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Optimal Conditions for the Catalytic Action of Iodine in the Sandell-Kolthoff Reaction

By

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With 11 Figures

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The Sandell-Kolthoff reaction[1], making use of the catalytic effect of iodine in the oxidation-reduction process between Ce^{4+} and As^{3+}, is widely used for iodine determination in biological material[2–4]. Nevertheless, optimal conditions for the reaction, viz. length of time, temperature, p_H of medium, effect of chlorides and of the ceric-arsenic ratio, vary from one method to another. Several modifications indicate various concentrations of the reagents employed, without, in most cases, giving plausible reasons which would justify the results arrived at. Errors and unreliability of the methods may further be increased through an empirical approach to the problem.

Before putting our modification to a practical application, we made, in an earlier study[5], a thorough investigation of the optimal conditions for the alkaline ashing method. The present study was undertaken to determine the optimal conditions for the catalytic effect of iodine in the Sandell-Kolthoff reaction. The ultimate purpose of both these studies was to work out a universal method for iodine assay in biological material.

Experimental

Part I

Reagents: 1. Ceric ammonium sulphate, 0.005 N: Prepared by dissolving 3.17 g of the salt in 3.0 N sulphuric acid.

2. Acid mixture: Consists of 0.1 N arsenic trioxide, 1.0 M sodium chloride in 4.5 N sulphuric acid.

3. Brucine acetate, 1%: Prepared by dissolving the base in 0.1 *N* acetic acid.

4. Standard solutions: (a) Stock solution I. 118.1 mg of NaI (or 130.8 mg of KI) are dissolved in 1000 ml of water (1 ml ≡ 100 μg of I^-).

(b) Stock solution II. 2.5 ml of stock solution I is made up to 1000 ml volume with water (1 ml ≡ 0.25 μg of I^-).

(c) Working standards: 33.0 ml of 2 *N* sodium carbonate are added to 1.0, 2.0, 4.0, 6.0 and 8.0 ml of stock solution II, and the respective volumes are brought to 100 ml with water. 2.0 ml of these standard solutions contain 0.005, 0.010, 0.020, 0.030 and 0.040 μg of I^-.

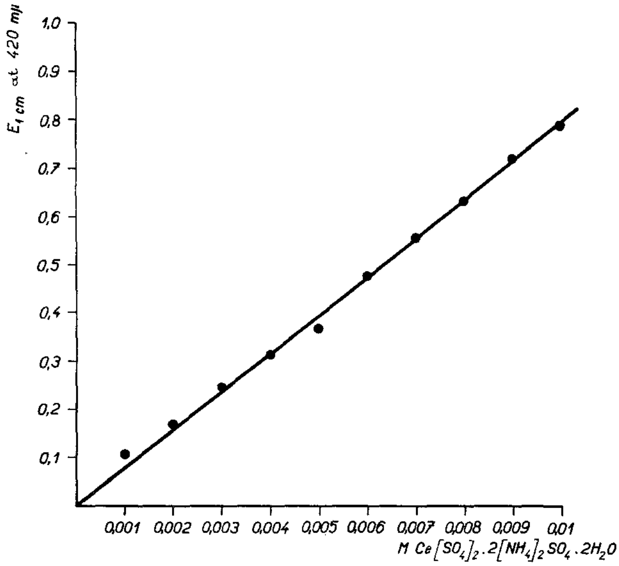

Fig. 1. Correlation between Ce^{4+} concentration and extinction

The blank is obtained by making up 33.0 ml of 2 *N* sodium carbonate to 100 ml with water.

Procedure: 2.0 ml of the acid mixture are added to 2.0 ml of the working standard and the whole is cooled for 10 minutes, after a thorough mixing, in an ice bath at + 4° C. Then 2.0 ml of an equally cooled solution of Ce^{4+} are added and after mixing, the whole is warmed in a water bath at 40° ± 0.1° C for 20 minutes. The samples are then transferred back to the ice bath at + 4° C. Ten minutes later, 0.5 ml of brucine solution are added and after a thorough mixing, the orange coloured solution is left to stand for 15 minutes at 100° C in a thermostated oven. The samples are measured on a spectrophotometer at 430 nm.

