposed. With substoichiometric reagent addition, the required element need not be isolated in a weighable form and the chemical yield need not be determined. Molybdenum is simultaneously separated not only from metal impurities but also from the bulk of matrices such as germanium.

J. STARÝ, J. RŮŽIČKA AND A. ZEMAN, *Anal Chim. Acta*, 29 (1963) 103–106

IODINE MONOCHLORIDE IN ANHYDROUS ACETIC ACID AS AN OXIDIZING AGENT

A systematic study of the redox reactions of iodine monochloride with various inorganic ions in glacial acetic acid medium is described. Sodium sulphite, mercury(I) perchlorate, antimony trichloride, arsenic trichloride and iron(II) perchlorate were examined. Potentiometric and amperometric methods were used to follow the reduction of iodine monochloride, which yields different products according to the type of reductant.

G. PICCARDI AND P. CELLINI, *Anal. Chim. Acta*, 29 (1963) 107–113

NON-AQUEOUS SPECTROPHOTOMETRIC DETERMINATION OF CITRIC ACID

A non-aqueous spectrophotometric method is described for the determination of citric acid in the presence of carboxylic acids and lactic acid. The method was developed as a result of critical evaluation of the Furth–Herrmann color reaction in a non-aqueous pyridine–acetic anhydride solution. The optimum conditions are described. The absorbance is read at 389 ± 2 mμ. The minimum concentration of citric acid that can accurately be determined is about 2 μg per sample.

T. K. CHOY, J. J. QUATTRONE, JR. AND MILTON ELEFANT, *Anal. Chim. Acta*, 29 (1962) 114–119

THE FLUORIMETRIC DETERMINATION OF FORMALDEHYDE

A sensitive fluorimetric procedure for formaldehyde has been developed. The method, which can measure 0.01 μg formaldehyde, is based on the Hantzsch reaction between acetylacetone, ammonia, and formaldehyde. The product, 3,5-diacetyl-1,4-dihydrolutidine, is colored yellow and fluoresces yellow green. Infra-red spectra indicates that this compound is ionic in dilute solution and aggregated in concentrated solution. The Hantzsch reaction may be extended for the assay of other aldehydes, amines, and β-diketones.

S. BELMAN *Anal. Chim. Acta*, 29 (1963) 120–126

THE USE OF PYRIDINE-2-AZO-*p*-DIMETHYLANILINE AS AN INDICATOR IN GLACIAL ACETIC ACID

Pyridine-2-azo-*p*-dimethylaniline is suggested as an indicator in the titration of amines (aniline, butylamine, *p*-phenylenediamine and ethylene diamine) with perchloric acid in glacial acetic acid medium. The precision obtainable is much better than that with crystal violet indicator. The pyridine dye can also be used satisfactorily in the titration of sodium carbonate and sodium acetate.

S. M. M. CASO, S. C. AND M. CEFOLA, *Anal. Chim. Acta*, 29 (1963) 127–133

THE DETERMINATION OF CHROMIUM IN LOW-ALLOY IRONS AND STEELS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

An atomic absorption spectrophotometric procedure for the determination of 0.001–0.50% chromium in low-alloy iron and steel is described. The sample is dissolved in phosphoric–sulphuric acid before atomisation. The method is rapid, preliminary separations are not required and the accuracy obtained with standard samples is well within the permissible range for routine determinations.

K. KINSON, R. J. HODGES AND C. B. BELCHER, *Anal. Chim. Acta*, 29 (1963) 134–138

CHROMATOGRAPHIC SEPARATION OF LEAD IN CONCENTRATED ZINC SULPHATE SOLUTIONS

(in French)

Spectrographic analysis of lead traces in concentrated zinc sulphate solutions is only possible after elimination of zinc and sulphate ions. Two methods of separation on ion exchangers are proposed: the first method consists in a separation of Pb from Zn on an anion exchanger after elimination of sulphate on a cation exchanger; in the second method, the two steps of the separation are realized on the same cation-exchange column. Both methods are satisfactory, but the second is faster and simpler.

The blank of the method is about 1.5 μg Pb (*i.e.* 0.15 mg Pb/l) for a separation made with 10 ml of electrolyte. The method is therefore suitable for accurate determinations of Pb contents as low as 1 mg/l of concentrated zinc sulphate electrolyte.

M. LECLERCQ ET G. DUYCKAERTS, *Anal. Chim. Acta*, 29 (1963) 139–144

COLORIMETRIC DETERMINATION OF PERSULFATE WITH ALCIAN BLUE

Alcian blue buffered at pH 2.5 is oxidized by persulfate. The plot of optical density at 615 mμ *vs.* concentration of persulfate (within the range of 10 to 80 μg) is linear. The assay is not affected by bromate and iodate.

E. VILLEGAS, Y. POMERANZ AND J. A. SHELLENBERGER, *Anal. Chim. Acta*, 29 (1963) 145–148

COMPLEX FORMATION OF IRON(III) WITH CHROME AZUROL S

The complex formation of iron(III) with 3″-sulpho-2″,6″-dichloro-3,3′-dimethyl-4′-hydroxyfuchson-5,5′-dicarboxylic acid (chrome azurol S) was studied by spectrophotometric, conductometric and potentiometric methods. The pure tetrabasic acid of the ligand was prepared from the impure trisodium salt (commercially available), and the dissociation constants of the ligand were redetermined. At 20° ± 1° and in the presence of 0.10 *M* potassium chloride the dissociation constants were $pk_1 < 0.0$, $pk_2 = 2.25 \pm 0.05$, $pk_3 = 4.71 \pm 0.03$ and $pk_4 = 11.81 \pm 0.03$.

In the pH range 2–4, four complexes were detected (the absolute stability constants at 20° ± 5° and at an ionic strength of 0.10 *M* are given in parentheses): a ring-formed dimer complex $[Fe(H_2O)_2]_2Ch_2^{2-}$ (log $k_{2,2} = 36.2$); a monomer of composition $[Fe(H_2O)_4]HCh^-$ or $[Fe(H_2O)_4]Ch^-$ (the absolute stability constant was calculated as log $k_{1,1} = 15.6$ for the latter composition); a complex $[Fe(H_2O)_4]_2Ch^{2+}$ (log $k_{2,1} = 20.2$) and, finally, a complex of composition $[Fe(H_2O)_2]H_xCh_2^{x-5}$ (the value of x being unknown). In addition, hydroxo complexes of the dimer were formed at higher pH values.

F. J. LANGMYHR AND K. S. KLAUSEN, *Anal. Chim. Acta*, 29 (1963) 149–167

GRAVIMETRIC SEPARATION OF TITANIUM AND ZIRCONIUM FROM NIOBIUM WITH PHENYLACETYLHYDROXAMIC ACID

Phenylacetylhydroxamic acid is used to separate titanium and zirconium from niobium in an oxalate medium at pH 6.5–7.5 in presence of ammonium chloride at room temperature. The method is accurate when the ratio of $(TiO_2 + ZrO_2) : Nb_2O_5$ is 10 : 1 to 1 : 1; when the niobium concentration is higher, reprecipitation is necessary.

Tantalum, citrate, tartrate, lactic acid, EDTA, and a large excess of oxalate interfere.

A. K. MAJUMDAR AND BIJOLI K. PAL, *Anal. Chim. Acta*, 29 (1963) 168–171

COMPLEX FORMATION AND FLUORESCENCE

PART I. COMPLEXES OF 8-HYDROXYQUINOLINE-5-SULFONIC ACID

The fluorescence of solutions of cation complexes with 8-hydroxyquinoline-5-sulfonic acid has been studied. The complexes formed with transition elements do not fluoresce. The additivity of the fluorescence at certain pH values has been used to indicate a new generally applicable analytical method for mixtures of cations which have similar reactions. The method is especially applicable to small or very dilute samples.

J. A. BISHOP, *Anal. Chim. Acta*, 29 (1963) 172–177

COMPLEX FORMATION AND FLUORESCENCE

PART II. THE USE OF 8-HYDROXYQUINOLINE-5-SULFONIC ACID AS AN INDICATOR

The use of fluorescent 8-hydroxyquinoline-5-sulfonic acid complexes in titrations was examined. The disappearance of the fluorescence of these metal complexes, upon titration with a stronger, non-fluorescing complexing agent, can be used to indicate end-points in both complexometric titrations and acid-base titrations.

J. A. BISHOP, *Anal. Chim. Acta*, 29 (1963) 178–181

STUDIES ON THE NAPHTHORESORCINOL REACTION OF TOLLENS

PART I. THE PRINCIPLES OF THE REACTION AND ITS EVALUATION

(in German)

Difficulties and sources of error which occur in TOLLENS' reaction are discussed. The reagent is best prepared by dissolving naphthoresorcinol in glacial acetic acid; such reagent solutions are more stable than those in water or ethanol. It is shown that the dyes formed by ageing aqueous naphthoresorcinol solutions and by the reaction of naphthoresorcinol with uronic acids are not identical.

W. WAGNER, *Anal. Chim. Acta*, 29 (1963) 182–189

THE CATHODE RAY POLAROGRAPHY OF COPPER

(Short Communication)

R. C. ROONEY AND A. M. UPPERTON, *Anal. Chim. Acta*, 29 (1963) 190–192

A KINETIC STUDY OF THE REDUCTION OF VANADIUM(V) CUPFERRATE BY CHLOROFORM

P. CROWTHER AND D. M. KEMP

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(Received January 1st, 1963)

The addition of sodium cupferrate to a solution of vanadium(V) in a hydrochloric acid medium produces an intense red precipitate which can be readily extracted into chloroform. Several workers[1,2] have reported that the red colour due to the vanadium(V) complex gradually fades after extraction, to give a light yellow solution. The fading of the colour can be attributed to interaction of the vanadium(V) with either the cupferron or the chloroform. The former possibility is apparently excluded by the stability of the colour of the suspended precipitate in the aqueous phase before extraction. Colour stability is also observed in organic solvents such as benzene, carbon tetrachloride, acetone, etc.

These facts point to the reduction of vanadium(V) by chloroform, similar to the well-known reduction of chromium(VI) by chloroform. It was the object of this investigation to study the kinetics of this reaction. As the reaction is accompanied by a marked colour change, it can be conveniently followed spectrophotometrically.

EXPERIMENTAL

Reagents

Vanadium(V) solution. 0.8935 g of vanadium pentoxide was dissolved in a minimum quantity of sodium hydroxide. The pH of the solution was adjusted to approximately 3 by the addition of perchloric acid, and its volume accurately diluted to 500 ml with distilled water. This solution was diluted ten times to give a vanadium concentration of 0.1 mg per ml.

Sodium cupferrate. Sodium cupferrate, prepared from ammonium cupferrate, was recrystallized from a mixture of ethanol and water. A fresh 0.1 *M* aqueous solution was prepared daily because of the unstable nature of cupferrate solutions.

Chloroform. Merck, G.R. grade chloroform, containing 1% ethanol, was used for the extraction of the vanadium(V) complex.

All other reagents were of A.R. or equivalent grade.

Apparatus

Absorbancy measurements were made in 1-cm cells on a Zeiss PMQII quartz spectrophotometer. pH values were obtained with a Metrohm E300 pH meter, using a calomel–glass electrode system.

Temperature control was effected by means of a thermostat bath of approximately 40-l capacity, the temperature of which could be controlled to better than 0.1°.

Procedure

Five ml of 2 *N* hydrochloric acid and 20 ml of vanadium(V) solution were pipetted into a 100-ml separating funnel; 3 ml of sodium cupferrate were then added, producing a dark red precipitate of vanadium(V) cupferrate. The separating funnel was well stoppered, inverted and immersed in the thermostat bath, with only the tap and delivery stem of the funnel protruding above the water level of the bath. A measuring cylinder containing 25 ml of chloroform, to be used for the extraction of the precipitate, was also immersed in the thermostat.

From preliminary experiments it was found that this acid concentration gave the best precipitation conditions and also resulted in the complete extraction of the vanadium(V) complex into the organic phase when the solution was shaken with chloroform.

Water from the thermostat was circulated through the cell chamber of the spectrophotometer. The sample cells, after cleaning and drying, were kept for approximately 30 min in the cell chamber before the commencement of a reaction.

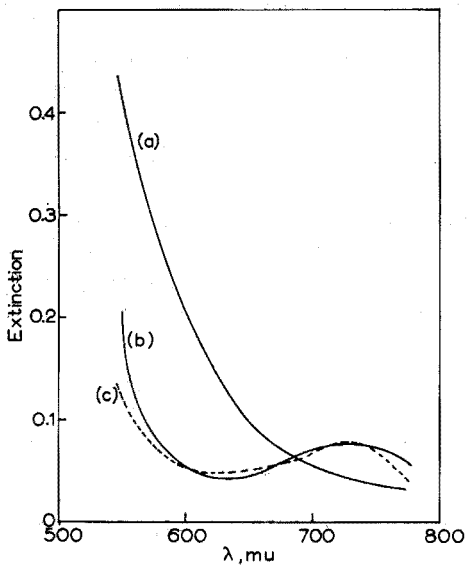

Fig. 1. Extinction against wavelength for various vanadium cupferrate solutions (see text). (a) Vanadium(V)-complex, (b) as (a), but 100 min after extraction, (c) Vanadium-(IV)-complex.

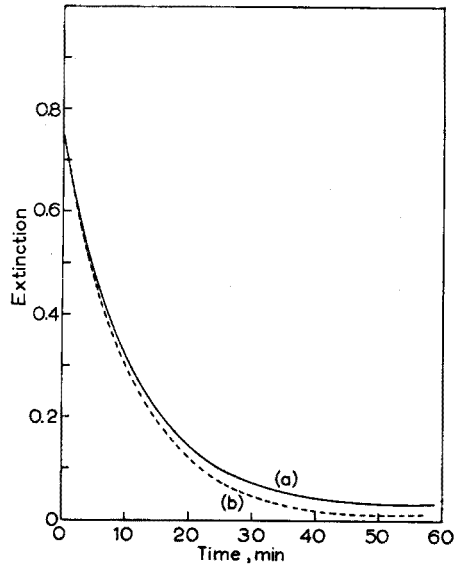

Fig. 2. Extinction against time (temperature 293.1° K). (a) observed extinction (E_T), (b) corrected extinction (E_a).

When thermal equilibrium had been attained, the chloroform was added to the aqueous solution in the separating funnel — the start of the reaction was taken from this time — and the two phases were shaken vigorously together for 10 sec. The organic phase, which now contained all the vanadium complex, was drawn off and filtered into a spectrophotometer cell. The progress of the reaction between the vanadium complex and chloroform was now followed by measuring the change in absorbancy with reference to a chloroform blank at 600 mμ (see Fig. 1). The first reading was taken 2 min after the start of the reaction and thereafter every min. The reaction required approximately 100 min to reach completion.

SPECTROPHOTOMETRIC MEASUREMENTS: THEORETICAL INTERPRETATION

In the reaction under investigation, the solution is initially coloured dark red due to a vanadium(V) cupferrate complex. This colour disappears, with the formation of a yellow coloured complex. By Beer's law

$$\log \frac{I_0}{I_t} = \varepsilon c t = E \tag{1}$$

where I_0 = the intensity of the incident light
I_t = the intensity of the transmitted light
c = concentration
E = extinction
ε = the extinction coefficient
t = thickness of the medium in cm.

As an extension to this law it may be assumed that for a mixture containing more than one absorbing component, the observed extinction is the sum of the extinctions due to the various absorbing components, provided that they do not interact with each other chemically. Thus,

$$\begin{aligned} \log \frac{I_0}{I_t} &= (\varepsilon_a c_a + \varepsilon_b c_b + \ldots + \varepsilon_n c_n)t \\ &= \left(\sum_{i=a}^{n} \varepsilon_i c_i\right) t \\ &= E_a + E_b + E_c + \ldots + E_n \\ &= E_T \end{aligned} \tag{2}$$

At the start of the reaction there is no yellow complex present and absorption, made with reference to a chloroform blank, is due entirely to the red complex. Similarly at the completion of the reaction the absorption is due to the yellow complex only. At any intermediate time the observed extinction E_T will be the sum of the contributions of the red and yellow complexes.

Since it is intended to follow the reaction between the vanadium(V) and chloroform, it is necessary to know the specific contribution of the vanadium(V) complex to the observed extinction. If the equations (1) and (2) are valid, the contribution of this complex to the extinction may be derived as follows.

The initial conditions at time $t = 0$ are:

$$\text{extinction due to the vanadium(V) complex} = E_a = E_T = E_0 \tag{3a}$$

and

$$\text{extinction due to the yellow complex} = E_b = 0 \tag{3b}$$

The final conditions at the completion of the reaction, denoted by time $t = \infty$ are,

$$E_b = E_T = E_\infty \tag{4a}$$

and

$$E_a = 0 \tag{4b}$$

From equations (1) and (2) it may be assumed that the rate of increase of E_b is directly proportional to the rate of decrease of E_a. E_b can thus be written as a linear function of E_a in the form,

$$E_b = -\frac{E_\infty}{E_0} E_a + E_\infty \tag{5}$$

Substituting equation (5) into equation (2) we get,

$$E_T = E_a + \left[E_\infty - \frac{E_\infty}{E_0} E_a\right] \tag{6}$$

and therefore

$$E_a = \frac{(E_T - E_\infty) E_0}{E_0 - E_\infty} \tag{7}$$

E_0 is obtained by extrapolation of E_T values to zero time (see Fig. 2) and E_∞ is E_T after practical completion of the reaction (approximately 100 min).

The excess of cupferron extracted into the chloroform layer does not interfere at 600 mμ; it does, however, slowly decompose, but significant interference is not observed until after about 2 h.

RESULTS

Order of the reaction and test of method

Algebraically if A_1 and A_2 are the concentrations of component A at times t_1 and t_2 respectively, then the mean value of $\mathrm{d}A/\mathrm{d}t$ is given by

$$\frac{\mathrm{d}\bar{A}}{\mathrm{d}t} = \frac{A_2 - A_1}{t_2 - t_1} \tag{8}$$

Consider the rate equation (of order n)

$$-\frac{\mathrm{d}A}{\mathrm{d}t} = k_A A^n \tag{9}$$

This may be written as

$$\log\left(\frac{-\mathrm{d}A}{\mathrm{d}t}\right) = \log k_A + n \log A \tag{10}$$

A plot of $\log(-\mathrm{d}A/\mathrm{d}t)$ against $\log A$ should give a straight line whose slope is n, the order of the reaction.

As E_a is proportional to the concentration of vanadium(V),

$$\log\left(\frac{-\mathrm{d}E_a}{\mathrm{d}t}\right) = \log \frac{E_{a_1} - E_{a_2}}{t_2 - t_1}$$

which has been plotted against $\log \bar{E}_a$ in Fig. 3. $E_{a_2} - E_{a_1}$ are the differences in E_a for successive minute intervals, and $\bar{E}_a$ is taken as $(E_{a_1} + E_{a_2})/2$.

Fig. 3 gives a straight line of slope 1.0, suggesting a first order reaction. The scatter of points is to be expected because of the necessarily small time intervals taken.

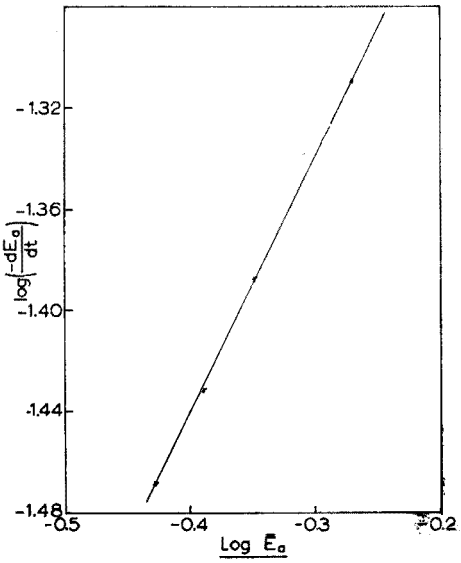

Fig. 3. Log–log plot of reaction rate against extinction. Slope, 1.0; temperature 293.1°K.

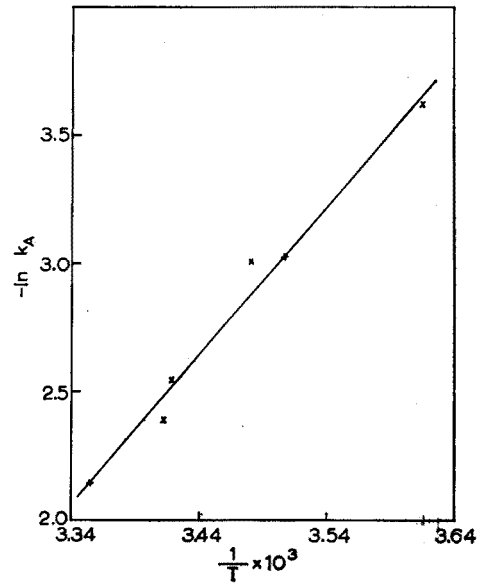

Fig. 4. Plot of $-\ln k_A$ against $1/T$.

The reaction has been studied at a number of different temperatures, and assuming that the reaction is of the first order, the rate constant $\bar{k}_A$ has been calculated for the different temperatures with the aid of the following equation derived from equation (10):

$$\bar{k}_A = \frac{E_{a_1} - E_{a_2}}{\bar{E}_a(t_2 - t_1)} \tag{11}$$

$\bar{k}_A$ is the average of a number of k_A values at a specific temperature, calculated for different E_a values corresponding to values of t_1, $t_2 < 9$ min (Table I), *i.e.* in the region where the correction due to the contribution of the yellow complex is very small.

TABLE I

VALUES OF THE RATE CONSTANT AT DIFFERENT TEMPERATURES

Temp. °K	$\bar{k}_A \cdot 10^2$
276.6	2.682
285.1	4.864
287.3	4.934
292.6	7.830
293.1	9.154
298.1	11.730

ENERGY OF ACTIVATION

Consider the Arrhenius equation

$$k_A = A \exp\left(\frac{-E}{RT}\right) \tag{12}$$

where A = constant
E = energy of activation
R = molar gas constant.
This equation may be written

$$\ln k_A = \ln A - \frac{E}{RT} \tag{13}$$

whence a plot of $-\ln k_A$ against $1/T$ should give a straight line of slope E/R. This is in fact found to be the case (Fig. 4) and the calculated activation energy is 11.45 kcal/mole.

DISCUSSION

It is not certain whether the colour change is due to reduction of vanadium(V) by chloroform or whether it is partly due to some other effect.

Preliminary experiments indicate it to be mainly due to a reduction to vanadium-(IV). Thus vanadium(V) cupferrate was extracted into chloroform, filtered immediately and the spectrum plotted against chloroform using a Zeiss PMQII quartz spectrophotometer with automatic 100-point adjustment (see Fig. 1a). This solution was left for 100 min and the spectrum again recorded (Fig. 1b).

The spectrum of a vanadium(IV) cupferrate solution in chloroform was also recorded (Fig. 1c). This was prepared by extracting vanadium(IV), obtained by reduction of vanadium(V) with sodium sulphite, into chloroform after addition of sodium cupferrate.

From Fig. 1 it is clear that the spectrum of vanadium(V) cupferrate in chloroform changes with time to a spectrum resembling that obtained for vanadium(IV). On the other hand, the colour of a vanadium(IV) cupferrate solution in chloroform also changes after extraction, though much less than that of vanadium(V) cupferrate (of the order of a few per cent below 600 mμ).

The true effect may thus be a combination of reduction of vanadium(V) and another as yet unknown reaction.

SUMMARY

The authors have carried out a kinetic study of the reduction of vanadium(V) cupferrate by chloroform. The reaction is accompanied by a colour change and can thus be followed spectrophotometrically. A theoretical interpretation of the results is given.

RÉSUMÉ

Les auteurs ont effectué une étude cinétique de la réduction du cupferrate de vanadium(V) par le chloroforme. La réaction étant accompagnée d'un net changement de coloration, elle peut être suivie spectrophotométriquement. Une interprétation théorique en est donnée.

ZUSAMMENFASSUNG

Beschreibung einer reaktionskinetischen Untersuchung der Reduktion von Vanadium-(V)-cupferrat durch Chloroform. Der Verlauf der Reaktion, die mit einer Farbänderung verbunden ist, kann spektrophotometrisch verfolgt werden. Es wurde festgestellt, dass die Farbänderung nur zum Teil auf einer Reduktion des Vanadium-(V) zu Vanadium-(IV) beruht und dass daneben eine noch nicht aufgeklärte Reaktion stattfindet.

REFERENCES

1 D. M. KEMP AND A. A. SMALES, *Anal. Chim. Acta*, 23 (1960) 397.
2 H. J. M. BOWEN, Wantage Radiation Laboratories, private communication.

SUBSTOICHIOMETRIC DETERMINATION OF TRACES OF MOLYBDENUM BY NEUTRON ACTIVATION ANALYSIS

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(Received January 7th, 1963)

The determination of traces of elements by destructive activation analysis is generally carried out as follows. To the dissolved irradiated sample a milligram amount of an isotopic carrier is added and the element required is isolated in a radiochemically pure form. For this purpose, many separation steps (which need not be quantitative) are necessary and the chemical yield of these procedures must be determined; thus, the end-product must be isolated in a well-defined chemical form suitable for weighing. In all cases, an excess of reagent (precipitating, extracting or chelating) is used.

The substoichiometric type of determination is based on the use of a smaller amount of reagent than that equivalent to the amount of the carrier added. This allows the number of separation steps to be substantially reduced and the determination of the chemical yield to be avoided.

THEORETICAL

The activity A of the element to be determined, induced in the sample by irradiation, is calculated from the relation

$$A = a\frac{x}{m} \tag{1}$$

where a is the activity of the recovered fraction of weight m and x is the amount of the carrier added. The same relationship applies for a simultaneously irradiated standard sample of the element to be determined.

The amount of the required element (y) originally present in the test sample is calculated from the activity induced by irradiation

$$y = y_s\frac{A}{A_s} \tag{2}$$

where y_s is the amount of the required element in the standard sample and the subscript s denotes the standard sample. For the case $x = x_s$ and $m = m_s$, it follows that

$$y = y_s\frac{a}{a_s} \tag{3}$$

This is the basic equation of the substoichiometric type of determination and is valid if the following two conditions are fulfilled:

(1) the amounts of carrier added to the test and standard samples after irradiation must be exactly the same ($x = x_s$);

(2) the amounts of the required element isolated from the test and standard samples must be exactly the same ($m = m_s$).

The second condition can be fulfilled by adding a smaller amount of the reagent than that which stoichiometrically corresponds to the amount of carrier added. The reagent used must react quantitatively with the required element to a compound which can readily be isolated from the excess of the unreacted element and from the other elements simultaneously present in the test solution. The optimum conditions for such a separation can be chosen on the theoretical basis described previously[1]. From this theory it is evident that for the determination of an element, it is very advantageous to use a reagent which forms a compound with a very high extraction constant, K_o; in such cases the determination will be very selective.

In a previous paper[2], the extraction of various metal oxinates was investigated. The data obtained at that time in conjunction with the above-mentioned theory[1] indicate that the substoichiometric determination of molybdenum with 0.02 *M* 8-hydroxyquinoline in chloroform can be carried out at pH values higher than 1.0. At this pH, molybdenum reacts quantitatively with 8-hydroxyquinoline, forming an extractable molybdenum oxinate, so that it is possible to extract always exactly the same amount of molybdenum from solutions of various concentrations. From the K_o values of the metal oxinates[2] it is evident that only palladium will interfere, and this can be easily removed by a preliminary extraction with dithizone. Other metal oxinates with much lower K_o values do not interfere even when present in great excess. The K_o values of vanadium(V), thallium(III), iron(III), zirconium(IV), gallium(III) and copper(II) are not very low, but these metals interfere only when present in great excess. Of these metals, vanadium, thallium and zirconium can be eliminated because of their nuclear properties. The non-interference of the appropriate amounts of iron, gallium and copper was experimentally verified at pH 1.3. Ultimately, a method was developed for the determination of trace amounts of molybdenum in germanium dioxide.

EXPERIMENTAL

Apparatus

A well-type scintillation counter with a NaI (Tl) crystal. A mechanical shaker. A pH-meter pH M 4B Radiometer (Copenhagen). Glass test tubes (40 ml) with ground stoppers.

Reagents

All reagents used were of A.R. purity. The 0.02 *M* 8-hydroxyquinoline solution used in all experiments was prepared by dissolving the appropriate amount of reagent in chloroform.

Irradiation

Samples of germanium dioxide and standards of ammonium molybdate were sealed in quartz ampoules and simultaneously irradiated in a reactor with a neutron flux of

TABLE I

(n, γ) REACTIONS OF NATURALLY OCCURRING MOLYBDENUM ISOTOPES

Naturally occurring isotope	*Abundance (%)*	*Thermal neutron activation cross-section*	*Isotope formed*	*Half-life*	*Mode of decay*
^{92}Mo	15.86	< 6 mb	^{93m}Mo	6.95 h	β^+, I.T.
^{98}Mo	23.75	0.45 b	^{99}Mo	66.0 h	β, γ
^{100}Mo	9.62	0.5 b	^{101}Mo	14.6 m	β, γ

10^{12} neutrons/cm^2/sec for 20 h and cooled for three days. The nuclear data for radioactive molybdenum isotopes formed during irradiation are summarized in Table I.

Preliminary experiments

The possibility of using the substoichiometric principle for the determination of molybdenum was verified by the following experiments.

Irradiated ammonium molybdate (0.263 mg) was dissolved in dilute ammonia and made up to a volume of 100 ml. To known aliquots of this solution were added exactly the same amounts of carrier (1 ml of 0.1 *M* ammonium molybdate solution). The acidity of the solutions was adjusted to approximately pH 1.3 with sulphuric acid and the solutions were simultaneously extracted for 10 min with 3 ml of 0.02 *M* hydroxyquinoline in chloroform. The activities of 2 ml of each of these extracts were measured under the same conditions. The aqueous phases were then again extracted with 3 ml of the same oxine solution. No differences between the activities of the first and second extracts were found, from which it follows that exactly the same amounts of molybdenum carrier were extracted. The activities are directly proportional to the amounts of irradiated molybdenum present, at least over the range 0–7 μg of molybdenum. The same results were obtained even in the presence of an excess of copper, iron and gallium. On the basis of these experiments a procedure was developed for the determination of molybdenum in germanium dioxide.

Procedure

The samples of germanium dioxide and standard samples were irradiated simultaneously and then treated in exactly the same way. The GeO_2 sample was dissolved in 3 ml of 8 *N* sodium hydroxide and the solution was neutralised by dropwise addition of 2 *N* sulphuric acid until a white precipitate appeared[3]. The precipitate was dissolved by addition of a few drops of the sulphuric acid and 1 ml of 0.1 *M* ammonium molybdate solution as carrier was added. The pH of the solution was adjusted to approximately 1.3. The solution was extracted with 3 ml of 0.02 *M* oxine solution in chloroform for 10 min. The organic phase was washed twice more with diluted sulphuric acid (0.001 *M*) and the 66-h ^{99}Mo activity of 2 ml of the extract obtained was measured in the well-type scintillation counter (*a*).

An appropriate aliquot of the irradiated standard of molybdenum (y_s) was treated in exactly the same way. The activity of 2 ml of the chloroform extract was measured under the same conditions. The quantity of molybdenum present as an impurity in GeO_2 was calculated from equation (3).

