Kinetic Spectrophotometric Determination of Total Iron by Sequential Injection Analysis

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A new, simple and highly sensitive kinetic spectrophotometric method for the determination of total iron by sequential injection analysis (SIA) has been proposed. The determination was based on the catalytic effect of potassium bromate on the oxidation of diaminoditolyl. The absorbance of the investigated chemical system, measured at the wavelength of 639 nm, was used to determine the amount of total iron. Chemical and physical conditions for the applied SIA method have been optimized and, after that, the calibration plot was constructed in the analyte concentration range of 0.035–0.700 µg mL⁻¹. Detection limit of iron was 5 µg L⁻¹. Relative standard deviation for 11 replicate determinations of 0.25 µg mL⁻¹ Fe(III) was 1.1% at a throughput of 27 samples per hour. The method was applied to the determination of total iron in tea, human hair and pharmaceutical formulation. The results obtained by the proposed method were compared with those obtained by FAAS. The t-test did not reveal any significant differences between the two methods at the confidence level of 95%.

Zaproponowano nową, prostą i wysokoczułą kinetyczną metodę spektrofotometrycznego oznaczania żelaza całkowitego za pomocą sekwencyjnej analizy wstrzykowej (SIA). Podstawą oznaczania jest katalityczne działanie bromianu potasu na utlenianie diaminoditolalu. Do oznaczania żelaza całkowitego wykorzystano absorbancję badanego układu chemicznego, mierzoną przy długości 639 nm. Zoptymalizowano chemiczne i fizyczne warunki SIA i wyznaczono wykres wzorcowy w zakresie stężeń analitu od 0,035 do 0,700 mg mL⁻¹. Wskaźnik wykrywalności żelaza wynosiła 5 µg L⁻¹. Względne odchylenie standardowe dla 11 próbek oznaczonych Fe(III) wynosiło 1.1% przy analizie 27 próbek na godzinę. Metodę zastosowano do oznaczania żelaza całkowitego w herbacie, włosach ludzkich i preparatach farmaceutycznych. Wyniki otrzymane zaproponowaną metodą porównano z otrzymanymi metodą FAAS. Test-t, przy poziomie ufności 95%, nie wykazał wyraźnych różnic obu metod.

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Sequential injection analysis (SIA) is the second generation of flow injection analysis (FIA). It was developed by Ruzicka and co-workers in 1990 [1]. Compared with FIA, SIA is advantageous with respect to repeatability, simplicity of performance, and low costs of application. Moreover, the manifold does not need to be changed if the flow parameters or the injection volumes are modified. This is due to the presence of the computer-controlled multi-port valve, which is the heart of the SIA system and connects particular manifold components. Various detection modes, including spectrophotometry [2–9] and atomic absorption spectrometry [10,11], have been coupled with the SIA system for the determination of iron. In spectrophotometric detection, the most commonly used chromogenic agents are tiron [2–4], 2,2-bipyridyl [5] and 1,10-phenantroline [8]. Kass et al. [2] described the application of SIA with spectrophotometric detection for the determination of iron in a zinc-processing plant. Determination was based on the formation of the blue-coloured complexes between Fe(III) and tiron. The linear dependence between the absorbance and the analyte concentration was observed for 0.2–3.0 g L$^{-1}$ of Fe(III). Mulaudzi et al. [3] also used tiron as a chromogenic reagent to determine Fe(III) and Fe(II) in pharmaceuticals and effluent streams after oxidation of Fe(II) to Fe(III) with hydrogen peroxide. The linear ranges were 0.15–100.00 and 0.30–80.00 mg L$^{-1}$ for Fe(III) and Fe(II), respectively. Oliveira et al. [5] described a determination procedure for Fe(II) in pharmaceutical formulations. The procedure was based on monitoring the absorbance at 523 nm, due to the formation of Fe(II)-2,2-depyridyl complex. The detection limit was 0.97 mg L$^{-1}$. Araujo et al. [6] proposed the SIA approach to the determination of Fe(III) in the fortified nutrition formulas for infants. Fe(III) forms coloured complex with thiocyanate. Thus, the amount of the analyte in the sample can be quantified spectrophotometrically. The calibration curve in this determination was linear in the analyte concentration range of 0.5–20 mg L$^{-1}$.

