How to Determine Column Volume and Porosity

Introduction

Measurement of the internal volume of an LC column is useful for a number of aspects of liquid chromatography, including determination of appropriate equilibration times, gradient re-equilibration times, setting appropriate injection volumes and performing gradient adjustments and translations. This Knowledge Note explains how the column volume can be calculated and experimentally measured.

Estimating Column Volume (V_M)

A convenient estimate of V_M in mL, can be obtained using the following equation.

$$V_M = 0.5 L d_c^2 / 1000$$

where L is the column length in mm and d_c is the column internal diameter in mm. This method provides an estimate accurate to within approximately 10% and is suitable for applications where an accurate measurement of V_M is not essential, e.g. establishing appropriate equilibration times or post-gradient re-equilibration times for a column.

Calculating V_M

For activities such as method transfer and translation, a more accurate calculation of V_M is necessary. The volume of the empty stainless steel column is easily calculated from $\pi r^2 L$. However, as the column is packed with packing material, only a fraction of the column volume is available to be occupied by mobile phase. Multiplying by the column porosity (ε) therefore provides an accurate value of V_M:

$$V_M = \pi \left(\frac{d_c}{2}\right)^2 L \varepsilon$$

It should be noted that different packing materials will have significantly different porosities, for example fully porous vs solid core (superficially porous, core shell or fused core) columns. ACE fully porous particles have a porosity of 0.63, whilst ACE UltraCore particles have a porosity of 0.55. Porosities of other manufacturer's columns may differ somewhat from these values. Tables 1 and 2 show calculated V_M values for commonly used ACE fully porous and solid core column formats.

Experimental Determination of Column Volume and Porosity

If the porosity of the column is unknown, or a very accurate determination is required, both V_M and ε can easily be determined experimentally by measuring the column dead time (t_0), through injection of an unretained analyte or solvent.

Injection of sample diluent or a pure mobile phase solvent (e.g. water) can often be used to generate a baseline disturbance at t_0 (Figure 1). Uracil or thiourea are commonly used as unretained t_0 markers in reversed-phase, whilst toluene can be used in HILIC. Ionic components have also been used as t_0 markers, although care must be taken to avoid ion exclusion effects from the packing material pores[1]. Whichever method is used,
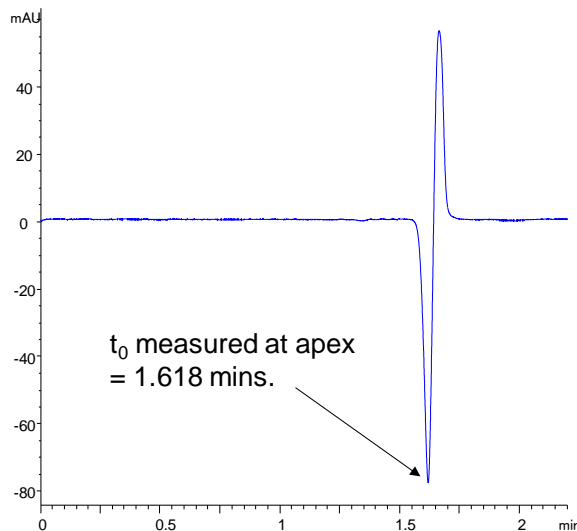

Figure 1: Experimental determination of t_0 using water injection on an
ACE Excel 5 C18, 150 x 4.6 mm.
Mobile phase: MeCN:H_2O (40:60) + 0.1% formic acid. Flow rate: 1.0 mL/min. Temperature: 30 °C. Detector: UV, 210 nm.

[1]C.A. Rimmer *et al. J. Chrom. A* **965** (2002) 219-232

it is important to be consistent in its application and measure t_0 at the same point of the baseline disturbance from run to run and column to column. V_M can be determined from the measured t_0 value by multiplying by the flow rate (F) in mL/min:

$$V_M = t_0 \times F$$

If required, the column porosity can be determined as the ratio of V_M to the volume of the empty column:

$$\varepsilon = \frac{V_M}{\pi \left(\frac{d_c}{2}\right)^2 L}$$

Small Column Formats

When measuring the column volume via determination of t_0, you are actually measuring the column volume plus the system extra column volume or ECV (i.e. the internal volume of the flow path within the system, comprised of Autosampler components, tubing, detector flow cell etc.). For details on how to measure ECV, please refer to AKN0017. For traditional format columns (e.g. 4.6 mm i.d.), the ECV is relatively insignificant compared to the column volume. For smaller format columns (e.g. ≤ 2.1 mm i.d.), the contribution of ECV to the measured t_0 can result in a significant over-estimation of V_M. In this case, the ECV (in units of mL) should be subtracted as follows to obtain an accurate value:

$$V_M = (t_0 \times F) - ECV$$

Column id (mm)	Column length (mm)							
	20	30	50	75	100	125	150	250
1.0	0.010	0.015	0.025	0.037	0.049	0.062	0.074	0.124
2.1	0.044	0.065	0.109	0.164	0.218	0.273	0.327	0.546
3.0	0.089	0.134	0.223	0.334	0.445	0.557	0.668	1.113
4.6	0.209	0.314	0.523	0.785	1.047	1.309	1.570	2.617

Table 1: Calculated column volumes in mL for common ACE 100 Å fully porous (ε = 0.63) column dimensions.

Column id (mm)	Column length (mm)							
	20	30	50	75	100	125	150	250
1.0	0.009	0.013	0.022	0.032	0.043	0.054	0.065	0.108
2.1	0.038	0.057	0.095	0.143	0.190	0.238	0.286	0.476
3.0	0.078	0.117	0.194	0.292	0.389	0.486	0.583	0.972
4.6	0.183	0.274	0.457	0.686	0.914	1.143	1.371	2.285

Table 2: Calculated column volumes in mL for common ACE UltraCore solid core (ε = 0.55) column dimensions.

Conclusion

This Knowledge Note has described how to estimate, or experimentally determine, column volume and porosity. These parameters are important for calculating equilibration times etc. and most important for accurate method translation.