Selective Extraction Using Preferential Transport Through Adsorptive Membranes

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Selective extraction of a protein from a mixture can be accomplished using an adsorptive membrane and low displacement recuperative parametric pumping. Low displacement recuperative parametric pumping can lead to the preferential transport of an adsorbing solute and the rejection of nonadsorbing solutes by the adsorptive membrane. Using a protein mixture consisting of lysozyme and myoglobin, we have found the conditions under which lysozyme is preferentially transported through an ion-exchange membrane cartridge while myoglobin is rejected by the membrane. Trends observed when parameters such as the desorbent concentration, feed concentration, and flow rate are varied agree with the predictions of a mathematical model. Comparison with facilitated diffusion shows that preferential transport can lead to higher solute fluxes, albeit at lower selectivity. Additionally, preferential transport can be used to transport a solute up a concentration gradient and to selectively extract a solute from a feed that contains suspended solids.
Key words: adsorptive membranes • facilitated diffusion • parametric pumping • uphill transport • integrated processes

INTRODUCTION

The recovery of products from fermentation broths is often complicated by the large number of dissolved chemicals and suspended particles present in the mixture. Although a series of separation steps can usually accomplish the product recovery, reduction in the overall number of separation operations is desirable because of the low product yields associated with some steps. In situ product recovery operations, techniques that reduce the overall number of steps, are gaining popularity due to their increased yield and productivity along with their reduction in the complexity of the separation train (Freeman et al., 1993).

Selective extraction using a membrane is one such technique. The membrane functions to selectively transport a particular species from one phase to another while rejecting solutes and suspended particles that cannot partition into the membrane. One typical selective extraction is accomplished by facilitated diffusion and is performed in an immobilized liquid membrane (ILM) (Armstrong and Li, 1988; Pellegrino et al., 1990; Tsai et al., 1995). The liquid immobilized in the membrane contains a carrier species that reacts with the desired solute and facilitates its transport across the membrane. Undesired solutes, on the other hand, do not react with the carrier and, therefore, diffuse to a lesser extent across the membrane. Facilitated diffusion of a desired solute may also be coupled to the transport of another solute so that the desired solute can be transported up a concentration gradient (Cussler, 1988).

Despite the advantages of facilitated diffusion in ILMs, the process is not widely used because of membrane instability. Membrane instability is caused by carrier loss from the liquid membrane and emulsion formation at the membrane interfaces (Neplenbroek et al., 1990). To improve membrane stability, researchers have studied another type of selective extraction: facilitated diffusion through fixed-site carrier membranes (Cussler et al., 1989; Lacan et al., 1995; Noble, 1991). Cussler et al. (1989) suggested that, if the carriers are close to each other, facilitated diffusion across fixed-site carrier membranes can take place by a "bucket brigade" mechanism in which solute is transported from one carrier to another by diffusion of the solute–carrier complex. Additionally, Noble (1991) suggested that if the carriers are not close enough for a "bucket brigade" mechanism, transport of solute between carriers can also take place by diffusion in the membrane phase.

In a previous study, we proposed a different technique and a different membrane system for selective solute extraction (Agrawal and Burns, 1996). The technique, performed in a macroporous adsorptive membrane, is referred to as preferential transport and is one of the two separation mechanisms of recuperative parametric pumping (Grevillot, 1986; Huang and Hollein, 1988; Sweed, 1984; Tsai et al., 1995; Wilhelm et al., 1966; Wankat, 1978). Preferential transport results from alternately flowing a protein solution and a desorbing solution into and out of the membrane, and is obtained when this cyclic volume of solution is less than the membrane void volume.

The mechanism of separation during preferential transport through an adsorptive membrane is shown in Figure 1. In the first half of a cycle, an adsorbing solution containing an adsorbing solute, *A*, and a nonadsorbing solute, *B*, is fed

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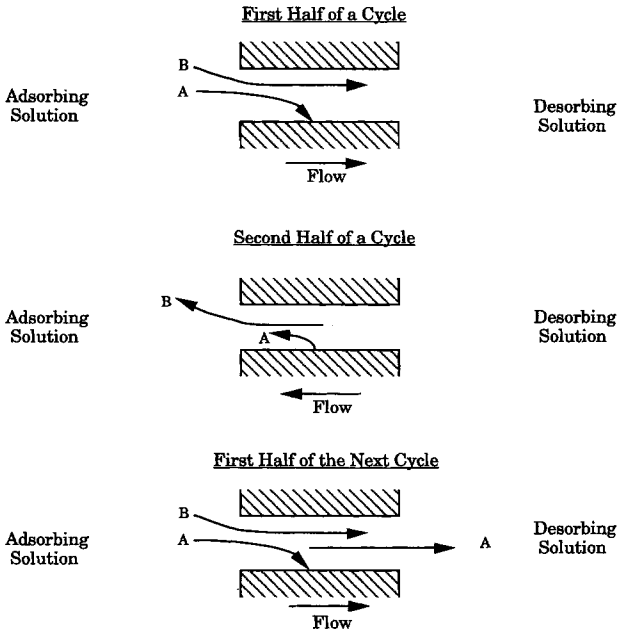

Figure 1. Preferential transport in an adsorptive membrane. In the first half of a cycle, the adsorbing solute, *A*, adsorbs to the membrane surface while the nonadsorbing solute, *B*, remains in the membrane void. In the second half of the cycle, *B* is "rejected" by the membrane while *A* desorbs but stays within the desorption front. In the first half of the next cycle, this desorbed *A* is preferentially transported across the membrane. (Reprinted with permission from *AIChE Journal* 42(1), January 1996, p. 136.)

into the membrane. During this half-cycle, *A* adsorbs to the surface of the membrane while *B* remains in the membrane void. In the second half of the cycle, a solute-free desorbing solution is fed into the membrane in the reverse direction. In this half-cycle, *B* is transported back to the membrane's left side while *A* desorbs but stays within the desorption front. This desorbed *A* is transported across the membrane in the first half of the next cycle. In this way, the nonadsorbing solute is "rejected" by the membrane while the adsorbing solute is preferentially transported through the membrane.

