Estimation of Effective Volume of HPLC Alkyl Bonded Phases by means of Macromolecular Probes

DUSAN BEREK[1] and STANISLAVA LABATOVA[2]

[1]Polymer Institute, Slovak Academy of Sciences, 842 36 Bratislava,
[2]Institute of Informatics, Slovak Academy of Sciences, 845 07 Bratislava
SLOVAKIA

Abstract: A method for estimation of volume of alkyl bonded phases for high performance liquid chromatography (HPLC) is presented. It is based on application of macromolecular probes, which are able to elute from the HPLC column without entering the bonded groups and thus to monitor their volume. The appropriate mathematical expression of results were looked for and it was concluded that the optimum represent polynomials of second degree.
Key –Words: Polymer Liquid Chromatography, polynomial regression, multi-agent systems

1 Introduction to Problem and Explanation of Backgrounds

High performance liquid chromatography (HPLC) belongs to the most important analytical tools of modern chemistry. HPLC separates complex mixtures of both low- and high- molecular species to allow conclusions on sample composition in terms of chemical structure and architecture of components, and – in the case of polymers – also in terms of their molar mass averages and distributions. HPLC is based on differences in retention of separated substances within appropriate *stationary phase.* Analyzed sample is introduced into a device usually *a column* filled with a stationary phase *a packing,* to be transported by means of *the mobile phase (eluent).* Volume of mobile phase needed to elute the particular substance from the column is measured. It is called *retention* or *elution volume* (V_R) and it reflects extent of retention of analyte within the column packing. Often, the time scale is used instead of volume supposing that the HPLC pumping system provides highly constant flow of mobile phase. The larger retention volume the greater is extent of attractive interaction between small molecules of analytes and the column packing. The behavior of macromolecules in the HPLC column is substantially different. In absence of attractive enthalpic interactions, polymer species enter *the pores* of the column packing in dependence of their *size* in mobile phase. Large macromolecules are able to permeate only large pores and elute from the column packing rapidly. Smaller polymer species can permeate greater fraction of the pore volume and they are retained longer. This is the entropy based separation principle of *size exclusion chromatography* (SEC).

Concentration of analytes leaving the HPLC columns is continuously monitored by means of appropriate *detectors,* which – depending on the nature of both analytes and eluent – measure light absorption, or refractive index, or optical rotation, or density etc. of the *column effluent*. Alternatively, the NMR signal and in case of polymer analytes, also viscosity or light scattering from column effluent are monitored. Provided the molecules of analyte do not evaporate, HPLC detectors can nebulize the column effluent, evaporate mobile phase and measure intensity of light scattered by the stream of "dry" particles of analyte. The time dependence of the *detector response* (the analyte concentration) is called a *chromatogram.* Chromatograms of complex low molecular mixtures represent a series of nearly Gaussian curves – *peak(s)*–, mutual distance of which is designated separation *selectivity.* Selectivity of separation of low molecular weight substances is largely controlled by the nature of both column packing and eluent. In the case of polymer separation, chromatogram represents only the envelope curve for it is not possible to fully separate hundred(s) of species present in the sample. Chromatograms of polymers are as rule deconvoluted mathematically. Limited separation selectivity in polymer HPLC is due

to the fact that the exclusion of particular macromolecules depends on the packing pore volume, size and size distribution.

In any case, the hearth of all HPLC systems is the column packing. In HPLC of small molecules, the most popular column packings are formed with silica particles, surface of which is covered by *chemically bonded* alkyl groups, usually linear octadecyl groups *(C-18 alkyl bonded phase).* The idealized structure of the alkyl bonded silica gel is schematically represented in Figure 1.

