



Review

Chromatographic selectivity triangles

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ABSTRACT

2010 marked the 50th anniversary of the use of selectivity triangles to characterize chromatographic phases. Such plots ultimately identify and quantify the blend of intermolecular interactions that occur between solutes and solvents/phases. The first chromatographic triangle was proposed by Brown and applied to GC stationary phases. Snyder then developed the influential solvent selectivity triangle (SST) based on the gas–liquid partition data of Rohrschneider. The SST was combined with simplex experimental designs to optimize RPLC separations. Subsequent criticisms of the work revolved around the inaccurate predictions that resulted from the SST. These inaccuracies ultimately relate to the inability of the SST to account for the effects of water on the interaction ability of organic solvents. Other criticisms focused on the selection of the three probe solutes (ethanol, dioxane, and nitromethane) that were used to define the apices of the SST. Here, the concerns include the lack of explicit consideration of dispersion interactions and the fact that the three probes do not represent any single intermolecular interaction but rather reflect a blend of intermolecular interactions. The SST approach was modified for NPLC by redefining the triangle apices to reflect the localization, general adsorption, and basicity of NPLC mobile phase modifiers. Because water is generally absent in NPLC, the triangle approach leads to better predictions for NPLC than for RPLC. In subsequent modifications of selectivity triangles, Fu and Khaledi have created a micellar selectivity triangle (MST) based on linear solvation energy relationships (LSERs) and Zhang and Carr have used the Dolan–Snyder hydrophobic subtraction model to create RPLC column selectivity triangles. We end this review by highlighting more recent methods for comparing selectivities and by discussing a new 3D visualization tool for classifying chromatographic systems as having similar or different fundamental energetics of retention and hence having similar or different selectivities.

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1. Introduction

The triangle has been used for tens of thousands of years to represent many rich and complex ideas. Triangles sitting on their base have represented the sun, maleness, and fire while downwards pointing triangles have symbolized the moon, femininity, and water [1]. Alchemists used a horizontal line through an upward triangle to symbolize air, and one through a downward triangle to symbolize earth, thus creating triangular symbols for the four elements: fire, earth, air, and water [2]. Therefore, chromatography could be represented with these symbols because water flowing through layers of earth can cause chemical separations.

While the symbolism of triangles has a long history, the application of triangles to chromatography goes back a mere 50 years. This is quite short in absolute terms, but it represents half the life of chromatography [3]. Chromatographers adopted triangles, prisms, and pyramids for explanatory purposes principally because they allow three or more column characteristics to be incorporated in two-dimensional images.

We begin this review with a brief description of the importance of selectivity in chromatography because many triangle schemes aim at understanding the selectivity of one phase relative to others. The first report of triangles in chromatography is then discussed to set the stage for all subsequent developments. We then examine Snyder's key solvent selectivity triangle and how it has been adapted in various ways for the various modes of chromatography (RPLC, NPLC, GC, MEKC). We end by departing from triangles and propose a different geometric figure, the cube, for examining and comparing selectivity. This shift can perhaps be best understood using an excerpt from Jennifer Michael Hecht's poem "On the Strength of All Conviction and the Stamina of Love" (from the Next Ancient World published by Tupelo Press, copyright 2001. Jennifer Michael Hecht. Used with permission) [4] in which she writes

But they didn't fill
the desert with pyramids.
They just built some. Some.

They're not still out there,
building them now.

.....

Yet we must not
diabolize time. Right?
We must not curse the passage of time.

In this, Hecht suggests that while the form of the pyramid had great symbolic and structural value, a time eventually came to seek new activities and new alternatives. Similarly, the chromatographic triangles that have been built have advanced our understanding of selectivity and guided our selections of mobile and stationary phases. In this review, we hope to shine light on those advances. But we also illustrate the limitations of the technique and propose a new alternative.

2. The importance of the separation factor

The separation factor, α (formerly know as the selectivity factor) is defined as k_B/k_A where k is the retention factor and A and B refer to two solutes for which B elutes after A. The general resolution equation, which relates the plate count (N), the separation factor, retention factors, and resolution (R), shows that resolution is highly dependent on the retention factor, particular at low α 's.

$$R = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k_B}{1 + k_{ave}} \right) \quad (1)$$

For example, a change in α from 1.1 to 1.2 nearly doubles the resolution, whereas it is necessary to increase the plate count four-fold for the same improvement in resolution. Thus, changes to a chromatographic system that differentially affect the retention of a critical pair of solutes are the key focus for improving separations. For purposes of this review we are taking 'system' to include the common variables such as temperature, stationary phase, and mobile phase composition that chromatographers frequently change in order to affect selectivity.

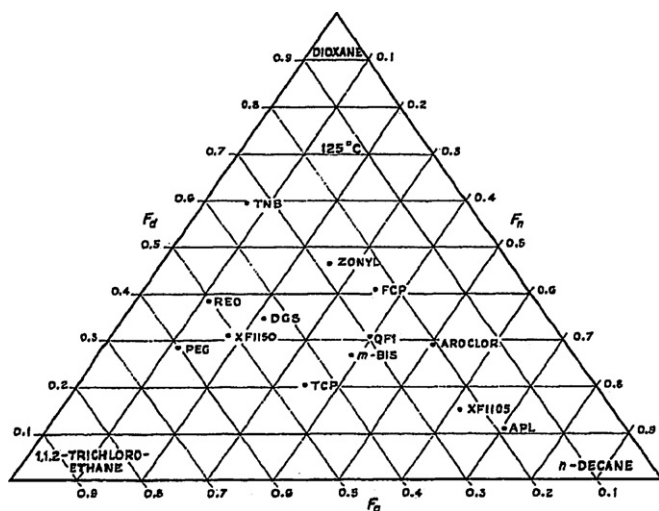


Fig. 1. Classification of GC stationary phases by Brown using dioxane, 1,1,2-trichloroethane, and n-decane as test solutes. F_n , F_a , and F_d are retention fractions measured at 125 °C. APL = Apiezon L (Metropolitan, Vickers); XF 1105, XF 1150 = cyanoethylated silicones (General Electric) with 5% and 50% cyano groups; AROCLOR = Aroclor 1262 (Monsanto) chlorinated biphenol; QF1 = fluorinated silicon (Applied Science Labs); *m*-Bis = *m*-bis-(*m*-phenoxyphenoxy)-benzene (Eastman); TCP = tricresyl phosphate (Albright and Wilson); Zonly = Zonyl E7 (Du Pont) pyromellitic perfluoro ester; DGS = diethylene glycol succinate (Research Specialties Co.); PEG = polyethylene glycol 1500 (Carbide and Carbon); REO = Reoplex 400 (Geigy); TNB = 1,3,5-trinitrobenzene; FCP = diester of tetrachlorophthalic acid and 1-H,1-H,5-H-octafluoro-1-pentanol. Reprinted from [6], with permission from Elsevier.

Comparisons of system selectivity try to help answer the question: when a given system fails to achieve a desired separation, what does the analyst try next? Because all chromatographic separations are ultimately based on a blend of intermolecular interactions (e.g., dipole–dipole, hydrogen bonding, and dispersion), using a system with similar blends of interactions as those demonstrated by the system that failed is unlikely to provide the desired results. Instead, systems that are substantially different in their intermolecular interactions must be sought. Thus, the questions of 1) how to characterize systems in terms of their interaction abilities and 2) how to differentiate one system from another naturally arise. Selectivity triangle schemes that classify, differentiate, and group chromatographic systems have been used to help answer this question. In this review, we analyze various selectivity triangle schemes and how they have been applied to RPLC, NPLC, GC, and MEKC systems.

3. The golden anniversary – 50 years of selectivity triangles

The year 2010 marked the 50th anniversary of the use of triangle schemes to classify chromatographic systems. We make this statement based on the fact that the earliest report along these lines that we could find was from Brown in 1960 [5,6]. He created a triangle to characterize GC stationary phases by defining a parameter, F_n , as

$$F_n = \frac{V_i}{V_n + V_a + V_d} \quad (2)$$

where F_n was called the ‘retention fraction’, V represented retention volumes, and i was n , a , or d which represented the retention volumes of non-polar, electron accepting, and electron donating solutes. The solutes chosen to represent n , a , and d were n-decane, 1,1,2-trichloroethane, and dioxane, respectively. Each phase was thus characterized by three parameters that varied from 0.00 to 1.00. The values were plotted at the apices of a triangle, resulting in Fig. 1.

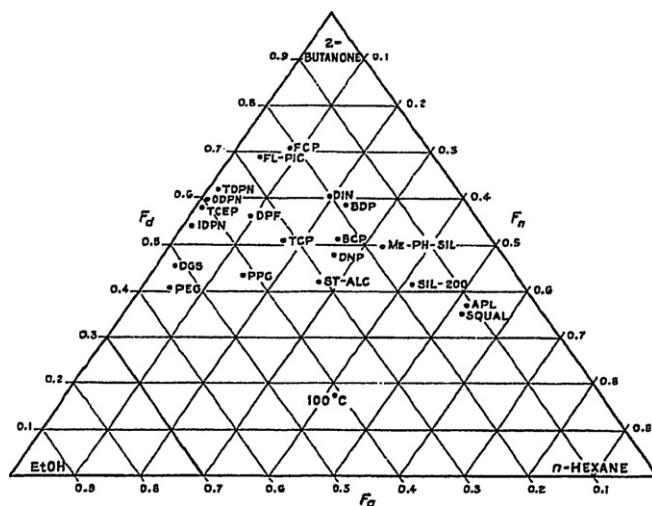


Fig. 2. Classification of GC stationary phases by Brown using 2-butanone, n-hexane, and ethanol as test solutes. F_n , F_a , and F_d are retention fractions measured at 100 °C and 67 °C from literature sources available at that time. APL = Apiezon L; SQUAL = squalane; Sil-200 = Silicone DC-200; Me-Ph-Sil = methylphenyl silicone (General Electric 81705); St-alc = stearyl alcohol; DNP = dinonyl phthalate; BCP = di-*n*-butyl tetrachlorophthalate; BDP = benzyldiphenyl; DIN = di-*n*-octyl ester of 4,4-dinitrodiphenic acid; TCP = tricresyl phosphate; PEG = polyethylene glycol; PPG = polypropylene glycol; DGS = diethylene glycol succinate; DPF = diphenyl formamide; IDPN = imino-dipropionitrile; ODPN = oxydipropionitrile; TDPN = thiodipropionitrile; TCEP = 1,2,3-tris-(2-cyanoethyl)-propane; FL-PIC = fluorine picrate; FCP = diester of tetrachlorophthalic acid and 1-H,1-H,5-H-octafluoro-1-pentanol. Reprinted from [6], with permission from Elsevier.

Brown also used different probe solutes. The results of creating the triangle based on n-hexane, ethanol, and 2-butanone are shown in Fig. 2. He noted that “the position of the triangular graph for a given phase is determined by the choice of the three test compounds, and these can be varied to suit a particular problem.” The influence of the choice of probe solutes is important and will be raised elsewhere in this review with regards to characterizing LC-related systems.

Interestingly, Brown then used an ‘inverse triangle’ (current authors’ description) to characterize the intermolecular interaction abilities of individual steroids. This was done by selecting three chemically different stationary phases – one neutral, one hydrogen bond (HB) accepting, and one HB donating – and using them to define the apices of a triangle. The solutes were then characterized by their affinity fraction, A_i , for each phase via the equation

$$A_i = \frac{V_i}{V_1 + V_2 + V_3} \quad (3)$$

where ‘ i ’ is one of the three columns represented by the numbers 1, 2, and 3. The three phases were SE-30 (silicone), NGS (neopentyl glycol succinate), and QF1 (fluorinated silicone).

Further, by taking the ratio of retention volumes of compounds relative to retention values of an n-alkane of the same size, Brown was able to make the plot shown in Fig. 3. The symbol G is used along the sides of the triangle because the ratio is ultimately related to the free energy of retention of the functional group.

Many of the ideas that Brown introduced would continue to appear in one form or another in subsequent papers using triangular plots to characterize chromatographic systems. Interestingly, though, the exception to this is the application of the triangles in an ‘inverse’ manner for the purpose of characterizing individual solutes.

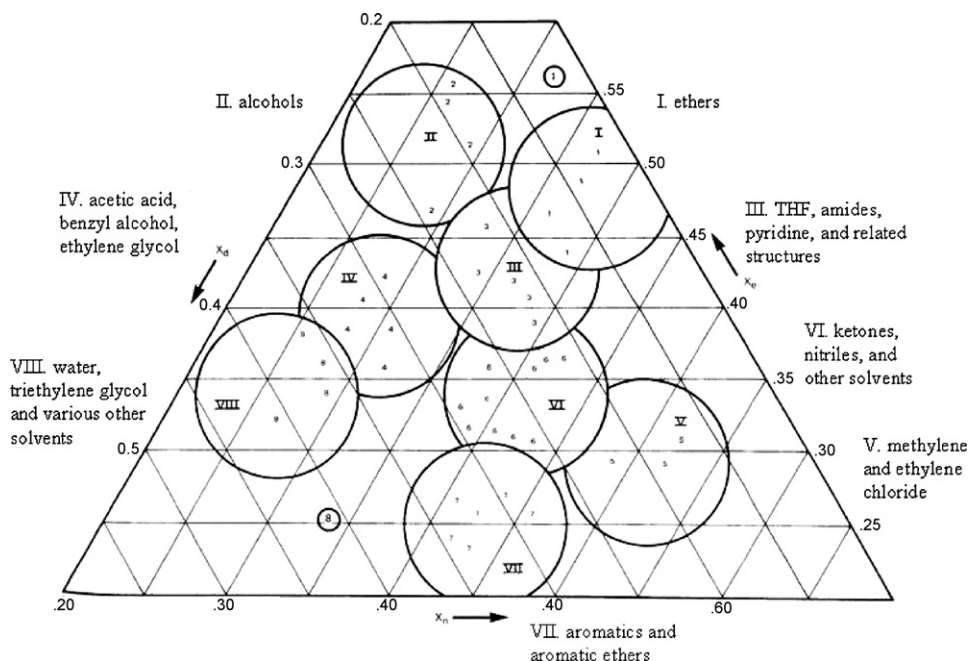


Fig. 4. Snyder's solvent selectivity triangle. Reproduced from the Journal of Chromatographic Science by permission of Preston Publications, a division of Preston Industries, Inc. The solvent listings along the axes were added by the present authors.

interactions. For example, he differentiates between the strength of a solvent and its selectivity by stating "The strength of a solvent depends on its "polarity", or ability to preferentially dissolve more polar compounds such as nitriles and alcohols. Solvent selectivity refers to the ability of a given solvent to selectively dissolve one compound as opposed to another, where the 'polarities' of the two compounds are not obviously different" [7].

While the 1974 publication explained the derivation of the parameters and subsequent triangle plot, the 1978 publication is notable because;

- (1) Most importantly, whereas the 1974 publication focused on common, volatile organic solvents related to LC, the 1978 publication was extended to include GC stationary phases,
- (2) Snyder offers a defense of using just three test solutes to classify solvents. Two additional solutes (methylethyl ketone and toluene) were examined as part of this analysis,
- (3) Assertions are made regarding the relative unimportance of dispersion interactions to selectivity, and
- (4) Snyder defends the groupings by showing the overall deviations of X_i values from their averages are generally within 0.03 units (one SD), or 0.015 if groups are further subdivided.

Thus, the 1978 publication simultaneously corrected, refined, bolstered, and expanded the SST scheme presented in the 1974 publication.

4.2. The SST and GC phases

As noted above, Snyder extended the SST to GC phases [10] by using the conversion

$$\log K''_{g,i,corr} = \frac{b}{100} \Delta I_i \quad (6)$$

where 'i' is ethanol, dioxane, or nitropropane, $\Delta I_i = I_{n,PH} - I_{n,SQ}$ where PH stands for the phase of interest and SQ represents squalane, and 'b' is the logarithm of the retention time increment per methylene unit added to a solute and is specific to the phase being studied. It was further noted that

$\Delta I_{\text{nitromethane}} = 1.18 \Delta I_{\text{nitropropane}}$, allowing for $\Delta I_{\text{nitropropane}}$ to be calculated if it were not in the data sets of Rohrschneider [8] or McReynolds [12,13] for various phases. Based on these $\log K''_g$ values, the parameters for X_i could be calculated for GC phases. Snyder presented X_i values for diethylhexyl sebacate, diisodecyl phthalate, tricresyl phosphates, carbowax 20, diethyleneglycol succinate, and tris-cyanoethoxypropane.

Klee et al. [14] developed a selectivity triangle for GC phases defining X_i as

$$X_i = \frac{\Delta I_i}{\Delta I_e + \Delta I_n + \Delta I_d} \quad (7)$$

In an interesting modification of the SST for GC phases, they used the sum of the three ΔI_i values to add another dimension to the triangle plot as shown in Fig. 5. This was done to indicate the overall polarity of phases in addition to the relative importance of the various specific interactions. Klee et al. also noted that for the best range of GC selectivities, it would be ideal to have phases with large $\sum \Delta I$ values in combination with points near the apices of the triangles, with the implication being that at that time, such a range of phases was not available.

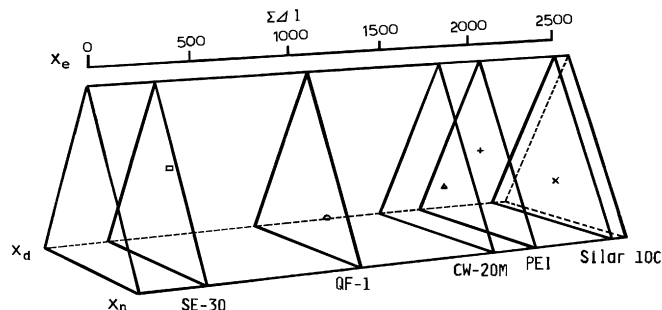


Fig. 5. A selectivity prism in which the sum of retention indices ($\sum \Delta I$) for ethanol, nitromethane, and dioxane is used to add another dimension to a selectivity triangle defined using those same solutes (see text for definitions of X_e , X_d , and X_n). CW-20M = Carbowax 20M.

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4.3. Teas diagrams

While the rest of this review focuses on the development, application, and analysis of chromatographic selectivity triangles, we briefly note here that Teas [15] published a solvent triangle in the years between the appearance of Brown's and Snyder's work. His triangle was based on the work of Hansen [16] who used solubility parameter and regular solution theory to define three solvent parameters, δ_d , δ_p , and δ_h to quantify the dispersion, polarity, and hydrogen bonding properties of solvents, respectively. Teas used these three parameters as the axes for his solvent triangle. He applied his triangle to make predictions about which solvents or solvent mixtures would solubilize polymeric resins. It is interesting to note that Teas diagrams (as they are called) are used in the field of art restoration to guide the selection of solvents to remove varnishes from old paintings [17]. For example, a Teas diagram was used in the 1994 restoration of Johannes Vermeer's *The Girl with a Pearl Earring* [18].

5. Impact of Snyder's solvent characterization scheme

5.1. The chromatographic optimization factor

A number of publications using Snyder's solvent triangle as a basis for optimizing chromatographic separations were published in the 1980s. The main impact of Snyder's work was in defining three solvents that were deemed to have different selectivities. For example, in RPLC, methanol, acetonitrile, and tetrahydrofuran were located in fairly distinct regions of the solvent triangle. For this and other reasons, these solvents were used in addition to water to optimize separations, often to the exclusion of other solvents.

Consistent with this, Glajch et al. proposed the chromatographic optimization factor (COF) as the basis for triangles related to maximizing separations [19]. The COF is defined as

$$\text{COF} = \sum_{i=1}^k A_i \ln \frac{R_i}{R_{id}} + B(t_M - t_L) \quad (8)$$

where R_i is the resolution of the i th pair of solutes in a mixture, R_{id} is the ideal desired resolution, t_M is the maximum acceptable analysis time, and t_L is the experimental time. A_i is an arbitrary weighting factor that allows greater emphasis on some critical pairs relative to others. B is also an arbitrary weighting factor. The function is constrained so that if $R_i > R_{id}$ then R_i is set equal to R_{id} , and if $t_M > t_L$, t_M is set equal to t_L . Using these definitions and constraints, the COF goes to zero for separations that meet all of the requirements. Negative values indicate less desirable separations – the larger the negative, the less desirable. This approach grew out of the chromatographic response functions (CRF) of Morgan and Deming [20] and subsequent improvements proposed by Watson and Carr [21]. Glajch et al. acknowledge limitations of the COF as the basis for solvent optimization. For example, it does not explicitly take note when peak elution order changes with different mobile phases. Furthermore, separations with overlapping peaks can have the same COF value as those with the expected number of peaks because the model does not 'know' how many peaks should be found – it simply measures the separation of the observed peaks.

