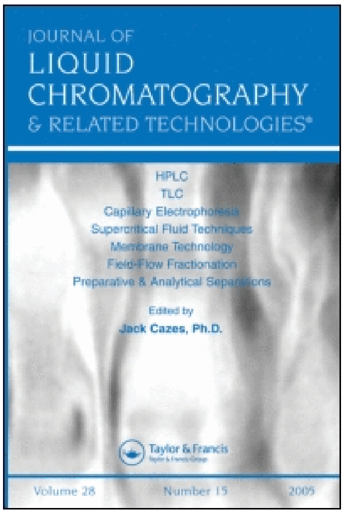

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A CORRELATION BETWEEN *Z* AND *S* IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A correlation between the stoichiometric parameter *Z* in the stoichiometric displacement model for retention (SDM-R) and the parameter S (denoted S_m in the new correlation) in Snyder's empirical equation for isocratic elution reversed-phase high performance liquid chromatography (RPLC) was derived with Taylor translation. The correlation is $S_m = Z/(2.3\Phi_m)$, where Φ_m is the intermediate value of the activity of the stronger organic solvent in the mobile phase. With the correlation, a more accurate conversion between Z and S can be made, and the physical meaning of the empirical parameter S can be explained by means of that of parameter Z. The correlation was validated with experiment data and data from the literature for a variety of RPLC systems with different solutes and mobile phases. The results indicated that for all solutes, especially for homologues, and mobile phases, a good linear relationship exists between the two parameters. The values of S and S_m were generally found to be similar, but the accuracy of S was generally influenced by the changing composition of the mobile phase. The reason why S_m is more accurate and easily obtained than S_g (the S value obtained from two or more gradient runs) was also explained.

INTRODUCTION

Reversed-phase high performance liquid chromatography (RPLC) has a very important place in liquid chromatography (LC). For example, in recent decades, about 70% of research publications in LC are related to RPLC. However, there are still many problems with the retention mechanisms and related applications of RPLC need to be resolved. These problems have been illustrated in detail in many of the review papers on the retention mechanism of small molecules in a special volume of the Journal of Chromatography.[1] With the recent development and improvement of theoretical models in HPLC, the capacity factor (k'), which was only expressed as a single parameter, has been gradually developed into models with more parameters. The most noticeable two of them are parameter Z in the stoichiometric displacement model for retention (SDM-R)[2] and parameter S in Snyder's empirical equation.[3] Both have been referred to as new characterization parameters providing more information about the retention mechanisms in HPLC.[4,5] Some special discussion has been made about them.[6-8]

One of the popular quantitative models which describe the relationship between solute retention and the constituents of the mobile phase in RPLC is the SDM-R:[2]:

$$\log k' = \log I - Z \log a_D \qquad (1)$$

where k' is the capacity factor, a_D is the molar concentration of the stronger organic solvent in the mobile phase, and Z and logI are constants. Equation 1 is a double logarithm linear equation with a_D changing in a relatively wide range. The physical meaning of logI is: the affinity of 1 mol of solute to the stationary phase. That of Z can be described as the total moles of solvent released at the contact region between the stationary phase and the solute interface when 1 mol of solvated solute is absorbed. The parameter Z has been referred to be a new characterization parameter in RPLC,[6-8] even in other types of LC.[9~12]

An empirical equation put forward by Snyder et al.[3] is also widely used in RPLC:

$$\log k' = \log K_w - S\phi \qquad (2)$$

where ϕ is the volume fraction of the solvent in the mobile phase, K_W is the value of logk' for pure water as the mobile phase ($\phi = 0$), and $\log K_W$ and S are constants when ϕ changes in a relatively narrow range. Therefore, Equation 2 can be approximately referred to as a linear equation. The parameter S has also been used as a new characterization parameter in RPLC.[13] However, since

Equation 2 is an empirical equation, parameter S is lacking a physical meaning. This somewhat affects the application of S in RPLC.

