
ARTICLES

Limit of Detection in Test Methods of Analysis with Visual Indication: Affecting Factors¹

E. A. Reshetnyak*, N. A. Nikitina*, L. P. Loginova*, and V. M. Ostrovskaya**

* Department of Chemistry, Karazin National University, pl. Svobody 4, Kharkov, 61077 Ukraine

** Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences,
Leninskii pr. 31, Moscow, 119991 Russia

Received January 11, 2005

Abstract—A statistical approach to evaluating the limit of detection (c_{\min}) was used for studying 12 test systems with visual indication based on reagent indicator paper, polyurethane foam, gelatin films, powdered methylsilicic acid, and reagent solutions. It was proposed to characterize the quality of test systems by relative width of the region of an unreliable response. The dependence of the characteristics of test systems on the material of the support, the type of analytical reaction, the conditions of color observation in the test system, and the number of observations were discussed.

The limit of detection (c_{\min}) is the most important performance characteristic of test procedures with visual indication. The analysts who develop these procedures evaluate this property in different manners, and the values of c_{\min} have not been specified in the majority of procedures. In the list of test systems manufactured by the Russian ecoanalytical association Ekoanalitika in 2004, the limits of detection were specified for only 4 of 55 systems.

Previously [1, 2], an algorithm was proposed for the reliable evaluation of c_{\min} . This algorithm included special steps for checking the adequacy of the experimental material. To test hypotheses on the type of distribution of analyte detection frequencies $P(c)$ in the region of unreliable response, numerical methods and the following set of criteria was proposed: χ^2 and Kolmogorov–Smirnov (λ) tests, the asymmetry ratio (\tilde{A}), and the coefficient of excess (γ_2).

In this work, we used this approach for evaluating the limits of detection in 12 test systems, which were either described in the literature or developed within the framework of this study. The aim of this work was to find the dependence of c_{\min} and other properties of test systems with visual indication on the following factors: the material of the support, the type of the analytical reaction, the conditions of color observation in the test system, and the number of observations.

Substrate paper for rapid tests, polyurethane foam, methylsilicic acid, and gelatin film were used as support materials in the test procedures. The complexation of metal ions with inorganic and organic ligands, the formation of associates and double salts, chemisorp-

tion, and redox reactions were used as analytical reactions.

Indicator paper for the test determination of cobalt(II) based on its reaction with 2-nitroso-1-naphthol in the presence of diphenylguanidine and NiSO_4 (these reagents enhance the contrast of a color change) is well known [3]. In this study, we extended this reaction to other materials (polyurethane foam and methylsilicic acid) and estimated the detection limit for this reaction in solution.

Butylrhodamine was chosen as an analytical reagent for the test determination of 2,4-dichlorophenoxyacetic acid (2,4-D). Butylrhodamine, which was proposed as a reagent for the extraction–photometric determination of 2,4-D [4], forms a bright pink associate with 2,4-D.

EXPERIMENTAL

All solutions were prepared using twice-distilled water and reagents and chemicals of analytical grade or better.

Reagent indicator papers. The following five types of reagent indicator paper (RIP) strips were used: RIB-Cadmium-Test [1, 5, 6], RIB-Iron(II)-Test [1, 5, 6], RIB-Iron(III)-Test [5, 6], RIB-Nitrite-Test [5], and RIB-Metal-Test II for the determination of total heavy metals (Cd^{2+} , Co^{2+} , Cu^{2+} , Fe^{3+} , Hg^{2+} , Ni^{2+} , and Zn^{2+}) [1, 2, 5]. Without preconcentration, a RIB strip was immersed in solution for 1–2 s and removed; the color was observed before drying. Under conditions with preconcentration, the test solution was passed through the RIB reaction zone with a pocket indicator device in accordance with a maximum preconcentration version [5]. The RIB was slightly dried for 5 min, and a color change was observed in the reaction zone.

¹ Presented at the 2nd All-Russia Symposium “Test Methods in Chemical Analysis” (Saratov, 2004).

Polyurethane foam. Test systems for the detection of cobalt(II) with KSCN and for the chemisorption detection of nitrites were prepared from polyurethane foam (TU 6-305-1-87, OST 6-19-37.039-85) in accordance with published procedures [7, 8].

