inlet and higher at the outlet. The effect is a function of molecular size within the polymer class. **Figure 4** is an example of both approaches.

See also: **II/Chromatography: Liquid:** Mechanisms: Reversed Phases; Mechanisms: Size Exclusion Chromatography; **III/Gradient Polymer Chromatography: Liquid Chromatography. Peptides and Proteins:** Liquid Chromatography.

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Thin-Layer (Planar) Chromatography

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The use of thin-layer chromatography (TLC) in polymer analysis was first mentioned in 1968. Belenkii and Inagaki with their co-workers described separations according to the composition of random styrene–methyl methacrylate and styrene–methyl acrylate copolymers with molecular weights varying from 40 to 200 kDa. Since then polymer TLC has been developed intensively and other researchers have also begun to work actively in this field.

The mechanisms of polymer TLC have been investigated and the methods for the determination of molecular weight (MW) and molecular weight distribution (MWD) of homopolymers, such as polystyrene (PS), poly(ethylene oxide) (PEO) and poly(methyl methacrylate) (PMMA), have been developed. Impressive results were obtained for separations in accordance with different features of the polymer architecture. This investigation of structural heterogeneity of styrene–methyl methacrylate (S-MMA) copolymers have made it possible to separate random, block and alternating copolymers as well as two- and three-block copolymers. The stereoregular heterogeneity of PMMA and polybutadiene (PB) has also been determined. Styrene (S) and butadiene (BD) block copolymers have been studied and deuterated and hydrogenous PS separated.

In the vast majority of studies silica gel has been employed as a sorbent with the occasional use of alumina. In 1976 Belenkii and co-workers reported that critical conditions exist on passing from size-exclusion to adsorption chromatography of polymers. The first reviews on polymer TLC appeared in 1977. In 1980 Glöckner demonstrated the importance of gradient elution for polymer separations, and in 1982 Armstrong and co-workers used reversed-phase plates to separate homopolymers according to MW. More recent reviews have been by Glöckner (1987) with the most comprehensive review presented by Gankina and Belenkii in 1991.

Here the behaviour of macromolecules and small molecules are compared and the mechanism of chromatographic processes and the analysis of different types of polymer heterogeneity considered.

Behaviour of Macromolecules under TLC Conditions

TLC is one of the most efficient methods used for the fractionation of polymers and the analysis of their heterogeneity. The chromatographic behaviour of polymers differs from that of low MW compounds in many ways that can be revealed even in the analysis of narrow-dispersity homopolymers. Unlike low MW compounds, polymers are characterized by physical heterogeneity, i.e. they are a mixture of macromolecules with different degrees of polymerization (polymer homologues). The concept of 'molecular weight' is replaced by the expression 'average MW', which is a statistical average value. In addition to physical heterogeneity characterized by molecular weight distribution (MWD), chromatographic

methods of analysis also enable the determination of chemical, functional, structural, stereoregular and topological polymer heterogeneity.

Macromolecules are characterized by low diffusion coefficients in solution and by even lower coefficients in pores. This adversely affects the kinetics of entrance and exit of macromolecules in and out of sorbent pores. Macromolecules consisting of many repeat units are, moreover, characterized by multicentre adsorption, which makes the sorption–desorption process slower and more complex. Both these factors hinder interphase mass transfer and, hence, chromatographic zones become broader in the longitudinal direction. At the limit, tails and tracks appear on chromatograms in both adsorption and adsorptionless chromatography (**Figures 1** and **2**). In TLC both these factors become apparent as the mobile phase velocity is increased as a result of the increase of sorbent particle size. Hence, it must be borne in mind that in polymer analysis the eluent velocity is subject to greater limitations than in the analysis of low MW substances. To avoid the appearance of false zones and tails in polymer analysis, sorbents with a particle size of 5–8 μm must be used.

Specific properties of polymers are a consequence of the large size of their molecules. According to current concepts, a long flexible chain molecule in a dilute solution is coiled. The coil size is comparable to that of the pores. In the absence of adsorption the molecules enter the pore when the coil size is smaller than that of pores. When the coil size is too large, the molecule is excluded from the pore.

