CREATION OF A MODIFIED SOLID-PHASE MATRIX BASED ON GELATIN MEMBRANE FOR DETERMINATION METAL IONS

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Abstract

The immobilization of disodium salt of 4-hydroxy-3-(4-sulfonato-1-naphthyl- azo)-1-naphthalene sulfonate onto photographic membrane, the optimal conditions for the sorption of the reagent and the process of complexation on a layer of gelatin membrane were investigated. A new solid-phase spectrophotometric method for determination copper ions in model mixtures simulating real objects has been proposed and metrologically improved results have been obtained.

Keywords: *solid-phase spectrophotometry, gelatin membrane, organic reagent, optical density*

Introduction

Expanding the range of solid-phase reagents is a necessary condition for the delaboration of new methods of chemical analysis. Solid-phase reagents are obtained mainly by immobilization of reagents or fixing of it's functional analytical groups on the surface of various sorbents. The analysis procedure using a solid-phase reagent involves the extraction of the analyte into the modified sorbent phase due to it's interaction with the modifier-reagent, i.e. carrying out solid-phase extraction and further determination of the isolated component directly in the sorbent phase or in the eluate after its desorption. Concentration and separation of analytes can significantly to increase the sensitivity and selectivity of analytical determinations; This important advantage of solid-phase reagents has found application in diffuse reflectance spectroscopy, colorimetry, solid-phase spectrophotometry, fluorimetry, and visual test analysis (Zolotov, Yu.A., et al., 2007; Moliner-Martinez, Y., et al., 2005; Zaporozhets, O.A., et al., 2005; Ngeontaea, W., et al., 2007). Specific interest are solid-phase reagents (indicator agents) presented for monitoring of integral (generalized) indicators that make it possible to quickly assess the quality of natsural, technical, waste and drinking waters and other objects.

Solid-phase reagents are also used as indicators and test systems for determination of total acidity and hardness, total content of metals, phenols, nitrites and nitrates, Fe(II, III), reducing agents, polycyclic aromatic hydrocarbons, aromatic amines and pesticides, active chlorine, iodine, total antioxidant activity, and phosphorus content, chemical and biological oxygen consumption (Zaitsev, V. N. 1997; Tikhomirova, T. I., et al., 2011; Dmitrienko, S. G., et al., 2010; Dini, F., et al., 2011; Kim, H. N., et al., 2011; Amelin, V. G., et al., 2011; Takagai, Y., 2010; Vilozny, B., et al., 2011; Boling, M. et al., 2010; Savvin, S. B., et al., 2008; Dedkova, V. P., et al., 2011; Nan, Wu, et al., 2009; Comes, M., et al., 2008; Koncki, R., et al., 2001; Mohr, G. J., et al., 1999; Descalzo, A. B., et al., 2007).

For these same purposes in some cases (Nicole, L., et al., 2004; Amelin, V. G., et al., 2001; Choi, M. M. F., et al., 2001; Hashemi, P. A., et al., 2008; Sadananda, D., et al., 2011; Jiang, J., et al., 2009; Fries, K., et al., 2008; Dedkova, V. P., et al., 2010; Amelin, V. G., et al., 2009; Li, C. M., et al., 2009) gelatin membrane, hardened gelatin gel and other matrices are used.

Gelatin membrane. In addition to transparent synthetic polymer matrices, membranes based on the biological polymer gelatin are widely used in modern analytical chemistry as suitable means for the immobilization of analytical reagents.

By it's chemical nature gelatin is considered as polydispersion submolecular polypeptide (Fig. 1.), consisting of amina ocid residues, from which, on average, more than 30% are bound on the basis glycine (El-Sheikh, A. H., Al-Degs, Y. S. 2013).

