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Determination of Trace Amounts of Iodide by an Inhibition Kinetic Spectrophotometric Method

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In this study, the reaction between Co(III)-EDTA and hypophosphite ion, catalyzed by Pd(II), was chosen as the indicator reaction. The inhibition kinetics of this catalytic reaction were investigated in the presence of iodide ion and the possibility of its analytical application was evaluated. Catalyzers other than $PdCl_2$ (Pt, Au and Ni salts) were assayed for the indicator reaction and it was observed that these catalysts have no effect on the reaction. The important variables that affected the reaction rate were investigated and the optimum conditions giving maximum sensitivity were established. The calibration graph, prepared following the inhibition kinetic method, gave a curve exhibiting a linear relationship (r = -0.9878) between the initial rate and iodide concentrations up to 35 ng.mL^{-1} I^-. Iodide was quantitatively be determined in the range 2-35 ng.mL$^{-1}I^-$ with a detection limit of 1.2 ng.mL$^{-1}I^-$ ($3S_b$/m criterion). The RSDs of the method (n = 5) for 7 and 14 ng.mL^{-1} are 1.19 and 0.81%, depending on the iodide concentration, respectively. The reaction was monitored spectrophotometrically by measuring the change in absorbance over time at 540 nm. Iodide in trace amounts had a strong inhibitory effect under optimum conditions. The possibility of the estimation of trace amounts of iodide based on its inhibitory effect was investigated. The method was applied to the determination of iodide in water, urine, table salt and some drug samples, and was compared with the modified Sandell-Kolthoff method. The determination of the iodide content of biological fluids is important both in malnutrition conditions and in metabolic and epidemiological studies of thyroid diseases. The main advantage of this proposed method for the determination of iodide in urine samples is that it does not necessitate an additional pretreatment step. The quantitative method developed, based on inhibition kinetics, is practical, fast and economical. For this reason, it is a technique open to research for the development of application fields (chemistry, biochemistry, environmental, pharmaceutical chemistry etc.).

Key Words: Co(III)-EDTA, inhibition kinetic, iodide, initial rate method and spectrophotometry.

Introduction

Iodine is an essential part of the thyroid hormones that play an important role in the development of brain function and cell growth. Iodine deficiency causes serious delays in neurological development. On the

other hand, an excess of iodine or iodide can cause goiter and hypothyroidism as well as hyperthyroidism; therefore, the determination of iodide in natural waters, foodstuffs and biological samples is important in environmental and biochemical terms. Not only a lack of iodine but also excessive iodine (>20 mg/day) in the diet may cause many disorders, including endemic goiter and hypothyroidism. Populations sufferring from iodine deficiency should be supported by more iodine, such as in the form of iodized salt, iodized oil and iodized bread. People and some older people who consume excess iodine suffer from thyroid disease who should consume no iodine and these should avoid excess iodine in their diet. That is why the iodine concentration range in the diet is very important for public health[1].

One method of protecting the population from iodine deficiency is the iodization of salt. Turkey is one country that suffers from iodine deficiency. The iodization of salt in Turkey has been mandatory since 1998 and the recommended iodine concentration is 50-70 mgKI/kg or 25-40 mgKIO_3/kg [2].

Several methods of iodine determination have been proposed, including selective electrodes[3], X-ray fluorescence (XRF)[4], inductively coupled plasma mass spectrometry (ICP-MS)[5,6] and atomic absorption spectrometry (AAS)[7]. Iodine has also been determined by catalytic methods[8,9]. There are main methods employed in this context. One is based on the redox reaction between cerium(IV) and arsenic(III), that was first demonstrated and exploited for the determination of iodide at the μg.mL^{-1} level by Sandell and Kolthoff [10,11]. Other authors later studied this reaction, especially the acid (nitric or sulfuric) used to adjust the pH and the mechanism of the reaction[12,13]. The other reaction is based on the catalytic action of iodide on the decomposition of the $FeSCN^{2+}$complex ion. This indicator reaction is characterized by an induction period, the length of which depends on the reagent concentration, pH and temperature[14,15].