Even dilute polymer solutions are characterized by considerable viscosity, this viscosity increases with concentration and MW. The chromatographic zone forms a region with high viscosity, past which the mobile phase flows. This leads to an increase in the size of chromatographic zones in the longitudinal direction (in the direction of mobile phase motion). Moreover, the longitudinal size of the zone increases both with concentration of the polymer in the zone and with increase in MW (Figure 2). Therefore, the shape of the polymer chromatographic zones is usually elongated in the direction of mobile phase motion, not only because of heterogeneity in MW and spreading as a result of slow interphase mass transfer, but also because of the viscous effect.

Polymers dissolve much more slowly than low MW compounds and their dissolution is preceded by swelling. Since, in TLC, dissolution precedes chromatography, the slower dissolution rate of polymers with high MW in the starting zone is manifested as tails on a plate at high eluent velocity (particle size 20 μm). Moreover, polymer solubility depends on MW, and the polymers usually become less soluble as MW increases.

The coil size (hydrodynamic volume) at a fixed MW is not constant. It depends on the thermodynamic quality of the solvent (thermodynamic quality expresses the measure of thermodynamic affinity of the solvent for the polymer) and also varies on passing from the mobile to the stationary phase.

There are several points of view about the mechanism of macromolecule adsorption. One view is that during adsorption the macromolecule diffuses from the solution into the sorbent pore, more or less retaining its globular shape. Another hypothesis states that in adsorption on the pore surface the molecule is uncoiled and lies flat on this surface. This becomes possible as a result of enthalpy gain when the chain segments interact with active centres on the sorbent surface. This gain exceeds the increase in the free energy because of entropy decrease. This process is accompanied by a considerable decrease in the

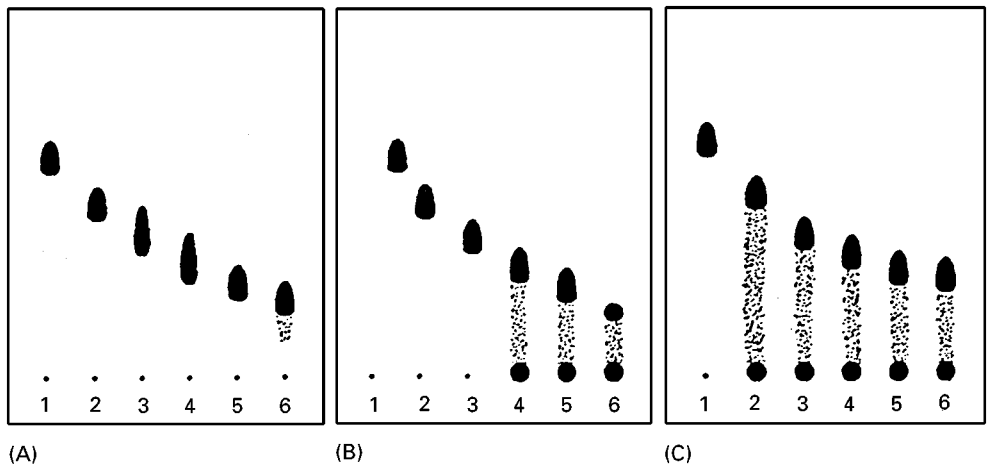

Figure 1 TLC of PS with MW (kDa) of (1) 20, (2) 111, (3) 200, (4) 498, (5) 865 and (6) 2610 on plates coated with silica gel KSKG with d_p (μm) of (A) 9.3, (B) 12.8 and (C) 19. Eluent: cyclohexane–benzene–acetone (12 : 1 : 1).

