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

# Enantiomeric Separation of Tryptophan by Ultrafiltration Using the BSA Solution System

S. Poncet[a]; J. Randon[a]; J. L. Rocca[a]
[a] LABORATOIRE DES SCIENCES ANALYTIQUES (CNRS UMR 5619), UNIVERSITÉ C. BERNARD, VILLEURBANNE CEDEX, FRANCE

**To cite this Article** Poncet, S. , Randon, J. and Rocca, J. L.(1997) 'Enantiomeric Separation of Tryptophan by Ultrafiltration Using the BSA Solution System', Separation Science and Technology, 32: 12, 2029 — 2038
**To link to this Article: DOI:** 10.1080/01496399708000752
**URL:** http://dx.doi.org/10.1080/01496399708000752

SEPARATION SCIENCE AND TECHNOLOGY, **32**(12), pp. 2029–2038, 1997

# Enantiomeric Separation of Tryptophan by Ultrafiltration Using the BSA Solution System

S. PONCET, J. RANDON,* and J. L. ROCCA
LABORATOIRE DES SCIENCES ANALYTIQUES (CNRS UMR 5619)
UNIVERSITÉ C. BERNARD, LYON 1, BAT. 308
43 BOULEVARD DU 11 NOVEMBRE 1918, 69622 VILLEURBANNE CEDEX, FRANCE



## ABSTRACT

The optical resolution of racemic tryptophan was performed by ultrafiltration using the BSA solution system. The pH of the feed solution had a strong influence on the complexation constants between BSA and tryptophan, especially for L-tryptophan. The complexation constant for L-tryptophan reached a maximum value at pH 9 ($K_L$ = 110,000), varying by 2 orders of magnitude in the range from pH 6 ($K_L$ = 1000) to pH 11 ($K_L$ = 21,000). Smaller variations of the complexation constant of D-tryptophan were observed. Based on these data, the recovery and the purity of the permeate were optimized by a proper control of the physicochemical parameters of the feed solution (essentially pH and initial concentrations). In one stage, 91% purity with a 89% recovery of D-tryptophan has been easily obtained with a high permeation rate ($6.3 \times 10^{-4}$ mol·m$^{-2}$·s$^{-1}$ at 1.5 bar).

## INTRODUCTION

The separation of optical isomers from the racemate is increasingly needed in biology, pharmacology, and the food industry. Separations of racemic mixtures are mainly performed by chromatography and diastereoisomeric salt crystallization, even if large-scale separations using these conventional methods are limited by many technical problems. However, scale-up could also be achieved using a membrane separation process

* To whom correspondence should be addressed. Telephone: (33) 04-72-43-10-79. FAX: (33) 04-72-43-10-78. E-mail: randon@univ-lyon1.fr

which is generally an energy-saving technique. Many separations have been reported (1–11), and high selectivity has been obtained with both liquid and solid membranes, but because the concentration gradient is the only driving force in most of these separations, the solute flux is very small. An alternative route giving high flux and high selectivity is described in this paper for DL-tryptophan resolution.

Several attempts have been made to separate racemic tryptophan (12–18) using solid membranes or a solution system (Table 1).

Solid membranes have been generally used in a diffusion cell (without transmembrane pressure). In such a situation the purity is as high as 0.93 (except for a complete resolution reported by Maruyama), but the membrane permeability is very low. To increase solute transfer through the membrane, a transmembrane pressure has been applied, increasing the volume flux and therefore the production rate. However, this modification has always led to an important loss of selectivity, and a compromise between the production rate and the purity had to be found.

In the solution system (2, 12) the addition of a chiral compound to a racemic mixture allows the formation of diastereoisomers. The choice of



