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HIGH-TEMPERATURE GAS-LIQUID CHROMATOGRAPHY

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Two trends have arisen in the gas chromatography of high-boiling substances. The first is based on the use of

moderate working temperatures, but with sensitive detectors having a low noise level. The second is based on the use of conditions leading to a decrease in retention volumes, *i.e.* increase in working temperatures, operation under vacuum, decrease in the percentage of stationary phase, etc. The future of gas chromatography will naturally depend on the progress and synthesis of the two trends.

The present review deals with problems relating to separations at high working temperatures. The reason for dealing with the high-temperature method separately is not only that the method is particularly promising for further extension of the boundaries of gas chromatography, but also that it requires the use of special materials and the application of special principles in the design of apparatus.

As early as 1952, James and Martin[1] suggested that gas–liquid chromatography might be used for the analysis of mixtures of substances with high boiling points. The use of gas–liquid chromatography at temperatures above 200° was first described in 1953 by Cropper and Heywood[2,3] for the analysis of the methyl esters of aliphatic acids with up to 22 carbon atoms. Subsequently a number of papers were published on the use of gas–liquid chromatography at temperatures up to 260° [4,5] and above[6–8].

The extension of the method of gas–liquid chromatography to the region of high temperatures involves a number of serious difficulties. The majority of solvents used as stationary phases are too volatile or unstable, or often both, at high temperatures. Increase in temperature, moreover, leads to a decrease in the separation factor. Gas–liquid chromatography at temperatures in the range 250°–350° involves special problems associated with the design of the apparatus, the choice of materials, seals, etc. When the separate parts of the apparatus are connected *via* glass-metal junctions, the difference in the expansion coefficients may lead to leaks in the apparatus; this does not usually occur at low temperatures. The most difficult problem, however, appears to be the choice of stationary liquid phase, with thermal stability and minimum volatility. At present there are no definite, generally accepted technical conditions which a liquid for use as stationary phase should satisfy when tested for volatility. It has been recommended[9] that when a given substance is used in a chromatographic column for 3 months its retention volume under comparable conditions should not change by more than 2%, but this criterion is not quite precise, since the retention volume may alter for other reasons, for which it is difficult to make allowance and which are not related to the volatility of the stationary phase.

Tuey[8] takes as limiting temperature the temperature at which the column loses 50% of the stationary phase in 1000 h of continuous operation at a carrier gas velocity of 15 ml min^{-1}. The tests were carried out in a column containing 2 g of stationary phase, so that this limit corresponds to a volatility of about 1×10^{-3} g per litre of gas.

Harvey and Chalkley[10] state that the upper temperature limit of operation of the stationary phase should be at least 100° below its boiling point. In any case, although this has not been proved experimentally, the vapour pressure of the liquid of the stationary phase should not exceed 1 mm Hg at the working temperature, otherwise the column soon ceases to function.

Despite the difficulties associated with the choice of stationary phase for high-temperature chromatography, a number of substances have been studied and have proved suitable for work in the temperature range 250°–350°. Keppler *et al.*[11] tested some substances which have found application as stationary phases at high temperatures (polyethylene, silicone high-vacuum greases and elastomers, Apiezon greases, etc.). These substances (deposited on Celite 545) gave the following figures for the loss (g h^{-1}) from the column at a gas flow rate of 15 ml min^{-1} in the range 210°–250° (the percentage of the stationary phase was different in each case):

Silicone elastomer DS No. 123	3
Apiezon grease M	5
Silastomer DS No. 156	8
Polyethylene (average mol. wt. 30 000)	8

Different opinions have been put forward regarding the mechanism of the loss (migration) of stationary phase from the chromatographic column. Franke[12] suggested a purely mechanical model of stationary phase removal and recommended that the working life of the column be prolonged by placing at its outlet a short section containing solid carrier without the stationary phase. In the author's opinion, the migrating liquid would saturate the surface of the solid carrier in this section and hence retard slightly its removal from the column.

Van de Craats[12] considers that the removal of stationary phase from the column takes place entirely as a result of its volatilisation and that there are no grounds for assuming that the stationary phase will be held back in the section of the column filled with dry solid carrier.

Some of the loss of stationary phase is apparently due to the dissolution, in the stationary phase, of components of the specimen when the latter is present in high concentrations in the gas phase, particularly in an overloaded column. This leads to a decrease in the viscosity of the stationary phase and hence to an increase in its mobility.

No matter which mechanism is suggested to explain the loss of stationary phase from a chromatographic column, it undoubtedly involves both purely mechanical removal of liquid and a volatilisation–condensation cycle. A slight vapour pressure of the stationary phase at a given temperature will cause it to be moved by the gaseous phase, as are the components of the specimen.

