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APPLICATION OF COLORIMETRY AS AN ANALYTICAL TOOL*

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ABSTRACT. Colorimetry method was applied for estimation the precision of the measurement results of chromaticity characteristics of colored sorbates on paper, polyurethane foam and silica gel using a portable photometer-reflectometer. The relative standard deviation of measurement results is not exceed 1.0%; the trueness and precision of results of determination with the photometer-reflectometer are not worse than results obtained using a scanner or a spectrophotometer with an attachment for diffuse reflectance. A two-parameter colorimetry was allied to study the possibility of the determination separately of two metals in a mixture with the reagent indicator papers and gelatin films. It was established that application of two-parameter colorimetry has significant limitations; the relative standard deviation of the results of separately two-colorimetric determination may exceed 50%. Colorimetry method and statistics of observations were used for the chromaticity evaluation of uniformity of color scales prepared using transparent gelatin films, polyurethane foams and indicator papers. For choosing an optimal scale for visually test determinations should be given the preference to the visual method – statistics of observations.

Keywords: *colorimetry, chromaticity coordinates, test-system, sorbent*

INTRODUCTION

Colorimetry method is widely used in solid-phase spectroscopy and test analysis. The quantitative expression of color coordinates and chromaticity characteristics are used for optimization of the conditions of reagent immobilization on the sorbent phase, investigation of the acid-base and complexing properties of the immobilized reagents, prediction of the increase in the selectivity of analytical reactions involving solid-phase reagents [1, 2]. By combining the sorption processes with concentration and selecting the most sensitive chromaticity functions, the detection limit of analytes is reduced

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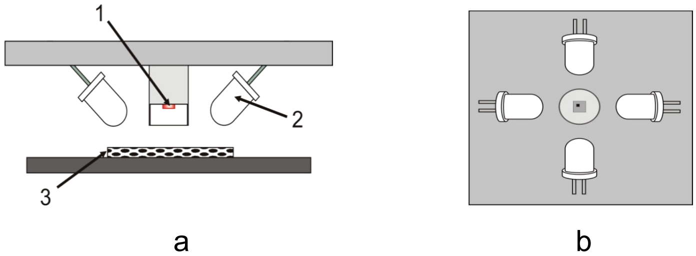
on 1.5-2 orders [2, 3]. The colorimetry method [4, 5] is applied in the visual analysis to test uniform chromaticity of color scales, prepared by using the non-transparent sorbents (silicas, silica gels, paper, ion-exchange resins) [2, 6, 7]. The chromatic characteristics of *L, a, b*, and total color difference ΔE in the CIELAB system is calculated on the basis of the color coordinates received from diffuse reflectance spectra (in the study of non-transparent media) or the transmission spectra (in the case of transparent matrix) [4, 5, 8].

The fast, objective and automated method of estimating the chromaticity characteristics of colored samples in the RGB system has appeared with the prevalence of digital photography, scanners, and computer image processing software [3, 9-16]. Advances in miniaturization of optical transducers allowed to create new optical elements - color sensor based on light-emitting diodes to detect the signal from colored solid surfaces [17-21].

The colorimetry method as «analytical tool» was applied in this paper as follow: for the precision estimation of results from the chromaticity characteristics measurements of colored sorbents using a portable photometer-reflectometer; for the investigation of a possibility of using of sorption-colorimetry two-component analysis of metal ions with reagent indicator papers; for evaluation of chromaticity uniformity of color scales prepared using sorbents of different nature.

RESULTS AND DISCUSSION

The portable photometer application. The design of the device measuring system includes four light-emitting diodes (LEDs), which are located on the four sides of the color sensor and sent to the sample angularly 45º [21] (Figure 1). This makes a possibility to scan colored solid samples of different porosity, including powdered sorbents.



a b

Figure 1. Arrangement of a portable photometer:
a - side view (1 – sensor, 2 – LED, 3 - specimen), b - bottom view.

The porous polyurethane foam (PUF), the dense reagent paper (RIP) and the silica gel were chosen to study the dependence of the precision of results of chromaticity measurements from the density of the sorbent and sorbate colors. Many-coloured products of known heterophase reactions (Table 9, the test-systems 1, 2, 5, 12, 13) [11, 22, 23] were chosen. The diffuse reflectance spectra of the sorbates are overlapped almost the entire visible wavelengths range. The absorption maxima were: 622 nm and 488 nm for the thiocyanate complexes of the Co^{2+} ions and Fe^{3+} adsorbed on PUF; 558 nm for the immobilized complex of Cu^{2+} ions with formazans on paper; 540 and 595 nm for the cationic forms of Neutral Red (NR) and Malachite Green (MG) adsorbed on silica gel.

The dependence of brightness of R-, G-, B-channels from the analyte concentration in solution (Figure 2) was obtained as a result of scanning of the stained samples of PUF, RIP and silica with a photometer. For each system was chosen the channel whose brightness decreased most sharply with increasing of the analyte concentration: for the complexes of Co(II) and Cu(II) and MG - this channel is R, for the complexes of Fe(III) and indicator NR - channel G.

