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### Gel Permeation Chromatography: The Effect of Sample Size on Efficiency

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## NOTE

### Gel Permeation Chromatography: The Effect of Sample Size on Efficiency\*

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#### Abstract

Various reported studies of the effect of sample size on efficiency in gel permeation chromatography are not in complete agreement. In particular there is conflicting experimental evidence as to the existence of a lower limit in sample size below which no further increase in efficiency is observed. This study reports efficiency studies in terms of plate number and resolution for polystyrene in chloroform for sample sizes less than those commonly studied. The results support the existence of such a lower limit on sample size.

#### INTRODUCTION

There is some uncertainty as to the effect of sample size on separation efficiency in gel permeation chromatography. A number of authors have concluded that while large sample sizes decrease efficiency, there exists a limiting sample size below which a further decrease in sample size gives no increase in efficiency (see, for example, Refs. 1-4). As detectors have been improved, the sample sizes that can be separated and detected successfully have decreased. Using a more sensitive detector, Maldacker and Rogers (5) found resolution increasing continuously with decreasing sample size until the limit of detection was reached. Therefore, further studies on this effect were undertaken and are reported here.

\*Part XXXII of a series on Column Fractionation of Polymers.

## EXPERIMENTAL

Experimental conditions for the chromatographic analyses are given in Table 1. The polymer samples were narrow molecular weight distribution anionically polymerized polystyrenes (Pressure Chemical Co., Pittsburgh, Pennsylvania). In the first series of measurements the various molecular weight samples were prepared in chloroform at concentrations of 8, 2, 0.5, and 0.725 mg/ml, respectively. The sample size injected was 0.125 ml. The second series consisted of 0.5 mg/ml each of polystyrenes of molecular weights 670,000, 97,200, and 20,400 in chloroform. Sample sizes injected ranged from 0.8 to 0.0125 ml.

## DISCUSSION

Plate numbers and resolutions were calculated in the conventional manner.

Within experimental error, little or no effect of sample size on the elution curves was observed for molecular weights up to 200,000. Above this molecular weight, increases in elution volume and peak width were observed with increasing concentration at relatively high concentrations.

Plate numbers as a function of concentration are plotted in Fig. 1. The injection volume was 0.125 ml in all points given in Fig. 1. A single molecular weight species was injected. At sample sizes below about 70  $\mu$ g there is scatter in the calculated plate values but there appears to be no systematic variation of total number of plates with sample size. Resolution for various pairs of samples vs sample size is given in Fig. 2. Again, within experimental error the values are essentially constant at the lower con-

TABLE I  
Experimental Conditions

Instrument	DuPont Liquid Chromatography Model 820
Injector	Waters Associates valve injector with modified loop
Detector	Ultraviolet, sensitivity from 128 to $1 \times 10^{-2}$ absorbance units
Columns	Four 1 m $\times$ 0.45 cm i.d. stainless steel tubes packed with CPG 75 $\text{\AA}$ + CPG 370 $\text{\AA}$ + CPG 1250 $\text{\AA}$ + CPG 1850 $\text{\AA}$ , 200-400 mesh.
Flow rate	1.032 ml/min
Solvent	Chloroform
Temperature	Ambient