When gas–liquid chromatography is carried out at high temperatures, no attempt is made to work with a high percentage of stationary phase on the solid carrier; its concentration is usually reduced to 10% and less. Calculations show that only a small percentage (by weight) of liquid is sufficient to cover the surface of the solid carrier completely; any liquid in excess of this amount, deposited on the carrier but not held to it by adhesive forces, will soon be removed from the column at high temperatures.

Gerrard *et al.*[9] have studied the factors determining the maximum temperature at which a given substance can be used as stationary phase. They state that when polymeric substances (polypropylene glycol sebacate, silicone oils, etc.) are used as stationary phase, their thermal decomposition is more important than their volatility. The authors studied the thermal decomposition of two silicone oils recommended for use as stationary phase at high temperatures (240° and above): Embaphase silicone oil and E-301 elastomer, which proved to be stable up to 260° and 300° respectively. The time dependence of the loss in weight of a specimen of silicone oil at 260° is shown in Fig. 1.

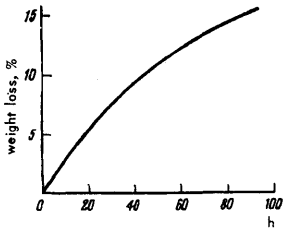


Fig.1. Weight loss of a specimen of silicone oil with time at 260°.

Analogous studies of stationary phases for high temperatures were made by Baxter and Keen[13], who determined the upper temperature limits at which the following substances can be used: Apiezon L (350°), high-vacuum silicone grease (250°), polyethylene (300°), asphaltenes (300°), and polyphenyl tars with molecular weights of 800 and 2100 (400° and 450° respectively).

When gas–liquid chromatography is used for preparative purposes in large-diameter columns, the choice of stationary phase is subject to more stringent requirements than when the process is carried out in analytical columns. Gerrard and coworkers put forward an equation for calculating the limiting permissible volatility of the stationary phase in a preparative column:

$$V = \frac{10Cw}{ft},$$

where V is the volatility (mg per litre of gas), C the maximum permissible percentage of stationary phase in the sample, w the weight of material condensed in the trap (mg), t the period of trapping (min), and f the flow rate of the gas stream (ml min^{-1}).

In the temperature range 250°–350° most workers generally use as stationary phase high-vacuum silicone greases of different grades. It has been found, however, that the separating power of columns with this stationary phase depends to a considerable extent on the quality of the grease. Cropper and Heywood[3] note that, in the analysis of esters of aliphatic acids, a much improved separation could be achieved by preliminary treatment of the silicone grease with ethyl acetate, subsequent addition of ethyl alcohol, and decantation of the solvent. This treatment removed the oily fractions from the grease.

Felton[6] states that columns containing silicone grease as stationary phase should be subjected to preliminary "treatment" ("conditioning"), in which the column is heated at 400° for 2–3 h while the carrier gas is passed through. If this preliminary treatment of the column is not carried out, the chromatogram usually shows, after the introduction of the specimen, a series of non-reproducible unstable peaks, whose formation is apparently due to the presence of volatile substances in the grease. The need for preliminary treatment of silicone grease was also pointed out by Cason and Miller[14].

In addition to silicone and Apiezon greases, asphaltenes have also been used as stationary phase at high temperatures for the separation of normal paraffins up to C_{38} at temperatures up to 320° [15] and for the analysis of phenylalkanes at 240°.[16] Asphaltenes have proved to be thermally stable and sufficiently non-volatile in the temperature range 300°–400°.

Recently there has been a tendency to use inorganic salts as stationary phase at high temperatures. Hanneman *et al.*[17] have successfully used a eutectic mixture of sodium, potassium, and lithium nitrates, deposited on brick, for the separation of normal paraffins up to C_{28} and polyphenyls at temperatures up to 400°. De Boer[18] has used a stationary phase consisting of 20% molten lithium chloride (m.p. 606°) deposited on sea sand, for the separation of an alloy of cadmium and zinc at 620°. Juvet and Wachi[19] have used a eutectic mixture of bismuth and lead chlorides for the separation of titanium and antimony halides at 520°.

In gas–liquid chromatography at high temperatures the thermal stability of the specimens being separated is not generally as important as expected. Kirkland[20] states that many organic compounds have a higher stability in the vapour than in the liquid state. Moreover, the inert carrier in gas chromatography reduces considerably the possibility of decomposition of the components of the specimen by oxidation, which is usually more marked than the actual thermal decomposition.