Figure 1. *Fragment of a gelatin molecule*
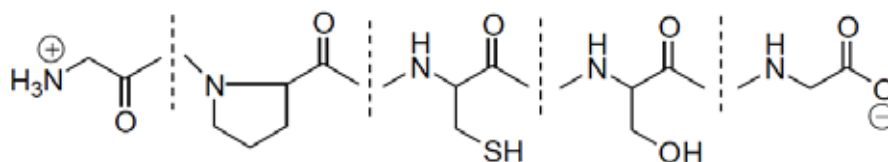
Gelatin is a fairly hydrophilic polymer. At gelatin action on aqueous solution, in it's mass, along with the solvent, dissolved chemical also are included. The penetration of water into the gelatin mass causes its isotropic swelling. Hydration of gelatin is achieved primarily through the formation of hydrogen bonds with water molecules. Hydration capacity is also provided by many anionic and cationic groups containing in gelatin macromolecules (Dinç, E., et al., 2002; Sahraei, R., et al., 2013).

In this work by immobilizing reagents in the gelatin layer of photographic membranes, solid-phase reagents were obtained for monitoring of various parameters of environmental objects. Gelatin layers of Agfa photographic membranes were used as a sorbent for the immobilization of HR for the determination of copper ions.

According to date of (Sahraei, R., et al., 2013; Kaur, A. D., Gupta, U. 2012; Unsal, Y. E., et al., 2012) spectrophotometric determinations with using of complexing and redox solid-phase reagents based on gelatin membranes, the sensitivity, they can be compared with standard methods of spectrophotometric analysis.

Experimental part

Reagents and solutions. $9.95 \cdot 10^{-4}$M (0.05%) aqueous solution of disodium salt of 4-hydroxy-3-(4-sulfonato-1-naphthyl-azo)-1-naphthalene sulfonate (HR) and $1.574 \cdot 10^{-2}$ M copper(II) solutions, prepared from metallic copper, a universal Britton-Robinson buffer solution was prepared by mixing H_3BO_3, CH_3COOH and H_3PO_4 at 0.04 M each and adding 0.2 M NaOH to achieve the appropriate pH value (Lurie, Yu. Yu. 1989) we used.

Matrices from photographic membranes were prepared by removing silver salts from the gelatin layer by using solutions of a special reagent (Razzoqova, S., et al., 2024; Khaitov, J.K., et al., 2024; Yunusov, Kh. E., et al., 2022).

Equipment. Absorption spectrums were recorded on a UV-VIS 500 spectrophotometer, and the optical density (OD) of solutions was measured on a KFK-3 concentration photocolorimeter. The pH of solutions was controlled by pH/ISE Meter pH meter with an accuracy of ± 0.05 units. A universal buffer solution was used to maintain constant pH (5.87–6.11). Based on the experimental data, pH = 5.95 was chosen as optimal.

Results and its discussion

The research results were studied by methods of physico-chemical analysis (photometry, spectrophotometry, solid-phase spectrophotometry).

Determination of the amount of immobilized reagent: For determination the amount of immobilized reagent on the matrix layer 0.05% solutions of HR were prepared and their concentration was calculated in mol/l. Comparative solutions of various concentrations were placed in a 25 ml volumetric flask, 5.0 ml of a universal buffer solution with a predetermined pH value 5.23 was poured on top, by double-distilled water was added to the mark and mixed. Then the optical density (OD) of the resulting solutions was measured on a UV-VIS 500 spectrophotometer in order of increasing concentration according to Table 1. Then the same solutions were placed in a glass beaker with a capacity of 50 ml, pre-cleaned photographic membranes with dimensions of 2×0.5 cm were placed and left for certain time. After 40 minutes the membranes were removed from the solution, washed with bidistilled water and dried at room temperature in air, after which the OD of the solution was determined in a glass beaker. The amount of immobilized reagent on the photographic membrane (Im-HR) was calculated based on the results shown in Table 1.

Table 1. *Results of calculating the amount of reagent immobilized on the membrane (pH =5.23, l = 520 nm, ℓ = 0.10 cm, n-3)*

