Strategies in Two-Dimensional Liquid Chromatographic Separation of Complex Polymer Systems

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SUMMARY: Complex synthetic polymer systems as for example copolymers exhibit distributions in at least two of the three basic molecular characteristics which are molar mass, chemical structure/composition and molecular architecture. Size exclusion chromatography (SEC) separates macromolecules according to their size in solution which simultaneously depends on all molecular characteristics. Therefore, multi-dimensional liquid chromatographic techniques are to be applied to independently assess all different distributions present in the sample. So far, two-dimensional separations have been attempted. In the first dimension separation column, selected liquid chromatographic mechanisms are intentionally combined to suppress effects of all but one molecular characteristic. Consequently, polymer species are separated exclusively or at least predominantly according to one single parameter. In the second dimension separation column, macromolecules are separated according to another molecular characteristic.

In this contribution the methods are briefly reviewed in which effect of polymer molar mass on polymer retention is suppressed. The resulting "one parameter separation systems" can be on-line or off-line connected to another separation system such as SEC to provide more detailed characterization of complex polymers. Besides, selected procedures for the re-concentration of diluted polymer solutions are concisely treated. These may be utilized for increasing the concentration of sample(s) leaving the first dimension separation column. Eventually, some arrangements for controlled sample re-introduction into the second dimension separation column are outlined.

Content

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1. Introduction

All presently available synthetic polymers exhibit non-uniform molar masses (MM). For the large majority of linear homopolymers which form a basis for numerous commodity plastics and rubbers, MM and its distribution can be reliably assessed by size exclusion chromatography (SEC). Many modern high performance polymers, however, possess also non-uniform chemical composition (CC) and/or molecular architecture (MA). In other words, all three basic molecular parameters assume particular distributions, MMD, CCD and MAD which can be described by specific distribution functions.

Besides above three basic molecular characteristics, several secondary molecular parameters can be defined. For example long-chain branched macromolecules may exhibit differences not only in the frequency and length of branches but also in their chemical composition or stereoregularity, etc.

Materials possessing more than one distribution of their molecular characteristics are called complex polymer systems. These are for example polymer blends, various kinds of copolymers, sequenced and functionalized polymers.

To determine the distributions in their molecular characteristics macromolecules must be separated. Presently, this is done mainly by the high performance liquid chromatography (HPLC) and mass spectrometry (MS), especially by matrix assisted laser desorption ionization time of flight MS.

HPLC separation of high polymers is dominated by size exclusion chromatography (SEC). SEC is based on entropic separation mechanism and discriminates macromolecules according to their size in solution. Therefore SEC can produce quantitative information on more than one distribution only if the functional dependence between measured parameters is known. Alternatively, approximative assumptions are done to receive at least semiquantitative data. The effective mean molar masses (MMM) and MMD of complex polymers are very often calculated directly from the SEC chromatograms applying the polystyrene based calibration dependences. Important quantitative conclusions are drawn from such data though they are in fact good only for the first-degree estimations such as “high vs. medium vs. low mean molar mass” or “narrower vs. broader or unimodal vs. bimodal molar mass distribution”. Often even bimodality in the molar mass distributions of complex polymers are overlooked though they are clearly signalized by the specific shape of SEC chromatograms.

To obtain reliable data on MMD, CCD and MAD of complex polymer systems, exclusion separation mechanism must be accomplished with further liquid chromatographic separation mechanisms. These additional HPLC mechanisms are based on enthalpic interactions of

macromolecules with column packing and/or with eluent. As a rule, macromolecules are separated in two or more basically independent separation steps using two or several different HPLC systems (columns, eluents or temperatures). These procedures are called two-, three- and multidimensional liquid chromatography of complex polymer systems. So far, practically only two-dimensional HPLC (2D-HPLC or 2D-LC) was successfully attempted and we shall discuss the corresponding approaches in this short review.

2. Two-dimensional high performance liquid chromatographic separation of macromolecules

The schematic representation of an on-line two-dimensional HPLC of polymers is shown in Figure 1. Sample is introduced in the conventional way into the first HPLC column "I" and separation is accomplished in the second HPLC column "II".

There are several general problems to be solved in the 2D-HPLC procedures:

i) the separation in the column #I must be performed predominantly according to one single molecular characteristic. The role of second characteristic must be suppressed. The more effective such suppression the easier are both the separation in the column #II and the corresponding data interpretation. Precision of the final results is also improved.

ii) sample fractions leaving column #I are fairly diluted and the reconcentration step "A" must be often introduced into the on-line working 2D HPLC chromatograph to allow a reliable sample detection.

iii) fractions leaving column #I must be introduced into column #II in the well defined way so that their retention volumes in the second dimension of separation can be precisely identified.

iv) sensitive and selective sample detection must be accomplished.

We shall briefly treat the first three items, i) to iii).

The 2D-HPLC polymer separation can be done also in the off line arrangement. Fractions from the HPLC column #I are collected, if necessary reconcentrated in bulk, and reinjected into HPLC column #II. This approach is material and labor consuming and has little chance to be widely applied. As to separation mechanism(s), the same general principles are valid for both off-line and on-line arrangements.

