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Simultaneous Determination of Copper and Iron in Biological Samples with 1-(2-Pyridylazo)-2-naphthol in Anionic AOT Micellar Solution Using Derivative Spectrophotometry

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A simple and selective derivative spectrophotometric method using 1-(2-pyridylazo)-2-naphthol (PAN) in *bis*-2-ethyl hexyl sulfosuccinate (AOT) micellar solution was developed for determination of copper and iron. The effect of various parameters on the sensitivity and selectivity of the method was investigated and optimum conditions were selected. The pH = 2.5 was chosen as an optimum in order to reduce the interference effects. At this pH, in addition to Cu(II) and Fe(III), only Ni(II), Co(II) and Co(III) may be react with PAN and interfere the determination of copper and iron. The concentrations of nickel and cobalt in the most samples usually are very low and thus have no interference on the determination of copper and iron. In the presence of AOT the absorption spectrum of Fe(III)-PAN complex shift to higher wavelengths and the overlapping with Cu-PAN spectrum was decreased. In this condition, in ordinary spectrophotometry, Cu(II) has no interference in the determination of iron, but Fe(III) completely interfere the determination of copper. In the first order derivative mode the spectra of Cu(II)-PAN and Fe(III)-PAN complexes completely resolved together and selectively can determine them. The zero and first order derivative spectrophotometric calibration curves were drawn at working wavelengths of 558 and 580 nm for copper and 600 and 630 nm for iron. Molar absorbtivity were found 1.82×10^4 and 2.75×10^4 L mol^{-1} cm^{-1}, respectively for Cu(II)-PAN and Fe(III)-PAN complexes. In derivative mode, the limits of detection (LOD) for Cu(II) and Fe(III) were 0.038 and 0.025 μg mL^{-1}, respectively. The method has been applied to human hair and serum samples successfully.

Key Words: Derivative spectrophotometry, 1-(2-Pyridylazo)-2-naphthol, AOT micellar solution, Copper, Iron.

INTRODUCTION

Copper is an essential constituent of about 30 enzymes and glycoproteins and is required for the synthesis of hemoglobin and for some biological processes. It also promotes iron absorption from the gastrointestinal system, which is involved in the transport of iron from tissues into plasma, helps to maintain myelin in the nervous system, is important in the formation of bone and brain tissues and is necessary for many other important functions[1-4].

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At low concentrations, iron is essential for a wide spectrum of biological functions, including oxygen transport, mitochondrial electron transfer and DNA synthesis. Above trace levels, however, iron has other roles. It has been stated that for all trace elements that considered to be essential, there exist a fairly narrow "concentration window" between the essential and toxic levels. Iron is a moderately toxic element when compared with other transition metals. However, the toxic doses of iron and its compounds can lead to serious problems, including depression, rapid and shallow respiration,coma, convulsions and cardiac arrest[5-9].

Thus, it is important and necessary to develop reliable, fast, selective and sensitive methods for the determination of copper and iron in biological samples. Several techniques such as inductively coupled plasma-atomic emission spectrometry (ICP-AES)[10,11], atomic absorption spectrometry using either flam (FAAS)[12,13], or electro-thermal atomization (ETAAS)[14,15], electroanalytical technique[16,17], UV-Visible spectro-photometry[18-21] have been used for the determination of copper and iron in different matrices. Among the most widely used analytical methods are those based on the UV-Visible spectrophotometric techniques, due to both the resulting experimental rapidity and simplicity and the wide application[22-25]. A survey of the literature reveals that less work has so far been reported regarding simultaneous determination of Cu(II) and Fe(III)[20,26,27]. Simultaneous determination of copper and iron in diverse matrixes is hampered due to spectral overlap of their complexes. Thus the determination of copper and iron with traditional UV-Visible spectrophotometric technique is difficult without any separation processes. In such cases spectrophotometry coupled with derivativization and analysis in a suitable micellar medium is useful in analyzing the complex matrixes. The high solubilizing capacity of micellar systems[28,29] and derivatization of spectral profiles not only offer a convenient solution to the resolution of absorption profiles of multicomponent systems[30,31], but also enhance the spectral details and analytical value of the normal photometric analysis[32-35].

