Journal of Chromatography B 1092 (2018) 207–219

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Review

Chromatographic test methods for characterizing alkylsiloxane-bonded silica columns for reversed-phase liquid chromatography

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ARTICLE INFO

Keywords:
Reversed-phase liquid chromatography
Column classification
Solvation parameter model
Hydrophobic-subtraction model
Prototypical test compounds
System maps
Alkylsiloxane-bonded silica stationary phases
Selectivity

ABSTRACT

Major obstacles to formulating a simple retention mechanism for reversed-phase liquid chromatography have a direct impact on the development of experimental methods for column characterization as they limit our capability to understand observed differences in retention at a system level. These problems arise from the heterogeneous composition of the stationary phase, the difficulty of providing a working definition for the phase ratio, and uncertainty as to whether the distribution mechanism for varied compounds is a partition, adsorption or mixed (combination) of these models. Retention factor and separation factor measurements offer little guidance as they represent an average of various and variable contributing factors that can only be interpreted by assuming a specific model. Column characterization methods have tended to ignore these difficulties by inventing a series of terms to describe column properties, such as hydrophobicity, hydrophilicity, silanol activity, steric resistance, etc., without proper definition. This has allowed multiple scales to be proposed for the same property which generally are only weakly correlated. Against this background we review the major approaches for the characterization of alkylsiloxane-bonded silica stationary phases employing prototypical compounds, the hydrophobic-subtraction model and the solvation parameter model. Those methods using prototypical compounds are limited by the lack of compounds with a singular dominant interaction. The multivariate approaches that extract column characteristic properties from the retention of varied compounds are more hopeful but it is important to be more precise in defining the characteristic column properties and cognizant that general interpretation of these properties for varied columns cannot escape the problem of a poor understanding of the distribution mechanism.

1. Introduction

Although the lexicon of available modes of liquid chromatography is now quite extensive, reversed-phase liquid chromatography (RPLC) remains the mode of choice for the separation of neutral and ionizable compounds in the biomedical and life sciences [1]. This dominance results from its capability to handle compounds of a wide range of size, polarity and ionicity. The combination of this versatility with rapid equilibration of the stationary phase with changes in mobile phase composition facilitating gradient elution and the availability of relatively straightforward, although largely empirical, approaches to method development add luster to the attraction [1, 2]. The inclusion of additives in the mobile phase allows exploitation of secondary chemical equilibria to control retention and selectivity by ion suppression, ion-pair formation, and various complexation mechanisms. Reversed-phase liquid chromatography is now so widely employed in pharmaceutical, biomedical and life science research, product development, and quality control one can only speculate as to how these fields might look today if reversed-liquid chromatography had not arrived on the scene at the same time as exponential growth occurred in these areas. It would be an exaggeration to say that this growth was possible only because of the development of reversed-phase liquid chromatography, but it is certainly true that it was one of the major enabling techniques that facilitated the rapid progress made.

From the beginning reversed-phase liquid chromatography has been synonymous with alkylsiloxane-bonded silica stationary phases, especially ocadecylsiloxane-bonded silica, or simply C18. Over time significant improvements in column performance, pH stability, and inertness with respect to the separation of basic compounds have been made [1, 3, 4]. More recent developments include sub-3 μm totally porous and core-shell particles and monolithic rods for fast separations or enhanced peak capacity [5–7]. Current developments in stationary phase chemistry are largely incremental and hidden from view to those, now the majority, of scientists who consider columns as a supply item and have passed the torch of innovation to the industrial sector for improvements through continuous product development. More than

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https://doi.org/10.1016/j.jchromb.2018.06.011
Received 31 March 2018; Received in revised form 3 June 2018; Accepted 4 June 2018
Available online 07 June 2018
1570-0232/