No.	V_{HR}, ml	$C_{HR} \cdot 10^{-4}$ mol/l	A, prior to immobilization	Ā, after immobilization	ΔĀ	$C_{HR} \cdot 10^{-4}$ mol/l after immobilization	$C_{Im\text{-}HR} \cdot 10^{-4}$ mol/l
1	2.2	0.876	1.8802	1.7611	0.1191	0.7569	0.1191
2	2.4	0.9552	1.9136	1.7897	0.1239	0.8311	0.1239
3	2.6	1.035	1.9790	1.8512	0.1243	0.9107	0.1243
4	2.8	1.114	1.9920	1.8627	0.1293	0.9847	0.1293
5	3.0	1.194	2.0091	1.8393	0.1696	1.0244	0.1696
6	3.2	1.274	2.4362	2.2516	0.1846	1.0894	0.1846
7	3.6	1.432	2.7440	2.4831	0.2609	1.1721	0.2609
8	4.0	1.592	2.9160	2.6531	0.2629	1.3291	0.2629
9	4.5	1.791	2.9391	2.6730	0.2659	1.5251	0.2659
10	5.0	1.99	2.9630	2.6845	0.2785	1.7115	0.2785
11	6.0	2.388	2.9810	2.7005	0.2805	2.1075	0.2805
12	6.5	2.587	2.9980	2.7125	0.2855	2.3015	0.2855
13	7.5	2.985	3.0120	2.7236	0.2884	2.6936	0.2914
14	8.0	3.184	3.1422	2.8531	0.2896	2.8944	0.2896
15	8.5	3.383	3.3037	3.0139	0.2898	3.0932	0.2898
16	9	3.582	3.7377	3.4252	0.2925	3.2825	0.2995
17	10	3.98	3.8643	3.5660	0.2987	3.6727	0.3073

Figure 2. *Proposed scheme for HR immobilization onto gelatin membrane layer*
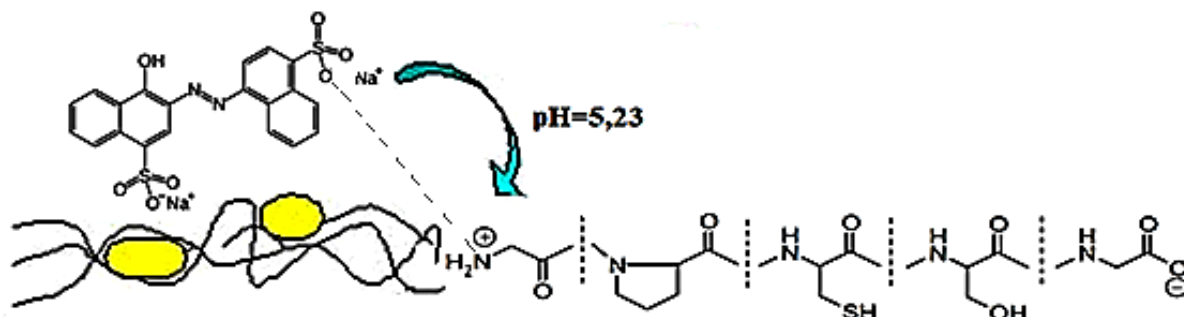
From (Fig. 1) it is clear that gelatin molecules are highly anisotropic and asymmetric. The polymer has an infinite network structure consisting from long chains of a limited number of interconnected molecules. The stabilization of this structure is formed mainly by hydrogen bonds and Van der Waals interactions. Gelatin is a typical ampholyte. The isoelectric point (pI) of alkaline gelatin is in the pH range 4.8–5.1, and the pI value of acidic gelatin is in the pH range 7.0-9.5. At such acidity all the main groups of gelatin become positively charged, while most of the carboxyl groups are overdissociated and have the same number of negative charge (Razzoqova, S., et al., 2024).

Study of the dependence of HR immobilization and copper(II) complexation with Imm-HR on the pH medium of the solution. Considering that one of the important conditions for the immobilization is the dissolution medium, universal buffer solutions with different pH values were prepared to select the optimal conditions for the immobilization of the reagent and the complexation of Cu(II) with HR. A 25 ml volumetric flask was filled with 1.0 ml of a 0.5% HR solution, with 5.0 ml of a universal buffer solutions with different pH values, filled to the mark with double-distilled water and mixed. The resulting solution was placed in a glass beaker with a volume 50 ml and the membrane was immersed in it. After 40 minutes the membrane was removed from the solution, washed with double-distilled water and dried at room temperature. The OD of the immobilized membrane was measured in various mediums (pH) in comparison with the immobilized membrane on KFK-3 at λ_{max}=520 nm, ℓ=0.10 cm. The results obtained are presented in (Table 2 and Figure 3).