RESULTS AND DISCUSSION

The results obtained (Table II) indicate that the germanium dioxide analysed contained less than 10^{-4} % of molybdenum. The sensitivity of the determination can be further increased by using a higher neutron flux, and by increasing the weight of sample taken and/or the irradiation time. The purpose of the present work was to

TABLE II

SUBSTOICHIOMETRIC DETERMINATION OF TRACES OF MOLYBDENUM

GeO_2 analysed (g)	0.0726	0.1496
The amount of irradiated molybdenum standard added to the irradiated GeO_2 sample	1.59	—
Activity[a] obtained from the analysed sample a	693	(7)
The amount of molybdenum in irradiated standard sample y_s (μg)	1.59	3.18
Activity[a] obtained from standard sample a_s	655	1044
The amount of molybdenum found in GeO_2 (μg)	1.68	0.02

[a] All activities are expressed in counts/min and are the mean values of 10 measurements corrected for background.

develop and verify a substoichiometric method for determination of molybdenum and this was accomplished. The published methods[4] for the determination of molybdenum by activation analysis are much more time-consuming than the proposed method.

The authors thank Dr. V. MAJER, D.Sc. for his interest in this work.

SUMMARY

A rapid, simple activation method for the selective determination of traces of molybdenum is proposed. With substoichiometric reagent addition, the required element need not be isolated in a weighable form and the chemical yield need not be determined. Molybdenum is simultaneously separated not only from metal impurities but also from the bulk of matrices such as germanium

RÉSUMÉ

Une méthode par activation est proposée pour le dosage de traces de molybdène. Ce dernier peut ainsi être séparé simultanément des impuretés métalliques (du germanium en particulier).

ZUSAMMENFASSUNG

Beschreibung einer Methode zur Bestimmung von Spuren von Molybdän durch Aktivierung. Die Methode eignet sich gleichzeitig zur Abtrennung von metallischen Verunreinigungen, besonders Germanium.

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IODINE MONOCHLORIDE IN ANHYDROUS ACETIC ACID AS AN OXIDIZING AGENT

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In the course of a systematic study of oxidation-reduction reactions in non-aqueous media, consideration was given to the behaviour of iodine monochloride. This compound has aroused a good deal of interest in the past with a view to its possible application in the field of organic chemistry. In the inorganic field, TOMÍČEK and his school[1] were the first to establish a stoichiometric relationship for the oxidations of sodium thiosulphate and arsenic trichloride in anhydrous acetic acid. Further reactions of iodine monochloride in non-aqueous media are described in the present paper.

EXPERIMENTAL

Apparatus and reagents

The oxidation-reduction reactions were followed using amperometric and potentiometric methods. The apparatus used was that described previously[2]. Analytical reagent-grade acetic acid was used.

The iodine monochloride solutions, about 0.025 *M*, were prepared by dissolving recrystallized ICl in anhydrous acetic acid; the iodine content was determined by iodometric titration in aqueous medium. Freshly prepared solutions were used and stored in the dark. The solutions of reducing agents were prepared as described in our previous communication[2].

RESULTS

Reaction of iodine monochloride with sulphites

Sodium sulphite dissolved in glacial acetic acid is easily oxidized in the cold by iodine monochloride and the reaction can be considered quantitative if a concentration of sodium acetate greater than molar is present. With the amperometric method at a potential difference (ΔE) of 0.8 V, two minima are found, one when the molar ratio of oxidant to reductant is 1:1, and the other when it is 2:1. With the potentiometric method and a saturated calomel electrode, the changes in potential with increasing ICl/SO_3^{2-} ratios are shown in Fig. 1. In the first part of the curve, before the first rise in potential, it was found that as the oxidant is gradually added, it reacts to form iodide; the solution remains colourless until point A is reached, *i.e.* when all the sulphite has been oxidized to sulphate. Further addition of iodine

monochloride causes the oxidation of the iodide ion, and the elementary iodine then combines with the excess of iodide in solution to form the complex triiodide ion, which is stable in the medium involved:

$$2\ I^- + ICl \rightarrow I_3^- + Cl^-$$

The yellowish brown colour of the triiodide ion can be clearly seen during the titration corresponding to the part of Fig. 1 extending from A to B. When all the iodine in

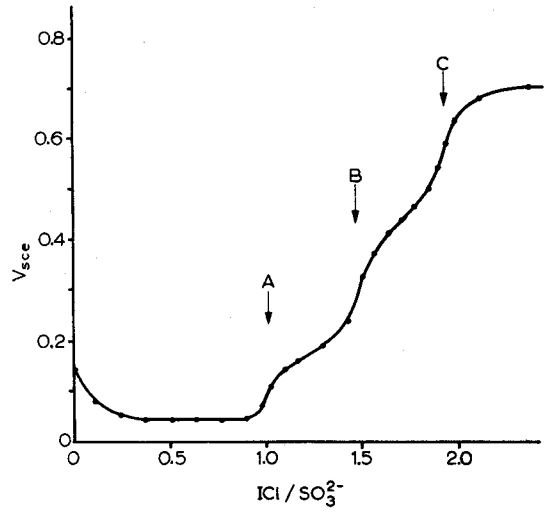

Fig. 1. Potentiometric titration of sodium sulphite with iodine monochloride.

solution has been transformed to triiodide a second jump in potential occurs and the colour of the solution undergoes a slight change to a bright red tint. The potential rises slowly until, when the ratio reaches 2:1, all the triiodide is converted to iodine:

$$I_3^- + ICl \rightarrow 2\ I_2 + Cl^-$$

At C the flattening of the curve indicates the presence of excess of iodine monochloride.

When the potentiometric titration is carried out in acetic acid containing increasing concentrations of water, steps A and C become more marked whereas B gradually becomes less marked until it is transformed into a slight fall. The disappearance of the B step is due to the much smaller stability of the triiodide ion in water ($pK = 3$)[3] than in organic solvents (in CH_3CN $pK = 7$)[4]. It is interesting to note that, in contrast to the precipitation that occurs on oxidation with bromine, the sulphate formed by oxidation of sulphite remains in solution.

Reaction of iodine monochloride with mercury(I)

Mercury(I) perchlorate is oxidized by iodine monochloride in glacial acetic acid even in the absence of sodium acetate. In fact, sodium acetate should be avoided because it leads to the precipitation of mercury(I) acetate which scarcely reacts with the oxidant. The course of the reaction can be followed by the amperometric

method provided that a certain amount of sodium perchlorate is added to the solution; this has the effect of diminishing the resistance-loss in the cell. With a potential difference of 0.8 V, the current remains almost constant throughout the titration until the equivalence point is reached, *i.e.* when 1 mole of oxidant is present for each two moles of mercury(I) (ICl/Hg = 0.5). The iodine monochloride oxidizes the mercury(I) and is itself reduced to iodide, so that the solution remains colourless until the equivalence point is reached. With the potentiometric method a sudden rise in potential occurs at the same point (Fig. 2). From the upper of the two curves in Fig. 2 it can be seen that the oxidation-reduction potential of the system Hg(I)/Hg (II) in glacial acetic acid is relatively so high as not to show the reduction of the iodine monochloride to iodide. On the other hand, it must be kept in mind that mercury(II) forms with the halogens, and especially with iodide, fairly stable complexes so that its presence would tend to promote heterolytic dissociation of iodine[5]:

$$I_2 \rightleftarrows I^+ + I^-$$

After the equivalence point, the solution becomes brown in colour owing to the presence of iodine; thereafter, no further points of interest appear.

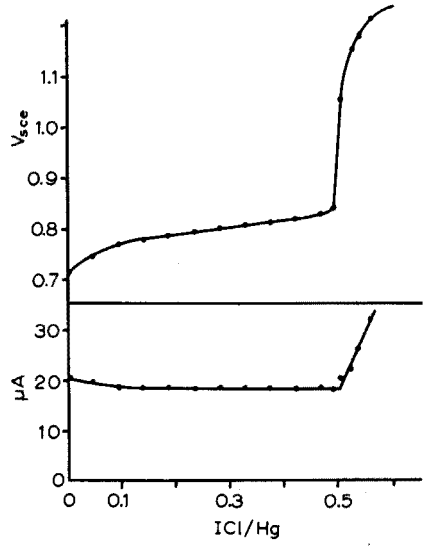

Fig. 2. Titration of mercury(I) perchlorate with iodine monochloride. Upper, potentiometric; lower, amperometric.

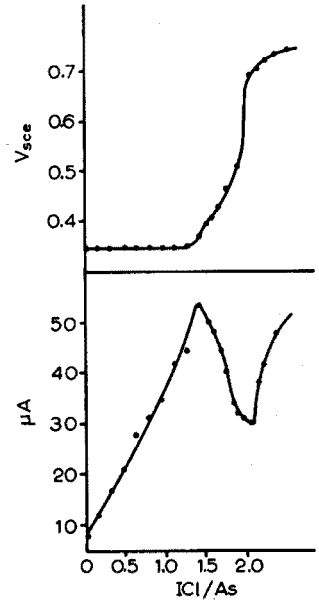

Fig. 3. Titration of arsenic trichloride with iodine monochloride. Upper, potentiometric; lower, amperometric.

Reaction of iodine monochloride with arsenic(III)

Unless anhydrous sodium acetate up to a concentration of 1.2 *M* is added to the solution, the oxidation of arsenic(III) does not go to completion in glacial acetic acid. The reaction takes place between 2 molecules of iodine monochloride (which yields iodine) and 1 molecule of arsenic(III):

$$2\ I^+ + As(III) \rightarrow 2\ As(V) + I_2$$

The experimentally determined equivalence point differs by less than 2% from the theoretical in both the amperometric and potentiometric methods. Both the curves shown in Fig. 3 also show a current maximum and a potential jump at the same point (ICl/As = 1.5) where the reaction, assuming the formation of the triiodide complex, may be written schematically:

$$3\ I^+ + 2\ As(III) \rightarrow 2\ As(V) + I_3^-$$

Both from the high value of the redox potential of the system As(III)/As(V) and from the yellowish brown colour formed at the beginning of the titration, it must be concluded that elementary iodine is liberated. Thereafter, the formation of triiodide must again be attributed to the dissociation of iodine and to its stability in glacial acetic acid.

Reaction of iodine monochloride with antimony(III)

Antimony trichoride is oxidized by iodine monochloride in glacial acetic acid in the cold. The reaction is very slow but quantitative results are obtained in solutions which are about 1 *M* in sodium acetate. Fig. 4 shows the curves for the potentiometric and amperometric titrations. It is seen that there is only a single equivalence point, corresponding to the molar ratio of 2:1 for ICl/Sb.

The oxidation of antimony entails the reduction of the iodine monochloride to elementary iodine, as can be seen from the characteristic red colour of the solution after the first addition of oxidant. Provided that the titration is performed slowly the equivalence point can be readily determined by either method with an error of about 1%.

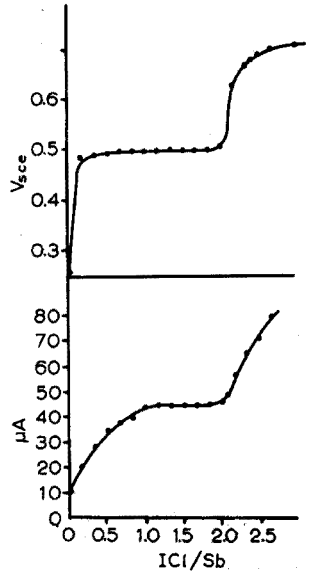

Fig. 4. Titration of antimony trichloride with iodine monochloride. Upper, potentiometric; lower, amperometric.

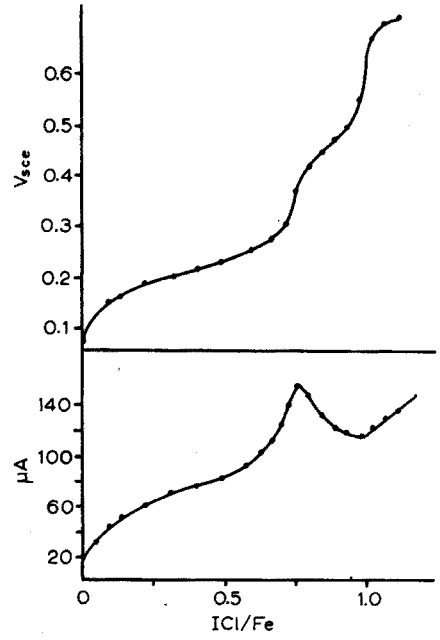

Fig. 5. Titration of iron(II) perchlorate with iodine monochloride. Upper, potentiometric; lower, amperometric.

Reaction of iodine monochloride with iron(II)

Iron(II) can also be oxidized quantitatively by iodine monochloride in the presence of sodium acetate. The reaction is rapid in this medium, but the precautions which are necessary in the oxidation with bromide[2] are also essential here. The oxidation is complete when one mole of oxidant has been added for each mole of reductant; iodine monochloride is converted to iodine. During the titration the solution becomes intensely brown because of the presence of basic iron(III)/acetate. Figure 5 shows, as in the case of the iodine monochloride–arsenic(III) reaction, a maximum current peak when three-quarters of the total iron(II) has been titrated; this corresponds to the formation of the triiodide complex. Potentiometrically (Fig. 5, upper curve), two potential jumps are seen corresponding to the formation of triiodide and iodine; both are very clear and in agreement with the theoretical values.

TABLE I

IODINE MONOCHLORIDE FOR TITRIMETRIC DETERMINATION OF SOME REDUCING AGENTS

Reducing ion	CH_3COONa *(M)*	*mg*		*Error*	
		Taken	*Found*	*mg*	%
SO_3^{2-}	1.0	14.09	13.98	−0.11	−0.8
	1.2	14.09	14.16	+0.07	+0.5
	1.2	31.85	31.50	−0.35	−1.1
	1.4	14.09	14.13	+0.04	+0.3
	1.6	31.85	31.65	−0.20	−0.6
Hg_2^{2+}	0.4	14.32	14.43	+0.11	+0.7
	0.8	11.32	11.40	+0.08	+0.8
	—	85.02	84.81	−0.21	−0.2
	—	85.02	85.44	+0.42	+0.5
	—	85.02	85.95	+0.93	+1.1
	—	85.02	84.68	−0.34	−0.4
As^{3+}	1.0	3.66	3.59	−0.07	−1.9
	1.2	3.66	3.68	+0.02	+0.5
	1.2	3.66	3.59	−0.07	−1.9
	1.6	3.66	3.70	+0.04	+1.1
	1.6	3.66	3.63	−0.03	−0.8
Sb^{3+}	0.6	9.72	9.63	−0.09	−1.0
	1.0	6.52	6.57	+0.05	+0.8
	1.0	6.52	6.53	+0.01	+0.2
	1.2	6.47	6.44	−0.03	−0.5
	1.2	6.52	6.55	−0.03	−0.5
Fe^{2+}	0.8	38.62	38.02	−0.60	−1.5
	0.8	38.62	37.81	−0.81	−2.0
	0.8	38.62	38.14	−0.48	−1.3

DISCUSSION

The experimental results, which are shown on Table I, demonstrate how iodine monochloride can be used titrimetrically in the determination of certain reducing agents. The facts that oxidation by iodine monochloride can go through two separate and distinct stages according to the type of reductant used, and that triiodide is

very stable in glacial acetic acid, lead to a behaviour pattern which is strongly dependent on the nature of the various systems to which the method is applied. Although the use of the aqueous calomel electrode as a reference does not yield accurate absolute values for the redox potentials in glacial acetic acid, it is nevertheless possible to obtain comparable values between the various reductants used. In the case of sodium sulphite, two redox systems are apparent, I^-/I_3^- and I_3^-/I_2, coupled with the lowest redox potential of all the systems studied. In the case of iron(II) salts and of arsenic trichloride only the second jump in potential is observed because the reductants convert the iodine in the oxidant directly to triiodide. Antimony(III), which has a rather high redox potential, reduces I^+ to elementary iodine, as can be seen from the single jump in the potentiometric curve. Mercury(I) is also oxidized by iodine monochloride which is reduced to iodide, as is shown by the colour of the solution and the molar ratio at the equivalence point. Whilst this conclusion about the reduction to iodide cannot rest solely on the redox potentials, it is suggested that under the conditions used, mercury(II) combines with iodide and chloride to form mixed complexes similar to those observed in aqueous solutions. Thus, the iodinating power of acid solutions of elementary iodine is strongly promoted by mercury(II), probably by displacement of the equilibrium in the heterolytic dissociation of iodine. For example, iodination of antipyrine is only possible in the presence of mercury(II) salts[5]. Similarly, this explains why the oxidation of mercury(I) is possible with iodine monochloride in the absence of sodium acetate. The curves obtained by both the amperometric and potentiometric methods in the oxidations by iodine monochloride lead to identical conclusions. In all cases in which the triiodide complex is formed, it can be seen that the amperometric curve passes through a point of maximum current precisely when the concentration of the triiodide complex is maximal. This is quite comprehensible when consideration is given to the principle upon which the method is based and to the fact that the I_2/I^- system in glacial acetic acid is, as in water, reversible. The absence of a maximum in the cases of mercury(I) and antimony(III) signifies that at any rate during the titration the formation of triiodide does not occur. In the case of sulphites, the first part of the amperometric curve shows a rise in current which culminates in a blunt maximum. The sulphite/sulphate system is irreversible in acetic acid (as in the case of the reactions with bromine), and the unusual behaviour of this system may be attributable to the kind of oxidant used. Actually, if sodium sulphite is titrated with iodine solutions under the same conditions, a quite similar curve is obtained.

SUMMARY

A systematic study of the redox reactions of iodine monochloride with various inorganic ions in glacial acetic acid medium is described. Sodium sulphite, mercury(I) perchlorate, antimony trichloride, arsenic trichloride and iron(II) perchlorate were examined. Potentiometric and amperometric methods were used to follow the reduction of iodine monochloride, which yields different products according to the type of reductant.

RÉSUMÉ

Une étude systématique a été effectuée sur les réactions redox du monochlorure d'iode, en milieu acétique glacial, avec divers composés minéraux (sulfite de sodium, perchlorate de mercure(I), chlorure d'antimoine(III), chlorure d'arsenic(III) et perchlorate de fer(II)). La réduction du monochlorure d'iode, donnant divers produits, suivant le réducteur, a été suivie potentiométriquement et ampérométriquement.

ZUSAMMENFASSUNG

Beschreibung einer systematischen Untersuchung der Redox Reaktion zwischen Jodmonochlorid und Natriumsulfit, Quecksilber-(I)-perchlorat, Antimon-(III)-chlorid, Arsen-(III)-chlorid und Eisen-(II)-perchlorat. Der Verlauf der jeweiligen Reaktion wurde potentiometrisch und amperometrisch verfolgt.

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NON-AQUEOUS SPECTROPHOTOMETRIC DETERMINATION OF CITRIC ACID

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Numerous chemical methods for the estimation of citric acid by the Furth–Herrmann reaction[1] have appeared in the literature[2–7]. The accuracy of these methods, however, restricted is by some of the features of this color reaction. The recommended procedures are exothermic in water, and for maximum reproducibility the reaction temperature must be kept constant and below 60°, so that the color will develop rapidly. MARIER AND BOULET[5] proposed the addition of pyridine to the sample before acetic anhydride, thereby allowing the color to develop first, and obviating the need for timing addition of pyridine. The reaction temperature is controlled by placing the sample tubes in a water bath immediately after addition of the anhydride[5,7,8]. This method was found to give satisfactory results, but is tedious and requires great care in controlling the temperature.

In order to avoid this tedious procedure, a non-aqueous method for the determination of citric acid was developed and is described here. The only other work mentioned in the literature for this determination in an anhydrous medium is that of GRONVALL[3], who used the Furth–Herrmann reaction for the rough estimation of the citric acid content of eye fluids. The poor results obtained by this method were due to the presence of proteins. The difficulty in determining the citric acid content in milk and in other dairy products is also attributed to the presence of protein. Precipitation with trichloroacetic acid or ion-exchange separation are the principal methods of deproteinization now in use. The results of the citric acid determination were found to be somewhat erratic, both in aqueous and in non-aqueous systems when the first method was used to deproteinize the sample, since the reagent was found to intensify the color produced. Variation of the colorimetric readings of the eluate when the deproteinization was carried out by the second method were also observed. It was therefore necessary to develop a method for the complete deproteinization of the sample without the use of a chemical reagent which would interfere with the determination. The procedure described, which makes use of a Florisil-packed chromatographic column, was found to give satisfactory results.

In our experiments no temperature rise was observed when carefully dried citric acid samples were reacted with anhydrous acetic anhydride and pyridine. The ultraviolet spectra of these solutions showed three maxima, at 460, 406 and 389

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$\pm$ 2 mμ. A plot of the absorbances at 406 and 389 mμ concentration gave a straight-line relation. A maximum absorptivity of about 1000 was recorded at 389 mμ (many times the value that HARTFORD[4] and other workers[2-6] reported for the aqueous mixture) and is indicative of the higher sensitivity obtainable with a non-aqueous system. The reaction was complete after about 30 min. The principal virtues of this non-aqueous method are the reproducibility, simplicity and speed with which the determination can be performed.

EXPERIMENTAL

Reagents

Pyridine (Spec. grade), anhydrous acetic anhydride (reagent grade) and anhydrous methanol (reagent grade) were obtained from Eastman. Anhydrous citric acid was U.S.P. grade.

Standard citric acid solution. Accurately weigh 100.0 mg of anhydrous citric into a 100-ml volumetric flask and dissolve to the mark with pyridine.

Standard aqueous citric acid solution. Accurately weigh 100.0 mg of citric acid into a 100-ml volumetric flask and dissolve to the mark with distilled water.

Apparatus

Spectrophotometer: Beckman recording Model DK. Colorimeters: Beckman DU and Evelyn. Florisil column: glass chromatographic column (25 cm $\times$ 20 mm) packed with Florisil, 60/100 mesh (Floridin Co., Tallahassee, Florida).

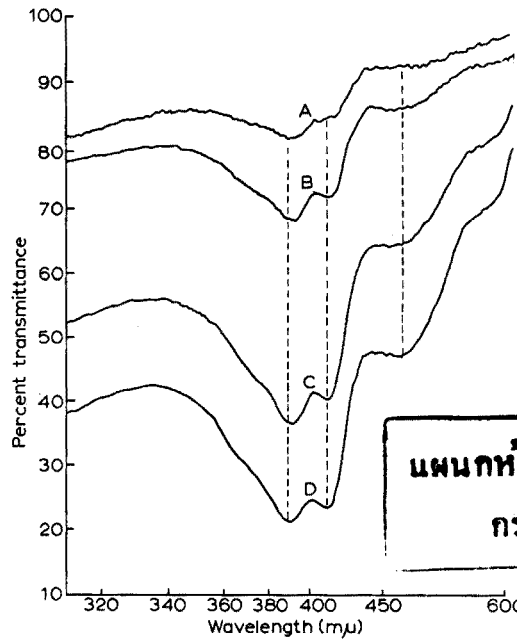

Fig. 1. Absorption spectra of citric acid solutions. A, 0.010 mg; B, 0.030 mg; C, 0.050 mg; D, 0.080 mg per sample).

Procedures

Absorption spectra. To a 1-ml sample of standard citric acid solution, add 3 ml of pyridine and 2 ml of anhydrous acetic anhydride. Allow to stand at room temperature for 20 min. Transfer the solution to a 10-mm quartz cell and record the absorbances over the region from 300 to 600 mμ.

Maximum absorption peaks were observed at 460, 406 and 389 ± 2 mμ, as shown in Fig. 1. A 2:1 mixture, by volume, of pyridine to acetic anhydride was used as a blank. Figure 2 shows the straight-line relation obtained by plotting absorbance at 389 and 406 mμ *vs.* concentration of citric acid over the range from 0.010 to 0.100 mg.

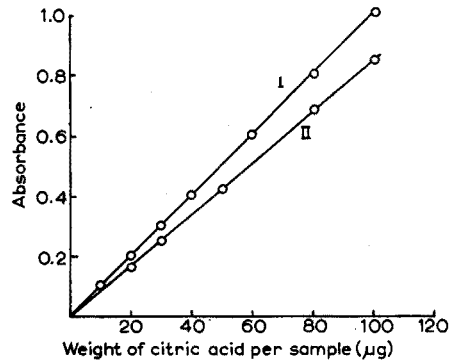

Fig. 2. Absorbance *vs.* concentration of citric acid solutions: absorbance read I, at 389 mμ; II, at 406 mμ.

Method for aqueous solutions. In order to determine the concentration of citric acid in the standard aqueous citric acid solutions of varying dilutions, the samples must first be dried. Add 4 ml of pyridine to a 2-ml sample of the standard in an evaporating dish, heat to near dryness in a steam bath, and then add 1 g of sodium sulfate to take up any trace of water. Next, swirl the residue with small amounts of pyridine and pass through a glass wool plug into a dry test tube which has previously been marked at the 8-ml position. Then fill the tube to the 8-ml mark with pyridine, add 4 ml of anhydrous acetic anhydride and allow to stand at room temperature for 30 min. Read the absorbances at 406 and 389 ± 2 mμ.

TABLE I

ABSORBANCE READING OF AQUEOUS CITRIC ACID SOLUTION

Citric acid taken (mg per 6-ml solution)	*Absorbance*	
	389 mμ	*406 mμ*
0.010	0.102	0.084
0.020	0.204	0.166
0.030	0.303	0.253
0.050	0.502	0.419
0.080	0.802	0.670
0.100	1.004	0.846

Table I gives the results of this determination for several concentrations of citric acid. The absorbances at 389 ± 2 mμ of these solutions are appropriate for routine use; rough colorimetric estimations may be made with an Evelyn Colorimeter equipped with a No. 400 filter. A 2:1 mixture, by volume, of pyridine to acetic anhydride was used as a blank. The Evelyn readings and the corresponding absorbances for the various concentrations of citric acid are tabulated in Table II.

TABLE II

THE ABSORBANCES OF THE CITRIC ACID SOLUTION USING AN EVELYN COLORIMETER

Citric acid taken (mg per 6-ml solution)	*Evelyn reading*	*Absorbance*
0.009	80^2	0.0942
0.018	65^3	0.1871
0.037	45^1	0.347
0.055	30^3	0.512
0.074	21^1	0.673
0.092	15^3	0.803

The citric acid content of an unknown sample can be determined by reading the concentration from a plot of the standard citric acid concentration *vs.* absorbance, or by using the formula

$$\text{mg citric acid} = \frac{\text{A sample} \cdot X \text{ (dilution factor)}}{A^*_{389} \cdot Y \text{ (weight of sample)}}$$

where A^*_{389} is the absorbance reading of a 6-ml solution containing a known concentration of citric acid.

Method for protein-containing samples. Shake a 10-g sample of a dairy product with 40 ml of methanol and then centrifuge. Pass the supernatants through the Florisil-packed chromatograph column with 2 g of anhydrous sodium sulfate on top of the column which has been prewashed with two 25-ml aliquots of 2% H_2SO_4 in 10:1 methanol–water solution. Wash the column with four portions of 25 ml of methanol; collect the eluate and washings. Pipette an aliquot containing about 20 to 200 μg of citric acid into an evaporating dish. Heat on a steam bath until dry. Dissolve the residue in pyridine, and perform the color reaction as described above.

RESULTS

The above method was first applied to the determination of citric acid in the presence of large amounts of lactic acid. The results are shown in Table III. Each determination represents the average of duplicate runs. Clearly, lactic acid does not interfere with the determination.

Table IV gives the results of the determination of citric acid in the presence of large amounts of common carboxylic acids. Each determination represents the average of triplicate runs.

When the citric acid content of simple solutions was determined, the reaction in non-aqueous media was found to give very satisfactory results. When the method was applied to the determination of citric acid in milk and other dairy products, the

TABLE III

DETERMINATION OF CITRIC ACID IN THE PRESENCE OF LACTIC ACID

Citric acid taken (mg)	*Citric acid found (mg)*		
	A[a]	*B*[a]	*C*[a]
0.0101	0.0102	0.0103	0.0103
0.20	0.0205	0.0205	0.0204
0.030	0.0305	0.0307	0.0308
0.050	0.0506	0.0508	0.0510
0.080	0.0808	0.0806	0.0809
0.100	0.1009	0.1010	0.1013

[a] Column A: the same amount of lactic acid was added.
Column B: a 5-fold amount of lactic acid was added.
Column C: a 10-fold amount of lactic acid was added.

TABLE IV

DETERMINATION OF CITRIC ACID IN THE PRESENCE OF A MIXTURE OF CARBOXYLIC ACIDS[a]

Citric acid taken (mg)	*Citric acid found (mg)*		
	A[b]	*B*[b]	*C*[b]
0.010	0.0098	0.0097	0.0098
0.020	0.0197	0.0197	0.0196
0.030	0.0295	0.0293	0.0290
0.050	0.0492	0.0490	0.0482
0.080	0.0788	0.0786	0.0780
0.100	0.0987	0.0985	0.0982

[a] A mixture of equal amounts, by weight, of acetic, propionic, butanoic, valeric and caproic acids.
[b] Column A: a 2-fold amount of acid mixture was added.
Column B: a 4-fold amount of acid mixture was added.
Column C: a 10-fold amount of acid mixture was added.

TABLE V

DETERMINATION OF CITRIC ACID IN DAIRY PRODUCTS

	Citric acid found (mg)			*Citric acid found (mg)*	
	Sample 1	*Sample 2*		*Sample 1*	*Sample 2*
Milk	0.0485	0.0423	Buttermilk	0.1243	0.1363
	0.0480	0.0426		0.1236	0.1375
	0.0474	0.0415		0.1252	0.1372
Skim milk	0.0658	0.0675	Milk + known amount	0.254	0.267
	0.0661	0.0686	(0.210 mg) of citric acid	0.256	0.269
	0.0654	0.0672		0.252	0.266

reproducibility of the analysis depended on the method used for the deproteinization. When a solution of trichloroacetic acid was used to precipitate the protein from milk by the method of BABAD AND SHTRIKMAN[2], the results obtained were somewhat erratic. When the deproteinization was carried out by the column chromatography tech-

nique described, a high degree of reproducibility was attained. Table V shows this degree of reproducibility for the determination of citric acid in samples of milk and dairy products. Each analysis was done in triplicate. This method has also worked satisfactorily with a number of other biological fluids.

The authors wish to express their thanks for the advice of Messrs. N. J. ALICINO and A. E. BERNSTEIN, and the assistance of Messrs. P. MASSY and J. CARUCCIO.

SUMMARY

A non-aqueous spectrophotometric method is described for the determination of citric acid in the presence of carboxylic acids and lactic acid. The method was developed as a result of critical evaluation of the Furth–Herrmann color reaction in a non-aqueous pyridine–acetic anhydride solution. The optimum conditions are described. The absorbance is read at 389 $\pm$ 2 mμ. The minimum concentration of citric acid that can accurately be determined is about 2 μg per sample.

RÉSUMÉ

Une méthode est décrite pour le dosage de l'acide citrique, en présence d'acides carboxyliques et de l'acide lactique, par spectrophotométrie, en milieu non-aqueux. Elle est basée sur la réaction colorée de Furth–Herrmann, en milieu pyridine–anhydride acétique. La concentration minimum d'acide citrique pouvant être déterminée avec précision est d'environ 2 μg.

ZUSAMMENFASSUNG

Es wird eine spektrophotometrische Methode in nicht-wässrigem Medium beschrieben zur Bestimmung der Citronensäure in Gegenwart von Milchsäure und anderen Carboxylsäuren. Die Methode beruht auf der Farbreaktion nach Furth–Hermann Pyridin–Essigsäureanhydrid lösung. Die kleinste noch genau bestimmbare Menge Citronensäure beträgt etwa 2 μg.