In the other cases, polyurethane foam pellets were successively washed with 0.1 M H_2SO_4 , water to pH 5–6, and acetone; squeezed between filter paper sheets; and dried in air. Test systems for detecting cobalt(II) by the reaction with 2-nitroso-1-naphthol were prepared by supporting appropriate reagents onto polyurethane foam pellets (Table 1). For this purpose, purified pellets were placed in a solution of reagents, and the mixture was shaken on a mechanical vibromixer for 30 min. The pellets were removed from the solution and dried in air. The prepared pellets were yellowish orange.

Prewashed polyurethane foam pellets were used as test systems for the detection of 2,4-D.

At low analyte concentrations, color changes in polyurethane foam pellets are difficult to observe. For example, it is difficult to observe the onset of the appearance of yellow and blue colors in white polyurethane foam (the detection of NO_2^- and Co^{2+} with SCN^-). Color errors are due to light scattering from the cellular surface of polyurethane foam. The reproducibility of the results is impaired if observations are performed under direct sunlight. Therefore, all of the observations with polyurethane foam were performed under conditions of diffuse daylight illumination.

Procedure for the extraction-adsorption detection of 2,4-D with butylrhodamine. An aliquot portion of the test solution was placed in a 25-mL volumetric flask; 2.5 mL of an acetate buffer solution (pH 3.6) and 0.5 mL of a 0.1% aqueous solution of butylrhodamine were added, and the contents were diluted with water to the mark. A 10-mL portion of the resulting solution was transferred to a separatory funnel; 10 mL of toluene was added, and the contents were intensely shaken for 1 min. After phase separation, the toluene layer was removed and transferred into a flask with a ground-glass stopper, and a pellet of polyurethane foam was dipped into the liquid. The flask with the contents was shaken on a mechanical vibromixer for 15 min. The pellet was removed and dried in air. In the presence of 2,4-D in the solution, the pellet became pink, and the color intensity increased with the concentration of 2,4-D. The above operations were also performed with a blank solution; in this case, a pale pink pellet was obtained. This was likely due to the background pollution of water and chemicals.

Procedure for the adsorption detection of cobalt(II) by its reaction with 2-nitroso-1-naphthol. A pellet with supported reagents was placed in 25 mL of the test solution; the contents were shaken for 30 min, and the pellet was dried in air.

If the solution contained cobalt(II), the yellowish orange color of pellets changed to lilac. The color

Table 1. Composition of reagent solutions for the detection of cobalt

Support	Reagent concentration in solution, M		
	2-nitroso-1-naphthol	diphenylguanidine	NiSO_4
Paper [3]	1.4×10^{-2}	4.7×10^{-4}	6.5×10^{-3}
Polyurethane foam	5.0×10^{-5}	1.0×10^{-6}	6.5×10^{-5}
Methylsilicic acid	5.0×10^{-4}	1.6×10^{-5}	2.3×10^{-4}
Solution	3.0×10^{-4}	6.0×10^{-6}	4.0×10^{-4}

intensity increased with the concentration of cobalt(II) in solution.

Methylsilicic acid. The pharmaceutical preparation Enterosgel (ZAO Kreoma-Pharm, Kiev) with a weight fraction of methylsilicic acid equal to 70% was used. The preparation was dried to constant weight at 150°C. The test system for the detection of cobalt(II) was prepared by supporting appropriate reagents (Table 1). In preliminary experiments, we found that, with the use of ordinary procedures for supporting the reagents, the adsorbent surface was inconvenient for tests because it is poorly wetted with aqueous solutions. This disadvantage was removed by treating the adsorbent with a surfactant solution. The preparation of the test system included the following operations: two grams of the adsorbent was placed in 25 mL of a reagent solution (Table 1), and the mixture was stirred with a magnetic stirrer for 30 min. The adsorbent was filtered off and dried at 40°C. The dry adsorbent powder was shaken 30 min with 25 mL of a 0.012 M Triton X-100 (non-ionic surfactant) solution, filtered off, and dried at 40°C. The resulting yellowish orange powder was kept in a dark vessel. The properties of the adsorbent remained unchanged for a month.

