METHODS FOR THE QUANTITATIVE DETERMINATION OF FORMALDEHYDE AND THEIR USE IN THE ANALYSIS OF NATURAL COMPOUNDS

S. V. Isai and V. E. Vas'kovskii

UDC 661.727.1+047.1+543.420.62+547.281

In recent years, attention to the development and perfection of analytical methods for the chemistry of natural compounds has greatly increased. The methods used in this field can be divided into two groups: those used for the analysis of substances of several classes (to these must be assigned, in the first place, methods of determining heteroatoms) and narrowly specialized methods permitting the analysis of a single class or even a single compound.

The first group undoubtedly includes methods for the quantitative determination of formaldehyde, which are widely used in structural investigations of substances of various classes, especially carbohydrates, and in the quantitative analysis of carbohydrates, lipids, and some other groups of natural compounds. Voluminous literature exists on methods of determining formaldehyde [1-4], but most of the reviews are somewhat old, and in the overwhelming number of current publications the use of one of the known methods of analysis is described for concrete purposes.

Although the present review is given primarily for the attention of specialists in the field of the chemistry of natural compounds and biochemistry, it briefly describes the whole analytical chemistry of formaldehyde. However, in this emphasis is placed on such sections as colorimetric methods of analysis and the determination of formaldehyde in the presence of periodate, i.e., those which are most important in working with natural compounds. The review covers the literature up to and including 1971.

Existing methods of determining formaldehyde can be roughly divided into three groups: a) gravimetric, b) volumetric, and c) physicochemical.

The gravimetric methods [1, 2] are based on the formation of insoluble organic derivatives which can be collected on a filter and weighed. The organic reagents most widely used in the gravimetry of formaldehyde include 2,4-dinitrophenylhydrazine and dimethylcyclohexanedione (dimedone, methone [2]); the second reagent is used more frequently.

The gravimetric method is extremely lengthy: it requires not less than 12 h for complete precipitation. Neither of the precipitants mentioned above give quantitative results at low concentrations of formaldehyde. At the present time, gravimetric methods are used comparatively rarely.

Volumetric methods of determining formaldehyde are widely used, thanks to their simplicity and accuracy, particularly in industrial laboratories [6-9]. Of the many methods of volumetric analysis, we may mention those which (in the opinion of the majority of authors) are simple, reliable, and economical in relation to time, apparatus, etc.: 1) the sulfite method; 2) the method using aluminum chloride; 3) the cyanohydrin method; 4) the methone (dimedone) method; 5) the alkali–peroxide method; 6) the iodometric method; and 7) the mercury method.

Methods 1-4 are based on those reactions characteristic for aldehydes and ketones which are most sensitive in relation to formaldehyde (thanks to its high reactivity) and are least sensitive for ketones and higher aldehydes. Such compounds as methanol, ethanol, and acetone do not interfere with the determination but in return the result of the analysis is affected by the presence of even small amounts of acetalde-

Pacific Ocean Institute of Bioorganic Chemistry, Far-Eastern Scientific Center, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 465-480, July-August, 1973. Original article submitted August 20, 1972.

hyde and formic and acetic acids. Some authors [10, 11] single out the cyanohydrin method as the most reliable in the analysis of formaldehyde in the presence of acetaldehyde, benzaldehyde, and other higher aldehydes and ketones. Some modifications of this method are mainly directed to increasing its specificity [12, 13].

Methods 5-7 are based on oxidizing processes. In the opinion of the majority of workers, neither methanol, ethanol, nor acetone interferes with the determination, while acetaldehyde gives high results. The iodometric method is suitable for the determination of small amounts of formaldehyde only in solutions practically free from any organic impurities capable of being oxidized.

Of the physicochemical methods used for the determination of formaldehyde we may mention in the first place the polarographic and colorimetric methods. The polarographic method was proposed as early as 1935 [14]. It permits the determination of formaldehyde in low concentrations with a high accuracy. Acetaldehyde and higher aldehydes of the aliphatic series do not interfere with the determination, since they are reduced at a higher potential than formaldehyde; methanol and ethanol, and acetone and benzaldehyde, do not interfere, either. Several modifications of this method are known [15-17]. Very important is the maintenance of an accurate temperature in the recording of the polarogram: a rise in the temperature by 1°C changes the height of the half-wave by 6.5%. A defect, particularly for routine analyses, is the necessity for preliminary distillation in order to free the formaldehyde from substances interfering with the analysis. At the present time, polarographic methods are used fairly rarely [18-21].

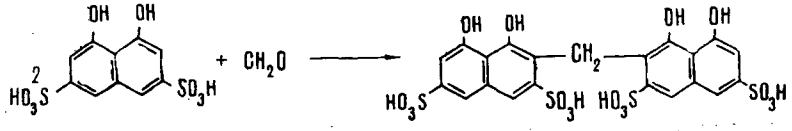
Colorimetric methods are the most widely used for the determination of formaldehyde. We shall devote special attention to them.

Schiff's test or Denigès' method – the first method for the colorimetric determination of small amounts of formaldehyde [2, 22] – is based on the fact that in an acid medium a fuchsine–bisulfite reagent gives a characteristic coloration with formaldehyde. This test is specific in the presence of both acetaldehyde and other aliphatic aldehydes, but it does not permit formaldehyde to be distinguished from acrolein and glycolic acid. The selectivity of the reagent can be raised by changing the pH of the solution. If the solution is strongly acid, the reagent is more sensitive to formaldehyde, and if it is alkaline it is more sensitive to higher aldehydes. The sensitivity of the method is substantially affected by the quality of the fuchsine used, and also by the method of preparing the reagent. Some authors [23] recommended the use of a smaller amount of the Schiff reagent than is required by a molecular ratio to the aldehyde. Proportionality between the density of the coloration and the concentration of the aldehyde is observed only in a relatively narrow range. The method permits the determination of 0.05 mg of formaldehyde.

The variants of the method relate mainly to the modification of the Schiff reagent [24, 25]. Veksler [26] has proposed a spectrophotometric variant of this method. He has shown that the temperature of the reaction is a fundamental factor.

Schiff's method, in spite of its long history and wide use, is one of the most complex methods. Its performance requires the daily preparation of standard solutions and analysis in strong sulfuric acid; the performance of one analysis requires a minimum of 6 h.

The Chromotropic Acid Method. In 1937, Eegriwe [27] proposed the use of 1,8-dihydroxynaphthalene-3,6-disulfonic acid (chromotropic acid) for the quantitative determination of formaldehyde. Heating a solution of formaldehyde with chromotropic acid in 72% sulfuric acid at 60°C for 10 min gives a purple coloration. The bearer of the coloration is the product of the condensation of formaldehyde with two molecules of chromotropic acid.



According to Eegriwe, this reagent does not form a coloration with many aliphatic and aromatic aldehydes (acetaldehyde, propionaldehyde, isobutyraldehyde, butyraldehyde, benzaldehyde, etc.) or with acetone, glucose, and glycerol and formic, glycolic, gallic, and levulinic acids. However, when a considerable amount of glyceraldehyde is present in the reaction solution a yellow color with a green fluorescence appears, and furfural gives a dark-brown coloration.

Thanks to its specificity, this reagent rapidly came into use also for quantitative determinations of formaldehyde [28].