Several SIA-based methods for the determination of iron provide high detection limit [2–7]. In contrast, the one proposed by Rubi et al. [9], coupled with photometric detection of the Fe(III) analyte, is characterised by low detection limit. To overcome this obstacle, preconcentration was required.

There is still a great interest in the development of a simple, sensitive and selective SIA method for the determination of iron in real samples without preconcentration. This was the aim of this work. The method was based on the catalytic effect of iron on the oxidation of diaminoditolyl by potassium bromate. Diaminoditolyl served as a new chromogenic agent. The method has occurred to be more sensitive than those reported elsewhere [2–11]. It was applied to the determination of iron in tea, human hair and pharmaceutical formulation, which has not been reported yet in the literature [2–9]. The results were compared with those obtained by FAAS.
EXPERIMENTAL

Apparatus

A Fialab instrument, Model 3500 (FIAlab Instruments Inc. USA) was used in the SIA measurements. Its scheme is shown in Figure 1. The solution was introduced to the system using a 2.5 mL-in-volume syringe pump and an 8-port rotary valve. The length of the holding coil (0.5 mm I.D.) was 200 cm. The reaction coil was 100 cm long, with an internal diameter of 0.5 mm. Other tubings were of 0.5 mm I.D. and were made of PTFE. The entire system was computer-controlled (Legend Cooperation, China) via the FIAlab software (FIAlab for Windows 5.0 Revision E). An USB2000–UV–VIS spectrophotometer (Ocean Optics, Inc., USA) equipped with a 10 mm light path optic fibre SMA Z-flow cell was used for the absorbance measurements. A H.H.S 11–21 thermostat (Shanghai No. 5 Medical Apparatus and Instruments Factory, China) was used for the heating at the controlled temperature. A pH S–3C pH-meter (Shanghai Weiye Instrument Factory, China) served for pH measurements.

Figure 1. Schematic diagram of the SIA system for the determination of iron. SP – syringe pump, SPV – syringe pump valve, SV – selection valve, HC – holding coil, RC – reaction coil, D – detector, C – carrier, W – waste solution, S – sample solution, R1 – 0.22 mol L⁻¹ KBrO₃, R2 – mixed solution (diaminoditolyl + HCl-NaOAc buffer)

Reagents

All reagents were of analytical grade purity. Doubly distilled water was used throughout. 1000 µg mL⁻¹ stock solutions of Fe(III) and Fe(II) were prepared by dissolving 0.8636 g of NH₄Fe(SO₄)₂•12H₂O (Beijing Chemical Plant, China) or 0.7022 g of Fe(NH₄)₂(SO₄)₂•6H₂O (Beijing Shuanghuan Chemical Reagent Plant, China) in 4 mL of 1:3 H₂SO₄ solution. The volume was made up to 100 mL with water. Working standard solutions were prepared freshly before use by serial dilutions of the stock solutions with water. NaOAc-HCl buffer solution of pH = 3.77 was prepared by adding 30 mL of 1.0 mol L⁻¹ sodium acetate solution (Wenzhou Chemical Reagent Plant, China) to 55 mL of water and adjusting the pH to 3.77 with the appropriate volume of 1.0 mol L⁻¹ HCl solution (Shanghai Haifan Industrial Corporation, China). 5.0 × 10⁻² mol L⁻¹ stock solution of diaminoditolyl was prepared by dissolving 2.6536 g of the compound (Shanghai Experiment Reagent Co. Ltd, China) in 25 mL of 1.0 mol L⁻¹ HCl solution. The volume was made to 250 mL with water in a standard flask. The solution was stored in
a refrigerator at 4°C. A 0.25 mol L\(^{-1}\) potassium bromate stock solution was prepared by dissolving 41.75 g of KBrO\(_3\) (Shanghai Second Reagent Factory, China) in 1 L of water. Working solution was prepared by the appropriate dilution. A mixed solution of dianimoditoly and NaOAc-HCl buffer was prepared by placing 2.2 mL of 5.0 \times 10^{-2} \text{mol L}^{-1} dianimoditoly solution and 4.5 mL of NaOAc-HCl buffer (pH = 3.77) in 10 mL-in-volume flask. The contents were diluted to the mark with water. Boiled and cooled doubly distilled water was employed as the carrier in order to avoid bubbling in the reaction coil.

