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REVIEW

Determination of Ascorbic Acid by Molecular Spectroscopic Techniques

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Abstract—The determination of ascorbic acid by spectrophotometry, chemiluminescence and fluorescence techniques, sorption–spectroscopy, and visual-test methods is considered. Data on the reagents in use, analytical ranges, detection limits, and interfering substances are presented. Special attention is focused on solid-phase reagents for the spectroscopic and rapid visual-test determination of ascorbic acid in various samples. The bibliography includes 65 references.

The widespread use of ascorbic acid in medical practice and the food industry is responsible for a variety of determination methods for this substance. The importance of ascorbic acid (vitamin C) for the human immune system and for the prevention of various diseases is a matter of common knowledge. Thus, ascorbic acid is a constituent of multivitamin preparations, food additives, and pharmaceuticals; it is added as an antioxidant to foods, juices, and beverages.

Ascorbic acid exhibits reducing properties. Ascorbic acid is rapidly oxidized in aqueous solutions at pH > 6.0 in the presence of atmospheric oxygen. This process is catalyzed by Cu(II) and Fe(III) compounds [1]. Thus, the necessity of the quality control of preparations, in particular, drugs, containing ascorbic acid is beyond question.

Along with traditional titrimetric analysis [2], highly sensitive physicochemical techniques, such as spectroscopy, chromatography, and electroanalysis, were proposed for the quantitative determination of ascorbic acid. The aim of this work was to summarize the domestic and foreign experience in the determination of ascorbic acid by molecular spectroscopic techniques over the last 15 years. These techniques are preferable to analytical laboratories because of their high sensitivity and good reproducibility and the availability of instrumentation.

SPECTROPHOTOMETRIC METHODS

Spectrophotometric methods for the determination of ascorbic acid can be subdivided into the following two groups: methods based on measuring the intrinsic absorption of ascorbic acid and methods based on measuring the light absorption of products that result from the reduction of various regents by ascorbic acid.

Direct UV spectrophotometry belongs to the former group. Abdel-Hamid *et al.* [3] proposed a procedure for determining ascorbic acid and thiamine in the presence of their degradation products. They used the first-derivative spectral peaks at λ = 215 nm for ascorbic acid and 254 nm for thiamine hydrochloride. Calibration graphs were linear over concentration ranges of 1.5–3.0 mg/L for ascorbic acid and 0.9–2.4 mg/L for thiamine. Brodnjak-Voncina and Dobcnik [4] used absorption at 245 (ascorbic acid) or 253.1 nm (thiamine hydrochloride) as an analytical signal. Matrix effects were taken into account with the use of reference solutions prepared by boiling with a 1 M alkali solution (ascorbic acid) or a borate buffer solution with pH 10.0 (thiamine hydrochloride). Calibration graphs were linear over the following ranges, mg/L: 2–50 for ascorbic acid and 3–30 for thiamine hydrochloride.

The determination of ascorbic acid in nonalcoholic beverages, fruits, fruit juices, and drugs requires the preliminary removal of carbon dioxide and solids by degasification and centrifugation, respectively [5–7]. The determination sensitivity was improved by the successive addition of Cu(II) (for the catalytic oxidation of ascorbic acid) and a Trilon B solution [6, 7]. The calibration graph was linear up to an ascorbic acid concentration of 120 mg/L when the absorbance was measured at 267 nm.

A procedure for determining ascorbic acid in pharmaceuticals by flow-injection analysis (FIA) was proposed [8]. This procedure is based on the measurement of the intrinsic absorption of ascorbic acid at λ = 245 nm in an acidic antioxidant medium (a 2-mercaptoethanol solution in H_2SO_4). To prepare a reference solution, a sample was treated with NaOH and held at 298 K for 10 min for the complete degradation of the ascorbic acid by the dissolved oxygen. The ascorbic acid concentration in a sample was found by a calibration graph plotted on the ΔA_{245}–c_{Asc} coordinates, where ΔA_{245} is the difference between the absorbance of test solutions before and after treatment with NaOH and c_a is the concentration of ascorbic acid. The calibration graph was linear up to an ascorbic acid concentration of 20 mg/L.

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The productivity of analysis was up to 180 samples per hour.

As a rule, classical reduction procedures for the spectrophotometric determination of ascorbic acid with the use of aniline, 2,6-dichlorophenolindophenol, Folin's reagent, and hydroxylamine [9] include a time-consuming and labor-intensive stage of converting ascorbic acid into a chromophoric product. Moreover, these methods are characterized by a low selectivity. For example, the determination of ascorbic acid with Folin's reagent is based on the reduction of a mixture of molybdophosphoric and uranophosphoric acids to molybdenum blue. Other reducing agents (amidopyrine, phenol, tannin, reducing sugars, hydroquinone, pyrocatechol, and resorcinol) interfere with the determination.

Several improved procedures with the use of the above reagents, in particular, 2,6-dichlorophenolindophenol, were proposed [10–14]. The determination is based on the decolorization of the reagent on the reduction by ascorbic acid. The interference of Fe(II) and Fe(III) (<400 μmol) was removed by the addition of Trilon B [11]. The sulfhydryl groups of cysteine or glutathione were masked by the pretreatment of a sample with acrylonitrile in a phosphate buffer solution at pH 7.0 [12]. In the determination of ascorbic acid in canned food [13], the reagent was added in excess. Unreacted 2,6-dichlorophenolindophenol was extracted with an organic solvent (butyl acetate or amyl acetate), and the light absorption of the extract was measured at 540 nm. The method determined ascorbic acid in the range of 4–8 mg/L. A kinetic procedure was proposed for the analysis of orange juice, parsley, and potato samples [14]. The parsley and potato samples were homogenized with a solution of $H_2C_2O_4$. The resulting suspensions and orange juice were centrifuged, and a 4×10^{-5} M 2,6-dichlorophenolindophenol solution in 0.2 M $H_2C_2O_4$ was added to the supernatant liquids of all samples. The light absorption was measured at 522 nm. The analytical range of ascorbic acid was 3.5–88 mg/L.

A number of methods for the determination of ascorbic acid were proposed based on the reaction of Fe(III) reduction followed by the detection of the resulting Fe(II). Thus, Arya and Mahajan [15] described an extraction–spectrophotometry procedure based on the extraction of Fe(II) with chloroform as a complex with quinaldic acid and pyridine followed by the photometric measurement of the extract. The calibration graph was linear in the ascorbic acid concentration range of 2.5–25 mg/L. The procedure [16] is different in that the Fe(II) formed by the reaction with ascorbic acid was extracted with a trioctylamine solution in chloroform as a complex with picolinic acid and the absorption of the extract was measured at $\lambda = 470$ nm. Another version of this procedure [17] is based on the extraction of an Fe(II) complex with picolinic acid and pyridine. The light absorption of the yellow complex was measured at 400 nm. The calibration graph was linear in an ascorbic acid concentration range of 0.4–5.6 mg/L. The procedures [15–17] were applied to the analysis of pharmaceuticals, biological samples, and foods, and satisfactory results were obtained.

