Substituting $\psi = \psi^{(0)} + \mu\psi^{(1)}$ into the kinematic boundary condition gives the free surface equation

$$d_\tau + A_\pm(d)d_\xi + \mu\{W_e[N_\pm(d)d_\xi^2 + C_\pm(d)d_{\xi\xi}] + R_eB_\pm(d)d_{\xi\xi} + D_\pm d_{\xi\xi\xi\xi}\} + 0(\mu^2) = 0 \quad \text{(A2)}$$

where

$$B_\pm(d) = -0.5q^6(\ln Q)^3 + 5q^4(\eta_0^2 - q^2)(\ln Q)^2/8 + q^2\eta_0^2(17q^2 - 7\eta_0^2)(\ln Q)/16 + 59q^6/192 + 16\eta_0^6/192 - 15q^4\eta_0^2/64 - 9q^2\eta_0^4/64$$

$$D_\pm(d) = -2\mu^2W_eM_\pm(d)$$

$$M_\pm(d) = 3q^3/16 + \eta_0^3Q/16 - \eta_0^2q/4 + q^3\ln Q/4$$

In the limit of $\eta_0 \to \infty$, Equation (A2) reduces to the corresponding equation obtained by Benney (1965) for the film flow down an inclined plane. See also Lin (1974). Equation (A2) may be compared with (6.42) of Atherton & Homsy (1975).

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Enzyme Separation by Parametric Pumping

Affinity chromatography and parametric pumping were combined to reduce trypsin concentration in an aqueous solution. The trypsin inhibitor, chicken ovomucoid, was covalently bonded to Sepharose 4B beads. A solution was cycled over this packing in a column at a low *p*H in one direction followed by a high *p*H in the other direction. Trypsin retention was favored at the high *p*H and elution at the low *p*H. The decrease in trypsin concentration at one end of the column was fitted to an equation derived from the equilibrium parametric pumping model and was a function of the *p*H limits imposed on the column. Separation was much less than predicted by equilibrium data, but the equation based on trypsin reduction agreed with the α-chymotrypsin-trypsin separation.

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SCOPE

Parametric pumping is a separation technique based on the periodic movement of a fluid phase over a solid adsorbent bed and a coupled energy input into the system to effect the separation. The most common form of parametric pumping is one in which a packed bed of adsorbent, undergoing a cycling temperature change, is subjected to a synchronous, alternating, axial flow. The variation of adsorption selectivity with temperature and the synchronized relative motion of the fluid over the fixed phase makes possible the enrichment of a given component at one end of the column and its depletion at the other end. In the initial announcement, Wilhelm et al. (1966) described the separation of an aqueous NaCl solution into two fractions; one enriched and the other depleted in NaCl. The potentiality of the technique was demonstrated by Wilhelm and Sweed (1968) when they obtained a separation factor of 10^5 in a toluene-*n*-heptane-silica gel system under total reflux conditions. Using the same system, Chen et al. (1972, 1973) have obtained separations with both continuous and semi-continuous product withdrawal. Other separations accomplished by parametric pumping include argon-propane and ethane-propane (Jenczewski and Myers, 1970) boron isotopes (Schroeder and Hamrin, 1970), and Na^+ and K^+ in aqueous solution (Sabadell and Sweed, 1970). The latter separation was achieved by a recuperative-mode, *p*H pump in which *p*H control was maintained by acid addition at one of the end reservoirs.

Affinity chromatography is a new separation method for macromolecules such as enzymes of particular importance because of its great specificity. One example of this method employs an inhibitor attached to a solid support which will selectively and reversibly bind an enzyme

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from a mixture. The process consists of passing an enzyme mixture through a column containing the inhibitor-solid which removes the desired enzyme followed by an elutriation step yielding the purified enzyme. To illustrate, chicken ovomucoid (CHOM) was covalently bonded (CVB) to Sepharose beads (Feinstein, 1970, Robinson et al., 1971). CHOM is an egg white protein (MW = 28,000) which is a highly specific inhibitor for bovine trypsin. Retention of trypsin occurred in a CHOM-Sepharose column at a pH of 8 when a mixture of α-chymotrypsin and trypsin were passed through. Elutriation at pH = 2 followed.

