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SEPARATION OF PEPTIDES BY MULTIMODE CHROMATOGRAPHY ON A COLUMN PACKED WITH VINYL ALCOHOL COPOLYMER GEL

NORIKO HIRATA*, MASAO KASAI, YUZO YANAGIHARA and KOHJI NOGUCHI

Gel Separation Development Department, Asahi Chemical Industry Co., Ltd., 1-3-2, Yakoo Kawasaki-ku, Kawasaki-shi 210 (Japan)

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SUMMARY

Several mechanisms of peptide separation in high-performance liquid chromatography were observed to occur on the Asahipak® GS-320 packed with vinyl alcohol copolymer. Neutral rather than acidic mobile phases were employed as they were found to result in higher retention of many peptides on the GS-320. For neutral peptides, the retention volume corresponded to the Rekker's hydrophobic fragmental constant, with a correlation coefficient of 0.71. Peptides with acidic residues eluted early, as an effect of ionic exclusion; those with basic residues were retained longer, owing to an ion-exchange effect. The ionic interactions were shown to involve the carboxylic group present on the gel polymer. The net result was found to be excellent separation of hydrophilic as well as hydrophobic peptides, related to differences in their isoelectric points. The combination of these complex mechanisms, together with the size-exclusion effect of the GS-320 gel for separation of proteins and other large molecules and for analysis with a mobile phase high in acetonitrile content, makes possible high-resolution isocratic analysis of peptides, which cannot be achieved on octadecylsilica or ion-exchange columns.

INTRODUCTION

In its most common form, high-performance liquid chromatographic (HPLC) analysis of peptides is performed on reversed-phase columns of octadecylsilica (ODS) gels, with gradient elution by aqueous solutions of trifluoroacetic acid and acetonitrile. The ODS gels, based on silica with bonded alkyl groups, interact most strongly with peptides under acidic conditions and exhibit excellent separation of many peptides based on the difference in their hydrophobicity. The Asahipak® GS-320, packed with a vinyl alcohol copolymer gel, is known for its highly efficient separation of polysaccharides and polyethylene glycols with aqueous eluents by a size-exclusion effect, in what may be referred to as gel permeation chromatography [1]. As previously reported, however, it has also been

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found to exhibit an adsorption effect in the separation of insulin, angiotensin, and dipeptides with neutral buffer solution as eluent [2].

Here we describe a study of the relation between the chromatographic behaviour of peptides on the GS-320 and their molecular masses, isoelectric points and hydrophobic fragmental constants, and a comparison of its separation mechanism in neutral eluent with that of the ODS column. The separation mechanisms are also discussed with reference to specific applications.

EXPERIMENTAL

Chromatography was performed with a Hitachi 638-30 high-speed liquid chromatograph (Hitachi, Tokyo, Japan), equipped with a Jasco (Japan Spectroscopic, Tokyo, Japan) supplier-100-IV UV detector operated at 220 nm. The columns shown in Table I were employed. The mobile phase consisted of aqueous 50 m*M* ammonium acetate buffer (pH 6.7) or 0.05% trifluoroacetic acid (pH 2.4), with or without acetonitrile. Proteins and peptides were obtained from Sigma (St. Louis, MO, U.S.A.), Peptide Institute (Osaka, Japan) or Serva (Heidelberg, F.R.G.). Their primary structures and properties relevant to this study are shown in Table II. The hydrophobic fragmental constants of the peptides were calculated by summming the Rekker [3] values of their amino acids. The isoelectric points were taken from the Handbook of Biochemistry [4], where listed, or otherwise calculated from Manabe's program [5]. Titration curves were obtained by washing the gel with diluted aqueous hydrochloric acid and then distilled water, dispersing the gel in aqueous 0.1 m*M* sodium chloride and titrating the dispersion with aqueous 20 m*M* sodium hydroxide.

Methylation of polymer-constituent carboxyl groups was performed by reaction with boron trifluoride–methanol complex, containing ca. 14% boron trifluoride by mass, at room temperature for 16 h or more.