**Sample preparation**

**Human hair and tea samples.** Human hair and tea samples were prepared according to the reference [12]. Hair were washed and dried under the infrared lamp. 0.8 g hair sample and 0.6 g teas sample were accurately weighed and placed in a 50 mL dry beaker. 15 mL of the concentrated nitric acid were added. The beaker was left overnight in a fume cupboard. Next day, the contents were heated almost to dryness at low temperature. After that, the sample was cooled and a certain amount of the concentrated HClO\(_4\) was added. The obtained solution was heated until it became transparent (in case the solution was not transparent, more HClO\(_4\) was added to digest the sample). The heating was continued to remove the excess of acids. After that, the sample was cooled to the room temperature and some water was added. The contents were heated again to dissolve the crystals precipitated at the end of digestion. Finally, the solution was transferred into a 25 mL flask and the contents were diluted to the mark. At the same time the blank test was performed. For this purpose an aliquot of the sample solution was placed into a 10 mL flask and its absorbance was measured according to the requirements of the procedure. The results were compared with those obtained by FAAS. The latter was performed using a Z–5000 Polarized Zeeman Atomic Absorption Spectrophotometer (Hitachi, Japan) with an air-acetylene flame. Instrumental parameters and operating conditions were set as recommended by the manufacturer.

**Tabellae ferrosi sulfatis.** Three tablets of tabellae ferrosi sulfatis were accurately weighed and finely powdered. A 0.5 g powder sample was precisely weighed, dissolved in distilled water, and filtered. The solution volume was made up to 100 mL with distilled water. The obtained solution was diluted appropriately to the concentration falling into the linear range.

**Procedure**

The procedure for the determination of iron was based upon the following steps: aspiration of 170 \(\mu\)L of 0.22 mol L\(^{-1}\)KBrO\(_3\) solution (1); aspiration of 80 mL of the sample or the standard containing iron in the concentration range 0.035–0.700 \(\mu\)g mL\(^{-1}\) (2); aspiration of 110 \(\mu\)L of the dianimoditoly – NaOAc-HCl buffer mixture (3) (in steps (1)-(3) the flow rate was 25 \(\mu\)L s\(^{-1}\)); propulsion of 290 \(\mu\)L of the carrier through the reaction coil at the flow rate of 50 \(\mu\)L s\(^{-1}\) and stop for 60 s (4); propulsion of 1000 L of the carrier through the reaction coil at a flow rate of 30 \(\mu\)L s\(^{-1}\) for the measurement (5). The absorbance of the oxidised dianimoditoly product was monitored continuously at the wavelength of 639 nm.

**RESULTS AND DISCUSSION**

Dianimoditoly can be oxidised in the presence of highly concentrated oxidising agents, such as KBrO\(_3\). After oxidation it is transformed to the blue compound, which
absorbs maximally at 639 nm. However, the redox reaction occurs very slowly. When trace iron is present in the system, the redox reaction rate increases, indicating that iron catalyses the reaction between diaminotolyl and KBrO₃. Furthermore, it is observed that there is a good linear dependence between the measured absorbance and the concentration of iron.

**Optimisation of the method**

In order to achieve maximum sensitivity of the system, the one-factor-at-a-time method was used to optimise experimental parameters. Iron concentration was 0.3 µg mL⁻¹, while other parameters were set as described in the procedure.

**Physical parameters**

The aspiration sequence of the sample and the reagents into the holding coil had no influences on the maximisation of the sensitivity. The following sequence was adopted: KBrO₃ – sample mixed solution. Compared to the holding and reaction coils of 0.7 I.D., those of 0.5 I.D. ensured higher sensitivity. Therefore, the later were used in the subsequent study. It was important to provide good mixing of the sample and the reagent zone. For this purpose, the influence of the flow rate of the SIA system on the analytical signal ΔA was evaluated (ΔA is the absorbance difference between the blank and iron). It has been shown in Figure 2 that the flow rate influenced ΔA, as the sample/reagents zone was moving through the reaction coil. ΔA increased with the increasing flow rate. After reaching a maximum at the flow rate of 50 µL s⁻¹, ΔA started to decrease. Thus, the flow rate of 50 µL s⁻¹ was chosen as optimal.

