Journal of Chromatography, 250 (1982) 119–123
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,194

Note

Separation of amino acids by charge-transfer interaction chromatography in aqueous systems

KAZUHIKO ISHIHARA*, TSUKASA IIDA, NAOHIRO MURAMOTO and ISAO SHINOHARA
Department of Polymer Chemistry, Waseda University, Tokyo 160 (Japan)
(Received May 4th, 1982)

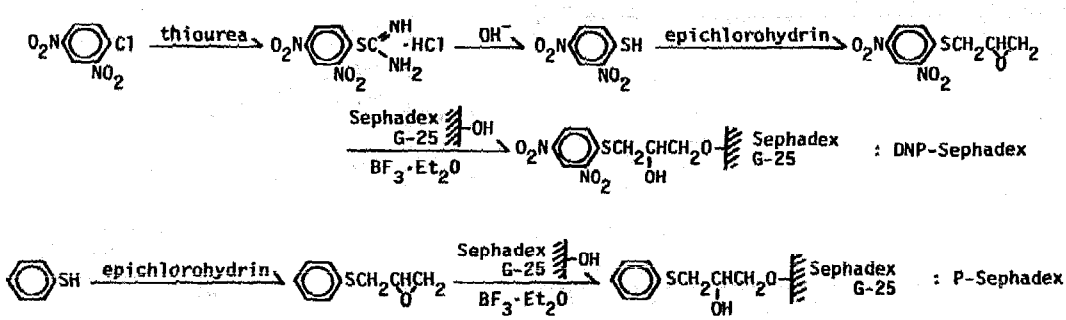
Most biosubstances have electron-donating groups such as an amino group or a heterocyclic group containing a nitrogen atom[1]. Thus, it is expected that the use of a polymeric adsorbent containing an electron acceptor which specifically interacts with the electron donating groups will enable the separation of the biosubstances. Such methods using the so-called charge-transfer interaction between electron donor and acceptor have mainly been investigated in organic media[2–4]. Porath and co-workers[5–9] studied charge-transfer interaction chromatography in aqueous media. However, the contribution of the charge-transfer interaction between substrates and adsorbents has not been clearly established.

In this article, chromatography of amino acids in aqueous media using a polymeric adsorbent containing the dinitrophenyl group, which is a strong electron acceptor, has been studied, and the interactions between the adsorbent and amino acids are discussed with attention centered on the charge-transfer interaction.

EXPERIMENTAL

Syntheses of polymeric adsorbents

The polymeric adsorbents were synthesized according to the following scheme:



The degrees of substitution of Sephadex (Pharmacia; cross-linked dextran) by dinitrophenyl (DNP) and phenyl (P) groups were 140 and 170 μmol per gram of dry adsorbents respectively, as determined by elemental analysis. Unsubstituted Sephadex was used as a control adsorbent.

0021-9673/82/0000–0000/$02.75

Chromatography of amino acids

The polymeric adsorbents were packed into a glass column (20 × 0.5 cm I.D.) by the slurry method, the temperature being kept constant by a thermostat. The column was equilibrated with an eluent, the total bed volume being 4.0 ml in each case. A 0.5-ml volume of sample solution was introduced into the column and eluted at a rate of 4.0 ml/h. Three amino acids, tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe), were used as substrates. The concentrations of amino acids in the eluate were determined spectrophotometrically at 280 nm (Trp), 276 nm (Tyr) and 259 nm (Phe).

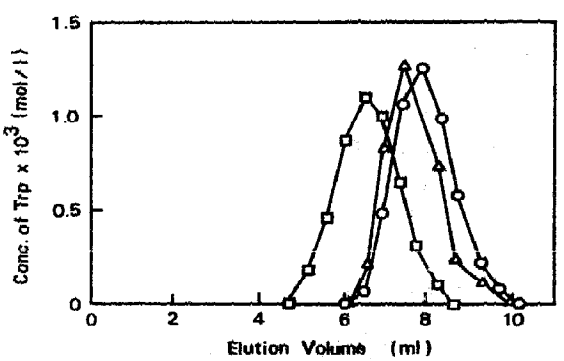
RESULTS AND DISCUSSION

Fig. 1 shows the elution patterns of Trp on these polymeric adsorbents at 10°C. The elution volumes, V_E, of Trp on DNP-Sephadex, P-Sephadex and Sephadex were 7.9, 7.5 and 6.5 ml, respectively. In general, an elution parameter, K_d, is defined by following equations

$$V_E = V_0 + K_d V_I$$
$$V_T = V_0 + V_I + V_G$$

where V_0 = void volume, V_I = internal volume, V_T = total bed volume and V_G = gel volume[10,11]. When the retention of a substrate is only caused by molecular sieving, $0 < K_d \leq 1$. From Fig. 1, however, the values of K_d were obviously larger than 1.0 for all the adsorbents used, because the value of V_T was 4.0 ml. Therefore, it is considered that the retention of Trp may be caused by other interactions in addition to molecular sieving, and the intensity of the interactions between the adsorbents and Trp decreases in the order DNP-Sephadex > P-Sephadex > Sephadex.