1-(2-Pyridylazo)-2-naphthol (PAN) forms highly coloured and stable complexes with number of metals. The absorption spectra of Fe(III)-PAN and Cu(II)-PAN complexes overlap compactly and the determination of these metals is difficult without any separation processes even by derivative spectrophotometry. To the best of our knowledge, there is no report for the simultaneous of determination for Cu(II) and Fe(III) in micellar anionic AOT media solution. The aim of this work was to develop an appropriate condition for which the absorption spectra of these complexes resolve together, enough and determination of copper and iron without any separation process. For this purpose, AOT micellar medium was used. In the presence of AOT, the spectrum of Fe(III)-PAN complex shift to higher wavelength and the overlapping with Cu-PAN spectrum was decreased. It seems this anionic surfactant enters in the structure of Fe-PAN complex and causes a shift in the absorption spectrum of it while no effect on Cu-PAN complex.

EXPERIMENTAL

Ordinary and derivative spectra were recorded with a Shimadzu UV-1650-PC spectrophotometer utilizing glass cells. pH measurements were made with a metrohm 645 pH-meter equipped with a combined glass electrode. The atomic absorption data were recorded on a Shimadzu model AA-670G atomic-absorption spectrometer.

All chemicals (E. Merck) were of analytical reagent grade and were used without further purification. All solutions were prepared in triply distilled water. Solutions of AOT (0.04 M), sodium dodecylsulfate (0.2 M) and Triton-X-100 (5 % v/v) were prepared in 100 mL volumetric flasks. Stock standard solutions of Cu(II) and Fe(III) at a concentration of 1000 μg mL^{-1} were prepared by dissolving 0.2904 g of $FeCl_3$ and 0.387 g $Cu(NO_3)_2 \cdot 3H_2O$, respectively, in 0.01 M HCl solution and diluting to 100 mL. Working standard solutions were obtained by appropriate dilution of the stock standard solution. A solution of 0.1 % PAN was prepared by dissolving appropriate amounts of this reagent in absolute ethanol. A buffer aqueous solution, pH = 2.5, was prepared with 0.5 M glycine.

Procedure: 1.5 mL of 0.04 M AOT solution, 0.5 mL of 0.5 M glycine buffer solution (pH = 2.5), 0.5 mL of 0.1 % (w/v) PAN solution and required volume of sample solution containing copper and iron was added into a 10 mL volumetric flask and the flask was adjusted to the mark with triply distilled water. After 3 min standing for completion of complexation reaction, absorbances of the above solution were measured against a reagent blank solution at 558 and 600 nm and were then considered for the determination of copper and iron using calibration graphs prepared in the same manner.

The first order derivative spectra of the sample were recorded against its reagent blank in the wavelength rang of 450-700 nm. Calibration curves in first order derivative mode (at 580 nm for copper and 630 nm for iron) were prepared by measuring analytical signals *versus* analyte concentration. The concentrations of copper and iron in sample solutions were determined using linear regression equations obtained with standard solutions. First-derivative spectra of Cu(II)-PAN and Fe(III)-PAN complexes preferred to ordinary spectra, because working wavelengths determination were more precise and has no spectral overlap.

Sample preparation

Serum sample: An 8 mL portion of serum sample was placed in a 100 mL beaker and 2 mL of concentrated HNO_3 was added. The contents in the beaker were heated at 110 ºC to dryness. To the obtained residue, 2 mL of concentrated HNO_3 was added and the mixture was heated at 150 ºC for 2 h. Then 2 mL of concentrated HNO_3 and 1 mL of $HClO_4$ (70%) was added and repeatedly heated at 150 ºC for 2 h. All of the heating processes were carried out under a hood with necessary precautions. To obtain a white residue, 4 mL of 0.01 M HNO_3 was added and heated at 110 ºC for 1 min. The resulted clear solution was neutralized by a 0.1 M NaOH solution.

The obtained solution was transferred into a 10 mL volumetric flask and analyzed by proposed procedure.

