Note

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Error incurred in gel permeation chromatography by using the elution peak volume in lieu of the elution mean volumes in the calculation of K_{av}

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The model of chromatography suggested by Lapidus and Amundson[1] says that the concentration profile in a packed bed follows the equation

$$D\frac{\delta^2 c}{\delta Z^2} = U\frac{\delta c}{\delta Z} + \frac{\delta c}{\delta t} + \frac{1\delta n}{\alpha\delta t} \tag{1}$$

where D = axial dispersion coefficient
  c = concentration in moving phase
  Z = axial position, $0 \leqslant Z \leqslant L$
  t = time, $t \geqslant 0$
  U = fluid velocity through bed interstices
  α = fractional bed void volume

and

$$n = Kc \tag{2}$$

which is the condition of equilibrium between mobile phase concentration, c, and immobile phase concentration, n (K = partition coefficient). Substituting eqn. 2 into eqn. 1 yields

$$\bar{D}\frac{\delta^2 c}{\delta Z^2} = \bar{U}\frac{\delta c}{\delta Z} + \frac{\delta c}{\delta t} \tag{3}$$

where

$$\bar{D} = D/\left(1 + \frac{K}{\alpha}\right) \tag{4}$$

$$\bar{U} = U/\left(1 + \frac{K}{\alpha}\right) \tag{5}$$

If a moving frame of reference is adopted wherein

$$t = Z - \bar{U}t \tag{6}$$

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then eqn. 3 becomes

$$\frac{\delta c}{\delta t} = \bar{D}\frac{\delta^2 c}{\delta x^2}$$

where x is a coordinate which moves with the center of mass of the concentration, c. The conditions on this equation are

$$\text{at } t = 0, c = H(x)c_0\frac{Q}{V} \tag{7}$$

where $c_0 H(x)$ is a pulse function of concentration c_0 and V is the column volume. The solution to this problem is then[2]

$$\frac{cV}{c_0 Q} = \frac{L}{(4\pi\bar{D}t)^{1/2}} \exp - (x^2/4\bar{D}t) \tag{8}$$

which may be rearranged to

$$\frac{cV}{c_0 Q} = \frac{\exp - \left\{\left(1 - \frac{v}{V}t\right)^2/4\left(\frac{v}{V}t\right)\left(\frac{\bar{D}}{\bar{U}L}\right)\right\}}{\left\{4\pi\left(\frac{v}{V}t\right)\left(\frac{\bar{D}}{\bar{U}L}\right)\right\}^{1/2}} \tag{9}$$

where v = the interstitial fluid volume flow-rate
Q = sample volume
L = the column length.

It is important to note that

$$\frac{\bar{D}}{\bar{U}L} = \frac{D}{UL} \tag{10}$$

so that this term is independent of K. In order to estimate the error caused by using peak concentrations to mark the eluent volume instead of the mean concentration, it is necessary to compute the difference between the time when the peak arrives and the mean passes. The peak concentration arrives at

$$\left(\frac{v}{V}t\right)_{\text{peak}} = \left\{\left(\frac{\bar{D}}{\bar{U}L}\right)^2 + 1\right\}^{1/2} - \frac{\bar{D}}{\bar{U}L} \tag{11}$$

while the mean concentration arrives at

$$\left(\frac{v}{V}t\right)_{\text{mean}} = 1.0 \tag{12}$$

so that the difference between the two is

$$\Delta\left(\frac{v}{V}t\right) = \left(\left(\frac{\bar{D}}{\bar{U}L}\right)^2 + 1\right)^{1/2} - \left(\frac{\bar{D}}{\bar{U}L} + 1\right) = ERR \tag{13}$$

which ensures that the peak and the mean volumes are always different. This term is a measure of the fractional error incurred by using the peak elution volume and, according to eqn. 10, it has no dependence on K. Defining

$$K_{\text{av}} = \frac{V_e - V_0}{V_t - V_0} \tag{14}$$

where V_e is the mean concentration *elution* volume, V_τ is the total column volume accessible to small molecules and V_0 is the column void volume.

The corresponding peak elution volumes are then

$$
\left.
\begin{aligned}
V_e^p &= (1 - ERR)V_e \\
V_\tau^p &= (1 - ERR)V_\tau \\
V_0^p &= (1 - ERR)V_0
\end{aligned}
\right\}
\qquad (15)
$$

where *ERR* is a function only of the column parameters and not of the partition coefficient so that

$$
K_{av}^p = \frac{V_e^p - V_0^p}{V_\tau^p - V_0^p} = \frac{(1 - ERR)V_e - (1 - ERR)V_0}{(1 - ERR)V_\tau - (1 - ERR)V_0} = K_{av}
\qquad (16)
$$

so that there is no net error incurred by using peak concentration volumes to measure K_{av} so long as all the volumes are measured according to the peak value.

It is important to consider the error that is routinely made in gel permeation chromatography. Both Bio-Rad[3] and Pharmacia[4] define V_τ as the volume occupied by the entire gel phase plus the interstitial bed volume. Using this definition of V_τ automatically introduces an error into the calculation of K_{av} because no solute may occupy the solid part of the gel phase. Also, because experimental measurement of V_e and V_0 is usually done using peak volumes the total error present in much of the reported data is

$$
\frac{\Delta K_{av}^*}{K_{av}} \approx \frac{V_{gel}}{V_\tau - V_0} + \frac{ERR\ V_\tau}{V_\tau - V_0}
\qquad (17)
$$

where the first term on the right is approximate and is roughly the percent of solids in the gel phase, usually between one and ten percent and the second term represents the error incurred by using the mean total volume with peak elution and void volumes. This term may often be on the order of a few percent and these two terms are *never* offsetting. Also, this total error is large enough and of the correct sign to account for the difference between the theoretical and experimental measurement of K_{av} (ref. 5).

REFERENCES

1 L. Lapidus and N. Amundson, *J. Phys. Chem.*, 56 (1956) 984.
2 O. Levenspeil and W. K. Smith, *Chem. Eng. Sci.*, 6 (1957) 227.
3 Bio-Rad Laboratories, *Gel Chromatography*, Bio-Rad Publications, Richmond, CA, 1971.
4 Pharmacia Fine Chemicals, *Gel Filtration in Theory and Use*, Pharmacia Publications, Uppsala 1974.
5 G. Ackers, *Advan. Protein Chem.*, 24 (1970).

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{}^* \Delta K_{av} = \frac{V_e^p - V_0^p}{V_\tau - V_0^p} - \frac{V_e^p - V_0^p}{V_\tau^p - V_0^p}, \text{ where } V_\tau \approx V_{gel} + V_\tau.
$$