Part II

In the present study the optimal conditions were determined for the oxidation-reduction reaction, which catalyses iodine, and which runs as follows:

1. $2\,Ce^{4+} + 2\,I^- \rightarrow 2\,Ce^{3+} + I_2$ (slow process).

2. $I_2 + As^{3+} \rightarrow 2\,I^- + As^{4+}$ (rapid process).

The theoretical explanation for this reaction has been formulated by *Glasstone*[6] and by *Kontaxis* and *Pickering*[7]. According to their results it is possible, in plotting the course of the calibration curve, to plot the linear relation, log extinction/iodine concentration, if the following conditions are fulfilled:

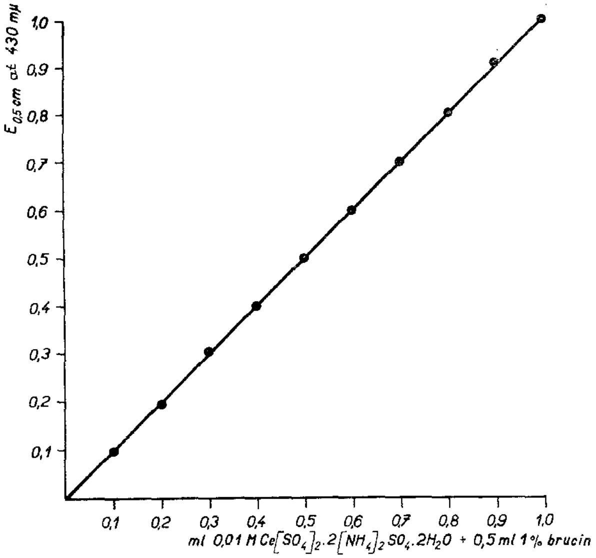

Fig. 2. Correlation between brucine, oxidised by Ce^{4+}, and extinction

A. The solution being measured must conform to Beer's Law.

B. The reduction of Ce^{4+} must be rate-limiting.

C. The catalytic coefficient, K, must be linearly related to iodine concentration.

We have therefore investigated the parameters of the reaction which would allow the above conditions to apply to our working procedure. The following factors were studied: behaviour of ceric-ammonium sulphate and of brucine oxidised by Ce^{4+} in relation to Beer's Law, maximum absorption curve, effect of sulphuric acid and sodium chloride concentrations and of the $As^{3+} : Ce^{4+}$ ratio on the rate constant of the reaction, the effect of an ice-bath (+ 4° C) on the stability of the reaction and the colour stability of brucine oxidised by Ce^{4+}; further, the possibility of reading the results off one single curve; and finally, the correlation between the rate constant of the reaction and iodide concentration.

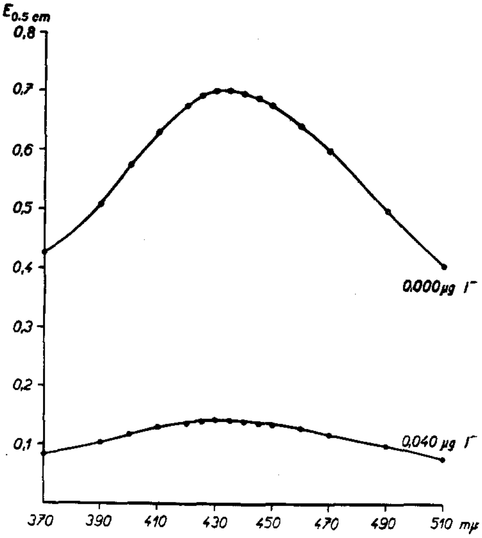

Fig. 3. Absorption curve of brucine, oxidised by Ce^{4+}. Standards of 0.000 and 0.040 μg of I^- in 2.0 ml processed as described

To obtain the rate constant, K, the samples were warmed at 40.0 ± 0.1° C for periods of 5, 10, 15 and 20 minutes. K was calculated from the equation for the first order reaction

$$K = \frac{2.303}{t} \log \frac{a}{a - x},$$

where a is the concentration of Ce^{4+} at the beginning, and $a - x$ represents its concentration at the end of the reaction.

