Progress in Liquid Chromatography of Synthetic Electroneutral Polymers

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Summary: Recent achievements and anticipated future progress in high performance liquid chromatography of synthetic electroneutral polymers (polymer HPLC) are briefly reviewed. Basic retention mechanisms of polymer HPLC are explained and corresponding separation procedures are discussed. Advantages, drawbacks and pitfalls are presented of the most important polymer HPLC method, namely size exclusion chromatography. Principles of polymer HPLC methods combining various separation mechanisms within one chromatographic column (coupled procedures) or in a set of different chromatographic columns (two- and multi-dimensional procedures) are outlined.

Keywords: high performance liquid chromatography; non-charged polymers; synthetic

1. Introduction

The end-use properties of polymeric materials basically depend on

i) Molecular characteristics of macromolecules

ii) Arrangement of macromolecules in system

iii) Nature and amount of additives

The primary molecular characteristics of macromolecules are **molar mass, chemical structure** and **physical architecture.** The term chemical structure includes nature of monomeric units, further overall chemical composition in the case of copolymers and polymer blends, as well as nature and amount of functional groups in modified, for example oxidized or functionalized polymers and oligomers. The term physical architecture of polymer species describes the way how the monomeric units or entire chains are arranged in macromolecules, and refers for example to various kinds of copolymers, to linear and branched macromolecules including stars and combs, further to stereoregular species, as well as to head-to-head and head-to-tail, and cis- vs. trans- ordered macromolecules, etc. Secondary molecular characteristics represent combinations of primary characteristics, for

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example block copolymers with blocks built of different statistical copolymers, or branched structures with side chains differing in their stereoregularities, etc.

All presently available man-made polymers exhibit distribution in their molecular characteristics. Therefore, molecular characteristics are expressed by their mean (averaged) values and by parameters describing both width and shape of their distributions.

Knowledge of polymer molecular characteristics is important for assessment of existing, and for design of new polymeric materials. Unfortunately, an unambiguous determination of polymer molecular characteristics is often rather difficult and relations between molecular characteristics and end-use properties of polymeric materials are too complex. Therefore, full molecular characterization of synthesis products in basic research laboratories is, unfortunately, not common. Many scientific papers entitled "Synthesis and characterization... " present bulk rather than molecular characteristics or they deal only with averaged molecular characteristics provided for example by nuclear magnetic resonance and other spectrometric methods, by elemental analysis, and further by viscometry or light scattering. Alternatively, conclusions are based on a semi-quantitative and often superficial evaluation of size exclusion chromatography (SEC) data directly applying polystyrene calibration. The latter values should be designated "polystyrene equivalent molar masses" and they can hardly be considered in quantitative conclusions on polyreactions. Surprisingly, industrial development and quality control laboratories often rely on more advanced molecular characterization of polymers than many basic research institutions.

In order to assess distributions in their molecular characteristics, polymers must be separated. Various separation methods are presently used for polymeric materials but the field is clearly dominated by liquid chromatography. Application of some other important separation methods is limited to specific groups of polymers. For example, very useful method of temperature rising elution fractionation is suitable mainly for polyolefins. In the recent few years, methods of polymer mass spectrometry (MS) underwent an intensive development and it seems that HPLC - MS hyphenation will help solving many – though certainly not all problems of polymer molecular characterization.

In this short, critical review, we shall present recent progress in polymer HPLC and demonstrate the necessity of further method development. Instead of dealing with conventional features of polymer HPLC, we shall discuss selected unusual and often overlooked aspects of these important methods. Isocratic, non-gradient procedures will be stressed. Selected books and review papers will be quoted and some new, so far non-published observations will be presented.

General reviews, which compile numerous applications of polymer HPLC can be found in several monographs, for example in the recent book by Pasch and Trathnigg (Ref. 1).

2. Retention Mechanisms in Polymer HPLC

As known, differences in transport velocities of sample components are responsible for selectivity of their chromatographic separation. Progression of separated species along a HPLC column is accelerated or decelerated by various processes which are based on interactions between mobile phase, analytes and column filling. It is useful to qualitatively classify HPLC retention on the base of a simple thermodynamic consideration. It holds for chromatographic retention volume of any analyte, V_R

$$V_R \sim f(K) = f\left(\frac{C_S}{C_M}\right) \sim \exp\left(\frac{-\Delta G}{RT}\right) = \exp\left(\frac{\Delta S}{R} - \frac{\Delta H}{RT}\right) \qquad (1)$$

where K is distribution constant of analyte expressed as ratio of its concentration in the (quasi) stationary phase, C_S, and in the interstitial mobile phase, C_M. ΔG is the Gibbs function, ΔS and ΔH are entropy and enthalpy changes connected with transfer of analyte molecules from mobile into (quasi) stationary phase or vice versa, R is the gas constant and T is temperature. Correspondingly, retention mechanisms in any kind of chromatography can be divided into entropic and enthalpic ones. ΔS changes are large in liquid chromatography of macromolecules due to conformational (Ref. 2) and orientation (Ref. 3) contributions to the mixing processes.