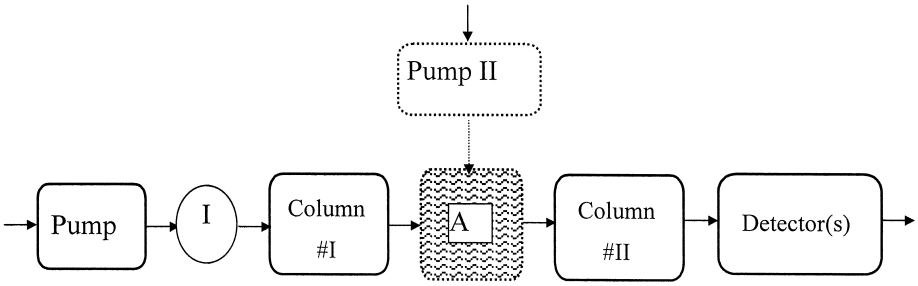


Figure 1. Schematic representation of a two dimensional HPLC system. I is sample injector. A is the sample storing, re-injection and reconcentration device which can be also used for sample matrix/eluent exchange. Pump II and the device A can be abandoned in some 2D-HPLC systems (see the following paragraphs).

2.1. Coupled procedures in HPLC of polymers

One of the basic problems of two-dimensional HPLC of complex polymers represents the first separation step in which macromolecules are separated predominantly or exclusively according to one characteristic and the effects of remaining characteristic(s) is suppressed (see paragraph the 2/i). With a few exemptions, this is done by controlled combination of appropriate separation mechanisms within one single column or column system that is by **coupling** HPLC separation mechanisms.

One of the first attempts toward two-dimensional HPLC of complex polymers was reported by Balke (Refs. 1,2). He called his approach "orthogonal" HPLC of polymers. Various acrylic and methacrylic copolymers were treated. Balke forwarded fractions from a stop-and-go (interrupted flow) operated "ideal" SEC system in which polymer retention volumes were not affected by any enthalpic interaction into an enthalpic interactions enforced second SEC system. The selectivity of such 2D-HPLC separations was remarkably high but, evidently, quantitative interpretation of data was difficult because the retention volumes in both HPLC systems were simultaneously affected with molar mass, chemical composition and possibly also by architecture (blockines) of copolymers.

In his excellent book, Glöckner (Ref. 3) reviewed several approaches to 2D cross-fractionation of macromolecules especially those in which the sample retention was controlled mainly with phase separation processes that is by polymer-solvent and polymer-polymer interactions. In this case, however, polymer retention was often affected also with

interactions between sample molecules and column packing. Consequently, macromolecules might be separated according to more than one characteristic.

A chance for coupling the HPLC separation mechanisms to suppress the effect of polymer molar mass can be evidenced by the following simplified consideration.

Retention volume V_R of a sample in any kind of HPLC separation is a function of its distribution constant **K. K** is expressed as a ratio of sample concentration in the (quasi)stationary phase C_S and in the interstitional mobile phase C_M. In SEC, the quasi stationary phase is represented by the pore volume and also by the outer surface of column packing particles from which macromolecules are partially or fully excluded. The stationary phase in the interactive (enthalpic) HPLC mainly includes outer and inner column packing surface on which the analyte adsorption or phase separation takes place. Alternatively, it is the chemically bonded phase or the quasi- stagnant phase formed by solvent molecules which is available for partition of analytes.

One can write equation 1

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

where ΔG is the Gibbs function, ΔS and ΔH are the changes in entropy and enthalpy of sample molecules transferred from mobile into (quasi) stationary phase and vice versa. R is the gas constant and T is temperature. For the macromolecular samples, both ΔS and ΔH are large. ΔS results from conformational (Ref. 4) and possibly also from orientational (Ref. 5) changes of macromolecules confined in the pores or excluded from the packing surface. ΔH of macromolecules is a sum of the enthalpic interactions of their segments with the column packing.

For entropy driven HPLC systems, typically SEC or hydrodynamic chromatography, retention volumes **increase** with **decreasing** sample molar mass (M). At the same time, retention volumes **increase** with **increasing** M of sample if the prevailing separation mechanism is based on enthalpic interactions. The appropriate coupling of exclusion and (enthalpic) interactions may lead to their **mutual compensation** which, in turn, results in the situation when retention volumes of macromolecules with given chemical structure and architecture **do not depend on the sample molar mass**. Of the interaction mechanisms, adsorption and partition are mainly used for the compensation type couplings. In both cases, the extent of enthalpic interactions between macromolecules and column packing is controlled by the

eluent nature and temperature. Adsorption of macromolecules on the given packing surface depends on the **strength** of interaction between eluent and adsorbent. We have **strong** and **weak** eluents. Strong eluents exhibit high affinity toward column packing and compete with polymer segments for the interactive sites within packing. The terms strong and weak solvent are, however, relative only. Eluent of a given strength may promote desorption of one kind of macromolecules (a desorli eluent) but, at the same time, it can allow adsorption of another kind of macromolecules (an adsorli eluent). Partition of macromolecules between mobile and stationary (bonded) phase depends not only on the solvent strength but also on its **thermodynamic quality** for dissolved macromolecules. Considering the latter parameter, we have **good** and **poor** solvents, as well as **nonsolvents** for a given polymeric sample. The relations between solvent strength and solvent quality are rather complex, also because the thermodynamic partition of macromolecules is often accompanied by adsorption. To achieve an efficient sample partition, eluent must be a thermodynamically (very) poor solvent for macromolecules. As result, another enthalpic mechanism may be present, namely phase separation (polymer precipitation), which further complicates the retention control.