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THE FLUORIMETRIC DETERMINATION OF FORMALDEHYDE

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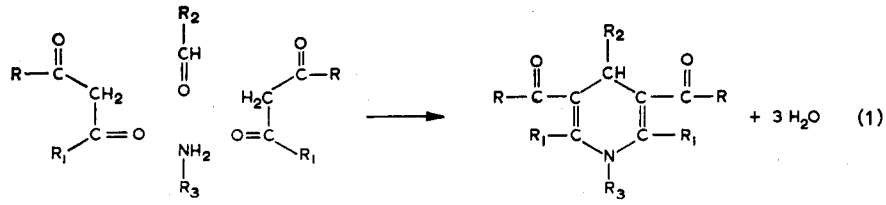
Highly sensitive analytical procedures for formaldehyde determination are useful in the study of biological systems[1] and air pollution[2]. Many biological substances such as steriods[3,4], sugars, and hydroxy amino acids[5] quantitatively release formaldehyde when oxidized by periodate or bismuthate. The assay of other compounds such as methanol[6] and formic acid[7] can also be performed by oxidation or reduction to formaldehyde. Our studies on the formaldehyde reaction with the liberated amino groups of deoxyribonucleic acid treated with mutagens and carcinogens have emphasized the need for such methods.

A very sensitive fluorimetric method has been developed in our laboratory which can measure as little as 0.01 μg formaldehyde. This is about ten times more sensitive than the colorimetric methods recently developed by SAWICKI and co-workers[8].

The colorimetric procedure of NASH[9] is based on the Hantzsch reaction between acetylacetone, ammonia, and formaldehyde which form 3,5-diacetyl-1, 4-dihydrolutidine (DDL). The fluorimetric determination of this compound is based on NASH's observation that it fluoresces.

The Hantzsch reaction requires a β-diketone, an aldehyde and an amine as shown in equation (1):



(1)

The quantitative nature of the reaction suggests that any one of the reactants may be determined. The extension of this method to the assay of other aldehydes, amines, and β-diketones is indicated by our observation that ammonia, methylamine, and amino acids react as well as acetaldehyde and acetoacetic ester reported by NASH[9].

EXPERIMENTAL

Reagents

Acetylacetone. This reagent, freshly distilled, should be colorless and non-fluorescent. It will slowly develop a green fluorescence at room temperature.

Formaldehyde. Analytical reagent 37% formaldehyde which contains 12% methanol as preservative.

Apparatus

Fluorimeter. A Farrand model A fluorimeter equipped with a primary 405 mμ interference filter and a secondary Corning 3-71 filter was used in this work. Fluorescence spectra were obtained with a Farrand automatic recording spectrofluorimeter equipped with a high intensity 150-watt Hanovia Xenon arc. Absorption spectra were obtained with a Beckman DU spectrophotometer. Infra-red spectra were obtained with a Perkin-Elmer infra-red spectrophotometer Model 221G.

Analysis of DDL

Crystalline DDL was prepared according to NASH[9] who reported a m.p. of 208°. We could not obtain a sharp melting point even after 2 recrystallizations from ethanol. The compound softened and shrank at 130° and melted over the range 190–200° Calculated for $C_{11}H_{15}NO_2$: C, 68.39; H, 7.72; N, 7.22. Found: C, 68.13, 68.19; H, 7.90, 7.68; N, 7.25, 7.23.

Formaldehyde determination

The freshly prepared reagent[9] consists of 2 *M* ammonium acetate and 0.02 *M* acetylacetone at pH 6. The reagent is mixed with an equal volume of formaldehyde solution and incubated for 10 min at 58° or for 60 min at 37°. The lower temperature is preferred for low (<0.05 μg/ml) concentrations of formaldehyde because of the slightly increased blank fluorescence which occurs at 58°. The fluorescence is read after cooling to room temperature.

RESULTS

Figure 1 shows the nature of the fluorescence which is linear from 0.005 μg/ml to about 0.4 μg/ml formaldehyde. The curve deviates slightly from linearity from 0.4 μg/ml to 1 μg/ml. Concentrations above 1 μg/ml can be determined colorimetrically and were therefore not determined fluorimetrically.

The fluorescence intensity was unchanged after 3 h and decreased 5% after 16 h at room temperature.

Four ml is the minimum convenient volume for use with the 75 × 10 mm culture tubes which require 1 ml for analysis. Since the dilution factor is 2, the minimum amount for formaldehyde that can be determined is 0.01 μg in 2 ml.

Absorption and fluorescence spectra of 3,5-diacetyl-1,4-dihydrolutidine

Crystalline DDL[9] was dissolved in ethanol and diluted with 1 *M* ammonium acetate (pH 6) for spectral analysis.

The absorption spectrum of DDL (Fig. 2) which has a visible maximum at 412 mμ is identical to that of the compound formed in solution in the region 350–400 mμ.

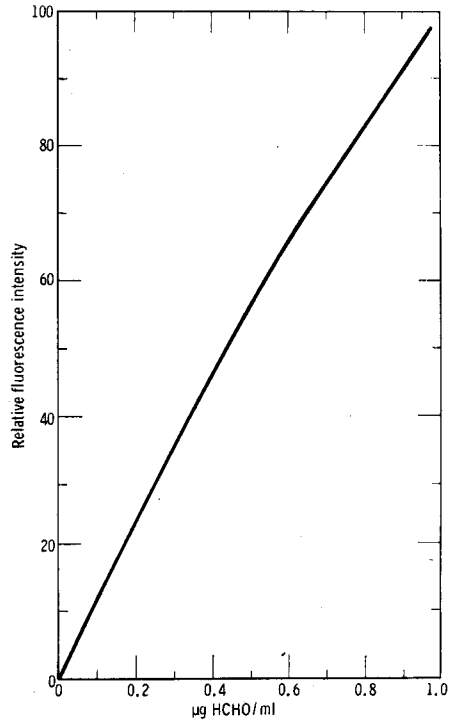


Fig. 1. Relationship between fluorescence intensity and formaldehyde concentration. The fluorescence intensity includes subtraction of blank values and is in arbitrary units to include the range of 0.005–0.97 μg HCHO/ml.

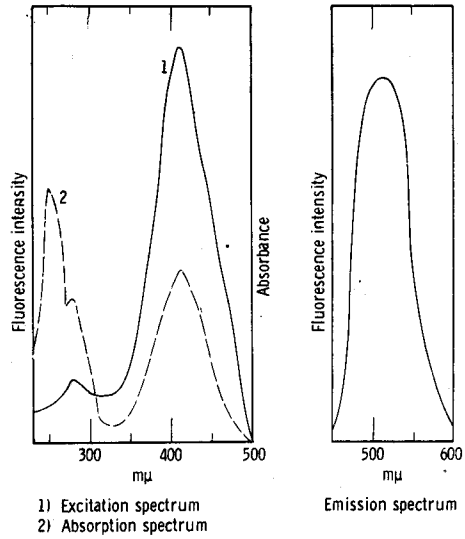


Fig. 2. Spectra of 3,5-diacetyl-1,4-dihydrolutidine. Concentration of DDL = 2 μg/ml in 1 *M* ammonium acetate (pH 6.)

The high absorbance of the reagent interferes with the spectral examination at shorter wavelenths.

These results are in agreement with NASH who also reported the molar absorbance at 412 mμ to be 8,000 based on formaldehyde for the compound formed in solution whereas the crystalline DDL had a molar absorbance of 7,700. He attributed the lower value to the incomplete solubility of the crystals in aqueous solution. The compound is completely soluble in ethanol, however, and when prepared as described above, the molar absorbance of DDL at 412 mμ was also 8,000.

The absorbance obeys Beer's law and was used to standardize formaldehyde solutions.

The fluorescence excitation and emission spectra are shown in Fig. 2. The respective maxima are 410 mμ and 510 mμ.

Theoretically, the fluorescence excitation maxima correspond with the absorption maxima. The comparison spectra (Fig. 2) show close agreement with two of these bands. Correspondence with the intensities of the maxima is also expected. The relatively low intensity of the ultraviolet excitation maximum may be attributed to the low energy output of the Xenon arc in this region[10].

Application of the method

The reaction of formaldehyde with purine and pyrimidine amino groups of nucleic acids has been used as a measure of the fraction of these groups which are hydrogen bonded and therefore unreactive to formaldehyde[11]. We have been studying the effect of mutagenic and carcinogenic agents which alter the melting profile of deoxyribonucleic acid[12]. These changes are associated with the rupture of hydrogen bonds within the nucleic acid.

Two differently treated samples of deoxyribonucleic acid were reacted with formaldehyde and then separated from the free reagent by passage through a Sephadex G-100 column. Details of this procedure will be published elsewhere. The isolated nucleic acid was analyzed for bound formaldehyde by the fluorimetric procedure. Control samples to which known amounts of formaldehyde were added to nucleic acid solution indicated that nucleic acid did not interfere with the analysis.

The results of these experiments were that heat-denatured deoxyribonucleic acid had 85% of its amino groups bound to formaldehyde while carcinogen (2-amino-1-naphthol) treated nucleic acid had 32% of its amino groups bound to formaldehyde. The latter value represents the degree of denaturation which was also calculated from the lower absorbance rise at 260 mμ when the nucleic acid was heated. This method gave a value of 30% denaturation which compares favorably with the formaldehyde method.

Modification of the reagent for ammonia determination

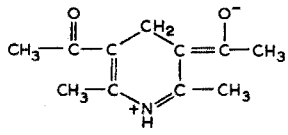
The Hantzsch reaction can be used for the fluorimetric determination of ammonia. The reagent was modified to consist of 1 *M* sodium acetate pH 6, 0.8 *M* acetylacetone and 2 *M* formaldehyde. The reagent was mixed with an equal volume of ammonia solution and heated at 100° for 10 min. The solution was cooled to room temperature and read in the fluorimeter. As little as 1 μg NH_3/ml was detected.

Structure and properties of DDL

The elemental analyses for DDL are given in duplicate (EXPERIMENTAL) because NASH reported a variation of 66.7 to 69.7 for carbon in replicate analyses. The analyses established our product as pure DDL. The odd melting behaviour must therefore be due to structural changes produced by heating.

The dipolar structure I was suggested by NASH for DDL because of its insolubility in ether, its high melting point, and the negligible effect of pH on the absorption spectra over the range pH 4–10. The strong yellow color and fluorescence also support this structure in dilute solution, which is undoubtedly stabilized by resonance with the equivalent structure II.

Some insight into the structure of this compound in solution and in the solid was obtained from infra-red spectra. The N-methyl derivative of DDL (DDL-N-methyl) was prepared by substituting methylamine for ammonia in the DDL synthesis and its I.R. spectra also examined. This derivative has a similar yellow color and yellow-green fluorescence as DDL.



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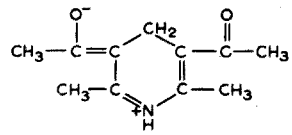

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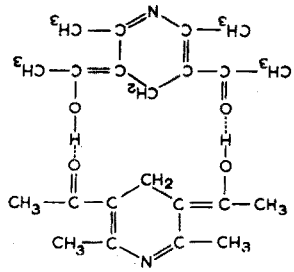

III

The infra-red data (Table I), discussed below, in conjunction with the other properties of DDL strongly suggest that the structure I pre-dominates in dilute solution and that the dimer III, or higher aggregates, predominate in concentrated solution and in the solid.

The bands in the region 3225–3450 cm^{-1} indicate a hydrogen bonded hydroxyl group[13–15]. It is unlikely that they are due to —N—H because they are also present in DDL-N-methyl which must have a similar structure. The band at 3370 cm^{-1} in dilute solution shifts to lower frequencies in concentrated solution. This suggests a vinyl alcohol (—C=C—OH)[16] which is more extremely hydrogen bonded in concentrated solution[17].

A most significant feature of the I.R. data is the appearance of the band at 2495 cm^{-1} in dilution. This is undoubtedly due to the $N\rangle H^+$ ion[18] which is absent in the concentrated solution and solid.

The two bands near 1620 and 1670 cm^{-1} cannot be definitely assigned but must be

due to conjugated —C=O, C—C, and —C=N-groups[13,14,16]. The additional strong band near 1600 cm^{-1} in the solid suggests increased conjugation of —C=O[17,19] probably associated with increased hydrogen bonding.

The two bands in the region 1210–1260 cm^{-1} are probably due to the —C—O-group[14,20–22]. The band at 1250 cm^{-1} is strong in dilute solution and weak concentrated solution. This may be attributed to the increasing contribution of —C—O— ion in dilute solution.

TABLE I

COMPARISON OF SOME I.R. BANDS OF DDL AND DDL-N-METHYL[a]

1% DDL in dimethyl sulfoxide (cm^{-1})	*5% DDL in dimethyl sulfoxide (cm^{-1})*	*1% DDL in solid KBr (cm^{-1})*	*0.5% DDL-N-methyl in solid KBr (cm^{-1})*
3400 (W)	3450 (W)	3400 (W)	3400 (S)
3370 (W)	3280 (M)	3330 (M)	3300 (S)
	3225 (M)		
2495 (M)	Absent	Absent	Absent
1680 (M)	1670 (S)	1680 (S)	1675 (S)
1620 (M)	1600 (S)	1630 (M)	1600 (S)
1250 (S)	1260 (W)	1570–1600 (S)	1260 (W)
1220 (S)	1210 (S)	1230 (S)	1220 (S)

[a] W=weak; M=medium; S=strong.

DISCUSSION

The Hantzsch reaction as described by NASH provides a very versatile and sensitive method for formaldehyde determination. Fluorimetric analysis as described in this paper can be used for concentrations of 0.01 μg/ml to 1 μg/ml, and colorimetric analysis can be used for higher concentrations.

An additional advantage of this procedure is that formaldehyde determination can be made in the presence of biological materials such as nucleic acids which do not interfere with the reaction.

The application of the Hantzsch reaction to the fluorimetric determination of any of the three reactants has been mentioned. Optimum conditions would, of course, have to be determined for the particular assay. The detection of microgram quantities of ammonia, for example, required high concentrations of formaldehyde and acetylacetone and heating at high temperature.

This work was supported by grants from Allied Chemical Corporation and Public Health Service. The author is grateful to Dr. B. L. VAN DUUREN for the analysis and interpretation of the infra-red data, and thanks Dr. W. TROLL and Dr. N. NELSON for their helpful interest and ESTHER RINDE and ELLEN LEVINE for their able technical assistance.

SUMMARY

A sensitive fluorimetric procedure for formaldehyde has been developed. The method, which can measure 0.01 μg formaldehyde, is based on the Hantzsch reaction between acetylacetone, ammonia, and formaldehyde. The product, 3,5-diacetyl-1,4-dihydrolutidine, is colored yellow and fluoresces yellow green. Infra-red spectra indicate that this compound is ionic in dilute solution and aggregated in concentrated solution. The Hantzsch reaction may be extended for the assay of other aldehydes, amines, and β-diketones.

RÉSUMÉ

L'auteur a mis au point une méthode fluorimétrique sensible pour le dosage du formaldéhyde. Ce procédé, basé sur la réaction de Hantzsch, entre acétylacétone, ammoniaque et formaldéhyde, permet de déterminer 0.01 μg de formaldéhyde. Le produit de réaction, diacétyl-3,5-dihydro-1,4-lutidine, est jaune avec une fluorescence vert jaune. Cette réaction peut s'appliquer à d'autres aldéhydes, amines et β-dicétones.

ZUSAMMENFASSUNG

Beschriebung einer empfindlichen fluorometrischen Methode zur Bestimmung von Formaldehyd. Die Methode, die noch 0.01 μg Formaldehyd zu erfassen erlaubt, beruht auf der Reaktion nach Hantzsch zwischen Acetylaceton, Ammoniak und Formaldehyd. Das Reaktionsprodukt, 3,5-Diacetyl-1, 4-dihydrolutidin, ist gelb gefärbt und zeigt gelbgrune Fluoreszenz. Die Reaktion kann auch zur Bestimmung von anderen Aldehyden, von Aminen und β-Diketonen angewandt werden.

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THE USE OF PYRIDINE-2-AZO-*p*-DIMETHYLANILINE AS AN INDICATOR IN GLACIAL ACETIC ACID

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The need for indicators which function effectively in non-aqueous titrations is well known, and this prompted the present study of the easily synthesized dye, pyridine-2-azo-*p*-dimethylaniline. This compound was first produced by FAESSENGER AND BROWN[1] using two simple and distinct methods. As shown by SEAMAN AND ALLEN[2], BALLCZO[3] and many others, conventional indicators have many limitations in regard to complexity of color changes in non-aqueous media, as well as precision under certain conditions. In the present paper, the behavior of the above-mentioned pyridine dye in glacial acetic acid medium is described.

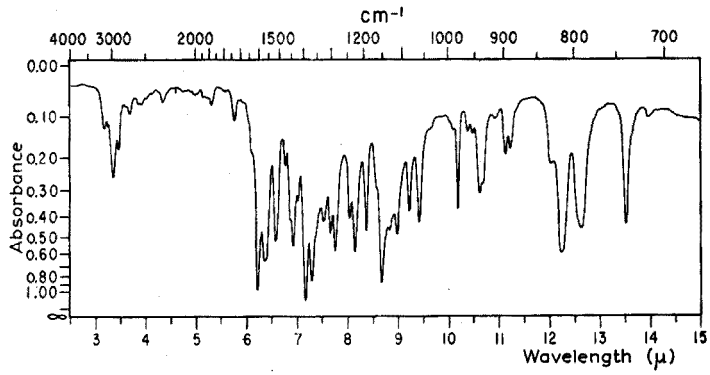

Fig. 1. Infrared spectrum of pyridine-2-azo-*p*-dimethylaniline.

The infrared spectrum (Fig. 1) readily confirms the structure of the dye. Characteristic of the pyridine moiety of the molecule are the absorption bands at 6.1, 7.1 and 8.2 μ. The N = N stretching frequency can be found at 6.2 and 7.2 μ with the tertiary aromatic amine absorbing in the 7.2 to 7.6 μ region of the spectrum.

EXPERIMENTAL

Instruments

The Precision-Shell Dual Titrometer, equipped with magnetic stirrers, was used for all potentiometric titrations. The glass–calomel electrode system was used exclusively and for these determinations the electrode pair attained equilibrium rapidly after the addition of each increment of titrant. The glass electrodes were permitted to stand in a 0.1 *N* solution of hydrochloric acid between titrations. Both electrodes were dried thoroughly before use and the calomel electrode was protected against contamination, between titrations, by cleaning the sleeve as recommended by LYKKEN *et al.*[4].

The spectrophotometric infrared studies were carried out with the Perkin-Elmer, Infracord, Model 137.

Reagents

*Pyridine-2-azo-*p*-dimethylaniline.* 1.0% in absolute methanol.

Crystal violet. 0.2 g was dissolved in 100 ml of glacial acetic acid.

Purified benzene. Benzene was dried over sodium for several days, refluxed over fresh sodium for several days, and then distilled.

Perchloric acid. 0.1 *N* in glacial acetic acid.

Potassium acid phthalate (primary standard grade, Merck) was dried at 145° for 3 h.

Sodium carbonate, reagent grade, labelled assay: 99.98%.

Sodium acetate, reagent grade, labelled assay: 99.90%.

Procedures

(*a*) *Standardization of perchloric acid.* Since perchloric acid is the acidic titrant in

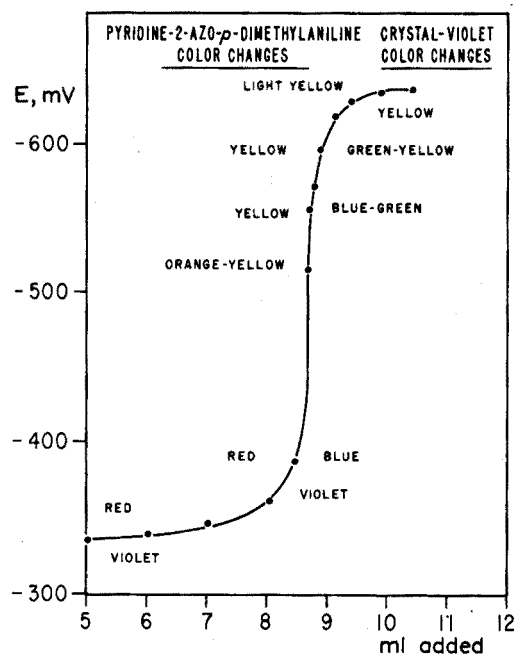

Fig. 2. Titration of potassium acid phthalate with 0.1 *N* perchloric acid; potentiometric curve with color changes for indicators.

many titrations using a glacial acetic acid medium and crystal violet as the indicator, the effectiveness of pyridine-2-azo-*p*-dimethylaniline compared with that of crystal violet was tested in this area.

The routine procedure for the standardization of perchloric acid was followed[5]. Exactly 0.8 milliequivalent of potassium acid phthalate was weighed into titration beakers and 10.0 ml of glacial acetic acid were added to dissolve the sample; heating on a hot plate for 2 min was necessary to complete the dissolution. After cooling, three drops of each dye were added to two successive samples, which were then titrated with the perchloric acid to determine the precise color at the potentiometric end-point. Fig. 2 shows the potentiometric curve for the titration, indicating the color changes for both crystal violet and pyridine-2-azo-*p*-dimethylaniline. It can be seen that the color change of crystal violet from violet to blue occurs slightly before the potentiometric end-point. On the other hand, the change of color for the pyridine dye from red to orange-yellow corresponds more precisely to the potentiometric end-point. Fig. 3 illustrates more clearly the color change of pyridine-2-azo-*p*-dimethylaniline at the end-point of the titration.

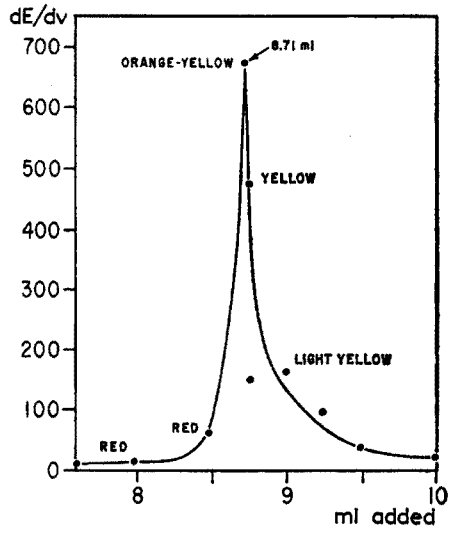

Fig. 3. Color changes of pyridine-2-azo-*p*-dimethylaniline in the titration of potassium acid phthalate with perchloric acid.

TABLE I

VISUAL STANDARDIZATION OF PERCHLORIC ACID AGAINST POTASSIUM ACID PHTHALATE IN GLACIAL ACETIC ACID

Indicator	*Determinations*	*Mean normality*	*Std. dev. of a single det. (%)*
Crystal violet	10	0.1110	0.33
Pyridine-2-azo-*p*-dimethylaniline	10	0.1111	0.32

Several series of visual titrations were performed using the two indicators for the standardization of perchloric acid. The analyses were evaluated statistically and the results are shown in Table I. It will be noticed that both indicators give excellent precision and accuracy in this titration and are apparently of equal value.

The presence of water was found to cause deleterious effects in non-aqueous systems using either indicator. Indicator response was less sharp, the loss of sharpness being proportional to the water content, and was of approximately equal magnitude with both crystal violet and the pyridine dye. Water was seen to cause a spreading of and decrease in the rate of change of potential with volume at the end-point, in a plot of dE/dV *vs.* ml of titrant. In addition, it was found that a small percentage of water was tolerated to a greater extent in an acetic acid medium, while in basic media any presence of water produced more complex deviations which tended to increase the error [5,10].

(*b*) *Titration of amines.* Pyridine-2-azo-*p*-dimethylaniline was tested for its effectiveness in an area where crystal violet is known to give a variety of color changes at the end-point, namely, in the titration of various amines.

Each of the amines listed below, in 0.1 equivalent weight portions, was dissolved separately as follows:

Aniline, *n*-butylamine, *p*-phenylenediamine, in 1000 ml of benzene.

Ethylenediamine, in 1000 ml of glacial acetic acid.

Aliquots (5 ml) of each solution of the amine were taken, and to each aliquot were added 5.0 ml of glacial acetic acid. Each sample was then titrated potentiometrically with perchloric acid in order to evaluate the exact color of crystal violet and pyridine-2-azo-*p*-dimethylaniline at the equivalence point. This was followed by a series of 10 visual titrations for each amine. The results of the visual titrations are given in Table II, together with the color changes of each indicator at the equivalence point.

TABLE II

TITRATIONS OF AMINES IN BENZENE–GLACIAL ACETIC ACID WITH 0.1 *N* PERCHLORIC ACID IN GLACIAL ACETIC ACID

Amine	*Indicator*	*Color at end-point*	*Determinations*	*Mean normality*	*St. dev. of a single det. (%)*
Aniline	Crystal violet	Blue to light blue	10	0.1126	0.40
	Pyridine dye	Red to orange-yellow	10	0.1131	0.20
Butylamine	Crystal violet	Blue to green	10	0.1007	0.50
	Pyridine dye	Red to orange-yellow	10	0.1002	0.29
p-Phenyl-enediamine	Crystal violet	Violet to blue	10	0.1067	0.30
	Pyridine dye	Red to orange-yellow	10	0.1065	0.16
Ethyl-enediamine	Crystal violet	Violet to blue	10	0.1348	0.20
	pyridine dye	Red to orange-yellow	10	0.1349	0.14

It was found in the potentiometric determination of aniline that crystal violet changes in color from blue to a lighter blue at the equivalence point. Pyridine-2-azo-*p*-dimethylaniline gave the same red to orange-yellow color change as in the perchloric acid standardization procedure. The color change of crystal violet at the end-point is difficult to perceive and requires much practice, while that of the pyridine dye is clear-cut and relatively easy to distinguish. This is reflected in the standard deviation for the determinations, which was 0.20% when the pyridine dye was used, compared with 0.40% for crystal violet.

In the *n*-butylamine titration the color change of crystal violet at the end-point was from blue to green. This change was more difficult to detect than that occurring in the aniline determination, with a resultant lessening of precision. Table II records the standard deviation at 0.50% when crystal violet was the indicator. Pyridine-2-azo-*p*-dimethylaniline, with the usual red to orange-yellow change at the equivalence point, showed a standard deviation of 0.29%.

The potentiometric evaluation of *p*-phenylenediamine at the equivalence point, using crystal violet as the indicator, showed a color change from violet to blue, while the pyridine dye again changed from red to orange-yellow. For crystal violet the standard deviation was 0.30%, which corresponds to that obtained by FRITZ[6], PIFER[7], and many others[8]. Pyridine-2-azo-*p*-dimethylaniline increased the precision of this titration, giving a standard deviation of 0.16%.

Ethylenediamine was found to give the best precision in this series of titrations, but since it is insoluble in benzene, and since the indicators did not give sharp color changes when the amine was dissolved in methanol, glacial acetic acid was chosen as the medium. In this solvent, crystal violet changes from violet to blue in color at the equivalence point, and pyridine-2-azo-*p*-dimethylaniline shows the usual red to orange-yellow color change. These indicators, when used in the determination of ethylenediamine, showed a standard deviation of 0.20 and 0.14 % respectively.

It will be noticed that when the color change of crystal violet was from violet to blue, the corresponding standard deviation was less than when complex forms of blue to green predominated at the potentiometric end-point. Crystal violet showed a variation of from 0.20 to 0.50% in standard deviation, while the standard deviation of pyridine-2-azo-*p*-dimethylaniline varied from 0.14 to 0.29%. SEAMAN AND ALLEN[2] recommend permanent color standards for improving the precision of determinations using crystal violet as indicator.

TABLE III

ASSAY OF INORGANIC SALTS BY TITRATION WITH 0.2 *N* PERCHLORIC ACID IN GLACIAL ACETIC ACID

Salt	*Indicator*	*Determinations*	*Purity (%)*	*Std. dev. (%)*
Sodium Carbonate	Crystal violet	3	100.6	0.15
Sodium carbonate	Pyridine-2-azo-*p*-dimethylaniline	3	100.02	0.13
Sodium acetate	Crystal violet	6	99.91	0.38
Sodium acetate	Pyridine-2-azo-*p*-dimethylaniline	6	99.77	0.23

When pyridine-2-azo-*p*-dimethylaniline was used in potentiometric titrations, the dye became bleached after the end-point had been passed. This could be prevented, or at least delayed, by tightening the sleeve of the calomel electrode. In no instance did the bleaching interfere with the potentiometric end-point, which occurs previously. The bleaching effect did not occur in the visual titrations, even when the solutions were allowed to stand for the same period of time.

(*c*) *Titration of inorganic salts.* Pyridine-2-azo-*p*-dimethylaniline was evaluated for use in potentiometric titrations of inorganic salts. Reagent-grade sodium carbonate and sodium acetate were dried and ground so as to pass through a No. 100 mesh sieve. Samples of from 1 to 2 milliequivalents were accurately weighed and dissolved in acetic acid, with heat when necessary. These were titrated with 0.2 *N* perchloric acid in glacial acetic acid, the indicators being crystal violet and pyridine-2-azo-*p*-dimethylaniline in separate titrations. The results are presented in Table III. In accuracy and precision, the pyridine dye is seen to compare well with crystal violet in the non-aqueous titrations of inorganic salts.

CONCLUSION

In non-aqueous media it is possible that the pyridine dye has an acid–base equilibrium similar to that of its aqueous solutions, as indicated by an analogous response of color changes to pH. In aqueous solution at an alkaline pH, the dye appears yellow and is probably in the azo form. As the pH of the system is lowered, the azo form of the dye picks up a proton, which probably attaches to the more basic part of the molecule at the pyridine nitrogen and the resulting color is red. In solutions of lowest pH, the molecule binds a second proton, which may attach either to the electron pair of the nitrogen atom in the aniline moiety, as suggested by KLOTZ AND LOH MING[9], or even at the azo linkage of the pyridine moiety. With the binding of two protons, the color becomes yellow once again.

When dissolved in acetic acid medium, the dye gives a red color probably corresponding to the binding of one proton and similar to the aqueous system above where the pH is lowered. This would seem to be consistent with the increased supply of protons from the acetic acid. When potassium acid phthalate is dissolved in acetic acid, the pH is made more alkaline and the red color of the pyridine dye becomes more pronounced. If perchloric acid is added to neutralize the base, the dye becomes orange-yellow in color, then bright yellow with the next drop of acid.

Pyridine-2-azo-*p*-dimethylaniline gives excellent accuracy and precision in non-aqueous titrations, and surpasses that obtained with standard indicators, such as crystal violet, in the titration of certain amines, and in the non-aqueous titration of sodium carbonate and sodium acetate. Its acid–base equilibria limit its use to titrations whose equivalence point is in the acid range.

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SUMMARY

Pyridine-2-azo-*p*-dimethylaniline is suggested as an indicator in the titration of amines (aniline, butylamine, *p*-phenylenediamine and ethylenediamine) with perchloric acid in glacialacetic acid medium. The precision obtainable is much better than that with crystal violet indicator. The pyridine dye can also be used satisfactorily in the titration of sodium carbonate and sodium acetate.

RÉSUMÉ

La pyridine-2-azo-*p*-diméthylaniline est proposée comme indicateur pour le titrage d'amines (aniline, butylamine, *p*-phénylènediamine et éthylènediamine) par l'acide perchlorique, en milieu acide acétique glacial. La précision est supérieure à celle obtenue avec le "violet cristal", comme indicateur. Le colorant proposé ici peut également être utilisé pour le titrage du carbonate de sodium et de l'acétate de sodium.

