4. Quoted from "Colloid Science", Vol. II, edited by H. R. Kruyt, chapter XII (1949).
5. V. A. Kargin, S. P. Papkov, and Z. A. Rogovin, Zhur. Fiz. Khim., 10, 607 (1937); 13, 206 (1939).
6. S. M. Lipatov, "Problemy Ucheniya o Liofil'nykh Kolloidakh" (Problems of the Science of Lyophilic Colloids), Minsk, 1941.
7. S. P. Papkov, Zhur. Fiz. Khim., 18, 560 (1944).
8. S. M. Lipatov, "Doklad na Vsesoyuznoi Konferentsii po Kolloidnoi Khimii, Tbilisi" (Lecture at the All-Union Conference on Colloid Chemistry, Tiflis), Izd. Akad. Nauk SSSR, 1958.
9. S. M. Lipatov, "Fiziko-khimiya Kolloidov" (The Physical Chemistry of Colloids), Goskhimizdat, 1948.
10. S. E. Bresler, Discuss. Faraday Soc., No. 25, 158 (1958).
11. H. Morawetz, Discuss. Faraday Soc., No. 25, 122 (1958).
12. J. Neuman, J. Phys. Chem., 60, 648 (1956).
13. A. Doolittle, Ind. Eng. Chem., 38, 535 (1946).
14. P. I. Zubov, Z. N. Zhurkina, and V. A. Kargin, Kolloid. Zhur., 9, 109 (1947).
15. P. I. Zubov, Z. N. Zhurkina, and V. A. Kargin, Kolloid. Zhur., 9, 367 (1947).
16. P. I. Zubov, Doctoral Thesis, Karpov Institute of Physical Chemistry, 1948.
17. D. Bourgoin and M. Joly, Kolloid Z., 136, 25 (1954).
18. N. F. Proshlyakova, P. I. Zubov, and V. A. Vargin, Kolloid. Zhur., 20, 199, 202 (1958).
19. J. Bisschops, J. Polymer. Sci., 17, 81 (1955).
20. E. Walker, in the book "Fibres from Synthetic Polymers" (Translated into Russian), Inostr. Lit., 1957, chapter 13.
21. R. B. Richards, Trans. Faraday Soc., 42, 16 (1946).
22. A. A. Tager, Uspekhi Khim., 27, 481 (1958).
23. Yu. S. Lipatov, P. I. Zubov, and E. A. Andryushchenko, Kolloid. Zhur., 21, 598 (1959).
24. D. Bourgoin and M. Joly, Kolloid Z., 146, 121 (1956).
25. A. Lottermoser, Z. phys. Chem., 141, 129 (1929).
26. R. Fischer, Kolloid Z., 51, 342 (1930).
27. S. M. Lipatov and S. M. Fel'dman, Kolloid. Zhur., 3, 703 (1937); 6, 806 (1940).
28. S. I. Meerson and S. M. Lipatov, Kolloid. Zhur., 17, 230 (1955).
29. A. V. Nikolaev, R. E. Neiman, and O. V. Neiman, Kolloid. Zhur., 19, 121 (1957).
30. S. M. Lipatov and S. I. Meerson, Kolloid. Zhur., 17, 230 (1955).
31. S. M. Lipatov and S. I. Meerson, Kolloid. Zhur., 19, 390 (1957).
32. I. Eldridge and I. Ferry, J. Phys. Chem., 58, 992 (1954).
33. P. Flory, "Principles of Polymer Chemistry", Cornell Univ., Ithaca, New York, 1953.
34. Yu. S. Lipatov, P. I. Zubov, and M. A. Andryushchenko, Vysokomol. Soed., 1, 425 (1959).
35. A. Walter, J. Polymer. Sci., 13, 207 (1954).
36. H. Boedtker and P. Doty, J. Phys. Chem., 58, 968 (1954).
37. P. Flory and R. Garnett, J. Amer. Chem. Soc., 80, 4836 (1958).
38. E. Heymann, Trans. Faraday Soc., 32, 462 (1936).
39. P. Derksen, Thesis, Amsterdam, 1935 (see Ref. 4).
40. S. M. Lipatov, "Uchenie o Kolloidakh" (The Science of Colloids), Gizlegprom, Moscow, 1933.
41. S. P. Papkov, Kolloid. Zhur., 19, 333 (1957).
42. V. I. Sharkov and R. K. Boyarskaya, Dokl. Akad. Nauk SSSR, 108, 99 (1956).
43. P. Flory, J. Amer. Chem. Soc., 63, 3083 (1941).
44. W. Stockmayer, J. Chem. Phys., 11, 45 (1943); 12, 125 (1944).
45. S. M. Lipatov and P. M. Lapin, Kolloid. Zhur., 3, 723 (1937).
46. N. A. Polyak, Thesis, Institute of Chemistry of the Academy of Sciences of the Byelorussian SSR, Minsk, 1952.
47. L. Aricz, Kolloid-Beih., 7, 40 (1915).
48. R. Krischnamurty, Proc. Roy. Soc., 129, 490 (1930).
49. G. Beyer, J. Phys. Chem., 58, 1050 (1954).
50. V. A. Kargin and N. F. Bakeev, Kolloid. Zhur., 19, 133 (1957).
51. V. A. Kargin and G. L. Slonimskii, Zhur. Fiz. Khim., 15, 1022 (1941).
52. V. A. Kargin and G. L. Slonimskii, Uspekhi Khim., 24, 785 (1955).
53. P. Flory, J. Phys. Chem., 46, 132 (1942).
54. P. I. Zubov, Z. N. Zhurkina, and V. A. Kargin, Kolloid. Zhur. 16, 109 (1954).
55. S. M. Lipatov, Zhur. Obshch. Khim., 3, 694 (1933).
56. I. Bello, H. Riese, and I. Vinograd, J. Phys. Chem., 60, 1299 (1956).
57. V. A. Kargin, G. L. Slonimskii, and A. I. Kitaigorodskii, Kolloid. Zhur., 19, 120 (1957).
58. V. A. Kargin, N. F. Bakeev, and Kh. Vergin, Dokl. Akad. Nauk SSSR, 122, 97 (1958).
59. Guth, James, and Mark, "Sbornik Khimiya Bol'shikh Molekul" (Collection, the Chemistry of Large Molecules), Translated into Russian, No. 1, Inostr. Lit., 1948.
60. S. Sheppard and S. Sweet, J. Amer. Chem. Soc., 43, 539 (1921).
61. H. Freundlich, "Thixotropy" (Translated into Russian), GONTI, 1939.
62. M. Miller and J. Ferry, J. Phys. Chem., 55, 1387 (1951).
63. J. Ferry and J. Eldridge, J. Phys. Chem., 53, 184 (1949).
64. N. Hirai, J. Chem. Soc. Japan, Pure Chem. Sect., 74, 64 (1953).
65. N. Hirai, J. Chem. Soc. Japan, Pure Chem. Sect., 74, 236 (1953).
66. L. V. Ivanova-Chumakova and P. A. Rebinder, Kolloid. Zhur., 18, 683 (1956).
67. Bungenberg de Jong, Kolloid-Beih., 36, 123 (1932).
68. J. H. Northrop and M. Kunitz, J. Gen. Physiol., 8, 317 (1926).
69. I. S. Kistler, J. Phys. Chem., 36, 53 (1932).
70. A. A. Trapeznikov, "Trudy Vsesouznoi Konferentsii po Kolloidnoi Khimii" (Proceedings of the All-Union Conference on Colloid Chemistry), Izd. Akad. Nauk SSSR, 1956.
71. A. Tobolsky, J. Chem. Phys., 14, 93 (1946).

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SODIUM NITROPRUSSIDE AND ITS USE IN ANALYSIS

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INTRODUCTION

Sodium nitroprusside, first prepared in 1850[1], has been used for over a century as a reagent in the qualitative and

quantitative analysis of organic and inorganic compounds. Its use is not yet fully developed, however, and has been increasingly investigated by analysts in recent years. The compound and its analytical applications feature little in Soviet and foreign literature; it is still not widely enough used in analysis, although it is cheap and available in any analytical laboratory, being manufactured in quantity in the Soviet Union. A review of problems connected with the increased analytical use of known reagents is of considerable scientific and practical interest[2], and in this connection a survey is presented of sodium nitroprusside and its analytical applications.

I. PREPARATION

Most of the preparative reactions described are between sodium ferro- or ferri-cyanide and nitric acid[3-7] or sodium nitrite[8-13]. Present-day preparations[14] likewise involve passage of oxides of nitrogen through sodium ferro- or ferri-cyanide solution[8], and the reaction between sodium nitrate and potassium ferrocyanide solution in the presence of sulphuric acid and ferric chloride[15]. Nitroprussides are formed when potassium ferrocyanide and potassium chlorate interact[16], and when potassium ferricyanide and calcium hypochlorite are heated together in solution[17]. Instead of the hexacyanoferrate complex, mercury[18], sodium[19], and potassium[9] cyanides may be used as starting materials, together with various iron compounds. Sodium nitroprusside was originally prepared from gaseous thiocyanates, formed by heating a mixture of tar and sulphur[20]. Electrolysis of potassium ferrocyanide solution also yields nitroprussides[17].