TABLE 1
Reported Separations of DL-Tryptophan by a Membrane Process

| Ref. | System | Feed solution | Purity | Permeability | Comments |
|---|---|---|---|---|---|
| 12 | BSA solution | 0.049 mM<br>0.6 mM BSA | D 0.87 | Unknown | pH 7 |
| 13 | Membrane: Poly[p-(L-(−)-menthoxycarbonyl)phenyl acetylene] | 0.5 wt% | D 0.70 | $5 \times 10^{-16}$ m$^2$·s$^{-1}$ | $\Delta P$ = 0 bar |
| 14 | Membrane: Plasma-polymerized *l*-menthol onto cellulose acetate | $10^{-3}$ mol·L$^{-1}$ | D 0.88 | $6.2 \times 10^{-13}$ mol·m·m$^{-2}$·s$^{-1}$ | $\Delta P$ = 0 bar |
| | | $10^{-3}$ mol·L$^{-1}$ | D 0.71 | $1.5 \times 10^{-12}$ mol·m·m$^{-2}$·s$^{-1}$ | $\Delta P$ = 15 bar |
| 15 | Membrane: Poly {γ-[3-(pentamethyldisiloxanyl) propyl]-L-glutamate} | 0.1 wt% | L 0.58 | $1.3 \times 10^{-12}$ mol·m·m$^{-2}$·s$^{-1}$ | $\Delta P$ = 10 bar |
| 16 | Membrane: Poly(L-glutamate) having (*n*-nonylphenoxy)oligo (oxyethylene) | $2 \times 10^{-3}$ mol·L$^{-1}$ | D 0.60 | $5.5 \times 10^{-15}$ mol·m·m$^{-2}$·s$^{-1}$ | $\Delta P$ = 0 bar<br>$T$ = 40 |
| | | $2 \times 10^{-3}$ mol·L$^{-1}$ | D 1.00 | $1 \times 10^{-16}$ mol·m·m$^{-2}$·s$^{-1}$ | $\Delta P$ = 0 bar<br>$T$ = 34 |
| 17 | Membrane: (+)-Poly{1-[dimethyl(10-pinanyl)solyl]-1-propyne} | 0.1 wt% | L 0.93 | $1.9 \times 10^{-13}$ mol·m·m$^{-2}$·s$^{-1}$ | $\Delta P$ = 0 bar |
| | | 0.1 wt% | L 0.57 | $6.2 \times 10^{-12}$ mol·m·m$^{-2}$·s$^{-1}$ | $\Delta P$ = 20 bar |

the chiral selector has to be made by following two criteria: the complexation criterion (one enantiomer of the racemic mixture more easily forms a complex as compared to the other enantiomer) and the size criterion (the size of the chiral selector, and so the size of the diastereoisomer complex, are big enough to be retained by the membrane). So the free molecules (mainly those of the less complexed enantiomer) went through the membrane. In the following stage the other enantiomer was collected by modification of the feed solution parameters. The only attempt made for tryptophan resolution using such a technique (12) has been performed using bovine serum albumin (BSA) at physiological pH, but no data about permeability and yield were reported.

Chiral columns are widely used in chromatography. BSA protein has been grafted onto such solid supports as a silica or polymer matrix and packed in chromatographic columns (see, for example, Refs. 18–20). Even if the grafting can slightly modify the protein conformation, most of the data can be transposed to a solution system with free BSA molecules. Protein columns (grafted with bovine or human serum albumin) have been used for the enantiomeric separation of amino acids. The influence of several parameters such as the pH, the ionic strength of the mobile phase, and the temperature have been reported (18–21). The capacity factors of D- and L-tryptophan are very sensitive to these mobile phase parameters, which means that the complexation of both enantiomers with BSA proteins can be dramatically changed, especially by pH modification.

Therefore, the physicochemical parameters of the feed solution have to be optimized in the membrane separation process in order to get the highest selectivity, recovery, and production rate.



## EXPERIMENTAL

### Reagents

Fatty-acid-free bovine serum albumin (A-6003), D-tryptophan [T-9753], L-tryptophan [T-8659], and DL-tryptophan [T-3300] were purchased from Sigma Chemicals. α-Cyclodextrine [alpha W6] was from Wacker. Other chemicals were of reagent grade and were used without further purification.

### Filtration Device

All filtration experiments were carried out using a dead-end stirred filtration cell (Sigma Aldrich S2278). The system was set up at 20°C with a transmembrane pressure of $1.5 \times 10^5$ Pa and a rotation speed of 600 rpm. All the feed solutions were prepared just before the filtration experiments, and the distilled water was prefiltrated through a 0.2-μm membrane.

A polysulfone membrane (Millipore PLGC10000) with a molecular weight cutoff of 10000 $g \cdot mol^{-1}$ was used. Such a membrane has a BSA rejection coefficient higher than 99.5%.

### Enantiomeric Analysis

Quantitative analysis of tryptophan enantiomers was performed by capillary zone electrophoresis using a Spectraphoresis 1000 system from Thermo Separation Products fitted with a fused silica capillary (length 44 cm). The background electrolyte was an α-cyclodextrine 50 mM solution with a 50-mM $NaH_2PO_4/H_3PO_4$ buffer, pH 2.2. Analysis of tryptophan was carried out at 20 kV, and the UV detection was performed at 278 nm. The practicable concentration range was from $5 \times 10^{-6}$ to $10^{-4}$ M.