TABLE I

COLUMNS USED IN THE STUDY

Column	Supplier	Column size	Material	Main separation mechanism
Asahipak GS-320	Asahi Chemical (Kawasaki, Japan)	500×7.6 mm I.D.	Vinyl alcohol copolymer	Size exclusion
Asahipak GS-320H	Asahi Chemical	250×7.6 mm I.D.	Vinyl alcohol copolymer	Size exclusion
Asahipak ES-502C	Asahi Chemical	100×7.6 mm I.D.	Vinyl alcohol copolymer	Cation exchange
TSK gel ODS-120T	Tosoh (Tokyo, Japan)	150×4.6 mm I.D.	Octadecyl silicated silica gel	Hydrophobic adsorption
YMC pack AM-312	Yamamura Chemical (Kyoto, Japan)	150×6.0 mm I.D.	Octadecyl silicated silica gel	Hydrophobic adsorption

TABLE II

CHARACTERISTICS OF SAMPLES

Substance	Primary structure	M_r	pI*	Hydrophobicity (Σf)
1. Human serum albumin (HSA)		69 000	4.7- 5.2**	
2. Myoglobin		17 500		
3. Lysozyme		14 300	11.0-11.4**	
4. Insulin (bovine pancrease)	Gly-Ile-Val-Glu-Gln-Cys-Cys-Ala-Ser-Val-Cys-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-|Tyr-Cys-Asn Phe-Val-Asn-Glu-His-Leu-Cys- Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys- Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala	5750	5.3- 5.8**	32.80
5. Insulin B chain S-sulphonate	Phe-Val-Asn-Glu-His-Leu-Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala	3496	6.05	24.05
6. β-Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu	3465	9.93	16.80
7. Insulin A chain S-sulphonate	Gly-Ile-Val-Glu-Gln-Cys-Cys-Ala-Ser-Val-Cys-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-Asn	2530	3.12	11.04
8. α-Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr	1746	6.85	6.94
9. Neurotensin	Pyr-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu	1673	9.85	12.12
10. Mastoparan	Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2	1479	12	14.57
11. Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	1348	12	8.61
12. Angiotensin I	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu	1297	7.7**	10.61
13. [Val^5]-angiotensin I	Asp-Arg-Val-Tyr-Val-His-Pro-Phe-His-Leu	1283	7.7**	10.08
14. Asn^1,Val^5-angiotensin I	Asn-Arg-Val-Tyr-Val-His-Pro-Phe-His-Leu	1282	9.85	9.05
15. Lys-bradykinin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	1188	12.50	8.87
16. Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	1060	12.50	8.35
17. Angiotensin II	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe	1046	7.75	8.85
18. [Val^5]-angiotensin II	Asp-Arg-Val-Tyr-Val-His-Pro-Phe	1032	7.75	8.32
19. Oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2	1007	7.7**	5.66
20. Angiotensin III	Arg-Val-Tyr-Ile-His-Pro-Phe	931	9.85	8.87
21. Des-Arg^9-bradykinin	Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	904	11.05	7.65
22. Leu-Trp-Met-Arg-Phe-Ala		883	11.05	8.85
23. Leu-Trp-Met-Arg-Phe		812	11.05	8.32
24. Leu-Trp-Met-Arg		665	11.05	6.08
25. Val-Glu-Glu-Ala-Glu		576	3.02	1.78
26. [Met^5]-enkaphalin	Tyr-Gly-Gly-Phe-Met	574	5.90	5.02
27. [Leu^5]-enkaphalin	Tyr-Gly-Gly-Phe-Leu	556	5.90	5.93
28. Leu-Trp-Met		509	5.96	5.38
29. Substance P fragments 1-4	Arg-Pro-Lys-Pro	497	11.44	3.24
30. Val-Gly-Ser-Glu		390	3.29	0.83
31. Ala-Gly-Ser-Glu		362	3.29	−0.37

(Continued on p. 74)

TABLE II (continued)

Substance	Primary structure	M_r	pI*	Hydrophobicity (Σf)
32. Ser-His-Asp		357	4.95	−0.81
33. Glu-Gly-Phe		351	3.29	2.17
34. Glu-Ala-Glu		347	3.12	0.39
35. Liver cell growth factor	Gly-His-Lys	340	9.95	0.29
36. Tyr-Leu		316	5.96	3.69
37. Met-Ala-Ser		307	5.96	1.05
38. Arg-Asp		289	6.75	0.68
39. Glu-Glu		276	3.12	−0.14
40. Gln-Gln		274	5.96	−2.18
41. Asp-Lys		261	6.71	0.50
42. Met-Thr		250	5.96	0.82
43. $(Gly)_4$		246	5.96	0
44. Asp-Leu		246	3.10	1.92
45. His-Ser		242	7.81	−0.79
46. Ala-His		226	7.81	0.30
47. Gly-Phe		222	5.96	2.24
48. Glu-Gly		204	3.29	−0.07

*Values calculated by Manabe's program [5].
**Values listed in Handbook of Biochemistry [4].

