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Analytical chromatography. Methods, instrumentation and applications

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Abstract. The state-of-the-art and the prospects in the development of main methods of analytical chromatography, *viz*., gas, high performance liquid and ion chromatographic techniques, are characterised. Achievements of the past 10 – 15 years in the theory and general methodology of chromatography and also in the development of new sorbents, columns and chromatographic instruments are outlined. The use of chromatography in the environmental control, biology, medicine, pharmaceutics, and also for monitoring the quality of foodstuffs and products of chemical, petrochemical and gas industries, *etc*. is considered. The bibliography includes 153 references.

I. Introduction

In the end of the 20th – beginning of the 21st century, analytical chemistry constantly faced the problems of the on-line environmental control, the monitoring of the quality and safety of foodstuffs and beverages and the quality and identity of drugs, as well as the problems of global safety associated with the destruction of chemical weapons, the prevention of terrorists' threats by the timely control over toxic agents and explosives in airports, railway stations and other crowded places and the detection of strong drugs. Chromatographic techniques played the key role in the solution of these problems.

Chromatography was discovered by the Russian botanist M S Tsvet in 1903.[1] In addition to being the pioneer of this method, he also developed in detail the methodological fundamentals of chromatography. Suffice it to say that Tsvet examined more than 100 different adsorbents. The procedure of chromatographic analysis he proposed was used for 50 – 60 years virtually without changes. His chromatographic columns were highly efficient. He also was the first to demonstrate the high effectiveness of multicolumn versions of chromatography. In 1931, R Kuhn, A Winterstein and E Lederer[2] repeated, in effect, the Tsvet's experiments and convinced in their great potential. Since that, the rapid spread of the chromatographic techniques in European countries has begun.

In the first 50 years, chromatography was used either for the separation and isolation of pure substances or as a research method. Its wide use as an analytical technique started only in the second part of the 20th century.

In the period from 1937 to 1972, fourteen Nobel Prizes were awarded for the studies in chemistry, biology, physiology and medicine with chromatography as the key method. Undoubtedly, chromatography has contributed to the progress in many fields of science, technology and industry; this is why it is ranked among 20 outstanding discoveries of the twentieth century that had the greatest impact on the mankind life.

A combination of chromatographic techniques allows one to solve most analytical problems. Gases and volatile substances are well determined by gas chromatography; high-molecular compounds can be determined by high-performance liquid chromatography (HPLC); mixtures of ions (both cations and anions) are separated by ion chromatography and ion-pair chromatographic techniques and also by capillary electrophoresis; the separation of macromolecules (polymers, biopolymers) is carried out by the gel-permeation and exclusion chromatography; for the separation of optical isomers, the methods of chiral chromatography were developed, while the analysis of microparticles uses the methods of force-field fractionation.

The progress in the major chromatographic techniques (gas, liquid, ion chromatography) was very rapid. The literature contains numerous publications that describe the state-of-the-art and the prospects in the development of chromatographic techniques,[3–5] the reviews devoted to the 20th anniversary of HPLC,[6] the 25th anniversary of ion-exchange chromatography,[7] the 30th anniversary of affinity chromatography,[8] 40 years of gas-chromatographic instrumentation industry,[9] 90 years of chromatography,[10] 100 years of chromatography.[11–13] The constant evolution of chromatographic methods was also surveyed.[14–16] The place of chromatography among other analytical methods was discussed.[17]

The present review briefly analyses the most remarkable advances achieved over the past 10 – 15 years in the theory and general methodology of chromatography as well as in the development of new sorbents and instruments for chromatographic studies.

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II. Advances in the theory and general methodology

At present, the main efforts in the field of chromatographic theory are directed towards the problems associated with the selectivity of separation. First of all, the relationship between the structure of molecules and their retention on sorbents of different chemical nature is studied. Attempts are undertaken to develop a retention theory that would be general for the gas, liquid and supercritical fluid chromatographic techniques and also to advance the methods of theoretical modelling. Recently, the geometric factor was invoked for the enhancement of the selectivity of chromatographic separation, which involves the use of cyclodextrins, crown ethers, liquid crystals, molecular sieves, *etc*.

The degree of resolution R in chromatography is determined by the relationship

$$R \simeq \frac{1}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{K_e}{1+K_e}\right)\sqrt{N}\,,$$

where α is the separation factor equal to the ratio of the adjusted retention times for the separated substances, K_e is the retention factor equal to the ratio of the net retention volume to the free column volume, N is the number of theoretical plates. The comprehensive theory of elution made it possible to design columns with the efficiency close to the theoretical one (for both gas and liquid chromatography). Especially high efficiency is characteristic of the capillary versions of chromatography. Taking the average efficiencies (the number of theoretical plates per metre, t.p. m^{-1}) of packed columns to be 1.5×10^3 (for GC) and 5×10^4 (for HPLC) and the efficiency of capillary columns to be 4×10^3 (GC), 3×10^5 (HPLC) and $>1\times10^6$ (capillary electrophoresis and capillary electrochromatography), it is possible to assess the maximum separation potential of columns in chromatography. The packed columns in GC allow only mixtures with $\alpha>1.05$ to be separated, while capillary columns are capable of separating mixtures with $\alpha>1.005$ in GC and $\alpha>1.003$ in capillary electrophoresis.

The studies of the retention mechanism in the reversed-phase chromatography on bonded phases are carried on. For this purpose, spectral methods are invoked. A very promising direction is the optimisation of separation by using expert systems, which includes the optimisation of gradient elution. Considerable progress was achieved in the theory of separation of optical isomers (in both gas and liquid chromatography).[18, 19]

The development of the theory of chromatography under critical conditions for the gel-filtration method is a considerable step forward.[20] Advances have been made in the establishment of a link between the retention parameters and the biological and chemical activity of molecules.[21] This offers much promise in the field of pharmaceutics at the quest for new drugs.

In recent years, the effect of the temperature on the retention in HPLC was studied, which led to the development of the high-temperature HPLC characterised by high resolution and fast performance. The elaboration of heat- and hydrolysis-resistant polymeric sorbents for this chromatographic version is in progress. At present, instruments are being developed that would allow the programmed control over the temperature in this method.† Of great importance is the optimisation of separation with simultaneous variations in the temperature and the eluent rate.[25]

Databases containing retention indices in HPLC in which nitroalkanes and alkylbenzenes are used as standard substances are compiled.[26]

The effect of the electric field on the retention and elution of compounds in the columns packed with carbonaceous sorbents was studied,[27] as well as the effect of the magnetic field on the retention in columns packed with magnetic particles.[28]

† For the first application of high-temperature chromatography (at 200 °C) for analytical purposes, see Refs 22–24.