## TRYPTOPHAN BINDING

Several sites are available on BSA protein for D- and L-tryptophan binding (21–24). L-Tryptophan is bound predominantly to one site in a highly stereospecific manner and to a secondary binding site with a very low order of magnitude. The secondary site is also involved for D-tryptophan binding. On this site, which does not have any stereospecific recognition for tryptophan molecules, both enantiomers bind competitively with the same complexation constant.

To describe BSA–tryptophan binding, the following relations were used.

Nonstereospecific adsorption of tryptophan:

$$\text{BSA} + \text{L} \leftrightarrow \text{BSA—L}, \qquad K = \frac{[\text{BSA—L}]}{[\text{BSA}][\text{L}]} \tag{1}$$

$$\text{BSA} + \text{D} \leftrightarrow \text{BSA—D} \qquad K = \frac{[\text{BSA—D}]}{[\text{BSA}][\text{D}]} \tag{2}$$

Stereospecific adsorption of L-tryptophan:

$$\text{S} + \text{L} \leftrightarrow \text{S—L}, \qquad K_L = \frac{[\text{S—L}]}{[\text{S}][\text{L}]} \tag{3}$$

$$[\text{L}]_0 = [\text{L}] + [\text{BSA—L}] + [\text{S—L}] \tag{4}$$

$$[\text{D}]_0 = [\text{D}] + [\text{BSA—D}] \tag{5}$$

$$[\text{BSA}]_0 = [\text{BSA}] + [\text{BSA—L}] + [\text{BSA—D}] \tag{6}$$

$$[\text{S}]_0 = [\text{BSA}]_0 = [\text{S}] + [\text{S—L}] \tag{7}$$

## RESULTS AND DISCUSSION

To determine $K_L$ and $K$ complexation constants, BSA protein was added to a racemic tryptophan solution (Fig. 1) and this solution was filtrated using a PLGC membrane with a molecular weight cutoff about 10,000 g·mol$^{-1}$. Therefore, free D- and L-tryptophan (MW 204 g·mol$^{-1}$) were able to go through the membrane. On the other side, BSA (MW 66,700 g·mol$^{-1}$) and its complex with tryptophan were rejected. D- and L-Tryptophan were analyzed in the permeate by capillary zone electrophoresis. Figure 2 illustrates the evolution of the concentration of both enantiomers from the initial feed solution (before BSA addition) to the permeate.

### Complexation Constants

Both $K$ and $K_L$ complexation constants were determined from the filtration of racemic tryptophan ($2 \times 10^{-4}$ M) with BSA ($1.5 \times 10^{-4}$ M) in 0.1 M KCl at 20°C. After the measurements of free D- and L-tryptophan in the permeate, the measured $K$ and $K_L$ values using Eqs. (1)–(6) were plotted versus pH (Fig. 3). For both constants, a maximum occurred at pH ranging from 9 to 10.

Tryptophan has two acidic functions with p$K_a$ equal to 2.42 and 9.44. Consequently, the apparent increase of the $K_L$ complexation constant from pH 6 to pH 9 can not be attributed to tryptophan modification since it is primarily a dipolar ion over this pH range. A change in protein conformation resulting from the dissociation of protonated groups can be responsible for this variation. Above pH 9.6 the decrease in the complexation



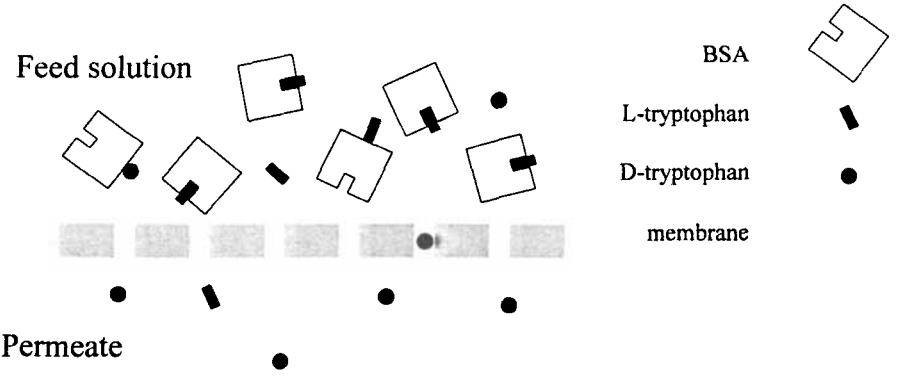


FIG. 1 Principle of separation of a racemic mixture (tryptophan) by an ultrafiltration membrane using a stereospecific complexant (BSA).

