Kinetic determination of Se(IV) in pharmaceutical samples

S. S. MITIĆ, J. I. VUČETIĆ#, S. M. MILETIĆ and D. A. KOSTIĆ

*Department of Chemistry, University of Niš, YU-18000 Niš, and Faculty of Chemistry, University of Belgrade, YU-11001 Belgrade, Yugoslavia

(Received 9 December 1999, revised 1 April 2000)

A kinetic method is described for the determination of Se(IV) based on its inhibiting effect on the Fe(III) catalysis of the oxidation of C₆H₅COONa with hydrogen peroxide. The detection limit is 0.06 µg cm⁻³. The relative error ranges between 2.1 and 9.5% for the concentration interval 0.26 to 2.6 µg cm⁻³. Kinetic equations are proposed for the investigated process. The effects of certain foreign ions upon the reaction rate were determined in order to assess the selectivity of the method. The method was applied for the determination of Se(IV) in pharmaceutical samples.

Keywords: kinetic method, Se(IV), pharmaceutical samples.

INTRODUCTION

Selenium is an essential microelement. Investigations have been performed to determine supplementation with a moderate amount of selenium in the form of Se enriched yeast has an effect on lipid peroxidation in the tissues of rats after oxidative stress experimentally induced by ionising radiation. The results showed that even 150 days after exposure to radiation stress Se intake had a positive effect in decreasing lipid peroxidation as the malonaldehyde (MDA) concentration in rat tissues, especially in the front and hear brain. Se supplementation was also shown to decrease the natural elevation of MDA that appears in the brain with ageing.¹,²

The average dietary intake of Se with diet by young healthy population of Serbia during the 1996–1997 period confirmed that average daily intake of Se is low (26 µg).³

Micro amounts of selenium in hyman hair were satisfactorily determined by using a spectrophotometric method based on the catalytic effect of Se(IV) on the oxidation of Neutral Red with KIO₄. The sensitivity of the method is 8 ng/cm³.⁴

The method for the determination of Se(IV) in mineral water is based on the action of Se(IV) upon the oxidation of Nile Blue A by hydrogen peroxide in the

# Serbian Chemical Society active member.
presence of phosphate buffer (pH 10.8) in ethanolic solution. The sensitivity of the method is 0.95×10⁻² ng/cm³.⁵

Micro amounts of selenium in health care products were determined by monitoring photometrically the decolorization of Methyl Orange at 525 nm, in the oxidation of hydrazine with KBrO₃. The sensitivity of the method is 0.5 µg/cm³.⁶

A new kinetic method for the determination of Se(IV), with a sensitivity of 0.26 µg cm⁻³, is described in this paper. The oxidation of C₆H₅COONa with hydrogen peroxide in an acetic acid solution gives a colored product. The reaction is catalysed by traces of Fe(III). We have observed that small amounts of Se(IV) strongly inhibit the catalysis of this reaction by Fe(III). The rate of the reaction is inversely proportional to the Se(IV) concentration. This fact was used as the basis of a kinetic method for the determination of ultramicro amounts of Se(IV).

EXPERIMENTAL

Apparatus

A spectrophotometric method was used for following the investigated reaction rate. The dependence of the absorbance (A) on time (t) was measured using a Perkin-Elmer Lambda 15 spectrophotometer, connected to a thermostating bath. The pH was measured by means of a radiometer PHM 29b pH meter and a combined glass-calomel electrode, GK 2311C. The solutions were thermostated at 25±0.1 °C before the beginning of the reaction.

Reagents

The stock FeCl₃ solution (1×10⁻³ mol dm⁻³) was prepared by dissolving FeCl₃ in water. The C₆H₅COONa solution (1×10⁻³ mol dm⁻³) was prepared by dissolving C₆H₅COONa in water. The acetic acid solution (1 mol dm⁻³) was prepared from 99.0 % glacial acetic acid. The hydrogen peroxide solution (1 mol dm⁻³) was prepared from the 34 % reagent. The SeCl₄ (1×10⁻³ mol dm⁻³) was prepared by dissolving SeCl₄ in water.

All chemicals were of analytical reagent grade and were supplied by Merck unless indicated otherwise. The solutions were made using deionized water. All the stock solutions were stored in polyethylene containers. The working solutions of Fe(III) and H₂O₂ were prepared immediately before use.