ZUSAMMENFASSUNG

Für die Titration von Anilin, Butylamin, *p*-Phenylendiamin und Aethylendiamin mit Perchlorsäure in Eisessig wird Pyridin-2-azo-*p*-dimethylanilin als Indikator vorgeschlagen. Die erhaltenen Werte sind genauer als bei Verwendung von Kristallviolett als Indikator. Der Pyridinfarbstoff kann auch für die Titration von Natriumcarbonat und Natriumacetat verwendet werden.

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THE DETERMINATION OF CHROMIUM IN LOW-ALLOY IRONS AND STEELS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

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Chromium is an important element in many low-alloy irons and steels and satisfactory quantitative procedures include volumetric[1-2], colorimetric[3-8], flame emission photometric[9] and emission spectrographic methods. WALSH[10] has indicated that atomic absorption spectrophotometry is practically interference free, but no quantitative methods for chromium based on this technique have been reported in the literature. Using atomic absorption spectrophotometry and an air–acetylene flame, GATEHOUSE AND WILLIS[11] and ALLAN[12] respectively have reported chromium sensitivities (1% absorption at 3579 Å) of 0.15 p.p.m. and 0.20 p.p.m.; ALLAN[12] has reported a sensitivity of 0.05 p.p.m. with a rich air–acetylene flame. Although the present authors could not reproduce this sensitivity with the equipment used in this study, atomic absorption spectrophotometry proved to be an excellent method for the determination of chromium in low-alloy irons and steels.

EXPERIMENTAL AND DISCUSSION

A Hilger large quartz E492 spectrograph was used with an E.M.I. 9558-QB photomultiplier tube, a modulated argon-filled chromium hollow-cathode lamp[13] and a tuned amplifier[13].

An examination of the spectrum of the chromium lamp in the range 3570–3610 Å showed three chromium absorption lines (3579, 3593 and 3605 Å) and four argon lines (3577, 3582, 3588 and 3606 Å). The dispersion of the spectrograph was 10 Å /mm and the argon lines disturbed the chromium absorption sensitivity under certain circumstances. With an entrance slit of 0.04 mm, the combined spectrum shown in Fig. 1 was obtained. The 3579 Å chromium line was found to be approximately 25% stronger in absorption than the 3593 Å chromium line and the weaker 3605 Å chromium line could not be resolved from the 3606 Å argon line. The resolution shown in Fig. 1 could not be used on a practical basis because it could not be reproduced readily from day to day.

When an entrance slit of 0.1 mm was used, the spectrum shown in Fig. 2 was obtained. The absorption sensitivity of the 3593 Å chromium line was found to be the same as that obtained under the conditions of higher resolution. The 3579 Å chromium line no longer exhibited the highest absorption sensitivity because of interference by the 3577 Å argon line; this argon line also caused deviations from linearity in the absorption-chromium concentration curve. These results would indicate that an inert

gas filling other than argon may be incorporated advantageously in future lamps so that the higher absorption sensitivity of the 3579 Å line may be utilized. Improved chromium lamps presently being manufactured do not exhibit argon interference[14].

The 3593 Å chromium line was considered to be the most suitable line for quantitative absorption studies. A preliminary investigation to determine the influence of varying solvent acids and iron concentration on the absorption sensitivity of chromium was carried out with an air-acetylene flame and a 10-cm slot burner; the results are shown in Fig. 3.

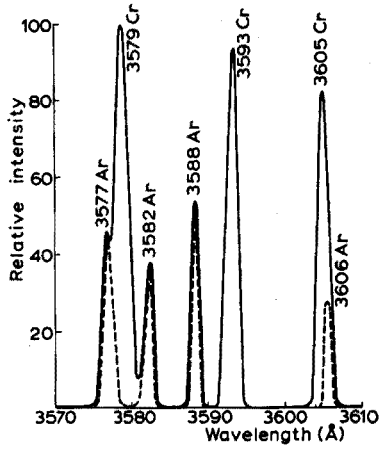

Fig. 1. The relative intensities of significant chromium and argon lines in the wavelength range 3570–3610 Å. Lamp current: 25 mA; spectrograph dispersion: 10 Å/mm; entrance slit: 0.04 mm; ——— chromium + argon spectrum; ----- argon spectrum.

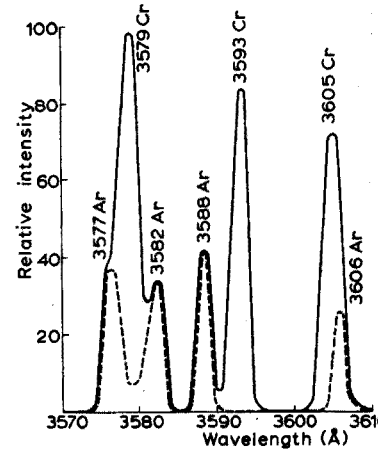

Fig. 2. The relative intensities of significant chromium and argon lines in the wavelength range 3570–3610 Å. Lamp current: 20 mA; spectrograph dispersion: 10 Å/mm; entrance slit: 0.10 mm; ——— chromium + argon spectrum; ----- argon spectrum.

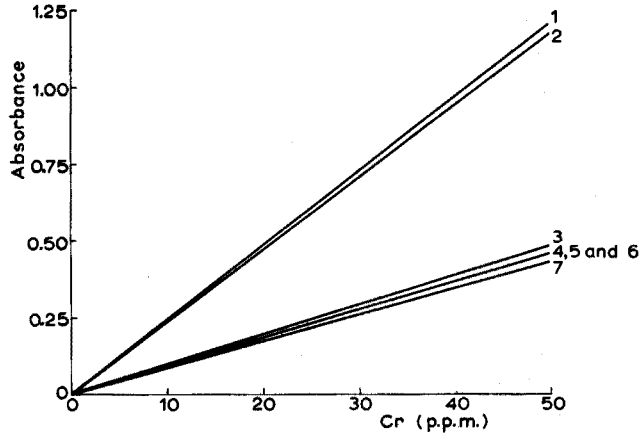

Fig. 3. The influence of solvent acids and iron concentration on the absorption sensitivity of chromium. Curve 1, aqueous; Curve 2, 10% (v/v) HCl; Curve 3, 1.0 g Fe^{3+} in 10% (v/v) HCl; Curve 4, 0.6 g or 1.0 g or 2.0 g Fe^{3+} in 6% (v/v) H_2SO_4; Curve 5, 0.6 g or 1.0 g or 2.0 g Fe^{3+} in 6% (v/v) H_3PO_4; Curve 6, 0.6 g or 1.0 g or 2.0 g Fe^{3+} in 3% (v/v) H_3PO_4–3% (v/v) H_2SO_4; Curve 7, 1.0 g Fe^{3+} in 6% (v/v) H_3PO_4–6% (v/v) H_2SO_4.

The results indicated that iron caused a major reduction in absorption sensitivity and that there were no significant reductions in absorption sensitivities, when hydrochloric, phosphoric, sulphuric or phosphoric–sulphuric acid media were used. Phosphoric–sulphuric acid is a suitable solvent mixture which has the advantageous property of retaining tungsten and other acid hydrolysable elements in solution; investigations showed that 30 ml of 15% (v/v)H_3PO_4–15%(v/v)H_2SO_4 would complex at least 200 mg of tungsten.

The chromium sensitivity (1% absorption) was 0.5 p.p.m. and this sensitivity was relatively independent of lamp current. Many low-alloy irons and steels contain chromium in the range 0–0.5% and an acceptable reproducibility is ±0.01%. This specification could be met by dissolving a 1-g sample in 30 ml of 15%(v/v)H_3PO_4–15%(v/v) H_2SO_4 and diluting to a final volume of 100 ml. Under these conditions with a lamp current of 20 mA, a calibration curve was prepared for the range 0–0.50% and found to be linear. In the calibration series, chromium(III) derived either from spectrographically pure chromium or potassium dichromate exhibited equivalent absorbances and therefore the cheaper potassium dichromate was used to prepare the calibration curves.

A comprehensive interference study was carried out at the 0.005, 0.200 and 0.500% chromium levels, the possible interfering elements being added as spectrographically pure metals. The interference of certain elements (Mo, W, Ni) varied with flame type and the height of the absorption path above the base of the burner. The most useful flame type was a slightly rich air–acetylene flame (10.4 l/min air and 2.4 l/min acetylene at S.T.P.) and the most useful absorption path was 8 mm above the base of the burner flame. Any interference greater than 0.005% Cr was considered to be significant. Under the selected conditions, no interference was encountered from 5% Ni^{2+}, 5% Mn^{2+}, 5% Cu^{2+}, 5% Co^{2+}, 5% W^{6+}, 2% Al^{3+}, 2% V^{5+}, or 1% Mo^{6+}.

However, higher percentages of nickel, tungsten and molybdenum caused interference at the 0.50% chromium level; 20% Ni^{2+} or 20% W^{6+} gave results which were 0.020% low and 5% Mo^{6+} gave results which were 0.030% low. These interferences can be accommodated by preparing calibration series which contain the same approximate composition of Ni^{2+}, W^{6+} or Mo^{6+} as the materials being analyzed. Many alloys which contain high percentages of Ni, W or Mo also contain greater than 0.5% Cr and the determination of chromium in such highly alloyed steels and methods of overcoming interferences are being investigated.

The results obtained indicated that with the same conditions as in the interference study, chromium in low-alloy irons and steels could be satisfactorily determined by atomic absorption spectrophotometry.

Reagents

AnalaR orthophosphoric (sp.gr. 1.75), sulphuric (sp.gr. 1.84) and nitric (sp.gr. 1.42) acids. Chromium solution (1 ml ≡ 0.5 mg Cr). Dissolve 1.4142 g of potassium dichromate ($K_2Cr_2O_7$; AnalaR, dried for 2 h at 150°) in water and dilute to 1 l. Johnson and Matthey spectrographically pure iron (chromium < 1 p.p.m.).

Recommended procedure

Transfer 1 g of iron or steel to a 125-ml conical beaker. Prepare a calibration series for the range 0.00–0.50% Cr, by making suitable additions of chromium solution to

several 1-g samples of spectrographically pure iron. Add 30 ml of 15% (v/v) phosphoric–15%(v/v) sulphuric acid, simmer to dissolve and oxidise by dropwise additions of nitric acid. The solution of certain classes of alloyed steels may be assisted by the use of aqua regia. Evaporate to fumes and fume gently for 1 min. Extract the residue with 30 ml of water and digest for 5 min to ensure solution of all soluble salts.

If filtration is necessary, filter through a Whatman 541 filter paper into a 100-ml graduated flask, rinse the beaker with several washes of hot 2% (v/v) sulphuric acid and wash the filter several times with hot 2% (v/v) sulphuric acid. Allow to cool and dilute to the mark. Measure the absorbance by atomising the solution in a 10-cm slot burner using a slightly rich air–acetylene flame and operating the chromium hollow-cathode lamp at 20 mA, with the light path 8 mm above the base of the burner flame. The chromium content is read directly from the calibration graph.

RESULTS

A series of standard low-alloy irons and steels were analyzed by the proposed procedure and the results, reported to the nearest 0.005% chromium, are listed in Table I.

TABLE I

CHROMIUM CONTENT OF STANDARD IRONS AND STEELS IN THE RANGE 0–0.50% CHROMIUM

Sample	*Type*	*Nominal composition*	*% Chromium*	
			Observed	*Certificate*
N.B.S. 55d	O.H. iron	—	0.005	0.005
N.B.S. 10 f	Bessemer steel	0.6% Mn	0.025	0.023
N.B.S. 4i	Cast iron	0.8% Mn	0.105	0.104
B.C.S. 252	Low-alloy steel	4% Ni, 0.5% V	0.200	0.20
B.C.S. 253	Low-alloy steel	3% Ni, 1% Mo 0.4% Mn, 0.5% Cu	0.350	0.35

TABLE II

CHROMIUM CONTENT OF STANDARD IRONS AND STEELS USING AN EXPANDED ABSORBANCE SCALE

Sample	*Type*	*Nominal composition*	*% Chromium*	
			Observed	*Certificate*
Johnson-Matthey iron	Sponge grade II	—	0.005	0.005
N.B.S. 55d	O.H. iron	—	0.007	0.005
N.B.S. 8h	Bessemer steel	0.5% Mn	0.024	0.022
N.B.S. 10f	Bessemer steel	0.6% Mn	0.026	0.023
N.B.S. 4i	Cast iron	0.8% Mn	0.105	0.104

Chromium hollow-cathode lamps exhibit low noise[11] characteristics and the absorbances of samples which contain 0–0.10% chromium can be measured on an expanded absorbance scale with the circuit described by DAVID[15]. The expanded absorption scale permits a significant discrimination of 0.001% chromium. With the help of this expanded scale, a series of standard low-alloy irons and steels were analyzed; the results are reported in Table II.

The results obtained on standard samples indicate that the proposed procedure is a

suitable alternative to many of the established procedures for the determination of chromium in low-alloy irons and steels.

Appreciation is expressed to W. F. PICKERING for helpful discussion and to the Chief Research Officer, The Broken Hill Proprietary Co. Ltd for permission to publish this work.

SUMMARY

An atomic absorption spectrophotometric procedure for the determination of 0.001–0.50% chromium in low-alloy iron and steel is described. The sample is dissolved in phosphoric–sulphuric acid before atomisation. The method is rapid, preliminary separations are not required and the accuracy obtained with standard samples is well within the permissible range for routine determinations.

RÉSUMÉ

Une méthode est proposée pour le dosage spectrophotométrique par absorption atomique du chrome (teneur: 0.001 à 0.5%) dans le fer et l'acier. L'échantillon est dissous dans un mélange d'acide phosphorique et d'acide sulfurique et atomisé. La méthode est rapide; aucune séparation préalable n'est nécessaire. La précision obtenue est satisfaisante pour des dosages de routine.

ZUSAMMENFASSUNG

Ein atomabsorptions-spektrophotometrisches Verfahren zur Bestimmung von 0.001–0.50% Chrom in niedriglegiertem Eisen und Stahl wird beschrieben. Die Methode ist rasch und erfordert keine vorherigen Trennungen. Die Genauigkeit, welche mit Standardproben erzielt wurde, liegt innerhalb der Grenzen für Routine-Bestimmungen.

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SÉPARATION CHROMATOGRAPHIQUE DU PLOMB CONTENUE DANS DES SOLUTIONS CONCENTRÉES DE SULFATE DE ZINC

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Le dosage de traces de plomb inférieures au mg/l, dans des solutions concentrées en sulfate, ne peut pas s'effectuer directement par les méthodes habituelles de polarographie ou de spectrographie; la séparation du plomb, préalable au dosage, peut être envisagée par divers procédés; WATANABE ET FUKUSHIMA[1] d'une part ont proposé l'extraction du dithizonate de plomb dans le chloroforme et le dosage colorimétrique de la phase organique et KOJIMA[2] a, d'autre part, étudié les possibilités de séparation du plomb par coprécipitation au moyen de sulfate de baryum. Après redissolution, le dosage s'effectue par colorimétrie du dithizonate.

La méthode à la dithizone étant assez délicate dans son application à des solutions aussi complexes que les électrolytes industriels de sulfate de zinc, nous nous sommes proposé de mette au point un procédé de séparation sur échangeur d'ions permettant d'obtenir le plomb en milieu chlorhydrique, conditions appropriées pour un dosage spectrographique ultérieur.

Méthodes de séparation Pb–Zn

Nous avons expérimenté deux méthodes:

(*a*) Fixation du zinc et du plomb sur résine cationique et élimination des ions sulfates; élution de la totalité du plomb et d'une partie du zinc par HCl; séparation Pb–Zn sur résine anionique.

Ce procédé est basé sur les données de la littérature concernant les coefficients de distribution[3,4].

En milieu neutre, Pb et Zn sont fixés quantitativement sur résine cationique acide et le lavage à l'eau permet l'élimination totale des sulfates. L'élution avec une solution de HCl 2 *N* fournit le plomb et le zinc (Figs. 1 et 2).

Cet éluat, passé sur une colonne de résine anionique équilibrée avec HCl 2 *N*, conduit à une fixation totale des deux métaux ($K_d^{Pb} = 50$, $K_d^{Zn} \simeq 1000$; K_d = coefficient de distribution). L'élution sélective du plomb s'obtient au moyen de HCl 8 *N* (Fig. 3) ($K_d^{Pb} < 1$, $K_d^{Zn} \simeq 100$).

(*b*) Après fixation de Pb et Zn en milieu neutre sur résine cationique et élimination des sulfates par l'eau, comme dans le 1^er^ procédé, élution sélective du plomb au moyen de HCl *N*, en utilisant une colonne de capacité et de longueur suffisantes pour obtenir une séparation complète du zinc (Figs. 4 et 5). Le traitement ultérieur par HCl 8 *N*

produit, d'une part, une élimination du zinc et, d'autre part, une régénération de la résine pour l'expérience suivante.

La mise au point des méthodes de séparations a été faite en utilisant la technique des traceurs radioactifs: ^{112}Pb et ^{65}Zn.

Réactifs et traceurs

Echangeurs d'ions. Dowex 50 (200–400 mesh, 4% DVB) séparation sur cationique, et 200 mesh, 8% DVB, séparation sur anionique et Dowex 2 (100–200 mesh, 8% DVB).

HCl 12 N. U.C.B., p.a.

Zn $SO_4 \cdot 7H_2O$. U.C.B., p.a., servant à préparer des électrolytes à 125 g Zn/l.

Traceurs. (1) ^{65}Zn obtenu par activation neutronique de zinc extra pur (99.999%) ($T^{1/2} = 250$ jours; émetteur-γ = 1.11 MeV; $\beta^+ = 0.35$ MeV). (2) ^{212}Pb ($T^{1/2} = 10.6$ h; $\beta^- = 0.36$ MeV et 0.59 MeV; $\gamma = 0.43$, 0.71, 0.238 MeV) (membre de la famille du ^{232}Th). Il donne par émission β^- le ^{212}Bi de 60.5 min ($\beta^- = 2.256$ MeV; $\gamma = 0.73$, 1.80 MeV).

Nous avons préparé régulièrement notre solution de ^{212}Pb à partir d'une solution stock contenant 10 g de nitrate de thorium, en nous inspirant de la méthode décrite par GORSUCH[5]. La solution de thorium dans HCl 2 *N*, vieille de quelques jours, est passée sur colonne de Dowex 2 (100–200 mesh, 8% DVB) de 1.5 × 11.5 cm; dans ces conditions, Po, Pb, Bi et Tl sont fixés tandis que les isotopes à vie longue passent. Le plomb est ensuite récupéré par lavage avec HCl 8*N* (20 ml d'éluat). La pureté du ^{212}Pb obtenu a été testée par mesure de sa période et de l'activité résiduelle après 10 périodes (0.5 à 1‰).

L'activité en plomb, mesurée au scintillateur γ, est de l'ordre de 10^5 d.p.m.

Modes opératoires pour la séparation Pb–Zn des solutions de sulfate

Comme nous l'avons signalé ci-dessus, la mise au point des procédés de séparations chromatographiques sur échangeurs d'ions a été faite sur des solutions synthétiques de sulfate de zinc concentrées (125 g Zn/l) contenant des traces de plomb, en marquant la solution au moyen de ^{65}Zn et ^{212}Pb.

(*a*) *Séparation Zn–Pb sur résine anionique.* 10 ml de solution synthétique neutre de

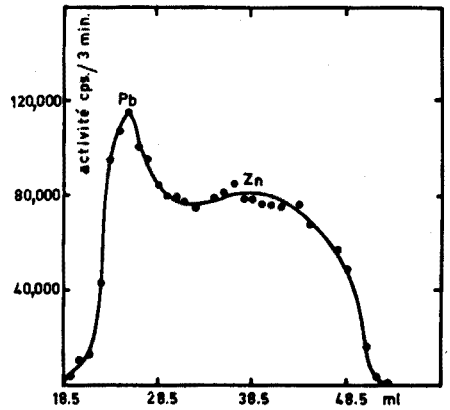

Fig. 1. Elimination des ions SO_4^{2-} sur colonne cationique Dowex 50. Elution du plomb et du zinc par HCl 2*N* (traceurs ^{212}Pb et ^{65}Zn).

sulfate de zinc et plomb marqués sont versés sur une colonne de Dowex 50 de 1.5 × 21.5 cm. Laver avec 20 ml d'eau désionisée (élimination des sulfates). Eluer au moyen de HCl 2 *N*; écarter les 18 ml de tête; recueillir les 20 ml suivants (la colonne cationique est régénérée par traitement avec 20 ml HCl 8 *N*, lavage à l'eau désionisée jusqu'à élimination de l'acidité). Les 20 ml d'éluat sont versés sur la colonne anionique Dowex 2 (2 × 12.5 cm) équilibrée avec HCl 2 *N*. Après lavage avec 10 ml de HCl 2 *N*, on élue avec HCl 8 *N*; les 25 ml de tête sont écartés; on recueille les 55 ml suivants contenant le plomb. La colonne est régénérée par passage de 225 ml de H_2SO_4 3.6 *N*, de HCl 2 *N* jusqu'à disparition des ions sulfates. Les Figs. 1, 2 et 3 montrent les différentes phases de la séparation.

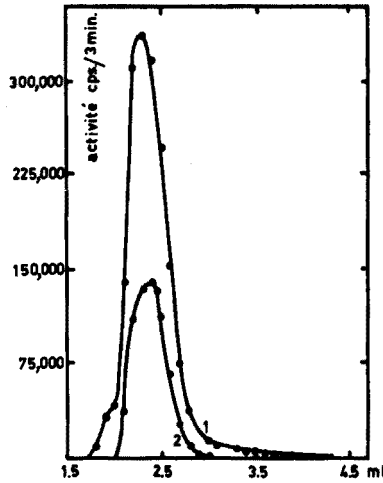

Fig. 2. Elimination des ions SO_4^{2-} sur résine cationique. Courbe d'élution du plomb (traceur ^{212}Pb) par HCl 2 *N*. (1) et (2) mesurés à deux temps différents.

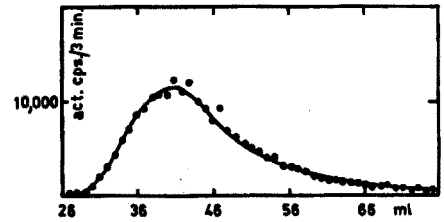

Fig. 3. Séparation Pb–Zn sur colonne anionique Dowex 2. Courbe d'élution du plomb (traceur ^{212}Pb). Elution par HCl 2 *N*.

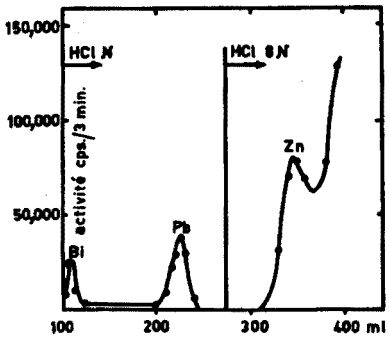

Fig. 4. Séparation Pb–Zn sur résine cationique Dowex 50. Courbes d'élution du plomb et du zinc. Volume traité: 10 ml.

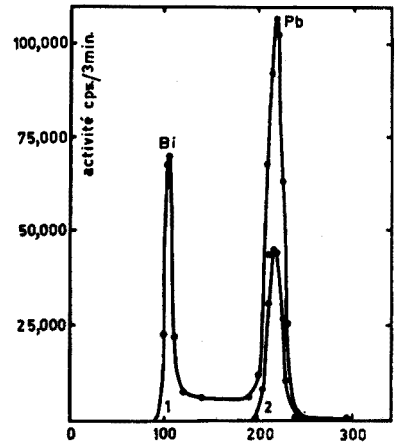

Fig. 5. Séparation Pb–Zn d'un électrolyte sur résine cationique Dowex 50. Courbes d'élution du plomb par HCl *N*. (1) avant décroissance du ^{212}Bi; (2) après décroissance du ^{221}Bi.

(*b*) *Séparation Pb–Zn sur résine cationique.* 10 ml de solution synthétique neutre de sulfate de zinc et plomb marqués sont versés sur une colonne de Dowex 50 (3.1 × 27.0 cm); après lavage avec 75 ml d'eau désionisée, éluer le plomb au moyen de HCl *N*: l'élution du plomb nécessite 240 ml de HCl *N*; les 150 ml du début sont écartés et l'on recueille les 90 ml suivants contenant le plomb (Figs. 4 et 5). Le zinc de la colonne est ensuite élué au moyen de HCl 8*N*; la colonne est enfin rincée à l'eau et elle est prête pour une nouvelle séparation.

Dosage du plomb récupéré par spectrographie d'émission

Le dosage spectrographique du plomb séparé a été effectué par le laboratoire central de la Vieille Montagne (Angleur) par la méthode visuelle après incorporation dans la base cuivre: à cet effet, la solution chlorhydrique contenant le plomb est traitée par HNO_3 et évaporée à sec pour l'élimination des chlorures; le résidu est additionné de 200 mg de cuivre extra pur et mis en solution par HNO_3. Cette solution est évaporée à sec et le résidu est dosé spectrographiquement sur électrode de graphite. La limite de dosage pour le plomb est de l'ordre du μg; la précision de la méthode visuelle est de l'ordre de 10%.

RÉSULTATS EXPÉRIMENTAUX

Les deux méthodes conduisent, d'une part, à une élimination très poussée du zinc et des sulfates et, d'autre part, à une récupération quantitative du plomb. Les résultats du Tableau I permettent de se faire une idée du caractère quantitatif de la récupération du plomb dans la première méthode: ce contrôle est basé sur la mesure de l'activité récupérée. Les chiffres de la dernière colonne de ce Tableau semblent indiquer une perte de l'ordre de 4%; cet écart est cependant du essentiellement au fait que la mesure de la solution d'élution est rapportée à une activité étalon de dix à quinze fois plus petite, ce qui introduit une erreur par défaut de l'ordre de 2–3%, comme il a été montré par l'étude des courbes de décroissance d'échantillons de ^{212}Pb.

TABLEAU I

RÉCUPÉRATION DU PLOMB APRÈS SÉPARATIONS SUR RÉSINE ANIONIQUE

Essai No.	*Activité étalon (Cps/3 min)*	*Facteur de compariason*	*Activité introduite (Cps/3 min)*	*Activité récupérée (Cps/3 min)*	*Pourcentage récupéré*
1	8510	× 15	127,650	123,046	96.4[a]
	6995	—	104,925	102,605	97.8
2	9617	× 12	115,404	110,026	95.3[a]
	9524	—	114,288	110,014	96.3
	8885	—	106,620	103,536	97.1
	8961	—	107,532	102,840	95.6
3	9651	× 12	115,812	110,328	95.3[a]
	9580	—	114,960	110,350	96.0
	8300	—	99,600	96,297	96.7
	8277	—	99,324	95,577	96.2
4	22,490	× 12	268,344	255,311	95.2[a]
	22,114	—	265,368	255,478	96.3
	21,277	—	255,324	245,488	96.2
	20,980	—	251,760	243,033	96.5

[a] Valeurs plus faibles de l'activité obtenue pour la première mesure lorsque l'équilibre entre les produits de filiation et le ^{212}Pb n'est pas encore atteint.

D'autre part, l'activité résiduelle, après plusieurs jours, montre que la séparation du zinc est très bonne (> à 99.999%).

Nous sommes cependant d'avis que la seconde méthode est plus satisfaisante: la récupération est également quantitative (Tableau II) et elle est plus simple puisqu'elle n'exige qu'une seule colonne; le pic est plus étroit.

Ces valeurs, obtenues sur un électrolyte industriel, montrent que dans les limites des erreurs de la méthode spectrographique, la récupération du plomb ajouté avant la séparation chromatographique est complète.

TABLEAU II

RÉCUPÉRATION DU PLOMB APRÈS SÉPARATION SUR RÉSINE CATIONIQUE

Volume électrolyte traité (ml)	*Ajoute de Pb avant séparation (µg)*	*Dosage spectrographique (µg)*	*Valeur trouvée pour l'ajoute (µg)*
10	0	2.8	
10	2	4.8	2.0
10	5	8.0	5.2
10	5	8.0	5.2
10	10	12.0	9.2

TABLEAU III

DOSAGE DU PLOMB DANS UN ÉLECTROLYTE INDUSTRIEL

Volume électrolyte traité (ml)	*Purification*	*Dosage spectrographique (µg)*
10	—	2.9
10	par Zn	1.2
10	par $BaSO_4$	1.7

Il reste évidemment à savoir si les 2.8 µg Pb trouvés dans 10 ml d'électrolyte industriel sont réels ou dûs au blanc de la méthode de séparation chromatographique: pour cela, deux fractions d'électrolyte industriel ont subi une purification poussée: la première par cémentation pendant 2 h au moyen de poudre de zinc (10 g de Zn par litre de solution) et la seconde par entrainement du plomb par un précipité de $BaSO_4$ (20 g de $BaSO_4$ par litre d'électrolyte).

L'ajoute d'une trace de ^{212}Pb a permis de savoir que 99% du Pb de l'électrolyte industriel sont éliminés dans ces conditions.

Le Tableau III fournit les résultats des dosages spectrographiques de ces deux solutions, comparés à celui obtenu avec un échantillon n'ayant pas subi une nouvelle purification.

Etant donné ces résultats, il est clair que les valeurs trouvées pour les deux électrolytes purifiés correspondent en fait au blanc de la méthode de séparation du plomb: à savoir en moyenne 1.45 µg Pb pour 10 ml d'électrolyte; dans ces conditions, la teneur dans l'électrolyte industriel utilisé est de 0.15 mg Pb/l de solution. Il est vraisemblable que ce blanc provient du plomb se trouvant dans la résine et dans les acides utilisés lors de la séparation.

Ces chiffres montrent que la méthode chromatographique proposée pour la séparation des traces de plomb contenues dans les solutions concentrées de sulfate de zinc en vue du dosage spectrographique n'est applicable, avec quelque précision, qu'aux cas où cette teneur est de l'ordre ou dépasse le mg de Pb/l.

Nous tenons à remercier Mr. G. SEMPELS, chef des laboratoires de la Vieille Montagne, pour les résultats des analyses spectrographiques effectuées dans son laboratoire.

RÉSUMÉ

Le dosage spectrographique de traces de plomb contenues dans une solution très concentrée de sulfate de zinc (bain d'électrolyse) n'est possible qu'après élimination des ions Zn^{2+} et SO_4^{2-} et passage en milieu chlorhydrique. Deux méthodes de séparations chromatographiques sur échangeurs d'ions ont été mises au point: la première consiste à séparer les ions sulfates par passage sur résine cationique et élimination des ions Zn^{2+} sur résine anionique; dans la seconde, les deux opérations se font par passage sur une résine cationique; les deux méthodes se sont avérées satisfaisantes du point de vue du caractère quantitatif de la séparation du plomb, encore que la seconde soit plus simple et plus rapide.

Le blanc de la méthode est de l'ordre de 1.5 μg Pb, ce qui équivaut à une teneur de 0.15 mg Pb/l d'électrolyte au cas où la séparation s'effectue sur 10 ml. Dans ces conditions, la méthode de dosage est applicable, avec quelque précision, à des électrolytes contenant du plomb en teneur de l'ordre de 1 mg/l.