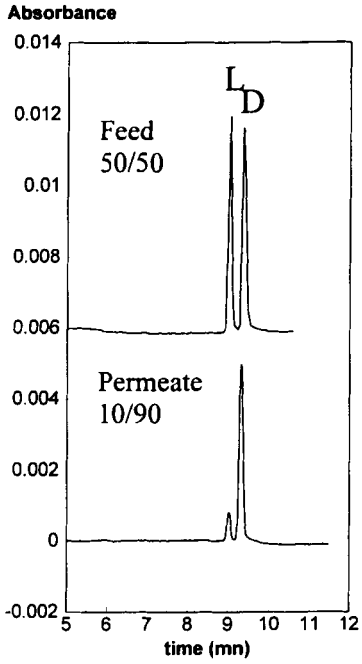


FIG. 2 Electropherogram of the racemic feed solution, $10^{-4}$ M for each enantiomer, and of the filtrate obtained at pH 9 with [BSA] = $1.5 \times 10^{-4}$ M.



constant is presumably the consequence of the negatively charged tryptophan above $pK_{a2}$ creating strong electrostatic repulsion forces between tryptophan and BSA protein.

The same behavior was observed for the nonstereospecific interaction of tryptophan $K$ as a function of pH. A slight shift of the maximum to higher pH values was obtained, and the complexation constant $K$ was smaller by 1 or 2 orders of magnitude.

## Purity and Recovery of Tryptophan

The purity and recovery were respectively determined by reference to the D enantiomer as

$$P = \frac{[D]}{[D] + [L]}; \qquad r = \frac{[D]}{[D]_0} \tag{8}$$

Because of the variation of $K$ and $K_L$ complexation constants, the purity and recovery of D-tryptophan were very sensitive to the pH of the feed solution (Fig. 4).

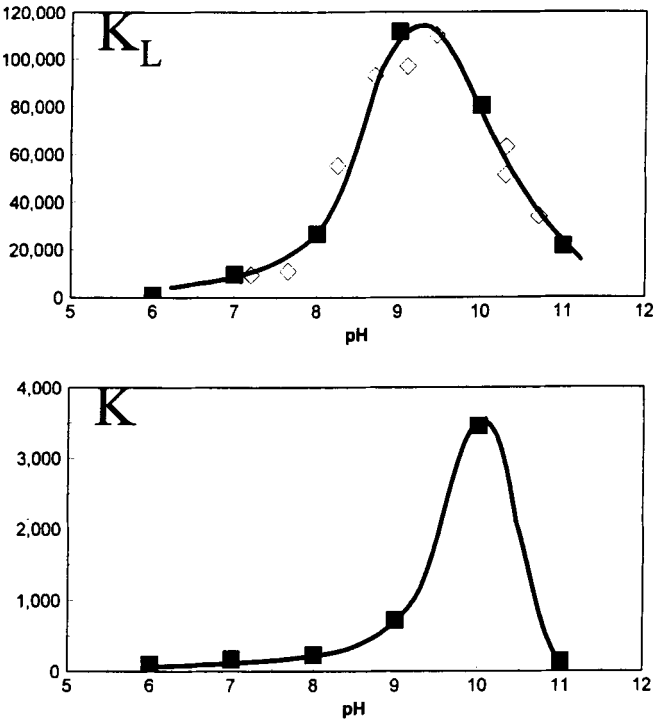


FIG. 3 Influence of pH on the complexation constant of tryptophan with BSA protein. $K_L$-stereospecific complexation constant with the L-enantiomer. $K$-nonstereospecific complexation constant. Feed solution: [BSA] = $1.5 \times 10^{-4}$ M, [D-try] = [L-try] = $10^{-4}$ M, $T$ = 20°C, $\Delta P$ = 1.5 bar, $\nu$ = 600 rpm. (◇) Data from Ref. 21.