Procedure for the adsorption detection of cobalt(II) by its reaction with 2-nitroso-1-naphthol on methylsilicic acid. Three drops of the test solution were applied to a 10- to 15-mg portion of the prepared adsorbent. The powder acquired a lilac color, whose intensity was proportional to the concentration of cobalt.

Detection of cobalt(II) by its reaction with 2-nitroso-1-naphthol in solution. Equal volumes of reagent (Table 1) and test solutions were placed in a 15- to 20-mL test tube; the test tube was closed with a stopper, and the contents were stirred. After 10 min, the color was observed from the side under diffuse daylight illumination. In the presence of cobalt(II), the orange color of solutions changed to lilac. The twofold dilution of the test solution was taken into account while comparing the performance characteristics of this reaction with those of analogous reactions on supports.

Gelatin films. Gelatin films were used as support materials for the detection of total heavy metals (Cd^{2+} , Co^{2+} , Cu^{2+} , Pb^{2+} , Ni^{2+} , and Zn^{2+}) with 4-(2-pyridyl-

lazo)resorcinol (PAR) [9, 10]. The gelatin coatings of a commercial photographic film for offset printing from Agfa and an MZ-3 positive film from Svema were used. Before the experiments, silver halides were completely removed from the films to obtain transparent colorless samples, which were modified by keeping them in a 1×10^{-3} M PAR solution in aqueous ethanol for 1 h. The resulting yellow film was washed with distilled water, dried in air, cut into segments of a required size, and stored at room temperature in a dark closed place. Upon dipping the film into a solution containing the above metal ions at pH 5 (acetate buffer solution), the yellow color of the film changed to red.

Evaluation of the limit of detection of an analyte in the procedures of test analysis with visual indication. The algorithm for evaluating c_{\min} was described previously [1] and implemented as follows:

(1) A group of 10–15 independent observers determined the region of an unreliable response.

(2) In the found region of an unreliable response, 7–12 concentrations (c_k) were chosen with the step Δ_c , which was greater than the triple standard deviation $3s_v$ of the concentration of prepared solutions.

(3) Two samples of 45–55 results of three or four series of observations were chosen for each value of c_k (from series to series, the reaction was repeated with a new test system using new portions of the solutions). The frequencies of detection were calculated for the concentration c_k in each series: $P(c_k) = n_k/N_k$, where n_k is the number of positive observations, and N_k is the total number of tests in the series. Crude errors were detected using the Q -test and excluded. The frequencies were averaged over series, and $\overline{P(c_k)}$, dispersion (s_k^2), and the standard deviation (s_k) of the frequency were found.

(4) The correspondence of the distribution of $\overline{P(c_k)}$ values to distribution functions commonly used in such studies [3, 11–13] (normal, lognormal, exponential, and Weibull distributions)² was checked for the first of the above samples. The distribution parameters and statistic parameters χ_{expt}^2 , λ_{expt} , \tilde{A} , and γ_2 were calculated as described elsewhere [2]. The calculated values of χ_{expt}^2 and λ_{expt} were compared with the 5% points of χ^2 and λ distributions at the number of degrees of freedom $f = j - Z$ (j is the number of concentrations, and Z is the number of parameters of the distribution function). The hypothesis on the correspondence of an empirical distribution to a particular type of distribution was

accepted if $\chi_{\text{expt}}^2 < \chi_{P,f}^2$ and $\lambda_{\text{expt}} < \lambda_{P,f}$ and the sample estimates \tilde{A} and γ_2 are close to zero. If these conditions were met for several distribution types, a distribution with the lowest values of all of the calculated tests was preferred. Upon choosing the type of distribution, the first estimate of the limit of detection $c_{\min,1}$ was calculated at the confidence probability $P(c) = 0.95$.

(5) To obtain the second estimate of the limit of detection, the results of the two $\overline{P(c_k)}$ samples were combined, which were previously compared in terms of F - and t -tests. If the samples were found inhomogeneous, the experiment was supplemented to reach the possibility of combining. From the combined sample of about 100 elements, the next estimate of the limit of detection $c_{\min,\text{comb}}$ was obtained. If the difference of $c_{\min,\text{comb}}$ from the previous estimate $c_{\min,1}$ was no greater than $\pm 3s_v$, it was taken as the final limit of detection.

