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# Development of Modified Stokes Expression to Model the Behavior of Expanded Beds Containing Polydisperse Resins for Protein Adsorption

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**Abstract**−Expanded bed behavior was modeled by using the Richardson-Zaki correlation between the superficial velocity of the feed stream and the void fraction of the bed. A polydisperse material, Chelating excellose® (70-210 μm in diameter, 1.21 g/cm$^3$ in density), which has $Ni^{2+}$ ions for the selective binding of histidine-tagged proteins, was used as the resin. A method to modify the Stokes expression to express the terminal settling velocity of the resins by introducing two empirical parameters, the effective diameter of the resins and an exponent for $(\rho_p-\rho)/\mu$ term, was developed. Combined use of the Richardson-Zaki correlation and the modified Stokes expression was successful in modeling the bed expansion by incorporating physical properties of feed streams and the resins.

Key words: Expanded Bed, Modeling, Scale-up, Stokes Expression

## INTRODUCTION

Before initiating selective separation of proteins by using packed-column chromatography methods, such as gel-filtration chromatography or ion-exchange chromatography, traditional recovery of extra-cellular proteins from fermentation broths or intra-cellular proteins from cell homogenates starts with the clarification steps: protein solutions containing solid particles should be treated with centrifugation or filtration steps to remove cells or cell debris followed by dialysis and concentration steps to remove untargeted small molecules and decrease solution volume [Lee et al., 2004; Shim et al., 2003]. Those preliminary steps for protein recovery are the main causes for low yields, prolonged process time, and high operational costs. These problems become more serious as the scale of protein recovery process increases.

Expanded bed adsorption (EBA) technology, in which a feed stream is applied from the bottom of the column containing proper resins for protein adsorption at a flow rate enough to maintain the resins well expanded but with low back-mixing, enables the simultaneous achievement of clarification, concentration, and selective separation [Chase and Draeger, 1992; Hjorth, 1997; Hu et al., 2001]. Solid particles contained in the feed stream can pass through the expanded bed; therefore, the clarification steps become unnecessary. EBA technology is especially proper for the separation of genetically engineered proteins possessing histidine tags. Proteins with histidine tags can be easily separated from solution by the specific adsorption on the metal containing resins.

EBA technology, however, lacks such thorough understanding about its hydrodynamic behavior as has been available for the conventional packed-column technology [Janson, 2001]. Therefore, in order to utilize EBA technology for industrial-scale protein purification processes, a method to describe the behavior of the expanded bed incorporating the properties of resins and feed streams should be provided. This model will be used to design the equipment and to preset the optimal values of operating parameters.

## MATHEMATICAL APPROACH

In this study, we compared two methods to describe the expanded bed behavior. One is the traditional dimensional analysis method, which has been successfully applied for the design of various fluid-processing equipments. The other is the Richardson-Zaki equation, which predicts a linear correlation between logarithms of the superficial fluid velocity ($v_o$) and the void fraction of the bed ($1-\phi_s$) for fluidized beds by

$$\log v_o = n \log (1-\phi_s) + \log v_t \qquad (1)$$

where $\phi_s$ and $v_t$ are the solid fraction of the expanded bed and the Stokes terminal settling velocity of the particle at infinite dilution ($\phi_s$=0), respectively [Richardson and Zaki, 1954]. The superficial velocity ($v_o$) is obtained by dividing the volumetric flow rate of the feed stream by the cross-sectional area of the column. In theory, the Stokes terminal settling velocity is applied when the particle's Reynolds number is less than 1 and expressed as a function of the properties of particle and solution by

$$v_t = g\,(d_p)^2(\rho_p-\rho)/18\mu \qquad (2)$$

where g is the gravitational acceleration, $\rho_p$ and $\rho$ are the densities of solid particle and solution, respectively, and $\mu$ the solution viscosity. Due to the poly-dispersity of the resins used for expanded beds, however, $v_t$ values cannot be theoretically determined by Eq. (2) but are estimated by Eq. (1) using the experimentally measured values of $v_o$ and $\phi_s$. The solid fraction, $\phi_s$, of the expanded bed at a height H can be obtained by

$$\phi_s = (\phi_s)_o H_o/H \qquad (3)$$

where $(\phi_s)_o$ and $H_o$ are the solid fraction and the height of the sedimented bed at zero flow rate of the feed stream, respectively. Combining Eq. (3) and Eq. (1) results in an explicit representation of the

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bed-expansion by

$$H/H_o=(\phi_s)_o/[1-(v_o/v_t)^{1/n}] \quad (4)$$

Though, the Richardson-Zaki equation was successful in modeling the behavior of expanded beds [Reichert et al., 2001; Thelen and Ramirez, 1997], no further attempts were made to correlate estimated values of n and $v_t$ with the properties of resins and feed streams.