III. Development of new sorbents and instruments for chromatographic studies

1. Gas chromatography

The major achievements in GC include the synthesis of stable sorbents with chemically bonded (immobilised) phases for packed and capillary columns and the development and application of capillary columns of relatively large diameters (0.32 and 0.53 mm) and high capacities that contain bonded liquid-phase films with a thickness of up to 5 μm (and even up to 16 μm)[29] and also of porous layer open tubular columns PoraPLOT with a fixed adsorption layer of silica or alumina, zeolyte, carbonaceous sorbents and porous polymers.[30]

For such stable columns, the databases (libraries) of the retention parameters can be created to help the identification of unknown components in a mixture. A combination of these databases with those of mass spectra allows valuable information to be retrieved.[31] By and large, the development of stable chromatographic columns resulted in the higher reliability and accuracy of the chromatographic analyses. Determination of microimpurities on capillary columns can be made using the methods that allow introducing large samples into the columns (on-column injection, cryofocusing, sampling with the solvent blow-out, *etc*.).[32] The electronic control over the carrier-gas rate and the pressure was beneficial for the stable operation of chromatographs. This allowed an automated programme for maintenance of the retention time upon changeover of columns to be developed.[33]

Recently, the analytical practice was supplemented with polycapillary columns,‡ which allow quick separation of mixtures to be carried out without programming the column temperature.[34] The use of adsorption polycapillary columns was documented.[35]

2. Liquid chromatography

The recent progress in analytical liquid chromatography was associated with the development and the use of methods of high-performance capillary chromatography that combines the chromatographic and electrophoretic separation methods.[36] It is due to the coupling of various methods of liquid chromatography and capillary electrophoresis that the nucleotide sequence in DNA was decoded and the programme 'Human genome' was completed.[37]

The potentials of liquid chromatography are substantially extended with the introduction of chiral,[38] immunoaffinity,[39] high-temperature[40] and perfusion[41] chromatographic techniques. The quick chromatographic analysis of proteins on non-porous particles deserves attention.[42]

The active development of new highly stable and selective sorbents based on dioxides of zirconium and titanium, porous polymers and carbonaceous adsorbents, which are stable over the whole pH range, is in progress.[43] For HPLC, a wide spectrum of sorbents are developed and manufactured. Dozens of companies all over the world produce more than 300 sorbent types. Most often (in 70% of cases), HPLC columns are packed with silica gels both pure and grafted with non-polar and polar groups (their fraction is 70%). Styrene–divinylbenzene copolymers, polymethacrylates, celluloses, *etc*. are used less often (20%), as well as porous carbonaceous sorbents and porous oxides of titanium and zirconium (4%) and alumina (1%).

The reversed-phase HPLC version, which employs silica gel sorbents with immobilised alkyl groups (the alkyl chain length varies from C_1 to C_{34}, C_{18} and C_8 being the most common), finds wide application in the analytical practice (more than 70% of chromatographic analyses are carried out using this LC version).

Depending on the synthetic method, the C_{18} sorbents can be either monomeric or polymeric. Polymeric C_{18} sorbents are synthesised from trifunctional silanes in the presence of water, while monomeric sorbents are prepared from monofunctional

‡ The discovery by Novosibirsk scientists [USSR P. 2986181 (1980)].

silanes. In certain cases, particularly, in the separation of complex mixtures of isomers, polynuclear aromatic hydrocarbons, the polymeric C_{18} sorbents are preferred. The retention capacity and the selectivity of monomeric C_{18} with respect to non-polar and weakly polar compounds depend on the number of immobilised alkyl chains (or on the total content of carbon per the sorbent mass unit, which varies from 8% to 20%). Small percentage of carbon leads to the small sorption capacity, fast separation and the rapid establishment of the equilibrium during the gradient elution.

However, silica-gel-based sorbents with immobilised alkyl groups have many drawbacks. They are unstable in acidic and alkaline media, *viz*., their Si–O–Si bonds are hydrolysed at pH < 3 and silica gel is dissolved at pH > 10 (especially at high temperatures). These sorbents are non-selective with respect to the separation of polar compounds and isomers, while the characteristics of basic compounds determined on these sorbents substantially depend on the purity, geometry and chemical nature of silica gels as well as on the method used for the immobilisation of alkyl groups, *etc*.

In recent years, great efforts are made to overcome these drawbacks. First of all, the manufacturing of original silica gels is modernised. The production of special grade silica gels (containing minute amounts of heavy metals) with spherical particles, the composition of which remains invariant from batch to batch, is organised. Unfortunately, not all hydroxyl groups in silica gels are completely substituted. The residual hydroxyl groups can take part in parasitic interactions with small polar molecules, giving rise to asymmetric peaks. It was proposed to block the OH groups with bulky isopropyl or isobutyl groups (the sorbent Zorbax Stable Bond). It is also possible to block hydroxyl groups by bonding the silica gel with bidentate reagents where two neighbouring alkyl groups are linked by a bridge of 3–4 methylene groups. These bridges shield the OH groups, making silica gels much more stable and capable of operating even at high pH (Zorbax Extend-C_{18}). For the sorbents based on silica gels that contain immobilised alkyl groups, the selectivity with respect to polar compounds can be enhanced by modifying their alkyl chains with polar groups, particularly, carbamide groups.

Silica gels with the average pore size of 80, 100 and 120 Å are used in the separation of low-molecular compounds, those with pores of $\geqslant$300 Å are applied for the separation of high-molecular compounds and biopolymers. Thus, a silica gel modified with C_{30}-alkyl groups was used for the separation of polynuclear aromatic compounds, oligonucleotides, fullerenes and alkylbenzenes.

Among other sorbents applied in the reversed-phase chromatography, the polyethylene glycol-based sorbents, which are used in the separation of phenolic compounds, deserve attention, as well as the perfluorinated sorbents for the analysis of halogen-containing compounds.

Polymeric sorbents based of poly(divinylbenzene) with 500-Å and 1000-Å pores were proposed (Jordi).

Today, hundreds of sorbent types are manufactured. To choose a sorbent for reversed-phase chromatography, different estimation procedures were developed.

The development of columns for turbulent chromatography should be assigned to the achievements of recent years. In columns 1-mm in diameter packed with 50-μm particles, high-velocity flows are employed; large molecules of proteins are not retained, while small molecules have time enough to diffuse into pores and are retained.

In the recent decade, considerable progress was achieved in the synthesis of new types of sorbents and the construction of columns for HPLC. Particularly, sorbents for perfusion chromatography and monolithic columns with a unified porous block based on polymeric materials or silica gels were developed.

The first application of perfusion chromatography dates back to 1990; its theory was developed later. Perfusion chromatography is conventionally used in the preparative isolation of substances (specifically, proteins) in their pure state and, to a lesser extent, for analytical purposes (particularly, for the analysis of food proteins). Perfusion columns are considered as intermediate between the conventional packed columns and monolithic ones. In perfusion columns, the eluent flows through the sorbent grains rather than around them as in conventional packed columns. In perfusion chromatography, styrene–divinylbenzene copolymers are used as the sorbents. Such sorbents have the shape of beads ~20 μm in diameter with two kinds of pores, namely, large pores (6000–8000 Å), along which the eluent percolates through the sorbent, and diffusion pores (800–1500 Å), through which the substances easily penetrate into the sorbent. Due to the enhanced mass transfer in perfusion columns, high eluent rates can be used, which reduces the separation time. This is especially important for large molecules. At present, perfusion sorbents are produced for various types of chromatography, namely, for reversed-phase, normal-phase, ion, hydrophobic and affinity versions.

