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# Simultaneous Second Derivative Spectrophotometric Determination of $Fe^{2+}$ and $Fe^{3+}$ with 2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol

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Owing to the presence of iron in environmental and biological materials, and the lack of sufficient understanding of the role of the two oxidation states of this element, the determination of both $Fe^{2+}$ and $Fe^{3+}$ is of great importance. 2-(5-Bromo-2-Pyridylazo)-5-Diethylaminophenol (5-Br-PADAP) was chosen as ligand. The optimal pH for complex formation with both $Fe^{2+}$ and $Fe^{3+}$ was pH 7.0, and the suitable buffer was 1 M ammonium acetate solution. Both complexes were formed immediately and were stable for at least 24 hours. The ordinary, first- and second-derivative spectra of 5-Br-PADAP complexes of $Fe^{2+}$ and $Fe^{3+}$ were recorded for these ions either alone or in binary mixtures. Second derivative spectra were selected for evaluation, because working wavelength determination was more precise and spectral overlap was less than in other spectra. Two wavelengths at which $^2$D-absorbance of one valency of iron had a substantial value as opposed to the zero value of the other were 611 nm for $Fe^{2+}$, and 577 nm for $Fe^{3+}$. Thus calibration curves drawn with $^2$D values as a function of concentration at these wavelengths were used to determine $Fe^{2+}$ and $Fe^{3+}$ concentrations. The relative standard deviation for the analysis of $Fe^{2+}$(1.12 mg/l) individually was 1.03%, and for its admixture with $Fe^{3+}$ (5.6 mg/l) was 4.36%. The relative standard deviation for analysis of $Fe^{3+}$(1.12 mg/l) individually and for its admixture with $Fe^{2+}$ (6.72 mg/l) were 3.93% and 1.52%, respectively. The linear range in $^2$D evaluation was between $1.4\times10^{-6}$ - $3.4\times10^{-5}$ M for both $Fe^{2+}$ and $Fe^{3+}$.

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Iron is the fourth most abundant element in the earth's crust occurring in nearly all types of rock and soil minerals as both $Fe^{2+}$ and $Fe^{3+}$. Iron also plays a central role in the biosphere, serving as the active center of proteins responsible for $O_2$ and electron transfer and of metalloenzymes such as oxidases, reductases, and dehydrases.[1] Also, a great deal of attention has been paid to biogeochemistry of iron in seawater, recently. It is known that the fixation of carbon dioxide by primary production of phytoplankton in oceans may possibly lower the concentration of carbon dioxide in air. The hypothesis that Fe limits primary production in the ocean[2,3] and $Fe^{2+}$ is probably the preferred nutrient for phytoplankton[4] combined with the property of $Fe^{3+}$ being an effective catalyst for the autoxidation of $SO_2$ to $SO_4^{2-}$ in clouds (associated with acid rain)[5,6] stimulate the iron research. Also, $Fe^{2+}$ is required for proper transport and storage of oxygen in higher animals by means of haemoglobin and myglobin, while the oxidized forms, methaemoglobin and metmyoglobin, which contain $Fe^{3+}$, will not bind oxygen. As a result, iron determination and speciation methods in environmental and biological samples have been developed with high sensitivities.

The determination of the oxidation state of iron in a variety of samples is generally achieved by complexation with specific chelating agents followed by spectrophotometric measurement. 1,10–phenanthroline,[7] bathophenanthroline (4,7-diphenyl-1,10-phenanthroline),[8] ferrozine (3-(2-pyridyl)-5,6-bis(4–phenylsulfonic acid)-1,2,4-triazine)[9] and 2,4,6-tris(2'-pyridyl)-1,3,5-triazine (TPTZ)[10] are the common chelating agents for $Fe^{2+}$. Also, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT) is utilized for determination of $Fe^{2+}$ in natural waters.[11] After the determination of $Fe^{2+}$, $Fe^{3+}$ is reduced to $Fe^{2+}$ using proper reducing agent such as ascorbic acid or hydroxylamine hydrochloride and the total iron is determined. Tiron (4,5-dihydroxy-1,3-benzene disulfonic acid)[12] and 1-(2-pyridylazo)-2-naphthol (PAN)[13] are the chelating reagents for $Fe^{3+}$. In that case, it is necessary to oxidize $Fe^{2+}$. So, methods mentioned above are sequentially rather than simultaneous. Pehkonen et al. have developed a simultaneous spectrophotometric method of determination of both valencies of iron using bis-2-pyridyl ketone benzoylhydrazone (DPKBH).[14]