In 1945, MacFadyen [29] and Bricker and Johnson [30] simultaneously and independently of one another proposed the use of chromotropic acid for the spectrophotometric analysis of formaldehyde. Their procedures differ in some experimental details. MacFadyen confirmed Eegriwe's statements concerning the specificity of the chromotropic acid reaction for formaldehyde. He also found that such compounds as methanol, acetaldehyde, and formic acid interfere with the determination of formaldehyde if they are present in amounts greater than 10:1. It was shown that important factors for the development of the coloration are the concentration of the sulfuric acid, the temperature, and the time of the reaction. According to MacFadyen the reaction is performed in 9-10 M sulfuric acid with heating on the boiling-water bath for 30 min. Bricker and Johnson performed the reaction in concentrated sulfuric acid with heating at 100°C for at least 30 min. They recommended for the performance of the reaction that a ratio of reagent to formaldehyde of 500:1 should be used. These authors showed that any compounds forming formaldehyde on hydrolysis by sulfuric acid give a purple coloration. Higher aliphatic alcohols inhibit the formation of the coloration, and acetaldehyde, acrolein, and β-hydroxypropionaldehyde give a yellow-brown coloration with chromotropic acid and therefore appreciably interfere with the determination of formaldehyde. The development of the coloration is prevented by diacetone alcohol and methyl ethyl ketone.

The methods described by these authors are fast and accurate and permit the determination both of free formaldehyde and of that formed on acid hydrolysis. The minimum detectable amount is approximately 1 μg of aldehyde in 1 ml of solution. The accuracy of the method is $\pm$ 5%.

All subsequent methods for determining formaldehyde with chromotropic acid are modifications of these methods. Bricker and Vail [31] showed that in the presence of a large excess of chromotropic acid it is possible to eliminate the influence of many compounds interfering with the determination of formaldehyde. Their modification reduces to the evaporation of the mixture under investigation with chromotropic acid at 170-200°C for 20 min (in an oil bath or on a hot-plate) with subsequent addition of sulfuric acid and heating the resulting solution under the conditions described previously. It was found that the recommended modification permits the determination of one part of formaldehyde in the presence of 20,000 parts of such solvents as chloroform, carbon tetrachloride, methanol and other lower alcohols, methyl ethyl ketone, pyridine, and benzene.

Recently, Still et al. [32] have shown that ethanol interferes with the determination of formaldehyde with chromotropic acid. Their results contradict the old statements of Bricker and his colleagues. Other authors [33] have shown an adverse influence of phenolic compounds.

Christofferson [34] has somewhat modified the method of Bricker and Vail [31] by considerably lowering the temperature of evaporation of the solution under investigation with the chromotropic reagent (to 110°C) and using 18 M sulfuric acid; however, under these conditions it was found that the measurement of the optical density of the solution can be performed only 5-44 h after the beginning of the reaction. Yet another modification of the chromotropic acid method was published in 1970 [35]. The maximum sensitivity (0.055 μg/ml of CH_2O, which is 36 times better than the sensitivity of Bricker and Vail's method) is achieved by planning the experiment with the aid of simplex optimization. The analysis of one sample requires less than 10 min, and the mean coefficient of deviation is 1.3%. Unfortunately, other aldehydes interfere with the determination.

The chromotropic acid method is one of the simplest, but it is fairly severe in the conditions of its performance. It requires high temperatures and prolonged heating with concentrated sulfuric acid. Consequently, any compounds forming formaldehyde under such conditions will give a positive reaction with chromotropic acid. Furthermore, many other substances give a coloration. It is true that some modifications [36, 37] have made this method highly specific for formaldehyde since they permit interfering impurities to be removed during the reaction or their influence to be eliminated in some other way.

We may note that in the use of chromotropic acid an important factor is its purity. A technical preparation is unsuitable because of the impurities that it contains. However, its purification is difficult and does not always give a good sample. Furthermore, the solutions of the reagent are unstable on storage. All these form serious defects of the method. Nevertheless, it is the most widely used in the analysis of formaldehyde.

The Phenylhydrazine Method. The oxidation of a phenylhydrazine complex was first used for the determination of formaldehyde by Schryver [38]. He used potassium ferricyanide as the oxidizing agent, and the determination was performed in an acid medium.

Later, this reaction was used by Desnuelle and Naudet [39]. Roberts [40] who carefully investigated it, showed that the coloration of the solutions formed in an acid medium was too unstable. It is unsuitable for accurate investigations.

This method began to be widely used after the work of Tanenbaum and Bricker [40], who used it to determine the free aldehyde in the presence of the bound aldehyde and of many organic compounds interfering with the chromotropic acid procedure. A similar problem had been solved previously by the use of the Schiff reagent (the defects of this method are described above). Tanenbaum and Bricker, in preliminary investigations, used the phenylhydrazine complex of diazobenzenesulfonic acid as the oxidizing agent, and the reaction was performed in an acid medium. They found that under such conditions some aliphatic aldehydes gave a crimson coloration, while with ketones no characteristic coloration whatever was produced, and aromatic aldehydes reacted slowly. Furthermore, it was observed that the coloration with formaldehyde was more stable than that with the higher aldehydes. However, in this form the procedure had a number of defects, of which the main one was the poor reproducibility of the results. The authors connected this with the instability of the diazobenzenesulfonic acid. To improve the method, they tested a whole series of oxidizing agents and for a number of reasons they chose potassium ferricyanide. The time necessary for the performance of one analysis is 30 min, and the accuracy of the method is of the order of 2%.

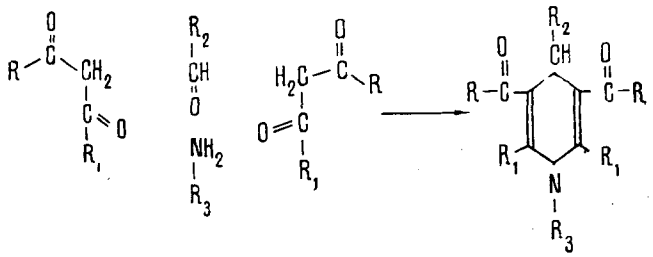
The method described was later improved by Stanković [41]. The essence of the modification consists in the salting out of isopropanol (which is used to improve the solubility of the reagents) from the solution. In this process, the colored complex passes into the organic layer. The sensitivity of the method is increased – it is possible to determine 0.02-1.0 mg of formaldehyde in one liter. The time for one determination is of the order of 25 min. The analysis can be performed in the presence of many substances (methanol, ethanol, phenol, etc.); the presence of hexamethylenetetramine in the mixture is impermissible. Acetaldehyde, sulfite ion, and salts of divalent iron interfere with the determination, beginning at low concentrations.

Mari et al. [42] showed that it is more convenient to use the oxygen dissolved in the reagents as the oxidizing agent. In these circumstances, the procedure is considerably less laborious. The reaction is performed in an alkaline medium. These authors investigated the influence of various factors on the process. They showed that the maximum coloration develops in approximately 80 min.

According to Mari et al. [42], the reaction is specific and sensitive for formaldehyde; thus, 10 μg of it give an absorption magnitude of 0.450 under the conditions of the experiment, while 50 μg of acetaldehyde give only 0.030. Moreover, the absorption for 10 μg of formaldehyde in the chromotropic acid procedure of Bricker and Johnson is only 0.200. The accuracy of this modification is 5-10% and the minimum amount of formaldehyde detectable is 1 μg.