RESULTS AND DISCUSSION

Table III lists the retention volumes found for various proteins and peptides with acidic and neutral aqueous eluents containing 5% acetonitrile on the GS-320 column and 20% acetonitrile in the ODS column. On the ODS column, the retention volumes were lower with acidic than with neutral eluents for all peptides except Asn[1], Val[5]-angiotensin I, Lys-bradykinin, and Leu-Trp-Met-Arg. On the GS-320 column, in contrast, neutralization of the mobile phase increased the retention volume for most of the peptides. The relation of peptide retention volumes to isoelectric points and hydrophobic fragmental constants, as shown in Figs. 1–5, indicates that the peptide separation mechanism of the GS-320 column is different from that of the ODS column. With both the acidic and the neutral eluent, the peptide retention volumes on the ODS column clearly correlated with their hydrophobic fragmental constants, thus implicating hydrophobic adsorption as the main mechanism of separation. The molecular masses of the peptides were also found to correlate with the retention volumes on the ODS column, apparently because of the general correspondence between hydrophobic constants and molecular size (Fig. 2). As shown in Fig. 3, on the other hand, no correlation was found between retention volumes and isoelectric points.

Figs. 4–6 show the chromatographic behaviour of peptides on the GS-320 column with neutral eluent, on the basis of their molecular masses, hydrophobic fragmental constants and isoelectric points. As shown in Fig. 4, many substances were found to elute much later than would be expected simply on the basis of molecular size. Fig. 5 shows the relationship between the hydrophobic fragmental

TABLE III

RETENTION VOLUMES OF PEPTIDES ON GS-320H AND ODS COLUMNS

Conditions for Asahipak GS-320H column (250 mm×7.6 mm I.D.): mobile phase, 0.05% trifluoroacetic acid (pH 2.4) or 50 m*M* ammonium acetate (pH 6.7)–acetonitrile (95:5); flow-rate, 1.0 ml/min; sample concentration, 1.0 mg/ml; sample volume, 2 μl; detection, UV at 220 nm; temperature, 30°C. Conditions for TSK gel ODS-120T column (150×4.6 mm I.D.): mobile phase, 0.05% trifluoroacetic acid (pH 2.4) or 50 m*M* ammonium acetate (pH 6.7)–acetonitrile (80:20); flow-rate, 0.6 ml/min; other conditions as for GS-320H.

Substance	GS-320H		ODS	
	pH 2.4	pH 6.7	pH 2.4	pH 6.7
1. Human serum albumin	4.07	4.48	–*	–
2. Myoglobin	4.24	5.57	–	–
3. Lysozyme	4.71	–	–	–
4. Insulin (bovine pancrease)	–	7.82	–	–
5. Insulin B chain S-sulphonate	–	12.19	–	–
6. β-Endorphin	5.48	17.39	–	–
7. Insulin A chain S-sulphonate	–	5.44	–	1.82
8. α-Endorphin	7.48	8.37	12.82	6.08
9. Neurotensin	8.74	14.35	13.09	7.12
10. Mastoparan	5.84	40.79	–	–
11. Substance P	7.60	71.00	73.22	–
12. Angiotensin I	6.57	20.35	47.15	31.25
13. [Val^5]-angiotensin I	6.43	17.64	26.40	19.12
14. Asn^1,Val^5-angiotensin I	6.25	28.71	23.14	26.59
15. Lys-bradykinin	6.24	20.52	6.68	6.68
16. Bradykinin	7.13	19.57	9.08	8.67
17. Angiotensin II	7.89	12.55	15.92	5.81
18. [Val^5]-angiotensin II	7.48	11.52	9.48	4.01
19. Oxytocin	10.73	16.75	7.27	No data
20. Angiotensin III	7.55	23.08	14.64	9.78
21. Des-Agr^9 bradykinin	10.20	15.96	20.89	9.66
22. Leu-Trp-Met-Arg-Phe-Ala	26.40	119.74	53.47	No data
23. Leu-Trp-Met-Arg-Phe	36.21	112.37	76.92	No data
24. Leu-Trp-Met-Arg	11.88	32.16	7.17	10.10
26. [Met^5]-enkephalin	No data	No data	9.61	4.62
27. [Leu^5]-enkephalin	38.51	10.73	13.01	3.89
28. Leu-Trp-Met	50.28	27.60	20.45	9.37
33. Glu-Gly-Phe	10.41	7.39	3.93	1.83
36. Tyr-Leu	13.63	10.93	4.32	2.38
37. Met-Ala-Ser	7.33	7.50	2.05	1.80
41. Asp-Lys	6.42	7.02	1.88	1.67
42. Met-Thr	7.66	7.77	2.12	1.82
43. $(Gly)_4$	6.90	7.23	1.86	1.69
45. His-Ser	6.44	7.80	1.87	1.73
47. Gly-Phe	9.79	9.08	3.55	2.09