In order to derive a physical meaning for S by means of that of Z and then to use it to characterize the retention behavior more accurately, Kunitani et. al.[14] derived a mathematical equation which can relate S to *Z* as the following:

$$Z = 2.3\overline{\Phi}S_g \tag{3}$$

where Sg is the value of S that could be determined by two or more gradient runs. The term ϕ is a constant independent of changes of ϕ and it could be determined from the following equation:[15,16]

$$\overline{\Phi} = \Phi_0 + [t_g - t_0 - t_D - 0.3(t_G/\Delta\Phi S_g)](\Delta\Phi/t_G) \tag{4}$$

where ϕ_0 is to the composition of the mobile phase at the beginning of a linear gradient elution, tg is the retention time of the solute, t_0 is the column dead-time for a small solute, t_D and t_G are the dwell time of the linear gradient system and the gradient time, respectively, Δ ϕ is the change in ϕ during the gradient run, and ϕ is the value of ϕ as the solute just passes the center of the column.

It should be pointed out that Sg and ϕ in Equation 3 should correspond to S and ϕ in Equation 2. However, ϕ in Equation 2 is a variable, while ϕ in Equation 3 is a constant. In addition, there is parallel relationship between Sg and Z, but that does not exist between Z and S. Also S in Equation 2 is only approximately a constant for its value may change within the range of ϕ, while Sg is a constant that only depends on the selected linear gradient elution.

Since then, the form Z (S) or S (Z) are often seen in the literature;[13,17,18] S has been used more widely than before. If the Z values and Sg values found in the literature could be converted between them, the applications of both of the parameters can become even more extensive. However, Z is determined by several isocratic elution runs, while Sg is determined by gradient runs. It is therefore difficult to convert the two parameters between each other according to Equation 3. Furthermore, different gradient elution modes need to be selected to obtain Sg. For some kinetic reasons, the conformation of some proteins could change so much that the elution order of them is changed with different gradient conditions.[14] In this circumstance, errors in Sg could not be avoided.

Some small solutes, such as alkanes, could only be detected by utility detectors such as the refractive index detector and conductance detector which could not be used in gradient elution. So, Sg could not be determined in this circumstance. This limits the application of Equation 3. However, this problem can be resolved if the Z values determined with isocratic elution can be converted to S values. It is, therefore, desirable to derive an equation so that Z and S could be related and the quantitative values of Z and S in the literature or from experiments can be readily converted to each other. In this paper, a correlation between parameter Z and S was derived from the SDM-R and validated with experimental data and data from the literature for a variety of small solute RPLC systems.

THEORY

The stoichiometric displacement model for retention (SDM-R) is a theoretical model that can be used for various chromatographic systems (except size exclusion chromatography).[2] The retention mechanism in RPLC is explained through the interactions among the components in a liquid-solid adsorption system which can be described by the following five thermodynamic equilibria: (1) Solute-solvent, (2) solute-adsorbent, (3) solvent-adsorbent, (4) re-solvation of solute -adsorbent complex, and (5) desorption of solvated solute-adsorbent complex. The net result is that a stoichiometric amount of solvent molecules must be released at the interface between the solute and the stationary phase (adsorbent) when a solvated solute molecule is adsorbed by the stationary phase:

$$PD_m + n\bar{L}D \Leftrightarrow P\bar{L}_n D_{(m-q)} + (n+q)D \qquad (5)$$

where D is the displacer or the stronger organic solvent in RPLC, P is the solute, PD_m is the solvated solute, L represents a "mean active site" (defined as an equivalent adsorption site with one solvent molecular adsorption or desorption on stationary phase surface)[19] by which the solute interacts with the stationary phase, and $PL_nD_{(m-q)}$ represents a mean active site-solute-solvent complex. The parameter m is the number of moles of the displacer solvated by one mole of solute and q is the number of moles of the displacer decreased when one mole of solutes is adsorbed by the stationary phase. Finally, the parameter n is the number of moles of ligands on the stationary phase covered by one mole of solute. The stoichiometric parameter Z is the sum of parameters n and q (Z = n + q).

