Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Affinity Separations in Magnetically Stabilized Fluidized Beds: Synthesis and Performance of Packing Materials

C. H. Lochmuller[a]; Larry S. Wigman[a]
[a] P. M. GROSS CHEMICAL LABORATORY DUKE UNIVERSITY, DURHAM, NORTH CAROLINA

To cite this Article Lochmuller, C. H. and Wigman, Larry S.(1987) 'Affinity Separations in Magnetically Stabilized Fluidized Beds: Synthesis and Performance of Packing Materials', Separation Science and Technology, 22: 11, 2111 — 2125
To link to this Article: DOI: 10.1080/01496398708068603
URL: http://dx.doi.org/10.1080/01496398708068603

SEPARATION SCIENCE AND TECHNOLOGY, 22(11), pp. 2111-2125, 1987

Affinity Separations in Magnetically Stabilized Fluidized Beds: Synthesis and Performance of Packing Materials

C. H. LOCHMÜLLER and LARRY S. WIGMAN

P. M. GROSS CHEMICAL LABORATORY
DUKE UNIVERSITY
DURHAM, NORTH CAROLINA 27706

Abstract

A magnetically stabilized fluidized-bed separator designed to test the use of pellicular, ferromagnetic affinity chromatography packing materials has been developed. A wire wound solenoid was used to produce the magnetic field. The ferromagnetic packing material is comprised of a magnetite-containing, polyurethane gel coated onto polystyrene beads. The gel contains free carboxyl groups. These were carbodiimide-coupled to soy trypsin inhibitor and the material used for trypsin purification. Narrow-band affinity chromatography was carried out in packed-bed, fluidized-bed, and magnetically stabilized, fluidized-bed separators. Pressure drop, capacity, dilution, and peak asymmetry were evaluated for each type of separator. The three types provide comparable efficiency but the fluidized separators exhibit a much lower pressure drop. As might be expected, fluidized-bed separators perform well for affinity chromatography (large k') but poorly for size exclusion chromatography.

INTRODUCTION

With the recent developments in recombinant DNA technology, large-scale fermentation, and monoclonal antibody formation, biochemicals once available only in microgram quantities are now readily available for research purposes. As research and development with these biochemicals progress, so does the need for efficient large-scale purification methods to meet the increasing demand for new biochemical and biomedical products. Fluidized-bed separators are routinely operated on a large scale and can be operated as a continuous process if desired (*1*).

Affinity chromatography offers a solute-specific alternative to filtration techniques and general chromatographic methods such as ion exchange and size exclusion (*2*). Affinity chromatography relies on specific biological interactions much like those modeled for enzyme–substrate interaction and enzyme inhibition. A "lock and key" model for trypsin–soy trypsin inhibitor is shown in Fig. 1.

In affinity chromatography, separations are achieved under mild aqueous conditions that preserve bioactivity and minimize denaturation, hence affinity chromatography can produce biochemicals in a highly active form. This high activity/mg makes affinity purified biochemicals useful as primary standards in assay procedures and kinetic studies. The capacity of an affinity matrix is based on its biologically-available surface area. Since relatively high surface area is desirable, two types of materials are useful for affinity matrices: porous and pellicular particles. Porous particles achieve high surface area because of the large internal surface of the pores. Two types of porous particles often used as affinity matrices are natural polysaccharide gels and synthetic macroreticular polymers. Natural polysaccharide gels are subject to microbial attack and are too fragile for high-pressure operation, thus limiting or eliminating their use in large-scale separators. However, these types of materials are commonly used on the laboratory scale (*3–5*). Synthetic macroreticular polymers such as the glycol methacrylates are suitable for high-pressure operation and are not biodegradable (*6–8*), but this type of material is expensive to produce and cost is a major factor in engineering scale-up.