Hair sample: Appropriate amounts of hair sample were washed with water and acetone and dried at 100 ºC. One gram of this hair sample was placed in a 100 mL beaker and 12 mL of concentrated HNO_3 and 2 mL of $HClO_4$ (70 %) was added. The contents in the beaker were heated on a hot plate (100 ºC 45 min, 150 ºC 45 min). Then, the solution was cooled to 70 ºC and 5 mL of hydrogen peroxide (30 %) was added. The mixture was heated at 200 ºC to dryness. To obtain a white residue, 5 mL of 0.01 M HNO_3 was added and heated at 110 ºC for 1 min. The resulted clear solution was neutralized by a 0.1 M NaOH solution and the volume was made up to 10 mL in a volumetric flask. Five milliliters of this solution was transferred into a 10 mL volumetric flask and analyzed by proposed procedure.

RESULTS AND DISCUSSION

The effect of various parameters on the sensitivity and selectivity of the method was investigated. pH is one of the most important parameters for this determination. The influence of pH on the analytical signals of Fe(III)-PAN complex at a constant concentration was investigated in the range of 1-5. The obtained results showed that the absorbance increase up to 1.8 for Cu(II) and 2.2 for Fe(III) and remains practically constant up to 5. The pH = 2.5 was chosen as an optimum in order to reduce the interference effects. At this pH, in addition to Fe(III) and Cu(II), only Ni(II), Co(II) and Co(III) may be react with PAN and interfere the determination them. The concentrations of nickel and cobalt in most of the samples usually are very low and thus have no interference on the determination of iron. The absorption spectra of Fe(III)-PAN and Cu(II)-PAN complexes are investigated in various micellizing agents such as Triton-X-100, SDS and AOT (Figs. 1-3). Also the first order derivative spectra of Fe(III)-PAN and Cu(II)-PAN complexes in AOT micellar solution are shown in Fig. 4. Results showed that the absorption spectra of Cu(II)-PAN and Fe(III)-PAN complexes compactly overlap in Triton-X-100 and SDS micellar solutions. It can be seen that in the presence of AOT the absorption spectrum of Fe(III)-PAN complex shift to higher wavelength and the overlapping with Cu-PAN spectrum was decreased. It seems this anionic surfactant enters in the structure of Fe-PAN complex and causes a shift in the absorption spectrum of it, while no effect on Cu-PAN complex. In AOT micellar solution, in ordinary spectrophotometry, Cu(II) has no interference on the determination of iron, but Fe(III) completely interfere the determination of copper. In the first order derivative mode the spectra of Cu(II)-PAN and Fe(III)-PAN complexes completely resolved together and selectively can determine them. Thus, the method is very selective for determination of copper and iron. Varying the concentration of AOT (0.00-0.02M), showed an increase in absorbance (at λ_{max}) of the Cu(II)-PAN and Fe(III)-PAN complexes up to 0.004 M and remains practically constant up to 0.02 M (Fig. 5). Subsequent studies were carried out at 0.006 M of AOT. The effect of PAN concentration was also studied and the PAN concentration of 2×10^{-4} mol L^{-1} was chosen as optimum.

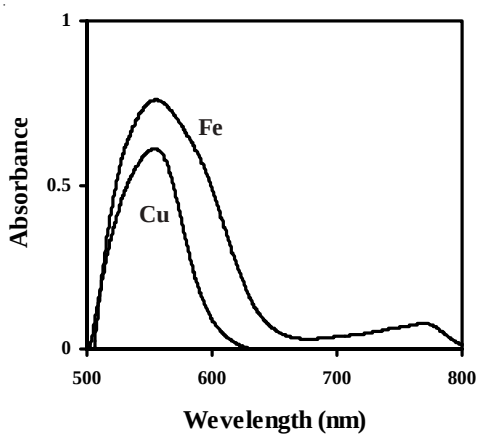

Fig. 1. Absorption spectra of Cu(II)-PAN and Fe(III)-PAN complexes in Triton-X-100 micellar solution. Conditions: 2 μg mL^{-1} of Cu(II), 2 μg mL^{-1} of Fe(III), 1.5 % (v/v) Triton-X-100, 2×10^{-4} mol L^{-1} PAN and pH = 2.5