The coupled HPLC techniques for molar mass independent retention of macromolecules were extensively discussed in the recent review (Ref. 6) and we shall present here only the basic ideas. Sample adsorption will be considered as the model enthalpic interactive mechanism because it is not too much complicated by presence of other enthalpic interactions mentioned above, viz. partition and phase separation. On the other hand, partition generally represents a milder enthalpic separation mechanism than adsorption and can be easier finely tuned.

The coupling of separation mechanisms can be done either under **isocratic** or under continuous/stepwise/local eluent **gradient** conditions and further at a constant temperature or applying a temperature gradient. The overall retention is easier to control when eluent composition and temperature affect preferably or exclusively only one separation mechanism. We shall discuss the isothermic approaches to the coupling of separation mechanisms.

2.1.1. Isocratic compensation techniques

(Inter)active column packing is applied under isocratic and isothermic conditions. Eluent properties and temperature are adjusted in such a way that enthalpic interactions and exclusion just compensate (see the paragraph 2.1). We speak about "liquid chromatography under critical conditions" (LCCC) or, when adsorption is the leading enthalpic mechanism, about LC at the critical adsorption point (LC CAP). It should be noted that these critical conditions have nothing common with supercritical state and supercritical liquid

chromatography – though as shown by Yun, Olesik, and Marti (Ref. 7) chromatographic critical conditions can be achieved also in supercritical eluents.

LCCC method was proposed in Sankt Petersburg in seventies of the last century. Belenkii, Gankina and Tennikov (Refs. 8,9) made basic experimental observations in both TLC and column LC arrangement and proposed plausible explanation of molar mass independent retention under "critical" conditions. Gorbunov and Skvortsov (Refs. 10,11) theoretically elucidated behavior of macromolecules at critical conditions and outlined some practical applications of this phenomenon. Several other authors utilized LCCC method to separate complex oligomers and polymers [for reviews see (Refs. 12,13)]. In the first approximation we can say that macromolecular chains which elute at critical conditions are "chromatographically invisible" because they elute at constant retention volume which is roughly equal to the total volume of liquid within column. At the same time, functional groups in oligomers (Ref. 12) and/or chemically or physically different chains or their parts in polymer blends (Ref. 13), block- (Ref. 13) and graft- (Ref. 14) copolymers or in stereoregular polymers (Ref. 15) are eluted under non-critical conditions and their characteristics can often be independently assessed in conventional manner. LCCC can be on-line accomplished with a second dimension LC separation, for example with an SEC characterization of the chains which were "invisible" in the LCCC step (Refs. 13,16). Alternatively, polymers which possess both MMD and tacticity distribution can be first SEC separated according to molar mass irrespectively from their tacticity and obtained fractions are subject to LC CAP discrimination according to stereoregularity (Ref. 17).

LCCC represents a challenging method but its wide application is hampered with several drawbacks, which must be explained, suppressed – or corrected for (Refs. 18,19):

- LCCC may produce broadened, ill shaped or even split peaks
- LCCC sample recovery may decrease with the increasing sample molar mass especially if macromolecules are excluded from the packing pores. This drawback is mitigated when narrow pore column packings are abandoned. In this latter case, however, selectivity of the SEC separation is sacrified for nonadsorbed species in the lower MM region
- repeatability of measurements is often limited due to high sensitivity of many LCCC systems toward minute variations in the eluent composition (e.g. due to humidity absorption and preferential evaporation from the eluent container), further in the column temperature (e.g. due to viscous heat dissipation), and in the packing surface properties (e.g. due to irreversible adsorption of some sample components, and various impurities in the course of previous analyses)

- the interactivity of one part of polymer chain can be affected with presence of the non-interactive polymer segments, for example by the non-adsorbed blocks in a block copolymer (Ref. 20). As result the interacting chain may lose its critical behavior and become "chromatographically visible"
- applying Monte Carlo model calculation Cifra and Bleha (Ref. 5) introduced some doubts concerning the polymer molar mass range for which the full ΔS and ΔH compensation and molar mass independent retention can be obtained
- system peaks appear on the LCCC chromatograms monitored by non-specific detectors. They are caused mainly by preferential solvation of dissolved macromolecules with one component of mixed eluent (Ref. 21). Sample peaks complicate solute detection. In the LCCC of copolymers, solvated macromolecules are successively separated from their initial solvent which composition was altered due to preferential solvation. As result, polymer retention may change in the course of experiment. This drawback can be mitigated by inserting a narrow pore non-interactive SEC column between the sample injector and the LCCC column. This additional SEC column separates solvated macromolecules from their initial ("micro-dialysed") solvent so that polymer sample travels along the LCCC column all the time surrounded with the original (mixed) eluent. This allows easier and more precise sample retention control. The latter procedure is termed **LC at the theta exclusion-adsorption.** (Ref. 22)