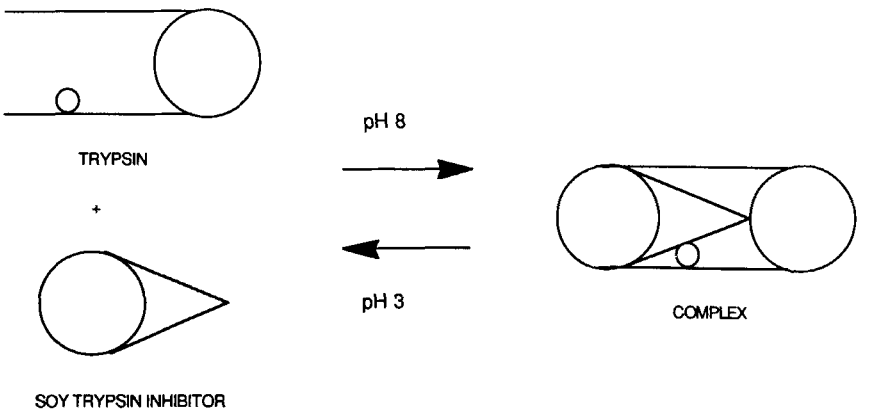


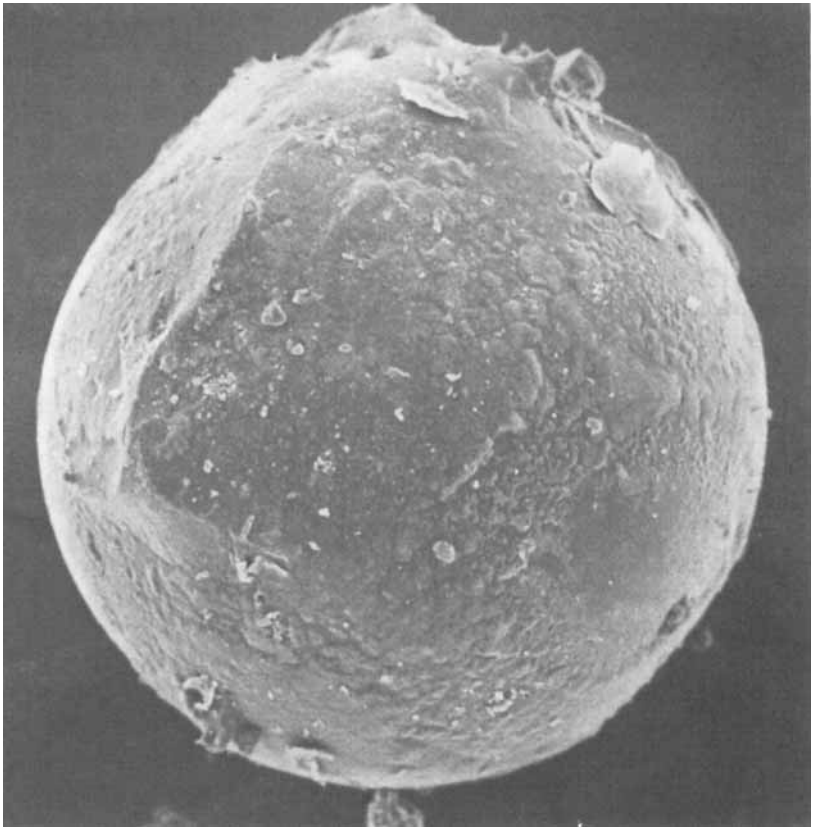
FIG. 1. A "lock and key" model of trypsin–STI (soy trypsin inhibitor).

The surface area of pellicular supports is confined to the outermost surface and, hence, pellicular particles for large-scale separations must be designed to be physically smaller and with higher coupling group density than porous particles to maintain useful capacity. Small particle size leads to higher operating pressure drops in packed beds, which limits throughput, thus making pellicular supports less attractive.

In the work reported here, a fluidized-bed separator has been examined in a test of maintaining capacity/column volume with fine pellicular particles while minimizing pressure drop. A fluidized-bed separator uses the force of a fluid flowing upward to balance the force of gravity on the packing material. Such systems are much like packed beds. The flow must be properly distributed, and the column leveled perpendicular to the horizon or the mobile phase will channel through the bed, resulting in poor performance. Three states of operation are encountered and dependent on flow rate (*9*): 1) The *dense pack* state when the force due to flow is less than the bed weight. 2) The *quiescent state* when the force due to flow is greater than the bed weight. 3) The *boiling bed* state when the force due to flow is much greater than the bed weight. The quiescent state is best suited for use in separators because of the low pressure drop and minimum solute dilution. In this state, narrow bands of solute can be eluted much like those from a packed bed because most of the plate height efficiency is retained. Fluidized beds operated in the boiling bed state perform more like batch extractors or stirred-bed reactors. This is because axial mixing is so extensive that the plate number is essentially one.

