Development of Extractive Spectrophotometric Methods for the Determination of Iron(III) with Dimercaptophenole and Heterocyclic Diamines

Kuliev Kerim Avaz, Verdizade Nailya Allahverdi and Mamedova Shafa Aga
Department of Analytical Chemistry, Azerbaijan State Pedagogical University, U. Gadjibekovstreet 68. Baku, AZ 1000, Azerbaijan.
kerim.kuliev.69@mail.ru*

(Received on 10th March 2017, accepted in revised form 16th January 2019)

Summary: Dimercaptophenole (DM) is proposed as a new sensitive reagent for the sensitive extractive spectrophotometric determination of Fe(II). DM in the presence hydrophobic amins reacts with Fe(II) in the pH range 5.3 -7.2 to form a coloured complex. Chloroform, dichloroethane, and carbon tetrachloride appeared to be the best extractants. The absorption spectrum of Fe (II)-DM-Am complexes in chloroform shows maximum absorbance at 552-583 nm. It was observed that the color development was instantaneous and stable. Linear calibration graphs were obtained for 0.03-4.2 μg mL^{-1} of Fe.The molar absorptivity calculated was found to be (3.08-4.33) × 10^4 dm^3 mol^{-1} cm^{-1} and the sensitivity of the method as defined by sandal's was 1.29-1.82 ng cm^{-2}. The stoichiometry of the complex is established as 1:1:2 (M : L : Am) by equilibrium shift method, and confirmed the methods of relative yield, Asmus straight line and the intersection curves. It may be satisfactorily applied for the determination of Fe(III) with present method. The results of the prescribed procedure applied for the determination of the micro amounts of iron in pharmaceutical, biological, water, food and in plant samples are presented.

Keywords: Iron, Spectrophotometric determination, Chloroform, Di*mercapto*phenole.

Introduction

In living organisms, iron is a basic trace mineral that catalyzes oxygen in exchange processes. Iron deficiency is seen as a disease organism (chlorosis in plants and anemia in animals). Excess too bad: iron compounds are deposited in the tissues of the eyes and the lungs, causing them to siderosis [1].

Iron is an absolute necessity for many life forms, including humans and many kinds of bacteria. Iron is a part of different food products. Fe(II) is a cofactor in heme enzymes such as catalase and cytochrome C, and in non-heme enzymes such as aldolase and tryptophan oxygenase. Is an essential component involved in oxygen transport. It is also essential for the regulation of cell growth, and differentiation of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity. Iron is an essential trace element that makes up the sea water, soil and can be counted among the biogenic elements. Its excess content in some waters is an indicator of water pollution due to human factors [2, 3].

Iron plays an important role in human life (4.0-4.5 grams per adult human body) participating in processes hematopoiesis, intracellular metabolism and regulation of redox processes. It is therefore necessary to ensure delivery of this element in the human body, and c using complex multivitamin preparations with trace elements (healthy person needs. Furthermore, there are a number of products of therapeutic purposes, which include various additives containing iron. A significant portion of trace elements needed by the body in certain concentrations can be toxic properties. Therefore, the analytical control of their contents should be carried out with sufficiently reliable methods. Modern requirements to the objects of the analysis of the environment and food products include the development of new highly sensitive and rapid methods for the determination of toxic metals. The multicomponent of analysis objects, low concentrations of metal contaminants to solve such problems, necessitates the use of combined methods of analysis, concentrating the concentration step. One of the promising methods for identifying metals in complex objects is the spectrophotometric analysis methods [4, 5].

Reagents containing OH-groups and nitrogen donor atoms are considered most suitable for the determination of Fe(III). Techniques for the photometric determination of iron (III) in the form of MLC these reagents in the presence of various third components have high sensitivity and selectivity [6].

For spectrophotometric determination of iron in various samples suggested leuco xylene cyanol [7], 1,2-dihydroxy-3,4-diketocyclobutene

*To whom all correspondence should be addressed.

(squaric acid) [8], thiocyanate [9], 9-(4-carboxypheny1) - 2,3,7-trihydroxy1-6-flurone[10], 2^1,3,4^1,5,7-Pentahydroxyflavone [11], 4-nitrocatechol and 2,3,5-triphenyl-2H-tetrazolium chloride [12], 4-nitrocatechol (4NC) and 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide [13],N-ethyl-2-methyl-3-hydroxypyridin-4-on [14], di(2-pyridyl)-N,N-di[(8-quinolyl)amino]methane [15-17], 2-hydroxy-1-naphthaldehyde-p-hydroxybenzoichydrazone [18] and 2-(2,3-dihydroxy -4-oxocyclobut-2-enylidene) hydrozinecarbothiamide [19]2-amino-4-(3-nitrophenylazo)pyridine-3-ol [20]. Most of these methods are either insensitive or take a long time to improve the color. Oxy*mercapto*phenolate and di*mercapto*phenolate complexes of *d*-elements (Mo, V, Co, Cu) are insoluble in chloroform, while mixed-ligand complexes with hydrophobic amines and aminophenols easily dissolve in various organic solvents [21-25]. The real work is devoted to studying of reaction of a complex formation of iron(III) with di*mercapto*phenoles (DM) in the presence of hydrophobic amines (Am) and the development of new extraction-spectrophotometric methods for the determination of iron.. From *dimercapto*phenols 2, 6-*dimercapto*phenol (DMP), 2, 6-*dimercapto*-4-methylphenol (DMMP) and 2, 6-dithiol-4-ethylphenol (DMEP) were used. in the presence of hydrophobic amines (Am). As hydrophobic amine 1,10-phenantroline (Phen), 4,7-diphenyl-1,10-phenanthrolin (ebatophenantroline (BPhen)) and 2,2'-bipiridina (Bip) were used.

Experimental

Instruments

The absorbance of the extracts was measured using a Shimadzu UV mini 1240 spectrophotometer (Japan) and KFK 2 photocolorimeter (USSR). Glass cells with optical path of 10 or 5 mm were used. pH of aqueous phase was measured using an I-120.2 potentiometer with a glass electrode. Muffle furnace was used for dissolution of the samples. The process of thermolysis of the compounds was studied using derivatograph system «Shimadzu TGA-50H»(Japan). The iR spectra were recorded on a spectrophotometer of the Specord M80 (Germany).^{1}H-NMR spectra were recorded on "Bruker" Fourier Transform (300.18 MHz) in C_6D_6.

Reagents and solutions

Stock solution (1.00 mg mL^{-1}) of Fe(II) was prepared by dissolving a weighed amount of purified-grade (Merck pro analysis grade) $FeSO_4 \cdot 7H_2O$ in 100 ml of doubled distilled deionized water. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with deionized water as and when required. Concentrations were checked using the standard potassium permanganate solution [26].