From the Mass Action Law, Equation 5 can be described by the following in activities:

$$K_5 = \frac{a_{p\bar{L}D_m} a_D^Z}{a_{\bar{L}D}^n a_{PD}} \qquad (6)$$

Although the above equilibrium can show the activity relationship among solute, solvent and the stationary phase, there is not a clear quantitative relationship between the activities of these components and the resulting chromatographic retention. In LC solute retention is expressed by the capacity factor (k'). Moreover, the relationship between the k' and the partition coefficient (P) of a solute is: $k' = P\varphi$, where φ is the column phase ratio defined thermodynamically. It refers to the k' value when the partition coefficient of the solutes equals unity or the free energy change of the solute on the liquid-solid interface is zero. The phase ratio is independent of solute.[20]

In liquid chromatography, it can be assumed that the solvation between the solute and the solvent is so strong that only the solvated states of the solute, $PL_nD_{(m-q)}$ on the stationary phase and PD_m in the mobile phase, exist. The amount of un-solvated solute is, therefore, negligible. Since the interactions between solute or solvent and the stationary phase take place through the mean active sites distributed on the surface of the stationary phase, the activities of the adsorbed solutes and solvent can be related to the activity of the mean active sites (N_o). In analytical chromatography, $a_{LP} \approx N_0$. Therefore, from Equation 6.

$$k' = \varphi \frac{K_5 N_0^n}{a_D^Z} \tag{7}$$

And its logarithm form is:

$$\log k' = \log(K_5 N_0^n \varphi) - Z \log a_D \tag{8}$$

Equation 8 is mathematically exactly the same as that of the SDM-R. Its Taylor expansion at a_{Dm}, which is the intermediate value of the activity of the stronger organic solvent in the mobile phase, can be represented as:

$$\begin{aligned} \log k' &= \log(K_5 N_0^n \varphi) - Z \log a_{Dm} - Z\left(\frac{1}{2.3 a_{Dm}}\right)(a_D - a_{Dm}) \\ &= \log(K_5 N_0^n \varphi) + Z\left(\frac{1}{2.3} - \log a_{Dm}\right) - \frac{Z}{2.3 a_{Dm}} a_D \end{aligned} \tag{9}$$

The selection of the intermediate value of the activity of organic solvent in the mobile phase for a linear gradient elution minimizes the deviation that may be caused by the change of the activity in the mobile phase.

Equation 2 can be re-written into the form of Equation 10:

$$\log k' = \log K_W + S\Phi = \log K_W + \frac{SM}{1000\rho} \cdot a_D \tag{10}$$

where M is the molar concentration and ρ is the density of the solvent, respectively. Equations 9 and 10 have the same mathematical forms and from comparison, $\log K_W$ in Equation 10(denoted $\log K_{Wm}$, corresponding to a_m) can be written as:

$$\begin{aligned}\log K_{Wm} &= \log(K_5 N_0^5 \varphi) + Z(\frac{1}{2.3} - \log a_{Dm}) \\ &= \log K_5 + n\log N_0 + \log\varphi + Z(\frac{1}{2.3} - \log a_{Dm})\end{aligned} \tag{11}$$

and S in Equation 10 (denoted S_m) can be related to Z in Equation 9 as:

$$S_m = \frac{1000\rho}{2.3a_{Dm}M} \cdot Z \tag{12}$$

or

$$S_m = \frac{Z}{2.3\phi_m} \tag{12a}$$

Equation 12 gives a correlation between the parameters Z and S. However, it should be pointed out that there are differences among S, Sm and Sg. Parameter S is the slope of the linear plot by Equation 2 which is determined as an empirical parameter from several experimental data with isocratic elution. Similarly, Sg is obtained from two or more gradient runs. Finally, S_m is defined as the slope corresponding to the intermediate concentration of the strong solvent in the mobile phase (ϕ_m). Parameter S has no physical meanings while Sg and Sm have physical meanings by means of that of Z. The accuracy of the correlation between the two parameters (S_m and S) is tested by quantitative experimental data and data from the literature below.

EXPERIMENTAL

Equipment

All separations were carried out on a chromatographic system equipped with a Backman-Altex (Berkeley, CA, USA) Model 7120 injection valve fitted with a 100-μL loop, a Varian Model UV-500 detector operated at 254nm, and a Fisher Recordall series 5000 recorder (Austin, TX, USA).

Supports

SynChropak™ supports of 6 μm particle diameter were obtained from SynChrom (Liden, IN, USA). A column of 50 × 4.1mm I.D. was slurry-packed by using 2-propanol as the solvent.

Mobile Phases

HPLC grade water was prepared in the laboratory. The mobile phases were prepared with the pumping system of the chromatographic system by mixing a weak solvent, A (methanol/water: 20/80 vol), with a strong solvent, B (methanol/water: 80/20), in a suitable proportion to achieve the required mobile phase composition. The mobile phase flow-rate was 1.0 mL/min. The column was equilibrated with 40 mL of mobile phase before any retention measurement was made.