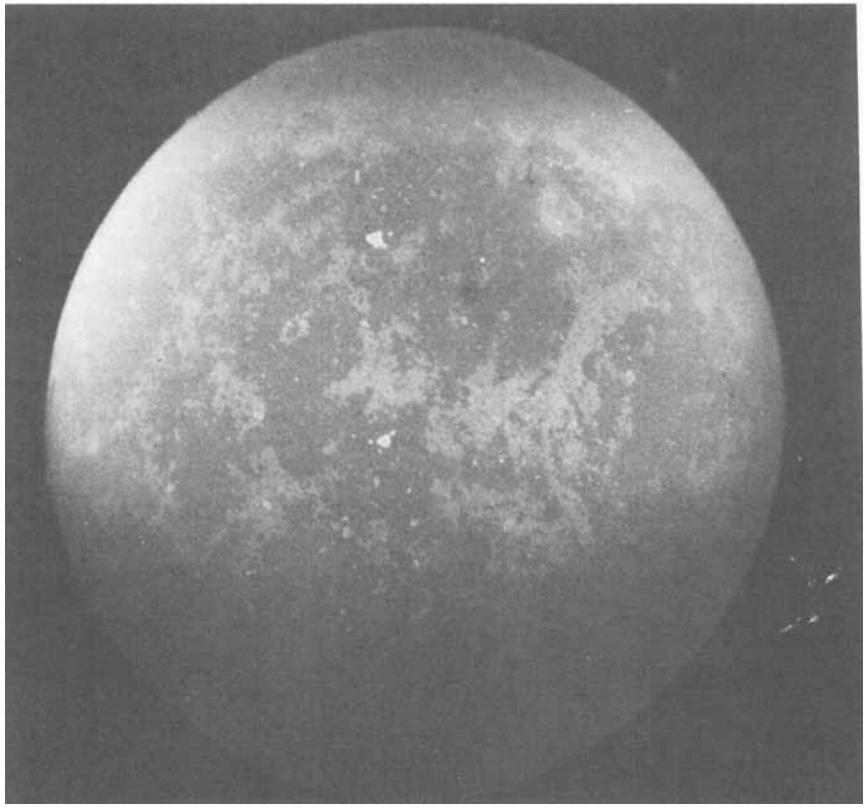
The experiments reported here differ significantly from typical fluidized-bed experiments (*1*, *10*, *11*) in that peaks were eluted in narrow bands rather than batch basis desorption. The performance characteristics of a properly designed fluidized-bed were evaluated by studying solute retention, peak broadening, and peak asymmetry in different flow domains and for different capacity factors. Two extreme cases were examined: $k' \approx 0$, using size exclusion chromatography, and $k' \approx \infty$, using affinity chromatography. These two cases define the effect bed expansion, relative to k', has on separation.

A magnetically stabilized fluidized-bed separator uses a ferromagnetic packing material and a magnetic field. The magnetic field extends the quiescent state of operation to higher flow rates, decreases bed expansion, increases the column capacity, and minimizes channeling by inhibiting axial motion of the packing material. Although magnetic stabilization has been used for gas fluidized systems (*12–14*), it has not been used for liquid fluidized systems. The ferromagnetic pellicular material developed for this experiment was designed to be inexpensive and useful under



(a)

FIG. 2. Scanning electron micrograph of (a) coated matrix (80×), (b) uncoated matrix (120×). Monolayer of gold vapor deposited on surface.



(b)

medium pressures (to 500 psi). The cost is minimized because an inert core is used and the active chemicals are limited to a thin coating on the particle surface. This material is the first reported use of Hypol polyurethanes for affinity chromatography and the first pellicular affinity matrix designed for column chromatography. The material is visibly coated as shown by electron photomicroscopy in Fig. 2.

EXPERIMENTAL

Chromatography

The chromatographic system was controlled by a Spectra-Physics Model 3500B gradient pumping system equipped with a Rheodyne 7010 sampling valve (0.25 mL) and a Gow-Mac Model 80-250 U.V. detector. The affinity matrix and size exclusion resin were contained in a 9 mm by 250 mm glass column with adjustable plunger. Some measurements were done using a 15 mm by 500 cm glass preparative column. For affinity chromatography a procedure similar to that of Hearn et al. (*15*) was employed. A 500-m*M* NaCl–50-m*M* tris[(hydroxymethyl)aminoethane] (TRIS), pH 8.0 mobile phase was used to bind trypsin and elute impurities, then a 3.0-m*M* HCl–500-m*M* NaCl mobile phase was used to elute purified trypsin. A sample of 5 mg/mL trypsin was chromatographed on the 15-mm column.

Reagents

Amberlite XAD-2 resin was purchased from Rohm and Hass. The 1-ethyl-3,(3-dimethyl aminopropyl),carbodiimide · HCl (EDC), purified casein, trypsin (type III), soy trypsin inhibitor (type IIs) (STI), and *p*-aminobenzoic acid (PABA) were purchased from Sigma. Hypol hydrogel was obtained from W. R. Grace. Magnetic Black S magnetite pigment from BASF Wyandotte and 37–75 μm Styragel (type 200A) were purchased from Waters Associates. All other chemicals were reagent grade.