II. STRUCTURE

The accepted general formula $R_2[Fe(CN)_5NO]$ is based on many studies[4,8,9,15,21-33], but the structure is not clear. Some authors consider the NO group to be charged negatively[34], others positively[35]; this is considered in recent monographs on co-ordination compounds[36,37]. Bailar[37] considers the possibilities of the NO group bearing positive, negative, or zero charge, and suggests that it is doubly bonded to the metal atom. Grinberg[38] relates nitroprussides to the pentacyanomonoammines, and suggests that the molecule NO is covalently bonded to the iron(III) atom. Sas[39] considers the iron atom to be divalent, one CN group being bonded to it through nitrogen and the rest through carbon. There is clearly no firm indication yet of the structure of the ion, the valency of the ion, or its bonding with the ligands.

III. PROPERTIES OF SODIUM NITROPRUSSIDE

The compound crystallises readily from aqueous solutions as ruby-red prisms of specific gravity 1.71, which dissolve in water (1 : 2.5) at 16° to give a dark red solution, but are not soluble in absolute ethanol[1,14]. They belong to the bipyramidal class of orthorhombic symmetry[40], and their crystallographic parameters, coefficient of expansion, hardness, refractive index, and magnetic susceptibility are known[21]. Sodium nitroprusside has two molecules of water of crystallisation and the crystals do not deliquesce in air[1]. At 98°–99°, 1.5 water molecules are lost at first, and the rest gradually. In air at 15°–17°, the anhydrous salt readily reverts to the dihydrate[21]. The dihydrate is also reported[14] not to lose water at 100°, to lose half of it at 115°, and to decompose on further heating[21]. Hofmann[24] considers loss of water to be complete after 6 h at 110°, further loss of weight at 160° being due to decomposition. At 200° cyanogen and oxides of nitrogen are evolved, leaving a residue of the ferrocyanide type[41]. Heating to 440° in a sealed vessel causes yellow-brown crystals of formula $Na_2Fe(CN)_4$ to be formed[25].

The absorption spectra of sodium nitroprusside solution have two maxima in the ultra-violet[21], and one in the infra-red[42]. From values of cryoscopic constants[21,32] and the conductivity of solutions at different temperatures, the mobility of the nitroprusside ion has been calculated[21].

The colour of solutions kept in colourless and brown bottles changes to dark blue in sunlight, but does not change in darkness[21,43,44]. Investigations of the mechanism of this change have led to varying results; the products are considered by some authors to be Prussian blue and oxides of nitrogen[44], by others to be Prussian blue and hydrogen cyanide[21,45], or sodium cyanide, nitric oxide, and Prussian blue[46]. The photosensitivity of sodium nitroprusside solution is twenty times greater than that of potassium ferricyanide solution, and may be increased about another twenty times by the addition of ferric chloride; the products of photo-decomposition in the latter case have been shown to be diverse[46-47]. The sensitivity to sunlight of the nitroprussides of uranium, silver, iron, and mercury has been studied[48]. Crystals of sodium nitroprusside decompose to sodium nitrate and cyanide, hydrogen cyanide and ferrous cyanide[49].

Chlorine attacks sodium nitroprusside solutions at room temperature, slowly in darkness, but more rapidly in sunlight to give cyanogen chloride, ferric chloride, sodium chloride, and hydrochloric acid[50]. Hydrogen peroxide and sodium nitroprusside react to give oxygen[51] and sodium pentacyanoaquoferrate(III)[52]. Alkaline potassium permanganate solution gives ferric cyanides and nitrates[53]. Bromine at 120°–140° gives hydrogen cyanide, carbon dioxide, sodium bromide, and ferrous and ferric pentacyano-complexes[15].

Boiling aqueous solutions of sodium nitroprusside evolve nitric oxide at a rate sharply increased by the passage of a stream of oxygen[54]. Thus, oxidants decompose sodium nitroprusside entirely or cause other pentacyano-complexes of iron to be formed.

Hydroxylamine converts sodium nitroprusside to complexes of composition dependent on the pH and temperature[24,55,56]. Sodium nitroprusside is reduced by alkaline phenylhydrazine solution to sodium pentacyanoaquoferrate(II), by sodium bisulphite to sodium pentacyanomonoammineferrate(II)[24], and by metallic sodium in alkaline media to a compound resembling a ferrocyanide in its composition[15]. The products from sodium nitroprusside and sodium amalgam and the zinc–copper couple have been studied[15,57]. Hydrogen does not affect sodium nitroprusside[15]; attempts to hydrogenate it in the presence of platinum black gave no definite results[58]. The successive reactions between sodium nitroprusside and hydrogen sulphide have been studied[21,59,60]. Reducing agents usually lead to the formation of other complex compounds.

Inorganic acids, and their mixtures with ethanol or potassium permanganate, decompose sodium nitroprusside[57,61]. Caustic alkalis and alkaline-earth hydroxides cause an intense yellow colour, due to $Na_4[Fe(CN)_5NO_2]$;[21,62]

the corresponding silver salt has been studied[61]. Spectroscopic study of the reaction with sodium hydroxide indicates the equilibrium[63]:

$$[Fe(CN)_5NO]^{2-} + 2OH^- \rightleftharpoons [Fe(CN)_5NO_2]^{4-} + H_2O.$$

On heating solutions containing sodium nitroprusside and sodium hydroxide, iron and sodium cyanides, nitrates, and other decomposition products are formed[1,15,22,23]. Masal'skii[64] showed that ferrous complexes are formed, of composition depending on the temperature and the concentration of alkali. The yellow coloration is not produced by solutions of ammonia, bicarbonates, or carbonates[62]; ammonia gives sodium cyanamide[65,66], sodium pentacyanomonoammineferrate(II)[67,68], or disodium ammonium pentacyanomonoammineferrate(II)[67]. Sodium nitroprusside rendered anhydrous at 160° takes up two molecules of ammonia[69].

Sodium nitroprusside reacts with potassium cyanide to give potassium ferro-[15,24] or ferric-cyanide[8], but does not react with phosphites, hypophosphites, or antimonites[24].

Potassium, silver, copper, iron, zinc, calcium, and ammonium nitroprussides were first prepared and studied by Playfair[1]. Cadmium, mercury, cobalt, and nickel nitroprussides were prepared later[70], and the product of the reaction between sodium nitroprusside and mercuric oxide was studied[71]. The reactions between sodium nitroprusside and copper, nickel, and cadmium salts in the presence of pyridine yield the compounds[72]:

$$[CuPy_2][Fe(CN)_5NO], \quad [NiPy_4][Fe(CN)_5NO],$$
$$\text{and} \quad [CdPy_2][Fe(CN)_5NO].$$

Methyl, ethyl, and propyl nitroprusside derivatives have been prepared and studied[61]. Tetramethyl and tetraethyl nitroprussides, prepared from the corresponding amines, form sparingly soluble ruby-red prisms[73]. The alkylation of nitroprussic acid yielded tetramethylammonium, diethyl, pyridinium, and benzidinium nitroprussides[74]. The mechanism of formation, and the properties, of compounds from sodium nitroprusside and arylhydroxylamines[75], methylamine, ethylenediamine, and pyridine in the presence of ammonia[76] have been studied. Compounds of sodium nitroprusside with barium formate[77], hexamethylene tetramine[78], and urea[79] are described. The properties have been studied of crystalline compounds formed from nitroprussides and the potassium salts of alkynes[80].

IV. ANALYSIS OF SODIUM NITROPRUSSIDE, AND ITS USE AS AN INDICATOR

The nitroprusside ion may be detected by various microchemical reactions[81]. Sodium nitroprusside is estimated volumetrically with cadmium nitrate[82], and its argentimetric estimation is used for potentiometric titration[83]. Mercurimetric estimation is used[84], and a determination based on the reaction between sodium nitroprusside and sodium tetracyanonickelate is described[85]. Polarography has been applied successfully[86-88].

Sodium nitroprusside is used as an indicator in the volumetric determinations of halides[89,90], cyanides[89], and nitroprussides of alkali metals[82], the titration of ammonium mercaptide with potassium ferricyanide[91], and the estimation of mercuric acetate in non-aqueous solvents[92]. Its use as an indicator is proposed in the mercurimetric estimation of chloride formed on the hydrolysis of chlorobutanol[93], and it is used in conjunction with certain dyes as an indicator in the estimation of reducing sugars[94].

V. ANALYSIS OF INORGANIC COMPOUNDS

The cyanide ion may be detected through the formation of the nitroprusside ion[95]. Very sensitive microchemical tests for zinc[96] and palladium[97] depend on the precipitation of the nitroprussides with sodium nitroprusside, the zinc salt forming characteristic crystals. The formation of metal nitroprussides is used for the volumetric estimation of nickel[98] and copper[99] and the gravimetric estimation of silver[100]. Errors are avoided in the conductimetric titration of heavy metal ions with nitroprusside if ethanol is present[101].

The intense yellow colour given by sodium nitroprusside and caustic alkalis or alkaline-earth hydroxides serves to indicate the presence of these compounds[21]. At room temperature, arsenious oxide dissolved in 20% sodium hydroxide solution gives an intense dark red colour with sodium nitroprusside, although sodium arsenite gives no colour under these conditions[102,103].