1. Conformity to Beer's Law: *Thompson* et al.[8] found a linear correlation between the absorbancy and concentration of Ce^{4+} with a Beckman DU spectrophotometer only. *Acland*[9] observed this linear correlation with a Unicam SP 600 instrument.

In our study, 2.0 ml of the acid mixture and 2.0 ml of Ce^{4+} solution, at concentrations ranging from 0.001 to 0.01 M, were added to 2.0 ml of a blank standard solution. The colour intensity was immediately measured on a Zeiss spectrophotometer at 420 nm and that of brucine, oxidised by Ce^{4+}, was measured at 430 nm.

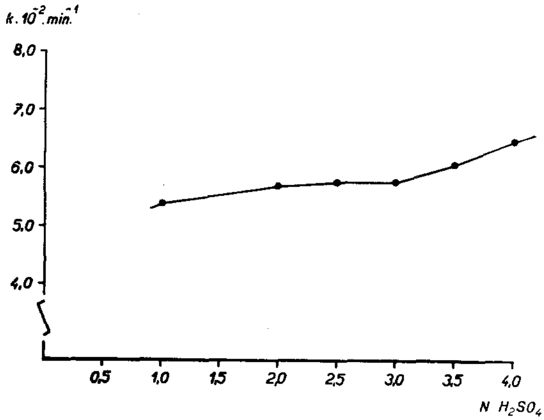

Fig. 4. Effect on the rate constant of sulphuric acid in the reacting medium

A linear correlation was found between the concentration of Ce^{4+}, or of brucine oxidised by Ce^{4+}, and the extinction (Figs. 1, 2). The Zeiss spectrophotometer was therefore regarded as suitable for these measurements.

2. Absorption curve for brucine, oxidised by Ce^{4+}. The absorption curve was measured in the range of 370–510 nm, following the process with the blank standard and the standard containing 0.040 μg of iodide. Maximum absorption occured at 430 nm (Fig. 3).

3. Effect of sulphuric acid concentration on peak absorption of brucine, oxidised by Ce^{4+}.

The effect of sulphuric acid concentration on the absorption intensity of Ce^{4+} has been observed by several investigators[8,10]. It was therefore presumed that this same factor may likewise operate, when the colour intensity of the brucine oxidised by Ce^{4+} is being measured.

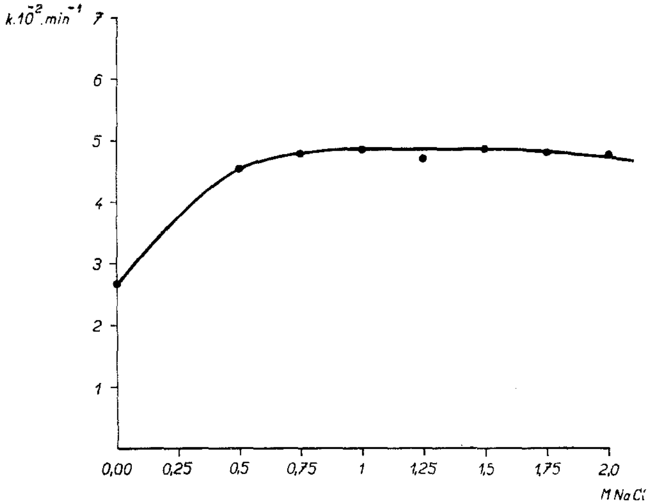

Fig. 5. Effect on the rate constant of sodium chloride concentration

In our procedure, 2.0 ml of the acid mixture were added to 2.0 ml of blank, the concentration of the sulphuric acid being so adjusted that after the addition of 2.0 ml of the Ce^{4+} solution, the resulting normalities in the reacting solution were 1.0, 2.0, 2.5, 3.0, 3.5 and 4.0. It was found that maximum absorption of the brucine oxidised by Ce^{4+} becomes a function of the H_2SO_4 normality and has the following values: 470, 440, 430, 430, 430, 420 nm.