(6) At a significant difference between the first and second estimates ($c_{\min,\text{comb}}$ and $c_{\min,1}$), the experiment was supplemented to obtain an additional sample of $\overline{P(c_k)}$ values, which was added to that combined after testing in terms of F - and t -values. The new combined sample of 150 results was used for the next approximation in the estimation of c_{\min} . The observations were supplemented until the difference between two sequential estimates c_{\min} became smaller than $\pm 3s_v$.

RESULTS AND DISCUSSION

Region of an unreliable response. The widths of these regions for various test systems were considerably different (Table 2). In accordance with the commonly accepted procedure, the boundaries of this region can be experimentally evaluated to within the step Δ_c . Of course, test systems with narrow regions of an unreliable response are more convenient for practical applications. Komar' [14] noted that the halfwidth of the region is related to the stability of the results: the smaller this halfwidth, the higher the stability of the given process to all external actions and the better the reaction in an analytical sense. The quantitative description of the region in the units of r (probable deviation) is based on the hypothesis that observations are consistent with a normal distribution [14]. We calculated the values of r for systems 2, 6, 8, and 12 as described by Komar' [14]. The range widths $\pm 3r$ were 6×10^{-4} mg/L, 0.40 mg/L, 0.011 mg/L, and 4.8 μM , respectively. The resulting estimated values are very close to the widths of the experimental regions of an unreliable response (Table 2); the differences do not exceed the step Δ_c . Hence, it follows that the quality of a test system can be characterized by the experimentally found width of the region without calculating normal distribution parameters.

² Another theoretical distribution, the Poisson distribution function, was used in previous publications [1, 2]. Because this function described none of the empirical distributions of frequencies, it was excluded from the list of conceivable distribution functions.

Table 2. Characteristics of test systems

Sys-tem	Analyte/reagent	Support	Color change*	Region of an unreliable response, $c \times 10^2$, mg/L	Relative width of the region of an unreliable response	Δ_c , 10^2 mg/L	Number of concentration levels	c_{\min} , 10^2 mg/L
1	Co ²⁺ /nitrosonaphthol	Solution	Orange to lilac	4.0–14.5	2.6	1.5	8	14
2	ΣM/RIB-Metal-Test-II	Paper	Light yellow to brownish violet	0.37–0.43	0.16	0.01	7	0.44
3	Fe ²⁺ /RIB-Iron(II)-Test		Light yellow to blue	1.25–3.25	1.6	0.25	9	3.4
4	Fe ³⁺ /RIB-Iron(III)-Test		Light yellow to violet	3.0–5.0	0.67	0.2	10	5.3
5	Cd ²⁺ /RIB-Cadmium-Test		Light orange to reddish brown	0.17–0.31	0.82	0.01	10	0.32
6	NO ₂ ⁻ /RIB-RIB-Nitrite-Test		White to purple	5–50	9.0	5	10	50
7	Co ²⁺ /nitrosonaphthol	Polyurethane foam	Orange to lilac	4.30–5.30	0.23	0.06	7	5.8
8	Co ²⁺ /SCN ⁻		White to light blue	2.0–3.2	0.60	0.2	7	3.1
9	NO ₂ ⁻		White to yellow	2–7	2.5	1	7	7.8
10	2,4-D/butylrhodamine		Light pink to bright pink	2–19	8.5	2	10	18
11	Co ²⁺ /nitrosonaphthol	Methylsilicic acid	Orange to lilac	9–50	4.5	3	13	60
12	ΣM/PAR	Gelatin	Yellow to red	$(2.0–6.5) \times 10^{-6}$ mol/L	2.3	0.5×10^{-6} mol/L	10	6.1×10^{-6} mol/L

However, to compare different test systems, primarily, under the conditions of evaluating their performance characteristics, it seems reasonable to characterize the width of the region of an unreliable response by relative rather than absolute values. This approach is consistent with the current practice of constructing color scales for test determinations, in which analyte concentrations are changed in a geometric rather than arithmetical progression. As a relative width of the region of an unreliable response, we took a ratio of the difference between the upper and lower boundaries to the lower concentration boundary of the region of an unreliable response. Table 2 summarizes the calculated values. The narrowest regions of an unreliable response characterize test systems 2 and 7, whereas the widest regions characterize systems 6 and 10. Of the two test systems 6 and 9, system 9 was found to be more stable for the detection of NO₂⁻. With the use of RIB-Metal-Test II indicator papers, a narrowing of the region of an unreliable response was found in a mode with preconcentration; the relative width of the region of an unreli-

able response decreased from 0.9 [2] to 0.16. The dependence of the relative width of the region of an unreliable response on the character of color changes was not found within the limits of available experimental data.