In order to examine the influence of temperature on the retention of Trp, the chromatography was carried out at different temperatures between 10°C and 70°C. Fig. 2 shows the relation between the value of V_E/V_T and the elution temperature. The value of V_E/V_T is used instead of K_d as a measure of the retentive power of adsorbents



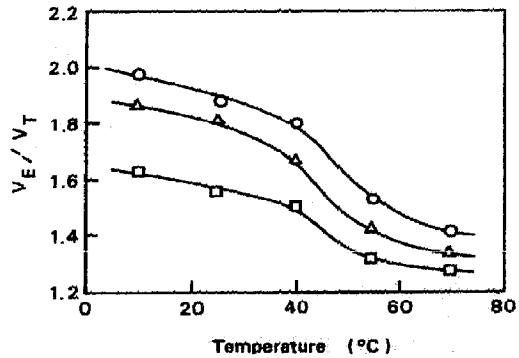


Fig. 1. Chromatography of tryptophan on DNP-Sephadex (○), P-Sephadex (△) and Sephadex (□) at 10°C in 0.07 *M* phosphate buffer at pH 7.0.

Fig. 2. Temperature dependence of the values of V_E/V_T for tryptophan on DNP-Sephadex (○), P-Sephadex (△) and Sephadex (□) in 0.07 *M* phosphate buffer at pH 7.0.

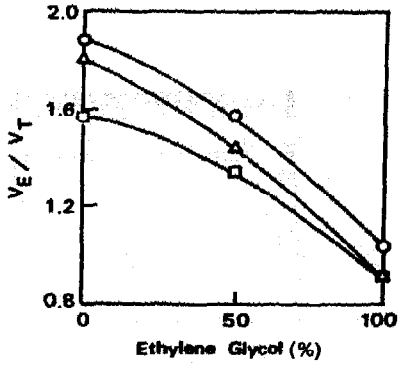


Fig. 3. Relation between the values of V_E/V_T for tryptophan and ethylene glycol content of the eluent at 10°C on DNP-Sephadex (○), P-Sephadex (△) and Sephadex (□).

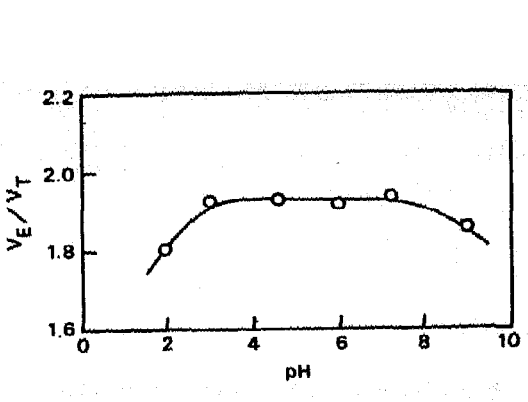
since the relation between V_E/V_T is K_d is linear, and the larger this value is the stronger is the retentive power[9]. It was found that the values of V_E/V_T increased in the order Sephadex < P-Sephadex < DNP-Sephadex in the temperature range 10–70°C, and they gradually decreased with increasing temperature.

When column chromatography using hydrophilic gels is carried out, normal retention forces such as hydrogen bonding and van der Waals forces are weakened by a rise in temperature, owing to a decrease in enthalpy[12]. In the present case, the value of V_E/V_T decreased drastically at about 50°C. According to Némethy[13], hydrophobic interactions become weaker at temperatures greater than 58°C because the ordered structure of water is broken down. Therefore, it is considered that the drastic change was caused by a decrease of the hydrophobic interaction between Trp and the adsorbents, *i.e.*, hydrophobic interactions are probably involved in the retention of Trp.

The decrease in the values of V_E/V_T for Trp when an ethylene glycol (EG) was added to the eluent is indicated in Fig. 3. Since an EG disrupts the ordered structure of water, this again suggests that the decrease in V_E/V_T is due to a decrease in the hydrophobic interaction between Trp and the adsorbents. The difference between the values of V_E/V_T on DNP-Sephadex and on P-Sephadex was nearly the same with or without EG, and in the case of 100% EG —where there is no hydrophobic interaction— the value of V_E/V_T on P-Sephadex is the same as that on Sephadex; the value of V_E/V_T on DNP-Sephadex is large. From these results, the retentive power of P-Sephadex, which is larger than that of Sephadex in aqueous media, seems mainly due to the hydrophobic interaction between the phenyl groups and Trp. Moreover, it is suggested that the retentive power of DNP-Sephadex for Trp is enhanced compared with that of P-Sephadex by the introduction of the nitro groups.

