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ARTICLES

Solid-Phase Reagent for Analgin and Ascorbic Acid on the Basis of a Copper(II) Complex with Tetrabenzotetraazacyclohexadecine Immobilized by Adsorption on Silica Gel

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Abstract—The reactions of a number of natural organic reducing agents with a copper(II) complex with tetrabenzo[*b, f, j, n*][1, 5, 9, 13]tetraazacyclohexadecine adsorbed on silica were examined. A solid-phase reagent was proposed for the sorption–spectrophotometric determination of Analgin and ascorbic acid (c_{min} = 0.91 and 0.06 mg/L, respectively) and for the visual test determination of the above compounds (c_{min} = 0.50 and 0.025 mg/sample, respectively).

Analgin and ascorbic acid are widely used in medical practice. As an analgesic, antipyretic, and anti-inflammatory agent, Analgin is a constituent of many combined pharmaceuticals. Ascorbic acid is a constituent of polyvitamins, other pharmaceuticals, beverages, vegetables, and fruits; it is frequently used as a food antioxidant [1]. Thus, the development of simple and rapid procedures for the monitoring of Analgin and ascorbic acid, in particular, in their medicinal forms, is a topical problem.

Analgin and ascorbic acid exhibit reducing properties [2]. Therefore, many procedures for the photometric determination of Analgin and ascorbic acid are based on their oxidation with various reagents, in particular, Folin's reagent (a mixture of molybdophosphoric and uranophosphoric acids) (c_{min} = 38.4 and 5.0 μg in 0.05 L, respectively), chloramine (Analgin), and 2,6-dichloroindophenol (c_{min} = 50 μg in 0.05 L) (ascorbic acid) [3, 4]. The chemiluminescence determination of Analgin (c_{min} = 0.8 μg/L) is based on the prereduction of vanadate with Analgin followed by the determination of vanadium(IV) by the reaction of 4-diethylaminophthalhydrazide oxidation [5]. These procedures are characterized by low detection limits. However, they are time-consuming and labor-intensive procedures and require the use of toxic organic solvents in a number of cases [2–4].

It is well known [6, 7] that the use of immobilized reagents, in particular, for determining organic compounds, makes it possible not only to improve the sensitivity, selectivity, and rapidity of analysis but also to reduce its labor intensiveness. These procedures are primarily based on complexation, ion association, or ion exchange reactions. Redox reactions at the surfaces of modified sorbents have been developed to a smaller degree.

Thus, a procedure was proposed for the sorption–photometric determination of ascorbic acid with the use of heteropoly acids immobilized on foamed polyurethane (c_{min} = 0.1 mg/L) [8]. The duration of a single determination is 1.5 h. However, the solid-phase reagent is unstable; its spectral characteristics remained unchanged for a time shorter than two weeks on keeping away from light. An indicator tube was also developed for determining ascorbic acid [9]; it is based on the reduction of ion associates of thiazine dyes and triodide ions. The analytical range is 0.9–14 mg/L. Indicator tubes and indicator powders with the use of noncovalently immobilized quinone imine indicators were also proposed for the determination of ascorbic acid (0.3–1000 mg/L) [10].

Tetrabenzo[*b, f, j, n*][1, 5, 9, 13]tetraazacyclodecinecopper(II) nitrate ($Cu(TAAB)(NO_3)_2$) adsorbed on silica gel (R-SG) is one of the most sensitive solid-phase reagents for the semiquantitative visual determination of ascorbic acid (5 mg/sample; V = 0.10 L) [11]. Its other advantages are its stability in time, ease of immobilizing at the surface of silica gel by adsorption from aqueous solutions, good reversibility, and the possibility of regeneration [12–14].

We found no published data concerning the application of R-SG to the sorption–spectrophotometric determination or visual test determination of organic reducing agents, in particular, ascorbic acid and Analgin.

In this study, we examined the reactions of Analgin and ascorbic acid with R-SG in order to develop sensitive and rapid procedures for the sorption–photometric

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determination and visual test determination of these substances.

