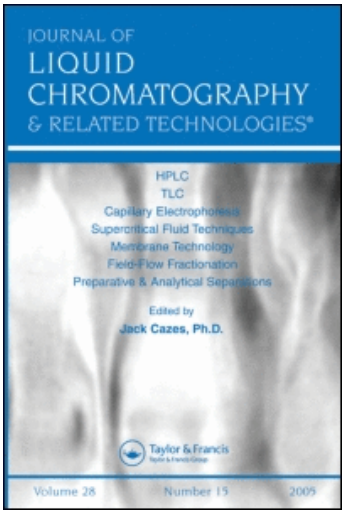

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Retention Behavior of Poly(L-Tryptophan)S and Poly(D,L-Tryptophan)S in Reversed-Phase Liquid Chromatography

C. H. Lochmüller[a]; Chun Jiang[a]; Math Elomaa[b]
[a] P. M. Gross Chemical Laboratory Department of Chemistry, Duke University, Durham, North Carolina [b] Department of Polymer Chemistry, University of Helsinki, Helsinki, Finland

To cite this Article Lochmüller, C. H. , Jiang, Chun and Elomaa, Math(1994) 'Retention Behavior of Poly(L-Tryptophan)S and Poly(D,L-Tryptophan)S in Reversed-Phase Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 17: 14, 3179 — 3189
To link to this Article: DOI: 10.1080/10826079408013197
URL: http://dx.doi.org/10.1080/10826079408013197

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(14&15), 3179-3189 (1994)

RETENTION BEHAVIOR OF POLY(L-TRYPTOPHAN)S AND POLY(D,L-TRYPTOPHAN)S IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

C. H. LOCHMÜLLER[1]*, CHUN JIANG[1],
AND MATTI ELOMAA[2]
[1]*P. M. Gross Chemical Laboratory*
Department of Chemistry
Duke University
Durham, North Carolina 27708
[2]*University of Helsinki*
Department of Polymer Chemistry
Meritullinktu 1 A
SF-00170 Helsinki, Finland

ABSTRACT

The reversed-phase liquid chromatographic retention behavior of poly(l-tryptophan)s and poly(d,l-tryptophan)s) whose molecular weight ranged from 5.4 kD to 37.25 kD is examined using a C-8 chemically bonded stationary phase and binary mobile phases of tetrahydrofuran (THF)-water and ternary mobile phases of THF-water-methanol. The retention of poly(l-tryptophan)s is compared with that of poly(d,l-tryptophan)s. Linear-Solvent-Strength (LSS) model is examined in describing the retention behavior. The effect of adding a third solvent to the binary mobile phase is also discussed.

INTRODUCTION

The use of reversed-phase liquid chromatography (RPLC) in polymer separation and characterization has drawn considerably large interest since 1980s [1-14]. The reversed-phase chromatographic method, which involves no size-exclusion, complements the widely used size-exclusion chromatographic method in which polymers are separated according to their sizes. It has been shown that using RPLC it is possible to separate a variety of polymers, especially copolymers [15] which has been a difficult task with size-exclusion chromatography. However, the retention mechanism of polymers in RPLC remains unclear and several models have been suggested [3,6,7,8,9,10,11,12,13]. A true and clear retention mechanism is needed in order to fully exploit the power of RPLC, such as optimizing separation and predicting retention. Central to the debate on polymer retention mechanism is that whether or not isocratic retention can be obtained. Lochmüller and McGranaghan [14] found that retention behavior of poly(styrene) that is similar to that found in small molecules could be obtained only when the sample was adequately mixed with the mobile phase using a low dispersion, crocheted mixer before the solute-column contact occurred. They reported that isocratic retention of polystyrenes of molecular weight ranged from 2000 Daltons to 2,800,000 Daltons could be obtained with binary mobile phases of tetrahydrofuran/H_2O and dichloromethane/ acetonitrile. Finite, non-zero k' values and linear relationships in the plots of log k' versus the volume percentage of tetrahydrofuran and dichloromethane were observed [14].