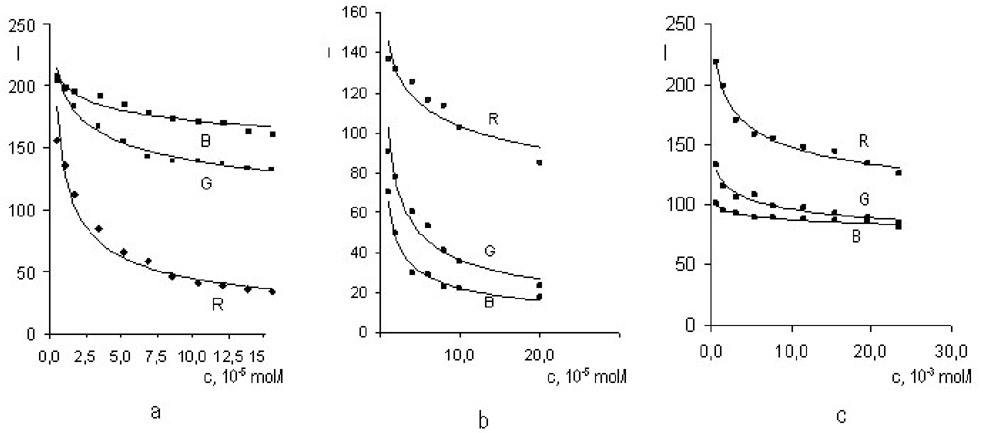


Figure 2. Dependence of brightness of R-, G-, B-channels from the analyte concentration in solution for: a – thiocyanate complexes of Co^{2+} ions adsorbed on PUF; b – indicator Neutral Red absorbed on silica; c – complexes of Cu^{2+} ions with formazans on paper.

All experimental curves describe a decreasing power function according to eq. 1:

$$I\ (R, G, B) = a \cdot c^{-t} \qquad (1)$$

where: a, t - curve equation parameters; c - analyte concentration (Table 1).

Table 1. The meanings of the parameters of graduation curve (eq. 1)

Test system	**Color channel**	**Concentration range, mol/l**	**Parameters**		**R^2**
			a	t	
RIP-Cu-Test	R	$(0.8–12)\cdot10^{-3}$	48.3±0.200	0.15±0.02	0.972
Co^{2+}-SCN-PUF	R	$(0.5–15)\cdot10^{-6}$	0.610±0.009	0.48±0.07	0.964
Fe^{3+}-SCN-PUF	G	$(0.4–15)\cdot10^{-6}$	0.850±0.006	0.36±0.02	0.984
NR-Silica	G	$(0.1–1)\cdot10^{-5}$	0.206±0.003	0.47±0.04	0.969
MG-Silica	R	$(0.1–1)\cdot10^{-5}$	0.191±0.002	0.67±0.03	0.989

The precision of chromaticity characteristics measurement results was studied in conditions of repeatability. The characteristic of precision by "repeatability" was evaluated for the same test samples by five-times measuring of the selected color channel brightness. In the case of evaluation of intermediate precision, the results of parallelly measurements (R, G) were the average of nine identical samples prepared for 10-12 concentrations of the working range.

The data from Table 2 show that the precision characteristics for colored PUF are slightly worse than that for RIP and silica. The largest scatter of the results was observed during the brightness measuring of the red channel for the system Co^{2+}-SCN-PUF. For all systems the value of relative standard deviation of measurement of R-, G- and B-components of the color of sorbates remained practically unchanged over the entire concentration range.

Table 2. The precision characteristics of the R-, G-, B-color components measurement results of different nature sorbents

Sorbent	**Repeatability, s_r% (n=5)**	**Intermediate precision, s_r% (n=9)**
PUF	0.10-0.6	0.1-0.7
Paper	0.08-0.3	0.1-0.3
Silica	0.06-0.4	0.1-0.4

The trueness of analysis results were tested using the standard solutions (Table 3, last column). The results are in good agreement with each other, there is no systematic error. The accuracy of our data is not worse than the results obtained using an office scanner or using a spectrophotometer with an attachment for diffuse reflectance [11, 24].

Table 3. The results of trueness checking using standard solutions

Test-system	**$c(M^{z+})$, mg/l**				
	Concen-tration	**Recovery (n=3, P=0.95)**			
		Diffuse reflectance		**RGB**	
		SF-2000 [24]	**Spectron** [11]	**Scanner** [11]	**Photometer**
Cu^{2+}-RIP	125	125±1 (s_r,%=0.2)	-	-	126±1 (s_r, %=0.2)
Co^{2+}-PUF	0.45	0.44±0.05 (s_r,%=3)	-	-	0.46±0.01 (s_r, %=0.5)
	0.40	-	0.40±0.03 (s_r,%=3)	0.50±0.10 (s_r,%=9)	-
Fe^{3+}-PUF	0.15	0.14±0.01 (s_r,%=2)	-	-	0.16±0.01 (s_r,%=1.5)

The relative error of the Fe(III) determination with a portable photometer (Table 3) was lower than using a portable spectrophotometer equipped with a microcell and emitting in the wavelength range 440-640 nm. The content of Fe(III) was controlled in the range of 0-0.2 mg/l with an error of ~2.4% [17].

With microspectrophotometer [18] and reflective sensor [19], the content of Fe(III) in aqueous media was measured with a relative standard deviation about 5%.

Thus, a portable photometer [21] on its characteristics is not worse than laboratory spectrophotometers (measurement of diffuse reflectance) and allows to measure the chromaticity characteristics of colored sorbents of different nature with relative standard deviation which is not exceed 1.0%.