The phenylhydrazine method is very sensitive but is specific for formaldehyde only in Mari's modification, which, in spite of its positive features, has not found wide use. Indeed, Tanenbaum and Bricker's method, in spite of its serious defects (in addition to lower aliphatic aldehydes, lower aliphatic alcohols also interfere with the reaction), is used far more frequently [43-46].

The acetylacetone method, which was proposed by Nash [47] in 1953, is based on the Hantzsch reaction – the formation of lutidine derivatives from a β-diketone, an aldehyde, and an amine. On condensation with acetylacetone and ammonia, formaldehyde gives 3,5-diacetyl-2,6-dimethyldihydrolutidine, which has a yellow color [47, 48].



Nash made a detailed investigation of the reaction conditions – the influence of the pH of the medium, the composition and stability of the reagent and of the colored complex formed with formaldehyde, the stability of the coloration, and the influence of various substances. It was found that for low concentrations of formaldehyde the reaction takes place quantitatively at pH 5.5-6.5. Ammonium acetate and phosphate were

used as the amine components of the reaction, the latter proving to be more suitable. It was shown that the diacetyldimethyldihydrolutidine is formed only if the formaldehyde and acetylacetone are present in small amounts while the ammonium acetate is present in enormous excess. In practice, the optimum concentrations of these components were as follows: ammonium acetate 0.1-1.0 M, and acetylacetone 0.1-0.001 M. The reagent (acetylacetone in ammonium acetate buffer) is stable for at least two weeks. The colored complex does not change its color for two days. The influence of the majority of substances is reduced to a minimum because of the mild conditions of the analysis.

Under the conditions of the reaction, acetaldehyde also forms diacetyldihydrolutidine derivatives, but the absorption maximum of their solution is in the 388-nm region, and not at 412 nm as in the case of formaldehyde. Furthermore, the rate of formation of the colored complex from acetaldehyde is very small in comparison with formaldehyde (at the same concentration). Some amines may compete with the ammonia in the Hantzsch reaction, although the rates of these reactions are also low. Acetone, chloral, furfural, and glucose do not interfere with the reaction. Periodates and sulfites destroy the color to an appreciable extent, especially sulfites which decrease the results by 90% in very low concentrations (0.001 M).

Hasegawa has modified this method for the microdetermination of formaldehyde, introducing the extraction of the colored complex with an organic solvent [49]. The influence of various factors on the reaction was checked. The most suitable of the solvents tested (benzene, cyclohexane, chloroform, xylene, butan-1-ol, and n-hexane) was butanol. At 60°C, the maximum coloration develops in 10 min. This author showed that there are no fundamental changes in the absorption of the solutions in the pH range from 4 to 8. The colored complex is stable for several days. This method permits the determination of formaldehyde in the presence of colored compounds. Hasegawa also investigated the influence of some substances on the determination of formaldehyde and showed that a 500-fold excess of acetone or of phenylacetaldehyde, a 50-fold excess of p-chlorobenzaldehyde or propionaldehyde, and a fivefold excess of acetaldehyde do not interfere with the determination. In Hasegawa's opinion, ketones and aromatic aldehydes should not interfere with the development of the coloration, and the influence of aliphatic aldehydes decreases with an increase in the chain length.

Belman has proposed a fluorimetric modification of Nash's procedure which permits the determination of 0.01 μg of formaldehyde [50].

The acetylacetone method of determining formaldehyde is undoubtedly the best method for the majority of cases. It is extremely selective and sensitive. The formation of the complex takes place in a neutral medium, which makes it particularly valuable for the analysis of free aldehydes in the presence of bound aldehydes and also for the investigation of biological materials. In addition to the methods for the colorimetric determination of formaldehyde that have been considered, many others have been proposed with different degrees of sensitivity and specificity [1, 2, 4]. We shall limit ourselves simply to mentioning them, since they have not found wide use and, unfortunately, to a large extent are more suitable for the qualitative detection of formaldehyde than for its quantitative analysis. Various reactions of formaldehyde are used: with phloroglucinol, with resorcinol, with alkali-metal and copper salts, with hydroxamic, nitrohydroxamic, and benzenesulfohydroxamic acids, with alkaloids, with an ammoniacal solution of silver nitrate, and with ferric chloride in concentrated hydrochloric acid. The main disadvantage of these methods is their low specificity.

Below we shall consider methods that have appeared in the literature in the last decade and have not appeared in earlier reviews. We shall not dwell on them in detail, since none of them, in our opinion, possesses the advantages of Nash's method and they are not so widely used as the chromotropic acid and phenylhydrazine methods.

In the first place we may mention the work of Sawicki and his colleagues [51-53] For the colorimetric determination of formaldehyde they have proposed 2-hydrazinobenzothiazole, p-nitrobenzothiazonium fluoroborate [52], and 3-methylbenzothiazolin-2-one hydrazone (MBTH) [53]. Each of these reagents is nonspecific for formaldehyde, since they all give a positive reaction with other aliphatic, aromatic, and heterocyclic aldehydes. Another disadvantage is the high values of the absorption for blank experiments.

Kamata modified the MBTH method by the photometry of the colored complex after extraction with a solvent [54]. As extractant he proposed a mixture of chloroform and acetone (1 : 1). The colored complex, after it has passed into the organic solvent, is stable for 2 h. In extraction, Kamata made use of salting out with sodium chloride, since without this more than 10% of the colored product remains in the aqueous medium. The strict maintenance of certain time intervals is important. Thus, the extract must be

dried rapidly, otherwise the absorption decreases. One of the most important advantages of Kamata's method is the fact that the presence of 1 mg of sugar in the sample gives a hardly distinguishable absorption while, for example, formaldehyde cannot be determined by the chromotropic acid method in the presence of appreciable amounts of carbohydrates.

Sawicki et al. have proposed another two spectrophotometric methods of determining the free formaldehyde from various organic compounds during chemical reactions. They are based on the reaction of formaldehyde with 6-amino-1-naphthol-3-sulfonic acid (J acid) [55] and with 6-anilino-1-naphthol-3-sulfonic acid (phenyl J acid) [56]. Both methods are 2-2.5 times more sensitive than the chromotropic acid method. As with chromotropic acid, the reaction with J acid is performed in the presence of sulfuric acid, and therefore any compounds giving a coloration under these conditions will interfere with the determination.

The authors mentioned immediately suggested four modifications of the determination of formaldehyde with J acid, distinguished from one another by the concentrations of sulfuric acid used and the conditions of heating the reaction mixture.

In the performance of the determination with phenyl J acid, the choice of solvent in which the reaction is performed proves to be very important. Thus, the use of water as solvent led to a substantial scatter in the results of the determination. The best results were obtained in methylcellosolve.

In working with phenyl J acid, sulfuric acid is necessary only to dissolve the main reagent. However, this amount is sufficient for the determination of the free formaldehyde liberated on acid hydrolysis.

These authors consider both methods to be highly specific for formaldehyde (like the chromotropic acid method), referring to the work of Kamel and Wizinger [57], according to whom the specificity of the reaction between formaldehyde and the reagents mentioned is the result of the steric effect of the two sulfo groups.