Also in this report, a simplex design [22] involving ten test runs, shown in Fig. 6, was used to optimize a three-solvent system (represented by A, B, and C) for a solute mixture of nine substituted naphthalenes. In this figure, A, B, and C were mixtures of methanol/water (MEOH, 63:37%, v/v), tetrahydrofuran/water (THF, 39:61%, v/v), and acetonitrile/water (ACN, 52:48%, v/v), respectively. Seven of the runs (labeled 1–7) were used to make predictions of separations while the remaining three runs (8–10) were used to test the accuracy of the predictions. In subsequent optimiza-

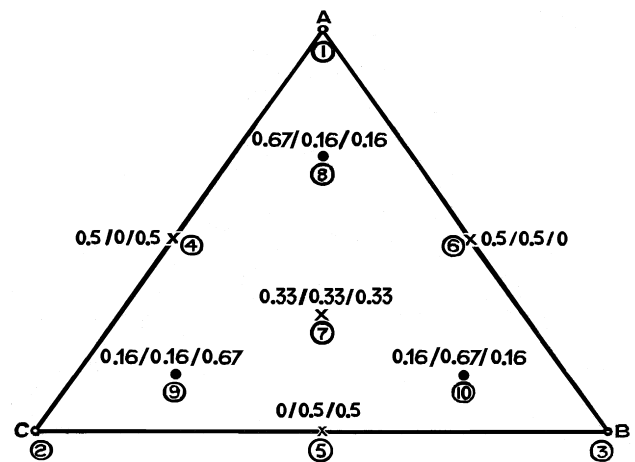


Fig. 6. Simplex experimental design involving seven training mobile phases (1–7) and three mobile phases used to test the accuracy of the predictions. A, B, and C were mixtures of A = methanol/water (63:37%, v/v), B = tetrahydrofuran/water (39:61%, v/v), and C = acetonitrile/water (52:48%, v/v). Reprinted from [19], with permission from Elsevier.

tion schemes involving three mobile phases, the three confirmatory analyses were dropped, leaving a seven-run optimization design. In the COF in this study, $B = 0$ and $A = 1.0$, indicating that time of analysis was not a concern and the separation of all adjacent pairs was taken to be equally important. The COF was evaluated at three R_{id} values of 1.2, 1.8, and 2.4 and plotted in a triangle scheme shown in Fig. 7. The optimum separation was found with 61%ACN : 39%THF.

5.2. Overlapping resolution mapping for RPLC

Due to limitations of the COF and difficulty extending it to mixtures with more solutes, the authors developed overlapping resolution maps (ORM) [19]. The ORM compares the resolution of every pair of peaks in a chromatogram obtained for each solvent mixture tested. A contour triangle map is used to estimate the resolution for each pair in all compositions. Any area of the map with a resolution less than the desired resolution for that pair is shaded in and areas with "excess" resolution are left clear. The maps for all adjacent pairs of compounds are overlaid and any area that remains unshaded provides a solvent composition that could separate the mixture with the desired resolution. Such an analysis

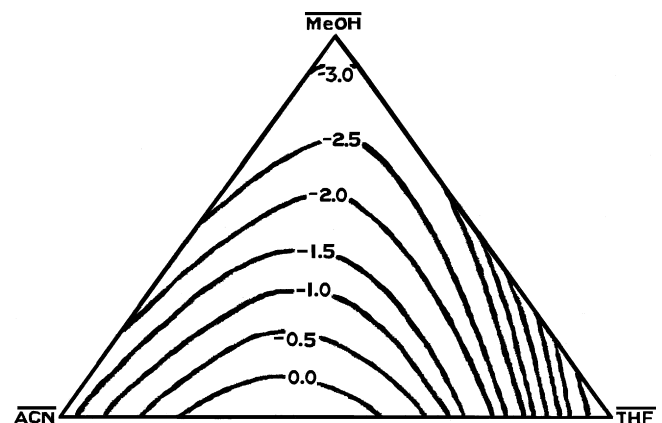


Fig. 7. Chromatographic optimization factor (COF) plot based on simplex experimental design for the separation of nine substituted naphthalenes. MEOH : methanol/water 63:37% (v/v); THF : tetrahydrofuran/water, 39:61% (v/v); and ACN : acetonitrile/water, 52:48% (v/v). Conditions: 15 cm × 4.6 cm Zorbax-C₈ column, 2.0 mL/min, 40 °C, UV photometer, 254 nm. Reprinted from [19], with permission from Elsevier.

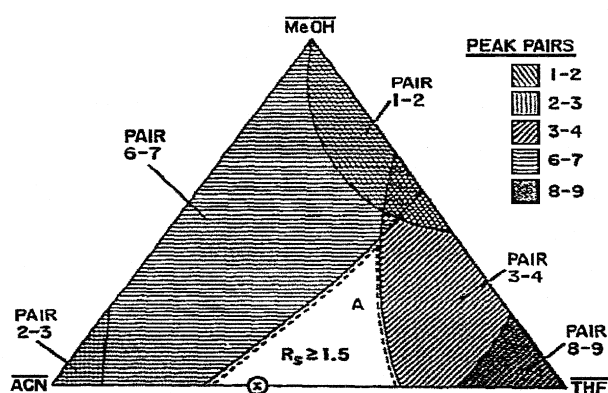


Fig. 8. Overlapping resolution maps (ORM) based on simplex experimental design for the separation of nine substituted naphthalenes. Shaded areas represent solvent mixtures that will not resolve the indicated pair to a resolution of 1.5. The five most difficult solute pairs to resolve are shown in the plot. MeOH : methanol/water 63:37%, (v/v); THF : tetrahydrofuran/water, 39:61% (v/v); and ACN : acetonitrile/water, 52:48% (v/v). Conditions: 15 cm × 4.6 cm Zorbax-C₈ column, 2.0 mL/min, 40 °C, UV photometer, 254 nm. Reprinted from [19], with permission from Elsevier.

was performed with the retention data for the nine naphthalene derivatives to yield the triangle plot in Fig. 8 [19]. On this plot, the optimum solvent mixture that was predicted by the COF method (designated with a ⊗) is included in the solvent mixture region generated by ORM. The authors go on to demonstrate their method using a literature data set of fifteen benzene derivatives [23].

For this approach to work, it is necessary to perform peak matching for each of the seven starting runs in order to identify any peak cross-overs. Then the retention times and peak widths (or calculated peak widths) can be used to calculate the resolution of any critical solute pairs for every composition within the triangle.

This approach was based on the optimization of only three solvents (mixed with the fourth solvent, water). The choice of optimizing three parameters was based on the conclusion from the original SST work that only three general solvent characteristics affect selectivity. Better resolution may be achieved by including more solvents or optimizing any additional variables such as temperature that also influence selectivity. However, including additional variables inflates the number of 'training chromatograms' required by the simplex design, with a subsequent increase in the labor and time required to optimize the separation.

In 1983, Glajch and Kirkland noted that the effects of different stationary phases, temperature, pH, ionic effects, and secondary equilibria such as ion-pairing could be incorporated into LC optimization schemes [24]. This publication includes a 3D visualization involving triangle schemes (see Fig. 9). It resulted from adding the actual predicted resolutions in the third dimension rather than just shading in regions below a certain threshold value as in the 2D triangle plots shown in the previous figure.

5.3. Overlapping resolution mapping for NPLC

Glajch et al. extended the ORM approach to optimizing the NPLC separation of thirteen substituted naphthalenes on bare silica particles [25]. The selection of the three mobile phase additives (methylene chloride, acetonitrile, and methyl tert-butyl ether, all mixed in hexane) was based on a new triangle scheme designed to account for effects that are important in NPLC. Specifically, basic polar solvents (e.g., methyl tert-butyl ether, MTBE) localize on the solid surface through direct hydrogen bonding with the surface. Other solvents with diminished basicities, such as acetonitrile (ACN), also localize on the surface but in a different manner than do the basic polar solvents. Both types of solvent localization create

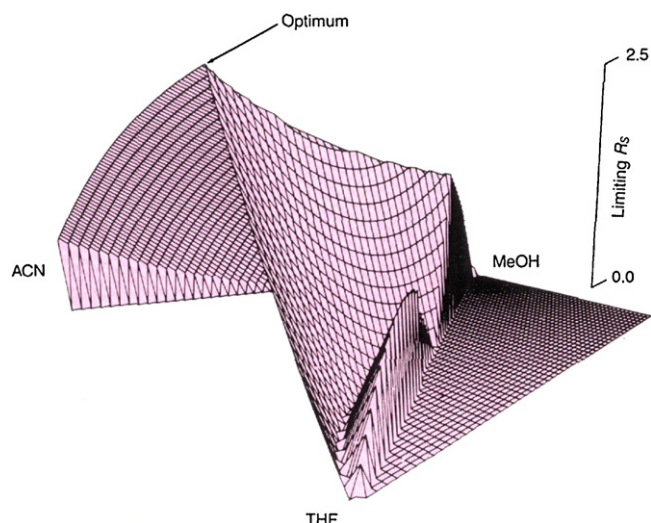


Fig. 9. A three-dimensional rendering of the ORM created by displaying the resolution for the most poorly resolved solute pair in the vertical direction. Based on the data from the previous figure. Reprinted with permission from [24]. Copyright 1983 American Chemical Society.

competition for surface sites with solutes which can also localize. The differences in the specific type of localization yield different effects on selectivity. A third class of solvents which do not demonstrate localization effects, but rather appear to adsorb to the surface in a more general manner was also identified. The three solvent properties (i.e., non-localizing, localizing basic, and localizing non-basic) were used as the apices to create an NPLC-specific triangle. Methylene chloride, MTBE, and ACN were used to represent the three properties, respectively, in a simplex optimization scheme. We go into more detail about the influence of localization effects on selectivity below. What is important to note here is the application of optimization schemes based on selectivity triangles to normal phase separations.

5.4. Gradient elution overlapping resolution mapping

Kirkland and Glajch extended the ORM approach to include gradient elution [26]. They did so by adding a third dimension – solvent strength – to the two-dimensional triangle plots. In the 2D plots, all three apices were selected to have comparable solvent strengths. Therefore, all that varied within the triangle space was selectivity. By turning the triangle into a prism (see Fig. 10), solvent strength was added along the third dimension such that any vertical slice of the prism parallel to the ends of the prism reflects mobile phase systems of comparable solvent strength. Varying solvent strength and selectivity allows gradient elution separations to be optimized in much the same manner as described for isocratic optimizations. Seven mobile phase gradients were used to collect resolution data for fourteen compounds. Estimates of resolution at other gradients were obtained via quadratic equations based on the original seven compositions and used to create resolution contour maps for individual pairs of solutes. An overlapping resolution map (now 3D) then indicates the position along the gradient and the solvent composition that yields the maximum predicted resolution. While each slice of the prism represents a different solvent strength, proceeding through the prism along any one line of solvent strength (e.g., line 7 in Fig. 11) does not change the selectivity of the mobile phase [26]. Thus, analyses using such gradients were termed 'isoselective multisolvent gradient elution' (IMGE). The authors note that the chromatogram obtained with the predicted gradient achieved a resolution of 2.0 or greater for the fourteen compounds in under fifteen minutes (15 cm × 0.46 cm column, Zorbax C-8, 3.0 mL/min,

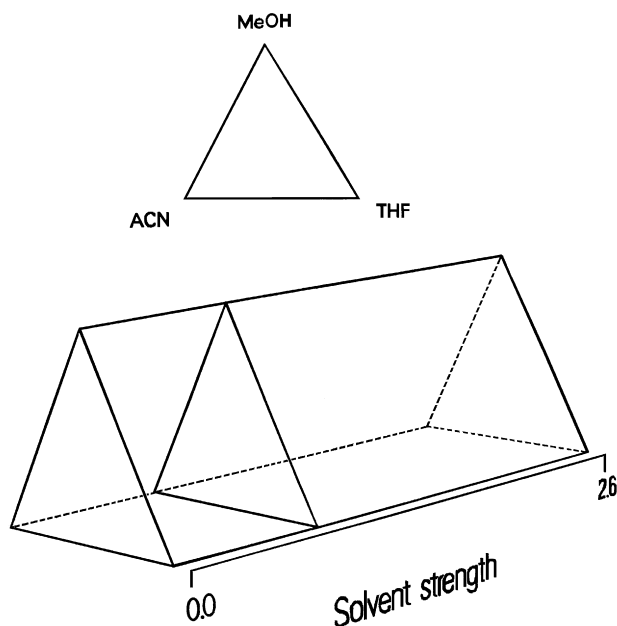


Fig. 10. Solvent strength prism for gradient elution with an isocratic selectivity triangle for one solvent strength. Apices are methanol (MeOH), acetonitrile (ACN), and tetrahydrofuran (THF). Reprinted from [26], with permission from Elsevier.

35 °C, particle size not shown) and that this was better than any of the seven gradients used to establish the analysis.

It is more typical in gradient elution to simultaneously vary selectivity and solvent strength. Kirkland and Glajch used the term 'selective multisolvent gradient elution' (SMGE) to describe this approach [26]. Visual inspection of the seven initial chromatograms resulted in a gradient depicted in Fig. 12. The chromatogram obtained with this gradient yielded even better separation for all peaks and resulted in a different elution order for some of the pairs. The authors did note, however, abrupt baseline changes corresponding to the abrupt changes in mobile phase composition depicted in the figure. Nonetheless, with seven training gradients selected based upon Snyder's original selectivity triangle (to select the three organic solvents) and simplex experimental design protocols, the authors were able to systematically select a quaternary mobile phase gradient that allowed for complete separation of all compounds.

5.5. Additional work on optimizations

Shortly after Kirkland and Glajch published the prism scheme for optimizing gradients elution, Sticher and co-workers [27]

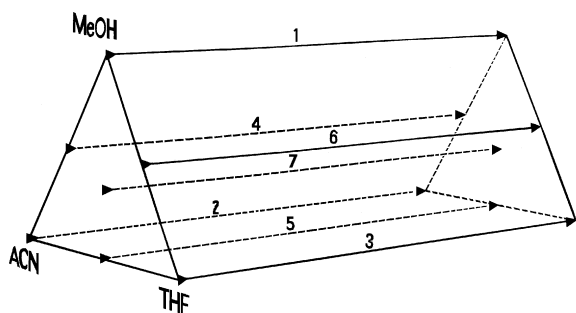


Fig. 11. Experimental design for seven gradient elution chromatograms to obtain data for optimization calculations. See original reference for solvent compositions. Reprinted from [26], with permission from Elsevier.

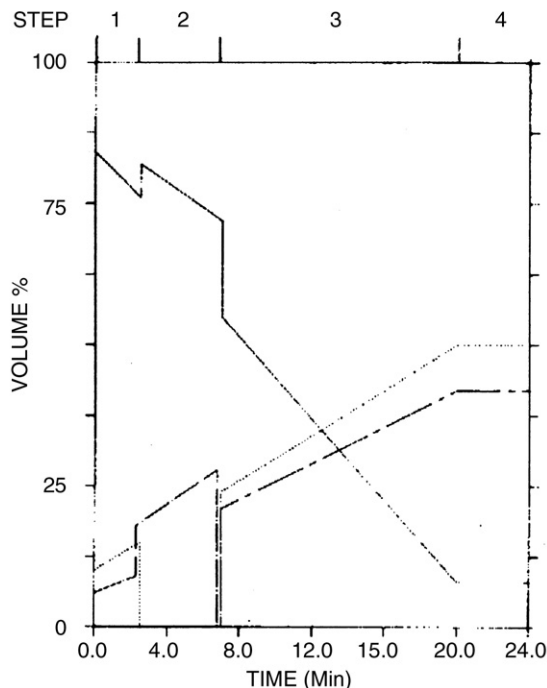
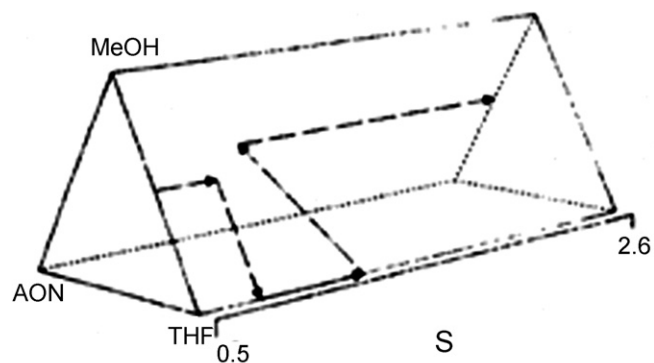


Fig. 12. Representation of solvent program for step-selectivity gradient solvent system. (—) Water; (···) methanol; (---) acetonitrile; (- - -) tetrahydrofuran. Reprinted from [26], with permission from Elsevier.

presented an approach that slightly reduced the complexity of calculation and the number of training chromatograms required (down to four) to obtain optimal solvent strength and selectivity for isocratic separations. They demonstrated this approach with a relatively simple mixture of four flavonoid glycosides. Different groups have suggested from four to ten or more training experiments. The choice naturally depends on the accuracy of the predictions that is required. More training experiments will be required for greater accuracy, separations with larger numbers of analytes, and separations involving analytes with closely related structures.

Whereas Glajch, Kirkland, Squire, and Minor's ORM approach strives to obtain a solvent mixture that maximizes COF (related to $\ln[R_i/R_{id}]$) for all components (if weighting factors are not used), O'Hare and co-workers modified this approach to focus on relative retention rather than on absolute retention as a function of solvent composition [28,29]. In their reports, the parameter that is related to solvent composition is $\ln(RT_o/RT_n)$ where RT_o is the retention time of an internal standard and RT_n is the retention time of various components. Separate polynomial equations are obtained for each compound in the mixture based on seven training chromatograms selected in a manner akin to that used by Glajch et al. based on

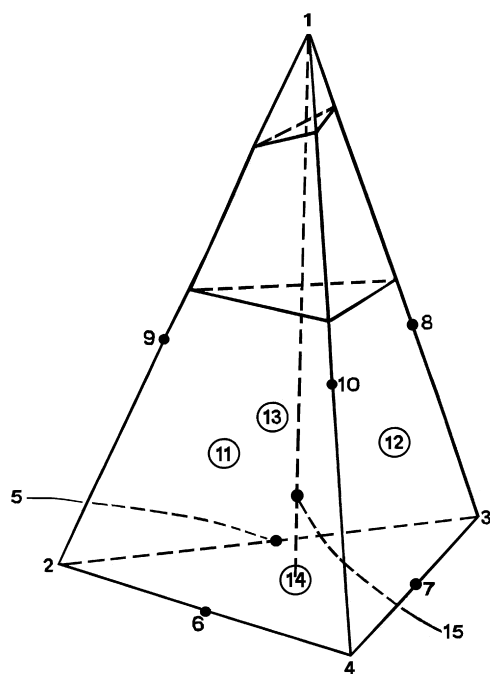


Fig. 13. Statistical design for optimizing isocratic elution using four solvents. The numbers indicate individual mobile phases in which retention data for all solutes must be obtained. The authors note that in RPLC, some water-rich and water-poor phases can be eliminated, reducing the number of training chromatograms to twelve.

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simplex designs. The authors stated interest was in analyzing mixtures of adrenocortical steroids, with a primary requirement “to separate and measure aldosterone without interference from other unrelated steroids [that were in the mixture] together with the resolution of 18-hydroxysteroid congeners of aldosterone, 18OH-B and 18OH-A.” This goal necessitated the shift from overall resolution to one that required specific attention on critical solutes, hence the emphasis on individual retention times rather than on resolution mapping for all components obtained via an ORM. The authors acknowledge that ORM can be adjusted to focus on critical analytes by excluding solvent selectivity areas corresponding to pairs of minor importance, but they noted some problems associated with this for their particular sample of interest. Using their approach, they were able to identify a mobile phase composition that achieved their goals.

Interestingly, they extend their analysis to consider the requirements of optimizing four-component systems (the above studies have four-components – water, methanol, THF, and ACN – but each apex of the triangle upon which the approach is based is actually a mixture such as water/MeOH, etc.). A four component system could include the four pure solvents, or perhaps involve another water/solvent mixture such as water/dioxane. If a four-component system were considered, simplex optimization dictates the need for fifteen training chromatograms as shown in Fig. 13 [28]. The time requirements and subsequent complexity of the data analysis for such an optimization become much more cumbersome than those for ternary systems and often are unnecessary, particularly because most of the theoretical optimizations we have focused on here result in isocratic mobile phases and therefore do not take advantage of the practical benefits of gradient elution.

