*Journal of Chromatography*, 172 (1979) 394–396
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 11687

**Note**

# Stability of a Sepharose 6B-CL column in 6 *M* guanidine–HCl

DON MAHURON

*Neurosciences Division, Research Institute, The Hospital for Sick Children, Toronto, Ontario (Canada)*

(Received December 28th, 1978)

Gel filtration of oxidized or reduced and alkylated proteins in 6 *M* guanidine–HCl is an accurate method for determining the molecular weights of the polypeptide chains in purified proteins. It is a particularly useful method when the protein in question contains carbohydrates because the more common method of polypeptide chain molecular-weight determination using polyacrylamide disc gel electrophoresis (PAGE) in SDS, gives inaccurate results when there is any significant amount of carbohydrate on the protein. The error arises from the difference in the binding of SDS to the carbohydrate groups compared to the protein chains[1].

One drawback to guanidine–HCl column chromatography as opposed to PAGE in SDS is the time and effort needed to pack and calibrate the column. Furthermore, in guanidine–HCl, Sepharose 6B columns have a finite lifetime of *ca.* 1 year[2]. In previous work I have found a continuous slowdown in the elution rate of the usual Sepharose 6B column in 6 *M* guanidine–HCl, to such an extent that the column becomes useless in about a year[3]. The manufacturers note that guanidine–HCl has a "structure-breaking" effect on non-cross-linked Sepharose 6B[4].

A 90 × 1.5 cm I.D. column of Sepharose 6B-CL in 6 *M* guanidine–HCl has been in use in our laboratory for over a year and has shown no detectable change in either the elution rate or the calibration curve of the standard proteins. The elution rate for this column is *ca.* 4.5 ml/h compared to the initial elution rate for an identical size and head pressure (15 cm) column of Sepharose 6B of 3 ml/h[2].

## MATERIALS AND METHODS

The protein standards used were: phosphorylase *b* (93,000), bovine serum albumin (68,000) aldolase (40,000), lactate dehydrogenase (36,000), chymotrypsinogen (25,500), and ribonuclease A (13,600). About 3 mg per 0.5 ml each of the performic acid oxidized proteins[5] were applied to the column. All standard proteins and the guanidine–HCl were obtained from Sigma (St. Louis, Mo., U.S.A.). The guanidine–HCl was recrystallized once from boiling absolute ethanol and benzene[6]. Sepharose 6B-CL was obtained from Pharmacia (Uppsala, Sweden). The column used was made by Glenco (Houston, Texas, U.S.A.).

The column was prepared and operated as described by Mann and Fish[2]. Fractions of *ca.* 2.0 ml were collected. Exact volumes were calculated by weighing each tube before and after the fractions were collected. The difference in grams was

taken as the "volume" of each fraction. The distribution co-efficient, $K_d$, was then calculated using the expression:

$$K_d = \frac{V_e - V_0}{V_i - V_0}$$

$V_e$ represents the elution "volume" of the protein, $V_0$ the elution "volume" of Blue Dextran 2000, and $V_i$, the elution "volume" of vitamin B12. A calibration curve was constructed by plotting $(K_d)^{1/3}$ against (molecular weight)$^{0.555}$, and calculating the best-fit straight line[1] (Fig. 1). Protein concentration was estimated by measuring the absorbance at 280 nm of each fraction.

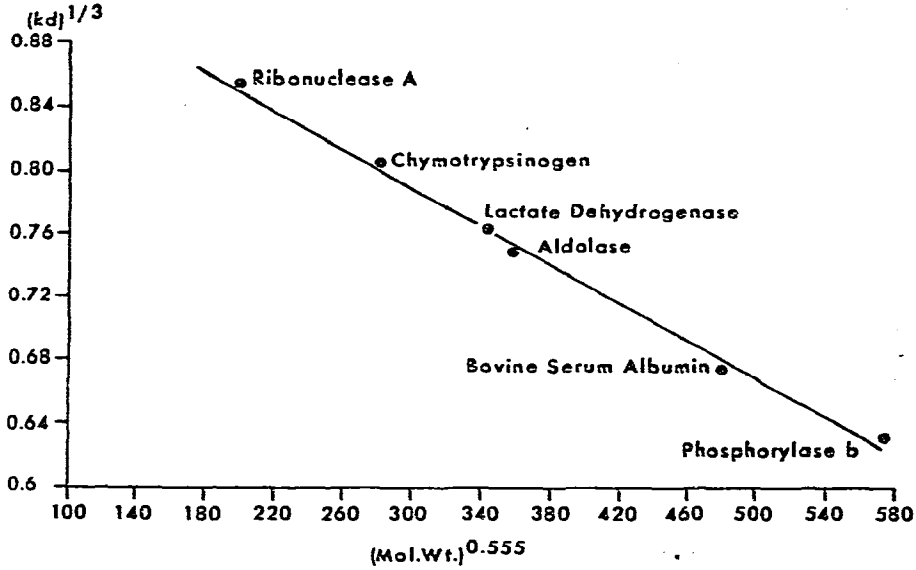


Fig. 1. Plot of $(K_d)^{1/3}$ against (molecular weight)$^{0.555}$ for standard proteins. Data obtained with samples run on the column after 1 year in use.

## RESULTS AND DISCUSSION

The standard curve obtained with Sepharose 6B-CL (Fig. 1) is similar to the standard curve obtained from Sepharose 6B[1], with a standard deviation of the $y$ values ($(K_d)^{1/3}$) of 0.005 (calculated from least squares analysis). Sepharose 6B-CL, however, has a higher elution rate, 4.5 ml/h compared to the initial rate for Sepharose 6B of 3.0 ml/h for identical columns. A protein polypeptide of 25,000 daltons or more is eluted in *ca.* 30 h. After running for 1 year, my Sepharose 6B-CL shows no change in elution rate or calibration curve (Table I) unlike previous experience with Sepharose 6B. The "structure-breaking" effect of guanidine–HCl described for the regular Sepharose 6B does not seem to be present with cross-linked gel. Thus, once poured and calibrated, a Sepharose 6B-CL column should last indefinitely.

TABLE I

ELUTION DATA AT STARTUP AND AFTER ONE YEAR

| *Protein* | *Elution "volume"* | |
|---|---|---|
| | *1977* | *1978* |
| Bovine serum albumin | 97.0 | 96.6 |
| Aldolase | 107.6 | 107.8 |
| Chymotrypsinogen | 117.5 | 117.4 |
| Ribonuclease A | 127.5 | 127.7 |
| Elution rate | 4.5 ml/h | 4.5 ml/h |

By using florescein labeling the amount of protein required to identify the peak can be reduced by at least 1000-fold[2], thus eliminating the only other major advantage the PAGE in SDS method had over 6 *M* guanidine–HCl gel filtration for polypeptide chain and subunit molecular-weight determinations.

ACKNOWLEDGEMENT

This work was supported by grant PG4 from The Medical Research Council of Canada.

REFERENCES

1 J. P. Gegrest and R. L. Jackson, *Methods Enzymol.*, 28 (1972) 54.
2 K. C. Mann and W. W. Fish, *Methods Enzymol.*, 26 (1972) 28.
3 D. J. Mahuran, R. H. Angus, L. V. Braun, S. S. Sin and D. W. Schmidt, *Can. J. Biochem.*, 55 (1977) 1.
4 Pharmacia Fine Chemicals, *Sepharose CL for Gel Filtration and Affinity Chromatography*, Rahms and Lund, Sweden, 1975.
5 C. H. W. Hers, *Methods Enzymol.*, 11 (1967) 199.
6 Y. Nozaki, *Methods Enzymol.*, 26 (1972) 43.