Sorption-colorimetry determination of metal ions in their mixture using reagent indicator paper. The majority of analytical reagents used in photometry and visual colorimetry for metals control in aqueous media are not selective. Developers of test systems, for instance, indicate: "test system is designed for total content of metal ions estimation" [22, 25-30]. However, analysts are often interested in the separate determination of metals; in this case we have to talk about how to increase the selectivity of reactions, reagents, test systems and processes. Selectivity of analytical reactions can be improved by varying the determination conditions (pH, temperature) or by adding of masking agents.

The various parameters so called coefficients or factors of selectivity are used to the quantitative expression of the selectivity. They are equals to a ratio of some parameters of interfering component to the same parameter of the target component. There is the ratio of ion concentrations in potentiometry, the distribution coefficients in separation methods, ion mobility in migration methods, the molar absorption coefficients in spectrophotometry [31].

The authors of [2, 32-36] have suggested to use a two-parameter colorimetry to improve the selectivity of the metal ions determination, which form a similar color products of heterophase reactions. The colored metal complexes were adsorbed on the modified silica and from the diffuse reflectance spectroscopy data color coordinates of A and B were calculated to implement the method of separate colorimetric determination of analytes. The dependences of changing of color coordinates A from B varying the concentration of metal ions in solution were obtained. The conclusion about the possibility of separate determination of metals was made on the basis of the separation factor α, which was calculated by the equations:

$$\alpha = |\mathrm{arctg}\ k_{M1} - \mathrm{arctg}\ k_{M2}|, \text{ if } |\mathrm{arctg}\ k_{M1} - \mathrm{arctg}\ k_{M2}| < 90°;$$

$$\alpha = 180° - |\mathrm{arctg}\ k_{M1} - \mathrm{arctg}\ k_{M2}|, \text{ if } |\mathrm{arctg}\ k_{M1} - \mathrm{arctg}\ k_{M2}| \geq 90°, \quad (2)$$

k_{M1}, k_{M2} - the slope of the graphic dependences of changing of color coordinates A from B at different concentrations of M_1 and M_2 respectively.

The separate determination of metals is possible if $\alpha \geq 25°$ [2] in the case of diffuse reflectance measurements on a colorimeter "Spektroton" (or other spectrophotometer with the same class of accuracy, for example, SF-2000).

The content of metals was found by solution of the system, composed as a vector sum of analytical signals according to the equations:

$$\Delta L_\Sigma^2 = (a_{11} + b_{11}x_1)^2 + (a_{12} + b_{12}x_2)^2,$$
$$\Delta S_\Sigma^2 = (a_{21} + b_{21}x_1)^2 + (a_{22} + b_{22}x_2)^2, \qquad (3)$$

where x_1, x_2 - the unknown concentrations of metal ions M_1 and M_2 in solution; a_{ij} and b_{ij} (i, j = 1, 2) - the equation parameters of dependences of the color differences in brightness (ΔL) or saturation (ΔS) from x_1 and x_2 [2].

The two-parameter colorimetry was applied to study the possibility of separate determination of two metals, immobilized in the form of colored complexes on paper or in a gelatin film. The non-selective reagents - formazan (Figure 3), 4-(2-pyridylazo)-resorcin (PAR) and 1-(2-pyridylazo)-2-naphtol (PAN) (test systems 6, 8 and 11 from Table 9) were used.

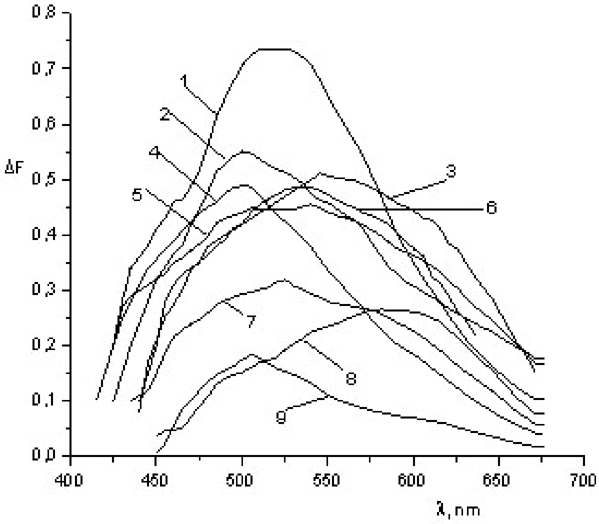


Figure 3. The absorption spectra of the sorbed complexes of metal ions with formazans on indicator paper RIP-Metal-test: 1 – Cu^{2+}, 2 – Co^{2+}, 3 – Mn^{2+}, 4 – Ni^{2+}, 5 – Fe^{3+}, 6 – $\sum M^{n+}$, 7 – Zn^{2+}, 8 – Cd^{2+}, 9 – Pb^{2+} (in solutions $c(M^{n+}) = c(\sum M^{n+}) = 8.0 \cdot 10^{-3}$ mol/l, pH 5).

For all immobilized metal complexes were calculated chromaticity characteristics and the dependences B from A were ploted on the basis of the absorption or reflection spectra (Figure 4, for RIP-Metal-test the similar dependences in the range of concentrations of M^{n+} from 0.5 to 8.0 mmol/l) were ploted.