The former reaction has been adopted as a standard method for iodide determination in natural and waste waters and in food and biological samples[16,18]. However, a high inter-laboratory relative standard deviation has frequently been reported for this method[16,18−20]. This might be partly attributed to the limitations of the method to quantitatively detect or tolerate iodate ions (IO_3^-) that are found in natural waters and/or formed during the ashing of food samples[16,17]. Therefore, a reverse-phase ion-pair liquid chromatographic method has replaced this method for iodide determination down to 20 ng.mL^{-1}, where iodide has been detected electrochemically with a silver working electrode at 0-50 mV [16]. However, the need for a low-cost or economical, rapid, more sensitive and selective method is still present.

A detailed study of the appropriate conditions for the inhibitory effect of iodide on the Pd(II)-catalyzed reduction of Co(III)-EDTA by the hypophosphite ion in a weak acid medium was performed. The reaction was monitored spectrophotometrically at the maximum wavelength of the Co(III)-EDTA complex (540 nm) by means of measuring the change in the absorbance over time with the tangent method or the initial rate method.

Experimental

Apparatus

Absorbance measurements were made on an analytically sensitive Jasco-UV/Visible 550 double beam spectrophotometer attached to a computer in a 1.0 cm quartz cuvette. The absorbance of the reaction mixtures was measured at 540 nm (at the wavelength at which the Co(III)-EDTA solution exhibited maximum ab-

sorbance). The absorbance-time curves (A = f(t)) can also be recorded in order to obtain the slope (tanα) with the same instrument.

A Grant LTD-6G model thermostatic bath (operating in a temperature range between –20 and 100 °C) was used to control the reaction temperature to a ±0.1 °C accuracy.

In additional, high precision micropipettes of 50, 500 and 1000 μL (Volac, UK) were used for handling or pipetting the solutions.

Reagents

Analytical reagent grade chemicals and doubly distilled water were used throughout the experiments.

Co(III)-EDTA stock solution, 0.04 M: 40 mL of 0.1 M Co(II) nitrate (Merck) and 40 mL of 0.1 M Na_2H_2EDTA (Merck) were pipetted into a 250 mL beaker. Then 2.4 g dipotassium peroxodisulfate (Merck) was added and the solution adjusted to pH 6 with ammonia solution (1+1, v/v, d:0.88 g/mL) and boiled gently for about 20 min in order to decompose the excess peroxodisulfate. The solution was made up to volume with doubly-distilled water in a 100 mL calibrated flask.

Sodium hypophosphite stock solution, 3 M: 7.95 g of $Na_2H_2PO_2.H_2O$ (Riedel-deHaen) was dissolved in water and diluted to 25 mL in a calibrated flask. This solution was prepared each week and stored in a dark bottle and place.

Palladium dichloride (Sigma), 5.0 x 10^{-3} M: prepared in 0.2 M HCl and stored in a dark bottle and place.

Standard iodide solution, 1000 mg.L^{-1}: prepared by dissolving 0.1308 g of potassium iodide (Merck), dried at 105 °C for 2 h, in water and dilution with water to 100 mL in a calibrated flask. Working solutions were prepared daily by appropriate dilution with water.

Citrate-phosphate-borate buffer solution, pH 2-12 (Geigy, 1963): solution A, 7.0 g of citric acid (Merck) was dissolved in water and diluted with water to 100 mL. Then 2.43 mL of H_3PO_4 (Merck, 85%, d = 1.7 g/mL) was diluted with water to 100 mL. The 2 solutions were mixed and then 343 mL of 1 M NaOH was diluted with water to 1 L. Solution B, 0.1 M HCl (Riedel de-Haen).

Potassium tetra chloro-platinate(II) stock solution, 1.0 x 10^{-3} M: K_2PtCl_6 (Ege University). The working solution was prepared daily.

Chloro auric acid stock solution, 1% (w/v): $HAuCl_4.4H_2O$ (Merck). The working solution was prepared daily.

Palladium nitrate stock solution, 1% (w/v): $Pd(NO_3)_2$ (BDH laboratory reagents, England) was prepared by dissolving diluted HNO_3.

Platinum dichloride stock solution, 7.5 x 10^{-4} M: 0.5 g $PtCl_2$ (Merck) was dissolved in HCl and diluted with water to 250 mL.

Ammonium hexachloroplatinate solution, 0.01 M: 0.12 g of $(NH_4)_2PtCl_6$ (synthesized by University of Ege, Faculty of Sciences, Department of Chemistry) was dissolved in 25 mL of 0.2 M HCl.