6. Failures of and modifications to the selectivity triangle

In this section we discuss challenges to the SST that appeared in the literature. The problems that were found when applying the

SST to RPLC arise largely because of the effects of water, although challenges were also made to the application of triangles to NPLC and GC. In RPLC, water is present in varying amounts in the mobile phase. This has three main effects: (1) increasing water content increases the overall polarity of the mobile phase and thereby alters the selectivity of the separation, (2) the water modifies the ability of the organic mobile phase additives to interact with solutes and these alterations affect different solutes to different extents and (3) the water itself interacts differently (i.e., selectively) with different solutes. None of these effects is captured in the SST because the SST was based on pure organic solvents, not solvents modified with water.

The work of Carr and co-workers [30,31] and El Seoud and co-workers [32] illustrates some of these complexities. Non-linearities in the frequency of maximum absorbance of solvatochromic dyes vs. percent water in methanol/water and acetonitrile/water mixtures are observed. These non-linearities are attributed to both microheterogeneity and to preferential solvation effects [30–32]. Furthermore, the nature and extent of these effects depend both on the organic modifier and the composition of the mixture. For example, acetonitrile/water mixtures were found to be dominated by solvent clustering between 30% and 80% acetonitrile [32–37]. In methanol/water mixtures, however, Shulgin and Ruckenstein [38] assert that if any clusters exist, they are small. While solvent clustering may not be extensive in methanol/water mixtures, the spectroscopic studies of Carr and co-workers [30,31] and El Seoud and co-workers [32] suggest that preferential solvation of solutes may still occur. Regardless of which effects exist within specific aqueous mixtures, neither the effects of microheterogeneity nor preferential solvation on solute retention are incorporated in the SST. Thus, the SST may not produce accurate predictions of selectivities when aqueous mobile phases are used. Examples of this are discussed below.

The failures of the SST arising from the presence of water in RPLC do not carry over to NPLC because the water content in NPLC mobile phases is generally minimized. Thus, predictions of NPLC mobile phase selectivity based on triangle schemes, when specific solvent localization and basicity effects are taken into account, are generally much more reliable than those in RPLC.

The other major challenges considered in this section revolve around (1) the influence of interfacial adsorption and inadequate retention of the test solutes in GC and (2) the number and specific nature of the test solutes used to create selectivity triangles. The focus here is on the importance of incorporating dispersion interactions and the influence that using different test solutes has on the position of solvents within the triangle (i.e., their overall classification and grouping).

6.1. Steroids and polystyrene oligomers

While Snyder's solvent selectivity triangle had an important impact on LC solvent selection as demonstrated by the above optimization methods, others discussed the limitations and failures of the approach. For example, West described the failure of the solvent selectivity triangle to group solvents according to their selectivity for resolving aromatic compounds and steroids using RPLC [39,40]. Lewis et al. made the same observation for polystyrene oligomers [41].

In his work related to steroid separations in RPLC, West noted that the slopes of steroid retention factor (measured using 2-ketoalkanes as standards akin to Kovats GC-based retention indices using n-alkanes) vs. volume fraction of organic solvent showed considerable variability for solvents from the same selectivity group. Specifically, he noted the average slope for twelve steroids was 2.3 times greater for 1-propanol than for methanol, which are in the same solvent group in the triangle. He also noted that the slopes

were sometimes more similar for solvents in different groups than within groups. For example, the average slope (again over twelve steroids) for 2-methoxyethylacetate (Group VI) was closer to that for tetrahydrofuran (Group III) than it was for acetonitrile (also Group VI). Other such examples are provided in his article [40].

The resolution of particular pairs of steroids in aqueous mobile phases with different organic modifiers of comparable solvent strength was also studied. For spironolactone and ethisterone, the resolution obtained with 2-ethoxyethanol, 2-methoxyethanol, and tetrahydrofuran (all in Group III) was 0.68, 1.15, and 3.26, respectively. It should be noted that the mobile phase composition was adjusted such that the first peak eluted with a retention factor of 2.00 ± 0.03 to ensure comparable mobile phase strengths. Within Group VI solvents, the resolution with dioxane, 2-methoxyethylacetate, and acetonitrile was 1.70, 0.87, and 0.59, respectively. West also notes that the resolution obtained with solvents from different groups is often more similar than that obtained with solvents within the same group. For example, the resolution of prednisone and hydrocortisone in THF ($R_s = 1.81$) and 2-methoxyethanol ($R_s = 1.93$), both from Group III, was more similar in ethanol ($R_s = 1.88$) from Group II than in another group III solvent, 2-ethoxyethanol ($R_s = 2.54$). Similar observations were made for spironolactone and ethisterone.

West states that these observations “contradict the theory of the solvent selectivity triangle concept” [40] and then goes on to suggest that the discrepancies result from the fundamental assumption that dispersion interactions do not play an important role in determining solvent selectivity for solutions of polar solvents. Certainly, given the structural similarity of the steroids in this study, it is reasonable to suggest that their overall characteristics regarding polarity and hydrogen bonding are comparable enough that even small differences in dispersion interactions in the solvents, if not accurately corrected for, could play a critical role in solvent selectivity.

Focusing on dispersion only, however, neglects the more important effects that water has on solvent selectivity. Specifically, Snyder's groupings are based on Rohrschneider's data, which were collected for pure solvents. In contrast, West used binary mixtures of solvents with water as the diluent. It is well known that water is hardly an ‘inert’ solvent and can significantly alter the properties of bulk organic solvents. Furthermore, it does so in different ways depending on the organic solvent and the percent composition of the mixture as discussed above with regards to preferential solvation and microheterogeneity. These variations could very well cause a difference between the group that a pure solvent would be in compared to that of aqueous mixture of the same solvent. The adjustment of solvent strength to obtain a retention factor of 2.00 for the earliest eluting peak is an arbitrary choice and required different amounts of water for different solvents. Clearly, the amount of water and its alterations of organic solvent characteristics will significantly impact retention of polar and hydrogen bonding solutes as compared to its impact on non-polar compounds. Thus, varying amounts of water will influence the selectivity of the separation in ways that the SST scheme for pure solvents does not incorporate and cannot accurately predict.

West does not comment directly on the influence that different amounts of water in the mobile phase have on selectivity. But in recognition of the possibility that dispersion plays an important role in selectivity, and also in consideration of the assumption that the stationary phase does not affect separations, he states

“Perhaps these assumptions have resulted in an oversimplified approach to characterizing selectivity, or perhaps the three test solutes that were used to establish the solvent triangle do not adequately encompass all of the important characteristics that

contribute to experimentally observed selectivity for more complex molecules.” [40]

In fairness to Snyder's selectivity triangle, it must be pointed out that it was not intended to be used in the way West applied it. It was a general scheme for classifying solvents to facilitate the selection of solvents that are broadly different in the way they interact with a wide range of solutes of varying chemical characteristics. It was not designed to predict the best solvent for resolving individual pairs of closely related solutes. Nevertheless, West's findings call into question the overall similarity of some of the solvents in various groups, as well as highlight the potential effects of water and dispersion interactions on selectivity (see below for more on the topic of dispersion).

The work of Martire and co-workers [37] is interesting as it relates to West's criticism that the SST fails to account for the role of the stationary phase. Using alkylbenzenes as test solutes, activity coefficients from the literature, and experimental measures of retention volumes, Martire et al. calculated contributions to the methylene unit selectivity arising from the mobile and stationary phases as a function of percent modifier in methanol/water and acetonitrile/water mobile phases. They show that the stationary phase contribution with both modifiers is comparable in magnitude and essentially constant from 5% to 60% water. The contribution from the mobile phase, however, varies significantly over that range, and is considerably larger than the stationary phase contribution at all compositions. Tan and Carr provide a comparable result based on the analysis of mobile and stationary phase cohesive energy densities for systems involving methanol, acetonitrile, and tetrahydrofuran. They state that “As the fraction of water is increased, the cohesive energy density of the mobile phase increases substantially. However, changes in the cohesivity of the bonded phase, which are largely controlled by the sorbed solvent, are minor” [42]. These results suggest that assuming a constant (and relatively unimportant) contribution to solvent selectivity arising from different modifications of the stationary phase due to different organic additives may be a reasonable approximation. Here again, it is important to remember that the SST ultimately deals with solvent selectivity. Thus, while the stationary phase clearly makes an important contribution to the overall retention of solutes, stationary phases modified with different solvents may be comparable enough in their characteristics that differences in the mobile phases alone are more important to overall selectivity differences. If this is the case, West's concerns about the role of the stationary phase may be overstated. We note here, however, that the changing structure of the stationary phase and the modification of the alkyl chains and surface silanol groups by sorbed solvents is clearly an important aspect of RPLC retention. Tan and Carr [42] provide an extensive discussion of the influence of sorbed water and modifier on mobile and stationary phase properties and how they contribute to changes in solute retention. An analysis of the effects of solvent sorption in general, and of their work in particular, is outside the scope of this review, but the reader is encouraged to consult their article.

In fairness to West, it must be noted that he acknowledged the possibility that the structural similarity of the steroids and polystyrene oligomers used in previous studies was the major factor behind the discrepancies between groupings and selectivities that he observed. To address this, he conducted another study with sixteen aromatic compounds (13 monosubstituted and three positional isomers) using aqueous mobile phases of twelve solvents ranging in P' values from 3.9 to 7.2 from three groups in the solvent triangle (II, III, and VI). The binary mobile phases were adjusted to yield retention factors of 4.00 ± 0.04 for benzene in an effort to keep solvent strength constant. Again West used retention indices based on 2-ketoalkanes to measure retention. He noted that the retention

indices of some compounds in some solvents were more comparable in solvents from different groups than in solvents within the same group, leading him to state “in general, there was very little or no correlation between retention indices and the solvents grouped according to the selectivity triangle concept” [39]. He also measured resolution of various compound pairs, noting

“the results of this study confirmed that solvents in the same selectivity group seldom give similar resolution, even at consistent solvent strength. . . Numerous examples of extreme variation of R with the solvent groups are evident, with resolution frequently being more alike for solvents classified in different groups than for those within a given group.”

Here again, different amounts of water were required to achieve comparable solvent strengths for the elution of benzene. As noted above, water preferentially alters the selectivity of polar and hydrogen bonding solutes. It does so through the three mechanisms discussed in the introduction to this section, namely, a general increase in mobile phase polarity with increasing water, modification of the solvent interaction abilities, and direct interaction with solutes. This suggests that an expansion of Snyder's triangle to include mixed solvents would provide valuable chemical insight into the effects of water on the properties of common organic solvents. It would also increase the predictive power of the triangle with practical implications for RPLC.

West, however, might reject this idea as his writings indicate a fundamental objection to the construction of the triangle, namely that “it is constructed using data that does not correlate with resolution” and that specifically “the use of fractions of summed retentions actually serves to hide differences in selectivity by masking absolute differences in retention units” [39]. He notes that these criticisms extend to the classification of NPLC solvents and GC stationary phases as well. West proposes instead that his approach (not discussed here but developed in his publication) using differences in retention indices, which clearly shows the selectivity differences between solvents, correlates better with experimentally observed resolution and this provides better predictions and better separations.

In contradiction to West's claims, Snyder et al. [43] cite a presentation given by Starcevic at the 15th International Symposium on Column Liquid Chromatography (Ref. [23] in the cited work) that the selectivities for a different series of compounds did correlate with predictions from the SST. Snyder et al., however, do not specify the series, and the authors of the present article did not find any publications by Starcevic to support the claims.

6.2. A note about dispersion

We mentioned above that the SST is based on the assertion that dispersion interactions in solutions of polar solvents do not contribute significantly to solvent selectivity. It is important to note that this is very different than saying that dispersion interactions do not contribute to overall gas/liquid partitioning or chromatographic retention. In fact, Snyder used n-alkanes of varying size to try to remove dispersion interactions in the formation of the SST. However, a brief examination of the overall importance of dispersion interactions is warranted.

Using regular solution theory [44,45], it can be shown that dispersion interactions do not cancel when considering solvent selectivity for gas–liquid partitioning. Specifically, when comparing the selectivity for two non-polar solutes (e.g., pentane and hexane) offered by two different non-polar solvents (e.g., benzene and toluene), selectivity differences between the two solvents exist. According to regular solution theory, these differences arise from (1) differences in the molar volumes of the solutes, (2) difference in the solutes' solubility parameters, (3) differences in the product of

the molar volume and the dispersion-related solubility parameter for the solutes, and (4) differences in the solvents' solubility parameters. In other words, according to this theory, dispersion effects do not cancel as they relate to solvent selectivity. The extent of their importance depends on the combination of solutes and solvents being considered. We did some simple calculations involving various combinations of hexane, pentane, benzene, and toluene as solutes and solvents. The most dramatic effect was observed using hexane and toluene as solutes and pentane and benzene as solvents. In this case, our calculations using regular solution theory suggest that the selectivity for these solutes in pentane will be nearly four times greater than in benzene. Using benzene and toluene as solutes and hexane and pentane as solvents led to the result that the selectivity in pentane will be only 1.0026 times greater than the selectivity in hexane. So even from these systems, in which dispersion is the only dominant intermolecular interaction, it is difficult to state how important dispersion interactions are to determining solvent selectivity. It can be said that they do not cancel, but the magnitude of their effect varies with specific systems.

Two things must be noted. First, we have considered systems in which dispersion is the main intermolecular interaction. It may be that the contributions of dispersion to solvent selectivity are quite small compared to the contributions from dipole–dipole and hydrogen bond interactions when polar and hydrogen bonding solutes and solvents are considered. Second, the above results were based solely on regular solution theory with no further normalization or attempts to cancel dispersion interactions. Snyder, however, in the development of the triangle, corrected Rohrschneider's partition data for differences in solvent molecular weight and then normalized the results to the partition coefficient for a hypothetical alkane of the same volume. Following this, a constant derived by considering the partitioning of solutes in saturated alkanes was used to compensate for incomplete cancellation of dipole induced–dipole interactions, entropy, and other effects. In these ways, the data treatment involved many steps that are not present in regular solution theory. Thus, while according to theory, dispersion interactions should play a role in solvent selectivity, Snyder took many steps to reduce or eliminate their influence.

It is also worth examining the work of Meyer and co-workers in this discussion of dispersion interactions. They quantified the relative importance of various intermolecular interactions in a series of papers that examined the cohesive energies (E_c) of polar organic liquids [46–49]. By examining the densities of polar organic compounds (e.g., 2-ketones) compared to paraffins, the authors were able to estimate the contributions of orientation (dipole–dipole), induction (dipole-induced dipole), and dispersion energies to the cohesion of the bulk solvents, defined as “the energy required to separate the component molecules to infinity without changing the average internal energy of the individual molecules.” While the authors' interest seemed to lie more in emphasizing the (sometimes overlooked) importance of induction effects, their results are relevant to our present discussion of the relative importance of dispersion interactions.

The results for the 2-ketones are shown in Table 2. It is clear that dispersion accounts for the majority of the interaction energies. For example, for 2-propanone, 71.2% of the cohesive energy arises from dispersion forces. This goes up to over 90% for 2-undecanone. Comparable results and trends were observed for n-alkylacetates, n-alkyl nitriles, and 1-chloroalkanes. It should be noted that Kersten and Poole [50] caution that Meyer's methodology is not well established and potentially overestimates the contribution of dispersion energies to the overall energy of interaction between molecules. However, they do not explain why this is so and they acknowledge that better alternatives were not available at that time. This caution notwithstanding, it is reasonable to conclude from Meyer's work

Table 2

Cohesive energy for a 2-ketones and the percent of dispersion, induction, and orientation forces contributing to the overall energy.

Compound	Temp. (°C)	E_c	% dispersion	% induction	% orientation
2-Propanone	14	7.08	71.2	15.5	13.3
	51	6.81	68.8	16.2	15.0
2-Butanone	40	7.62	77.6	14.4	8.0
	80	7.19	76.2	15.3	8.3
2-Pentanone	51	8.29	81.9	13.3	4.8
	93	7.84	80.2	14.0	5.8
2-Heptanone	78	9.99	85.5	11.1	3.5
	122	9.24	85.5	11.9	2.6
2-Nonanone	96	11.48	89.5	9.6	0.9
	143	10.71	88.8	10.3	0.9
2-Undecanone	111	13.09	91.6	8.4	–
	162	12.09	91.0	9.0	–
2-Tridecanone	123	14.89	92.6	7.4	–
	176	14.02	92.0	8.0	–

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that dispersion interactions play a significant role in retention, even for polar solutes in polar systems.

As mentioned earlier in this review, Tan and Carr [42] extensively analyzed the effects of dispersion on retention and how these effects change as a function of mobile phase modifier and composition. They state that the “the contribution of the presumed highly unfavorable cavity formation process in water is actually smaller than thought compared to the net favorability of forming dispersive interactions with the stationary phase.” They then use free energies of methylene group transfer from the gas phase to water (+159 cal/mol) and to hexadecane (–634 cal/mol) to indicate the importance of dispersion interactions to the retention of solutes in RPLC. They also provide a thorough dissection of the linear solvation energy relationships (LSER) that they used to quantify changes in the relative importance of dispersion, dipole–dipole, and hydrogen bonding interactions to overall solute retention. They consider their results in light of the amount of water and modifier sorbed into the stationary phase for aqueous methanol, acetonitrile, and tetrahydrofuran mobile phases from 20 to 50% (v/v). Overall, they stress the importance of dispersion interactions between solutes and the stationary phase. They also examine the relative cohesive energy densities of the mobile and stationary phases which contribute to retention via the cavity formation process. Cavity formation processes, however, also reflect dispersion interactions in that interactions between components within the mobile phase or within the stationary phase must be broken or rearranged in order to create cavities to accommodate solutes. Different organic solvents and different compositions will clearly have different effects on the cohesive energy densities of the mobile and stationary phase that could, depending on their magnitudes, contribute to solvent selectivity.

Given the work of Meyer et al. and Tan and Carr, to classify solvents, it is important to accurately account for dispersion interactions. Failure to do so may overlook important differences between solvents and their ability to interact with solutes. Thus, if the procedure used by Snyder yields only approximate cancellations of dispersion effects, the “excess” dispersion effects must be distributed (in some unknown fashion) throughout the remaining three solvent parameters in the SST. This complicates the interpretation of these parameters and perhaps also leads to some of the unusual groupings noted in the literature.

6.3. Further challenges to the SST – interfacial adsorption in GC

Kersten and Poole [50] characterized fifteen GC polymeric phases and found that the relative positions of the phases within the triangle change depending on the test solutes used to define the apices of the triangle. They further asserted that the SST for

GC phases fails because the Kovats Retention Index, upon which the GC solvent triangles are based, does not account for interfacial adsorption of the test solutes and n-alkane standards and because of inadequate retention of ethanol, nitromethane, and dioxane (the three probe solutes) on phases of low polarity. After correcting for interfacial adsorption effects (see the publication for more details on how they did this), the authors calculated X_e , X_n , and X_d values according to the methodology first described by Snyder and plotted the data as shown in Fig. 14.

Using a free energy-based parameter, $\sum \delta(\Delta G_K^0)_{SQ,i}^{PH}$ where $i = e, n, \text{ and } d$, SQ stands for squalane, and PH is the phase of interest, they replotted the data as shown in Fig. 15 [50,51]. The phases generally shift to the right compared to the plot based on P_i . The authors attributed this to a decreased contribution from proton-donor forces as measured by the free energy-based parameter and suggested this arises either because none of the phases examined have strong proton-donor properties or because dioxane is an insensitive probe for measuring proton-donor interactions. In either case, the authors challenged the basis for the construction of the SST as applied to GC phases.

6.4. Further challenges to the SST – test solute selection

6.4.1. Kersten and Poole – GC

Kersten and Poole examined the use of other probe molecules [50]. Specifically, they commented on the use of butanol in place of

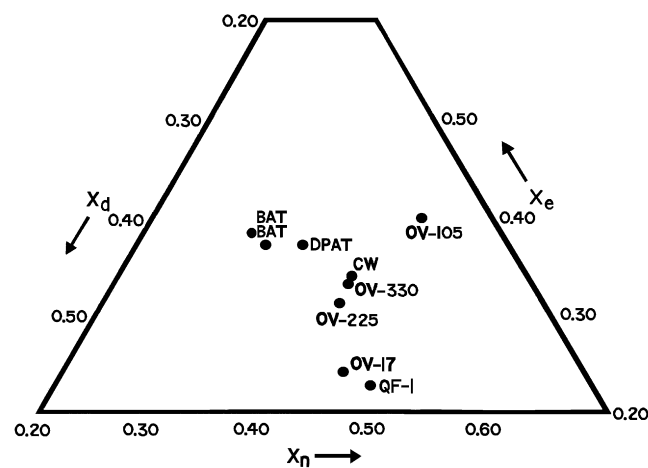


Fig. 14. GC solvent selectivity triangle from Kersten and Poole after correcting retention indices for interfacial adsorption effects. Reprinted from [50], with permission from Elsevier.