Type of the distribution function of detection frequencies in the region of an unreliable response. The possibility of describing an experimental distribution of $P(c)$ by a particular distribution function depends on the number of observations and the width of the region of an unreliable response. In test systems with a narrow region of an unreliable response (relative widths of 0.16 and 0.23), we found that the experimental distribution of $P(c)$ even at 50 observations was consistent with either of the hypotheses on the type of distribution (Table 3, systems 2 and 7). The accepted hypotheses were not rejected as the number of observations in these systems was increased. Polyurethane foam was found the only support material for which the type of distribution can be concluded in all cases based on even 50 observations.

Table 3. Types of distribution functions that adequately describe experimental data

System	Analyte/reagent	Support	Total number of observations (<i>N</i>)					Sufficient number of observations	Chosen distribution function
			50	100	150	200	250		
1	Co ²⁺ /nitrosonaphthol	Solution	None	None	All	All	–	150–200	Weibull
2	ΣM/RIB-Metal-Test-II	Paper	All	All	The same	All	All	50–100	Weibull
3	Fe ²⁺ /RIB-Iron(II)-Test		None	None	Normal, exponential	Normal, exponential	–	150–200	Normal
4	Fe ³⁺ /RIB-Iron(III)-Test		Lognormal, exponential	Weibull, lognormal, exponential	All	–	–	100–150	Exponential
5	Cd ²⁺ /RIB-Cadmium-Test	Polyurethane foam	None	all	The same	–	–	100–150	Normal
6	NO ₂ [–] /RIB-RIB-Nitrite-Test		Normal	Weibull, normal	"	–	–	100–150	Normal
7	Co ²⁺ /nitrosonaphthol		All	the same	"	All	All	50–100	Exponential
8	Co ²⁺ /SCN [–]		Weibull, lognormal, normal	"	All other than exponential	All other than exponential	–	50–100	Normal
9	NO ₂ [–]		All	"	All	All	All	100–150	Normal
10	2,4-D/butylrhodamine	Methyl-silicic acid	Weibull, lognormal, normal	"	The same	–	–	100–150	Weibull
11	Co ²⁺ /nitrosonaphthol		None	none	Weibull, lognormal, normal	Weibull, lognormal	Weibull, normal	150–200	Weibull
12	ΣM/PAR		The same	normal	Weibull	Weibull, normal	–	100–150	Weibull

Note that differences in experimental distributions for various test systems correlate with the relative width of the region of an unreliable response. This can be clearly seen in a comparison between test systems 3 and 4. The relative widths were different (1.6 and 0.67) at the same absolute width (0.02 mg/L) of the region of an unreliable response. The characters of experimental data distribution were also found to be different: the type of distribution for system 3 with a greater relative width of the region of an unreliable response was found only from 150 observations, whereas even 50 observations were found to suffice for system 4.

The following behavior was observed for the other test systems: the greater the sample size, the smaller the number of hypotheses on the type of distribution to be accepted (Table 3). The compliance with none of the tested types of theoretical distribution primarily manifests itself in overestimated λ values as compared with

the 5% point of theoretical distribution. For great samples, hypotheses on exponential (systems 8, 11, and 12) and lognormal (systems 3 and 12) distributions were unsupported more frequently than the others were.

Tables 2 and 3 summarize the selected types of distribution and the evaluated limits of detection. The evaluated limits of detection were similar when experimental data could be described by several distribution functions (Table 4).