Nobrega and Lopes [18] used hexacyanoferrate(III) ($\lambda = 700$ nm; $\varepsilon = 3.0 \times 10^4$ L mol^{-1} cm^{-1}) for the detection of Fe(II) formed by the reaction with ascorbic acid. Under conditions of FIA, an alkaline oxalate solution was used for washing off a colored product adsorbed on the walls of a cell. Calibration graphs were linear for 5–100 μM ascorbic acid. The productivity was 140 determinations per hour, and the consumption of reagents was no higher than 0.5 mL per determination. Ferreira *et al.* [19] proposed the photometry of iron(II) as a complex with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol ($\varepsilon_{560} = 1.31 \times 10^5$ L mol^{-1} cm^{-1}; ($\varepsilon_{748} = 5.69 \times 10^4$ L mol^{-1} cm^{-1}). The developed method is highly sensitive: the detection limit is 0.015 (560 nm) or 0.044 mg/L (748 nm). Koch and Peisker [20] and Lau and Luk [21] determined the concentration of Fe(II) by the reaction with *o*-phenanthroline in a sodium acetate buffer solution in the presence of EDTA (for masking an excess of Fe(III)). Background correction was achieved by the pretreatment of a sample with a Cu(II) solution [21]. In the extraction–spectrophotometric determination of ascorbic acid [20], $NaClO_4$ was added to the reaction mixture; the resulting mixture was extracted with nitrobenzene for 30 s; the extract was separated from an aqueous phase and dried. The absorbance was measured at 515 nm with reference to nitrobenzene. The detection limit of ascorbic acid was 0.012 mg/L at a sample volume of 25 mL. The analytical range was 2–10 μg of ascorbic acid in a sample.

Yamane and Ogawa [22] described a procedure to determine the ascorbic acid in fresh fruit juices and fruit drinks by flow-injection analysis. The solutions of Fe(III) and *o*-phenanthroline were passed at flow rates of 0.55 and 0.25 mL/min, respectively, and 100 μL of the test solution was injected into the flow. The absorbance at 510 nm was measured in a continuous mode. The detection limit was equal to 0.04 mg/L; the calibration graph was rectilinear up to an ascorbic acid concentration of 1.8 mg/L. The productivity of the procedure was about 70 determinations per hour.

Besada [23] analyzed tablets and ampoule solutions containing vitamin C as described below. A solution containing *o*-phenanthroline, HCl, and $FeNH_4(SO_4)_2$ was added to a sample; the mixture was allowed to stand for 1 min and then diluted with distilled water to a total volume of 25 mL. The light absorption was measured at 510 nm. The calibration graph for ascorbic acid was linear in the range of 0.8–2.4 mg/L. Nicotinic acid; nicotinamide; thiourea; methionine; starch; glucose; fructose; mannose; sucrose; citric acid; and Ca^{2+}, Mg^{2+}, and Cu^{2+} caused no interference with the determination.

Skaltsa *et al.* [24] proposed to detect iron(II) formed in a reaction with ascorbic acid as a complex with 2,4,6-tri(2-pyridyl)-1,3,5-triazine. Ascorbic acid was determined in plants as described below. A sample of a comminuted plant material was mixed with an acetate buffer solution for 3 min in a nitrogen atmosphere, and the mixture was filtered through a paper filter. The filtrate and a standard solution of ascorbic acid were treated with a solution of ascorbate oxidase, stirred, and thermostatted at 310 K for 15 min. Next, equal volumes of a mixture containing an acetate buffer solution, 2,4,6-tri(2-pyridyl)-1,3,5-triazine, HCl, and $FeCl_3$ were added to each solution. The absorbance of both solutions was measured at 593 nm. The calibration graph was rectilinear over an ascorbic acid concentration range of 10–100 mg/L.

Procedures based on Cu(II) reduction followed by the detection of univalent copper were proposed for the determination of ascorbic acid. Thus, Baker and Lowe [25] bound Cu(I) ions by neocuproine in a colored complex and measured the light absorption of this complex at 450 nm. The method determined 2–20 μg of ascorbic acid. Cysteine, *N*-ethylmaleimide, and iodoacetic acid interfered with the determination.

Tutem *et al.* [26] proposed a simple method for determining ascorbic acid by a reaction with Cu(II) in the presence of 5-(4-hydroxy-3-methoxybenzylidene)rhodanine in a water–dioxane mixture at pH 3.8. The photometry of the product was performed at 473 nm ($\varepsilon = 2.6 \times 10^4$ L mol^{-1} cm^{-1}). Beer's law was obeyed in the concentration range 1.1–6.6 mg/L. The method was applied to the analysis for ascorbic acid in pharmaceutical preparations.

A procedure for the photometric determination of ascorbic acid in trace amounts by the reduction of Ag(I) (gelatin complex) in an aqueous solution at pH 7.5–10.0 with the formation of a yellow silver sol was described [27]. For this purpose, a solution of $AgNO_3$ was mixed with a gelatin solution and pH 8.0 was adjusted by adding a solution of NaOH. A sample of ascorbic acid was added, and the absorbance at 415 nm was measured after 20 min. The linearity range was 1–10 mg/L. Glycine, alanine, sucrose, fructose, citric acid, tartaric acid, oxalic acid, maleic acid, succinic acid, and various reducing agents caused no interference with the determination. Pal [28] photochemically reduced Ag(I) with ascorbic acid in a Triton X-100 solution to form a yellow silver sol. The absorbance at 415 nm was measured ($\varepsilon = 1.43 \times 10^4$ L mol^{-1} cm^{-1}). Ascorbic acid can be determined within a range of 0.4–13 mg/L. The method was proposed for the analysis of pharmaceutical preparations containing vitamin C.

Lazaro *et al.* [29] described the FIA determination of ascorbic acid with spectrophotometric measurement. The determination was based on the oxidation of HI with chloramine T in an acidic medium followed by the photometric determination of a complex of I_2 with starch. In the presence of ascorbic acid, I_2 was also consumed in the oxidation of ascorbic acid to decrease the concentration of the colored complex. The determination was performed as described below. A solution of ascorbic acid was mixed with H_2SO_4 and a starch–KI solution; the mixture was injected into the flow of a chloramine T solution, which was passed through a coil and directed to a flow cell. The light absorption was measured at 650 nm. The decrease in the absorbance was proportional to the concentration of ascorbic acid in the range of 15–150 mg/L. The same indicator system was proposed for the simultaneous determination of ascorbic acid and sulfite ions in soft drinks. For this purpose, two microsamples, one of which was premixed with a NaOH solution, were synchronously introduced at different points of a flow. As the result of the successive mixing of each sample zone with the flow of a reagent (a chloramine T solution containing a KI–starch indicator mixture at pH 10.82) and then with the flow of an H_2SO_4 solution, both ascorbic acid and SO_3^{2-} or only SO_3^{2-} reacted with the reagent. The amount of resulting colored products was measured by photometry at 581 nm as peaks whose heights were proportional to the total concentration of ascorbic acid and SO_3^{2-} or to the concentration of SO_3^{2-} in samples. The analytical range for ascorbic acid and SO_3^{2-} was 4–140 mg/L.

A spectrophotometric procedure was proposed for the FIA determination of ascorbic acid; this procedure is based on the generation of triiodide ions or a triiodide complex with starch directly in a flow-injection system [31]. A decrease in the absorbance of solutions at 380 and 580 nm was proportional to the concentration of ascorbic acid. The solutions of KI–starch and KIO_3 in H_2SO_4 were passed through a coil (3 m × 0.7 mm) at a flow rate of 1.5 mL/min and mixed with an aqueous sample flow. The mixed flow was passed through a coil (130 cm × 0.5 mm) and directed to a flow cell for photometry. The linearity range of calibration graphs for ascorbic acid varied from 0.1–7 to 2–40 mg/L, depending on the concentration of iodate. The productivity of this procedure was 300 samples per hour. The procedure was tested in the analysis of pharmaceutical preparations containing vitamin C and pineapple juice and jam.