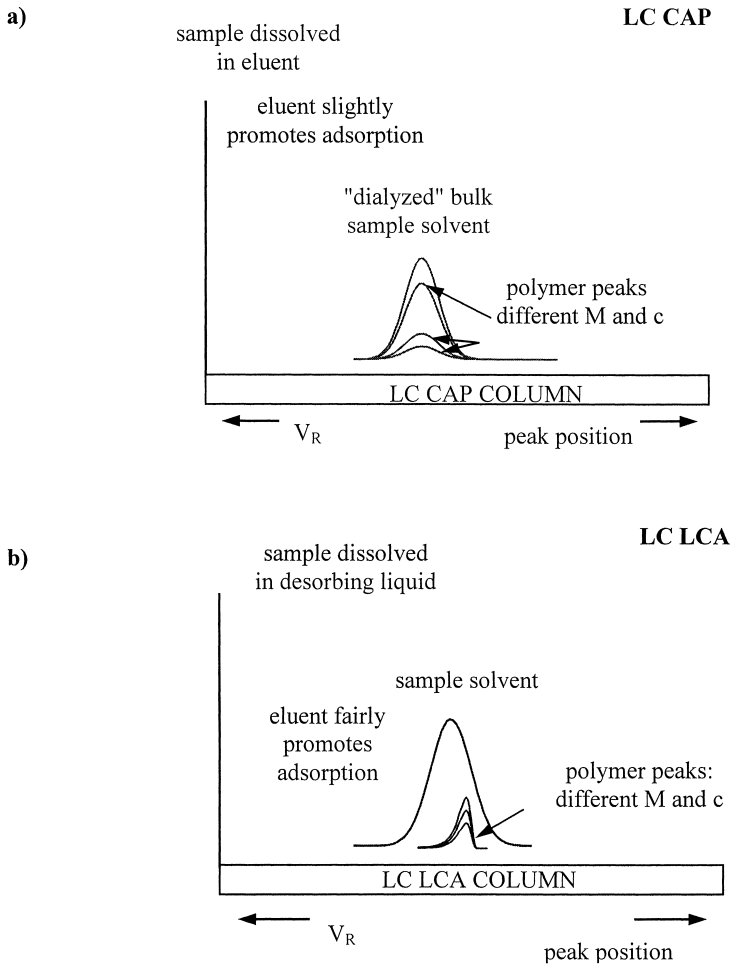
2.1.2. Local gradient compensation techniques

The sample elution is generally isocratic but local zones within eluent are created by introducing pulses of liquids with the adsorption strength different from that of eluent. These procedures are also called "the barrier methods".

LC under limiting condition of adsorption (LC LCA) (Refs. 23,24). Eluent rather strongly promotes adsorption so that macromolecules would be retained within the column if injected in eluent. Instead, the sample is dissolved and injected in a liquid which promotes its desorption (a desorli). Due to their exclusion, macromolecules tend to move faster along column than small molecules of their initial solvent. However, polymer species cannot leave the desorli zone because otherwise they would be retained by adsorption. In this way, eluent creates a continuous "impermeable barrier" for the polymer sample which accumulates near the front of sample solvent zone. Consequently, macromolecules of all sizes elute within the zone of their initial solvent.

LC under limiting conditions of desorption (LC LCD) (Ref. 25). Eluent prevents sample adsorption. Sample is dissolved and injected in an adsorption promoting liquid (adsorli). Here again, macromolecules are accelerated by their exclusion. However, the initial sample solvent forms a local "impermeable barrier" which cannot be surmounted by macromolecules. As result, macromolecules with different MM are eluted just behind their original solvent adsorli barrier.

The principles of both isocratic and local gradient compensation methods are schematically depicted in Figure 2. It should be noted that both LC LCA and LC LCD can produce compressed (focused) narrow polymer peaks. This is advantageous for the two-dimensional liquid chromatography of macromolecules.



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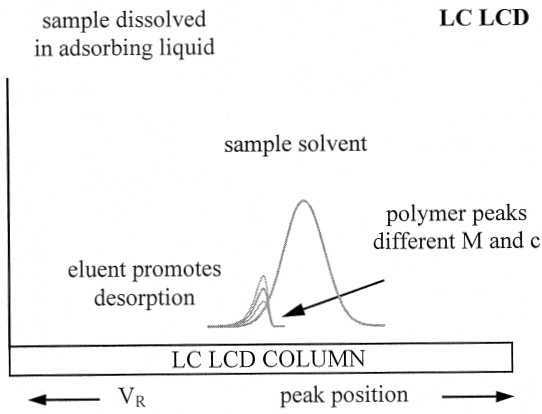


Figure 2. Schematic representation of the isocratic and local gradient compensation approaches. The simultaneous effect of partial or total exclusion of macromolecules from the column packing pores and their adsorption is a prerequisite for the molar mass independent polymer elution.

a) Liquid chromatography at the critical adsorption point (LC CAP). System peak reflects the excess of one sample solvent component apart of polymer coils that is in the bulk volume of solvent. The sign of system peak depends on the measured property of eluent component, which was extracted by macromolecules due preferential solvation. System peak is separated from the peak of macromolecules in SEC (Ref. 21). In contrast, macromolecules travel together with their bulk solvent in the case of LC CAP.

b) Liquid chromatography under limiting conditions of adsorption (LC LCA). Macromolecules travel within the zone of their initial solvent which prevents their adsorption. Eluent represents the barrier which is impermeable to macromolecules.

c) Liquid chromatography under limiting conditions of desorption (LC LCD). Macromolecules travel just behind the zone of their initial solvent which promotes their adsorption and thus forms an "impermeable barrier".

Notice the opposite direction of the peak position within column and the retention volume. For detailed explanation see the text.

The local gradient compensation methods are more robust than the "critical" liquid chromatography (Ref. 25). The experimental conditions can be varied in the relatively broad range. Still, it must be kept in mind that the desorli zone in the LC LCA and the adsorli zone

in the LC LCD procedures are broadened and diluted in the course of their passage along the LC column. Consequently, it may happen that the desorli zone in LC LCA is no more able to prevent polymer adsorption and, on the contrary, the adsorli zone in LC LCD loses its barrier properties. The latter situation opens an interesting possibility to combine the LC LCD and SEC elution in the course of one single elution experiment provided the adsorli zone efficiency can be effectively controlled. Both LC LCA and LC LCD can be performed also in the pulsed arrangement. In this case a series of zones with different desorbing or adsorbing strengths is produced during the same chromatographic run.

