Short communication

Fresenius Z Anal Chem (1989) 334:270 —
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Spectrophotometric titration of μg -amounts of iron(III) ion in human serum by means of phosphoorganic complexon (EDHBP)

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Spektralphotometrische Titration von μg -Mengen Eisen(III)-ion in menschlichem Serum mit Hilfe von EDHBP

Ethylenedi(aminosalicylphosphonic)acid (EDHBP) as phosphoorganic complexon is a hexaprotonic ligand.

The EDHBP compound with iron(III) ion is a very stable complex and its stability constant has been estimated as $\log K_{\text{Fe(III)EDHBP}} > 40$ [1, 2]. We have determined the stability constant for iron(III) ion to be $\log K = 41.5$. This complex is stable in the pH range 0–13 [2]. The coloured complex has $\lambda_{\text{max}} = 480 \text{ nm}$ and $\epsilon_{\text{max}} = 9.9 \cdot 10^3 \text{ mol}^{-1} \cdot \text{cm}^{-1}$.

The stability constant for iron(II) ion is estimated as $\log K_{\text{Fe(II)EDHBP}} = 16.20$ [1]. We have determined the stability constant for iron(II) ion to be $\log K = 16.25$. This complex is not coloured.

The big difference observed between the values of stability constants for iron(III) and other metals ($\log \text{CuL} = 24.0$, $\log \text{NiL} = 18.2$, $\log \text{MgL} = 12.0$) is responsible for the lack of cationic interferences in the determination of iron(III). Any influence of anions was not observed while determining iron(III) on a indicator paper [2] due to lower values of stability constants of complexes of other anions. Ascorbic acid forms with iron(III) ions a complex with the stability constant of $\log K = 33.6$ [3]. Sulphosalicylic acid forms a stable complex with iron(III) ion with the stability constant of $\log K = 32.6$ [3]. Citric acid has $\log K = 28.4$ and oxalic acid $\log K = 18.49$ when they react with iron(III) ion [3]. The results of a microgram spectrophotometric titration of iron(III) ion in human serum by means of EDHBP are reported below (Fig. 1, Table 1).

The presented method allows the determination of iron(III) in the presence of iron(II) in human serum. Speciation can be performed due to the selectivity of the EDHBP reagent. Comparing the spectrophotometric titration method with a spectrophotometric method we can conclude the following: 1. the spectrophotometric method of titration is simple and more easily applicable; 2. the results obtained indicate a high precision and sensitivity of this method when compared with other methods (Table 1).

Fig. 1. Spectrophotometric titration of iron(III) ion in human serum using EDHBP. *a* Determination curve of iron(III) ion, *b* determination curve of iron(III) ion after iron(II) ion oxidation

Procedure

Iron(III) ion determination

a) 1 ml of human serum was transferred into 9 ml 0.05 mol/l of oxalic acid. The solution was mixed and transferred into a glass cell, placed in the spectrophotometer Specol (C. Zeiss) cell compartment and immediately titrated with a solution of EDHBP (0.001 mol/l). The latter was added portion-wise (0.1 ml at a time). Changes of absorbance were read at λ_{max} 480 nm. The titration endpoint was taken from the plot $A = f(c)$ (Fig. 1, curve a).

b) Determination of total iron as iron(III) ion. 1 ml of human serum was transferred into 5 ml of 0.05 mol/l of oxalic acid and 4 ml of 10% H_2O_2 was added. After 15 min of mixing the glass cell was placed into a Specol. The solution was titrated with 0.001 mol/l EDHBP complexon solution (0.1 mol added at a time). Changes of absorbance were read at λ_{max} 480 nm and the titration end point was found on the plot $A = f(c)$ (Fig. 1, curve b).

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Received November 25, 1988

Table 1. Comparison of the results of iron determination with EDHBP, sulphosalicylic acid [4] and α, α' dipyridyl [4]

Reagents	Method	Iron(III) ion determination in $\mu\text{g}/10 \text{ ml}$		Iron(II) ion determination after reduction in $\mu\text{g}/10 \text{ ml}$
		Fe(III) ion found	Fe(III) found after oxidation	
EDHBP	Spectrophotometric titration	1. 1.50	1. 15.40	
		2. 1.90	2. 19.41	
		3. 1.12	3. 11.51	
Sulphosalicylic acid	Spectrophotometric titration		1. 16.50	
			2. 20.50	
			3. 12.05	
α, α' dipyridyl	Spectrophotometric titration			1. 15.50
				2. 19.45
				3. 11.56

1., 2., 3., Samples of human serum