4. Effect of sulphuric acid concentration on the rate constant of the reaction.

The majority of authors do not take into account the influence of the sulphuric acid concentration on the catalytic effect of iodine. *O'Neil* and *Simmons*[10] use a concentration of 1.4 *N*, *Thompson* et al.[8] use 1.15 *N*, because they had found that at this normality, minor changes in its concentration have no effect on the percent transmittance of the yellow colour of Ce^{4+}. *Strickland* and *Maloney*[11], who followed the direct effect of sulphuric acid on the rate constant of the reaction, found optimal results at 2.5 *M*.

Consequently, the effect of 1.0, 2.0, 2.5, 3.0, 3.5 and 4.0 *N* concentrations of H_2SO_4 on the course of the catalytic iodine reaction was tested. Since it was found (point 2 above), that maximum absorption of brucine oxidised by Ce^{4+}, becomes a function of the p_H of the medium, the yellow colour of Ce^{4+} was measured. The procedure included the addition of Ce^{4+} to individual samples in 30 seconds, warming for 5, 15 or 20 minutes at 40 ± 0.1° C, and measurement after the same time intervals at 420 nm. From the results in Fig. 4, it may be inferred that the optimal concentration of H_2SO_4 in the reagent medium is 2.5 *N*.

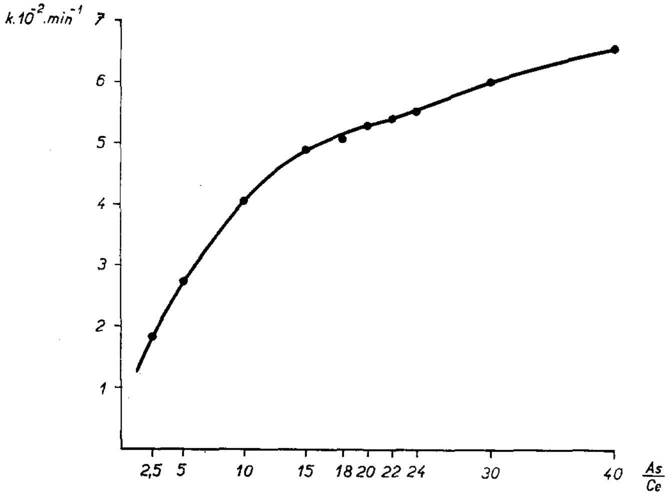

Fig. 6. Effect on the rate constant of As^{3+}/Ce^{4+} ratio

5. Effect of sodium chloride concentration on the rate constant. The stimulating effect of chlorides on the catalytic action of iodine, has been reported by several authors[8,10,12], and hence this relationship was also examined.

The concentration of NaCl in the acid mixture was varied over the range 0.00–2.00 *M* and the optimum concentration was found to be 1.00 *M* (Fig. 5).

6. Effect of the ratio $As^{3+} : Ce^{4+}$ on the rate constant.

This ratio represents one of the most important factors in the catalytic action of iodine. Although this point has been investigated in the past[10,12], the majority of authors use a more or less empirically selected concentration of arsenic and cerium. In most cases this ratio varies between 5 and 10 : 1.

In the present study the concentration of arsenic trioxide in the acid mixture was varied. The ratio $As^{3+} : Ce^{4+}$ in the reagent solution varied over the range 2.5–40.0 : 1. The optimal concentration of As_2O_3 in the acid mixture is 0.1 *N*, when the ratio $As^{3+} : Ce^{4+}$ in the reagent solution is 20 : 1 (Fig. 6).