![Figure 2. The influence of the flow rate on ΔA and precision of ΔA.](image)

0.3 µg mL⁻¹ Fe(III); for other data see experimental section
The effect of the KBrO$_3$ solution volume on $\Delta A$ was investigated between 120 and 190 $\mu$L. $\Delta A$ increased with the increase of the volume from 120 to 160 $\mu$L. Up to 190 $\mu$L no further changes were observed. Finally, a volume of 170 $\mu$L was adopted in further studies. Also a volume of 110 $\mu$L of the mixed solution was used.

The effect of the sample volume on $\Delta A$ was also evaluated. The sample volume was changed from 60 to 100 $\mu$L, as shown in Figure 3. For the volume equal to 70 $\mu$L and larger, $\Delta A$ remained nearly constant. For this reason, the sample volume used was 80 $\mu$L.

![Figure 3](image)

Figure 3. The influence of the sample volume on $\Delta A$ and precision of $\Delta A$. $\triangleleft$– (AA); $\triangle$– (RSD) (n = 3); 0.3 $\mu$g mL$^{-1}$ Fe(III); for other data see experimental section

Reaction time and temperature were of the utmost importance to provide the best analytical results from the kinetic analysis. Stopped flow can be used to increase the measured absorbance [12]. The effect of the stopped flow timing on $\Delta A$ was studied in the range of 33–80 s. Generally, $\Delta A$ increased with the increase of the stopped flow timing. A 60 s was selected for the stopped flow timing in order to achieve a compromise between the reliable sample throughout and sensitivity. $\Delta A$ was also increased with the increasing temperature in the range of 71–96°C. A temperature of 92°C was selected for the further measurements.

**Chemical parameters**

In order to choose an optimum reaction medium, the effect of HCl–NaOAC, HCl–KCl, KH$_2$PO$_4$–NaOH, HOAC–NaOAC and Tris–HCl mixtures on $\Delta A$ was studied. Both catalytic and non-catalytic reactions processed slowly in KH$_2$PO$_4$–NaOH,
Kinetic spectrophotometric determination of total iron

HOAC–NaOAC and Tris–HCl. The sensitivity was better in HCl–NaOAC and HCl–KCl media. HCl–NaOAC provided the best sensitivity among all the media mentioned above. Thus, HCl–NaOAC was chosen as an optimum medium. The effect of the pH on ΔA was tested in the pH range of 2.58–4.18. ΔA reached maximum at about pH = 3.77 and this pH value of HCl–NaOAC buffer was adopted. Also the effect of the buffer volume on ΔA was studied. Different volumes, ranging from 1.5 to 6.0 mL were applied. ΔA remained constant in the range of 4.0–5.0 mL and decreased for larger volumes. Thus, 4.5 mL of the buffer solution in a final volume of 10 mL were used.

The effect of the oxidants, such as H₂O₂, KIO₄, KIO₃, KClO₃, KClO₄ and KBrO₃, on ΔA was evaluated. Both catalytic and non-catalytic reactions hardly processed in H₂O₂, KClO₃, and KClO₄ solutions. Non-catalytic reaction was very fast in the presence of KIO₄, which resulted in large background signals. KBrO₃ provided better sensitivity and reproducibility than KIO₄. Therefore, the former was finally used as the oxidant. KBrO₃ was used in different concentrations ranging from 0.18–0.24 mol L⁻¹. ΔA remained constant for the concentrations larger than 0.21 mol L⁻¹. Thus, 0.22 mol L⁻¹ concentration of KBrO₃ was applied.

ΔA remained nearly constant for the concentration of diaminoditolyl ranging from 0.8 × 10⁻² to 1.3 × 10⁻² mol L⁻¹. Thus, a concentration of diaminoditolyl equal to 1.1 × 10⁻² mol L⁻¹ was used.

Interference study

The interfering effects of various foreign ions, possibly affecting the SIA – spectrophotometric determination of iron(III) were studied. The samples were spiked with the known amounts of foreign ions and, after that, the absorbance was measured according to the requirements of the standard procedure.