In the entropy controlled polymer HPLC, typically in size exclusion chromatography and in hydrodynamic chromatography, macromolecules are partitioned between interstitial mobile phase and (quasi) stationary phase situated within pores of column filling or on the surface of particulate column packing, further on the walls of channels in monoliths or (capillary) columns. The driving force for distribution of macromolecules is difference in concentration between both phases while resistance against distribution is connected mainly with conformational entropy changes of macromolecules due to their spacial confinement (Ref. 4). One speaks about **entropic partition** or about **exclusion processes** (further only **exclusion).**

Enthalpic interactions in chromatographic systems result from attractive and repulsive forces between all constituents of chromatographic system and lead to adsorption, partition, phase

separation, as well as to ion, and bioaffinity effects. Former three retention mechanisms can be applied in HPLC of synthetic electroneutral species. For their successful utilization, it is necessary to understand their principles. Unfortunately, differences among particular enthalpic retention mechanisms are overlooked in literature and this results in misunderstandings and wrong statements, such as "... a nonsolvent for polymer sample must be added to eluent to enhance adsorption of macromolecules."

By definition, the term **adsorption** denotes distribution of solute molecules between a volume of solution and a solid surface or an interface. Solute molecules compete with solvent (eluent) molecules for active sites within column filling surface or interface. In other words, extent of adsorption of given macromolecules on given surface / interface is strongly affected by solvent – surface/ interface interactions, i.e. by solvent (eluent) **strength. Strong** eluents intensively interact with filling surface/ interface and suppress adsorption. A liquid, which fully prevents adsorption in a given polymer/ filling system is called **a desorli.** A weak solvent which promotes full adsorption in a given polymer/filling system is denoted **an adsorli.** No polymer elution is observed in an adsorli, irrespectively of applied volume of mobile phase. Evidently, an adsorli solvent for a polymer A/ filling S system may be a desorli for polymer B/ filling S system. Eluent strength depends on temperature and, especially in mixed eluents, also on pressure (Ref. 5). Column fillings for adsorption chromatography comprise for example various oxides, mainly SiO_2, and materials, in which appropriate polar groups such as $-NH_2$, -CN or $-O-CH_2-CHOH-CH_2OH$ are chemically bonded for example on silica gel surface via propyl-spacers. Solvent strength in adsorption chromatography can be semiquantitatively described by Snyder parameter, ε^o (Ref. 6).

The term **partition** describes distribution of solute molecules between two chemically different liquid phases, that is we deal with volume/volume effects. Unlike exclusion, **enthalpic partition** (further **partition**) is largely controlled by the difference in affinity of analyte macromolecules toward both phases. Experimental work with two immiscible free liquids (stagnant and mobile phase) is difficult and therefore most polymer HPLC experiments are done with chemically bonded phases. This means that one "liquid phase" is immobilized on the surface of column filling. Unfortunately, the choice of appropriate bonded phases is rather limited and it is so far dictated solely by requirements on columns for HPLC of small molecules. Commercially available are aliphatic groups up to C30, mainly linear C18 groups bonded on silica carriers and sometimes also on densely crosslinked synthetic polymers. Evidently, "thickness" of these bonded phases is much smaller than dimensions of macromolecules in solution. Development of column fillings, which would contain various

bonded synthetic macromolecules may substantially influence polymer HPLC applying partition mechanism.

As mentioned, the decisive driving force for partition of macromolecules is difference in their "solubility" in both phases. To reach extensive retention based on partition, solute must be better soluble in solvated bonded phase than in eluent. With a certain simplification, one can say that eluent must "push" macromolecules into stationary phase. Solubility of macromolecules in liquids can be characterized by various parameters, for example by Flory polymer – solvent interaction parameters, χ, or by exponent **a** in the Kuhn-Mark-Houwink-Sakurada viscosity law. Accordingly, one speaks about (thermodynamic) **quality of solvents** to distinguish between **good** and **poor** solvents, as well as about **nonsolvents.** If eluent does not effectively block active sites on the bonded phase surface that is if eluent is not strong enough from the point of view of solute adsorptivity, partition of macromolecules may be accompanied with their extensive adsorption. We deal with a complicated **hybrid retention mechanism.**

Evidently, solvent strength and solvent quality are two different and mutually rather independent parameters. As to adsorption retention mechanism a desorli for a given polymer/ column filling can be either a good solvent or a nonsolvent. The same holds for an adsorli. For example in the case of poly(methyl methacrylete) (PMMA)/ bare silica gel system, methanol and tetrahydrofuran (THF) are desorli while toluene, chloroform and hexane are adsorli. THF, toluene and chloroform are good solvents for PMMA while methanol and hexane are nonsolvents.