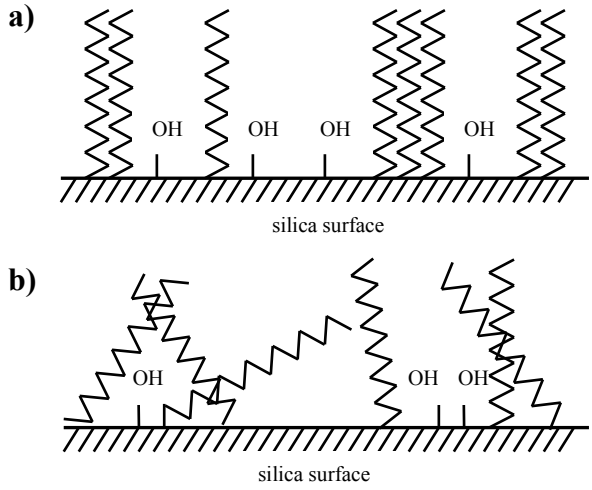

Figure 1. Schematic representation of the alkyl bonded silica gel HPLC column packing

a) Fully stretched alkyl groups
b) Partially collapsed alkyl groups

Note presence of free silanols on the silica gel surface

The real silica C-18 bonded phases do exhibit enormous variability. Presently, there are about 700 such materials on the market sold under different names. It is estimated that about half of them mutually differ in the pore size and shape of starting silica gel, density of surface coverage with alkyl groups, as well as in the concentration of free surface silanols. The actual spatial organization of bonded alkyl groups may differ due to different bonding densities and also due to interaction with eluent. They may assume different structure from a rather stretched (Figure 1a) to a collapsed one (Figure 1b).

Separated substances may *adsorb* on the *surface* silanols and/or undergo the *enthalpic partition (absorption)* in favor of the bonded phase *volume* (Figure 2). It is evident that extent of enthalpic partition of analyte depends not only on its compatibility with ("solubility" within) the bonded phase but also on the *effective volume of bonded phase.* This is why it is of interest to assess the latter characteristic of bonded phases.

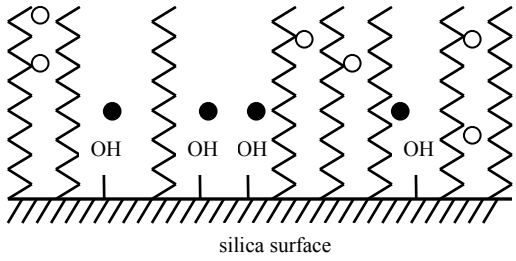

Figure 2. Schematic representation of adsorption (●) and enthalpic partition (○) of low molar mass analytes within the alkyl bonded phase.

Several authors measured volume of alkyl bonded phase in dry state using for example measurements of surface area by B.E.T. (nitrogen or argon adsorption) [1] or positron annihilation lifetime spectroscopy [2]. They have shown that the bonded alkyl groups are largely stretched and their collapse is evident only for longer chains such as C-18. Measurement of bonded phase volume in presence of eluent is rather complex. A probe must be identified, which does neither adsorb on free silanols nor partitionate (absorb) in favor of bonded phase. Difference in retention volumes of such probe measured with the bare, non-bonded silica and with the bonded material prepared from the same silica gel would give an estimate of bonded phase volume. So far, these attempts exhibited limited applicability because of lacking suitable low molecular probes. Similarly, it is rather problematic to assess separately only *silanophilic activity* (adsorption on free silanols) or only *nonpolar interactivity* (enthalpic partition) in favor of alkyl groups.

Recently [3,4] application of macro-molecular probes was proposed and tested to solve the latter task. Polar polymer species such as poly(methyl methacrylate)s or poly(2- vinyl pyridine)s eluted from the alkyl bonded silica gels in mobile phases of low- to medium-polarity *(weak solvents)* for example toluene or

tetrahydrofuran (THF), respectively, exhibited adsorption on free silanols (Figure 3a) but practically negligible enthalpic partition. Activity of free silanols for different alkyl bonded silica gels could be compared on the base of such experiments [3].