Pantaler and coauthors [3, 11] gave preference to a particular distribution based on small samples (20–70 observations); this was likely due to the fact that the graphical methods used revealed only crude differences between experimental and theoretical distributions. At the same time, conclusions on the type of distribution drawn by eye from graphical depictions at a great number of observations [12, 13] were supported when data

Table 4. Results of tests for consistency of the empirical distribution of frequencies of cobalt detection by the reaction with KSCN on polyurethane foam in the region of an unreliable response with theoretical distributions (values higher than the theoretical values of $\chi^2_{f=5}$ ($\alpha = 5\%$) = 11.1 and $\lambda_{f=5}$ ($\alpha = 5\%$) = 0.56 and the selected type of distribution function are boldfaced)

Theoretical distribution	Characteristic	Total number of observations (<i>N</i>)			
		50	110	160	220
Normal	χ^2_{expt}	5.48	0.49	0.32	0.54
	λ_{expt}	0.42	0.14	0.20	0.26
	\tilde{A}	-0.72	-1.01	-0.41	-0.27
	γ_2	-1.21	-1.01	-1.81	-1.46
	c_{min} , mg/L	0.030	0.028	0.031	0.031
Lognormal	χ^2_{expt}	5.45	0.71	0.55	0.80
	λ_{expt}	0.45	0.22	0.27	0.33
	\tilde{A}	-0.43	-0.48	-0.32	-0.50
	γ_2	-1.09	-0.82	-1.78	-1.49
	c_{min} , mg/L	0.029	0.030	0.031	0.030
Weibull	χ^2_{expt}	5.59	0.59	0.46	0.70
	λ_{expt}	0.42	0.21	0.24	0.30
	\tilde{A}	-0.77	-0.71	-0.36	-0.43
	γ_2	-1.31	-0.48	-1.83	-1.51
	c_{min} , mg/L	0.029	0.030	0.030	0.030
Exponential	χ^2_{expt}	6.70	2.06	2.98	3.26
	λ_{expt}	0.66	0.49	0.57	0.61
	\tilde{A}	-0.48	-0.07	-0.21	-0.52
	γ_2	-1.22	0.08	-1.19	-1.13
	c_{min} , mg/L	0.031	0.030	0.030	0.031

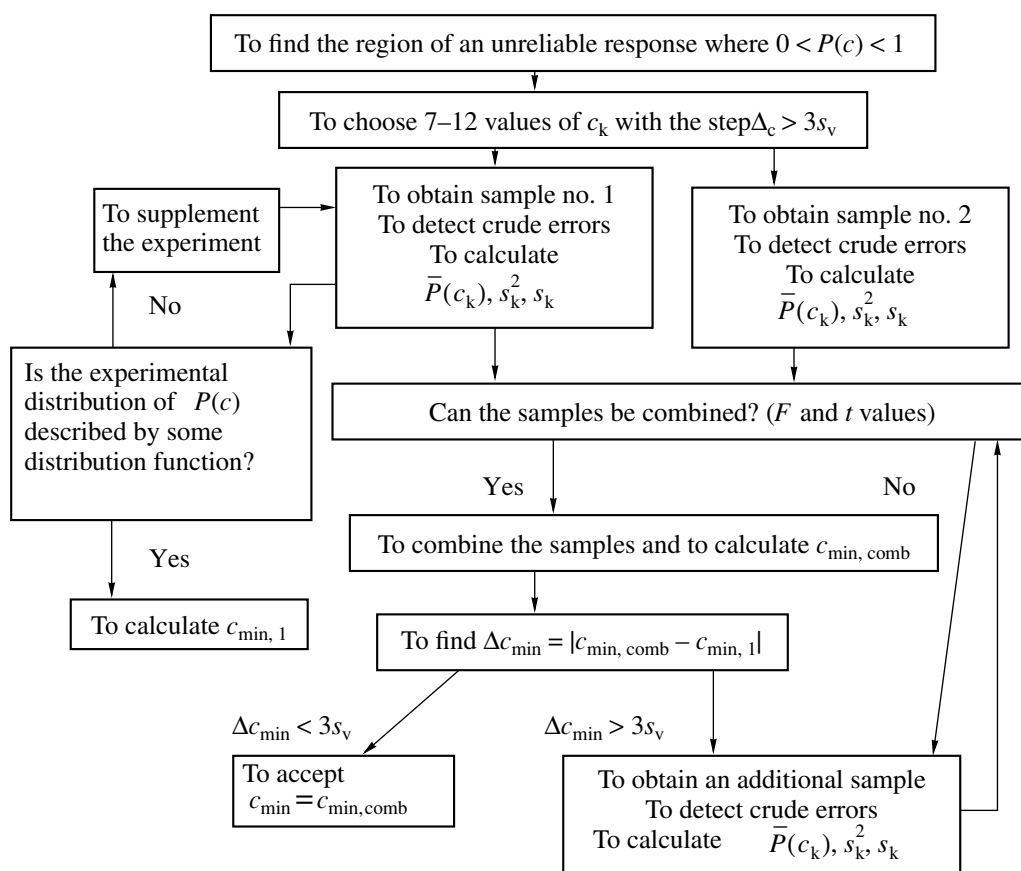
were treated in accordance with the approach used in this work.