All the polyethylene containers and the glassware used were cleaned in aqueous HCl (1:1) and then thoroughly rinsed with deionized water.

Procedure

A measured amount of hydrogen peroxide was stored in one compartment of a special vessel, the Fe(III) solution was placed in the second compartment, C₆H₅COONa in the third compartment and acetic acid (acetic acid and Se(IV)) and water (up to a total volume of 15 cm³) in the fourth compartment. The spectrophotometer cell was rinsed well and filled with the solution. The absorbance at 540 nm was measured every 30 s over a period of 5–8 min after the addition of hydrogen peroxide. The measurement were made at 25±0.1 °C.

RESULTS AND DISCUSSION

Kinetic studies

A differential variant of the tangent method was used for processing the kinetic data, because a linear correlation exists between the absorbance and time dur-
ing the first 5 to 8 min after the addition of hydrogen peroxide. The reaction rate was followed by the change in the values of the tangent of the angle (tan α) of the slope of the linear part of the kinetic curve to the abscissa in the co-ordinates A−t, because \( \tan \alpha = \Delta A/\Delta t \).

In order to determine the lowest possible determinable concentration of Se(IV), the conditions needed to be optimised. Therefore, the dependencies of the rates of both the catalytic and the inhibited reactions on the concentration of each of the reactants were determined.

![Graphs](image)

**Fig. 1.** Dependence of the rate of the catalysed 1) and inhibited 2) reaction on the acetic acid concentration. Initial concentrations: \( c_{\text{CH}_3\text{COONa}} = 2\times10^{-4} \text{ mol dm}^{-3} \), \( c_{\text{H}_2\text{O}_2} = 0.266 \text{ mol dm}^{-3} \), \( c_{\text{Fe(II)}} = 2 \times 10^{-4} \text{ mol dm}^{-3} \), \( c_{\text{Se(IV)}} = 5.2 \mu\text{g cm}^{-3} \), \( T = 295.5 \text{ K} \).

**Fig. 2.** Dependence of the rate of the catalysed 1) and inhibited 2) reaction on the \( \text{C}_6\text{H}_5\text{COONa} \) concentration. Initial concentrations: \( c_{\text{Fe(II)}} = 2 \times 10^{-4} \text{ mol dm}^{-3} \), \( c_{\text{CH}_3\text{COOH}} = 2 \times 10^{-2} \text{ mol dm}^{-3} \), \( c_{\text{H}_2\text{O}_2} = 0.266 \text{ mol dm}^{-3} \), \( c_{\text{Se(IV)}} = 5.2 \mu\text{g cm}^{-3} \), \( T = 295.5 \text{ K} \).

Figure 1 shows the influence of pH on the rate of both reactions. It can be seen that the greatest difference between the reaction rates occurs at \( c_{\text{CH}_3\text{COOH}} = 6.67 \times 10^{-3} \text{ mol dm}^{-3} \), when Se(IV) maximally decreases the catalytic reaction rate. For further work an acetic acid concentration of \( 2 \times 10^{-2} \text{ mol dm}^{-3} \) was selected.

The dependence of \( \tan \alpha \) on the \( \text{C}_6\text{H}_5\text{COONa} \) concentration is shown in Fig. 2, which shows that the difference in the rates of the inhibited and catalytic reactions increases with increasing \( \text{C}_6\text{H}_5\text{COONa} \) concentration. For further work a \( \text{C}_6\text{H}_5\text{COONa} \) concentration of \( 3.33 \times 10^{-4} \text{ mol dm}^{-3} \) was selected.

The correlation between \( \tan \alpha \) and the Fe(III) concentration is shown in Fig. 3. Both reactions are first order with respect to the Fe(III) concentration. A Fe(III) concentration of \( 2 \times 10^{-4} \text{ mol dm}^{-3} \) was selected, for further work, because at higher concentrations the linear part of the kinetic curve \( (A−t) \) is rather short.
Fig. 3. Dependence of the rate of the catalysed 1) and inhibited 2) reaction on the Fe(III) concentration. Initial concentrations: $c_{\text{CH}_3\text{COOH}} = 2 \times 10^{-2}$ mol dm$^{-3}$, $c_{\text{H}_2\text{O}_2} = 0.266$ mol dm$^{-3}$, $c_{\text{C}_6\text{H}_5\text{COONa}} = 3.3 \times 10^{-4}$ mol dm$^{-3}$, $c_{\text{Se(IV)}} = 5.2 \mu$g cm$^{-3}$, $T = 295.5$ K.