The chelating agents were utilized for preparing chelating resins. Recently with combination of on-line chelating resin preconcentration and spectrophotometric,[15-17] spectrometric[17,18] or chemiluminescence[19,20] detection, a number of analytical methods for iron with high sensitivities have been developed. Hyphenated techniques such as liquid chromatography often coupled with sophisticated detection systems such as inductively coupled plasma atomic emission or mass spectrometry[21,22] require expensive instruments and well-trained operators. The simultaneous determination of $Fe^{2+}$ and $Fe^{3+}$ as their 4,7-diphenyl-1,10-phenanthroline chelates is also possible by ion-paired RP-HPLC.[23] A few capillary electrophoresis methods for iron speciation have also been developed.[24,25] In addition, flow injection analysis (FIA) for especially spectrophotometric determination of both valencies of iron has gained much interest in recent years because of its simplicity, high reproducibility and the possibility of coupling different detection systems, operated in parallel or sequentially.[26-28] Electroanalytical methods have also been described.[29, 30] Kinetic-catalytic methods extensively studied have high sensitivity and sufficient accuracy. But, the interdependence of reaction variables in kinetic methods is commonly rather complicated, so a large number of experimental runs are required to optimize the experimental analytical conditions. Moreover, kinetic methods generally determine the total iron, rather than differentiating its valencies.[31,32]

2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) forms highly colored and stable complexes with a number of metals. For this reason, Br-PADAP was used as both a colorimetric reagent[33, 34] for many metal ions and a post-column reagent.[35, 36] for the determination of $Fe^{2+}$ and $Fe^{3+}$ along with many metal ions after cation exchange chromatographic separation.

Derivative spectrophotometry in the UV-Vis region is a useful technique in extracting qualitative and quantitative

information from overlapping bands of the analyte and interferents due to incompletely resolved peaks.[37-39] A number of studies were reported showing the advantage of higher selectivity of derivative spectrophotometry than normal (zero-order) spectrophotometry.[33, 34, 40, 41]

Both $Fe^{2+}$ and $Fe^{3+}$ forms colored complexes with 5-Br-PADAP and ordinary spectra of these complexes overlaps greatly. So, it was aimed to develop a simple, rapid, highly selective and sensitive simultaneous (not sequential) determination method of $Fe^{2+}$ and $Fe^{3+}$ by making use of second derivative spectra of complexes which was not reported earlier.

## Experimental

*Instruments and reagents*

Ordinary and derivative spectra were recorded with a Varian Cary 1E spectrophotometer utilizing quartz cells. pH measurements were made with a Metrohm E-512 pH-meter equipped with a glass electrode.

All chemicals (E. Merck) were of analytical reagent grade and were used without further purification. A $5.0x10^{-4}$ M solution of 5-Br-PADAP was prepared in methanol. $Fe^{2+}$ ($1.0x10^{-2}$ M) and $Fe^{3+}$ ($1.0x10^{-2}$ M) solutions were prepared from $(NH_4)_2Fe(SO_4)_2.6H_2O$ and $NH_4Fe(SO_4)_2.12\ H_2O$, respectively in 0.1 M HCl solution and were diluted as required. A 1 M $CH_3COONH_4$ solution was used as buffer solution of pH 7.0. The interferent solutions were prepared from $CrCl_3.6H_2O$, $AlCl_3.6H_2O$, $MgCl_2.6H_2O$, $CaCl_2.2H_2O$, $BaCl_2$, $K_2Cr_2O_7$, $ZnCl_2$, $Na_2HPO_4$, NaCl, KCl, $NH_4F$, $NaHCO_3$, $(NH_4)_2SO_4$, $NaNO_3$.