The standard Fe (III) solution (1.00 mg mL-1) was prepared by dissolving a quantity of weighed (Fe (NO 3) 3, 10H 2 O) in distilled deionized water added to 100 ml of 1-2 mL of nitric acid (1: 1). Concentrations were checked using standard potassium permanganate solution [26]. Working solutions were prepared just prior to use by diluting the standard solution with redistilled deionized water Solutions of DM and Am in chloroform (0.01M) were used. The extractant was purified chloroform.

Acetate buffers of pH 4, 5 and 6 were prepared by mixing 0.05 M solutions of CH_3COONa and acetic acid. 4. Phosphate buffers of pH 7 and 8 were prepared by mixing 0.05 M solutions of KH_2PO_4 and 0.01 M NaOH. Borate buffer of pH 9 was prepared by mixing 90 ml of 0.05 M solution of $Na_2B_4O_7$ and 10 ml of 0.5 M H_3BO_3 solution.

The stock solution of various metal ions and anions were prepared by dissolving the appropriate metal salts in distilled water or with suitable dilute acids and making up to a known volume.

Procedure

General procedure for determination of Fe(II)

Aliquots of Fe(II) solution, DP solution (up to 2.0 mL), Am solution (up to 2.0 mL) and buffer solution (pH ranging from 3.0 to 9) were introduced into 100-mL separatory funnels. Then 1.0 mL of chloroform was added and the funnels were shaken for a defined period of time (up to 8 min). The solution was 25 mL. The solutions were diluted with distilled water to a total volume of 25 mL.When the equilibrium was reached, the organic layer was separated from the aqueous layer. A portion of the organic extract was filtered through a filter paper into a cell and the absorbance was read against a blank.

Determination of Fe(II) in pharmaceutical samples

0.5 - 1.0 gm sample of pharmaceutical product was dissolved in boiling with 10 ml of aquaregia. The resulting solution was evaporated to dryness and the residue was dissolved in 10 ml of 1N HCl filter, if required and solution was diluted to 100

ml with doubly distilled water. The working solution was prepared by appropriate dilution of stock solution. To an aliquot of this solution 1ml was analyzed for iron by the procedure as described earlier

Determination of iron in biological samples

Human blood (2-5 mL) and urine (20-30 mL) were collected in polyethylene bottles. Immediately after collection, they were stored in a salt ice mixture and later, at the laboratory, were kept at -20 ^{o}C. The samples were taken into a 100 mL micro-Kjeldahl flask. A glass bead and 10 mL of concentrated nitric acid were added and the flask was placed on the digester under gentle heating. When the initial brisk reaction was over, the solution was removed and cooled following. Then 1 mL of concentrated sulfuric acid was added carefully, followed by the addition of 2 mL of concentrated HF, and heating was continued for at least 30 min, followed by cooling. The solution of flask was then neutralized with dilute NH_4OH solution in the presence of 1-2 mL of a 0.01% tartrate or EDTA solution. The resultant solution was then transferred quantitatively into a 50-mL calibrated flask and made up to the mark with deionized water. A suitable aliquot (1-2-mL) was pipetted into a 10-mL calibrated flask. An appropriate aliquot of the solution (1-2 mL) was pipetted into a 10 mL calibrated flask and the iron content was determined as described above.

Determination of iron in food samples

An air-dried food sample (25-50 g; egg -1 piece). incinerated in a muffle furnace at a temperature 550-750^{o} S. 2 g of the resulting ash is dissolved in a mixture of 15 ml of HCl and 5 ml of conc.HNO_3. To completely remove the HNO_3 sample 2-3 was treated with 3-4 ml of conc. HCl. The solution was concentrated to 2 mL and diluted with distilled water, filtered into a volumetric flask of 100 ml.

Results and Discussion

DMwere synthesized according to the procedure [27]. DM was recrystallised using aqueous ethanol. Its solution (0.01 M) was prepared in chloroform. Structure of ligand was confirmed by using NMR and IR spectra (Table-1) [28-30].

In acid and neutral media (pH 1.2-7.0) The reagents DM with Fe(II, III) forms red coloured complexes, which non extracted into organic phase. When hidrophobamins (Am) were introduced into the system, the extraction of these compounds into the organic phase as a mixed-ligand complex (MLC) was observed. The results of various studies are discussed below. It was found that the spectrophotometric characteristics of the MLC of Fe(III) and Fe(II) were identica, *i.e*., in the interaction with DM, Fe(III) was reduced to Fe(II).

Effect of pH

Iron extraction with DP and Am was studied at a pH range of 1-9 and the percentage extraction of Fe (II) was observed to be maximum at pH 5.3-7.2. The effect of pH on the intensity of the color reaction is shown in the Fig. 1. Hence further analytical investigations were carried out in media of pH 6. The presence of a second ligand led to a shift of optimal acidity complexation more acidic region, pH_{opt}wider than in the case of two-component compound. In the beginning extraction of Fe(II) enhanced with the increase in the acidity of the initial solution. the further increase in acidity lead to the gradual decrase of recovery, which was obviously associated with a decrease in the concentration of the ionized form of DM. At pH 9 complexes are practically not extractable, which is apparently associated with increased concentrations of complexes $[Fe(DM)_2]^{4-}$ and $[Fe(DM)_3]^{7-}$, which are not extracted.

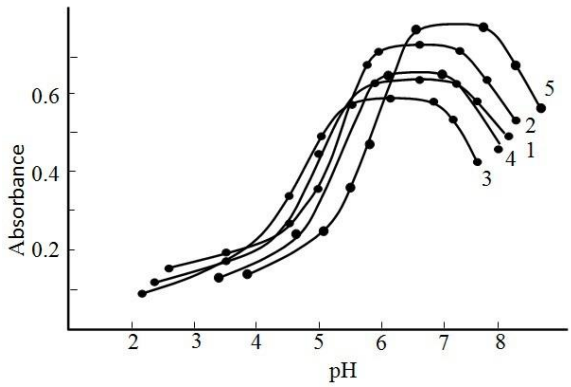

Fig. 1: Absorbance of mixed-ligand complexes as a function of the pH of the aqueous phase
1 - Fe(II)-DMMP-Phen, 2 - Fe(II)-DMMP - BPen, 3 - Fe(II)-DMMP –Bip, 4-Fe-DMEP-Phen, 5-Fe– DMEP-BPhen
C_{Fe} = 3.57×10^{-5} M;C_{DP} = (6-8)×10^{-4} M;C_{Am} = (8.0-8.8)×10^{-4} M, λ=540nm, *l* = 0,5 см

Absorption spectrum

The absorption spectrum of Fe(II)-DM-Am in chloroforme shows the maximum absorption at 552-583 nm. The absorption due to reagent at this wavelength is nearly negligible. Hence the absorption measurements were carried out at 540 nm.

Table-1: The research results of IR and NMR spectroscopy.

