Moment Analysis of Experiments in Gel Permeation Chromatography

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Gel permeation chromatography is a form of liquid chromatography which separates components primarily on the basis of molecular size with porous particles in packed columns. The process is used extensively in laboratory biochemical separations and has been tested for large-scale operations, for example, the desalting of cheese whey. Separation depends on axial dispersion, mass transfer to the particle surface, diffusion inside the particle pores, and in some cases adsorption. The same transport processes are common to many chromatographic procedures and are included in a theory of chromatography proposed by Kubin (1965) and Kucera (1965). The theory involves analysis of the moments of elution curves to determine values of bed and transport properties and has been applied mainly to gas chromatography (Schneider and Smith, 1968; Suzuki and Smith, 1971).

We have used the Kubin-Kucera technique to analyze aqueous solution pulse-response data of gel chromatography (Mehta, 1973). Data are presented here for four nonadsorbing packing-solute systems: (1) solid glass beads and sodium chloride; (2) polyacrylamide gel and Blue Dextran 2000, a large polysaccharide sterically excluded from the gel pores; (3) gel and NaCl, which has complete access to the gel interior; and (4) gel and a mixture of NaCl and a protein, β-lactoglobulin, which is also excluded.

The working equations for the pulse analysis are expressed as differences between moments at the entrance and exit of the column. Neglecting adsorption we have for the first absolute moments

$$\Delta\mu_1' = L\,(1 + \delta_0)/v, \qquad (1)$$

and for the second central moments

$$\Delta\mu_2 = 2L\,[\delta_1 + D_0\,(1 + \delta_0)^2/\alpha v^2]/v\,. \qquad (2)$$

For nonporous packing or for solutes excluded from the pores, the internal void volume is effectively zero ($\beta = 0$).

Downflow elution of sample pulses was carried out in glass or acrylic plastic columns carefully packed with spherical polyacrylamide gel beads (Bio-Gel P-6) or nonporous glass beads (3M Superbrite). Elutriation was used to classify the gel into size fractions which were within $\pm$ 10% of the mean diameter. Inlet and outlet pulses were monitored on strip chart recorders using either internal or external detectors. External flow-through conductivity cells connected by tubing to the inlet and outlet of the column indicated salt concentration; similar cells for ultraviolet absorption were used with Blue Dextran and β-lactoglobulin. To determine the effect of the inlet and outlet tubes and fittings, some data were taken with electrodes inside the column. Entrance and exit electrode probes consisted of a 1/32-in. diam. platinum wire sealed in a hollow acrylic plastic support rod of O.D. 1/8 in. The probes were positioned radially in the column perpendicular to the eluant flow with the electrode faces parallel. For gel columns these internal electrodes were surrounded by short sections of 120 μm nonporous glass beads so that only the fluid in the interstitial space between particles influenced the conductivity reading. The glass and gel packings were separated by thin porous filters. The packing length between the sets of probes was 37.40 cm. The 1.60-cm part of this length packed with glass beads contributed negligibly to the moments. We found that external conductivity cells designed with no stagnant regions and attached to the column with short lengths of tubing provide data nearly as accurate as internal electrodes.

Shape imperfection of the pulses was not a significant problem for the 132 pulses studied. Fifteen of the output pulses exhibited slight tailing caused by backmixing in an external uv cell. For the internal electrodes there was no observable tail for any output pulse. Twenty of the NaCl output pulses exhibited a leading edge, that is, the converse of the tailing phenomenon. Giddings (1965) has reported that the fluid velocity in the region near the wall is greater than the average fluid velocity over the remainder of the bed. We infer that the leading edge is caused by solute traveling faster in the wall region and arriving at the outlet detector sooner than the bulk of the pulse traveling at the average velocity. This channeling effect for gel was eliminated when a silane-treated glass column was employed. Calculations of moments are reported here with the tails and leading edges trimmed. Moments were evaluated by numerical integration of inlet and outlet recorder responses.

PULSES OF NaCl IN A COLUMN PACKED WITH NONPOROUS GLASS BEADS

Pulses of NaCl (1.08 ml, 2.50% wt./vol) were injected onto an 85.0-cm bed of 450 μm beads in a 1.27 I.D. acrylic column with internal electrodes. Values of α calculated from first moment differences for each velocity provided a mean value of $\alpha = 0.384 \pm 0.005$, in agreement with the range of values given by Giddings (1965).

In the Reynolds number range (0.26 to 0.97) of these experiments, correlations between N_{Pe} and N_{Re} (Wilhelm, 1962; Gunn, 1969 and 1971; De Ligny, 1970) specify a nearly constant Peclet number, implying that the axial dispersion coefficient D_0 is proportional to velocity. Thus we take $D_0 = \lambda v$, where the parameter λ may depend on column packing quality, column-to-particle diameter ratio, etc. Substitution of this expression into Equation (2) shows that a plot if $\Delta\mu_2$ versus $2L/v^2$ is a straight line passing through the origin with a slope equal to λ. From such a plot we calculate $\lambda = 0.065$ and hence $N_{Pe} = 0.69$. This value is higher than Wilhelm's (1962), $N_{Pe} = 0.4$, but in general agreement with Gunn's (1969) curve.