A year before nitroprussides were first synthesised, Gmelin[21] observed the colour given by sulphides with the product from nitric acid and "red blood salt" [presumably potassium ferricyanide (Ed. of Translation)], so that the formation of the sulphide–nitroprusside colour was called Gmelin's reaction. Playfair described this reaction in the first description of the synthesis of nitroprussides[1]. Conditions for the analytical use of the reaction were later studied in detail; the optimum concentrations of the reactants were established, the reaction was studied in neutral, alkaline, and acid solution, and in the presence of glycerol, ethanol[104], and phosphates[105], and the influence of respective concentrations was investigated[106]. The colour is given by hydrogen sulphide only with alkaline solutions[104], so that to test for this gas in air, filter paper moistened with ammoniacal sodium nitroprusside solution has been proposed; a purple-violet colour is produced[107]. The sulphide–nitroprusside colour is given in the presence of not only caustic alkalis and ammonia, but also carbonates, borates, silicates, and phosphates, so that it may be used to indicate alkalinity[108]. The colour given by hydrogen sulphide and sodium nitroprusside in the presence of magnesium or calcium carbonate is used to detect sulphides in mineral water[109]. Alkaline solutions of polysulphides mixed with sodium nitroprusside transmit purple light, but reflect blue light[10]. The sensitivity of the sulphide–nitroprusside reaction has been recorded[21,110,111]. The mechanism of the reaction has been much studied; Scagliarini and his coworkers[112-116] investigated it, as well as the products of the decomposition of the coloured complex, the composition of which they showed to accord with the formula $Na_4[Fe(CN)_5NOS]$. Other workers[33,117] studied the reaction mechanism, and suggested the structure[117]:

$$Na_4\left[Fe(CN)_4\left(S{=}S\begin{matrix}S\\S\end{matrix}\right)\right].$$

Scagliarini confirmed the theoretical basis for this structure[118]. Later work[119] showed the coloured complex of Gmelin's reaction to be $(Na_6[Fe_2(CN)_8S_2])_n$. Gmelin's colour reaction is used for the potentiometric estimation

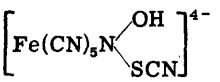
of sulphides[112,120] or the spectrophotometric estimation of small quantities of sulphide ion[121].

The rose colour given by sulphites and sodium nitroprusside is intensified by ferrocyanides and zinc salts[122]. The effects on this reaction of reagent concentrations, pH, and other cations have been studied in detail[123]. The detection of sulphur dioxide in gases has been described[124], using zinc nitroprusside suspension on a glass rod. The sulphite–sodium nitroprusside colour is used to detect sulphur dioxide in drops of sulphite cellulose extract[125], and for the photometric estimation of small amounts of sulphite[121]. The additive formation of the coloured ion $[Fe(CN)_5(NO)(SO_3)]^{4-}$ has been suggested[116,126].

Mixtures of sodium thiosulphate and nitroprusside solutions remain colourless in darkness, but direct sunlight produces a dark blue colour[21], used to detect thiosulphates[95]. A drop method for this detection has been proposed, so that sodium nitroprusside may be used for the consecutive drop analysis of a mixture of sulphide, thiosulphate, and sulphite ions in the presence of sulphate[127].

A yellow colour results from sodium nitroprusside and sodium tetrathionate in the presence of alkali metal carbonates, becoming green after several hours; the change is more rapid in sunlight, while the colour disappears again in darkness[128]. Sodium nitroprusside and alkaline sodium hydrosulphite solutions give a red colour[129].

The colour reaction between potassium thiocyanate and sodium nitroprusside has been studied in alkaline and acetic acid solutions; formation of the ion



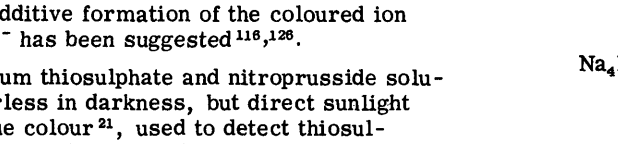
was suggested by Tarugi[130]. Data concerning the reaction mechanism were reviewed critically in the light of the part played by the NO group in the nitroprusside ion[131], but the criticism was refuted by Tarugi, who had investigated the mechanism[132].

The literature indicates that the use of sodium nitroprusside for the qualitative and quantitative analysis of cations is based on the formation of insoluble nitroprussides. A series of anions, all sulphur-bearing, give colorations with sodium nitroprusside, sometimes only after exposure to sunlight. Studies of reaction mechanism indicate that the anions become attached to the NO group, or replace it.

VI. ANALYSIS OF ORGANIC COMPOUNDS

Sodium nitroprusside is widely used as a reagent for organic analysis. Copper nitroprusside, obtained from it, was used in 1857 to detect artificial essential oils, particularly turpentine[133]. Small quantities of pyruvic acid are detected and estimated colorimetrically with sodium nitroprusside[134]; in alkaline solution the latter undergoes a colour reaction with cyclic hydrocarbons, suggested as due to co-ordination of these to the nitroprusside ion[135]. Isophorone is estimated colorimetrically by means of its colour reaction with sodium nitroprusside[136]. Halogen derivatives of some aliphatic amines are detected by means of the colour formed on successive addition to their solution of sodium nitroprusside, caustic alkali, hydrochloric acid, and ferric chloride[137]. An alkaline solution of cane sugar and sodium nitroprusside becomes yellow-brown on boiling, while glucose and invert sugar give this colour at room temperature or at 50°–60°.[138] Sodium nitroprusside is used in the microanalysis of organic compounds containing reactive methylene groups[139]. Sodium nitroprusside solution, hydrogen peroxide, and benzene react in sunlight to give a green colour in the benzene layer, due to o-$C_6H_4(OH)(NO)$.[140]

The use of sodium nitroprusside as a reagent for aldehydes and ketones has been studied in detail. Alkaline formaldehyde solution gives an amber colour[7], due to the compound[129]:



If alkaline formaldehyde solution is treated with sodium nitroprusside and phenylhydrazine in hydrochloric acid, a blue coloration appears which becomes red on addition of ferric chloride and concentrated hydrochloric acid, and then orange[141]; formaldehyde is estimated in this way[142]. A colour reaction was later demonstrated between sodium nitroprusside and formaldehyde in the presence of hydroxylamine[143]. Legal[144] discovered the colour given by acetone and sodium nitroprusside in alkaline or acetic acid solution, and Bitto[145] showed that many aldehydes and ketones may be detected thus; acetyl compounds are also detected[146], but compounds in which the carbonyl group is directly bonded to –OH, –OR, –OM, $-NH_2$, and –Cl groups give a negative reaction[147]. Cambi[148,149] separated and examined the product from sodium nitroprusside and acetone, and suggested that acetone and some other ketones form coloured compounds by addition to the NO group. Other workers investigated the mechanism of the Legal reaction[150,151], the conditions for its occurrence being studied in detail with aliphatic and cyclic aldehydes and ketones[152], acetone[153], acetaldehyde[7,151,153], 2-oxopropan-1,3-diol, methylglyoxal[154], and acetophenone[155]. Stanley[156] established the sensitivity of the reaction, and the absorption maxima of the coloured solutions, for many ketones. Rosenthaler[157] used the Legal reaction to detect citral, citronellol, vinyl acetate, and vinyl ether, and Tanabe and Kamiya[158] used it to detect benzimidazole, quinoxaline, quinazoline, adenine, methionine, and uric acid. It is used to verify the authenticity of pharmaceutical Chloretone [1,1,1-trichloro-2-methyl-2-propanol] and Lobeline [N-methyl-2-(β-hydroxy-β-phenylethyl)-6-phenaryl-piperidine] preparations, which are degraded to ketones by strong alkali[159]. Methods of macro- and spot-test analysis with sodium nitroprusside have been proposed for Novarsenol [Neosalvarsan; Neoarsphenamine; sodium 3,3′-diamino-4,4′-dihydroxyarsenobenzenemethanalsulphoxylate], differentiating this from organic arsenicals of similar structure[160]. The applicability of the Legal reaction to the analysis of compounds which are not aldehydes or ketones is therefore evident.

The sodium hydroxide used in the Legal reaction may be replaced by other bases; aldehydes give a colour reaction with sodium nitroprusside and trimethylamine[161], while the colour given by sodium nitroprusside, ethylamine or ethylenediamine, and acetone distinguishes the latter from formaldehyde and acetaldehyde[162]. Diethylamine and piperidine intensify or modify the colour produced in alkaline solution by sodium nitroprusside and 2-oxopropan-1,3-diol or methylglyoxal[154]. Sodium nitroprusside and piperidine are used to detect acrolein, acetaldehyde, and some other aldehydes[163,164]; compounds containing the CH_3CO-, C_2H_5CO-,

and C_3H_7CO- groups give a red colour under these conditions, but the C_6H_5CO- group gives no colour[166]. A blue colour is given by a mixture of nitroprusside, piperazine, and piperidine and ethyl, propyl, or allyl aldehydes[167]. Some ketones may be detected with sodium nitroprusside and hydroxylamine[143]. Sodium nitroprusside is used to detect ketones mixed with alcohol[168] and in the chromatographic analysis of acetone[169], and has been examined in detail as a means of detecting acetone in pharmaceuticals containing volatile oil of mustard [allyl isothiocyanate (Ed. of Translation)][170-174].