The difficulties associated with gas–liquid chromatography at high temperatures have led research workers to seek ways of reducing the temperature of the process. In many cases chromatography at high temperatures has been carried out at reduced pressures, on the erroneous assumption that, by analogy with vacuum distillation, the rate of displacement of a component with low volatility in a chromatographic column should increase at low pressures. Later, however, it was shown that this velocity depends only to a very slight extent on the absolute pressure in the column[21] being proportional to the pressure drop in the column and dependent to a marked extent on the distribution coefficient of the component between the gaseous and liquid phases. Although operation at reduced pressures does not increase the efficiency of the column or accelerate the analysis, it does have some advantages, of which the following may be noted. (1) There is an increase in the sensitivity of the katharometer due to the increase in the concentration of the specimen in the carrier gas at low pressures. Fig.2 shows the dependence of the sensitivity factor (expressed as the ratio of the peak height in cm to the weight of the corresponding component in the specimen in mg) on the pressure at the outlet from the column. The

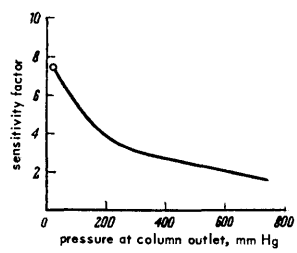


Fig.2. Dependence of the sensitivity factor of a katharometer on pressure.

katharometer was used on the peak for methyl palmitate (stationary phase — silicone grease[7]). (2) If the pressure at the column inlet is less than atmospheric, it is easier to achieve rapid volatilisation of the specimen, and this is very important for a good separation. (3) The katharometer apparently gives a more linear response at low pressures.

In columns in which the packing has a high hydraulic resistance, operation under inefficient conditions is unavoidable at reduced pressures, due to the marked increase in the velocity gradient of the gas. This explains the tendency of some workers, when carrying out gas–liquid chromatography at reduced pressures, to decrease the hydraulic resistance of the column packing by choosing an appropriate solid carrier.

In the analysis of mixtures of hexadecanol and octadecanol and of brominated technical methyl oleates Cropper and Heywood[3] were able, by replacing Celite by NaCl crystals (with 5% silicone grease), to reduce the pressure at the column inlet from 40 to 4 mm Hg, and that at the column outlet from 20 to 2 mm Hg, with a corresponding decrease in the temperature of the process from 265° to 190°–200° and consequent avoidance of thermal decomposition of the specimen.

In the determination of the esters of aliphatic acids up to C_{18} at reduced pressures Sörensen and Söltoft[22] used as solid carrier crystals of NaCl, and also small rings of stainless steel wire gauze (stationary phase silicone oil). The data on the pressure drop in the column (Table 1) show that the hydraulic resistance of the gauze rings is less than that of the NaCl crystals by a factor of 12–13.

In a short communication Hishta *et al.*[23] report that they were able to carry out the process at temperatures 200°–250° lower than the boiling point of the compound. At these temperatures many organic compounds have a vapour pressure which is still sufficient to enable gas–liquid chromatography to be carried out. The retention times of the compounds were decreased considerably by decreasing the quantity of stationary phase on the solid carrier to 0.05–0.2% instead of the 10–30% usually employed. The adsorption effect of the solid carrier, at this low concentration of stationary phase, was reduced to a minimum by using as carrier glass microbeads 200 μ in diameter instead of the carriers usually employed (brick, Celite). Because of the marked decrease in the quantity of stationary phase, it was necessary to decrease the size of the specimen to several tenths of a milligramme, to prevent saturation of the gas phase by the vapour of the specimen. The specimens were injected in the form of solutions in a volatile solvent. A flash evaporator was used (at 375°) for rapid volatilisation of the specimen. The authors obtained chromatograms for an extensive range of high-boiling substances using a 2 m column packed with glass microbeads + 0.05% silicone oil. Table 2 gives some of the compounds studied, their melting and boiling points, and the temperatures at which the process was carried out. As pointed out by the authors themselves, the efficiency of this column is lower than that of ordinary analytical columns. The authors give examples of chromatograms only for pure compounds and mention only one example of a separation, that of a mixture of isomeric eicosanes at 127° (b.p. of n-eicosane 205° at 15 mm Hg). Unfortunately the authors give no details of their work. Further data appear for an estimation of the value of this method for separating high-boiling compounds.

TABLE 1.

Solid carrier	NaCl	Rings	Rings
Length of column, m	2	2	2
Temperature, °C	200	200	180
Gas velocity, ml min^{-1}	8	8	8
Pressure at inlet, mm Hg	72	24.5	24
Pressure at outlet, mm Hg	20	20	20
Pressure drop, mm Hg	52	4.5	4

TABLE 2.