In monolithic columns, the sorption layer represents a continuous rigid porous rod. Polystyrene, styrene–divinylbenzene copolymers, polyacrylates, polymethacrylates (PMA), poly(glycidyl methacrylate), poly(ethylene dimethacrylate), *etc*. are used as the materials (Table 1). Monolithic columns of large diameters (> 5 cm) are manufactured for preparative purposes. Special monolithic columns are developed for the separation of enantiomers as well as for polypeptides and polynucleotides. A new generation of monolithic columns based on silica gels of the Chromolith type were developed. In 2001, a series of analytical monolithic columns (Chromolith Perfomance RP-18e, Chromolith Flash RP-18e, *etc*., Merk, column size 100 × 4.6 mm, 50 × 4.6 mm, 25 × 4.6 mm) was rewarded with a gold metal at the Pittsburg Conference as the best product. These columns can operate at high eluent flow rates without sacrifice in separation, moreover, the input pressure is substantially lower as compared with packed columns. Chromolith RP-18e columns are suitable for the separation of acidic, basic and non-polar compounds.

Table 1. Monolithic chromatographic columns used in reversed-phase chromatography.

Name	Shape	Manufacturing company	Material
CIM-Disk	disk	BIA Separations	PMA and PS
CB Silica Plate	"	ConChrom	silica gel
Onyx	cylinder	Phenomenex	silica gel
SWIFT	"	ISCO	PMA and PS
Chromolith	"	Merck	silica gel
Monoliths	"	LC Packing	PS

For the sorption of only one type of molecules or a group of related molecules, the sorbents with molecular-size pores (SMSP) were developed. The major method of SMSP preparation is polymerisation of methacrylic acid or ethylene dimethacrylate. These selective sorbents are applied for the separation and preconcentration (such separation relies on the principle of molecular recognition). They selectively bind non-polar supertoxic agents, particularly, 2,3,7,8-tetrachlorodibenzodioxine, and are well suited for the selective concentration of noxious substances (*e.g*., pesticides), as well as for the concentration and separation of medicinal drugs. Special SMSPs allowed successful separation of phenylalanine and tryptophane.

A template technology for the production of macroporous carbon sorbents with the surfaces resembling that of graphitised thermal carbon black was developed. Macroporous 5-μm silica gel beads were used as the templates. The pores in such particles were filled with a phenol–formaldehyde polymer, after which the polymer was carbonised by heating to 1000 °C in an inert medium so that the pores turned out to be filled with carbon. Then, silica gel was dissolved in 5 M NaOH to produce a macroporous carbon sorbent, a mirror replica of the porous silica gel. Unfortunately, in

this stage, the carbon sorbent contained too small pores (measuring 30 Å), which were hardly suitable for HPLC, and also had the amorphous surface (specific surface area of ~ 400 m^2 g^{-1}). Graphitisation at temperatures above 2000 °C led to the total restructurisation of the carbon sorbent, particularly, to the disappearance of micropores and the formation of a uniform crystalline surface. Upon the graphitisation, only those pores retained that were present in the original silica gel. Fractionation was the final technological step.

The commercially produced sorbents called 'Hypercarb' (Shendon) have the specific surface of ~ 120 m^2 g^{-1}, the average pore diameter is ~ 250 Å, the volume of pores amounts to 75% and the particle size is 5 or 7 μm. This sorbent is stable throughout the pH range (0–14), it is neither dissolved, nor hydrolysed, nor swelled in the solvent and withstands pressures up to 400 atm. Columns packed with Hypercarb are highly efficient, *viz*., up to 60 000 theoretical plates.

At present, the Hypercarb sorbent is widely used for the separation and the analysis of different mixtures, namely, aromatic compounds, various pharmaceuticals, cations and anions, as well as structural isomers. Hypercarb has no drawbacks typical of the C_{18} sorbent in reversed-phase chromatography; particularly, polar compounds are well retained in Hypercarb-packed columns. For example, the retention factor is 1.8 for phenol, 2.35 for resorcinol and 2.7 for 1,3,5-trihydroxybenzene. The latter two compounds could not be separated on the C_{18} column. In addition, Hypercarb can be used for the separation of carbohydrates and compounds containing carboxyl and amino groups.

In recent years, size-exclusion chromatography was actively developed, which was favoured by the introduction into the chromatographic analysis practice of Sephadexes, *i.e*., dextran gels cross-linked with epichlorohydrin. Sephadexes are extensively used in medicine.

To date, the HPLC techniques are actively used in proteomics,[44] in the metabolism studies,[45] as well as for the determination of the components of a single cell.[46]

3. Ion chromatography [47, 48]

The main achievements of ion chromatography include the development of fast monolithic columns for superfast separation of substances, particularly, inorganic anions ($H_2PO_4^-$, Cl^-, NO_2^-, Br^-, NO_3^-, ClO_3^-, I^-, SO_4^{2-}) and columns for the simultaneous separation and detection of anions and cations; synthesis of sorbents for micropacked and capillary columns; the extension of the array of detection systems used in ion chromatographs (up to 10 types), particularly, the development of pulsed amperometric detectors for the determination of carbohydrates, amino acids, biogenic amines and alcohols; the development of the eluent generator (the add-only-water system) and a 10-port valve for the analysis of samples with widely different concentrations; the use of non-metallic materials (ceramics, PEEK plastics, sapphire, PTFE, *etc*.) in pumps and liquid tracts in ion chromatographs; the introduction into practice of a 'heart-cut' system for the partial disposal of the sample to prevent its entering into the column; the increase in the sensitivity and stability of detection systems in the determination of ions down to the ppt level (1 ppt $= 1 \times 10^{-10}$%).

4. Thin-layer chromatography

Conventional thin-layer chromatography (TLC) represents a popular, simple and cheap chromatographic technique used for the detection and determination of different classes of substances.[49–51] In TLC, the separation is usually carried out on glass, metal or plastic plates measuring 100×100 mm and covered with an adsorbent layer 50–300 μm thick and containing 3–15 μm grains. At present, the following four types of plates are used most often: classical, high-performance, microplates and preparative.

Silica gels as such and containing immobilised alkyl groups, alumina, cellulose, cyclodextrins, Florisil (magnesium silicate), inorganic ion-exchangers, *etc*. are used most often as the adsorbents. The main advantages of TLC include the simultaneous parallel separation of several samples (up to 10 and more), high efficiency, the possibility of two-dimensional separation and the fast and cheap determination. Yet another positive feature is the possibility to observe the separation process, in particular, see the remainders of a non-soluble sample at the application point. The drawbacks of TLC include the exposedness of the separation plates to atmosphere, which may result in the decomposition of easily oxidisable and hydrolysable compounds.

