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Gas-chromatographic Analysis of Nucleic Acids

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Problems of the gas-chromatographic analysis of the components of nucleic acids are examined and data for the conditions governing such studies are presented. Since the investigation of the composition of nucleic acids involves, apart from gas chromatography, their isolation from natural sources and the preparation of volatile derivatives, particular attention is devoted to the problem of the preparation of samples for analysis. The fundamental trends in the practical applications of the method are discussed.
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I. INTRODUCTION

The unflagging interest in nucleic acids (NA) is due to their relation to such most important problems of vital activity as heredity and the development and regulation of various functions. One of the most important aspects of the study of NA involves the investigation of their composition, i.e. the separation, identification, and quantitative determination of purine and pyrimidine NA derivatives. For this purpose, studies have been made of a wide variety of fractionation methods, including ion-exchange chromatography[1-4], paper chromatography[5-8], paper electrophoresis[9-12], and thin-layer chromatography[13-15]. With their aid, most of the studies on the composition of NA have been carried out. The characteristics of the above methods have been widely discussed in a series of detailed reviews[16-21].

The majority of the methods employed require milligrams of material for investigation if a radioactive tracer is not employed. Since biological samples are frequently very small (micrograms of material), there was a need for methods which would yield satisfactory quantitative results under these conditions also. The attention of investigators was therefore attracted to liquid and gas–liquid chromatography. Gas–liquid chromatography (GLC), which ensures rapid separation of the test systems, selectivity, and sensitivity of the analysis, is becoming increasingly popular in the study of various biological substances. A GLC technique for numerous biological components has now been developed: aliphatic acids, cyclic compounds, alcohols, aldehydes, ketones, essential oils, steroids, lipopolysaccharides, amino-acids, and others[22-24].

The first attempt to use the GLC analysis in the study of NA was undertaken in 1962.[25] Since the NA components are inert and involatile in the temperature range used in gas chromatography, the authors used their acetyl, methyl, and isopropylidene derivatives. In 1964 MacGee[26] investigated *N*-methyl derivatives of purine and pyrimidine bases. However, the data presented were unsuitable for quantitative analysis, because numerous derivatives were obtained for each NA component after esterification (thus, four such derivatives obtained for adenine alone). Despite the fact that MacGee[27] was able to obtain certain quantitative data in 1966, this method for the analysis of NA has not been widely used. Progress in the application of GLC in the analysis NA became possible when silylating agents began to be used to prepare their volatile derivatives.

The majority of the studies on NA by gas chromatography have now been carried out using their trimethylsilyl (TMS) esters, their acetylation and methylation being used only in a few investigations[28-33]. Silylated NA derivatives were first obtained by Hancock and Coleman[34] who used for this purpose hexamethyldisilazane (I) and chlorotrimethylsilane (II) in pyridine. They authors obtained numerous derivatives of the constituents and asymmetric peaks were recorded on the chromatogram. In the same year Hancock[35] reported a successful separation of various adenosine derivatives and showed that, after silylation, they can be used for quantitative analysis.

In 1966–1968 Hashizume and Sasaki[36-38] published studies devoted to the separation and quantitative determination of TMS derivatives of the bases, nucleosides, and nucleotides obtained using compounds (I) and (II) as silylating agents. The possibility of using this method for the analysis of bases forming part of the composition of TNA and RNA was also demonstrated in these investigations. Gehrke and Ruyle[39] proposed a quantitative method for the GLC analysis of TMS derivatives of NA obtained in the reaction with *N*,*O*-bis(trimethylsilyl)acetamide(III) and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide(IV), which had been used earlier to modify aminoacids[40]. Subsequently *N*-trimethylsilylimidazole, *N*-diethyl-*N*-trimethylsilylamine, *N*-methyl-*N*-trimethylsilylacetamide and their various combinations were used to silylate NA derivatives[41-44]. All the reagents enumerated above are used nowadays to prepare TMS derivatives of NA. Silylation of the NA components made it possible to obtain quantitative data by the gas chromatography of microamounts of the test material, which permits a wide scale application of the method.

The present review deals with problems of the preparation of NA samples for GLC analysis, the experimental technique, and aspects of its practical applications.