Reagent	IR (KBr)	^{1}H NMR (300,18 MHz, C_6D_6)
DMP	3470 см$^{-1}$ ν (OH), 3050 см$^{-1}$ ν(CH), 2580 см$^{-1}$ ν(SH), 1580 см$^{-1}$ ν(C_6H_5).	δ 5.48 (s, 1H - OH), δ 3.57 (s, 2H - 2SH), δ 7.28 (s, 2H Ar-H), δ 6.95 (s, 1H - Ar-H).
DMMP	3460 см$^{-1}$ ν (OH), 3050 см$^{-1}$ ν(CH), 2570см$^{-1}$ ν(SH), 2962 и 2872 см$^{-1}$ ν(-CH_3), 1555см$^{-1}$ δ(C_6H_5), 1390 см$^{-1}$ δ_{as} (-CH_3).	δ 5.24 (s, 1H- OH), δ 3.32(s, 2H - 2SH), δ 7.11 (s, 2H Ar-H), δ 2.38 (s, 3H –CH_3).
DMEP	3460 см$^{-1}$ ν (OH), 3050 см$^{-1}$ ν(CH), 2575см$^{-1}$ ν(SH), 2965 и 2874 см$^{-1}$ ν(-CH_3), 1555см$^{-1}$ δ(C_6H_5), 1460 см$^{-1}$ δ_{as} (-CH_2-CH_3).	δ 5.29 (s, 1H- OH), δ 3.38(s, 2H - 2SH), δ 7.15 (s, 2H Ar-H), δ 2.59 (s, 2H –CH_2-), δ 1.22 (s, 3H –CH_3).

The absorption spectrum of Fe (II)- DM shows the maximum absorption at 515 nm. Contrast of reactions was high: initial reagents - are colourless, and complexes – are intensively painted (Fig. 2). Molar coefficients of absorption make (3.08-4.33) × 10^4dm^3 mol^{-1}·cm^{-1}.

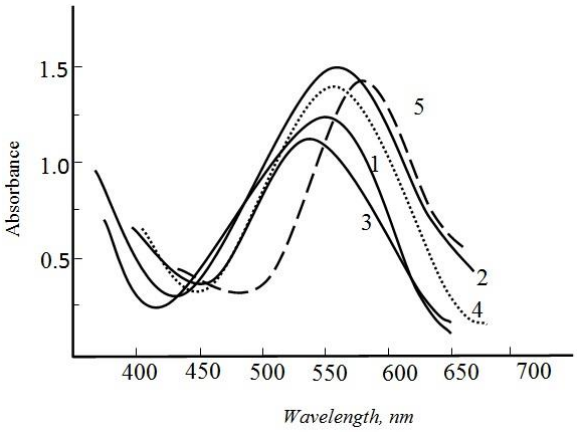

Fig. 2: Absorption of mixed-ligand complexes 1-Fe(II)-DMP-Phen, 2 -Fe(II)-DMP-BPen,3-Fe(II)-DMP- Bip, 4-Fe-DMEP-Phen, 5-Fe–DMEP-Phen
C_{Fe} = 3.57×10^{-5} M;C_{DP}= (6.0-8.0)×10^{-4} M;C_{Am} = (8.0-8.8)×10^{-4} M, acetate buffers of pH 4- 6, *l*= 1 см

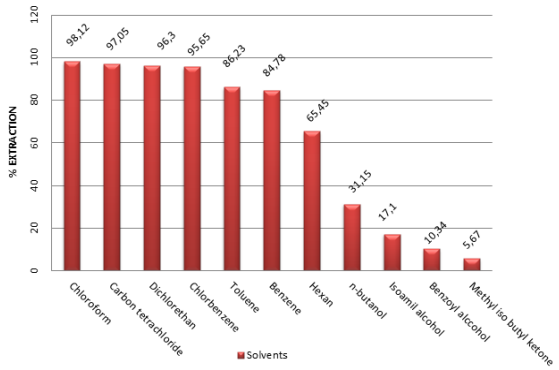

Fig. 3: Effect of solvents on extraction of Fe - DMEP-Bip.

Influence of solvents

The suitability of diluents was investigated using organic solvents such as chloroform,ethyl acetate, isoamyl alcohol, xylene, hexane, diethyl ether, toluene, n-butanol, carbon tetrachloride, MIBK, nitrobenzene, etc. (Fig. 3). With in compounds of one class extraction ability decreases with increasing number of carbons in the solvent molecule. The best solvents are chloroform, dichloroethane and carbon tetrachloride. The extraction of iron(II) was quantitative with DP and Am in chloroform. Hence, chloroform was used for further extraction studies as it gave better and quicker phase separation. After a single extraction with chloroform, 97.6 - 98.3% of iron was extracted as an ion associate (in a case the dichloroethane and carbontetrachloride was removed 95.5 - 96.5% of iron). After separation of the two phases, Fe(II) in each phase was determined by 1,2-phenanthroline method [31].

Effect of salting out agents and of temperature

The presence of 0.1 M salts of various alkali and alkaline metals does not show any effect over the absorbance value of Fe(II)-DM-Am complexes extract. Therefore, no salting out agent was required during the extraction.

Effect of various temperatures (10-80 ^{o}C) on the Fe(II)-DM-Am system was studied. The Fe(II)-DM-Am system attained maximum and constant absorbance at room temperature (25 ^{o}C).

Effect of reagents concentration

Various volumes of 0.01 mol L^{-1} reagent solution were added to the sample solution containing 50 µg of Iron at respective pH values. The absorbance remained nearly constant when the volume of the reagents solution (DM and Am) used was more than 2 ml. Therefore, 2.5 ml of 0.01 mol L^{-1}reagent and 2.5 ml of 0.01 mol L^{-1} Am was chosen for the quantitative determination of the metal. For the formation and extraction of MLC are provided by (6.0-8.0) ×10^{-4} M DM and (8.0-8.8) × 10^{-4} M Am. It was found that the presence of excess of the reagent solution does not alter the absorbance of the color reaction.

Effect of equilibration time and stability of the complex

The study of change in absorbance with variation in equilibrium time for extraction of extraction of the complex into organic solvent shows that equilibration time of 8 min is sufficient for the quantitative extraction of Iron. The study of stability of colour of the Fe(II)-DM-Am complex with respect to time shows that the absorbance due to extracted species is stable up to 48 hours, after which slight decrease in absorbance is observed. Throughout the experimental work, for practical convenience, the measurements have been carried out within one hour of extraction of iron. Unlike homogeneous ligand complexes, mixed ligand complexes Fe (II) with DM and Am do not decompose within a month after extraction in closed vessels.

The composition of mixed- ligand complexes and the mechanism of complex formation

The stoichiometry of the complexes formed a method of equilibrium offset and the relative yield confirmed the methods of the Asmus straight line and intersection curves [32]. The data shown in Fig. 4 shows that the composition ratio MLC Fe: DM: Am = 1: 1: 2.