EXPERIMENTAL

Reagents. Silica gel L 100/250 (SG) from Chemapol (Czech Republic) was washed with HCl and then with distilled water, filtered off, and dried at 80°C for 8 h.

The solutions of Analgin and ascorbic acid of chemically pure grade were prepared by dissolving accurately weighed portions of the substances in freshly boiled (for the removal of dissolved oxygen) and cooled distilled water. $Cu(TAAB)(NO_3)_2$ was synthesized according to a published procedure [15]. Carbonate buffer solutions of $NaHCO_3$–Na_2CO_3 were used for adjusting a constant pH value (7.5–12) [14].

The silica gel was modified as follows: 100-mL portions of aqueous $Cu(TAAB)(NO_3)_2$ solutions (concentrations, $n \times 10^4$ M: 0.45, 4.5, and 7.5) were placed in 250-mL Erlenmeyer flasks with 15 g of silica gel, and the contents were stirred for 1 h. Next, the sorbent was filtered off and dried in air. The sorbent capacity ($n \times 10^6$ mol/g) was 0.3, 3.0, or 5.0.

Instrumentation. Absorption spectra in the visible region were recorded on SF-26, Perkin-Elmer Lambda 40 UV/VIS Spectrometer, and Specord M40 instruments. The acidity of solutions was measured on an EV-74 multipurpose potentiometer using a glass electrode.

Procedures. The spectral characteristics of the solid-phase reagent were examined in accordance with recommendations [16]. For this purpose, the suspensions of modified and unmodified sorbents were transferred from solutions to cuvettes 0.1 cm in thickness with the use of a pipette. To attain equal degrees of compaction of the sorbents in the cuvettes, the contents of both cuvettes were stirred simultaneously. The light absorption of a suspension of R-SG was measured with reference to a suspension of silica gel 5 min after stirring. The effect of the inhomogeneity of sorbents on the measured signal was taken into account using the method of two wavelengths [17]. In this case, $\Delta A = A_{660} - A_{950}$, where A_{660} and A_{950} are the absorbances of suspensions at 660 and 950 nm (background level), respectively, was taken as a measure of light absorption by the system.

The degree of reduction of R-SG was calculated as $\alpha = \Delta A/\Delta A_{max} \times 100\%$, where ΔA_{max} is the value of ΔA at the complete reduction of the solid-phase reagent.

The kinetics of protolytic equilibrium in the silica gel (R-SG)–aqueous reductant solution system was studied at a pH value optimum for the reduction of the immobilized complex. For this purpose, a weighed portion of the sorbent was added to a 6 mM solution of a reductant at pH 10.1 ± 0.1 in the absence or in the presence of a buffer solution, and the mixture was stirred with a magnetic stirrer until equilibrium was attained; the pH value was measured at regular intervals of 30 s.

The pH dependence of the degree of reduction of the immobilized complex with Analgin and ascorbic acid was studied as follows: 10 mL of a 1.2×10^{-2} M Analgin or 6×10^{-3} M ascorbic acid solution was stirred with 0.1 g of R-SG ($a = 3 \times 10^{-6}$ mol/g) until equilibrium was attained (2–5 min). The required pH values were adjusted by the addition of 0.1 M H_2SO_4, $NaHCO_3$, and Na_2CO_3 solutions in different proportions. The sorbent suspension was transferred to a cuvette, and photometric measurements were performed at 660 and 950 nm 5 min after the termination of stirring.

RESULTS AND DISCUSSION

The determination of ascorbic acid with the use of R-SG is based on the following redox reaction, which takes place at pH 9–11 [13]:

$$CuTAAB^{2+} + \bar{e} \rightleftharpoons CuTAAB^{+}$$

yellow ($\lambda'_{max} = 268$, $\lambda''_{max} = 298$ nm) blue ($\lambda = 660$ nm).