II. PREPARATION OF SAMPLES FOR THE GAS-CHROMATOGRAPHIC ANALYSIS OF NUCLEIC ACIDS

1. Hydrolysis of Nucleic Acids

For the GLC analysis of NA, it is necessary in the first place to hydrolyse the polymer to various monomers: purines, pyrimidines, nucleosides, or nucleotides (depending on the

method of depolymerisation). The characteristics of the silylation method show that the best procedure involves decomposition to the bases or nucleosides carried out with the aid of various acids and enzymes[45–47]. The method of hydrolysis of NA by $HClO_4$ has come to be widely used. This method, followed by purification of the product by ion-exchange chromatography, has been employed in the preparation of samples for GLC.[36,37] However, the method of hydrolysis of NA by trifluoroacetic acid or a mixture of trifluoroacetic and formic acids must be regarded as more useful, since the two acids can be separated from the sample by simple evaporation and there is therefore no need for an additional purification of the sample. The depolymerisation of the NA in this way greatly simplifies the procedure involving the preparation of the sample and increases the overall reliability and sensitivity of the method. Purines and pyrimidines, prepared in this way, have been studied successfully by GLC.[48,49]

2. Silylation of Nucleic Acids

Detailed information about the properties of the silylating agents and about the nature of their interaction with the NA component has been published in a number of reviews[50–52]. According to Muni and Altschuler's data[53], the formation of the TMS esters of NA derivatives involves the substitution of an active proton by a trimethylsilyl group. This phenomenon decreases the possibility of the formation of hydrogen bonds and increases the stability of the mixture. The silylated derivatives of purines and pyrimidines are less strongly bound and are more volatile compared with their unmodified analogues.

The conversion of NA into volatile components can be regarded as ideal when it proceeds in accordance with the "one base (nucleoside, nucleotide)–one derivative" principle. However, such a ratio cannot always be maintained for each component of the test mixture. In certain cases purines and pyrimidines containing a functional amino-group each form two derivatives[52,54]. The cause of this is a whole series of factors: the nature of the silylating agent, the solvent, the reaction temperature, and certain others[55]. At the same time the presence of two peaks corresponding to a single base (nucleoside, nucleotide) on the chromatogram does not interfere with the quantitative analysis and is fully reproducible[39,56,57]. Cytosine and the corresponding nucleoside and nucleotide most frequently give rise to two such compounds[48,56]. According to the results of the mass-spectrometric analysis of TMS-cytosine, the two peaks of its derivatives correspond to di-TMS-cytosine and tri-TMS-cytosine[58].

The available information about the proposed structures of various TMS-nucleosides and TMS-nucleotides as well as the isomers has been published in a number of communications[44,59–63]. According to the data obtained, cytidine forms tetra-TMS-cytidine and penta-TMS-cytidine. The reactions of minor derivatives of cytosine (5-methylcytosine) and adenine (6-methylaniline) with compound (III) or (IV) also each gave rise to two TMS-derivatives[56,64,65]. It is noteworthy that, when other silylating agents or other modification conditions are used, it is possible to obtain only one derivative of the base[49,66,67].

Much attention is devoted nowadays to the selection of both the best silylating agent and of the optimum esterification method. The data for the silylation conditions and the agents employed are compiled in Table 1. Among the substances enumerated which are used to prepare the TMS esters of NA, compounds (III) and (IV) are the most popular in view of their high modifying activity in relation to NA compared with other silylating agents[44,54,68,69].

It has been suggested that the optimum conditions for the interaction of bases and nucleotides with compound (III) involve heating of the reaction mixture for 45 min at 150 °C in the presence of 100 moles of the above agent in excess[39]. It has also been suggested that, in the treatment of nucleosides with compound (III), the latter be present in excess (225 moles) and that the mixture be heated for 2 h at 120 °C.[44] In the optimisation of the conditions in the modification of bases and nucleosides using compound (IV) best results were obtained on heating the mixture for 15 min at 150 °C in the presence of 225 moles of the silylating agent in excess[57,70]. The same amount of compound (IV) was chosen as optimal also in the silylation of nucleotides. An increase of the volume of the silylating agent led in this case to the appearance in the mixture of the corresponding TMS-bases. Deoxycytosine gave rise to a TMS-cytosine peak on the chromatogram also under all other silylation conditions using both compound (III) and compound (IV).[44]

The optimum conditions in the esterification of nucleotides and dinucleotides using compound (IV) as the silylating agent have been studied by Patel and Gehrke[71], who concluded that, for a more successful modification and chromatography, mono- and di-nucleotides from biological samples must be converted into the corresponding nucleosides or bases. According to the data of Miller et al.[54] optimum silylation using compound (IV) is attained in the case of bases by heating at 150 °C in the presence of 100 moles of compound (IV) in excess for 15 min, while in the case of nucleosides the heat treatment should last 30 min. It is noteworthy that NA are silylated also at room temperature but less efficiently[42,44,49]. The silylation of NA has been studied quantitatively by Řehák and Pacáková[72], who established, using spectrophotometric and radioisotope methods to follow the process, that if the latter takes place at 150 °C over a period of 45 min in the presence of 50 moles of compound (IV) in excess, then at least 95% of the initial amount of the bases investigated are converted into TMS-derivatives.