## EXPERIMENTAL

In this study, chelating excellose® (70-210 μm in diameter, 1.21 g/cm$^3$ in density, purchased from Bioprogen, Inc., Korea), which has $Ni^{2+}$ ions enabling the selective binding of histidine-tagged proteins, was used as the resin. The column has an internal diameter of 2.54 cm and a height of 75 cm. The column was first packed with acid-washed glass beads (425-600 μm in diameter, from Sigma) to a height of 2 cm; then the resin was packed above the glass beads to a height of approximately 6 cm. This method of packing the resins ensures an even flow distribution to minimize channeling inside the bed. In order to vary the solution properties, such as density and viscosity, aqueous buffers (50 mM $NaH_2PO_4$, 300 mM NaCl, 10 mM imidazole, pH 7) containing different concentrations of glycerol were used as feed streams.

## RESULTS AND DISCUSSION

The effects of superficial velocity ($v_o$) of the feed stream containing different amounts of glycerol on the bed expansion ($H/H_o$) were measured. The bed expansion factor was limited below 3 as recommended by Reichert et al. [2001] for proper performance of expanded beds. The viscosity of each glycerol-aqueous buffer was estimated by interpolating the data provided by Poker and Janjic [1987]. As shown in Fig. 1, the expansion of the bed becomes greater as the solution viscosity increases at a fixed flow rate of the feed

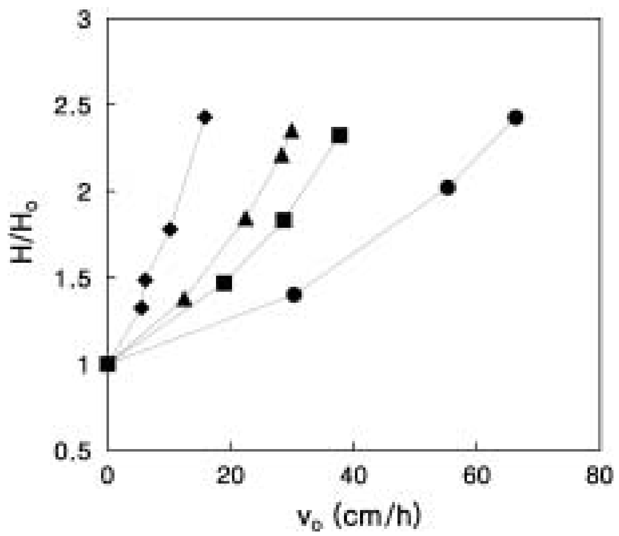


**Fig. 1. Effects of the superficial velocity of fluid on the bed expansion of the resins (chelating excellose®, 70-210 μm in diameter, 1.21 g/cm$^3$ in density) in glycerol-aqueous buffer mixtures. The concentrations of glycerol (v/v) are 0% (●), 10% (■), 20% (▲), and 30% (◆).**

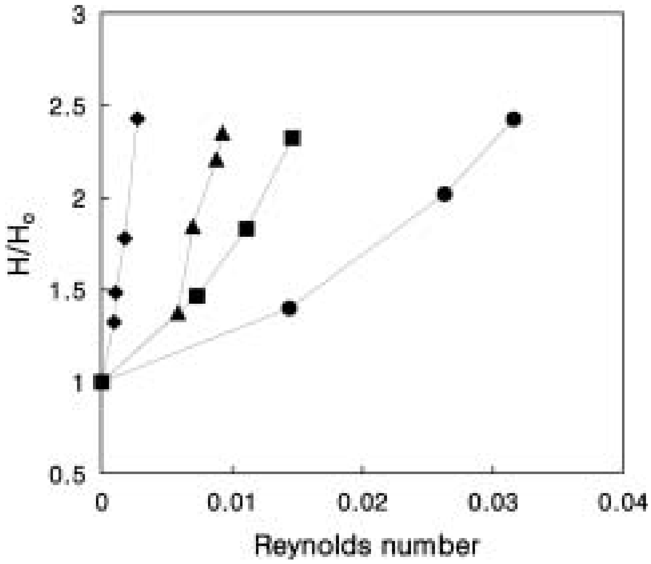


**Fig. 2. Bed expansion of the resins (chelating excellose®, 70-210 μm in diameter, 1.21 g/cm$^3$ in density) in glycerol-aqueous buffer mixtures as functions of the Reynolds number of the resins ($d_p$=160 μm). The same symbols as in Fig. 1 are used for glycerol-aqueous buffer mixtures.**