Standard potassium iodate stock and working solutions: the stock iodine calibrator (A) was prepared by dissolving 168.6 mg of KIO_3 (Merck) with water in a 100 mL volumetric flask, resulting in an iodine concentration of 7.87 x 10^{-3} M (1000 μg.mL^{-1} iodine). For stock B solution, 1.0 mL of stock A solution was diluted in 100 mL of water; the iodine concentration was 78.74 μmol.L^{-1} (10 μg.mL^{-1} iodine). The

sulfuric acid solution was 25 M, the ceric ammonium sulphate solution was 0.0158 M and the arsenious acid solution was 0.0253 M.

General procedures

Determination of the reaction rate

The indicator reaction was performed using the values in the literature without any inhibitor [21] and the rate of this reaction was monitored spectrophotometrically.

Then 0.2 mL of 0.04 M Co(III), 1.0 mL of CPB buffer and 0.3 mL of 5.0 x 10^{-5} M $PdCl_2$ were placed, in that order, in the spectrophotometric cells and diluted to 2.6 mL with water. The cells were placed in a thermostatic bath at 25 ± 0.1 °C for 10 min. Then 0.4 mL of 3 M sodium hypophosphite was added to the test cell and the spectrophotometer recorder was started. The final concentrations in the cell were as follows:

2.67 x 10^{-3} M Co(III), 0.4 M $H_2PO_2^-$, 0.57 μg.mL^{-1} Pd(II) and pH 3.2.

The kinetic study on the catalytical reaction with and without iodide as an inhibitor was performed by the initial rate method, where the initial slope of the reaction was measured as described by Yatsimirskii [22] and the slope of the curve (dA/dt = tanα) was taken as a measure of the initial reaction rate. The absorbance-time curves (the A = f(t) curve) were recorded at 540 nm in order to obtain the slope (tanα, min^{-1}) for each sample run. The calibration graph was obtained by plotting the change in the catalyzed reaction rate for the sample solution versus the standard iodide concentration as inhibitor.

For the determination of iodide in iodized table salt, water and urine samples, the samples were analyzed either directly by using a suitable aliquot of a sample solution or using a suitable aliquot of its diluted solution.

Results and Discussion

It was observed experimentally that iodide ion considerably decreased the catalytic effect of Pd(II) on the reaction of the Co(III)-EDTA/$H_2PO_2^-$ system in an acidic medium, so that it proceeded much slower in the presence of iodide. This reaction can be monitored spectrophotometrically by measuring the decrease in absorbance versus time as a function of time at 540 nm. The reaction rate of the catalyzed reaction decreased as the amount of iodide as inhibitor increased. The rate of decrease of the reaction rate (tanα) was suppressed by iodide ion in that the net reaction rate with a decreased slope is much smaller compared to the catalyzed reaction. The reaction is fast in the absence of iodide ion and slow in the presence of iodide ion.

The strong inhibitory effect of iodide on the reaction rate can be explained by the stable complex formation between Pd^{2+} and iodide ions (as $[PdI_4]^{2-}$), the latter replacing the chloride ions in $PdCl_2$. The formation of the $[PdI_4]^{2-}$ complex blocks the reduction of Pd^{2+}, or at least delays it. Therefore, trace amounts of iodide could easily be determined using this system due to the relation between the decrease in the reaction rate and the iodide concentration.

Effect of reaction variables on the inhibited reaction

To obtain the maximum sensitivity in the determination of iodide, we studied the effect of several variables on the rate of catalyzed and inhibited catalyzed reactions.

The effect of the Co(III) concentration on the catalyzed and inhibited catalyzed reactions was studied in the concentration range (0.65-6.5) x 10^{-3}M while the iodide concentration was kept constant at 10 ng.mL^{-1}. The influences of Co(III) concentration on the reaction rate and hence on the inhibition % for iodide were separately measured with the same method at varying Co(III) concentrations. The experiments were repeated 3 times for the same Co(III) concentrations and their average values were taken into consideration. Inhibition % variance was calculated with the following equation:

Inhibition % = (rate $_{withoutinh.}$-rate $_{withinh.}$) / rate $_{withoutinh.}$ x 100

The best result was obtained at 3.2 x 10^{-3}M of Co(III). This concentration had both the maximum percentage inhibition and the maximum sensitivity (the maximum difference between the reaction rates with and without iodide; $\Delta(\Delta A) = \Delta A_{cat.} - \Delta A_{inh.}$). When the concentration of Co(III) was more than 3.2×10^{-3} M, the inhibition % was decreased proportionally. Therefore, it was chosen as the optimum Co(III) concentration for the next studies (Figures.1 and 2).