Selective solute removal using preferential transport in an adsorptive membrane offers many advantages over facilitated diffusion in an ILM. During preferential transport, the desired solute flux is based on convection rather than diffusion, so higher solute fluxes can be obtained. Furthermore, an adsorptive membrane is more stable than an ILM; in an adsorptive membrane, the adsorption sites are covalently bound to the membrane, thereby alleviating the carrier loss problem. The problem of emulsion formation is also eliminated because the solution in an adsorptive membrane is aqueous. The aqueous solution in an adsorptive membrane may make it more suitable to process solutions containing solutes, such as proteins, that may denature upon exposure to the organic phase of an ILM.

This study focuses on designing selective separations based on the principle of preferential transport through adsorptive membranes. We will first present experimental and theoretical results on the selective transport of lysozyme from a mixture of lysozyme and myoglobin through an ion-exchange membrane cartridge. Next, we will show how preferential transport can be optimized by presenting results on the effect of process variables such as desorbent concentration, feed concentration, and flow rate. We will then compare solute fluxes and selectivities obtained by preferential transport with those obtained by facilitated diffusion. Additionally, we will demonstrate that, similar to facilitated diffusion, preferential transport can also be used for transporting a solute up a concentration gradient and for selectively extracting a solute from a feed that contains suspended solids.

MATHEMATICAL MODEL

The mathematical model for recuperative parametric pumping in adsorptive membranes has been presented in detail elsewhere (Agrawal and Burns, 1996), and will be discussed only briefly in this section. In the model, the membrane is considered isothermal and homogeneous with a uniform cross-sectional area, A, porosity, ϵ, and thickness, L. The mass balances of a solute, i, and of the desorbent, d (if the adsorption of the desorbent is negligible), for the adsorbing stroke (first half of a cycle), are given by Eqs. (1) and (2), respectively.

$$\frac{\partial C_i}{\partial t}+\frac{1}{\epsilon}\frac{\partial Q_i}{\partial t}=E_i\frac{\partial^2 C_i}{\partial^2 l}-u_a\frac{\partial C_i}{\partial l} \tag{1}$$

$$\frac{\partial C_d}{\partial t}=E_d\frac{\partial^2 C_d}{\partial^2 l}-u_a\frac{\partial C_d}{\partial l} \tag{2}$$

where C_i and Q_i are the concentration of the species i in the pore and on the surface, respectively; C_d is the concentration of the desorbent in the pore; E_i and E_d are the axial dispersion coefficients for species i and desorbent, respectively; and u_a is the interstitial velocity during the adsorbing stroke. The mass balances for the desorbing stroke are also given by Eqs. (1) and (2), except that the velocity during the adsorbing stroke, u_a, is replaced by the velocity during the desorbing stroke, u_d, and the dispersion coefficients are evaluated at u_d.

If local equilibrium is assumed and multicomponent effects can be neglected, the concentrations of the solute in the pore, C_i, and on the surface, Q_i, can be related using single component Langmuir isotherms obtained at various desorbent concentrations. Such a Langmuir isotherm formalism is given by:

$$Q_i=\frac{Q_{m,i}K_{m,i}C_i}{1+K_{m,i}C_i} \tag{3}$$

where the adsorbent capacity, $Q_{m,i}$, and equilibrium constant, $K_{m,i}$, are functions of the desorbent concentration. These functions can be written (Antia and Horvath, 1989):

$$K_{m,i}=K_{m,i}^{\max}f(C_d) \tag{4a}$$

$$Q_{m,i}=Q_{m,i}^{\max}g(C_d) \tag{4b}$$

where C_d is the concentration of the desorbent, $K_{m,i}^{\max}$ and

$Q_{m,i}^{max}$ are the values of the equilibrium constant and membrane capacity of solute i, respectively, at zero desorbent concentration; and f and g are experimentally determined functions.

Eqs. (1)–(4), combined with the appropriate initial and boundary conditions, were dedimensionalized and yielded the following dimensionless parameters: $\alpha_i = K_{m,i}^{max} C_{i,0}$, which is the isotherm linearity parameter for species i; $\delta_i = Q_{m,i}^{max}/\epsilon C_{i,0}$, which is the dimensionless membrane capacity for species i; and $Pe_i = u_a L/E_i$, which is the Peclet number for species i. The model was then used to predict the transport of proteins through the membrane by solving these equations using finite difference techniques and the appropriate boundary conditions (Agrawal and Burns, 1996). All parameters used in the model were determined independently.

MATERIALS

Lysozyme (L 6876), myoglobin (M 1882), *Saccharomyces cerevisiae* (baker's yeast, YSC-2), and sodium azide (S 2002) were purchased from Sigma Chemical Co. (St. Louis, MO). Potassium chloride (3040-01), dibasic anhydrous sodium phosphate (3828-01), and concentrated hydrochloric acid (9535-01) were purchased from J. T. Baker Inc. (Phillipsburg, NJ). Water was distilled in a Barnstead (Boston, MA) glass still (A 1040) and deionized in a Barnstead Nanopore II deionizer (D 3700). The buffer used in all the experiments was 0.02 M Na_2PO_4 at pH 7.5. The membrane chromatography cartridge (CICM10H01) was purchased from Millipore Corp. (Bedford, MA). This cartridge is a stack of cation-exchange membranes with a bed volume of 1.4 mL (0.5 × 1.9 cm i.d.) and a porosity of 0.825. The cartridge provides a 1.2-μm macroporous network of cellulose mo

exchange capacity of about 0.68 mEq (manufacturer's data). In addition, 0.22-μm Millex-GV filters (SLGV 025 LS) were purchased from Millipore. Prior to use in experiments, all solutions were filtered using 0.22-μm Durapore filters (GVWP 047 00), which were also purchased from Millipore.

METHODS

Solute Concentration Measurements

The concentrations of lysozyme and myoglobin were determined by measuring absorbance at 280 nm and 410 nm, respectively, whereas the concentrations of KCl were determined by measuring conductivity on a VWR Scientific Digital Conductivity Meter (Model 604). The spectrophotometer used for measuring protein concentration in batch quantities was an HP 8452A diode array spectrophotometer equipped with a cell of path length 10 mm and chamber volume of 1 mL. The monitors used for continuous measurements of protein concentration were Pharmacia UV-1 monitors equipped with flow cells of path length 10 mm and chamber volume 8.7 μL.