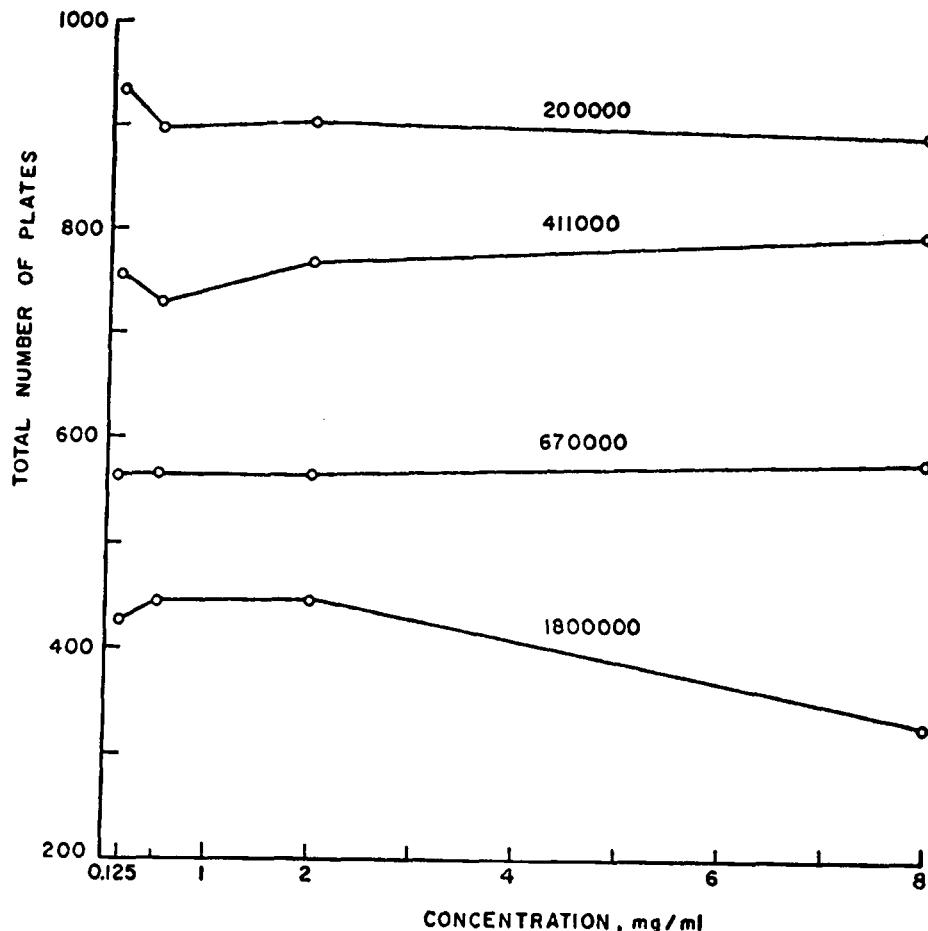


FIG. 1. Concentration effect on total number of plates, Injection volume 0.125 ml.

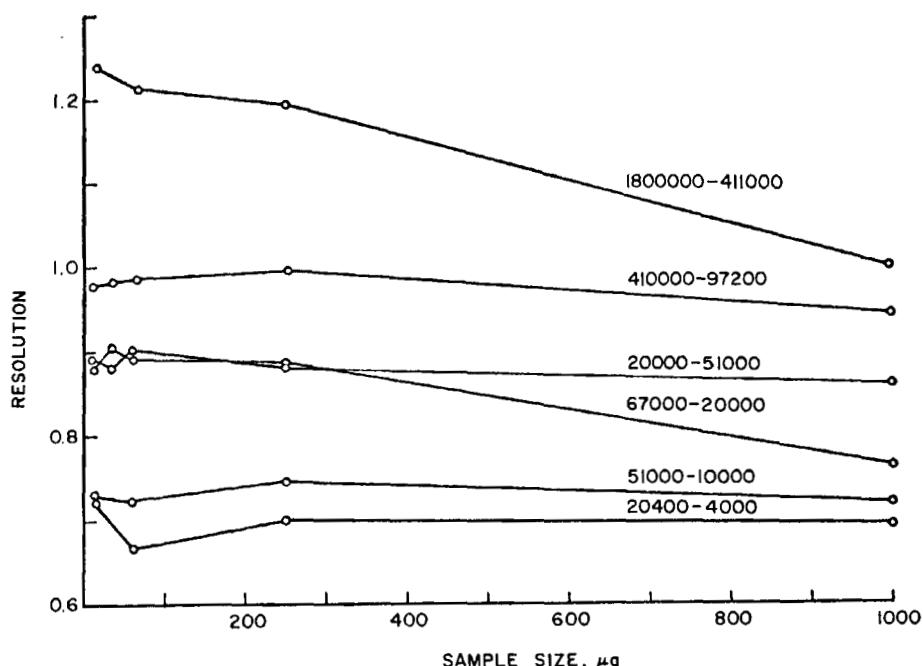


FIG. 2. Resolution of polymer pairs of various sample sizes calculated from the elution curves of single polymer solution.

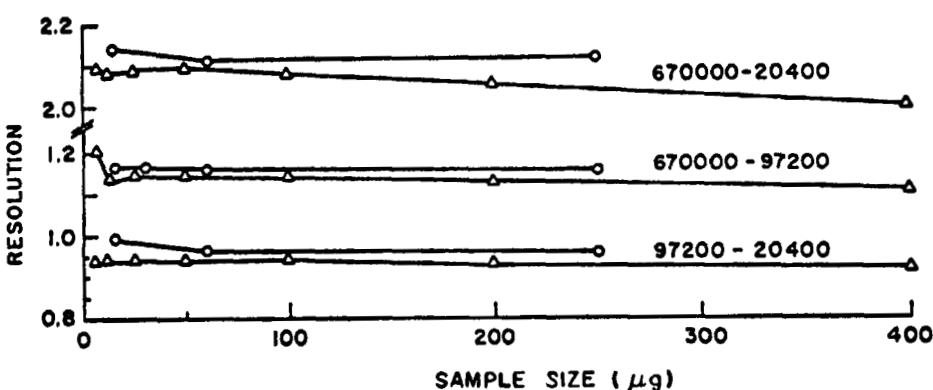


FIG. 3. Resolution and sample size: ( $\Delta$ ) samples containing a single molecular weight species, ( $\circ$ ) samples containing three molecular weights.

centrations. Results from samples containing multiple molecular weights are compared with those injected individually in Fig. 3. The agreement between resolution measured by the two methods is good, and within experimental error no dependence of resolution on sample size is observable at sample sizes below about 50  $\mu\text{g}$ .

These data support the conclusion that there is a limiting sample size in gel permeation chromatography below which the column efficiency is independent of sample size. It should be noted that this conclusion is not in conflict with that of Maldacker and Rogers because the sample sizes used in this study were much smaller than theirs.

### Acknowledgment

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