In addition to the methods described by Sawicki et al, several others have been proposed. Thus, Custa [58] has used the reaction with *l*-tyrosine hydrochloride in 50% sulfuric acid. Heating to 150-160°C is necessary to develop a green coloration. In these circumstances, for the determination of formaldehyde at least 40 mg of it must be present in the sample. In this form, the method can hardly be considered suitable for the chemistry of natural compounds.

Dokladova and Stankova [24] have proposed a method based on the formation of the addition product of sulfur dioxide to formaldehyde. As the SO_2 donor they use the complex $[HgCl_2SO_3]^{2-}$ (dichloridosulfitomercuriate) the reaction of which with parafuchsine hydrochloride forms the colored quinoid form of parafuchsine methyl sulfonic acid (p-rosaniline methyl sulfonic acid). The sensitivity of the method can be changed by varying the concentration of the complex formed. This method is in fact a modification of Schiff's test. Later, the same authors proposed another variant of this method [25].

Ekberg and Silver [59] recommend the determination of low concentrations of formaldehyde in the presence of ethanol with the aid of the peptone proteose. They consider that their rapid method has a substantial advantage for their purposes over the chromotropic acid method [60] and Sawicki and Hauser's method [51]. The colored complex formed in this method is stable for 1 h. Small amounts of acetaldehyde, butyraldehyde, propionaldehyde, and diacetone alcohol interfere with this method, while they do not interfere in the chromotropic acid method.

Korenman et al. have proposed four color reactions for formaldehyde [61]. They are based on the reactions of formaldehyde with derivatives of chromotropic acid and azo dyes containing free hydroxy groups. The concentration of the reagents and the time of heating the reaction mixture have marked effects on the intensity of the coloration.

Bailey [62] has developed a method for determining formaldehyde which is based on its catalytic action in the oxidation of β-phenylenediamine with hydrogen peroxide.

In the development of new procedures, some authors make use of various modifications of known methods. Of such procedures we may mention the following: the spectrophotometric determination of formaldehyde with dimedone [63]; the determination of formaldehyde in cellulose formals and methylcellulose formals by a modification of Schiff's method [64]; and the automatic spectrofluorimetric determination of formaldehyde by a modification of Nash's method [65].

It can be seen from the literature information considered in our review that at the present time colorimetric methods (especially spectrophotometric and spectrofluorimetric methods) are most widely used

for the determination of formaldehyde as being the most accurate and rapid and, which is particularly important, requiring only small amounts of the starting material for their performance, which is essential in the solution of many chemical and biochemical problems.

It can be seen from a short account of the characteristics of the colorimetric methods that the most selective for formaldehyde are the chromotropic acid, the phenyl J acid, and the acetylacetone methods. Of these the most widely used is the chromotropic acid method, although it is considerably inferior to the other two in many respects. The results of a comparison of the chromotropic acid and the phenyl J acid methods shows that while their selectivities for formaldehyde are similar, the latter is considerably superior to the chromotropic acid method both in sensitivity and in the mildness of the conditions of performing the analysis. Nevertheless, it has scarcely come into use so far.

The acetylacetone method, with a selectivity as high as the two just mentioned, is superior to all known methods in the mildness of the conditions of analysis. However, it is little used. Consequently, at the present time the problem of the development of new and the modernization of old methods of determining formaldehyde is an urgent one. This is particularly important for the solution of the problem of the analysis of products of periodate oxidation.

All the methods given permit the determination either of free formaldehyde or of formaldehyde formation in the hydrolysis and oxidation of organic compounds. A special case is the analysis of formaldehyde produced by the oxidation of substances with periodate [66-70], since periodates and iodates fundamentally influence the course of color reactions.

We shall consider in more detail methods of determining formaldehyde formed as the result of periodate oxidation – one of the most important methods in the chemistry of natural compounds.

In this section we shall not dwell in detail on the methods of determining formaldehyde themselves, since this would be to repeat what has already been said. It is more desirable to consider their features connected with periodate oxidation and relating to the elimination of the periodate and the iodate, which interfere with the color reactions.

Some authors determine formaldehyde after its preliminary distillation from the reaction mixture [28, 71]. The quantitative distillation of micro amounts of a substance is a difficult problem. Furthermore, the products of the oxidation of a number of substances are unstable and may decompose on distillation, forming either an additional amount of formaldehyde or substances preventing its determination.

In the majority of cases, however, various precipitants and reducing agents are used to eliminate periodate and iodate ions.

Various methods are used to determine formaldehyde freed from interfering substances [47, 72, 73], but the chromotropic acid method [67] or a modification of it is the one used more frequently. Success has been achieved in the use of the latter method after the appropriate selection of reagents quantitatively precipitating periodate and iodate.

Fleury et al. [74] have shown that it is possible to eliminate iodine-containing ions in the form of insoluble salts. However, the use for this purpose of barium hydroxide and silver acetate did not give good results because of the impossibility of selecting a pH of the medium which would simultaneously be the optimum for the determination of the formaldehyde and for the precipitation of the interfering ions. Precipitants that have been used are salts of mercury, zinc, magnesium, aluminum, beryllium, titanium, bismuth, calcium, etc., but the desired effect was not achieved with these ions either.

The most promising results have been given by the use of lead salts as precipitants [72]. It has been shown that it is not any lead salt that can be used for this purpose: for example, the nitrate interferes with the reaction and the acetate is more suitable but the latter gives a yellow coloration with a solution of chromotropic acid. The most successful reagent proved to be lead dithionate, thanks to its high solubility and the capacity of the dithionate ion for decomposing in an acid medium into sulfate and sulfur dioxide. The lead sulfate can be eliminated by centrifuging, and the sulfur dioxide does not interfere with the determination of the formaldehyde – on the contrary, it protects the chromotropic acid from the oxidizing action of light and air. Strontium hydroxide may also be used as precipitant [75].

The use of ion-exchange resins for the elimination of periodate and iodate ions is known [67]. Here the resin must be carefully prepared for the elimination of various impurities that may interfere with the determination. Treatment of the reaction mixture with the ion-exchange resin leads to a sharp change in its pH. The formaldehyde may be partially lost by adsorption on the resin.

In addition to precipitants, reducing agents may be used for eliminating interfering ions. Among these, arsenite, sulfites, iodides, and chlorides have found the widest use.

Unfortunately, the arsenite ion is the most widely used [67-69, 77]. When arsenite is used, the presence of large amounts of mineral acids or alkalis is impermissible. It has been established that the reduction of periodate with arsenite takes place quantitatively at pH 8 and room temperature. At this pH value, the presence of iodate (IO_3^-) does not interfere with the determination. However, under such conditions the reaction requires several hours for its completion. The addition of iodide to the reaction mixture decreases the time of the reaction to a few seconds (at pH 8).

Since this reducing agent is unsuitable for working under acid conditions, it cannot be used for the chromotropic acid method. Furthermore, chromotropic acid gives a coloration with arsenite [66]. The arsenic oxide present in the reaction mixture forms a colloidal suspension if solutions containing chromotropic acid are cooled below 60°C. It is not surprising that the literature contains statements according to which the use of arsenite as reducing agent does not always give reproducible results [69]. There are also reports that the use of arsenite does not always give good results even in association with other methods of determining formaldehyde [65]. Very recently, it has been proposed to replace arsenite as reducing agent by ascorbic acid, as a more accessible and nonpoisonous reagent [78].