2.1.3. Continuous gradient compensation techniques

An alternative to the isocratic and local gradient compensation method represents continuous **eluent gradient polymer liquid chromatography** (EGPLC) which is also called gradient polymer elution chromatography™ (Trade Mark of Waters Company) (Ref. 26). The basis of EGPLC was laid by the group of Inagaki (ref. 27) who used the TLC arrangement with adsorption as the separation mechanism. Teramachi (Ref. 28) applied as first the column liquid chromatographic elution and Glöckner (Ref. 3) pioneered the phase separation approach.

EGPLC represents a very important approach to analysis of complex polymers. So far, two modes of EGPLC have been elaborated. In both cases, macromolecules are injected into a retaining liquid (a retainer) which prevents their elution. It may be e.g. an adsorli or a nonsolvent. Respectively, sample is either adsorbed or precipitated near the column inlet and stops moving. Subsequently, an eluent gradient is applied with increasing concentration of the component (a displacer or a releaser) which promotes sample elution due to its desorption, or partition in favor of eluent, or redissolution. In the former case we have a continuous **eluent gradient liquid adsorption chromatography** (EGLAC) (Ref. 29) (Figure 3) while we speak about **high performance precipitation LC** (Ref. 3) or about precipitation – redissolution LC in the latter case. Unwanted and non-controlled adsorption and partition effects, as well as slow process of redissolution (Ref. 30) may complicate quantitative evaluation of results in the precipitation – redissolution LC. On the other hand, separation selectivity of this approach is high if well chosen solvent/nonsolvent systems are applied. The important drawback of precipitation – redissolution LC of macromolecules is the dependence of retention volumes on more than one single molecular characteristic.

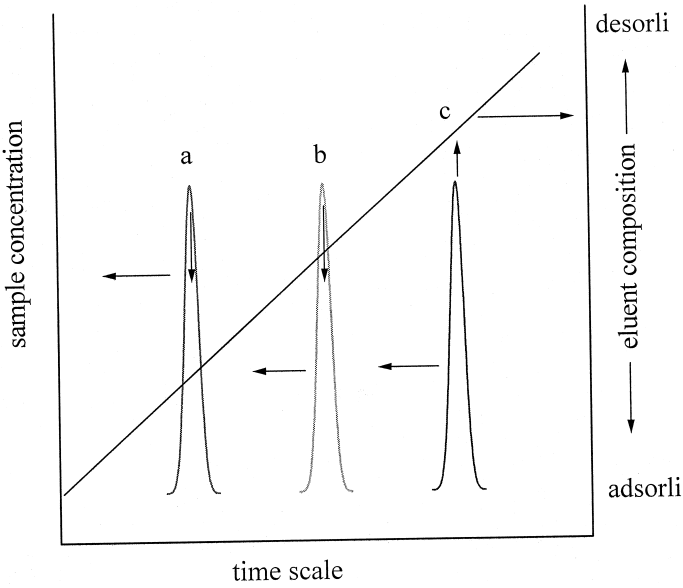


Figure 3. Schematic representation of the principle. a, b and c are the statistical copolymers with different CC and narrow CCD. Each peak contains species with different molar masses (broad MMD). For the sake of simplicity, linear shape of the eluent gradient is considered. Macromolecules are excluded from the packing pores and have a tendency to move faster along column than the eluent gradient. Each copolymer composition elutes at the gradient composition which is no more permeable that is near the critical adsorption point in the case of adsorption and partition based enthalpic retention mechanism. Sample elution near the phase separation onset takes place in the case of precipitation – redissolution HPLC. The situation may be, however, complicated with adsorption/partition processes and therefore molar mass independent retention may be deteriorated.

On the contrary, adsorption and partition based EGPLC often produces molar mass independent retention volumes for homo- and copolymers. This makes it an important partner for the 2D-HPLC of macromolecules. Brun (Refs. 31,32) explains the molar mass independent retention in adsorption and partition based EGPLC applying analogy with the LCCC. Macromolecules of homopolymers, statistical and graft copolymers with given chemical composition and interactivity are eluted exactly at the eluent composition which

corresponds to their critical point and therefore their retention volumes do not depend on their molar mass.

Alternatively, the molar mass independent retention in EGPLC can be explained considering similarity between EGLAC and LC LCD (Ref. 33). In both cases, macromolecules are accelerated by their exclusion and they tend moving faster than do eluent molecules which freely penetrate even the narrowest pores of the HPLC column packing. However, polymer species cannot surmont the eluent "barrier" – a particular eluent composition within continuous gradient which promotes their retention. As result, the (co)polymer molecules with particular interactivities accumulate within the gradient locus near the critical point which just allows their elution. Eventually, macromolecules leave the column independently of their molar mass – provided the pores of column packing are narrow enough to effectively exclude polymer species (cf. Figure 2).

An important feature of EGPLC is the fact that already short columns may afford selective separations so that the peak broadening does not represent a serious problem. Moreover, peak compression (focusing) processes are operative (Refs. 6,34) in EGPLC systems and the column sample capacity is usually surprisingly high. This often enables to skip the sample reconcentration when applying EGPLC as the first separation step.

Certainly, separation of macromolecules by means of eluent gradient elution will take place also without compensation processes (Ref. 35). Typically, this is the case for oligomers. On the other hand, EGPLC of high polymers on non-porous column packings seems not to be efficient enough (Ref. 36).

