
Junichi Odo,*† Yu Inomata,† Haruhiko Takeya,** Setsuko Miyanari,** and Hitoshi Kumagai**

*Department of Biological Chemistry, Faculty of Science, Okayama University of Science, Ridai-cho, Okayama 700-0005, Japan
**Chemical Technology Laboratory, Cosmo Research Institute, Satte, Saitama 340-0193, Japan

An iron(III) complex of thiacalix[4]arenetetrasulfonate on a modified anion-exchanger (Fe³⁺-TCAS₄₋₅₀₀) has shown high peroxidase-like activity at pH 5 – 6 for the reaction of quinoid-dye formation between 3-methyl-2-benzothiazolinone hydrazone and \( N \)-(3-sulfopropyl)aniline in the presence of hydrogen peroxide. Utilizing the peroxidase-like activity of Fe³⁺-TCAS₄₋₅₀₀ for this reaction, a method using Fe³⁺-TCAS₄₋₅₀₀ was applied for the spectrophotometric determination of hydrogen peroxide. The calibration curve by the method using Fe³⁺-TCAS₄₋₅₀₀ was linear over the range from 1 to 10 µg of hydrogen peroxide in a 1 ml sample solution. The apparent molar absorptivity for hydrogen peroxide was \( 2.4 \times 10^4 \) l mol\(^{-1}\) cm\(^{-1}\), which was about 80% of that by peroxidase under the same conditions. This determination method of hydrogen peroxide using Fe³⁺-TCAS₄₋₅₀₀ was applied for the determination of glucose in diluted normal and abnormal control serum I and II.

(Received March 19, 2001; Accepted October 9, 2001)

Calix[n]arenes are of much interest because of their high selectivity and specificity for forming host-guest complexes.²,³ In analytical chemistry, various calix[n]arene derivatives have been developed by modifying either the upper or lower rim in order to apply them as analytical reagents for separations⁴-⁷ and sensors⁸-¹² of various ions, molecules, and so on. It has been shown that recently developed thiacalix[n]arenes have a very interesting characteristic: an ability to form very stable metal complexes without modifying the upper and/or lower rims.¹³ Accordingly, much attention has been focused on the activity of thiacalix[n]arenes and their metal complexes. However, calix[n]arenes as effective analytical reagents with enzyme-like activity have not yet been developed.

On the other hand, in clinical analyses, various enzymes were frequently utilized for determining trace amounts of some vital compounds in numerous components of vital fluids with high selectivity and specificity.¹⁴ However, these enzymes in clinical analyses generally have some disadvantages regarding stability, purity, handling and storage. To improve these disadvantages, many artificial mimetic instead of these enzymes has been developed.¹⁵-¹⁷ However, most of them have not always been developed for the purpose of analytical reagents for clinical analyses, although they have exhibited high activities as effective catalysts. So far, only a few artificial mimetic have been developed.¹⁸,¹⁹ to the best of our knowledge, which were easily prepared and showed high enzyme-like activities. Moreover, they were actually usable instead of acting as enzymes in clinical analyses. As an example, an anion-exchanger modified with Fe³⁺-thiacalix[4]arenetetrasulfonate (Fe³⁺-TCAS₄₋₅₀₀) has recently been developed.¹⁹ However, the methods using Fe³⁺-TCAS₄₋₅₀₀ and other artificial mimetic¹⁸ were still not sufficient for determining vital compounds with regard to the sensitivity and/or reaction conditions, based on the concept that the amount of the vital sample (especially blood) should be minimized in clinical analyses. To improve this disadvantage, it is necessary to develop a method with higher sensitivity; that is, the higher is the sensitivity of method, the greater are the amounts of samples that can be minimized.

A previous method using Fe³⁺-TCAS₄₋₅₀₀,¹⁹ which we recently developed for determining H₂O₂ and glucose in blood, still has some disadvantages regarding its sensitivity and reaction conditions; its sensitivity is still not sufficient (apparent molar absorptivity: \( 3 \times 10^3 \) l mol\(^{-1}\) cm\(^{-1}\)), and its reaction solution must be adjusted to be strongly alkaline (pH 10 – 11) and to be incubated for a relatively long time (60 min). In this study, in

Scheme 1  Color reactions (1) and (2).
an attempt to improve especially the low sensitivity of the method using Fe$^{3+}$-TCAS$_{A}$-500, the activity of Fe$^{3+}$-TCAS$_{A}$-500 for the color reaction (2) between 3-methyl-2-benzothiazolinone hydrazone (MBTH) and N-(3-sulfopropyl)aniline (HALPS) in the presence of H$_2$O$_2$, instead of color reaction (1) between 4-aminoantipyrine (4-AAP) and phenol used in the previous method, was investigated. Moreover, the activity of Fe$^{3+}$-TCAS$_{A}$-500 was applied to the determination of H$_2$O$_2$ and serum glucose using color reaction (2).

