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RECENT PROGRESS IN GEL PACKING MATERIALS AND DETECTORS FOR MODERN LIQUID CHROMATOGRAPHY IN JAPAN

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SUMMARY

Developments in rigid polymer gels for size exclusion, ion-exchange and reversed-phase chromatography are described, especially products which are commercially available in Japan. New detectors for high-performance liquid chromatography are reviewed.

HISTORICAL INTRODUCTION

In 1958 I set up The Research Group of Automatic Liquid Chromatography in Japan, the aim of which is to modernize liquid chromatographic techniques and to promote liquid chromatographic research. It meets once a year and has held workshops with leaders in liquid chromatography every summer since 1963.

The first contribution by this group to the automation of liquid chromatography was in developing the Hitachi Model KLF-1 automatic liquid chromatograph which is equipped with a two-wavelength spectrophotometric detector, and was reported in 1961[1] and 1962[2]. This instrument had an automatic fractionation mode and the detector enabled the selection of two wavelengths from 200 to 750 nm for simultaneous spectrophotometric detection and recording after colour development (chemical reaction).

I had previously published a book on automatic amino acid analysis[3]. An automatic amino acid analyzer (Hitachi Models KLA-1 and KLA-2)[4], equipped with three constant-flow delivery pumps and a three-wavelength flow-photometric detector utilizing a continuous-flow mode, was produced by myself and my colleagues immediately afterwards. The photometric detector was used at 570, 440 and 640 nm. The wavelength of 570 nm was most effective for detection of ninhydrin colour reaction with ordinary alpha-amino acids, 440 nm was for imino acids, proline and hydroxyproline and 640 nm for measurements of one-third the intense absorbance of large amounts of eluted amino acids.

Since 1964, The Research Group has published a series of books on automatic and modern liquid chromatography[5–11] and since 1977, data books[12] on high-performance liquid chromatography (HPLC), under the sponsorship of The Japan Society for Promotion of Science and of The Promotion Bureau of Science and Technology.

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In 1973, further development of a coulometric detector was reported by Muto and Takata[13] and of a spectrofluorimetric detector for HPLC by Hatano *et al.*[14]. These efforts[15,16] eventually led to the U.S.–Japan Seminar of Advanced Techniques of Liquid Chromatography, held at Boulder, Colorado, in 1978[17] and to Micro-capillary Techniques in Liquid Chromatography, held at Honolulu in Hawaii in 1982[18] under the sponsorship of The National Science Foundation, U.S. and of The Japan Society for Promotion of Science. The 18th International Symposium on Advances in Chromatography was held at Tokyo in 1982[19], and The International Symposium on High Performance Liquid Chromatography was held at Kyoto in 1985[20], under the sponsorship of The Japan Society for Promotion of Science and of The Science Council of Japan. These meetings were also co-sponsored by The Chemical Society of Japan, The Japan Society for Analytical Chemistry, The Japanese Biochemical Society, The Agricultural Chemical Society of Japan, Japan Analytical Instrumental Manufacturer's Association and the Nissan and the Inoue Science Foundations. Several books on modern liquid chromatography have been published by members of this Research Group[21–26].

Recently, a quite unique detector, an electron spin resonance spectrometric detector, was linked with a high-performance liquid chromatograph[27], which enabled the separation not only of stable free radicals but also trapped spin adducts of unstable radicals, produced in chemical reactions and biological processes. A review paper on liquid chromatographic detectors[28] was read in Melbourne. Developments in ion chromatography were reported by Hanaoka *et al.*[29]. Several distinguished works on miniaturization of columns and instruments have been reported by Ishii and co-workers since 1977[30–33], on microbore packed columns by Tsuda and Novotny[34] and by Rokushika and Hatano[35]. The combination of a high-performance liquid chromatograph with a mass spectrometer[36,37], with ion-selective electrodes[38] and with spectroscopic detection system[39] were proposed.

GEL PACKING MATERIALS AND COLUMN TECHNOLOGY

Various porous polymer gels for size exclusion and ion-exchange chromatography have been reported[40–44] These aqueous and non-aqueous materials are commercially available as Hitachi gels from Hitachi, Shodex gels from Showa Denko Co., as Shim-pack gels from Shimadzu, as TSK gels from Toyo Soda Co., as Asahi gels from Asahi Kasei Co., as Fine-pak gels from Jasco Co. and as MCI gels from Mitsubishi Kasei Co.