Similarly to the isocratic LC procedures, also EGPLC can be relatively easily on-line or off-line combined with the size exclusion chromatography so that we arrive at a two-dimensional separation procedure, as well.

2.2. Sample reconcentration

As mentioned in paragraph 2/ii fractions leaving column #I are often rather diluted and must be reconcentrated. In the off-line arrangement, eluent is evaporated from each fraction collected. This is a time consuming work. In the on-line systems, alternative approaches must be seeked for. An elegant procedure would involve the vacuum induced pervaporation of eluent using narrow-bore capillaries with walls permeable to small eluent molecules but not for macromolecules.

Of the chromatographic reconcentration methods, LC LCA and LC LCD procedures exhibit some potential thanks to the polymer zone focusing processes which are operative especially in the very narrow pore column packings.

A prospective sample reconcentration procedures represent those based on the full sample retention and following quantitative elution using a small-to-micro-sized devices to prevent excessive mixing of fractions. They can be termed full retention-elution methods.

These are in principle non-chromatographic or chromatography-like procedures which resemble solid phase extraction of small molecules. The basic difference is, however, rendered by the possibility to quantitatively, and under given experimental conditions irreversibly trap polymeric samples within an appropriate "mini-column" so that no break-through is observed. Next, experimental conditions (usually eluent composition) are abruptly changed and whole sample, or its specific part is quantitatively released. All three above mentioned enthalpic mechanisms can be utilized in full retention-elution procedures but it seems that the highest potential exhibits adsorption and desorption combination. In this case we speak about

2.2.1. Full adsorption-desorption approach

Full adsorption-desorption (FAD) procedure was successfully tested with various medium– and highly-polar polymers using nonporous silica as the FAD column packing (for review see Refs. 37,38). Samples were displaced from the FAD column in the form of narrow zones by the eluent switching (adsorli to desorli) or by the short pulse of desorli. Applying optimized adsorli/desorli system, FAD column was able to separate multicomponent polymer blends in the wide range of molar masses of blend constituents (Ref. 39). Also some minor macromolecular admixtures (<1%) could be quantitatively discriminated from the major components before their further analysis (Ref. 40). FAD column can be directly connected to the SEC column thus representing first and independent step in the "quasi" 2D-HPLC (see paragraph 3).

Full retention – elution procedure can be also applied to effective reconcentration of diluted polymer solutions (Refs. 41,42). In the 2D-HPLC of macromolecules, fractions leaving column #I can be treated in this way (for schematic representation see Figure 4). Reconcentration factor as high as 600 was easily obtained applying optimized FAD system (Ref. 42).

FAD allows also both sample storing and sample matrix/eluent change. Sample storing is important if the second dimension of separation - usually SEC - is too slow to match the

sample throughput within column #I. The sample solvent/eluent change may become necessary if eluent used in the column #I is incompatible with the column #II and/or the detector(s). The varying eluent composition in gradient methods may cause important problems in the second separation step, as well as in the sample detection e.g. by viscometry or by the light scattering measurement.

Full retention-elution procedures may help solving also the problem of controlled sample re-introduction into the column #II (see paragraphs 2/iii, and 2.3). An example of the five FAD column set used for sample storing and reconcentration, as well as for eluent exchange is show in Figure 4.

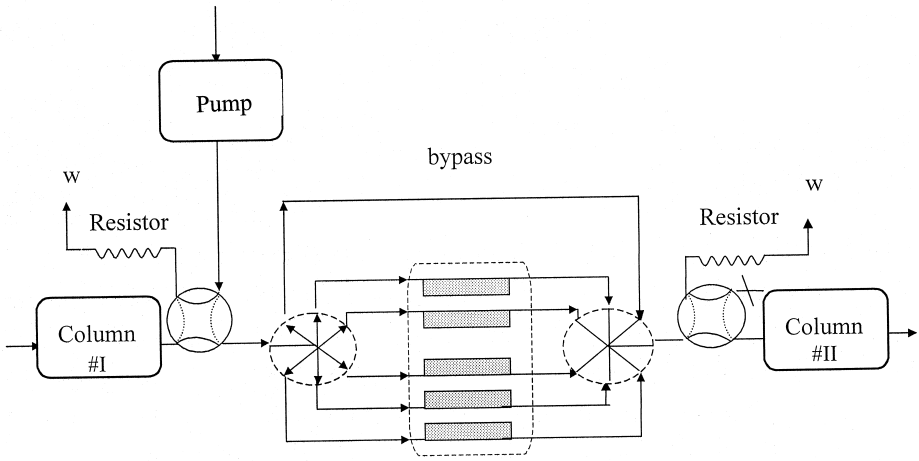


Figure 4. Sample storing, reconcentration and matrix/eluent exchange using a series of the full adsorption – desorption columns.