Preferential Transport Apparatus

The recuperative parametric pumping apparatus is shown in Figure 2. The apparatus consisted of the following: the membrane cartridge, two HPLC pumps (HPX), two three-way solenoid actuated pinch valves (G-98301-22), two two-way solenoid actuated pinch valves (G-98301-01), a time delay relay (G-20602-30) connected to a DC power supply (6284A, Hewlett Packard), two UV monitors connected to a computer, and four beakers. Two 75-psi back pressure regu

f valves (U-456),

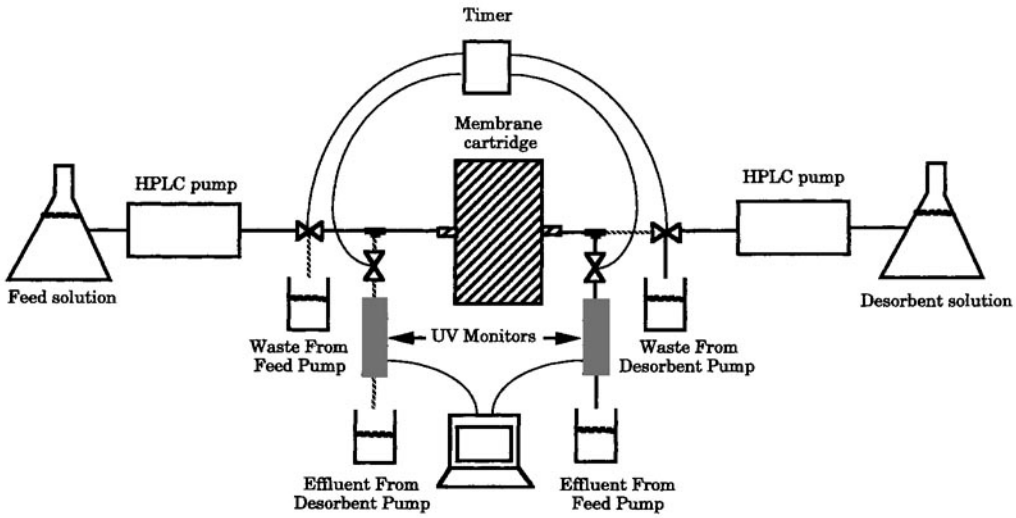

Figure 2. Apparatus used for preferential transport. During the first half-cycle, feed flows through the membrane and the right UV monitor into a beaker, while the desorbent flow is diverted into a waste beaker (shown by dark lines). During the second half-cycle, desorbent flows through the membrane and past the left UV monitor into a beaker, while the feed flow is diverted into a waste beaker.

and two 0–100-psi pressure gauges (9225) were attached in between the HPLC pumps and the three-way solenoid actuated pinch valves (not shown in Fig. 2). All tubing used in the apparatus was of 1/16-inch. o.d. The HPLC pumps, backpressure regulators, and pressure relief valves were purchased from Rainin Instrument Co. (Emeryville, CA). The solenoid valves and the time delay relay were purchased from Cole-Parmer Instrument Co. (Chicago, IL). The pressure gauges were purchased from Alltech Associates, Inc. (Deerfield, IL).

Preferential Transport Experiments

Before the experiment was begun, the membrane, the UV monitors, and all the tubing were equilibrated with the buffer solution, and the desired cycle time was set on the timer. The experiment then proceeded as follows. During the first half-cycle, protein flowed through the membrane and the right UV monitor into a beaker while the desorbent flow was diverted into a beaker (effectively shutting off the desorbent pump). During the second half-cycle, desorbent flowed through the membrane and the left UV monitor and into a beaker while the protein flow was diverted to a beaker (effectively shutting off the protein pump). These cycles were continued until there was no change in the amounts of protein collected from one cycle to the next in the two effluent streams. The parameter values listed in Table I were used in the experiments unless otherwise noted.

The experiments described above were conducted under several different conditions. Similar experiments were conducted in which desorbent concentration, feed concentration, and pump flow rates were varied. Experiments to study transport up a concentration gradient were conducted with the desorbing solution containing various concentrations of lysozyme. The integrated separation experiments were also conducted in the same way as described earlier except for the following modifications. Measured amounts of yeast were suspended in a buffer solution by continuous stirring on a magnetic stirrer for at least 2 h. A feed solution was then prepared by adding lysozyme and myoglobin to the yeast suspension. Also, a 0.22-μm Millex-GV filter was attached in series onto the feed side of the membrane cartridge.

Table I. Typical parameters. Unless otherwise indicated, these parameters were used in all experiments and simulations.

Experimental parameters	
Lysozyme concentration in feed	C_0 = 4.0 mg/mL
Ionic strength (KCl + buffer concentration)	I = 0.37 M
Ratio of stroke volume to membrane void volume	V_s/V_m (or $V_{s,d}/V_m$) = 0.8
Ratio of adsorbing stroke volume to desorbing stroke volume	$V_{s,a}/V_{s,d}$ = 1.02
Cycle time (adsorbing stroke + desorbing stroke)	1 min
Parameters determined independently	
Membrane void volume	V_m = 1.16 mL
Peclet number	Pe = 2100
Equilibrium constant at 0 M KCl	K_m^{max} = 69.8 mL/mg
Membrane saturation capacity at 0 M KCl	Q_m^{max} = 18.2 mg/mL
Function relating equilibrium constant to ionic strength	$f = 0.0024*I^{-1.55}$
Function relating membrane capacity to ionic strength	$g = 0.0046*I^{-1.38}$

Membrane Cleaning and Storage

After each experiment, the membrane was rinsed with 0.5 M KCl until there was no detectable protein in the eluent. Typically, this occurred after flowing about 30 mL of 0.5 M KCl. At the end of the day, the membrane cartridge was flushed with 25 mL of distilled and deionized water and then with 10 mL of a 0.02% sodium azide solution in deionized water before storage at 4°C.