Polystyrene gels are copolymers of polystyrene–divinylbenzene, and polyester gels comprise polyacrylate, polymethacrylate or polyvinyl acetate. Polyacrylamide has been used as a base material for soft gel packings. The other gels are hydroxymethyl, carboxyl, quaternary ammonium and sulphonated derivatives of the copolymers. These gels have large surface areas, over 300 m^2/g, pore sizes of 40–1000 Å and particle sizes of 5–20 μm. All of these gels can be used with both aqueous methanol and non-aqueous hexane–methanol mobile phases in normal or reversed-phase chromatography[45].

The separation behaviours of biologically active molecules on these gel columns are characteristic and the biological activities of molecules are completely recoverable. Moderate separations and effective recoveries can be obtained on size

exclusion gel columns. Ion-exchange resins have been used for separation of relatively small molecules such as amino acids and nucleic acid constituents. Recently, improved ion exchangers have been introduced for separation of large molecules such as proteins and nucleic acids. Progress in the separation of biomolecules has also been reported in reversed-phase liquid chromatography. Developments in high-performance columns[46] and packing materials[47] have been described.

GEL PACKING MATERIALS FOR SEPARATION OF PROTEINS AND NUCLEIC ACIDS

Proteins and nucleic acids are polyvalent ionic compounds with high molecular weights and which sometimes show oligomeric association in solution. The ionic dissociation of such molecules enables their separation by ion exchange, the high molecular sizes by size exclusion and the large hydrophobic molecular skeletons by reversed-phase chromatography.

Porous polymer gel packings for HPLC columns can be classified into three groups: wholly inorganic silica, wholly organic synthetic polymer and silica chemically bonded to organic molecules. For high resolution, the gel packing should comprise homogeneous spherical fine particles with extremely small particle diameters, homogeneous pore size and even homogeneous pore depth. Among these properties, it is important that the pore size is relatively large for separation of large biomelecules and loading capacity is as large as possible for the preparation of large amounts of biomolecules, *e.g.*, capacity of a gel packing with a pore size of 300 Å is about 50–100 kilodaltons). The column size, length and internal diameter, is relatively less important for the separation of large biomolecules, contrary to the separation of small molecules such as amino acids and nucleic bases. Gel packing materials for size exclusion chromatography are classified into soft gel, semi-hard gel and hard gel depending upon their mechanical strength. The polystyrene–divinylbenzene copolymer is a semi-hard gel and is useful for high-performance size exclusion chromatography. All TSK gel Type H, Shodex gel A, Shimadzu gel HSG and Hitachi Gelko GL materials are polystyrene gels and are used for non-aqueous gel permeation chromatography of synthetic polymer compounds, the molecular weights of which are $< 10^8$. TSK gel Type PW (polyacrylate), Type Phenyl-PW (polyacrylate phenyl derivative), Shodex OH-pak (glyceryl methacrylate), Asahi-pak Typ GS (polyvinyl alcohol), Shim-pack HSG-W and Hitachi GL-W520 materials are used for aqueous gel filtration chromatography of proteins and nucleic acids, the exclusion limits of which are $< 10^5$. Toyo-Pearl Type HW is an hydrophilic vinyl polymer and is used for the exclusion limit ranges from 10^3 to 10^7, for preparation of biopolymers[48].

It is important that the biological activities of molecules such as enzymes and hormones should not be lost during the separation procedures. The wholly organic synthetic polymer gel might be most effective for the separation of such compounds.

SIZE EXCLUSION GELS FOR PROTEIN AND NUCLEIC ACID SEPARATION

Porous synthetic polymer gels of methacrylate, amide and polyvinyl resin, and porous silica gels, have been used for aqueous gel filtration of proteins and nucleic acids. Pressure-resistant and less adsorptive aqueous polymer gels are presented in Table I[49,50]. The most suitable pore size for the separation of proteins is probably

TABLE I

HARD POLYMER GELS (COMMERCIALLY AVAILABLE IN JAPAN) FOR AQUEOUS SIZE EXCLUSION CHROMATOGRAPHY OF PROTEINS AND NUCLEIC ACIDS