Ciesielski [32] described a procedure for the simultaneous determination of cysteine, glutathione, and ascorbic acid traces in a mixture with the use of the iodine–azide reaction induced by analytes. A sample was introduced into a solution containing KI and a phosphate buffer solution with pH 5.8. The ascorbic acid content was found from a decrease in the light absorption (350 nm). Another sample was added to a solution containing NaN_3 and KI (pH 8.0–8.5), and the resulting solution was mixed with formaldehyde, which blocked the effect of cysteine. After 1 min, HCl and I_2 solutions were introduced, and photometric measure-

ments were performed after 5 more minutes. The glutathione content was found by a decrease in the absorbance. A third sample was added to a solution containing NaN_3, KI, and HCl (pH 5.8). A solution of I_2 was added to the resulting solution, and the light absorbance of the solution was measured after 1 min. The cysteine content of the sample was calculated from the data obtained. The method made it possible to determine 10–30 μg of ascorbic acid, 0.05–0.25 μg of cysteine, and 0.4–2 μg of glutathione in 10 mL of the solution.

A simple and selective procedure was developed for the determination of ascorbic acid with diazotized 1-aminoanthraquinonezinc chloride (Fast Red AL salt) in an alkaline medium (λ = 630 nm; $\varepsilon = 4.07 \times 10^3$ L mol^{-1} cm^{-1}) [33]. The calibration graph was rectilinear within an ascorbic acid concentration range of 5–25 mg/L. Other vitamins and dehydroascorbic acid caused no interference with the determination. This procedure was applied to the determination of ascorbic acid in pharmaceutical preparations and juices.

A procedure based on the reduction of ascorbic acid with a ternary Mo(VI)–Sb(III)–P(V) heteropoly acid was described [34]. Optimum conditions of the determination are the following: reagent concentrations (10^{-4} M) of 3.0 for PO_4^{3-}, 74 for MoO_4^{2-}, and 0.45 for Sb(III); H^+ concentration of 0.5 M; and a temperature of 298 K. The color ($\varepsilon_{710} = 3.5 \times 10^3$ L mol^{-1} cm^{-1}) was developed in 35 min. The linearity of the calibration graphs was retained within the limits of 1–50 mg/L; the detection limit was 0.2 mg/L. The procedure was tested in the analysis of vitamin C tablets.

A procedure was proposed for the spectrophotometric determination of ascorbic acid with a tungstophosphate–vanadate reagent, which consisted of a mixture of tungstophosphoric acid and sodium vanadate with an acetate buffer solution (pH 1.5) [35]. The concentration of ascorbic acid was directly proportional to the value of $\Delta A_{360} = A_1 - A_2$, where A_1 and A_2 is the absorbance of the reagent in the absence and in the presence of ascorbic acid. The procedure was tested in the determination of ascorbic acid (≥50–60 μg) in tablets, syrups, and fruit juices.

A procedure was proposed for the determination of ascorbic acid in pharmaceuticals, fruit juices, vegetables, fruits, and infant milk powder is based on the reduction reaction of 4-chloro-7-nitrobenzofurazane in an alkaline medium with the formation of a colored product (λ_{max} = 582 nm) [36]. The calibration graph for ascorbic acid was linear over a range of 5–20 mg/L. Dehydroascorbic acid, all other vitamins, mineral components of multivitamin preparations, rutin, salicylamide, acetylsalicylic acid, paracetamol, caffeine, etc. did not interfere with the determination.

Dabrowski and Hinterleitner [37] described the spectrophotometric determination of ascorbic acid and its derivatives in the extracts of animal tissues and feed. For this purpose, five series of solutions were prepared. 2,6-Dichlorophenolindophenol was added to the first series of solutions. Potassium bromate was added to the second series of solutions to oxidize ascorbic acid. 2,4-Dinitrophenylhydrazine was added to the fourth series of solutions to convert ascorbic acid into dehydroascorbic acid. Two series of blank solutions containing degraded ascorbate were used for taking into account interferences from other sample components. A 20% 2,4-dinitrophenylhydrazine solution in 12 M H_2SO_4 was introduced into all of the series; the solutions were held at 333 K for 3 h and centrifuged. The absorbance of the solutions was measured at 524 nm. The detection limit of ascorbic acid was equal to 0.4 mg/L.

Al-Tamrah [38] developed a procedure for the determination of ascorbic acid in pharmaceutical preparations. This procedure is based on the selective oxidation of ascorbic acid with an excess of $K_3[Fe(CN)_6]$ or KIO_4. The concentration of the unreacted oxidant was determined spectrophotometrically by the reaction with phthalophenone (λ_{max} = 553 nm). The detection limit was equal to 0.1 mg/L; the calibration graph was linear up to an ascorbic acid concentration of 7.0 mg/L. Many reductants caused no interference with the determination.

Abdel-Badei and El-Sawi [39] proposed a thymolphthalin reagent for the spectrophotometric determination of ascorbic acid. The reagent was prepared by mixing weighed portions of thymolphthalein, zinc dust, and NaOH with water followed by heating under reflux for 2 h; the mixture was filtered, and the filtrate was diluted to a required volume. A sample containing 0.4–1.6 μg of ascorbic acid was heated with a $K_3[Fe(CN)_6]$ solution at pH 12–13 in a boiling-water bath for 1 h. After cooling, the thymolphthalin reagent was introduced, and the resulting mixture was diluted to 25 mL. After 15 min, the absorbance of the test (A_2) and blank (A_1) solutions was measured at 592 nm. The calibration graph on the coordinates of $\Delta A = A_1 - A_2$ was linear in an ascorbic acid concentration range of 16–64 μg/L.

Thompson [40] described a FIA procedure for the spectrophotometric determination of ascorbic acid in the aqueous solutions of pharmaceutical preparations. This procedure is based on a decrease in the reaction rate of 4-aminoantipyrine and 3,5-dichloro-2-hydroxybenzenesulfonate oxidation by H_2O_2 catalyzed by peroxidase (λ_{max} = 510 nm). The detection limit was equal to 0.4 mg/L; the calibration graph was linear in an ascorbic acid concentration range of 0.4–18 mg/L. The productivity was 80 determinations per hour. In the determination of ascorbic acid at a concentration of 9.0 mg/L, equal amounts of $Ca(H_2PO_4)_2$, citric acid, $FeCl_3$, thiamine hydrochloride, and uric acid caused no interference. The interference of iron(II) was removed by the precipitation of iron(II) oxalate in the treatment of samples with oxalic acid to prevent the aerobic oxidation of ascorbic acid. The procedure was tested in the analysis of multivitamin preparations.