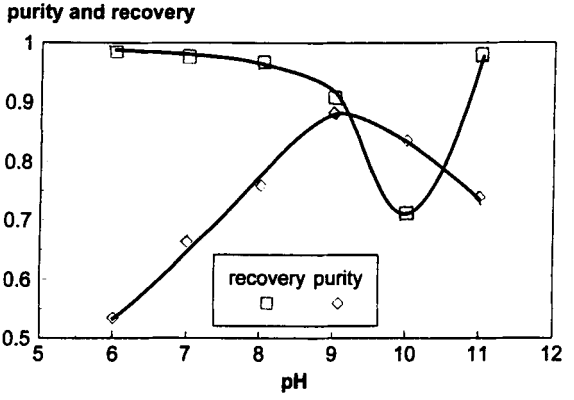


FIG. 4 Influence of pH on the purity and recovery of D-tryptophan. Feed solution: [BSA] = $1.5 \times 10^{-4}$ M, [D-try] = [L-try] = $10^{-4}$ M, $T$ = 20°C, $\Delta P$ = 1.5 bar, $\nu$ = 600 rpm.

The purity was from 0.54 at pH 6 up to its maximum of 0.9 at pH 9. Then the purity decreased to 0.75 at pH 11. From pH 6 to 9, both $K$ and $K_L$ complexation constants increased (see Fig. 3.) but the ratio between them also increased. Then L-tryptophan was proportionally more bonded to BSA, and so the purity increased. Above pH 9, the decreasing $K_L$ value led to a lower purity.

The D recovery showed an opposite tendency. High recovery was obtained at extreme pH values (6 and 11), and a minimum was observed around pH 10. Because the filtration experiments were performed with a constant initial concentration of BSA protein, the increased complexation at pH 9 led to a smaller quantity of free tryptophan and so lower recovery.

One of the most interesting results was obtained by filtration of [D-try] = [L-try] = $10^{-4}$ M, [BSA] = $1.5 \times 10^{-4}$ M at pH 9, giving in one stage a 91% purity with a 89% recovery of the D-tryptophan. The production rate was $6.3 \times 10^{-4}$ mol·m$^{-2}$·s$^{-1}$ at 1.5 bar. In this type of filtration the production rate can be increased by raising the transmembrane pressure. The selectivity of the separation process was only dependent on the $K_L$ and $K$ complexation constants.



## Optimization

The optimization of racemic separation of tryptophan using BSA complexing agent must take into account several parameters, especially the pH and the tryptophan and BSA concentrations. The complexation constants were very sensitive to the pH of the feed solution. At fixed tryptophan and BSA concentrations, this parameter had a strong influence on purity and recovery, as described above.

At fixed pH, concentrations have to be defined so that purity or/and recovery are acceptable for the intended application. An example is given for pH 9 with $K = 700$ and $K_L = 110{,}000$ in Fig. 5. Recovery and purity were plotted as a function of the initial concentration of each enantiomer for several BSA concentrations.

For a constant BSA concentration, the recovery is low for the lower tryptophan concentration whereas the purity is at the maximum. An increase of the tryptophan concentration leads to the opposite tendency for recovery and purity: when more tryptophan is in solution, proportionally less quantity is complexed, so the recovery is at the maximum, but conversely the purity falls.

For a constant tryptophan concentration, an increase of the BSA concentration leads to lower recovery but higher purity. However, this concentration cannot be too high because a dynamic BSA membrane layer will be created on the original membrane surface, giving another selectivity and a lower volume flow.

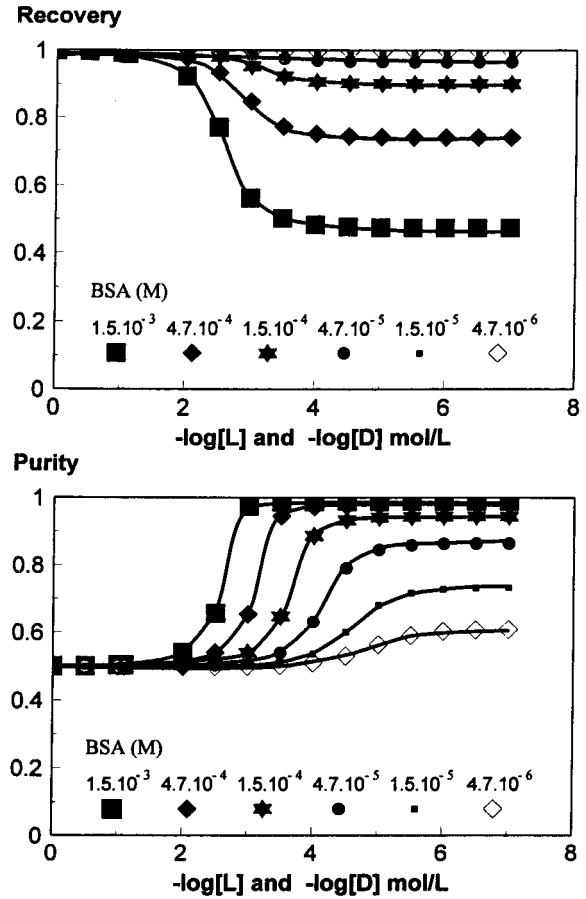


FIG. 5 Influence of BSA and tryptophan concentrations on the purity and recovery of D-tryptophan. pH 9, $K_L$ = 110,000, $K$ = 700.