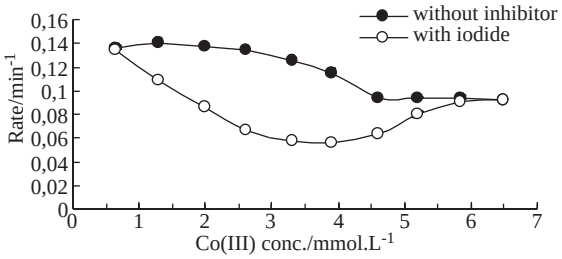

Figure 1. Influence of Co(III) concentration on catalyzed and inhibited catalyzed reaction rates with and without iodide. Conditions: 0.4 M, $H_2PO_2^-$; 0.57 μg.mL^{-1}, Pd(II); 3.2, pH; 10 ng.mL^{-1} I^- and 25 ± 0.1 °C at 540 nm.

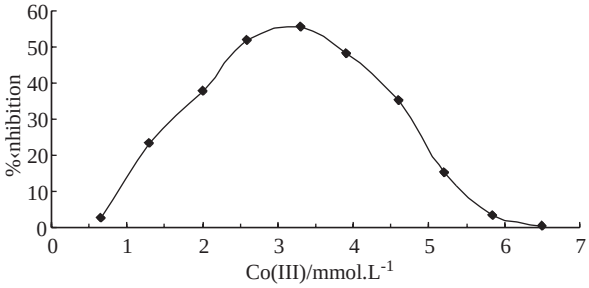

Figure 2. Influence of Co(III) concentration on the inhibition % in the presence of iodide as an inhibitor. Conditions: 0.4 M, $H_2PO_2^-$; 0.57 μg.mL^{-1}, Pd(II); 3.2, pH and 25 ± 0.1 °C at 540 nm.

The effect of the hypohosphite concentration on the catalyzed and inhibited catalyzed reaction rates was studied in the concentration range 0.1-1.0 M while the iodide concentration was kept constant at 10 ng.mL^{-1}. The experiments were repeated 3 times for the same hypophosphite concentrations and their average values were taken into consideration. The rate of sample reaction (or the rate of the inhibited catalyzed reaction) and the rate of blank reaction (or the rate of the catalyzed reaction) increased with increasing hypophosphite concentrations. However, the rate of sample reaction increased up to 0.4 M and at higher concentrations the reaction rate and inhibition % slowly decreased. In addition, above a 0.4 M hypophosphite concentration, the inhibition % is kept approximately constant; thus a concentration of 0.4 M was used as optimum concentration for the next studies (Figures 3 and 4).

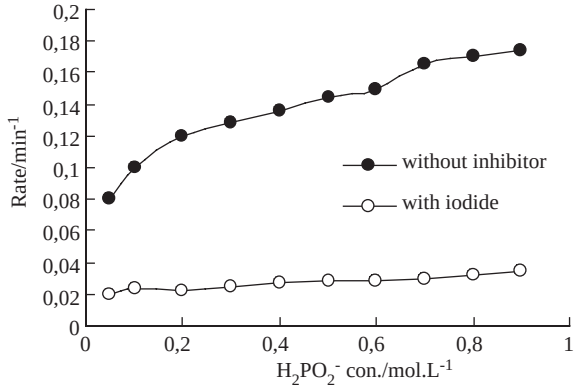

Figure 3. Influence of $H_2PO_2^-$ concentration on catalyzed and inhibited acatalyzed reaction rates with and without iodide. Conditions: 2.67 x 10^{-3} M Co(III); 0.57 μg.mL^{-1}, Pd(II); 3.2, pH; 10 ng.mL^{-1}I$^-$ and 25 $\pm$ 0.1 °C at 540 nm.