*Not eluted within 120 min.

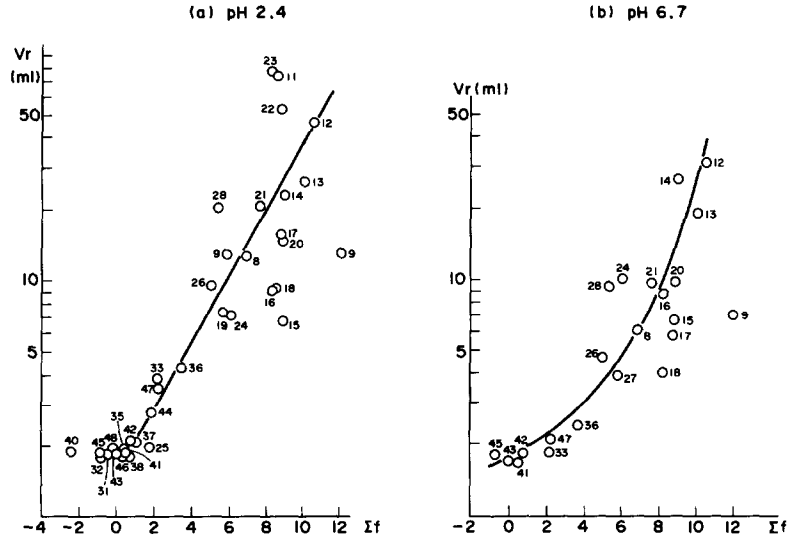


Fig. 1. Relationship between peptide hydrophobicity and retention volume on the ODS column. Numbers indicate peptides as listed in Table II. (a) pH 2.4; correlation = 0.74; (b) pH 6.7; correlation = 0.77 (except No. 7). Conditions as in Table III.

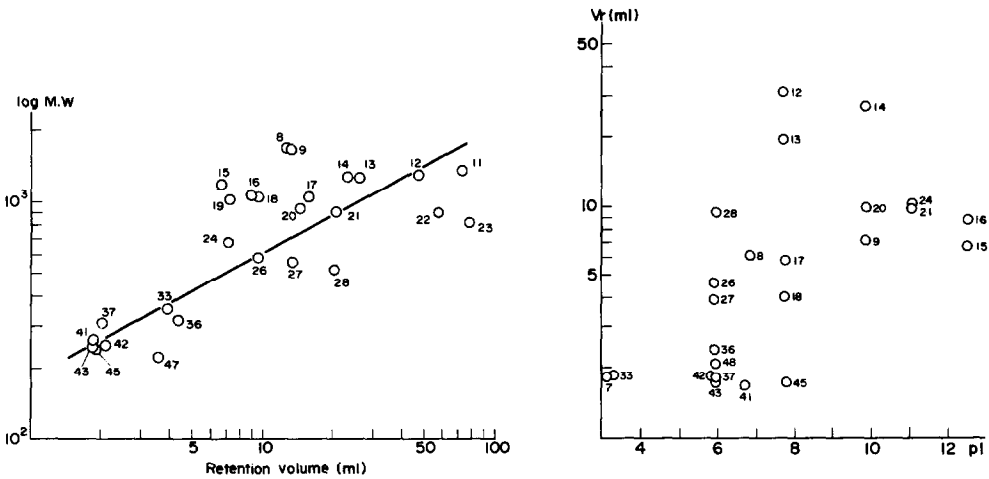


Fig. 2. Relationship between peptide molecular mass and retention volume on the ODS column with 0.05% trifluoroacetic acid (pH 2.4)–acetonitrile (80:20). Numbers indicate peptides as listed in Table II. Conditions as in Table III.