As it can be seen from Figure 4a none of the pair of metal ions in their total presence in solution can not be separately identified by reaction with PAR in a gelatin film; all the graphics are almost parallel. In the case of reaction M^{2+} with PAN on paper (Figure 4b), the angle between the graphic dependences was more than 25° for couples: Co^{2+} ion - either M^{2+} that is cobalt falls out of the total amount of metal in spectroscopic measurements and it can be determined separately. In the visual colorimetric measurements in the case of close concentration of metal ions the green color of the cobalt (III) complex with PAN is not visible on the background of the red complexes of other metals.

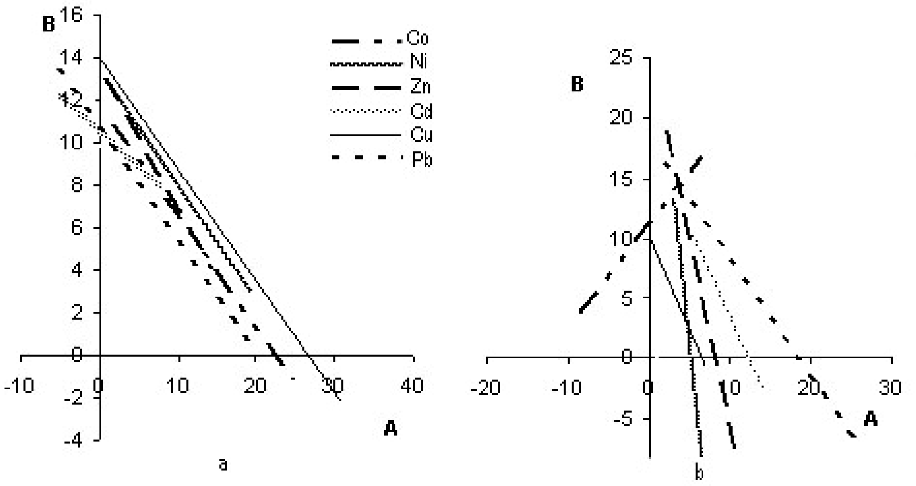


Figure 4. The change of color coordinates of immobilized complexes from metal ions concentration in a solution (from $1 \cdot 10^{-5}$ to $3.2 \cdot 10^{-4}$ mol/l):
a) metal ion complexes with PAR in gelatin film;
b) metal ion complexes with PAN on RIP.

The separation factor values for all pairs of metal ions adsorbed on the RIP-metal-test are shown in Table 4. According to the data for some pairs of α>25°, they can be separately determined.

Table 4. The separation factor values for pairs of metal ion complexes adsorbed on the RIP-metal-test

	Cu^{2+}	Pb^{2+}	Zn^{2+}	Cd^{2+}	Ni^{2+}	Co^{2+}	Mn^{2+}	Fe^{3+}
Cu^{2+}	—	69.3°	52.6°	52.5°	37.7°	2.2°	48.3°	51.0°
Pb^{2+}	69.3°	—	16.7°	16.8°	31.6°	71.5°	21.1°	18.3°
Zn^{2+}	52.6°	16.7°	—	0.1°	15.0°	54.8°	4.4°	1.7°
Cd^{2+}	52.5°	16.8°	0.1°	—	14.9°	54.7°	4.3°	1.5°
Ni^{2+}	37.7°	31.6°	15.0°	14.9°	—	39.9°	10.6°	13.3°
Co^{2+}	2.2°	71.5°	54.8°	54.7°	39.9°	—	50.5°	53.2°
Mn^{2+}	48.3°	21.1°	4.4°	4.3°	10.6°	50.5°	—	2.6°
Fe^{3+}	51.0°	18.3°	1.7°	1.5°	13.3°	53.2°	2.6°	—

The examples of the single results of two-component determination of metal ions in model solutions with indicator papers are presented in table 5. For selected pairs the colored test samples were obtained, diffuse reflectance was measured, color coordinates were calculated and the system of the equations (3) was solved using the program Mathcad 2001. The system of the equations of fourth degree has four roots, two of which are often negative. However, the four positive roots or three negative and one positive root can satisfy of equations solution. We are faced with this situation (Table 5) and in this case the analysis is meaningless. For more good examples the difference between the concentration values of injected and found metal ions varied from 2 to 50%. Thus, the application of two-parameter colorimetry has significant limitations.

Table 5. The results of two-component colorimetry determination of metal ions in model solutions with indicator paper

RIP	**Concentration in model solution**				**Recovery, %**	
	M_1	M_2	$c(M_1)$, μmol/l	$c(M_2)$, μmol/l	M_1	M_2
RIP-PAN	Co	Zn	6.0	6.0	123	103
			4.0	4.0	103	negative
		Ni	4.0	4.0	147	102
			2.0	2.0	70	154
		Pb	2.0	2.0	65	80
			8.0	8.0	4 positive results	
RIP	M_1	M_2	$c(M_1)$, mmol/l	$c(M_2)$, mmol/l	M_1	M_2
RIP-Metal-Test	Cu	Ni	$1.7\cdot10^{-3}$	$3.8\cdot10^{-3}$	135	126
			$4.2\cdot10^{-3}$	$1.7\cdot10^{-3}$	114	129
		Zn	$4.2\cdot10^{-3}$	$1.5\cdot10^{-3}$	93	107
			$1.5\cdot10^{-3}$	$1.5\cdot10^{-3}$	93	135
			$3.9\cdot10^{-3}$	$3.8\cdot10^{-3}$	28	100