RESULTS AND DISCUSSION

Selective Extraction Using Preferential Transport

During preferential transport, an adsorptive membrane selectively transports an adsorbing protein through the membrane while rejecting nonadsorbing solutes. An experimental apparatus that can be used to obtain this transport is shown in Figure 2. To obtain preferential transport, a protein feed solution is pumped through the membrane and the right UV monitor for a set time period. Next, a desorbent is pumped through the membrane in the opposite direction. During both pumping operations, the effluent from the membrane is monitored using UV monitors. This cyclic procedure is continued until there is no change in the amounts of protein collected from one cycle to the next in the two effluent streams. It is important to note that the volume of solution pumped in each half cycle is less than the void volume of the membrane.

Figure 3 shows the concentrations of lysozyme, the adsorbing protein, and myoglobin, the nonadsorbing protein, leaving the desorbing side of the membrane (the right side in Fig. 2) versus the number of cycles. Results are presented in terms of fractional fluxes (F_i), where F_i is defined as the ratio of the amount of protein i transported through the membrane in a cycle to the amount of protein i pumped into the membrane in that cycle. The figure shows that, after an initial phase of about 30 cycles, the system attains a state of coherence after which the fluxes of both proteins reach constant values. Coherence is analogous to steady state (Helfferich, 1986) in that there is no change in the system from one cycle to the next. In other words, after coherence is attained, there is negligible accumulation of protein within the membrane from cycle to cycle.

Once coherence is attained, lysozyme is selectively and continuously extracted from the protein mixture by the

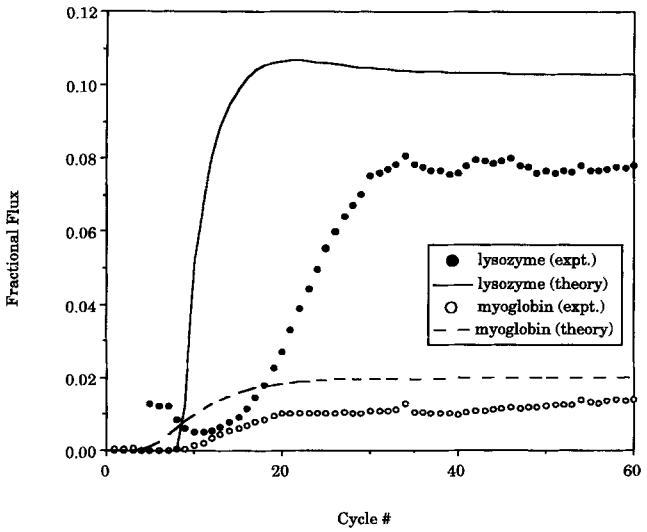

Figure 3. Preferential transport of lysozyme from a mixture of lysozyme and myoglobin. After an initial phase of about 30 cycles (30 min), the fluxes of both lysozyme and myoglobin reach constant values and coherence is attained. The coherent flux of lysozyme, which is the adsorbing protein, is more than six times higher than the coherent flux of myoglobin, the nonadsorbing protein.

membrane. In other words, the coherent fractional flux of lysozyme (0.078, from Fig. 3) is greater than the coherent fractional flux of myoglobin (0.012, from Fig. 3). These results indicate that the membrane preferentially transports lysozyme over myoglobin with a selectivity (the ratio of the coherent fluxes) of 6.5. These measurements were found to be reproducible within experimental error and control experiments with only lysozyme in the feed showed that the lysozyme flux is not significantly affected by the presence of myoglobin (data not shown). The experiments clearly show that the adsorbing protein is preferentially transported through the membrane over the nonadsorbing protein, even though the size of each protein (<0.005 μm) is much smaller than the membrane pore size (≈1.2 μm).

These experimental results have been compared with results from the mathematical model. Figure 3 shows that the model predicts the same trend as the experimental results with an increase in protein fluxes followed by constant fluxes. It is important to note that the mathematical model results are strictly predictive because the model does not contain any adjustable parameters; the model results are based on experimental parameters that are independently determined (see Table I). In light of the fact that the model is purely predictive, theoretically predicted coherent fluxes are in satisfactory agreement with the experimentally measured coherent fluxes.

The diagrams in Figure 4 show the simulated coherent solute waves in the liquid phase for the experimental conditions used to obtain Figure 3. At the end of the desorbing stroke, the adsorbing protein present in the membrane void forms a large peak around the front of the desorbing wave (Fig. 4a). Most of the peak is in the adsorbing phase ahead of the desorbent front, while a part of the peak is within the desorbing phase. During the following adsorbing stroke, a portion of the peak that was in the adsorbing phase readsorbs to the membrane, whereas the part of the peak that was within the desorbent front travels through the membrane (Fig. 4b); not all of the peak that was present in the desorbent phase is transported across the membrane.

Optimizing Preferential Transport

Clearly, the adsorbing solute fractional flux is a function of several process variables and optimizing these variables is critical in designing selective separations. Optimizing preferential transport implies maximizing the fractional flux of the adsorbing protein while minimizing the fractional flux of the nonadsorbing protein. The process variables that can be varied to optimize preferential transport separations are the stroke volumes, desorbent concentration, protein concentration, interstitial velocity, and dispersion.

In an earlier study, we have already presented experimental and theoretical data on the optimization of stroke volumes (Agrawal and Burns, 1996). The study showed that, for our experimental system, the ratio of the adsorbing stroke volume to the membrane void volume ($V_{s,a}/V_m$) that gives maximum adsorbing protein flux is 0.8. Lower $V_{s,a}/V_m$ values led to lower amounts of protein in the desorbed peak, whereas higher $V_{s,a}/V_m$ values led to rejection of the peak