The infrared spectrum of the ligand (DMMP) and Phen was compared with the spectra of its Fe(II)-DMMP-Phen complex (Fig.5). Were observed bands at 1593-1448 cm^{-1} (C=C), 1518 cm^{-1} (C=N), 3028 cm^{-1} (Ar).In complexes band at 1525 cm^{-1} was shifted to higher regions,1615 cm^{-1} for Fe(II) complex , suggesting the coordination of nitrogen of Phen to metal atom in complexation [29, 30].

The disappearance of the pronounced absorption bands in the 3250-3620 cm^{-1} with a maximum at 3475 sm^{-1} observed in the spectrum of DMMP, says that the -OH group is involved in the formation of the complex). The observed decrease in the intensity, absorption bands in the area 2570 sm^{-1} shows that one of the -SH groups involved in the formation of coordination bond in the ionized state. Detection of the absorption bands at 1380 cm^{-1} indicates the presence of a coordinated phenantroline.

New bands were observed between 400 - 600 cm^{-1} region in the complex, which were absent in the spectrum of ligand. The bands between 410-445 cm^{-1} were assigned to stretching frequencies of υ(M-S) and the band between 540-590 cm^{-1} have been assigned to the stretching frequencies υ(M-N) respectively.

The iron content in the complexes was determined after their decomposition aqua regia photometrically using sulfosalicylic acid [31]. The purity of the compound was checked by the elemental analysis. Elemental analysis individually selected complexes are given in Table-2.

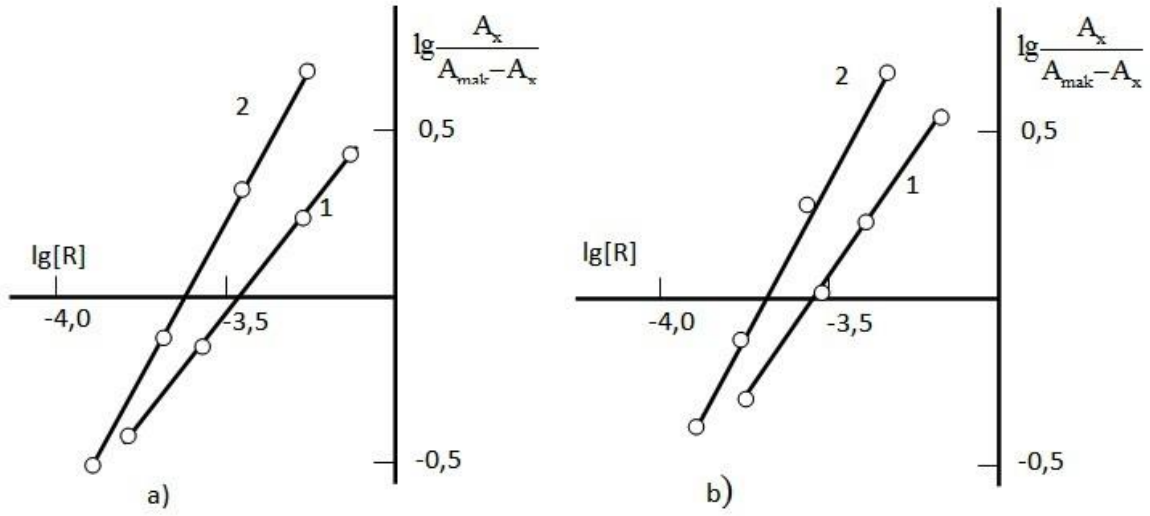

Fig. 4: Deterination of the ratio of components by equilibrium shift method for Fe -DMMP-Phen (a) иFe - DMEP-Dip (b).
Fe :DP; 2. Fe : Ам.
$C_{Fe} = 3.57\times10^{-5}$ M;C_{DP}= (6.0-8.0)×10^{-4} M;$C_{Am} = (8.0\text{-}8.8)\times10^{-4}$ M, pH=6(acetate buffers), 540nm, l=1см.

Table-2: Elemental analysis of some complexes.

Compound	%	S	H	C	Fe
Fe- DMEP -Phen	Found	10.57	1.31	15.85	9.37
	Calculated	10.66	1.33	15.99	9.33
Fe- DMEP -BPhen	Found	7.12	0.92	10.61	6.25
	Calculated	7.08	0.89	10.66	6.21
Fe- DMEP -Bip	Found	11.62	1.42	17.37	10.10
	Calculated	11.58	1.45	17.33	10.14

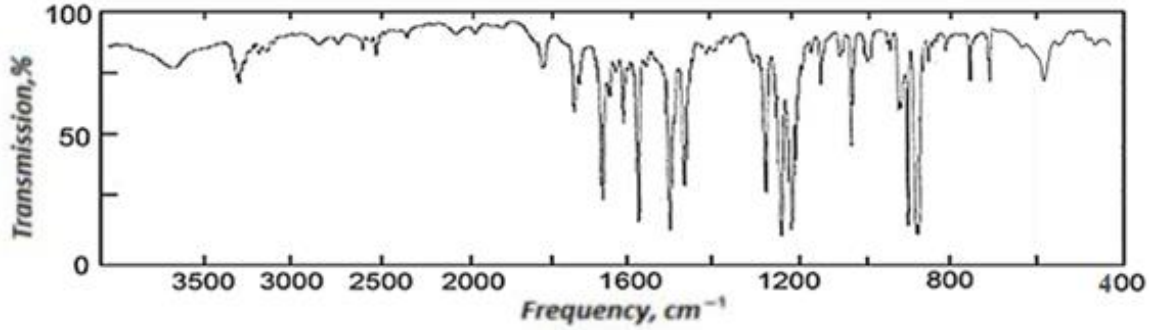

Fig. 5: IR spectrum of Fe-DMMP-Phen system.

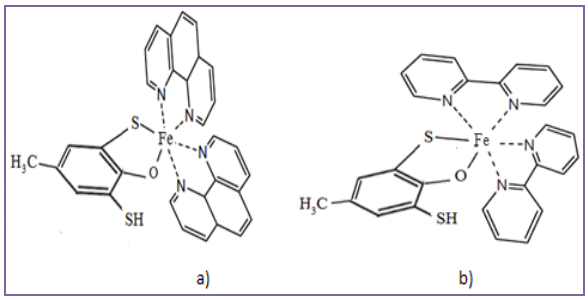

Fig. 6: Structure of complexesFe(DTMP)$(Phen)_2$ and Fe(DTMP)$(Bip)_2$.

Thermogravimetric study of the complex Fe-DMMP-Phen shown that thermal decomposition of the complex takes place in two stages: at 430-500 °C- decomposed Phen (weight loss -41.66 %), and at 500-540 °C- DMMP (weight loss -39.35%). The final product of the thermolysis of the complex is Fe_2O_3 .Given the molar ratio of components in the complexes, the complexing form of central ions, monomeric complexes in the organic phase, IR spectroscopic data, thermogravimetric studies and chemical analysis, it can be assumed that the Fe(II) with DM and Am forms mixed ligand complexes (Fig. 6).