In clinical analyses, for the determination of H$_2$O$_2$ and vital compounds, such as glucose and uric acid, the color reactions between 4-AAP and phenol or between MBTH and phenol were previously used in the presence of H$_2$O$_2$ and peroxidase. However, at present, some modified Trinder’s reagents instead of phenol are frequently used, because of the high molar absorptivity and preferable wavelength for measuring the quinoid dye produced through the color reaction. In this study, HALPS of commercially available Trinder’s reagents was chosen because of the highest molar absorptivity and preferable wavelength for measuring the quinoid dye produced in combination with MBTH in the presence of H$_2$O$_2$.

**Experimental**

**Reagents and apparatus**

Sodium thiacalix[4]arenetetrasulfonate (TCAS, Fig. 1) was prepared according to methods described in the literature. DEAE cellulofine A-500 (an anion-exchanger of cellulose-type with diethylaminoethyl groups), purchased from Seikagaku Kogyo Co., was washed with water several times and dried over P$_2$O$_5$ under reduced pressure. Peroxidase (POD, from horseradish), 3-methyl-2-benzothiazolinone hydrazone (MBTH), and control serum I (normal) and II (abnormal) were purchased from Wako Pure Chemical Industries Co. Glucose oxidase (GOD, Aspergillus niger) and N-(3-sulfopropyl)aniline (HALPS) were from Dojindo Laboratories and Sigma Chemical Co., respectively. All other chemicals were of either analytical or reagent grade and were used without further purification.

The anion-exchanger modified with Fe$^{3+}$-TCAS (Fe$^{3+}$-TCAS$_{A}$-500) was prepared according to method A, described in the literature. The modified anion-exchanger (Fe$^{3+}$-TCAS$_{A}$-500) contained 100 $\mu$mol of Fe$^{3+}$-TCAS per 1 g of dry ion-exchanger.

The absorption spectra and absorbances were recorded on a Shimadzu UV-1600 PC double-beam spectrophotometer with a 10 mm quartz cell.

**Procedure**

Method using the MBTH-HALPS system for H$_2$O$_2$: The determination of H$_2$O$_2$ by this method was carried out by measuring the absorbance at 582 nm of the quinoid dye produced through color reaction (2). Fe$^{3+}$-TCAS$_{A}$-500 (20 mg, 100 $\mu$g/ml) was added to a mixture containing the sample solution (1.0 ml, 1 – 10 $\mu$g of H$_2$O$_2$) and the reagent solution A (5.0 ml); the mixture was incubated at room temperature for 30 min. After Fe$^{3+}$-TCAS$_{A}$-500 was filtered off, the absorbance at 582 nm of the supernatant was measured against the reagent blank solution.

In a method using POD instead of Fe$^{3+}$-TCAS$_{A}$-500 as a reference, a mixture of the sample solution (1.0 ml), POD solution (0.5 ml, 25 U/ml) and reagent solution B (4.5 ml) was incubated at room temperature for 30 min. The absorbance at 582 nm of this reaction solution was measured against the reagent blank solution.

Reagent solutions A and B, which were used in these methods, consisted of a 1:1:3 and 1:1:2.5 (v/v) mixture of 0.1 M sodium acetate–0.1 M acetic acid for pH 2 – 5, 0.1 M KH$_2$PO$_4$–0.05 M Na$_2$B$_4$O$_7$ for pH 5 was used for the reagent solutions A and B.

In both methods for glucose, a 0.1 M acetate buffer solution of pH 5 was used for the reagent solutions A and B.

Fe$^{3+}$-TCAS$_{A}$-500 (20 mg) was added to a mixture containing the sample solution (0.5 ml, 5 – 50 $\mu$g of glucose), the GOD solution (0.5 ml, 32 U/ml) and the reagent solution A (5.0 ml); the mixture was incubated at room temperature for 30 min. After Fe$^{3+}$-TCAS$_{A}$-500 was filtered off, the absorbance at 582 nm of the supernatant was measured against the reagent blank solution.