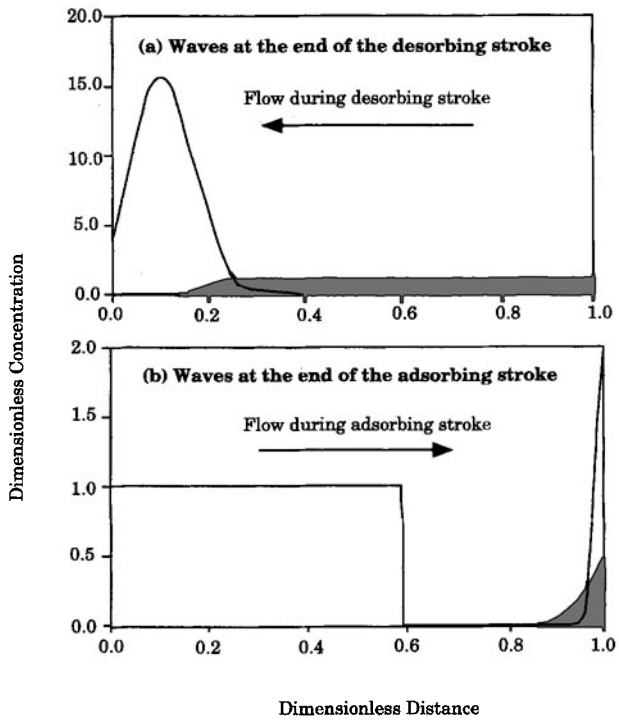

Figure 4. Simulated adsorbing protein and desorbent waves at coherence. (a) During the desorbing stroke (flow is to the left), the adsorbing protein (shown as a single curve) forms a peak around the front of the desorbent wave (shown as a shaded curve). (b) During the adsorbing stroke (flow is to the right), the adsorbing protein peak travels through the membrane and a fraction of the peak is transported across the membrane.

toward the feed side. The study also showed that, for our experimental system, the ratio of the adsorbing stroke volume to the desorbing stroke volume ($V_{s,a}/V_{s,d}$) that gave high adsorbing protein flux while maintaining low nonadsorbing protein flux was 1.02. It is important to note that, as $V_{s,a}/V_{s,d}$ increases, the adsorbing protein flux increases because the fraction of the peak that is transported to the right side (Fig. 4b) increases. However, as $V_{s,a}/V_{s,d}$ increases, the net convective flux through the membrane also increases, resulting in a concomitant increase in the nonadsorbing protein flux.

In this study, we keep the parameters discussed above ($V_{s,a}/V_m$ and $V_{s,a}/V_{s,d}$) constant and present data on the effect of other process variables such as desorbent concentration, feed concentration, and interstitial velocity.

Desorbent Concentration

Desorbent concentration is very important in preferential transport separations because protein movement in the membrane is coupled to the desorbent movement in the membrane. The desorbent concentration at a particular location in the membrane affects the equilibrium constant as well as the adsorption capacity at that location. Therefore, the desorbent concentration in the desorbent stream as well as that in the feedstream has a strong influence on the adsorbing solute flux.

Figure 5 shows that an optimum desorbent concentration is required in the desorbing stream to maintain a high value of the adsorbing solute flux. This optimum desorbent concentration for our experimental system is 0.35 M. An optimum desorbent concentration exists because, at high desorbent concentration, the entire membrane is nonadsorbing; alternatively, for low desorbent concentration, the entire membrane is adsorbing.

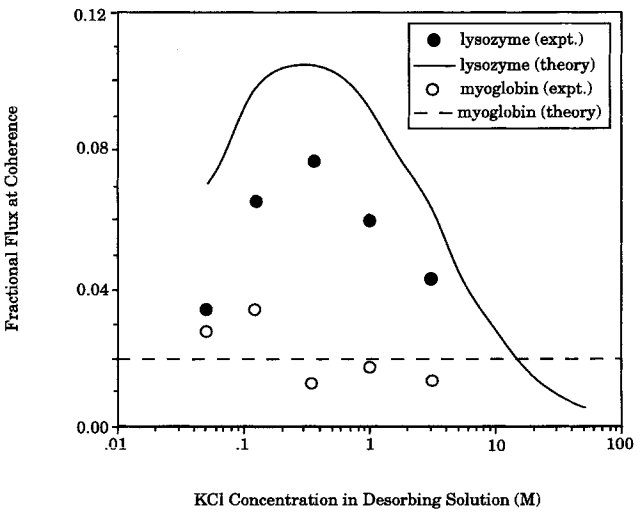

Figure 5. Effect of desorbent concentration in the desorbing solution. There is an optimum KCl concentration in the desorbing solution that leads to the highest lysozyme flux. Myoglobin flux is relatively independent of KCl concentration in the desorbing solution.

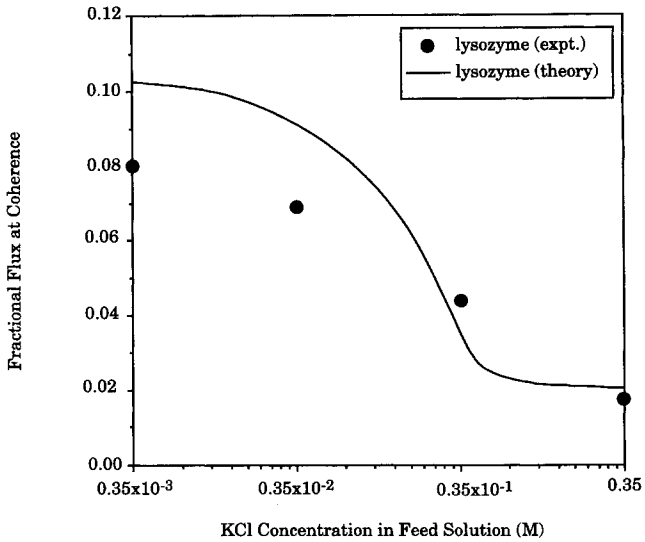

Figure 6. Effect of desorbent concentration in the feed solution. Lysozyme flux does not decrease appreciably when the feed contains only traces of the desorbent (KCl concentration in feed solution $<0.35 \times 10^{-2}$ *M*); however, lysozyme flux decreases significantly when the concentration of KCl in the feedstream is high (KCl concentration in feed solution $>0.35 \times 10^{-1}$ *M*). The KCl concentration in the desorbing solution was kept constant at 0.35 *M*.

In certain situations, the concentration of the desorbent in the feed may be nonzero. For example, a feed originating from cell culture or fermentation may contain salts that lower the membrane affinity and capacity [see Eqs. (4a) and (4b)]. In such situations, it is important to consider the adsorbing protein flux when the feed contains a nonzero concentration of the desorbent. Figure 6 shows that the lysozyme flux does not decrease appreciably when the feed contains only traces of desorbent. For example, when the concentration of KCl in the feedstream is only 0.0035 *M* (1% of the concentration of KCl in the desorbent stream), lysozyme flux is lowered by only about 10%. However, the flux of lysozyme decreases significantly when the concentration of KCl in the feedstream is high. For example, when the concentration of KCl is 0.035 *M* (10% of the concentration of KCl in the desorbent stream), lysozyme flux is drastically lowered by about 50%. This suggests that a feed containing high salt concentrations may need to be pretreated before a protein can be selectively removed from it by preferential transport. Alternatively, for such a feed, an affinity membrane and another modulator of adsorption and desorption (such as pH) could be considered.

