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This Week's Citation Classic

Whitaker J R. Determination of molecular weights of proteins by gel filtration on Sephadex. *Anal. Chem.* **35**:1950-3, 1963.
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An excellent linear correlation between the logarithm of molecular weight of a protein and the ratio of its elution volume, V, to the void volume, V_o, of the column was found for chromatography of proteins on Sephadex G-100 and G-75, cross-linked dextrans. [The *SCI*® indicates that this paper has been cited over 1,100 times since 1963.]

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"The research was the result of a need to determine the molecular weights of ficin, papain, and bromelain on which I was working and the relative inaccessibility of an analytical ultracentrifuge. Stimulus for the research was provided by the work of P. Andrews published in *Nature* in 1962 on use of agar columns for molecular weight determination of some proteins.[1] Molecular weight determination on agar columns proved unsuccessful largely because of the ion-exchange properties of the acidic components of agar and the nonuniformity of the material. Early in my investigations, particular attention was given to the effect of proper equilibration of the column with buffer, suppression of the smaller number of ionic (carboxyl) groups on Sephadex, and calibration of the column with essentially spherical proteins in order to avoid shape effects. I was ecstatic with the initial excellent results with standard proteins in terms of reproducibility, agreement with known molecular weights, and ease of determination. My enthusiasm was severely dampened when I discussed the results with colleagues more versed in protein chemistry than I. They assured me that molecular weight determinations by gel filtration were not to be taken seriously and that gel filtration could never replace the ultracentrifuge for molecular weight determinations.

"For some six months I let the investigation lapse as I pondered the merits of bringing the data to a publishable stage. Toward the end of the summer of 1962, having decided my colleagues could be wrong, I went on to complete the work and to submit it for publication. Fortunately, the editor of *Analytical Chemistry* considered it worthy of publication.

"The paper recognized the method would not give correct molecular weights for certain types of proteins. By chance, I had included oxomucoid, lysozyme, and hemoglobin in the research. Results with these proteins indicated that proteins containing appreciable carbohydrate would give apparent molecular weights higher than the true value and that proteins which readily dissociate into subunits or absorb to the gel would give apparent molecular weights lower than the true value. Interestingly, the proteins ficin, papain, and bromelain tor which I developed the method do not behave normally in gel filtration. For reasons which still elude me, these proteins give apparent molecular weights some 20 percent too low on either Sephadex or Bio-gel columns.

"The method caught on rapidly for a number of reasons. Gel filtration proved to be a rapid, highly reproducible, and inexpensive method of determining molecular weights with as much confidence as by ultracentrifugation and with the additional advantage that molecular weights can be determined on crude preparations provided the protein of interest has measurable biological activity. Its success has been due to the contributions of many other scientists–P. Andrews, G.K. Ackers, etc. –who early recognized the potential of the method and who later provided a theoretical basis for understanding its performance and use not only in determining molecular weights but also shapes of molecules.[1,2]

"J. Porath, P. Flodin, and co-workers at Pharmacia in Sweden developed cross-linked dextrans to serve as inert stabilizing media for electrophoresis. Little did they anticipate they would prove to be such powerful analytical tools for separation and molecular weight determinations.[3-5] A recent review of this field has been published by Ackers in *Proteins*."[6]

1. **Andrews P.** Estimation of molecular weights of proteins by gel filtration. *Nature* **196**:36-9, 1962.
2. **Steere R L & Ackers G K.** Restricted-diffusion chromatography through calibrated columns of granulated agar gel: a simple method for particle-size determination. *Nature* **196**:475. 1962.
3. **Flodin P.** *Dextran gels and their applications in gel filtration.* Uppsala, Sweden: Pharmacia, 1962. 85 p.
4. **Flodin P & Porath J.** Molecular sieve processes. (Heftmann E. ed.) *Chromatography.* New York: Reinhold, 1961. p. 328-43.
5. **Granath K A & Flodin P.** Fractionation of dextran by gel-filtration method. *Makromol. Chem.* **48**:160-71, 1961.
6. **Ackers G K.** Molecular sieve methods of analysis. (Neurath H & Hill R L. eds.) *Proteins.* New York: Academic Press, 1975. Vol. 1. p. 1-94.