Compound	B.p., °C	Temperature of column, °C	Compound	B.p., °C	Temperature of column, °C
n-Octacosane	412	200			
Anthracene	354	100	Octadecane	210 at 15 mm Hg	200
Chrysene	448	190	1,3-Diphenylbenzene	363	200
Anthraquinone	380	175	Methyl oleate	216 at 20 mm Hg	148
p-Dibromobenzene	218	103			

In gas–liquid chromatography at high temperatures the great majority of workers have used thermal-conductivity detectors — katharometers — of varying design. Other detecting devices have been used in only a few cases: Martins' gas balance[5,24], hydrogen flame detectors[25,26], an emission detector[27], etc. Glass and metallic katharometers have been proposed. Glass conductimetric cells have been used by Sörensen and Söltoft[22] (up to 240°), Keppler *et al.*[11] (up to 300°), Dal Nogare and Safranski[8] (up to 300°) and others[28]. Metallic katharometers[11,29] with a taut platinum filament are of complicated construction and are generally used in the lower temperature range up to 250°. Felton and coworkers[6,30] constructed a katharometer in which the sensing elements were made from the glow plugs from a model aeroplane engine. The authors report that these katharometers are of simple construction and operate successfully in the range 400°–500° with a sensitivity of the same order of magnitude as that of ordinary katharometers with a taut platinum wire.

The question of retaining the high sensitivity of the katharometer at high temperatures is a special problem. It has been found that at high temperatures a large number of substances, for example alcohols, give negative peaks. A chromatogram for methanol[11] (Fig.3) shows that, with increase in the temperature of the sensing wire of the katharometer, the start and finish of the peaks become more negative, until the positive peak disappears completely. The usual explanation of peak inversion is that dehydrogenation of the compound takes place on the surface of the hot platinum wire. This dehydrogenation on the surface of a hot platinum wire has been observed for a large number of compounds, including ethanol (at 186°), butanol (at 220°), glycol (at 230°), cyclohexane (at 245°), ether (at 215°), etc.[11].

In gas–liquid chromatography, considerable difficulties may also be associated with the introduction of the specimens on the column[31] and their rapid volatilisation, the thermostatic control of the column and detector, etc. Since the components of a specimen with high boiling point are usually either solids or very viscous liquids, it is often necessary, when introducing them on the column, to

heat both the specimen itself and the syringe to a sufficiently high temperature to enable the specimen to pass readily through the needle of the syringe. Another method of introducing specimens, particularly solids, is by injecting their solution in a suitable solvent. This method is often inconvenient, since the emergent peak of the solvent may complicate the interpretation of the resulting chromatogram, although sometimes it is possible to select a solvent which can serve as an internal standard in quantitative analysis.

Dubsky and Janàk[32] have suggested using thin-walled capillaries of Wood's metal for the introduction of liquid and solid specimens at high column temperatures (up to 500°). The capillary containing the specimen was placed in the cold section of the column over a special tap. When the tap was turned, the capillary fell into the high-temperature zone, where the casing of the capillary melted and the specimen volatilised.

In the analysis of high-boiling specimens it is necessary to have at the column inlet a heated section, normally filled with glass wool, to ensure rapid volatilisation of the specimen. Sometimes volatilisation devices of special design are used for this purpose, for example the flash evaporator described by Taylor[33,34], operating at temperatures up to 425°.

Temperature control is less important in high-temperature chromatography than in low-temperature chromatography. Normally the temperature of the column is kept constant only to within ± 1°. Preference is given to separate thermostatic control of the column and detector, since this permits more flexible control of the process and makes it possible to keep the temperature of the detector 50°–70° higher than that of the column. The latter is necessary to prevent condensation of the components in the detector. Water or oil thermostats cannot be used for the thermostatic control of columns above 200°. In some cases it is possible to use vapour jackets containing the vapour of high-boiling liquids at known pressure, but the choice of thermally stable liquids suitable for this purpose is limited. Lewis and Patton[35] and Hawkes[24] used a vapour jacket containing dimethyl phthalate vapour (b.p. 283°) for thermostatic control of the column. Air thermostats, with and without circulation, have been most widely used for thermostatic control. Air thermostats

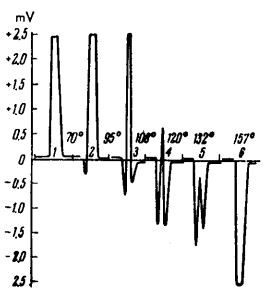


Fig.3. Influence of the temperature of the wire in the sensing element of a katharometer on peak inversion.

with circulation, which make it possible to alter the temperature rapidly and to keep it constant with sufficient accuracy, have been described by Ashbury *et al.*[29], Dubsky[36], and others.

Table 3 gives some of the most characteristic results on the analysis of different substances by gas–liquid chromatography at high temperatures, together with the working conditions and the chief parameters relating to the columns.