We experimentally found that the reaction of the immobilized complex with an ascorbic acid solution under these conditions is also accompanied by a color change of the reagent. Figure 1 demonstrates the absorption spectra of the solid-phase reagent before and after reactions with Analgin and ascorbic acid. It can be seen that the light absorption of the modified silica gel at 660 nm increases with the concentration of the reductant in solution. To optimize the conditions for determining Analgin and ascorbic acid, we examined the reduction of R-SG depending on the pH of the solution, the capacity of the sorbent, the sample volume, and the reductant concentration in the solution.

It is well known that the redox potential of the test system essentially depends on the pH of solution [16]. We experimentally found that the addition of silica gel or R-SG to a solution with pH 9–11 results in an increase in the acidity of the medium. This is likely due to the sorption of an alkali by silica [18]. Protolytic equilibrium in this system is attained in 1–10 min. Because Analgin and ascorbic acid can be partially oxidized with atmospheric oxygen during this time interval, it was necessary to minimize the equilibration time. Figure 2 indicates that a decrease in the pH of solution upon adding a sorbent was observed in all of the systems: silica gel–H_2O (curve *1*), silica gel–reductant

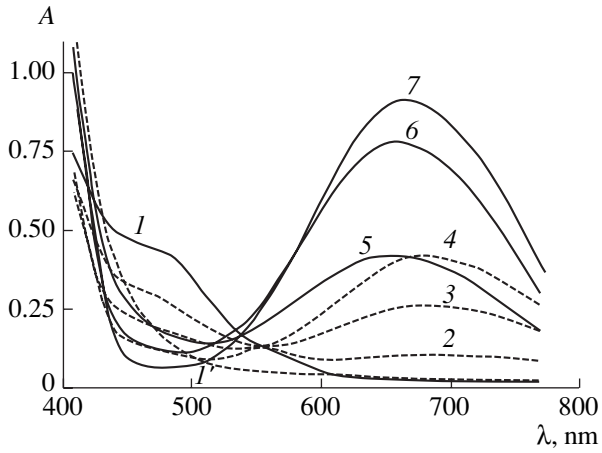

Fig. 1. Absorption spectra of a suspension of R-SG (*1*, *1'*) before and after treatment with (*2–4*) Analgin and (*5–7*) ascorbic acid solutions. Concentrations ($n \times 10^4$, M): (*2*) 1.6, (*3*) 12.6, (*4*) 120, (*5*) 1.14, (*6*) 2.27, and (*7*) 5.68. pH: (*1–4*) 10.5 and (*1', 5–7*) 9.3. m = 0.1 g; V = 10 mL.

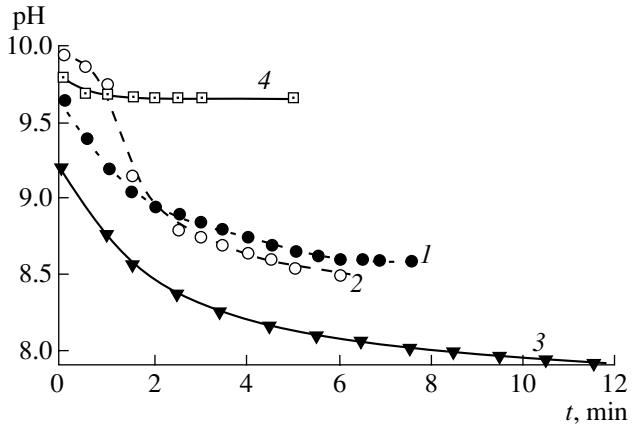

Fig. 2. Kinetic curves of pH changes on the addition of a sorbent in the systems: (*1*) silica gel–H_2O, (*2*) silica gel–ascorbic acid solution, and R-SG–ascorbic acid solution (*3*) in the absence and (*4*) in the presence of a carbonate buffer solution. All solutions had pH 10.6 before the addition of the sorbent. The ascorbic acid concentration was 6×10^{-3} M; m = 0.1 g; V = 10 mL; T = 293 K.