Since arsenite could not in fact be used in the chromotropic acid method, some authors have proposed to use sulfite to reduce periodate [79], since it does not affect reactions with chromotropic acid. However, in its original variant this procedure possessed no smaller defects than the one using arsenite [72]. With the use of sulfite as reducing agent, the iodide formed in the reaction was eliminated in the form of the silver salt, large amounts of relatively expensive silver salt being required and, moreover, colloidal silver being formed photochemically, which interfered with the photometry.

Speck and Forist [79] have proposed to use sulfur dioxide in an acidic medium for the elimination of periodate. In this process, however, the dye that chromotropic acid forms with formaldehyde passes into the leuco form. They found that this leuco form is unstable and may be decomposed by passing air into the reaction mixture to eliminate SO_2. This stage complicates the procedure for determination.

Mitchell and Percival [80] have used sodium bisulfate for the destruction of periodate. They started from unpublished reports by Rees. In their paper, the authors show that with well-devised blanks this method enables results to be obtained which agree well with the results of gravimetric determination.

While sulfites do not interfere appreciably in the chromotropic acid method, they cannot be used for the acetylacetone method. In very low concentrations (0.001 M) sulfites cause the loss of 90% of the formaldehyde determined [47].

The iodide ion has also been used as reducing agent [68, 69, 75]. In this case, both strongly acid and strongly alkaline reaction solutions can be analyzed. In acid solution, both periodate and iodate are reduced to free iodine.

The use of stannous chloride as reducing agent has been described [81-87]. Investigations in which this reducing agent is used are slight modifications of the chromotropic acid procedure. Modifications of the phenylhydrazine [88] and acetylacetone [89] methods for the determination of formaldehyde after periodate oxidation have been developed in our laboratory.

A detailed investigation of the phenylhydrazine method has shown that when periodate is used as oxidizing agent an excess of it within a definite range of concentrations does not interfere with the determination of formaldehyde [88]. However, this procedure is not completely satisfactory, primarily because of the substantial influence of various impurities (lower aldehydes, especially acetaldehyde). This impelled us to change to the acetylacetone method, the advantages of which have been described. We have shown that to eliminate an excess of periodate it is possible to use polyols – butane-2,3-diol, rhamnose, and inositol [89]. Butanediol and rhamnose are more suitable for a rapid reaction, but if for any reason the introduction of acetaldehyde into the reaction mixture is undesirable it is possible to use inositol.

Methods for determining formaldehyde can be used for two purposes: to deduce the structure of compounds containing α-glycol groupings in the molecule and to determine substances of various classes quantitatively. The first direction is the most important for the chemistry of carbohydrates and has been examined in detail in available reviews [66-70].

Among the quantitative determinations based on the analysis of formaldehyde, the simplest case is the analysis of polyols. In the periodate oxidation of various carbohydrates, formaldehyde is formed from free pentoses and hexoses, and also from pentoses and hexoses present at the reducing end of a poly- or oligosaccharide chain (if this chain is attached to the hexose in the 2, 3, or 4 position and to the pentose in the 2 or 3 position). However, the oxidation of free or bound sugars takes place comparatively slowly and can be complicated by side reactions. Consequently, as a rule, it is best to reduce the free or bound polysaccharide to the corresponding polyol before oxidation. Analysis by the method of reduction–periodate oxidation–determination of formaldehyde is of great importance for determining the molecular weights of oligo- and polysaccharides [90]. In the investigation and determination of carbohydrates by this procedure, various methods are used. The dimedone [91-93] and phenylhydrazine [94] methods are used comparatively rarely. The chromotropic acid method is the most widely used [95-100]. Although Nash's method has been described in a handbook of carbohydrate chemistry [67], only recently have papers appeared in the literature describing the results of the use of this method or modifications of it [101-102]. Thus, the use of a modification of Nash's method for the automatic spectrofluorimetric analysis of carbohydrates after periodate oxidation is known [65].

The methods of determining formaldehyde may find and are finding wide use in the chemistry of lipids [103]. In actual fact, glycerolipids, sphingolipids, and glycolipids, i.e., practically all classes of lipids, contain fragments from which, after appropriate transformations, the $\diagup CH-CH_2$ (OH OH) grouping that is necessary for analysis can be obtained. Such fragments may be glycerol and other polyols, sphingosine bases ($R-\underset{OH}{CH}-\underset{NH_2}{CH}-CH_2OH$), and monosaccharide residues.

Methods of determining sphingolipids can be divided into two groups. The first includes methods based either on the determination of sphingosine nitrogen [104] or on the color reaction between the free amino group formed in the acid hydrolysis of sphingosine bases and a reagent giving a colored complex. Such reagents are Methyl Orange (sodium p-dimethylaminoazobenzenesulfonate) [105], fluorodinitrobenzene [106], trinitrobenzenesulfonic acid [107, 108], and 1-aminonaphthalene-4-sulfonic acid [109]. A second group of methods is based on the determination of the degradation products formed after the oxidation of the sphingosine bases by periodate or by lead tetraacetate. While lead acetate is used comparatively rarely, since it leads to overoxidation and to other side reactions [110-111], periodate is widely used. Its advantage consists in the possibility of performing oxidation in various media: aqueous [112], acetic acid [113], aqueous methanol [112], and other mixed solvents [114]. Recently, Baumann and Schmid [111] have shown that the oxidation of lipids takes place specifically in absolute pyridine at room temperature. The use of pyridine enables side reactions to be excluded, and many lipids are readily soluble in it; furthermore, amino alcohols are more easily cleaved in a basic medium.

The products of degradation by periodate oxidation are determined either by gas chromatography (long-chain aldehydes, N-acetyl-O-trimethylsilyl derivatives of free bases [111, 112, 115, 116]), or colorimetrically. In the latter case, the formaldehyde formed is determined most frequently by chromotropic acid method [117, 118], and more rarely by other methods [119].

Although in the determination of triglycerides gas chromatography [120-123] or other physical methods [124] have been used ever more frequently in recent years, until now the main method for their quantitative analysis has been the determination of formaldehyde after saponification and periodate oxidation, and this by the most various methods. The phenylhydrazine method is used comparatively rarely [125-126], and the chromotropic acid method is used most frequently [130-141].

The acetylacetone method has become widely used for the analysis of triglycerides in all its possible modifications: colorimetric [142-144], spectrophotometric [145, 146], fluorimetric [143, 147-152]. The fact that it is just on the basis of the acetylacetone method that a semiautomatic procedure for determining triglycerides is used [145, 147-154] shows the high reliability and stability of the method. Recently, Weigel [153] has published a paper on the determination of glycerophosphatides as glycerol. The basis of the method is the thermal cleavage of the glycerophosphates to glycerol, the oxidation of the glycerol with periodate, and the subsequent determination of the resulting formaldehyde by automatic acetylacetone method.

For the colorimetric determination of triglycerides, some authors use 2-hydrazino-3-methylbenzothiazoline [154] or "sulfophosphovanillin" [155]. Hoeflmayr et al. determined triglycerides from the difference between the total amount of esters remaining after the separation of free cholesterol and phosphatides and the cholesteryl esters [156, 157]. In recent years, enzymatic methods, which permit the determination of individual triglycerides, have come into use for the structural analysis of the triglycerides [158-163].