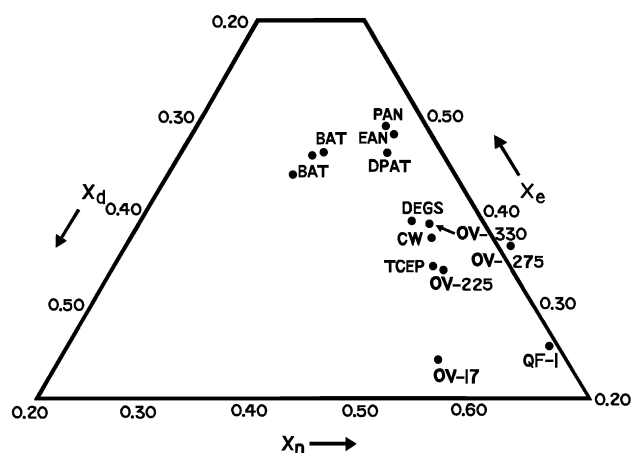


Fig. 15. GC solvent selectivity triangle calculated using the free energy-based parameter, $\delta(\Delta G_{K,SO,i}^0)^{PH}$, to define the sides of the triangle. Reprinted from [50], with permission from Elsevier.

ethanol, nitropropane in place of nitromethane, and 2-pentanone or pyridine in place of dioxane. They provided evidence that changing the three probe solutes would change the position of the GC phases within the triangle in an unpredictable manner. Unfortunately, they do not actually replot the selectivity triangle based on these new probes to visually demonstrate the changes in position. Changes in position, particularly if groupings change as a result, would call into question the utility of the SST approach as a means of classifying solvents. They also perhaps reinforce the concerns of West examined earlier.

6.4.2. Shah, Na, and Rogers – GC

Shah et al. had earlier noted the sensitivity of the position of GC phases within the triangle to the choice of test solutes [52]. They used Klee's definition of X_i values (defined earlier in this review) and characterized the same six phases. Fig. 16 shows a comparison of the results from the two reports. In general, the agreement is acceptable.

When butanol rather than ethanol was used as the test probe, the position of the phases changed dramatically. For example, the value of X_e for SE-30 changed from 0.534 to 0.246, which clearly would change its grouping. By examining the series ethanol,

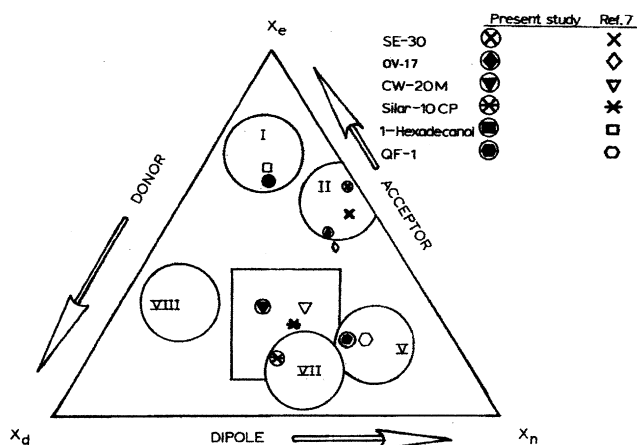


Fig. 16. Selectivity triangle comparing locations of six GC stationary phases reported by Klee et al. [14] with those from Shah et al. [52] to ascertain reproducibility of positions within the triangle using ethanol, dioxane, and nitromethane as probe solutes to determine X_e , X_d , and X_n , respectively. Reprinted from [52], with permission from Elsevier.

Table 3

Effect of test probes on the X_e , X_d , and X_n values of SE-30.

Probes	X_e	X_d	X_n
Ethanol, dioxane, nitromethane	0.534	0.119	0.346
Propanol, dioxane, nitromethane	0.403	0.310	0.287
Butanol, dioxane, nitromethane	0.246	0.225	0.528

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propanol, and butanol as test probes for SE-30, they found that as the chain length increased, X_e decreased monotonically and X_d and X_n had more complex changes as shown in Table 3. More polar phases such as QF-1 and CW-20M were less affected by the changes.

The authors show that the changes in the position can be reduced by dividing the corrected retention time of the alcohol homolog by the corrected retention time of its corresponding n-alkane. However, the authors also noted changes in positions when nitromethane was replaced by acetonitrile or nitropropane as the polarity indicator. Normalizing for changes such as switching from nitropropane to acetonitrile would be more difficult as there is not an underlying homolog series in common as there is for the n-alkohols.

The success of the normalization procedure suggests that the number of methylene units in the test compounds is important, which in turn suggests that dispersion or induction effects are not being completely removed by subtracting the retention index on squalane. It should be further noted that in this study, no attempt was made to account for solute interfacial adsorption or inadequate retention of the test molecules. Kersten and Poole demonstrated that this can alter retention indices and consequently X_i values [50]. Some of the observed changes with increasing probe chain length may therefore be due to changes in the relative contributions of adsorption and absorption (i.e., partitioning) to the retention of the probes and the n-alkanes.

6.4.3. Betts – GC

Betts also published a GC triangle using yet another set of probe solutes [53]. Based on his work, he ultimately recommended that three GC phases are essentially all that are required for most separations (SE-30, polysiloxane; QF-1, trifluoropropyl; and XE-60, cyanoethyl) – three that had been identified in 1969 as being among the most used phases around that time, 15 years before Betts published his findings [54]. Betts' somewhat vehement response to Klee et al.'s prediction that a computerized optimization for making new mixed GC stationary phases would eventually be in place [14] was "There are already far too many; let us not mix them!" Betts also cites McReynolds, who, based on his own work, wrote "It is hoped that this data will help reduce the number of liquid phases being used" [12]. He said this because of his finding that many phases show similar characteristics. It would be interesting to hear Betts' thoughts on today's era of two-dimensional GC and LC separations (which in some ways can be [incorrectly] thought of as mixed phases of a sort) and the hundreds of commercially available LC and GC phases.

6.4.4. Cooper and Lin – RPLC

Based on Snyder's selectivity triangle, Cooper and Lin [55] selected toluene, phenol, aniline, and nitrobenzene to test the relative importance of proton donor, proton acceptor, and dipole characteristics of RPLC mobile and stationary phases. Toluene was used as a reference compound and the slopes of plots of $\ln k$ vs. volume fraction of organic modifier obtained with toluene were subtracted from comparable slopes for the other compounds. The intention was to isolate just the retention of the functional groups. In some ways, this is similar to Snyder's approach of correcting partition coefficients of solutes by subtracting the partition

coefficient of an alkane of the same size. Unfortunately, the authors do not convert their findings into values that can be plotted in a triangle and thus the results of this study using different probe solutes cannot be readily compared to other studies using Snyder's probes.

6.4.5. Smith – RPLC

The last paper we will note in this section regarding Snyder's selection of probe solutes is that of Smith [56]. In this work, principal components analysis of RPLC data on eight columns with three different mobile phase compositions was used to determine the number and identity of test compounds needed to account for the variance in retention indices using alkyl aryl ketones as standards. Six potential test solutes were studied (aromatic analogs of Rohrschneider's and McReynold's standards). Smith ultimately concludes that toluene, nitrobenzene, 2-phenylethanol, and *p*-cresol give optimal discrimination between mobile and stationary phases. This is an interesting result in that four compounds were found to be necessary, in contrast to Snyder's suggestion that three suffice. It is also interesting that toluene is included in the four. This suggests that dispersion and/or dipole-induced dipole interactions are important in discriminating/characterizing different mobile and stationary phases, which is consistent with Meyer et al.'s work discussed above. If this is correct, then mobile or stationary phases with greater ability to participate in these interactions could show greater selectivity for non-polar and polarizable compounds. This seems to be an argument against Snyder's assertion that dispersion effects are negligible in solutions of polar solvents [10]. Snyder does acknowledge that "there is no doubt that the inclusion of additional test solutes improves the ability to predict solute retention behavior and to carry out fine-tuning of the solvent selectivity based on second-order effects" but doing so "appears more to confuse than to clarify our understanding of solvent selectivity for a given application." Snyder does continue on in his article to consider X values for toluene and shows them to have little variation between different solvents. One could postulate, then, that in Smith's study, toluene would be the least important of the four probes in terms of its contribution to the primary principal components that recreate the data set and that it is there to 'fine-tune' the model for minor effects of dispersion forces. This is not the case, however, as shown by the principal components analysis results in Table 4.

It would be interesting to see the effects of dropping toluene from the data that was fed into the principal components analysis. Unfortunately, Smith did not perform this analysis. It is also interesting to note the apparent redundancy of 2-phenylethanol and *p*-cresol as test probes, which suggests that resonance and induc-

tive effects make the behavior of these two solutes different enough so as to provide distinct chemical information.

7. Re-evaluation of the SST using solvatochromism and linear solvation energy relationships (LSERs)

7.1. Reevaluating the SST using solvent solvatochromic parameters

In 1989, Snyder participated in a reevaluation of the solvent triangle [57]. This work produced three major results:

- (1) More thermodynamically rigorous corrections for dispersion and entropy (cavity formation) effects produced only slight modifications to the relative position and groupings of solvents compared to the original SST, and the modifications that did result could be rationalized chemically,
- (2) The selectivity parameters (X_e , X_d , and X_n) were shown to be composite values comprised of dipolar, hydrogen bond acidity, and hydrogen bond basicity effects, and
- (3) The three original probe solutes used to develop the SST were acknowledged to be "inefficient" choices in terms of their ability to discriminate between solvents.

We will leave the interested reader to explore points 1 and 3 in the publication and focus on the second point.

To analyze the meaning of the selectivity parameters, the authors plotted values of X_e , X_d , and X_n for various solvents vs. the solvent parameters β , α , and π^* , respectively. The parameters π^* , α , and β , are measures of solvent dipolarity/polarizability, hydrogen bond donating ability, and hydrogen bond accepting ability, respectively [58–62]. They are derived largely from spectroscopic shifts of aromatic compounds that are sensitive to their chemical environment and hence are sometimes referred to as solvatochromic parameters. Given that they are based on spectroscopic data, they are derived from data entirely independent from that used to define P' and X_i values. Furthermore, through judicious selection of multiple solvatochromic probes, the π^* , α , and β scales were very carefully constructed to measure only the solvent interaction ability of interest and to exclude contributions from other possible interactions (e.g., α is a measure of just a solvent's HB donating ability with very little or no contribution from polarity or HB accepting ability). It is also worth noting that dispersion interactions play almost no role in solvatochromism. Dipole-induced dipole effects arising from solvent polarizability do contribute, though, and dispersion interactions tend to be collinear with polarizability. Dipole-induced dipole interactions tend to be much smaller

Table 4

Factors calculated with principal components analysis of the retention indexes of six reference compounds.

Eluent	Component	Weighting ^a	Contribution from reference standard						
			PhMe	PhOEt	PhNO ₂	MeOBz	PhEtOH	<i>p</i> -Cresol	
a	All sets	1	76.1	0.35	0.29	0.36	-0.01	0.15	0.80
		2	14.2	-0.02	-0.10	-0.37	0.21	0.90	0.05
		3	8.2	0.75	0.46	-0.27	0.15	-0.06	-0.36
b	70:30 MeOH/H ₂ O	1	66.6	0.62	0.49	-0.17	0.07	0.30	0.49
		2	30.3	-0.46	-0.35	-0.02	-0.05	0.58	0.58
		3	1.8	-0.17	-0.01	-0.97	-0.07	-0.03	-0.12
c	50:50 MeCN/H ₂ O	1	67.8	0.51	0.45	0.13	0.09	0.75	0.71
		2	30.3	-0.55	-0.32	0.29	-0.08	0.50	0.50
		3	1.3	-0.08	-0.04	-0.78	-0.53	-0.04	0.30
d	40:60 THF/H ₂ O	1	66.0	0.71	0.16	-0.46	-0.02	-0.43	-0.25
		2	20.2	0.23	-0.16	-0.52	-0.21	0.63	0.45
		3	10.6	-0.50	-0.45	-0.53	-0.24	-0.15	-0.43

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^a Percent of overall variance attributed to this component. Components 4–6 have been omitted because they contribute so little (<4%) to overall variance.

in magnitude than dispersion interactions, as indicated by Meyer's work discussed above.

Plots of X_i values for a variety of solvents vs. their corresponding π^* , α , or β values are shown in Fig. 17 [57]. Assuming π^* , α , β to be 'pure' scales in terms of measuring only a single intermolecular interaction ability (which is known not to be case for π^*), if X_i values for a set of solvents correlate strongly with the corresponding solvatochromic parameters, then the X_i values would also be said to be quite pure. However, as the authors point out, there is "disappointingly little correlation of the selectivity factors with the individual solvatochromic parameters." They note, however, that in reality, it is not the X_i values that should be correlated with the solvatochromic parameters, but rather PX_i values. Still they found little correlation between parameters suggesting that the two methods of characterizing solvents were not measuring the same attributes. To understand better what the Snyder parameters represented, the authors next used multi-parameter linear regressions to correlate PX_i with the solvatochromic parameters π^* , α , β , and δ , where δ is a term added to account for polarizability effects not incorporated in π^* [63]. The correlations thus took the form

$$PX_i = SP_0 + s\pi^* + d\delta + a\alpha + b\beta + h\alpha\beta \quad (9)$$

where SP_0 is an intercept term. The parameter δ is set equal to 0.00, 0.500, or 1.00 for aliphatic, halogenated, and aromatic solvents, respectively. The results of the regression are shown in Table 5.

As the authors point out, these results indicate that all three original test solutes have appreciable dipolar interactions with solvents as indicated by their large positive s -coefficients. Furthermore, dioxane and ethanol are both sensitive to solvent hydrogen bond acidity (positive a -coefficients), negating the assumption that X_d is the primary measure of solvent HB donating ability in the SST. It was also concluded that the assumption that ethanol is the main probe of solvent basicity is correct as evidenced by its large β -coefficient compared to that for dioxane and nitromethane. In this way, the authors showed that

- (1) X_i is a composite of solvent dipolarity/polarizability, HB acidity, and HB basicity,
- (2) X_d reflects a blend of solvent dipolarity and HB acidity, and
- (3) X_n mainly reflects dipolarity with smaller contributions from HB acidity and basicity.

The authors go on to propose that triethylamine ($\pi^* = 0.14$, $\alpha = 0.00$, $\beta = 0.71$) and trifluoroethanol ($\pi^* = 0.73$, $\alpha = 1.51$, $\beta = 0.00$) be used to probe solvent HB acidity and basicity, respectively. This is based on their relatively high β/π^* and α/π^* ratios. While the α -value for trifluoroethanol is high, a π^* value of 0.73 is also quite high (given that the scales generally range from 0.00 to 1.50), such that it is questionable as to how much using this probe would help in determining a pure basicity contribution to the SST free from dipolar interactions.

Table 5

LSEF coefficients showing the contributions of polarity, dispersion, and hydrogen bonding effects on the PX_i values of several probe solutes.

Solute	s	d	a	b	h	SP_0	S.E.
2-Butanone	1.65 (0.06)	-0.18 (0.05)	0.89 (0.24)	- ^a	-0.79 (0.04)	-0.18 (0.04)	0.103
Ethanol	1.32 (0.15)	-0.24 (0.10)	1.31 (0.38)	1.88 (0.17)	-1.59 (0.24)	0.00 (0.06)	0.173
Toluene	0.97 (0.03)	-0.13 (0.02)	0.13 (0.09)	- ^a	-0.54 (0.11)	0.01 (0.02)	0.053
p-Dioxane	1.44 (0.06)	-0.06 (0.05)	1.07 (0.18)	- ^a	-1.14 (0.23)	0.02 (0.04)	0.11
Nitromethane	2.29 (0.13)	-0.34 (0.08)	0.49 (0.27)	0.55 (0.14)	-1.27 (0.39)	0.04 (0.05)	0.138

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Standard deviations of the coefficients are in parentheses.

^a These coefficients were found to be not significantly different from zero and were omitted in the final fit.

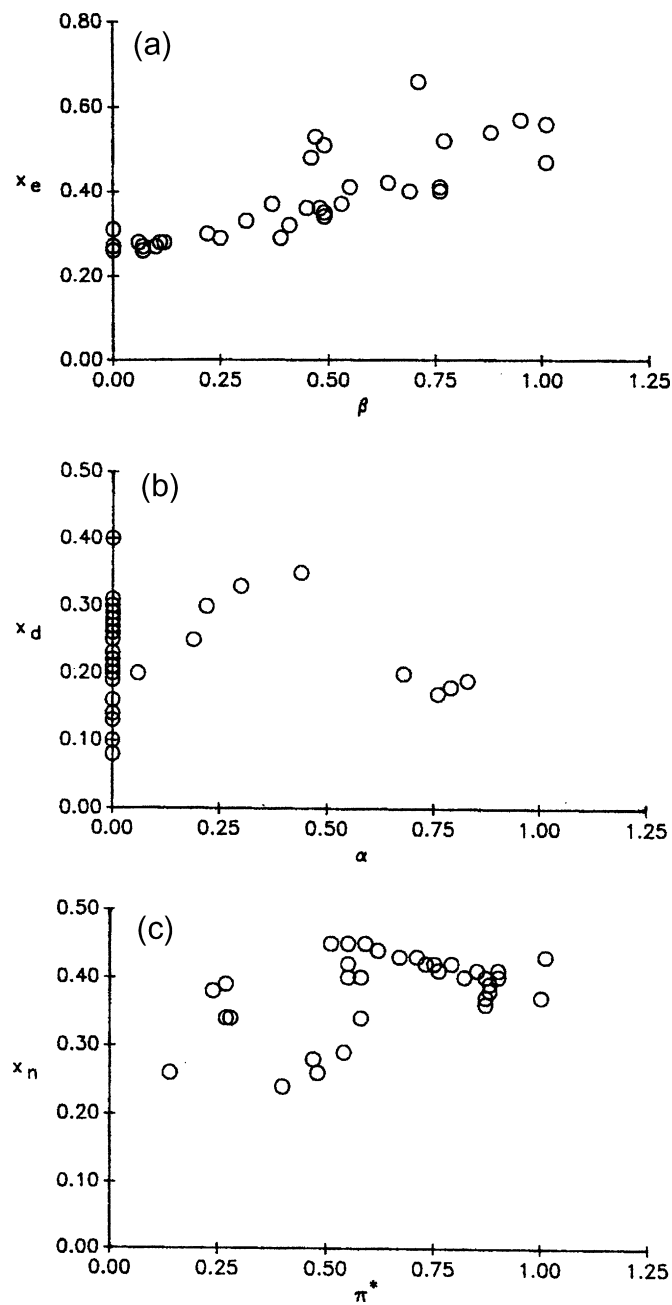


Fig. 17. Plots of Snyder parameters vs. related Kamlet–Taft solvent parameters to compare the similarity (or lack thereof) of solvent properties measured by each. (a) X_e vs. β , (b) X_d vs. α , and (c) X_n vs. π^* . Reprinted from [57], with permission from Elsevier.

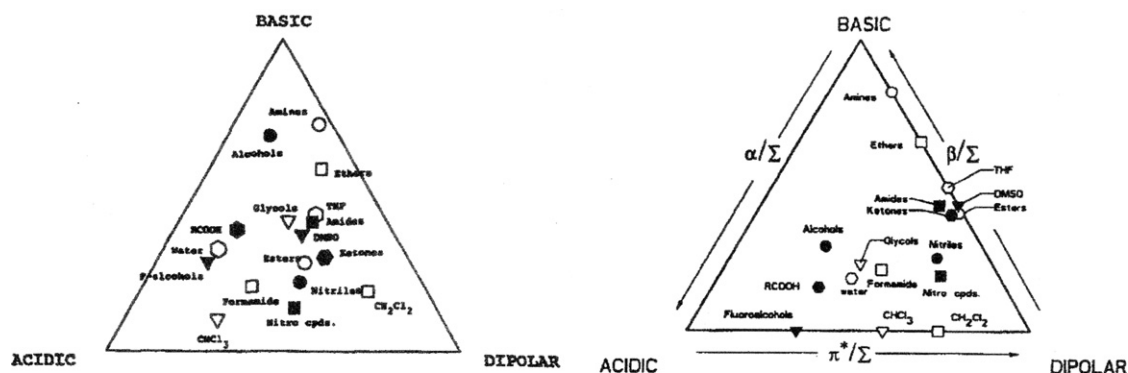


Fig. 18. Solvatochromic parameter-based plot of properties of aliphatic solvent classes and some specific solvents (left). Further simplification of the plot obtained by averaging X_i values for solvents of a given type (amines, alcohols, etc.) (right). Reprinted from [43], with permission from Elsevier.