Phase separation in polymer solutions is also connected mainly with interactions of solvent molecules with macromolecules. Precipitation/ redissolution processes can lead to very selective HPLC separation of polymers (Ref. 7). Unfortunately **two** phases with different compositions are often formed when decreasing solvent quality (for example by temperature changes or by adding a nonsolvent to a polymer solution). Moreover, phase separation processes are usually slow and, as rule, they are accompanied by adsorption and/or by partition processes. These are reasons why phase separation based HPLC procedures may produce excessively broadened, distorted or even split peaks. Therefore, we shall further concentrate our discussion on adsorption and partition enthalpic retention mechanisms.

Besides above-mentioned solvent – column filling and solvent – polymer enthalpic interactions, one has to consider also polymer – filling enthalpic interactions. These are semi-quantitatively described by **segmental interaction energy**, ε_s (Ref. 8). As mentioned, polymer – filling interactions are directly affected by presence of solvent. The **effective**

segmental interaction energy, ε, depends on solvent strength (especially in the case of adsorption of macromolecules) and on solvent quality (in the case of polymer partition). ε is influenced also by temperature and possibly also by pressure. Due to conformational barriers and steric hindrances in pores of a column filling, **interaction energy for entire macromolecule,** ε_t, is as rule smaller than the sum of segmental interaction energies.

The role of enthalpic interaction in polymer HPLC can be schematically represented by plots of **log** $\mathbf{V_h}$ vs. $\mathbf{V_R}$ or more simply by plots of **log M** vs. $\mathbf{V_R}$, where V_h is **hydrodynamic volume** of macromolecules, defined with relation

$$V_h = [\eta]M \tag{2}$$

[η] is the limiting viscosity number of polymer in eluent. M is the most abundant molar mass present in polymer sample. This Benoit's concept of hydrodynamic volume corrects differences in polymer – solvent interactions, at least for linear and long chain branched macromolecules with flexible chains (Ref. 9).

The schematic plots of **log** $\mathbf{V_h}$ vs. $\mathbf{V_R}$ for exclusion, as well as for exclusion – adsorption and exclusion – partition combinations are shown in Figs. 1a and 1b. It is evident

that retention volumes for macromolecules of the same size increase with increasing ε values. At a certain, "critical" $\varepsilon = \varepsilon_{cr}$ value, compensation of entropic (exclusion) retention mechanism with enthalpic ("interactive") retention mechanisms is attained. In other words, effect of entropic retention mechanism, which increasingly "accelerates" transport of macromolecules with rising size along the column (V_R's decrease with increasing V_h, or M) equals to ΔH effects, which increasingly decelerate macromolecules as their V_h, or M rise (V_R's increase with increasing V_h, or M). The area of effective interaction energy near to ε_{cr} is utilized in "critical chromatography" which is termed also liquid chromatography in domain of entropy – enthalpy transition (cf. paragraph 5).

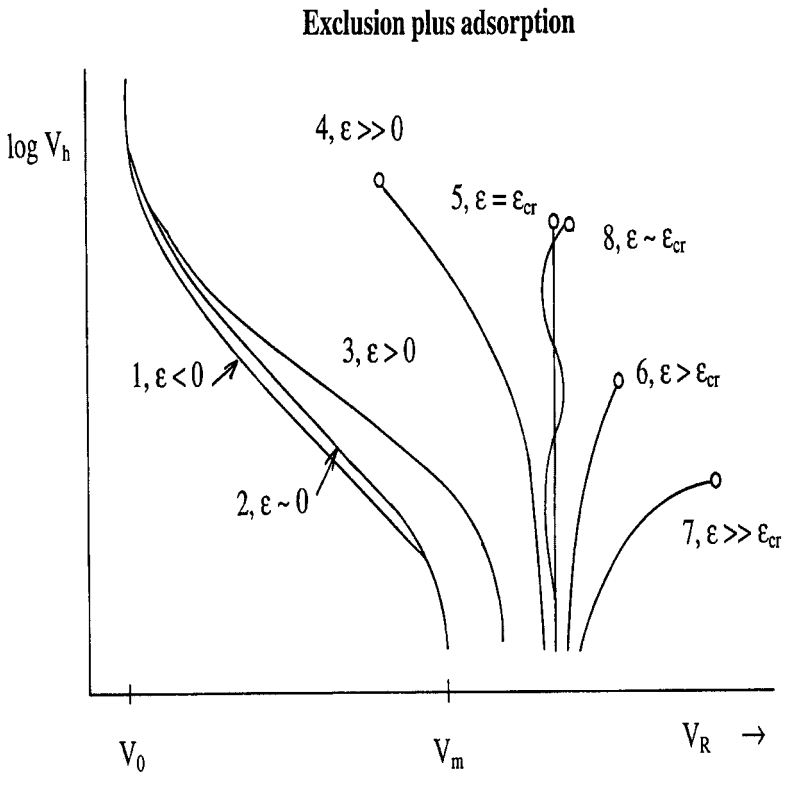
Several features are different in the case of exclusion – adsorption combination (Fig. 1a) compared with exclusion – partition combination (Fig. 1b). Origin of these differences is so far not fully understood.