Table 1. The effect of foreign ions on the determination of 0.3 µg mL⁻¹ Fe(III)

<table>
<thead>
<tr>
<th>Foreign ions</th>
<th>Concentration µg mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₃⁻</td>
<td>1400</td>
</tr>
<tr>
<td>K⁺</td>
<td>340</td>
</tr>
<tr>
<td>SO₄²⁻, Mo(VI), Co⁶⁺, Cr⁺⁺⁺</td>
<td>170–190</td>
</tr>
<tr>
<td>NO₂⁻, Pb²⁺, Br⁻, Sr²⁺</td>
<td>90–100</td>
</tr>
<tr>
<td>W(VI), CO₃²⁻, Al³⁺, Na⁺</td>
<td>60–80</td>
</tr>
<tr>
<td>Ca²⁺, Zn²⁺, Hg²⁺, Ag⁺</td>
<td>30–44</td>
</tr>
<tr>
<td>V(V), Cr(III), Cu²⁺, NH₄⁺, Bi⁵⁺, PO₄³⁻, F⁻, As(III), Cd²⁺, Mn²⁺, Ni²⁺</td>
<td>1–10</td>
</tr>
</tbody>
</table>
The results were compared with those obtained for the standard solution. The tolerance limit was defined as the concentration of the added species causing a relative error lower than ±5%. The results of the interference studies are presented in Table 1. According to these results, the most of metal ions do not disturb in the course of analysis, even if present at high concentrations. This proves good selectivity of the method.

**Analytical characteristics**

Under optimum conditions linear calibration plots for Fe(III) and Fe(II) were constructed in the concentration range of 0.035–0.700 µg mL\(^{-1}\). The linear regression equations are as follows: \(\Delta A = -0.0766 + 2.73C\) (µg mL\(^{-1}\)), \(r = 0.9997\) (n = 3) for Fe(III), and \(\Delta A = -0.0689 + 2.74C\) (µg mL\(^{-1}\)), \(r = 0.9994\) (n = 3) for Fe(II). The sensitivity of the proposed method towards Fe(II) and Fe(III) was almost equal, as it can be seen from the respective regression equations – relative error was less than 10%. That is probably due to the oxidation of Fe(II) by bromate. Therefore, the proposed method can be applied to the determination of total iron. Detection limit calculated as 3 times the standard deviation of the method was 5 µg mL\(^{-1}\), which is lower than the results reported in the literature [2–11]. Relative standard deviation obtained for 11 replicate determinations of 0.25 µg mL\(^{-1}\) Fe(III) in 80 µL-in-volume injections was 1.1%. One complete analysis lasts 131 s, which results in a frequency of 27 samples per hour.

**Application**

The applicability of the proposed method was evaluated by determining iron in tea, human hair and pharmaceutical formulation. The results were compared to those obtained by FAAS. The t-test was applied to examine whether these two methods differ significantly at the 95% confidence level. The results are presented in Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proposed method(^a) (n = 6)</th>
<th>R.S.D %</th>
<th>FAAS(^a) (n = 2)</th>
<th>t(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tea 1</td>
<td>233</td>
<td>0.7</td>
<td>234</td>
<td>1.44</td>
</tr>
<tr>
<td>Tea 2</td>
<td>233</td>
<td>1.4</td>
<td>235</td>
<td>1.92</td>
</tr>
<tr>
<td>Human hair</td>
<td>83.6</td>
<td>1.2</td>
<td>84.1</td>
<td>1.34</td>
</tr>
<tr>
<td>Tabellae ferosi sulfatis(^c)</td>
<td>53.9</td>
<td>1.4</td>
<td>53.6</td>
<td>0.95</td>
</tr>
</tbody>
</table>

\(^a\) Tea: µg g\(^{-1}\); human hair: µg g\(^{-1}\); Tabellae ferosi sulfatis: mg/tablet.

\(^b\) Theoretical value = 2.57, n = 6, confidence level 95%.

\(^c\) Obtained from Shanghai Huanghai Pharmaceutical, Ltd.
Kinetic spectrophotometric determination of total iron

According to these data it can be concluded that the proposed method provides reproducible, accurate and reliable results. The determination results of iron in tabellae ferrosi sulfatis are indicative for the equal sensitivity of the method towards Fe(II) and Fe(III).

CONCLUSIONS

The proposed method based on the SIA with spectrophotometric detection is simple, easy and automated. It provides low consumption of the sample and the reagents, as well as the volume of the disposable waste is only 1.29 mL in one full cycle. Therefore, the method can be considered as a contribution to the development of a „green” analytical chemistry. The method was successfully applied to the determination of total iron in tea, human hair and pharmaceutical formulation, with a relative standard deviation better than 1.4%. Compared to FAAS, the proposed SIA method is cost-effective.

REFERENCES


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