Protein Concentration

Concentration of the adsorbing protein in the feed ($C_{A,O}$) affects both the linearity of the adsorption isotherm and the membrane capacity. Low feed concentrations result in relatively linear isotherm behavior and low preferential transport. As our theoretical results showed earlier, nonlinear behavior is one of the conditions necessary for obtaining

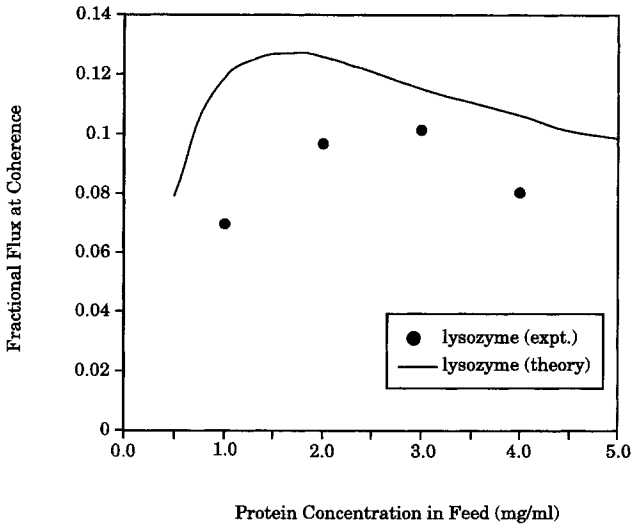

Figure 7. Effect of protein concentration in the feed. In the range of feed concentrations studied experimentally (1 mg/mL $\leq C_{A,O} \leq$ 4 mg/mL), there is little effect of feed concentration on the fractional flux; this experimental observation agrees with theoretical predictions. On the other hand, theoretical predictions show that, at low ($C_{A,O} \leq$ 0.5 mg/mL) and high ($C_{A,O} \geq$ 10 mg/mL) concentrations, the fractional flux is low.

preferential transport (Agrawal and Burns, 1996). As the feed concentration increases, the preferential transport of the adsorbing solute increases and approaches a maximum value. This maximum absolute flux as a function of feed concentration is limited by the membrane capacity and results in an eventual decrease in fractional flux through the membrane with increasing feed concentration. Thus, for any given conditions, there will be an optimum feed concentration to obtain the maximum preferential transport.

The theoretical results were experimentally verified and the experimental and theoretical results are shown in Figure 7. In the experiments, the feed concentration ($C_{A,O}$) was varied while holding all other parameters constant. The figure shows that, in the range of feed concentration studied experimentally (1 to 4 mg/mL), there is little effect of feed concentration on the fractional flux; this experimental observation agrees with theoretical predictions. However, theoretical predictions show that, at low concentrations ($C_{A,O} \leq$ 0.5 mg/mL) and high concentrations ($C_{A,O} \geq$ 10 mg/mL), fractional flux is low (results not shown).

The feed concentration also affects the number of oscillations the system takes to attain coherence. Specifically, as the feed concentration decreases, the number of oscillations required to attain coherence increases. For example, in our experiments we found that, for a feed concentration of 4 mg/mL, about 30 oscillations were required to attain coherence, whereas for a feed concentration of 1 mg/mL about 70 oscillations were required to attain coherence. The larger number of oscillations required to attain coherence for low feed concentration is attributable to the larger number of cycles necessary for protein to saturate the fraction of the membrane that is not reached by the desorbent during the desorbing half-cycle.

Interstitial Velocity and Dispersion

The interstitial velocity can indirectly influence the fractional flux because it affects dispersion in the media. In our membrane system, however, the fractional flux was essentially independent of the velocity used. From the dimensionless model equations (Agrawal and Burns, 1996), the dispersion coefficient was only present in the Peclet number ($Pe = uL/E$). Using the correlation developed by Dolan (1987), the dispersion coefficient can be shown to be a linear function of velocity ($E = 2ud_p$), and, therefore, the Pe number ($Pe = L/2d_p$) is independent of velocity. Thus, the fractional flux is also independent of velocity.

Figure 8 confirms this prediction. The experimental results were obtained at five different interstitial velocities: 0.36, 0.73, 1.45, 2.18, and 3.63 cm/min. It is important to note that, while the total adsorbing and nonadsorbing protein fluxes vary significantly with Pe, the fractional fluxes obtained at the various velocities remains constant within experimental error. The experimental error probably arises due to the variations in $V_{s,a}/V_{s,d}$ values that are experimentally obtained at the different interstitial velocities; earlier it was shown that small variations in $V_{s,a}/V_{s,d}$ values lead to significant errors in fractional fluxes (Agrawal and Burns, 1996). That protein fractional fluxes remain constant with increasing velocity suggests that increasing the velocity from 0.36 to 3.63 cm/min does not change the membrane Pe number significantly; this result agrees with the results of Gerstner et al. (1992).

Theoretical results show that selectivity of preferential transport separations can be increased by using lower dispersion media (media having higher Pe). Because preferential transport can be used as a separation mechanism in

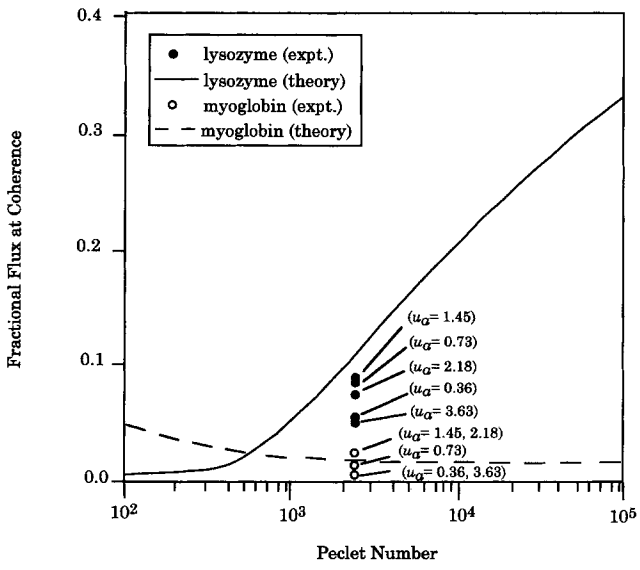

Figure 8. Effect of interstitial velocity and dispersion. The experimental results were obtained at five different interstitial velocities: 0.36, 0.73, 1.45, 2.18, and 3.63 cm/min. The adsorbing and nonadsorbing protein fluxes obtained at these velocities remain constant within experimental error.