The detection of spots on a plate involves the use of different methods including mass spectrometry, Raman spectrometry, spectrophotometry with diode matrices, plasma-ionisation detectors, fluorescence indicators. Electrochemical detection is also used.

In recent years, the TLC techniques were constantly developed and improved. New TLC versions were proposed, namely, polyamide, thin-film, high-performance, rotation, two-dimensional, micellar, flow-through, *etc*.[52, 53] Densitometers were developed for the quantitative TLC determination of substances. Chiral plates are used for the separation of optical isomers.

The standardisation of TLC procedures, namely, pretreatment of adsorbents, preparation of plates, the processing of the results of separation favoured the introduction of TLC into the wide practice of analytical laboratories in many industrial fields.

IV. The main advantages of analytical chromatography

According to Zolotov,[54] chromatography pertains to the hybrid analytical methods. In chromatographs, the analysed mixture is preliminarily separated on a column due to the different sorption ability of the mixture components, then, at the column outlet, a sensitive element, *viz*., a detector, registers each of the separated components.

Insofar as the separation capacity of modern chromatographic columns is extremely high (tens and even hundreds of components of a mixture can be separated), the modern chromatography has no rivals in the analysis of complex natural and industrial multicomponent mixtures. In principle, it allows the separation of any mixtures of molecules including all kinds of isomers, particularly, enantiomers, to be performed. However, chromatography has to pay a price for this advantage, namely, this analytical method is indirect rather than direct. The point is that the initial concentration of a substance in the original mixture by no means remains unchanged. Following the injection to the chromatograph, a component is diluted with the eluent and then is spread over the column due to diffusion and kinetic factors. At the outlet, the detector measures a concentration different from the initial one. This situation calls for the calibration in order to establish the relationship between the initial concentration of a substance and that at the outlet from the column.

Yet another peculiarity of analytical chromatographic techniques is their periodicity.

Let us consider the advantages of chromatography over other analytical methods.

1. Versatility and high separation capacity

Chromatographic methods allow virtually all kinds of mixtures to be separated and analysed.§ Modern chromatographic techniques exhibit high separation capacities. They make it possible to separate multicomponent mixtures including complex mixtures formed by all kinds of isomers and also certain isotopes and isotopically substituted compounds.

Gas chromatography allows more than a thousand of components in gasoline fractions to be separated from a single injection; two-dimensional high-performance TLC makes it possible to separate hundreds and even thousands of components; capillary

§ It is evident that in this case only those mixtures can be analysed that can be separated in principle.

electrochromatography allows the separation of more than a thousand of proteins (the majority of scientists working in the proteomics field are confident that coupling of HPLC with tandem mass spectrometry would provide a rapid breakthrough in proteomics).

2. Low detection limits (high sensitivity)

Among the advantages of chromatography is its compatibility with almost all analytical methods known. However, this cannot be the grounds for the comparison of chromatography with other modern analytical methods, because chromatography employs all the achievement of these methods. At the same time, it is within the latter methods that the sensitive flow-through cells were developed specially designed for the chromatographic applications and the procedures were modernised to fit chromatographic techniques.

For chromatographic techniques, various detection systems, both versatile and selective (specific) were developed. Table 2 shows the detectors used most often in gas chromatographs. In addition to those listed in the table, the Hall electrolytic, chemiluminescence, infrared, fluorescence, helium ionisation, radioactive and ultrasonic detectors are used, as well as densitomers. In total, more than 50 types of detectors are known. The most sensitive of them make it possible to determine impurities at a level of $10^{-5}\% - 10^{-7}\%$ without preconcentration.

Selective detectors have a great advantage in the determination of impurities in complex mixtures. It is possible to provide the conditions where the impurities would be determined, while the basic substances in the mixture would not, for instance, in the determination of chlorine-containing organic pesticides in the environment by means of an electron-capture detector.

In HPLC, the detection schemes based on almost all determination methods are used. Table 3 shows the detectors used in high-performance liquid chromatographs. Under favourable conditions, the electrochemical and fluorescence detectors allow determining impurities at a level of pico- or femtograms ($10^{-12} - 10^{-15}$ g) without preconcentration.

Unfortunately, even most sensitive detection systems fail to determine microamounts of certain environmental pollutants, for example, supertoxicants. In this case, the methods of preliminary concentration of samples are used, which makes its possible to reduce the detection limits by two – four orders of magnitude and determine certain contaminants, *e.g.*, dioxins, at the ppt level.

In the past decade, the interest in the concentration methods increased [they were the subject of a monograph [55] and several reviews (*e.g.*, see Refs 56 – 59)]. The conventional methods of concentration are modernised, new methods are proposed and new instruments are developed.

Earlier, the liquid – liquid extraction has been used for the preconcentration; however, since this method required very expensive special-grade solvents and sometimes did not provide the complete extraction, which necessitated the regeneration or utilisation of great amounts of solvent wastes, its current application in the classical version progressively decreases. Today, the following new methods of liquid extraction are used: the extraction with small amounts of solvents (merely several millilitres) in a special vessel; the extraction with small amounts of solvents at elevated pressures and temperatures under dynamic conditions (the instrumentation resembles that used in liquid chromatographs); the extraction with a single drop of an organic solvent in water; the extraction with superhot water[¶] under elevated pressure (the dynamic version is possible). In the latter case, the utilisation presents no special problems, because water is 'friendly' to the environment.

At present, the solid-phase extraction occupies the first place in the popularity, because this provides more efficient (nearly 100%) extraction of microcomponents, makes the automation of the preconcentration procedure possible and allows direct coupling with chromatographs in the on-line mode. Various polymeric, carbonaceous and inorganic sorbents (polar, non-polar and ion-exchange) were developed for the solid-phase extraction.[60] Many companies manufacture filters of large diameters and 96-cartridge kits for performing concentration from large volumes.

The determination of impurities in aqueous media effectively employs the solid-phase microextraction, *i.e.*, the preconcentration on a sorption-layer-coated rod, which is drawn out from the tip of a special syringe. In those cases where selective solid-phase extraction is required solely for the determination of a certain class of compounds or even a single compound, the methods of ion-pair [61] and immunoaffinity [62] solid-phase extraction are used. In recent years, technologies for the synthesis of porous polymers with molecular-scale pores of fixed sizes have been actively

[¶] High temperatures increase the solubility of non-polar compounds in water.

Table 2. Technological characteristics of detectors used most frequently in GC.