Characterization of rigid polymers with size-exclusion chromatography is difficult because the calibration curve that is derived from flexible polymers is no longer applicable [16,17,18,19]. If a polymeric sample contains both rigid and

flexible polymers, it is even more difficult to assign the peaks in the size-exclusion chromatogram. RPLC can be a good method for characterizing rigid polymers because of its partition/adsorption retention mechanism and its independence of calibration. It is also highly desirable to be able to control the retention with current available separation optimization knowledge developed from small molecules, such as the "third solvent" strategy [20]. In this report, Poly(l-tryptophan)s and poly(d,l-tryptophan)s are chosen as model polymers because (1). the former is a rigid and helical polymer which is rod-like, and the later a flexible and globular one; (2). a very small amount (less than 0.5 nanogram) of poly(tryptophan)s can be detected with fluorescence detection and this insures that solution of poly(tryptophan) is close to infinitely dilute; (3). poly(tryptophan)s are actually synthetic peptides and this study may shed some light on the future study of separation of synthetic peptides and proteins. It is found that poly(l-tryptophan)s show longer retention times than poly(d,l-tryptophan) of the same molecular weight. For all poly(tryptophan)s samples, linear plots of the logarithm of the capacity factor (k') versus volume fraction (ϕ) of the strong solvent are obtained. The LSS model is examined via inter-relating isocratic and gradient elution. With the volume fraction of THF kept constant, the effect of adding a small amount of methanol in THF-water mobile phase on the retention time is discussed.

EXPERIMENTAL SECTION

HPLC. A Perkin-Elmer Series 4 liquid chromatograph and 420B autosampler (Perkin-Elmer, Norwalk, CT) was used. Detector used was a Perkin-Elmer 850-

10LC Fluorescence Spectrometer with excitation wavelength at 280 nm and emission wavelength at 350 nm. Flow rate was 1 mL/min. A sample loop of 5 μL was used. Retention data were collected using a Nelson Analytical Chromatography package (Nelson Analytical, Inc., Cupertino, CA). Samples were dissolved in the mobile phase collected right before injection. The concentrations of all samples were 50 μg/mL. Before each injection the samples were vigorously agitated by a shaker for 10-20 minutes. Capacity factor k' was calculated by the equation of $k'=(t_r-t_o)/t_o$, where t_r is the retention time of polymer peak maximum and t_o is the void time. The standard deviation of k' calculated from three measurements was within 2% error.

Materials. Columns used were 25cmX4.6mm ones with Partisil 10 C8 bonded phase (Whatman, Clifton, NJ). HPLC grade methanol, tetrahydrofuran (THF) and water were used as received. Poly(tryptophan) standards were purchased from Sigma (St. Louis, MO, USA). Their molecular weights are: poly(l-tryptophan) 5,400 Daltons (Mw/Mn=1.45), 115,000 Daltons (Mw/Mn=1.24), 37,250 Daltons (Mw/Mn=1.11) and poly(d,l-tryptophan) 5,700 Daltons (Mw/Mn=1.47) and 14,500 Daltons (Mw/Mn=1.42).

RESULTS AND DISCUSSION

In reversed-phase chromatography of polymers, a pair of good and poor solvents are often used [1-15]. In this study, THF and H_2O were used. THF is the "good" solvent and H_2O is the "poor" or hostile one. The sample chromatograms from isocratic elution are shown in Figure 1. It is interesting to observe that at the same mobile phase composition, poly(l-tryptophan) 11.5 kD has longer retention

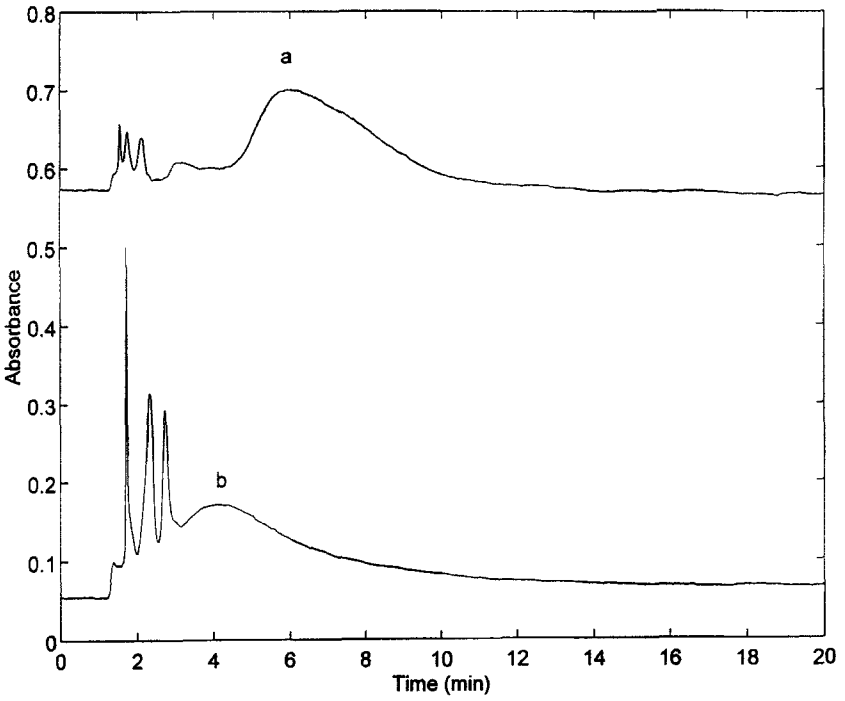

FIGURE 1. Chromatograms of poly(tryptophan)s Mobile phase THF:water 77:23, (a). 11.5 kD poly(l-tryptopahn), (b). 14.5 kD poly(d,l-tryptophan). The sharp peaks are from oligomers.