Enzyme Assay

The enzymatic activity of collected fractions was measured by casein digestion based on a procedure developed by Laskowski (*16*). A 5-mg

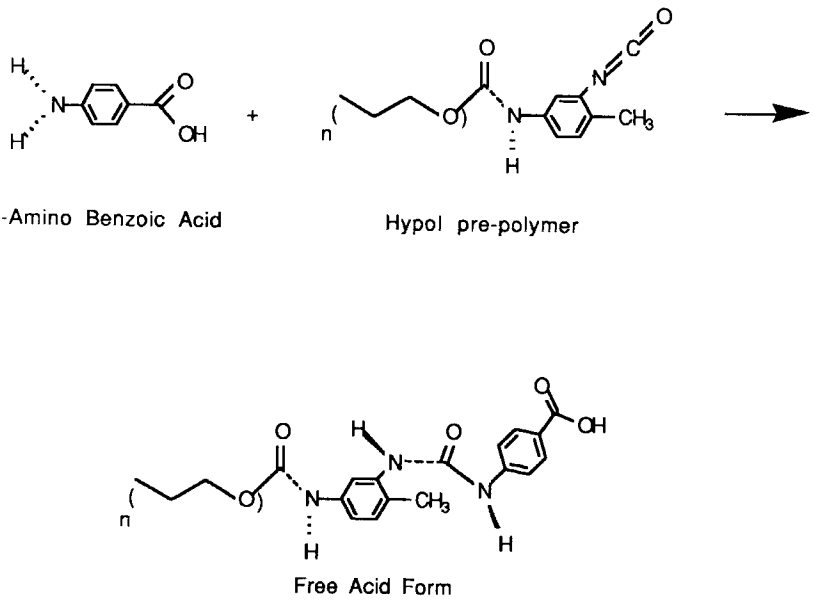
trypsin sample of known activity served as the reference for unit activity.

Magnetic Field

The magnetic field was controlled by adjusting the current passing through a 2.5-in. long solenoid of 1156 turns of #34 AWG copper wire. The current was controlled by a Lambda Model LP 412 power supply. Unless otherwise noted, a 6.8 gauss field was used.

Affinity Matrix Preparation

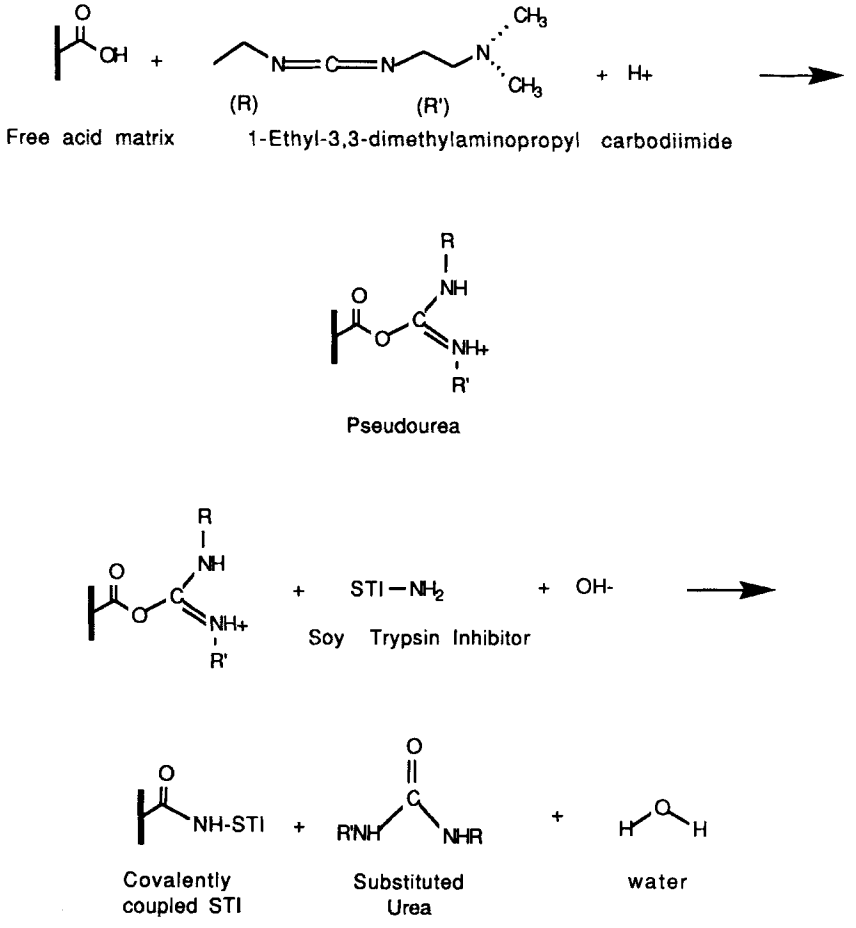
A solution containing 0.08 g PABA and 6 mL methyl ethyl ketone was prepared, and 10 mL of Hypol hydrogel added and reacted for 5 min. Then 4 mL toluene, 2 g Magnetic Black, and 10 mL Amberlite XAD-2 were added and stirred until well mixed. The coated XAD-2 was sieved out, transferred to 100 mL water, and mechanically stirred for 20 min. The water was decanted, the product washed three times with 100 mL water, then with ethanol, and finally dialyzed overnight in water. The synthesis is summarized in Scheme 1.