A method using POD instead of Fe$^{3+}$-TCAS$_{A}$-500 as the reference, a mixture of the sample solution (0.5 ml), the GOD solution (0.5 ml, 32 U/ml), the POD solution (0.5 ml, 25 U/ml) and the reagent solution B (4.5 ml) was incubated at room temperature for 30 min. The absorbance at 582 nm of this reaction solution was measured against the reagent blank solution.

In both methods for glucose, a 0.1 M acetate buffer solution of pH 5 was used for the reagent solutions A and B.

**Results and Discussion**

The activity of Fe$^{3+}$-TCAS$_{A}$-500 for color reaction (2)

In order to elucidate whether Fe$^{3+}$-TCAS$_{A}$-500 will show activity for color reaction (2) or not, the peroxidase-like activity...
of Fe⁴⁺-TCAS₅₀₀ was evaluated by the method using the MBTH-HALPS system for 7 µg of H₂O₂ in buffer solutions of various pH. Figure 3 shows the absorbances at 582 nm of the quinoid dye produced through color reaction (2) by Fe⁴⁺-TCAS₅₀₀ at each pH. In the previous method,⁹ Fe⁴⁺-TCAS₅₀₀ exhibited high activity in alkaline buffer solutions (pH 10 – 11) for color reaction (1) using 4-AAP and phenol. However, for color reaction (2) in this study, Fe⁴⁺-TCAS₅₀₀ exhibited high activity in acidic buffer solutions, especially at pH 5 – 6. Accordingly, because the optimum pH for the method by MBTH-HALPS system is closer to neutral than that for the previous method, the method using the MBTH-HALPS system is better than that using the 4-AAP-phenol system for preparing the reaction solution.

As previously described, thiacalix[n]arenes have the characteristic to form very stable metal complexes without any modification of their upper and/or lower rims,¹³ because the –S– of the epithio groups of thiacalix[n]arenes is able to coordinate to metal ions. In the case of TCAS, not only the –S– of the epithio groups and the phenolic oxygen atoms, but also the –SO₃⁻ groups of TCAS, may coordinate to the Fe⁴⁺ ion. However, the –SO₃⁻ groups may be bound to the anion-exchanger by ionic-bonding between the –SO₃⁻ groups and the –NH(C₂H₅)₂⁺ groups of the anion-exchanger. It may be reasonable that the Fe⁴⁺ ion is coordinated to the –S– of the epithio groups and the phenolic oxygen atoms of TCAS. According to an X-ray analysis of a Zn²⁺ complex of p-t-butylthiacalix[4]arene by Iki et al.,²² actually, the Zn²⁺ ion was coordinated to the –S– of the epithio groups and the phenolic oxygen atoms. In the case of Fe⁴⁺-TCAS on a modified anion-exchanger, similarly, the –S– of the epithio groups and the phenolic oxygen atoms of TCAS may be responsible for coordination to the Fe⁴⁺ ion. Based on the result of EDTA titration, the Fe⁴⁺ ion was coordinated to TCAS on the modified anion-exchanger with a molar ratio of 1:1. Moreover, no elution of Fe⁴⁺-TCAS from the modified ion-exchanger was observed under the reaction conditions in this study.

Figure 3 shows that the activity of Fe⁴⁺-TCAS₅₀₀ for color reaction (2) may be applicable for the determination of H₂O₂ in place of peroxidase. To establish the optimum conditions for determining H₂O₂ using Fe⁴⁺-TCAS₅₀₀, the following experiments for some factors which may influence the activity of Fe⁴⁺-TCAS₅₀₀ were carried out using the MBTH-HALPS system for 7 µg H₂O₂ in an acetate buffer solution of pH 5.

**Effects of the concentration of chromogen**

As shown in Figs. 4 and 5, the concentrations of MBTH and HALPS affected the activity of Fe⁴⁺-TCAS₅₀₀. However, because the absorbances at 582 nm were almost maximum and constant over 0.1 mg/ml of MBTH and 0.5 mg/ml of HALPS, 0.1 mg/ml of MBTH and 1.0 mg/ml of HALPS were selected for the optimum concentrations of the chromogen.

**Effects of the incubation temperature and incubation time**

Only a small increase in the absorbances at 582 nm was observed with increasing the incubation temperature from 20 to 50°C. Room temperature was selected as the incubation temperature.