High-temperature gas–liquid chromatography has been applied by various workers to the analysis and separation of organic compounds of different classes: the methyl esters of aliphatic acids from C_6 to C_{34}[5,7,11,29,37-44], aliphatic monobasic and dibasic acids[45], high-molecular-weight fluoro-esters of camphoric acid[6,34], amines[46], amino-acids[47], phenols and cresols[24,48,49], normal paraffins and polyphenyls[17], phenylnaphthylamines[34], phenyl-substituted benzenes[33] and thiodan[50], n-tetradecane, hexadecane, and octadecane[25], etc. The method has been applied successfully to the analysis of petroleum wax and lubricating oil fractions[7,15], coal tar and its products[51,52], plasticisers of the phthalic ester type[35], and compounds of complex composition – lipids, steroids, and sterols[53-56].

Adlard and Whitham[7] have used high-temperature chromatography for the analysis and separation of the methyl esters of aliphatic acids up to C_{35}, paraffinic hydrocarbons up to C_{40}, gas oil C_{10}–C_{19}, wax C_{19}–C_{36}, lubricating oil fractions, and some industrial insecticides and antioxidants at temperatures up to 300°. Some pure substances and wax and lubricating oil fractions with very similar boiling points were isolated for further study on a mass spectrometer. Considerable difficulties were encountered through condensation of the high-molecular-weight components in the gas leaving the column in the form of a mist. Satisfactory condensation was achieved by using low pressure (40–200 mm Hg at the column outlet and 360–645 mm Hg at the inlet) and traps consisting of a large number of concentric tubes.

The authors also analysed some liquids recommended for use as stationary phases at temperature up to 150°, namely benzylbiphenyl (at 200°), dinonyl phthalate (at 254°), and squalane (at 290°). In all cases the stationary phase used for the analyses was E-301 silicone oil (on Sterchamol). For hydrocarbon mixtures at temperatures up to 270°, Apiezon grease L gave better separation, but a disadvantage was the fact that on this stationary phase the light fractions or decomposition products were often separated with great difficulty from the chief components of the specimen and contaminated the fractions intended for subsequent spectral analysis. Fig. 4 gives as an example the chromatogram for a purified low-melting paraffin wax, obtained at 270°.

In the analysis of petroleum wax fractions (normal paraffins from C_{20} to C_{38}) at 320° Ogilvie *et al.*[15] used asphaltenes as stationary phase, with a special procedure for depositing them on the solid carrier (brick). A 0.7 m steel column was filled with brick (20–40 mesh fraction), wound into a spiral, and, together with the pre-column heater and katharometer, fused into an aluminium block fitted with an electric heater. The calculated quantity of asphaltene (10 wt.%) was dissolved in sufficient ethyl ether to fill the free volume of the column. The solution was passed into the column under pressure of nitrogen. The apparatus was then heated slightly with the simultaneous passage of helium through the column, and finally a small

TABLE 3.