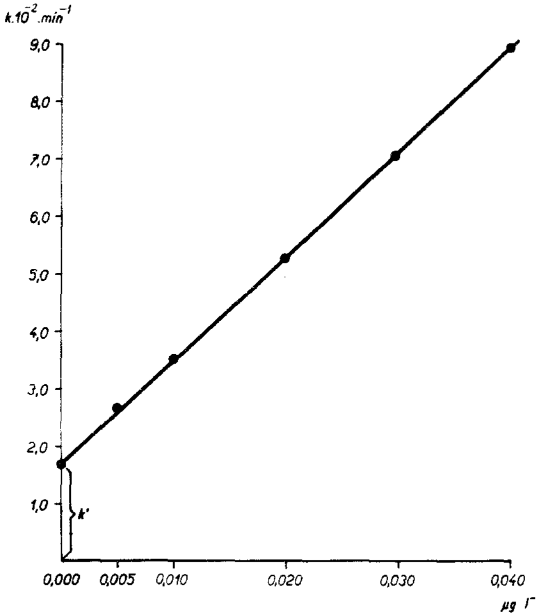

Fig. 7. Correlation between the rate constant and iodine concentration. *k'* = value of rate constant for iodine present as contaminating agent in the reagents used

7. Effect of ice bath (+ 4° C) on the stability of the reaction. The use of ice baths is recommended by *Grossmann* and *Grossmann*[13]. Their conclusions, however, have not been borne out by experimental results, hence *Acland*[9] considers the interruption of the catalytic action of iodine as attractive though rather unnecessary. In our opinion, however, inhibiting of the iodine action would mean a great simplification and enhanced precision in the procedure.

The present study included also the question whether the catalytic reaction takes place at + 4° C. This temperature is maintained both before the addition of 2.0 ml of the Ce^{4+} solution to the samples, and also after the immersion of the latter into a warm bath, before adding brucine.

The test was carried out as follows: Ten calibration curves were processed in the same run in such a way that the cooling time in the ice bath was 2.5 minutes, 5.0 minutes or 10.0 minutes. The average deviations for the 60 samples of various standards and for the blank were ± 1.56% for 2.5 minutes ± 1.87% for 5.0 minutes interval and ± 1.27% for 10.0 minutes interval (Table I).

8. Colour stability of brucine oxidised by Ce^{4+}.

Magee and *Spitzy*[14] do not recommend interruption of the catalytic reaction of iodine by the addition of brucine, although other investigators[13,15] favour this step, since brucine oxidised by Ce^{4+} gives a relatively stable colour intensity.

Table I. Mean values and standard deviation for ten calibration curves processed in one batch (20 minutes at + 40° C). Different time periods were used for the cooling of samples in the ice bath (10, 5 or 2.5 minutes at + 4° C)

μg I^-	10 minutes		5 minutes		2.5 minutes	
	E* ± S. D.**	% S. D.	E ± S. D.	% S. D.	E ± S D.	% S. D.
0.000	0.700 ± 0.008	1.14	0.690 ± 0.007	1.01	0.691 ± 0.007	1.01
0.005	0.576 ± 0.006	1.04	0.556 ± 0.009	1.62	0.565 ± 0.010	1.77
0.010	0.466 ± 0.007	1.50	0.441 ± 0.006	1.36	0.457 ± 0.006	1.31
0.020	0.302 ± 0.002	0.66	0.278 ± 0.006	2.16	0.294 ± 0.007	2.38
0.030	0.196 ± 0.003	1.53	0.176 ± 0.009	5.11	0.188 ± 0.003	1.59
0.040	0.127 ± 0.003	2.36	0.106 ± 0.005	4.72	0.116 ± 0.003	2.59
$\bar{x}$	0.394 ± 0.005	1.27	0.374 ± 0.007	1.87	0.385 ± 0.006	1.56

* = Extinction of brucine oxidised by Ce^{4+}.
** = Standard deviation (S. D.).