Relative stability MLC increases with differences in the properties of dissimilar ligands. Ligands with feedback π-bond optimally combine with oxygen-containing reagents.

Constant of stability of complexes are determined by method of crossing of curves [32]. The sizes of equilibrium constant K ecalculated on a formula lgK_e= lg D - 2lg [Am] were presented in Table-1.

Using the nazarenko method, Fe (II) in the complexes was found to be Fe^{2+}. The number of protons replaced with iron in a DP molecule was found to be one [33, 34]

Additional experiments by the Akhmedly's method [35] showed that the complex exists in monomeric form in the organic phase (the obtained coefficient of polymerization γ was equal to 1.04 - 1.12).

In conclusion the analytical parameters pertaining to the proposed method are given in Table-3.

Effect of foreign ions and masking substances

The influence of the presence of diverse ions on the absorbance value of Fe(II)–DMEP-BPhen complex system was studied with 50 μg Fe(II) in the presence of foreign ions. The results are summarized in Table-4. The ions which show interference in the spectrophotometric determination of Iron were overcome by using appropriate masking agents. Large amounts of alkali and alkaline-earth metals and REE do not interfere with the determination of iron.

Co(II). Ni(II), Cu(II), V(IV,V), W(VI), Mo(VI), Ti(IV), Mn(II) and etc. interfere determination of Fe(II). The interfering effect of Zn (II), Mn (II), Co (II), Ni (II), Cd (II), and Ag (I) in the determination of iron is eliminated by precipitation of Fe (III) with ammonia. Among the anions studied, thiosulphate, oxalate, citrate and thiocyanate show severe interference at all levels of concentration. In order to eliminate interference of V(V) and Mo(VI), H_2O_2 and NaF were used as masking agents, respectively. In the presence of a small amount of a 0.01 M EDTA solution, the ions Ti (IV), V(IV), Nb(V), Ta(V) and Mo(VI) do not interfere the determination of Fe(II) with DMEP and BPhen.

Table-3: Optical characteristics mixed-ligand complexes of Fe(II) with DM and Am.

Compound	pH	λ, nm	Δλ, nm	ε×10^{-4}	lgβ	lgK$_p$	lgK$_{ex}$
Fe-DMP-Phen	5,5-6,8	558	288	3.25	14.68		
Fe- DMP -BPhen	5,3-6,6	565	295	4.05	15.05		
Fe- DMP -Bip	5,3-6,5	552	282	3.08	14.62		
Fe- DMMP -Phen	5,9-7,2	565	291	3.42	18.46	5.71	21.2
Fe- DMMP -BPhen	5,8-7,0	574	300	4.22	18.45	5.86	21.3
Fe- DMMP -Bip	5,7-6,8	562	288	3.15	18.12		
Fe- DMEP -Phen	5,8-7,1	572	296	3.60	18.10	5.82	21.1
Fe- DMEP -BPhen	5,7-6,8	583	307	4.33	17.23	5.94	21.2
Fe- DMEP -Bip	5,6-6,6	568	292	3.26	16.79		20.4

Table-4: Influence of interfering ions on the determination of Fe(II) as MLC with DMEP and BPhen (50.0 µg Fe added)

Ion	Molar excess of the ion	Maskingagent	FoundFe, µg	RSD(%)
Co(II)	25	Ascorbic asid	49.8	4
Ni(II)	25		49.8	2
Cd(II)	190		49.5	4
Bi(III)	200		50.3	2
Cu(II)	25	Sodium thiosulphate	49.2	4
Zr(IV)	50		49.8	3
W(VI)	20	Oxalicacid	49.6	5
Hg(II)	40	$Na_2S_2O_3$	50.5	5
Ti(IV)	40		49.7	3
V(IV)	50	H_2O_2	50.4	3
Mo(VI)	15	SodiumFluoride	49.4	4
Mn(II)	50			
Cr(III)	40	Triethanolamine	49.6	5
Nb(V)	50	SodiumFluoride	50.2	4
Ta(V)	50	SodiumFluoride	49.6	6
UO_2^{2+}	50	Acetylacetone	49.2	3
Ag(I)	25	Potassium bromide	49.5	4
Ammonium(I)	1000			
Acetate	100		50.5	6
Tartarate	300		50.3	5
Sulphate	130		49.8	2
Thiourea	25		49.7	5
Fluoride	110		50.3	6
Thiosulphate	36		49.8	6
Oxalate	50		49.8	4
Thiocyanate	50		49.6	5
Iodide	100		50.4	5
Ascorbicacid	400		49.9	3
Nitrate	800		50.6	4
Cyanide	100		49.8	3
Citrate	50		50.6	5
EDTA	110		50.3	5
Salisilasid	50		49.6	5

The proposed method compares favourably with the existing ones (Table-5) and offers the advantages of better simplicity, rapidity, sensitivity and selectivity.

Calibration curve and sensitivity

We observed absorbency to be in accordance with Beer's Law for complex extracts with chloroform in intervals of concentration of iron between 0.02 - 4.2 µg mL^{-1}. This shows that the Beer's law is obeyed in this range. Comparison of the analytical capabilities of the studied reagents and heterocyclic diamine showed that contrast and sensitivity of reaction with DMEP more than others. Reactions using batofenantrolina significantly more sensitive than phenanthroline and dipyridyl [40].

Complexes of metals withphenanthroline and batofenantroline stronger and more intensely colored than the complexes with dipyridyl. Apparently, the expansion through the inclusion of another aromatic nucleus in the case phenanthroline creates energetically more favorable orbitals for the formation of the reverse π-bond [41].

The detection limit indicates the smallest amount of analyte which can be detected with a reasonable degree of confidence under specified conditions. The equations of the obtained straight lines and some important characteristics concerning the application of the ternary complexes for extractive-spectrophotometric determination of Fe(II) are listed in Table-6.

Table-5: Comparative characteristics of the procedures for determining of iron.

Reagent	pH (solvent)	λ, nm	ε×10^{-4}	Linearrange (μg mL^{-1})	References
2-Hydroxy-1-naphthaldehyde-p-hydroxybenzoichydrazone	4.5-5.5	405	5.60	0.055–1.373	[18]
Ferron	3.5 (*n*-amylalcohol))	510	0.38	0.5-2.5	[36]
1-Nitroso-2-naphtol	4.0	420	2.57	0.5-6.0	[37]
Sulfosalicylicacid	1.2	528	0.38	-	[38]
Phenanthroline	2-9 (Isoamylalcohol))	512	1,10	-	[38,39]
Batofenantroline	4-7(Chloroform - ethanol)	533	2,24	-	[38,39]
methylthymolblue	5.0	628		2-6	[40]
SalicylicAcid	6.1	460		3.75-37.5	[41]
DMMP+Bip	5.7-6.8 (Chloroform)	562	3.15	0.05-3.6	Thiswork
DMEP+Phen	5.8-7.1 (Chloroform)	572	3.60	0.04-3.8	Thiswork
DMEP+BPhen	5.7-6.8 (Chloroform)	583	4.33	0.03-4.2	Thiswork

Table-6: Analytical characteristics of some ternary complexes of Fe with DP and hydrophobic Amine.