i) In the regime of strong enthalpic interactions (high ε values), large macromolecules can "de-coil" and reptate into narrow filling pores. The same macromolecules would be fully excluded at $\varepsilon \sim 0$. One speaks about "confined enthalpic interaction", which causes full retention of large macromolecules within pores – even at ε

values which would still allow complete elution of smaller polymer species. Confined enthalpic interaction is rather pronounced in the case of adsorption. It strongly reduces sample recovery in polymer HPLC. Elution of macromolecules, which were retained within column by confined adsorption is rather tedious (Ref. 10). Fully retained macromolecules can affect both enthalpic and entropic (exclusion) (Ref. 11) properties of HPLC columns for later injected macromolecules ("history of SEC columns")

ii) Exclusion – partition combination can lead to highly selective separations (Ref. 12) (see Fig. 1b, curve 4).

ii) Very small variations in ε may completely change overall retention of macromolecules in the area near to ε_{cr}. This refers both to exclusion – adsorption and exclusion – partition combinations. In practice, eluent composition variations as small as ±0.1 %, presence of traces of humidity in eluent, and temperature changes ± 0.5 °C may affect



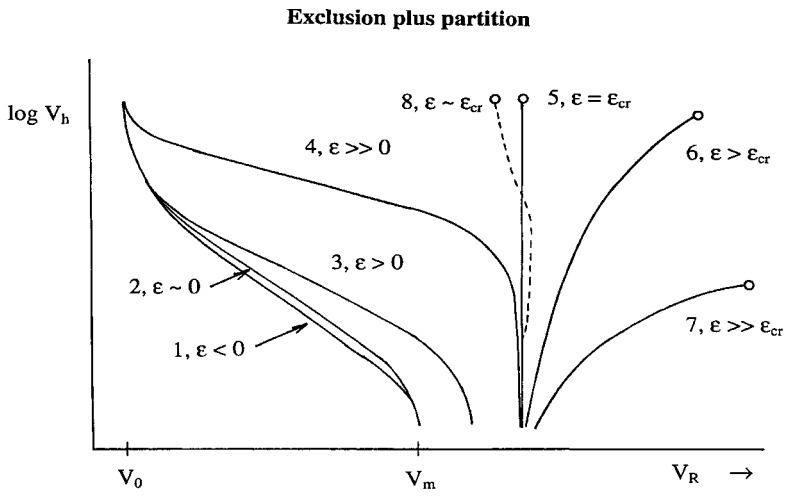


Figure 1. Schematic representation of dependences of log(hydrodynamic volume of macromolecules, V_h) versus retention volume, V_R in polymer HPLC for combination of exclusion with adsorption (a), and exclusion with partition (b). ε is the effective segmental interaction energy between polymer species and column filling. $\varepsilon \sim 0$ (curves 2) holds for purely entropic retention mechanism ("ideal size exclusion chromatography"), while $\varepsilon = \varepsilon_{cr}$ (curves 5) and $\varepsilon \sim \varepsilon_{cr}$ (curves 8) for "critical chromatography". **O** depicts a situation when macromolecules start to be fully retained within column. This is especially pronounced for systems exhibiting confined adsorption. For further explanation see the text.

shape of the log $V_h - V_R$ curve and cause transition from enthalpy dominated to entropy dominated situation. This results in often observed experimental problems in liquid chromatography of macromolecules under critical conditions (LCCC) (paragraph 5).

3. Size Exclusion Chromatography - SEC

SEC is the most popular method for molecular characterization of synthetic polymers. It is fast, relatively simple and cheap. Moreover SEC is well repeatable (it exhibits good precision) - and it easily creates illusion that a product of synthesis is good...

At the same time, SEC suffers from limited inter-laboratory reproducibility: results are not accurate. This was recently shown in a series of round robin tests organized under auspices of IUPAC (Ref. 13). Four commercial polymers were included: polystyrenes, polyamides, polyethylenes and Na–poly(acryl amide)s, as well as oligomeric epoxy resins. Data produced

in several laboratories exhibited remarkable scatter – it ranged from 100 to 2000% for particular samples of high polymers. It is evident, that

- skill of SEC operators is often not satisfactory. This refers mainly to students who perform occasional SEC measurements in the "switch on – inject – switch off" mode
- standardization of measurements and data processing is needed. This includes for example rules for base line laying (cf. Fig.2), assessment of peak limits, as well as agreement on the maximum acceptable base line drift.