7.2. Solvatochromic SST for solvents

As an extension of the ideas presented in the article just discussed, Snyder et al. reconstructed the SST using the Kamlet–Taft solvatochromic parameters to define the apices [43]. To do so, each solvent parameter (π^* , α , β) was normalized to the sum (Σ) of the three parameters for each individual solvent. The normalized parameters were used as the apices of the triangle.

In order to facilitate the comparison between the solvatochromically based triangle and Snyder's original SST, the authors averaged X_i values for solvents within a class (e.g., amines, alcohols, etc.). They also only compared aliphatic solvents, largely because all but one of the aromatic solvents studied had $\alpha = 0.00$ and thus they clustered along the right vertex of the triangle. The original and 'new' plot, thus simplified, are shown in Fig. 18 [43]. The authors state

"the relative positioning of different solvents. . . is similar in that solvents which are more basic, acidic, or dipolar in [the original] are also more basic, acidic, or dipolar [in the new plot]. A further examination. . . however, shows that solvents of similar acidity or basicity are better grouped in the solvatochromic approach. Thus, amines and ethers show up as distinctly basic, as compared to the alcohols in [the original plot]. The alcohols, glycols, formamide, carboxylic acids, water, and chloroform show up as acidic solvents in [the new plot]. The acidity of these latter solvents seems inadequately expressed in [the original]."

They go on to suggest that these problems (and a few others) in the original SST can likely be attributed to the fact that nitromethane, ethanol, and dioxane do not provide pure measures of polarity, basicity, and acidity, whereas the solvatochromic parameters were designed to do just that. Furthermore, the solvatochromic parameters are averages obtained with several probes for the determination of each π^* , α , and β value. This reduces some of the probe-specific effects that are inherently embedded in the construction of the SST.

As a final, and rather eloquent, explication of why it is that the original three probes represent blends of interactions, the authors provide their solvatochromic parameters, reproduced here in Table 6 [57]. These values make it clear that all three solutes are polar and that dioxane and ethanol are both good hydrogen bond acceptors (high β values). Thus, any parameters derived from this triad of solutes will necessarily represent blends of interactions.

Given all of the challenges that had come before this work, the solute-dependent nature of the original SST was not a novel revelation (as the authors acknowledge via their citations). What was new, however, was the use of the solvatochromic parameters to

explain the exact nature of the dependence of the original SST on probe solute selection. The use of solvatochromic parameters to reconstruct the SST was also new.

7.3. Solvatochromic SST and practical RPLC considerations

It is worth extending our discussion of Snyder et al.'s examination of the SST as it relates to solvent selection in RPLC [43]. As presented earlier, the SST was used as the basis for many optimization schemes. However, while the SST was proposed as a guide to aid in solvent selection, it does not provide guidelines regarding the effect of increasing the percent water in each of the solvents on chromatographic selectivity. Along those lines, the authors noted that the "SST approach assumes that solvent strength can be varied (by varying the percent water in RP-HPLC) *without* changing selectivity" (italics ours). But water is certainly not a passive diluent, as the authors attest by pointing to changes in the π^* , α , β values of solvents modified with varying amounts of water [30,31,64,65]. We agree with their statements that the "SST approach to adjusting solvent strength and selectivity in RP-HPLC is overly simplified" and that "it is all but impossible to vary the mobile phase strength via a change in the water content without also changing some other significant solvent-selectivity property" [43]. Specifically, they go on to note that previous studies show that, with respect to selectivity, water simultaneously affects the mobile phase cohesivity (i.e., the ease of cavity formation to accommodate solutes), polarity, and HB acidity, with only minor changes in basicity. They suggest, therefore, that for RPLC purposes, the SST should be reconstructed using surface tension, or some other cohesivity-related property, polarity, and hydrogen bond acidity to define the apices, thus eliminating hydrogen bond basicity. The fact that the mobile phase also modifies the stationary phase properties is also noted as a complication in predicting selectivity changes between different solvents and solvent compositions. These effects were comprehensively explored by Tan and Carr [42]. Using linear solvation energy relationships, they demonstrate changes in the relative contributions to solute retention of cavity formation, dispersion, dipolarity, and hydrogen bonding interactions as a function of mobile phase

Table 6
 π^* , α , and β values of Snyder's original test probes showing that they participate in a blend of intermolecular interactions and do so to varying extents.

	π^*	α	β
Dioxane	0.45	0.00	0.79
Ethanol	0.29	0.29	0.52
Nitromethane	0.67	0.06	0.16

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properties present in the analyte mixture. In this case, if both X_i and Y_i for the solutes make substantial contributions to retention, then “effective” or useful changes in selectivity could result from changing from one system to the other. So the key to obtaining useful selectivity is to have *ratios*, not necessarily absolute magnitudes, of LSER coefficients that are different. For this reason, the GC selectivity triangle discussed previously would have been better presented if ratios of LSER coefficients had been used to define the apices rather than absolute magnitudes.

8. Recent uses of selectivity triangles – MEKC, RPLC, and NPLC

8.1. MEKC selectivity triangles based on LSERs

Based on all of the work over the years that has been summarized above, Fu and Khaledi have recently characterized pseudo phases used in electrokinetic chromatography (EKC) [82,83]. The phases included elution buffers modified with micelles, polymers, vesicles, liposomes, mixed micelles, polymer/surfactant mixtures, and organically modified pseudo phases. This is the first report that the present authors are aware of in which an SST approach was applied to micellar and related systems.

Their triangle is ultimately based on LSERs of the form

$$\log k = c + \nu V + bB + aA + sS + eE \quad (17)$$

where V , B , A , S , and E are measures of a solute's volume, HB basicity, HB acidity, dipolarity/polarizability, and excess polarizability, respectively [84,85]. The values of c , ν , b , a , s , and e are determined by linear regression analysis and were either taken from literature reports or measured by the authors.

The definition of the apices of their triangle is somewhat more complex than others discussed in this review. First, the authors calculated the ratio of LSER coefficients as suggested by the Carr publication discussed above [81]. They choose to normalize to the ν -coefficient, which is commonly done because the ν -coefficient is often one of the largest in LSERs, particularly those involving solute transfer into or out of aqueous phases. The symbol I_i is given to each possible ratio (a/ν , b/ν , s/ν , e/ν), where the subscript ‘ i ’ represents any one of the individual ratios. Next, the I_i values are converted to U_i values using

$$U_i = \frac{I_i - I_{\text{low}}}{I_{\text{high}} - I_{\text{low}}} \quad (18)$$

where the ranges of I_{high} and I_{low} were selected to incorporate (but not equal) the high and low values for each ratio for the 74 systems studied. For example, $I_{\text{high}} = -1.50$ and $I_{\text{low}} = 0.00$ for b/ν , even though for the 74 systems, the actual lowest value was -1.38 and the highest was -0.23 . While the actual I_{high} and I_{low} values were adjusted for each ratio being considered, the difference between them (i.e., the range) was kept constant at 1.50. Defining U_i in this way provided quantities that were all positive (unlike LSER coefficients which can be positive or negative) and which ranged from 0.00 to 1.00.

Next, in a manner similar to that in Brown's original work, the U_i values were normalized according to

$$X_i = \frac{U_i}{U_a + U_b + U_s} \quad (19)$$

such that $X_a + X_b + X_c = 1.00$. These three X_i values then served as the apices of the micellar selectivity triangle (MST). With four LSER coefficient ratios, there are obviously several different triads that can be used to define a triangle and the authors explored these options. They ultimately recommended one based on $X_b + X_s + X_a$. Using this scheme, the 74 systems were plotted as shown in Fig. 20 [82].

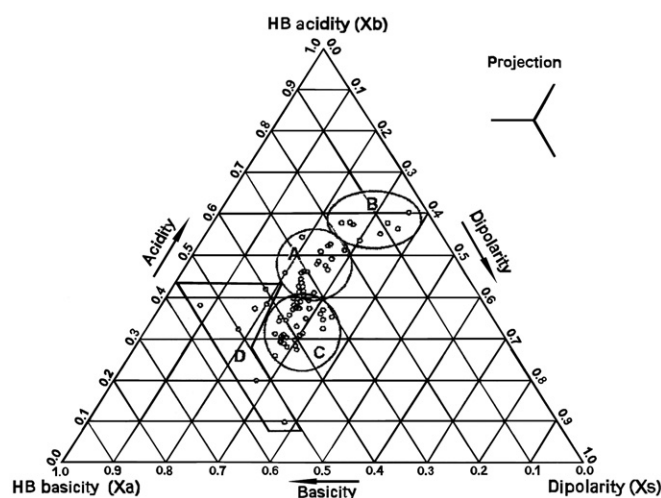


Fig. 20. Micellar selectivity triangle based on LSERs. See reference for groupings. Reprinted from [82], with permission from Elsevier.

As a critical test of the methodology and the reproducibility of using LSERs in this manner, Fu and Khaledi collected fourteen different literature reports of LSERs for sodium dodecyl sulfate (SDS) and plotted them in a triangle shown in Fig. 21. It is reassuring that they generally cluster together. Reasons that a few of the results are outliers can be offered based on differences in experimental conditions.

Returning our attention to Fig. 20, the authors identified four different groupings of systems labeled A, B, C, and D. As with other selectivity triangles, the suggestion is that if a system in one grouping does not achieve the desired separation, then switching to another system *within* the same group is unlikely to produce dramatic changes in elution order or selectivity. Rather, it would be better to change to a system in a different group that might have different blends of intermolecular interactions and therefore might offer different selectivity.

8.1.1. Analysis of the MEKC selectivity triangle

As with the original Snyder triangle, some challenges can be made to this classification scheme. First, the vertices are labeled with “polarity”, “basicity”, and “acidity” as if they are absolute mea-

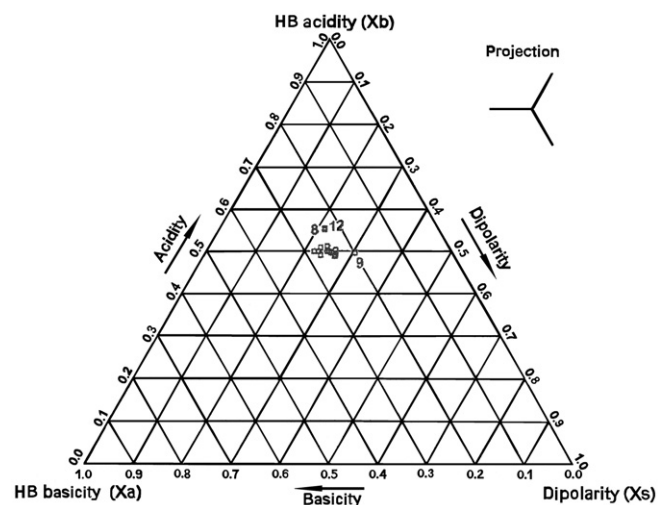


Fig. 21. Micellar selectivity triangle showing data points based on fourteen literature reports for sodium dodecyl sulfate LSERs. Reprinted from [82], with permission from Elsevier.

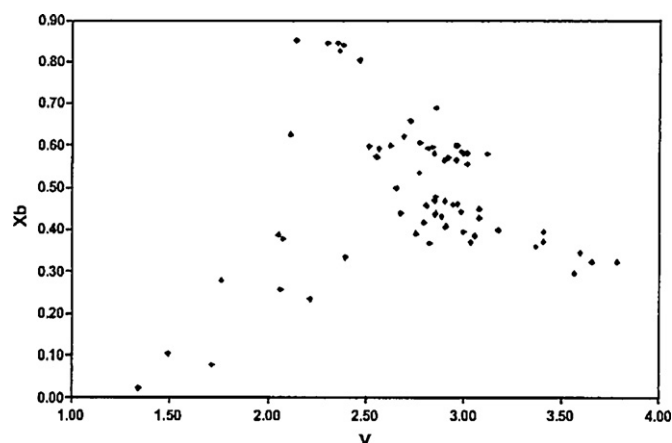


Fig. 22. Plot presented to show a poor correlation between X_b and ν -coefficients, suggesting that the X scales in the MST are not influenced by differences in ν -coefficients for the various pseudophases. Reprinted from [82], with permission from Elsevier.

tures, whereas in reality they are quite complex parameters. Their complexity can be recognized by first considering that they are ratios of LSER coefficients. Then, they are normalized to an arbitrary high and low value, and then get renormalized to the sum of three such normalized parameters. This means that they are not truly direct measures of any single selectivity characteristic of the system. For example, when the authors state that “Group B is mainly comprised of fluorinated micelles and could be considered the strongest hydrogen bond donor and weakest hydrogen bond acceptor among all the micelle systems” or “In general, the components in group C are slightly stronger hydrogen bond acceptors and weaker hydrogen bond donors than those in group A”, these statements would only be true if the ν -coefficients for all of the systems were the same. However, the ν -coefficients in their collection vary from 1.49 to 3.78 (excluding the value of 3.94 for octanol/water partitioning which was included in their study as a bulk phase model of micelle/water partitioning). This is a relatively large range for LSER coefficients and quite comparable to the overall range for the b -coefficients in this study (-0.47 to -3.86). Even if the ν -coefficients were the same, it is difficult to make these statements because X_i is a relative measure of the property under consideration compared to the sum of three different properties. Thus, all that really can be said, for instance, is that the contribution of the acidity/cavity formation ratio compared to the overall sum of basicity/cavity formation, acidity/cavity formation, and polarity/cavity formation is highest for systems in group B. Even this, however, is an oversimplification because the solute parameter V (solute size) models both cavity formation (endoergic) and dispersion (exoergic) effects. So the interpretation of the ν -coefficient is itself not simple. Thus, at best, the descriptors along the sides of the triangle are oversimplifications and serve, perhaps, as first approximations or convenient labels of what are quite complex measures of the systems’ characteristics.

To demonstrate that the X scales are *not* influenced by the magnitudes of the ν -coefficients in the manner suggested in the paragraph above, Fu and Khaleli show a plot of X_b vs. ν , which has generally scattered data for the 74 systems (see Fig. 22) [82].

We have developed the related plot of b/ν vs. b shown in Fig. 23. If all of the ν -coefficients were identical, such a plot would result in a straight line. However, it is clear that several systems fall well below the general correlation. The outliers correspond to the seven AGENT polymeric micelles and Elvacite 2669. These eight systems have the six lowest ν -coefficients of all 74 systems and the other two are numbers nine and twelve when all 74 ν -coefficients are listed in

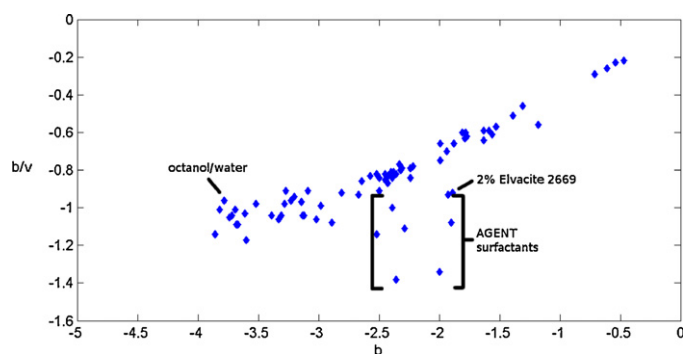


Fig. 23. Plot of b/ν vs. b to show the influence of ν -coefficients on the interpretation of pseudophase properties as defined by l -values in the development of the MST.

ascending order. This suggests that the smaller ν -coefficients (perhaps arising from easier cavity formation or weaker solute/micelle dispersion interactions in these micelles compared to others) are leading to the high magnitudes of b/ν ratios. Thus, it is perhaps not an acidity effect but rather a different effect that places them in the group in which they reside within the triangle. This suggestion is supported by arbitrarily replacing the actual ν -coefficients for these systems with the average ν -coefficient calculated using all of the systems. If the plot is remade (Fig. 24), it is clear that the eight ‘outliers’ fit into the general correlation. So the apparent enhanced magnitude of the AGENT and Elvacite 2669 systems (in terms of larger negative b/ν ratios) is more a result of low ν -coefficients. Similarly, the apparently smaller-than-expected magnitude of the b/ν ratio of octanol/water partitioning is a result of a larger-than-average ν -coefficient, as demonstrated by replacing its ν -coefficient with the average and replotting it as shown in Fig. 24. These concerns do not negate the use of the MST, but they complicate the chemical understanding of why the systems fall where they do within the triangle.

In terms of *absolute strengths* of interactions, it is useful to look at the interpretations of LSERs. In general, the solute partitioning is defined as the transfer of the solute from the aqueous phase into the pseudo phase. Thus, negative coefficients indicate the solutes partition less as their solute parameters increase. For example, a negative b -coefficient indicates that the aqueous phase is a stronger hydrogen bond donor than is the pseudo phase (which typically makes sense given the ability of water to donate hydrogen bonds). The magnitude of the coefficient indicates the degree to which this is true. The larger the magnitude, the weaker the hydrogen bond donating ability of the pseudo phase. A coefficient of zero indicates that the pseudo phase is just as strong a donor as the aqueous phase, and a positive coefficient indicates that the pseudo phase is a better

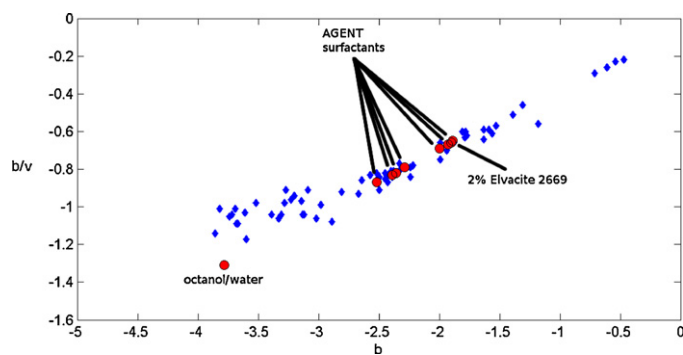


Fig. 24. Same plot as in the previous graph except that the ν -coefficients for the AGENT surfactants, Elvacite 2669, and octanol-water have been replaced with the average ν -coefficient for the set of 74 pseudophases.

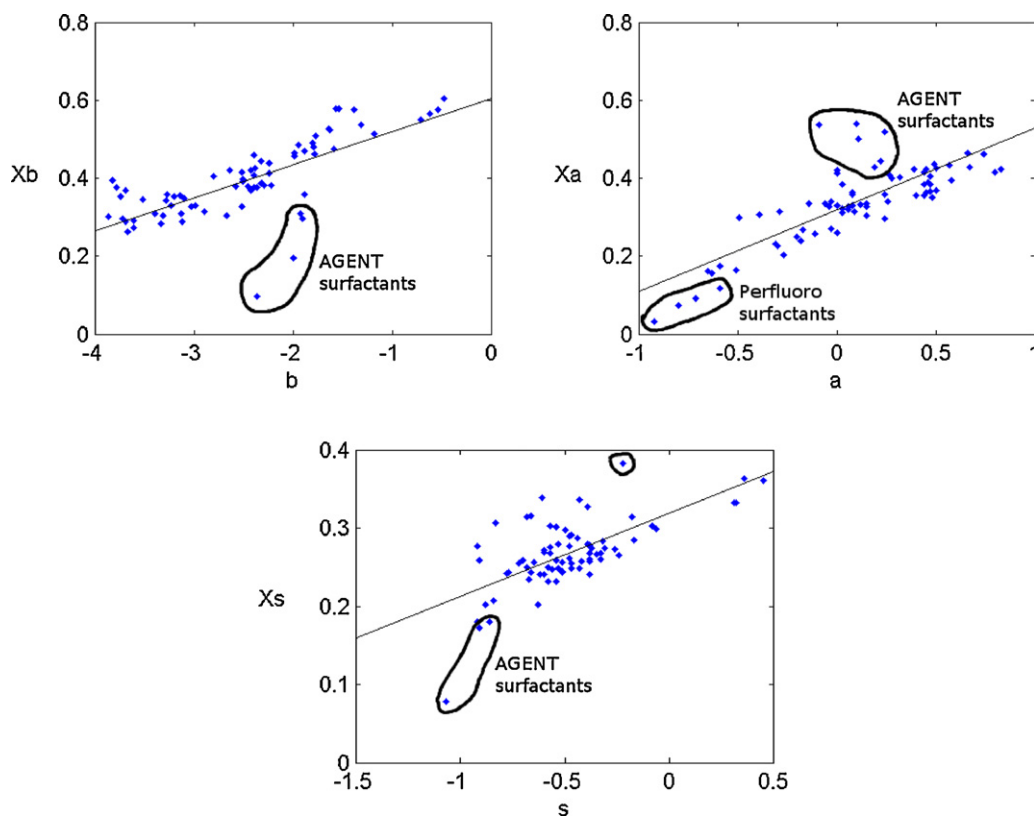


Fig. 25. Plots of X_i vs. i to compare the triangle parameter (X_i) to the LSER (i) parameter and the complications that can arise when interpreting X_i values. (a) X_b vs. b , (b) X_a vs. a , (c) X_s vs. s .