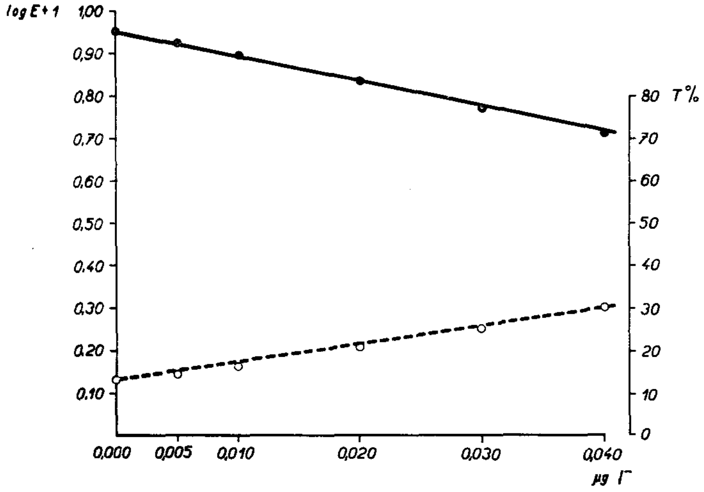

Fig. 8. Relation between log extinction and iodine concentration, and between percent transmittance and iodine concentration after 5 minutes of warming at + 40° C

This problem was examined as follows: When the orange colour had developed, the samples were heated for various periods at 100° C in a thermostated oven. It was found that after 15 minutes of heating the colour became stable enough to allow measurements to be made at any time up to 48 hours following colour development.

9. Reading off a single calibration curve.

Statistically significant differences ($P < 0.001$), between the extinction values of the calibration curves were found even though obtained from the same reagents on the same day. It is therefore advisable to process all the working standards along with every series of samples.

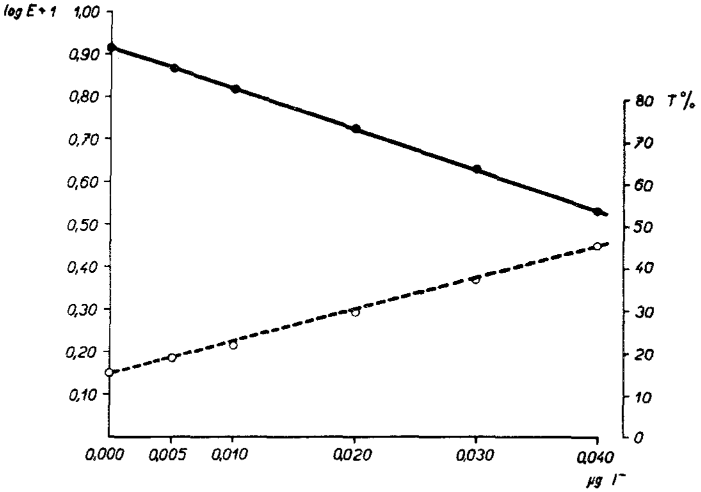

Fig. 9. Relation between log extinction and iodine concentration, and between percent transmittance and iodine concentration after 10 minutes of warming at + 40° C

10. Relation between the rate constant and iodine concentration. The purpose of this study was to verify the third assumption (point C

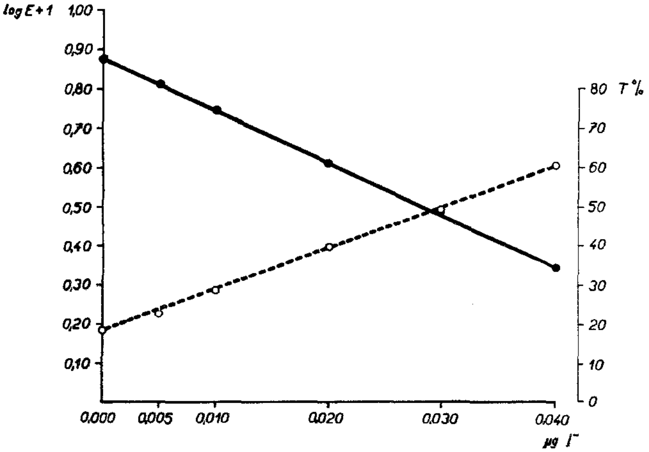

Fig. 10. Relation between log extinction and iodine concentration, and between percent transmittance and iodine concentration after 15 minutes of warming at + 40° C

above), which would justify the use of the calibration curve as a linear correlation of log extinction to iodine concentration. To this end, the standards for the whole calibration curve were processed as described above.