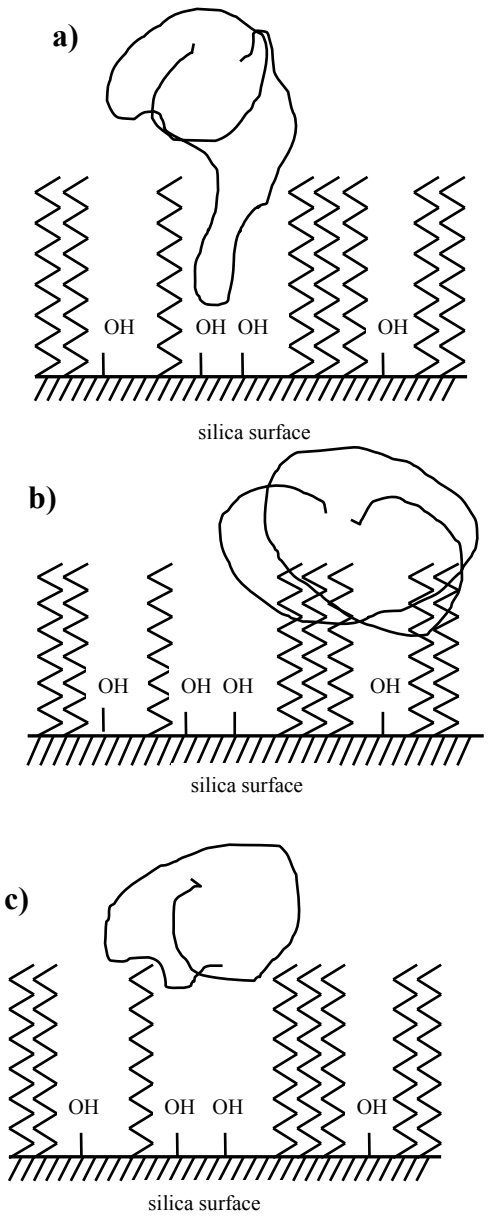

Figure 3. Schematic representation of adsorption (a), and enthalpic partition (b) of polymer coil onto/within the alkyl bonded silica gel HPLC column packing. No attractive interaction between polymer coils and column packing is present in the case 3c.

On the contrary, low polarity polymers such as polystyrenes or poly(n-butyl methacrylate)s eluted in their thermodynamically *poor solvents* e.g. in the mobile phases containing dimethyl formamide did not adsorb on free silanols but they preferred staying in the solvated bonded phase, they were partitioned in favor of column packing (Figure 3b) [4]. Finally, polymers of medium or high polarity neither adsorb on silica surface nor partition in favor of bonded phase if eluted in their good solvents, which suppress interactions between polymer coils and column packing. These species "slide" on the surface of bonded phase just touching alkyl groups (Figure 3c). In this way appropriate macromolecular probes "feel" the effective volume of bonded phase. The latter can be estimated from the difference in retention volumes of macromolecules of the same nature and size – that is in the same eluent and at the same temperature – measured with bare silica gel and with the alkyl bonded phase [5]. It is however, important that the pore geometries of both starting and bonded silica gel are identical. If starting silica gel is not accessible the bonded groups can be removed by hydrolysis or pyrolysis under mild conditions so that silica matrix remains uncharged.

2 Experimental Results

The appropriate experimental systems for above measurements are polystyrenes in THF and dextran species in methanol/water mobile phases. It was revealed [6] that adsorption of above macromolecular probes within bare silica gels, as well as their partition in favor of alkyl bonded silica gels was negligible. The typical examples of semilogaritmic dependences of V_R measured with series of macromolecular probes with different molar masses are shown in Figures 4 and 5 and dependences of ΔV_R vs. log M are depicted in Figures 6 and 7. ΔV_R is the difference of retention volume determined with bare silica gel and with bonded phase using polymer probe of the same molar mass. The task of present study was to find mathematical expression for the log M vs. V_R and log M vs. ΔV_R dependences as an important step to

quantitative evaluation of HPLC bonded phase volume.

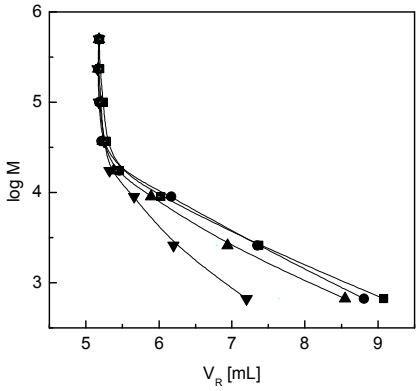

Figure 4. Dependences of retention volume of polystyrenes in THF eluent on molar mass of probe. (■) bare silica gel; (●) C-4; (▲) C-8; (▼) C-18 alkyl bonded phases.