A special case in the chemistry of lipids is the analysis of glyceryl ethers. Generally, glyceryl ethers are determined in the unsaponifiable fraction of lipid extracts [164, 165]. It is known that they are a component part both of neutral lipids and of phospholipids. In the majority of previous investigations the isolation of the glyceryl ethers from the phospholipid fraction included its dephosphorylation, saponification, and extraction of the unsaponifiable products, the analysis of which gave information on the presence of ethers in them [165-169]. However, in recent years reduction with lithium tetrahydroaluminate is being used ever more frequently for the isolation of glyceryl ethers [167, 170-172]. Other questions relating to the analysis of glyceryl ethers have been considered in reviews [173, 174].

In the majority of cases, the chromotropic acid procedure (the defects of which we have discussed) is used for the analysis of glyceryl ethers. The periodate oxidation is most frequently performed in ethanol [175] or aqueous ethanol with the addition of other solvents [176]. Marinetti has used 90% acetic acid for this purpose [177].

We have shown the applicability for the analysis of glyceryl ethers of a modification of the acetylacetone method that we have developed [89]. In this, the periodate oxidation of the preparatively isolated glyceryl ether fraction was performed in aqueous isopropanol.

As can be seen from the last sections of this review, which have been devoted to the analysis of natural compounds using methods of determining formaldehyde, up to the present time the chromotropic acid procedure has been the most widely used. However, in the near future it will probably be replaced by other methods, especially Nash's method. This has already practically taken place in individual fields, such as the determination of triglycerides.

We have briefly discussed a small fraction of the questions relating to the prospects for the development of the analytical chemistry of formaldehyde. There is no doubt that this analysis is an important problem in the chemistry of natural compounds.

LITERATURE CITED

1. J. G. Reynolds and P. D. Irwin, Chem. Ind. (London), 419 (1948).
2. J. F. Walker, Formaldehyde, Reinhold, New York (1953).
3. G. Dryhurst, Periodate Oxidation of Diol and Other Functional Groups, Pergamon Press, Oxford, Vol. 2 (1970), p. 139.
4. F. D. Snell and C. T. Snell, Colorimetric Methods of Analysis, 3rd ed., Van Nostrand, Princeton, Vol. 3 (1953), p. 225.
5. R. Ohme and E. Schmitz, Z. anal. Chem., 220, 105 (1966).
6. P. N. Norkus and S. P. Stul'gen, Zh. Analit. Khim., 23, 130 (1968).
7. Z. Sláma, Chem. prům., 18, 200 (1968).
8. S. Ebel, M. Brüggeman, and E. Rosswog, Z. anal. Chem., 240, 326 (1968).
9. E. N. Zelenina, Methods of Analytical Chemistry, Preparation of Reagents [in Russian], No. 15 (1968), p. 156.
10. A. Mutschin, Z. anal. Chem., 99, 346 (1934).
11. G. Romijin, Z. anal. Chem., 36, 19, 21 (1877).
12. E. Schulek, Ber., 58, 732 (1925).
13. E. Pfeihl and G. Schroth, Z. anal. Chem., 134, 333 (1952).
14. F. G. Jahoda, Časopis českoslov. Lékarnictva, 14, 225 (1934); Chem. Zentr., I, 1091 1935.
15. M. J. Boyd and K. Bambach, Ind. Eng. Chem., Anal. Ed., 15, 314 (1943).
16. G. C. Whitnack and R. W. Moshier, Ind. Eng. Chem., Anal. Ed., 16, 496 (1944).
17. B. Warshawsky and P. J. Elving, Ind. Eng. Chem., Anal. Ed., 18, 253 (1946).
18. A. I. Vashkyalis, Zh. Analit. Khim., 22, 334 (1967).
19. V. Novack, Chem. prům., 19, 25 (1969).
20. Yu. S. Ignat'ev, M. P. Strukova, L. E. Volkova, and L. A. Mikheeva, Zh. Analit. Khim., 25, 1389 (1970).

21. Yu A. Davydovskaya and M. M. Rotenshtein, in: Methods of Analyzing Chemical Reagents and Materials [in Russian], Vol. 19 (1971), p. 52.
22. G. Denigès, Compt. Rend., 150, 529 (1910), cited in ref. [2].
23. G. Damkohler and W. Eggersgluss, Z. Physikal. Chem., (B), 51, 157 (1942).
24. J. Dokladova and O. Stankova, Mikrochim. Acta, 725 (1965).
25. J. Dokladova and O. Stankova, Mikrochim. Acta, 257 (1968).
26. R. I. Veksler, Zh. Analit. Khim., 1, 301 (1946).
27. E. Eegriwe, Z. anal. Chem., 110, 22 (1937).
28. M. J. Boyd and M. A. Logan, J. Biol. Chem., 146, 279 (1942).
29. D. A. MacFadyen, J. Biol. Chem., 158, 107 (1945).
30. C. E. Bricker and H. R. Johnson, Ind. Eng. Chem., Anal. Ed., 17, 400 (1945).
31. C. E. Bricker and W. A. Vail, Anal. Chem., 22, 720 (1950).
32. R. H. Still, K. Wilson, and B. W. J. Lynch, Analyst, 93, 805 (1968).
33. J. Knabe, Arch. Pharm., 297, 312 (1964).
34. K. Christofferson, Anal. Chim. Acta, 31, 233 (1964).
35. M. J. Houle, D. E. Long, and D. Smeete, Anal. Letters, 3, 401 (1970).
36. H. Neumark, J. Sci. Food Agric., 16, 668 (1965).
37. A. M. Afanas'ev, E. P. Kalyazin, et al., Zh. Analit. Khim., 26, 189 (1971).
38. S. B. Schryver, Proc. Roy. Soc., 82B, 226 (1910).
39. P. Desnuelle and M. Naudet, Bull. Soc. Chim. France, 12, 871 (1945).
40. M. Tanenbaum and C. E. Bricker, Anal. Chem., 23, 354 (1951).
41. V. Stanković, Chem. Zvesti, 16, 683 (1962).
42. R. Mari, M. Fevé , and M. Dzierzinsky, Bull. Soc. Chim. France, 1395 (1961).
43. L. Josimovik and O. Gal, Anal. Chim. Acta, 36, 12 (1966).
44. G. I. Benzina, Gigiena i Sanitariya, No. 6, 100 (1968).
45. W. Greenfield, R. Berg, and R. Moore, Clin. Chem., 15, 1180 (1969).
46. K. Moehler and G. Denbsky, Z. Lebensm.-Unters, u.-Forsch., 142, 109 (1970); Chem. Abstr., 73 (1970).
47. T. Nash, Biochem. J., 55, 416 (1953).
48. T. Nash. Nature, 170, 976 (1952).
49. K. Hasegawa, Ann. Rep. Sankyo Res. Lab. (Tokyo), 15, 87 (1963).
50. S. Belman, Anal. Chim. Acta, 29, 120 (1963).
51. E. Sawicki and T. R. Hauser, Anal. Chem., 32, 1434 (1960).
52. E. Sawicki and T. W. Stenley, Mikrochim. Acta, 510 (1960).
53. E. Sawicki, T. R. Hauser, T. W. Stenley, and W. Elbert, Anal. Chem., 33, 93 (1961).
54. E. Kamata, Bull. Chem. Soc. Japan, 38, 2005 (1965).
55. E. Sawicki, T. W. Stenley, and J. Pfaff., Anal. Chim. Acta, 28, 156 (1963).
56. E. Sawicki, T. R. Hauser, and S. McPherson, Anal. Chem., 34, 1460 (1962).
57. M. Kamel and R. Wizinger, Helv. Chim. Acta, 43, 594 (1960).
58. L. A. Custa, Rev. Asoc. Bioquim. Arg., 30, 48 (1965); R. ZhMim 16G208 (1966).
59. D. R. Ekberg and E. C. Silver. Analyt. Chem., 38, 1421 (1966).
60. W. R. Frisell and C. G. Mackenzie, Methods Biochem. Anal., 6, 63 (1958).
61. K. M. Korenman, V. G. Ganina, É. P. Grigor'eva, and L. A. Esenkova, Trudy po Khimii i Khim. Tekhnologii, 2, No. 28, 130 (1969).
62. B. W. Bailey and J. M. Rankin, Anal. Chem. 43, 782 (1971).
63. E. Sawicki and R. A. Carnes, Mikrochim. Acta, 148 (1968).
64. S. Ramanathan, J. Rivlin, O. A. Stamm, and H. Zollinger, Text. Res. J., 38, 63 (1968); Chem. Abstr., 68, 60419g (1968).
65. H. Cho Tun, J. F. Kennedy, M. Stacey, and R. R. Woodbury, Carbohydr. Res., 11, 225 (1969).
66. W. R. Frisell and C. G. Mackenzie, Methods Biochem. Anal., 6, 63 (1958).
67. J. C. Speck, Methods in Carbohydrate Chemistry, 1, 441 (1962).
68. J. M. Bobbit, Adv. Carbohydr. Chem., 11, 1 (1956).
69. J. R. Dyer, Methods Biochem. Anal., 3, 111 (1965).
70. K. B. Wiberg, Oxidation in Organic Chemistry, Academic Press, New York, Vol. 5A (1956), p. 367.
71. A. C. Corcoran, J. Lab. Clin. Med., 37, 297 (1950).
72. J. F. O'Dea and R. A. Gibbons, Biochem. J., 55, 580 (1953).
73. R. E. Reeves, J. Amer. Chem. Soc., 63, 1476 (1941).