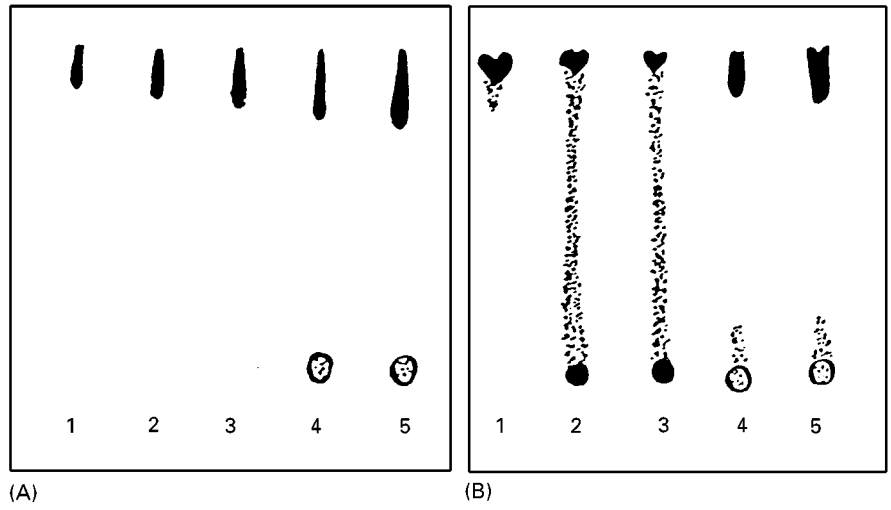

Figure 2 Adsorptionless TLC of PS with MW (kDa) of (1) 20, (2) 111, (3) 200, (4) 498 and (5) 867 on silica gel plates with sorbent particle size d_p (μm) of (A) 6.5 and (B) 19.0. Mobile phase: toluene.

volume occupied by the macromolecule, i.e. by an increase in density. As a result, the macromolecules are adsorbed on the surface of small pores inaccessible to them in a size-exclusion regime. Consequently, the change in free energy ΔG, when a macromolecule enters a pore is the sum of the change in entropy ΔS and enthalpy ΔH of the system ($\Delta G = \Delta H - T\Delta S$). The entropy change is caused by a decrease in the number of conformations of the macromolecule inside the pore over that in solution. The value of ΔH is due to the interaction of chain units with the pore walls.

It has been established experimentally, and confirmed theoretically, that in polymer chromatography the adsorption region and the molecular-sieve region are separated by a critical point at which the change in the conformational free energy of the macromolecule on passing from the mobile into the stationary phase is equal to zero. At this point the macromolecules undergo first-order phase transition. Under critical conditions homopolymers are not separated according to molecular weight; the pore structure of the sorbent and its specific area do not influence the behaviour of macromolecules. Hence, the number of separation mechanisms for polymers is greater than that for low MW compounds: besides adsorption chromatography ($-\Delta G > 0, k_d > 1$) at least two other chromatographic regimes are possible:

- $-\Delta G < 0$, $k_d < 1$ – size-exclusion chromatography (SEC);
- $-\Delta G = 0$, $k_d = 1$ – adsorption chromatography under the critical conditions (ACCC), where k_d is the distribution coefficient.

It is possible to pass from adsorption regime to size exclusion and vice-versa by varying temperature and eluent composition or by modifying the adsorbent. These facts experimentally confirm a single mechanism of the liquid chromatography of polymers.

Chromatographic Regimes Applied to the TLC of Polymers

Adsorption–Exclusion Regimes

Adsorption TLC Adsorption TLC (ATLC) is used more extensively in polymer analysis. Enthalpy change is the dominant factor in the mechanism of adsorption separation of macromolecules. By selecting appropriate chromatographic conditions, adsorption activity of macromolecules can increase with increasing MW or the fraction of adsorption-active polar groups (for copolymers or homopolymers with functional groups).

ATLC is used to separate homopolymers according to their MW, functional groups and stereoregularity. It can also be used for the separation of copolymers according to composition and for the analysis of the MWD of oligomers with complete separation into oligomer homologues.

The most complex problem in ATLC is mobile phase selection. It is solved for the separation of homopolymers according to MW. In this case, the eluent for corresponding oligomer homologues is selected first (using, for example, the 'Prizma' model). Subsequently, a small amount of the adsorption-active component, the displacer, is added.