stream. To find a method to represent the bed-height expansion in a unifying way by using a combined parameter of operating conditions, we performed dimensional analysis for the expansion of the bed. The bed-expansion ($H/H_o$) can be expressed by an intrinsic function of the various operating conditions, such as the fluid velocity (V), resin diameter ($d_p$), solution viscosity ($\mu$), and solution density ($\rho$), by the following equation.

$$H/H_o=f(V, d_p, \mu, \rho) \quad (5)$$

Dimensional analysis of Eq. (5) proposes that the bed expansion may be represented by Eq. (6) using the particle Reynolds number ($Re_p=d_pV\rho/\mu$) as a single combined parameter incorporating various operating conditions.

$$H/H_o=g(Re_p) \quad (6)$$

Fig. 2, however, shows that Eq. (6) was not successful in exhibiting the expanded bed behavior in a unifying way under various operating conditions. The average diameter of the resins was used to calculate the $Re_p$ values.

Therefore, we applied the Richardson-Zaki correlation. Although Coulson et al. [1991] provided $(\phi_s)_o$ values for spherical particles of various sizes, we directly measured $(\phi_s)_o$ value for the resin as follows. In the column, the resin was settled in distilled water. The water contained inside the void fraction of the sedimented bed was drained off by slightly pressurizing the column from above with $N_2$ gas. The volume of the drained water was measured and divided by that of the total sedimented bed to determine the void fraction, $\varepsilon$, of the bed. Finally, $(\phi_s)_o$ value determined by $1-\varepsilon$ was 0.848.

Fig. 3 shows the Richardson-Zaki plots for the experimental data in Fig. 1. In glycerol-aqueous buffer mixtures, expansion of the bed follows the Richardson-Zaki relation very satisfactorily. All the linear lines in Fig. 3 have approximately the same slope (n) values with different intercepts. The average value of the slopes, $n_{av}$, was 1.71.

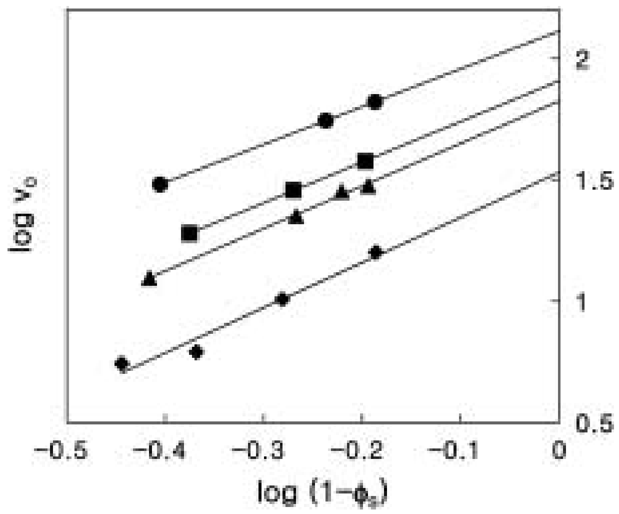


**Fig. 3. Richardson-Zaki plots for the bed expansion. The same symbols as in Fig. 1 are used for glycerol-aqueous buffer mixtures.**

**Table 1. Physical properties of glycerol-aqueous buffer mixtures and the parameter values of Richardson-Zaki correlation**

| Glycerol (v/v %) | Density $(g/cm^3)^a$ | Viscosity $(cp)^b$ | n | $v_t$ (cm/h) |
|---|---|---|---|---|
| 0 | 1.01 | 0.89 | 1.56 | 130 |
| 10 | 1.04 | 1.25 | 1.68 | 81 |
| 20 | 1.06 | 1.67 | 1.72 | 65 |
| 30 | 1.11 | 2.50 | 1.86 | 34 |

[a]Density values were directly measured.
[b]Viscosity for each solution was estimated by interpolating viscosity data for glycerol-water mixtures provided by Poker and Janjic [1987].