time than its counterpart 14.5 kD poly(d,l-tryptophan)s within the range of mobile phase compositions used in the experiments. The difference in retention time between 5.4 kD poly(l-tryptophan) and 5.7 kD poly(d,l-tryptophan)s is small. It has been reported that the predicted order of solute retention is: rigid-rod solutes>plate solutes>flexible chain solutes [21]. There have been many reports and debates on whether the retention mechanism for small molecules is an adsorption process ("Solvophobic" model), or a partition process, or both [22]. For the high molecular weight polymer case, if retention only involves adsorption mechanism

in which solute-stationary phase interaction only takes place at the surface of the stationary phase, the retention of poly(l-tryptophan)s should be shorter than that of poly(d,l-tryptophan)s because the average surface area of the polymer solute in contact with the stationary phase of poly(l-tryptophan) is smaller than that of poly(d,l-tryptophan). The bulky globular poly(d,l-tryptophan)s may not be able to enter the bonded phase of alkyl chains, whereas a part of the rod-like poly(l-tryptophan)s may get intercalated in the bonded alkyl chains. If the molecular weight is low, there may not be dramatic difference in retention time as we observed in 5.4 kD and 5.7 kD poly(tryptophan)s. Therefore, the rationale can be suggested as that high molecular weight poly(d,l-tryptophan)s may experience a mechanism of adsorption, on the other hand, poly(l-tryptophan)s and low molecular weight poly(d,l-tryptophan)s may have a combined partition and adsorption mechanism. The results here support that for RPLC of polymers the stationary phase plays a very important role. More mechanistic study can be done by varying the alkyl chain length of stationary phase and studying the retention of polymers of different size and/or shapes.

In Figure 2 are shown the plots of log k' versus the volume fraction of THF (ϕ) in binary THF-water mobile phase. These plots fit linear relationships (log k'= A-Sϕ) where A is the intercept and S is the slope.

Linear-Solvent-Strength has been applied to optimization of separation and prediction of retention [23,24,25]. From the LSS theory, gradient retention time t_g is given by:

$$t_g = t_o \log[2.3k_o/k'] + t_o + t_D \qquad 1$$

where t_D is the dwell time of the gradient system (the time between the beginning of the gradient at the pump and its reaching the inlet of the column), k'

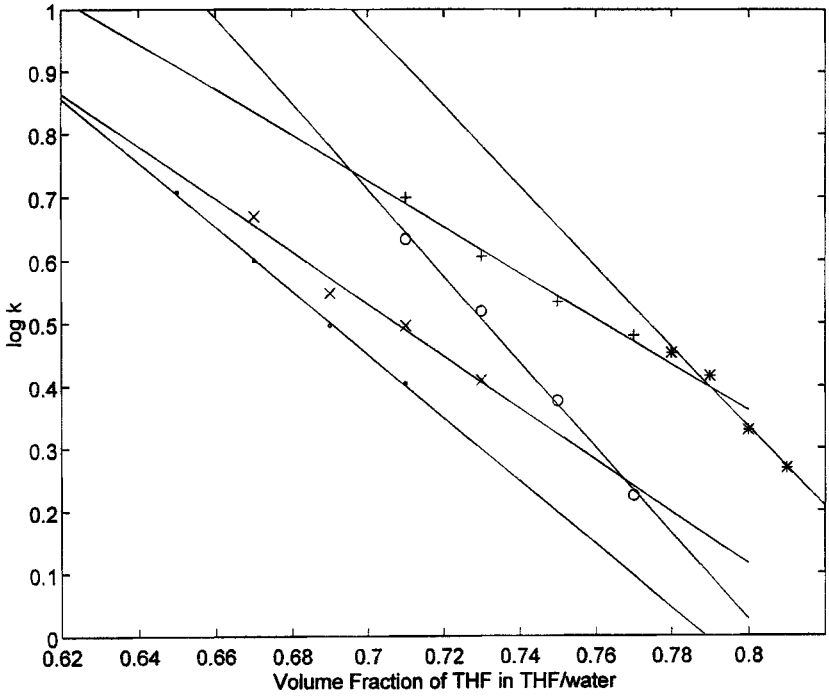

FIGURE 2. Plots of log k' versus volume fraction of THF Poly(l-tryptophan)s: (*) 37.25 kD; (+) 11.5 kD; (x) 5.4 kD; Poly(d,l-tryptopahn)s: (o) 14.5 kD; (·) 5.7 kD.