There exists both striking similarity and difference between FAD and EGLAC. In both cases the sample is initially fully retained within column packing by adsorption and, subsequently, its constituents are successively released and eluted. The displacer composition which is needed for desorption of macromolecules from a given FAD column depends on polymer adsorptivity that is on both its molar mass and chemical composition. As result, macromolecules of different molar masses and compositions may jointly leave the FAD column. Therefore the full adsorption – desorption procedure is hardly suitable for effective separation of copolymers built of monomeric units which exhibit similar affinity toward the FAD column packing (Refs. 43,44). On the contrary, retention of high molar mass

copolymers is often independent of their molar mass in eluent gradient polymer liquid chromatography (cf. paragraph 2.1.3) and therefore EGLAC separation of copolymers exclusively according to their composition is very attractive. Further, many times repeated adsorption-desorption processes render to EGLAC much higher separation selectivity than that attained in FAD. It seems that the initial stage of displacement of macromolecules near the EGLAC column inlet represents an important pre-fractionation step similar to FAD processes. In the course of further EGLAC elution, macromolecules with different molar masses rearrange their elution order to accumulate within particular eluent segment near its critical composition (cf. paragraph 2.1.3). As result the polymer zones are compressed and the resulting peaks are focused. These latter processes resemble those present in the LC LCD column. The above elution rearrangements of macromolecules need a certain minimum column length. Below this limit, EGLAC separation is ineffective because the molar mass and chemical composition interfere similarly to FAD. On the contrary, above this minimum column length, no rearrangement/accumulation takes place any more and a longer EGLAC column only contributes to the peak broadening due to diffusion and mixing processes. Below the minimum column length, FAD can be considered EGLAC with a very steep, local or continuous gradient.

2.3. Sample re-introduction

The direct connection between the columns #I and #II is evidently the simplest way to introduce sample into second dimension separation column in an on-line 2D HPLC. Unfortunately, the repeatability of sample retention volumes in coupled HPLC methods may be generally rather limited. Therefore it is often necessary to reintroduce the sample that is the effluent from the column #I into the column #II in a better controlled way (see paragraph 2/iii). Various arrangements are possible. A defined effluent segment from the column #I can be forwarded into column #II via a simple four port two way valve (Figure 5).

In the same way, eluent the column #II can be exchanged. Column #I can work in the stop-and-go mode because zone broadening due to diffusion in liquids confined within packed bed is surprisingly low (Ref. 45). Some possibilities for sample re-introduction into the column #II without interruption of elution in the column #I are depicted in Figure 6a-c. Their operation model is evident from the schemes. The arrangements in Fig. 6 a, b utilize two loops in connection with one two-way eight-port valve (Ref. 13) or with two six-port valves, respectively. While one loop is filled by effluent from the column #I, content of the second loop is injected into the column #II. The sizes of both columns and their elution rates must be

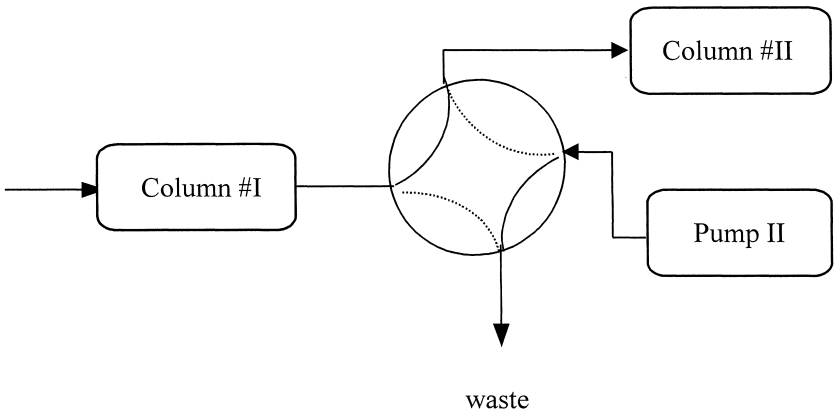
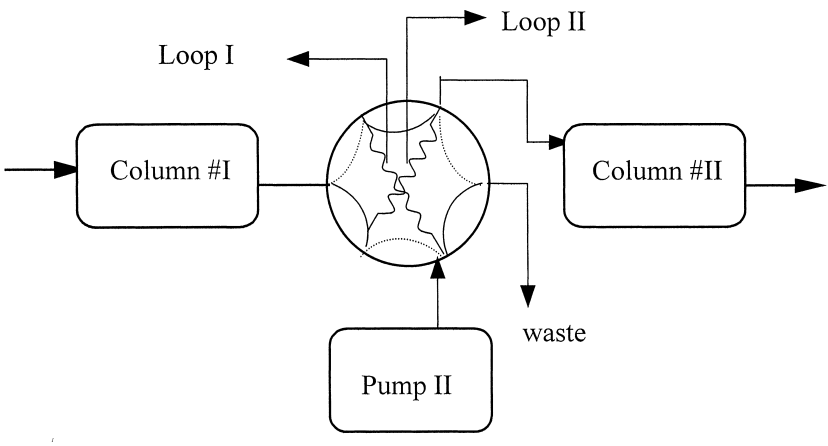


Figure 5. Sample (fractions from the column #1) reintroduction into column #2. Simultaneously, eluent is exchanged. Column #1 works in the stop-and-go regime.

exactly matched. If this is inconvenient or even impossible, the arrangement shown in Fig. 6c with several loops can be used. The column #I effluent can be stored in these loops. If the loops in the latter arrangement are substituted by a set of FAD columns, fractions leaving column #I can be also reconcentrated (cf. paragraph 2.2.1 and Figure 4). In this case, sample separation in the column #I can be repeated and corresponding fractions can be combined within FAD columns to increase amount of sample re-injected into column #II.

a)