The rate constants, given in Fig. 7, were calculated from these results. Since a linear correlation was found to exist between the rate constant *K*, and the iodine concentration, the calibration curve may be plotted from the relationship, log extinction/concentration of iodine. Figs. 8, 9, 10 and 11 show calibration curves of log extinction/iodine concentration and of percent of transmittance/iodine concentration, after 5, 10, 15 or 20 minutes warming in a bath (40 ± 0.1° C).

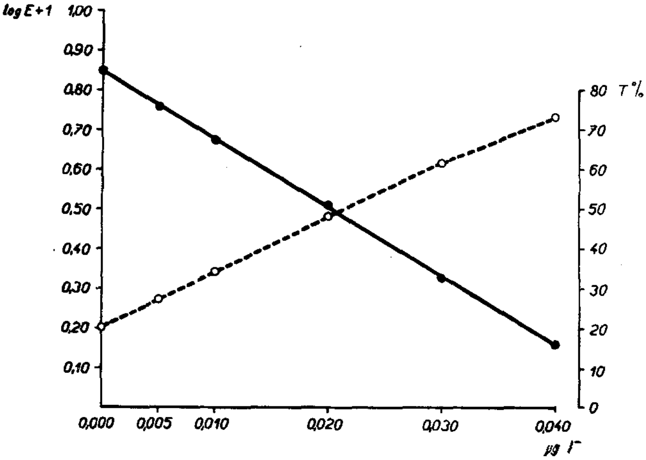

Fig. 11. Relation between log extinction and iodine concentration, and between percent transmittance and iodine concentration after 20 minutes of warming at + 40° C

Theoretically, this relationship[6, 7] log extinction/iodine concentration is linear for each time interval, whereas the relation, percent of transmittance/iodine concentration loses its linearity 20 minutes after warming. Hence, in agreement with *Acland*[9], using any other relation except that of log extinction/iodine concentration, for plotting the calibration curve, lacks a theoretical basis.

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Summary

Optimal condition were determined for the determination of ultramicroquantities of iodine by the Sandell-Kolthoff reaction. Solutions of 1.0 *M* sodium chloride, 0.1 *N* arsenic trioxide and 0.005 *N* ceric-ammonium sulphate in the ratio of $As^{3+}/Ce^{4+} = 20$ were used. The optimum concentration of sulphuric acid in the reacting medium was found to be 2.5 *N*. Justification was given for the use of an ice bath (+ 4° C) and of brucine to obtain a stable colour. Evidence is provided for the possibility of plotting the calibration curve from log extinction against iodine concentration.

Zusammenfassung

Die optimalen Bedingungen für die Bestimmung von Ultramikromengen Jod mit Hilfe der Sandell-Kolthoff-Reaktion wurden ausgearbeitet. 1-m Natriumchloridlösung sowie 0,1-n arsenige Säure und 0,005-n Cer-Ammoniumsulfatlösung werden im Verhältnis $As^{3+} : Ce^{4+} = 20$ verwendet. Das Reaktionsgemisch soll an Schwefelsäure am besten 2,5-n sein. Die Verwendung eines Eisbades (+ 4° C) und von Brucin zur Erzielung einer konstanten Farbe wird begründet. Die Möglichkeit der Ermittlung einer Eichkurve durch Auftragung des Logarithmus der Extinktion gegen die Jodkonzentration wird gezeigt.

Résumé

On a mis au point les conditions optimales du dosage de quantités d'iode à l'échelle ultramicro par la réaction de *Sandell* et *Kolthoff*. On a utilisé des solutions de chlorure de sodium 1,0 *M*, d'anhydride arsénieux 0,1 *N* et de sulfate de cérium et d'ammonium 0,005 *N* dans le rapport $As^{3+}/Ce^{4+} = 20$. On a trouvé que la concentration optimale en acide sulfurique dans le milieu réagissant était de 2,5 *N*. On a justifié l'emploi d'un bain de glace (+ 4° C) et de la brucine pour l'obtention de la stabilité de la couleur. On a montré la possibilité de construire la courbe d'étalonnage à partir de la densité optique au lieu de la concentration en iodure.

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