The number of observations required for the evaluation of performance characteristics. From 250 to 300 observations were obtained at each concentration level (to 16 series of observations) for half of the test systems; 140–160 observations were performed for each of the other test systems. As can be seen in the above discussion, the experiment should be supplemented primarily because of the fact that we failed to determine the type of distribution in a small sample. In this context, we supplemented the previously developed algorithm. A schematic diagram of the algorithm is given below with consideration for supplements (figure). The second reason for supplementing the experiment remained the difference in evaluated limits of detection. Thus, for RIB-Iron(III)-Test, an exponential distribution was concluded based on even 50 observations; however, the difference between the first and sec-

ond estimates of the detection limit of Fe(III) was greater than $3s_v = 0.002$ mg/L (Table 5). The third estimate (at $N = 150$) differed insignificantly from the second. It was taken as the ultimate result.

For test systems with a narrow region of an unreliable response (relative widths of 0.16 and 0.23), the estimate of c_{min} obtained from 50 observations was equal to the final estimate within the limits of error.

Dependence of the limit of detection on the material of the support. This dependence was studied using the detection of cobalt(II) with 2-nitroso-1-naphthol as an example. The limits of detection decreased in the order methylsilicic acid > reagent paper [3] > solution > polyurethane foam and were equal to 0.60, 0.44 [3], 0.14, and 0.058 mg/L, respectively. Thus, polyurethane foam should be considered optimal material for the test detection of cobalt using the above test reaction. This



Schematic diagram of the algorithm for evaluation the detection limits of procedures for test analysis with visual indication.

test system is also characterized by the narrowest relative width of the region of an unreliable response.

Dependence of the limit of detection on the type of reaction. This dependence manifested itself in the cases of both reagent papers and polyurethane foam. The effect of this factor cannot be reduced to the stability of the resulting compound. Thus, 2-nitroso-1-naphthol, which is a more sensitive reagent for cobalt in

spectrophotometry [15, transl., p. 566], is second to thiocyanate under conditions of visual test detection: the detection limit of cobalt with thiocyanate is almost two times lower (Table 2, test systems 7 and 8).

It is likely that the type of reaction affects the type of $P(c)$ distribution to a greater extent than the type of support material. Thus, the frequencies of detection of cobalt(II) with 2-nitroso-1-naphthol in solution and on methylsilicic acid were described by the same Weibull distribution function, and those on polyurethane foams were described by an exponential distribution function, which is a particular case of the Weibull distribution.

The specificity of a chemisorption reaction (test system 9) was also reflected on the performance characteristics of the system. Although the relative width of the region of an unreliable response was great, the correspondence of the experiment with theoretical distributions was revealed even in small samples. The first estimate of the limit of detection almost does not require further refinements. It is likely that these special features are due to the absence of the step of supporting reagents (support modification) and related errors.

Table 5. Adequacy of the experiment size for evaluating the detection limit of iron(III) with the use of RIB-Iron(III)-Test ($3s_v = 0.002$ mg/L; $\chi^2_{f=9}$ ($\alpha = 5\%$) = 16.9; $\lambda_{f=9}$ ($\alpha = 5\%$) = 0.43). Theoretical distribution: exponential

Characteristic	Total number of observations (N)		
	50	100	150
χ^2_{expt}	1.76	1.87	0.55
λ_{expt}	0.24	0.27	0.16
\tilde{A}	0.37	1.06	0.55
γ_2	-0.55	1.66	-0.08
c_{min} , mg/L	0.050	0.054	0.053

ACKNOWLEDGMENTS

We are grateful to R.P. Pantaler for providing us with chemicals required for this study.

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