

Mathematic Expression of Enthalpy Partition in Coupled Polymer Liquid Chromatography

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Abstract: Modeling and algorithms are presented in this contribution that enable analysis and solution of the decision problems of polymer liquid chromatography. The process of liquid chromatography is highly nonlinear. In case of complex nonlinear system description the associated decision model has multiple locally optimal solutions. Numerous local cases where local solution is unknown exist in our work. Therefore multiextremal decision model is very complex and standard optimization strategies are not applicable to its solving.

Key Words: Extent of enthalpic interactions in coupled methods of polymer liquid chromatography – case of enthalpic partition; modeling; cubic regression curve; approximation; nonlinear systems; multi-agent system; decision-making.

1 Introduction to Liquid Chromatography

Liquid chromatography is presently the most common tool for separation and molecular characterization of synthetic polymers. For example, size exclusion chromatography (SEC) (gel permeation chromatography) enables precise determination of molar mass averages and distributions of linear synthetic macromolecules. SEC also produces *estimates* of these characteristics for complex polymers, which exhibit more than one distribution in their molecular characteristics namely beside molar mass also in chemical structure (composition, functionality) and in physical architecture (stereoregularity, cis – trans isomerism, etc.). Separation mechanism of SEC is based on the entropy-controlled partition of macromolecules

between volume of pores and interstitial volume in a column packed with porous particles and flushed with appropriate mobile phase, eluent. Entropic partition of macromolecules is connected with their exclusion from the column packing process. Extent of retention of macromolecules in column depends on relation between sizes of pores - their diameters and volumes - and sizes of macromolecules. As result, SEC separates polymer species according to their *size* in solution and processing of data obtained that is chromatograms, leads to values of their molar masses. Sizes of macromolecules in solution, however, depend on all their molecular characteristics, not only on their molar mass. Therefore, for precise characterization of complex polymers as to their molar mass, chemical structure and/or architecture, macromolecules must be separated exclusively or at least prevailingly according to one molecular

characteristic and effect of other characteristics is to be eliminated or at least suppressed. Alternatively, effect of one parameter is to be enhanced so that role of other characteristics can be neglected. To do so, entropic retention mechanism of SEC has to be combined with enthalpic retention mechanisms such as adsorption, enthalpic partition and solubility (phase separation). Resulting separation procedures are called "coupled methods of polymer high performance liquid chromatography" (coupled polymer HPLC)[1]. Evidently, macromolecules in the coupled polymer HPLC are retained simultaneously with at least two different retention mechanisms, one of them is in all cases entropic partition. Second and some time several other retention mechanism(s) are added to either suppress or to enhance effect of one molecular characteristic - this is usually molar mass of polymer species - on their retention. As result of entropic partition, retention volume of macromolecules, V_R , that is volume of eluent needed to elute given polymer species from the SEC column decrease with molar mass of macromolecules. Enthalpic retention mechanisms as rule lead to an opposite effect that is to an increase of retention volume of polymer sample with its molar mass. A situation is looked for, in which entropic and enthalpic effects mutually compensate so that effect of polymer molar mass on its retention within HPLC column vanishes. This contingency is called "critical conditions" [2] and the resulting method is quoted "critical chromatography" (LC CC). It is necessary to stress that LC CC has nothing common with HPLC with supercritical mobile phases or "supercritical HPLC". Under critical conditions, retention of macromolecules within HPLC column does not depend on their molar mass so that the analyzed sample is separated mainly or exclusively according to its second molecular characteristic, which can be thus assessed without interference or with only a small effect of molar mass. In contrast to LC CC, the situation can be identified, in which enthalpic retention mechanism augments selectivity of polymer separation according to molar mass and retention volumes of macromolecules rapidly decrease with their molar mass. In this case one speaks about enthalpy assisted SEC "(ENA SEC)"[3]. This latter case will be discussed in present contribution. So far, there does not exist a theory, which would quantitatively describe contribution of entropy and enthalpy on the separation process of macromolecules. The appropriate chromatographic systems for given

polymer species comprising specific porous column packing, mobile phase and temperature must be looked for in a series of tedious chromatographic experiments. Lacking theory of coupled polymer HPLC also prevents prediction of sensitivity of LC CC and ENA SEC toward small variations in eluent composition and temperature, which complicates application of both methods.