Fig. 3. Relationship between peptide isoelectric point and retention volume on the ODS column with 50 m*M* ammonium acetate (pH 6.7)–acetonitrile (80:20). Numbers indicate peptides as listed in Table II. Conditions as in Table III. Correlation factor = 0.37.

constants of the peptides and their retention volumes, with aqueous eluent containing 50 m*M* ammonium acetate and 5% acetonitrile (v/v). The retention volumes of the neutral peptides (Fig. 5a) correlated closely with their hydrophobic constants, thus confirming hydrophobic interaction to function as an effective

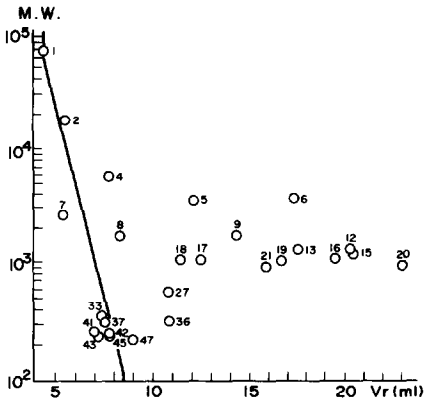


Fig. 4. Relationship between peptide molecular mass and retention volume on the Asahipak GS-320H column with 50 m*M* ammonium acetate (pH 6.7)-acetonitrile (95:5 v/v). Numbers indicate peptides as listed in Table II. Other conditions as in Table III.

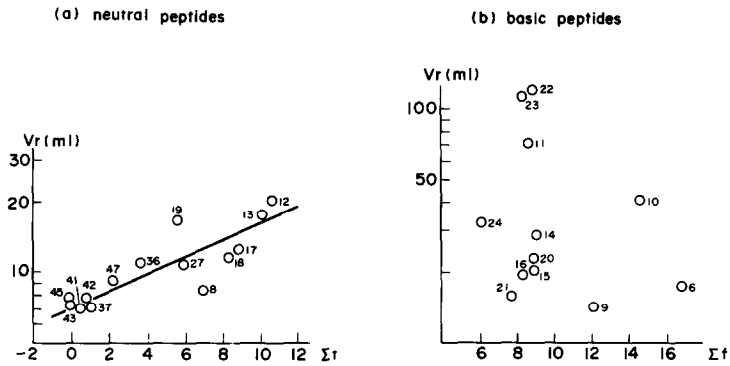
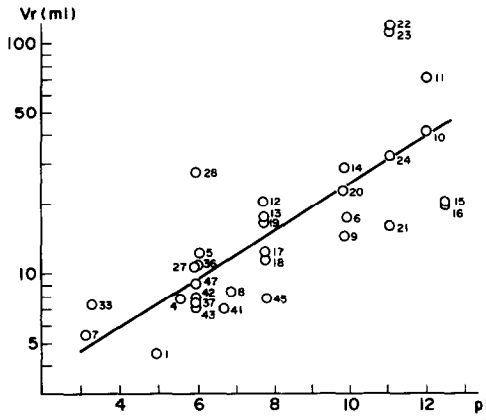


Fig. 5. Relationship between hydrophobicity of neutral or basic peptides and retention volume on the Asahipak GS-320H column. Conditions as in Fig. 4. Numbers indicate peptides as listed in Table II. (a) Neutral peptides; correlation = 0.71; (b) basic peptides; correlation = 0.06.

separation mechanism on this column, though more weakly than on the ODS column as indicated by the low acetonitrile concentration at which the correlation is observed and the low slope of the correlation curve. For basic peptides, on the other hand, no such correlation was found (Fig. 5b), thus indicating that their separation is effected by some mechanism other than hydrophobic interaction.

On the same column, electrostatic interaction also appears to function as a separation mechanism, as shown by the correlation between peptide retention volumes and isoelectric points in Fig. 6. The basis for such a mechanism is indicated by the titration curves for unmethylated and methylated GS-320 gel, shown in Fig. 7. The difference between the curves may be attributed to the presence of carboxyl groups on the gel polymer and the effect of methylation on these groups. These results thus indicate that, in chromatographic analysis of peptides on the GS-320 column with neutral buffer solution as the mobile phase, electrostatic interaction with the carboxyl groups on the gel polymer occurs, with retardation



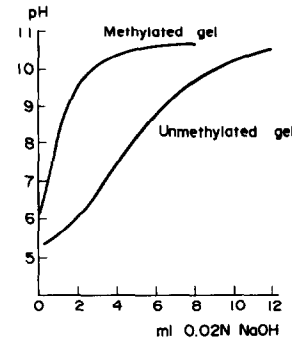


Fig. 6. Relationship between peptide isoelectric point and retention volume on the GS-320H column. Conditions as in Fig. 3. Correlation factor = 0.58. Numbers indicate peptides as listed in Table II.