Table 1. Performance characteristics of spectrophotometric methods for the determination of ascorbic acid

Reagent	Method	Linearity range, mg/L	c^*_{min}, mg/L	Reference
Quinaldic acid–pyridine–Fe(III)	Extraction–spectrophotometry	2.5–25	2.5	[15]
o-Phenanthroline–Fe(III) in the presence of Trilon B	Extraction–spectrophotometry	0.08–0.4	0.012	[20]
o-Phenanthroline–Fe(III)	Extraction–spectrophotometry FIA	0.04–1.8	0.04	[22]
o-Phenanthroline–Fe(III)	Spectrophotometry	0.8–4.8	0.8	[23]
2,6-Dichlorophenol–indophenol	Extraction–spectrophotometry	4–8	4	[13]
2,6-Dichlorophenol–indophenol	Kinetic spectrophotometry	3.5–88	3.5	[14]
2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol–Fe(III)	Spectrophotometry	0.015–2.4	0.015	[19]
		0.044–2.4	0.044	
Hexacyanoferrate(III)–thymolphthalein + Zn + NaOH	Spectrophotometry	0.016–0.064	0.016	[39]
Hexacyanoferrate(III)–phthalophenone	Spectrophotometry	0–7	0.1	[38]
Hexacyanoferrate(III)–Fe(III)	Extraction–spectrophotometry FIA	0.9–18	0.9	[18]
Picolinic acid–pyridine–Fe(III)	Spectrophotometry	0.4–5.6	0.4	[17]
2,4,6-Tri(2-pyridyl)-1,3,5-triazine–Fe(III)	Spectrophotometry	10–100	10	[24]
Ag(I)–Triton X-100	Spectrophotometry	0.4–13	0.4	[28]
Ag–gelatin complex	Spectrophotometry	1–10	1	[27]
Cu(II)–5-(4-hydroxy-3-methoxybenzylidene)rhodanine	Spectrophotometry	1.1–6.6	1.1	[26]
Neocuproine–Cu(II)	Spectrophotometry	2–20	2	[25]
Folin's reagent	Spectrophotometry	0.08–10	0.04	[10]
I_2	Extraction–spectrophotometry FIA	0.1–0.7	0.1	[31]
I_2	Extraction–spectrophotometry FIA	4–140	4	[30]
I_2	Extraction–spectrophotometry FIA	15–150	15	[29]
KI + NaN_3	Spectrophotometry	1.0–3.0	1.0	[32]
Vanadotungstophosphoric acid–$NaVO_3$	Spectrophotometry	2–2.4	2	[35]
Mo(VI)–Sb(III)–P(V) heteropoly acid	Spectrophotometry	1–50	0.2	[34]
2,4-Dinitrophenylhydrazine	Spectrophotometry		0.4	[37]
Diazotized 1-aminoanthraquinonezinc chloride (Fast Red AL salt)	Spectrophotometry	5–25	5	[33]
4-Chloro-7-nitrobenzofurazan	Spectrophotometry	5–20	5	[36]
〃	Spectrophotometry	1.5–3.0	1.5	[3]
〃	Spectrophotometry	2–50	2	[4]
〃	Spectrophotometry	15–1125	15	[5]

* Limit of detection.

Table 2. Performance characteristics of fluorescence methods for the determination of ascorbic acid

Reagent	Method	Linearity range, mg/L	c^*_{min}, mg/L	Reference
Luminol–Fe(III)	Chemiluminescence FIA	1.76×10^{-6}–0.176	1.76×10^{-7}	[42]
Luminol–H_2O_2–VO_3^-	Chemiluminescence	0.01–0.02	0.01	[41]
Lucigenin	Chemiluminescence	0.09–0.70	0.05	[44]
Lucigenin	Chemiluminescence	2–14	0.17	[45]
2-Hydroxynaphthaldehyde thiosemicarbazone–Mn(II)	Kinetic fluorescence	0.026–0.05	0.026	[50]
2,3-Diaminonaphthalene	Fluorescence	2–30	0.4	[48]
1,2-Diamino-4,5-dimethoxybenzene	Fluorescence	1.3–200	1.3	[49]

* Limit of detection.

Table 1 summarizes some analytical characteristics of the described procedures.

CHEMILUMINESCENCE AND FLUORESCENCE METHODS

Pharmaceutical preparations are usually supplied to a human body in small amounts; they occur in blood in even lower concentrations, or they are eliminated from the body in urine. In this context, fluorescence and chemiluminescence methods are promising for the determination of biologically active substances. Table 2 summarizes the performance characteristics of fluorescence and chemiluminescence procedures for the determination of ascorbic acid.

Lukovskaya and Mitropolitska [41] used vanadometry for the chemiluminescence determination of ascorbic acid. The method is based on the reduction of vanadate ions by ascorbic acid in an acid medium followed by the detection of the resulting vanadyl using a chemiluminescence reaction of luminol with oxygen. The detection limit of ascorbic acid was equal to 0.01–0.02 mg/L.

Alwarthan [42] proposed a flow-injection procedure for the determination of ascorbic acid. The procedure is based on the prereduction of Fe(III) followed by the detection of the resulting Fe(II) using a chemiluminescence reaction of luminol oxidation by hydrogen peroxide. Optimum conditions of the determination are the following: reagent concentrations of 0.1, 3.0, and 30 mM for luminol, Fe(III), and H_2O_2, respectively; pH 1.5 in the initial solution; flow rate of 0.1 mL/min; and an optimum reactor length of 200 cm. The procedure makes it possible to determine ascorbic acid in the range of 1.76×10^{-6}–0.176 mg/L. The detection limit is 1.76×10^{-7} mg/L. Oxalate, sulfate, and sulfide ions interfered with the determination; nitrate, chloride, and nitrite ions interfered to a lesser extent. Tartrate, glucose, fructose, and riboflavin did not interfere. The procedure was tested in the analysis of pharmaceutical preparations and fruit juices.

A chemiluminescence procedure with the use of the ferriheme-catalyzed reaction of luminol oxidation with H_2O_2 at pH 8.97 was proposed for the determination of ascorbic acid in aqueous solutions and biological fluids [43]. The concentration of ascorbic acid was determined by a calibration graph plotted on the *t*–*c* coordinates, where *t* is the induction period of reaction. The calibration graph was rectilinear up to an ascorbic acid concentration of 10.5 mg/L. The selectivity of analysis was attained by the use of an appropriate enzyme.

The method proposed by Dubovenko and Kuyan [44] is based on the property of ascorbic acid to induce a chemiluminescence reaction of lucigenin in an alkaline solution. Under optimum conditions (a lucigenin concentration of 50–100 μM; pH 13), the detection limit of this method was 0.05 mg/L. Co(II), Cu(II), Mn(II), and Pb(II) ions at total concentrations of $\leq 10^{-6}$ M did not interfere with the determination of ascorbic acid in a range of 0.09–0.70 mg/L.

Veazey and Nieman [45] applied the above reaction to the determination of ascorbic acid in blood and urine over concentration ranges of 2–14 and 10–100 mg/L, respectively. The limit of detection of ascorbic acid was equal to 0.17 mg/L. Reducing agents (creatinine, uric acid, glutathione, glucuronic acid, lactose, and glucose) interfered with the determination of ascorbic acid.

The addition of a surfactant (0.15 mmol) improved the performance characteristics of this procedure [46].

Visser [47] described a microfluorimetric method for determining ascorbic acid in food products. A homogenized sample was treated with a trichloroacetic acid solution and filtered through a paper filter. The filtrate was treated with activated carbon and filtered once again. A sodium acetate solution was added to the filtrate to adjust pH 5.5–6.0, and an *o*-phenylenediamine solution was added after 30 min. The resulting mixture was held for 30 min in the dark, and the fluorescence intensity was measured. A reference solution was prepared in a similar manner with the addition of boric acid. The linearity range of the calibration graph was 1.87–145 mg/100 g.