SCHEME 1. Acid group derivitization of Hypol Hydrogel prepolymer using PABA.

Coupling to Soy Trypsin Inhibitor

The dialyzed pellicular matrix was filtered, 40 mL water and 100 mg soy trypsin inhibitor were added, and magnetically stirred. The pH was adjusted to 4.5 with concentrated HCl, 690 mg EDC added, and the reaction allowed to proceed at room temperature for 2 h. The pH was raised to 8.0 with strong NaOH solution and reacted at 4°C for 2 days. The final matrix was washed with pH 8 and pH 3 solutions before use. The coupling reaction is summarized in Scheme 2.



SCHEME 2. Carbodiimide coupling of STI to the affinity matrix.

RESULTS AND DISCUSSION

A fluidized bed can be scaled up and operated with a low pressure drop but, because the bed expands upon fluidization, the separator's performance decreases with increasing flow rate, relative to an equivalent packed-bed separator. This loss of performance with increasing flow is a major problem, limiting the widespread use of fluidized-bed separators. A logical explanation of the performance loss is that k' decreases and some peak broadening is encountered because, as the volume of the mobile phase increases at constant volume of stationary phase, an effect occurs which is akin to using much larger particles in a packed bed. The exact effect of bed expansion was investigated as the first experiments performed. In a fluidized-bed separator the bed expands as a function of flow rate in a nearly linear fashion throughout the quiescent state, as shown in Fig. 3.

Maximizing fluidized-bed separator performance by minimizing bed expansion and extending the quiescent state of operation while maintaining a low pressure drop are the primary goals of this research. Although proper column leveling and flow distribution are essential to achieving these goals, a magnetic field and ferromagnetic particles were also found to be useful. The magnetic field adds stability and decreases

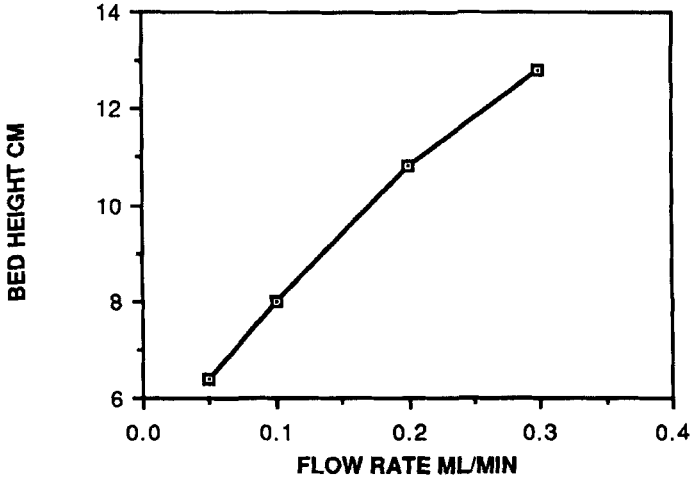


FIG. 3. Effect of flow rate on bed height in a 9-mm diameter column packed with Styragel (37–75 μm) and fluidized with THF.

bed expansion as a function of field strength. The nature of stabilization is not linear with field strength and is flow-rate dependent. This flow-rate dependence is shown by Fig. 4. The pressure drop across a fluidized bed is about the same as that of a magnetically stabilized fluidized bed even though the bed expansion is significantly less. The pressure drop is also considerably less than that of a packed bed, as shown in Table 1.

Capacity factor, more than any other single factor, determines whether a chromatographic system is suitable for fluidized-bed separators. Systems with small capacity factors in packed beds are not suited for fluidized separation conditions because of the increased mobile phase/stationary phase volume ratio caused by bed expansion; hence, systems such as size-exclusion chromatography ($k' = 0$ to 1) and "analytical" reversed-phase chromatography ($k' = 1$ to 10) are not ideal candidates for transfer to this technology. Systems with large k' values such as those found in ion-exchange chromatography, activated-carbon-adsorption chromatography, and affinity chromatography are easily adapted. Ion exchange and carbon adsorption are already used on a large scale (but not in narrow-band elution) (*1*, *11*).