Fig. 7. Titration curves of unmethylated and methylated GS-320 gel.

of basic, positively charged peptides by ion exchange and accelerated elution of acidic, negatively charged peptides by ion exclusion.

Application to multimode chromatography

These results suggest that multimode chromatography for peptide analysis can be performed on the GS-320 column, by combining its size-exclusion, hydrophobic adsorption, ion-exchange and ion-exclusion mechanisms. This was attempted, and found to be effective, in the following applications. Isocratic separation of a mixture of high-molecular-mass proteins and low-molecular-mass dipeptides was attempted on the GS-320 and ODS columns, with the results as shown in Fig. 8. On the GS-320, with a single eluent used throughout, clear separation and high recovery rates (Table IV) were obtained for all samples. As indicated by Fig. 9, human serum albumin, myoglobin, insulin B chain, α-endorphin, Leu-enkephalin and Gly-Phe were apparently separated by size exclusion, whereas the separation of Tyr-Leu, Met-enkephalin, bradykinin and Leu-Trp-Met-Arg involved other mechanisms.

Two separate analyses were necessary on the ODS column, as proteins elute with eluents containing ca. 50% or more acetonitrile, while peptides tend to elute simultaneously at such high acetonitrile concentrations. As shown in Fig. 8, the separation on the ODS column, even with two analyses, was incomplete for the peptides and practically non-existent for the two proteins, and the elution order was different from that observed on the GS-320 column, apparently reflecting a difference in separation mechanisms. Fig. 10 shows the separation of dipeptides with low hydrophobicity on the GS-320 and ODS columns. As shown, the hydrophilic dipeptides Glu-Glu, Glu-Gly and Gln-Gln eluted on the GS-320 with clear separation, in order of increasing isoelectric point. On the other hand, the three dipeptides with the same isoelectric point, Gln-Gln, Gly-Phe and Tyr-Leu, eluted in the order of increasing hydrophobicity, also with good separation. The same

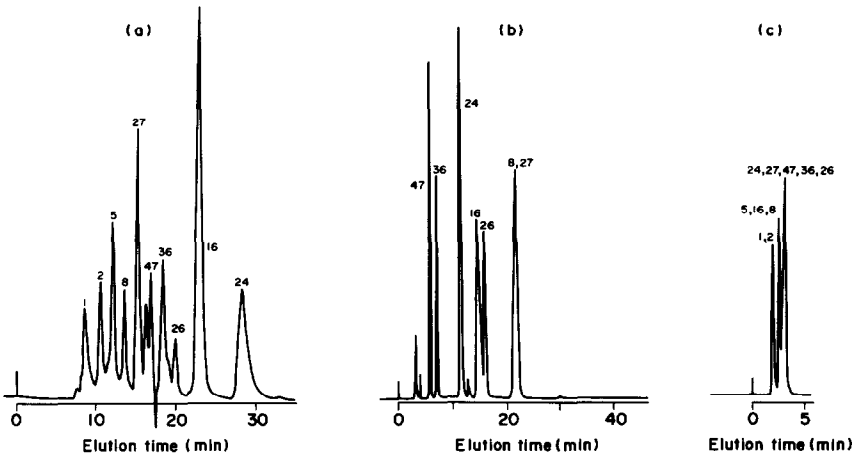


Fig. 8. Separation of protein and peptide mixture. Numbers indicate peptides as listed in Table II. Columns: (a) GS-320 (500×7.6 mm I.D.); (b) and (c) TSK gel ODS-120T (150×4.6 mm I.D.). Mobile phases: (a) 50 m*M* ammonium acetate (pH 6.7)–acetonitrile (80:20, v/v); (b) 0.05% trifluoroacetic acid (pH 2.4)–acetonitrile (80:20); (c) 0.05% trifluoroacetic acid (pH 2.4)–acetonitrile (50:50, v/v). Samples as shown in Table IV. Flow-rates: (a) 1.0 ml/min; (b) and (c) 0.6 ml/min. Detection, UV at 220 nm; temperature, 30°C; sample concentration, 1.0 mg/ml total. Volume injected: (a) 25 μl; (b) and (c) 10 μl.