Dichloroethane, hexane, benzene, acetonitrile, pyridine, dimethylformamide, toluene, and certain other substances have been used as solvents for the preparation of volatile NA derivatives. Pyridine and acetonitrile are most widely used nowadays. Comparative data for the solvents employed are listed in Table 1. According to the available information about the influence of various solvents on the silylation process, acetonitrile must be regarded as the best[44,65]. Data on the efficiency of the silylation of nucleosides using various solvents are presented in Table 2.

Apart from all the factors enumerated, the preparation of TMS ethers of purines and pyrimidines and the results of their subsequent GLC analysis can be influenced by various salts in water, which sometimes remain in the NA sample after its isolation or hydrolysis. The studies carried out to elucidate this problem have shown that the NA components respond in different ways in the presence of particular salts. When compounds such as Na_2SO_4 and $CaSO_4$ were added to the reaction mixture, the results of the GLC analysis remained unchanged, while the presence of added $CaCl_2$ caused some decrease of the guanine peak[57]. The Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cu^{2+} cations added to the reaction mixture in the silylation of guanine and hypoxanthine had a negative influence on the esterification process, apparently owing to the decrease of the volatility of TMS-derivatives[43]. The presence of NH_4^+ cations did not affect this process.

In their study of the influence of salts such as NaCl, KCl, NH_4Cl, $(NH_4)_2SO_4$, $MgCl_2$ $CaSO_4$, $CaCl_2$, and Na_2SO_4 and the silylation and chromatography of nucleosides, Patel and

Table 1. Experimental conditions in the silylation and gas-chromatographic analysis of purines and pyrimidines*.

Components investigated	Silylating agent	Solvent	Silylating conditions		Carrier	Liquid phase, %	Type of detector	Carrier gas	Refs.
			t, °C	τ, min					
Nucleotides	(I):(II) = 2:1	Pyridine	155	60	Diasolid, 30-50 mesh	DC-430 (5)	TCD	Nitrogen	36
Bases	(I):(II) = 2:1	Pyridine	155	60	Zeolite 540, 80-100 mesh	DC-430 (5)	TCD	Helium	38
Bases, nucleosides	(I):(II) = 2:1	Pyridine	155	60	Diasolid, 30-50 mesh	Apiezon L (5); SE-30 (2); SE-52 (5); QF-1 (1); DC-430 (5); LAC-2R446 (1)	TCD	Helium	66
Bases, nucleotides	(I):(II) = 2:1	Pyridine	155	60	Ditto	DS-430 (5)	TCD	Helium	74
Nucleosides	(III)	Pyridine	120	120	Gas Chrom Q, 100-120 mesh	OV-17 (4)	FID	Argon	75
Bases, nucleosides	(III)	Pyridine	150	45	Gas Chrom Q, 100-120 mesh	SE-30 (3)	FID	Nitrogen	64
Bases, nucleosides	(IV) + 1.0% (II)	Pyridine	60	960	Chromosorb W, 80-100 mesh	OV-1 (3)	FID	Helium	67
Bases	(IV)	Pyridine	150	45	Chromosorb W, 60-80 mesh	OV-17 (3)	FID	Nitrogen	72
Bases	(IV)	Acetonitrile-dichloroethane (1:1)	150	15	Chromosorb W, 80-100 mesh	OV-101 (10)	FID	Nitrogen	76
Bases	(II), (III), (IV) etc.	Pyridine	room		Diatoport S	OV-17 (3)	FID	Helium	43
Nucleosides	(IV)	Acetonitrile	150	15	Supelcoport, 100-120 mesh	SE-30 (4) SE-30 (3); OV-17 (3); OV-11 (4)	FID	Nitrogen	70
Bases	(IV)	Acetonitrile	150	15	Ditto	SE-30 (10); OV-3 (5); OV-7 (5)	FID	Nitrogen	65
Bases, nucleosides	(III), (IV)	Pyridine	150	45,20, 30	Chromosorb W, 100-120 mesh	OV-17 (3) OV-101 (3)	FID	Argon	54, 77
5-Fluorouracil, florafur, 6-azauridine, allopurinol	(IV)	Pyridine	150	20	Chromosorb W, 100-120 mesh	OV-101 (3) OV-17 (3)	FID	Argon	51
Nucleosides	(III), (IV), trimethylsilylimidazole	Acetonitrile	**	**	Supelcoport, 100-120 mesh	OV-11 (4)	FID	Argon	44
Nucleosides	(IV) + 1.0% (II)	Pyridine	75	180	Chromosorb W, 80-100 mesh	SE-30 (5)	FID	Helium	68
Nucleosides	IV + 1.0% (II)	Pyridine	75	180	Gas Chrom Q, 100-120 mesh	SE-30 (3)	FID	Helium	78
Bases	(IV)	Acetonitrile	150	15	Supelcoport, 100-120 mesh	SE-30 (10)	FID	Nitrogen	73
Nucleosides	(III)	Hexane	150	40	Chromosorb W	OV-1 (1)	FID	Helium	79
Nucleosides	(IV)	Acetonitrile:dichloroethane = 1:1	150	15	Chromosorb W, 80-100 mesh	OV-7 (3)	FID	Helium	80
Bases	(III), (IV)	Pyridine	room	–	Gas Chrom Q, 100-120 mesh	SE-30 (3); OV-225 (2); OV-17 (3); OV-210 (2)	FID	Nitrogen	49
Nucleosides	(II) + (IV)	Pyridine	80	60	Gas Chrom P, 100-120 mesh	SE-30 (1); OV-17 (1)	FID	Nitrogen	59