The color scales construction. The comparison color scale is applied to quantitative estimation of the analyte content in the objects of analysis. Scales are a set of test samples (standards) corresponding exactly to known analyte content [1, 22]. Concentration of the determined component on the color scale increases in geometric progression. This deals with a human perception of color and exponential dependency of color characteristics from the analyte concentration. The coefficient of geometric progression, which is the ratio of adjacent concentrations on the scale ($q=c_{n+1}/c_n$), is chosen equal two or three. There are also other recommendations, for example, in the work [37] was proposed to build scale in accordance with the Fibonacci number, in this case every subsequent concentration on a scale equals the sum of the two previous concentrations, progression approaches asymptotically to a geometric with $q=1.618$.

The scale should be with uniform chromaticity and well perceived by the human eye. This is a basic requirement for the color scale for visual colorimetry. The color scale uniform chromaticity prepared by using non-transparent sorbents (silica, paper, ion-exchange resin) is checked using a colorimetry method [2, 6]. The general color discrimination ΔE is selected as main analytical parameter to record the change in color of modified sorbents. On the basis of ΔE experimental dependency from the analyte concentration the color scale with a constant step $\Delta E=10$ is built. It is considered that the error of test technique is minimized and the scale will be distinguished by eye of any person [2, 7].

However, the criterion $\Delta E \geq 10$ was not strictly enough to visual discrimination of color differences of adjacent scale standards. The total color difference varied on 5-7 reference units [26] in case of Al (III) determination with chromazurol S immobilized on a cellulose chromatography paper using color scale with q=2. The authors [37] affirm that the value of $\Delta E=5$ is the natural border of eye color perception if the scale is constructed in accordance with a Fibonacci series.

Thus there is no general consensus about how to build a color scale and how to estimate its quality; it is not clear what criterion corresponds to the boundary color discrimination; no recommendations for testing uniform chromaticity scale, prepared using transparent sorbent.

To study these problems seven test systems were chosen: Co^{2+}-SCN-PUF, Fe^{3+}-SCN-PUF, NO_2^--PUF, RIP-Co-Test, Co^{2+}-NRS-gelatin film, Al^{3+}-EChC-gelatin film and ΣM^{n+}-PAR- gelatin film (Table 9). The four color scales with the coefficients q=1.5, q=2, q=3 and q≈1.618 (Fibonacci series) were prepared three times for all test systems.

On the basis of colorimetry measurements for all sorbents were obtained the values of total color difference ΔE and for each concentration range on a scale the difference according to eq. (4) was calculated:

$$\Delta E^* = \Delta E_{n+1} - \Delta E_n. \qquad (4)$$

It was found which of the scales were constructed more correctly using the value of color discrimination step on the scale (ΔE^*).

Along with the colorimetry method for estimation of quality of prepared color scales, statistics of observations was applied. The scales were placed on a sheet of white paper in order of analyte concentration increasing and compared the color intensity of the adjacent standard samples of the scale. Fifty independent observers were asked: "Do you see any difference between the color of adjacent samples of the scale?" After that the frequency of detection of the color discrimination was calculated:

$$P(c) = n/N \qquad (5)$$

where: n - number of positive responses, N - total number of observations.

The scale with P(c) ≥ 0.9 was chosen, that is more than 90% of observers have seen the color difference of adjacent scale standards. This scale should provide high accuracy of test determinations.

The examples of the results of visual and instrumental methods of investigation of the color scales construction correctness for the two test systems based on polyurethane and gelatin film are presented in Tables 6 and 7. In the case of the Fe(III) determination in the form of $Fe(SCN)_4^-$ complexes sorbed on PUF you should choose a scale with coefficient 2 or 3 in the Fe^{3+} concentration range 0.02-0.80 mg/l. In this range on a scale with q=2 color discrimination step ΔE*=4-6, on a scale with q=3 color discrimination step ΔE*=7-8 (Table 6). The intensity of color tablets increased sharply above these concentrations and the value of the difference ΔE* increased too. In the case of the Co(II) determination as blue thiocyanate complexes was observed a similar situation. The uniform chromaticity scale with coefficient 2 was observed in the Co^{2+} concentration range of 0.03-0.5 mg/l (ΔE*≈4); at higher concentrations the value of ΔE* increased dramatically. Scales with coefficients q=1.5 and q=1.618 were invalid at a Co^{2+} concentration less than 0.5 mg/l.

Table 6. The meanings ΔE* and the frequency of detection of the color discrimination of adjacent standards of color scales for test-system Fe^{3+}-SCN-PUF.