Compound	Beer's law range (μg· ml^{-1})	The equation of calibration curves	Limit of detection (LOD): ng ·mL^{-1}	Limit of quantification (LOQ): ng ·mL^{-1}	Sandell'ssensitivity (μg· cm^{-2})
Fe-DMP-Phen	0.03-3.6	0.049+0.265x	11	36	1.72
Fe-DMP-BPhen	0.02-3.8	0.055+0.334x	10	33	1.38
Fe-DMP-Bip	0.04-3.6	0.042+0.249x	11	36	1.81
Fe-DMMP-Phen	0.03-4.0	0.037+0.286x	10	33	1.64
Fe-DMMP-BPhen	0.03-4.2	0.045+0.354x	9	30	1.32
Fe-DMMP-Bip	0.05-3.6	0,025+0,315x	10	33	1.77
Fe-DMEP-Phen	0.04-3.8	0.045+0.230x	10	33	1.55
Fe-DMEP-BPhen	0.03-4.2	0.071+0.351x	9	30	1.29
Fe-DMEP-Bip	0.04-4.0	0.063+0.260x	10	33	1.72

Applications

Various commercial samples and synthetic mixtures containing fe (II) were prepared and analyzed according to the recommended procedure and the results were compared with those obtained by the standard method. The proposed methods facilitate the separation of Fe (II) from pharmaceutical samples, soils, blood and urine samples, water samples in some food samples. The results were in good agreement with the results obtained with the known standard method (Table-7-11)

Table-7: Determination of Fe(II) in Pharmaceutical Samples.

Pharmaceutical Samples	Fe(II) found	RSD(%)
Dexorange	32.65 mg	3.5
Autrin capsule	98.53 mg	4.8
Fefol	149 mg	3.9
Dexorange	49.8 μg/ml	3.2
Autrin capsule	1.31 mg/ml	3.1

Average of three determinations

Table-8: Determination of iron in soils n=6, P=0,95.

Метод	$\overline{X}$, %(10^{-3})	S(10^{-3})	RSD(%)	$\overline{X} \pm \frac{t_P \cdot S}{\sqrt{n}}$
Marine soil				
AAS	2.72	0.106	3.9	(2.72±0,11) ·10^{-3}
Sulfosalicylate	2.70	0.113	4.2	(2.70±0,12) ·10^{-3}
DMMP+Phen	2.68	0,094	3.5	(2.68±0,10) ·10^{-3}
DMMP+BPhen	2.69	0,073	2.7	(2.69±0,08) ·10^{-3}
Agriculturesoil				
Sulfosalicylate	3.68	0.128	3.5	(3.68±0,13) ·10^{-3}
DMP+Phen	3.64	0.146	4.0	(3.64±0,15) ·10^{-3}
Industrial soil				
Sulfosalicylate	5.10	0.153	3.0	(5.10±0,16) ·10^{-3}
DMP+Phen	5.12	0.143	2.8	(5.12±0,15) ·10^{-3}
River soil				
Sulfosalicylate	3.25	0.107	3.3	(3.25±0,13) ·10^{-3}
DMP+Phen	3.30	0.102	3.1	(3.30±0,10) ·10^{-3}

Table-9: Determination of iron in blood and urine samples.

Method	$\overline{X}$	S	RSD(%)	$\overline{X} \pm \frac{t_P \cdot S}{\sqrt{n}}$
Bloodcows(mg/ml)				
Sulfosalicylate	1.92	0.034	1.8	1.92±0.036
DMP+Phen	1.75	0.038	2.2	1.75±0.040
DMP+BPhen	1.88	0.047	2.5	1.88±0.050
DMP+Bip				
Normal adult blood(Male) (mg /m l)				
Sulfosalicylate	0.88	0.025	2.8	0.88±0.026
DMMP+Phen	0.87	0.022	2.5	0.87±0.023
DMEP+BPhen	0.89	0.020	2.3	0.89±0.021
Normal adult urine(Male)(mg / l)				
Sulfosalicylate	0.26	0.0065	2.5	0.26±0.0068
DMP+Phen	0.28	0.0064	2.3	0.28±0.0070
DMP+BPhen	0.29	0.0070	2.4	0.29±0.0073

Table-10: Determination of iron in some environmental water samples.

Method	$\overline{X}$	S	RSD(%)	$\overline{X} \pm \frac{t_P \cdot S}{\sqrt{n}}$
Tapwater (μg / ml)				
Sulfosalicylate	0.17	0.0056	3.3	0.17±0.0061
DMEP+BPhen	0.22	0.0062	2.8	0.22±0.0065
DMEP+Bip	0.19	0.0055	2.9	0.19±0.0058
River water(μg/ml)				
Sulfosalicylate	0.049	0.00147	3.0	0.049±0.0016
DMP+BPhen	0.045	0.00130	2.9	0.045±0.0014
DMMP+BPhen	0.046	0.00142	3.1	0.046±0.0015
Sea water(μg/ml)				
Sulfosalicylate	0.007	0.00027	3.8	0.007±0.00028
DMEP+BPhen	0.008	0.00029	3.6	0.008±0.00030

Table-11: Determination of iron in some food samples.(n = 6, P = 0.95).