An isocratic, size-exclusion/reversed-phase experiment was designed to test the performance of fluidized-bed separators for systems with small

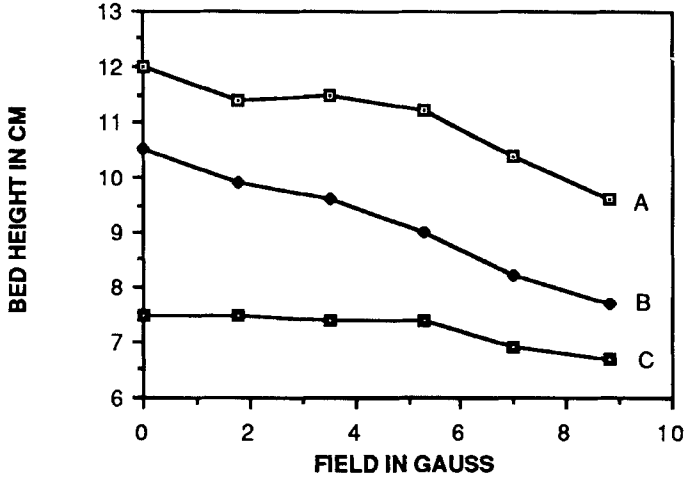


FIG. 4. Effect of magnetic field strength on bed height in a 15-mm diameter column packed with 13 mL of ferromagnetic affinity matrix and fluidized with water at (A) 10, (B) 5, and (C) 3 mL/min.

TABLE 1
Pressure Drop Performance at 2.8 mL/min

	Packed bed	Fluidized bed	Magnetically stabilized fluidized bed
Pressure drop, psi	170	130	130
Bed height, cm	7.0	11.7	10.4

capacity factors. Water was gradually added to a THF mobile phase as a way to increase the capacity factor by adsorption. The two solutes used in this experiment were baseline resolved using a packed-bed separator, but upon fluidization the resolution was completely lost. The loss of resolution can be attributed to a loss of capacity, increased peak asymmetry, and band broadening. As the capacity factor is increased by increasing mobile phase water content, less column capacity is lost upon fluidization. This effect is illustrated in Fig. 5.

An unretained solute, $k' = 0$, was used as a probe to study asymmetry and dilution and to minimize the complications of solute–sorbent mass transfer effects on peak shape. Peak dilution and asymmetry can be minimized by minimizing bed expansion. This is accomplished either by operating in the quiescent bed state at low flow rates or by magnetic-field stabilization. Peak dilution, measured by σ, has a linear dependence on

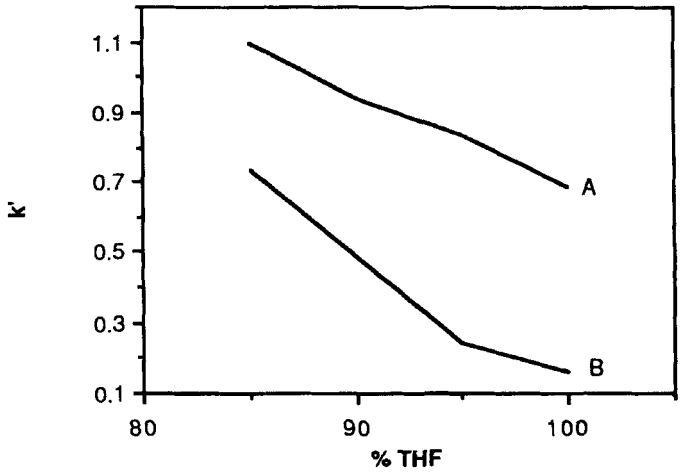


FIG. 5. Effect of mobile phase composition on capacity factor in a 9-mm column packed with 6 mL Styragel (37–75 μm).