TABLE IV

RECOVERY OF PEPTIDES ON GS-320 COLUMN

Sample concentration, 1.0 mg/ml each; volume injected, 20 μl. Other conditions as in Fig. 7.

Compound	M_r	Recovery (%)
1. Human serum albumin	69 000	89
2. Myoglobin	17 500	77
5. Insulin B chain	3496	92
8. α-Endorphin	1684	93
27. Leu-enkephalin	556	94
47. Gly-Phe	222	104
36. Tyr-Leu	316	99
26. Met-enkephalin	556	94
16. Bradykinin	1188	103
24. Leu-Trp-Met-Arg	665	104

elution order was observed on the ODS column, but with little or no separation of the first three dipeptides and excessive separation of the latter three. These results indicate that the predominant separation mechanism in the ODS column is a hydrophobic interaction considerably stronger than that of the GS-320, with little or no ionic interaction, and that this precludes separation of hydrophilic peptides. They indicate further that the GS-320 can effectively separate such peptides, through a mechanism of electrostatic interaction.

Fig. 11 shows the separation of seven small-molecule peptides with low hydrophobicity on the GS-320 column, and also on ODS and ion-exchange columns.

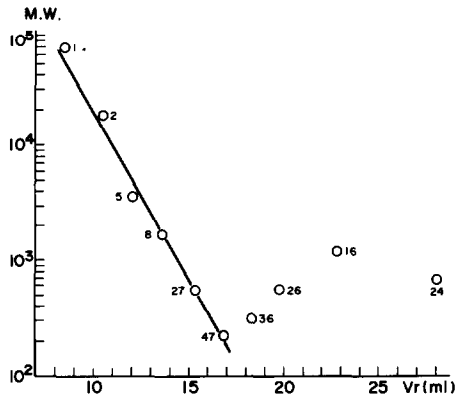


Fig. 9. Relationship between peptide molecular mass and retention volume on the GS-320 column. Samples as in Table IV. Conditions as in Fig. 8.

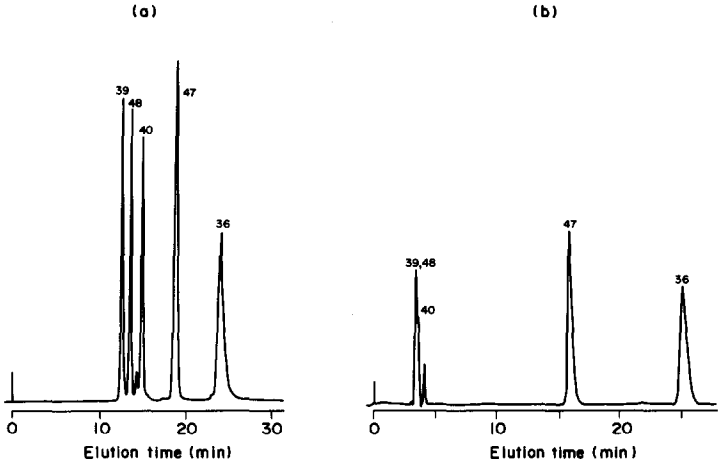


Fig. 10. Separation of dipeptides. Numbers indicate peptides as listed in Table II. Columns: (a) GS-320 (500×7.6 mm I.D.); (b) TSK gel ODS-120T (150×4.6 mm I.D.). Mobile phases: (a) 50 m*M* ammonium acetate (pH 6.7); (b) 0.05% trifluoroacetic acid (pH 2.4)–acetonitrile (90:10, v/v). Flow-rates: (a) 1.0 ml/min; (b) 0.6 ml/min. Volume injected: (a) 15 μl; (b) 10 μl. Other conditions as in Fig. 8.