packed beds as well as membranes, packed beds (*Pe* in the range 3×10^4 to 10^6) (Phillips et al., 1988) may lead to more selective separations than adsorptive membranes. However, in packed beds other factors may limit separation performance. For example, packed beds with large diameter beads may lead to high mass transfer resistances and packed beds with low diameter beads may lead to large pressure drops. Therefore, it may be more beneficial to develop adsorptive membranes that have dispersion in the same range as that in packed beds to maintain the low mass transfer resistance (Brandt et al., 1988; Briefs and Kula, 1992; Suen and Etzel, 1992) and low pressure drop (Josic et al., 1992; Tennikova et al., 1991) qualities of membranes.

Unique Applications of Preferential Transport

One of the unique applications of preferential transport is that one solute can be selectively extracted from a mixture containing both dissolved chemicals and suspended solids. The operation of preferential transport in this mode is most similar to facilitated diffusion, but the fluxes that can be obtained are several orders of magnitude higher with preferential transport. After comparing the fluxes of these two types of selective transport, we will present results on the transport of a protein up a concentration gradient and the extraction of a protein from a complex mixture.

Comparison with Facilitated Diffusion

The maximum protein flux obtainable by preferential transport through adsorptive membranes can be compared to the maximum possible flux obtainable by facilitated diffusion through immobilized liquid membranes. The total flux obtainable during preferential transport is given by:

$$J_A = F_A \frac{u_a}{2} C_{A,0} \tag{5}$$

where J_A is the total protein flux, F_A is fractional flux at coherence, u_a is the solvent interstitial velocity during the adsorbing stroke, and $C_{A,O}$ is the concentration of the desired protein in the feed. The maximum possible flux obtainable during facilitated diffusion is under the conditions of negligible mass transfer on the feed and receiving sides of the membrane and fast reactions between the protein and carrier in the membrane. Under these hypothetical conditions, the maximum possible protein flux obtainable during facilitated diffusion is given by:

$$J_A = \frac{D_A}{L} P C_{A,0} \tag{6}$$

where D_A is the diffusivity of the protein–carrier complex, L is the membrane thickness, P is the partition coefficient, and $C_{A,O}$ is the concentration of the desired protein in the feed solution.

Typical flux values can be calculated from Eqs. (5) and (6) by plugging in typical values for each term. A typical value for preferential transport flux is 0.8 mg/cm^2/min (F_A = 0.1, $u_{s,a}$ = 4 cm/min, $C_{A,O}$ = 4 mg/mL), whereas a typical value for facilitated diffusion flux is 0.07 mg/cm^2/min ($D_A = 10^{-6}$ cm^2/s, L = 100 μm, P = 3, $C_{A,O}$ = 4 mg/mL). The protein fluxes obtained by facilitated diffusion experimentally are at least two orders of magnitude lower than this calculated value (typically 10^{-4} mg/cm^2 per minute) (Armstrong and Li, 1988; Tsai et al., 1995). Therefore, in typical situations, transport by preferential transport is several orders of magnitude faster than transport by facilitated diffusion.

Although transport of proteins is faster by preferential transport than by facilitated diffusion, the selectivity obtained by preferential transport will probably be lower than that obtained by facilitated diffusion; the selectivity obtained in our system is on the order of 5, while selectivity obtained in facilitated diffusion systems can be as high as 10 (Armstrong and Li, 1988). Selectivity in preferential transport can be increased by using low dispersion media (Fig. 8) and lower $V_{s,a}/V_{s,d}$ values (Agrawal and Burns, 1996).

Transport up a Concentration Gradient

An interesting property of preferential transport, similar to coupled facilitated diffusion (Cussler, 1988), is that it can be used to transport solutes up a concentration gradient (i.e., from a solution of low concentration to a solution of high concentration). To study this property, experiments were conducted in which protein was present at 1 mg/mL in the feed side and 2 mg/mL in the desorbing side. For myoglobin, which is the nonadsorbing protein, the value of the fractional flux was −0.03, indicating that transport of the nonadsorbing protein is, as expected, from a solution of higher concentration to a solution of lower concentration. On the other hand, for lysozyme, which is the adsorbing protein, the value of the fractional flux was +0.04, indicating that transport of the adsorbing protein is from a solution of lower concentration to a solution of higher concentration.

Transport of an adsorbing protein up a concentration gradient can be explained very simply for the hypothetical case of no dispersion and high desorbent concentration. For such a hypothetical case, protein present in the desorbent stream remains within the desorbing phase. For example, in Figure 4a, if protein were present in the desorbent stream, it would remain in the shaded area representing the desorbing phase and would be transported back to the desorbing side during the adsorption stroke.

In real cases, the basic mechanism is the same as explained above; however, protein fractional flux will decrease as protein concentration in the desorbing stream increases. Figure 9 shows that lysozyme fractional flux decreases slightly with increasing lysozyme concentration in the desorbing solution. We could only conduct experiments with a maximum of 2 mg/mL of lysozyme in the desorbent solution because of the sensitivity of our measurements. Specifically, our fractional flux measurements are based on the difference of protein concentration in product (effluent