HB donor than the aqueous phase. When one lists the b -coefficients for all of the systems from smallest negative (i.e., strongest donor) to largest negative (i.e., weakest donor) the perfluorinated surfactants are numbers 1–5 and 11 in a list of all of the systems. Thus, on the absolute scale, they are the strongest HB donors just as they are in the triangle, so the normalization process does not appear to distort their position with regards to their HB donor strength. However, the AGENT pseudo phases are seen as some of the weakest HB acids on the triangle, which is inconsistent with their absolute values when compared to all of the other systems.

It is possible to argue that the normalization of b/ν values to high and low values and then to the sum of U_i values as dictated by the methodology removes the effect of the small ν -coefficients such that the X_i values that are ultimately plotted in the triangle accurately reflect the relative intermolecular interaction strengths of the mobile phases. Fig. 25a–c shows plots of X_i vs. i for $i = b, a$, and s . These plots make it clear that the way in which the apices of the triangle are defined, combined with the magnitude of the ν -coefficients, can produce over- or underestimated strengths of specific classes of phases. For example, the AGENT surfactants have underestimated X_b and X_s values given their absolute magnitudes for the corresponding b - and s -coefficients. These underestimated X_b and X_s values are offset by overestimated X_a values. These values do not result because of enhanced acidity and polarity of these surfactants, but rather because of their small ν -coefficients relative to most systems. The fact that their basicity (X_a) is overestimated is a mathematical artifact that arises from the requirement for the sum of all X values to equal 1.00. If some parameters are underestimated, then others will necessarily be overestimated.

To further complicate the analysis of the triangles, we note that group B is seen to have the lowest dipolarity according to the labels on the triangle. This contradicts what is observed based on absolute values of the s -coefficients. In fact, the perfluoro surfactants are the only pseudo phases that have positive s -coefficients, indicating that

they actually interact more strongly with polar solutes than does water (or more precisely, the aqueous phases used in these studies). Thus, it appears that the normalization process is not accurately simultaneously reflecting all of the properties of these phases.

As a final illustration of the problem of interpreting the axes, when the authors plot $X_b + X_s + X_e$ (instead of $X_b + X_s + X_a$) the perfluoro surfactants switch from having the lowest ‘dipolarity’ values to the highest (figure not shown).

Of course, it must be noted that some of the above conclusions about the MST are drawn based on a consideration of the absolute values, and not ratios of LSER coefficients which, as pointed out above, are the more critical parameters to consider when interested in selectivity differences between systems. Nevertheless, while they do not negate the utility of the triangle, they do show that the statements made about the various systems and the labeling of the sides of the triangle are at best oversimplifications.

8.2. RPLC column selectivity triangle based on the hydrophobic subtraction model

Quite recently, Zhang and Carr [86] published multiple triangles based on the Snyder–Dolan hydrophobic subtraction model of column selectivity [87] which takes the form

$$\log \left(\frac{k_i}{k_{EB}} \right) = \eta' H - \sigma' S^* + \beta' A + \alpha' A + \kappa' C \quad (20)$$

where the column parameters H , S^* , A , B , and C are obtained via multiparameter linear least squares regression of $\log(k_i/k_{EB})$ against the known solute descriptors η'_i , σ'_i , α'_i , β'_i , and κ'_i for a set of solutes, i , analyzed with a given mobile phase and stationary phase for different columns. H , S^* , A , B , and C provide measures of solute–column interactions. Specifically, they represent hydrophobicity, steric resistance, HB acidity, HB basicity, and cation–exchange activity, respectively, of the mobile/stationary

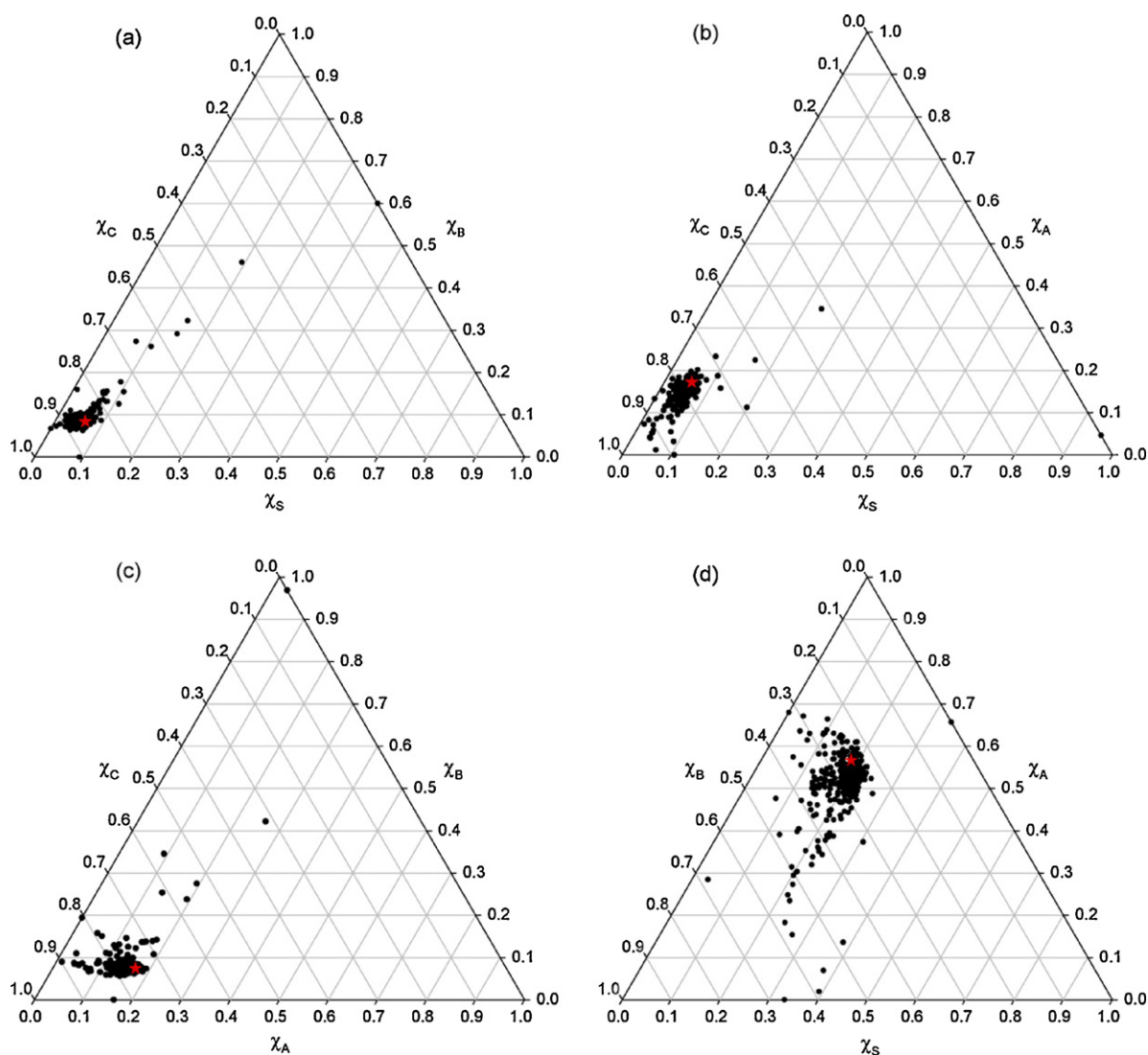


Fig. 26. RPLC selectivity triangles based on the Snyder–Dolan hydrophobic subtraction model for 366 stationary phases and a single mobile phase with a particular weighting factor (see reference for details). (a) S^* – B – C triangle, (b) S^* – A – C triangle, (c) A – B – C triangle, (d) S^* – A – B triangle. Reprinted from [86], with permission from Elsevier.

phase combination being studied. Data for sixteen solutes analyzed on 366 commercial RPLC phases were collected and analyzed using the hydrophobic subtraction model. The resulting H , S^* , A , B , and C values were used to construct selectivity triangles according to the following methodology.

First, ratios of the coefficients were calculated (in accord with the earlier discussion which showed that it is the ratios, not the absolute values of coefficients, that must be compared in order to compare the selectivities of two different chromatographic systems). The authors selected H as the parameter to which other coefficients were normalized.

The authors then defined a parameter X_i as

$$X_i = (I - I_{\min})\phi_i \quad (21)$$

where $I = S^*/H$, A/H , B/H , or C/H and ϕ_i is a weighting factor. To develop a triangle, three χ_j values were defined as

$$\chi_j = \frac{X_j}{X_{S^*} + X_B + X_C} \quad (22)$$

where $j = S^*$, B , or C .

Clearly, with four different I -ratios, four different sets of three I -values are possible. Thus, four different triangles were plotted as

shown in Fig. 26. These triangles used a weighting factor defined in such a way as to yield the same quantitative effect on phase selectivity (defined as the standard error in a $\log k$ vs. $\log k$ plot for retention of sixteen solutes on the different phases) for an equivalent numerical change in two different normalized phase properties (see the original reference for more details). A brief examination of the triangles developed using this weighting shows that the C parameter (ionized silanol effects) dominates the three triangles in which it appears. The effect is to cluster all but the most dissimilar phases. Such clustering makes it impractical to use the triangles to select phases of differing properties, or to distinguish one group of columns from another as is usually done with triangles. The authors thus sought a different weighting scheme, using instead the definition

$$\phi_i = \frac{1}{I_{\max} - I_{\min}} \quad (23)$$

Such that X_i becomes

$$X_i = \frac{I - I_{\min}}{I_{\max} - I_{\min}} \quad (24)$$

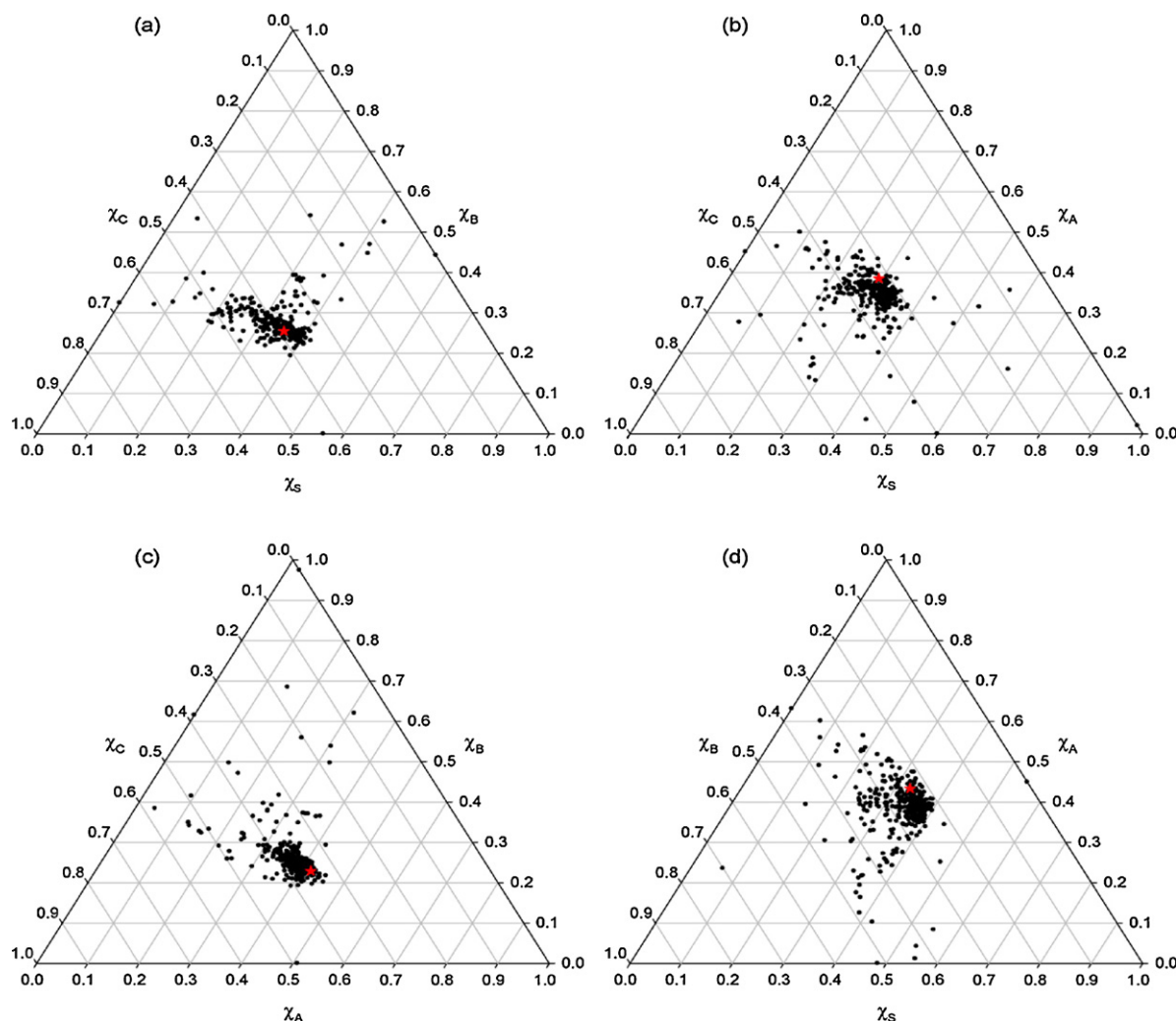


Fig. 27. RPLC selectivity triangles based on the Snyder–Dolan hydrophobic subtraction model for 366 stationary phases and a single mobile phase with a different weighting factor compared to the previous plot (see reference for details). (a) S^*B-C triangle, (b) S^*A-C triangle, (c) $A-B-C$ triangle, (d) S^*A-B triangle. Reprinted from [86], with permission from Elsevier.

which is akin to one of the steps in the development of the micellar selectivity triangle (MST) of Fu and Khaledi discussed elsewhere in this review.

The use of this weighting factor resulted in the four selectivity triangles shown in Fig. 27. With these triangles, the authors find that type-B silicas derivatized with alkyl chains are generally grouped together in the center. Phases derivatized with cyano, phenyl, fluoro, or polar embedded groups and those based on type-A silicas generally show larger differences in coefficients and fall outside of the central cluster. All phases of a given chemical compositional class certainly do not fall in the same region of the triangle.

The authors also comment on some surprising findings. For example, the three chemically different columns (ACE AQ – a polar embedded phase; Betasil Phenyl-hexyl – a phenyl phase, and Bondclone C18 – a type A alkyl silica) are near each other in the triangle. This suggests that their selectivities are comparable, as was verified by high correlation coefficients and small standard error values for regressions of $\log k$ on one column vs. $\log k$ on another for the set of sixteen variegated test solutes.

Zhang and Carr make two important points about the phases they studied, stating that “a huge fraction of the available space [in the triangles] is under populated and certain regions are extremely over populated” [86]. So their first conclusion is that on one hand, many of the phases are quite comparable to one another, meaning

they may not be truly needed as they do not add to our ability to achieve separations. Their second, and perhaps more important conclusion, is that commercially available stationary phases are not exploring all possible blends of intermolecular interactions and thus not providing a full range of selectivities. It is interesting to note that this is the same conclusion that Brown reached 50 years ago, and which Betts reiterated in 1986, regarding GC stationary phases as discussed above.

8.2.1. Analysis of the RPLC selectivity triangle

Given the similarity of this approach based on the hydrophobic subtraction model with that of Fu and Khaledi’s based on LSERs, many of the same potential advantages and disadvantages exist. For example, both approaches rely on entire sets of solutes, as opposed to just three probe solutes, to define the apices of the triangle. This increases the probability that the results are more broadly representative and would apply to a broad range of solutes.

A disadvantage is that to create the plots, one parameter of the model is ignored. Thus, two phases that are identical in three of the four parameters may appear to have similar selectivities in one of the triangles. Therefore, use of any one triangle may be blind to a major difference in selectivity of two phases which is very evident in a different triangle. This is particularly important if the solute set includes compounds that differ in the property that is comple-

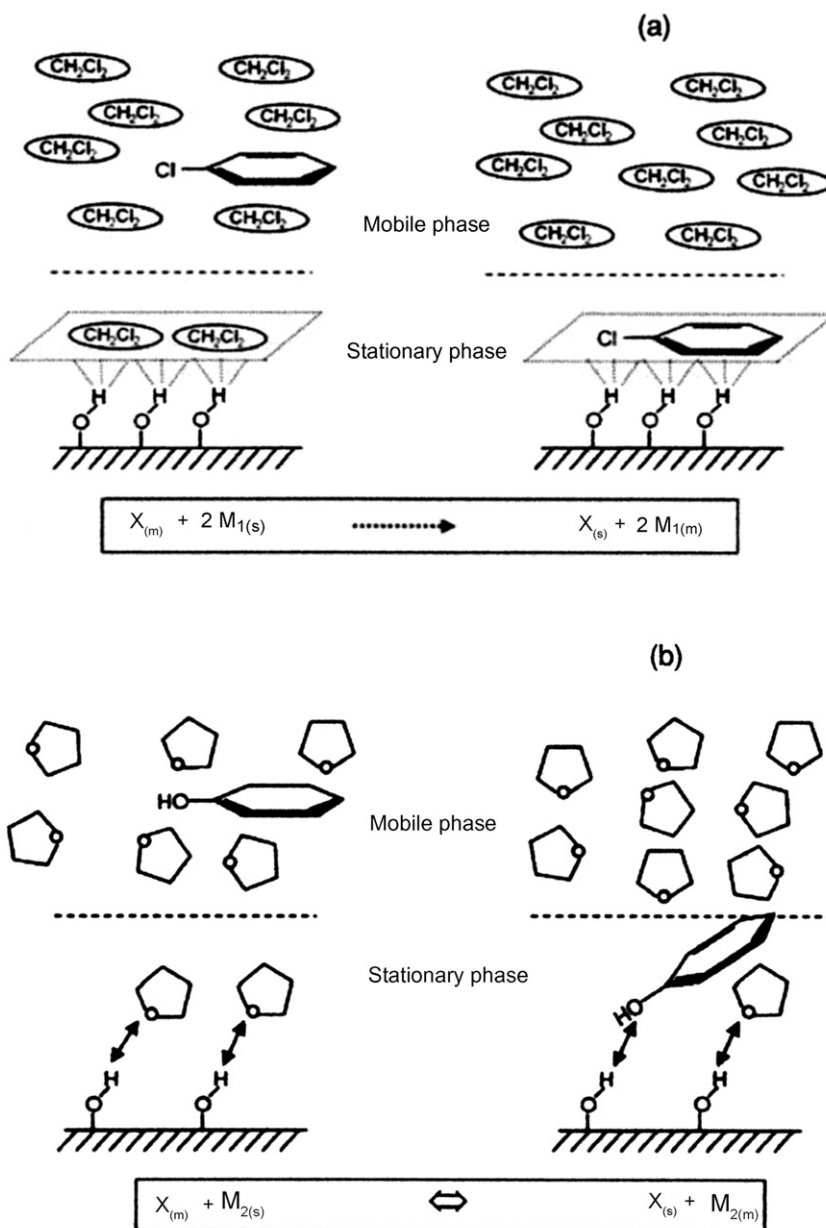


Fig. 28. Illustration of solute and solvent general adsorption compared to localization. (a) Non-localized retention of chlorobenzene on silica with dichloromethane as mobile phase; (b) localized retention of phenol, with tetrahydrofuran as mobile phase. Reprinted with permission from [88].

mentary to the one that is not plotted (i.e., solute HB acidity paired with considerations of system HB basicity). The limitation of three parameters is overcome by creating four different triangles, but the different plots can lead to different conclusions regarding similar and different phases. So the need for multiple plots can complicate the use of the selectivity triangles for selecting orthogonal (or similar replacement) phases.

Lastly, as pointed out above, the use of normalized parameters complicates the chemical interpretation of the parameters. Zhang and Carr are careful in their manuscript to avoid labeling the sides of the triangles with absolute descriptors as Fu and Khaledi did. In fact, they explicitly recognize that the normalization complicates interpretations of the derived parameters. This does not preclude the use of the triangles in a practical sense but does limit the degree to which chemical meaning can be ascribed to conclusions based on them regarding similar and different phases.