*Procedures*

For single $Fe^{2+}$ determination, 1 ml of sample solution containing preferably $1.0x10^{-5}$–$2.4x10^{-4}$ mmol (0.56–13.4 μg) $Fe^{2+}$ was taken; 5 ml of $5.0x10^{-4}$ M 5-Br-PADAP solution (10-fold of maximum amount of $Fe^{2+}$) followed by 1 ml of 1 M $CH_3COONH_4$ solution were added to yield a final volume of 7 ml.

For single $Fe^{3+}$ determination, 1 ml of sample solution containing preferably $1.0x10^{-5}$–$2.4x10^{-4}$ mmol (0.56–13.4 μg) $Fe^{3+}$ was taken; 5 ml of $5.0x10^{-4}$ M 5-Br-PADAP solution (10-fold of maximum amount of $Fe^{3+}$) followed by 1 ml of 1 M $CH_3COONH_4$ solution were added to yield a final volume of 7 ml.

For simultaneous determination of $Fe^{2+}$ and $Fe^{3+}$, 1 ml of sample solution containing preperably $2.0x10^{-5}$– $2.4x10^{-4}$ mmol total metal (as $Fe^{2+}$ plus $Fe^{3+}$) was taken; 5 ml $5.0x10^{-4}$ M 5-Br-PADAP followed by 1 ml 1 M $CH_3COONH_4$ solution were added to yield a 7 ml of mixture solution.

The second derivative absorbance values ($^2D$) of the above mixtures at 577 nm and 611 nm were measured against a reagent blank after 10 min of mixture preparation. The concentrations (C) of $Fe^{2+}$and $Fe^{3+}$ were estimated from the linear calibration curves of $^2D$ versus C drawn with standard solutions of a suitable concentration range.

*Interference analysis*

The standard procedure for the determination of $Fe^{2+}$ and $Fe^{3+}$ was followed with the exception that 0.5 ml of the interferent solution was added along with 0.5 ml iron solution.

*Analysis of water samples*

The proposed method was applied to tap water and coastal seawater samples. After the filtration through a 0.45 μm membrane filter, the method of standard additions was applied to both water samples.

## Results and discussion

*Absorption spectra*

The ordinary and second derivative spectra of $Fe^{2+}$- and $Fe^{3+}$-5-Br-PADAP complexes were recorded for individual ions and for mixtures covering the $Fe^{2+}$:$Fe^{3+}$ molar ratio range between 1:20 - 20:1. The ordinary and second derivative spectra for $Fe^{2+}$ and $Fe^{3+}$ are presented in Figs. 1 and 2, respectively. The spectra in Figs. 1 and 2 show that both metal-5-Br-PADAP complexes exhibit ordinary (A) and second-derivative absorbance ($^2D$) at the extremum wavelengths of the other iron complex. Second derivative spectra were selected for evaluation, because working wavelength determination was more precise and spectral overlap was less than in ordinary spectra. Two wavelengths at which $^2D$-absorbance of one valency of iron had a substantial value as opposed to the zero value of the other were 611 nm for $Fe^{2+}$, and 577 nm for $Fe^{3+}$.

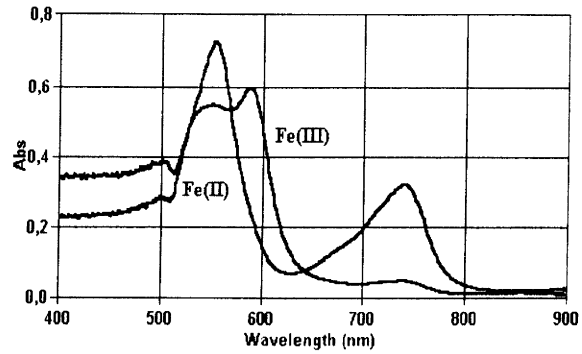


Fig. 1 Ordinary spectra of $Fe^{2+}$ and $Fe^{3+}$ complexes with 5-Br-PADAP. $[Fe^{2+}]_{final}$ = $1.0x10^{-5}$ M, $[Fe^{3+}]_{final}$= $6.6x10^{-6}$ M, $[5\text{-Br-PADAP}]_{final}$ = $3.57x10^{-4}$ M