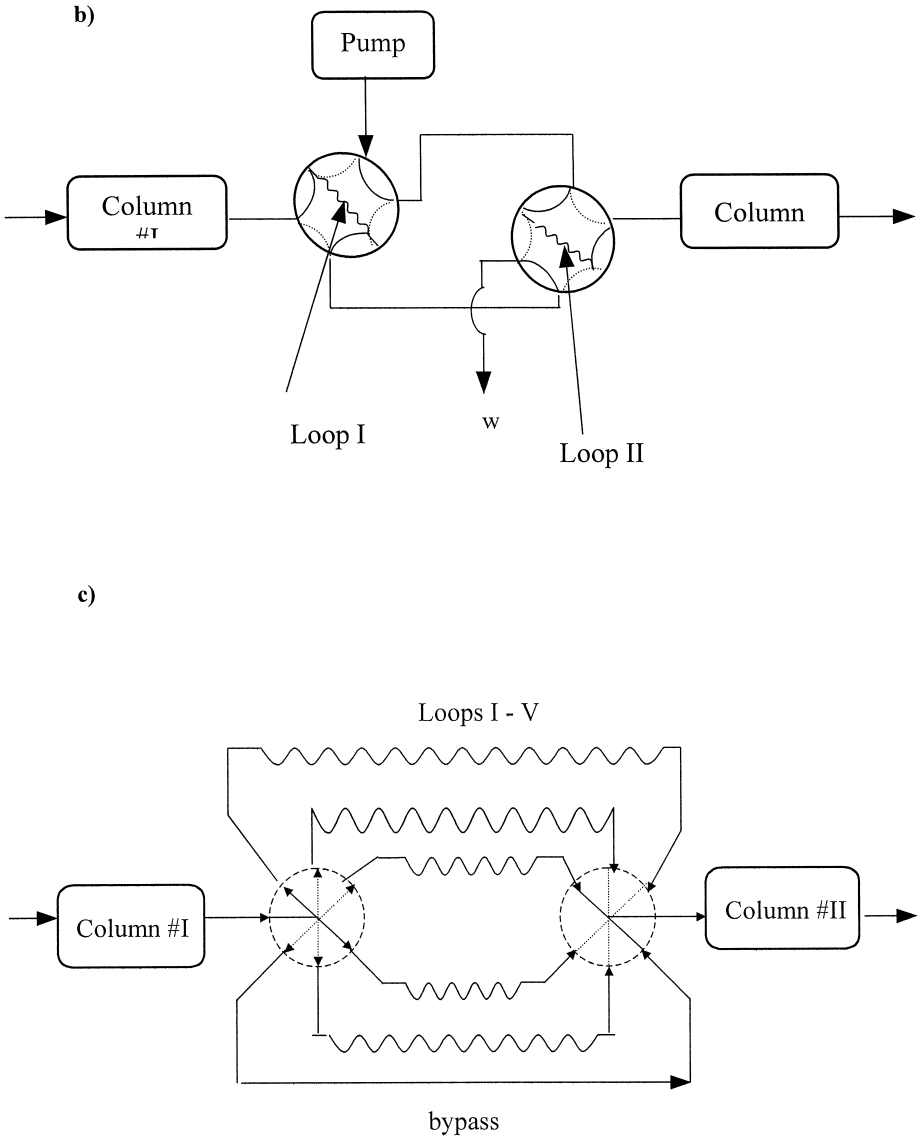


Figure 6. Sample reintroduction in 2D-HPLC of polymers:
a) Two-loop system utilizing one eight-port two-way valve.
b) Two-loop-system utilizing two six-port two-way valves.
c) Multi-loop-system utilizing two multi-port valves.

3. Quasi Two-dimensional liquid chromatographic separation of macromolecules

In the quasi two-dimensional liquid chromatography, the non-chromatographic or chromatography-like separation procedures are combined with the actual LC separation. A typical example of the quasi 2D LC of macromolecules represents the combination of a full retention-elution procedure, for example the full adsorption-desorption with the size exclusion chromatography (Refs. 37,38,46). Polymer blend constituents successively leaving the FAC column are directly forwarded into an on-line SEC instrument. SEC column has to be previously equilibrated with the same displacer which is applied to selectively elute macromolecules possessing similar chemical nature and adsorptivity from the FAD column. SEC provides molar mass both averages and distributions of all blend components discriminated by the FAD column (Ref. 39). Full retention procedure can be used also to selectivity remove interfering macromolecules from the polymer mixture before SEC analysis (Ref. 47). In this way, also amount and molecular characteristics of parent homopolymers present in copolymers can be quickly and efficiently determined (Ref. 48). The FAD/SEC combination usually does not require any sample reconcentration or special valves for sample reintroduction.

Further important opportunities for polymer characterization render quasi two-dimensional procedures which combine for example temperature rising elution fractionation or hydrodynamic chromatography or field-flow fractionation, etc. with size exclusion chromatography. On the opposite end of this scale are the methods combining liquid chromatography of macromolecules with mass spectrometry which can be considered a "separation technique", too. These combinations lie, however, beyond the scope of this short review.

4. Conclusion

Two-dimensional high performance liquid chromatography represents an important tool for molecular characterization of complex polymer systems. Though the quantitative and precise assessment of polymer molecular characteristics seems to be more popular among the people who control the large-scale polymer production than among the polymer synthesists engaged in the basic research, the progress in the 2D-HPLC method development continues. Further impact is anticipated to appear when the present fast and extensive cover research in polymer synthesis will turn towards details and when more exact information on the product characteristics will be needed in polymer synthesis research. Progress in the two-dimensional high performance liquid chromatography of macromolecules is also strongly dependent on

intensification of theoretical knowledge and on further development of tailored HPLC column packings and detectors.

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