*Notation: (I) = hexamethyldisilazane, (II) = chlorotrimethylsilane, (III) = *N*,*O*-bistrimethylsilylacetamide, and (IV) = *N*,*O*-bis(trimethyl)trifluoroacetamide; TCD = thermal conductivity detector and FID = flame ionisation detector.

**Different values for different silylating agents.

Gehrke established that thymidine is most sensitive to them on modification with compound (IV). If a mixture of the above salts was involved in the reaction, an appreciable decrease of the relative weight sensitivity coefficients of all the nucleosides was observed. According to the authors[71], these solvents can decrease the degree of silylation of nucleosides by reducing their solubility and the molar excess of compound (IV) as a result of the formation of derivatives of TMS anions such as TMS-SO_4 and TMS-PO_4 and the decomposition of the TMS-nucleosides in the sample. A similar response to the presence of these salts apparently occurs also in the silylation of the bases.

Various workers obtained data according to which the maintenance of anhydrous conditions is obligatory for the successful GLC analysis of NA derivatives. After the silylation of guanine and hypoxanthine by compound (III), the peaks of these substances were not observed on the chromatograms when 100 μl of water had been added to a sample containing 5 mg of each substance[43]. According to the data of Gehrke and Lakings[57], the presence of 5 μl of water in a 200 μl sample of the bases reduced the second peak corresponding to cytosine on the chromatograph up to its complete disappearance. On further increase of the concentration of water, the first peak also diminished as well as the peak corresponding to guanine. In the reaction of nucleosides with compounds (III), (IV), and trimethylsilylimidazole a negative influence of water on the silylation process was observed. The necessity to maintain anhydrous conditions in the GLC analysis of NA has also been reported by other workers[53].

The retention of the TMS derivatives of NA from the instant of their preparation until the gas-chromatographic

investigation is of practical importance. Thus the TMS derivatives of bases stored at room temperature for five days yielded a clearly reproducible result in their GLC analysis[57]. TMS derivatives of nucleosides stored under the same conditions remained stable for 48 h. After more prolonged storage of the samples, numerous peaks, corresponding to the TMS-bases of the initial nucleosides and other products of the decomposition of the TMS-derivatives of nucleosides, appeared on their chromatogram[70]. At the same time there are data[53] according to which the majority of the silylated purines and pyrimidines are chromatographically stable for a year and more when stored in a tightly closed vessel in the presence of an excess of the silylating agent at 3–5 °C.

Thus reliable methods have now been developed for the quantitative preparation of volatile derivatives of bases and nucleosides suitable for GLC analysis.