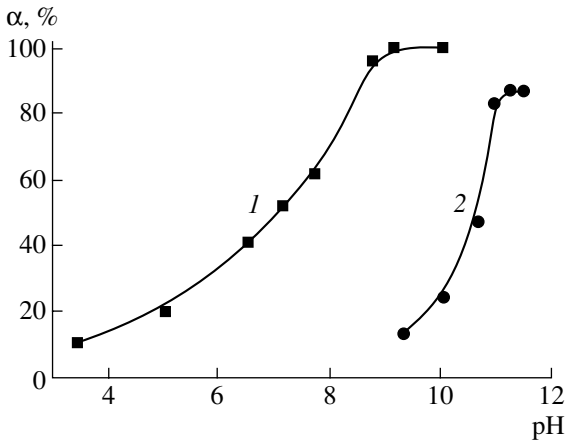

Fig. 3. Degrees of reduction of R-SG (3×10^{-6} mol/g) with (*1*) a 6×10^{-3} M ascorbic acid solution and (*2*) a 1.2×10^{-2} M Analgin solution as functions of pH in solution. m = 0.1 g; V = 10 mL; $a = 3 \times 10^{-6}$ mol/g.

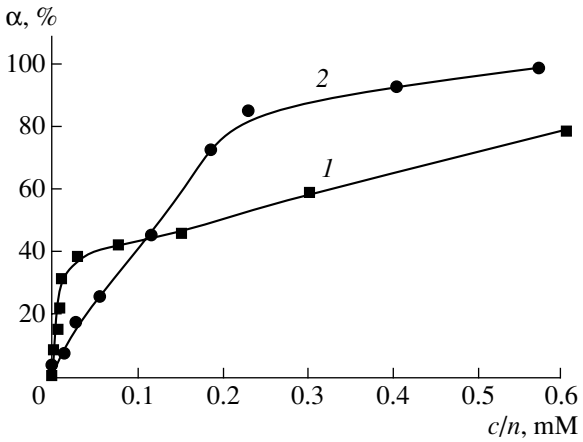

Fig. 4. Degrees of reduction of R-SG as functions of (*1*) Analgin and (*2*) ascorbic acid concentrations in solution. n = 1 (ascorbic acid) or 20 (Analgin). pH: (*1*) 10.5 and (*2*) 9.3. m = 0.1 g; V = 10 mL; $a = 5 \times 10^{-6}$ mol/g.

solution (curve *2*), and R-SG–reductant solution (curve *3*). The time taken to attain protolytic equilibrium in the silica gel (R-SG)–aqueous solution systems in the absence and in the presence of a reductant is almost equal and only depends on the ratio between the sorbent weight and the solution volume. The use of a buffer solution makes it possible to shorten this time to 1 or 2 min (Fig. 2, curve *4*). The subsequent experiments in neutral and alkaline regions were performed in carbonate buffer solutions with pH 7–12.

Figure 3 shows the pH dependence of the degree of reduction of R-SG in the presence of reductants. It can be seen that a range of pH 9–10 or pH ≥ 11 is optimum for the determination of ascorbic acid or Analgin, respectively. Thus, Analgin causes no interference with the determination of ascorbic acid at pH ≤ 9.

A study of α as a function of the surface concentration of the complex on silica gel demonstrated that a sorbent with the capacity $a \geq 5 \times 10^{-6}$ mol/g is optimum for the determination of both Analgin and ascorbic acid. Under optimum conditions, the value of α is independent of solution volume at $V \leq 50$ mL.

The degrees of reduction of the immobilized complex as functions of Analgin and ascorbic acid concentrations in solution (Fig. 4) can be linearly approximated over the concentration ranges 0.010–0.200 and

Table 1. Equations of calibration curves on the ΔA (chromaticity function)–concentration coordinates for (I) Analgin and (II) ascorbic acid (concentration ranges, mg/L: (I) 0.9–70 (ΔA), 5.0–40 (chromaticity functions); (II) 0.06–40.5 (ΔA), 0.5–4.0 (chromaticity functions); $n = 3$; $P = 0.95$)