is the average capacity factor of gradient elution and k_0 is the capacity factor from pure weak solvent. Since

$$k'_1 = t_G/(\Delta\phi S t_0) \quad (2)$$

where t_G is the gradient time and $\Delta\phi$ is the change of mobile phase composition. With two gradient runs, k'_1 can be calculated as:

$$k'_1 = [t_{g1}-(t_{g2}/\beta)-(t_0+t_D)(\beta-1)/\beta]/(t_0 \log\beta) \quad (3)$$

$$\beta = t_{G2}/t_{G1} \quad (4)$$

Given a known k'_1, S can be calculated as:

$$S = t_{G1}/(\Delta\phi k'_1 t_0) \quad (5)$$

TABLE 1: Predicted S Values from LSS Model and Actual S Values *

	37.25 kD (l)	11.5 kD (l)	5.4 kD (l)	14.5 kD (d,l)	5.7 kD (d,l)
predicted S	6.68	4.2	4.5	7.5	5.5
actual S	6.42	3.6	5.1	6.94	5.1

* In the table, (l) stands for poly(l-tryptophan)s, (d,l) for poly(d,l tryptophans).

If the gradient retention time from two runs is known, S can be calculated by equation 5. The predicted and actual S and t_r values are listed in Table 1. The prediction is moderately accurate, considering the fact that the plot of log k' versus ϕ is not perfectly linear over a large range of mobile phase composition and LSS model is a empirical model.

Mobile phase plays an important role in RPLC [20,22]. Many optimization techniques center around using combinations of different mobile phase. Using ternary solvent mixture as mobile phase to precisely control the elution strength and polarity of mobile phase has been studied for a long time [20]. However, to our best knowledge there is no report on studying the effect of ternary mobile phase on the polymer retention. In this work, methanol is added into THF-water mobile phase as the third solvent. Methanol is a poor solvent for poly(tryptophan)s, but it is a better solvent than water. This is verified by mixing the same amount of poly(l-tryptophan) 11.5 kD with the same volume of methanol, and water and taking fluorescence intensity measurements of the supernatant. The normalized fluorescent intensities at 350 nm indicate that methanol and acetonitrile are better solvents than water, e.g., the intensity at 350nm for methanol solution is 7 times larger than that for water. While the volume fraction of THF is kept constant, the fraction of water is decreased with the increase of the fraction of methanol. Since methanol is a better solvent than

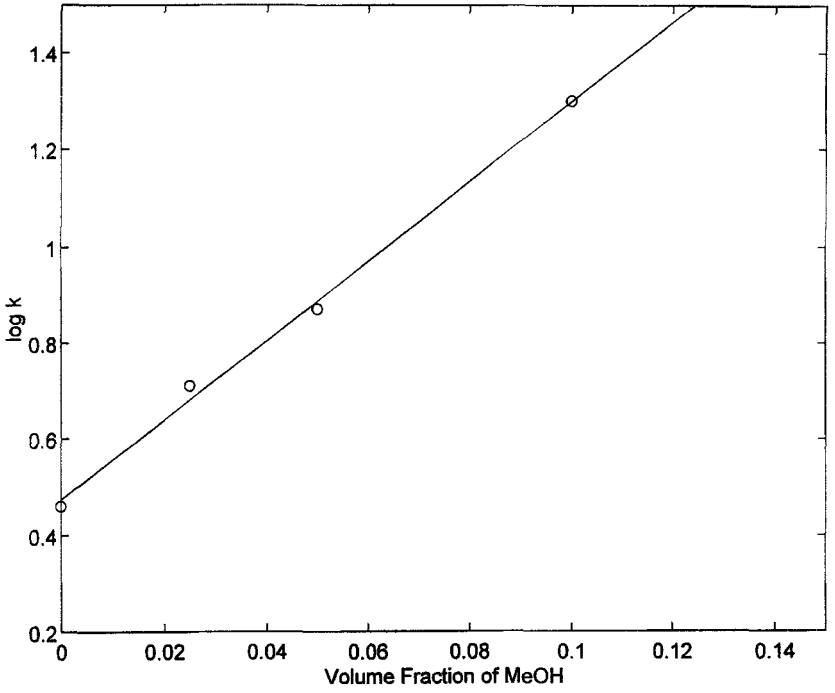

FIGURE 3. Plot of log k' versus volume fraction of methanol in THF-water-methanol tenery mobile phase.