In the previous method,¹⁹ the reaction solution was incubated for 60 min for the maximum and constant absorbance of the quinoid dye in color reaction (1). However, in the method using the MBTH-HALPS system the absorbances at 582 nm became almost maximum and constant above 15 min of incubation time, as shown in Fig. 6. Thus, 30 min was sufficient for the incubation time, indicating that Fe⁴⁺-TCAS₅₀₀ showed
Table 1 Effect of foreign substances on the activity of Fe$^{3+}$-TCAS$_{A,500}$ by the method using the MBTH-HALPS system

<table>
<thead>
<tr>
<th>Substance Added/µg</th>
<th>Error, %</th>
<th>Substance Added/µg</th>
<th>Error, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin 70</td>
<td>+3.7</td>
<td>Fe$^{3+}$ 70</td>
<td>0.0</td>
</tr>
<tr>
<td>NaF 70</td>
<td>–1.6</td>
<td>K$^+$ 70</td>
<td>+2.3</td>
</tr>
<tr>
<td>EDTA 70</td>
<td>–5.7</td>
<td>Cu$^{2+}$ 70</td>
<td>+3.7</td>
</tr>
<tr>
<td>Glycine 70</td>
<td>+0.5</td>
<td>PO$_4^{3-}$ 70</td>
<td>–0.7</td>
</tr>
<tr>
<td>Ascorbate 70</td>
<td>–75.9</td>
<td>CO$_3^{2-}$ 70</td>
<td>+3.3</td>
</tr>
<tr>
<td>Citrate 70</td>
<td>–4.6</td>
<td>NH$_4^+$ 70</td>
<td>+2.9</td>
</tr>
<tr>
<td>Albumin (HSA) 1 × 10$^4$</td>
<td>–3.6</td>
<td>Br$^-$ 70</td>
<td>+1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I 70</td>
<td>+2.9</td>
</tr>
</tbody>
</table>

In a pH 5 buffer solution for 7 µg H$_2$O$_2$.

a. Error, % = 100 × (H$_2$O$_2$(found) – H$_2$O$_2$(added))/H$_2$O$_2$(added).

In these experiments, the reagent solution, which consisted of a 1:1.25 (v/v) mixture solution of 0.5 mg/ml MBTH, 5.0 mg/ml HALPS and an acetate buffer of pH 5, instead of the reagent solution B, was applied for the method using the MBTH-HALPS system for H$_2$O$_2$. The calibration curve between 10 and 100 µg of H$_2$O$_2$ in the sample solution (1.0 ml) was linear up to almost 70 µg (2.1 µmol) of H$_2$O$_2$. In contrast, the catalytic activity of Fe$^{3+}$-TCAS$_{A,500}$ became weakened at over 70 µg (2.1 µmol) of H$_2$O$_2$. In terms of the repetitive use of Fe$^{3+}$-TCAS$_{A,500}$ separated from the reaction solution, a slight decrease in its catalytic activity was observed; that is, the activity of Fe$^{3+}$-TCAS$_{A,500}$ for 70 µg (2.1 µmol) of H$_2$O$_2$ after 5-times repeated use was over 80% of the activity obtained during the first run. Taking into account that 20 mg of Fe$^{3+}$-TCAS$_{A,500}$ (100 µmol/g) added in sample solution contained 2 µmol of Fe$^{3+}$-TCAS, it is reasonable that Fe$^{3+}$-TCAS$_{A,500}$ shows catalytic activity for reaction (2). Moreover, the catalytic activity of Fe$^{3+}$-TCAS$_{A,500}$ remained constant even after being allowed to stand for over 6 months at room temperature in a desiccator.

In this way, as one of characteristics of Fe$^{3+}$-TCAS$_{A,500}$ is that Fe$^{3+}$-TCAS$_{A,500}$ can be used repeatedly after being separated from the reaction mixture, while POD is difficult to be separated.