Detector	Detection limit /g s^{-1}	LDR [a]	Detector type	Determined compounds
Flame-ionisation (FID)	2×10^{-12}	10^7	selective	organic compounds ionisable in the hydrogen flame
Heat conductivity (DHC, katharometer)	4×10^{-10} (see [b])	10^6	universal	all compounds differing in heat conductivity from that of the carrier gas
Electron-capture (ECD)	1×10^{-14}	$10^3 - 10^4$	selective	mainly organohalogen compounds
Photoionisation (PID) [c]	2×10^{-12}	10^7	"	all compounds with ionisation potential below 10.7 eV
Thermoionic (TID)	4×10^{-13} (for N), 2×10^{-13} (for P)	10^4	"	heterocyclic compounds containing N and P atoms
Flame-photometric (FPD)	2×10^{-11} (for S), 9×10^{-13} (for P)	10^3 10^4	specific	S- and P-Containing compounds
Atomic-emission (AED)	$2 \times 10^{-11} - 1 \times 10^{-13}$	10^4	universal	all compounds containing 12 basic elements (H, C, O, S, N, P, *etc.*)
Mass spectrometric (MSD) [d]	$1 \times 10^{-9} - 1 \times 10^{-11}$	10^5	"	all compounds

[a] LDR is linear dynamic range; [b] in g ml^{-1}; [c] UV radiation; [d] identifies a compound from its mass spectrum.

Table 3. Technological characteristics of detectors applied in HPLC.

Detector	Detection limit /mol litre^{-1}	Selectivity	Advantages and drawbacks
Coulonometric	10^{-11}–10^{-12}	high	three-dimensional signals can be recorded; complete oxidation or reduction of the determined substance
Amperometric	10^{-10}–10^{-11}	"	low cost; selectivity with respect to electroactive compounds
Mass spectrometric	10^{-10}	very high	allows identification of unknown compounds; high cost
Fluorescence	10^{-7}–10^{-10}	high	many mixtures should be derivatised; high cost
Chemiluminescence	10^{-7}–10^{-10}	very high	high cost; limited application (only for N- and S-containing compounds)
Spectrophotometric with diode arrays	10^{-6}–10^{-7}	high	possibility of recording three-dimensional signals; high cost
Spectrophotometric	10^{-5}–10^{-7}	low	low cost, versatility
Light-scattering-based	10^{-4}–10^{-5}	"	versatility
Refractometric	10^{-5}	"	versatile detector very sensitive with respect to the temperature and eluent rate variations

developed, aimed at the extraction of the molecules with dimensions corresponding to the pore sizes.

The supercritical fluid extraction is a very promising method;[63] however, its instrumentation is very expensive. The supercritical fluid extraction utilises substances at the temperatures and pressures above the critical points. Carbon dioxide is used most often. The potential prospects of the application of the extraction with supercritical CO_2 in the recycling of nuclear fuel wastes were demonstrated.

The preconcentration of light admixtures from solutions is carried out most often by gas (head-space) extraction, which is adequately automated. The method itself and its instrumentation are constantly improved. In particular, the procedure of gas extraction at high temperatures and coupling of gas extraction with solid-phase microextraction were developed as well as other procedures.[64] Among the dynamic versions of extraction, mention should be made of the purge-and-trap methods[65] and also the membrane extraction,[66] which allows the continuous isolation of microcomponents from the flows.

3. Reproducibility of determination and express separation

Modern chromatographs make possible achieving good reproducibility of measured quantities. For the best instruments, the relative standard deviation of the retention parameters on capillary columns can reach record-breaking values, *viz*., 0.1% and even 0.01%.

For ordinary chromatographs, the root-mean-square deviation of retention parameters and peak heights and areas lies normally within 1%–3%. For the determination of microimpurities, these values are, naturally, larger.

Chromatographic techniques are used most often for the determination of microcomponents (10^{-1}%–10^{-10}%). For the impurity contents within 10^{-1}%–10^{-5}%, the relative standard deviations of commercial chromatographs are quite comparable with those provided by other methods.

Most often, the duration of separation and determination is within 5–30 min. In this period, tens and even hundreds of substances are separated and determined. Recalculated per a single substance, the analysis is in the range of minutes. At present, the methods for rapid separation, which allow several dozen of components to be separated in two minutes, are developed for GC, HPLC, TLC and capillary electrochromatography.[67–70]

V. Chromatographic instrumentation

The advances of analytical chromatography are largely associated with the modernisation of the chromatographic instrumentation. The wide distribution of one or another chromatographic technique in the analytical practice was closely related with the commercial production of the corresponding chromatographs. Thus the development by Moore and Stein in 1956 of an amino acid analyser based on ion-exchange chromatography, post-column derivation and photometric detection gave impetus to its wide application in the analysis of amino acid mixtures. The commercial production of gas chromatographs in many countries in 1955–1960 determined the boom in the application of gas chromatography in numerous fields of science and technology. The development in 1965–1970 of high-pressure pumps, flow-through liquid detectors, columns packed with stable fine-grain sorbents and other parts of modern liquid chromatographs made it possible to convert the classical liquid chromatography into a rapid high-performance technique that meets the analytical goals.

In the end of the 20th century, the chromatographic instrumentation represented an individual, sufficiently large industrial branch. Hundreds of companies all over the world produced different types of chromatographs and accessories for the total sum of above USD 10 billions.

The chromatographic instrumentation has reached a very high level; the control over the analysis and the processing of its results are carried out on the basis of microprocessors, most procedures are automated. These instruments employ the latest achievements of electronics, fine mechanics, pneumatics, planar technology and optics.

The commercial production of gas chromatograph–mass spectrometers (GC-MS) is the major achievement in chromatographic instrumentation. Recently, mass spectrometers have occupied a prominent place among detectors for gas chromatographs, they are constantly modernised and their cost decreases. Annually, 3000–3500 such detectors are sold, 80% of which are quadrupole and the other are equipped with ion traps. Their considerable part (above 25%) are used in the environmental control.[71] Table 4 shows the major types of gas chromatographs, which include analytical laboratory instruments, industrial, portable, preparative and miltidimensional chromatographs, chromatographs as the gas analysers, GC-MS, GC-IRS and radiochromatographs.[1,72]

Table 4. Basic types of gas chromatographs.

Basic types of chromatographs	Functions	Advantages, specific features
Laboratory	for stationary laboratories	wide set of accessories and options
Industrial, automatic	for monitoring industrial processes	in most cases, explosion-proof construction
Two-dimensional, multi-dimensional	for complete separation of complex multicomponent mixtures	separation on twin columns of different selectivity
Chromatographs mass spectrometers	for identification of already separated compounds	quantitative and qualitative analysis of unknown mixtures
Compact	for mobile and stationary laboratories	economy without reducing analytical characteristics, low weight (10 – 25 kg)
Portable, mobile, field	for on-the-site analysis	high rate analysis with autonomous power and gas supply, low weight (5 – 15 kg)
Chip-based microchromatographs (silicon-based technology) personal, pocket-size	the same	rapid analysis, complete autonomy, small weight (0.2 – 3 kg); used only for simple tasks
Special microchromatographs	for space studies	automated analysis, shock- and shake-resistant

To extend their analytical potential, the gas chromatographs are also equipped with sets of additional options, namely, instruments for the sample preconcentration (units for solid-phase, solid-phase micro-, supercritical and gas extraction, systems for purge-and-trap, *etc*.), supplemental sampling systems (autosamplers, cryofocusing systems, gas valves), facilities for the pyrolysis, pre- and postcolumnn derivatisation (*e.g*., methanators, *etc*.).