PULSES OF BLUE DEXTRAN 2000 AND OF SYNTHETIC WHEY IN GEL COLUMNS

A moment analysis of Leung's (1971) chromatography data with external electrodes extends the previous results. Pulses of Blue Dextran (0.70 ml, 0.10-0.15% wt./vol) or synthetic whey (7.0 ml) were injected onto 1.50 cm I.D. columns packed with 40 cm of Bio-Gel P-6. The synthetic whey solution consisted of 0.70 g β-lactoglobulin and 0.60

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g NaCl in 100 ml of water. Since Bio-Gel P-6 excludes solutes whose molecular weight is greater than ~ 6,000, we may consider that Blue Dextran (M.W. ~ 2×10^6) and β-lactoglobulin (M.W. ~ 36,000) are totally excluded. Sodium chloride ions, on the other hand, easily diffuse into the pores. For input pulses of synthetic whey, therefore, the protein pulse was eluted prior to the NaCl pulse.

Calculations based on Equation (1) provide individual values for α for the different gel particle diameters, Table 1. With decreasing gel particle size the bed void fraction decreases but tends to stay within the limits reported by Giddings (1965), that is, 0.43 to 0.36. The value of α for glass beads (450 μm) was 0.38, indicating that glass beads pack more tightly than gel of the same size.

From NaCl data in the synthetic whey we calculated a value for internal void fraction β. Values for $\Delta\mu_1'$ lay on a straight line with slope 0.93. Using a mean value of α from the results of Table 1 ($\alpha = 0.40$), we calculated $\beta = 0.88$ from Equation (1). This value agrees with rough determinations of the gel particle porosity (Lang, 1970) by tracer dilution and pycnometer methods ($0.82 < \beta < 0.93$). We know of no other determinations of β for hydrated gel.

The data of 510 μm and 430 μm gel were suitable for determining δ_1 for the NaCl pulses of synthetic whey. A plot of $\Delta\mu_2 v/2L$ versus $1/v$ was a straight line with intercept $\delta_1 = 10.80$ for 510 μm and 7.60 for 430 μm gel. These values were used to calculate pore diffusivities as in the next section.

PULSES OF NaCl IN GEL COLUMNS

A 1.27-cm I.D. acrylic column with internal electrodes was packed with either 230 μm or 130 μm Bio-Gel P-6. The combination of plastic column and packing gave rise to a leading edge at the exit detector which was unchanged by lengthening the column but increased with increasing particle size. The results reported here are based on pulses with leading edges curtailed. Plots of $\Delta\mu_1'$ produced straight lines with little scatter. Substituting $\alpha = 0.368$ for 130 μm and $\alpha = 0.385$ for 230 μm gel (Table 1) gave values of β equal to 0.839 and 0.834. We average these values of β with that obtained in the previous section, and use the result, $\beta = 0.86 \pm 0.03$, in subsequent calculations.

Table 1. Bed Void Fractions, Internal Diffusion Coefficients, and Tortuosity Factors for Bio-Gel P-6

$2R(\mu m)$	α	δ_1(s)	$kR \times 10^5$ (cm²/s)	$D_i \times 10^5$ (cm²/s)	q
510	0.424 ± 0.013	10.80	19.1	0.44	2.87
430	0.411 ± 0.007	7.60	19.0	0.48	2.65
230	0.385 ± 0.024	2.85	13.5	0.42	3.01
130	0.368 ± 0.011	0.60	11.8	0.48	2.62

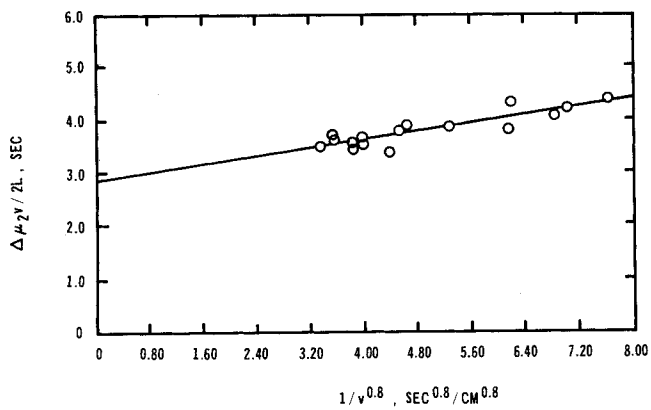

Fig. 1. Second central moments for NaCl pulses on 230 μm gel with $D_0 = \lambda v^{1.2}$ ($L = 37.4$ cm).

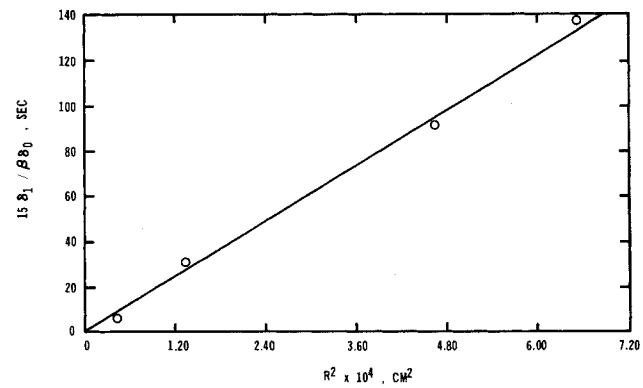

Fig. 2. Chromatographic data for NaCl pulses on four sizes of gel particles.