Type	*Gel material*	*Trade-name*	d_p *(μm)*	*Pore size (Å)*	*Exclusion limit*	*Supplier*
Synthetic polymer	Hydrophilic methacrylate	MCI gel CQP10	10	100		Mitsubishi-Kasei
		30	10	300		
	Glyceryl methacrylate	Shodex OH-PakQ 801	10		2×10^3	Showa Denko
		802	10		5×10^3	
		OH-pakB 803	15		1×10^5	
		804	15		5×10^5	
		805	15		5×10^6	
		806	15		10^7	
	Hydrophilic acrylate polymer	TSK gel PW				Toyo Soda
		G-1000	10		10^3	
		2000	10		5×10^3	
		3000	10		5×10^4	
		4000	10		3×10^5	
		5000	10		1×10^6	
		6000	10		10^7	
		TSK gel PW_{XL}				
		G-2500	6			
		3000	6		8×10^5	
		4000	10		4×10^6	
		5000	10		1×10^7	
		6000	13		2×10^8	
		$GM\text{-}PW_{XL}$	13		2×10^8	
		G-Oligo-PW	6			
		G-DNA-PW	10			
	Hydrophilic vinyl polymer	Toyo Pearl EW-35			5×10^3	
		Toyo Pearl HW series (6 grades)			1×10^4 ~ 5×10^7	
	Polyvinyl alcohol	Asahi-pak GS310, 320	9		4×10^4	Asahi Kasei
		GS510, 520	9		3×10^5	
	Hydrophilic porous polymer	Gelko GL-W520			6×10^3	Hitachi
		530			5×10^4	
		550			2×10^6	
		Shim-Pack HSG-30W				Shimadzu
Porous silica	Glycerylpropyl-silica	Shodex Protein WS 802	9	150	2×10^4	Showa Denko
		803	9	300	8×10^5	
	Hydroxy-silica	Shim-pack Diol-150	5	150	2×10^5	Shimadzu
		Diol-300	5	300	1×10^6	
	Hydrophilic silica	MCI GEL CQS10	9–11	90 ~ 110		Mitsubishi-Kasei
		S30	9–11	250 ~ 350		
		TSK gel SW				Toyo Soda
		G-2000	10	130	6×10^4	
		3000	10	240	3×10^5	
		4000	10	450	1×10^6	

100–500 Å and the exclusion limits of the gel packings should be selected according to the nature of the sample mixtures.

Glycerylpropylsilica is slightly adsorptive, leading to deviations from a linear

relationship between retention volumes and logarithms of molecular weights. Such gels have been improved for sufficient recoveries of biological activities of proteins[51].

A successful separation of lipoproteins on TSK gel columns was reported and compared with the results achieved by ultracentrifugation, and the medical significance of the separated lipoproteins was classified in relation to liver disease[52].

The separation of oligonucleotides has been reported[53] using the Asahipak GS320 columns. Oligonucleotides in a mixture of three kinds of deoxyhexamer (dCGTCCA, dTGTCCA and dGGTCCA), and of ribosomal ribonucleic acids (rRNA) in a mixture of 23S (MW 1 100 000), 16S (MW 550 000) and 5S (MW 160 000) rRNA particles were separated on Asahi-pak GS320 and GS520 columns respectively. It has been shown that these gel packings are very useful in the developing fields of genetics and biotechnology.

ION EXCHANGERS FOR PROTEIN AND NUCLEIC ACID SEPARATION

The most useful ion exchanger for separation of proteins and nucleic acids is sulphonated polystyrene, which has been used for analysis of the amino acid constituents of proteins, nucleic bases, nucleosides and nucleotides of nucleic acids. The automatic amino acid analysis carried out on the ion-exchange resin column at Moore's laboratory in 1958 was a significant event in the fields of instrumental liquid chromatography and of protein chemistry. Protein separation had been performed by ion-exchange chromatography since 1954. However, the strongly acidic cation exchanger adsorb proteins and nucleic acids irreversibly owing to the hydrophobicity of their polystyrene structures. Porous glass chemically bonded to aminopropyltrimethoxysilane also adsorbs non-specifically owing to residual silanol groups on its surface. High-performance ion-exchange separations are performed by using ion exchangers coated with a thin epoxy resin layer or with polyamine films. Three kinds of ion exchangers are now available; pellicular coated ion exchanger on inactive carrier; wholly porous silica containing introduced ion-exchangeable groups and synthetic polymer gels containing ion-exchangeable groups. Ion exchangers for rapid and high-performance separations should be mechanically strong enough to withstand a linear velocity of 1 mm/sec, exhibit reversible adsorption for high recoveries, have large exchange capacity for preparative separations (pore diameter 30–100 nm, pore depth 0.5–1.0 ml/g), be stable over wide ranges of pH, be homogeneously spherical in shape with diameter 5–10 μm and, of course, be in expensive even for large preparative columns. The ion exchangers available for separation of proteins, amino acid and nucleic acids are presented in Table II[45,51].