SUMMARY

Spectrographic analysis of lead traces in concentrated zinc sulphate solutions is only possible after elimination of zinc and sulphate ions. Two methods of separation on ion exchangers are proposed: the first method consists in a separation of Pb from Zn on an anion exchanger after elimination of sulphate on a cation exchanger; in the second method, the two steps of the separation are realized on the same cation-exchanger column. Both methods are satisfactory, but the second is faster and simpler.

The blank of the method is about 1.5 μg Pb (*i.e.* 0.15 mg Pb/l) for a separation made with 10 ml of electrolyte. The method is therefore suitable for accurate determinations of Pb contents as low as 1 mg/l of concentrated zinc sulphate electrolyte.

ZUSAMMENFASSUNG

Die Bestimmung von Spuren von Blei in konzentrierten Zinksulfatlösungen durch spektrographische Analyse ist nur möglich nach Eliminierung der Zink- und Sulfationen. Hierzu werden zwei Methoden unter Verwendung von Ionenaustauscherharze vorgeschlagen: Bei der ersten Methode werden die Sulfationen mit einem Kationenaustauscherharz und Zink und Blei mit einem Anionen-Austauscherharz getrennt. Bei der zweiten Methode gelingt die Trennung in zwei Stufen mit einer Kolonne von Kationenaustauscherharz. Beide Methoden ergeben gute Resultate. Die zweite Methode ist jedoch rascher und einfacher. Der Blindwert der Methode beträgt etwa 1.5 μg Pb bei Anwendung von 10 ml Elektrolyt.

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COLORIMETRIC DETERMINATION OF PERSULFATE WITH ALCIAN BLUE*

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The literature reveals a large number of qualitative colorimetric tests for the persulfate ion. The well-known benzidine test[1] is based on the formation of a quinoidal oxidation product of benzidine. The benzidine derivatives, 2,7-diaminofluorene and 2,7-diaminophenyloxide, are even more sensitive than benzidine. Various workers have reported the use of fuchsine, brucine, strychnine, iodides, aniline sulfate, leuco-methylene blue, *o*-tolidine, indigo and leuco-fluorescein to detect persulfate[2-4]. This report deals with a simple, quantitative, fairly rapid method, based on decolorization of Alcian blue in a buffered solution, to determine persulfate. Alcian blue, which is a water-soluble derivative of copper phthalocyanine, is a quaternary methyl-*p*-toluene sulfonate of copper tetra-4-pyridylphthalocyanine. The compound was obtained from extensive research aimed at producing a dye which combines the brilliant turquoise blue of copper phthalocyanine and sufficient solubility, fastness to light, and affinity for cellulosic fibre to warrant its use in dyeing fabrics[5]. STEEDMAN[6] is credited with introducing Alcian blue as a biological stain to detect mucin.

EXPERIMENTAL

Materials

All the reagents employed were analytical grade. Alcian blue (C.I. 74240[7]) was purchased from Matheson, Coleman and Bell, Norwood, Ohio**.

Assay

Unless stated otherwise, 50 mg of dye was dissolved in 160 ml of water and, after either acetic or hydrochloric acid was added to give a pH of 2.5, the solution was diluted to 200 ml. Five-ml aliquots of the dye solution were mixed with 5 ml of solutions containing from 10 to 100 μg of oxidant and allowed to stand at room temperature for 2 h. The mixture was diluted to 20 ml with distilled water, and the optical density was measured in a Bausch and Lomb Spectronic colorimeter at 615 mμ.

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** Mention in this report of a trade product, equipment or a commercial company does not imply its endorsement by the U.S. Department of Agriculture over similar products or companies not named.

RESULTS AND DISCUSSION

A number of important factors in an analytical assay procedure were studied. Alcian blue had a maximum optical density at 615–620 mμ. The dye was found to obey the BEER–LAMBERT law for the concentration range employed. The effect of time on the reaction of Alcian blue with persulfate is summarized in Fig. 1. (The decrease in optical density was small beyond 60 min and negligible beyond 120 min.) Measurements were made after a reaction period of 2 h, but a shorter time might be employed in concentrations below 80 μg of persulfate. To test the reversibility of the decolorization of Alcian blue, an excess of cysteine or glutathione was added to mixtures of the dye and persulfate after a reaction period of 2 h. It was not possible to restore the color by adding a reducing agent to the dye decolorized by oxidant. Adding the reducing sub-

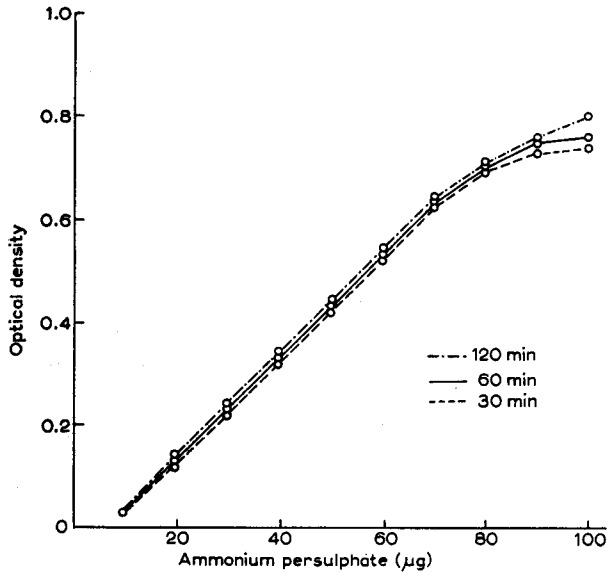

Fig. 1. Effect of reaction time on decolorization of Alcian blue by ammonium persulfate. Results are reported as decrease in optical density compared with that of the control.

TABLE I

EFFECTS OF VARIOUS OXIDANTS ON DECOLORIZATION OF ALCIAN BLUE

Oxidant	*Optical density Oxidant level (μg)*					
	0	25	50	100	250	500
Persulfate	0.73	0.56	0.32	0.06	—	—
Bromate	0.73	0.74	0.73	0.73	—	—
Iodate	0.73	—	—	—	0.74	0.73
Ferricyanide	0.73	0.80	0.75	0.51	—	—
Dichromate	0.73	0.80	0.80	0.80	—	—
Permanganate	0.73	0.31	0.04	0.01	—	—
Benzoyl peroxide*	0.73	0.58	0.37	0.03	—	—

* Dissolved in a little alcohol before addition.

TABLE II

EFFECT OF pH ON DECOLORIZATION OF ALCIAN BLUE BY OXIDANTS

Oxidant name and level (μg)	*Optical density*		
	pH 2.5 (acetic acid)	*pH 2.5 (hydrochloric acid)*	*pH 0.7 (hydrochloric acid)*
Ammonium persulfate			
0	0.85	0.85	0.74
20	0.70	0.70	0.61
40	0.49	0.50	0.44
60	0.31	0.30	0.30
80	0.15	0.13	0.22
100	0.05	0.04	0.17
Potassium bromate			
0	0.85	0.85	0.75
20	0.85	0.85	0.75
40	0.85	0.85	0.74
60	0.85	0.85	0.60
80	0.85	0.85	0.66
100	0.85	0.85	0.57
Potassium iodate			
0	0.81	0.81	0.75
20	0.81	0.81	0.75
40	0.81	0.81	0.75
60	0.81	0.81	0.75
80	0.81	0.81	0.75
100	0.81	0.81	0.75

stance, on an equimolar basis, simultaneously with the oxidant, almost completely prevented decolorization by persulfate. Cysteine, homocysteine, glutathione, sodium glutathione, cysteine hydrochloride and ascorbic acid effectively blocked the action of persulfate on Alcian blue; cystine, as expected, had no effect. As the results pointed to the oxidative nature of the reaction, a number of oxidants were tested (Table I). It was noted, however, that while potassium iodate was ineffective at any pH tested, bromate had a decolorizing action at a low pH (Table II). That the effect was due to pH and not to the acid used was shown when acetic acid or hydrochloric acid, at levels to give the same pH, gave comparable results in decolorization. The use of mixtures of ammonium persulfate and potassium bromate showed that an 8-fold level of potassium bromate did not affect the decolorizing action of persulfate.

The use of equimolar concentrations of potassium or ammonium persulfate gave comparable results (Table III). Commercial dyes are known to vary in their actual dye content when purchased from various manufacturers or even among lots from the same manufacturer. While these differences do not affect linearity or relative response, they are undesirable. It has been found, however, that if the dyes are adjusted to give the same optical density by diluting dye solutions which have concentrations slightly above that recommended, the variations among dye stock solutions can be overcome.

The financial support of the senior author by the Rockefeller Foundation is gratefully acknowledged.

TABLE III

EFFECT OF EQUIMOLAR CONCENTRATIONS OF POTASSIUM AND AMMONIUM PERSULFATE ON DECOLORIZATION OF TWO LOTS OF ALCIAN BLUE

Oxidant name and level (μg)	*Optical density*	
	Lot 1	*Lot 2*
Potassium persulfate		
0	0.78	0.68
11.8	0.75	0.66
23.6	0.68	0.58
47.2	0.52	0.41
70.8	0.35	0.25
94.4	0.20	0.12
118.0	0.08	0.07
Ammonium persulfate		
0	0.78	0.68
10	0.75	0.65
20	0.66	0.56
40	0.46	0.37
60	0.28	0.21
80	0.14	0.09
100	0.06	0.07

SUMMARY

Alcian blue buffered at pH 2.5 is oxidized by persulfate. The plot of optical density at 615 mμ *vs.* concentration of persulfate (within the range of 10 to 80 μg) is linear. The assay is not affected by bromate and iodate.

RÉSUMÉ

Une méthode est proposée pour le dosage colorimétrique des persulfates (10 à 80 μg), basée sur l'oxydation du "bleu alciane", au pH 2.5. Les bromates et les iodates ne gênent pas.

ZUSAMMENFASSUNG

"Alcian-Blau" wird durch Persulfate bei einem pH von 2.5 oxydiert. Diese Reaktion wurde zu einer colorimetrischen Bestimmungsmethode für Persulfate angenandt. Bromate und Jodate stören nicht.

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COMPLEX FORMATION OF IRON(III) WITH CHROME AZUROL S

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In analytical chemistry chrome azurol S (3''-sulpho-2'',6''-dichloro-3,3'-dimethyl-4'-hydroxyfuchson-5,5'-dicarboxylic acid) has been applied as a metal indicator and as a reagent for the photometric determination of metals. Very few metal complexes with chrome azurol S have been studied with regard to composition and stability. SILVERMAN AND SHIDELER[1] employed the method of continuous variation to determine the mole ratio of the beryllium–chrome azurol S complex, and found it to be 1 : 1. MACNULTY AND WOOLLARD[2] utilized the reaction of chrome azurol S with aluminium for the determination of fluoride, and stated that under the conditions applied the aluminium–chrome azurol S complex appeared to have the formula $Al(dye)_2$. In a preceding paper in this series which dealt with methods for determining fluoride, MACNULTY, HUNTER AND BARRETT[3] gave the structural formula of a reagent designated as chrome azurol S. This molecule was, however, not 3''-sulpho-2'',6''-dichloro-3,3'-dimethyl-4'-hydroxyfuchson-5,5'-dicarboxylic acid, the difference being that both methyl groups and one chlorine atom were omitted. The probable explanation of this discrepancy is an incorrect reproduction of the structural formula (see below).

In the present investigation, the complex formation of iron(III) with chrome azurol S was studied by spectrophotometric, conductometric and potentiometric methods. A method was also worked out for the preparation of the pure tetrabasic acid of the ligand.

Chrome azurol S

In strong acid solution the ligand was believed to be present as the zwitterion shown in Fig. 1. This structure is in accordance with that previously suggested by

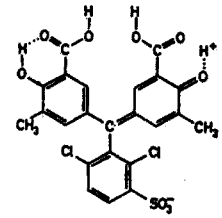

Fig. 1. Proposed structure of H_4Ch in strong acid solution.

MALÁT[4]. Owing to the comparatively long distance between the two bidentate groups, it was considered improbable that both would be engaged in complex formation with

the same metal ion. The ligand was therefore believed to be only bidentate. As indicated in Fig. 1, intramolecular hydrogen bonding may be present.

EXPERIMENTAL

Apparatus

For extinction measurements a Zeiss spectrophotometer PMQ II (5.000-cm cells) and a Beckman DB recording spectrophotometer (4.000-cm cells) were employed.

The conductivity was measured with a Philips conductivity measuring bridge PR 9500 and a cell GM 4221.

For the potentiometric titrations and for the measurement and regulation of pH a Beckman Zeromatic pH meter, with glass (entire pH range) and calomel electrodes, was used.

The temperature of solutions measured conductometrically and potentiometrically was maintained constant with an ultrathermostat.

The potentiometric titrations were made in a double-walled titrating vessel, water from the thermostat being circulated through the jacket.

Reagents

The trisodium salt of the tetrabasic acid is produced commercially under different trivial names, the most common of which is chrome azurol S. In the present investigation the commercial trisodium salt (J. R. Geigy) could be used for some measurements without purification; this product is designated below as CAS(I). For other measurements the pure tetrabasic acid was prepared; this product is designated as CAS(II). Iron(III) solutions were prepared from spectrographically standardized iron sponge (Johnson, Matthey and Co., Ltd) containing the following metallic impurities (given in p.p.m.): manganese – 3; nickel – 2, sodium – 2; copper, magnesium, silicon and silver – each element < 1. All other chemicals were of reagent-grade quality.

Preparation and investigation of the ligand

In a communication to the authors, the producer of CAS(I) specified the following contents of inorganic impurities: 10.8% SO_4^{2-}, 0.79% Cl^-, 0.1% $NaNO_2$, and traces of CO_3^{2-}. By evaporating a sample of CAS(I), first in the presence of concentrated sulphuric acid, and then in the presence of ammonium carbonate, the content of sodium was calculated to be 4.8 moles against a theoretical content of 3.0 moles.

No procedures for the purification of CAS(I) or the preparation of CAS(II) could be found in the literature, nor could the producer indicate suitable methods. However, a simple method for the preparation of CAS(II) was found to be as follows: 4 g of CAS(I) were dissolved in 20 ml of distilled water, and the tetrabasic acid was precipitated by adding 20 ml of concentrated hydrochloric acid. The precipitate was filtered on a glass filter crucible, washed with 1 : 1 hydrochloric acid solution and dried in vacuum over solid potassium hydroxide. The acid was redissolved in 25 ml of distilled water by heating to about 60°, reprecipitated by adding 25 ml of concentrated hydrochloric acid to the cooled solution, filtered, washed and dried as described above. After a second dissolution and reprecipitation, the final product was first dried over solid potassium hydroxide, and then to constant weight over phosphorus pentoxide.

The purity and structure of the preparation were checked by alkalimetric titration, elementary analysis and visual and infrared spectrophotometry.

The titrimetric determination of the equivalent weight indicated the presence of two molecules of water (a characteristic titration curve of CAS(II) is reproduced in Fig. 6); repeated determinations over a period of 6 months gave a constant value. By elementary analysis the following data were obtained: C, 47.51%; H, 3.48%; S, 4.39%; Cl, 13.96%; ignition residue, 0.77%; O (by difference), 29.89%. Theoretical percentages for $C_{23}H_{16}O_9SCl_2 \cdot 2\ H_2O$ are: C, 48.00%; H, 3.50%; S, 5.57%; Cl, 12.32%; O, 30.60%.

The infrared spectrum of CAS(II) exhibited absorption at 1036, 1626 and 1681 cm^{-1}. The absorption at 1036 cm^{-1} was probably due to the sulphonic acid group, but impurities of sulphate would also absorb at this wave number. The content of impurities of sulphate was probably low, as indicated by the low ignition residue obtained by elementary analysis. An unbounded quinone carbonyl double bond would be expected to give an absorption at about 1660 cm^{-1}. A frequency shift to 1626 cm^{-1} was explained by the presence of strong inter- or intramolecular hydrogen bonding with the carbonyl oxygen. Double bonds of a quinoid ring may give absorption at about 1625 cm^{-1}, but if these rings accounted for the present absorption at 1626 cm^{-1}, the spectrum would also have been expected to exhibit absorption at 1660 cm^{-1}. The presence of the two carboxylic acid groups explained the absorption at 1681 cm^{-1}.

The investigations of CAS(II) led to the conclusion that the chemical composition of the preparation was $C_{23}H_{16}O_9SCl_2 \cdot 2\ H_2O$ (in formulae abbreviated to H_4Ch).

Standard solutions

A standard solution of iron(III) (about $2 \cdot 10^{-2}$ *M*) was prepared by dissolving 1.1678 g of sponge metal in 1 : 3 hydrochloric acid. After dissolution 1.7 ml of concentrated nitric acid were added and the solution was heated. Complete oxidation to the trivalent state was checked with a freshly prepared solution of potassium ferricyanide. The solution was finally diluted to 1000 ml with distilled water. The iron concentration was determined gravimetrically and was found to be $1.989 \cdot 10^{-2}$ *M*. A 10^{-3} *M* solution of iron(III) was prepared by transferring 50.28 ml to a 1000-ml volumetric flask and diluting to volume with distilled water. The pH of this solution was 2.2.

The acid content of the standard iron solution was found by pipetting into two beakers 25 ml of the 10^{-3} *M* iron solution, adding to one beaker 2.50 ml and to the other 2.60 ml of a 0.01 *M* solution of ethylenediaminetetraacetic acid (disodium salt)* and titrating potentiometrically with a 0.05896 *N* carbonate-free sodium hydroxide solution from a calibrated 5-ml burette. By this titration the total amount of acid originating partly from the iron solution and partly from the acid formed by complex formation was determined. The content of acid in the iron solution could then be calculated. As pointed out by SCHWARZENBACH[12], the two hydrogen ions released in this complex formation are titrated before the appearance of any hydroxo complexes of iron(III).

A standard solution of CAS(I) (about $5 \cdot 10^{-4}$ *M*) was prepared by dissolving 0.0756 g in distilled water and diluting to 250 ml. Similarly, a 10^{-3} *M* solution of CAS(II) was prepared by dissolving 0.1463 g in distilled water and diluting to 250 ml.

A stock aqueous 1.0 *M* solution of potassium chloride was prepared. The pH meter

* 2.5 ml of this solution was exactly equivalent to the amount of iron(III) present, whereas 2.6 ml provided a slight excess; the end-point was the same in both cases.

was standardized by means of solutions of 0.05 M potassium hydrogen phthalate (pH = 4.00) and 0.05 M borax (pH = 9.22).

Ionic strength

During the photometric and potentiometric measurements, the ionic strength was kept relatively constant by maintaining a concentration of 0.10 M potassium chloride and low concentrations of ligand and metal ion.

Temperature

The photometric and conductometric measurements were made at 20° and 25°, respectively. Potentiometric titrations were carried out at 25° and 60°.

SPECTROPHOTOMETRIC MEASUREMENTS

Absorption curves of CAS(II)

Absorption curves of aqueous solutions of CAS(I) were plotted by MALÁT[5]. In the ultraviolet region he found that four different products from three different firms all exhibited two maxima at about 350 mμ. The pH of these solutions was 7.38. The absorption curve of a $0.5 \cdot 10^{-5}$ M solution of CAS(II) was recorded. The solution had a pH of 7.40 and was 0.10 M in potassium chloride. The curve exhibited the same maxima and minimum as those found by MALÁT[5].

Determination of the dissociation constants of CAS(II)

The dissociation constants of CAS(II) were determined previously by MALÁT[4]. He employed a spectrophotometric method and used buffers containing carboxylic acid. During their investigation of the beryllium–chrome azurol S complex, SILVERMAN AND SHIDELER[1] observed that the presence of carboxylic acids seemed to reduce the colour

TABLE I

DISSOCIATION CONSTANTS OF CHROME AZUROL S

	MALÁT[4]	*Present investigation*
Equilibrium I: $HCh^{3-} \leftrightharpoons H^+ + Ch^{4-}$	11.47	11.81
Equilibrium II: $H_2Ch^{2-} \leftrightharpoons H^+ + HCh^{3-}$	4.86	4.71
Equilibrium III: $H_3Ch^- \leftrightharpoons H^+ + H_2Ch^{2-}$	2.45	2.25
Absorption maximum (in mμ) of:		
Ch^{4-} (blue)	590	598
HCh^{3-} (yellow)	430	427
H_2Ch^{2-} (red)	500	492
H_3Ch^- (orange)	480	463
H_4Ch (pink)	—	542
H_4Ch^+ (pink)[a]	540	—
Isosbestic points (in mμ) for:		
Equilibrium I	490	488
Equilibrium II	458	456
Equilibrium III	466	478

[a] MALÁT[4] stated that the ion H_4Ch had a colour identical with that of the ion H_3Ch^-, and that the pink colour was caused by the ion H_5Ch^+. However, the pink colour could just as well originate from H_4Ch.

intensity of solutions of the reagent, and therefore advised against the use of such buffers. In view of this observation a redetermination of the constants in the absence of carboxylic acid was desirable. The spectrophotometric method was also applied in the new investigation, but the pH was regulated, by means of a pH meter, with strong acid or base. The experimental details were as follows. Into a series of 100-ml volumetric flasks, 2 ml of standard solution of CAS(I) and 10 ml of 1.0 *M* potassium chloride solution were pipetted. After dilution to a volume of about 75 ml, diluted hydrochloric acid or sodium hydroxide solutions were added to a predetermined pH value. After the solutions had been diluted to volume, a final measurement of pH was made and the flasks were placed in the thermostat at 20°. The extinctions were measured at the wavelengths of the absorption maximum of the two ionic species in equilibrium, and the constants were calculated in the usual way. The equilibria considered, the constants calculated and the wavelengths of the absorption maximum of the different species

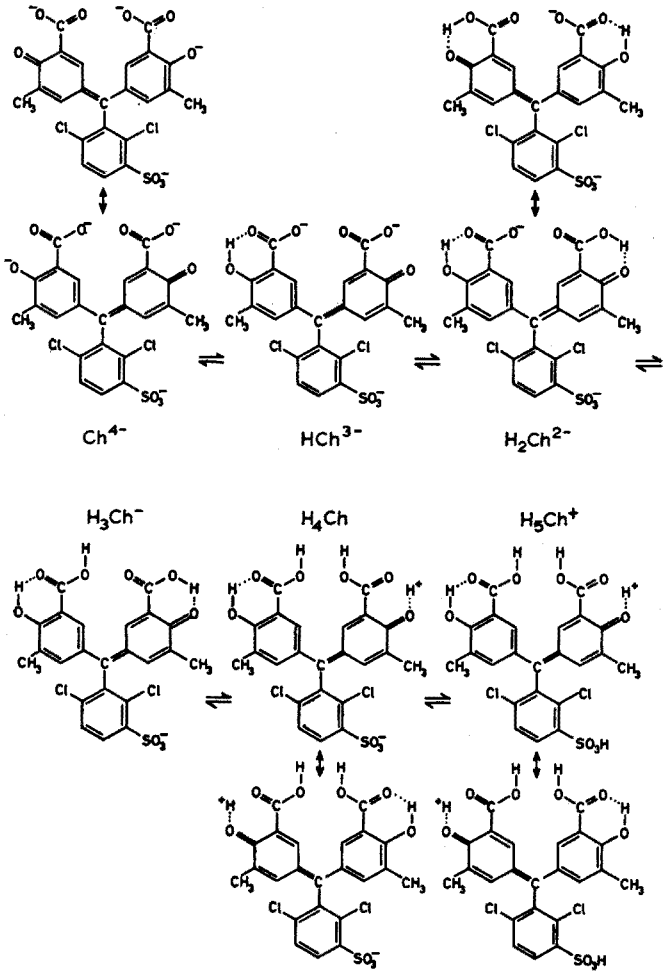

Fig. 2. The equilibria between the different possible species of the ligand.

are given in Table I together with the data obtained previously by MALÁT[4]. It should be noted that MALÁT used an ionic strength of 0.2 *M* and measured at room temperature.

The present constants do not agree closely with those of MALÁT. On the other hand, nothing indicated any effect due to carboxylic acid. The constants derived in the present investigation were used in all calculations.

The possible forms of the ligand and the probable equilibria between these forms are surveyed in Fig. 2. As seen from the figure, resonances may also be present.

Absorption curves of mixtures of CAS(II) and iron(III)

The complex formation was first studied by recording absorption curves of four series of solutions. In each series the ratio of metal ion to ligand was kept constant, while the pH varied. The solutions were prepared by pipetting into 100-ml volumetric flasks the proper volumes of the standard solutions of CAS(II) and of iron(III), adding 10 ml of inert salt solution, adjusting the pH and diluting to volume. All measurements were made in 4.000-cm cells against blanks containing the same amount of ligand and inert salt and adjusted to the same pH.

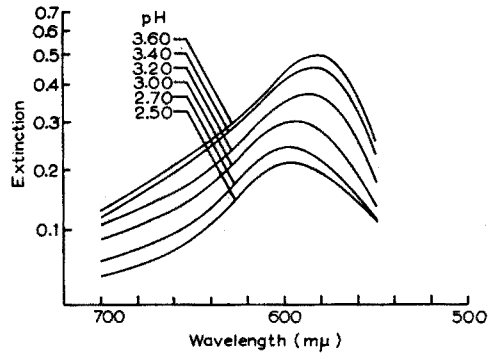

Fig. 3. Absorption curves at different pH values of solutions containing the reactants in equimolar amounts. Ligand solution added to metal solution.

Fig. 3 shows a series of curves containing metal ion and ligand in equimolar amounts. The curves at pH 2.50, 2.70 and 3.00 all exhibited a maximum at 596 mμ. With increasing pH the maximum shifted to shorter wavelengths, ending at 582 mμ for the solution adjusted to pH 3.40 and 3.60. From Fig. 3, the preliminary conclusion was drawn that two complexes were present, one predominating at lower (absorption maximum 596 mμ) and another at higher pH values (absorption maximum about 582 mμ).

In Fig. 4 curves are given for solutions containing an excess of metal ion (mole ratio of metal ion to ligand 4 : 1). In the pH range 2.20 to 2.70 the maximum of these curves moved from 604 to 610 mμ. The curve at pH 3.00 also exhibited the latter maximum. By increasing the pH to 3.20 and further to 3.50, the maximum shifted to lower wavelengths. In this series a third complex (absorption maximum 610 mμ) seemed to be present.

In the preparation of the two preceding series, the ligand solution was added to the

metal solution. For mixtures containing an excess of ligand, however, the order of addition proved to be of importance. In one series containing an excess of ligand (mole ratio of iron(III) to ligand, 1 : 4) and prepared as described above, the complexes present were identical with those detected in solutions of equimolar strength. In

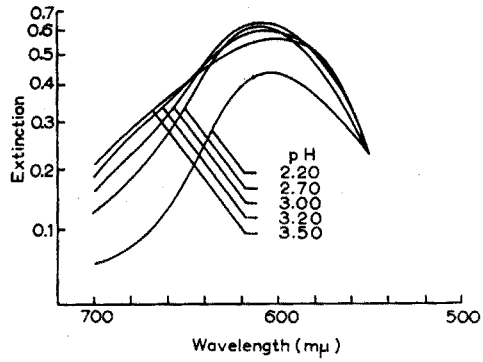

Fig. 4. Absorption curves at different pH values of solutions containing metal and ligand in the mole ratio 4 : 1. Ligand solution added to metal solution.

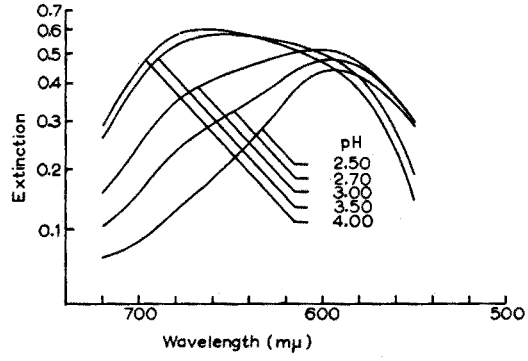

Fig. 5. Absorption curves at different pH values of solutions containing metal and ligand in the mole ratio 1 : 8.3. Metal solution added to ligand solution.

another series with an excess of ligand (mole ratio of metal ion to ligand, 1 : 8.3) and prepared by adding iron(III) solution to ligand solution, a new complex appeared, as seen from the curves in Fig. 5. At high acidities these curves exhibited a maximum at about 595 mμ, which was considered to be identical with that detected at low pH in the first series (see Fig. 3). With increasing pH values, the shoulder seen at about 680 mμ developed into a new maximum at 670 mμ; this maximum was believed to be due to a fourth complex.

The preliminary spectrophotometric investigations thus indicated the presence of four complexes.

The method of continuous variation

The method of continuous variation was employed to elucidate the composition of the two complexes which were detected by varying the pH of solutions containing the

reactants in equimolar amounts. Solutions for photometric measurement were prepared by pipetting into 100-ml volumetric flasks 10 ml of inert salt solution, a volume v_1 of standard iron(III) solution (10^{-3} M) and a volume v_2 of standard CAS(II) solution (10^{-3} M). For all mixtures $v_1 + v_2 = 0.80$ ml. After adjusting the pH to 3.40, the solutions were diluted to the mark with distilled water and measured at 570 mμ in 5.000-cm cells against a blank of distilled water. Similarly, a second series of solutions containing varying amounts of standard CAS(II) solution was measured under the conditions given above. On the basis of the latter series, the extinction data of the first series were corrected for the contribution from the amount of ligand added.

The measurements were then repeated on two new series, the only difference being that the pH was changed to 2.50.

The two curves of continuous variation (at pH 3.40 and 2.50) both exhibited a distinct maximum at the mole fraction 0.5, corresponding to the mole ratio 1 : 1.

The "straight-line method"

The straight-line method of ASMUS[6] is applicable for the determination of the composition of weak complexes. In the present investigation, the method was found suitable for the determination of the number of ligands of the complex predominating in solutions containing an excess of iron(III). In a previous paper[7], the present authors described the application of the method at pH 3.40 to the system iron(III)–chrome azurol S and found that under the conditions prescibed by ASMUS and in the presence of an excess of iron(III), the number of ligands was one. It was also demonstrated that the straight-line method did not distinguish between mono- and polynuclear species, and consequently the composition of the complex with one ligand could only be indicated by the formula Fe_xCh (the value of x being unknown). The straight-line method was also employed in the presence of a constant excess of ligand and varying amounts of iron(III). In the pH range 3.0–3.5 and by adding ligand solution to the solution of iron(III), the number of metal atoms was found to be two, corresponding to the formula Fe_2Ch_y (the value of y being unknown). The latter series of measurements were then repeated using the same order of addition, but changing the pH to 2.70. This time the number of metal atoms attached to the ligand was found to be one, corresponding to the formula $FeCh_z$ (the value of z being unknown).

CONDUCTOMETRIC TITRATIONS

Solutions of CAS(II) were titrated conductometrically with standard alkali in the absence and presence of iron(III); inert salt was not added. All conductance data were corrected for the change of volume during titration by using the formula

$$L = \frac{1}{R} \cdot \frac{V + v}{V}$$

in which L is the conductance, R the resistance measured, V the original volume and v the volume added.

In the titrations of the ligand, 25 ml of standard solution of CAS(II) were pipetted into a 150-ml beaker placed in the thermostat. After thermal equilibrium was reached, standard alkali was added in small increments and the corresponding resistances were recorded as soon as constant values were obtained.

Titrations in the presence of iron(III) were performed by pipetting 25-ml aliquots of standard iron(III) solution and standard CAS(II) solution and titrating as described above.

The titration curves are reproduced in Fig. 6; two scales are given on the abscissa, one indicating the volume (in ml) of alkali added and another giving the number of moles of base added per mole of metal present. The latter scale starts at the point (2.017 ml of base added) corresponding to the volume of alkali needed to neutralize the amount of acid originating from the iron solution. To facilitate comparison, the titration curve of the ligand solution also starts at this point.