Interval Δc(Fe^{3+}), mg/l	**P, % (N=50)**	**ΔE***	**Interval Δc(Fe^{3+}), mg/l**	**P, % (N=50)**	**ΔE***
q=2			q=3		
0-0.02	100	4	0-0.02	100	4
0.02-0.04	92	4	0.02-0.06	100	8
0.04-0.08	93	4	0.06-0.18	98	7
0.08-0.16	95	6	0.18-0.54	100	8
0.16-0.32	98	4	0.54-1.62	100	16
0.32-0.64	100	6	-	-	-
0.64-1.28	100	14	-	-	-
q=1.5			Fibonacci series		
0-0.02	100	4	0-0.02	100	4
0.02-0.03	68	2.5	0.02-0.03	70	3
0.03-0.05	60	3	0.03-0.05	63	2.5
0.05-0.07	44	2	0.05-0.08	85	3.5
0.07-0.10	72	2	0.08-0.13	89	4
0.10-0.15	94	6	0.13-0.21	86	3
0.15-0.23	96	4	0.21-0.34	87	3
0.23-0.34	90	6	0.34-0.55	85	3
0.34-0.51	89	3	0.55-0.89	91	5
0.51-0.77	88	4	-	-	-

The samples of indicator papers on the scales with coefficients 2 and 3 were more differentiated for a test system based on the indicator paper RIP-Co-Test, the condition P(c)>90% satisfied with ΔE*≥4. The scale with q=1.5 was less reproducible in color test samples perception, on the scale constructed in accordance with Fibonacci series the value of P(c) has not reached 90%. Thus, the value of ΔE*=4 can be taken as a boundary for visual color discrimination of red and blue colors shades on the PUF, red color shades on a yellow paper and the value of ΔE*=3 - for color discrimination yellow color shades on the PUF (Table 8). If you follow the recommendations of [2] to create uniform color scales (ΔE*=10) the concentration of Fe^{3+}, Co^{2+} and NO_2^- ions would be increased by more than 3 times, that is unreasonably for practical purposes.

The colorimetry method was not managed to apply for estimation of the color scales quality prepared using transparent gelatin films. The value of the total color difference of Al(III) with Eriochrome Cyanine R (EChC) immobilized complex practically unchanged until the middle of the scale concentration ranges (up to Al^{3+} concentration ~0.5 mg/l), and the difference ΔE* was equal zero (Table 7).

Table 7. The meanings ΔE* and the frequency of detection of the color discrimination of adjacent standards of color scales for test-system Al^{3+}-EChC-gelatin film

Interval Δc(Al^{3+}), mg/l	**P, % (N=50)**	**ΔE***	**Interval Δc(Al^{3+}), mg/l**	**P, % (N=50)**	**ΔE***
q=2			q=3		
0-0.14	90	1	0-0.14	90	1
0.14-0.27	92	0	0.14-0.41	98	0
0.27-0.54	95	0	0.41-1.23	100	1
0.54-1.08	98	0	1.23-3.69	100	1
1.08-2.16	100	1	-	-	-
q=1.5			Fibonacci series		
0-0.14	90	1	0-0.14	90	0
0.14-0.20	60	0	0.14-0.16	26	0
0.20-0.30	54	0	0.16-0.30	86	0
0.30-0.46	96	0	0.30-0.46	90	0
0.46-0.70	94	1	0.46-0.76	96	0
0.70-1.05	80	1	0.76-1.22	100	2
1.05-1.57	92	0	1.22-1.98	86	1
1.57-2.36	92	1	-	-	-

The difference ΔE* did not exceed 2 at higher concentration of the complex. Similar results were obtained for the test systems for determination of Co^{2+} ions with the Nitroso-R-salt (NRS) and the sum of metal ions with PAR. The difference ΔE* exceeded zero value for $c(Co^{2+})$~1.5 mg/l and $c(\Sigma M^{2+}) \sim 1 \cdot 10^{-5}$ mol/l.

Statistics of observations allowed to choose an optimal color scale. More than 90% of observers distinguish adjacent color reference samples of the scale over the whole concentrations range for the three studied test systems. For optimal color scales have been chosen scales with coefficients q=2 and q=3.

Thus, it is impossible to formulate common recommendation and common criteria for the construction of color scales using different sorbents. For choosing an optimal scale for visual test determinations should be give preference to the visual method. In each case should choose the geometric progression of the concentration and the background color, which will provid color distinguishing of the adjacent standard samples of the scale more than 90% of a large number of observers (~50).

Table 8. The results of optimal color scale choice

Test-system	**ΔE***	**Efficient scale**			
		q=2	**q=3**	**q=1.5**	**q=1.62**
Fe^{3+}-SCN-PUF	4	+	+	-	-
Co^{2+}-SCN-PUF	4	+	+	-	-
NO_2^- -PUF	3	+	+	-	+
RIP-Co-Test	4	+	+	-	-
Co^{2+}-NRS-gelatin film	-	+	+	-	-
ΣM^{2+}-PAR-gelatin film	-	+	+	-	-
Al^{3+}-EChC-gelatin film	-	+	+	-	-

CONCLUSIONS

A mini photometer-reflectometer [21] allows to measure the chromaticity characteristics of colored sorbents of different nature with relative standard deviation which is not exceed 1.0%.

Application of two-parameter colorimetry has significant limitations; after separation or masking of interfering components in real samples, the relative standard deviation of the results of separate two-colorimetric determination may exceed 50% or this method of analysis might has no solutions.

Colorimetry method is not suitable for estimation of the quality of color scales prepared using transparent gelatin films. For choosing an optimal scale for visual test determinations should be give preference to the visual method.