Table 2. The effect of various solvents (relative weight sensitivity coefficient) on the silylation of nucleosides by compound (IV).[70]

Compound	Aceto-nitrile	Pyri-dine	Dimethyl-formamide	Methylene chloride	Chloroform	Benzene	Toluene	Hexane
Pyrimidines								
Thymidine	0.50	0.48	*	0.48	0.44	0.36	0.38	0.43
Uridine	0.78	0.75	0.67	0.76	0.74	0.32	0.33	0.56
Cytidine	0.37	0.35	0.01	0.34	0.31	0.16	0.17	0.28
Purines								
Inosine	0.78	0.78	0.76	0.38	0.33	0.05	0.16	0.48
Adenosine	1.01	0.97	0.99	0.58	0.51	0.54	0.56	0.85
Xanthosine	0.78	0.73	0.78	0.38	0.34	0.13	0.19	0.40
Guanosine	0.70	0.70	0.70	0.30	0.28	0.12	0.19	0.40

*The peak was not obtained.

III. ANALYTICAL CONDITIONS

1. Liquid Phases

Non-polar and moderately polar liquid phases have been used successfully for the separation of TMS derivatives of NA. With their aid, it is possible to separate TMS derivatives of the bases, nucleosides, and the phosphates (Table 1). Among non-polar liquids, phases such as SE-30, OV-1, OV-101, DC-430, and certain others are employed. Among moderately polar phases, those containing phenyl groups have been used: OV-3, OV-17, XE-60, OV-7, and others. The characteristics of these phases are quoted in a handbook[81]. The non-polar liquid phase SE-30, which is a methylsiloxane polymer, has been most frequently used for the separation of NA derivatives.

The concentration of the liquid vary within fairly wide limits—from 0.2 to 10%.[39,65,72] Universally prescibed concentrations are lacking for each phase. In choosing the concentration of the liquid phase, one must bear in mind that the presence of a large amount of the latter entails an increase of the separation time, while the phase present in a small amount cannot fully compensate for the inertness of the carrier, which impairs the quality of the separation and decreases the sensitivity of the method. The selection of the liquid phases and their concentrations is related to the nature of the mixtures separated and the purposes of the investigation. Thus in the separation of ribonucleosides, best results were obtained using phases such as 4% of OV-11 and 3% of OV-17.[70] The best separation of the minor bases was achieved on 0.5% of OV-3.[65] The separation of 5-methylcytosine from cytosine in preparation of bases obtained from calf thymus DNA and bacteriophages was observed on the following liquid phases: 3% of SE-30 and 2% of OV-225.[49] According to Muni and Altschuler[53], a satisfactory separation of the TMS derivatives of purines and pyrimidines was attained using 3–5% of the SE-30 and OV-17 phases.

Substances lacking catalytic activity, exhibiting a minimal adsorption capacity, and having highly homogeneous surfaces, dimensions, and shapes are used as carriers for the liquid phase. Such substances are in the first place diatomaceous carriers of the type of Chromosorb W, Supelcoport, Anachrome, Gas Chrom Q, etc. (Table 1). Silylated carriers are frequently used[54,56].

2. Conditions of the GLC Analysis of NA

A relatively wide set of GLC analytical conditions can be used in the study of NA. After the liquid phase, the carrier, and the column length have been chosen, the temperature and the flow rate of the gas are usually regulated in such a way that the required separation can be achieved during a satisfactory time. The GLC analysis of NA can be carried out under both isothermal conditions and with temperature programming. The choice of the conditions in the investigation depends in many respects on its aim. Multicomponent systems are mainly separated using temperature programming[38,64,65,67,75]. The separation of the TMS derivatives of the bases, nucleosides, and nucleotides is as a rule carried out in the temperature range from 80° to 325 °C.[38,65,67,75,78]

The chromatographic columns are in most instances 1–2 m long and have an internal diameter of 2–4 mm. Both glass and metallic columns are used for the chromatography of the TMS derivatives of NA. However, it is believed[82] that the entire GLC analysis should be carried out in glass apparatus, since the TMS derivatives decompose on contact with heated metallic components. An analogous behaviour has been observed also in the gas chromatography of aminoacids[23].

A flame ionisation detector and more rarely thermal conductivity, argon ionisation, and electron capture detectors are used in most studies. The carrier gas is chosen depending on the type of detector employed: nitrogen, argon, and helium (Table 1). Since the TMS derivatives of purines and pyrimidines are highly sensitive to moisture, thorough drying of the gas supplied is necessary.