The TSK gel 2SW is suitable for separation of peptides, nucleotides and other relatively small molecules, and also of oligomers. TSK gel 3SW and Shodex gel Ax-pak U424 are used for separation of proteins and enzymes. The polyacrylate or polymethacrylate polymer gels are better for separation of proteins and enzymes rather than the polystyrene–divinylbenzene copolymer gels owing to lower adsorption of proteins. The TSK and Shodex gels comprise fine particles of small diameter, 5–10 μm. However, the macroporous gels with larger particle diameters of 30–70 μm are better for preparative separations of larger biomolecules.

The TSK gel 5PW series have large pore sizes of about 1000 Å and are very efficient in separating proteins and nucleic acids. Beautiful separation patterns of

TABLE II

HARD POLYMER GELS (AVAILABLE IN JAPAN) FOR ION-EXCHANGE CHROMATOGRAPHY OF PROTEINS, AMINO ACIDS AND NUCLEIC ACIDS

Type	*Material trade-name*	*Functional group*	d_p *(μm)*	*Exchange capacity (mequiv./g)*	*Pore size (Å)*	*Supplier*
Wholly porous silica	TSK gel DEAE 2SW	$-N(C_2H_5)_2$	5	>0.3	130	Toyo Soda
	CM 3SW	$-COOH$	10	>0.3	240	
	DEAE 3SW	$-N(C_2H_5)_2$	10	>0.3	240	
	Shodex Axpak U424	Polyamine	10	>0.3	300	Showa Denko
Porous polymer gel	TSK gel SP5PW	$-SO_3-$	10,15	>0.3	1000	Toyo Soda
	DEAE 5PW	$-N(C_2H_5)_2$	10,15	>0.3	1000	
Polystyrene–divinylbenzene	Hitachi resin 2600 series	$-SO_3-(Na^+)$	13–17			
	Shodex CX pak	$-SO_3-(Na^+)$				Showa Denko
	Shim pack ISC-07	$-SO_3-$	7			Shimadzu
	MCI gel CK	$-SO_3-$	5,7			Mitsubishi-Kasei
	Jasco AA pak	$-SO_3$	5			Jasco

mixtures of oligo deoxynucleotides have been obtained on TSK gel DEAE-PW500[53]. The mixtures contained eight kinds of oligodeoxynucleotides from the hexamer to the 26-residue oligomers: dCATGGT, dCTAAATC, dCGGGATTTGA, dCGACCCGGGT, dCATCTTCATGGC, unknown sequential 16-residual oligomer, dCCIAAITCCATCCAICCITAIGC, dCCIAAITCCATCCAICCCATITAITC, where D = deoxy, C = cytidine, A = adenine, G = guanine, I = inosine and T = thymine. Some polystrene–divinylbenzene copolymers in Table II, such as the Hitachi 2600 series, etc., are efficient ion exchangers for analysis of amino acids.

REVERSED-PHASE GELS FOR PROTEIN AND NUCLEIC ACID SEPARATION

Hydrophobic and hydrophilic interactions among chemically bonded reversed-phase packings, aqueous mobile phase solvents with organic modifiers and amphoteric molecules of proteins and nucleic acids are extremely complicated in solution and result in complex separation behaviours on reversed-phase gel columns. Many kinds of reversed-phase packings have been used for efficient separation of nucleic bases, nucleosides and nucleotides (Table III)[50].

Cytochrome *c*, myoglobin, ribonuclease, lysozyme, α-chymotrypsin and α-chymotrypsinogen were separated on the TSK gel Phenyl-5PW[49]. This gel packing is an aqueous polymer chemically bonded to phenyl groups. It is very efficient for protein separation, although the separation mechanism is likely to be more complicated than solvophobic interaction in the reversed phase.