Solutes

All the following 24 solutes were purchased from Aldrich: Benzene, Bibenzyl, Biphenyl, m-Diisopropylbenzene, p-Diisopropylbenzene, 2,2-Diphenylpropane, 3,3'-Dimethyl-diphenyl, 4,4'-Dimethylbi-phenyl, methylene, m-xylene, n-Amylbenzene, Naphthalene, n-Propyl-benzene, Pentamethyl-benzene, 4-phenyltoluene, P-Xylene, Sec-butylbenzene, 1,2,3,4-Tetramethyl-benzene, 1,2,3,5-Tetramethylbenzene, 1,2,4,5-Tetramethylbenzene, Toluene, 1,2,3-Trimethyl-benzene, 1,2,4- Trimethylbenzene, o-Xylene. All the solutes were dissolved in pure methanol to 1.0 mg/mL.

RESULTS AND DISCUSSION

In order to test the accuracy of Equation 12a, the values of Z and S were calculated by using Equation 1 and 2, respectively, according to the values of k' and different ϕ under isocratic elution obtained from our experiments and references.[21~24] The values of S_m were obtained by using Equation 12a from the values of Z and the intermediate point of the organic solvent concentration ϕ_m and by plotting S vs. $Z/2.3\phi_m$. All data were calculated by using Microsoft Excel spread sheets. When the correlation coefficient was over 0.9999, it was taken as unity.

Small Polar Homologues

The plots of S vs. Sm (Z/2.3 ϕm) for two polar homologues in different mobile phases are shown in Fig.1.

Plot a is for nine n-alkanol homologues[21] when acetonitrile is the organic solvent and ϕ change from 50% to 80%. The correlation coefficient for Plot a was 1.000. The slope and intercept were 1.0104 and 0.0247, respectively. It is shown that the correlation between S and S_m is very high and they can be expressed by the function y = x. Because the Z value for a solute is a constant obtained from five to seven experimental points under isocratic elution, while S is only approximately constant, the use of ϕ_m avoids the errors that can arise from the effects on S due to a too broad range of ϕ in the mobile phase; S_m cal-

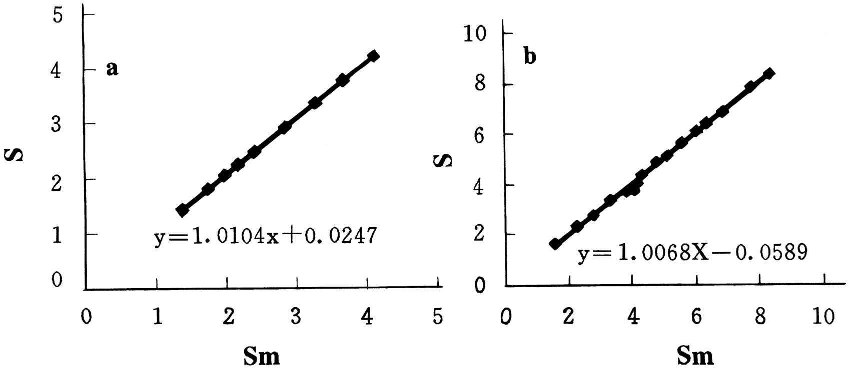

Figure 1. Plots of S vs. S_m ($Z/2.3\phi_m$) for two small polar homologues in two of mobile phases. (a) The original data was taken from Ref. 21. The 9 n-alkanol homologues (the carbon number is: 4, 5, 6, 7, 8, 9, 12, 14, 16, respectively) on YMC-phenyl stationary phase, were eluted with acetonitrile-water (v/v) from 50%~80%. (b) The original data was taken from Ref. 22, the 16 alkylphthalimides homologues eluted with methanol-water on Lichrosorb RP-18 stationary phase.

culated by using Equation 12a can be regarded as more accurate than S, or at least comparable with S.

Plot b is for 16 n-alkylphthalimide homologues with a methanol-water mobile phase.[22] The corresponding concentration range of methanol for each solute is quite different with each other. When the alkyl group changes from H- to octadecyl-, the largest $\Delta\phi$ is from 40% to 100%, and the smallest is from 90% to 100%. The correlation coefficient for Plot b was 0.9980. The slope and intercept were 1.0068 and -0.0589, respectively. This is still close to the function y = x. The changing range of methanol, $\Delta\phi$, does not seem to influence the slope and intercept, but has a slight effect on the closeness of the linear relation between S and S_m for this homologue.

