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ON THE CALCULATION OF THE STATIONARY PHASE VOLUME IN REVERSED PHASE CHROMATOGRAPHY

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ABSTRACT

The volume phase ratio (stationary/mobile, V_S/V_M) in reversed phase liquid chromatography (RPLC) is a necessary quantity for obtaining thermodynamic information such as chromatographic partition coefficients and changes in entropy and Gibbs free energy as a solute partitions from the mobile to the stationary phase. Previous methods of calculating this ratio have used only the surface area of the bonded stationary phase or have overestimated the stationary phase volume by including that of the inert silica support. A new method is presented for the calculation of the stationary phase volume, V_S , which only requires measurements of carbon load and the total weight of packing in the column. A primary advantage of this method is that knowledge of the packing surface area is not necessary. When calculated by this method, V_S is the actual volume of the alkyl chains bonded to the support surface, but neglects intercalated solvent volumes.

INTRODUCTION

Reversed phase liquid chromatography (RPLC) is currently one of the most widely practiced methods of analysis, and the contribution of both mobile phase and stationary phase interactions to retention is becoming better understood. Although much work has been done on measuring the volume of the mobile phase, V_m [1-4], measurement of V_s , the volume of the stationary phase has remained more ambiguous [5-10]. In determining a method for the measurement of V_s , a convention for defining V_s must be chosen, since the choice of the phase ratio must be compatible with the definition of K that is in agreement with the molecular mechanism of retention. Guiochon et al. have defined the stationary phase volume as that fraction of the column volume that is not occupied by the mobile phase [5]. While this choice is certainly convenient and can be readily determined, it is at best a crude measure, as similar (or even identical) values of V_s would be obtained for stationary phases made from the same bulk silica but with different bonding densities of alkyl chains, or possibly even of different chain lengths. Any determination of stationary phase volume based solely on mobile phase volume measurements is fraught with difficulty as such a measurement may not be sufficiently sensitive to ascertain bonding density or small chain

length differences. Although Armstrong and Nome [11] have used this definition of V_s in their determination of partition coefficients in micellar liquid chromatography, they admit that the use of the entire volume of the column packing for the volume of the stationary phase is merely for convenience, adding "One should keep in mind, however, that only the surface bonded layer is actually involved" [11].

Melander and Horvath have suggested defining the phase ratio as the ratio of the surface area of the adsorbent (m^2) divided by the column dead volume (cm^3) [12]. While this approach is an improvement in definition, it again fails to account for certain variations in the structure of the bonded phase and it implies that adsorption is the sole mechanism in RPLC retention. The major drawback to this proposed phase ratio convention, however, lies in the accurate measurement of the two parameters involved. Although many methods have been used to calculate column dead volumes, chromatographers have been unable to embrace any one of these methods as being accurate and consistent enough for precise work [1-4]. Other problems exist as well. The surface area of the adsorbent is usually found by use of the BET analysis method. It should be noted that the surface area of the adsorbent must be determined after derivatization with

the alkyl ligand, as the surface area of the derivatized silica will be significantly different from that of the underivatized support. Although use of the BET method for surface area determination is widespread, this method is inappropriate in assessing that surface area of derivatized silica packings which is chromatographically significant. The BET method measures the area of surface that is accessible to a small molecular probe such as nitrogen. Yet in an irregular surface such as porous silica, there may exist many pores which are large enough to allow nitrogen in, but which are too small to allow the passage of any larger molecules of chromatographic interest. Chromatographic support surface area data based on BET analysis is usually overestimated, and the amount of overestimation is by no means a constant, depending on the base silica structure and the derivatization method. Horvath states that "...any estimation of 'stationary phase volume' on the basis of BET surface area of the support is likely to be inaccurate" [12]. Due to the errors in determining both adsorbent surface area and column dead volume, there will consequently be a large error propagated in the subsequent calculation of the phase ratio if Melander and Horvath's convention is used.

Sander and Field have estimated the phase ratio by constructing physical models of the bonded phase using

manufacturer's data regarding silanol surface coverage and percent carbon loading [7]. This approach is quite reasonable from a theoretical standpoint, as it accounts for variation in bonding density and alkyl chain length. However, the construction of such physical models is quite time consuming.