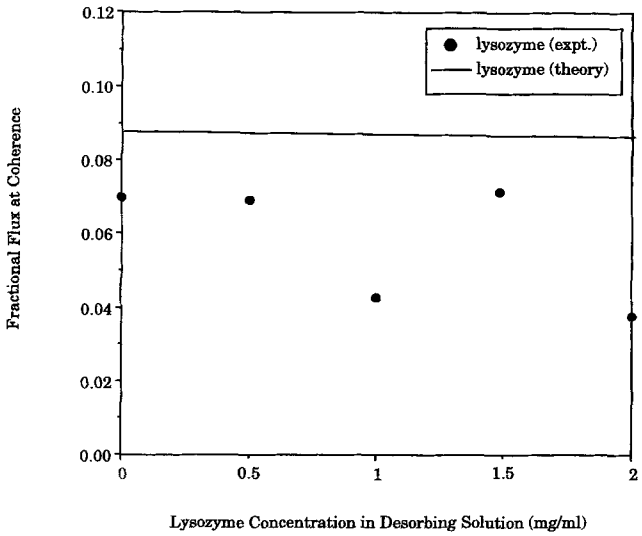

Figure 9. Transport of lysozyme up a concentration gradient. Lysozyme is transported from a feed solution of 1 mg/mL to solutions of varying lysozyme concentration in the desorbing solution. There is a net transport of lysozyme to concentrations higher than 1 mg/mL, i.e., up a concentration gradient. The experimental data showed large scatter due to the sensitivity of our measurements.

from feed pump, Fig. 2) and desorbing solution and this difference is small when the protein concentration in the desorbing solution is high. Theoretical simulations show that protein can be preferentially transported up much higher concentration gradients. For example, theoretical simulations indicate that, for our experimental system, a feed solution of 2 mg/mL has a lysozyme flux of 0.126 when the desorbing solution is solute-free and a flux of 0.122 when the desorbing solution has a concentration of 10 mg/mL of lysozyme.

Selective Extraction from a Complex Mixture

By using preferential transport, an adsorbing protein can be selectively removed from a complex mixture because the adsorbing protein is transported across the membrane while nonadsorbing solutes and cells (larger than membrane pore size) are rejected by the membrane. To investigate the selective removal of a protein from a feed containing suspended particles we used the same experimental set-up as described earlier. Yeast (0.1% dry weight) was added to the feed containing lysozyme and myoglobin, and a 0.22-μm filter was placed in series with the ion-exchange membrane cartridge. Thus, the oscillatory flow took place about the filter as well as the adsorptive membrane. By putting the filter in series we ensured that the adsorptive membrane remained unclogged; however, the coherent lysozyme flux with the in-series filter should be lower than that obtained without the filter.

Figure 10 shows experimental results in which lysozyme is selectively removed from a mixture of lysozyme, myoglobin, and yeast cells. As expected, the coherent lysozyme flux with the filter (Fig. 10, $F \approx 0.06$) is slightly lower than that without the filter (Fig. 3, $F \approx 0.08$). In separate experiments (data not shown), we found that there is negligible increase in pressure drop as long as the oscillatory flow is maintained, implying that yeast accumulation on the membrane surface is negligible.

The results of Figure 10 illustrate the basic concept of integrating filtration, adsorption, and desorption into a single step by using preferential transport through adsorptive membranes. This concept of integrated separation combines the concept of preferential transport discussed in this article and the concept of the alleviation of cake formation using oscillatory flow discussed by other researchers (Belfort, 1989; Redkar and Davis, 1995). Such an integrated separation can be extended to other protein–membrane systems and process configurations. For example, in some situations, selectivity could be enhanced using an affinity membrane. Preferential transport could also be used to selectively extract a solute from a fermentation broth; the broth could be oscillated through an adsorptive membrane and then returned to the fermentor.

CONCLUSIONS

Selective extraction can be obtained using preferential transport through adsorptive membranes. Such a selective extraction is obtained only after a state of coherence is attained after which time an adsorbing solute can be continuously removed from a mixture. Separations during preferential transport can be optimized by using an optimum desorbent concentration, feed concentration, and interstitial velocity. The experimentally observed effect of these process variables agreed with theoretical predictions.

In typical situations, transport by preferential transport is several orders of magnitude higher than transport by facilitated diffusion. Also, similar to facilitated diffusion, preferential transport can be used to transport a solute up a concentration gradient and to selectively extract a solute

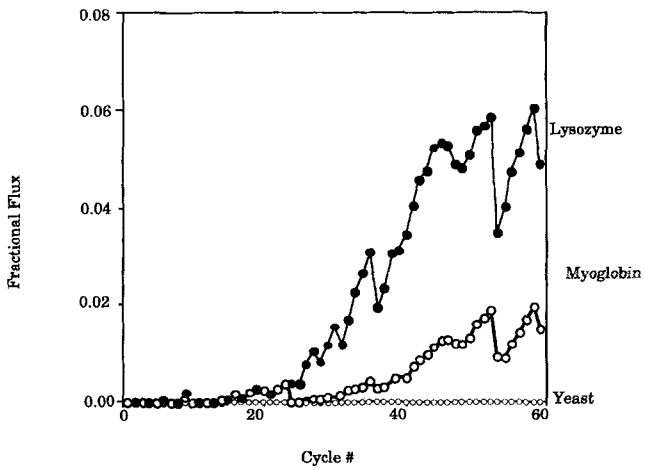

Figure 10. Selective extraction from a complex mixture. Using preferential transport, lysozyme was continuously removed from a mixture of lysozyme, myoglobin, and yeast. The total time for the run was 1 h.

from a feed that contains suspended solids. Therefore, preferential transport has the potential to lead to a continuous integrated separation process that can combine the steps of filtration, adsorption, and desorption.

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NOMENCLATURE

A	an adsorbing protein
B	a nonadsorbing solute
C	concentration in solution (mol/L)
d_p	diameter (cm)
D	diffusion coefficient (cm^2/s)
E	dispersion coefficient (cm^2/s)
F	dimensionless flux (amount transported/amount fed per oscillation)
J	flux (mg/min)
K_{eq}	equilibrium constant (M^{-1})
l	distance in the axial direction (cm)
L	membrane thickness (cm)
n	cycle number
P	partition coefficient
Pe	Peclet number
q	flow rate (mL/min)
Q	concentration on the surface (mg/mL)
t	time (s)
u	velocity (cm/s)
V	volume (mL)

Subscripts

O	feed
a	adsorbing
d	desorbing
i	a nonadsorbing solute
m	membrane
max	maximum
s	stroke

Greek letters

α	affinity parameter of the Langmuir isotherm (dimensionless)
δ	feed concentration/maximum capacity

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