Exclusion TLC Entropy change is the dominant factor in the mechanisms of exclusion separation of macromolecules (ETLC). The chromatographic mo-

bility of macromolecules is determined by the ratio of the size of macromolecules to pore size and increases with the increasing hydrodynamic volume of the macromolecules. The exclusion effect is seen in TLC when two conditions are obeyed: the adsorption activity of the sorbent is suppressed and its pores are filled with the solvent. The interparticle volume should remain free. Pore filling can be accomplished either by pre-elution with subsequent removal of the solvent from the interparticle volume: the solvent passes along the plate before sample spotting; or else capillary condensation takes place during preliminary plate saturation with solvent vapour in a saturated chromatographic chamber. In the latter case the samples are spotted before saturation (or pore filling). Solvents or their mixtures in which analytes migrate along a dry sorbent layer with the front are used as eluents. The pore size distribution of the sorbent is of great importance and resolution in ETLC is lower than that in ATLC.

Adsorptionless TLC Adsorptionless (or viscometric) TLC is a type of exclusion TLC. It is carried out when the mobile phase moves along the dry sorbent layer. In this case the polymer is concentrated near the eluent front because the pores of the sorbent are accessible to it.

Moreover, a viscous polymer solution in the chromatographic zone plays the role of a kind of plug along which the solvent flows. Hence, the chromatographic zone acquires a droplike elongated shape and distinct boundaries. The zone length increases with both polymer concentration and its MW. For a fixed polymer quantity, the length of the chromatographic zone (L) is a function of intrinsic viscosity ($[\eta]$, at infinite dilution). This dependence is obeyed for polymers of different nature, i.e. it is of universal character and can be obtained with the aid of any type of polymer standards (**Figure 3**). To determine $[\eta]$, it is necessary to plot the calibration dependence $L = f[\eta]$ by using four or five polymer standards in the required MW range at $C = \text{const}$. Subsequently, the length of the chromatographic zone is determined under the same conditions. Viscosity average MW value can be easily determined according to the Mark–Kuhn–Houwink equation with the aid of its coefficients available from reference books.

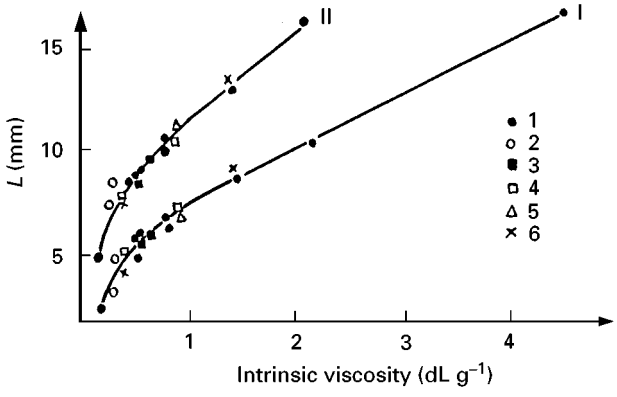

Figure 3 Dependence of zone length (L) on intrinsic viscosity for (1) PS, (2) PMMA, (3) polycarbonate, (4) polyisoprene (PI), (5) poly(vinyl chloride) and (6) poly(α-methyl styrene) with MW (kDa) of 20.8, 111, 200, 498, 867, and 2610 (1); 60.6 and 75 (2); 36.4 and 50.2 (3), 32.5 and 109 (4); 75 (5); and 97 and 610 (6) at a fixed polymer quantity (μg) of (I) 4 and (II) 10. Binder: gypsum; layer thickness, 500 μm.