Mixture being separated	Volume of specimen, ml	Column dimensions		Column packing		Carrier gas		Temperature of column, °C	References
		diameter, mm	length, mm	inert solid carrier	stationary phase	nature	velocity, ml min^{-1}		
1	2	3	4	5	6	7	8	9	10
Methyl esters of aliphatic acids ($C_{12}-C_{26}$)	0.006—0.014	4.0	1.13	Celite 545 20—30 mesh	Apiezon L, silicone oil, silicone grease, 17%	nitrogen	20—30	257	5
Methyl esters of aliphatic acids	0.01	6.0	3.6	Celite 545 80—100 mesh	Reoplex 400, 30%	helium	75	240	37
Methyl esters of aliphatic acids	0.005	6.0	2.4	Chromosorb 30—60 mesh	poly(vinyl acetate), 15%	helium	83	205	38
Methyl esters of aliphatic acids ($C_{18}-C_{24}$)	0.021	6.0	0.86	brick 52—85 mesh	E-301 silicone grease	nitrogen	23	295	7
Methyl esters of aliphatic acids (C_6-C_{22})	—	5.0	1.0	Sterchamol	Apiezon M, 25%	hydrogen	180	265	39
Fluoro-esters of camphoric acid	0.01	6.0	2.0	Celite 60—100 mesh	silicone grease, 25%	helium	25	325	6
Methyl esters of resin acids		4.0	3.7	Celite	Apiezon grease, polyesters, 28.4%	helium	100	270 225	57
Methyl esters of aliphatic acids ($C_{12}-C_{22}$)		5.0	0.9	Celite	silicone grease, 50%	nitrogen	8	230	2
Plasticisers of the ester type	0.01	5.0	0.6 1.2	Celite 545 50 mesh	Apiezon oil, 30%	helium	50	285	35
Primary aliphatic amines (C_8-C_{18})		4.0	1.37	NaCl crystals 40—60 mesh	silicone grease, 5%	helium	23	330	46
			1.525	Chromosorb W 50—60 mesh	silicone oil, 26%	helium	45	340	
n-Paraffins ($C_{16}-C_{26}$) Polyphenyls (biphenyl, *m*-terphenyl, etc.) Phenyl-*m*-terphenyl	0.001—0.0015	6.0	15.0	C-22 brick 42—60 mesh	eutectic mixture of $NaNO_3$, KNO_3, and $LiNO_3$, 28.6%	helium	35	250—400	17
n-Tetradecane, n-hexadecane, n-octadecane	0.00175		1.2	Celite	Apiezon grease, 20%	hydrogen	40	298	25
Phenyl-substituted benzenes	0.005		1.2		Apiezon grease L	helium	90	300	33
Phenol, 2-hydroxybiphenyl 3-hydroxybiphenyl, 4-hydroxybiphenyl, diphenylsulphone				Celite 545	Apiezon grease, 33%	nitrogen	18	290—295	24
Lipids	1	4.0	1.5	brick 42—60 mesh Sterchamol 80—100 mesh	silicone adhesive or grease, 5—25%	helium	60—90	245—300	53
Steroids	0.05	2.0	1.0	Celite 545	polyethylene glycol isophthalate	argon	50—100	220—280	53
Petroleum wax fractions: 1. n-paraffins ($C_{20}-C_{30}$) 2. n-paraffins ($C_{20}-C_{38}$)	0.1—0.05	9.5	7.0	C-22 brick 20—40 mesh	asphaltenes, 10%	helium	42—50	282—320	15
Heavy oil tar fractions		6.0—8.0	2.5	brick 60—80 mesh	silicone grease, 23%	helium	50	40° above b.p. of specimen	51
Cadmium—zinc alloy	0.18	13.0	0.5	sea sand	$ZnCl_2$, 20%	helium	150	620	18
1. Purified low-melting paraffin wax	0.025	6.0	0.86	Sterchamol 52—86 mesh	silicone grease	nitrogen	21	270	7
2. Raw low-melting paraffin wax, lubricating oils	0.025	6.0	0.86	Sterchamol 52—86 mesh	silicone grease	nitrogen	21	270	—
Phenylalkanes ($C_{12}-C_{26}$)		6.35	3.66	firebrick 42—60 mesh	asphaltenes, 28.6%	helium	35	240 270 320	16
Biphenyl and *o*-, *m*-, and *p*-terphenyls	—	—	2	Chromosorb 30—60 mesh	Polyphenyl resin (M-2100), 20%	helium	48—125	375—445	13
Mono-, di-, and tri-ethylene glycol	—	—	2		Apiezon L	helium		200	58
Brominated esters of stearic and oleic acids	—	—	2.44	Celite 545 60—80 mesh	Poly(diethylene glycol glutarate), 10%	argon	60	205	59
Determination of phenyl and chloromethylphenyl ethers in chloromethylation products	—	6	4.58	Chromosorb 30—60 mesh	bis[α-(x-tolyl)-p-Tolyl] ether	helium	130	250	60

quantity of pentane and ether was introduced into the column to remove volatile substances. In the analysis the wax specimens were injected into the column by means of a syringe, in the molten state or in solution in a volatile solvent. The components of the wax were identified by comparing the retention times with those for known normal paraffins and also by means of a mass spectrometer. The authors state that the apparatus can be used for the analysis of high-boiling substances with molecular weights above 500.

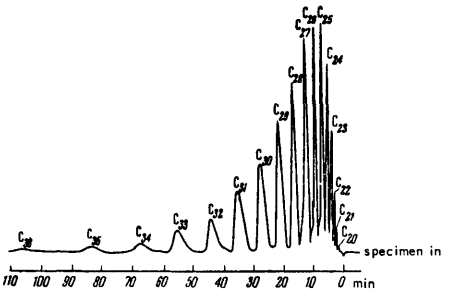


Fig.4. Chromatogram for purified low-melting paraffin wax. Volume of specimen 25 μl; column length 0.86 m; column diameter 6 mm; packing — Sterchamol (52-85 mesh) + E-301 silicone grease; nitrogen velocity 22 ml min^{-1}; pressure at outlet 63 mm Hg; pressure at inlet 383 mm Hg.