Tailored column packings as to their selectivity and compatibility with both sample and eluent should be used in exact SEC measurements. For example, some polystyrene/ divinylbenzene column packings exhibit unexpectedly high enthalpic interactivity toward polar macromolecules. Consequently, polymer retention volumes extensively increase due to adsorption if eluent is not strong enough (Refs. 14, 15). At the same time, retention volumes of the non-polar polymers in too polar eluents rise due to partition of macromolecules in favor of non-polar matrix of column packing (cf. Fig. 1b).

Further progress in SEC will include improved pumps and mainly detectors, which represent an important weak-point of all polymer HPLC methods. Widely used refractive index detectors seem to approach theoretical limits of their sensitivity. Evaporative light scattering detectors urgently need amelioration of their nebulization systems. Introduction of flow-through viscometers and light scattering photometers, as well as their combinations dramatically improved reliability of SEC data. Unfortunately, flow-through osmometers are so far commercially not available. Hyphenations SEC – mass spectrometry will substantially improve sample detection and overall data interpretation.

Apart from standardization, improved column packings, pumps, and detectors, also simple and efficient methods for band broadening corrections, as well as high speed/ high sample throughput procedures are needed in SEC. The latter issue is especially important for combinatorial material science.

New and improved SEC applications will also include procedures to reduce shearing degradation for ultra-high molar mass species, as well as advanced methods for determination of various physico-chemical parameters of macromolecules and their solutions like theta conditions, (preferential) solvation, diffusion in porous bodies, etc. Complexing (associating, aggregating and micellizing) macromolecules, as well as fast decomposing polymers will be characterized by ameliorated procedures based on exclusion retention mechanism.

SEC separates macromolecules according to their size in solution. In the case of **complex polymers,** which exhibit distribution not only in their molar mass but also in at least one further molecular characteristic - that is in their chemical structure and/or in their physical

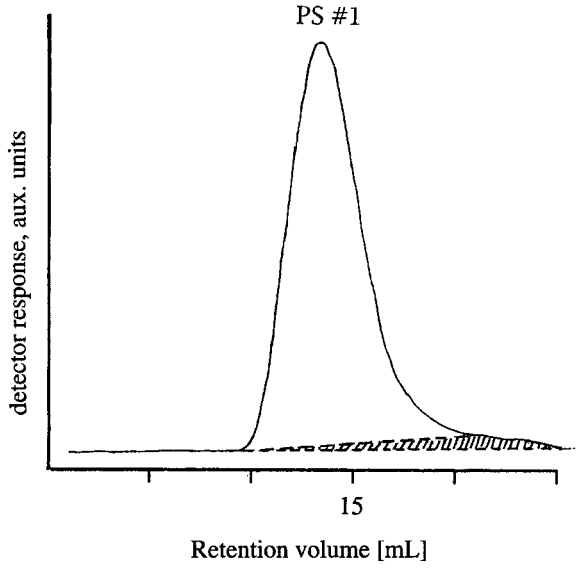


Figure 2. Chromatogram of a broad polystyrene sample, containing larger amount of low molar mass material. (Sample # 1 measured in the framework of the IUPAC round robin test, Ref.13.). Depending on the base line setting, differences in calculated number average molar mass reached 800%. Evaporative light scattering instrument was used for detection so that system peaks, peaks of air and low molecular impurities were no visible.

architecture, size of macromolecules in solution depends on **all** these characteristics. If the mutual dependence of particular characteristics is unknown, straightforward determination of molar mass distribution from SEC alone is hardly possible and iteration procedures are to be applied. For more exact molecular characterization of complex polymers, however, coupled and two-dimensional procedures should be applied (paragraphs 5 and 6).

4. Methods Dominated by Enthalpic Retention Mechanisms

As shown in paragraph 2, isocratic polymer HPLC with dominating enthalpic retention mechanism is possible only for oligomers. Several practical examples are presented for example in Ref. 1.

High polymers can be separated in prevailingly enthalpic mode using eluent gradient or temperature gradient procedures. These options will be briefly outlined in paragraph 5.

Dominating enthalpic retention mechanisms can be relatively easily applied for separation of many complex polymer systems, mainly polymer blends in the "HPLC-like" systems. Multitude of short retention-elution processes, which is typical for chromatography, is substituted by a single **full** retention and a selective but **full** elution step. One speaks about **full retention-elution (FRE) procedures.** Small HPLC-like FRE columns (down to a few microliters in volume) are packed with **nonporous**, particulate, interactive materials. Alternatively, appropriate monoliths can be used. Experimental conditions are chosen (eluent, temperature) under which entire sample or its part is fully retained within FRE column. Next, experimental conditions are abruptly changed, for example, a displacing liquid is introduced into FRE column either by eluent switching or by injection of narrow zone of appropriate displacer. As result, a part of sample is eluted. The released fraction is transported into an on-line SEC column for molecular characterization. The most appropriate retention mechanism seems to be adsorption and one speaks about **full adsorption – elution/ SEC method** (FAD/ SEC) (Ref. 16). It allows selective separation and independent SEC characterization of multicomponent polymer blends including those of chemically similar constituents (Ref. 17), as well as polymer systems containing minor ($\leq$ 1%) polymer admixtures (Ref. 18). Selective full retention procedure can be applied to remove interferences from sample. This can be done either within the separation column itself (Ref.19) or by means of an appropriate on-line full retention microcolumn.