On the GS-320, the peptides eluted in the order of increasing isoelectric point. As shown by the accompanying plot of molecular mass against elution time, the acidic peptides (25, 34, 30) eluted earlier than would be predicted from a calibration curve based simply on molecular mass, and the basic peptides (35, 29) later, indicating elution acceleration by ion exclusion for the former group and retardation by ion exchange for the latter. On the ODS column the same peptides generally eluted in the order of increasing hydrophobicity, indicating this to be the predominant mechanism. On the ES-502C ion-exchange column, the elution order was the same as that on the GS-320, thus showing that the main mechanism for these peptides on the GS-320 is electrostatic interaction. The clearer, better balanced separation by the GS-320 may be attributed to an ion-exchange group

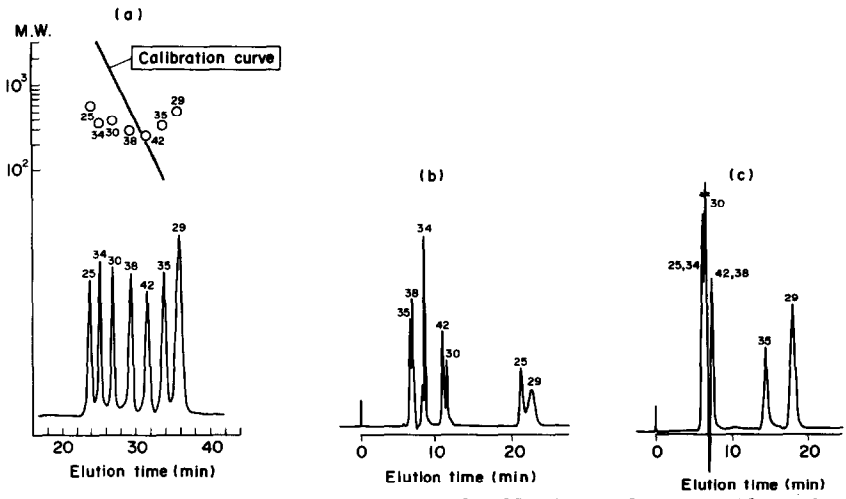


Fig. 11. Separation of hydrophilic oligopeptides. Numbers indicate peptides as listed in Table II. Columns: (a) GS-320 (500×7.6 mm I.D.); (b) YMC pack AM-312 (150×6.0 mm I.D.); (c) ES-502C (100×7.6 mm I.D.). Mobile phases: (a) 50 m*M* ammonium acetate (pH 6.7); (b) 0.05% trifluoroacetic acid (pH 2.4)-acetonitrile (95:5, v/v); (c) 50 m*M* ammonium acetate (pH 6.7) containing 200 m*M* sodium chloride. Flow-rates: (a) 0.5 ml/min; (b) 1.0 ml/min; (c) 0.5 ml/min. Volume injected, 15 μl. Other conditions as in Fig. 10.

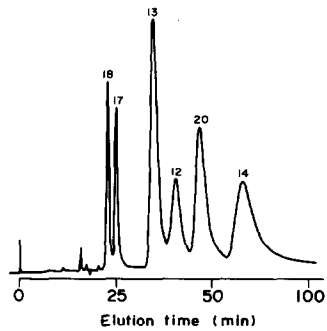


Fig. 12. Separation of angiotensins on the GS-320 column. Numbers indicate peptides as listed in Table II. Mobile phase, 50 m*M* ammonium acetate (pH 6.7)-acetonitrile (95:5); flow-rate, 1.0 ml/min; volume injected, 20 μl. Other conditions as in Fig. 10.

concentration in the gel packing, which is more appropriate than that of the ES-502C for separation of these peptides.

The separation of angiotensins on the GS-320 column is shown in Fig. 12. The results show that for these strongly hydrophobic peptides, also, the primary separation mechanism on the GS-320 is one of electrostatic interaction, while a mechanism of hydrophobic interaction is also present and serves to separate peptides with the same isoelectric point.

CONCLUSION

The Asahipak GS-320 exhibits a combination of size-exclusion, hydrophobic interaction and electrostatic mechanisms, which can be readily used for analysis

of peptides with neutral eluents (pH 6.7). The resulting multimode chromatography is in many cases more effective than the single-mode chromatography of ODS and ion-exchange columns, for mixtures of substances varying in either hydrophobicity or isoelectric point. It also allows efficient separation of hydrophilic peptides, which is difficult or impossible on ODS columns. All of these separations can be obtained on the GS-320 with isocratic elution. The stability of the GS-320 gel in neutral aqueous solutions containing inorganic salts, due to its polymeric structure, also facilitates multimode chromatography. The column may, therefore, be expected to find an expanding range of applications in HPLC, for separation of peptides and many other biological substances on the basis of both hydrophobicity and electrostatic charge.

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