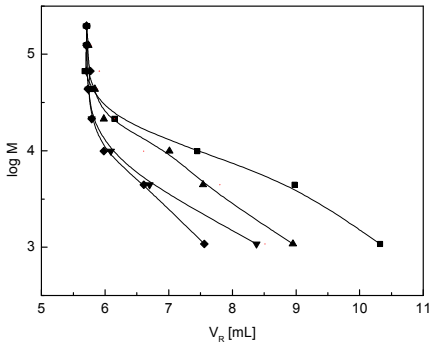

Figure 5. Dependences of retention volume of dextrans in methanol/water 20/80 wt./wt. eluent on molar mass of probe. (■) bare silica gel, (▲) C-4; (▼) C-8; (◆) C-18 alkyl bonded phases.

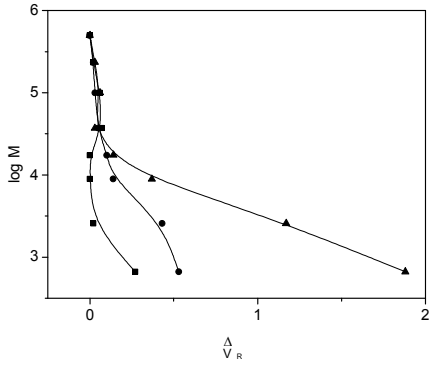

Figure 6. Dependence of ΔV_R on molar mass of polystyrene probes in THF (▲) C-18 (●) C-8; and (■) C-4 alkyl bonded phases.

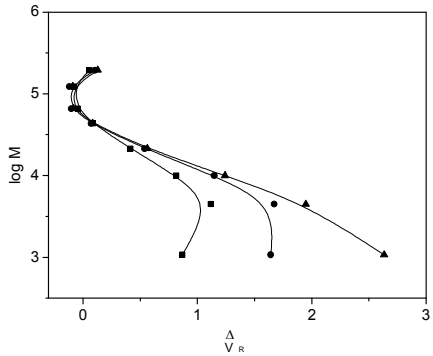

Figure 7. Dependence of ΔV_R on molar mass of dextran probes in methanol/water 20/80 wt./wt. eluent (▲) C-18;(●) C-8; and (■)C-4 alkyl bonded phases.

3 Optimal Design for Polynomial Regression

From mathematical point of view for solution above problem we can write:

$$Y_i = f(x_i) + \varepsilon_i ; \quad i=1,2,...,n \qquad (1)$$

where x_i is value of independent variable of experiment
Y_i is measured response of experiment
f is unknown function
ε_i is random error
n is a number of measurements

We would be willing to assume that function f is sufficiently smooth over the range of interest, so we represent the function f adequately by a polynomial. If we assume that function

$$f(x) = a_0 + a_1 x + a_2 x^2 + ... + a_m x^m \qquad (2)$$

is a polynomial of degree m, where a_i are unknown, we will call the setup model P_m. Let $\boldsymbol{Y}$, $\boldsymbol{a}$, $\boldsymbol{\varepsilon}$ denote column vectors, $\boldsymbol{Y} = (Y_1, Y_2, ..., Y_n)$, $\boldsymbol{a} = (a_0, a_1, ..., a_n)$, $\boldsymbol{\varepsilon} = (\varepsilon_1, \varepsilon_2, ..., \varepsilon_n)$ and $\boldsymbol{X}$ denote a matrix n x m+1 given by:

$$x = \begin{bmatrix} 1 & x_1 & x_1^2 & \cdots & x_1^m \\ 1 & x_2 & x_2^2 & \cdots & x_2^m \\ \vdots & \vdots & \vdots & \cdots & \vdots \\ 1 & x_n & x_n^2 & \cdots & x_n^m \end{bmatrix} \qquad (3)$$

then the model P_m can be written as :