Table 2. *Immobilization of HR on a layer of photographic membrane depending on the pH medium (λ_{max} = 520 nm, ℓ = 0.10 cm, n = 3)*

pH	3.05	3.44	3.95	4.23	4.83	5.05
A	0.021	0.056	0.099	0.119	0.135	0.177
pH	5.23	5.48	5.65	5.95	6.05	6.22
A	0.182	0.176	0.151	0.144	0.076	0.044

Table 3. *Immobilization of Cu(II) complex with HR on a layer of photographic membrane depending on the pH medium (λ_{max} = 630 nm, ℓ = 0.10 cm, n = s3)*
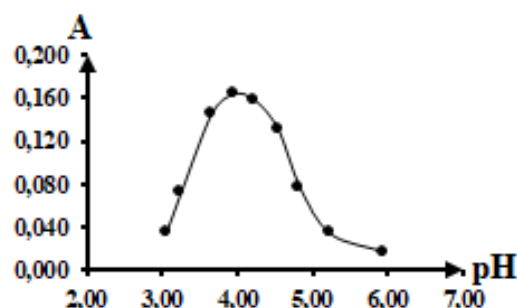
pH	3.05	3.25	3.66	3.95	4.23	4.54	4.83	5.05	5.95
A	0.034	0.072	0.146	0.164	0.158	0.131	0.077	0.034	0.017

Figure 3. *Study of HR immobilization on a layer of photographic membrane depending on the pH medium*

Figure 4. *Immobilization of the Cu(II) complex with HR on a layer of photographic membrane depending on the pH medium*
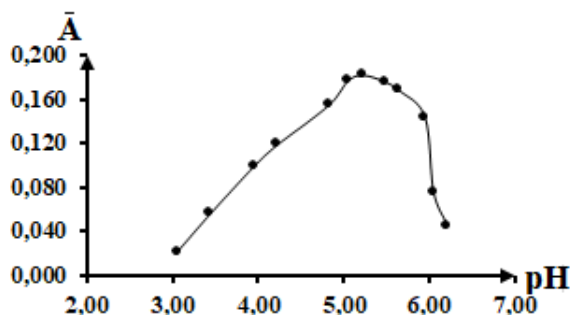
In 25 ml volumetric flask 1.0 ml of Cu(II) solution with a concentration of 100.0 µg/ml and 5.0 ml of a universal buffer solution with different pH were added, the flask was filled to the mark with double-distilled water and mixed. The resulting solution was placed in a glass beaker with a volume of 50 ml and the immobilized membrane was immersed in it. After 30 minutes the membrane was removed and washed with double-distilled

ion water. The OD of the complex of the immobilized membrane with the Cu(II) ions in comparison with the immobilized membrane was measured at KFK-3 (λ_{max} = 630 nm, ℓ = 0.10 cm. The results obtained are presented in (Table 3 and ion Figure 4).

Determination of stability of HR immobilized on photographic membrane and it's complex with Cu(II), in time. To determine the stability of the immobilized HR and obtain reproducible results, the stability of the optical density of the immobilized membrane over time was studied.

The methodology of work. 1.0 ml of 0.5% HR solution was added to a measuring flask with a capacity of 25 ml, 5.0 ml of a universal buffer solution pH = 5.23 was added and the flask was filled to the mark with bidistilled water,and was mixed. The resulting solution was placed in a glass beaker with capacity 50 ml and the membrane was statically immersed in it. After a certain time the membrane was removed from the solution, washed with distilled water and dried at room temperature. Thus, the OD of the immobilized membrane compared to the pure membrane was measured in KFK-3 at λ = 520 nm, ℓ = s0.10 cm. The results are presented in (Table 4 and on Figure 5).

Table 4. *Stability of the immobilization of the reagent on the photographic membrane over time (HR–0.5%, pH =5.23, l = 520 nm ℓ = 0.10 sm, n = 3)*

t_{min}	5	10	15	20	25	30	35	40	45	50	55
A	0.123	0.045	0.058	0.087	0.111	0.132	0.152	0.182	0.182	0.182	0.182
t_{min}	60	65	70	75	80	85	90	95	100	105	110
A	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182

In order to obtain reproducible results at the determination of copper (II) with HR, the stability of the OD solution of a complex compound formed on a layer of immobilized membrane was studied.