For the range of Reynolds number (0.06 to 0.12) of the experiments with 230 μm gel, Gunn's (1969) theoretical curve specifies that N_{Pe} is proportional to $N_{Re}^{-0.2}$, and therefore $D_0 = \lambda v^{1.2}$. Similarly for the 130 μm gel ($0.07 < N_{Re} < 0.22$) one finds $D_0 = \lambda v^{1.3}$. Thus for 230 μm gel a plot of $\Delta\mu_2 v/2L$ versus $1/v^{0.8}$ should be a straight line with intercept δ_1, Figure 1. For 130 μm gel $\Delta\mu_2 v/2L$ is plotted versus $1/v^{0.7}$. The slopes of these graphs equal $\lambda(1 + \delta_0^2)/\alpha$, and the intercepts equal δ_1.

For the range of Reynolds number of these experiments and for packed bed systems similar to ours, Miyauchi (1972) has provided a correlation for the mass transfer coefficient k, $N'_{Sh} = 1.0\, N'^{1/3}_{Pe}$. These dimensionless numbers are based on a hydraulic radius, which for cylindrical packed columns is given by $R_h = AR$ where $A = d\alpha/[3d(1 - \alpha) + 4R]$. Thus kR is only weakly dependent on v_0 and is evaluated for the average velocity of the runs for a given size gel, Table 1.

Rearranging the definition of δ_1 yields an expression from which D_i is calculated, Table 1. We may define a tortuosity factor in the standard manner, $q = \beta D_{AB}/D_i$, and calculate the values of q in Table 1. The averages are $D_i = (0.45 \pm 0.05) \times 10^{-5}$ cm²/s and $q = 2.8 \pm 0.3$.

The definition of δ_1 provides that a graph of $15\delta_1/\beta\delta_0$ vs. R^2 should be a straight line if kR is much larger than D_i and varies little with R. Furthermore, if the assumption of negligible adsorption is valid, the line should pass through the origin. Figure 2 is confirmation that these conditions hold.

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NOTATION

d = inside diameter of the column, cm
D_{AB} = binary molecular diffusion coefficient, cm²/s
D_i = effective internal diffusion coefficient, cm²/s
D_0 = effective axial dispersion coefficient, cm²/s
k = mass transfer coefficient, cm/s
L = length of bed or distance between two measuring stations, cm
N_{Pe} = $2Rv_0/D_0$, axial Peclet number
N_{Pe}' = $2R_h v_0/D_0$, axial Peclet number based on hy-

draulic radius R_h

N_{Re} = $2Rv_0\rho/\mu$, Reynolds number based on sphere radius

N'_{Sh} = $2kR_h/D_{AB}$, Sherwood number based on hydraulic radius R_h

q = internal tortuosity factor

R = average particle radius, cm

R_h = hydraulic radius, cm

v = linear interstitial velocity, cm/s

v_0 = αv, linear superficial velocity, cm/s

α = bed void fraction

β = internal porosity of the particle

δ_0 = $\beta(1-\alpha)/\alpha$

δ_1 = $(\beta^2R^2/15)\ (1/D_i + 5/kR)\ (1-\alpha)/\alpha$

$\Delta\mu_1'$, $\Delta\mu_2$ = differences between moments at inlet and outlet; s, s^2

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The Grid Region in a Fluidized Bed Reactor

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The current models for gas fluidized bed reactors as described by Kunii and Levenspiel (1969) do not distinguish between the region near the inlet grid and the rest of the reactor. It is well known, however, that the grid region plays a critical role in determining reactor performance, particularly in large beds. Industrial pilot plant experiments by Cooke et al. (1968) have shown that with fast reactions most of the conversion can take place in the first half-meter of bed height. Changing the grid can have a significant effect. Cooke et al. found that increasing the grid hole size caused a drop in conversion and Hovmand et al. (1971) showed that increasing the number of holes in the grid caused a marked improvement. These observations imply that the contacting efficiency near the grid is greater than in the rest of the bed.

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In this note, we present a simple model which accounts explicitly for the grid region and allows easy analysis of the effect of grid modifications.

Recent experiments by Behie (1971, 1972) have characterized the heat and mass transfer in the region above a perforated plate grid in a pilot scale fluidized bed. Grid hole diameters from 6.35 to 25.4 mm were used with jet velocities between 15.3 and 91.5 m/s, giving data which are typical of industrial conditions. The data could be represented adequately by the simple transfer resistance model described below.

THE MODEL

The gas enters the reactor as high speed jets which penetrate a distance h before breaking up into bubbles. That part of the bed below h is called the grid region and is assumed to contain no bubbles. It is assumed that the jets are well mixed radially with plug flow in the axial direction. The fluidized emulsion in the neighborhood of the distributor is assumed to be perfectly mixed by the