The main achievements involve the development of portable field chromatographs,[73] portable industrial devices in the explosion-proof versions, thermostats with new methods of heating (microwave, IR-radiation), instruments with the temperature programming facilities for the direct heating of columns, new methods and instruments for feeding large samples into capillary columns (cryofocusing, solvent blow-out, programmed heating, on-column introduction), and also the development and introduction into analytical practice of large-diameter (0.53 mm) capillary columns with thick bonded layers of stationary phases (up to 5 μm) and capillary adsorption columns with layer thickness of up to 10 – 20 μm of the PoraPlot type,[74, 75] the use of electronic control over the gas flow rate and pressure, *etc*.

The development of instruments for the analytical liquid chromatography started 15 years later than that for gas chromatography; nonetheless, its current production and market exceed those of gas chromatographs. Among liquid chromatogaphs, mention should be made of high-pressure liquid chromatographs (closed systems), gradient, isocratic, ion, exclusion, microcolumn, counter-current, multidimensional and high-temperature chromatographs, instruments for HPLC-MS, *etc*. (nearly 20 types of instruments). Commercial liquid chromatographs can be equipped simultaneously with several detection systems, *viz*., optical, electrochemical, fluorescence, mass spectrometric, elemental, gas-chromatographic, immune, *etc*. The lowest detection limits (below several femtomoles or attomoles) are typical of electrochemical (coulometric and amperometric) liquid chromatographs.

Special mention should be made of the development of chromatographs for HPLC-MS. This method is actively developed since 1990. At present, sufficiently reliable and sensitive systems based on the electrospray ionisation at ambient pressure are developed, which are suitable for wide analytical application. The 2001 Nobel Prize was awarded for the development of these systems and their use in biochemical studies. The HPLC-MS technique has found the wide application in phamaceutics, medicine, in the analysis of herbs, in biology and biotechnology (particularly, in proteomics). As the mass analysers in HPLC-MS, the quadrupole, ion, time-of-flight, magnetic (sector), and other traps are used.[71, 72]

VI. Major application fields of analytical chromatography

Undeniably, the chromatographic methods occupied the leading position among other analytical methods in the past decade.[76]

According to publications of the last 20 years, gas chromatography was largely applied for the analysis of environmental objects (12.5%), foodstuffs and beverages (10%), medicinal drugs (10%), for clinical studies (4%), in forensic medicine (3.5%) and for the determination of enantiomers (2.5%). Moreover, it was widely used in the gas, chemical and petrochemical industries, although only few publications were devoted to these issues.

The detection and identification of biological compounds (in particular, amino acids, proteins, lipids, carbohydrates, vitamins, steroids, *etc*.) is the main application field of HPLC (> 50% publications). Among the other application fields, mention should be made of the analysis of medicinal drugs (16%) and the products of chemical and petrochemical industries (more than 10%), the separation and determination of enantiomers (5.5%), the analysis of inorganic compounds (mainly the determination of anions and cations by the ion technique), the analysis of foodstuffs and beverages (4.5%), in the environmental control (3%), clinical analyses (2.8%) and in toxicology (1.3%).

At present, there are virtually no fields in industry, science and technology that do not apply chromatographic techniques. Here, we list the most important advances achieved with the use of chromatographic methods:

— decoding of the human genome;

— separation of multicomponent protein mixtures for the proteomics purposes;

— analysis of the components of a single cell;

— studies in the field of pharmacokinetics (studies of the effect of drugs on the human organism);

— early recognition of diseases (detection and identification of biochemical markers);

— separation of optical isomers, analysis of the enantiomeric purity of medicinal drugs;

— doping-control of sportsmen;

— forensic and medical tests, detection of drugs;

— environmental control, which includes the monitoring of supertoxicants (dioxins, polychlorinated biphenyls, polynuclear aromatic compounds at the ppt level);

— purity control of foodstuffs and beverages, including the detection of most dangerous mycotoxins, nitrosamines, pesticides, *etc.*;

— studies of the components in smells and aromas of foodstuffs and beverages;

— analysis of natural gas, gas condensate, gasoline, kerosene, mineral oils and crude oil; simulation of oil distillation;

— separation and determination of metal ions, including transuranic ions;

— complete analysis of the polluted urban atmosphere;

— determination of Freons in the stratosphere;

— detection and identification of insect pheromones;

— analysis of atmospheres and soils of other planets (Venus, Mars and Moon);

— studies of isotopically substituted compounds.

Below, some of these achievements are discussed in detail.

1. Environmental analysis [77–81]

Gas chromatography coupled with mass spectrometry is used for the detection and quantitation of organic impurities in the atmosphere of large industrial cities. The impurities are first concentrated on non-polar sorbents that do not retain moisture, then, desorbed and collected at the top of a capillary column (cryofocusing), separated with temperature programming and, finally, detected and identified using a mass spectrometric detector.

Harmful gases in the atmosphere (NO_2, SO_2, SO_3, HCl, HF, Cl_2, NH_3, *etc.*) are determined using gas and ion chromatographs. Petrochemicals, being the abundant contaminants of water and soil, are determined most often using certified HPLC methods. Residual pesticides in the environment are determined in most cases by GC, HPLC and TLC techniques. Thousands of analyses are carried out at plant protection stations, veterinary laboratories and various environmental centres.

Carcinogens, namely, certain chlorine-containing organic compounds † and aromatic compounds, phthalates, polyamines, *etc.*, are the most hazardous environmental pollutants. Their detection and quantitation in water, air and soil involves most often the use of GC and HPLC techniques. For example, polynuclear organic compounds (*e.g.*, 3,4-benzopyrene) are determined in water and soil by HPLC, while benzene, toluene, ethylbenzene and xylenes in the urban atmosphere are determined by means of GC equipped with photoionisation detectors. Phenol and its derivatives can be determined at the MPC level with a liquid chromatograph 'TsvetYauza' equipped with an amperometric detector. This instrument was also used for the determination of cyanide ions in drinking water.[81]

All major anions in drinking water are determined using ion chromatography (in 10–15 min at a level of $1\times10^{-4}\%$–$1\times10^{-5}\%$), while volatile chlorine-containing organic compounds are determined by the GC method combined with head space analysis. Ion chromatography is also used in the determination of nitrates and heavy metals in water and soil.

2. Quality control of foodstuffs and beverages

The quality and safety control of foodstuffs is at present among the topical problems of analytical chemistry. In certain sense, it is even more important that the problem of environmental control, because, according to scientists, more than 70% of toxic impurities get into a human organism with food, 20%, with water and 10%, with inspired air.[82] In addition to technological contaminants that get into foodstuffs through soil, water and air, the foodstuffs can also contain natural contaminants that either are formed during their improper storage, due to failures in the food procession technology or get into them through the wrapping, *etc.*[83, 84]

Currently, the safety of people (especially in Russia ‡ largely depends on the cleanness and quality of foodstuffs, spirits and non-alcoholic beverages, because many contaminants possess carcinogenic, mutagenic, teratogenic and immunosuppressive properties. In addition to direct poisoning, the contaminated foodstuffs can reduce the immunity and cause hereditary changes. In some cases, they can directly cause diseases (allergic, oncological, cardiovascular, *etc.*) as a result of the changes in the biochemical reactions in the organism.