5. Coupled Methods in Polymer HPLC

Combination, coupling of different retention mechanisms in HPLC of macromolecules is aimed either at increasing separation selectivity or at suppressing separation of macromolecules according to one molecular characteristic.

Increased selectivity of separation can be reached for example by extending V_R range, which is available for HPLC separation. Typical examples represent separations of oligomers possessing interactive functional groups, for example end groups (Refs. 20 - 22). Enthalpic interactions of macromolecules with column filling may substantially increase V_R's of the lowest members of homological series where the role of end groups is most pronounced. By contrast, shift of V_R for large macromolecules is small. The resulting elution order still corresponds to SEC but selectivity of separation increases. This principle can be used also for

improvement of selectivity of polymer separation employing adsorption (Fig. 1a, curve 3) but the effect is usually low. On the contrary, controlled combination of exclusion and partition can lead to large augmentation of separation selectivity also for polymers with high molar masses (Fig. 1b, curve 4). This behavior was recently observed in systems polystyrene or poly(n-butyl methacrylate) plus tetrahydrofuran/ dimethyl formamide mixed eluents plus silica C18 column packings (Ref. 12).

Coupling of entropic and enthalpic mechanisms is so far more frequently applied for suppressing separation according to molar mass (c.f. paragraph 2, "critical chromatography"). Full entropy – enthalpy (adsorption or/ and partition) compensation allows separation and independent characterization of binary polymer blends and to some extend also of block copolymers (Refs. 1 and 23). Recent results of Chang et al (Ref. 24) cast, however some doubt on general exactness of such measurements for block copolymers. Numerous experimental limitations of LCCC are mentioned in paragraph 2, see also Ref. 25.

Entropy – enthalpy ("critical") compensation is utilized in an original method developed by Chang (Ref. 26) and termed **temperature gradient interaction chromatography** (TGIC). In TGIC, elution of macromolecules is controlled by column temperature. TGIC allows highly selective separation of homopolymers and also some complex polymers.

LCCC principle renders very good results in separation of oligomers according to nature, amount and topology of their functional groups (Refs. 1, 27)

A special group of coupled procedures in polymer HPLC represent **barrier methods** (Ref. 28). Rapid transport of partially or fully excluded macromolecules along HPLC column is hampered by a slowly moving "impermeable barrier" of small molecules. The progression of small molecules is slowed down by their permeation through most of the filling pores. All three above discussed enthalpic retention mechanisms can be applied, namely adsorption, partition and phase separation. The low molecular barrier can be either eluent itself or a narrow zone of another appropriate liquid, for example an adsorli. In the former case, transport of macromolecules along column is rendered possible by means of a zone of displacer. The displacer zone prevents adsorption, partition, or precipitation of polymer. Macromolecules should stay within transporting zone of displacer. It is convenient if the displacer is identical with sample solvent. The resulting procedures are termed **liquid chromatography under limiting conditions** (LCLC) (Refs. 28, 29). They are in principle isocratic and utilize local eluent changes, "local gradients".

It seems that the barrier principle can also explain the overall retention mechanism in the eluent gradient polymer HPLC (EGLC) (Ref. 30). EGLC is presently the most selective

method for separation of macromolecules according to their composition (Refs. 1,7,31). It is often applied to various kinds of copolymers and polymer blends. Initial eluent promotes full polymer retention due to its adsorption, partition or precipitation. Consequently, sample is immobilized near column inlet soon after its injection. Next, eluent composition is gradually changed so that concentration of displacer increases. If the overall shape and steepness of eluent gradient is well optimized, highly selective, molar mass independent elution of many complex polymer systems can be attained. In the case of adsorption and partition, macromolecules are eluted nearly at the "critical composition" of mobile phase (Refs. 32, 33). We suppose that that in EGLC, eluent represents a barrier with continuously changing strength or/ and thermodynamic quality. Macromolecules of particular composition (and retentivity) accumulate on certain loci of barrier, irrespectively on their molar mass (Ref. 30). This process also brings about important peak focusing (Ref. 34), which results in surprisingly high both efficiency and sample capacity of short EGLC columns.