In this work, we have attempted to mechanistically express individual contribution of enthalpic partition to retention volumes in coupled polymer HPLC. Enthalpic partition (absorption) of macromolecules between mobile phase and chemically bonded alkyl phase [4] (a volume – volume distribution of solute molecules) by definition differs from more widely applied adsorption of macromolecules on the solid surface (a volume – surface distribution of solute). Alkyl groups of different lengths are chemically bonded to appropriate solid surface, usually to porous silica gel. Silica gels carrying dimethyl octadecyl groups (silica C18 phases) are most commonly used in HPLC of small molecules. These materials in fact contain 20 carbons per one bonded group. Several hundred (!) different C18 silica phases are presently on the market. These materials found application also in polymer HPLC. Besides C18 also C1 (in fact C3), C4 (that is C6), C8 (C10), C14 (C16), C22 (C24) and C30 (C32) are commercially available. Existence of enthalpic partition can be in the first approximation explained by the fact that in some systems macromolecules prefer staying in the solvated alkyl phase rather than in mobile phase. This is a somewhat unexpected situation in case of alkyl-bonded phases because very few synthetic polymers dissolve in alkanes, especially in high alkanes such as octadecane (C18). However, if macromolecules are eluted in a thermodynamically poor solvent, which they do not like, they may be pushed into solvated alkyl phase and thus partitioned in its favor [4]. If enthalpic partition is extensive, macromolecules stay retained within HPLC column and do not elute at all. On the other hand, if the same polymer species are eluted from the same HPLC bonded phase using a thermodynamically good solvent, macromolecules prefer staying in solvent and do not partition in favor of bonded phase. They elute under "ideal SEC" conditions or, in other words, their retention within HPLC column are governed solely by entropic, exclusion processes. This means that extent of enthalpic partition of macromolecules within given bonded phase is to a large extent controlled by interaction

of eluent with macromolecules that is by the “quality” of mobile phase for polymer species. If a certain polymer is eluted from the alkyl-bonded phase in a good solvent (mobile phase), retention will be mainly entropic (SEC) while addition of a poor solvent (mobile phase) would increase contribution of enthalpic partition. Enthalpic contribution is reflected in increasing polymer retention volume, V_R . In order to assess extent of its enthalpic partition, polymer is eluted first in a good solvent and later in a mobile phase, which contains a poor solvent or a no solvent. Macromolecules behave under ideal SEC condition in the former eluent while their retention is affected by enthalpic partition in the latter eluent. Difference of V_{RS} in poor and good eluent (ΔV_R) can be ascribed to the effect of enthalpic partition in poor eluent. In order to assess the molar mass effect on enthalpic partition a series of polymer samples with known molar masses and narrow molar mass distribution is injected into HPLC column. The dependences $\log M$ vs. V_R are constructed and compared. ΔV_R values obtained by subtraction of V_R (SEC) from V_R (coupled) for the same M indicate contribution of enthalpic partition to total retention volume of macromolecules. This approach is of course only approximate since it does not consider changes in size of macromolecules and in volume of alkyl-bonded phase when eluent composition changes. It was, however, shown that size of polystyrene (PS) macromolecules do not drop substantially in mixed solvent (tetrahydrofuran (THF – a good solvent) plus dimethylformamide ((DMF – a poor solvent for PS) containing as much as 81% of DMF [5]. Similarly bonded alkyl groups on silica gel surface assume a rather stretched and stable conformation even under very harsh conditions when eluent is a strong no solvent for alkanes, such as mixture of water and methanol containing 80 vol.% of water [6]. Similar situation can be anticipated also in mixed mobile phases THF plus DMF. Interphase adsorption on the surface of alkyl-bonded groups is not separately considered in our present approach. It may augment enthalpic partition [7]. Further important simplification lies in neglecting differences in the contributions of net exclusion to V_R for SEC eluted macromolecules and for those undergoing enthalpic partition. Still, we believe that even this simplified approach can produce valuable information about *tendencies* in behavior of macromolecules in coupled polymer HPLC methods. It was of interest to evaluate extent enthalpic partition by ΔV_R values for

entire spectra of polymer molar masses and for different eluent thermodynamic quality for polymer considering different bonded alkyl phases.