A procedure was developed for the fluorescence determination of ascorbic acid based on the reaction with 2,3-diaminonaphthalene at pH 10.2–10.5 [48].

The fluorescence intensity was measured in a quartz cuvette (l = 1 cm) at 520 nm (excitation at λ_{max} = 400 nm). The detection limit was 0.4 mg/L; the calibration graph was linear over a range of 2–30 mg/L.

The determination of the total ascorbic acid and dehydroascorbic acid in blood plasma was based on the oxidation of ascorbic acid with iodine to dehydroascorbic acid followed by its detection by the reaction with 1,2-diamino-4,5-dimethoxybenzene [49]. The fluorescence intensity was measured at 458 nm (excitation at λ_{max} = 371 nm). The detection limit was equal to 1.3 mg/L at a sample volume of 4 μL. The calibration graph was linear to an ascorbic acid concentration of 200 mg/L.

The kinetic fluorimetric determination of ascorbic acid traces is based on the Mn(II)-activated reaction of 2-hydroxynaphthaldehyde thiosemicarbazone oxidation with atmospheric oxygen [50]. The calibration graph was rectilinear in an ascorbic acid concentration range of 0.025–0.050 mg/L. The determination was performed as described below. A sample solution was introduced into an ethanolic solution containing the reagent, the catalyst, and NH_3; the mixture was stirred and thermostatted at 303 ± 0.1 K for 15 s. The change in the fluorescence intensity at 450 nm (with excitation at λ_{max} = 390 nm) with time was monitored. The concentration of ascorbic acid in the sample was found from the initial rate of reaction or by the tangent method. In the determination of 1 nM ascorbic acid, 25-fold amounts of Ca(II), Mo(VI), oxalates, glucose, cysteine, and glutathione; 15-fold amounts of phosphates, sulfites, and chlorides; and 10-fold amounts of tartrates and Mg(II) did not interfere. Trilon B and $P_2O_7^{2-}$ interfered with the determination. The method was applied to determine ascorbic acid in pharmaceuticals, fruit juices, and urine.

SORPTION–SPECTROPHOTOMETRIC METHODS

This group of methods uses the same principles as in the spectrophotometric determination of ascorbic acid. A higher sensitivity is attained by the preconcentration of the analyte or reaction products in a sorbent phase from a relatively large volume of the test solution. As distinct from liquid extraction preconcentration, sorption methods do not require the use of toxic organic solvents; hence, they are more environmentally friendly.

Diaz and coauthors [51, 52] proposed a FIA method with the subsequent solid-phase spectrophotometric detection of ascorbic acid by the intrinsic absorption at 267 nm. Ascorbic acid was extracted from solution with an anion-exchange gel layer of the sorbent Sephadex QAE A-25 placed in a quartz flow cell 1 mm thick. An acetate buffer solution was used as a mobile carrier; this buffer solution was also used for the regeneration of the sorbent. The calibration graphs were linear in the ascorbic acid concentration ranges of 1.0–20.0 (300-μL samples), 0.5–10.0 (600-μL samples), and 0.2–6.0 mg/L (1000-μL samples). The detection limits were equal to 0.04, 0.03, and 0.02 mg/L, respectively. The productivity of this method was 28, 24, or 21 determinations per hour, respectively. The relative standard deviations (n = 10) were no higher than 0.01%. The method was tested in the analysis of pharmaceutical preparations, confectioneries, and urine samples.

Fernandes *et al.* [53] proposed a FIA system with the use of a column (2.79 mm in diameter and 50 mm in length) packed with silica gel modified with aminopropyl groups for the analysis of ascorbic acid preparations. The light absorption was measured at 265 nm. The limit of detection was equal to 0.35 mg/L. The calibration graph was linear in a range of 1.8–18 mg/L; the productivity of this method was 30 determinations per hour.

Dmitrienko *et al.* [54] proposed molybdosilicic heteropoly acid immobilized on polyurethane foam for the sorption–spectrophotometric determination of ascorbic acid. In contact with an aqueous ascorbic acid solution (pH 5), the immobilized heteropoly acid was desorbed and reduced in the solution to form blue products. On the subsequent acidification with an HCl solution to pH 2, the reduced species was quantitatively extracted with an unmodified sorbent. The distribution coefficient was equal to 7.5×10^5 cm^3/g. The detection limit was 0.1 mg/L; the time taken for a single determination was 75 min. The analytical range of the method was 0.3–2.4 mg/L; RSD < 7%. In the determination of 1.5 mg/L ascorbic acid, a 200-fold amount of sulfite ions caused no interference. Among the advantages of this method are the use of modified polyurethane foam as a ready-made analytical form and the applicability to the analysis of turbid solutions. The method was tested in the analysis of orange juice and a Zuko nonalcoholic drink.

A procedure for the determination of ascorbic acid by solid-phase spectrophotometry was based on the reduction of Fe(III) ions with ascorbic acid followed by the detection of the resulting Fe(II) by the reaction with ferrozine [55]. The colored product was adsorbed by a dextran-like anion-exchange gel, and its absorbance was measured at 567 and 800 nm. The analytical range for ascorbic acid was 5–90 μg/L; the limit of detection was equal to 0.91 μg/L. The relative standard deviation was no higher than 0.01%. The method was applied to determine ascorbic acid in fruit juice, pharmaceuticals, and conservation liquids from canned foods.

A solid-phase reactor containing copper(II) phosphate immobilized on a polymeric polyester matrix was developed for the flow-injection determination of ascorbic acid [56]. The test solution was passed through the reactor (a carrier solution was an acetate buffer with pH 4.5), and Cu(I) formed in the reaction with ascorbic acid was washed off to a liquid phase, where it was mixed with a bathocuproine solution. The light absorption of the resulting chelate was measured at 480 nm.

The calibration graph was linear over an ascorbic acid concentration range of 0.9–7.0 mg/L. The detection limit was 0.05 mg/L. The throughput of the system was 80 determinations per hour.

Zaporozhets *et al.* [57] developed a simple and rapid procedure for the sorption–spectrophotometric determination of ascorbic acid in the pharmaceutical preparation Ascorbic Acid (0.1 g) with Glucose and Aspirin UPSA. A copper(II) complex with tetrabenzo[*b*,*f*,*j*,*n*][1,5,9,13]tetraazacyclohexadecine immobilized at the surface of Silica gel L 100/250 (SG) (the capacity $a = 5 \times 10^{-6}$ mol/g) by adsorption, underwent reduction on the treatment with an ascorbic acid solution, and its color changed from yellow to blue. The light absorption of a suspension of modified SG was measured in a 10-mm cuvette with reference to a suspension of unmodified silica gel at 660 and 950 nm. The concentration of ascorbic acid in the sample was calculated by a calibration equation in the ΔA–c coordinates, where $\Delta A = A_{660} - A_{950}$. The calibration graph was linear in a range of 1.8–40 mg/L; the limit of detection was 0.06 mg/L at a sample volume of 50 mL. Amino acids (glycine, aspartic acid, and glutamic acid) and saccharides did not interfere with the determination of ascorbic acid.