Adsorption TLC under critical conditions In adsorption TLC under critical conditions (ATLC), the changes in enthalpy during polymer interaction with the sorbent surface are compensated for by increasing entropy of the macromolecule when it enters the pore: $-\Delta H = T\Delta S$. ATLC under critical conditions is used to analyse heteropolymers: polymers and oligomers containing functional groups, block copolymers (AB and ABA types) and graft comb-like copolymers. Using ATLC it is possible to separate linear, cyclic and branched structures. In this regime one of the components of the copolymer undergoes chromatography under critical conditions and remains 'invisible' – having no effect on separation. Another component of the copolymer, the characteristics of which should be determined, takes part in the chromatographic process according to an adsorption or a size-exclusion mechanism. Critical conditions can be implemented either by using an adsorbent, the pores of which are filled with the eluent (analogously to ETLC) or by eluent migration along a dry sorbent layer. In the former case critical conditions are indicated by the absence of a separation according to MW for homopolymers with the same chemical composition as that component of the copolymer which should become 'invisible'. In the latter case the indication of critical conditions is the absence of separation (R_F values are equal) of homopolymers differing in MW but containing the same functional groups (**Figure 4**).

Precipitation–Extraction Regimes

Precipitation TLC Precipitation TLC (PTLC) was first suggested and its mechanism investigated by Kamiyama and Inagaki in 1971. The classical examples are the separation of PMMA according to MW using a chloroform–methanol (29 : 71) mixture as the mobile phase.

In PTLC mixtures of polymer, solvent and adsorption-active precipitant (a thermodynamically poor solvent) are used as the mobile phase. Separation

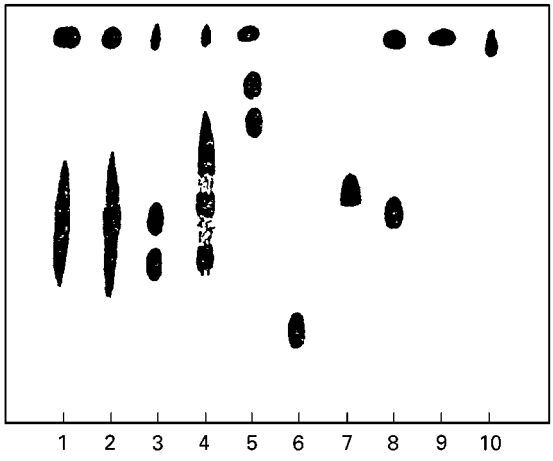

Figure 4 ATLC of reaction mixtures, obtained by the reaction of PSLi with (1–4) methyl acetate, and (5) methyl benzoate; of tertiary alcohols (6) $(C_8H_{17})_2$–C(OH)–CH_3 and (7) $(C_8H_{17})_2$–C(OH)–C_6H_5, of (8) polymer ketone PS–CH_2–$C(C_6H_5)_2$–$COCH_3$ and its PS precursor with MW 0.4 kDa and PS standards with MW (kDa): (9) 0.4 and (10) 40. Reaction mixtures (1–5) contain: PS precursors with MW (kDa): (1, 5) 0.4, (2) 0.6, (3) 6.4 and (4) 13.5, polymer ketone PS–CO–R with the same MW as that of PS precursors, and tertiary polymer alcohol $(PS)_2$–C(OH)–R with double MW, where R is $-CH_3$ or $-C_6H_5$. Conditions are close to the critical point for PS: adsorbent is silica gel KSKG; mobile phase is toluene–CCl_4 (1 : 4) mixture.

occurs as a result of changes in the dissolution properties of the mobile phase along the chromatographic plate, for instance, as a result of partial evaporation of mobile phase components demixing during chromatography. The dissolution properties of the mobile phase can also be changed by delivering it with a changing composition to the plate. The initial mobile phase should contain the adsorption-active component at a concentration completely preventing polymer adsorption.

During PTLC when the individual polymer species with different MW pass along the plate they undergo a continuous series of precipitations and extractions. The elementary PTLC process is the separation of a polymer solution into the dilute phase, which is transported with the solvent flow, and the concentrated gel phase which is precipitated on the surface of sorbent particles.

PTLC is used to separate homopolymers and random copolymers by MW and to separate a block copolymer from the corresponding homopolymers. The analysis of oligomers with the aid of PTLC is not effective. The working range of MW exceeds 10 kDa.