Parameter	I		II	
	$a + bc$	r	$a + bc$	r
ΔA	$(1.643 \pm 0.006) \times 10^{-2}c$	0.998	$(0.029 \pm 0.002) + (1.875 \pm 0.001) \times 10^{-2}c$	0.998
ΔE	$(1.11 \pm 0.6) + (0.71 \pm 0.02)c$	0.995	$(14.4 \pm 0.3)c$	0.998
ΔL	$(1.6 \pm 0.3) - (0.37 \pm 0.01)c$	0.994	$(1.1 \pm 0.1) + (6.37 \pm 0.06)c$	0.998

0.5–12 mM (Analgin) or 0.01–0.23 mM (ascorbic acid). The calibration functions are given in Table 1. The detection limits of Analgin and ascorbic acid are equal to 0.9 mg/L (45.5 μg/sample) and 0.06 mg/L (3 μg/sample), respectively, at a sample volume of 50 mL.

A study of the interference from organic reducing agents demonstrated that amino acids (glycine, aspartic acid, and glutamic acid) and saccharides do not interfere with the determination of Analgin and ascorbic acid. Quercetin, pyrocatechol, hydroquinone, and tannin in commensurable concentrations interfere with the determination. Thus, R-SG is more selective for organic reducing agents than well-known Tollens' and Nessler's reagents and 1,2-dinitrobenzene [2, 4].

Standard color scales were developed for the visual semiquantitative determination of Analgin and ascorbic acid in solution. The colorimetry method [19, 20] was used for substantiating the linearity ranges of the scales and for taking into account the human factor of vision in the certification of test procedures under laboratory conditions. Chromatic characteristics were calculated in the equal-contrast colorimetric CIE system (1976), which is based on the use of the chromaticity coordinates *L*, *a*, and *b*. The calculations were performed with reference to a standard light source C (natural lighting).

To prepare a scale, a series of solutions of volume 10 mL containing different amounts of Analgin or ascorbic acid with pH 10.5 or 9.3, respectively, were stirred with 0.1-g portions of R-SG ($a = 5 \times 10^{-6}$ mol/g) for 5 min. The absorption spectra of the sorbents were measured as described above. Figure 5 shows ΔE (curves *1*) and ΔL (curves *2*) as functions of reductant concentration. It can be seen that ΔE and ΔL are linear functions up to 40 and 4 mg/L for Analgin and ascorbic acid, respectively (at a maximum sample volume of 50 mL). The equations of calibration curves in these coordinates are given in Table 1. Based on these results, standard color scales were developed with the use of the CorelDraw 7 program. Analgin or ascorbic acid can be developed by the visual test method using these scales in the concentration range 0.1, 0.8, 1.5, and 2.2 or 0.025, 0.06, 0.10, 0.14, 0.18, and 0.20 mg/sample with RSD ≤ 30%.

Standard solutions of Analgin and ascorbic acid were analyzed for evaluating the performance characteristics of the developed procedures. Table 2 summarizes the results. It can be seen that the sorption–spectrophotometry and test procedures are characterized by satisfactory precision and accuracy.

Determination of Analgin in a 50% solution for injections. An aliquot portion of the solution (0.05 mL) was placed in a 50-mL volumetric flask and diluted with distilled water to the mark. An aliquot portion of the resulting solution (0.9 mL) and 8.0 mL of a buffer solution with pH 10.5 were placed in a 20-mL beaker, and distilled water was added to a total volume of 10 mL; the contents were stirred with 0.100 ± 0.001 g of the solid-phase reagent with a capacity of 5×10^{-6} mol/g for 5 min. The suspension was transferred to a cuvette 0.1 cm in thickness, and the absorbance at 660 and 950 nm was measured or the absorption spectrum was recorded; the chromaticity functions ΔE and ΔL were calculated.