This study was undertaken to combine the two processes of parametric pumping and affinity chromatography. In particular, the objectives were

1. To determine the feasibility of operating a pH-parametric pumping system for enzyme separation.
2. To measure the separation and compare with the equilibrium model.

CONCLUSIONS AND SIGNIFICANCE

Twenty-one parametric pumping runs were carried out using a 26-mm I.D. X 400-mm chromatographic column packed with Sepharose CVB CHOM. Trypsin removal was studied at pH gradients of 3 and 6 and 4 and 6 and from an α-chymotrypsin-trypsin mixture at the latter pH gradient. The gradient was achieved at the ends of the column by using hollow-fiber dialysis cells containing buffered solutions of the desired pH. Separations were fitted to an equation of the form $\log_{10}(\langle y_B \rangle / y_0) = \alpha n$ where n is the number of cycles. Values of α were -0.01572 (pH gradient of 3 and 6) and -0.00689 for the 4 to 6 gradient. Separations were much less than those predicted from equilibrium data determined for the system using the linear equilibrium model for parametric pumping appropriate to Region 1 operation; however, they indicate the viability of parametric pumping as an enzyme separation method.

THEORY

Pigford et al. (1969) achieved a theoretical breakthrough in explaining parametric pumping by assuming local equilibrium and a linear equilibrium relationship. These assumptions allowed the partial differential conservation equation to be separated into a pair of ordinary differential equations. Expressions for the top reservoir concentration and the bottom reservoir concentration as well as the ratio of these two quantities (the separation factor) were given. They were functions of n, the number of cycles, and b, an equilibrium constant change parameter. The latter depends on both the system equilibrium properties as well as the operating conditions of the para-pumping system. The expression given by Pigford et al. (1969) for the bottom composition is

$$\frac{\langle y_B \rangle}{y_0} = \left(\frac{1-b}{1+b}\right)^n \tag{1}$$

This can be rearranged to yield

$$\log \frac{\langle y_B \rangle}{y_0} = \alpha n \tag{2}$$

where

$$\alpha = \log\left(\frac{1-b}{1+b}\right) \tag{3}$$

and

$$b = \frac{1-10^{\alpha}}{1+10^{\alpha}} \tag{4}$$

Chen et al. (1972) have generalized this expression by allowing for dead space in the end reservoir to get

$$b = \frac{1-10^{\alpha}+C_2(1-10^{\alpha})}{1+10^{\alpha}-C_2(1-10^{\alpha})} \tag{5}$$

These expressions are applicable for Region 1 operating conditions. Operation in this region is assured when the reservoir volume is equal to or less than the column void volume. Equation (2) shows a plot of the log of the concentration ratio in the bottom reservoir to that in the column initially vs. the cycle number results in a straight line of slope α. Either Equation (4) or (5) can be used to calculate b from the slope depending on the importance of the reservoir dead volume.

Another method of evaluating b is from equilibrium experiments. Linear equilibrium is represented by

$$C_s = K(\text{pH})\, C_f \tag{6}$$

To get a dimensionless m comparable to that of Pigford et al. (1969), one uses

$$m(\text{pH}) = \frac{(1-\epsilon)}{\epsilon} K(\text{pH}) \tag{7}$$

The quantity b can be calculated from two values of m; say $m(\text{pH}=2) = m_1$ and $m(\text{pH}=8) = m_2$

$$b = \frac{|m_1 - m_2|}{2 + m_1 + m_2} \tag{8}$$

Another parameter of importance is

$$m_0 = \frac{m_1 + m_2}{2} \tag{9}$$

Values of these parameters b and m_0 allow the prediction of separation factor as a function of the number of cycles for parametric pumping operation.

EXPERIMENT

Preparation of CHOM-Sepharose

Sepharose 4B (Pharmacia Fine Chemicals) was activated by cyanogen bromide in 200-ml batches according to the procedure described by Cuatracassas et al. (1968). Details are presented by Burkhead et al. (1973). The column was packed to a length of 0.191m with CHOM-Sepharose, and the void fraction was $\epsilon = 0.18$ as determined by draining the interstitial water.