From the above two sets of results with different mobile phases of acetonitrile/water and methanol/water, S and Sm are almost identical for the two homologues.

Small Non-Homologue Solutes

The S vs S_m relationship for non-homologue solutes was tested by using data from a special set of experiments designed in this paper. In total, 24 nonpolar aromatic compounds were chosen as solutes. Methanol was used as the organic solvent in the mobile phase. Obviously, $\Delta\phi$ for this group of solutes needed to be quite difference from each other. The results are shown in Fig. 2a which indicates that the relationship between S and S_m was less accurate than that for the homologue series. Although the correlation coefficient was only 0.9907, the slope and intercept were 1.127 and 0.4516, respectively. The slope and the intercept value was significantly higher than that in Fig. 1, indicating a more significant deviation from the function y = x than that shown in Fig. 1.

In the same way, the linearity between S and S_m for 6 polycyclic hydrocarbon compounds as solutes and methanol/water as mobile phase[21] is shown in

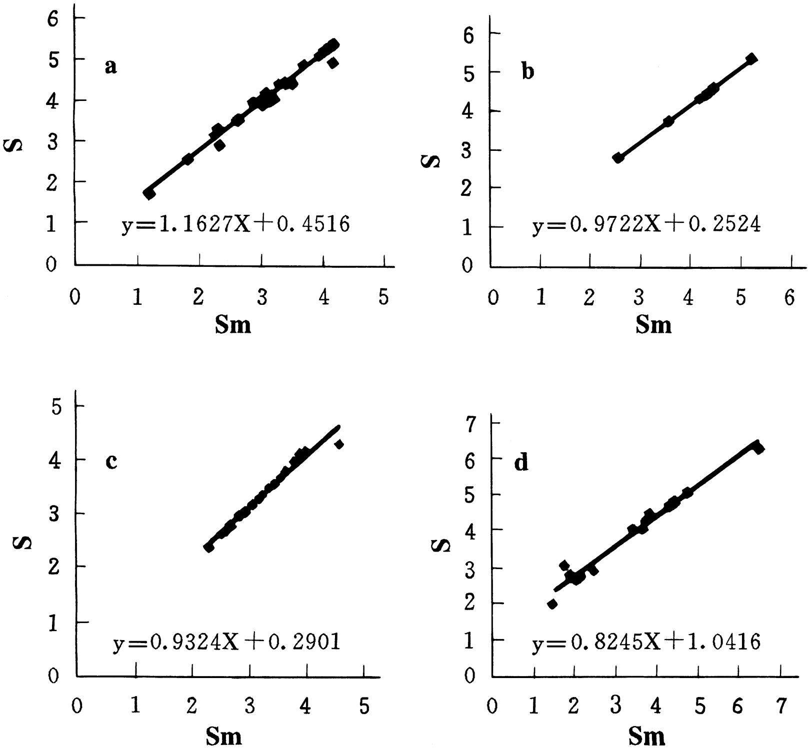

Figure 2. Plots of S vs. S_m ($Z/2.3\phi_m$) for non-homologues solutes system. (a) 24 non-polar benzene and its derivatives. (b) 6 polycyclic hydrogen compounds eluted with methanol water mobile phase on Nucleosil-C18 stationary. The original data was taken from Ref. 21. (c) 22 Aromatic derivatives. The original data comes from Ref. 23. Details were given in Table 1. (d) 16 different types of small molecular including non-polar and polar aromatic derivatives and amino acids, eluted with methanol-water on YQC-CH stationary phase. The original data comes from Ref. 24.