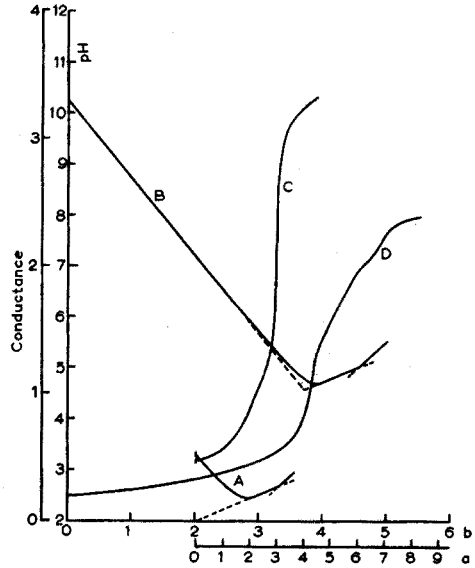

Fig. 6. Conductometric and potentiometric titration curves. Curve A: Conductometric curve of the ligand. Curve B: Conductometric curve of an equimolar mixture of the reactants. Curve C: Potentiometric curve of the ligand. Curve D: Potentiometric curve of an equimolar mixture of the reactants. b = ml of base added. a = moles of base added per mole of iron(III).

The ligand curve

Up to the first intersection point (about two equivalents of base added), the slope of the curve, compared with that of hydrochloric acid, indicated the neutralization of acid of medium strength. In the interval comprising 2–3 equivalents of base added, the conductance increased again, the slope of the curve indicating the neutralization of a weak acid. After the addition of three equivalents, the "alkali line" appeared. The proton of the strongly basic phenolic group (dissociation constant $10^{-11.81}$, was thus not titrated.

In the range up to the first intersection point the strongly acid protons were presumably neutralized. The third equivalent of base then neutralized the proton attached to the quinoid carbonyl group and adjacent carboxylic acid group.

The titration curve of the equimolar mixture of CAS(II) and iron(III)

The first, not clearly indicated, intersection point (about two ml of base added)

corresponded to the neutralization of acid originally present in the iron solution. The next intersection point appeared after the addition of four equivalents of alkali. The slope of the curve in this range differed only slightly from that of the preceding part, indicating that a relatively strong acid was neutralized. The complex formation thus seemed to liberate four hydrogen ions.

As mentioned above, steric considerations led to the preliminary assumption that the ligand was bidentate. According to this hypothesis, the formation of a monomer complex consisting of one iron(III) atom and one ligand molecule would only account for the liberation of two hydrogen ions. Other hydrogen ions may be released by the formation of hydroxo complexes, but owing to the considerably higher basicity of the aqua iron(III) ion, the curve would then be expected to exhibit an intersection point after the addition of three equivalents of base* and a slope corresponding to the "salt line" beyond this point.

The release of four equivalents of strong acid could only be explained by the presence of a dimer complex formed according to the following reaction:

$$Fe(H_2O)_6^{3+} + H_4Ch \rightleftharpoons \tfrac{1}{2}\, Fe_2(H_2O)_4Ch_2^{2-} + 4\, H_2O + 4\, H^+$$

In the range 4–6 equivalents of alkali added, the conductance increased again, probably owing to the formation of a hydroxo complex:

$$\tfrac{1}{2}\, Fe_2(H_2O)_4Ch_2^{2-} + 2\, OH^- \rightleftharpoons \tfrac{1}{2}\, Fe_2(OH)_4Ch_2^{6-} + 2\, H_2O$$

Fig. 6 (curve B and D) indicates that hydroxo complex formation started at pH about 3.5, *i.e.* before the second intersection point was reached.

After the third intersection point the "alkali line" was obtained.

Titrations of solutions containing iron(III) and ligand in the mole ratio 1 : 3 did not disclose the presence of any new species. The solution was found to contain a mixture of equal amounts of ligand and the dimer complex detected in the 1 : 1 mixture. This result was consistent with the information obtained from the photometric measurements, *viz.* that a comparatively large excess of one of the reactants was needed to change the composition of the complex predominating in 1 : 1 mixtures.

POTENTIOMETRIC TITRATIONS

Solutions of CAS(II) were also titrated potentiometrically with standard base in the absence and presence of iron(III).

In the former titrations 25 ml of standard CAS(II) solution, 25 ml of distilled water and 5.55 ml of potassium chloride solution were pipetted into the specially made titration vessel. After thermal equilibrium was reached, standard alkali was added in small increments and the pH was recorded as soon as constant values were obtained. The titration curve is reproduced in Fig. 6.

A solution containing metal ion and ligand in equimolar amounts was prepared by pipetting 25 ml of standard CAS(II) solution, 25 ml of standard iron(III) solution and 5.55 ml of inert salt solution. The mixture was titrated as described above, and the resulting curve is given in Fig. 6.

The ligand curve exhibited an inversion at about two equivalents and a distinct end-point at three equivalents of base added. This result was in accordance with that obtained by conductometric titration.

* Corresponding to two strongly acid protons originally present and one proton released by complex formation.

The titration curve of the equimolar mixture of ligand and iron(III) showed one inflection corresponding to the addition of slightly more than four equivalents of alkali. On further addition of base, the time needed to reach equilibrium conditions increased considerably. No precipitation took place during the titration at 25°, but at 60° iron(III) hydroxide precipitated at pH about 7.

The inflection obtained after the addition of about four equivalents of base was again explained by the formation of a dimer complex and the liberation of four hydrogen ions. This result therefore confirmed the conclusions reached on the basis of the conductometric investigations. (The reaction suggested for the formation of the dimer complex is given in the preceding section.)

As to the possible formation of hydroxo complexes, no conclusions could be reached from the 1 : 1 curve.

A titration curve was also plotted for a solution containing iron(III) and ligand in the mole ratio 1 : 2. This curve, however, did not exhibit any distinct end-points and is not reproduced in Fig. 6.

RESULTS AND DISCUSSION

Composition of the complexes

The following conclusions were drawn about the compositions of the different complexes detected in the system.

The conductometric and potentiometric measurements demonstrated that in solutions of pH 3–4 and containing iron(III) and ligand in equimolar amounts, a dimer complex of two iron(III) atoms and two ligand molecules predominated. When these results were compared with those of the preliminary photometric measurements, it was concluded that the dimer was identical with the complex exhibiting an absorption maximum at 582 mμ. Taking into consideration also the results obtained from the photometric measurements, it was further concluded that the dimer was identical with the complex designated Fe_2Ch_y (the real value of y thus being two).

When the pH of solutions containing the reactants in equimolar amounts was changed to below 3, the photometric measurements showed the presence of another complex with a different absorption maximum, but the same mole ratio (1 : 1). It was concluded that on increasing the acidity, the dimer split up into two identical monomers consisting of one iron(III) atom and one ligand molecule. The absorption maximum of the monomer was 596 mμ. Taking into consideration the results obtained by the straight-line method, it was concluded that the monomer was identical with the compound designated $FeCh_z$ (the real value of z being one).

As demonstrated by the conductometric titrations, the appearrance of hydroxo groups in the dimer became detectable at about pH 3.5. In hot solutions (60°) iron(III) hydroxide started to precipitate at pH about 7.

In the presence of comparatively large excesses of one of the reactants, other complexes were detected by spectrophotometry.

A compound different from the two previously found appeared in solutions of pH 2.5–3.5 containing an excess of iron(III). The use of the straight-line method showed that this complex contained one ligand molecule. These results pointed strongly to the composition Fe_2Ch^{2+}, one iron(III) ion being attached to each of the two bidentate groups of the ligand. Recalling the results obtained from the use of the straight-line method, it was assumed that Fe_2Ch^{2+} was identical with the complex previously desig-

nated Fe_xCh (the value of x thus being two). It was considered highly improbable that more than two iron(III) atoms would enter into complex formation with one ligand molecule.

For solutions containing an excess of ligand, the order of addition proved to be of importance. Mixtures in the pH range 3.0–3.5 prepared by adding an excess of ligand

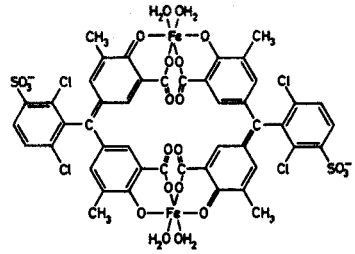

Fig. 7. Proposed structure of the dimer $[Fe(H_2O)_2]_2Ch_2^{2-}$.

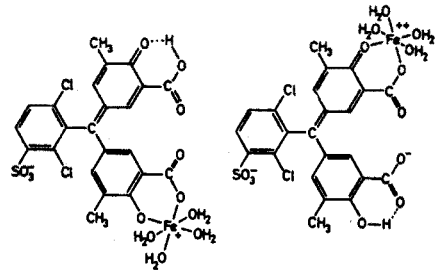

Fig. 8. Possible structures of the monomer $Fe(H_2O)_4HCh$.

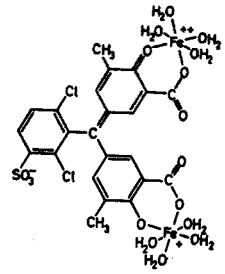

Fig. 9. Suggested structure of $[Fe(H_2O)_4]_2Ch^{2+}$.

solution to iron(III) solution contained mainly the dimer; inverting the order of addition gave different results. At high acidities, the monomer predominated, but at higher pH values a new complex appeared, probably with the composition $FeCh_2^{5-}$.

Thus, when a small amount of ligand solution was added to a solution of iron(III), the complex formed initially was probably Fe_2Ch^{2+}. Further addition of ligand resulted in the formation of the dimer $Fe_2Ch_2^{2-}$. Owing to the high stability of the dimer,

the addition of an excess of ligand did not cause the formation of any new complexes.

However, if a small amount of iron(III) solution were added to a solution of ligand, the formation of the complex $FeCh_2^{5-}$ would be favoured. It seems highly probable that this complex predominated in solutions of relatively high pH, and that it was transformed into the monomer by lowering the pH.

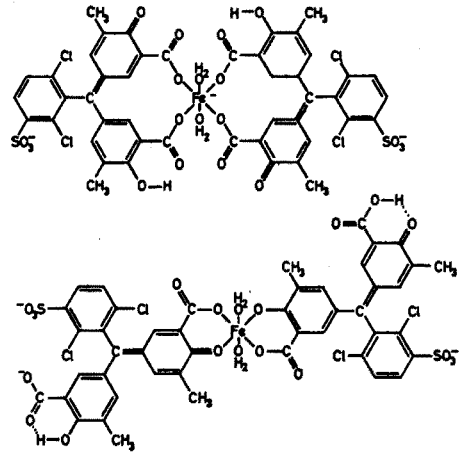

Fig. 10. Probable structures of $Fe(H_2O)_2H_2Ch_2^{3-}$

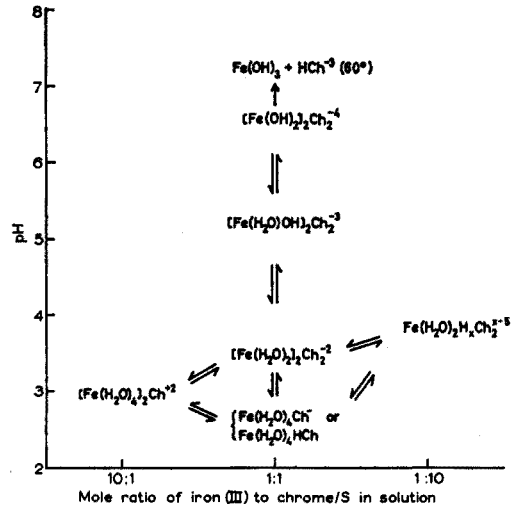

Fig. 11. A survey of the species detected in the system iron(III)–chrome azurol S as a function of pH and the mole ratio between the reactants.

The structures shown in Figs. 7, 8, 9, and 10 are suggested for the four complexes detected.

A survey of the complexes detected and their mutual relationship is shown in Fig. 11. In this figure the complexes are given roughly as a function of the mole ratio between the reactants and of pH.

Stability constants

From the experimental data on the different complexes it was also possible to obtain information on their stability.

The stability constant of the dimer $Fe_2Ch_2^{2-}$ could be calculated from the potentiometric titration curve of the equimolar mixture of the reactants. Many of the methods normally applied for calculating the stability from potentiometric data are based on the assumption that only mononuclear species are present. A method of calculation developed by SCHWARZENBACH[8] was found applicable, when the assumption was made that the dimer was the only complex present. By keeping a relatively high concentration of reactants and by selecting points on the titration curve in the pH interval 2.8–3.2, it was highly probable that the dimer predominated strongly.

Assuming that the dimer was formed by the reaction

$$2\ Fe^{3+} + 2\ Ch^{4-} \rightleftharpoons Fe_2Ch_2^{2-}$$

the absolute stability constant would be

$$k_{2,2} = \frac{[Fe_2Ch_2^{2-}]}{[Fe^{3+}]^2[Ch^{4-}]^2}$$

Owing to the protolytic reactions of Ch^{4-} and Fe^{3+}, a series of other equilibria had to be considered: the equilibria between the species Ch^{4-}, HCh^{3-}, H_2Ch^{2-}, H_3Ch^{-}, H_4Ch and H_5Ch^{+}, and further between the ions Fe^{3+}, $FeOH^{2+}$, $Fe(OH)_2^{+}$ and $Fe_2(OH)_2^{4+}$. (Water molecules are omitted in all formulae.) The dissociation constants of the ligand were determined in the present investigation, and the equilibrium constants ($\varkappa_{1,1} = 9.0 \cdot 10^{-4}$, $\varkappa_{1,2} = 4.9 \cdot 10^{-7}$ and $\varkappa_{2,2} = 1.22 \cdot 10^{-3}$) for the reactions of iron(III) with water molecules were taken from a paper by HEDSTROM[9]. The following equations were set up following the method of SCHWARZENBACH[8], but were extended to include the protolytic reactions of iron(III).

For each point on the potentiometric titration curve the following equations were valid when $[Fe]_t$, $[Ch]_t$ and $[H]_t$ represented the total concentration of iron(III), ligand and protons attached to the ligand, respectively, and when $[Fe]_t = [Ch]_t = C$

$$[Fe]_t = C = 2[Fe_2Ch_2^{2-}] + \alpha'[Fe^{3+}]$$

$$[Ch]_t = C = 2[Fe_2Ch_2^{2-}] + \alpha[H_3Ch^{-}]$$

$$[H]_t = Cg = \beta[H_3Ch^{-}] - \beta'[Fe^{3+}]$$

The partition coefficients were defined by:

$$\alpha = 1 + \frac{k_2}{[H^+]} + \frac{k_2k_3}{[H^+]^2} + \frac{k_2k_3k_4}{[H^+]^3}$$

$$\beta = 3 + 2\frac{k_2}{[H^+]} + \frac{k_2k_3}{[H^+]^2}$$

$$\alpha' = 1 + \frac{\varkappa_{1,1}}{[H^+]} + \frac{\varkappa_{1,2}}{[H^+]^2} + 2\frac{\varkappa_{2,2}}{[H^+]^2}[Fe^{3+}]$$

$$\beta' = \frac{\varkappa_{1,1}}{[H^+]} + 2\frac{\varkappa_{1,2}}{[H^+]^2} + 2\frac{\varkappa_{2,2}}{[H^+]^2}[Fe^{3+}]$$

For practical reasons, the constant of the equilibrium

$$2\,Fe^{3+} + 2\,H_3Ch^- \rightleftharpoons Fe_2Ch_2^{2-} + 6\,H^+$$

defined by

$$k'_{2,2} = \frac{[Fe_2Ch_2^{2-}][H^+]^6}{[Fe^{3+}]^2[H_3Ch^-]^2}$$

was first calculated, and then $k_{2,2}$ was found by

$$k_{2,2} = k'_{2,2} \cdot k_2^2 \cdot k_3^2 \cdot k_4^2$$

The different data extracted from the titration curve and those calculated are given in Table II.

TABLE II

DATA BASED ON POTENTIOMETRIC TITRATION FOR THE CALCULATION OF THE ABSOLUTE STABILITY CONSTANT OF THE DIMER $Fe_2Ch_2^{2-}$

v (in ml)	2.10	2.20	2.30	2.60	2.90	3.10
pH	2.84	2.86	2.88	2.97	3.11	3.22
a	0.196	0.432	0.667	1.375	2.083	2.554
C (10^{-3} M)	0.4337	0.4329	0.4322	0.4299	0.4277	0.4263
h (10^{-3} M)	1.445	1.380	1.318	1.072	0.776	0.603
g	0.472	0.380	0.283	0.131	0.102	0.032
α	4.944	5.132	5.329	6.345	8.426	10.64
β	10.84	11.21	11.60	13.59	17.67	21.97
α'	1.851	1.903	1.958	2.259	2.961	3.828
β'	1.085	1.160	1.240	1.686	2.775	4.177
$-\log k'_{2,2}$	2.35	2.04	1.59	1.64	1.89	1.39
$\log k_{2,2}$	35.19	35.50	35.95	35.90	35.65	36.15

The absolute stability constant of the dimer $Fe_2Ch_2^{2-}$ was found to be $\log k_{2,2} = 35.8$ at 25° and at an ionic strength of 0.10 M.

According to ASMUS[6], it is possible to determine stability constants of mononuclear complexes from the curves plotted in connections with the use of the straight-line method. For a dimer complex ($m = n = 2$) it follows[7] that the conditional constant at the hydrogen concentration $[H^+]$ may be calculated from the expression

$$k_{(2,2)H} = \frac{V^3 N}{4a_o v_o b_o^2}$$

(The designations used in this equation are those originally introduced by ASMUS[6].)

From a series of measurements at pH 3.50 using a constant excess of ligand and varying amounts of iron(III), a straight line was obtained for $n = 2$. (The experimental details of these measurements were given previously[7].) The straight line cuts the ordinate axis at a distance from the origin corresponding to $N = 6.2$ ml^{-2}. The other data were: $a = 10^{-3}$ M, $v_o = 1$ ml, $b_o = 10^{-3}$ M and $V = 100$ ml. From these data the conditional constant was calculated to be $1.55 \cdot 10^{15}$.

The absolute stability constant was defined by

$$k_{2,2} = k_{(2,2)H} \cdot \alpha_H^2 \cdot \alpha'^2$$

In this equation α_H was calulated from

$$\alpha_H = 1 + \frac{[H^+]}{k_4} + \frac{[H^+]^2}{k_3 k_4} + \frac{[H^+]^3}{k_2 k_3 k_4}$$

neglecting the concentrations of $[H_4Ch]$ and $[H_5Ch^+]$.

For the calculation of α', the equation given above was used.

With $\alpha_{pH\ 3.50} = 3.7 \cdot 10^9$ and $\alpha'_{pH\ 3.50} = 8.75$ the absolute stability constant at room temperature and in the presence of 0.10 M potassium chloride was found to be $\log k_{2,2} = 36.2$.

Finally, it was possible to establish the value of the stability constant of the dimer from curves of continuous variation.

Following the directions given by FOLEY AND ANDERSON[10], the conditional constant of the dimer was given by

$$k_{(2,2)H} = \frac{y}{(a_1 - 2y)^2(b_1 - 2y)^2} = \frac{y}{(a_2 - 2y)^2(b_2 - 2y)^2}$$

In Table III data resulting from two curves of continuous variation are given. The conditional constant at pH 3.40 was found to be $3.66 \cdot 10^{15}$. By calculations identical with those described above, $\alpha_{pH\ 3.40}$ and $\alpha'_{pH\ 3.40}$ were determined to be $5.88 \cdot 10^9$ and $6.33 \cdot 10^9$, respectively. The absolute constant was then $\log k_{2,2} = 36.7$.

TABLE III

DATA FOR THE CALCULATION OF THE CONDITIONAL CONSTANT OF THE DIMER $Fe_2Ch_2^{2-}$ AS DESCRIBED BY FOLEY AND ANDERSON[10]

pH = 3.40. Wavelength = 570 mμ. The cut through the curves was made at an extinction of 0.190

Total concentration (10^5) of reactants	*Mole fraction*	$a \cdot 10^5$	$b \cdot 10^5$	$y \cdot 10^5$
0.8	0.349	0.2795	0.5205	0.03375
1.0	0.198	0.1980	0.8020	

The curves of continuous variation and of the straight-line method may also be used to determine the stability constant of the monomer. As described above, the monomer was found to predominate in solutions of low pH containing an excess of ligand and prepared by adding the ligand solution to the ion(III) solution. In the following calculation of the absolute stability constant the monomer was assumed not to contain protons. In formulae the monomer is designated Fe(ChS).

The conditional constant of the monomer at the hydrogen concentration $[H^+]$ was defined by

$$k_{(1,1)H} = \frac{[Fe(ChS)]}{[Fe^{3+}][ChS]}$$

and the absolute stability constant by

$$k_{1,1} = k_{(1,1)H} \cdot \alpha_H \cdot \alpha'$$

the meaning of α_H and α' being given above.

When the straight-line method was applied to a series of solutions prepared as described above and maintained at pH 2.70, a straight line was obtained for $n = 1$. In the presence of a monomer ($m = n = 1$) the conditonal constant is given by

$$k_{(1,1)H} = \frac{VN}{b_o}$$

the designations used in this expression being identical to those introduced by ASMUS[6]. From the $n = 1$ curve, N was found to be 0.19 ml^{-1}. The other data were: $V = 100$ ml and $b_e = 10^{-3}$ M. $k_{(1,1)H}$ was calculated to be $1.9 \cdot 10^4$, and with $\alpha_{pH\ 2.70} = 1.8 \cdot 10^{11}$ and $\alpha'_{pH\ 2.70} = 1.57$, log $k_{1,1}$ was 15.7.

Curves of continuous variation were also plotted for series of solutions kept at pH 2.70 and prepared to contain mainly the monomer. A method described by SCHWARZENBACH[11] and based on the determination of the gradient at one of the end-points and the extinction at a certain mole fraction was employed.

On the basis of the experimental data in Table IV the conditional constant at pH 2.70 and the absolute constant were calculated to be $k_{(1,1)H} = 1.1 \cdot 10^4$ and log $k_{1,1} = 15.5$, the values of $\alpha_{pH\ 2.70}$ and $\alpha'_{pH\ 2,70}$ being $1.8 \cdot 10^{11}$ and 1.57, respectively.

TABLE IV

DATA FOR THE CALCULATION OF THE CONDITIONAL CONSTANT OF THE MONOMER (FeCh OR FeHCh) FROM A CURVE OF CONTINUOUS VARIATION[a]

pH = 2.70. Wavelength = 570 mμ. Total concentration of reactants $0.80 \cdot 10^{-5}$ M

Mole fraction	*Extinction*	*Conditional constant·10^{-4}*
0.575	0.114	1.10
0.425	0.110	1.16
0.312	0.090	1.02
0.250	0.077	1.03

[a] The gradient was found to be 0.36.

A reservation should be made at this point, *viz.* that the absolute stability constant of the monomer (recommended average value log $k_{1,1} = 15.6$) is valid only if the complex does not contain protons. As for the dimer, the value of the constant of the monomer was established at room temperature and at an ionic strength of 0.1 M.

As described above, solutions containing mainly Fe_2Ch^{2+} may be prepared by maintaining an excess of iron(III) and a pH value in the range 2.5–3.5 When the straight-line method was applied under such experimental conditions[7] a straight line was found for $n = 1$. For the present complex Fe_2Ch^{2+} ($m = 2$, $n = 1$) the conditional constant at the hydrogen concentration [H$^+$] may be calculated from the equation

$$k_{(2,1)H} = \frac{V^2N}{4a_o v_o b_o}$$

the designations being identical to those adopted by ASMUS[6].

As demonstrated by the authors[7], the straight line for $n = 1$ was practically unaffected by the value of m. In the present case, the value of N was derived from the set ($m = 1$, $n = 1$) and was found to be 1.08 ml^{-1}. The other data being $V = 100$ ml, $a_o = 10^{-3}$ M, $b_o = 10^{-3}$ M and $v_o = 4$ ml. At pH 3.40 the conditional constant was $6.7 \cdot 10^8$. The values of $\alpha_{pH\,3.40}$ and $\alpha'_{pH\,3.40}$ were $5.88 \cdot 10^9$ and 6.33, respectively. The absolute constant was then $\log k_{2,1} = 20.2$.

Table V summarizes the methods employed in the present section and the absolute stability constants calculated for three of the four complexes detected in the system.

TABLE V

METHODS EMPLOYED AND RESULTS OBTAINED FOR THE ABSOLUTE STABILITY CONSTANTS OF THREE IRON(III)–CHROME AZUROL S COMPLEXES

(Ionic strength = 0.1 M. Temperature = 20° ± 5°)

Complex	*Calculations based on*	*Absolute stability constant (log k)*	*Recommended average value (log k)*
$Fe_2Ch_2^{2-}$	Potentiometric titration	35.8	36.2 ± 1.1
	Straight-line method	36.2	
	Curves of continuous variation	36.7	
$FeCh^-$ or $FeHCh$	Straight-line method	15.7	15.6
	Curves of continuous variation	15.5	
Fe_2Ch^{2+}	Straight-line method	20.2	20.2

NOTE. In a paper recently published by SETH AND DEY (*J. Indian Chem. Soc.*, 39 (1962) 773), the complex formation at pH 3.0 of iron(III) with chrome azurol S is described. These authors report the presence of one complex with mole ratio 1 : 1. Reference should also be made to another, recent paper by SRIVASTAVA AND DEY (*J. Inorg. Nucl. Chem.*, 25 (1963) 217), who studied the system beryllium chrome azurol S, and found a complex with mole ratio 1 : 1.

SUMMARY

The complex formation of iron(III) with 3″-sulpho-2″,6″-dichloro-3,3′-dimethyl-4′-hydroxyfuchson-5,5′-dicarboxylic acid (chrome azurol S) was studied by spectrophotometric, conductometric and potentiometric methods. The pure tetrabasic acid of the ligand was prepared from the impure trisodium salt (commercially available), and the dissociation constants of the ligand were redetermined. At 20° ± 1° and in the presence of 0.10 M potassium chloride the dissociation constants were: $pk_1 < 0.0$, $pk_2 = 2.25 \pm 0.05$, $pk_3 = 4.71 \pm 0.03$ and $pk_4 = 11.81 \pm 0.03$.

In the pH range 2–4, four complexes were detected (the absolute stability constants at 20° ± 5° and at an ionic strength of 0.10 M are given in parentheses): a ring-formed dimer complex $[Fe(H_2O)_2]_2Ch_2^{2-}$ ($\log k_{2,2} = 36.2$); a monomer of composition $[Fe(H_2O)_4]HCh$ or $[Fe(H_2O)_4]Ch^-$ (the absolute stability constant was calculated as $\log k_{1,1} = 15.6$ for the latter composition); a complex $[Fe(H_2O)_4]_2Ch^{2+}$ ($\log k_{2,1} = 20.2$) and, finally, a complex of composition $[Fe(H_2O)_2]H_xCh_2^{x-5}$ (the value of x being unknown). In addition, hydroxo complexes of the dimer were formed at higher pH values.

RÉSUMÉ

Les auteurs ont étudié la formation de complexe du fer(III) avec le "chrome azurol S" (acide sulfo-3″,dichloro-2″,6″-diméthyl-3,3′-hydroxy-4′-fuchsone-dicarboxylique-5,5′ ou "H_4Ch"), par spectrophotométrie, conductométrie et potentiométrie. A un pH de 2 à 4, quatre complexes ont pu être décelés: $[Fe(H_2O)_2]_2Ch_2^{2-}$, $[Fe(H_2O)_4]HCh$ ou $[Fe(H_2O)_4]Ch^-$, $[Fe(H_2O)_4]_2Ch^{2+}$ et $[Fe(H_2O)_2]H_xCh_2^{x-5}$. Les constantes de stabilité sont indiquées. En plus, des hydroxocomplexes du dimère sont formés à des pH valeurs plus élevés.

ZUSAMMENFASSUNG

Die Komplexbildung von Eisen-(III) mit "Chrom azurol S" (3''-sulfo-2'',6''-dichloro-3,3'-dimethyl-4'-hydroxyfuchson-5,5'-dicarboxylsäure) wurde unter Anwendung von Spektrophotometrie, Konduktometrie und Potentiometrie untersucht. In einem pH Bereich von 2–4 konnten 4 Komplexe nachgewiesen werden: $[Fe(H_2O)_2]_2Ch_2^{2-}$; $[Fe(H_2O)_4]HCh$ oder $[Fe(H_2O)_4]Ch^-$, $[Fe(H_2O)_4]_2Ch^{2+}$ und $[Fe(H_2O)_2]H_xCh_2^{x-5}$. Die Stabilitätskonstanten werden angegeben. Bei höheren pH Werten werden ausserdem Hydroxokomplexe des Dimeren gebildet.

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GRAVIMETRIC SEPARATION OF TITANIUM AND ZIRCONIUM FROM NIOBIUM WITH PHENYLACETYLHYDROXAMIC ACID

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The presence of titanium and zirconium renders much more difficult the chemical separation of niobium and/or tantalum. These difficulties are attributed to similarities in the atomic and covalent radii, first ionisation potentials, electro-negativity, etc.

Several gravimetric methods have been suggested for the separation of zirconium from titanium[1-6] but niobium interferes with all of them. The barium fluorozirconate method[7] allows the determination of zirconium in presence of either of the elements niobium or titanium. Zirconium can be separated from niobium with salicylhydroxamic acid[8] but titanium interferes. The selenious acid method[9] gives a separation of niobium from titanium but zirconium interferes strongly.

In the present paper, a rapid and simple method for the separation of titanium and zirconium from niobium even when the first metals are present in large excess, is outlined. Titanium and zirconium, instead of being kept in solution with a complexing agent, are precipitated and niobium is held in solution.

It was mentioned in an earlier paper[10] that tantalum can be quantitatively precipitated from an oxalate solution with phenylacetylhydroxamic acid, leaving niobium in solution, and that titanium and zirconium interfere seriously. Further investigations showed that this reagent can be used for the quantitative precipitation of titanium and zirconium in presence of ammonium chloride at room temperature, provided that a large excess of reagent is present. Niobium up to a certain amount remains in solution. Based on these observations, a successful method was developed for the separation of titanium and zirconium from niobium. The optimum pH ranges for the quantitative precipitation of titanium and zirconium are respectively, 6.0–7.5 and 6.5–8.5, the effective range for the separation of both titanium and zirconium from niobium being 6.5–7.5.

A single precipitation yields a quite satisfactory separation when the ratio of $(TiO_2 + ZrO_2):Nb_2O_5$ varies from 10:1 to 1:1. Higher concentrations of niobium necessitate reprecipitation. Tantalum partially coprecipitates, and even double precipitation fails to prevent the contamination. Citrate, tartrate, lactate, glycerol and EDTA must be absent. A large excess of oxalate ion tends to keep titanium and zirconium partly in solution.

EXPERIMENTAL

Apparatus

The pH measurements were carried out with a Marconi pH-meter.

Reagents

All the chemicals used were of reagent quality.

Phenylacetylhydroxamic acid solution. A freshly prepared hot aqueous 2% (w/v) solution of phenylacetylhydroxamic acid was used as the precipitant.

Wash water. 100 ml of water containing 0.2 g of the reagent and 2.0 g of ammonium chloride and adjusted to pH 6.5–7.5.