The Legal reaction is used to identify organic compounds which may be oxidised or reduced to aldehydes. Acetaldehyde vapour, from the reduction of chloral with hydrogen, is identified with filter paper moistened with sodium nitroprusside and piperidine[175]. Feigl[176] modified this to a spot reaction by using filter paper moistened with sodium nitroprusside solution containing morpholine, and used this to detect the decomposition products of nitroethane[177], *N*-ethyl and propyl derivatives[178], traces of ethanol[179], and acetic acid impurity in formic acid[180]. Acrolein in air has been detected[181,182] by its well-investigated colour reaction with sodium nitroprusside in the presence of piperidine[163]. Isopropanol is detected, after oxidation to acetone with potassium dichromate solution, with sodium nitroprusside in the presence of ammonia and ammonium chloride[183]. The oxidation products of some alcohols are detected with sodium nitroprusside, piperazine, and piperidine[167]. Formic acid and sodium bisulphite react to give dithionous acid, which gives a green or dark blue colour with sodium nitroprusside[184]. The structure is described of the coloured reaction products from acetic acid and ethyl acetate and sodium nitroprusside in the presence of ammonia and aliphatic amines[185]. Butyric acid is detected by oxidation with hydrogen peroxide to acetoacetic acid, identified by the Legal reaction[186]; lactic acid may be detected with sodium nitroprusside after dehydration[187] or oxidation of silver lactate to acetaldehyde with iodine[187]. Viala and Grignon[188] identified lactic acid in pharmaceuticals by oxidation with bromine water to pyruvic acid, which gives a colour reaction with sodium nitroprusside[189]. Citric acid is identified after conversion to acetonedicarboxylic acid with acetaldehyde and concentrated sulphuric acid[190].

These colour reactions with aldehydes and ketones in the presence of aliphatic and heterocyclic amines are used to detect the latter. The colour reactions between sodium nitroprusside and methylamine, dimethylamine, ethylamine, and other aliphatic amines, in the presence of acetone, have been studied together with the structures of the coloured compounds[191]. A spot test for identifying secondary aliphatic amines with sodium nitroprusside in the presence of acetaldehyde[192] is used to detect secondary aliphatic and heterocyclic amine impurities in primary and tertiary amines[193] and to identify secondary amines and primary alkylamines[194]. Aqueous–alcoholic sodium nitroprusside and propionaldehyde or acetaldehyde are used for the systematic identification of amines[195].

A characteristic colour change occurs when a weakly alkaline solution of diethylamine, sodium nitroprusside, and potassium ferricyanide is heated and then cooled[196]. Sodium nitroprusside is used together with potassium ferricyanide and phenylhydrazine to identify 1-phenyl-2-methylaminopropane, and together with acetone to identify 1-phenyl-2-aminopropane[197]. The NO group is replaced when sodium nitroprusside reacts with alkaline solutions of hydrazine, phenylhydrazine, ethylamine, and diethylamine[198]. An alkaline or acetic acid solution of phenylhydrazine, glucose, and sodium nitroprusside acquires a characteristic colour[199].

Sodium nitroprusside gives colour reactions with some organic compounds containing sulphur. After hydrolysis with sodium hydroxide solution, carbon disulphide and mustard oil form coloured compounds with sodium nitroprusside similar to those of sulphides[200]. The method of detecting mercaptans with sodium nitroprusside[21] has been modified for the detection of small amounts[201], and the estimation[187], of sulphydryl derivatives, and is used for detecting organic sulphides in petroleum[202] and to test the authenticity of the sulphur-bearing pharmaceutical Unithiol [sodium propan-2,3-dithiol-1-sulphonate][203]. No colour reaction is given with aromatic sulphides[204].

Hofmann investigated and proposed a structure for the coloured compound formed by sodium nitroprusside and thiourea[27]; Cambi[205] reviewed this work critically and proposed a different structure. A solution of sodium nitroprusside, thiourea, and mercaptans assumes a characteristic colour in sunlight[206,207]. Okuma[208] used a colour spot test for thiourea and thiosemicarbazide with sodium nitroprusside and hydroxylamine. A more specific and sensitive test using sodium nitroprusside alone was developed for thiosemicarbazide later, whereby it may be distinguished from thiourea and thiosemicarbazones[209]; this test may be modified for application to the pharmaceutical thiosemicarbazones Thibone [Thiacetazone, or 4′-formylacetanilidethiosemicarbazone (Ed. of Translation)] and Cutisone [4-formylcumenylthiosemicarbazone][210]. Sodium nitroprusside is used in spot tests for thioethers and thioketones in pharmaceuticals and biological matter, which yield sulphides on fusion with sodium hydroxide[211]. The same test is used for onion and garlic phytoncidal preparations[210]. Organic compounds containing sulphur may be detected on filter paper, after reduction with sodium in liquid ammonia, with sodium nitroprusside[212].

A ruby red colour is given by alkaline creatinine solution and sodium nitroprusside[213], which disappears on acidification with acetic acid, and on subsequent heating becomes green[145] and then blue; a blue deposit is finally produced[214]. The reaction mechanism[215], the optimal conditions, and the effect of pH have been investigated[216]. Creatine, guanidine, and glycine give no colour reactions[213]. A solution of sodium nitroprusside, turned yellow-brown by sunlight, gives a red colour with creatine, guanidine, and their methyl derivatives[217].

Sodium nitroprusside has been examined as a reagent for phenols and primary aromatic amines. Aqueous or alcoholic phenol solutions, mixed with concentrated sulphuric acid and crystalline sodium nitroprusside, assume various colours according to the nature of the phenol, changed by addition of ammonia[218]. Colour reactions with phenols in the presence of caustic alkalis have been described[129]; ammoniacal pyrocatechol solution gives a cherry red colour[219]. Phenol derivatives and sodium nitroprusside give blue or green colours in the presence of caustic alkalis and hydroxylamine[220]. Aqueous sodium nitroprusside solution, illuminated with ultra-violet light, is used as a macro- and spot-reagent for aniline[221], whereby a simple method of analysis has been devised for antifebrin and phenacetin in medical preparations; the latter is detected in them by this means[222]. Sodium nitroprusside and potassium ferricyanide are used for the macro- and spot-detection of novocaine[223]. For the analysis of sulphanilamide preparations, mixtures of sodium

nitroprusside, sodium hydroxide, and potassium permanganate [224] or diphenylamine, sodium nitroprusside, and concentrated sulphuric acid [225] are proposed. Reduction products of some sulphanilamide preparations may be detected with sodium nitroprusside paper [226]. A later colour reaction for sulphanilamide is used for the systematic qualitative analysis of such preparations [227].

Sodium nitroprusside is used as a reagent for heterocyclic compounds and some biologically active natural products; α-substituted pyrroles give characteristic colours [228], and alkaline indole solution assumes a muddy brown colour which acetic acid converts to blue [114]. Methyl indole gives a violet colour under these conditions only when the acetic acid solution is boiled [187]. The reaction of sodium nitroprusside with antipyrine [229], and its coloured products with antipyrine [230,231] and pyramidone [232] in the presence of iron salts, are described. Regitine [2-(*N'*-*p*-tolyl-*N'*-*m*-hydroxyphenylaminomethyl)-2-imidazoline] has been detected with sodium nitroprusside [233], and the method has been modified for the identification of imidazole derivatives [234] and their colorimetric estimation in pharmaceuticals [235]. Hydantoin reacts with alkaline sodium nitroprusside solution to give the coloured compound $Na_3[(CN)_5FeNOCH.NH.CO.NH.CO]$, which is hydrolysed to parabanic acid [oxalylurea] and hydroxylamine [236]. 2,3-Dimethylbenzothiazole gives an intense dark blue colour with alkaline sodium nitroprusside [237].

Wachsmuth [238] suggested that the colour reaction between sodium nitroprusside and isonicotylhydrazide be used to test the authenticity of pharmaceutical preparations of Tubazid [Isoniazid or isonicotinic acid hydrazide] [239]. Sodium nitroprusside is used for the spectrophotometric estimation of isonicotinic nitrile [240], the colorimetric estimation of pyrazinamide [241], and the gravimetric estimation of pharmaceutical preparations of Chlorpromazine (Aminazine) [2-chloro-10-(3-dimethylaminopropyl)-phenothiazine] [242]. The microchemical reaction of sodium nitroprusside with Demerol [ethyl-1-methyl-4-phenylpiperidine-4-carboxylate] has been described [243].