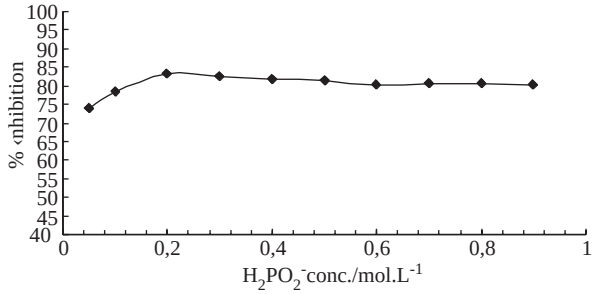

Figure 4. Influence of $H_2PO_2^-$ concentration on the inhibition % in the presence of iodide as an inhibitor. Conditions: 2.67 x 10^{-3}M, Co(III); 0.57 μg.mL^{-1}, Pd(II); 3.2, pH and 25 $\pm$ 0.1 °C at 540 nm.

The effect of pH on catalyzed and inhibited catalyzed reactions was studied in the pH range 2.0-5.4. Iodide concentration was kept constant at 10 ng.mL^{-1}. The rate of the catalyzed and inhibited catalyzed reactions increased with increasing pH values up to 3.2. At higher pH values the rate of the catalyzed and inhibited catalyzed reactions decreased significantly. Therefore, a pH value of 3.2 was selected as an optimum pH value in order to compromise between the sensitivity (inhibition %) and the reaction rate (Figures 5 and 6).

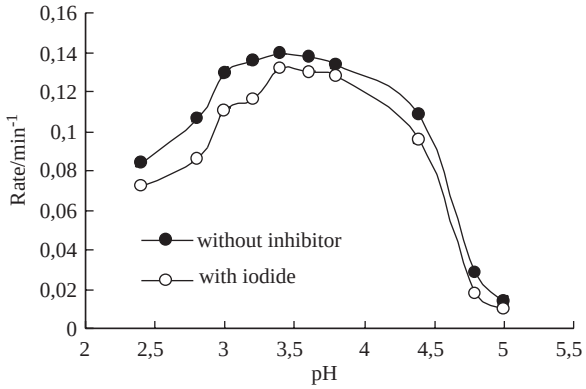

Figure 5. Influence of pH on catalyzed and inhibited catalyzed reaction rates with and without iodide. Conditions: 2.67 x 10^{-3}M, Co(III); 0.4 M, $H_2PO_2^-$; 0.57 μg.mL^{-1}, Pd(II); 10 ng.mL^{-1}I$^-$ and 25 $\pm$ 0.1 °C at 540 nm

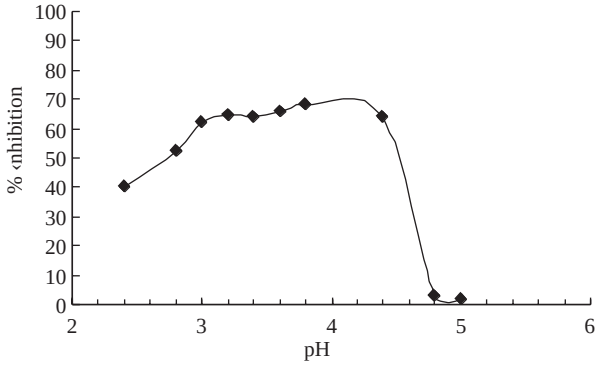

Figure 6. Influence of pH on the inhibition % in the presence of iodide as an inhibitor. Conditions: 2.67 x 10^{-3}M, Co(III); 0.4 M, $H_2PO_2^-$; 0.57 μg.mL^{-1}, Pd(II) and 25 ± 0.1 °C at 540 nm.

The effect of the Pd(II) concentration on the catalyzed and inhibited catalyzed reaction rate was studied in the concentration range 0.08-1.20 μmol.L^{-1}, while the iodide concentration was kept constant at 10 ng.mL^{-1}. The experiments were repeated for the same Pd(II) concentrations and their average values were taken into consideration. The rate of the catalyzed reaction increased strongly while the rate of the inhibited catalyzed reaction increased slowly in this concentration range. The best result was obtained at 0.33 μmol.L^{-1}Pd(II), which was chosen as the optimum concentration because this concentration had the maximum inhibition % (Figures 7 and 8).