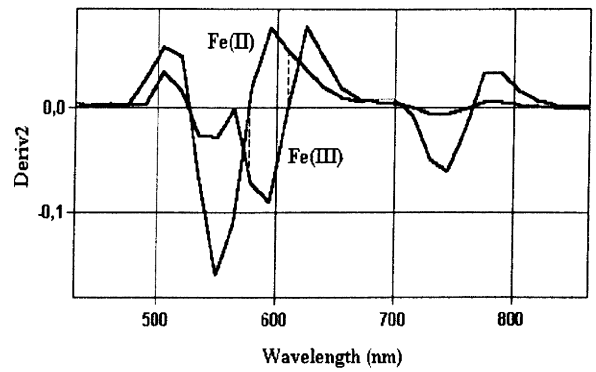


Fig. 2 Second derivative spectra of $Fe^{2+}$ and $Fe^{3+}$ complexes with 5-Br-PADAP. $[Fe^{2+}]_{final}$= $1.0x10^{-5}$ M, $[Fe^{3+}]_{final}$= $6.6x10^{-6}$ M, $[5\text{-Br-PADAP}]_{final}$ = $3.57x10^{-4}$ M; $\Delta\lambda$=15 nm.

*Selection of optimal parameters for analysis*

The $^2D$ values obtained with 3.9 μg $Fe^{2+}$ and 2.6 μg $Fe^{3+}$ were sufficiently high for 15 nm of $\Delta\lambda$.

The pH of $Fe^{2+}$- and $Fe^{3+}$-5-Br-PADAP mixtures were varied between 3.0-11.0 by the use of $1.0x10^{-3}$ M HCl or $1.0x10^{-3}$ M NaOH solutions. Since maximum absorbance was obtained at about pH 7.0, this value was selected as the working pH. 1M $CH_3COONH_4$ solution was used as the buffer solution at this pH.

The colored complexes of $Fe^{2+}$ and $Fe^{3+}$ with 5-Br-PADAP were formed immediately after mixing the solutions, and the colors formed were stable for at least 24h. For this reason, absorbances were measured 10 min after preparing the complexes.

5-Br-PADAP/$Fe^{2+}$ or $Fe^{3+}$ mole ratios were varied between 1-100 at pH 7.0. Above a ratio of 5, absorbances did not change appreciably. So, a ligand/metal ratio >10 was selected for further evaluations.

*The linear plot (calibration curve)*

The individual calibration curves drawn at the working wavelengths of 577 nm and 611 nm for $Fe^{3+}$ and $Fe^{2+}$, respectively, had the following linear equations with the corresponding correlation coefficients (r).

For $Fe^{2+}$,

$^2D_{611} = 5.74 \times 10^3 C_{Fe2+} + 0.001$ ( r = 0.9999) Eq. (1)

For $Fe^{3+}$,

$^2D_{577} = -9.93 \times 10^3 C_{Fe3+} + 0.015$ ( r = 0.9992) Eq. (2)

Table 1 Interference analysis for $Fe^{2+}$

| Interferent ion | Mole ratio | Error (%) |
|---|---|---|
| $Fe^{3+}$ | 20 | 0 |
| $Na^+$ | 1000 | 0 |
| $K^+$ | 1000 | 0 |
| $Ba^{2+}$ | 100 | 1.1 |
| $Ca^{2+}$ | 100 | -3.3 |
| $Mg^{2+}$ | 100 | 3.3 |
| $Zn^{2+}$ | 2 | 1.1 |
| $Al^{3+}$ | 100 | 1.4 |
| $Cr^{3+}$ | 2 | 2.9 |
| $Cr^{6+}$ | 100 | -1.4 |
| $HPO_4^{2-}$ | 1 | -4.2 |
| $HCO_3^-$ | 5 | 1.7 |
| $Cl^-$ | 1000 | 0 |
| $F^-$ | 1000 | -0.7 |
| $SO_4^{2-}$ | 1000 | 0 |
| $NO_3^-$ | 10 | 0 |

*Reproducibility and linear range*

The relative standard deviation for the analysis of $Fe^{2+}$ (1.12 mg/l) individually was 1.03%, and for its admixture with $Fe^{3+}$ (5.6 mg/l) was 4.36%. The relative standard deviations for analysis of $Fe^{3+}$ (1.12 mg/l) individually and for its admixture with $Fe^{2+}$ (6.72 mg/l) were 3.93% and 1.52%, respectively. The linear ranges in $^2D$ evaluation were between $1.4 \times 10^{-6}$-$3.4 \times 10^{-5}$M for both $Fe^{2+}$ and $Fe^{3+}$.