Dilute sodium nitroprusside solution is coloured pink by coniine [187]. The composition and properties have been studied of the compounds formed by sodium nitroprusside with strychnine, brucine, quinine, and quinidine [244]. Sodium nitroprusside is used in the microanalysis of strychnine [245] and brucine [246]. A colour reaction with sodium nitroprusside is used to detect methylnarceine impurity in narceine [247]. Feigl and Silva [248] used a micro-method of detecting ephedrine based on a reaction between its decomposition products and sodium nitroprusside solution containing morpholine. Solutions of vitamin B_1 and sodium nitroprusside give a greenish colour when alkaline, turning violet on acidification and then pale blue [249]. Sodium nitroprusside forms red compounds with cardiac glycosides, the aglycones of which contain an unsaturated five-membered lactone ring in the side chain (digitalis, strophanthus, and oleander glycosides); no colour reaction occurs with aglycones containing an unsaturated six-membered lactone ring (sea onion glycoside) [178]. This reaction is used also by the pharmacopeia [250] as a tentative test for the authenticity of crystalline cardiac glycosides and pharmaceutical preparations in which they are present. Dihydrostreptomycin is estimated colorimetrically with sodium nitroprusside and potassium ferrocyanide [251].

Division into three groups of the reactions between sodium nitroprusside and organic and inorganic compounds has been proposed: (1) those involving addition of inorganic ions or organic molecules to the NO group, giving complicated complex anions; (2) those involving replacement of the NO group by NH_3 or H_2O to give new pentacyano--complexes, which then form coloured compounds with organic substances; and (3) those involving replacement of the CN group, as with hydroxylamine [252]. The reactions considered above belong mainly to groups (1) and (3). The use in analysis of other iron pentacyano-complexes, first obtained by Hofmann [27] from sodium nitroprusside, has been considered. Sodium pentacyanoaquoferrate(II) and pentacyanoammineferrate(II) are used to analyse thioketones and thioaldehydes [253], aromatic nitroso- and nitro--compounds, hydrazine derivatives [254], aromatic and α- and β-unsaturated aldehydes [255], and derivatives of imidazole [256] and pyrazinamide [241]. Grote [206] showed that sodium pentacyanoaquoferrate(III) forms coloured compounds with sulphydryl derivatives; Grote's reagent is used for the colorimetric estimation of thiouracil [257], methylthiouracil [258], and other sulphur-bearing organic compounds [259]. Sodium pentacyanoaquoferrate(III) is used in the spot testing of primary aromatic amines [260]. Herington [261-263] studied the composition, properties, and mechanism of formation of the reaction products from sodium pentacyanoammineferrate(III) and aromatic amines. Iron pentacyano-complexes and hydrogen peroxide are used for the qualitative analysis of some sulphur-bearing organic compounds and primary aromatic amines [264] and, together with hydroxylamine, for detecting thiourea, thiosemicarbazide [208], and phenol derivatives [220].

It is clear from the above that sodium nitroprusside has been most thoroughly investigated as a reagent for aldehydes and ketones, and compounds which may be converted to them. It is also used in the analysis of hydrocarbons, phenols, amino-derivatives, sulphur compounds, heterocyclics, and other organic compounds. Irradiation of the solution with ultra-violet light may be used, or other reagents may be added — potassium ferro- or ferri-cyanide, hydrogen peroxide, phenylhydrazine, hydroxylamine, or ferric chloride. Other iron pentacyano-complexes may be used. Interaction of sodium nitroprusside and organic compounds involves either replacement of the NO group or the addition of the organic compound to the complex anion. Sodium nitroprusside cannot be regarded as a general reagent for defined groups of organic compounds since different conditions are needed for the analysis of each one, and varying colours are produced by the compounds in any group.

VII. BIOCHEMICAL ANALYSIS

Sodium nitroprusside is used to detect compounds bearing sulphydryl groups in tissues [265]. The influence of pH, and its optimal value, have been determined [216]; the reaction mechanism between sodium nitroprusside and cysteine has been studied [266]. This reaction has been studied spectrophotometrically [121] and used for the colorimetric estimation of cysteine in cystine [267]. Cysteine gives no colour with sodium nitroprusside in the presence of hydrogen peroxide [237]. Sodium nitroprusside solution (10%) is a reagent for the colorimetric estimation of methionine [268]. The optimum conditions for the colour reaction between glutathione and sodium nitroprusside have been investigated [216] and a structure suggested for the product [269]; the reaction is used to detect glutathione and its derivatives in the organs of white mice [270] and to estimate

glutathione in normal tissues and tumours[271]. Arnold[272] showed that proteins containing cysteine are slowly coloured purple-red by sodium nitroprusside; the use of this reaction was developed later[273]. The sulphydryl group of keratin, treated with mild alkali, gives a pale red-violet colour with sodium nitroprusside, in contrast to other thiols[274]. Albumin gives a colour after prolonged boiling with sodium hydroxide solution[21]. A crystalline compound has been obtained from sodium nitroprusside and albumin from human blood serum[275]. Sodium nitroprusside is used, together with benzidine, fuchsine, and hydrogen peroxide in blood tests[276,277]. On account of its great capacity for adsorbing arsine, sodium nitroprusside is used to complex the latter in blood[60]. Paper soaked in alkaline sodium nitroprusside solution is recommended for detecting thioglycollic acid in hair[278]. Urine passed after muscular exertion gives a characteristic colour with sodium nitroprusside in alkaline or acetic acid solution[279]; the use of this reaction in biochemical analysis has been described in detail[280-282]. Sodium nitroprusside is used for the detection and differentiation of some bacteria[283-285]. Legal's reaction[144] was first used by him to detect acetone in urine; modifications of this test have been proposed[286-291]. A review, critical of some of these modifications, was based on interference by sulphides[292-294]. Methods of detecting acetone in biological liquids by means of sodium nitroprusside and ferric chloride have been comparatively assessed[295]. The Legal reaction is used to estimate acetone in urine and other biological liquids[296,297].

Sodium nitroprusside is therefore used to detect and estimate sulphydryl derivatives and amino-acids, polypeptides and proteins containing sulphur; it is used for microbiological tests, and for blood and urine analysis.