8.3. NPLC selectivity triangle

In addition to the recent uses of triangles in MEKC and RPLC detailed above, Snyder has recently reviewed solvent selectivity and its applications to NPLC [88]. The first part of the review makes it clear that the main mechanisms of solute retention on polar surfaces – the work focuses on alumina and silica adsorbents – are (1) non-localized adsorption to the surface via displacement of mobile phase molecules and (2) localized interactions with the formation of specific interactions between analytes and the stationary phase. The former predominate for non-polar solutes and the latter for compounds containing polar function groups. Both types of interactions are depicted in Fig. 28.

Snyder focuses on the effects of the “B-solvent” – the more polar solvent in a mixed mobile phase. The A-solvent is typically something like n-pentane or cyclohexane. He points out that if a polar,

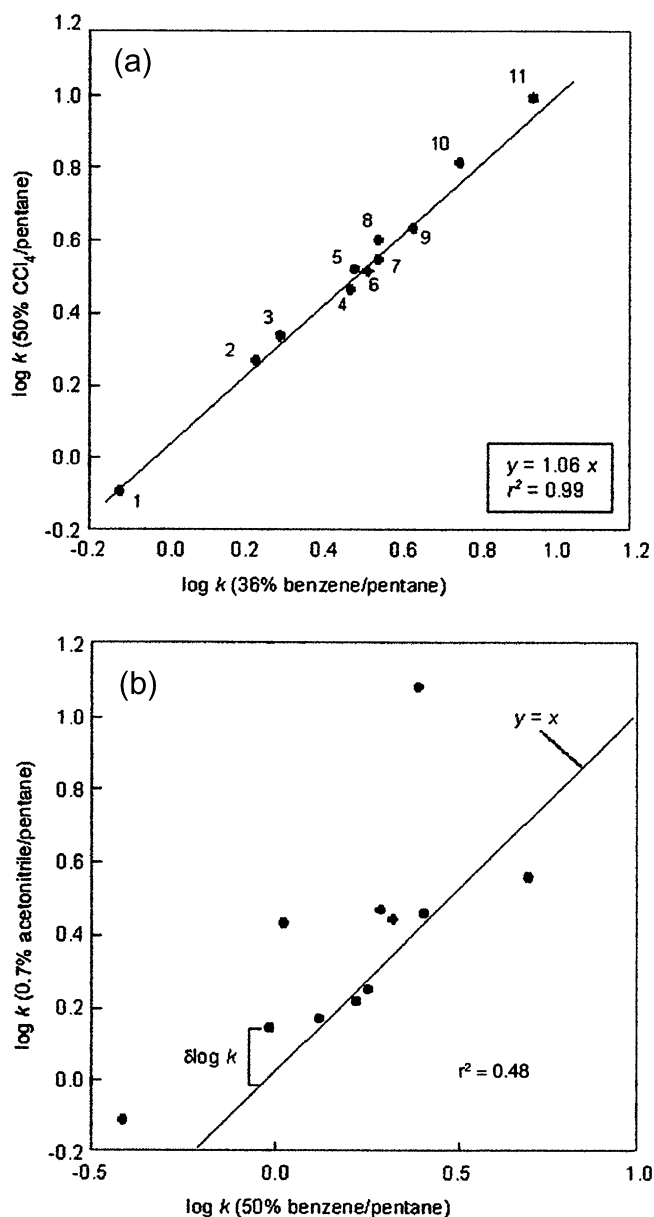


Fig. 29. Comparison of solvent-type selectivity for two equal-strength mobile phases; (a) non-localizing B-solvents (benzene and carbon tetrachloride), (b) one localizing B-solvent (acetonitrile). Alumina as adsorbent. See text for solutes and corresponding numbering.

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localizing solvent, like tetrahydrofuran (THF) is replaced with a less polar one, like CH_2Cl_2 , polar solutes will experience decreased competition for localized interactions with the surface, leading to preferential retention of polar solutes compared to nonpolar solutes and hence to a change in the selectivity of the separation.

In order to better understand the effects of the nature of the B-solvent on selectivity, Snyder first defines a solute-specific property, $\delta \log k$. $\delta \log k$ values are derived by correlations of $\log k$ values obtained using one mobile phase vs. $\log k$ values obtained using a second mobile phase with a different B-solvent (so-called κ - κ plots). Such plots are illustrated in Fig. 29 for eleven solutes. The $\delta \log k$ parameter is also shown in the figure. The mobile phases are adjusted such that their overall solvent strengths, as measured by the solvent strength parameter, ϵ , are comparable. The solutes are (1) 2-methoxynaphthalene; (2) 1,7-dimethoxyaniline; (3) 1-nitronaphthalene; (4) 2-chloroquinoline; (5) 1-methylnaphthoate;

(6) picene; (7) 1-cyanonaphthalene; (8) N-methylaniline; (9) 1-naphthaldehyde; (10) 1,5-dinitronaphthalene; and (11) 1-acetonaphthalene.

The first plot in this figure compares the two non-localizing B-solvents benzene and carbon tetrachloride. The $\log k$ values are highly correlated, meaning that the retention mechanisms with both phases are essentially identical. When a localizing B-solvent (acetonitrile) is compared to a non-localizing one (benzene), the correlation is poor, indicating different interactions are governing retention, creating potential selectivity differences with the different mobile phases. In these plots, it is clear that polar, localizing solutes such as 2-methoxynaphthalene, 2-nitronaphthalene, and 1-naphthaldehyde are each affected differently by the presence of acetonitrile in the mobile phase.

To further understand and differentiate the effects of different B-solvents, Snyder correlates $\delta \log k$ values from one κ - κ plot with $\delta \log k$ values from a second κ - κ plot for the eleven solutes shown in Fig. 29. The squares of the correlation coefficients for these $\delta \log k$ vs. $\delta \log k$ plots are shown in Table 7 (adapted from [89]). To aid in understanding the different results, Snyder uses the solvatochromic selectivity triangle shown in Fig. 30a to differentiate highly basic solvents (top shaded portion of the triangle) from weakly and non-basic solvents (those outside the top shaded portion). The specific B-solvents studied and their relative basicities according to the definitions used to establish the solvent triangle are shown in Fig. 30b, as are their average r^2 values from the $\delta \log k$ vs. $\delta \log k$ correlations.

It is clear from these results that those solvents classified as non- or weakly basic (e.g., nitromethane and acetonitrile) produce the strongest correlations whereas the strongly basic solvents (triethylamine, pyridine, ethyl ether, THF) have the lowest correlation coefficients. It is also clear from the data in the table that correlations between two non-basic solvents (upper left quadrant) produce stronger correlations than those between two basic solvents (lower right quadrant). Furthermore, correlations between a non-basic and basic solvent (upper right quadrant) are weaker than those between two non-basic solvents (upper left quadrant). All of this indicates that the basic solvents have some additional mechanism (or mechanisms) of interacting with solutes and/or the stationary phase that creates additional likelihood for selectivity differences to exist between them. Snyder goes on to offer evidence that part of those selectivity differences relates to the ability of those solvents to increase retention for proton-donating solutes, with the basic solvents increasing retention more than non-basic solvents. This arises because the basic solvents are concentrated on the stationary phase surface. These solvent molecules will preferentially interact with and increase the retention of donor solutes.

The practical upshot to all of these studies is that they provide guidance for optimizing NPLC separations. As detailed earlier in this review, seven ‘training’ chromatograms are used in a simplex optimization scheme when optimizing three parameters. An isocratic optimization scheme related to the use of basic localizing, non-basic, and non-basic localizing solvents in mobile phases of equal solvent strength is shown in Fig. 31 (see original publication for more details). Chromatograms obtained using some of the ‘training’ mobile phases are shown in Fig. 32a–c and the optimized chromatogram is shown in Fig. 32d. The training chromatograms show multiple overlapping peaks and considerably different selectivities for some solutes. By combining non-basic, basic, and localizing solvents, a minimum resolution of 1.3 was obtained for all components. Comparing chromatograms b and c and the resulting chromatogram in d shows the profound effect that the addition of ACN and CH_2Cl_2 to the mobile phase in (c) has on selectivity for solute pairs 6 + 10, 4 + 11, and 8 + 9 (an unfavorable influence for the last pair).

Table 7
Squares of correlation coefficients for $\delta \log k$ values from one κ - κ plot correlated with $\delta \log k$ values from a second κ - κ plot based on eleven solutes listed in the text studied in nine solvents.

Solvent	Non-basic				Basic				
	NM	ACN	ACT	EA	DMSO	TEA	THF	EE	PYR
Nitromethane NM	1.00	0.96	0.98	0.96	0.94	0.86	0.85	0.85	0.81
Acetonitrile ACN	0.96	1.00	0.96	0.96	0.92	0.92	0.81	0.81	0.76
Acetone ACT	0.98	0.96	1.00	0.98	0.92	0.88	0.88	0.90	0.85
Ethyl acetate EA	0.96	0.96	0.98	1.00	0.94	0.90	0.92	0.92	0.88
Dimethylsulfoxide DMSO	0.94	0.92	0.92	0.94	1.00	0.92	0.94	0.81	0.88
Triethylamine EA	0.86	0.92	0.88	0.90	0.92	1.00	0.85	0.79	0.83
Tetrahydrofuran THF	0.85	0.81	0.88	0.92	0.94	0.85	1.00	0.86	0.94
Diethyl ether EE	0.85	0.81	0.90	0.92	0.81	0.79	0.86	1.00	0.88
Pyridine PYR	0.81	0.76	0.85	0.88	0.88	0.83	0.94	0.88	1.00
Average r^2	0.90	0.89	0.92	0.93	0.91	0.87	0.88	0.85	0.85

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While much of the data upon which Snyder's review rests was obtained many years ago, the creation of and subsequent developments of the triangle classification system have helped provide a more complete understanding of the retention mechanism in NPLC and continues to serve as a guide for selecting initial chromatographic conditions in optimization schemes.

9. Future directions in comparing selectivity

9.1. A unifying method for comparing chromatographic selectivity

As suggested in Section 1, new methods for determining selectivity differences between chromatographic systems may help overcome some of the limitations of triangle schemes that we have described throughout this review. For example, Ishihama and Asakawa constructed vectors in five-dimensional space based on LSER coefficients. They used the angle between vectors to assess the similarity of two chromatographic systems [90]. Instead of using the angle between two vectors, Abraham and Martins used the distance between vectors as the metric for comparing two systems [91]. Lázaro et al. also used the distance between vectors, but only after normalizing the vectors to be the same length [92]. Fuguet et al. used principle component analysis of LSER coefficients for pseudostationary phases in electrokinetic chromatography, along with radial distribution plots, to assess selectivity differences between the phases [93]. Principle components analysis of multiple column parameters such as surface coverage, hydrophobic selectivity, shape selectivity, hydrogen bonding capacity and

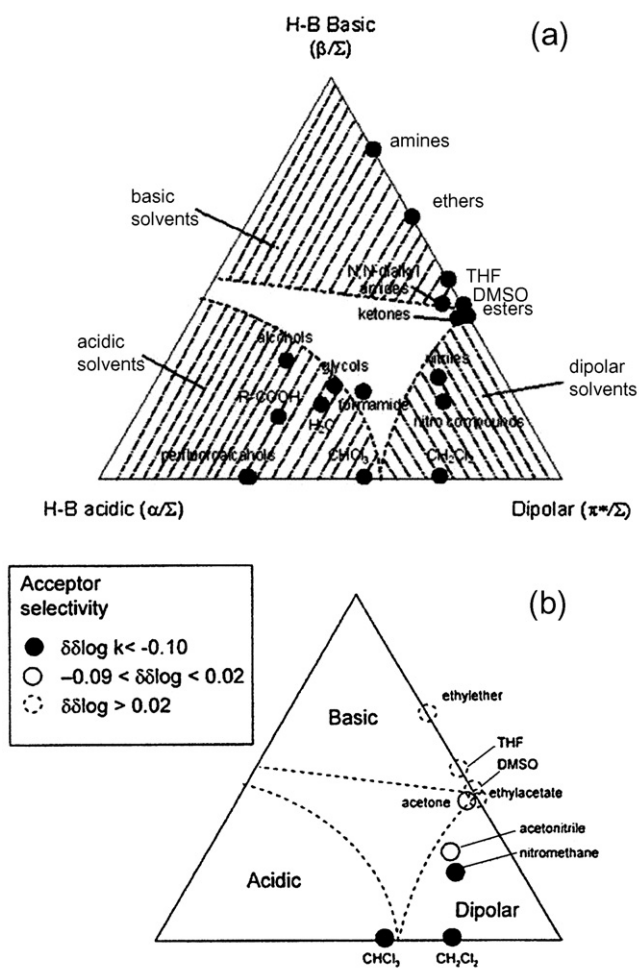


Fig. 30. Solvent-type selectivity as a function of the hydrogen-bond (H-B) basicity of the B-solvent. (a) The solvent-selectivity triangle adapted from Ref. [88]; (b) solvent-type selectivity as a function of B-solvent H-B basicity. Reprinted with permission from [88].

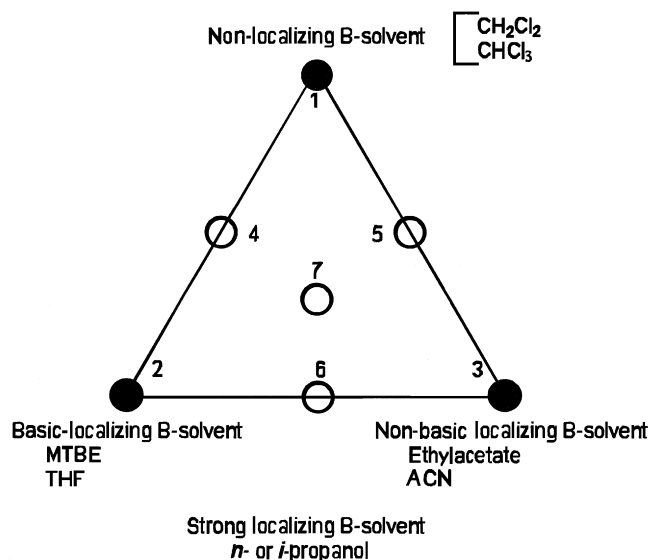


Fig. 31. Optimization scheme for NPLC using non-localizing, basic-localizing, and non-basic localizing B-solvents with A-solvents such as pentane, hexane, or dichloromethane for $\epsilon^0 > 0.30$. Reprinted with permission from [88].

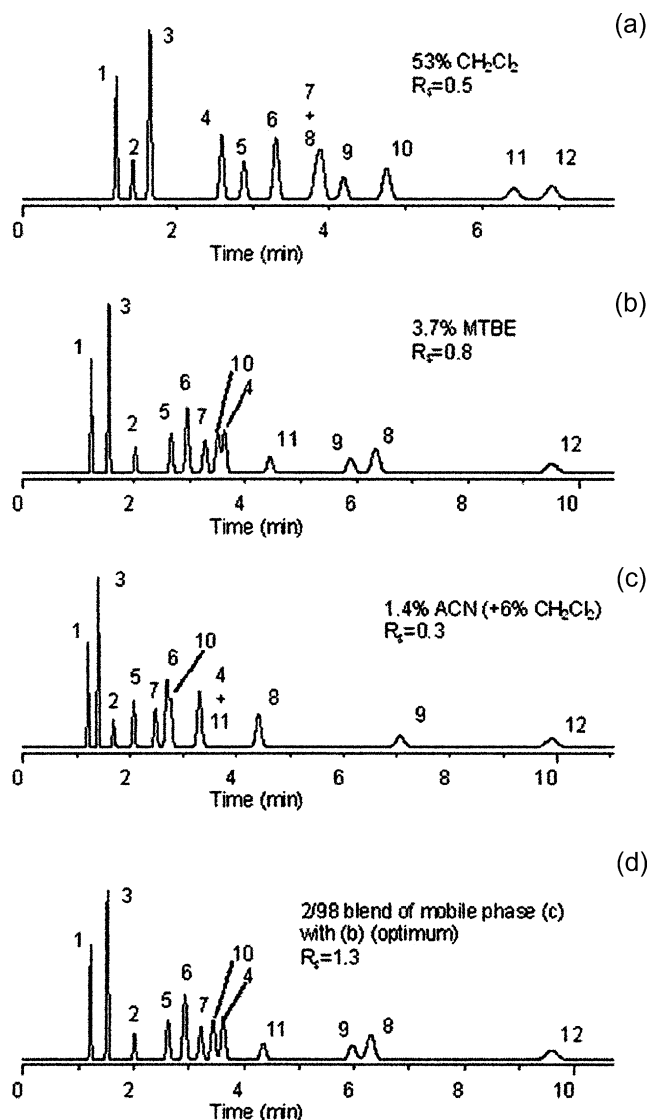


Fig. 32. Examples of the application of the scheme in the previous figure for the selection of an optimum mobile phase. Conditions: 150 × 4.6 Zorbax-SIL column; mobile phases shown in the figure (50% water-saturated). See original publication for details of chromatogram recreation based on retention data. Reprinted with permission from [88].

ion-exchange capacity at pH 2.7 and 7.6 was used by Euerby and Petersson to analyze and easily visualize the similarities and differences between hundreds of RPLC columns [94]. Neue et al. [95,96] recently presented a graphical method for quantifying and visualizing selectivity differences between chromatographic systems of varying pH, eluent type, and stationary phase. Correlations of gradient retention times for the same solutes on two different systems differing in one variable (pH, eluent, or stationary phase) are used to determine s -values, defined as

$$s = \sqrt{1 - r^2} \quad (25)$$

where r^2 is the square of the correlation coefficient. To visualize the data, prisms are constructed (see Fig. 33) in which the s -value that correlates the two systems are used as tie-lines. In this example, differences in stationary phase type (C18, RP18 with a polar embedded group, and a phenyl column) are compared, along with differences in methanol and acetonitrile as mobile phase modifiers. The pH of the solution has been held constant in all studies. Systems with the largest differences in their s -values represent those offering the

$$s^2 = 1 - r^2$$

$$s = \sqrt{1 - r^2}$$

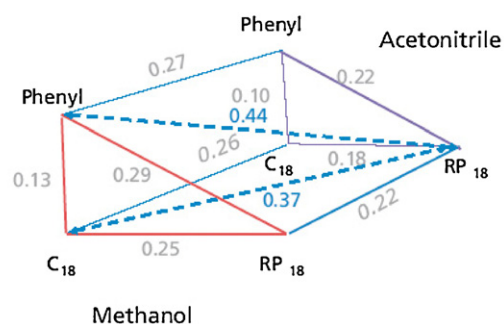


Fig. 33. Depiction of the selectivity differences between the surface chemistries of packings (C18, RP18 with an embedded polar group, and phenyl) and the organic mobile phases methanol and acetonitrile. Light grey values represent ammonium formate (pH 3). The values on each bar are the measured selectivity differences. Note that the largest selectivity differences are found along the diagonal lines. Shown are the selectivity differences between the packing with an embedded polar group with acetonitrile as the organic modifier and the phenyl and the C18 column with methanol as the organic modifier. Reprinted with permission from [96].

largest differences in selectivity (i.e., those most poorly correlated). Snyder et al. [96] point out that this method requires adjustment of the solute retention times such that they span comparable ranges and is only rigorously valid if all of the system variables are independent. Nevertheless, the presentation of this work was compelling and the approach offers an interesting method for comparing system selectivities.

We recently presented a 3D visualization cube to visually detect similarities and differences between separation systems [97]. Our method is based on the correlation of LSER coefficients. Examples of such correlations are shown in Fig. 34, which compares sodium dodecylsulfate with sodium tetradecylsulfate (STS) and with lithium perfluorooctanesulfonate (LiPFOS). In these analyses, as in Fu and Khaledi's [82] and Zhang and Carr's work [86], all of the LSER coefficients are ratioed to the ν -coefficient before the correlation is performed. Clearly, SDS and STS correlate strongly, while SDS

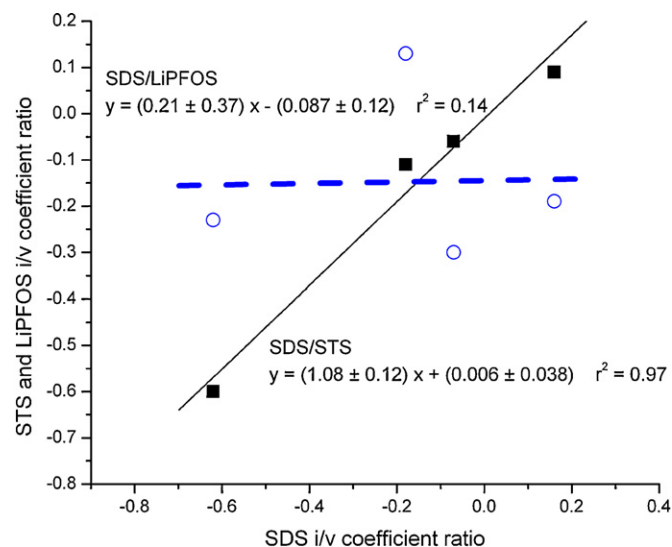


Fig. 34. A plot of the correlation of STS and LiPFOS vs. SDS. The axes are defined as the i/ν LSER coefficient ratio, where $i = a, b, e,$ or s . STS vs. SDS (■), LiPFOS vs. SDS (○). Reprinted with permission from [97]. Copyright 2010 American Chemical Society.