The monitoring of the foodstuff quality often involves using chromatographic techniques.[85, 86] The latter are used to control the quality and freshness of foodstuffs, determine the technological and natural contaminants, reveal counterfeit foodstuffs and determine synthetic dietary supplements and residual veterinary drugs in meat (Table. 5).

Table 5. Main applications of chromatographic techniques in the foodstuff monitoring.

Analysis goal	Determined compounds	Ref.
Determination of nutrition validity of products	food proteins	87
	amino acids	88
	lipids	89
	organic acids	90
	carbohydrates	91
	vitamins	92
	carotenoids	93
Determination of impurities in foodstuffs	pesticides	94
	mycotoxins including:	95
	aflatoxins	96
	patulin	97
	ochratoxins	98
	fumonizins	99
	zearalinon	100
	nitrosoamines	101
	polynuclear aromatic hydrocarbons	102
	dioxins	103
	biogenic amines	104
Determination of dietary supplements	antioxidants	105
	synthetic dyes	106
	sweeteners	107
	aromatisers	108
	bitter substances	109
Determination of residual veterinary drugs in foodstuffs	antibiotics (tetracycline, *etc.*)	110
	sulfamide drugs	111
	hormones	112
	anabolic steroids	113
	cytostatin	82

A new direction, the enantioselective analysis of food components, has appeared, which, based on the ratio of optical isomers of amino acids, hydroxy acids, *etc.*, makes it possible to infer whether a given foodstuff is natural or contains synthetic substitutes and additives. For instance, the development of a procedure of gas chromatographic separation and analysis for all fatty acid isomers has forced the manufactures to reduce several-fold the content of *trans*-isomers of unsaturated acids in margarine.

† Chlorine-containing organic compounds (polychlorobiphenyls, dioxins, *etc.*) are among twelve most dangerous contaminants.

‡ In recent years, the counterfeit foodstuffs and beverages (butter and vegetable oil, mineral water, juices, wine, brandy, vodka, *etc.*) became more frequent. The spread of retail trade has resulted in the improper storage of foodstuffs (without refrigeration) and their delivery at long distances in poorly equipped vehicles.

3. Early recognition of diseases by determination of biological markers

The successful curing of various dangerous diseases depends on their early recognition. Many diseases can be recognised by their markers, *i.e.*, substances contained in biological fluids and expired air.

Most often, the markers represent low-molecular biologically active compounds, namely, nucleosides, nucleotides, catecholamines, hydrocarbons, amino acid derivatives (*e.g.*, homocysteine, *etc.*), steroids, carbohydrates, indoles, porphyrins, organic acids, vitamins, *etc.*[114] Large molecules, for example, proteins and lipids, can also be the markers.

Chromatographic techniques allow many biochemical markers to be determined at the level of $10^{-9}-10^{-15}$ g (depending of the preconcentration and detection techniques used).[115, 116] The large volume of available information (due to the possibility of the separation and determination of tens – hundreds compounds per cycle), the low detection limits,§ the reliable instrumentation, the automated analysis that provides the complete test report, the data storage in the PC memory, the versatility (a single chromatograph can be used for carrying out numerous tasks), the preferential use of non-invasive samples (urine, saliva, tears, sweat, hair, expired air, *etc.*) make the chromatographic techniques irreplaceable in the analysis of markers.

GC and HPLC are the techniques used most often for the analysis of markers.

Naturally, the markers of dangerous, particularly, oncological diseases attract the keenest attention (Table 6).

Here, we consider the diagnostics of oncological diseases, namely, breast and lung cancer.

Table 6. Biochemical markers of oncological diseases.

Disease to be recognised	Disease markers	Ref.
Breast cancer	(1) modified nucleosides (1-methylguanosine, 2-methylguanosine, pseudouridine, 5,6-dihydrouridine) in urine (2) polyamines (spermine, spemidine, putrescine, cadaverine) in urine	117 – 119
Lung cancer	hydrocarbons(n-butane, n-pentane, n-heptane, 2-methylhexane, 3-methylhexane, 4-methyloctane, 5-methyldecane, 3-methyltridecane, 7-methyltridecane) in expired air	120
Malignant melanoma	cysteine and indole derivatives in urine	121
Ovarian carcinoma	polyamines in urine	119, 122
Oestrogen-initiated tumours	4-hydroxyoestradiol in urine	123
Carcinogenic diseases	5-hydroxyindoleacetic acid in urine	124
Cancerous growth (children)	fucose in urine	125
Neuroblastoma (children)	homovanillic acid and vanillyl mandelic acid in urine	126
Pheochromocytoma	catecholamines (adrenaline, noradrenaline and dopamine) in blood plasma or urine	127

§ Due to the low detection limit, only small amounts of biological samples are required for the analysis (often, several microlitres).

The specialists at the American Oncological Centre who studied the possibility of early recognition of cancer based on biological markers have found that high concentrations of modified nucleosides in the blood of oncological patients points to the presence of a tumour. Such an analysis allows the disease to be recognised at the earliest stage. The determination of nucleosides in urine can be performed with the use of HPLC. The results of the analysis of urine samples of 62 women suffering beast cancer (reliably confirmed) and 100 healthy women were described.[119] Their statistical processing confirmed the higher diagnostic value as compared with the analyses that used conventional markers of breast cancer, *viz.*, CEA and CA 15-3. The sensitivity and selectivity of the diagnostics from the analysis of modified nucleosides exceed 80%. The examination of the transformed nucleosides allows certain types of leucaemia, lung and brain cancer to be recognised.[117 – 119]

The prospects of the early recognition of lung cancer are associated with the analysis of expired air.[120] The air expired by the patients suffering lung cancer was found to contain higher concentrations of alkanes and methylalkanes such as n-butane, n-pentane, 3-methyltridecane, 7-methyltridecane, 4-methyloctane, 3-methylhexane, heptane, 2-methyhexane and 5-methyldecane. These hydrocarbons were present at the level of nano-(pico)moles; hence, their determination should involve their preconcentration on special adsorbents that do not absorb moisture. The chromatographic method of the diagnostics based on the analysis of the expired air has advantages over other methods due to its safety, low cost and high selectivity. Therefore, it was proposed for the early initial screening of oncological patients.