Taylor and Fawcett[33,34] analysed high-boiling substances (the methyl esters of aliphatic acids, phenyl-substituted benzenes, and α- and β-*N*-phenylnaphthylamines) on a Fisher–Gulf model 300 "partitioner" at 250°–400°. The stationary phases were silicone grease and Apiezon L. Rapid volatilisation of the specimen was achieved by means of the so-called flash evaporator, which can be used to volatilise specimens with boiling points up to 400°. The carrier gas was helium.

Felton[6] analysed fluoro-esters of camphoric acid (b.p. 320°–460°), isomers of *N*-phenylnaphthylamine (b.p. 400°) and tolyl isocyanates in the temperature range 250°–370°, and established that better separation is achieved in a column with silicone grease (20%), previously "conditioned" at 400° for 2–3 h with simultaneous passage of helium at 25 ml min^{-1}. In the katharometer the sensing elements were prepared from the glow plugs from a model aeroplane engine.

Cropper and Heywood[2,3] analysed a mixture of the methyl esters of aliphatic acids (C_{12}–C_{22}) in a 0.9 m column packed with equal quantities of Celite and silicone grease (internal standard — naphthalene) at 230° and reduced pressure (40 mm Hg at column inlet and 20 mm Hg at column outlet). Under these conditions the esters were separated efficiently, with the exception of the oleates and stearates. Subsequently, in the analysis of technical brominated methyl oleates and hexadecanol–octadecanol mixtures, difficulties were encountered due to the thermal decomposition of the specimens on the column. By replacing the Celite by NaCl crystals (with 5% silicone grease), the authors were able to reduce considerably the hydraulic resistance of the packing and thus to decrease the column temperature from 265° to 190°–200°. The separation achieved on this column was better than that on the column with Celite at a higher temperature. The authors used a katharometer with a coiled platinum wire in a small glass envelope, five times more sensitive than the katharometer with taut wire, used in earlier work.

In the analysis of the methyl esters of C_8–C_{18} aliphatic acids Sörensen and Söltoft[22] used solid carriers consisting of NaCl crystals and Dixon rings of wire gauze, with silicone oil as stationary phase. The different columns showed different efficiencies. The specimens were introduced in sealed ampoules. The sensing element of the katharometer consisted of two platinum spirals on a glass holder.

Dal Nogare and Safranski[8] have described an apparatus which gave trouble-free operation for one year at temperatures up to 350°. The authors analysed mixtures of compounds with boiling points in the range 150°–350° (polyethylene glycols, esters of phthalic and adipic acids, C_{10}–C_{18} hydrocarbons, etc.). The columns and katharometer were of glass and the stationary phases were silicone grease and polyethylene. Fig. 5 shows chromatograms for hydrocarbon mixtures.

Chromatogram *A*, obtained for 4 μl of a liquid mixture of C_{14}–C_{20} olefines and paraffins at 175°, shows that the separation was almost complete. By increasing the temperature and reducing the gas velocity, it was possible to separate the series of paraffins from C_{12} to C_{26} (Fig. 5, *B*), but indistinct peaks were obtained for the higher members of the series up to C_{36} (Fig. 5*C*). The authors obtained a chromatogram for DS-200 silicone oil at 350° (Fig. 6). The large number of peaks apparently corresponds to successive homologues.

Dupire[52] has separated fractions from coal tar and its products in glass columns up to 6 m long, packed with "Silocel" S-22 silicone grease. In the analysis of complex mixtures containing components boiling over a wide temperature range (50°–500°) it was necessary to carry out the separation at various increasing temperatures: 130°, 185° and 260°, and 360°. The components were identified from their retention times and infra-red spectra. Indene, naphthalene, and anthracene were used as internal standards.

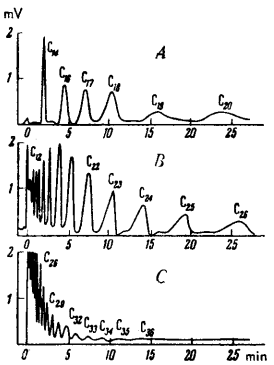


Fig.5. Chromatograms for hydrocarbon mixtures:
A) C_{14}–C_{20}; column temperature 175°; detector temperature 265°; helium, 124 ml min^{-1}.
B) C_{12}–C_{26}; column temperature 226°; detector temperature 302°; helium, 40 ml min^{-1}.
C) C_{12}–C_{36}; column temperature 285°; detector temperature 340°; helium 56 ml min^{-1}.

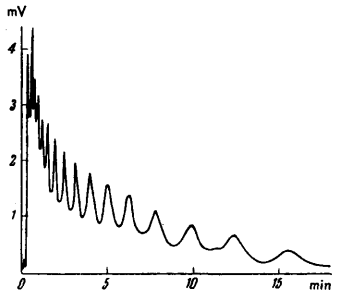


Fig.6. Chromatogram for DS-200 silicone oil.