Pumping Operation

The pumping system used in this research is shown schematically in Figure 1. Since constant temperature operation and a liquid, mobile phase was planned, a constant volume process was chosen. Because of the substrate used and the specialized column needed to hold it in order to prevent packing

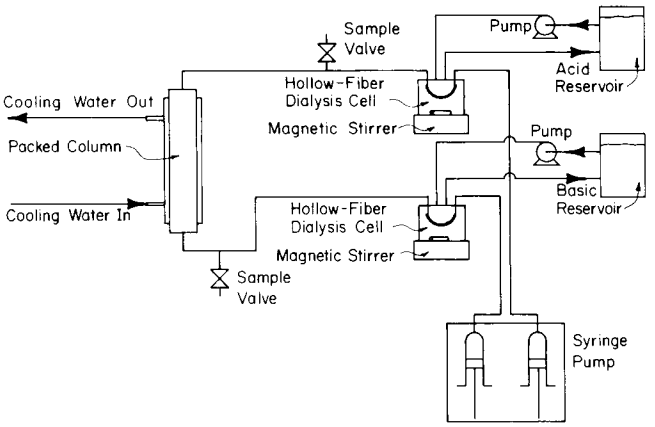

Fig. 1. Apparatus for recuperative, pH-mode parametric pumping operation.

up of the bed supports and flow stoppage, the recuperative mode was chosen for pH control. Direct control could be envisioned using dialysis tubing to contain the substrate surrounded by a cycling pH solution.

The movement of the liquid trypsin solution over the CHOM-Sepharose substrate was accomplished by use of an infusion-withdrawal syringe pump (Harvard 914) fitted with 50 ml glass syringes (Becton and Dickenson #2455). The syringes were connected to the column using Luer-lock fittings and adaptors and teflon capillary tubing. The lines and all necessary fittings at each end of the column contained a dead volume of 9 ml. The column used was a model K 26/40 ion exchange column manufactured by Pharmacia Fine Chemicals having an inside diameter of 26 mm and a length of 400 mm. The column was fitted with two variable length A-26 flow adaptors and R 25/26 column packing reservoir. Samples were withdrawn from the lines just ahead of the syringes through three-way, Luer-lock fitting valves, which were inserted into custom made telflon tees.

The change in pH of the system was performed by using a b/HFD-1 Dow dialysis cell in each line entering the column. The trypsin solution was allowed to pass through the tube bundles of these cells, while acidic and basic solutions (McIlvaines citrate buffer) were circulated on the outside of the tubes. These cells were magnetically stirred in order to assure proper mixing and circulation of the buffers around the tube bundles. Circulation was accomplished through use of two Cole-Parmer peristaltic pumps.

In order to check on the efficiency of this system, a $p\text{H} = 2.0$ solution was passed through the tube bundles at a flow rate of 0.0833 ml/s, while a $p\text{H} = 8.5$ solution was circulated on the reservoir side. The outlet pH of the tube bundle solution was found to be 8.0, thereby indicating good performance by the dialyzer cells.

Constant temperature of the system was maintained by use of a Haake constant temperature bath, which supplied water at 277°K to the cooling jacket of the column. The trypsin solution in the column was entirely in contact with surfaces made only of teflon, glass, or polyethylene during the entire operating cycle.

To start operation of the system, the column was equilibrated at a pH of 3.0. This was achieved by cycling a solution of 5 mg/ml trypsin through the column 15 to 20 times. Next the column was placed in a vertical position and the cooling water and trypsin solution lines attached. The acid side syringe was filled with 45-ml trypsin solution of 5 mg/ml and all air was bled from the syringes. The system was then allowed to run until the desired number of cycles had elapsed, at which time samples were taken of the top and bottom syringe solutions for analytical purposes. These samples were kept small (0.5 ml) so that several could be taken during each run without unduly disturbing the system.

At time zero, the acidic side syringe is filled and starts forward. The solution passes through the column, which is already equilibrated at these conditions. For this reason, the solution that emerges from the column and fills the basic side syringe has essentially the concentration of a 5 mg/ml solution that has been allowed to equilibrate with a column full of CHOM-Sepharose. This is the initial concentration for the basic side of the system.

When the cycle is half over, the pump drive reverses automatically by a micro-switch, and the solution flows back through the dialysis cell. Here the pH is adjusted to the high value where trypsin adsorption is preferred as the solution passes through the column. At the end of the syringe stroke, the pump again reverses, and one cycle is completed. This process is continued until the desired number of cycles is obtained with sampling occurring periodically.