EXPERIMENTAL SECTION

Four types of sorbents – porous polyurethane foam (PUF), dense reagent paper, silica and gelatin film and products of heterophase reactions with different colors have been chosen for investigations.

Reagents, materials and equipment

All solutions were prepared using distilled water and all chemicals were of reagent grade. The true concentrations of metal ions in initial salt solutions were determined using trilonometric ($Co(NO_3)_2$, $Cd(NO_3)_2$, $Ni(NO_3)_2$, $Zn(NO_3)_2$, $Pb(NO_3)_2$, $FeCl_3$, $Al(NO_3)_3$ [38]) and iodometric titration. Stock solutions of 1 mol/l NaF, 5 mol/l KSCN, 1 mol/l NaCl, 0.01 mol/l $NaNO_2$, 0.8 mol/l $Na_2B_4O_7$, 0.03 mol/l Nitroso-R-salt (NRS), 0.005 mol/l 4-(2-pyridylazo)-resorcin (PAR), 0.001 mol/l Malachite Green (MG), 0.001 mol/l Neutral Red (NR) and 0.5 mol/l Cetylpyridinium Chloride (CPCh) were prepared by dissolution of exactly weighed amounts of reagents in water. The solution of 1-(2-pyridylazo)-2-naphtol (PAN) with mass fraction 0.1% was prepared by dissolution of exactly weighed amounts of reagent in acetone. The solution of Eriochrome Cyanine R (EChC) with mass concentration 0.7 mg/l was prepared according to recommendations [39]. In the case of Ferrum (III) determination the solution of KSCN was purified from Fe^{3+} ions impurity using prepared PUF tablets.

The solutions of strong acids (H_2SO_4, HCl) and buffer solutions (acetic, borax) were used to create the required pH values.

Indicator paper. The specimens of reagent indicator paper on the basis of epoxidized cellulose [22]: RIP-Co-Test with immobilized 1-nitroso-2-naphthol; RIP-Cu-Test with covalently fixed 1,3-biphenyl-5-(6-methyl-4-oxo-3,4-dihydropyrimidine-2-yl)-formazan; RIP-Metal-Test on the basis of 1-(2-carboxyphenyl)-5-(benzoxazolyl-2-yl)-formazonylcellulose; RIP-Metal-Test II on the basis of irregular 1-(2-carboxyphenyl)-5-(4-methyl-6-metoxypyrimidine-2-yl)-formazan-6-cellulose [23] and also prepared by the technique [25] the indicator paper with adsorption fixed of 1-(2-piridilazo)-2-naphthol were used in this work.

Polyurethane foam. Test means for determination Co^{2+} and Fe^{3+} ions with KSCN and chemisorption determination of nitrite was prepared from the polyurethane sheet on the basis of mixed esters (brand 2240) according to the technique described in [1, 3]. The sorbent used in the form of tablets with a diameter of 16 mm, thickness of 7-10 mm, weight 0.03-0.05 g. Tablets of PUF was purified by keeping during 30 min in solutions of 0.1 mol/l H_2SO_4 (for Co^{2+} determination), 0.1 mol/l HCl (for Fe^{3+} determination), 5 mol/l HCl (for NO_2^- determination), then tablets were washed with water to pH 5-6, acetone, squeezed between sheets of filter paper, air dried and stored at room temperature in a darkness.

Gelatin films were used in the role of carrier material for the Co^{2+} determination with Nitroso-R-salt, Al^{3+} with Eriochrome cyanine R and metal ions sum (Zn^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}) with 4-(2-pyridylazo)-resorcin. Photographic films for offset printing manufactured by AGFA, with thickness of gelatin layer around 20 μm, fixed on the transparent triacetylcellulose support [40] were used for the producing of sensitive elements. The samples used were colorless and transparent with size 2.5×3.5 cm. previously the silver halogenides were completely removed from the films.

The Nitroso-R-salt extraction in a gelatin matrix was carried out from water solution with the NRS concentration of 0.003 mol/l and pH 2 (HCl); Eriochrome cyanine R – from the solution $5 \cdot 10^{-4}$ mol/l EChC, 0.01 mol/l CPCh and pH 5.5; 4-(2-pyridylazo)-resorcin – from the solution of 0.001 mol/l PAR. Samples of the films were immersed in a reagent solution for 30 min in the case of the NRS, for 20 minutes – EChC and for 45 minutes – in the case of the PAR, then the films were removed from the solution with tweezers and air dried in a Petri dish placed edgewise on a filter paper. Samples of the films with immobilized reagents were uniformly colored in yellow (NRS, PAR) and orange (EChC), they were stored at room temperature in a darkness.

We used macroporous *silica* grade L 100/250. Malachite Green and Neutral Red indicators were sorb from solution as cationic forms, creating a pH 9.18 and 3 respectively. Powder with weight of 0.2 g was poured in the indicator solution (25 ml) and the suspension was shaken on a mechanical mixer for 3 minutes in the case of the MG and 90 minutes in case of the NR.

Characteristics of test systems and conditions of testing procedure are shown in Table 9. All observations were carried out in daylight illumination in conditions of diffused light, the value of lighting in the room was 500-600 lux. Comparison of the color intensity of the adjacent reference samples of the scale and color comparison of control samples, which were obtained as a result of testing the control samples or model solutions, with the color scale were carried out by placing the scale and control samples on a sheet of white paper.