1. L. Playfair, Annalen, 74, 317 (1850).
2. A. K. Babko, Zavod. Lab., 25, 515 (1959).
3. A. Overbeck, Arch. Pharm., 122, 270 (1853).
4. I. Kyd, Annalen, 74, 340 (1850).
5. R. Wild, Arch. Pharm., 131, 26 (1855).
6. A. Schafarik, J. prakt. Chem., 90, 19 (1863).
7. F. Hyde. J. Amer. Chem. Soc., 19, 23 (1897).
8. E. Hadow, J. Amer. Chem. Soc., 2, 341 (1866); Annalen, 141, 125 (1867).
9. G. Städeler, Annalen, 151, 8 (1869).
10. M. Prud'homme, Compt. rend., 111, 45 (1890).
11. C. Deventer, Ber., 26, 589 (1893).
12. P. Schwarzkopf, Chem. Zentr., 11, 1536 (1912).
13. O. Baudisch, Ber., 62, 2700 (1929).
14. Yu. V. Karyakin and I. I. Angelov, "Chistye Khimicheskie Reaktivy" (Pure Chemical Reagents), Goskhimizdat, Moscow, 1955, p. 391.
15. W. Weith, Annalen, 147, 312 (1868).
16. E. Davy, J. prakt. Chem., 59, 502 (1853).
17. H. Jensen, J. Pharm. Chim., 11, 315 (1885).
18. Z. Roussin, Ann. Chim. Phys., 52, 30 (1858).
19. W. Manchot and E. Enk, Annalen, 470, 283 (1929).
20. L. Potsdamer, J. Ind. Eng. Chem., 11, 769 (1919).
21. "Gmelins Handbuch der anorganischen Chemie", Vol. 59, 111 Berlin, 1932, pp. 734, 900.
22. J. Gentele, J. prakt. Chem., 64, 193 (1858); Chem. Zentr., 843 (1858).
23. O. Pawel, Ber., 15, 2612 (1882).
24. K. Hofmann, Z. anorg. Chem., 12, 146 (1896).
25. E. Etard and G. Bemont, Compt. rend., 99, 1024 (1884).
26. N. Dhar, Z. anorg. Chem., 80, 43 (1913).
27. K. Hofmann, Annalen, 312, 1 (1900).
28. A. Miolati, Z. anorg. Chem., 25, 318 (1900).
29. G. Deniges, Bull. Soc. chim., 19, 79 (1916).
30. J. Friend, J. Chem. Soc., 109, 715 (1916).
31. E. Turner, J. Chem. Soc., 109, 1130 (1916).
32. G. Burrows and E. Turner, J. Chem. Soc., 115, 1429 (1919).
33. E. Justin-Mueller, J. Pharm. Chim., 22, 496 (1935).
34. L. Cambi, Z. anorg. Chem., 247, 22 (1941).
35. W. Hieber and F. Seel, Z. anorg. Chem., 249, 308 (1942).
36. F. Hein, Chemische Koordinationslehre, Leipzig, 1954, pp. 255, 363.
37. J. C. Bailar and D. H. Busch, "The Chemistry of the Co-ordination Compounds", New York, 1956, pp. 531, 538.
38. A. A. Grinberg, "Vvedenie v Khimiyu Kompleksnykh Soedinenii" (Introduction to the Chemistry of Complex Compounds), Goskhimizdat, Moscow–Leningrad, 1945, p. 152.
39. F. Sas, Anales real Soc. españ. Fís. Quím., 39, 55 (1943).
40. P. Cooke, Nature, 157, 518 (1946).
41. A. Perret and A. Gislon, Bull Soc. chim., 53, 283 (1933).
42. K. Shinra and I. Masuda, J. Chem. Soc. Japan, 75, 1211 (1954); Chem. Abs., 49, 10 055i (1955).
43. I. M. Kolthoff, Z. analyt. Chem., 62, 216 (1932).
44. A. Overbeck, Arch. Pharm., 124, 189 (1853).
45. T. Pavolini, Giorn. Farmac. Chim., 79, 119 (1930); Chem. Zentr., I, 259 (1931).
46. E. Justin-Mueller, Bull. Soc. chim., 2, 1932 (1935).
47. A. Vogel, N. Rep. Pharm., 20, 520 (1871); Chem. Zentr., 665, (1871).
48. H. Leffmann and C. Pines, Bull. Wagner Free Inst. Science, Philadelphia, 4, 41 (1929); Chem. Zentr., I, 3175 (1930).
49. R. Bahadur, Bull. of the College of Agric. Tokyo, 6, 177 (1904); Chem. Zentr., II, 248 (1904).
50. E. Davy, Ber., 11, 1834 (1878).
51. M. Qureshi, J. Phys. Chem., 35, 656 (1931); Chem. Zentr., I, 2440 (1931).
52. B. Lal, Proc. Indian Acad. Sci., 14A, 652 (1941); Chem. Abs., 36, 5429^2 (1942).
53. S. Cloëz and E. Guignet, Annalen, 108, 378 (1858).
54. O. Baudisch, Ber., 52, 35 (1919).
55. K. Hofmann and H. Arnoldi, Ber., 39, 2204 (1906).
56. J. Giral, Anales real Soc. españ. Fís. Quím., 42, 236 (1923); Chem. Zentr., III, 612 (1923).
57. K. Bhaduri. Z. anorg. Chem., 84, 95 (1913).
58. H. Gall and W. Manchot, Ber., 58, 482 (1925).
59. D. Venditori, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 22, I, 162 (1913); Chem. Zentr., I, 1501 (1913).
60. R. Meissner, Z. exp. Path. Ther., 13, 293 (1913); Chem. Zentr., II, 705 (1913).
61. K. Hofmann, Z. anorg. Chem., 11, 278 (1896).
62. S. Reichard, Z. analyt. Chem., 43, 275 (1904).
63. L. Cambi and L. Szegö, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 5, 737 (1927); Chem. Zentr., II, 1670 (1927).
64. V. L. Masal'skii and A. T. Chernyi, Zhur. Obshch. Khim., 6, 37 (1936).
65. K. Hofmann, Z. anorg. Chem., 10, 262 (1895).
66. K. Hofmann, Z. anorg. Chem., 11, 31 (1896).
67. W. Manchot, Ber., 45, 2869 (1912).
68. F. Ephraim and F. Moser, Ber., 53, 548 (1920).
69. W. Peters, Z. anorg. Chem., 77, 143 (1912).
70. T. Norton, Amer. Chem. J., 10, 222 (1888); Chem. Zentr., 920 (1888).
71. E. Montignie, Bull. Soc. chim., 3, 459 (1936); Chem. Abs., 30, 3348^7 (1936).
72. E. Vascautanu, Ann. sci. univ. Jassy, 27, 117 (1941); Chem. Abs., 37, 2291^6 (1943).

73. O. Bernheimer, Wien. Anz., 196 (1879); Chem. Zentr., 580 (1879).
74. G. Burrows and E. Turner, J. Chem. Soc., 119, 1450 (1921).
75. L. Cambi and T. Rucci, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 11, 443 (1930); Chem. Zentr., II, 378 (1930).
76. W. Manchot and P. Woringer, Ber., 46, 3514 (1913).
77. R. Weinland and A. Henrichsen, Ber., 56, 528 (1923).
78. E. Voyatzakis, Chem. Zentr., 203, 1365 (1936).
79. W. Fearon, Analyst, 62, 586 (1937).
80. R. Nast and F. Urban, Z. anorg. Chem., 289, 244 (1957).
81. L. Yanowski and W. Hynes, Mikrochem., 29, 1 (1941).
82. H. Fonzes-Diaçon and P. Carquet, Bull. Soc. chim., 29, 636 (1903).
83. O. Tomicek and Z. Rektorik, Coll. Czech. Chem. Comm., 5, 129 (1933); Chem. Abs., 27, 2907[3] (1933).
84. C. Tomicek and J. Kubik, Coll. Czech. Chem. Comm., 9, 377 (1937); Chem. Abs., 32, 81 (1938).
85. P. Rose, Z. analyt. Chem., 143, 195 (1954).
86. P. Lanza and A. Corbellini, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 14, 65 (1953).
87. I. M. Kolthoff and P. Toren, J. Amer. Chem. Soc., 75, 1197 (1953).
88. P. Zuman and M. Kabat, Chem. Listy, 48, 368 (1954).
89. E. Votocek, Chem.-Ztg., 42, 257 (1918); Chem. Zentr., II, 560 (1918).
90. F. König, Z. analyt. Chem., 68, 385 (1926).
91. A. Laur, Acta et Comment. Univ. Tartuensis, 12, 50 (1927); Chem. Zentr., II, 140 (1927).
92. O. Tomicek, Sborn. Celostatni Pracovni Konf. Anal. Chem., 1, 246 (1952); Chem. Abs., 49, 15 603b (1955).
93. S. Vach and F. Horak, Pharmazie, 10, 596 (1955).
94. I. Napoli, Indian. J. Appl. Chem., 15, 125 (1933); Chem. Zentr., I, 3989 (1933).
95. F. P. Treadwell and W. T. Hall, "Analytical Chemistry, Vol. 1 Qualitative Analysis" (Translated into Russian), Goskhimizdat, Moscow–Leningrad, 1946, p. 365.
96. H. Bradley, Amer. J. Sci., 22, 326 (1906); Chem. Zentr., II, 1873 (1906).
97. V. N. Ivanov, Zhur. Russ. Fiz.-Khim. Obshch., 54, 701 (1922–23).
98. G. Zuccari, Ann. Chim. appl., 3, 277 (1915); Chem. Zentr., II, 247 (1917).
99. G. Joret, Ann. Fals. Fraudes, 15, 354 (1922); 16, 47 (1923); Chem. Zentr., II, 295 (1923); IV, 42 (1923).
100. I. M. Kolthoff, Z. analyt. Chem., 62, 209 (1922).
101. E. Voyatzakis and D. Jannakoudakis, Compt. rend., 247, 1590 (1958).
102. A. Gutmann, Ber., 55, 3007 (1922).
103. A. Gutmann, Z. analyt. Chem., 66, 224 (1925).
104. J. Virgili, Z. analyt. Chem., 45, 409 (1906).
105. L. Rossi, Quimica e Industria, 3, 289 (1926); Chem. Zentr., I, 633 (1927).
106. W. Manchot and J. Düsing, Ber., 63, 1228 (1930).
107. H. Kral, Z. analyt. Chem., 36, 696 (1897).
108. L. Filhol, Compt. rend., 66, 1155 (1869).
109. A. Bechamp, Compt. rend., 62, 1087 (1866).
110. A. Bechamp, Z. analyt. Chem., 9, 77 (1870).
111. C. Reichard, Z. analyt. Chem., 43, 222 (1904).
112. G. Scagliarini and P. Pratesi, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 7, 75 (1928); Chem. Zentr., I, 737 (1929).
113. G. Scagliarini and P. Pratesi, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 13, 199 (1931); Chem. Zentr., II, 472 (1931).
114. G. Scagliarini and F. Gentile, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 17, 398 (1933); Chem. Zentr., II, 356 (1933).
115. G. Scagliarini and F. Monforte, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 20, 41 (1934); Chem. Abs., 29, 3622[8] (1935).
116. G. Scagliarini, Atti IV congr. chim. pura appl., 597 (1933); Chem. Abs., 29, 2872[9] (1935).
117. F. Sas, Anales real Soc. españ. Fís. Quím., 34, 419 (1936); Chem. Abs., 30, 5900[9] (1936).
118. G. Scagliarini, Gazzetta, 66, 693 (1936); Chem. Abs., 31, 2955[7] (1937).
119. V. Brustier and H. Garcia-Fernandes, Bull. Soc. chim., 1262 (1950).
120. G. Scagliarini and P. Pratesi, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 11, 193 (1930); Chem. Zentr., I, 3812 (1930).
121. R. Dworzak, K. Becht, L. Reitter, and E. Ruf, Z. anorg. Chem., 285, 143 (1956).
122. C. Boedeker, Annalen, 117, 193 (1861).
123. J. Fages, Compt. rend., 134, 1143 (1902).
124. E. Eegriwe, Z. analyt. Chem., 65, 182 (1924).
125. L. M. Kul'berg and N. I. Chechenev, Zavod. Lab., 14, 790 (1948).
126. G. Scagliarini, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 22, 155 (1935); Chem. Abs., 30, 6670[4] (1936).
127. V. G. Belikov, Nauch. Dokl. Vys. Shkoly, Khim. i Khim. Tekhnol., No. 1, 108 (1959).
128. A. Gutmann, Ber., 41, 300 (1908).
129. R. Brunner, Z. anorg. Chem., 190, 384 (1930).
130. N. Tarugi, Ann. Chim. appl., 16, 407 (1926).
131. L. Cambi, Ann. Chim. appl., 17, 55 (1927).
132. N. Tarugi, Ann. Chim. appl., 17, 519 (1927).
133. G. Heppe, Arch. Pharm., 139, 57 (1856).
134. L. J. Simon and L. Piaux, Bull. Soc. Chim. biol., 6, 477 (1924); Chem. Zentr., II, 1490 (1924); Chem. Abs., 18, 3200[2] (1924).
135. G. Scagliarini and E. Lucchi, Boll. sci. Fac. Chim. ind., Bologna, 58 (1940); Chem. Abs., 34, 2287[7] (1940).
136. R. Carpignano, Ann. Chim., 45, 81 (1955).
137. M. Passerini, P. Papini, and A. Longo, Sperimentale, Sez. Chim. biol., 2, 70 (1951); Chem. Abs., 46, 1084c (1952).
138. K. Lasch, Report. anal. Chem., 4, 376 (1884); Chem. Zentr., 893 (1884).
139. H. Ishidate and M. Akatsuka, Kagaku no Ryoiki, 3, 600 (1949); Chem. Abs., 46, 4463h (1952).
140. O. Baudisch, Science, 108, 443 (1948).
141. E. Rimini, Z. analyt. Chem., 42, 451 (1903).
142. C. Goldschmidt, J. prakt. Chem., 72, 536 (1905); Chem. Zentr., I, 402 (1906).
143. P. Pratesi, Giorn. chim. ind. appl., 13, 513 (1931); Chem. Zentr., I, 846 (1932).
144. E. Legal, Z. analyt. Chem., 22, 464 (1883).
145. B. Bitto, Annalen, 267, 372 (1892).
146. G. Deniges, Bull. Soc. chim., 15, 1058 (1896).
147. G. Deniges, Bull. Soc. chim., 17, 381 (1897).
148. L. Cambi, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 22, 376 (1913); Chem. Zentr., I, 1756 (1913).
149. L. Cambi, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 23, 812 (1914); Chem. Zentr., II, 1099 (1914).
150. F. Sas, Anales real Soc. españ. Fís. Quím., 32, 985 (1934); Chem. Abs., 29, 1355[3] (1935).
151. J. Zwikker, Pharm. Weekblad, 67, 560 (1930).
152. T. Pavolini, Boll. chim. farm., 69, 713 (1930); Chem. Zentr., I, 117[2] (1931).
153. H. Urk, Z. analyt. Chem., 70, 252 (1927).
154. C. Neuberg, Biochem. Z., 71, 154 (1915).
155. W. Küster, Z. physiol. Chem., 155, 157 (1926).
156. T. Stanley, Chem. Analyst, 47, 91 (1958).
157. L. Rosenthaler, Pharm. Acta Helv., 33, 269 (1958).
158. Y. Tanabe and A. Kamiya, Ann. Report Fac. Pharm., Kanazawa Univ., 6, 12 (1956); Ref. Zhur. Khim., 48 362 (1957).