Standard solutions. A standard solution of niobium oxalate was prepared by fusing a weighed amount of the Specpure pentoxide (Johnson, Matthey and Co., Ltd) with ten times its weight of potassium bisulphate and extracting the cool clear melt with a mixture of 5% (w/v) oxalic acid and 5% (v/v) sulphuric acid. After filtration and dilution with water to a known volume, the niobium content of the solution per ml was determined with N-benzoyl-N-phenylhydroxylamine[11,12].

Separate standard solutions of titanium and zirconium oxalates were prepared similarly by fusing their dioxides with bisulphate and then extracting. The titanium and zirconium contents of the solutions were determined as the dioxides after precipitation with ammonia.

Separation of titanium and zirconoum from niobium

Aliquots of the standard solutions of niobium, titanium and zirconium were mixed and diluted to 100 ml with water. After the addition of 5–6 g of ammonium chloride, a sufficient excess (20–25 times in excess of the total metal oxides present) of the reagent solution was added. The pH was then adjusted to 6.5–7.5. In this pH range, titanium and zirconium were quantitatively precipitated at room temperature while niobium remained in solution. The precipitate was then filtered through a filter paper, washed with wash water until free from sulphate, dried and ignited. The filtrate and washings were combined for the determination of niobium with N-benzoyl-N-phenylhydroxylamine[11,12].

RESULTS AND DISCUSSION

To study the effect of pH, an aliquot of the standard solution of titanium or zirconium oxalate was diluted to 100 ml with water, and 5–6 g of ammonium chloride and an

TABLE I

EFFECT OF pH ON THE SEPARATION OF ZIRCONIUM AND TITANIUM

pH	ZrO_2		TiO_2	
	Taken (mg)	*Found (mg)*	*Taken (mg)*	*Found (mg)*
5.5	12.6	5.0	9.3	9.0
6.0	12.6	8.2	8.6	8.5
6.5	12.6	12.6	8.6	8.6
7.0	12.6	12.6	8.6	8.6
7.5	12.6	12.6	8.6	8.4
8.0	12.6	12.6	8.6	7.4
8.5	12.6	12.4	8.6	6.0

aqueous 2% solution of the reagent were added. The pH was then adjusted to the required value. The precipitated complex was filtered, washed until free from sulphate, dried, ignited to oxide at 800°, cooled in a desiccator and weighed. The results are shown in Table I.

The oxalate concentration during the separation of titanium and/or zirconium should preferably be kept below 80 times the amount of total oxides.

The above procedure gives satisfactory separation by a single precipitation, when the ratio of $(TiO_2 + ZrO_2) : Nb_2O_5$ is about 10 : 1 to 1 : 1. For higher amounts of niobium, reprecipitation is necessary. Typical results are shown in Table II. Tantalum, citrate and tartrate seriously interfere. EDTA (disodium salt), glycerol, lactic acid and a very large excess of oxalate tend to keep titanium and zirconium partly in solution.

TABLE II

SEPARATION OF TITANIUM AND ZIRCONIUM FROM NIOBIUM

$Nb_2O_5 : TiO_2 : ZrO_2$ ratios	*$TiO_2 + ZrO_2$ taken (mg)*	*Nb_2O_5 taken (mg)*	*$TiO_2 + ZrO_2$ found (mg)*	*Nb_2O_5 found (mg)*
1 : 1 : 1	(5.6 + 6.4) = 12.0	6.4	12.0	6.4
2 : 1 : 1	(5.6 + 6.4) = 12.0	12.8	12.0	12.6
3 : 1 : 1	(5.6 + 6.4) = 12.0	19.2	13.6[a], 12.0[b]	19.0
1 : 4 : 1	(22.4 + 6.4) = 28.8	6.4	28.8	6.4
1 : 7 : 1	(43.2 + 6.4) = 49.6	6.4	49.4	6.4
1 : 1 : 7	(5.6 + 38.4) = 44.0	6.4	43.8	6.4
1 : 1 : 9	(5.6 + 51.2) = 56.8	6.4	56.6	6.4
1 : 10 : 0	(43.2 + 0.0) = 43.2	4.0	43.0	4.2

[a] Single precipitation.
[b] Double precipitation.

SUMMARY

Phenylacetylhydroxamic acid is used to separate titanium and zirconium from niobium in an oxalate medium at pH 6.5–7.5 in presence of ammonium chloride at room temperature. The method is accurate when the ratio of $(TiO_2 + ZrO_2) : Nb_2O_5$ is 10 : 1 to 1 : 1; when the niobium concentration is higher, reprecipitation is necessary. Tantalum, citrate, tartrate, lactic acid, EDTA, and a large excess of oxalate interfere.

RÉSUMÉ

Les auteurs ont utilisé l'acide phénylacétylhydroxamique pour la séparation du titane et du zirconium d'avec le niobium, en milieu oxalique (pH 6.5–7.5). La méthode est précise pour des rapports en $(TiO_2 + ZrO_2) : Nb_2O_5$ de 10 : 1 à 1 : 1. Pour des concentrations plus forte en niobium, une reprécipitation est nécessaire. Gênent: tantale, citrate, tartrate, acide lactique, EDTA et un grand excès d'oxalate.

ZUSAMMENFASSUNG

Zur Trennung des Titans und Zirkoniums von Niob wird Phenyl-acetylhydroxamsäure in Gegenwart von Oxalsäure verwendet. Die Methode ist genau bei Mengenverhältnissen $(TiO_2 + ZrO_2) : Nb_2O_5$ = 10 : 1 bis 1 : 1. Bei höherem Gehalt an Niob ist eine Doppelfällung notwendig. Tantal, Citrat, Tartrat, Milchsäure, EDTA und zu grosser Ueberschuss an Oxalat stören.

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Anal. Chim. Acta, 29 (1963) 168–171

COMPLEX FORMATION AND FLUORESCENCE

PART. I. COMPLEXES OF 8-HYDROXYQUINOLINE-5-SULFONIC ACID*

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(Received February 22nd, 1963)

While the fluorescence of 8-hydroxyquinoline compounds has been investigated rather thoroughly[1,2] the possibility of the use of the corresponding water-soluble complexes of 8-hydroxyquinoline-5-sulfonic acid has been more or less ignored. BAILAR AND LIU[3] have noted that the cadmium and zinc salts fluoresce in solution. MACDOUGALL[4] has used the magnesium compound as a means of determining small amounts of a pesticide. HOLLINGSHEAD[1] mentions the work of BAILAR AND LIU[3] but makes no mention of the fluorescence.

The acid itself has almost no fluorescence in dilute water solutions at any pH between 1 and 12, but it is best to run blanks to allow for any impurities.

EXPERIMENTAL

All fluorescence measurements were made using the Farrand Spectrofluorimeter, the DC Xenon arc lamp being used in connection with a Sola power pack. The phototube was the photomultiplier tube 1P28, which was connected through an RCA microammeter to a Varian strip recorder, type G-10. Since the maxima in the emission frequencies of these complexes lie in the blue-green region of the spectrum, a 440 mμ cut off filter was used after the solution cuvette.

UDENFRIEND[5] and PARKER AND REES[6,7] have discussed the instrumental errors involved in fluorescence analysis. In the work reported here, since it was desired to study the effects of pH change and complex formation, the instrument was used at constant slit width and the activation wave length was kept constant at 360 mμ. No correction for phototube response was made in using the fluorescence intensities, since all of the complexes involved had maxima at about the same wave length.

The solutions were prepared by dilution of more concentrated solutions, which had been made up by mixing standardized solutions of the inorganic salts with freshly prepared standard solutions of 8-hydroxyquinoline-5-sulfonic acid dissolved in the minimum amount of sodium hydroxide required for solution before dilution in a volumetric flask. Inorganic salts used were of reagent grade, and the 8-hydroxyquinoline-5-sulfonic acid was obtained from the Eastman Kodak Company.

DISCUSSION

Since the oxine group has a basic nitrogen, there are three possible reacting groups involved with 8-hydroxyquinoline-5-sulfonic acid ($OxSO_3H_2$): $OxSO_3H_3^+$, $OxSO_3H_2$,

* Part III, see *Anal. Chim. Acta*, 29 (1963) 85.

and $OxSO_3H^-$, all of which may dissociate. Two types of complexes are given in the Tables of "Stability Constants"[8], ML and ML_2^{2-}, indicating that the phenolic hydrogen is displaced in forming the complexes. Using the zinc compound as an example of the method of approach, a series of equations develops as follows:

$$Zn^{2+} + OxSO_3H_3^+ \rightarrow Zn(OxSO_3) + 3\,H^+ \quad (1)$$

$$K' = \frac{(ZnOxSO_3)(H^+)^3}{(Zn^{2+})(OxSO_3H_3^+)} = K_1 K_{a1} K_{a2} K_{a3} \quad (2)$$

$$ZnOxSO_3 + OxSO_3H_3^+ \rightarrow Zn(OxSO_3)_2^{2-} + 3\,H^+ \quad (3)$$

$$K'' = \frac{(Zn(OxSO_3)_2^{2-})(H^+)^3}{(ZnOxSO_3)(OxSO_3H_3^+)} = K_2 K_{a1} K_{a2} K_{a3} \quad (4)$$

The values for the constants for the complexes studied in this paper were taken from the "Stability Constants"[8].

The $ZnOxSO_3$ is apparently a zwitterion having a positive charge on the zinc and a negative charge on the sulfonic group.

By a series of approximations, the above equation may be used to determine the distribution of the species in a solution containing zinc(II) and $OxSO_3H_2$ at any pH. Such a distribution is shown in Fig. 1, and has been calculated for the other ions discussed in this paper.

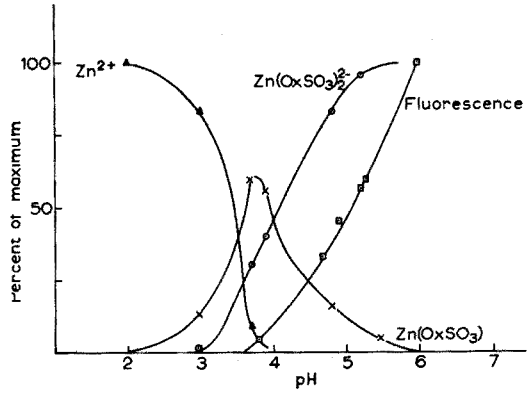

Fig. 1. Variation of composition and fluorescence of $Zn(OxSO_3)_2$ solutions with pH.

In Fig. 1, there is also shown the variation of fluorescence intensity with pH, of solutions containing zinc(II) and $OxSO_3H_2$, referred to the maximum intensity of fluorescence of the zinc complex (at pH 8). WILLIAMS has used this type of plot to show that there are two fluorescing species in the case of anthranilic acid[9]. In the present case, it can be seen by inspection of Figs. 1 and 2 that the fluorescence is entirely due to the second complex.

In Fig. 2, there is shown the variation of formation of the second complex with $OxSO_3^{2-}$ with pH for Cd, Zn and Mg along with the variation of fluorescence intensity of the same solutions with pH. MACDOUGALL[4] has investigated the change in fluorescense intensity of the Mg complex with pH. He reports that it increases to a maximum

and then decreases, probably owing to the formation either of hydroxy complexes or of the hydroxide. The present writer has confirmed this effect with the magnesium complexes, and has found a similar decrease in fluorescence intensity at high pH values for the Zn and Cd complexes. The complexes of the last two are stable at lower pH values, however, as can be seen from Fig. 2, and hence have a much wider pH range over which they can be used, than the magnesium complex.

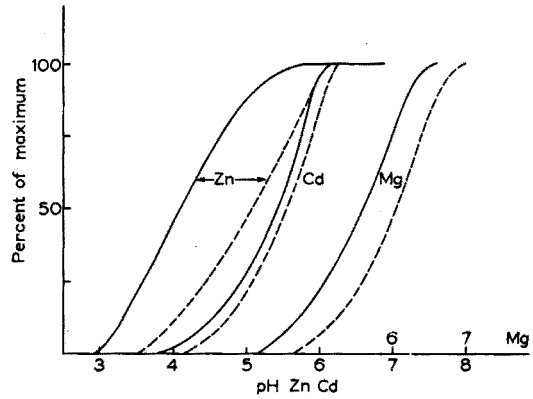

Fig. 2. Range of pH over which fluorescence increases for Zn, Cd and Mg–$OxSO_3$ salts. —— Formation of second complex; ---- Fluorescence increase.

From the analytical standpoint, solutions containing $OxSO_3H_2$ have the advantage that the solutions used (cation and $OxSO_3H_2$) have no fluorescence of their own. It is possible, therefore, to use graphs of the type shown (Figs. 1 and 2) to choose conditions for an analysis involving the measurement of fluorescence intensity. It is true that this intensity is affected somewhat by excess $OxSO_3H_2$ (Fig. 3), but this can be allowed for by using a standard amount of the complexing agent greatly in excess of that needed for formation of the complex. Al, Ba, Sr, Ca, Be, Cu, Ni, Co ions have also been tested for fluorescence in solutions of $OxSO_3H_2$. Results for these cations follow the re-

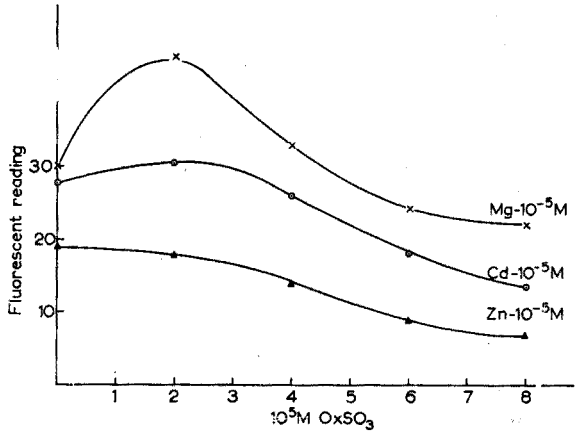

Fig. 3. Fluorescence quenching by excess $OxSO_3H_2$ added to solutions of 2 : 1 complex.

sults of STEVENS[2], who found that complexes formed by 8-quinolinol did not fluoresce when the cation involved was one of the transition metal ions.

In using these graphs for analytical work, there are two sets of conditions that may be chosen. The first is to select a pH such that one cation complex fluoresces and only one. This is relatively simple when the cations involved include only one that forms a fluorescent complex. Combinations of this type are shown in Fig. 4, in which a 10-fold excess of the complexing ligand was used, along with a pH such that the complex was all in the ML_2 form.

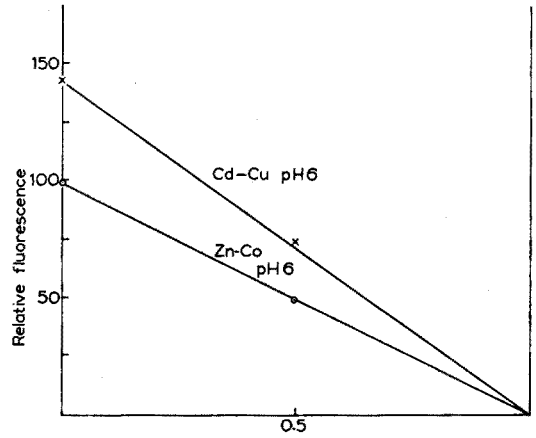

Fig. 4. Fluorescence of mixtures of $OxSO_3$ salts in presence of large excess $OxSO_3$. Constant total salt concentration. One fluorescent salt only.

If more than one cation forms a fluorescent complex, it is possible to set conditions such that only one of these will fluoresce. Thus, Fig. 2 indicates that it should be possible to determine zinc in the presence of magnesium at a pH of 4.5. It is true, however, that an increase in cation concentration will move the curve towards a lower pH, and

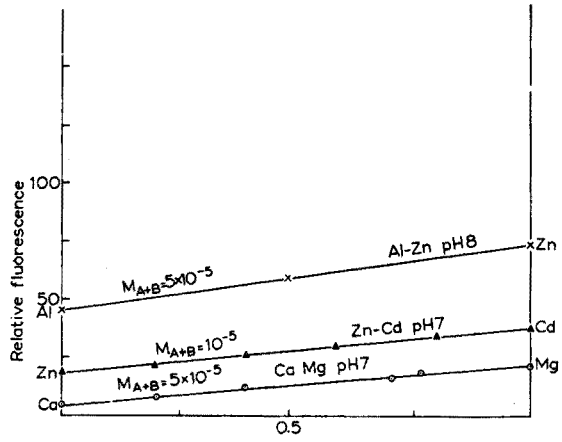

Fig. 5. Fluorescence of mixtures of $OxSO_3$ salts in presence of large excess $OxSO_3$. Constant total salt concentration. Both salts fluoresce.

therefore, an increase in the amount of magnesium accompanied by a decrease in the amount of zinc could cause overlapping and prevent the determination. It is evident, nevertheless, that it is possible to set conditions for such combinations in which the cation present in much smaller amount has the lower stability constant for its complex. Thus the effect of traces could be avoided.

By setting conditions such that both cation complexes will fluoresce at maximum intensity, it is possible to determine both cations, providing that the two fluorescence intensities are additive. This summation of fluorescence intensity is analogous to the additivity of solution densities in spectrophotometry. Just as the additivity of absorbances is possible only if both colored substances follow Beer's law, the additivity of fluorescence intensities is possible only if the solutions are of such low concentration that fluorescence intensity is proportional to concentration. In this case,

$$F_A + F_B = k_A C_A + k_B C_B.$$

Some combinations of this type are shown in Fig. 5, conditions being indicated on the graph.

The method used in setting up the graphs of Figs. 4 and 5 may be summarized as follows, to analyze for two ions A and B.

Dilute the sample to exactly 100 ml (or any convenient volume). Take an aliquot portion and analyze for both ions (M_{A+B}). This may be done by any method which will determine both, but an EDTA titration seems best for most cations. Take a second aliquot for dilution to 10^{-5} or 10^{-6} M in a 100-ml volumetric flask. To this sample in the volumetric flask add a definite volume of $OxSO_3H_2$ solution (0.01 M and at the pH to be used), which will be in large excess over what is needed to complex both ions. Add buffer of the desired pH (solution 1). Prepare a solution of ion A of the molarity used in solution 1 for the sum of the two ions, including in the solution the same amount of $OxSO_3H_2$ and the same amount of buffer as in solution 1 (solution 2). Prepare a similar solution for ion B (solution 3). Obtain fluorescence intensity curves for the solutions 1, 2 and 3. Finally calculate the amount of the ion present, assuming that ion A complex has a lower fluorescence at the peak than B.

$$\text{Milligrams of B} = \frac{F_{mix} - F_A}{F_B - F_A} (M_{A+B}) \text{ (Atomic wt. A) (ml of original sample)}$$

If the blank is assumed to be the same for all solutions, it may be ignored since only differences are used. In cases in which one of the two ions does not cause fluorescence (such as copper), the value obtained with such a solution may be considered to be the blank.

If it is desired to prepare a graph to be used as a permanent reference, the fluorescence may be referred to some reproducible standard, such as quinine sulfate.

This investigation was supported by research grant No. 8005 from the National Institute of Health, Public Health Service.

SUMMARY

The fluorescence of solutions of cation complexes with 8-hydroxyquinoline-5-sulfonic acid has been studied. The complexes formed with transition elements do not fluoresce. The additivity of the fluorescence at certain pH values has been used to indicate a new generally applicable analytical method for mixtures of cations which have similar reactions. The method is especially applicable to small or very dilute samples.

RÉSUMÉ

L'auteur a fait une étude sur la fluorescence des solutions de complexes de cations avec la sulfo-5-hydroxy-8-quinoléine. Les complexes formés avec les éléments de transition ne fluorescent pas. L'influence du pH a été examinée et permet d'envisager une nouvelle méthode analytique applicable à des mélanges de cations ayant des réactions similaires. Ce procédé convient spécialement pour de faibles quantités d'échantillon ou pour des échantillons très dilués.

ZUSAMMENFASSUNG

Die Fluoreszenz der Lösungen von Kationen-Komplexen mit 8-Hydroxychinolin-5-sulfosäure wurde untersucht. Komplexe, die mit den Übergangselementen gebildet werden, fluoreszieren nicht. Die Additivität der Fluoreszenz bei bestimmten pH-Werten wurde für eine neue, analytische Methode benutzt. Sie ist dann allgemein anwendbar, wenn Mischungen von Kationen mit ähnlichen Reaktionen vorliegen. Die Methode eignet sich besonders für kleine oder sehr verdünnte Proben.

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COMPLEX FORMATION AND FLUORESCENCE

PART II. THE USE OF 8-HYDROXYQUINOLINE-5-SULFONIC* ACID AS AN INDICATOR

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The first paper of this series[1] discussed the use of 8-hydroxyquinoline-5-sulfonic acid as a complexing agent, and the effect of pH on the fluorescence of its complexes. Curves were given showing that there is a rather sudden increase in intensity of fluorescence after a certain pH is reached, the pH depending on the particular complex and also on its concentration. The pH range over which the fluorescence develops is usually 1 to 1.5 pH units, there being no fluorescence at the lower pH value and fluorescence at the higher value. There is usually a range of pH over which the high fluorescence intensity is maintained, above which it falls off, probably due to formation of hydroxy complexes[2].

TABLE I

COMPLEXOMETRIC TITRATIONS

Ion	$M \cdot 10^{-4}$	*Volumes of CDTA*	*pH*
Mg	10	0.700; 0.720; 0.705	7
Mg	5	0.700; 0.710; 0.710	7
Cd	5		
Cd	10	0.710; 0.690; 0.710	7
Zn	10	0.695; 0.715; 0.720	7
Mg	5	0.700; 0.700; 0.705	7
Zn	5		
Be	10	No end-point; pale fluorescence	
Be	5	0.350; 0.355	7
Zn	5		
Be	5	0.360; 0.350	4
Zn	5		
Be	5	0.370; 0.360	7
Cd	5		
Be	5	0.350; 0.355	7
Mg	5		
Cu	10	No fluorescence	
Cu	5		
Cd	5	N.G.	7
Co	10	No fluorescence	7
Co	5	0.33 A; 0.83 B	7
Zn	5		

* Part III, see *Anal. Chim. Acta*, 29 (1963) 85.

SILL[3] has discussed the use of EDTA to prevent interference in fluorescence measurements. HOWERTON AND WASILEWSKI have discussed fluorescent titrations[4] and WILKINS[5] has discussed fluorescent indicators related to fluorescein complexone, as have KÖRBL AND SVOBODA[6]. It would seem that by choice of the correct pH it should be possible to use 8-hydroxyquinoline-5-sulfonic acid as a fluorescent indicator in titrations involving complexing agents of the EDTA type. To carry out such a titration, three restricting conditions must be observed.

(*1*) The complexing agent used for titration must form a much stronger complex than the complex of the cation with 8-hydroxyquinoline-5-sulfonic acid.

(*2*) The pH chosen must be such that fluorescence will occur.

(*3*) The concentration of the titrating agent should be great enough to cause a complete destruction of the small amount of fluorescing complex at the end-point.

With these restrictions in mind, a series of titrations were carried out, with results shown in Table I.

EXPERIMENTAL

Reagents

Inorganic salt solutions were prepared from reagent grade chemicals.

A 0.0140 *M* solution of 1,2-diaminocyclohexane-N,N'-tetraacetic acid (CDTA) was used as titrant; 10^{-3} *M* salt solutions were prepared. 8-Hydroxyquinoline-5-sulfonic acid solution (0.01 *M*) was prepared by dissolving the weighed amount of the compound in a minimum of sodium hydroxide, and diluting with water.

The CDTA was chosen instead of EDTA because it forms more stable complexes above pH 4, and the pH range for maximum intensity of fluorescence is between 4 and 8 for most complexes. The CDTA was obtained from Geigy.

Complexometric titrations

In carrying out the titrations, 10-ml samples were used. Five drops of 0.01 *M* 8-hydroxyquinoline-5-sulfonic acid were used in each sample, and the solution was buffered to the desired pH by adding 10 ml of buffer solution. Mixtures were made by using 5 ml of each salt solution. Results of the titrations are shown in Table I. Transition metal cations do not give fluorescent complexes.

In considering the results recorded in Table I, it becomes apparent that the results are dependent upon the extent of complex formation with the titrating agent, rather than dependent on the formation of the fluorescent complex, which is simply acting as indicator.

In cases in which both ions form fluorescent complexes with the indicator, and both form complexes with the titrating agent, the results are in very good agreement. In other cases, one ion (*e.g.* Be) apparently is not complexed by the titrating agent, with the result that the fluorescence disappears when the fluorescing ion-complex is destroyed. Actually the Be–$OxSO_3$ complex does have some slight fluorescence but there is a pronounced change at the volumes indicated in the Table. In the last case, the titration of the two ions overlaps, because their complexes are of about the same stability. Hence there is either complete interference (Cu–Cd) or partial interference. In the case of Co–Zn mixture, there was no fluorescence until volume A was reached, and the fluorescence disappeared at volume B. There was probably some interference owing

to absorption by the red cobalt solution also. Table I is a typical set of data and indicates the spread of results to be expected in check runs.

In considering these results, it must be remembered that the end-points were determined visually. Results reported in the previous paper on the fluorescence of mixtures[1] show that by using a spectrofluorimeter it is possible to show fluorescence of mixtures of Cd and Cu, and Co and Zn, and to use this fluorescence for the detection of the ions in the presence of each other.

Acid-base titrations

Since, as noted above, the appearance of fluorescence takes place over a very short pH range, there is the possibility that the complexes might be used as acid-base indicators, for titrations with end-points at the correct pH. It was decided to test this by titrating ammonia with hydrochloric acid solutions. When the pH fluorescence curves are consulted[1] it is found that the fluorescing cadmium complex forms over almost the exact pH range for this titration, while the Mg complex is formed at a more basic range, and the Zn complex forms in a more acidic solution. Freshly prepared 0.01 *M* ammonia was titrated with 0.0100 *M* hydrochloric acid, using a 10-ml microburet. Ten drops of freshly prepared solutions of 0.001 *M* $Cd(OxSO_3)_2$, $Zn(OxSO_3)_2$ and $Mg(OxSO_3)_2$ were used as indicators. These solutions were slightly basic as originally prepared, but were brought to a pH of 7 before being used as indicators. Checks were run using methyl red. Results are shown in Table II. Titrations were done under the mercury lamp.

TABLE II

$OxSO_3H_2$ COMPLEXES AS ACID-BASE INDICATORS

Indicator Methyl red	*Volume of HCl (ml) 6.480 and 6.460*	*Blank (ml) 0.020*
$Cd(OxSO_3)_2$	6.450; 6.500; 6.495; 6.500	0.040
$Mg(OxSO_3)_2$	6.000; 6.150; 6.025; 6.250	0.030
$Zn(OxSO_3)_2$	6.615; 6.620; 6.620; 6.615	0.130

As concluded from the graphs mentioned above, the Mg complex changed before the correct end-point had been reached and the Zn complex just after it. It is noted, however, that the zinc end-point is in good agreement when the blank is subtracted.

This investigation was supported by Research Grant No. 8005 from the National Institutes of Health, Public Health Service.

SUMMARY

The use of fluorescent 8-hydroxyquinoline-5-sulfonic acid complexes in titrations was examined. The disappearance of the fluorescence of these metal complexes, upon titration with a stronger, nonfluorescing complexing agent, can be used to indicate endpoints in both complexometric titrations and acid-base titrations.

RÉSUMÉ

L'auteur a examiné les possibilités d'emploi de la sulfo-5-hydroxy-8-quinoléine, comme indicateur en volumétrie. La fluorescence de ses complexes métalliques peut disparaître par addition d'un

réactif complexant plus fort et non-fluorescent. On pourra ainsi déceler le point visal de titrages complexométriques et alcali-acidimétriques.

ZUSAMMENFASSUNG

Es wird die Anwendbarkeit von fluoreszierenden Komplexen der 8-Hydroxychinolin-5-sulfosäure als Indikator für die Massanalyse geprüft. Die Abnahme der Fluoreszenz dieser Metallkomplexe bei der Titration mit stärkerem nichtfluoreszierenden Komplexreagenzien kann zur Anzeige des Endpunktes benutzt werden sowohl bei komplexometrischen als auch bei Säure-Basen-Titrationen.

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STUDIEN ÜBER DIE NAPHTHORESORCIN-REAKTION VON TOLLENS

I. GRUNDSÄTZLICHES ZUR AUSFÜHRUNG UND BEURTEILUNG DER REAKTION

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(Eingegangen den 1. Januar, 1963)

Die meistbenutzte Reaktion zur Erkennung wie auch zur mengenmässigen Erfassung von Uronsäuren ist die von BERNHARD TOLLENS vor über fünfzig Jahren aufgefundene und nach ihm benannte Naphthoresorcin*-Reaktion[1,2]. Sie ist in der Folgezeit sehr viel untersucht und abgeändert worden mit dem Ziel, die Probe empfindlicher und spezifischer zu gestalten (siehe[3,31]). Wesentliche Verbresserungen stammen besonders von CARL NEUBERG und seinen Schülern.

Natürlich hat es nicht an Versuchen gefehlt, die NR-Reaktion für quantitative Zwecke auszugestalten. Schon im Jahre 1909 zeigte C. TOLLENS, dass mit ihrer Hilfe eine ungefähre Bestimmung der Glukuronsäure im Harn möglich ist[5]. Diese Erfahrung blieb unverständlicherweise volle 25 Jahre unbeachtet! Erst 1935 wurde sie von SALT bestätigt[6]; seine Bemühungen um eine genaue Methode waren jedoch erfolglos. Dasselbe gilt für Versuche von VON FÜRTH UND PESCHEK, die auf der Suche nach einer Mikrobestimmung für Uronsäuren die NR-Reaktion wegen ihrer „Launenhaftigkeit" alsbald angeschlossen haben[7]. FLORKIN konnte 1937 erstmals über günstige Ergebnisse bei reinen Uronsäuren bzw. ihren Salzen berichten[8]; und wiederum ein Jahr später veröffentlichten MAUGHAN *et al.* ein für quantitative Reihenanalysen brauchbares Verfahren unter Verwendung einer wässrigen NR-Lösung[9].

Nun setzt eine Fülle von Arbeiten ein [10–45], die sich mit der kolorimetrischen Bestimmung von Uronsäuren teils in reinen Lösungen, teils in biologischen Flüssigkeiten befassen. Sie sollen hier jedoch nicht im einzelnen besprochen werden.

Schwierigkeiten und Fehlerquellen

Immer wieder wird in der biochemischen Literatur Klage geführt, dass die NR-Reaktion einen „launischen Charakter" aufweist, vor allem bei unzweifelhafter Anwesenheit von Uronsäuren häufig negativ ausfällt[3,4,7,31,46]. Die Gründe hierfür sind verschiedener Art. Wiederum hat sich NEUBERG um ihre Aufhellung besonders verdient gemacht.

(*I*) Versagen der qualitativen Reaktion trotz Anwesenheit von Uronsäuren bzw. zu niedrige Werte bei der Bestimmung:

(*1*) *Mangelhafte Hydrolyse von Uronosiden und Polyuronosiden.* Viele glykosidische Bindungen, an denen Uronsäuren beteiligt sind, werden mit Säure verhaltnismässig schwer aufgespalten. Dies gilt insbesondere für die hochmolekularen Stoffe Pektin,

* Im folgenden stets abgekürtzt als NR.