Narrow molar mass distribution polystyrenes (PS) were chosen for the work because of their availability. Good solvent for PS was tetrahydrofuran (THF) and poor solvent was dimethylformamide (DMF). THF prevents enthalpic partition while DMF promotes enthalpic partition of polystyrenes on alkyl-bonded silica gels. Bonded phases with C4, C8 and C18 were compared. All bonded phases were prepared from the same silica gel Kromasil 100 and delivered by Eka Chemicals, Bohus, Sweden. The effective mean pore diameter of kromasil 100 was 10 nm, and pore volume was 0.85 mL g^{-1} .

Outcomes of corresponding analyses are presented in this contribution. Mathematical processing of experimental data was attempted with aim to arrive at functional expressions for different experimentally measured systems were produced.

2 Advance of Evaluation of Measured Values from Coupled LC

The dependences V_R vs. $\log M$ represents complex non-linear system, so it may be solved by an associate decision model, which has multiple local optimum solutions.

As was suggested in [9], the system for evaluation of calibration curves in LC is constructed as Multi – Agent System (MAS). System consists from decision agent and agents for modeling different cases of CC. In this work the agent for CC is described for applications where situation is most suitable for modeling by polynomial of 3-th degree.

An important part in treatment of data obtained by SEC and enthalpy assisted SEC is modeling $\log M$ vs. V_R calibration curves. The aim is to better understand the process and to find optimal design of experiment. SEC experiments generate a set of points:

$$\{x_i, y_i\}, \quad i=1,2,\dots,m \quad (1)$$

These points can be fitted by polynomials

$$a_n x^n + a_{n-1} x^{n-1} + \dots + a_0, \quad n=1,2,3,\dots \quad (2)$$

that is by a straight line, quadratic or cubic, etc. polynomials, using the least square regression procedure. Application of the root squared error method (RMSE), given by (3) and considering

characteristic of polynomial shape was used for the decision-making agent, which associate model could be used to identify optimal solution.

$$E = \sqrt{\frac{\sum_{i=1}^m (y_i - y'_i)^2}{m}} \quad (3)$$

where y_i are experimental values and y'_i are values determined by regression polynomial in points x_i . On the basis of results and shape of cubic parabola decision agent suggests next processing, i.e. by the model of polynomial of 3-th degree (cubic parabola). The shape of cubic parabola depends on value:

$$D = 3a_2a_0 - a_1^2 \quad (4)$$

If $D < 0$ then polynomial has one maximum and one minimum. This characteristic was used in present work.

In case when E is sufficiently small and simultaneously $D \geq 0$, a model for CC can be used which is given by regression polynomial of lower degree.

As mentioned, ΔV_{Ri} obtained by subtraction of V_{Ri} (SEC) from V_{Ri} (coupled) for the same M indicated contribution of enthalpic partition. Valuable information is produced about tendencies in behavior of macromolecules in HPLC.

3 Results and Discussion

Schematic representation of the proposed approach to discrimination enthalpic and entropic contribution to retention volumes of macromolecules in coupled polymer HPLC is depicted in Figure 1. Curve A shows ideal SEC elution of PS species from an alkyl- bonded phase in a thermodynamically good solvent THF.