The property of quinone imine indicators changing their colors in redox reactions was used as the basis for the sorption–spectrophotometric determination of reducing agents, in particular, ascorbic acid [58]. Bindschedler's green dye immobilized on an SG-SO_3H ion exchanger was used to determining ascorbic acid. In the spectrophotometric determination, an acetate buffer solution (pH 4.0–5.5) was added to the sample of a test solution, and a weighed portion of the modified sorbent (a = 6.0 μmol/g) was introduced into the resulting solution. The mixture was stirred for 10 min. The absorbance of the indicator powder was measured at 720 nm. The ascorbic acid content was determined by a calibration graph. The linearity range was 0.3–5.0 mg/L; c_{min} = 0.1 mg/L.

A procedure was developed for the determination of ascorbic acid by solid-phase spectrophotometry with the use of a silicic acid xerogel simultaneously modified with the Vavele reagent (a mixture of 12-molybdophosphoric and 18-molybdophosphoric acids) and copper(II) [59]. The Vavele reagent was reduced by ascorbic acid with a change in color from yellow to blue; the modification of the xerogel with Cu(II) ions increased the rate of the reduction reaction and shortened the analysis time to 10–15 min. To determine ascorbic acid, 0.2 g of the indicator powder was mixed with an aliquot portion of the test solution and the mixture was shaken for 10 min. The absorbance of the modified xerogel was measured at 680 nm. The ascorbic acid content was determined by a calibration graph. The linearity range was 1–50 mg/L; the limit of detection was 0.3 mg/L. Oxalic acid (in a 500-fold amount), citric acid (in a 200-fold amount), and tartaric acid (in a 200-fold amount) did not interfere with the determination. The procedure was tested in the analysis of dried fruit juices.

VISUAL-TEST METHODS FOR THE DETERMINATION OF ASCORBIC ACID

Note that analytical methods that can be used not only in the laboratory but also on the sampling site came recently to the attention of researchers. The advantages of such visual-test methods consist in their simplicity, operating economy, and rapidity. An analysis of published data demonstrated that a relatively narrow range of visual tests was proposed for the determination of ascorbic acid.

An indicator paper was developed, which changed its color from greenish yellow to dark blue depending on the concentration of ascorbic acid [60]. The analytical range was 50–600 mg/L ascorbic acid. The indicator paper was prepared as described below. An alkaline solution containing molybdophosphoric acid, sodium dioctylsulfosuccinate, malonic acid, tartrazine, and cerium ammonium sulfate was applied to filter paper; the paper was dried and cut into sheets of a required size.

Merckoquant test strips (No. 110 023) were proposed for the semiquantitative determination of ascorbic acid in a concentration range of 0.05–2.0 mg/mL. With the reflectometric color detection (Reflectoquant, No. 116 981), the analytical range was 25–450 mg/L ascorbic acid [61].

Schwedt [62] proposed to use test strips for the reflectometric determination of ascorbic acid in a range of 60–800 mg/L. Coloring agents that are adsorbed on a cellulose layer of the test zone interfered with the determination.

The operation of indicator tubes and indicator powders is based on the property of quinone imine indicators to change their colors under conditions of redox reactions [58]. Bindschedler's green immobilized on an SG-SO_3H ion exchanger was used for determining ascorbic acid. In the spectrophotometric determination, an acetate buffer solution (pH 4.0–5.5) was added to the sample of a test solution and a weighed portion of the modified sorbent (a = 6.0 μmol/g) was introduced into the resulting solution. The mixture was stirred for 10 min. The absorbance of the indicator powder was measured at 720 nm. The ascorbic acid content was determined by a calibration graph. The linearity range was 0.3–5.0 mg/L; the detection limit was 0.1 mg/L. For the visual colorimetric determination of ascorbic acid, a weighed portion of the modified sorbent and an acetate buffer solution were placed in a test concentrator. The contents were stirred, and the concentrator was turned beak down. The color in the beak was compared to a scale, and the ascorbic acid content was determined. The analytical range was 5–100 mg/L; the limit of detection was 1.4 mg/L. Indicator tubes of molybde-

Table 3. Sorption–spectroscopy and visual-test determination of ascorbic acid

Indicator system in solution	Support or solid-phase reagent	Detection technique	Linearity range, mg/mL	c_{min}, mg/L	Test material	Reference
Ferrozine, Fe(III)	Dextran-like anion exchanger	Spectrophotometry	0.005–0.09	9.0×10^{-4}	Fruit juices, pharmaceutical preparations, and conservation liquids	[55]
″	Sephadex QAE A-25	Spectrophotometry FIA	1.0–20.0	0.04	Pharmaceutical preparations and confectioneries	[51]
			0.5–10.0	0.03		[52]
			0.2–6.0	0.02		
″	Cu(II) complex with tetrabezo[*b*,*f*,*j*,*n*][1,5,9,13]tetraazacyclohexadecine adsorbed on the surface of silica gel	Spectrophotometric	1.8–40	0.06	Pharmaceutical preparations	[57]
		Visual test	0.5–4.0	0.05		
Bathocuproine	Cu(II) immobilized on a polymeric polyester matrix	Spectrophotometry FIA	0.9–7.0	0.05	Standard solutions	[56]
″	Heteropoly acid immobilized on polyurethane foam	Spectrophotometry	0.3–2.4	0.1	Orange juice and Zuko drink	[54]
″	Bindschedler's green immobilized on SG-SO_3H	Spectrophotometric	0.3–5.0	0.1	Standard solutions	[58]
		Visual test	5–100	1.4		
		Visual test*	20–1000	7		
″	Vavele reagent and Cu(II) immobilized on xerogel	Spectrophotometric	1–50	0.3	Dried fruit juices	[59]
		Visual test	10–400	10		
″	Aminopropyl silica gel	Spectrophotometry FIA	1.8–18	0.35	Pharmaceutical preparations	[53]
[Methylene blue]$^+I_3^-$ complex, Triton X-100	Silochrome C-120	Visual test*	0.9–14, 17–650	0.9	Orange juices	[63]
[Methylene blue]$^+I_3^-$ complex, Triton X-100	Reflectoquant test strips No. 110 023	Visual test	25–450	25	Standard solutions and food products	[51]
″	Merckoquant test strips No. 110 023	Visual test	50–2000	50	Standard solutions and food products	[61]
″	Filter paper impregnated with molybdophosphoric acid	Visual test**	50–600	50	Standard solutions	[60]
″	Test sticks	Visual test	60–800	60	Food products	[62]

* Indicator tubes.
** Indicator paper.

num glass 50 mm in length and 1.8 mm in internal diameter were used. The tubes were packed with silica gel that was sequentially modified with sodium dodecylsulfonate (SG-SO_3H) and Bindschedler's green. The indicator tubes were dipped in a solution containing 5 mL of a sample and 0.2 mL of an acetate buffer solution. Once the solution was raised through the entire height of the tube, the tube was removed from the solution, and the length of a pale yellow zone was measured. The ascorbic acid content was determined by a calibration graph. The analytical range was 0.02–1.0 mg/mL; the limit of detection was 7 mg/L. Sulfite, thiosulfate, hydroquinone, and 4-aminophenol; ionic strength up to 0.5 M; 1000-fold amounts of sulfate, phosphate, chloride, and nitrate; and 100-fold amounts of Fe(II) and Cu(II) caused no interference with the determination.