A method for determining the stability of a complex formed on an immobilized membrane over time: 1.0 ml of Cu(II) ion solution with a concentration of 50.0 mkg/ml, 5.0 ml of universal buffer solution with pH = 5.23 were added to a 25 ml volumetric flask, then the flask was filled with bidistilled water to the mark and mixed.

Figure 5. *Time stability of the copper(II) complex formed on the immobilized membrane with HR (Cu(II) = 50 mkg, HR–0.05%, pH = 3.95 (λ_{max}= 630, ℓ = 0.10 nm, n = 3)*

t_{min}	5	10	15	20	25	30	35	40	45
A	0.0026	0.053	0.083	0.112	0.141	0.164	0.164	0.164	0.164
t_{min}	50	55	60	65	70	75	80	85	90
A	0.164	0.164	0.164	0.164	0.164	0.164	0.164	0.164	0.164

Figure 5. *Shows the dependence of the stability of the immobilization of the reagentHR on membrane from time*
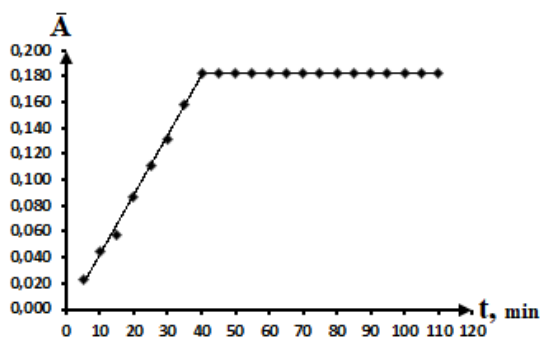
Figure 6. *Stability of the copper(II) complex formed on an immobilized membrane with HR from time*

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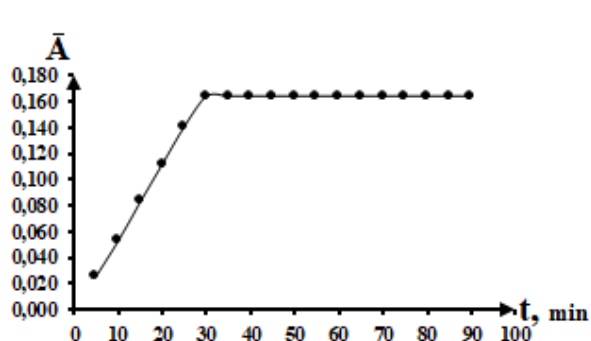
The resulting solution was placed in a glass beaker with a capacity of 50 ml and a membrane immobilized with HR was immersed in it. In this order, the OD of the complex was measured, compared with the immobilized membrane on KFK-3 at λ = 630 nm, ℓ = 0.10 sm. The results are presented in Table 5 and on (Figure 6).

The results of (Table 2.19 and Figure 2.12), as well as (Table 2.20 and Figure 2.13), have shown that the immobilization of the HR reagent on the membrane increased to 40 minutes and remained unchanged for thirty days or more. The results show that the OD of the complex formed on the immobilized membrane with HR has increased to 30 minutes and then remains unchanged for several days (even more than a month). This allows to get reproducible results.

Determination of the optimal absorption wavelength of Imm-HR and it's complex with copper (II) on the memrane.

Considering that each process molecule absorbs light of a certain wavelength during immobilizated , the immobilization of HR on the film and it's complex formed with copper(II) ions is carried out according to the method (Moliner-Martinez, Y., et. al., 2005).

The methodology of work:

1.0 ml of 0.5% HR solution was placed in 25 ml volumetric flask, 5.0 ml of a universal buffer solution with pH = 5.23 was poured, the flask was filled to the mark with bidistilled water and shaken. The resulting solution was placed in 50 ml glass beaker and the membrane was immersed in it.

Through 30 minutes the membrane was removed from the solution, washed with bidistilled water and dried at room temperature and the OD was measured at the optimal wavelength. The results are presented in (Table 6 and on Figure 7).