In order to investigate the contribution of electrostatic interactions, the chromatography on DNP-Sephadex was carried out at 10°C and various pH values. The results are shown in Fig. 4. The values of V_E/V_T were constant in the range pH 2.5–7.5, but decreased at pH > 7.5 or < 2.5 where Trp was negatively or positively charged, respectively. Thus, if the elution is carried out at a pH near the isoelectric point of Trp (5.89), the influence of electrostatic interactions is negligible.

Fig. 5 shows the elution pattern of an artificial mixture of Trp, Tyr and Phe on DNP-Sephadex. The elution volume increased in the order Phe < Tyr < Trp. The



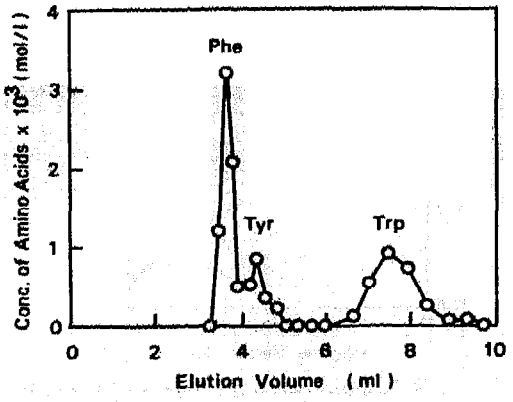


Fig. 4. pH dependence of the values of V_E/V_T for tryptophan on DNP-Sephadex at 10°C.

Fig. 5. Chromatography of an artificial mixture of tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) on DNP-Sephadex at 10°C in 0.07 *M* phosphate buffer at pH 7.0.

electrostatic interaction between DNP-Sephadex and the amino acids was negligible, because these amino acids are not charged (pH 7.0).

The values of hydrophobicity[14], energy of the highest occupied molecular orbital (HOMO), which is a measure of the electron-donating ability[15], and V_E/V_T obtained from Fig. 5 are listed in Table I. The order of the hydrophobicity is Tyr < Phe < Trp, so that the order of the values of V_E/V_T, Phe < Tyr < Trp cannot be explained only by the hydrophobic interaction. Therefore, the introduction of nitro groups into the adsorbent considerably affects the separation of amino acids. On the other hand, the order of the electron-donating ability of these amino acids —the lower the energy of the HOMO is the stronger is the donating ability— agreed with the order of V_E/V_T. Moreover, the dinitrophenyl group introduced into DNP-Sephadex is a very strong electron acceptor. From these results, it is suggested that the effect of the nitro groups observed in the case of DNP-Sephadex is based on the charge-transfer interaction.

TABLE I

PHYSICAL PROPERTIES OF AROMATIC AMINO ACIDS

	Phenylalanine	*Tyrosine*	*Tryptophan*
Hydrophobicity[14] (cal/mol)	2500	2300	3400
Energy of HOMO[15]	0.908	0.792	0.534
V_E/V_T*	0.91	1.07	1.91

* Calculated from the result in Fig. 5

In conclusion, the charge-transfer interaction and the hydrophobic interaction play an important rôle in the chromatography of amino acids on polymeric adsorbents containing the dinitrophenyl group as a ligand.

ACKNOWLEDGEMENT

We thank Dr. Naoki Negishi for valuable discussions.

REFERENCES

1 M. A. Slifkin (Editor), *Charge Transfer Interactions of Biomolecules*, Academic Press, London, 1971.
2 J. T. Ayres and C. K. Mann, *Anal. Chem.*, 36 (1964) 2185.
3 J. T. Ayres and C. K. Mann, *Anal. Chem.*, 38 (1966) 859.
4 J. T. Ayres and C. K. Mann, *Anal. Chem.*, 38 (1966) 861.
5 J. Porath and K. D. Caldwell, *J. Chromatogr.*, 133 (1977) 180.
6 J. Porath and B. Larsson, *J. Chromatogr.*, 155 (1978) 47.
7 J.-M. Egly and J. Porath, *J. Chromatogr.*, 168 (1979) 35.
8 M. A. Vijayalakshmi and J. Porath, *J. Chromatogr.*, 177 (1979) 201.
9 J. Porath, *Pure Appl. Chem.*, 51 (1979) 1549.
10 B. Gelotte, *J. Chromatogr.*, 3 (1960) 330.
11 P. Flodin, *J. Chromatogr.*, 5 (1961) 103.
12 H. Determann and K. Lampert, *J. Chromatogr.*, 69 (1972) 123.
13 G. Némethy, *Angew. Chem. Int. Ed. Engl.*, 6 (1967) 195.
14 C. C. Biogelow and M. Channon (Editors), *Handbook of Biochemistry and Molecular Biology*, CRC Press, Cleveland, OH, 1976.
15 B. Pullman and A. Pullman, *Rev. Mod. Phys.*, 32 (1960) 428.