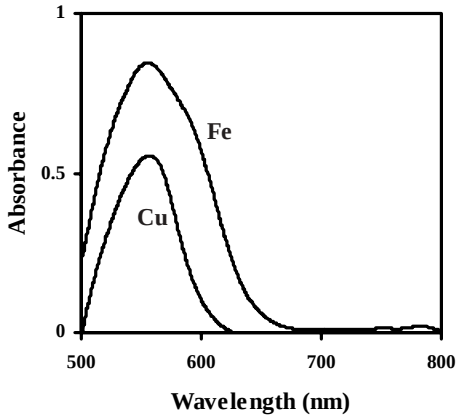

Fig. 2. Absorption spectra of Cu(II)-PAN and Fe(III)-PAN complexes in SDS micellar solution. Conditions: 2 μg mL^{-1} of Cu(II), 2 μg mL^{-1} of Fe(III), 0.02 mol L^{-1} SDS, 2×10^{-4} mol L^{-1} PAN and pH = 2.5

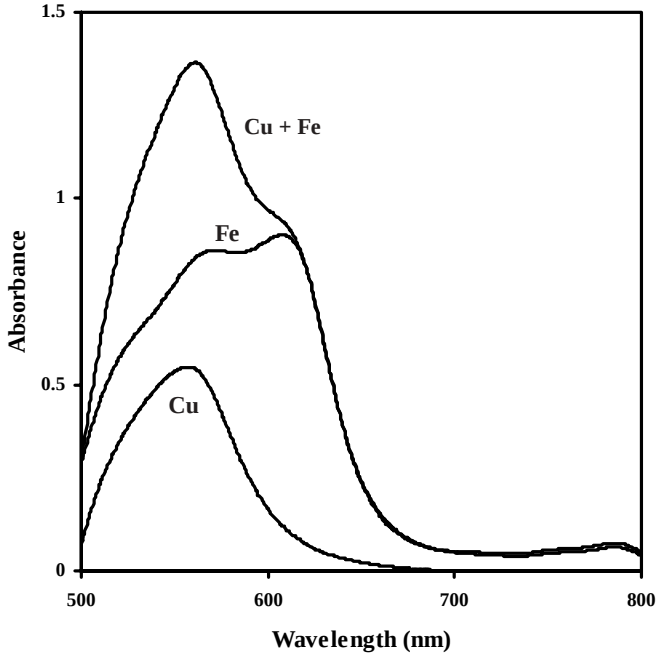

Fig. 3. Absorption spectra of Cu(II)-PAN, Fe(III)-PAN and mixture of Cu(II)-PAN + Fe(III)-PAN complexes in AOT micellar solution. Conditions: 2 μg mL^{-1} of Cu(II), 2 μg mL^{-1} of Fe(III), 0.006 mol L^{-1} AOT, 2×10^{-4} mol L^{-1} PAN and pH = 2.5

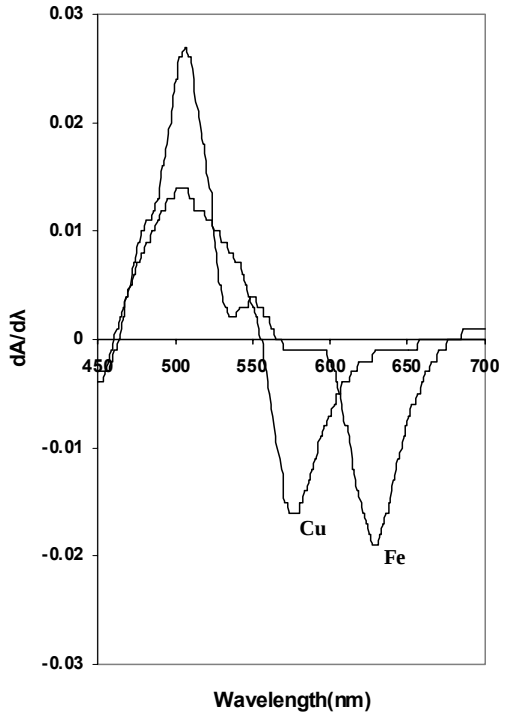

Fig. 4. First order derivative spectra of Cu(II)-PAN and Fe(III)-PAN complexes in AOT micellar solution. Conditions: 2 µg mL^{-1} of Cu(II), 2 µg mL^{-1} of Fe(III), 0.006 mol L^{-1} AOT, 2×10^{-4} mol L^{-1} PAN and pH = 2.5