ever this has resulted in a crowded market place where competing companies launch new products at regular intervals supported by marketing information that often does not make chemical sense and serves to confuse as much as inform the customer. Columns are certainly more reliable, reproducible and robust compared to yesteryear, as can be gleaned from batch-to-batch studies of similar columns over multiple years [8, 9], but with so many columns of notionally similar type to choose from, the identification of columns with properties that closely resemble each other (selectivity equivalent) or columns with significantly different selectivity (orthogonal selectivity, although orthogonal is somewhat an exaggeration when applied to RPLC columns) suitable for the screening phase of method development have never been more difficult to identify. For typical alkylsiloxane-bonded silica columns the manufacturer will usually provide information for the type of bonded ligand, type of silica substrate (type-A, or -B), particle shape, the mean particle size, and mean pore diameter. In some cases they may provide information for the particle size distribution; the pore size distribution and pore volume; the specific surface area; percent carbon loading and/or degree of surface coverage (bonding density), concentration and identity of major metal impurities; and whether or not the stationary phase has been endcapped. Type-A silica refers to first generation silica substrates characterized by a relatively high concentration of metal impurities and type-B silica to modern high purity silica substrates with very low metal contamination. However, it is neither easy nor straightforward to relate the above properties to chromatographic retention and selectivity. Methods used to determine these properties remain non-standardized and they are not necessarily comparable when taken from different sources [2, 3, 9–12], The mean particle size and size distribution may be based on number, area or volume depending on the type of instrument used for the measurement and are not directly comparable, although this parameter is strongly correlated with the column kinetic performance (plate count or plate height) and the column pressure drop. Compared to the number particle size distribution, the area and volume distributions are more effected by large particles resulting in a distribution skewed towards the fraction of larger particles present in the packing. The surface area, pore size and pore volume are typically determined by either nitrogen BET or mercury porosity measurements with the aid of different models. The values obtained depend on the model employed and how micropores are treated in evaluating the experimental data. The percent carbon loading is influenced by whether the packing is endcapped or not and the extent of this reaction. For column packings with different surface areas the surface coverage (bonding density) is a more useful parameter but is calculated assuming the surface is covered with a single type of bonded organic moiety, in other words, does not handle endcapped packings correctly. In general, surface area and mean pore diameter values are given for the silica substrate prior to bonding and are not the values for the chemically bonded phase. Manufacturers typically withhold information for how a column packing is endcapped, or provide only general information, such as “by trimethylsilyl groups”, using a “polar endcapping reagent” or “endcapped to insure water compatibility”. Thus, in summary, typical physicochemical column parameters are not generally suitable or trustworthy for column selection and are only useful in a qualitative sense for a broad classification of column types. There is little impetus for individual laboratories to re-determine these parameters with a common standard scale since this means destroying (unpacking) the column. Also, the necessary equipment for these measurements may not be available in many analytical laboratories. From a customer perspective a more meaningful solution is the development of column chromatographic tests utilizing standard substances and prescribed separation conditions, typical instrumentation for liquid chromatography, and one or more chromatograms for data interpretation.

2. Column chromatographic test methods

A number of column test methods have been proposed to assess different separation properties of alkylsiloxane-bonded silica stationary phases. The most prominent methods are the Tanaka [13], Engelhardt [14], the extended Engelhardt test proposed by Neue [9, 15] and the Hoogmartens' test (generally referred to as the Katholieke University Leuven method) [16, 17]. For these methods, specific compounds (prototypical compounds) with an assigned singular characteristic property are injected onto the column and their retention factors, separation factors, or in some cases peak asymmetry factors and column plate number, are determined with a specified mobile phase composition. These values are then used to rank columns according to the property inferred for the prototypical compounds. Typical column characteristic properties determined in this way are hydrophobicity (or hydrophobic selectivity), silanol activity (hydrogen-bonding interactions with neutral silanol groups), polar interactions, shape selectivity (or steric selectivity), metal contamination and ion-exchange capacity (electrostatic interactions with ionized silanol groups). Numerous studies have compared individual test methods leading to the selection of a smaller number of tests to define column properties. Euerby and coworkers [18–20], for example, used principle component analysis to define six test procedures to compare a large database of columns for the separation of pharmaceutical compounds. Likewise, Adams and coworkers [16, 17] selected four test parameters to rank columns in a large column database for the separation of pharmaceutical compounds. The object of these studies was to identify columns with similar separation properties (selectivity equivalent columns) that could be used as an alternative for a column specified in a method and to identify columns with significant selectivity differences for the column screening phase in method development.

A different approach to column characterization based on quantitative retention relationships is represented by the hydrophobic-subtraction model and the solvation parameter model. These are model-based, solute-independent approaches that isolate pre-defined characteristic column properties based on experimental retention factors for varied solutes with established capabilities (descriptor values) associated with the characteristic column properties. The hydrophobic subtraction model, Eq. (1), is an empirical model only applicable to retention in reversed-phase liquid chromatography [21–23]

$$\log\ k = \log\ k_{EB} + \eta'H - \sigma'S^* + \beta'A + \alpha'B + \kappa'C \quad (1)$$

in which k is the retention factor for a compound selected to facilitate calculation of the characteristic column properties; k_{EB} is the retention factor for ethylbenzene (a reference compound to account for differences in the phase ratio for compared columns); η', σ', β', α' and κ' are characteristic solute properties (descriptors); H, S^*, A, B and C are the characteristic properties of the system (column, mobile phase and temperature). To characterize column properties, typically isocratic retention factors for 16 specified compounds and ethylbenzene are determined on each column with a mobile phase of 50% (v/v) acetonitrile-phosphate buffer at pH = 2.8 or 7.0 and a temperature of 35°C. It is further assumed that the column parameters (H, S^*, A, B and C) are largely independent of mobile phase conditions except for pH and the solute parameters (η', σ', β', α' and κ') are varied to allow fitting of the retention factors to the model for different column types [24]. The contributions to retention from hydrophobic interactions is identified as η'H, from steric interactions $\sigma'S^*$, from hydrogen-bonding of basic solutes to silanol groups (stationary phase acting as a hydrogen-bond acid) β'A, from hydrogen bonding of acidic solutes to basic groups within the stationary phase (stationary phase acting as a hydrogen-bond base) α'B, and ion-exchange interactions of ionized (protonated) bases at a mobile phase pH = 2.8 $\kappa'C_{2.8}$ or pH = 7 $\kappa'C_{7.0}$. The column properties (H, S^*, A, B and C) are determined simultaneously by multiple linear regression analysis for the complete set of experimental retention factors for compounds selected to cover the descriptor space