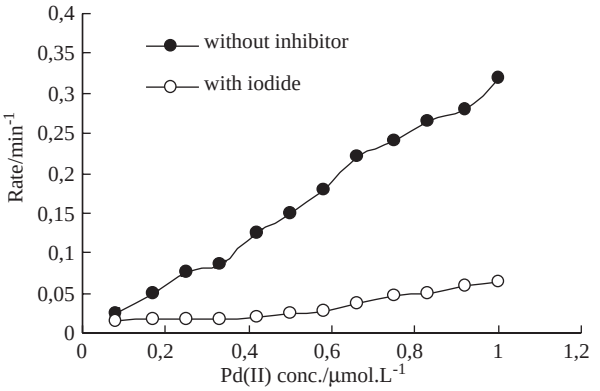

Figure 7. Influence of Pd(II) concentration on catalyzed and inhibited catalyzed reaction rates with and without iodide. Conditions: 2.67 x 10^{-3}M, Co(III); 0.4 M, $H_2PO_2^-$; 3.2, pH; 10 ng.mL^{-1}I$^-$ and 25 ± 0.1 °C at 540 nm.

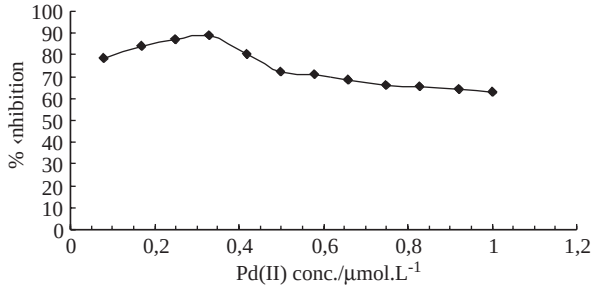

Figure 8. Influence of Pd(II) concentration on the inhibition % in the presence of iodide as an inhibitor. Conditions: 2.67 x 10^{-3}M, Co(III); 0.4 M, $H_2PO_2^-$; 3.2, pH and 25 ± 0.1 °C at 540 nm.

The effect of temperature on the initial rate of both the catalyzed and inhibited catalyzed reactions was studied in the range 10-55 °C at the optimum reagents concentration. The results are shown in Figure 9, which shows, that the rates of both the catalyzed and inhibited catalyzed reactions increase proportionally with increases in temperature. The results show that 25 °C is the best temperature since at higher temperatures the inhibition effect of iodide is reduced causing a large increase in the rate. Therefore, taking into consideration the laboratory conditions as well as the rate of reaction, 25 °C was selected as the optimum temperature.

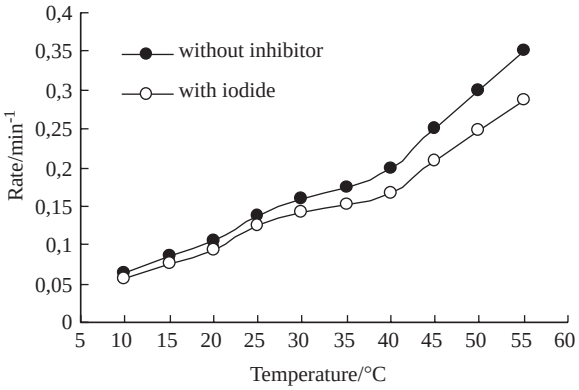

Figure 9. Influence of temperature on catalyzed and inhibited catalyzed reaction rates with and without iodide. Conditions: 2.67 x 10^{-3}M, Co(III); 0.4 M, $H_2PO_2^-$; 0.57 μg.mL^{-1}, Pd(II); 3.2, pH and 10 ng.mL^{-1}I$^-$ at 540 nm.

In additional, in order to obtain the activation energies of both of the reactions, the initial rates of the catalyzed and inhibited catalyzed reactions were measured with a temperature rise of 5 °C in the temperature range 20-45 °C and their lnk-1/T x 10^3 plots were drawn according to the Arrhenius equation ($k = Ae_a^{-E/RT}$). Afterwards, the corresponding activation energies were calculated from the slope of these plots. The results obtained from the temperature dependence of the initial reaction rates are 5.30 and 6.81 kcal.moL^{-1} with and without iodide as an inhibitor, respectively.

No attempt was made to maintain conditions of constant ionic strength as changes in ionic strength are concluded to have no significant effect on the inhibited catalyzed reaction rate.

Calibration

The calibration graph, which was prepared following the proposed method, gave a linear relationship (r = -0.9878) between the initial reaction rate and iodide concentration up to 35 ng.mL^{-1} iodide.

The least squares equation for the calibration graph is 100.tanα (min^{-1}) = 12.52-0.401 [I$^-$] where [I$^-$] is the iodide concentration expressed in ng.mL^{-1}.