The concentration of Analgin in a sample was calculated by the equations of calibration curves on the ΔA–*c*

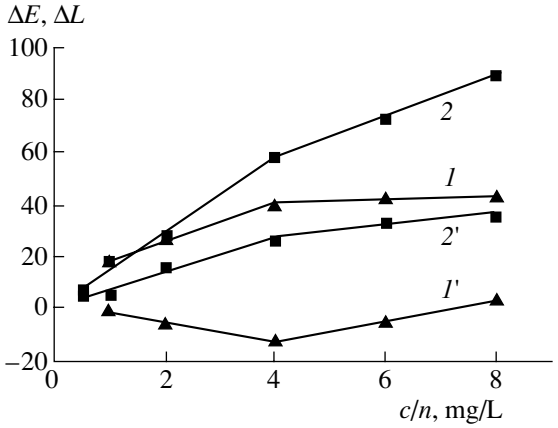

Fig. 5. (*1*, *1*') Total color difference ΔE and (*2*, *2*') color difference in brightness ΔL as functions of (*1*, *2*) Analgin or (*1*', *2*') ascorbic acid concentration in solution. $n = 1$ (ascorbic acid) or 10 (Analgin). pH: (*1*, *2*) 10.5 or (*1*', *2*') 9.3. $m = 0.1$ g; $V = 10$ mL; $a = 5 \times 10^{-6}$ mol/g.

Table 2. Determination of Analgin and ascorbic acid by the (I) sorption–spectrophotometry and (II) test methods. Sample: (1–3) 10 mL, (4) 0.9 mL, (5) 1.0795 mg, or (6) 1.8640 mg (n = 3; P = 0.95)

No.	Test material	Analgin content, mg/sample			Ascorbic acid content, mg/sample		
		added	found		added	found	
			I	II		I	II
1	Standard solution	0.218	0.218 ± 0.005	0.22 ± 0.03	0.060	0.060 ± 0.006	0.06 ± 0.02
2	″	0.498	0.498 ± 0.009	0.50 ± 0.03	0.100	0.100 ± 0.001	0.10 ± 0.02
3	″				0.140	0.14 ± 0.02	0.14 ± 0.02
4	Analgin solution for injections in ampules*	0.479 ± 0.002**	0.48 ± 0.01	0.46 ± 0.06	–	–	–
5	Ascorbic Acid (0.1 g) with Glucose, tablets	–	–	–	0.089 ± 0.001**	0.08 ± 0.01	0.08 ± 0.01
6	Aspirin UPSA, tablets***	–	–	–	0.112 ± 0.001**	0.11 ± 0.02	0.12 ± 0.02

* The preparation was diluted by a factor of 1000.

** Found by the standard method.

*** A 1.8640-g portion of the preparation (one tablet) contained ascorbic acid (0.200 g), acetylsalicylic acid (0.330 g), potassium bicarbonate, and potassium benzoate (manufacturer's data).

and $\Delta E(\Delta L)$–c coordinates (Table 1). Table 2 indicates that the results obtained using the developed determination procedure and the standard method [1] are consistent.

Determination of ascorbic acid in the preparations Ascorbic Acid (0.1 g) with Glucose (Darnitsa, Ukraine) and Aspirin UPSA (France). A weighed portion (0.0108 or 0.0186 g, respectively) of powder obtained by crushing ten tablets was dissolved in 25 mL of distilled water. If necessary, the solution was filtered through a blue ribbon paper filter for removing excipients. An aliquot portion of 2.5 mL and 7.0 mL of a buffer solution with pH 9.3 were placed in a 20-mL beaker, and distilled water was added to a total volume of 10 mL; the contents were stirred with 0.100 ± 0.001 g of R-SG ($a = 5 \times 10^{-6}$ mol/g) for 5 min. Next, the suspension was transferred to a cuvette 0.1 cm in thickness, and measurements were performed as described above.

The concentration of ascorbic acid in a sample was calculated by the equations of calibration curves on the ΔA–c and $\Delta E(\Delta L)$–c coordinates (Table 1). Table 2 indicates that the developed procedures are characterized by satisfactory accuracy and precision.

Thus, the proposed sorption–spectrophotometric determination procedures are highly competitive with well-known hybrid procedures [2–4, 8–10] in sensitivity. However, they are more rapid and do not require the use of organic solvents or extractants. The procedures can be recommended for the spectrophotometric and visual-test quality control of preparations with Analgin or ascorbic acid concentrations ≥0.91 or ≥0.06 mg/L, respectively.

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