Table 6. *The results of choosing the optimal absorption wavelength of a membrane immobilized by HR in comparation with pure membrane (C_{HR}– 0.5%; pH = 5.23; ℓ = 0.10; n = 3)*

λ, nm	320	330	340	350	360	370	380	390	400
A	0.033	0.046	0.05	0.052	0.054	0.052	0.05	0.048	0.046
λ, nm	410	420	430	440	450	460	470	480	490
A	0.045	0.042	0.042	0.043	0.051	0.072	0.093	0.119	0.143
λ, nm	500	510	520	530	540	550	560	570	580
A	0.161	0.177	0.182	0.172	0.152	0.13	0.111	0.077	0.029

A method for determining the optimal absorption wavelength of a copper(II) complex on an immobilized membranbe: 1.0 ml of Cu(II) solution with a concentration of 50.0 micrograms/ml, 5.0 ml of a universal buffer solution with pH = 3.95 were added to a measuring flask with a capacity of 25 ml and distilled water was added to the mark on the flask. The re-sulting solution was placed in a 50 ml glass and an immobilized membrane was immersed in it. After 30 minutes, the membrane was removed from the solution, dried in air and OD was measured in various light filters. As a comparison, a membrane immobilized by HR only without metal was used. The measurement results are presented in (Table 7 and Figure 7).

Table 7. *The results of determining the optimal absorption wavelength for the copper(II) complex with HR on an immobilized memebrane (C_{HR}–0.5%; Cu(II)–50 мкг; pH = 3.95; ℓ = 0.10; n = 3)*

λ, nm	320	330	340	350	360	370	380	390	400
A	0.048	0.06	0.071	0.094	0.116	0.125	0.131	0.135	0.138
λ, nm	410	420	430	440	450	460	470	480	490
A	0.139	0.136	0.133	0.13	0.126	0.118	0.112	0.103	0.096
λ, nm	500	510	520	530	540	550	560	570	580

A	0.092	0.088	0.085	0.089	0.098	0.105	0.117	0.13	0.142
λ, nm	590	600	610	620	630	640	650	660	670
A	0.157	0.162	0.164	0.164	0.164	0.164	0.163	0.162	0.161
λ, nm	680	6900	700	710	720	730	740	750	760
A	0.16	0.159	0.158	0.157	0.155	0.152	0.15	0.148	0.145

Figure 7. *Results of choosing the optimal absorption wavelength
of the immobilized HR and it's Cu(II) complex on the membrane*
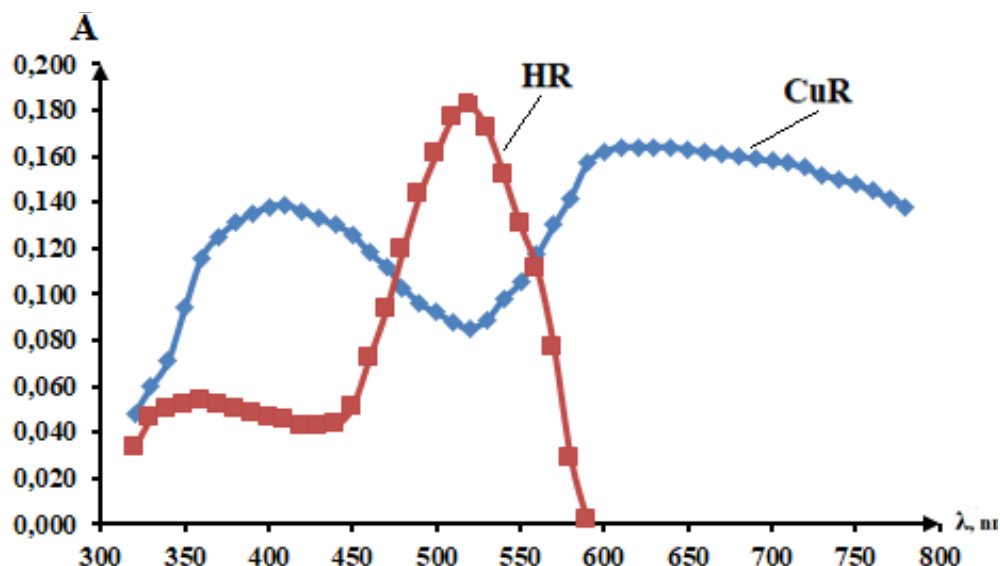
It can be seen from the results obtained that the immobilization of the membrane with a reagent obtained at pH = 5.23 and absorption compared to a pure membrane showed the highest optical densitys at λ_{max} = 520 nm. It was found that the maximum light absorption of the copper(II) complex on the immobilized membrane is observed at pH = 3.95 and has the highest optical density at λ_{max} = 630 nm.