A calibration plot changed linearly was obtained in the concentration range 2-35 ng.mL^{-1}I$^-$under the optimum conditions of all variables that affected the reaction rate (Co(III), 3.2 x 10^{-3} M; $H_2PO_2^-$, 0.4 M; Pd(II), 0.33 μg.mL^{-1}; pH, 3.2; without ionic strength and temperature, 25 ± 0.1 °C). The theoretical limit of the detection LOD = K.S_b/m was 1.2 ng.mL^{-1} of iodide, where K = 3, S_b is the standard deviation of blank signals and m is the slope of the calibration plot. The relative standard deviations for 5 replicate determinations of iodide were 1.19 and 0.81% for 7 and 14 ng.mL^{-1} of iodide, respectively.

Applications

In order to evaluate the analytical applicability of the inhibition kinetic method to the real samples, the proposed method was applied to the determination of iodide in 4 different samples; water, iodized table salt, urine and drug samples. To check the accuracy of the method, the known concentrations of iodide standard were directly spiked into the definite volumes of the samples or into samples prepared with dilution. The accuracy ratios of the analysis were checked by the procedure of standard iodide addition and compared to the results from the modified Sandell-Kolthoff method. In order to confirm whether they were quantitative and accurate, the measurements were repeated at least 5 times after standard iodide addition and the recovery of each method was separately determined. The results obtained by both the proposed inhibition kinetic method and the modified Sandell-Kolthoff method are shown in Tables 1 and 2.

These results show that there is a good agreement between the results obtained by the 2 methods. However, the direct determination of iodine in properly prepared dilutions of drug samples could not be accomplished by either method.

Table 1. Recoveries of iodide spiked into different samples with the inhibition kinetics method.

Sample	Added/ ng/mL	Found ± SD[a]/ ng/mL	RSD %	Recovery %
Tap water	25	25.20 ± 0.53	2.10	100.80
	35	34.07 ± 0.93	2.74	97.34
	50	48.34 ± 1.90	3.93	96.68
Urine	25	24.00 ± 1.18	4.94	96.00
	35	35.01 ± 0.10	0.28	100.03
	50	51.97 ± 1.01	1.94	103.94
Table salt	25	25.06 ± 0.15	0.61	100.24
	35	34.03 ± 1.10	3.25	97.23
	50	48.02 ± 2.22	4.63	96.04

[a]Mean and standard deviation of 5 replicate determinations.

Table 2. Recoveries of iodide spiked into different samples with the modified Sandell-Kolthoff method.

Sample	Added/ ng/mL	Found ± SD[a]/ ng/mL	RSD %	Recovery %
Tap water	25	25.05 ± 0.19	0.79	100.20
	35	34.99 ± 0.12	0.34	99.97
	50	48.99 ± 1.15	2.34	97.98
Urine	25	24.10 ± 1.01	4.18	96.40
	35	37.01 ± 2.25	6.09	105.74
	50	48.96 ± 1.17	2.40	97.92
Table salt	25	24.99 ± 0.18	0.74	99.96
	35	35.98 ± 1.11	3.08	102.80
	50	48.80 ± 1.39	2.85	97.60

[a]Mean and standard deviation of 5 replicate determinations.

Conclusions

The results of this study show that the Co(III)-EDTA-$H_2PO_2^-$-Pd(II) system can be successfully used for the quantitative determination of trace amounts of I^-.

The great advantage of this method that we introduce, as an alternative to the Sandell-Kolthoff method in iodide determination, is that no pretreatment steps are needed before measurement. In additional, a time-consuming alkaline ashing preparative procedure is necessary in order to apply the standard method.

Our method is more advantageous than the standard method because it is a simple, fast, practical and economical method operating with accessible reagents and equipment.

The inhibitory effect of iodide on the indicator reaction between Co(III)-EDTA and $H_2PO_2^-$ with Pd(II) catalyzed at pH 3.2 is very sensitive and the methods based on this indicator reaction have a detection limit as low as 1.2 ng.mL^{-1} of I^- and also an analytical working range of 2-35 ng.mL^{-1} of I^-. It is possible to determine the iodide at concentrations as low as 1.2 ng.mL^{-1}of I^- without any pre-concentration and pretreatment steps. Every effort was made to prevent possible interference due to the matrix effect. The interference effect was only controlled by the standard iodide addition method for the determination of the iodide contents of real samples. The proposed method is comparable with such other kinetic-catalytic methods and instrumental methods as NAA (0.1-0.2 μg.L^{-1}) [23,24], ICP-MS (1.0-9.0 μg.L^{-1}) [25,26], ICP-AES (40.0-470.0 μg.L^{-1}) [27,28] and IC (0.1-0.8 μg.L^{-1}) [29,30] with regard to simplicity, cheapness, detection limit, convenience and relative selectivity (in which the numerical values given in parentheses are the detection limits of each instrumental method). However, these instrumental methods have also several disadvantages, such as very high costs and the need for pre-concentration and/or separation.