159. V. G. Belikov, Med. Prom. SSSR, No. 6, 31 (1958).
160. V. G. Belikov, Izv. Vys. Shkoly, Khim. i Khim. Tekhnol., No. 1, 78 (1960).
161. M. Simon, Z. analyt. Chem., 38, 457 (1899).
162. H. Leffmann and C. Pines, Bull. Wagner. Free Inst. Science Philadelphia, 4, 39 (1929); Chem. Zentr., I, 3218 (1930).
163. L. Lewin, Ber., 32, 3388 (1899).
164. G. Heyl, Apoth.-Ztg., 28, 165 (1913).
165. G. Gough and H. King, J. Chem. Soc., 2426 (1928).
166. T. Higasi, Chem. News, 139, 244 (1929); Chem. Zentr., I, 302 (1931).
167. J. Sanchez, Semana med., 33, 640 (1926); Chem. Zentr., I, 1622 (1927).
168. L. Rosenthaler and G. Vegezzi, Mitt. Gebiete Lebensmitteluntersuch. und Hyd., 44, 475 (1953); Ref. Zhur. Khim., 29 243 (1955).
169. F. M. Shemyakin and O. S. Lobakhina, Zhur. Anal. Khim., 10, 65 (1955).
170. C. Leuken, Apoth.-Ztg., 20, 609 (1905).
171. E. Lücker, Apoth.-Ztg., 20, 725 (1905).
172. F. Barth, Apoth.-Ztg., 20, 758 (1905).
173. J. Ziegler, Apoth.-Ztg., 20, 779 (1905).
174. G. Fendler and C. Mannich, Apoth.-Ztg., 20, 788 (1905).
175. T. Jona, Z. analyt. Chem., 52, 230 (1913).
176. F. Feigl and C. Stark, Mikrochim. Acta, 996 (1955).
177. F. Feigl and D. Goldstein, Analyt. Chem., 29, 1521 (1957).
178. F. Feigl and E. Silva, Analyst, 82, 582 (1957).
179. F. Feigl and C. Stark, Chem. Analyst, 45, 39 (1956).
180. F. Feigl and C. Stark, Chem. Analyst, 45, 46 (1956).
181. I. M. Korenman and Ya. B. Reznik, Gigena, Bezopasnost' i Patalogiya Truda, No. 8–9, 108 (1930).
182. I. M. Korenman, M. G. Tenenbaum, and F. S. Forum, Lab. Prakt., No. 9–10, 18 (1933).
183. J. Rae, Z. analyt. Chem., 72, 392 (1927).
184. E. Comanducci, Z. analyt. Chem., 60, 424 (1921).
185. F. Sas, Anales real Soc. españ. Fís. Quím., 33, 247 (1935); Chem. Abs., 29, 4740[8] (1935).
186. G. Deniges, Z. analyt. Chem., 60, 426 (1921).
187. K. Bauer, "The Analysis of Organic Compounds" (Translated into Russian), Inostr. Lit., Moscow, 1953, pp. 162, 248, 312, 408, 440.
188. A. Viala and H. Grignon, Trav. Soc. pharmac. Montpellier, 17, 154 (1957); Ref. Zhur. Khim., 50 722 (1959).
189. C. Ward, J. Chem. Soc., 123, 2207 (1923).
190. B. Merck, Pharm. Ztg., 48, 894 (1903).
191. F. Sas, Anales real Soc. españ. Fís. Quím., 32, 1187 (1934); Chem. Abs., 29, 3312[7] (1935).
192. F. Feigl and V. Anger, Mikrochim. Acta, 1, 127 (1937).
193. L. M. Kul'berg, G. S. Al'terzon, and R. P. Vel'tman, "Kapel'nyi Analiz" (Drop Analysis), Goskhimizdat, Moscow–Leningrad, 1951, p. 397.
194. F. Brown and J. Wolt, Proc. Louisiana Acad. Sci., 18, 95 (1956); Ref. Zhur. Khim., 19 697 (1957).
195. I. Bertetti, Ann. Chim. (Italy), 43, 351 (1953); 44, 313 (1953); Ref. Zhur. Khim., 26 527, 26 528 (1955).
196. S. Okuma, J. Pharm. Soc. Japan, 74, 220 (1954); Chem. Abs., 48, 7482 (1954).
197. Nagasava and S. Okuma, J. Pharm. Soc. Japan, 74, 773 (1954); Ref. Zhur. Khim., 10 171 (1956).
198. E. Biesalsci and O. Hauser, Z. anorg. Chem., 74, 384 (1912).
199. W. Parri, Giorn. Farm. Chim., 73, 153 (1924); Chem. Zentr., II, 1788 (1924).
200. A. Oppenheim, J. prakt. Chem., 81, 305 (1860).
201. R. Fleming, Compt. rend., 104, 831 (1930).
202. J. Jimenez and L. Bermejo, Anales real Soc. españ. Fís. Quím., 31, 267 (1933); Chem. Zentr., II, 418 (1933).
203. A. N. Oboimakova and S. A. Malykhina, Aptechnoe Delo, 6, No. 2, 92 (1957).
204. L. Bermejo and J. Herrera, Anales real Soc. españ. Fís. Quím., 31, 48 (1933); Chem. Zentr., II, 418 (1933).
205. L. Cambi, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 24, 434 (1915); Chem. Zentr., I, 1141 (1916).
206. I. Grote, J. Biol. Chem., 93, 25 (1931).
207. I. Grote and J. Barnett, J. Chem. Educ., 10, 43 (1933); Chem. Zentr., I, 3153 (1933).
208. S. Okuma, J. Pharm. Soc. Japan, 74, 220 (1954); Chem. Abs., 48, 7482 (1954).
209. V. G. Belikov, Zavod. Lab., 25, 931 (1959).
210. V. G. Belikov, Med. Prom. SSSR, No. 4, 52 (1959).
211. V. G. Belikov, Zhur. Anal. Khim., 15, 612 (1959).
212. L. Jirousek, Chem. Listy, 52, 1553 (1958).
213. T. Weyl, Ber., 11, 2175 (1878).
214. E. Salkowski, Z. physiol. Chem., 4, 133 (1880); Chem. Zentr., 421 (1880).
215. G. Scagliarini and P. Pratesi, Atti Accad. naz. Lincei, Rend. Classe Sci. mat. nat., 10, 261 (1929).
216. P. Zimmet and J. Perrenoud, Bull. Soc. Chim. biol., 18, 1704 (1936).
217. O. Tieg, Ber. ges. Physiol., 36, 383 (1926); Chem. Zentr., II, 3103 (1926).
218. L. Ekkert, Z. analyt. Chem., 78, 235 (1929).
219. J. Sivadjian, J. Pharm. Chim., 13, 528 (1931).
220. S. Ohkuma, J. Pharm. Soc. Japan, 72, 872 (1952); Chem. Abs., 46, 9473b (1952).
221. V. G. Belikov, Zavod. Lab., 23, 1437 (1957).
222. V. G. Belikov, Uchenye Zapiski Pyatigorskogo Pharm. Inst., 2, 266 (1957).
223. V. G. Belikov, Uchenye Zapiski Pyatigorskogo Pharm. Inst., 2, 261 (1957).
224. A. Roux, Ann. pharm. franç., 6, 107 (1948); Chem. Abs., 42, 7488i (1948).
225. M. Filho and E. Aichinger, Tribuna farm. (Brazil), 21, 25 (1953); Chem. Abs., 47, 9572c (1953).
226. S. I. Burmistrov, Pharmatsiya, No. 2, 29 (1946).
227. V. G. Belikov, Uchenye Zapiski Pyatigorskogo Pharm. Inst., 3, 113 (1959).
228. G. Scagliarini, Atti Accad. naz. Lincei, Rend. Classe Sci. mat. nat., 24, 294 (1936); Chem. Abs., 31, 7421[7] (1937).
229. M. Schuyten, Chem. Zentr., II, 1362 (1901).
230. J. Foucry, J. Pharm. Chim., 17, 432 (1933).
231. S. I. Gusev and R. G. Beiles, Zhur. Obshch. Khim., 21, 1971 (1951).
232. J. Dubsky, E. Krametz, and J. Trtilek, Pub. faculte sci. univ. Masaryk, 223, 7 (1936); Chem. Abs., 30, 5901[6] (1936).
233. H. Laubie, Bull. Soc. pharmacie Bordeaux, 91, 109 (1953).
234. H. Laubie, Bull. Soc. pharmacie Bordeaux, 93, 67 (1955).
235. S. Slack and W. Mader, J. Amer. Pharmaceut. Assoc. (Sci. Edn.), 46, 742 (1957).
236. G. Travagli, Ann. Chim. appl., 29, 479 (1939).
237. A. Schoberl, M. Kawohl, and R. Hamm, Ber., 84, 571 (1951).
238. H. Wachsmuth, J. Pharm. Belg., 8, 561 (1953).
239. A. N. Oboimakova and S. A. Malykhina, Aptechnoe Delo, 5, No. 5, 62 (1956).
240. W. Czerwinski, Chem. Analit., 4, 47 (1958).
241. K. Kakemi, T. Uno, and H. Sezaki, J. Pharm. Soc. Japan, 76, 273 (1956); Ref. Zhur. Khim., 4857 (1957).
242. C. Analoguidis, Praktika Tis Akademias Afinon, 31, 273 (1957); Ref. Zhur. Khim., 65 404 (1959).
243. J. Levine, Ind. Eng. Chem., Analyt., 16, 408 (1944).
244. M. Greshoff, Pharm. Weekblad, 40, 541 (1903).
245. M. Wagenaar, Pharm. Weekblad, 66, 1073 (1929).
246. M. Wagenaar, Pharm. Weekblad, 66, 1070 (1929).
247. J. Zwikker, Pharm. Weekblad, 66, 445 (1929).
248. F. Feigl and E. Silva, J. Amer. Pharmaceut. Assoc. (Sci. Edn.), 47, 460 (1958).
249. H. Wachsmuth, J. Pharm. Belg., 5, 300 (1950).