74. P. Fleury, Mikrochim. Acta, 37, 863 (1951).
75. R. D. Guthrie, Methods in Carbohydrate Chemistry, 1, 435, 445 (1962).
76. M. A. Smith and B. R. Willeford, Anal. Chem., 26, 51 (1954).
77. M. Lambert and A. C. Neish, Can. J. Res., B28, 83 (1950).
78. N. A. Loseva, L. A. Abramov, and I. V. Domaradskii, Lab. Delo, 116 (1970).
79. J. C. Speck and A. A. Forist, Anal. Chem., 26, 1942 (1954).
80. W. E. A. Mitchell and E. Percival, J. Chem. Soc., 1423 (1954).
81. A. C. Corcoran and J. H. Page, J. Biol. Chem., 170, 165 (1947).
82. C. D. West and S. Rapoport, Proc. Soc. Exp. Biol. Med., 70, 141 (1949).
83. W. D. Hofman, Med. Ernähr, 5, 126 (1964).
84. A. C. Corcoran and J. H. Page, J. Biol. Chem., 146, 279 (1940).
85. J. Hamburger, R. Duizend, and M. Argant, Ann. Biol. Clin., 6, 353 (1948); Chem. Abstr., 43, 6271e (1949).
86. N. Argant. Bull. Soc. Chim. Biol., 31, 485 (1949); Chem. Abstr., 44, 685b (1950).
87. F. M. Paolloggy and C. Y. Jarrean, Presse Med., 75, 806 (1967).
88. S. V. Isai and V. E. Vas'kovskii, Izv. Sibirskogo Otd. Akad. Nauk SSSR, Ser. Khim., 142 (1969).
89. V. E. Vas'kovskii (Vaskovsky) and S. V. Isai (Isay), Anal. Biochem., 30, 25 (1969).
90. G. W. Hay, B. A. Lewis, F. Smith, and L. Hough, Methods in Carbohydrate Chemistry, 5, 357, 370 (1965).
91. D. J. Bell, J. Chem. Soc., 992 (1948).
92. D. Aminoff and W. T. J. Morgan, Biochem. J., 48, 74 (1951).
93. S. Mukherjee and H. C. Srivastava, J. Amer. Chem. Soc., 77, 422 (1955).
94. V. D. Harwood, R. A. Laidlaw, and R. G. J. Telfer, J. Chem., Soc., 2364 (1954).
95. L. Hough, B. M. Woods, and M. B. Perry, Chem. Ind., 1100 (1957).
96. A. M. Unrau and F. Smith, Chem. Ind., 330 (1957).
97. F. Smith and R. Montgomery, The Chemistry of Plant Gums and Mucilages, Reinhold, New York (1959).
98. F. W. Parrish and W. J. Whelan, Nature, 183, 991 (1959).
99. W. J. Whelan, Methods in Carbohydrate Chemistry, 4, 72 (1964).
100. R. Belcher, G. Dryhurst, and A. M. G. Macdonald, J. Chem. Soc., 4543 (1965).
101. A. F. Bochkov, I. S. Yazlovitskii, and N. K. Kochetkov, Izv. Akad. Nauk SSSR, Ser. Khim., 1812 (1968).
102. N. K. Kochetkov, A. F. Bochkov, and I. S. Yazlovitskii (Yazlovetsky), Carbohydrate Res., 9, 49 (1969).
103. M. Kates and M. K. Wassef, Ann. Rev. Biochem., 39, 323 (1970).
104. J. M. McKibbin and W. E. Taylor, J. Biol. Chem., 178, 29 (1949).
105. C. J. Lauter and E. J. Trams, J. Lip. Res., 3, 136 (1962).
106. E. Robins, O. H. Lowry, K. M. Eydt, and R. E. McCaman, J. Biol. Chem., 220, 661 (1956).
107. A. Yamamoto and G. Rouser, Lipids, 5, 442 (1970).
108. A. N. Siakotos, S. Kulkarni, and S. Passo, Lipids, 6, 254 (1971).
109. L. Coles and G. M. Gray, J. Lip. Res., 11, 164 (1970).
110. T. Sakagami, J. Biochem., 45, 313 (1958).
111. W. J. Baumann, H. H. O. Schmid, and H. K. Mangold, J. Lip. Res., 10, 132 (1969).
112. R. Wood and R. D. Harlow, J. Lip. Res., 10, 463 (1969).
113. P. Karrer and K. Pflaehler, Helv. Chim. Acta, 17, 766 (1934).
114. H. E. Carter, J. A. Rothfus, and R. Gigg, J. Lip. Res., 2, 228 (1961).
115. A. J. Polito, T. Akito, and C. C. Sweeley, Biochemistry, 7, 2609 (1968).
116. K. A. Karlsson, Lipids, 5, 878 (1970).
117. R. O. Brady and R. M. Burton, J. Neurochem., 1, 18 (1956).
118. R. C. Gavec and C. C. Sweley, J. Amer. Oil. Chem. Soc., 42, 294 (1965).
119. H. E. Carter, W. P. Norris, F. J. Glick, and G. E. Phillips, J. Biol. Chem., 170, 285 (1947).
120. T. W. Culp, C. R. Creger, A. A. Swanson, J. R. Couch, and R. D. Harlow, Exp. Eye Res., 7, 134 (1968); Chem. Abstr., 69, 1129k (1968).
121. M. Gold, Lipids, 3, 539 (1968).
122. R. Watts and R. Dils, Res., 9, 40 (1968).
123. U. Persmark and B. Toregard, J. Cromatogr., 37, 121 (1968).
124. R. A. Hites, Analyt. Chem., 42, 1736 (1970).
125. A. Randrup, Scand. J. Clin. Lab. Invest., 14, 578 (1962); Chem. Abstr., 58, 11809a (1963).
126. A. Jover, J. Lip. Res., 4, 228 (1963).