The dependence of the reaction rates on the concentration of H$_2$O$_2$ is shown in Fig. 4, in which it can be seen that the inhibited reaction is first order with respect to the H$_2$O$_2$ concentration, whereas the catalytic reaction is only first order with respect to the

Fig. 5. Dependence of the rate of the catalysed 1) and inhibited 2) reaction on the Se(IV) concentration. Initial concentrations: $c_{\text{CH}_3\text{COOH}} = 2 \times 10^{-2}$ mol dm$^{-3}$, $c_{\text{Fe(III)}} = 2 \times 10^{-4}$ mol dm$^{-3}$, $c_{\text{C}_6\text{H}_5\text{COONa}} = 3.3 \times 10^{-4}$ mol dm$^{-3}$, $c_{\text{H}_2\text{O}_2} = 0.266$ mol dm$^{-3}$. 1) $T = 295.5$ K; 2) $T = 298.0$ K; 3) $T = 300.5$ K.
H₂O₂ concentration up to 0.266 mol cm⁻³ and negative first order at higher concentrations. A H₂O₂ concentration of 0.266 mol dm⁻³ was selected for further work.

Under the optimal reaction conditions (c₁H₂O₂ = 0.266 mol dm⁻³, c₅CH₅COONa = 3.33×10⁻⁴ mol dm⁻³, cFe(III) = 2×10⁻⁴ mol dm⁻³, c₇CH₃COOH = 2×10⁻² mol cm⁻³) the Se(IV) concentration was varied from 0.26 to 2.6 μg cm⁻³.

Three calibration lines, obtained at different temperatures, are shown in Fig. 5, which can be used for the determination of the Se(IV) concentration in the interval mentioned.

The following kinetic equations for the investigated process were deduced on the basis of the graphic correlations obtained.

For the catalysed reaction:

\[ \frac{dc}{dt} = k_1 \times c^{-1}_{CH₃COOH} \times c_{C₅H₅COONa} \times c_{Fe(III)} \times c_{H₂O₂} \]

were \( k_1 \) is a constant proportional to the rate constant of the catalysed reaction.

For the inhibited reaction:

\[ \frac{dc}{dt} = k_2 \times c^{-1}_{CH₃COOH} \times c_{C₅H₅COONa} \times c_{Fe(III)} \times c_{H₂O₂} \times c^{-1}_{Se(IV)} \]

were \( k_2 \) is constant proportional to the rate constant of the inhibited reaction.

On the basis of these equations, the rate constants for the inhibited and catalysed reactions were calculated.

A linear relationship between the logarithm of the rate constant and the reciprocal of the absolute temperature was found for the inhibited as well as for the catalysed reaction. The activation energies were found to be 15.8 kJ mol⁻¹ for the inhibited reaction and 10.0 kJ mol⁻¹ for catalysed reaction.

The minimum concentration of Se(IV) which can be determined by this method may be calculated by the method given by Perez-Bendito and Silva.⁸ The detection limit was found to be 0.06 μg cm⁻³.

| TABLE I. Accuracy and precision of the Se(IV) determinations |
|-----------------|-----------------|--------|-----------------|-----------------|-----------------|-----------------|
| Taken \( × 10^7 \) g cm⁻³ | Found \( \times 10^7 \) g cm⁻³ | \( n \) | \( S \times 10^8 \) g cm⁻³ | \( G/\% \) | \( (x-\mu) \times 100/\mu/ \% \) |
| 2.6 | 2.66 | 5 | 2.06 | 9.5 | +2.3 |
| 13.0 | 12.7 | 5 | 4.18 | 4.08 | -2.3 |
| 26.0 | 26.2 | 5 | 4.47 | 2.11 | +0.76 |

\( x \) - Mean value; \( \mu \) - true value; \( n \) - number of determinations; \( S \) - standard deviation; \( G \) - relative error (= 100 \( t \) \( s/x \) \( \sqrt{n} \), where \( n = 5 \) and \( t \) is Student's \( t \) for 95% confidence)

The accuracy and precision of the measurements are presented in Table I. The relative error ranges from 2.1 to 9.5 % for Se(IV) concentrations from 0.26 to 2.6 μg cm⁻³.