Pektinsäure, Alginsäure, Hyaluronsäure, Heparin, *usw.* Einer Verschärfung der Hydrolysebedingungen steht aber der Umstand entgegen, dass Uronsäuren bei Gegenwart von Mineralsäure in der Hitze CO_2-Abspaltung erfahren, wodurch sich kleine Mengen dem Nachweis entziehen können. Niedermolekulare Glykoside dürften aber in jedem Falle für die qualitative Probe genügend schnell aufgespalten werden, obwohl im einzelnen beträchtliche Unterschiede bestehen (*vgl.*[52]); bei quantitativen Bestimmungen ist auf schwer hydrolysierbares Material unbedingt zu achten!

HEYNS UND KELCH[30] verfolgten den Hydrolysenverlauf von Phenol-β-D-glukuronosid unter den Bedingungen ihrer quantitativen Methodik (4 *N* HCl; 100°; 30 Min Erhitzungszeit) und stellten durch Bestimmung des freigemachten Phenols fest, dass nur 89.6% des eingesetzten Uronosids aufgespalten war. Ähnliche Erfahrungen sammelten HANSON *et al.*[18] mit *o*-Aminophenol-β-D-glukuronosid.

(*2*) *Decarboxylierung.* Beim Erhitzen mit starken Säuren auf 100 bis 120° werden die Uronsäuren decarboxyliert und gehen in Pentosen über, worauf TOLLENS UND LEFÈVRE bereits 1907 ein recht brauchbares Bestimmungsverfahren aufgebaut haben. Unbegreiflicherweise blieb diese Fehlerquelle lange Zeit unbeachtet, bis HEYNS UND KELCH[30] neuerlich zeigten, dass unter den üblichen quantitativen Reaktionsbedingungen (4 *N* HCl; 100°) nach halbstündigem Erhitzen ohne NR nur noch 86% der ursprünglichen Glukuronsäuremenge wiedergefunden werden! Wenn auch der Verlust durch Decarboxylierung bei der Anstellung der NR-Reaktion nicht dieses Ausmass erreicht, indem ja durch Farbstoffbildung laufend die (von Anfang an vorhandene oder im Hydrolysenverlauf neu gebildete) freie Uronsäure festgelegt wird, so können doch die Ergebnisse beträchtlich verfälscht werden. TEAGUE[34] vertritt deshalb *u.E.* mit Recht die Ansicht, dass alle Bestimmungen von Glukuronosiden, bei denen Glukuron und nicht das betr. Uronosid selbst zur Aufstellung der Eichkurve gedient hat, keine genauen Werte ergeben haben. In ähnlichem Sinne äusserten sich auch DEICHMANN UND DIERKER[21].

(*3*) *Beschlagnahme des NR durch Nebenreaktionen und Begleitsubstanzen.* (*a*) *Reaktion mit anderen Carbonylverbindungen.* Bei Anwesenheit Carbonylgruppen-haltiger Begleitstoffe kann das NR-Reagens in mehr oder weniger grossem Ausmass abgefangen werden, sodass es für die Umsetzung mit den Uronsäuren nicht mehr in ausreichendem Masse zur Verfügung steht. (*b*) *Selbstkondensation des NR.* Kleine Mengen NR werden durch die Bildung eines intensiv grün fluoreszierenden Farbstoffes (*vgl.* Nebenreaktionen, S. 187) der gewünschten Umsetzung entzogen. Sehr wahrscheinlich handelt es sich um eine Selbstkondensation unter den Bedingungen der TOLLENS-Reaktion, die naturgemäss nicht verhindert werden kann. (*c*) *Farbstoffbildung im Licht.* Eine Verringerung der NR-Konzentration als Folge von Lichteinwirkung wird dadurch mehr als wettgemacht, dass der hierbei entstehende rote Farbstoff (*vgl.* Nebenreaktionen, S. 186) im Messbereich um 570 mμ stark absorbiert. Es wird sich also der Einfluss im entgegengesetzten Sinne wie unter (*a*) und (*b*) geltend machen.

(*4*) *Verharzung bzw. Adsorption des gebildeten Farbstoffes.* Der aus NR und Uronsäure entstehende Farbstoff ist in wässrigem Medium unlöslich und scheidet sich gewöhnlich in Form von graublauen Flocken ab. Manchmal kommt es indessen zur Bildung harziger Schmieren und Krusten, an deren Aufbau ausser dem Farbstoff noch überschüssiges NR und etwaige sonstige Begleitstoffe mehr oder weniger stark beteiligt sind. Solche hochmolekularen Körper lösen sich nur noch unvollkommen oder

überhaupt nicht mehr in den vorgeschriebenen Extraktionsmitteln, sodass auch eingeschlossener oder adsorbierter unverharzter Farbstoff nicht mehr in Erscheinung tritt. Enthält die Probe nur wenig Uronsäure, dann kann die NR-Reaktion aus diesen Gründen völlig versagen.

(5) *Leicht kondensierendes Aglykon.* Bei Phenol-uronosiden kann es auch dadurch zu Störungen kommen, dass das Aglykon unter den Reaktionsbedingungen anstelle des NR mit der freigesetzten Uronsäure einen Farbstoff bildet. Dies ist beschrieben für die Glukuronsäurepaarlinge des *p*-Kresols[47], α- und β-Naphthols[48] *u.a.*

(*II*) Scheinbar positive Reaktion bei Abwesenheit von Uronsäuren bzw. zu hohe Werte bei der Bestimmung:

(1) *Störende Carbonyl-carbonsäuren.* Farbstoffe von gleicher oder doch sehr ähnlicher Lichtabsorption geben: Glyoxylsäure (und Abkömmlinge derselben wie Allantoin), Askorbinsäure, Mesoxalsäure (und Abkömmlinge derselben wie Alloxan) sowie Hydroxy-carbonyl-carbonsäuren, wie Hydroxy-brenztraubensäure, *usw.*

(2) *Anwesenheit anderer Kohlenhydrate.* Liegen grössere Mengen von sonstigen Kohlenhydraten vor, so können Fehlschlüsse und zweifelhafte Ausfälle bei der qualitativen Probe die Folge sein; bei der quantitativen Bestimmung werden fast durchweg zu hohe Werte vorgetäuscht (näheres siehe[52]!).

(3) *Zersetztes NR-Reagens.* Eine wässrige oder alkoholische NR-Lösung färbt sich allmählich gelb → orange → rot, besonders schnell dann, wenn Licht und Sauerstoff einwirken können. Aber auch nach Ausschaltung dieser Einflüsse tritt langsame Zersetzung ein. Der gebildete Farbstoff lässt sich leicht ausziehen, sogar mit Benzol oder Toluol, und da er bei 570 mμ eine beträchtliche Absorption aufweist (*vgl.* S. 187), hat er vielfach zu Irrtümern und Fehlern Anlass gegeben.

Über die Problematik des NR-Reagenses siehe den folgenden Abschnitt.

Reagentien und Nebenreaktionen

(*I*) *Naphthoresorcin-Reagens.* Von wesentlicher Bedeutung für die Genauigkeit und Zuverlässigkeit der TOLLENSschen Reaktion erweist sich die Anwendungsform des NR. Um die Verschiedenartigkeit und Unsicherheit der hier herrschenden Ansichten und Befunde aufzuzeigen, lässt es sich nicht vermeiden, eine gedrängte Darstellung dieser Problematik zu geben.

B. TOLLENS hat in seiner ersten Mitteilung[2] über seinen neuen Uronsäurenachweis ein *alkoholisches NR-Reagens* angegeben, das auch von C. TOLLENS für die erste halbquantitative Ausführungsform[5] übernommen wurde. Im NEUBERGschen Laboratorium ist aber bald die grosse Zersetzlichkeit von alkoholischen NR-Lösungen bemerkt worden; NEUBERG empfiehlt daher, bei Anstellung der qualitativen Probe *festes NR* zuzusetzen. Das erste brauchbare, wenn auch umständliche, quantitative Verfahren von FLORKIN verwendet eine Lösung von *NR in Aceton*[8].

MAUGHAN *et al.*[9] haben schliesslich eine *wässrige* Reagenslösung vorgeschlagen auf Grund der Beobachtung, dass umso mehr Farbstoff gebildet werd, je geringer die Alkoholkonzentration im Reaktionsgemisch ist. Sie stabilisieren die 0.2%ige Lösung in dest. Wasser durch 24-stündiges Erwärmen auf etwa 38° im verschlossenen Gefäss. Nach Filtration soll dann das Reagens etwa einen Monat stabil sein, wenn es bei 3–5° im Dunkeln aufbewahrt wird.

Ähnlich erwärmen HANSON *et al.*[18] die wässrige Lösung eine Stunde lang auf 37° und lagern sie dann lichtgeschützt bei 0°. Dieses Reagens soll etwa eine Woche haltbar sein.

STARY UND YUVANIDIS[32] endlich „erhitzen" die Lösung von NR in Wasser eine Min (Temperatur ist nicht angegeben) und finden, dass sie nur 24 Stunden brauchbar ist.

Der Sinn dieser Wärmebehandlung wurde von JARRIGE[23] so gedeutet, dass sich „ein bestimmtes Gleichgewicht zwischen NR und seinem Oxydationsprodukt" in der wässrigen Lösung einstellt. Eine Begründung für diese absonderliche Annahme führt er jedoch nicht an. Man kann sich nicht vorstellen, wie im dest. Wasser, *z.T.* sogar unter Luftabschluss eine Oxydation stattfinden soll. Doch wurde dieser Gedanke aufgegriffen, und wenig später erschien eine Arbeit von BISSET *et al.*[24], welche die Farbstoffbildung „irgendeinem instabilen Oxydationsprodukt des NRs" zuschreiben, ohne allerdings Belege hierfür beibringen zu können. Es wird lediglich angeführt, dass es sich nach ihren Befunden nicht um 3-Hydroxy-naphthochinon-(1.4) handeln könne, was aber nahezu selbstverständlich ist, da die Einführung einer neuen Sauerstoff-funktion durch blosse Alterung einer wässrigen NR-Lösung wohl nur wenig Wahrscheinlichkeit für sich hat. Überdies kann man 3-Hydroxy-naphthochinon-(1.4) („Naphthalinsäure") nicht als instabil bezeichnen; es stellt eine seit langem bekannte, durchaus beständige Verbindung dar.

Nach den Erfahrungen von BISSET *et al.* lassen sich die mit den unkontrollierbaren Alterungsvorgängen in der wässrigen Reagenslösung verbundenen Fehlerquellen vermeiden, wenn eine „künstliche Alterung" des NR mit einem schwachen Oxydationsmittel während der Farbentwicklung durchgeführt wird; als solches habe sich $H_3[Fe(CN)_6]$ bewährt. Wie man den Angaben im Original entnehmen kann, trifft auf 1 Mol NR nur 0.1 Mol Oxydationsmittel, sodass an eine stöchiometrische Redox-Reaktion nicht zu denken ist. Nun vermag die Hexacyano-eisen(III)-säure in der stark salzsauren Lösung zwar schwach oxydierend zu wirken[49]; man muss jedoch erwarten, dass bei unmittelbarer Zugabe der $H_3[Fe(CN)_6]$ zum Reaktionsgemisch (noch dazu vor dem Zusatz des NR) vor allem die leicht oxydierbare Aldehydgruppe der Uronsäure angegriffen wird. In der genannten Arbeit sind leider keine Beleganalysen angegeben, sodass eine wirkliche Beurteilung dieser „Oxydationsmethode" nicht möglich ist.

Schon vordem wollen übrigens MEYER *et al.*[15] durch Zusatz von Kalium-peroxydisulfat, $K_2S_2O_8$, erhöhte Farbintensitäten bei der quantitativen Bestimmung erzielt haben. Nachdem leider Beleganalysen auch hier fehlen, ja nicht einmal das Verfahren beschrieben wird, ist eine Stellungnahme zu dieser Angabe ausgeschlossen.

Der letzte Vorschlag in dieser Reihe stammt von SMITH[50], der ebenfalls die Alterung des wässrigen NR-Reagenses für einen Oxydationsvorgang erklärt und das Oxydationsprodukt für die Farbstoffbildung verantwortlich macht, wiederum ohne jeglichen Anhaltspunkt. Er bereitet das Reagens, indem er durch die wässrige Lösung von NR eine Stunde lang Sauerstoff leitet und den „Überschuss" an letzterem nach 24 Stunden mit Stickstoff verdrängt. Diese Variante wurde von den meisten neueren Bearbeitern übernommen, *u.a.* von HEYNS UND KELCH[30].

Im Gegensatz zu allen übrigen Bearbeitern verzichteten FUCHS UND TRAUNER-ADELPOLLER[22] auf jegliche Vorbehandlung; das Reagens wurde unmittelbar vor Gebrauch durch einfaches Auflösen in Wasser hergestellt. Auch in der sorgfältigen Arbeit von FISHMAN UND GREEN[38] fand eine täglich frisch bereitete, wässrige NR-Lösung ohne weitere Zusätze und ohne Alterungsprozess Verwendung.

Wir haben aus verschiedenen Gründen, die später[52] näher dargelegt werden, einer Lösung von *NR in Eisessig* den Vorzug gegeben. Solche Lösungen sind relativ stabil, auch ohne eine der oben erwähnten Vorbehandlungen; selbst nach wochenlangem

Aufbewahren bei Raumtemperatur im Dunklen lieferten sie mit reinen Uronsäuren zufriedenstellende Ergebnisse. Anders jedoch, wenn noch weitere Zucker anwesend sind; in diesen Fällen haben aus unbekannten Gründen stets die Versuche mit frisch bereiteten NR-Lösungen die geringsten Abweichungen aufzuweisen. Vergleichsweise wurde entsprechend der Vorschrift von MAUGHAN *et al.* das frisch bereitete Reagens 24 Stunden bei 38° gehalten. Dadurch traten aber keine charakteristischen Veränderungen gegenüber der nicht vorbehandelten Eisessiglösung des NR auf; insbesondere waren die Farbintensitäten nicht erhöht.

(*II*) *Nebenreaktionen.* Bei der TOLLENSschen Reaktion arbeitet man stets mit einem grossen Überschuss an NR, einmal um die Bildung des violetten Farbstoffes mit den Uronsäuren möglichst zu beschleunigen, zum andern um der Tatsache Rechnung zu tragen, dass das NR durch eine Anzahl von Nebenreaktionen der gewünschten Umsetzung entzogen werden kann. Diese in anderem Zusammenhang meist schon erwähnten Nebenreaktionen zerfallen in drei Klassen, von denen die letzte noch etwas eingehender zu behandeln ist: (*1*) Umsetzungen mit störenden Carbonyl-carbonsäuren, *usw.*; (*2*) Umsetzungen mit anderen Carbonyl-verbindungen; (*3*) Farbstoffbildung.

Farbstoffbildung durch Belichtung

Die Bildung eines stark störenden, gelbroten bis roten Farbstoffes durch Zersetzung alkoholischer NR-Lösungen am Licht wurde zuerst von NEUBERG beschrieben. Nach unserer Auffassung dürften die meisten positiven Fehldeutungen der TOLLENSschen Reaktion auf diese Ursache zurückzuführen sein; denn der mit Benzol und Chloroform, erst recht natürlich mit Äther oder Amylalkohol extrahierbare Farbstoff entsteht auch in wässriger NR-Lösung und bei Belichtung von festem NR, dagegen kaum in einer Eisessiglösung dieses Reagenses.

Lässt man eine frische, 0.2%ige, wässrige NR-Lösung in einem Gefäss aus gewöhnlichem Glase bei Raumtemperatur stehen, so überrascht die Schnelligkeit, mit der selbst zerstreutes Tageslicht einwirkt: Schon nach einer Stunde hat sich der zunächst gelbliche Farbton deutlich vertieft; nach eintägigem Stehen ist die Lösung goldgelb, und am vierten Tage bereits fallen aus der tief rotorangefarbenen Lösung braunviolette Partikel aus. Die gleichen Erscheinungen, nur in verzögerter Folge, beobachtet man bei der Alterung unter Lichtausschluss; es besteht also in dieser Beziehung kein grundsätzlicher Unterschied.

Nachdem der braunviolette Körper (im folgenden als „Farbstoff NR-Z" bezeichnet) offenbar die Endstufe des Zersetzungsprozesses darstellt und auf Grund seiner Färbung der stärkste störende Einfluss auf die TOLLENS-Reaktion zu erwarten ist, wurde er etwas genauer untersucht.

Der im Verlauf der nächsten Tage gebildete Niederschlag wurde abzentrifugiert, mehrfach mit Wasser ausgewaschen und getrocknet. Man erhält ein violettbraunes Pulver, dessen Löslichkeitsverhältnisse in Tabelle I zusammengestellt sind.

Ein Vergleich mit dem aus Uronsäuren + NR erhaltenen Farbstoff lehrt, dass von einer Identität beider Körper, wie sie TEAGUE[34] postuliert hat, nicht die Rede sein kann. Ihre grundsätzliche Verschiedenheit geht klar aus dem Absorptionsspektrum (Fig. 1) hervor, das für den Uronsäurefarbstoff eine höchst charakteristische Bande mit dem Maximum bei 565 mμ aufweist, für den Farbstoff NR-Z dagegen ein wenig ausgeprägtes Maximum bei $\sim$ 510 mμ. Bedeutsam ist auch der Verlauf der Extinktionskurven im violetten und nahen ultravioletten Gebiet.

Die Darstellung veranschaulicht ferner die erhebliche Absorption des störenden Farbstoffes NR-Z im Messbereich von 560–570 mμ.

TABELLE I

LÖSLICHKEITSVERHÄLTNISSE

Lösungsmittel	*Farbstoff NR-Z*		*Farbstoff aus Uronsäure + NR*	
	Löslichkeit	*Farbe der Lösung*	*Löslichkeit*	*Farbe der Lösung*
Wasser	Unlösl.	—	Unlösl.	—
Äthanol	Leicht lösl.	Rosa	Lösl.	Blau
Eisessig	Leicht lösl.	Rosa	Lösl.	Blau
Äthylacetat	Leicht lösl.	Tiefrosa	Lösl.	Rotviolett
Aceton	Leicht lösl.	Rosa	Lösl.	Rotviolett
Äther	Schwer lösl.	Rosa	Leicht lösl.	Violett[a]
Chloroform	Schwer lösl.	Rosa	Lösl.	Violett[a]
Toluol	Sehr schwer lösl.	Schwach rosa	Lösl.	Violett[a]
Toluol/Eisessig 4 : 1	Schwer lösl.	Rosa	Lösl.	Violett[a]

[a] Farbe der organischen Phase nach Extraktion des Farbstoffes aus dem Reaktionsgemisch.

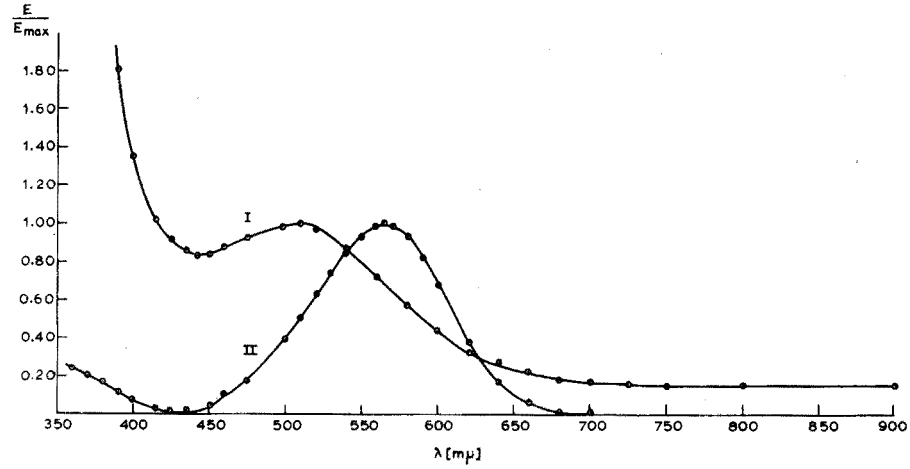

Fig. 1. $E/E_{max} = f(\lambda)$ für den Farbstoff NR-Z (I) und für den Farbstoff aus Uronsäure + NR (II).

Farbstoffbildung beim Erhitzen

Schon nach kurzem Erhitzen des Reaktionsgemisches[52] im Wasserbad, bevor noch wahrnehmbare Mengen des Uronsäurefarbstoffes entstanden sind, zeigt die Lösung eine prächtige grüne Fluoreszenz zunehmender Intensität, die auch neben dem später hervortretenden Uronsäurefarbstoff deutlich sichtbar ist. Beim Extraktionsvorgang tritt sie nicht in die Toluolphase über. TOLLENS UND RORIVE[1] haben diese Erscheinung in ihren ersten Versuchen mit NR schon beobachtet, erachten sie aber als spezifisch für die Anwesenheit von Pentosen oder Methylpentosen, was nach unseren Erfahrungen nicht stimmen kann; denn sie macht sich bei den Uronsäure-haltigen Proben wie bei den Leerwerten in der gleichen Weise geltend. Unter diesen Umständen sowie in Anbetracht der Leichtigkeit, mit der sich der fluoreszierende Farbstoff bildet, wird man an eine Selbstkondensation des NR denken müssen, wie sie vom Resorcin seit langer Zeit bekannt ist[51].

Farbstoffbildung durch Alterung der Reagenslösungen

Selbst unter Lichtabschluss ist das wässrige NR-Reagens sehr instabil und nur begrenzte Zeit verwendungsfähig. Wieweit in letzterem Punkte die Meinungen auseinandergehen, wurde im S. 184 bereits ausgeführt.

Beim Aufbewahren einer 0.2%igen Lösung von NR in reinstem Eissesig machen sich im Gegensatz zur wässrigen Lösung nur langsam Alterungsvorgänge bemerklich. Die frisch bereitete, eben wahrnehmbar beigefarbene Lösung zeigt nach achtwöchiger Lagerung im Dunkeln bei Raumtemperatur einen gelblichen Farbton. Wie ein Vergleich der beiden Extinktionskurven (Fig. 2) lehrt, hat am violetten Ende des Spektrums eine Verschiebung der Absorption nach längeren Wellen hin stattgefunden; im übrigen Bereich ist die Extinktion etwa im gleichen Verhältnis erhöht.

Es ergeben sich demnach keine Anhaltspunkte für die Bildung eines typischen Farbstoffes bei der Alterung einer NR-Lösung in Eisessig über mehrere Wochen.

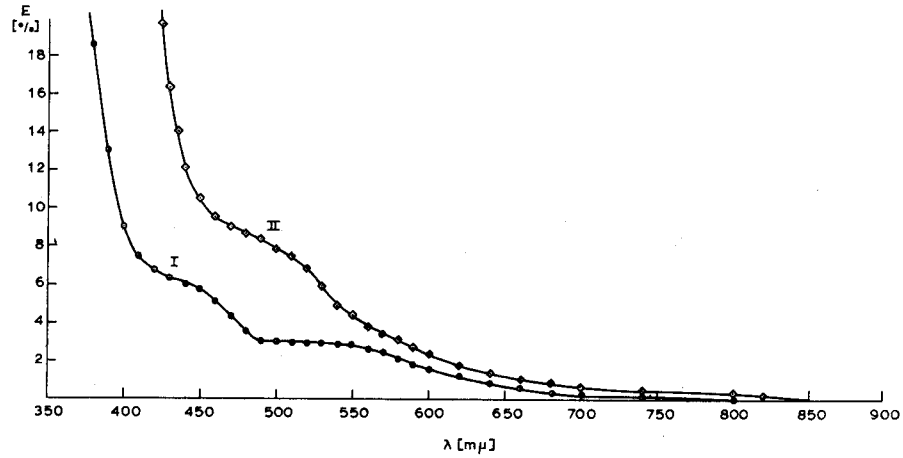

Fig. 2. $E = f(\lambda)$ für eine frisch bereitete (I) und für eine acht Wochen alte (II) NR-Lösung in Eisessig (0.2%).

ZUSAMMENFASSUNG

Die bei der TOLLENSschen Naphthoresorcin-Reaktion auftretenden Schwierigkeiten und Fehlerquellen werden zusammengestellt und besprochen. Als Reagens empfiehlt sich eine Lösung von Naphthoresorcin in Eisessig, welche an Haltbarkeit alle bisher vorgeschlagenen Lösungen in Wasser, Alkohol, *usw.* übertrifft. Es wird gezeigt, dass der im Verlauf der Alterung wässriger Naphthoresorcin–Lösungen gebildete Farbstoff nicht mit dem aus Uronsäuren und Naphthoresorcin entstehenden identisch ist.

SUMMARY

Difficulties and sources of error which occur in TOLLENS' reaction are discussed. The reagent is best prepared by dissolving naphthoresorcinol in glacial acetic acid; such reagent solutions are more stable than those in water or ethanol. It is shown that the dyes formed by ageing aqueous naphthoresorcinol solutions and by the reaction of naphthoresorcinol with uronic acids are not identical.

RÉSUMÉ

L'auteur a fait une étude sur les difficultés et les sources d'erreurs possibles de la réaction de TOLLENS. Il est préférable de dissoudre le naphtorésorcinol dans l'acide acétique; une telle solu-

tion est plus stable qu'une solution aqueuse ou alcoolique. D'autre part, on constate que les colorants formés par action de solutions de naphtorésorcinol dans l'eau ne sont pas identiques à ceux formés par la réaction du naphtorésorcinol avec des acides uroniques.

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Short Communication

The cathode ray polarography of copper

Recent work by one of us on the determination of copper by single cell and differential (subtractive) cathode ray polarography[1] showed that with copper concentrations in the range 1 μg/ml to 100 μg/ml curved calibration graphs were obtained. The base electrolytes used were 0.1 *N* hydrochloric acid, a sodium acetate–hydrochloric acid buffer of pH 4.6, and 0.25 *M* pyridine-pyridinium chloride. These results were contrary to our expectations, since most non-linear calibration graphs are caused by obvious chemical effects such as low solubility (lead in sulphate solutions) or lack of reagents (aluminium or iron with Solochrome violet RS). As it was not immediately clear where the effect with copper originated, it was decided to investigate further.

First, the linearity of the instrument itself was demonstrated, for both reversible and irreversible reactions. Calibration graphs were prepared for lead in the pH 4.6 buffer, and for arsenic in a base electrolyte containing 0.1 *M* sulphuric acid and 0.5 *M* potassium bromide. The results were very satisfactory, and showed quite good linearity in view of the concentration range covered. Only one point on each curve differed by more than 5% from its expected value, and these points corresponded to the lowest concentrations used, 0.1 μg/ml.

Calibration graphs for copper were then plotted, using the original base electrolytes. The curves were again non-linear; the results are shown in Table I. These figures show a definite trend for the calibration factor to decrease as the copper concentration is increased; the figures for the three base electrolytes were determined on three different days, using two different polarographs.

The effect of the copper to chloride ratio was investigated by measuring peak heights due to both 10^{-5} *M* and 10^{-3} *M* copper ions in 0.1 *M* sulphuric acid containing

TABLE I

CALIBRATION FACTORS[a] FOR COPPER BY CATHODE RAY POLAROGRAPHY

Copper	*Calibration factor*				
(μg/ml)	*N HCl*	*pH 4.6 buffer*	*pyridine/HCl*	*pH 4.6 buffer*[b]	*pyridine/H_2SO_4*[b]
0.1	98	133	150	114	68
0.3	99	128	125	120	74
0.7	98	130	125	120	76
1.0	96	125	120	118	72
3.0	85.5	106	95	116	74
7.0	79	74	88	115	74
10	74	64	89	115	72
30	57.5	51	81	115	72
70	52.5	43	66	120	74
100	52	40	58	115	74

[a] The calibration factor is the peak height in cm converted to maximum sensitivity and divided by the concentration.
[b] In chloride-free media.

increasing amounts of chloride. The results showed a change in calibration factor from 82 to 116 as the copper to chloride ratio was changed from 1 to 10^{-5}; and the two copper concentrations used showed very good agreement as to calibration factor at similar copper to chloride ratios. It appears that the effect on the original calibration curves was due to the differing copper to chloride ratios obtained when the chloride concentration was fixed and the copper varied over a range of 1000 to 1. In addition. the different ratios of lowest to high figures obtained in the buffered and acid solutions show some pH dependence.

That the effect was due to the chloride ion was confirmed by constructing calibration graphs for copper in buffer solutions free from chloride; a sodium acetate–sulphuric acid buffer of pH 4.6, and 0.25 *M* pyridine-pyridinium sulphate were used. The graphs obtained were linear; the results obtained are again shown in Table I.

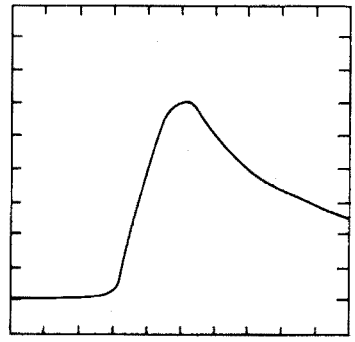

Fig. 1. 1 μg/ml Cu^{2+} in 0.1 *N* H_2SO_4. Sensitivity 1/15 maximum.

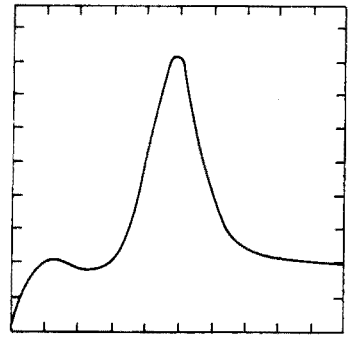

Fig. 2. 1 μg/ml Cu^{2+} in 0.1 *N* H_2SO_4 plus 1 *N* KCl. Sensitivity 1/25 maximum.

It was noted that the shape of the copper wave changed as the chloride to copper ratio increased; at low chloride levels it corresponded to the usual type of C.R. polarogram, but at high chloride levels became much more peaked. Typical polarograms are shown in Figs. 1 and 2. It is known that the shape of a C.R. polarogram is one of the characteristics of the depolariser present, and it is suggested that the cause of this effect is a change in the nature of the reducible species. The copper is thought

to be present as hydrated cations in non-chloride media, and as the chloride to copper ratio is increased the copper gradually becomes converted to an anionic chloride complex. Similar results would be expected from other elements forming anionic chloride complexes, and some evidence has been obtained to show that zinc exhibits the same effect, but to a much smaller degree. Anionic complex formation has been given as a reason for the reducibility of tin(IV) in chloride-rich media only[2], and it would appear likely that many elements may show an effect similar to that noted for copper.

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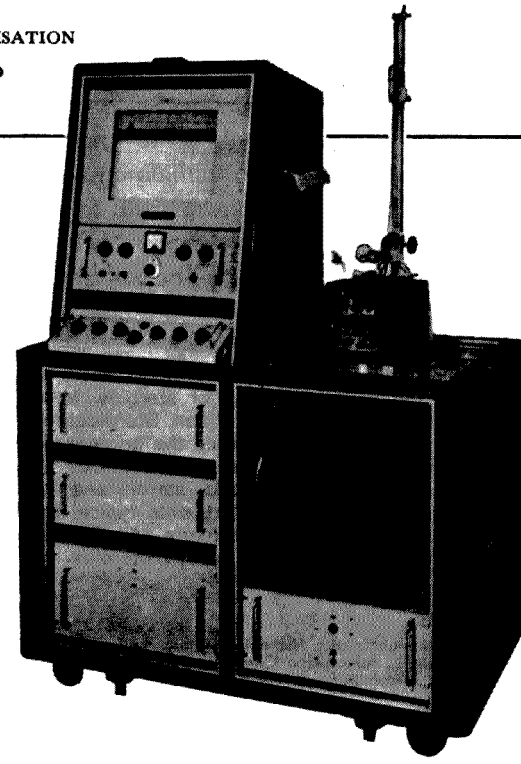

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