127. G. C. Buckley, J. M. Cutler, and J. A. Little, Can. Med. Assoc. J., 94, 886 (1966); Chem. Abstr., 64, 20176f (1966).
128. F. Galletti, Clin. Chim. Acta, 15, 184 (1967).
129. M. R. Lloyd and R. B. Goldrick, Med. J. Austral., 2, 493 (1968); Chem. Abstr., 70, 26285s (1969).
130. E. Van Handel and D. B. Zilversmit, J. Lab. Clin. Med., 50, 152 (1957); Chem. Abstr., 51, 18069d (1957).
131. L. A. Carlson and L. B. Madström, Clin. Chim. Acta, 4, 197 (1959).
132. E. Van Handel, Clin. Chem., 7, 249 (1961).
133. L. A. Karlson, J. Atheroschl. Res., 3, 334 (1963).
134. A. Kaplan and V. F. Lee, Proc. Soc. Expt. Biol. Med., 118, 296 (1965).
135. S. Laurell, Scand. J. Clin. Lab. Invest., 18, 686 (1966).
136. S. L. Kanter, Clin. Chim. Acta, 16, 177 (1967).
137. G. A. R. Deckoix and J. G. Gobert, Anal. Biochem., 25, 523 (1968).
138. K. Moline and E. J. Barron, Clin. Biochem., 2, 321 (1969); Chem. Abstr., 73, 11251r (1970).
139. M. Kraml and L. Cosyns, Clin. Biochem., 2, 373 (1969); Chem. Abstr., 73, 22087t (1970).
140. P. Haux and S. Natelson, Microchem. J., 16, 68 (1971).
141. H. P. Chin, S. S. Abdel-Meguid, and D. H. Blankenhorn, Clin. Chim. Acta, 31, 381 (1971).
142. M. J. Fletcher, Clin. Chim. Acta, 22, 393 (1968).
143. R. Lemaur and J.-P. LePalec, Ann. Pharm. Franc., 28, 257 (1970); RZhBkh, IF91 (1971).
144. F. Mill, A. Lawn, R. Phillips, and J. A. O'Malley, J. Amer. Med. Technol., 32, 693 (1970); Chem. Abstr., 74, 108002u (1971).
145. F. Dunsbach, Z. Klinische Chemie, 4, 262 (1966); Chem. Abstr., 66, 82993a (1967).
146. V. M. Sardesai and J. A. Manning, Clin. Chem., 14, 156 (168); RZhBkh,9F81 (1969).
147. D. G. Cramp and G. Robertson, Anal. Biochem., 25, 246 (1968).
148. J.-R. Claude and F. Corre, Ann. Biol. Clin., 26, 451 (1968); RZhBkh, 9F114 (1969).
149. M. E. Royer and H. Ko, Anal. Biochem., 29, 405 (1969).
150. G. Kessler and H. Lederer, Technicon Symposium "Automation in Analytical Chemistry," Mediad, Inc., New York (1966); Chem. Abstr., 67, 40942y (1967).
151. R. P. Noble and F. M. Campbell, Clin. Chem., 16, 166 (1970); Chem. Abstr., 74, 61510r (1971).
152. W. Godike and U. Gerike, Clin. Chim. Acta, 30, 727 (1970).
153. W. Weigel, Z. Physiol. Chem., 353, 113 (1972).
154. M. Pays, P. Malangeau, and R. Bourdon, Ann. Pharm. Fr., 25, 29 (1967); Chem. Abstr., 67, 47121p (1967).
155. D. D. Woodman and C. P. Price, Clin. Chim. Acta, 38, 39 (1972).
156. J. Hoeflmayr and R. Fried, Schweiz. Med. Wochenschr., 99, 1826 (1969); Chem. Abstr., 72, 28775s (1970).
157. J. Hoeflmayr, R. Fried, A. Cordua, and G. Knecht, Z. Klin. Chem. Klin. Biochem., 8, 134 (1970); Chem. Abstr., 73, 32217z (1970).
158. R. A. Timms, L. A. Kelly, J. A. Spirito, and R. G. Engstrom, J. Lip. Res., 9, 675 (1968).
159. T. V. Chernenko, A. L. Markman, and A. U. Umarov, Khim. Prirodn. Soedin., 76 (1969).
160. G. Berner and G. Guhr, Fette, Seif., Anstrichm., 71, 459 (1969).
161. U. B. Seiffert, Mikrochim. Acta, 456 (1969).
162. A. G. Vereshchagin, Usp. Khim., 50, 1995 (1971).
163. J. P. Mallon and C. Dalton, Anal. Biochem., 40, 174 (1971).
164. M. L. Karnovsky and W. S. Rapson, J. Soc. Chem. Ind., 65, 138, 425 (1946).
165. G. A. Tompson and P. Lee, Biochim. Biophys. Acta, 98, 151 (1965).
166. D. Todd and G. P. Rizzi, Proc. Soc. Exp. Biol. Med., 115, 218 (1964).
167. G. B. Ansell and J. N. Hawthorne, Phospholipids, Elsevier, Amsterdam, Vol. 3 (1964), p. 85.
168. D. G. Hanahan and R. Watts, J. Biol. Chem., 236, PC59, 191 (1961).
169. D. Subrahmanyam, Can. J. Biochem., 45, 1195 (1967).
170. R. Wood and F. Snyder, Lipids, 3, 129 (1968).
171. L. A. Horrocks and D. J. Cornwell, J. Lip. Res., 3, 165 (1962).
172. J. Ellingboe and M. L. Karnovsky, J. Biol. Chem., 242, 5693 (1967).
173. G. V. Marinetti, Lipid Chromatographic Analysis, Marcel Dekker, New York,Vol. 1 (1967), p. 339.
174. F. Smith, Progress in Thin-Layer Chromatography and Related Methods (1969).*
175. P. W. Albro and J. C. Dittmer, J. Chromatogr., 38, 230 (1968).
176. M. L. Karnovsky and A. Brumm, J. Biol. Chem., 216, 689 (1955).
177. G. V. Marinetti, J. Erbland, and E. Stotz, J. Amer. Chem. Soc., 81, 861 (1959).

*As in Russian original. The journal of this name did not begin publication until 1970 – Publisher.