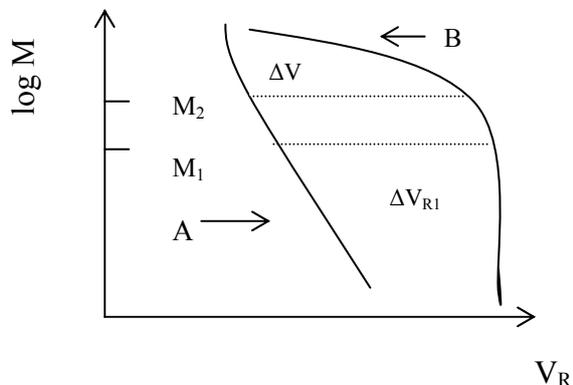


Figure 1: Schematic representation of enthalpic partition effect on retention volume of polymer

Curve A: Ideal SEC dependence, enthalpic partition is absent.

Curve B: Cooperative effect of exclusion and enthalpic partition to retention volume $\Delta V_{R,i}$ contribution of enthalpic partition to retention volume at mass M_i . M is polymer molar mass and V_R is corresponding retention volume of polymer species.

Retention mechanism of macromolecules is exclusion (entropic partition) and effect of enthalpic partition can be neglected. Poor solvent for PS, namely DMF pushes macromolecules into bonded phase and promote their enthalpic partition. As result, successive additions of DMF to THF eluent causes shifts of $\log M$ vs. V_R dependences to higher retention volumes. ΔV_R is in the first approximation considered contribution of enthalpic partition.

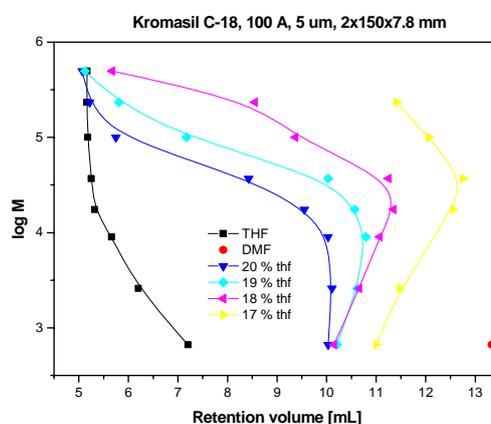


Figure 2: Examples of $\log M$ vs. V_R Dependences for polyst. in neat THF and mixed eluent THF+DMF

Typical experimental dependences of V_R on logarithm of polymer molar masses are shown in Figure 2. It demonstrates practical effects of DMF addition to eluent. V_R of polystyrenes increase in particular manner. Small amount of DMF added to THF eluent does not much affect retention volumes, while V_R are very sensitive above certain content of the DMF in eluent. Nearly critical conditions were reached at 20 wt.% of THF in eluent that is the situation when V_{RS} of polystyrenes do not depend on polymer molar mass.

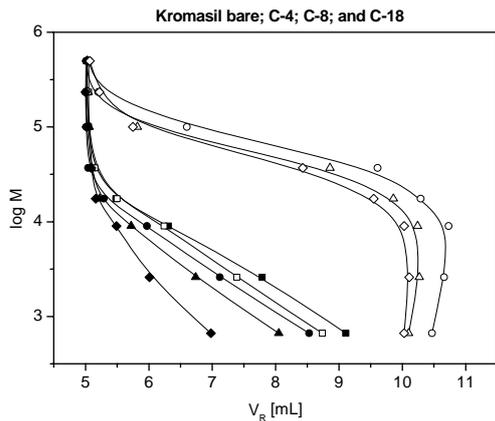


Figure 3: Dependences $\log M$ vs. V_R for polystyrenes and C18 bonded phases compositions of mixed eluents THF plus DMF

where

- Kromasil bare: ■ THF; □ mixed eluents DMF:THF 80:20 wt./wt. %
- Kromasil C-4: ● THF; ○ mixed eluents DMF:THF 80:20 wt./wt. %
- Kromasil C-8: ▲ THF; △ mixed eluents DMF:THF 80:20 wt./wt. %
- Kromasil C-18: ◆ THF; ◇ mixed eluents DMF:THF 80:20 wt./wt. %

Similar courses of $\log M$ vs. V_R dependences were observed for C4 and C8 column packing. Their direct comparison is however hardly feasible. Therefore $\log M$ vs. V_R dependences are plotted together for C4, C8 and C18 column packing in Figure 3.