Enzyme Analysis

Samples were analyzed for trypsin using a Beckman DB-G spectrophotometer by the method of Schwert et al. (1948). This method is good only for active trypsin, that is, trypsin that catalyzes the ionization of a phenolic hydroxyl group of n-benzoyl-L-arginine ethyl ester. About 75% of the stock trypsin solution was active (Burkhead et al., 1973).

RESULTS AND DISCUSSION

Parametric Pumping Runs

Three sets of runs were carried out. Group I consisted of Runs 1 through 13 which were made with a feed solution of 5 mg/ml trypsin and pH operating limits of 3.0 and 6.0. Four runs were eliminated due to insufficient initial equilibration and mechanical failure. The half cycle time was 225 s (450 s for total cycle), and the reservoir displacement rate was 0.178 ml/s.

Group II consisted of Runs 14 to 20, with Run 14 being rejected due to a system breakdown. These runs were also made with a feed solution of 5 mg/ml trypsin and a cycle time of 450 s, but the pH operating limits were 4.0 and 6.0.

A final run was made (No. 21) under the same operating conditions used for Group II, with the exception that the feed solution contained 5 mg/ml α-chymotrypsin in addition to the usual 5 mg/ml trypsin. This was done in order to see if the system would actually remove trypsin from an enzyme mixture.

In all of these runs, four top reservoir and four bottom reservoir samples were taken at various numbers of cycles from 1 to 40. From these concentrations normalized values were calculated by computing $<y_B>/y_0'$ where y_0' was the initial solution concentration measured. In early runs this value wasn't determined until 2 or 4 cycles had elapsed. The normalized values were fit according to Equation (2) using a least-squares program and allowing an intercept to occur. From the intercept the actual initial column concentration y_0 was found and the data were adjusted to force the least-squares line through the origin as shown in Figure 2. A second regression was made after eliminating 3 data points which were 2 standard deviations from the original fit. For the Group I runs with pH limits of 3.0 and 6.0, the correlation equation was found to be

$$\log [<y_B>/y_0] = -0.01572\, n \qquad (10)$$

with an $R^2 = 0.80$. A t-test was made on the slope, yielding a value of -10.2 showing that the slope was statistically significant for the 28 points.

In the next set of runs, Group II, the lower pH limit was raised from 3.0 to 4.0 to study the effect of pH limits on separation. From the equilibrium studies, this change produced a 53% increase in the equilibrium adsorption of trypsin on CHOM-Sepharose; therefore, it was expected to produce poorer trypsin removal. Such was the case as shown in Figure 3. The least-squares line was determined as described above and found to be

$$\log [<y_B>/y_0] = -0.00689\, n \qquad (11)$$

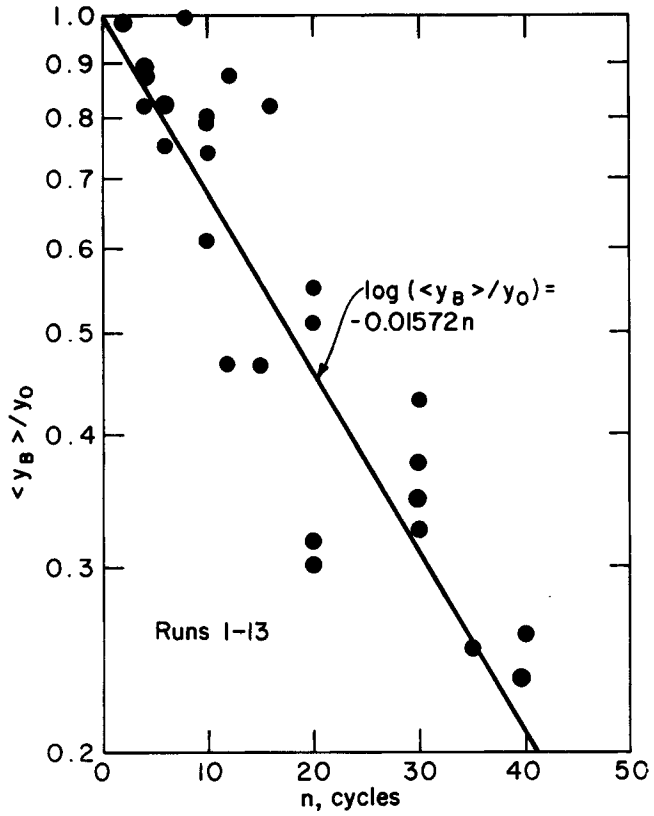

Fig. 2. Bottom reservoir concentration vs. cycles, low side pH = 3.0.