## CONCLUSION

The optical resolution of racemic tryptophan was performed by ultrafiltration using the BSA solution system. The pH of the feed solution had a strong influence on the complexation constants between tryptophan and BSA, especially for the L-enantiomer. The selectivity and the recovery were mainly controlled by this parameter. Depending on which of the main criteria (recovery, purity) has to be optimized, the concentrations of tryptophan and BSA as well as the pH of the feed solution must be carefully defined.

## REFERENCES


1. T. Masawaki, M. Sasai, and S. Tone, *J. Chem. Eng. Jpn.*, *25*, 33–39 (1992).
2. A. Higuchi, M. Hara, T. Horiuchi, and T. Nakagawa, *J. Membr. Sci.*, *93*, 157–164 (1993).
3. T. Shinbo, T. Yamaguchi, H. Yanagishita, K. Sakaki, D. Kitamoto, and M. Sugiura, *Ibid.*, *84*, 241–248 (1993).
4. W. H. Pirkle and W. E. Bowen, *Tetrahedron: Asymmetry*, *5*, 773–776 (1994).
5. T. Masawaki, S. Matsumoto, and S. Tone, *J. Chem. Eng. Jpn.*, *27*, 517–522 (1994).
6. E. Yashima, O, Noguchi, and Y. Okamoto, *J. Appl. Polym. Sci.*, *54*, 1087–1091 (1994).
7. E. Yashima, J. Noguchi, and Y. Okamoto, *Chem. Lett.*, pp. 1959–1962 (1992).
8. M. Yoshikawa, J. Izumi, T. Kitao, S. Koya, and S. Sakamoto, *J. Membr. Sci.*, *108*, 171–175 (1995).
9. T. Aoki, A. Maruyama, K. Shinohara, and E. Oikawa, *Polym. J.*, *27*, 547–550 (1995).
10. K. Shinohara, T. Aoki, and E. Oikawa, *Polymer*, *36*, 2403–2405 (1995).
11. J. T. F. Keurentjes, L. J. W. M. Nabuurs, and E. A. Vetger, *J. Membr. Sci.*, *113*, 351–360 (1996).
12. A. Higuchi, Y. Ishida, and T. Nakagawa, *Desalination*, *90*, 127–136 (1993).
13. T. Aoki, M. Kokai, K. Shinohara, and E. Oikawa, *Chem. Lett.*, pp. 2009–2013 (1993).
14. S. Tone, T. Masawaki, and T. Hamada, *J. Membr. Sci.*, *103*, 57–63 (1995).
15. T. Aoki, S. Tomizawa, and E. Oikawa, *Ibid.*, *99*, 117–125 (1995).
16. A. Maruyama, N. Adachi, T. Takatsuki, M. Torii, K. Sanui, and N. Ogata, *Macromolecules*, *23*, 2748–2752 (1990).
17. T. Aoki, K. Shinohara, and E. Oikawa, *Makromol. Chem., Rapid Commun.*, *13*, 565–570 (1992).
18. Z. Simek and R. Vespalec, *J. Chromatogr. A*, *685*, 7–14 (1994).
19. R. K. Gilpin, S. E. Ehtesham, and R. B. Gregory, *Anal. Chem.*, *63*, 2825–2828 (1991).
20. V. Tittlelbach and R. K. Gilpin, *Ibid.*, *67*, 44–47 (1995).
21. R. H. McMenamy and J. L. Oncley, *J. Biol. Chem.*, *233*, 1436 (1958).
22. B. Loun and D. S. Hage, *J. Chromatogr.*, *579*, 225–253 (1992).
23. J. Yang and D. S. Hage, *Ibid.*, *645*, 241–250 (1993).
24. J. Yang and D. S. Hage, *Ibid.*, *725*, 273–285 (1996).


*Received by editor September 24, 1996*
*Revision received December 1996*