$$\boldsymbol{Y} = \boldsymbol{X}\boldsymbol{a} + \boldsymbol{\varepsilon} \qquad (4)$$

where x_i's take at least $m+1$ distinct values and $n \geq m+1$. Then unique least squares estimator of $\boldsymbol{a}$ is given by

$$\boldsymbol{a^* = (X'X)^{-1}XY = (a_0^*, a_1^*, ..., a_m^*)} \quad (5)$$

Information matrix of design $M_n(\xi)$ denote matrix $(m+1)X(m+1$

$$M_n(\xi) = 1/n\, \boldsymbol{X'X} \quad (6)$$

whose ij-th element is:

$$m_{ij} = \int_X x^{i+j-2} d\xi \quad (7)$$

where ξ is probability measure on region of interest.

Optimal design of experiment minimizes some criterion optimality on the set of all information matrixes. In describing designs are used discrete probability measure ξ on region of interest.

Historically was first considered design criterion based on variance of estimated regression function known as G-optimal model for P_m if it minimize [7]

$$\max_{-1<i\leq 1} \operatorname{var}\left[\sum_{i=1}^{m} a_i x^i\right] \quad (8)$$

Criterion that we used is application root square error method (RSEM) given by

$$E = \sqrt{\frac{\sum_{i=1}^{n} (y_i - y_i')^2}{n}} \quad (9)$$

Using some level of importance it is possible to define compromise optimal criterion, which detects information about exactness of parameters and share of model, respectively.

System for evaluation of calibration curves in liquid chromatography is constructed as Multi – Agent System (MAS). System consists from decision agent and agent for modeling and processing different cases of CC. In this work the agent for processing CC is P_3 most reflected, as this agent is very suitable for our aims. The shape of cubic parabola depends on value $D = 3a_2a_0 - a_1^2$. If $D<0$ then polynomial P_3 has one maximum and one minimum, which characteristic is used for analysis for example for obtaining information about tendencies in behavior of macromolecules in HPLC.

4 Conclusions

It is evident that macromolecular probes can give basic information about volume of alkyl bonded phases for high performance liquid chromatography. The retention volumes V_R obtained with alkyl bonded silica gel decrease with the increasing length of alkyl chain (Figures 4 and 5). Consequently, the ΔV_R values, that is the differences between retention volumes of polymer probes measured with bare silica gel and with bonded phases increase with increasing length of bonded groups. The plots of log M vs.ΔV_R for the dextrans exhibit extremes at low molar masses of probes. This is likely due to penetration of smaller macromolecules into the bonded phase. Therefore, only results with molar masses above few thousands should be considered. Dependences of both log M vs. V_R and log M vs. ΔV_R can be well represented with the polynomials of second order. Pore excluded macromolecules give zero ΔV_R values because the effect of bonded phase present on the outer surface of column packing particles is negligible. The actual value of ΔV_R depends on the pore volume, which is accessible for macromolecules in solution and thus on the molar mass of the probe. In the next stage, it will be attempted to correct the ΔV_R values for the effect of both the pore size and the molar mass of probes and to calculate the actual volume of the bonded groups layer.

Acknowledgements: Financial support from the Slovak Grant Agencies APVV (Project 51-013 204) and VEGA (Projects 2/6016/26 and 2/7101/27). The authors are thankful to Mrs. J. Tarbajovska for her excellent technical assistance.

References

[1] I. Rustamov, T. Farcas, F. Ahmed, F. Chan. R LO Brutto, H.M.Mc Nair, Y.V. Kazakevich, J.Chromatogr. A **2001**, *913*, 49

[2] O. Šauša, J. Krištiak, D. Berek, *Chem. Pap.***2005**, *59*, 268
[3] D. Berek, J.Tarbajovska, *J. Chromatogr.* A, **2002**, *976*, 27
[4] D. Berek, *J. Chromatogr*. **2003**, *1020*, 219
[5] D. Berek, *PITTCON* **2007,** CD 430-7
[6] D. Berek, unpublished results
[7] S.M. Stigler, *Journal of American Stat. Ass.* Vol. 66, No.334, 1971, pp.311-318