TABLE 2
Dilution and Asymmetry Using an Unretained Solute (saturated $NaNO_3$)

	Flow rate (mL/min)	Bed height (cm)	σ (mL)	A/B 10%
Packed bed	2.4	5.4	2.9	0.52
Fluidized bed	4.0	13.6	9.2	0.15
	3.1	12.8	5.0	0.50
	2.2	11.4	4.7	0.48
	1.3	10.7	3.6	0.48
	0.44	9.8	3.4	0.36
Magnetically stabilized fluidized bed	4.0	11.7	4.2	0.27
	3.1	10.0	3.8	0.52
	2.2	9.3	3.3	0.48
	1.3	8.5	2.6	0.46
	0.44	7.8	2.8	0.60

bed expansion throughout the quiescent state. Dilution quickly increases upon entering the boiling bed state. This effect is shown in Table 2, where the highest flow rate reported is one in which the fluidized bed separator has just entered the boiling bed state. Magnetic-field stabilization decreases dilution and extends the quiescent state to higher flow rates. The linear relation between bed expansion and dilution is slightly perturbed at the lowest flow rate, shown in Table 2, because the magnetically stabilized fluidized bed is near the dense packed state. Peak asymmetry of a properly operated fluidized bed is much the same as for a packed bed except at high flow rates where tailing occurs. The tailing effect can be reduced by magnetic-field stabilization but is still pronounced. The asymmetry and dilution study is summarized in Table 2.

A ferromagnetic, pellicular affinity matrix covalently coupled to STI was prepared to test the performance of the fluidized-bed separator in a high capacity factor case. The association constant for trypsin–STI has been reported at 10^{11} (*17*). Under such high binding constant conditions, bed expansion causes little difference in peak retention, dilution, or asymmetry. The similarity between packed-bed separators, fluidized-bed separators, and magnetically stabilized fluidized-bed separators is shown by the affinity chromatography tracing in Fig. 6.

The small differences in capacity, band broadening, and peak asymmetry between a packed-bed separator and a fluidized-bed separator can further be reduced by magnetic-field stabilization. This effect is summarized in Table 3.

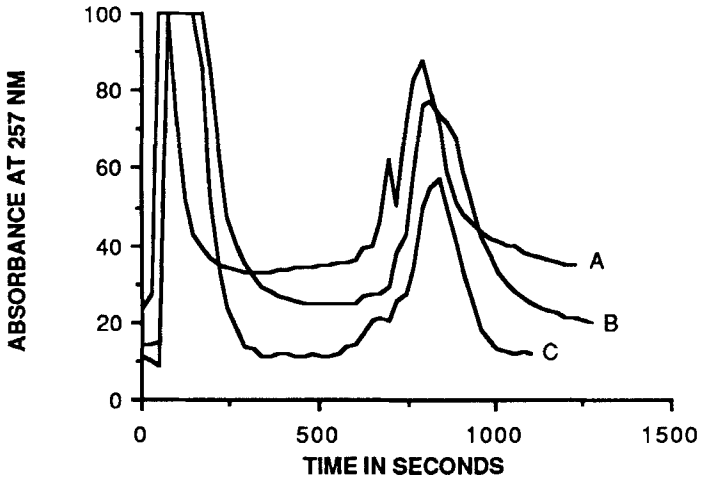


FIG. 6. Chromatographic purification of trypsin on 4 mL of a ferromagnetic affinity matrix. Conditions: (A) packed bed, (B) fluidized bed, (C) magnetically stabilized fluidized bed, flow rate 3 mL/min, 9 mm diameter column, mobile phase step gradient from pH 8 to 3 m*M* HCl after 10 min.

In order to confirm the identity of the peaks in the affinity chromatography tracings and to determine the relative protease activities, a preparative scale column was packed with the pellicular affinity matrix. A 20-mg sample of trypsin was chromatographed and fractions collected. The fractions were assayed for protease activity to confirm the presence of trypsin. Both the unretained and the retained peaks were shown to contain some trypsin, but the activity/mg of the purified peak had

TABLE 3
Capacity Study (using trypsin retention)

	Packed bed	Fluidized bed	Magnetically stabilized fluidized bed
Unretained, μg	0.991	0.112	0.105
Retained, μg	0.414	0.045	0.0412
%	29.4	28.6	28.7
Relative %	100	97.3	97.6

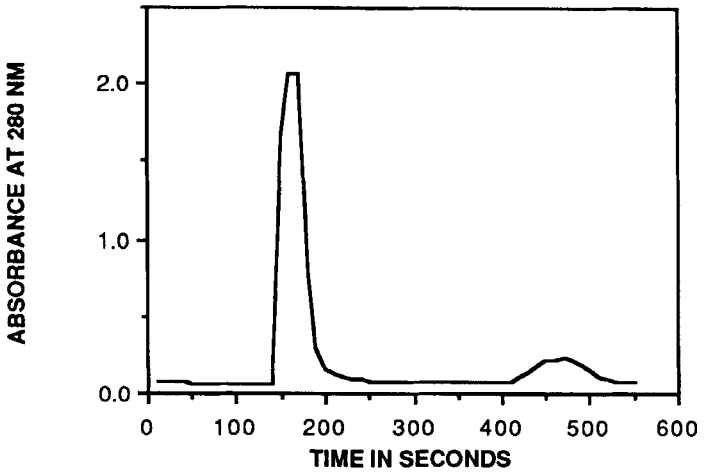


FIG. 7. Preparative chromatographic purification of trypsin on 10 mL of a pellicular affinity matrix. Conditions: flow rate 10 mL/min, 15 mm column diameter, mobile phase step gradient from pH 8 buffer to 3 m*M* HCl after 5 min.