Методика	Found, Fe(II)	S	S_r	$\overline{X} \pm \frac{t_p \cdot S}{\sqrt{n}}$
		Cherry(mg/kg)		
Sulfosalicylate	**5.2**	**0.13**	**2.4**	**5.2 ±0.131**
DMP+BPhen	**5.3**	**0.12**	**2.3**	**5.3 ±0.128**
DMMP+BPhen	**5,1**	**0.12**	**2.0**	**5.1±0.013**
DMEP+BPhen	**5.2**	**0.10**	**1.9**	**5.2±0.010**
		***whitecherries*mg/kg**		
Sulfosalicylate	**14.0**	**0.43**	**3.1**	**14.0±0.45**
DMP+BPhen	**14.2**	**0.35**	**2.5**	**14.2±0.37**
DMMP+BPhen	**13.8**	**0.36**	**2.6**	**13.8±0.38**
DMEP+Bip	**13.9**	**0.35**	**2.4**	**12.9±0.37**
		***red cherries*mg/kg**		
Sulfosalicylate	**20.4**	**0.714**	**3.5**	**20.4±0.75**
DMP+BPhen	**19.5**	**0.565**	**2.9**	**19.5±0.59**
DMMP+BPhen	**20.2**	**0.727**	**3.6**	**20.2±0.763**
DMEP+BPhen	**19.8**	**0.693**	**3.5**	**19.8±0.727**
		***strawberries*mg/kg**		
Sulfosalicylate	**11.6**	**0.475**	**4.1**	**11.6±0.5**
DMP+BPhen	**11.4**	**0.408**	**3.5**	**11.4±0.42**
DMMP+BPhen	**11.3**	**0.282**	**2.5**	**11.3±0.296**
DMEP+BPhen	**11.5**	**0.333**	**2.9**	**11.5±0.350**
		***briar*(mg/kg)**		
Sulfosalicylate	**13**	**0.403**	**3.1**	**13±0.423**
DMEP+BPhen	**14**	**0.357**	**2.5**	**14±0.367**
		***wildstrawberry*(mg/кg)**		
Sulfosalicylate	**12**	**0.348**	**2.9**	**12±0.366**
DMEP+Phen	**11**	**0.495**	**4.5**	**11±0.519**
		beef ((mg/g)		
Sulfosalicylate	**2.92**	**0.134**	**4.5**	**2.92±0.14**
DMMP+Phen	**2.95**	**0.127**	**4.3**	**2.95±0.13**
DMMP+Bip	**2.91**	**0.105**	**3.6**	**2.91±0.11**
DMEP+Bip	**2.93**	**0.117**	**3.8**	**2.92±0.12**
		cow's milk (mg/ml)		
Sulfosalicylate	**3.88**	**0.163**	**4.2**	**3.88±0.171**
DMMP+Bip	**3.93**	**0.123**	**3.3**	**3.93±0.136**
DMEP+Phen	**3.94**	**0.138**	**3.5**	**3.94±0.145**
		Egg(μg/kg)		
Sulfosalicylate	**70**	**1.75**	**2.5**	**70±1.83**
DMEP+Phen	**68**	**1.56**	**2.3**	**68±1.64**

Conclusion

The results obtained show that di*mercapto*phenole and hydrophobic amins in chloroform can be effectively used for quantitative extraction of iron from aqueous media. The proposed method is found to be quantitative as compared to other standard methods.

- Extraction of mixed ligand complexes is maximal at pH 5.3-7.2. The proposed method is quick and requires less volume of organic solvent.
- The equilibrium time required is 8 min and the complex is stable for 48 hrs. The results show good agreement with the standard method. The method is very fast, accurate and precise. Theproposed method shows good selectivity.
- The absorption spectrum of Fe (II)-DP-Am complexes in chloroform shows maximum absorbance at 552-583 nm. It was observed that the color development was instantaneous and stable. Linear calibration graphs were obtained for 0.03-4.2 μg mL $^{-1}$of Fe.
- A new, simple, sensitive, selective and inexpensive method has been developed with Fe-DP-Am complexes for the determination of iron in some pharmaceutical, biological, water, food and plant samples

References

1. M. Skurikhin. Problemy analiticheskoy khimii. T. VIII. Metody analiza pishevykh produktov. M.: Nauka. (In Russian). (1988).
2. Zh. M. Orozbayeva, A. A. Adysheva, K. A. Asanova. *Bioneorganicheskaya khimiya. Konspekty lektsiy*. Zhalal-Abad. (In Russian). (2009).
3. N. N. Osipov, A. K. Charykov. Ekstraktsionno-fotometricheskoye opredeleniye zheleza v morskoy vode v forme assotsiata ferroin-iona s pelargonovoy kislotoy. *J. Ana. Chem.,*. **35**, 2355 (1980).
4. N. N. Royeva, S. B. Savvin. Organicheskiye reagent dlya spektrofotometricheskogo opredeleniya rtuti. *J. Anal. Chem.*, **47,** 1750 (1992).
5. R. F. Gur'yeva, S. B. Savvin. Test-metod opredeleniya rtuti(I, II) na poverkhnosti polimernogo nositelya. *J. Anal. Chem.*, **58**, 1104 (2003).
6. E. Upor, M. Makhoni, D. Novak. *Fotometricheskiye metody opredeleniya sledov neorganicheskikh soyedineniy*. Moskow, Mir. (1985).
7. T. N. K. Kumar, H. D. Revanasidappa. Rapid and sensitive spectrophotometric determination of trace amounts of iron(III) using leuco Xylene cyanol FF. *Anal. & Bioanal. Chem*., **376**, 1126 (2003).
8. C. D. Stalikas, A. C. Pappas, M. I. Karayannis, P. G. Veltsistas. Simple and selective spectrophotometric method for the determination of iron(III) and total iron content, based on the reaction of Fe(III) with 1,2-dihydroxy-3,4 diketocyclobutene (Squaric acid). *Microchim. Acta*,, **142**, 43 (2003).
9. F. G. Martins, J. F. Andrade, A.C. Pimenta, L.M. Lourenco, J. R.M. Casto, V. R. Balbo. Spectrophotometric study of iron oxidation in the iron(II)/ thiocyanate/acetone system and some analytical applications. Eclét. Químic, **30**, 63 (2005).
10. L. Zaijun, F. You, L. Zhongyun, T. Jian. Spectrophotometric determination of iron(III)-dimethyldithiocarbamate (ferbam) using 9-(4-carboxyphenyl)-2,3,7-trihydroxyl-6-fluorone. *Talanta*, **63**, 647 (2004).
11. M. J. Ahmed., U. K. Roy. A simple spectrophotometric method for the determination

of iron(II) aqueous solutions. *Turk J. Chem.* **33**, 709 (2009).