In determining the stationary phase volume, the pertinent volume should be the volume of the alkyl chains bonded to the silica surface. Dill has performed statistical mechanical calculations based on a lattice interphase model of RPLC stationary phases which describes chromatographic retention in reversed phase systems [13]. After careful consideration of both partitioning and adsorption retention mechanisms in conjunction with his interphase model and available experimental evidence, Dill concludes that the principal retention mechanism for small nonpolar solutes in RPLC is partitioning. He states that two driving forces dominate the retention process; (i) the difference in the chemistry of the contacts of the solute with its surrounding molecular neighbors in the stationary and mobile phases, and (ii) the partial ordering of the grafted chains which leads to an entropic expulsion of the solute relative to that which would be expected in a simpler amorphous oil/water partitioning process. We have tested the validity of (i) against an extensive

data base of nearly 350 sets of experiments [14]. We have also tested the validity of (ii) by experimental measurements of the chromatographic partition coefficients of small nonpolar solutes as a function of stationary phase alkyl bonding density [15]. The partition coefficient increases as a function of alkyl bonding density until a certain critical bonding density has been reached wherein chain packing constraints become appreciable. At these higher bonding densities, solute partitioning decreases with increasing bonding density since the energy required to create a solute sized cavity in the stationary phase becomes increasingly prohibitive due to stationary phase chain configurational constraints. This indicates that partitioning is the dominant mechanism in RPLC retention; adsorption should be unaffected by the surface density of the bonded chains. These calculations and experiments have shown that in a well endcapped column chain interactions with solutes are the most important stationary phase contribution to solute retention. Therefore the calculation of V_s should give only the actual volume of the alkyl chains bonded to the support surface. The assumption here is that all of the bonded stationary phase volume is accessible to the solute.

RESULTS AND DISCUSSION

A simple method for calculating the stationary phase volume has been devised in our laboratory. The only measurements necessary are the carbon load of the packing and the actual weight of packing contained in the chromatographic column. In order to define the surface coverage of the bonded silica in an unambiguous and pertinent manner, the surface coverage should be expressed as the number of silane molecules attached to the surface, usually as micromoles of bonded silane molecules per square meter of silica surface, taking into account the increase in weight of the silica after the bonding reaction. These surface coverages are calculated from the percentage of carbon as obtained from elemental analysis of the bonded phase [16]. This calculation is quite straightforward for monoreactive silanes and for monochlorosilanes (the most commonly used monoreactive reagents) can be expressed by equation 1:

$$\alpha = \frac{(\%C) (10^6)}{(12.011)(n_c)(S)(100 - [(\%C)/(12.011)(n_c)](M - 35.5))}$$

where α is the surface coverage ($\mu\text{moles}/\text{m}^2$); %C is grams carbon per 100 grams bonded silica, as obtained from elemental analysis or via a gravimetric procedure [17]; n_c is the number of carbon atoms per mole silane; M is the molecular weight of the silane; and S is the surface

area of the native silica in m^2/g . The volume of the stationary phase, V_S , can be expressed by equation 2:

$$V_S = (\alpha)(S)(100 - [(\%C)/(12.011)(n_C)](M - 35.5))(v)(W_p)(10^{-6})$$

where v is the molar volume of the bonded alkyl group in cm^3/mole and W_p is the weight (g) of the bonded packing contained in the chromatographic column. Cheng has experimentally determined the densities of the bonded alkyl groups for some commonly used reversed phases and reported values of 0.8607 g/cm^3 , 0.8625 g/cm^3 , and 0.8638 g/cm^3 respectively for the octadecyldimethylsilyl, octyldimethylsilyl, and trimethylsilyl bonded groups [17]. Substitution of equation 1 and the density (ρ) and molecular weight (M , g/mole) of the bonded phase alkyl group for the molar volume in equation 2 results in the volume of the stationary phase, V_S (in cm^3), as expressed by the following formula (equation 3):

$$V_S = \frac{(\%C)(M)(W_p)}{(100)(12.011)(n_C)(\rho)} .$$

This method provides a more accurate calculation of V_S than has been previously possible. A principal advantage of this method is that the surface area of the packing is not used in determining V_S , which eliminates the errors associated with this measurement. The stationary phase volume that is calculated in this method is the volume that is important in the

chromatographic process, i.e. the actual volume of the bonded alkyl chains themselves. The precision is limited only by the carbon loading determination and by the measurement of the mass of packing in the column. Calculation of the volume of the stationary phase by this method will provide the means for a more accurate and uniform determination of the phase ratio. This in turn will allow investigators to more easily determine the associated thermodynamic parameters, allowing greater insight into the retention process in both reversed phase and micellar liquid chromatographic systems.

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