4. Pharmaceutical and pharmacological applications

The major application fields of chromatography in the analyses and examination of medicinal drugs (pharmaceutics) and also in the determination of drugs in biological fluids (pharmacology) are as follows:

— pharmacopeia control over the quality of drugs;[128]
— monitoring of impurities in drugs;[129]
— studies of the stability of drugs;[130]
— analysis of medicinal herb extracts;[131]
— analysis of drugs based on the herbs used in traditional Chinese medicine;[132]
— application of the LC-MS technique in the development of new drugs;[133]
— application of supercritical fluid chromatography for the separation of drug enantiomers;[134]
— the use of HPLC for the separation of drug enantiomers;[135]
— the use of HPLC in phamaceutical industry;[136]
— determination of natural phenolic compounds in plants (flavonoids, hydroxy acids, natural dyes, *etc.*);[137 – 140]
— analysis of drugs in the doping control;[141]
— clinical pharmacy;[142]
— chromatography in pharmacokinetics;[143]
— toxicologic analysis;[144]
— binding of drugs in the organism.[145]

The use of chromatography in pharmaceutics was also the subject of monographs.[146, 147]

High-performance liquid chromatography is the main method used in the analysis of medicinal drugs at pharmaceutical factories. Ion chromatography is widely used for the determination of ions in drugs, of anions and cations in special-grade water and water used for injections. Ion chromatography coupled with a pulsed amperometric detector allows mono-, di-, tri- and polysaccharides, alcohols and free acids to be determined (without preliminary derivatisation). Gas chromatography is used in the analysis of solvent residues that can be present in pharmaceutical products.

5. Chromatographic methods for the analysis of natural gases, gas condensates, mineral oils and crude oil

Chromatography is widely used in the gas, oil and oil-refining industries.[148–151] Analyses are carried out at all stages, *viz*., during the geological survey of gas and oil areas, gas and oil production, piping, at different stages of technological processing and, ultimately, for monitoring the finished products. Moreover, the chromatographic techniques are used for the monitoring of pollution of the environment (soil, water, air, bottom sediments, *etc*.) with mineral oils.

Modern high-performance capillary columns make it possible to completely separate the gasoline fractions into components (200–1000 components in a single sample). These analyses cannot be accomplished by other methods.

Chromatographic techniques (GC, HPLC, ion, gel-filtration chromatography) are used for the monitoring of natural and accompanying petroleum gases, gas condensates, gasoline and kerosene fractions, diesel fuel, lubricating oils, oil heavy fractions, pyrobitumen and even crude oil. The methods of the analysis of natural gases and different fractions of mineral oils are specified by the Russian State Standards (GOSTs).

Chromatography makes it possible to determine both individual (component-after-component) and group compositions; to simulate the distillation of petroleum and its derivatives according to their boiling points; to assess the environmental characteristics of petroleum derivatives, in particular, determine the benzene content, the total sulfur and nitrogen contents and the concentrations of heavy metals and polynuclear aromatic compounds.

High-performance liquid chromatography allows one to easily perform the 30-min separation of petroleum into the following four fractions: asphaltenes, saturated hydrocarbons, aromatic hydrocarbons and polar compounds. After such a grouping, the separation of saturated, aromatic and polar compounds into individual components is possible. The group type separation (to paraffins, naphthens and aromatic compounds) of gasoline is carried out on 13X-zeolite-packed columns, whereas their separation into individual components is accomplished on capillary columns. The chromatographic separation of oxygenates in gasoline is performed using a flame ionisation detector, which is selective with respect to oxygen-containing compounds. The operation of the detector is based on the conversion of oxygen-containing compounds into methane, which allows one to avoid the effect of the hydrocarbon background and determine oxygenates at the trace level.

Multidimensional gas chromatography ¶ is used for the separation of complex multicomponent mixtures in petrochemistry and also for the determination of the hydrocarbon content of natural and accompanying gases. The increase in the gas cost places more stringent requirements upon the reliability of the natural gas analysis, which is necessitated by the interpayments between the oil-production, oil-delivery and oil-consumption companies.

Chromatography plays the exceptionally important role in the chemical, pulp and paper and many other industrial fields. It is used, first of all, to exercise technological control (analyses at all technological stages) and maintain the optimum conditions and, secondly, to analyse rough materials, finished products and wastes. The technological control at hundreds of Russian plants involves the use of more than 50 thousand gas chromatographs. Thousands of gas, liquid and ion chromatographs are constantly operated at the laboratories and centres of Gosepidnadzor (State Sanitary and Epidemiological Control), environmentral control committees and centres, toxicological laboratories, organisations such as Vodokanal, laboratories of Goskomgidrometeotsentr (State Hydrometeorology Centre), veterinary laboratories, plant protection stations and the laboratories of forensic and medical examination, *etc*.

In recent years, the role the chromatography plays in power engineering increased, namely, it is used for the determination and evaluation of the calorific power of gases and in the analysis of pollutant emissions. Ultimately, chromatography finds application in archaeology and art (for studying old paints, varnishes, coatings, *etc*.).

Chromatography surpasses other analytical methods in the number of publications. More than 300 thousand books, reviews and papers were published (including 110 thousand on the liquid column chromatography, 65 thousand on the gas chromatography, 65 thousand on the planar chromatography and about 50 thousand on the capillary and planar electrophoresis.).

VII. Conclusion

In summary, we outline the main directions in the development of chromatography.

Upgrading the selectivity of separation due to the development of new sorbents. In GC, this is the synthesis of new liquid phases with bi- or trifunctional groups, the development of adsorption capillary columns of the PoraPlot type of different chemical nature, the use of the geometric factor for the increase in the selectivity. In HPLC, this is the synthesis of reversed-phase sorbents that contain immobilised polar groups in alkyl chains and also sorbents with molecular-size pores for the retention of molecules of both single and related types, the use of Hypercarb carbonaceous sorbents based on porous graphitised carbon black to enhance the selectivity of the separation of isomers.

Enhancement of the column efficiency due to the changeover to capillary columns. At present, more than 60% of studies are carried out with the use of capillary columns. The share of micropacked, capillary, and nanocolumns in HPLC has substantially increased. In capillary electrochromatography, the separation is carried out exclusively on capillary columns. In GC, HPLC and capillary electrochromatography, the efficiency of columns exceeds one million of theoretical plates.

The development of new rapid separation methods. In GC, the high separation rate is achieved due to the decrease in the column volume and also due to the high-gradient temperature changes (up to 1800 deg min^{-1}). Dozens of components can be separated in a minute. In HPLC, the high separation rates are achieved by using columns packed with fine non-porous particles (measuring 1.0–1.5 μm), monolithic columns and columns with perfusion sorbents and turbulent flows.

Miniaturisation of instruments is the common trend of all chromatographic techniques. The most significant progress is observed in GC, HPLC and ion chromatography. Portable field chromatographs, hand-held devices, and also chip-based instruments are developed.

Improvement of microprocessor programmes for the analysis-data processing and the control over the chromatograph parameters. Attention is drawn to the computer-assisted processing of the results of chromatographic analysis. The systems for recording, processing and storage of chromatographic information are constantly renewed and refined. The number of publications on the simulation of chromatographic processes increases, particularly, the publications devoted to the use of neural networks and the linear and non-linear regression analysis for the simulation and optimisation of these processes. The chromatographic databases are constantly replenished.

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