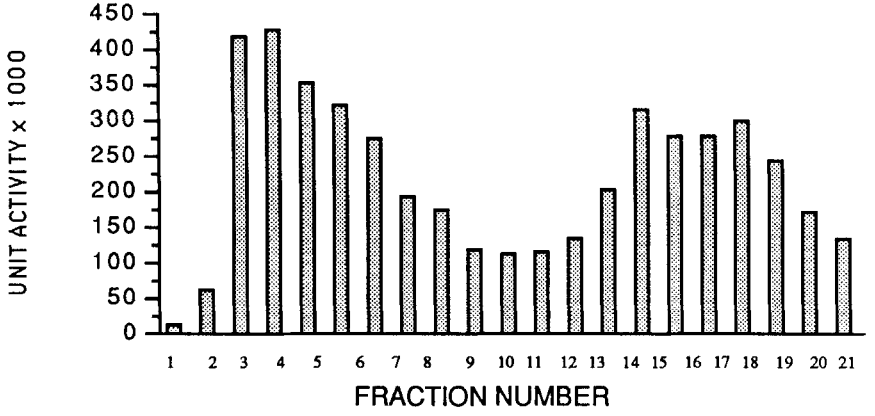


FIG. 8. Relative protease activity for fractions eluted from the preparative chromatographic purification of trypsin.

increased by 400%. The preparative column tracing and assay results are shown in Figs. 7 and 8.

To summarize, a fluidized-bed separator, with proper column leveling and flow distribution, operated in the quiescent state offers a way to use small pellicular packing materials with low pressure drop and still

maintain a high column capacity. Systems with high k', such as affinity chromatography, can readily be adapted to this new technique, but systems with low k', such as size exclusion, and not suitable because of the large performance loss. The performance difference between a fluidized and packed affinity chromatography separator is small and can further be reduced by magnetic stabilization.

REFERENCES

1. T. Kolini and M. Zumer, *Can. J. Chem. Eng., 57*(2), 183 (1979).
2. J. M. Curling, D. Low, and J. M. Cooney, *Am. Biotech. Lab., 2*(4), 32 (1984).
3. P. Cuatrecasas, M. Wilchek, and C. B. Anfinsen, *Biochemistry, 61,* 636 (1968).
4. G. S. Bethell, J. S. Ayers, W. S. Hancock, and M. T. Hearn, *J. Biol. Chem., 254*(8), 2572 (1979).
5. G. Redeuilh, C. Secco, and E. E. Baulieu, *Ibid., 260*(7), 3996 (1985).
6. J. Turkova, O. Hubalkova, M. Kirvakova, and J. Coupek, *Biochem. Biophys. Acta, 322,* 1 (1973).
7. H. Sasaki, R. Asada, Y. Kato, and T. Hashimoto, *Toyo Soda Kogyo, 1,* 49 (1983).
8. K. Shimura, M. Kazama, and K. Kasai, *J. Chromatogr., 292,* 369 (1984).
9. S. S. Zabrodsky, *Hydrodynamics and Heat Transfer in Fluidized Beds,* MIT Press, Cambridge, Massachusetts, 1966.
10. A. Buijs and J. A. Wesselingh, *J. Chromatogr., 201,* 319 (1980).
11. T. M. Keinath, *AIChE Symp. Ser., 73*(166), 1 (1977).
12. R. E. Rosensweig, J. H. Seigell, W. K. Lee, and T. Mikus, *Ibid., 74*(176), 8 (1978).
13. R. E. Rosensweig, *Science, 204*(4), 57 (1979).
14. A. Lucas, J. Casal, and L. Duigjaner, *Proc. Int. Conf. Fluid, 4th,* p. 129 (1983).
15. M. T. Hearn, G. S. Bethell, J. S. Ayers, and W. S. Hancock, *J. Chromatogr., 185,* 463 (1979).
16. M. Laskowski, *Methods Enzymol., 2,* 33 (1955).
17. H. Kitano, K. Nakamura, and N. Ise, *J. Appl. Biochem., 4,* 487 (1982).

Received by editor October 29, 1986