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Note

A single equation relating molecular weight, pore-size, and elution coefficient in the controlled pore glass chromatography of protein–sodium dodecyl sulfate complexes

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In a previous publication[1] the permeation chromatography of protein–SDS complexes on columns of controlled pore glass of narrow pore size was reported. It was shown that the plotting of the normalized peak position k (also called elution coefficient) of the eluting complexes *versus* the logarithm of the protein subunit molecular weight M resulted in a straight-line relationship over a molecular weight range from 17,000 to 385,000. A controlled pore glass of approx. 500 Å actual average pore diameter (for determination and definition see ref. 2) was suitable for resolving the full molecular weight range mentioned. Applying the described method to an unknown protein, it was necessary to calibrate a column of a given pore size with two or more proteins of known molecular weight in order to explore the linear relation of k *vs.* log M.

This note reports on an analysis of the previous experimental data which resulted in an equation relating the normalized peak-position of a protein–SDS complex to the actual average pore diameter (P) of the used glass and to the molecular weight of the protein. The equation does not contain other unknown numbers, and its use allows the calculation of approximate molecular weights without calibration with known proteins. In order to normalize the peak position of the unknown protein, it is still necessary to know the void volume (V_0) and total intrusion volume (V_t) of the used column. The determination of these parameters, however, is good practice for any permeation chromatography and should precede the use of any new column. V_0 and V_t are easily determined with tobacco mosaic virus (TMV) and with tryptophan using water or buffer (not SDS) as eluant. For experimental details, the reader is referred to ref. 1.

The empirical relationship satisfying the experimental points is

$$(k-1.234)^2+(y-1.234)^2=1.577 \quad (1)$$

where

$$y=(2.13\ M^{0.413}/P)^2 \quad (2)$$

k being the normalized peak position (elution coefficient) of the SDS–protein complex, M being the molecular weight of the protein, and P being the actual average pore size

in Ångstrom units (by mercury intrusion) of the controlled pore glass. The normalized peak position k is calculated from the elution volume V_e of the substance using the well-known relation $k=(V_e-V_0)/(V_t-V_0)$ whereby V_0 and V_t are void volume and total intrusion volume.

Fig. 1 combines elution data for thirteen different proteins on columns of four different pore diameters. The curve represents eqn. 1, given above.

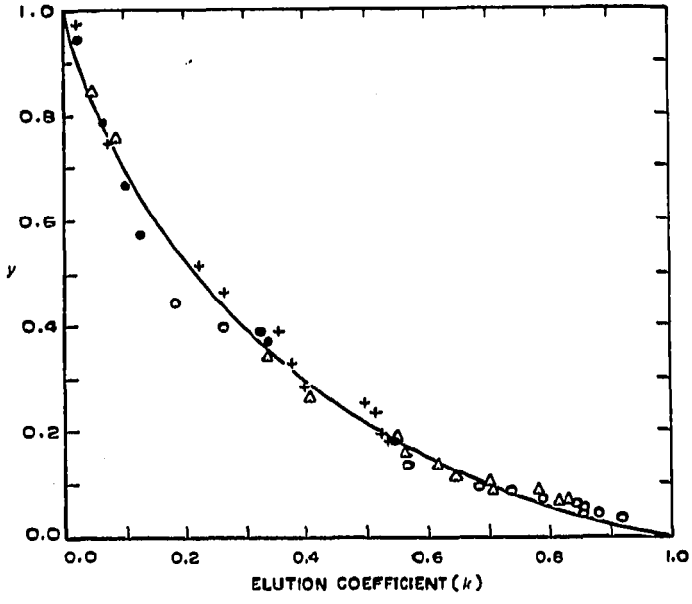

Fig. 1. Master curve, relating elution coefficient to protein molecular weight and pore-diameter of glass. Pore diameter (P): ●, 197 Å; +, 280 Å; △, 470 Å; ○, 650Å. The curve represents eqn. 1.

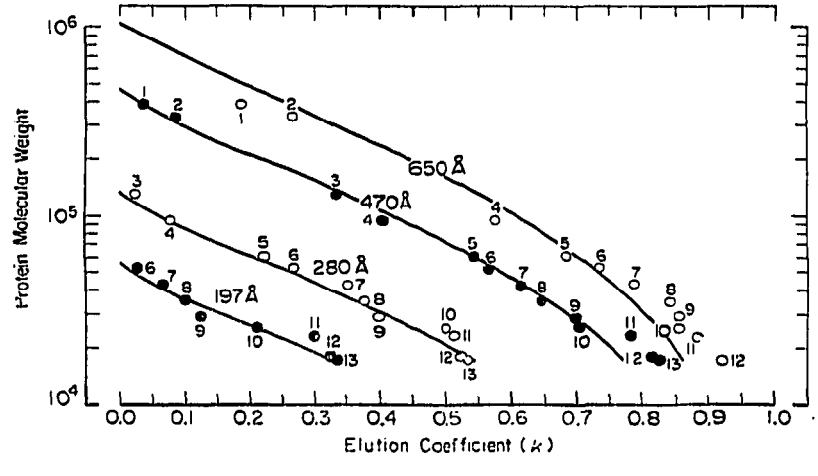

Fig. 2. Log protein molecular weight *versus* elution coefficient. The curves represent eqn. 3 for different pore diameters. Proteins are: (1) hemocyanin, (2) thyroglobulin, (3) β-galactosidase, (4) phosphorylase, (5) catalase, (6) glutamate dehydrogenase, (7) ovalbumin, (8) pepsin, (9) carbonic anhydrase, (10) α-chymotrypsinogen, (11) trypsin, (12) β-lactoglobulin, (13) myoglobin.

The equation obviously does not postulate a linear relationship between k and log M as was used in the plots of ref. 1. This is shown in Fig. 2 where the more familiar log M and k coordinates are used. The curves in Fig. 2 represent again eqn. 1, except that individual curves for different pore diameters were drawn. To do this, eqn. 1 is rearranged to

$$k = 1.234 - \sqrt{0.05475 - y^2 + 2.468y}. \quad (3)$$

The curvature introduced by the new equation is only slight, and, within the experimental uncertainty, the new equation represents the data as well as the original straight-line treatment.

While presently the new equation is strictly offered as a practical tool for molecular weight determination, the reader interested in chromatographic theory should be made aware that the term 2.13 $M^{0.413}$ found in y is the exclusion size of protein–SDS complexes, determined by an extrapolation technique suggested by this author[3] and briefly described in the previous paper[1]. It is believed that this exclusion size represents the largest linear dimension (in Ångstroms) of the protein SDS complex. The term y represents, therefore, the square of the ratio of the diameter of the molecule over the diameter of the pore.

REFERENCES

1 R. C. Collins and W. Haller, *Anal. Biochem.*, 54 (1973) 47.
2 W. Haller, *Nature (London)*, 206 (1965) 693.
3 W. Haller, in preparation.