Table 8
Interpretations of energetic similarity or difference and thus selectivity similarity or different for various combinations of r^2 , slope, and intercept from correlations of LSER coefficient ratios from one system vs. those for another system.

Possible results for correlation of s/v , a/v , b/v , and e/v for two systems				Interpretation	
r^2	Slope	Intercept	$\nu_1 = \nu_2$	Energetics	Selectivity
High	= 1.00	= 0.00	Yes	Homoenergetic	Ineffective
High	= 1.00	= 0.00	No	Homeoenergetic	Ineffective
High	\neq 1.00	= 0.00	Yes or No	Potentially heteroenergetic	Potentially effective
High	= 1.00 or \neq 1.00	\neq 0.00	Yes or No	Potentially heteroenergetic	Potentially effective
Low	= 1.00 or \neq 1.00	= 0.00 or \neq 0.00	Yes or No	Potentially heteroenergetic	Potentially effective

and LiPFOS do not. Given the structural similarity of SDS to STS and the dissimilarity of SDS and LiPFOS, these results are not surprising. Such plots are similar to Horvath et al.'s κ - κ plots [97,98] because they fundamentally compare solute retention on one phase to that on another. By using LSERs, though, the same compounds need not be run on each system as long as the solutes analyzed on each phase explore a wide range in type and strength of the intermolecular interactions that govern retention. This makes many more system comparisons possible.

According to Horvath, two systems that yield a κ - κ plot with a high correlation coefficient and unity slope would be termed "homoenergetic" [98]. Linear regressions such as those shown in Fig. 34 yield three statistical metrics: the slope, intercept, and correlation coefficient of the fit. We have shown [97] that for two systems whose correlation yields slope = 1.00, intercept = 0.00, and $r^2 = 1.00$, and whose ν -coefficients are equal, those systems will exhibit homoenergetic retention. This implies that the energetics of retention on both phases are identical and thus there is little to no difference in their selectivities. In other words, there is no real chance for what Zhao and Carr called 'effective selectivity' [81]. The two systems will yield the same order of elution and thus very comparable separations.

If the systems exhibit a high correlation and slope = 1.00, but the ν -coefficients for both LSERs are different such that $\nu_1/\nu_2 \neq 1.00$, the situation would be termed 'homeoenergetic', indicating a similar physico-chemical basis for separation but no chances for elution order changes [98]. Again Zhao and Carr would say there is no real chance for effective selectivity differences to exist between the two systems [81]. It is possible that the solutes will be spread out on one system more than the other, but the chance for fundamentally altering the separation does not exist.

Finally, correlations between systems that yield slopes \neq 1.00, intercepts \neq 0.00 and/or low correlation coefficients may exhibit "heteroenergetic retention" [98]. In other words, retention on one phase is not necessarily correlated with retention on the other. Potential differences in selectivity exist between the two systems. In fact, elution order changes, and hence 'effective selectivity differences' are possible. When two systems exhibit this kind of relationship, if the desired separation is not being achieved with one system then switching to the other system could improve the separation.

Thus, correlating the LSER coefficient ratios of one system vs. another and analyzing the slope, intercept, and correlation coefficient can yield information about the similarity or differences in selectivity for the two systems. The possible combinations and their interpretations are shown in Table 8. The key point in Table 8 is that retention on the two systems can be compared with three parameters: r^2 , slope, and intercept (a fourth dimension regarding the relationship between ν -coefficients is needed to differentiate homo- and homeoenergetic retention). Specifically, systems with (1) non-unity slopes, (2) non-zero intercepts, or (3) poor correlation coefficients offer the possibility for 'effective selectivity differences' (i.e., elution order changes, dramatic changes in relative retention, etc.). In other words, their retention mechanisms are different and

separations that fail on one system may be better on the other. Or, the two systems together are candidates to be used as 'orthogonal' systems in 2D separations. This, of course, assumes that the solute properties are such that they take advantage of the differences in the energetics of retention. The elution order of n-alkanes is likely to be the same in all systems because there is only one dominant mode of interaction amongst them. To exploit system differences, the solute set must differentially explore the interactions offered by the systems.

While the paragraph above focuses on finding chemically different systems, it is also important to point out the utility of the approach to finding comparable systems (those with high r^2 , unity slopes, and $\nu_1 = \nu_2$). Such systems can be used as replacements to yield comparable separations should such a need arise.

As noted elsewhere [97] this approach unifies three major concepts in selectivity: (1) the general LSER formalism, or any other multi-parameter model of retention such as the Snyder-Dolan hydrophobic subtraction model, (2) Zhao and Carr's concept that the ratios of LSER coefficients, not their absolute magnitudes, are the important parameters for comparing system selectivity, and (3) Horvath's κ - κ plots for classifying systems as homo-, homeo-, or heteroenergetic.

9.2. Visualizing the results using 3D plots

The above procedure requires that the LSER coefficient ratios be analyzed for each pair of systems of interest. For Fu and Khaledi's set of 74 MEKC systems, this yields 2701 different comparisons that can be performed. Performing the correlations is easily automated, but understanding the output could be daunting if one tries to simply look at the statistical output for this many correlations. A visualization method is needed. For that reason, we developed what we call a system selectivity cube (SSC) – a three-dimensional plot for which the axes are the slope, intercept, and correlation coefficient for system correlations. Every correlation of LSER coefficient ratios for two systems is then represented as a glyph in three-dimensional space. The name 'system selectivity cube' is meant to recognize the valuable contributions to chromatography arising from Snyder's solvent selectivity triangle. The development and characteristics of the SSC are detailed elsewhere [97], but an example of the 3D plot based on the LSERs gathered by Fu and Khaledi is shown in Fig. 35.

We highlight here a few of the capabilities of this visualization method:

- (1) The light green dot is a marker for the point with slope = 1.00, intercept = 1.00, and $r^2 = 1.00$ (in other words, highly correlated systems).
- (2) The cube can be rotated, shrunk, or enlarged using a mouse to help highlight certain axes or certain regions of the cube. An example is shown in Fig. 36.
- (3) Different colors represent correlations between systems within a group according to the groupings proposed by Fu and Khaledi

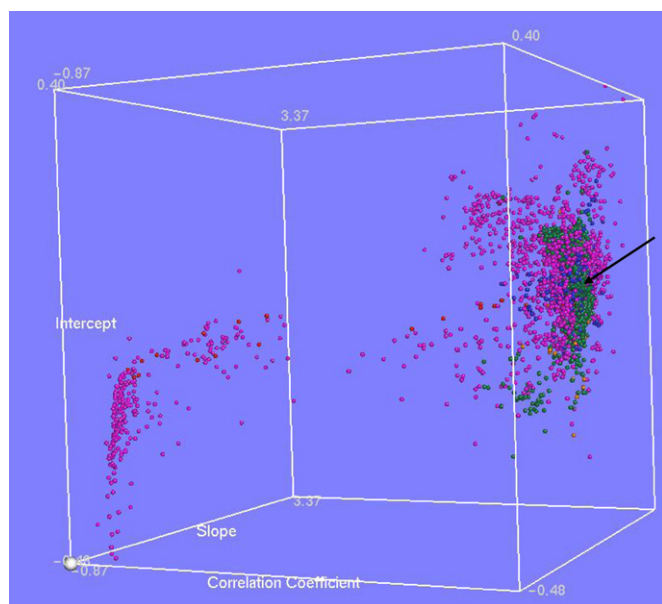


Fig. 35. Example of a 3D visualization made by plotting the slope, correlation coefficient, and intercept resulting from the correlation of LSER coefficient ratios for two systems. Each point represents the regression results obtained by correlating two systems. Data from Fu and Khaledi's MST compilation was used to generate this plot. See text for other details. The correlation coefficient axis goes from 0.00 (left) to 1.00 (right). The point representing an ideal homeoenergetic relationship has an $r^2 = 1.00$, intercept = 0.00, and slope = 1.00. It is therefore on the rightmost face of the cube, roughly in the center and indicated with an arrow. Reprinted with permission from [97]. Copyright 2010 American Chemical Society.

based on their selectivity triangle (blue = group A, red = group B, green = group C, orange = group D).

- (4) The pink glyphs represent comparisons between systems in different groups.
- (5) A user interface allows the user to

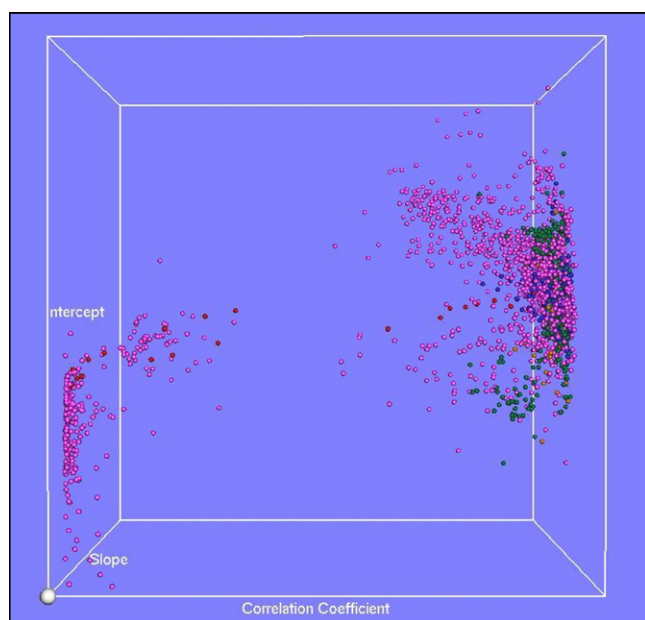


Fig. 36. Same plot as in the previous figure but rotated to make the correlation coefficient axis more prominent. The default axes values are the high and low values present in the data set but are not shown for clarity. The correlation coefficient axis goes from 0.00 (left) to 1.00 (right). Reprinted with permission from [97]. Copyright 2010 American Chemical Society.

- (A) Display only particular group correlations (e.g., correlations within group B).
- (B) Display (or not) the maximum and minimum values on the axes.
- (C) Change the maximum and minimum values for all of the axes. The large grey ball at one corner indicates the minimum for all three coordinates.
- (D) Display (or not) the light green marker that represents highly correlated systems.
- (E) Display the numeric coordinates of the points shown on the screen by hitting the 'output' button. It is important to note here that not only the slopes and intercepts are provided, but uncertainties in these values are also shown. Values of the correlation coefficients are also shown.
- (F) Display only comparisons of interest by selecting individual systems (e.g., specify system 12 correlated with systems 19, 22, 35, and 65).
- (G) Add another dimension of data by displaying the ν -coefficient ratio for each comparison. Recall that when the ν -coefficients of two systems are equal, different energetics exist than when the ν -coefficients have different magnitudes. Thus, we have built in an option to display 'spikes' on the dots – the 'spikier' the dot, the larger the ν -coefficient ratio is for the two systems represented by the glyph.
- (H) A free version of the analysis and visualization software is available at <http://artsci.drake.edu/urness/download/ssc.html>.

9.3. Advantages of the system selectivity cube

There are some significant benefits to the cube visualization compared to triangles:

- Basing the 3D comparisons on LSERs means that dozens, and sometimes hundreds of solutes have been used to generate the coefficients. This is likely more reliable than selecting only three representative solutes as was done in the early selectivity triangle schemes.
- Additionally, all of the LSER coefficients are simultaneously considered, unlike triangles which, even if based on LSERs, can only consider three parameters at a time.
- As shown above for the Fu and Khaledi and Zhang and Carr reports, four separate plots are required to represent all of the parameters when using triangles. With the new approach, a single plot incorporates all of the data.
- Furthermore, if two systems are the same in three of the four LSER parameters, they will appear in the same group in one of the triangles and potentially in different groups in the other three triangles. Here, if the fourth parameter is different enough to ruin the correlation, the systems will immediately appear to be different. See, for example, the plot for SDS vs. LiPFOS in Fig. 34.
- Unlike κ - κ plots which require the same solutes to be analyzed on both columns, with the LSER approach any representative set of solutes can be used to obtain the coefficients upon which the methodology relies.
- Lastly, and perhaps most importantly, this approach can be applied to any multi-parameter model of retention – not just LSERs. For example, we have started analyzing the large RPLC data set of Zhang and Carr discussed above which uses the hydrophobic subtraction model to understand retention.

9.4. Disadvantages of the system selectivity cube

Some of the advantages of the new approach are also disadvantages, depending on the analyst's goals. Here is a brief (and not

necessarily exhaustive) list of some of the important issues as we currently see them.

- LSER coefficients generally have large relative uncertainties due to imperfections in the model. Thus, the slopes and intercepts of the correlations may be unreliable as a basis for firmly classifying similar and dissimilar systems. Nevertheless, we believe the approach can certainly serve as a helpful guide in distinguishing systems.
- Another disadvantage is the severe data reduction that is taking place and the chemical information that is lost in the process. Hundreds of solutes are sometimes used to generate LSER equations for a system. The LSERs themselves generally have five or six fitting parameters (therefore 10–12 values for two systems). Thus, in comparing two systems, hundreds of individual data points that relate directly to retention and selectivity, and up to a dozen LSER terms that also relate to solute retention, are getting simplified down to three or four parameters (slope, intercept, correlation coefficient, and ν -ratio). These four parameters are not immediately related to solute retention. Thus, a lot of useful chemical information is not being used explicitly in this model. Such a process has an analogy in assigning semester grades at most U.S. academic institutions. Throughout a semester, students take multiple tests and quizzes, write lab reports, turn in homework, etc. – all of which contain very specific information about student performance. The scores on these individual pieces get weighted and then averaged together to produce an overall semester average. This average is further simplified to a single semester grade on a five-grade scale of A through F. So a lot of specific information is sacrificed for the simplicity of obtaining a single letter grade. Likewise, our approach sacrifices specific retention information for the sake of obtaining a simple three-parameter comparison of system selectivity.
- Another concern that this approach shares with the triangles is the loss of chemical insight regarding the actual type and strength of intermolecular interactions governing separations. The glyph merely tells the user if two systems are similar or different in their energetics of retention but without any details regarding the specific blend of interactions. For this, an analysis of the LSERs (or other model) would still need to be done.
- As noted above, different amounts of water in RPLC mobile phases can have dramatically different effects on selectivity, and the extent of those effects varies with the organic modifier. As a result, the coefficients of LSERs vary with percent water. Thus, multiple LSERs would be required to fully characterize one organic modifier. The proposed selectivity cube could handle this in terms of comparing one modifier at one composition either to the same modifier at a different composition or to a different modifier at the same percent composition. In fact, any number of combinations of modifier/composition could be compared, but the number of comparisons could be quite large and thus difficult to fully evaluate. Furthermore, because of the different effects of water on different solvents, such comparisons will always be system-specific and will not lead to general classifications regarding the organic modifiers.
- Somewhat related to this, two systems may be poorly correlated because of a single parameter. For example, the a/ν ratio could be positive for one system and negative for another, with all other ratios generally the same. This is likely to still lead to a poor correlation and be interpreted as arising from two dissimilar systems. Thus, differences in a single parameter may be overemphasized in this approach (see again the correlation of SDS vs. LiPFOS in Fig. 34). This would be particularly important if the user's solute set does not contain solutes that are hydrogen bond donating (i.e., no solutes with significant A values). Our approach would lead one to believe that the systems are different, but for such a solute

set, the two systems could provide nearly identical selectivities since the solutes cannot take advantage of the difference in the a/ν ratios of the two systems. This could be mitigated with proper weighting schemes, which we are considering. Ideally, the user would get to input the weighting schemes and in this way get to emphasize the solute characteristics they believe to be most important.

Thus, much work remains to figure out how best to use this approach and to apply it to other data sets.

We introduced the SSC and the other methods for analyzing selectivity summarized earlier as alternatives and possible complements to or replacements for selectivity triangles. This brings us back full circle to the poem in our introduction. At some point, people stopped building pyramids, but as Jennifer Michael Hecht urges “we must not curse the passage of time.”

10. Summary

Overall, we have traced the development of chromatographic selectivity triangle schemes over the past 50 years. Valid criticisms of some of the schemes were considered, and recent applications based on new models of retention were highlighted. Finally, newer methods for comparing system selectivity were presented.

Specifically, we started this review by examining the early origins of triangles as first applied to GC stationary phases. We then focused largely on Snyder's original SST because it has received the most attention of all of the triangles produced. We discussed the use of the SST in combination with simplex experimental designs to optimize LC separations. We have also discussed the complications that the presence of water in RPLC mobile phases causes when using selectivity triangles to make accurate predictions of selectivity. This stems from the effect that water has on the overall polarity of the mobile phase, the specific changes it induces in the organic additives, and the specific polar and hydrogen bonding interactions it has with solutes. Because of these effects, even mobile phases of comparable solvent strength yield different selectivities. Because the SST is based on pure solvents and does not incorporate the effects of water, this generally limits the accuracy of predictions of RPLC selectivity that are based on the SST.

With regards to GC, many groups have used the SST approach to characterize common stationary phase coatings. In the original development of the SST, Snyder tried to remove the dispersion effects by normalizing partition coefficients of polar solutes to those of hypothetical n -alkanes of comparable size. Furthermore, he based the SST apices on test solutes that do not explicitly represent dispersion interactions. In subsequent studies by other groups, changing the test solutes was observed to change the location of the phases within the triangle. The contributions of dispersion interactions to selectivity were of specific interest in these studies, as well as some studies involving RPLC.

Fifteen years after the publication of Snyder's original SST, solvatochromic parameters were used to show that the original X_e , X_d , and X_n values represented blends of two or more intermolecular interactions. In subsequent publications, a triangle based on solvatochromic solvent parameters was presented. This produced some advantages over the original version in terms of the chemical interpretation of the triangle because of the relative ‘purity’ of the parameters used to define the apices. Nevertheless, these parameters cannot account for the effects of water on the nature of the solvents. Thus, triangles based on solvatochromic parameters still suffer from the same complications regarding the practical application of triangles to predict RPLC selectivities.

More recently, selectivity triangles have been used in combination with LSERs to characterize pseudo phases used in MEKC

separations. The definition of the parameters, however, complicates understanding their chemical meaning and potentially influences the groupings produced within the triangle. Using similarly defined parameters, multiple triangles examining hundreds of commercially available RPLC phases have recently been published. These triangles, however, were based on the coefficients produced by the hydrophobic subtraction model of retention rather than on LSERs.

NPLC triangles were recently reviewed by Snyder. These triangles emphasized the importance of solvent interactions with the bare stationary phase – either through general non-localized or specific localized interactions. Furthermore, the influence of solvent basicity on selectivity was also considered. Because of the absence of water and its associated complications, predictions of selectivity based on NPLC triangles schemes are generally much more accurate than those in RPLC.

Lastly, we closed this review by discussing other methods for comparing the selectivity of separation systems, focusing on our system selectivity cube. This work unifies concepts that underpin selectivity triangles with theories regarding the homo-, homeo-, and heteroenergetic nature of retention on two different systems. A 3D visualization method for simultaneously viewing hundreds or thousands of system comparisons was discussed. Potential advantages and disadvantages of this new approach were briefly examined.

Given all of the advances that have occurred over the past half of a century in the development of selectivity triangles, it is natural to end this review by turning to the future. The theory behind the triangles seems to be well examined, and the advantages and disadvantages of various formulations are well understood. Perhaps it is time to shift the emphasis from creating more triangle schemes to rigorously evaluating how well they do the job of characterizing selectivity that they are designed to do. We therefore suggest that it would be helpful to have studies aimed at using some of the recent publications involving MEKC and RPLC to show how selectivity triangles can be used to guide the selection of pseudo phases or stationary phases for practical separations. In other words, perhaps we need more examination of how reliably the triangles identify different and comparable phases for actual mixtures of interest, not just general test mixtures used to develop LSERs and the hydrophobic subtraction model. More specifically, perhaps such schemes can be shown to be effective for identifying orthogonal phases that can be coupled in 2D RPLC separations. Relatedly, it is equally important to be able to identify systems that have comparable selectivities so that replacement systems can be readily adopted should some commercial phases become unavailable. Such studies, if successful, would then encourage the further development of fundamental triangles schemes for characterizing, selecting, and optimizing separation systems.

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