As mentioned, an important challenge for any HPLC method and for separation and characterization of complex polymer systems in particular are detectors. These must be able not only to monitor overall concentration of polymer species but also to assess relative concentration or architecture of particular constituents, or both. Though presently available infrared interfaces, mass spectrometers and NMR instruments produce valuable information their further improvement is needed. Moreover, new detection principles must be developed to improve reliability of coupled HPLC procedures.

Important problem, which limits development of reliable coupled methods of polymer HPLC represent appropriate, well defined macromolecular models.

6. Two- and Multi-Dimensional Polymer HPLC

Some coupled methods of polymer HPLC as for example LCCC, LCLC and EGLC, are designed so that effect of at least one molecular characteristic (usually molar mass of one kind of polymer chains) is suppressed. Consequently, macromolecules are separated according to molar mass of second kind of polymer chains or according to overall polymer composition (Refs. 1, 28). It is useful to on-line add further, different HPLC system to separate and characterize macromolecules according to the remaining molecular characteristics. So far only binary on-line separation steps were successfully attempted. A typical two-dimensional polymer HPLC system is schematically shown in Fig.3. If column #1 is well chosen and separates macromolecules for example according to their chemical

composition, column #2 may be a regular SEC system. Often, two different column packings and/ or two different eluents are applied. Mobile phases are delivered by two independent pumping systems, #1 and #2. Important problem for two-dimensional polymer HPLC represent sample reconcentration, storage and reinjection, as well as eluent switching. These steps are performed by means of the RSR unit (Fig. 3). Reconcentration step is needed if column #1 produces diluted fractions and sample storage is useful if column #1 has low sample capacity so that separation must be repeated and corresponding fractions combined.

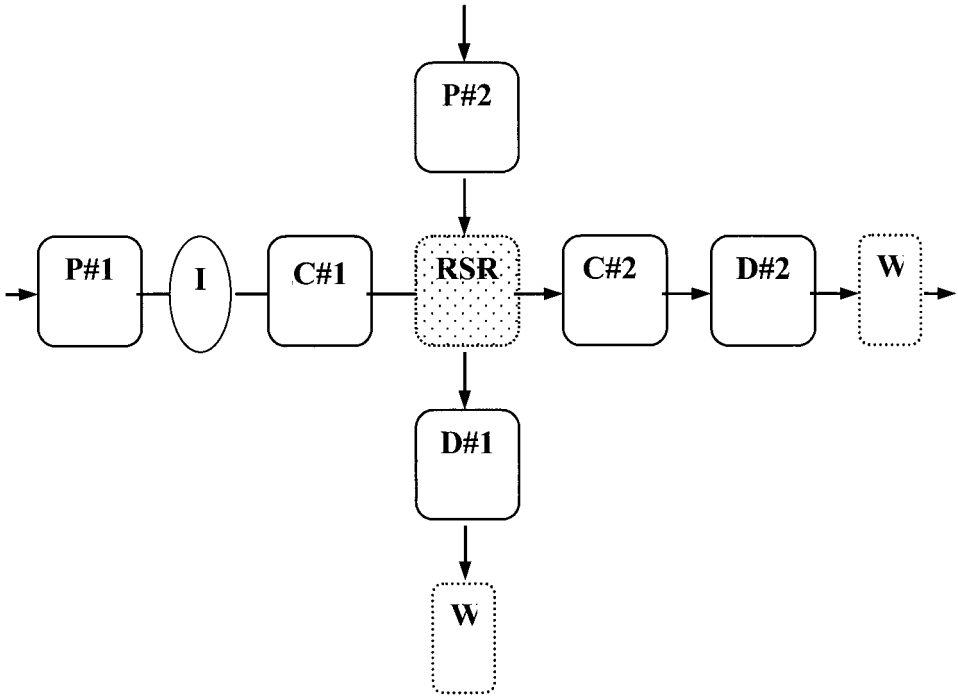


Figure 3. Scheme of a two-dimensional polymer HPLC system. **P** stands for pumps, **I** for injector, **C** for columns, **D** for detectors and **W** for waste. **RSR** device reconcentrates, stores, and reinjects fractions from column #1, and allows also their diverting into detector # 1, as well as switching mobile phase and exchanging solvent of fractions introduced into column #2. For further explanation see the text.

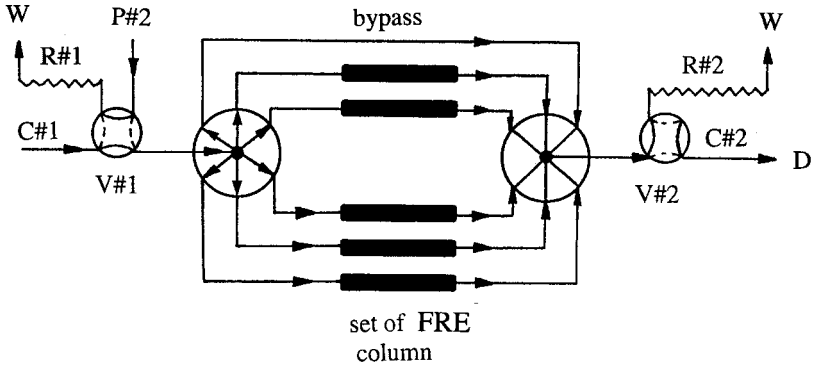


Figure 4. Schematic representation of a set of five full retention – elution columns - as an RSR system for two-dimensional polymer HPLC. For further explanation see the text.