Colorimetric measurement. Diffuse reflection of PUF tablets and RIP samples was measured using spectrophotometer SF-2000. Tablets and sample indicator paper were fixed on the attachment; the reflection was measured relative to white purified tablet of PUF or a sample of white dense paper in the wavelength range from 400 to 700 nm. Using the software that is supplemented to the spectrophotometer chromaticity characteristics and the values of total color difference (ΔE) were obtained.

Absorption (transmission) of gelatin films was registered using spectrophotometer Hitachi U 3202. The films were fixed in the holder in a cuvette compartment. Absorption spectra of the films with reagent or complex were measured against reference colourless film. The method of selected coordinates was chosen to calculate the X, Y, Z color coordinates [4, 5, 8]. Coordinates *L, a, b* and total color difference were calculated by formulas:

$$L = 116(Y/Y_0)^{1/3} - 16, \tag{6}$$

$$a = 500[(X/X_0)^{1/3} - (Y/Y_0)^{1/3}], \tag{7}$$

$$b = 200[(Y/Y_0)^{1/3} - (Z/Z_0)^{1/3}], \tag{8}$$

$$\Delta E = [(L_0 - L)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2}, \tag{9}$$

L, a, b, L_0, a_0, b_0 – color coordinates of sample with immobilized complex and sample with reagent respectively.

Color coordinates in the RGB system were obtained using a portable photometer-reflectometer, which has both optical filters in red, blue and green spectral regions (λ_R = 615 nm, λ_G = 540 nm, λ_B = 465 nm) and sensors without filters with a spectral range of sensitivity 400–700 nm [21]. The instrument has a small overall size 70×60×35 mm and a connection to USB, which makes the system suitable for colorimetric measurements of colored non-transparent samples (Figure 1).

Stained samples of silica gel, PUF and RIP were scanned with predetermined calibration of the photometer using white standard. Samples of PUF and RIP were placed at a white substrate and firmly pressed to the device screen. The powder of modified silica with a mass of ~ 0.1 g densely was poured into a white circular teflon cell (10 mm in diameter and 2 mm high), the upper layer of powder was leveled and the measurements were carried out. The brightness of R-, G-, B-channels was performed using computer programs Microsoft Excel and Origin 6.0. Brightness of channels varied in the range of 0-4500 that corresponds to a value range 0-225 in a case of desktop scanner.

LIST OF ABBREVIATIONS

ΔE – total color difference
LEDs – light-emitting diodes
PUF – polyurethane foam
RIP – reagent paper
NR – Neutral Red
MG – Malachite Green
PAR – 4-(2-pyridylazo)-resorcin
PAN – 1-(2-pyridylazo)-2-naphtol
NRS – Nitroso-R-salt
CPCh – Cetylpyridinium Chloride
EChC – Eriochrome Cyanine R

Table 9. The characteristics of the test-systems and testing conditions

№	Analyte	Reagent	Indicator system	Sorbent	Concentration range, mol/l	Time, min	Color changing of a sorbent
1	Co^{2+}	SCN^-	KSCN, 1.0 mol/l NaF, 0.01 mol/l H_2SO_4, pH 2	PUF	$(0.5\text{-}40)\cdot10^{-6}$	30	white – blue
2	Fe^{3+}	SCN^-	KSCN, 1.0 mol/l H_2SO_4, pH 2		$(0.4\text{-}20)\cdot10^{-6}$	15	white – red
3	NO_2^-	–	HCl, pH 0		$(0.15\text{-}3.0)\cdot10^{-5}$	25	white – yellow
4	Co^{2+}	Formazan	RIP-Co-Test, pH 6.6	Paper	$(1.6\text{-}22)\cdot10^{-4}$	5 sec	yellow – red
5	Cu^{2+}	Formazan	RIP-Cu-Test, HCl, pH 3		$(0.8\text{-}12)\cdot10^{-3}$	15	yellow – violet
6*	ΣM^{n+}	Formazan	RIP-Metal-Test		$(0.5\text{-}8)\cdot10^{-3}$	3 sec	yellow – dark grey
7*	ΣM^{n+}	Formazan	RIP-Metal-Test II		$(1\text{-}8)\cdot10^{-4}$	3 sec	yellow – dark blue
8*	ΣM^{n+}	PAN	$Na_2B_4O_7$, pH 9		$(5\text{-}32)\cdot10^{-6}$	15	yellow – red
9	Al^{3+}	EChC	CPCh, $1\cdot10^{-4}$ mol/l acetate buffer, pH 5 NaCl, 0.1 mol/l	Gelatin film	$(0.5\text{-}13.0)\cdot10^{-5}$	20	violet – dark blue
10	Co^{2+}	NRS	HCl, pH 2		$(0.5\text{-}16)\cdot10^{-5}$	20	yellow – reddish-brown
11*	ΣM^{n+}	PAR	acetate buffer, pH 5		$(0.3\text{-}40)\cdot10^{-5}$	15	yellow – red
12	MG	–	borate buffer, pH 9.18	Silica	$(0.1\text{-}1)\cdot10^{-5}$	3	white – blue
13	NR	–	HCl, pH 3		$(0.1\text{-}1)\cdot10^{-5}$	120	white – crimson

*In the case of the determination of total metal ion concentration in each solution created equal molar concentrations of metals

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