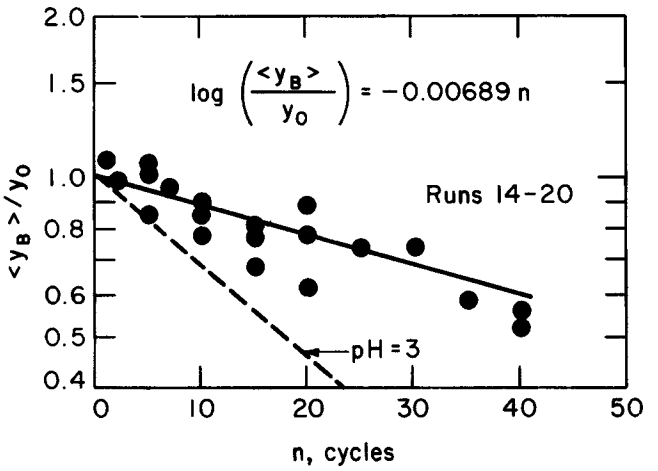

Fig. 3. Bottom reservoir concentration vs. cycles, low side pH = 4.0.

with $R^2 = 0.77$. This slope is clearly different from the correlation equation of Group I which is shown for comparison. A t-test made on the equation for Group II yielded a t-value of -7.87 indicating that the slope was significant past the 0.001 level for 21 data points. Confidence limits were also calculated for both slopes, and it was found that the 99% confidence intervals for the slopes did not overlap. The fact that these limits did not coincide indicated that there was a difference between the slopes which was statistically significant past the 99% level.

Figure 4 is a plot of Run 21 results and shows that there is definite separation taking place with a separation factor of 1.25 after 15 cycles. The trypsin concentration points from this run are seen to follow quite closely the results from Group II [Equation (11)]. It should be pointed out that Run 21 was made with limiting pH values of 4.0 and 6.0, and greater separation would be expected at a greater spread of pH values, such as 3.0 and 6.0.

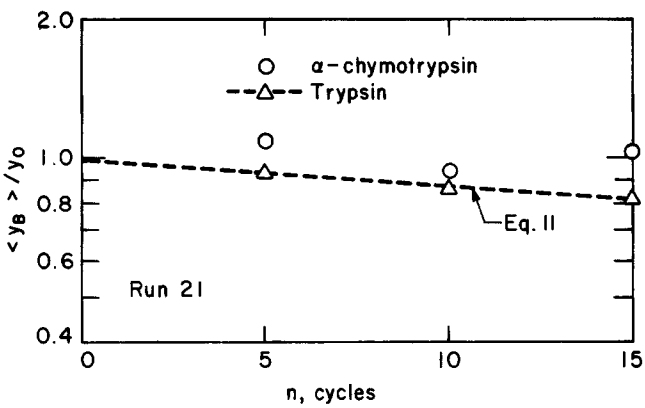

Fig. 4. Bottom reservoir concentration vs. cycles.

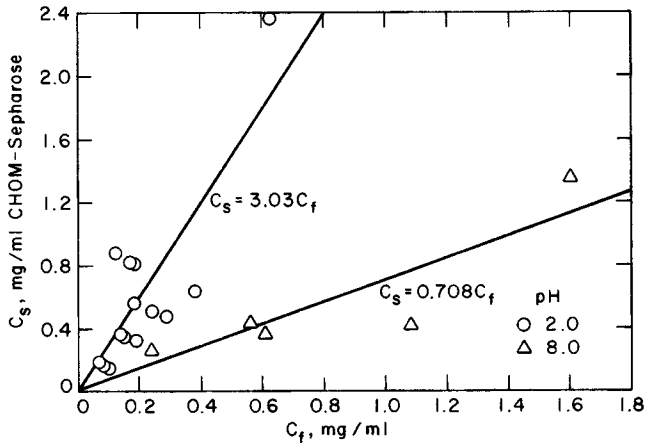

Fig. 5. Equilibrium in CHOM-Sepharose-trypsin system (T = 294 K).

