



# Lab Highlights

## WHAT'S THE VOID VOLUME OF YOUR LC COLUMN ?

The experimental determination of a "true" void or dead volume of an LC column may not be an exercise which many investigators may worry about; however, it is of great importance if one wishes to optimize the separation, identify solute bands or most importantly exploit the thermodynamic data ( $k'$ ). Calculation of the  $k'$  value is, of course, based on the "exact" knowledge of the volume of mobile phase within the column or, more classically, in a dynamic system, the volume or "breakthrough time" of the "unretained" components.

A variety of approaches have been suggested and practiced in attempting to define the void volume ( $V_0$ ) of a column. Some of the most common which have been suggested are: 1) Measuring  $V_0$  at the first perturbation in the baseline (as done in GC); 2) Injecting a "non-retained" component such as an inorganic salt ( $\text{NaNO}_3$ ),  $\text{D}_2\text{O}$ , or an organic UV absorbing compound such as acetone or uracil; and 3) Weighing the column with and without the eluent.

Not all these methods are applicable or practical for all columns. There has also been considerable controversy in the literature (1,2,3) on the determination of a "true"  $V_0$  or if indeed, a "true"  $V_0$  really can be calculated. Some recent work by Engelhardt (1) and Krstulovic (2) both suggest that deuterated solvents appear to be the best marker for determining  $V_0$ . Table I (1) shows some suggestions for  $V_0$  markers on both silica and reversed-phase columns. The authors caution, however, that depending on the type of chromatography, factors such as size exclusion effects, ionic exclusion, and interactions on the silica surface and adsorption of the marker may influence the value for  $V_0$ .

Alternately, Berendsen *et al.* (3) suggest using data derived from the linearization of a homologous series, a method widely applied to gas chromatography. Despite being a lengthy procedure, the authors (3) believe it would give the most accurate  $V_0$  for the reverse-phase system they studied.

Finally, as the determination of  $V_0$  is somewhat of a contested issue in LC, it should always be stated how the  $V_0$  required for  $k'$  calculations was determined.

**Table I: Suggestion of Inert Markers for Silica and Reversed-Phase Systems**

Eluent	Silica		Stationary Phase Reversed Phase		
	n-C <sub>7</sub> H <sub>16</sub>	CH <sub>2</sub> Cl <sub>2</sub>	H <sub>2</sub> O	CH <sub>3</sub> OH	CH <sub>3</sub> CN
	n-C <sub>6</sub> H <sub>14</sub> (RI)	CD <sub>2</sub> Cl <sub>2</sub> (RI)	D <sub>2</sub> O (RI)	CD <sub>3</sub> OH (RI)	CD <sub>3</sub> CN (RI)
		CHCl <sub>3</sub> (RI)			
		C <sub>2</sub> Cl <sub>4</sub> (UV, RI)		CH <sub>3</sub> NO <sub>2</sub> (UV, RI)	CH <sub>3</sub> NO <sub>2</sub> (UV, RI)
		CCl <sub>4</sub> (UV, RI)			
		C <sub>6</sub> H <sub>6</sub> (UV, RI)			
			Mixtures of D <sub>2</sub> O/CH <sub>3</sub> OH    D <sub>2</sub> O/CH <sub>3</sub> CN (RI)                (RI) identical with eluent composition		

1. Engelhardt, H., Müller, H. and Dreyer, B., *Chromatographia*, 19 (1984) 240-245.
2. Krstulovic, A. M., Colin, H. and Guiochon, G., *Anal. Chem.*, 54 (1982) 2438-2443.
3. Berendsen, G. G., Schoenmakers, P. J., DeGalan, L., Vigh, G., and Varga-Puchony, Z., *J. Liq. Chromatogr.*, 3, (1980) 1669-1686.