The study of the subordination to the Booger-Lambert-Beer law of the Cu(II) whiz HR complex immobilized on membrane.

The behavior of the solution of the complex formed on an immobilized membrane with HR was studied under selected optimal conditions.

Working methods: A Cu(II) solution with a concentration of 50 mkg/ml in various quantities was placed in a measuring flask with a capacity of 25.0 ml, 5.0 ml of a universal buffer solution with a pH of 3.95 was poured, supplemented with bidistilled water to the label of the flask, poured into a glass beaker, the immobilized membrane was immersed in the same solution, after 30 minutes the membrane was removed from the solution, They were washed with distilled water and dried at room temperature in air, then the membrane OD was measured on KFK-3, the light absorption thickness was ℓ =0.10 sm in a light filter with λ_{max} = 630 nm. The results are presented in (Table 8 and on Figure 8).

Table 8. *Investigation of the subjection to the Buger-Lambert-Ber law of the Cu(II)
c HR complex immobilized on membrane (C_{HR}– 0.5%; pH = 3.95; ℓ = 0.10; n = 3)*

No.	Aliquot part, ml	The amount of Cu(II) in the aliquot, mkg	A_{comp}
1	0.5	5.0	0.032
2	1.0	10.0	0.044
3	1.5	15.0	0.057
4	2.0	20.0	0.071
5	2.5	25.0	0.085

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No.	Aliquot part, ml	The amount of Cu(II) in the aliquot, mkg	A_{comp}
6	3.0	30.0	0.098
7	3.5	35.0	0.111
8	4.0	40.0	0.124
9	4.5	45.0	0.137
10	5.0	50.0	0.151
11	5.5	55	0.164
12	6.0	60	0.175
13	7.0	65	0.175
14	7.5	70	0.175

Figure 8. *The dependence of the optical density on the amount of added Cu^{2+}*
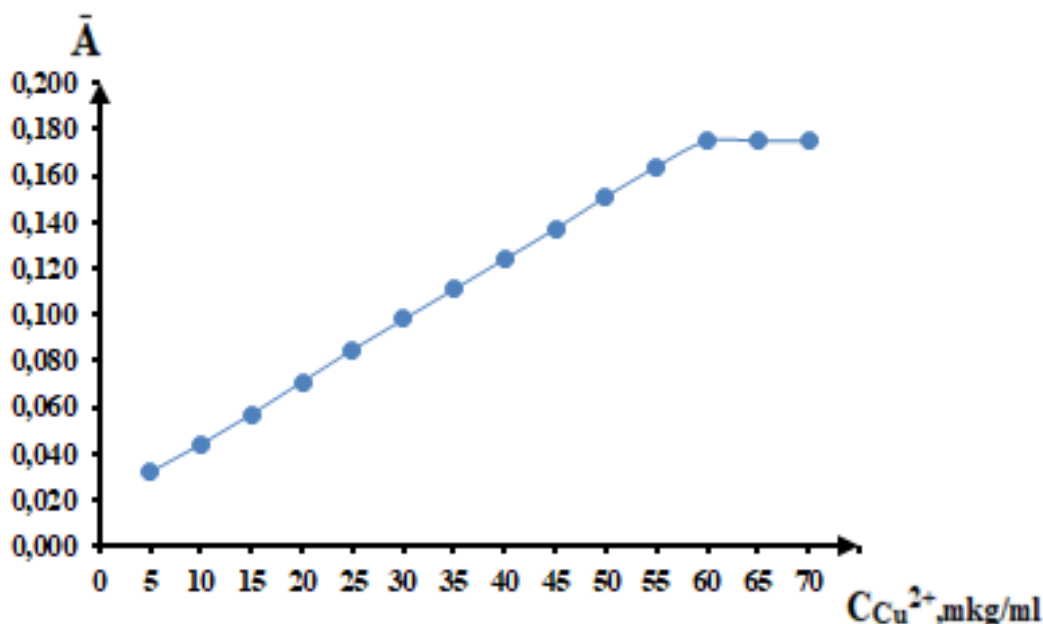
It can be seen from the results obtained that the area of subordination to the Buger-Lambert-Ber law was observed in the range of 5.0-55 micrograms of Cu(II) in 25.0 ml solution. At a higher concentration a deviation from the linear dependence was observed.