Guillet *et al.*[61] developed a method for determining the monomeric composition of methacrylate polymers and copolymers. The specimen being studied was decomposed on a heated spiral at the inlet to the chromatographic column and the resulting monomers were analysed at 255° in a column with Apiezon L on Celite.

Franc and Wurst[62] developed a method for the analysis of the technical mixture formed in the manufacture of phenylchlorosilanes, containing benzene, chlorobenzene, phenyldichlorosilane, biphenyl, and diphenyldichlorosilane, using a column packed with Chromosorb + 20% silicone elastomer at 240°. At the column outlet the components were burnt to CO_2 and water. The carbon dioxide was absorbed and the water was reduced to hydrogen, which was detected at 20° by means of a katharometer. The areas under the hydrogen peaks are proportional to the number of H atoms in the molecules of the compounds.

Cacace *et al.*[63] analysed high-boiling organic compounds containing ^{14}C in a column with Apiezon L at 400°. The components leaving the column were burnt to CO_2 and the radioactivity of the gas was measured by means of an ionisation chamber. The authors obtained chromatograms for the ethyl ester of phenylacetic acid containing ^{14}C.

Gudzinowicz and Smith[64] studied the separation of high-boiling aromatic hydrocarbons, solid at room temperature, on a modified standard chromatograph with an ionisation detector and radium source. At 303° low retention times were obtained for *p*-quaterphenyl (b.p. 520°), coronene (b.p. 577°), and hexaphenylbenzene (b.p. 525°). Chromatograms were obtained for the high-boiling resin formed when terphenyls are irradiated (22 peaks).

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High-temperature gas–liquid chromatography is now a fully-developed method for analysing complex mixtures of high-boiling organic substances, and is applicable at temperatures up to over 500°. Efforts are being made to extend this upper temperature limit. Work is also being carried out on the study of new conditions which would make it possible to analyse high-boiling substances at much lower temperatures than is possible at present. The use of high-temperature chromatography on a preparative scale, although involving considerable difficulties, may compete with molecular distillation in the laboratory.

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NEW METHODS FOR THE SYNTHESIS OF NUCLEOTIDE COENZYMES

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I. INTRODUCTION

Consideration of synthetic methods for obtaining nucleotide coenzymes is of considerable interest in view of their important role in metabolic processes and especially in view of the fact that most nucleotide coenzymes have been obtained artificially only during recent years by new methods of organic synthesis.

Nucleotide coenzymes combined with a specific protein form numerous enzyme systems, in which they catalyse various chemical reactions occurring in the living organism. Nucleotide coenzymes are the monomers of nucleotides (phosphoric esters of nucleosides) and are structurally similar or analogous to units in ribonucleic acids (RNA) or deoxyribonucleic acids (DNA), which are polymers of nucleotides.

The molecules of nucleotide coenzymes are based on those of nucleosides, *N*-glycosides of heterocyclic bases, linked *via* the ring nitrogen atom with the primary carbon of D-ribose, and in this respect all natural nucleotide coezymes have the β-configuration.

Complex nucleotide coenzymes can be regarded as made up of two simpler compounds joined by a pyrophosphate link: flavin-adenine dinucleotide (FAD) consists of adenosine-5′ phosphate and riboflavin-5′ phosphate, uridine diphosphate glucose (UDPG) of uridine-5′ phosphate and α-D-glucose-1-phosphate, nicotinamide-adenine dinucleotide (NAD)† of adenosine-5′ phosphate and nicotinamide mononucleotide, nicotinamide-adenine dinucleotide phosphate (NADP)† of adenosine-2′,5′ diphosphate and nicotinamide mononucleotide, and coenzyme A (CoA) of adenosine-3′,5′ diphosphate and the 4′ phosphate of D-pantetheine, etc.

Among the various methods for obtaining nucleotide coenzymes from nucleosides or simple nucleotides, the very complex specific methods for producing a pyrophosphate link, which we shall examine in the present paper, are of considerable interest.

In a simple nucleotide coenzyme molecule, which is a monoester of a polyphosphoric acid, the pyrophosphate link is produced either by direct phosphorylation of a nucleoside or by forming di- and tri-phosphates from the nucleoside monophosphate. In the molecule of a complex nucleotide coenzyme which is an unsymmetrical P^1P^2-diester of pyrophosphoric acid, the pyrophosphate link is produced mainly in two ways: (*a*) condensation of two different

† According to the nomenclature recommended by the Commission on Enzymes of the International Biochemical Congress and by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry–Chem.Abs., 55, 6558 (1961); Biokhimiya, 26, 563 (1961).