3. Quantitative Analysis

The quantitative determination of the bases, nucleosides, and nucleotides in the GLC analysis is carried out by an internal standardisation method. The internal standards employed are phenanthrene, pyrene, purene, acenaphthene, n-octacosane, n-tetratriacontane, n-nonadecane, and others[42,43,76,83,84]. Among these, phenanthene and pyrene are most widely used. The internal standard is added to the initial mixture and undergoes all the stages in the preparation of the TMS derivatives. The amount of test substance is calculated using the "relative molar sensitivity coefficient" or the "relative weight sensitivity coefficient"[65,66].

The reproducibility of the data obtained in the GLC analysis of NA has been estimated with the aid of the relative standard deviation calculated from the results of several measurements. Thus the standard deviation for the TMS

derivatives of the minor components, determined in biological liquids, is on average 3.8% according to the results of four measurements[65]. The results of the determination of the relative molar sensitivity coefficients as well as the standard deviations for various nucleosides are presented in Table 3.

The introduction of comparatively large concentrations of the test substance is undesirable if quantitatively reproducible data are to be obtained. Thus, when a flame ionisation detector was used, the introduction of more than 25 μg of the TMS derivative led to the appearance of an additional peak on the elution curve, which may be associated with the moderate cooling of the flame as a result of the combustion of the organosilicon substance[85]. The minimum amount of the TMS derivatives of purines and pyrimidines which can be determined by GLC using a flame ionisation detector is $(3-5)\times10^{-9}$ g, which corresponds to 3×10^{-11} moles of each of the bases introduced[39].

Qualitative investigation of the composition of the DNA bases of salmon sperm and calf thymus and the RNA bases of yeast and tobacco mosaic virus by GLC at a macrolevel (1 mg), semimicrolevel (100 μg), and microlevel (5 μg) yielded data identical with those obtained earlier for the same systems when the latter were studied with the aid of other fractionation methods[48,75].

Table 3. The reproducibility of the results of etherification and the GLC of the TMS derivatives of the methylated bases.

Compound	Retention time, min	Relative molar sensitivity coefficient	Standard deviations*
6-Methyladenine	13.2	0.58	0.024
6-Dimethyladenine	13.0	0.64	0.029
6-Isoadenine	18.4	0.86	0.025
2-Methyladenine	16.4	0.57	0.008
2-Methylguanine	17.2	0.58	0.017
2,2-Dimethylguanine	18.0	0.83	0.020
7-Methylguanine	19.6	0.39	0.320
5-Methylcytosine	9.6—12.0	0.73**	0.024
Thymine	6.8	0.63	0.025

*The standard deviation was determined from the results of four independent experiments.

**Two peaks were obtained, each being independently integrated, and were then added to determine the overall area and to calculate the relative molar sensitivity coefficient.

4. Qualitative Analysis

The gas-chromatographic analysis of the composition of NA is extremely sensitive; it ensures a high degree of resolution and detection of the components present at very low concentrations. The identification of the unknown TMS components of the mixture by GLC in a pure form is in most cases carried out by comparing their retention times with those of known standards. Another procedure involves the comparison of the behaviour of the TMS derivatives on different phases. In order to obtain data permitting the identification of the unknown components and in the first place those for which comparison standards are not already available, it is necessary to employ an additional technique. The possibility of employing spectrometric methods such as ultraviolet and infrared scanning, NMR, mass spectrometry, and certain others for the identification of the TMS derivatives of NA has therefore been investigated[64,66,86–88]. The combination of modern GLC with the above methods[64,72] greatly extends the scope of the analysis of NA.

The absorption by purines and pyrimidines in the ultraviolet and infrared is highly specific and is widely used for their identification and quantitative determination[89–94]. The study of the spectroscopic properties of the TMS derivatives of NA obtained by GLC showed that, although the ultraviolet absorption differs to a considerable extent from that observed for the intact purines and pyrimidines, it is nevertheless specific[53,64]. The ratios of the absorption at 260 and 280 nm and at 250 and 280 nm have been used as characteristics of the TMS derivatives of NA and of their unmodified precursors. The absorption of TMS derivatives of NA in the infrared is also fairly characteristic[64,66].

The specificity of the properties of the TMS-bases has been demonstrated also in their study by NMR.[64,66] However, one should note that the application of this method is limited, because a comparatively large amount of the starting material is required for the investigation.