Indicator tubes based on stable ion associates of thiazine dyes with the triiodide anion were developed [63]. Because of the interaction of such ion associates with reducing agents, a free dye was regenerated, which was subsequently adsorbed by Sylochrome C-120. The determination of ascorbic acid in fruit juices was performed as described below. An acetate buffer solution (pH 4.5), a suspension of the ion associate of triiodide with methylene blue, a Triton X-100 solution, and deionized water were added to a sample of filtered juice. After 5–7 min, the solution was passed through an indicator tube. Under analogous conditions, a calibration graph was constructed on the colored zone length–ascorbic acid concentration coordinates. The analytical ranges for ascorbic acid were 0.9–14 and 17–650 mg/L for tubes with i.d. of 1 and 2 mm, respectively. Of the compounds that occur in fruits and berries, only tannin in commensurable amounts interfered with the determination. The method was applied to the analysis of orange juices from different origins.

A standard color scale, which was based on the reduction reaction of a copper(II) complex with tetrabenzo[*b*,*f*,*j*,*n*][1,5,9,13]tetraazacyclohexadecine immobilized at the surface of Silica gel L 100/250 by adsorption, was proposed for the visual-test determination of ascorbic acid in solution [57]. To prepare the scale, a series of solutions with pH 9.3 containing various amounts of ascorbic acid (10-mL portions) were stirred with weighed portions of a solid-phase reagent ($a = 5 \times 10^{-6}$ mol/g) for 5 min. A sample of the test solution was treated analogously, and the developed color of the modified sorbent was compared to the scale. The method of colorimetry was used to substantiate the linearity range of the scale and to take into account the human factors of vision in the certification of test methods under laboratory conditions [64, 65]. In this case, the absorption spectra of the scale sorbents were measured by reflectometry and the colorimetric functions ΔE (total color difference) and ΔL (difference in the lightness) were calculated. This method made it possible to determine the concentration of ascorbic acid in a sample using a calibration graph plotted on the ΔE (ΔL)–*c* coordinates over a range of 0.025–0.200 mg with RSD ≤ 30%.

A visual-test procedure for determining 10–400 mg/L ascorbic acid was developed with the use of a silicic acid xerogel modified with the Vavele reagent and copper(II) as an indicator powder [59].

Table 3 summarizes some characteristics of solid-phase reagents for the spectrophotometric and visual-test determination of ascorbic acid.

CONCLUSIONS

An analysis of published data indicates that ascorbic acid can be determined by various methods of molecular spectroscopy. Chemiluminescence methods exhibit the highest sensitivity. With the use of these methods, the detection limits of ascorbic acid are at a nanogram level; therefore, these methods can be used for the analysis of biological samples (blood, urine, etc.). At higher concentrations of ascorbic acid, it is primarily determined by spectrophotometry. The required sensitivity is attained by solvent extraction or sorption preconcentration. The latter mode is more environmentally friendly, because toxic organic solvents are not required. Three groups of methods that exhibit the highest sensitivity and selectivity can be recognized among sorption–spectrophotometric techniques. The first group of methods is based on the reduction of metal ions by ascorbic acid to achieve lower oxidation states followed by the extraction of the metals as complexes with various chromophoric or chelating reagents [55, 56]. The second group includes methods based on the degradation of ion associates under the action of reducing agents followed by the extraction and detection of the liberated dye at the surface of a support [63]. The third group of methods is based on the interaction of the analyte with a reagent immobilized on the surface of a sorbent [54, 57–62]. In the latter, an immobilized chromophoric reagent is used as a ready-to-use analytical form. The advantages of these methods consist in that they are single-stage procedures and other organic reagents are not used in the course of analysis. Moreover, the immobilization of organic reagents on the surface of sorbents improves their stability. These reagents are convenient in storage and transportation; therefore, they can be used for preparing concentrates on the site of sampling. Analytical forms used in the third group of methods are also most promising for the development of simple and inexpensive visual tests for rapid analysis. A survey of published data demonstrated that only a narrow range of visual tests was proposed for the determination of ascorbic acid. The development of new test methods for determining ascorbic acid is a topical problem because instrumental analysis is not always economically sound. Moreover, the need for analytical methods that do not require specially equipped laboratories and highly skilled personnel increases.