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References

1. World Health Organisation (WHO), **Trace Elements in Nutrition and Health**, 49-71, (1996).
2. **Official Journal of the Turkish Republic**, 9 June 1998, 23397, 29.
3. S. Alegret, A. Florido, J.L.F.C. Lima and A.A.S.C. Machado, **Talanta, 8,** 825 (1989).
4. E.A. Crecelins, **Anal. Chem., 47**, 2034 (1975).
5. H. Vanhoe, F. van Allemeersch, J. Versieck and R. Dams, **Analyst, 118**, 1015, (1993)
6. P. Schrame and S. Hasse, **Microchim. Acta**, 116, 205 (1994).
7. G.E. Kirkbright and P.J. Wilson, **At. Absorp. Newsl., 13**, 140 (1974).
8. A. Tanaka, K. Obata and T. Deguchi, **Anal. Sci., 2**, 197 (1986).
9. B. Liang, S. Kawakuba, M. Iwatsuki and T. Fukasawa, **Anal. Chim. Acta, 282**, 87 (1993).
10. E.B. Sandell and I.M. Kolthoff**, J. Am. Soc., 56**, 1426 (1934).
11. E.B. Sandell and I.M. Kolthoff, **Microchim. Acta, 4**, 6 (1937).

12. G. Knapp and H. Spitzy, **Talanta, 16**, 1353 (1969).
13. Z. Zhu and Z. Gu, **Analyst, 118**, 105 (1993).
14. P.A. Rodriguez and H.L. Pardue, **Anal. Chem., 41**, 1369 (1969).
15. R.E. Moxon and E.J. Dixon, **Analyst, 118**, 105, 344 (1980).
16. K. Helrich (ed.) **Official Methods of Analysis of the Association of Official Analytical Chemists**, 15^{th} edn. AOAC, Arlington, VA, 4^{th} Suppl., 192, (1990).
17. E. Greenberg, LS. Clescieri and AD. Eaton (eds) **Standard Methods for the Examination of Water and Waste-Water**, 19^{th} edn. American Public Health Association, Washington, DC, (1995).
18. M.M. Heckwan, **J. AOAC. Int**., 62: 1045, (1979).
19. R. Pedriali, E. Giuliani, A. Margutti and E. Degli-Uberti, **Ann. Chim-Rome** 87: 449, (1997).
20. J.L. Garvin, N.S. Rosenholtz and A. Abdollahi, **J. Food Sci. 59**: 1135, (1994).
21. M.S. Garcia, C. Sanchez-Pedreno and M.I. Albero, **Analyst, 115**, 989-992, (1990).
22. K.B. Yatsimirskii, **Kinetic Methods of Analysis**, Pergamon, Oxford, (1966).
23. E.A. Arafa, F.H. Beshawa, A.I. Saleh and Das Ha, **J. Trace Microprobe T., 18**: 137, (2000).
24. X.L. Hou, H. Dahlguard, B. Rietz, U. Jacopsen, S.P. Nielsen and A. Arkinog, **Anal. Chem., 71**: 2745, (1999).
25. L.F. Sanchez and J. Szpunar, **J. Anal. Atom. Spectrom., 14**: 1697, (1999).
26. E.H. Larsen, P. Knuthsen and M. Hansen, **J. Anal. Atom. Spectrom., 14**: 41, (1999).
27. K.A. Anderson and P. Markowski, **J. AOAC. Int.**, 83: 225, (2000).
28. K. Kregel-Rothense, U. Richter and P. Heitland, **J. Anal. Atom. Spectrom., 14**: 699, (1999).
29. W. Hu, P.R. Haddad, K. Hasebe, K. Tanaka, P. Tong and C. Khoo, **Anal. Chem., 71**: 1617, (1999).
30. Y. Bichsel and U. von-Gunten, **Anal. Chem., 71**: 34, (1999).