water, one would expect that replacing water with methanol should decrease retention of poly(l-tryptophan) 11.5 kD. However, we observed the opposite. The plot of log k' versus the volume fraction of methanol shows a linear relationship (Figure 3). This unusual retention behavior was observed in small molecules by Lochmüller and co-workers[26]. They found that water actually was freed from the methanol-water associates when a small amount of acetonitrile or THF was added into the methanol-water mobile phase and the increased content of "free" water led to the increase of the retention time within a certain range. The small addition of methanol into THF-water mixture also frees water from the

associated THF-water complex and caused the increase in retention time. More study will be directed to understand the selectivity and optimization of separation of polymers with different mobile phases.

CONCLUSION

The retention behavior of poly(l-tryptophan)s and poly(d,l-tryptophan)s were examined. It is found that their retention behavior can be explained in terms of LSS model. It is observed that retention time for high molecular weight poly(l-tryptophan)s is longer than that of poly(d,l-tryptophan)s under the same mobile phase composition. When methanol is added into THF-water mobile phase, the retention time becomes larger. The mechanism and explanation are suggested for such retention behavior.

REFERENCES

1. Barth, H. G.; Mays, J. W. eds Modern Methods of Polymer Characterization, Wiley, New York, 1991.

2. Glöckner, G. Polymer Characterization by Liquid Chromatography; Elsevier, 1987.

3. Armstrong, D.W.; Bohem, R.E. J. Chromatogr. Sci., 22: 378 (1984).

4. Jandera, P.; Rozkosna, J. J. chromatogr. 362: 325 (1986).

5. Kirkland, J. J. J. Chromatogr., 125: 231 (1976).

6. Stadalius, M. A.; Quarry, M.A.; Mourey, T. H.; Snyder, L. R. J. Chromatogr., 358: 1 (1986).

7. Stadalius, M. A.; Quarry, M.A.; Mourey, T. H.; Snyder, L. R. J. Chromatogr., 358: 17 (1986).

8. Snyder, L.R.; Stadalius, M.A.; Quarry, M.A. Anal. Chem., 55: 1412 A (1983).

9. Glöckner, G.; VanDen Berg, J. H. M. Chromatographia, 19: 55 (1984).

10. Glöckner, G.; Van Den Berg, J. H. M. J. Chromatogr., 317: 615 (1984).

11. Armstrong, D.W.; Bui, K. H. Anal. Chem., 54: 706 (1982).

12. Glöckner, G.; Van Den Berg, J. H. J. Chromatogr., 352: 511 (1986).

13. Bohem, R. E.; Matire, D. E.; Armstrong, D. W.; Bui,K.H. Macromolecules, 17: No. 3, 400 (1984).

14. Lochmüller, C. H.; McGranaghan, M. B. *Anal. Chem.*, 61: 2449 (1989).

15. Barth, H. G., Janca, J., Polymer Analysis and Characterization, Wiley, New York, 1992.

16. Yau, W.W.; Kirkland, J. J.; and Bly, D.D., Modern Size Exclusion Chromatography, Wiley, New York, 1979.

17. Hager, D., J. Chromatogr., 187: 285 (1980).

18. Grubistic, Z.; Rempp, P., and Benoit, H., Polymer Lett., 5: 753 (1967).

19. Giddings, J. C.; Kucera, E.; Russell, C. P.; and Myers, M.N., J. Phy. Chem., 72: 4397 (1968).

20. Snyder, L.R.; Kirkland, J. J., Introduction to Modern Liquid Chromatography, Wiley, New York, 1979.

21. Matire, D. E. and Bohem, R. E., J. Phys. Chem., 87, 1045 (1983).

22. Lochmüller, C. H., and Wenzel, T. J., in Physical Methods of Chemistry, 2nd ed., Vol. X., eds. Rossiter, B. W. and Baetzold, R.C., pp 85-161, Wiley, New Nork, 1993.

23. Snyder, L.R.; Stadalius, M. A.; Quarry, M. A. Anal. Chem., 55, 1413A (1983).

24. Snyder, L. R.; Dolan, J. W. DrylabG Users Manual; LC Resources; California, 1986.

25. Quarry, M. A.; Grob, R. L.; Snyder, L. R. Anal. Chem., 58: 907 (1986).

26. Lochmüller, C. H.; Hamzavi-Abedi, M.A.; and Ou, Chu-Xiang, J. Chromatogr., 387: 105 (1987).

Received: January 19, 1994
Accepted: February 1, 1994