Study of the spectral characteristics of the copper(II) complex with HR immobilized on membrane.

The absorption spectrum of the copper(II) complex formed on the immobilized membrane with HR were measured under selected optimal conditions.

Working methods: standard Cu(II) solutions with a concentration of 50.0 micrograms/ml, 5.0 ml of a universal buffer solution with a pH of 3.95 and bidistilled water to the label of the flask were added to measuring flasks with a capacity of 25.0 ml. The resulting solution was placed in a 50 ml glass and a membrane immobilized with HR was immersed, after 30 minutes it was washed with distilled water and dried in air. The absorption spectra of a membrane immobilized by the reagent were compared with a membrane without immobilization. The results are presented on (Fig. 9 and in Table 9). According to the absorption spectrum shown on (Fig. 9), the maximum absorption region of the HR reagent in the membrane is located at $\lambda = 520$ nm, and the maximum absorption region of the copper complex in the membrane was observed at $\lambda = 630$ nm. The Mendelian sensitivity of the method was calculated using the formula:

$$S_{s.b.s} = \frac{Q \cdot \ell \cdot 0.001}{A \cdot 25} = \frac{50 \cdot 0.10 \cdot 0.001}{0.175 \cdot 25} = 0.00114 \, mkg/sm^2$$

Table 9. *Spectral description of HR and it's complex with copper (II)*
(Cu(II)–50 mkg/ml, ℓ = 3.0 cm, λ_{max} = 600 nm, pH = 8.40, n = 3

Colur	pH (HR)	pH (MeR)	λ_{max}, nm		$\Delta\lambda$	$C_{Cu(II)}$ mkg/ 25 ml	$C_{Cu(II)}$, mol/l	A	Sendel sensitivity of mkg/sm²
			MeR	HR					
Reddish -brown	5.23	3.95	630	520	110	50	$3.147 \cdot 10^{-5}$	0.175	0.00114

It was determined that the sensitivity of the delaborated method is equal to 0.00114 mkg/cm².

Figure 9. *Absorption spectrum of the HR reagent and it's complex with copper(II)*
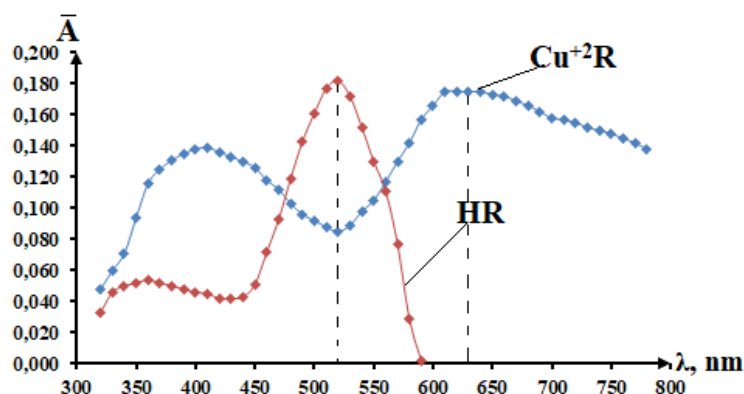
As can be seen from the results obtained, the contrast ratio of the reaction is 110 nm and the sensitivity of the Sendel sensivity 0.00114 mkg/ sm² respectively, which indicates on the high sensitivity of the delaborated method.

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