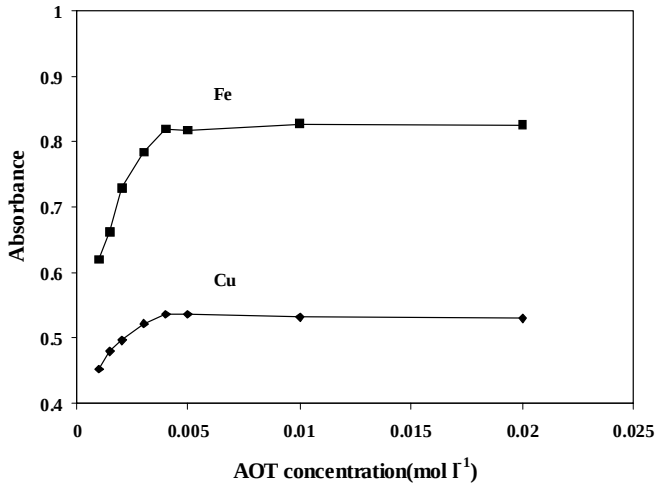

Fig. 5. Effect of AOT concentration on the absorbance (at λ_{max}) of the Cu(II)-PAN and Fe(III)-PAN complexes. Conditions: 2 µg mL^{-1} of Cu(II), 2 µg mL^{-1} of Fe(III), 2×10^{-4} mol L^{-1} PAN and pH = 2.5

Calibration graphs and analytical parameters: The zero and first order derivative spectrophotometric calibration curves were drawn for determination of copper and iron at working wavelengths. Table-1 gives the parameters of the calibration curves, the relative standard deviations obtained for 5 replicates subjected to the complete procedure and the detection limits.

TABLE-1
PARAMETERS OF THE CALIBRATION CURVES

Calibration equation	Wavelength (nm)	Linear range (μg mL^{-1})	R^2	RSD % (n=5)	LOD[a] (μg mL^{-1})	Molar absorptivity (L cm^{-1} mol^{-1})
$A = -0.057 + 2.86 \times 10^{-1} C_{Cu}$	558	0.31 - 5	0.9994	0.29	0.031	1.82×10^4
$dA/d\lambda = 0.0004 - 8.1 \times 10^{-3} C_{Cu}$	580	0.38 - 5	0.9995	0.22	0.038	-
$A = -0.037 + 4.30 \times 10^{-1} C_{Fe}$	600	0.20 - 5	0.9984	0.35	0.020	2.75×10^4
$dA/d\lambda = 0.005 - 1.24 \times 10^{-2} C_{Fe}$	630	0.25 - 5	0.9947	0.27	0.025	-

[a]Limit of detection. Calculated as 3 times the standard deviation of the blank signal.

Application: In order to confirm the usefulness of this proposed method, it was applied for the determination of copper and iron in human serum and hair samples. The results presented in Table-2 show that the proposed method can be successfully applied to the determination of copper and iron in biological samples. The results of analyses by the developed method were found to be in good agreement with those obtained by atomic absorption spectrometric method.

TABLE-2
DETERMINATION OF COPPER AND IRON IN HUMAN SERUM AND HAIR SAMPLES

Sample	Iron[a]		Copper[a]	
	Proposed method	AAS	Proposed method	AAS
Serum (μg mL^{-1})	2.3 ± 0.16	2.2 ± 0.08	0.75 ± 0.06	0.79 ± 0.03
Hair (μg g^{-1})	59.8 ± 3.30	59.1 ± 1.40	13.20 ± 0.70	12.60 ± 0.40

[a]Mean of 3 experiment ± standard deviation.

Conclusion

In the presence of AOT the absorption spectrum of Fe(III)-PAN complex shift to higher wavelength and the overlapping with Cu-PAN spectrum was decreased. In the first order derivative mode the spectra of Cu(II)-PAN and Fe(III)-PAN complexes completely resolved together and has no spectral overlap. A simple, sensitive and selective derivative spectrophotometric method was developed for determination of copper and iron in biological samples, using 1-(2-pyridylazo)-2-naphthol in AOT micellar solution. The method is very selective and no need for masking agents.

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