Extraction TLC The action of extraction TLC is opposite to that of precipitation TLC. Extraction TLC is based on selective dissolution and desorption of the polymer in the starting zone region. Desorbed polymer fractions are displaced to the eluent front. Eluent migration length is not significant. Polymer zones move along the plate in size-exclusion mode. Since the eluent moves along a dry sorbent layer, separation according to MW does not take place. In this method a single component mobile phase or binary solvent mixtures are used most often; gradient column chromatography is the closest analogue. Under conditions of extraction TLC the gradient is replaced by stepwise elution.

Using extraction TLC it is possible to separate compounds differing essentially in solubility or adsorption activity: isotactic and atactic polystyrene, isotactic and atactic PMMA, poly-1,4 trans- and 1,2-butadiene and to separate the S-MMA block copolymer from PS and PMMA.

Some Problems in Polymer Analysis

Determination of MW and MWD TLC, like other chromatographic methods, is not an absolute method. Therefore, in order to determine physical or chemical heterogeneity, polymer standards with known characteristics are necessary. Belenkii and Gankina state that in order to determine the MWD, it is necessary first to establish the dependence of R_F on MW with the aid of narrow-disperse standards, secondly to obtain a densitogram of the chromatographic zone, thirdly to establish the dependence of the recorded signal (I) on polymer mass (P) for different R_Fs and to determine $(dP/dI)_{R_F}$, fourthly to determine the distribution of polymer mass in the zone $P(R_F)$, and finally to calculate MWD according to the following equation:

$$P(M) = P(R_F)\left(\frac{dM}{dR_F}\right)_{R_F}$$

If the distribution, $P(M)$ is known, it is also possible to obtain the expressions for weight-average MW

$$\bar{M}_W = \frac{\int_0^\infty M^2 P(M)\, dM}{\int_0^\infty MP(M)\, dM}$$

number average MW:

$$\bar{M}_n = \frac{\int_0^\infty MP(M)\, dM}{\int_0^\infty P(M)\, dM}$$

and heterogeneity:

$$H = \frac{M_w}{M_n}$$

An important point increasing the precision of MWD determination is the correction for instrumental broadening in SEC. This is attained with the aid of

two-dimensional chromatography. In the first direction the chromatographic zone is separated according to MW and also undergoes chromatographic spreading. In the second direction the separation according to MW may be neglected. The dispersion of chromatographic spreading is equal to the difference between zone dispersion after the second and the first elution in the direction of the second elution. For correction, the calculated dispersion of chromatographic spreading is subtracted from the total dispersion of the polymer zone after the first elution in the direction of mobile phase migration.

Analysis of Copolymers

As polymer molecules very often contain functional groups or consist of chains differing in chemical nature, polymers are characterized by a mixed separation mechanism. The analysis of copolymers exhibiting chemical and structural heterogeneity requires more complex elution procedures: for example, stepwise, two-dimensional, gradient and continuous TLC. In most cases TLC is combined with column chromatography or pyrolysis–gas chromatography (GC), as well as with spectroscopic methods.

In the analysis of block and graft copolymers or branched homopolymers, the following problems should be solved:

- the diagnosis of a copolymer or a branched homopolymer;
- the determination of linear homopolymer present in the copolymer; and
- the investigation of MW and MWD of copolymers.

The first two problems can be solved by comparing the chromatographic mobility of the polymer being analysed with that of the corresponding linear homopolymers in appropriate solvents using ATLC or PTLC. An indispensable condition of separation is the difference in adsorption activity or solubility of A and B homopolymers. Difficulties can appear if their properties are similar and also when block copolymer complexes with one of the homopolymers are formed.

Additional proofs of copolymer presence can be obtained by double detection of the chromatographic spots, for example, by using reagents specifically staining homopolymers of different types and by spectroscopic methods *in situ*, or after elution of the polymer zone from the plate.

To evaluate the MW of block-copolymer components, one can use ATLC under critical conditions.