Retention volumes of Ps in neat THF systematically decrease with increasing length of bonded alkyl group. This is logical because longer alkyls occupy more pore space. $\log M$ vs. V_R dependences obtained with mixed eluent THF/DMF demonstrate effect of enthalpic partition. Unfortunately, even direct comparison of dependences on Figure 3 does not allow drawing conclusions on the role of the alkyl group length on enthalpic partition of PS macromolecules.

This is why we have calculated ΔV_R values for corresponding polymer molar masses as explained in Figure 1 for each column packing and for various mixed eluent compositions. The typical set of ΔV_R vs. $\log M$ data points is depicted in Figure 4. Their courses of corresponding dependences were expressed by polynomials of various degrees.

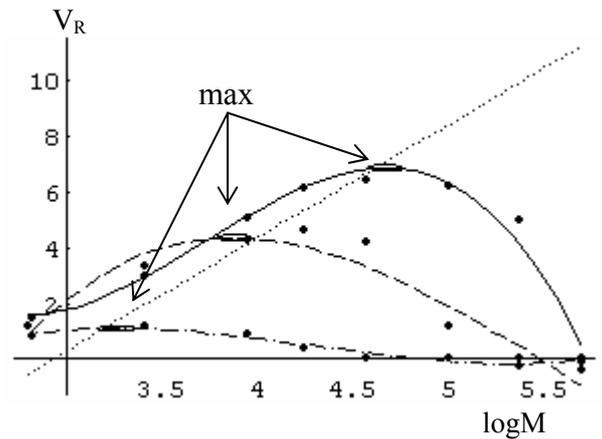


Figure 4: ΔV_R vs. $\log M$ for C4 where — for mixed eluent 18:82, ···· for mixed eluent 50:50, - - - for mixed eluent 20:80 THF/DMF

As evident from Figure 4, largest $\Delta V_{R,max}$ values were obtained for eluent THF/DMF containing 18% of THF and for C4 bounded phase. This surprising result can be understood as a proof that enthalpic partition takes place mainly in the upper layer of bonded phase and macromolecules do not deep permeate among alkyl groups.

The best fit represented by polynomials of third degree. The extremes of above curves were calculated and the corresponding values are summarized in Table I.

Kromasil	$\Delta V_{R,max}$	M_{max}	THF
C4	1.12	3.27	50
C4	4.34	3.88	20
C4	6.84	4.67	18
C8	0.54	3.29	50
C8	4.8	3.74	20
C8	6.47	4.44	18
C18	0.5	3.64	50
C18	3.48	4.59	20
C18	5.66	5.15	18
C18	7.87	5.71	17.5

Table 1.

4 Conclusions

Extend of enthalpic partition of model of polystyrene macromolecules within alkyl bonded silica gel column packing was assessed. Tetrahydrofoman was partition promoted mobile phase component while dimethylformamide suppressed enthalpic partition of model polystyrenes. Experimental $\log M$ dependences were processed mathematically for different both column packings and eluent compositions. Here ΔV_R is approximate net contribution of enthalpic

partition to retention of macromolecules with molar mass M within LC column. Extreme values of ΔV_R and M were determined, $\Delta V_{R, \max}$, and M_{\max} . As expected, $\Delta V_{R, \max}$ rose with concentration of DMF in eluent. However, values of $\Delta V_{R, \max}$, and M_{\max} lead to a surprising conclusion that extent of enthalpic interaction in C4 bonded phase is larger than in C8 and in C18 bonded phases.

This indicates that only very upper part of bonded phase takes part in the enthalpic partition process. Consequently, application of C18 bonded phase, which dominates many present coupled polymers HPLC method, is not relevant.

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