250. "Gosudarstvennaya Pharmakopeya" (National Pharmacopeia), Medgizdat, Moscow, 8th Edition, 1952.
251. O. Angeles and O. Vivina, Bol. Soc. quim. Peru, 21, 189 (1955); Ref. Zhur. Khim., 12 253 (1958).
252. M. Ishidate and T. Sakaguchi, J. Pharm. Soc. Japan, 70, 308 (1950); Chem. Abs., 45, 971e (1951).
253. H. Schwechten, Ber., 65, 1734 (1932).
254. P. Feigl, V. Anger, and O. F. Frehden, Mikrochem., 15, 181 (1934).
255. F. Feigl, V. Anger, and R. Zappert, Mikrochem., 15, 192 (1934).
256. S. Kikui Kikuchi, J. Pharm. Soc. Japan, 76, 1290 (1956); Ref. Zhur. Khim., 48 361 (1957).
257. R. Williams, B. Jandorf, and G. Kay, J. Lab. Clin. Med., 29, 329 (1944).
258. H. Genderen and K. Lier, J. Biochem. Biophys. Acta, 2, 482 (1948).
259. J. Hollman and T. de Jonge, Pharm. Weekblad, 83, 1 (1948).
260. V. Anger, Mikrochim. Acta, 2, 3 (1937).
261. E. Herington, Nature, 176, 80 (1955).
262. E. Herington, J. Chem. Soc., 2747 (1956).
263. E. Herington, J. Chem. Soc., 4683 (1958).
264. S. Ohkuma, J. Pharm. Soc. Japan, 72, 1101 (1952); Chem. Abs., 47, 448h (1953).
265. A. Heffter, Mediz.-Naturwiss. Archiv., 1, 81 (1907); Chem. Zentr., II, 823 (1907).
266. G. Scagliarini, Atti V congr. naz. chim. pura appl., 546 (1936); Chem. Abs., 31, 3407[9] (1937).
267. E. Abderhalden and E. Wertheimer, Arch. Physiol., 198, 122 (1923).
268. T. Carthy and M. Sullivan, J. Biol. Chem., 141, 871 (1941).
269. G. Scagliarini and G. Avoni, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 24, 215 (1936); Chem. Abs., 31, 4276[5] (1937).
270. P. Mattei and F. Dulzetto, Atti Accad. naz. Lincei, Rend. Classe Sci. fis. mat. nat., 8, 317 (1928); Chem. Zentr., I, 1118 (1929).
271. E. Kennaway and I. Hieger, Biochem. J., 21, 751 (1927).
272. V. Arnold, Z. physiol. Chem., 70, 300 (1910).
273. K. Besk and H. Urack, Z. Unters. Lebensm., 65, 399 (1933); Chem. Zentr., II, 467 (1933).
274. O. Ripa, Textile Res. J., 23, 776 (1953); Chem. Abs., 48, 1008h (1954).
275. J. Lewin, J. Amer. Chem. Soc., 73, 3906 (1951).
276. E. Goodpasture, J. Lab. Clin. Med., 4, 442 (1919).
277. M. Strumia, Arch. exp. Path. Pharmak., 5, 447 (1928).
278. W. Semco, U. S. P. 2 529 886 (14. 11. 1950); Chem. Abs., 45, 830d (1951).
279. V. Arnold, Z. physiol. Chem., 49, 397 (1906).
280. V. Arnold, Z. physiol. Chem., 70, 317 (1910)–(1911).
281. V. Arnold, Z. physiol. Chem., 83, 304 (1913)
282. H. Janagawa, Biochem. Z., 61, 256 (1914).
283. A. Callow and M. Robinson, Biochem. J., 19, 19 (1925).
284. F. Sander, Zentr. Bakt., 132, 465 (1934); Chem. Zentr., II, 3152 (1934).
285. R. Hoffling, Zentr. Bakt., 133, 113 (1934); Chem. Abs., 29, 1455[8] (1935).
286. W. Stepp, Biochem. Z., 127, 13 (1922).
287. H. Schaeffer, Amer. J. Pharm., 98, 643 (1926).
288. P. Horkheimer, Süddeut. Apoth.-Ztg., 69, 596 (1929).
289. P. Horkheimer, Süddeut. Apoth.-Ztg., 69, 798 (1929).
290. F. Leti, Ital. P. 354 174; Chem. Zentr., I, 609 (1940).
291. F. Sas, Rev. acad. cienc. Madrid, 31, 185 (1934); Chem. Abs., 29, 1845[5] (1935).
292. G. Rodillon, J. Pharm. Chim., 25, 57 (1922).
293. H. Otto and H. Kaiser, Süddeut. Apoth.-Ztg., 69, 601 (1929).
294. P. Fischer, Süddeut. Apoth.-Ztg., 69, 601 (1929).
295. E. Bigwood and W. Ladd, J. Biol. Chem., 58, 347 (1923).
296. N. Engfeldt, Biochem. Z., 159, 257 (1925).
297. M. Sitsen, Pharm. Weekblad., 62, 622 (1925).

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