Oligomers

Oligomers are built from the same monomer units as polymers, but their chain is much shorter (MW < 10 kDa). Polymers in dilute solutions are characterized by the following types of interactions: solvent–solvent, solvent–polymer segment, polymer segment–segment, solvent–surface, and polymer segment–surface, whereas for oligomer segment–segment interaction and local entropy effects are small. Oligomers without end groups are readily separated into oligomer homologues on polar adsorbents by adsorption chromatography. It is also easy to separate according to MW, oligomers with end groups for which the energies of interaction with adsorbent for the central (ε_c) and end (ε_e) units are similar. If the energy of interaction with silica gel is much lower for the central chain unit than for the end groups ($\varepsilon_c < \varepsilon_e$), separation according to the types of functionality will take place. Therefore, to separate according to MW it is necessary to use silica gel modified either chemically (for example, RP_{18}) or dynamically (for example, the separation of PEO oligomers in a pyridine–water mixture, 0.1 : 10).

Two-component mobile phases are known to separate some oligomers according to MW on silica gel: PS, PI, poly(propylene glycol), PEO and its various derivatives, oligoacrylates, etc. If the MW of one of the members of the homologous series on the chromatogram is known, corresponding standards are not necessary to calculate MWD.

For oligomers used in the production of synthetic polymers, the distribution according to the types of functionality (FTD) is very important. It characterizes the relative content of macromolecules with different functionalities in the oligomer. The functionality type of chemical compounds is determined by the number and nature of functional group. For macromolecular compounds the concept 'functionality' just as the concept 'molecular weight' has a statistical significance. In order to characterize distribution width according to functionality types the values of number-average ($\bar{f}_n$) and weight-average ($\bar{f}_w$) functionality are used:

$$\bar{f}_n = \sum n_i f_i / \sum n_i$$

$$\bar{f}_w = \sum n_i f_i^2 / \sum n_i f_i$$

where $n_i = p_i/MW_i$ (is the number of moles of macromolecules i with molecular weight MW_i, functionality f_i and weight p_i.

Oligomers are separated according to the number and nature of functional groups by adsorption chromatography under the critical conditions or under conditions close to critical. An example of this separation is shown in Figure 4. A scanning densitometer or a videodensitometer can be used for quantitative FTD determination.

Future Developments

TLC of polymers is more complex than that of small molecules for the following reasons: the size of sorbent pores and that of macromolecules are similar and co-operative effects exist which are characteristic of macromolecules as multicentred formations. For the same reasons, TLC of polymers is a very useful technique to investigate chromatographic mechanisms. The open sorbent layer in TLC makes it much easier to detect and investigate chromatographic artefacts than by column chromatography. The versatility of this method, the absence of restrictions with respect to solvents, and high sensitivity to functional groups (ATLC) make it possible to analyse effectively the compositional and structural heterogeneity of polymers.

Further development of this method involves investigations of mechanisms of adsorption and chromatography of macromolecules, more extensive use of chemically modified sorbents, more extensive application of quantitative methods of analysis and such promising methods as overpressured-layer chromatography (OPLC). The investigation of macromolecules with complex architecture and of reaction mixtures is not possible without perfecting complex methods of investigation. The combination of ATLC and column chromatography in different regimes and the combination of TLC with spectroscopic methods, especially with MALDI-TOF-MS are very promising.

See also: **II/Chromatography:** Size Exclusion Chromatography of Polymers. **III/Polymers:** Field Flow Fractionation; Supercritical Fluid Extraction.

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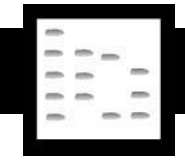
TERPENOIDS: LIQUID CHROMATOGRAPHY



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Introduction

Essential oils are secondary metabolites of plant origin and are complex mixtures of fragrance and flavour substances. They originate in roughly one-third of known plant families and are isolated from plant bodies by extraction or distillation procedures. The composition of the constituents are dependent on the isolation method used, the part of the plant body from which they are isolated and also on their thermal, pH and intrinsic chemical stability. Most essential oils are currently separated from nonvolatile materials by steam distillation. As for other classes of natural products, a fully satisfactory definition of essential oils is difficult to put into words. Although they are volatile plant materials, they do not leave a grease stain on paper.

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