COMPARISON OF RESULTS TO EQUILIBRIUM PARAMETRIC PUMPING MODEL

In order to compare the results obtained in these experiments with those based on equilibrium data, the factor b was calculated from Equations (5) and (8). Using the value of $\alpha = -0.01572$ from the Group I runs for pH limits of 3.0 and 6.0 and $C_2 = 9/40 = 0.225$, one gets from Equation (5) $b = 0.0223$. For the Group II runs (pH limits of 4.0 and 6.0) the value of $b = 0.0097$ which is indicative of the narrower pH range.

Equilibrium data were determined at pH-values of 2.0 and 8.0 and ambient temperature ($T \simeq 294$°K) as shown in Figure 5. The least-squares lines are shown and the slopes correspond to K (pH = 8) = 3.03 ml solution/ml CHOM-Sepharose ($R^2 = 0.87$ and $t = 8.8$) and $K(2) = 0.708$ ($R^2 = 0.92$ and $t = 6.0$). Using Equation (7) and $\epsilon = 0.18$, one finds m (pH = 8) = 13.8 and m (pH = 2) = 3.23. The value of b is found to be 0.555 from Equation (8) and $m_0 = 8.52$ from Equation (9). The equilibrium b-value is so much higher due to differences in temperature and pH limits between the parametric pumping runs and the equilibrium determination. Variations in enzyme activity and the amount of CHOM attached to the Sepharose from batch to batch are also causes for the differences and account for the scatter in the data.

The equilibrium b and m_0 values were used to calculate penetration distances, and it was found that ($L_2 = 28$) < ($L_1 = 99$) < ($h = 191$ mm) as required for Region I operation; however, the wide disparity between experiment and theory indicates the need for further study of macromolecule separation by parametric pumping-affinity chromatography processes.

ACKNOWLEDGMENTS

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NOTATION

b = dimensionless equilibrium parameter
C_2 = fraction of bottom reservoir dead volume to displacement, dimensionless
C_f = trypsin concentration in fluid phase, mg/ml
C_s = trypsin concentration on solid phase, mg/ml CHOM-Sepharose
h = packed height of column, mm
K = equilibrium constant, ml solution/ml CHOM-Sepharose
L_1, L_2 = penetration distance of concentration front, mm
m = equilibrium constant parameter defined by Equation (7), dimensionless
n = number of cycles
y_B = concentration of trypsin in bottom syringe, mg/ml
y_0 = initial concentration of trypsin in column, mg/ml
y_0' = initial concentration of trypsin measured, mg/ml

Greek Letters

α = slope of line given by Equation (2)
ϵ = column void fraction

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Catalytic Activity and Selectivity on Heterogeneous Surfaces with Mass Transfer

Effective reaction rates and selectivities on surfaces having active and inactive regions are calculated for systems in which diffusion of reactants and products through a boundary layer is significant. With a single reaction it is shown that rates are larger than those calculated assuming area weighted averages. The selectivity of production of an intermediate in a series of reactions on a heterogeneous surface is higher than on a homogeneous surface, and with parallel reactions selectivities may be lower or higher depending on the kinetics of the competing reaction.

Numerical examples from NH_3 oxidation on a Pt gauze and CO oxidation in the automotive converter indicate that these effects may be important in determining selectivities and conversions in some industrial reactors.

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SCOPE

Catalytic reactions on external surfaces have been studied theoretically by many investigators over the past two decades. Chung (1965) reviewed the literature in this field stressing the coupling of boundary layer and finite reaction rate. Since that time several additional papers have been published (Lindberg and Schmitz, 1969; Shirotsuka and Saro, 1969; Mihail, 1972). All these investigators have considered a uniformly active solid surface in contact with a gas. However, it is well known that most adsorbent surfaces exhibit heterogeneity with respect to their interactions with adsorbing gases. A recent paper by Rudzinski (1974) reviews this topic briefly, but to our