Sample (fraction) storage also enables easier matching of elution rates in both columns. The RSR system must also allow exact identification of elution start within column #2.

Several RSR systems are discussed in Refs.1 and 35. In a simple design, they include various configurations of multi-port HPLC valves. It seems that a set of full retention – elution microcolumns will meet exacting demands for RSR operation in many two-dimensional polymer HPLC systems (Fig.4).

Similar to coupled polymer HPLC procedures, detection of macromolecules leaving a two-dimensional HPLC system is a complicated task. Information about separation within column #1 can be obtained by detector(s) #1.

Separations of complex polymer systems using three-, etc. independent HPLC systems will be in future certainly also attempted. Complexity of such procedures will, however, grow exponentially with number of separation steps.

7. Conclusions

Methods of high performance liquid chromatography in their present stage of development allow molecular characterization of many polymeric materials of sufficient solubility. This refers mainly to size exclusion chromatography, which produces precise but so far not highly accurate data on polymer species exhibiting distribution only in their molar masses and/ or long chain branching. Important progress in SEC brought multiple "absolute" detectors, viz. on line light scattering photometers and viscometers. Further development of SEC is still

needed to improve its accuracy. This includes hardware, especially pumps, columns and detectors, further standardization of both measurement and data processing procedures, as well as new applications and acceleration of analyses.

It is expected that SEC will further dominate also characterization of complex polymer systems possessing multiple distributions of their molecular characteristics, though data obtained can be considered only semi-quantitative. In spite of this, highly valuable information showing mainly tendency, failure – or success of polymer syntheses are attained.

Development of other methods of polymer HPLC is still in its initial stage. These methods combine entropic (exclusion) and enthalpic retention mechanisms either within one HPLC system (coupled HPLC procedures) or within two or several different HPLC systems (two- and multi-dimensional HPLC). Important drawback of all these combined methods is a necessity to identify optimum experimental conditions for each polymer analyzed. The corresponding search is so far largely empirical because understanding of enthalpic retention mechanisms is so far insufficient. Moreover, most of coupled and two-dimensional HPLC HPLC methods are highly sensitive toward minute changes of experimental conditions. As result, both coupled and two-dimensional polymer HPLC methods are so far non-universal, as well as time and work intensive. Detection sensitivity and selectivity is to be improved for most complex polymeric materials of interest.

Hyphenation of polymer HPLC with mass spectrometry, NMR and other spectrometric, as well as separation procedures will further extend applicability of methods and bring further substantial improvement in both data precision and accuracy. Significant progress may imply polymer analysis HPLC on chips.

8. Terminological Notes

The term **high performance liquid chromatography** (HPLC) includes all chromatographic methods in which mobile phase is a **liquid.** Size exclusion chromatography (SEC) in its modern, high performance form belongs among HPLC methods. It is not adequate to speak about HPLC **and** SEC procedures.

Entropic partition of solute molecules takes place between chemically identical liquid phases. One of them, the quasi – stationary phase is situated within pores of the column filling, on the surface of column packing particles or on the surface of flow – through channels in monoliths. Another phase is eluent among particles or in channels of monoliths.

Enthalpic partition of solute molecules takes place between two chemically different liquid phases. One of them is usually a **bonded** phase while another one is interstitial (mobile) phase in a packing or eluent in channels of monoliths.

Enthalpic retention mechanisms can be largely and sometimes even completely suppressed in SEC. One speaks about **ideal SEC.** On the other hand, large entropic (exclusion) effects accompany **all** HPLC separations of macromolecules – even those dominated by enthalpic retention mechanisms. Therefore the term "non-exclusion polymer HPLC" is inappropriate.

In various areas of analytical chemistry, the term **hyphenated methods** describes combinations of different physico-chemical principles while the term **coupling** is reserved for direct combinations of similar or related physico-chemical principles. Accordingly, one can speak about hyphenation of polymer HPLC and mass spectrometry or about hyphenated HPLC detection, etc. - and about coupling of HPLC separation mechanisms.

Column filling is a general term, which includes any kind of stationary phase or its carrier within HPLC column that is either hard or soft, particulate porous or nonporous **column packing,** or a **monolit.** Polymer HPLC is dominated by columns filled with porous particulate packings though nonporous beads and monoliths were successfully applied in numerous fast separations of macromolecules.

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