12. K. Gavazov, T. Stefanova, G. TonchevaLiquid-liquid extraction and spectrophotometric characterization of a new ternary ion-association complex of iron(III). *J. Chem. Technol. Metall*.. **48**, 642 (2013).
13. G. K. Toncheva, T. S. Stefanova, K. B. Gavazov. Liquid-Liquid Extraction-Chromogenic Systems Containing Iron(III), 4-Nitrocatechol and Tetrazolium Salts. *Orient. J. Chem*., **31**, 327 (2015).
14. J. Dehkordi, L. Saghaie, N. Movahedi. A new spectrophotometric method for direct determination of iron(III) in serum. *DARU J. Pharm. Sci*., **16**, 76 (2008).
15. D. J. Sullivan. Collaborative study of a spectrophotometric determination of ferric, ferrous, and total iron in drugs by reaction with alpha, alpha'-dipyridyl. *J. Assoc Anal Chem*., **60**, 1350 (1977).
16. R. Escobar, J. M. Cano Pavon. Selective spectrophotometric determination of trace amounts of iron with di(2-pyridyl)-N,N-di((8-quinolyl)amino)methane: determination of iron in blood serum. *Analyst*, **108,** 821 (1983).
17. L. M. Kovalev, L. I. Kruglikovskaia, V. M. Ianova, O. G. Iashina. Determination of iron in saccharomycete yeasts by the dipyridyl method. Nauchnye Doki. Vyss. Shkoly *Biol. Nauki*, **12**, 96 (1984).
18. V. S. Devi, V. K. Reddy. Spectrophotometric determination of iron (II) and cobalt (II) by direct, derivative, and simultaneous methods using 2-hydroxy-1-naphthaldehyde-*p*-hydroxy benzoichydrazone. *Int. J. Anal. Chem*., **2012** (2012).
19. M. Saeidi, R. Aboutalebi, A. Darehkordi, A New Spectrophotometric Reagent for Fe (III): 2-(2, 3-Dihydroxy-4-oxocyclobut-2-enylidene) Hydrozinecarbothiamide and its application in real samples // *J. Chem*., **2013** (2012).
20. M. A. Kassem, A. S. Amin. Spectrophotometric determination of iron in environmental and food samples using solid phase extraction. *Food Chem*. **141**, 1941 (2013).
21. K. A. Kuliev, N. A. Verdizadeh, A. B. Gadjieva. Liquid-Liquid Extraction and Spectrophotometric Determination of Molybdenum with 2, 6-Dithiolphenol and its Derivatives in the Presence of Hydrophobic Amines. *Chem. Journal*, **5**, 45 (2015).
22. K. A. Kuliyev, N. A. Verdizadeh, A. B. Gadjieva, S. A Mamedova. Spectroscopic Investigation Complex Formation of Molybdenum with 2, 6-Dithiolphenol and Its Derivatives in the Presence of Hydrophobic Amines. *Int. J. Chem. Stud*, **4**, 42 (2016).
23. K. A. Kuliyev, N. A. Verdizadeh, G.S. Suleymanova. Spectrophotometric Determination of Cobalt (II) with 2, 6-Dithiolphenol and Its Derivatives in the Presence of Hydrophobic Amines. *American J. Chem*., **6**, 95 (2016).
24. K. A. Kuliyev, N. A. Verdizade. Spectroscopic Investiqation Complex Formation of Vanadium Using 2,6-Dithiolphenol and Hydrofob Amins. *American J. Chem*., **5**, 10 (2015).
25. A. Z. Zalov, G. I. Amanullayeva. Spectrophotometric determination of cobalt (II) in a liquid-liquid extraction system containing 2-hydroxy-5-iodothiophenol and diphenylguanidine. IJRDO-*J. Appl. Sci*.. **2**, 2 17 (2016).
26. P. P. Korostelev. *Preparation of solutions for chemical analysis works*. M. Publishing house of Academy of Sciences of the USSR. (1964)
27. A. M. Kuliev, S. R. Aliev, F. N. Mamedov, M. Movsumzade. Synthesis of aminomethyl derivatives of 2-hydroxy-5-*tert*-alkylthiophenols and their cleavage by thiols. *Russian J. Org. Chem*, **12,** 426 (1976).
28. R. Shrayner, R. F'yuzon, D. Kortin, T. Morrill. *Identifikatsiya organicheskikh soyedineniy*: Per. s angl. M. Mir, (1983).
29. K. Nakamoto. *IK-spectr KR neorqaniceskikh i koordinatsionnikh soedineniy (Infrared and Raman Spectra of Inorganic and Coordination Compounds).* M: Mir. (1991).
30. L. Bellami. *Infrakrasnie spectra slojnikh molecule (The infrared spectra of complex molecules*). M: Mir. (1991).
31. I Busev, V.G. Tiptsova, V. M. Ivanov. *Analytical Chemistry of Rare Elements***,** Mir Publication, Moscow, (1981).
32. M. I. Bulatov, I. P. Kalinkin. *Prakticheskoe Rukovodstvo po Fotokolorimetricheskim i Spektrofotometricheskim Metodam Analiza (Practical Guide on Photocolorimetric and Spectrophotometric Methods of Analysis)*, Moscow: Khimiya. (1972).
33. V. A. Nazarenko, E. A. Biriuk. Issledovanie khimizma reaktsii ionov mnogovalentnykh elementov s organicheskimi reagentami [Research of Chemism of Reactions of Ions of Multivalent Elements with Organic Reagents]. *J. Anal. Chem.*, **22**, 57 (1967).
34. V. A. Nazarenko. Vzaimodeistvie Ionov Mnogovalentnykh Elementov s Organicheskimi Reagentami [Interaction of Ions of Multivalent Elements with Organic Reagents]. Trudy komis. Po analit. khimii AN SSSR [Proc. of the

Commission on analytical chemistry of Academy of Sciences of the USSR]. Moscow, **17**, 22 (1969).

35. M. K. Akhmedly, A. E.Kly'gin, L. I. Ivanova, E. A. Bashirov. On the chemistry of interacttion of gallium ions with a number of sulphophtaleins. Russ, *J. Inorg, Chem*, **19**, 2007 (1974).
36. A. Shokrollahi, M. Ghaedi, H. R. Rajabi. Highly selective and sensitized spectrophotometric determination of iron (III) following potentiometric study // *Ann. Chim*, **97**, 823 (2007).
37. H. Abdollahi, M. Shariat Panahi, M. R. Khoshayand. Simultaneous spectrophotometric determination of iron, cobalt and copper by partial least-squares calibration method in micellar medium // *Iranian J. Phar. Research*. **2**, 207 (2010).
38. Z. Marczenko, M. K. Baltsejak. *Metodı Spectrophotometrii v UF i vidimoy oblastyax (Spectrophotometrically in the UV and visible regions in inorganic analysis).* M. Binom. Laboratoriya znaniy (2007).
39. F. Umland et al. *Kompleksnye soedineniya v analiticheskoi khimii: teoriya i praktikaprime-neniya [Complex compounds in analytical chemistry: theory and practice of application*]. M., Mir, (In Russian) (1975).
40. M. Totan, E. Antonescu, F. G. Gligor. Quantitative Spectrophotometric Determinations of Fe^{3+} In Iron Polymaltose Solution. *Indian J. Pharm. Sci.*, **80**, 268 (2018).
41. N. Rajendraprasad and K. Basavaiah. Modified Spectrophotometric Methods for Determination of Iron(III) in Leaves and Pharmaceuticals Using Salicylic Acid. *Indian J. Advances in Chem. Sci*., **4**, 302 (2016).
42. E. N. Dorokhova, G. V. Prokhorova. *Analytical chemistry (physical-chemical methods of analysis).* M.: Higher School (1991).
43. T. Pilipenko, M. M. Tananayko. *Raznoligandnyye i raznometal'nyye kompleksy i ikhprimeneniye v analiticheskoy khimii.* M.: Khimiya (1983).