Effect of foreign substances

In the presence of some substances that may intrinsically exist in blood, or be generally added to blood as an anticoagulant, their interference for the method using the MBTH-HALPS system was investigated. As shown in Table 1, human serum albumin (HSA) showed almost no interference, while it showed appreciable interference in the previous method. This difference of interference between the two methods may be attributed to the difference in the optimum pH for the reactions of both methods. Although pK$_a$ of the –NH(C$_2$H$_5$)$_2$+ group of DEAE cellulofine A-500 is not clear, the structure difference of free –NH(C$_2$H$_5$)$_2$+ groups on Fe$^{3+}$-TCAS$_{A,500}$ in the reaction solutions of both methods may influence the interaction between HSA and Fe$^{3+}$-TCAS$_{A,500}$ and, consequently, the activity of Fe$^{3+}$-TCAS$_{A,500}$. On the contrary, ascorbic acid showed appreciable interference, as shown in Table 1, although it showed almost no interference in the previous method. In the previous method, ascorbic acid in reaction solutions could be decomposed without producing H$_2$O$_2$ during the course of the reactions, because ascorbic acid is more rapidly oxidized by air in an alkaline solution. In the case of applying the present method for samples containing ascorbic acid, the addition of ascorbic acid oxidase to sample solutions in analogy with the case of methods using POD in clinical analyses would be above all

appreciably higher activity for color reaction (2) using MBTH and HALPS than that for color reaction (1) using 4-AAP and phenol in the previous method.  Effect of the amount of Fe$^{3+}$-TCAS on the modified ion-exchanger

The amount of added Fe$^{3+}$-TCAS$_{A,500}$ was adjusted so that the amount of Fe$^{3+}$-TCAS in the reaction system remained constant (2 µmol in this case) in all runs of this experiment. The amount of Fe$^{3+}$-TCAS on the modified ion-exchanger did not affect the activity very much between 25 and 125 µmol Fe$^{3+}$-TCAS per 1 g of dry ion-exchanger. Because the absorbance reached the maximum at 50 µmol, and remained at 125 µmol Fe$^{3+}$-TCAS per 1 g of dry ion-exchanger, 100 µmol/g of Fe$^{3+}$-TCAS$_{A,500}$ was selected.

Moreover, the effect of the amount of Fe$^{3+}$-TCAS$_{A,500}$ (100 µmol/g) on the activity was investigated. Since the absorbance at 582 nm became almost maximum and constant at between 10 and 25 µg and 25 µg, 20 µg of Fe$^{3+}$-TCAS$_{A,500}$ (100 µmol/g) was added to the reaction solution.

Calibration curve and sensitivity

Under the optimum conditions described above, a linear calibration curve was obtained over the range between 1 and 10 µg of H$_2$O$_2$ in the sample solution (1.0 ml) by the method using the MBTH-HALPS system. The correlation coefficient and the relative standard deviation (n = 7) were greater than 0.996 and 4.0%, respectively, for 7 µg of H$_2$O$_2$. The apparent molar absorptivity for H$_2$O$_2$ was 2.4 × 10$^4$ l mol$^{-1}$ cm$^{-1}$, which is about 80% of that for the corresponding reaction by POD and 8-times as large as that in the previous method using the 4-AAP-phenol system. Accordingly, the method using the MBTH-HALPS system is applicable for the determination of H$_2$O$_2$ with higher sensitivity than that of the previous method using the 4-AAP-phenol system.

Catalytic activity of Fe$^{3+}$-TCAS$_{A,500}$ for reaction (2)

As shown regarding the previous method, Fe$^{3+}$-TCAS$_{A,500}$ exhibited catalytic activity for color reaction (1). In order to elucidate whether Fe$^{3+}$-TCAS$_{A,500}$ will also exhibit catalytic activity for color reaction (2) or not, the activity of Fe$^{3+}$-TCAS$_{A,500}$ was investigated by the method using the MBTH-HALPS system for sample solutions containing 10 – 100 µg (0.29 – 2.9 µmol) of H$_2$O$_2$ and, moreover, the activity of Fe$^{3+}$-TCAS$_{A,500}$ after being separated from the reaction solution was repeatedly investigated for 70 µg (2.1 µmol) of H$_2$O$_2$. In this way, as one of characteristics of Fe$^{3+}$-TCAS$_{A,500}$ is that Fe$^{3+}$-TCAS$_{A,500}$ can be used repeatedly after being separated from the reaction mixture, while POD is difficult to be separated.