One of the most promising procedures for the study of mixtures of TMS derivatives of NA components is the combined GLC–mass-spectrometric method. The possibility of using it for this purpose was first demonstrated by McCloskey et al.[88] The mass spectra of the bases, nucleosides, and nucleotides, obtained on silylation with compounds (III) and (IV) as well as trimethylsilylimidazole, proved to be simple to interpret. Derivatives obtained from NA components by methylation, acetylation, trifluoroacetylation, trimethylsilylation, and the synthesis of 2′,3′-*O*-isopropylidene and 2′,3′-*O*-phenylborate ethers and esters have been investigated by this method[33,58,60,88,95]. The above combined method has been used successfully to investigate bacterial RNA and rare NA components[79,87,96,97]. Together with gas chromatography, a new mass-spectrometric method—mass-fragmentography has been employed in the analysis of TMS derivatives of NA. The application of other methods for the qualitative analysis of NA together with GLC greatly increases the accuracy of the latter.

It follows from the data presented that the purine and pyrimidine components of NA can be separated successfully by GLC. Various conditions for the GLC of nucleic acids, whereby they can be studied both quantitatively and qualitatively, have been developed. Bearing in mind the flexibility of the method, in view of the possibility of employing various liquid phases and conditions for the GLC itself, one may claim that virtually any NA component can be separated and identified with its aid.

IV. EXAMPLES OF THE APPLICATIONS OF THE GAS-CHROMATOGRAPHIC ANALYSIS OF NUCLEIC ACIDS

Gas–liquid chromatography has already found extensive applications and is arousing increasing interest, as shown, in particular, by the increase of the number of studies, carried out with its aid, which have been published in the last few years. The composition of the RNA of microorganisms, which are of theoretical and practical importance in agriculture, medicine, and industrial microbiology, has been studied by the above method[38,39,48,75,79,87,96,98,99]. Preparations of DNA from animal and bacterial cells have been investigated, particular attention being devoted to its minor components[38,48,49,74]. The biological importance of these compounds is now being vigorously investigated.

GLC has been employed in pharmacology for monitoring the chemical composition and purity of preparations and for the investigation of their distribution in the organism. The

applications of the method in this field are associated with the use of derivatives of the bases and nucleosides as therapeutic agents in the treatment of various diseases (hypertension, sugar diabetes, malignant neoplasts, etc.). In the study of therapeutic chemical preparations—standard and in biological liquids—with the aid of GLC their various volatile derivatives have been used[28-31,54,77,100-102]. The substances 6-mercaptopurine-nucleoside, 5-fluoro-2-deoxyuridine, ftorafur, investigated in these studies include 5-fluorouracil, 6-azauridine, allopurinol, β-phenylethylbiguanidine, and certain others.

The GLC analysis of NA has acquired major importance for clinical laboratory diagnosis. A method for the detection of minor NA components in biological liquids has now been developed in greater detail. It is known that the quantity of these compounds in such liquids increases in certain diseases as a result of the degradation of transport RNA.[103,104]. An increase of the content of these bases has also been observed in diseases such as accute leukaemia, psoriasis, melanoma, etc.[105,110]. The possibility of obtaining TMS derivatives of the purines and pyrimidines encountered in urine and of their analysis by GLC has been demonstrated[67,78]. Comparative GLC study of the minor derivatives of NA present in the urine of healthy and ill humans revealed diagnostically significant differences between them. Such studies have been carried out in instances of leukaemia, various malignant neoplasts, and other diseases[65,76,80,83,111,112].

The information presented in this review justifies the claim that GLC ensures a highly effective separation and identification of minimal amounts of the test substance combined with a high degree of reproducibility. The extensive possibilities of the employment of GLC in the manufacture of preparations containing NA components and in clinical laboratory diagnosis are due to the fact that, apart from the advantages already mentioned, the method can be fully automated.

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During the preparation of the manuscript for the press, certain studies of fundamental importance on the GLC of NA were published. Conditions were proposed for the GLC investigation of DNA bases under isothermal conditions[113,114] and the first successful attempt was made to separate the TMS derivatives of NA components using capillary columns[115]. The possibility of investigating certain pyrimidines by GLC without their preliminary conversion into volatile derivatives was demonstrated[116]. It is noteworthy that the method of pyrolytic GLC study of NA, which had been used earlier[117-119] but had not found extensive applications, is nowdays used successfully for the comparative analysis of biological samples containing NA.[120]

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