To assess the selectivity of the method, the influence of several foreign ions on the rate of the inhibited reaction rates was studied at a constant Se(IV) concentra-
tion of 1.3 μg cm⁻³ (Table II). It may be seen that SCN⁻ and C₂O₄²⁻ in a 1:1 ratio with Se(IV) interfere with the reaction. The other investigated ions have practically no influence on the determination of Se(IV) by this method.

**TABLE II. Tolerance levels of interferents in the kinetic determination of 1.3 μg cm⁻³ Se(IV) using the optimum conditions**

<table>
<thead>
<tr>
<th>Tolerance level cION/cSe(IV)</th>
<th>Ion added</th>
</tr>
</thead>
<tbody>
<tr>
<td>10²</td>
<td>Na⁺, K⁺, Ca(II), Mg(II), Cl⁻</td>
</tr>
<tr>
<td>10</td>
<td>NO₃⁻, SO₄²⁻, Sr(II),</td>
</tr>
<tr>
<td>1</td>
<td>CO₃²⁻, HCO₃⁻, PO₄³⁻, ClO₄⁻, Se(IV)</td>
</tr>
<tr>
<td></td>
<td>Zn(II), Cd(II), Co(II), Pb(II)</td>
</tr>
<tr>
<td></td>
<td>SCN⁻, C₂O₄²⁻-inhibited</td>
</tr>
</tbody>
</table>

**Determination of Se(IV) in pharmaceutical preparations**

The developed method was directly applied to the determination of Se(IV) in the following pharmaceutical preparations: a) “Oligogal-Se” tablets (ICN Galenika) and b) “Coenzyme-Q” tablets.

**Procedure:** The pharmaceutical samples for analysis were prepared by the following procedure. To the mass of five capsule add 5 cm³ of a mixture (2:1) conc. HNO₃ and HClO₄. The mixture was heated until the evolution of nitrogen oxides ceases and the white smoke of HClO₄ developed. The procedure was repeated after addition of a further 2 cm³ of acidic mixture to the sample. Add 2 cm³ of mixture to previous mixture and repeat the proceeding. The obtained solution must be colourless after complete dissolution of the tablets. A long in the employed reaction heating time was required because Se(IV) can be oxidized to Se(VI) which is inactive. The adjusted pH was between 5.5 and 6.0. The obtained solution was diluted with distilled water to the predetermined volume.

**TABLE III. Determination of Se(IV)/(mg/tablet) in pharmaceutical preparations**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Kinetic method</th>
<th>Rel. st. dev.</th>
<th>AAS method</th>
<th>Specified on the by manufactures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligogal-Se</td>
<td>0.098ᵃ</td>
<td>0.62ᵇ</td>
<td>0.975</td>
<td>0.1</td>
</tr>
<tr>
<td>Coenzyme-Q</td>
<td>0.972</td>
<td>0.64</td>
<td>0.987</td>
<td>0.1</td>
</tr>
</tbody>
</table>

ᵃMean for n = 5;ᵇStandard deviation

The tangent method was also applied to determine Se(IV) in the two pharmaceutical preparations specified above, the results being shown in Table III. The reproducibility was very good. The results obtained by the proposed method also agreed well with the values claimed on the labels.
ИЗВОД

КИНЕТИЧКО ОДРЕЂИВАЊЕ Se(IV) У ФАРМАЦЕУТСКИМ УЗОРЦИМА

С. С. МИТИЋ1, Ј. Ј. ИВЧЕТИЋ1, С. М. МИЛЕТИЋ2 и Д. А. КОСТИЋ2

1Филозофски факултет, С. Г. Хемија, Универзитет у Нишу, 18000 Ниш и 2Хемијски факултет Универзитета у Београду, 11000 Београд

Предложена је кинетичка метода за одређивање Se(IV) на бази инхибиторног дејства у реакцији оксидације натријумбензоата водоник-пероксидом која је катализована са гвожђем(III). Гранција детекције методе је 0,06 µg cm⁻³. Релативна грешка се креће од 2,1 до 9,5 % за интервал концентрација 0,26 до 2,6 µg cm⁻³. Предложене су кинетичке јединице за проучавани процес. Ради оцене селективности методе испитан је утицај извесног броја страних јона на брзину реакције. Метода је примењена за одређивање трагова Se(IV) у фармацеутским препаратима.


REFERENCES

1. Ј. Джуић, О. Јовановић-Станков, М. Мандић, М. Демајо, М. Врвић, Biol. Trace Element Res. 33 (1992) 197