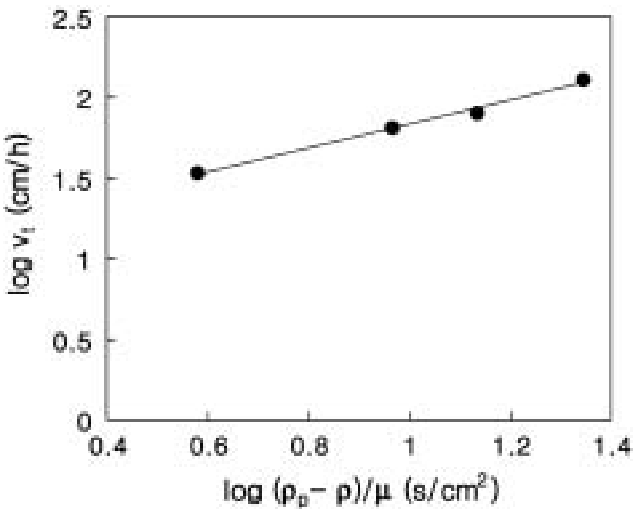


**Fig. 4. Linear correlation between log $v_t$ values determined from Richardson-Zaki plots with log $(\rho_p-\rho)/\mu$ values.**

Table 1 lists the values of n and $v_t$ in glycerol-aqueous buffer mixtures along with their density and viscosity values. Eq. (2) implies that log $v_t$ values can be linearly correlated with log $(\rho_p-\rho)/\mu$ values. Fig. 4 shows that this implication is true. The linear correlation in Fig. 4 as represented by Eq. (7) allows us to develop a modified Stokes expression as follows.

$$\log v_t=1.09+0.74\log(\rho_p-\rho)/\mu \tag{7}$$

Comparing Eq. (7) to Eq. (2) enables the introduction of two empirical parameters, the effective particle diameter, $(d_p)_e$, and an exponent, a, to modify the Stokes expression as in the following general equations.

$$v_t=g(d_p)_e^2[(\rho_p-\rho)/\mu]^a/18 \tag{8}$$

or

$$\log v_t=\log[g(d_p)_e^2/18]+a\log(\rho_p-\rho)/\mu \tag{9}$$

By comparing Eq. (7) to Eq. (9), values of $(d_p)_e$ and a are calculated to be 80 μm and 0.74, respectively. It is very reasonable that the effective diameter is close to the diameters of the smallest resins used, since the smallest resins would be at the top of the expanded bed, and therefore should be used as a basis in modeling the behavior of expanded bed.

Fig. 5 demonstrates that the calculated bed expansions, $(H/H_o)_{cal}$, using the modified Stokes expression (Eq. (8)) and Eq. (4) are in good agreement with experimentally measured values of bed expansion, $(H/H_o)_{exp}$. In this case, the n value in Eq. (4) is replaced by the average, $n_{av}$, of n values, the slopes of the linear plots in Fig. 3 for all glycerol-aqueous buffer mixtures.

## CONCLUSIONS

Conclusively, the explicit expression of the Richardson-Zaki correlation in combination with the modified Stokes expression for the terminal settling velocity developed in this study can be successfully used to model the behavior of expanded beds. Application of the method developed in this study to model the behavior of the ex-

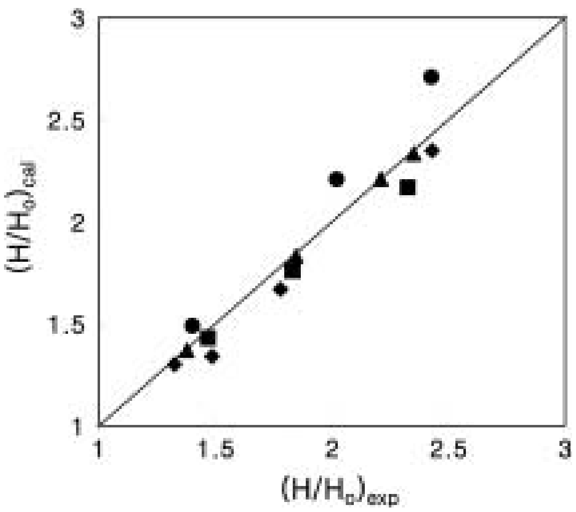


**Fig. 5. Comparison of the bed expansions calculated using the modified Stokes expression, $(H/H_o)_{cal}$, with those of experimentally determined value, $(H/H_o)_{exp}$. The same symbols as in Fig. 1 are used for glycerol-aqueous buffer mixtures.**

panded bed of various sizes with different feed streams is to be pursued in the future.

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