Table 1

Comparison Among Z, S_m, and S for 22 Aromatic Compounds from Reference 23*

Solute	Z	S	S_m
Benzene	3.62	2.95	2.86
Chlorobenzene	4.60	3.75	3.64
Toluene	4.36	3.56	3.45
Bromobenzene	4.82	3.93	3.81
Iodobenzene	5.06	4.13	4.00
Ethylbenzene	4.97	4.06	3.93
O-xylene*	5.81	4.25	4.59
Aniline	2.93	2.38	2.31
Phenol	3.27	2.66	2.58
Methoxybenzene	3.89	3.16	3.07
Benzylalcohol	3.21	2.62	2.54
Benzaldehyde	3.43	2.79	2.71
Nitrobenzene	3.72	3.03	2.94
Benzonitrile	3.75	3.05	2.97
Benzylcyanide	4.03	3.27	3.19
1-Chloro-4-nitrobenzene	4.23	3.45	3.35
1-Bromo-4-nitrobenzene	4.47	3.64	3.53
4-Nitrophenol	3.42	2.78	2.70
1,4-Dimethoxybenzene	4.02	3.27	3.18
2,4-Dinitrotoluene	4.06	3.33	3.22
4-Methoxybenzaldehyde	3.89	3.16	3.07
4-Hydroxybenzaldehyde	3.31	2.66	3.62

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* Denotes $\Delta\phi$, (%) changing between 50 ~ 70. Concentration range ($\Delta\phi$): 40 ~ 70% (v/v): ϕ_m = 55% (v/v). Column zorbax ODS, mobile phase: methanol-water.

Fig. 2b. For these solutes, the values of ϕ changed as follows: benzene (50%~95%), naphthalene and biphenyl (60%~95%), phenanthrene, anthracene and chrysene (70%~95%). Some of the ranges of ϕ are broad, and some are narrow. The slope and intercept for the plot were 0.9722 and 0.2524, respectively, and the correlation coefficient was 0.9996. Similar to the case in Fig. 2a, both the slope and the intercept deviated from the function y = x.

Fig. 2c shows the S vs. Sm relationship for 22 benzene and its derivatives with a mobile phase of methanol and water. The corresponding data is tabulated in Table 1. The original data was obtained from reference 23. Among this set of data, the values of ϕ for the solutes changed from 40% to 70%, except for, o-xylene (ϕ changed from 50% to 70%). As shown in Fig. 2c, the point for o-xylene caused a derivation from the entire linear plot, and resulted in the lower correlation coefficient 0.9865; slope and intercept were 0.7371 and 0.2901, respectively. If this point is excluded, the above three items became 0.9999, 1.0363, and -0.0196, respectively. The latter is much closer to the function y = x.

From the above discussion, the conclusion would be obtained that for small solutes of non-homologous systems, there still exists a good parallel relationship between S and S_m and it is close to the function y = x, as long as the values of ϕ for each solute are limited within a certain similar range.

Another example is shown in Fig.2d for sixteen non-homologue aromatic derivatives and amino acids used as solutes.[24] Methanol and water were used as the mobile phase. This set of solutes includes both non-polar and polar solutes.

The correlation coefficient (0.9833), slope (0.8245), and intercept (1.0416) values all indicated that there is a large deviation from the linear relationship as shown before. This is because of the fact that Z is a constant that is independent of the values of ϕ in the mobile phase, while S, which is the slope of log k' to ϕ in Snyder's empirical equation (Equation 2), can be significantly affected by the changes of ϕ in the mobile phase. This change in S caused by the variations in ϕ are reflected in the linearity between S and S_m shown in Fig. 2d.

CONCLUSIONS

A new correlation was derived to relate Z (stoichiometric parameter of the stoichiometric displacement model for retention of solute) and S (a parameter of Snyder's empirical equation). This relationship indicates that both Z and S can not only be converted to each other, but the physical meaning of S can also be reasonably taken from that of Z.

There is a close relationship between S and S_m ($Z/2.3\phi m$) in isocratic elution. This relationship is influenced by the ϕ_m for each solute in isocratic elution. The equation fits well to S and S_m values for various solutes, especially for homologues, and mobile phases.

The quantitative relationship between S and S_m could almost be expressed as the function y = x. The only condition is that the values of ϕ do not change significantly in the same solute systems. Therefore, not only can Sm and S be converted to each other, but the linearity between S and Z is also quite satisfactory. However, if the difference between the corresponding ϕ_m of the solutes in the system is not very significant, the accuracy of the calculated S by equation 2 is dominated by the excellent linearity between S and S_m but not the kind of the solutes employed.

The accuracy of S_m is independent of the gradient selection mode. Therefore, using S_m to characterize chromatography system can avoid the errors that may be caused by the gradient selection used to derive S_g,, especially, for some biopolymers and proteins.

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