*Interference analysis*

The possible interferences of a number of cations and anions on the determination of 2.8 μg $Fe^{2+}$ and $Fe^{3+}$ are depicted in Tables 1 and 2, respectively. Alkali and alkaline earths and some transition metal cations and common anions did not interfere with the proposed method. But, $Fe^{3+}$ determination was impossible in the presence of $Zn^{2+}$.

Table 2 Interference analysis for $Fe^{3+}$

| Interferent ion | Mole ratio | Error (%) |
|---|---|---|
| $Fe^{2+}$ | 20 | 0 |
| $Na^+$ | 1000 | 0 |
| $K^+$ | 1000 | 0 |
| $Ba^{2+}$ | 100 | -1.8 |
| $Ca^{2+}$ | 100 | 2.6 |
| $Mg^{2+}$ | 100 | 2.6 |
| $Al^{3+}$ | 100 | 3.4 |
| $Cr^{3+}$ | 2 | 1.4 |
| $Cr^{6+}$ | 100 | -2.5 |
| $HPO_4^{2-}$ | 1 | -3.6 |
| $HCO_3^-$ | 5 | -1.1 |
| $Cl^-$ | 1000 | 0 |
| $F^-$ | 1000 | -0.7 |
| $SO_4^{2-}$ | 1000 | 0 |
| $NO_3^-$ | 10 | 0 |

*Analysis of water samples*

The results obtained by applying standard addition recovery tests to a coastal seawater sample are presented in Table 3. The recoveries for spiked samples were in the range of 97-99% and 99-100% for $Fe^{2+}$ and $Fe^{3+}$, respectively.

The results obtained by the same manner with a tap water sample are also presented in Table 4. The recoveries for spiked samples were in the range of 95-100% and 96-98% for $Fe^{2+}$ and $Fe^{3+}$, respectively.

Table 3 Recovery of iron added to seawater sample

| | Added (μg) | | Found (μg) (within 95% confidence interval) | | Recovery (%) | |
|---|---|---|---|---|---|---|
| Samples | $Fe^{2+}$ | $Fe^{3+}$ | $Fe^{2+}$ | $Fe^{3+}$ | $Fe^{2+}$ | $Fe^{3+}$ |
| Seawater | - | - | - | - | - | - |
| 1 | 1.12 | 4.50 | 1.09± 0.038 | 4.52± 0.051 | 97 | 100 |
| 2 | 2.24 | 6.70 | 2.21± 0.041 | 6.61± 0.057 | 99 | 99 |
| 3 | 3.33 | 9.00 | 3.29± 0.043 | 8.92± 0.059 | 99 | 99 |

Table 4 Recovery of iron added to tap water sample

| Samples | Added (μg) | | Found (μg) (within 95% confidence interval) | | Recovery (%) | |
|---|---|---|---|---|---|---|
| | $Fe^{2+}$ | $Fe^{3+}$ | $Fe^{2+}$ | $Fe^{3+}$ | $Fe^{2+}$ | $Fe^{3+}$ |
| Tap water | - | - | 0.39 +0.045 | - | - | - |
| 1 | 0.56 | 4.50 | 0.92 ± 0.035 | 4.42± 0.047 | 95 | 98 |
| 2 | 1.12 | 2.24 | 1.47± 0.039 | 2.18± 0.051 | 96 | 97 |
| 3 | 2.24 | 1.12 | 2.63± 0.041 | 1.07± 0.049 | 100 | 96 |
| 4 | 4.50 | 0.56 | 4.91± 0.038 | 0.54± 0.045 | 100 | 96 |

While the sequential methods constituting most of the $Fe^{2+}/Fe^{3+}$ determination methods require oxidation or reduction steps after the determination of one valency of iron, the proposed method capable of simultaneous determination of both valencies of iron does not require any further processes. So, the proposed method for the simultaneous determination of $Fe^{2+}$ and $Fe^{3+}$ with 5-Br-PADAP is simple, rapid, highly sensitive, selective and reproducible.

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