REFERENCES

1. Dawson, R., Elliott, D., Elliott, W., and Jones, K., *Data for Biochemical Research*, Oxford: Clarendon, 1986. Translate under the title *Spravochnik biokhimika*, Moscow: Mir, 1991.
2. *Gosudarstvennaya farmakopeya* (USSR State Pharmacopoeia), Moscow: Meditsina, 1968, X ed.
3. Abdel-Hamid, M.E., Barary M.H., Hassan, E.M., and Elsayd, M.A., *Analyst* (Cambridge, U.K.), 1985, vol. 110, no. 7, p. 831.
4. Brodnjak-Voncina, D. and Dobcnik, D., *Vestn. Slov. Kem. Drus.*, 1986, vol. 33, p. 183.
5. Fung, Y.-S. and Luk, S.-F. *Analyst* (Cambridge, U.K.), 1985, vol. 110, no. 12, p. 1439.
6. Lau, O.-W., Luk, S.-F, and Wong, K.-S., *Analyst* (Cambridge, U.K.), 1986, vol. 111, no. 6, p. 665.
7. Lau, O.-W., Luk, S.-F., and Wong, K.-S., *Analyst* (Cambridge, U.K.), 1987, vol. 112, no. 7, p. 1023.
8. Verma, K.K., Jain, A., Verma, A., and Chaurasia, A., *Analyst* (Cambridge, U.K.), 1991, vol. 116, no. 6, p. 641.
9. Korenman, I.M., *Fotometricheskii analiz. Metody opredeleniya organicheskikh veshchestv* (Photometric Analysis: Methods of Determination of Organic Substances), Moscow: Khimiya, 1975.
10. Chen, Y.-L., Xiao J.-X., and Wang, Z.-W. Abstracts of Papers, *Pittsburgh Conf. on Analytical Chemistry and Applied Spectroscopy*, Chicago, 1994, p. 142P.
11. Davies, S.H.R. and Masten, S.J., *Anal. Chim. Acta*, 1991, vol. 248, no. 1, p. 225.
12. Srivastava, A., Kochar, R., and Verma, A., Abstracts of Papers, *Pittsburgh Conf. on Analytical Chemistry and Applied Spectroscopy*, New Orleans, 1985, S.1., s.a., 1160.
13. Shvartsman, G.A., Iorga, E.V., and Byrsanu, G.I., *Novye vidy produktsii iz plodov i vinograda* (New Products Made of Fruit and Grapes), Moscow, 1990, p. 140.
14. Karayannis, M.I. and Farasoglou, D.I., *Analyst* (Cambridge, U.K.), 1987, vol. 112, no. 6, p. 767.
15. Arya, S.P. and Mahajan, M., *Mikrochim. Acta*, 1997, vol. 127, nos. 1–2, p. 45.
16. Arya, S.P., Mahajan, M., and Jain, P., *Chem. Anal.* (Warsaw), 1998, vol. 43, no. 2, p. 231.
17. Arya, S.P. and Mahajan, M., *Anal. Sci.*, 1996, vol. 12, no. 6, p. 941.
18. Nobrega, J.A. and Lopes, G.S., *Talanta*, 1996, vol. 43, no. 6, p. 971.
19. Ferreira, S.L.C., Bandeira, M.L.S.F., Lemos, V.A., *et al.*, *Fresenius' J. Anal. Chem.*, 1997, vol. 357, no. 8, p. 1174.
20. Koch, S. and Peisker, S., *Z. Chem.*, 1990, vol. 30, no. 5, p. 184.
21. Lau, O.-W. and Luk, S.-F., *J. Assoc. Off. Anal. Chem.*, 1987, vol. 70, no. 3, p. 518.
22. Yamane, T. and Ogawa, T., *Bunseki Kagaku*, 1987, vol. 36, no. 10, p. 625.
23. Besada, A., *Talanta*, 1987, vol. 34, no. 8, p. 731.
24. Skaltsa, H.D., Tzakou, O.A., Kouparris, M.A., and Philianos, S.M., *Anal. Lett.*, 1987, vol. 20, no. 10, p. 1679.
25. Baker, W.L. and Lowe, T., *Analyst* (Cambridge, U.K.), 1985, vol. 110, no. 10, p. 1189.
26. Tutem, E., Ulkuseven, B., and Apak, R., *Anal. Lett.*, 1992, vol. 25, no. 3, p. 471.
27. Pal, T. and Maity, D.S., *Anal. Lett.*, 1985, vol. 18, no. 9, p. 1131.
28. Pal, A., *Indian J. Chem., Sect. B*, 1999, vol. 38, no. 4, p. 484.
29. Lazaro, F., Rios, A., Luque de Gastro, M.D., and Valcarcel, M., *Analyst* (Cambridge, U.K.), 1986, vol. 111, no. 2, p. 163.
30. Lazaro, F., Luque de Gastro, M.D., and Valcarcel, M., *Analysis*, 1987, vol. 15, no. 4, p. 183.
31. Hernandez-Mendez, J., Alonso-Mateos, A., Almendral-Parra, M.J., and Garcia-de-Maria, C., *Anal. Chim. Acta*, 1986, vol. 184, p. 243.
32. Ciesielski, W., *Chem. Anal.* (Warsaw), 1986, vol. 31, no. 1, p. 99.
33. Backheef, E.W., Emara, K.M., Askal, H.F., and Saleh, G.A., *Analyst* (Cambridge, U.K.), 1991, vol. 116, no. 8, p. 861.
34. Chen, Y.-L., Xiao, J.-X., and Wang, Z.-W. Abstracts of Papers, *Pittsburgh Conf. on Analytical Chemistry and Applied Spectroscopy*, Chicago, 1994, p. 143P.
35. Muralikrishna, O. and Murty, J.A., *Analyst* (Cambridge, U.K.), 1989, vol. 114, no. 3, p. 407.
36. Abdelmageed, O.H., Khashaba, P.Y., Askal, H.F., Saleh, G.A., and Rafaaf, I.H., *Talanta*, 1995, vol. 42, no. 4, p. 573.
37. Dabrowski, K. and Hinterleitner, S., *Analyst* (Cambridge, U.K.), 1989, vol. 114, no. 1, p. 83.
38. Al-Tamrah, S.A., *Anal. Chim. Acta*, 1988, vol. 209, nos. 1–2, p. 309.
39. Abdel-Badei, M.M. and El-Sawi, E.A., *Rev. Roum. Chim.*, 1987, vol. 32, no. 2, p. 141.
40. Thompson, B., *Anal. Chem.*, 1987, vol. 59, no. 8, p. 1119.
41. Lukovskaya, N.M. and Mitropolitska, E.V., *Zh. Anal. Khim.*, 1975, vol. 30, no. 5, p. 985.
42. Alwarthan, A.A., *Analyst* (Cambridge, U.K.), 1993, vol. 118, no. 6, p. 639.
43. Frew, J.E. and Jones, P., *Anal. Lett.*, 1985, vol. 18, no. 13, p. 1579.
44. Dubovenko, L. and Kuyan, O.A., *Visn. Kiiv. Univ., Ser. Khim.*, 1980, no. 21, p. 18.
45. Veazey, R.L. and Nieman, T.A., *Anal. Chem.*, 1979, vol. 51, no. 13, p. 2092.
46. Klopf L.L. and Nieman, T.A., *Anal. Chem.*, 1984, vol. 56, no. 8, p. 1539.
47. Visser F.R., *J. Assoc. Off. Anal. Chem.*, 1984, vol. 67, no. 5, p. 1020.
48. Yang, J.H., Tong, C.L., Jie, N.Q., Zhang, G.L., *et al.*, *Talanta*, 1997, vol. 44, no. 5, p. 855.
49. Iwata T., Hara S., Yamaguchi M., Nakamura M., *et al.*, *Chem. Pharm. Bull.*, 1985, vol. 33, no. 8, p. 3499.
50. Peinado, I., Toribio, F., and Perez-Bendito, D., *Analyst* (Cambridge, U.K.), 1987, vol. 112, no. 6, p. 775.
51. Diaz, A.M., Medina, A.R., and de-Cordova, M.L.F., *Fresenius' J. Anal. Chem.*, 1999, vol. 363, no. 1, p. 92.

52. Medina, A.R., de-Cordova, M.L.F., and Diaz, A.M., *J. Pharm. Biomed. Anal.*, 1999, vol. 20, nos. 1–2, p. 247.
53. Fernandes, J.C.B., Neto, G.D., and Kubota, L.T., *Anal. Chim. Acta*, 1998, vol. 366, nos. 1–3, p. 11.
54. Dmitrienko, S.G., Goncharova, L.V., and Runov, V.K., *Zh. Anal. Khim.*, 1998, vol. 53, no. 9, p. 914.
55. Barrales, P.O., de Cordova, M.L.F., and Diaz, A.M., *Anal. Chim. Acta*, 1998, vol. 360, nos. 1–3, p. 143.
56. Pereira, A.V. and Fatibello, O., *Anal. Chim. Acta*, 1998, vol. 366, nos. 1–3, p. 55.
57. Zaporozhets, O.A., Krushinskaya, E.A., Lipkovskaya, N.A., and Sukhan, V.V., *Zh. Anal. Khim.*, vol. 56, no. 6, p. 591.
58. Morosanova, E.I., Marchenko, D.Yu., and Zolotov, Yu.A., *Zh. Anal. Khim.*, vol. 55, no. 1, p. 86.
59. Morosanova, E.I., Reznikova, E.A., and Velikorodnyi, A.A., *Zh. Anal. Khim.*, vol. 56, no. 2, p. 195.
60. Svoboda, V. and Pulkrabkova, J., ČSSR Inventor's Certificate no. 224 488, 1981.
61. *Merckquant*, Darmstadt: Merck, 2000.
62. Schwedt, G., *Dtsch. Lebensm.-Rundsch.*, 1986, vol. 82, no. 4, p. 111.
63. Marchenko, D.Yu., Morosanova, E.I., Kuz'min, N.M., and Zolotov, Yu.A., *Zh. Anal. Khim.*, 1997, vol. 52, no. 12, p. 1287.
64. Kirillov, E.A., *Tsvetovedenie* (Color Science), Moscow: Legprombytizdat, 1987.
65. Morozko, S.A. and Ivanov, V.M., *Zh. Anal. Khim.*, 1997, vol. 52, no. 8, p. 858.