Effect of foreign substances

In the presence of some substances that may intrinsically exist in blood, or be generally added to blood as an anticoagulant, their interference for the method using the MBTH-HALPS system was investigated. As shown in Table 1, human serum albumin (HSA) showed almost no interference, while it showed appreciable interference in the previous method. This difference of interference between the two methods may be attributed to the difference in the optimum pH for the reactions of both methods. Although pK$_a$ of the –NH(C$_2$H$_5$)$_2$+ group of DEAE cellulofine A-500 is not clear, the structure difference of free –NH(C$_2$H$_5$)$_2$+ groups on Fe$^{3+}$-TCAS$_{A,500}$ in the reaction solutions of both methods may influence the interaction between HSA and Fe$^{3+}$-TCAS$_{A,500}$ and, consequently, the activity of Fe$^{3+}$-TCAS$_{A,500}$. On the contrary, ascorbic acid showed appreciable interference, as shown in Table 1, although it showed almost no interference in the previous method. In the previous method, ascorbic acid in reaction solutions could be decomposed without producing H$_2$O$_2$ during the course of the reactions, because ascorbic acid is more rapidly oxidized by air in an alkaline solution. In the case of applying the present method for samples containing ascorbic acid, the addition of ascorbic acid oxidase to sample solutions in analogy with the case of methods using POD in clinical analyses would be above all

appreciably higher activity for color reaction (2) using MBTH and HALPS than that for color reaction (1) using 4-AAP and phenol in the previous method.
maximum and constant absorbances of the quinoid dye.  It was shown that the absorbance change in the previous method was sufficient for the determination of glucose.  Moreover, the GOD-Fe$_3^+$-TCAS$_{A,500}$ method was applied for the determination of glucose in control serum I and II (0.5 ml) diluted 15 and 50 times with water, respectively.

Application for the determination of glucose

Glucose was determined using the GOD-Fe$_3^+$-TCAS$_{A,500}$ method by measuring the quinoid dye produced through reactions (3) and (2), catalyzed by GOD and Fe$_3^+$-TCAS$_{A,500}$, respectively.  In the previous method, because of optimum pH for the activity of GOD was very different from that of Fe$_3^+$-TCAS$_{A,500}$ for reaction (1), Fe$_3^+$-TCAS$_{A,500}$ was added separately to the reaction solution after GOD was added to the sample solution and incubated for 10 min.  However, in the present method, because of optimum pH for the activities of GOD and Fe$_3^+$-TCAS$_{A,500}$ could be added simultaneously to the sample solution.  This may result in a shortening of the incubation time compared to the previous method; that is, less than half that in the previous method was sufficient for the maximum and constant absorbances of the quinoid dye.

For a sample solution containing 5 - 50 µg of glucose in 1.0 ml, the calibration curve using the GOD-Fe$_3^+$-TCAS$_{A,500}$ method was straight.  The correlation coefficient and the relative standard deviation (n = 4) were greater than 0.995 and 2.5%, respectively, for 30 µg of glucose.  The apparent molar absorptivity for glucose was 2.13 × 10$^4$ l mol$^{-1}$ cm$^{-1}$, which is about 72% of that for the corresponding reaction by POD.  Moreover, the GOD-Fe$_3^+$-TCAS$_{A,500}$ method was applied for the determination of glucose in control serum I (normal) and II (abnormal), of whose sample solutions were applied after being diluted 15 and 50 times with distilled water, respectively.  Table 2 shows that the observed amounts of glucose by the GOD-Fe$_3^+$-TCAS$_{A,500}$ method were satisfactory for both of control serum I and II.

In conclusion, Fe$_3^+$-TCAS$_{A,500}$ showed high peroxidase-like activity for catalyzing color reaction (2) in an acidic buffer solution (pH 5 - 6).  The method using the MBTH-HALPS system has some advantages regarding its high sensitivity and improved reaction conditions (especially, optimum pH and incubation time), compared with the previous method using the 4-AAP-phenol system.  Accordingly, the method using the MBTH-HALPS system and Fe$_3^+$-TCAS$_{A,500}$ was useful for determining hydrogen peroxide, and may be applied to the determination of vital compounds in blood and urine in connection with their corresponding oxidase.

Table 2  Glucose amount in diluted control serum I and II

<table>
<thead>
<tr>
<th>Method</th>
<th>Glucose amount (mean ± SD, n = 4 each)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control serum I (µg/0.5 ml)</td>
</tr>
<tr>
<td>GOD-POD method</td>
<td>26.0 ± 0.7</td>
</tr>
<tr>
<td>GOD-Fe$<em>3^+$-TCAS$</em>{A,500}$ method</td>
<td>24.1 ± 0.9</td>
</tr>
</tbody>
</table>

a. The amount of glucose was 24.7 and 28.5 µg in control serum I and II (0.5 ml) diluted 15 and 50 times with water, respectively.

References

1. A part of this work was presented at the 61st Symposium on Analytical Chemistry, Nagoaka, May 2000, 45.