DETECTORS AND SYSTEMS FOR MODERN LIQUID CHROMATOGRAPHY

A new ion-selective electrode detector with an hydrophobic ion-exchange resin

TABLE III

CHEMICALLY BONDED REVERSED-PHASE PACKING MATERIALS AVAILABLE IN JAPAN

Polarity	*Trade-name*		*d_p (μm)*	*Functional group*	*Supplier*
None	Hitachi gel	#3050	10–15	Octadecylsilane	Hitachi
		#3053	4–6		
		#3056	4–6		
		#3063	5		
	Yana pak	ODS-N	5	Octadecylsilane	Yanaco
		ODS-A	7		
		ODS-T	10		
	Yanaco Pel	ODS	30–40		
	Fine-pak	SIL C_{18}	5,10	Octadecylsilane	Jasco
		C_{18}T	10	(capping)	
	ODS pak F	series	5,10	Octadecylsilane	Showa Denko
	Develosil	ODS	3,5,7,10	Octadecylsilane	Nomura Kagaki
			10–20,		
			15–30		
	Cosmosil	$5C_{18}$	5	Octadecylsilane	Nakarai Kagaku
		$5C_{18}$-P	5		
	TSK gel	ODS-120	5,10	Octadecylsilane	Toyo Soda
		120T	5,10	(capping)	
	Fine pak	SIL C_8	10	Octylsilane	Jasco
	Cosmosil	$5C_8$	5	Octylsilane	Nakarai Kagaku
	Develosil	C_8	3,5,7,10	Octylsilane	Nomura Kagaku
			10–20		
			15–30		
	Shim-pack	PC_8	10	Octylsilane	Shimadzu
	Cosmosil	5PH	5	Phenyl	Nakarai Kagaku
	Fine pak	SIL C_2	10	Ethyl	Jasco
	Fine pak	SIL C	10	Dimethylsilane	Jasco
	Yanapak	DMS	5	Dimethylsilane	Yanaco
	Cosmosil	5TMS	5	Trimethylsilane	Nakarai Kagaku
	Shim-pack	TMS	5	Trimethylsilane	Shimadzu
	TSK gel	TMS-250	10	Trimethylsilane	Toyo Soda
Weak	Fine pak	SIL OH	10	Hydroxyl	Jasco
	TSK gel	OH-120	5,10	Hydroxyl	Toyo Soda
Medium	Fine pak	SIL CN	10	Nitrile	Jasco
	Yana pak	CN	10	Nitrile	Yanaco
	Yanaco Pel	CN	35	Nitrile	Yanaco
	Cosmosil	5CN-R		Nitrile	Nakarai Kagaku
	Yanaco Pel	PA		Polyamide	Yanaco
High	Shim-pack	PNH_2	10	Amino	Shimadzu
	Yanaco Pel	NH_2	35	Amino	Yanaco
	Yana pak	NH_2	10	Amino	
	Shodex	NH pak		Amino	Showa Denko
	Fine-pak	SIL NH_2	10	Aminopropyl	Jasco
	TSK gel	NH_2-60	5,10	Aminopropyl	Toyo Soda
	Bile pak		10		Jasco
	Catechol pak		5		Jasco

membrane has been reported[38]. Hydrophobic anion-exchange resin membranes based on homogeneous cross-linked polystyrene membrane were used. The membranes showed good selectivity and high sensitivity for HPLC. A highly sensitive twin-electrode voltammetry detector for HPLC was developed by Kurahashi and co-workers[54], and a dual electrochemical detector for micro-HPLC by Goto *et al.*[55]. The latter had two working electrodes (anode and cathode) and was employed for the selective detection of catecholamine. A rapid determination could be performed by means of cyclic semi-differential voltammetry. The detector was utilized for the selective determination of catecholamine in human urine. Miniaturization of a electrical conductivity detector for a new micro-packed column ion chromatograph with a hollow-fibre suppressor has been achieved using a new micro-scale detection cell[35]. Other types of detectors are a streaming current detector and a spray detector for detection of nanogram amounts of carboxylic acids[56]. The spray detector showed almost equally high sensitivities for the detection of underivatized free and conjugated bile acids and their sulphates. The detection limit was about 100 μg, and a linear response was obtained for the injected amounts in the range 0.1–10 μg.

A time-resolved laser fluorescence detector was developed for highly sensitive detection in micro-HPLC by Imasaka *et al.*[57]. A sub-nanosecond tunable dye-laser pumped by a transversely excited atmospheric pressure nitrogen-laser was combined with the time-resolved detection system, which consisted of a microchannel plate photomultiplier, a sampling oscilloscope and a microcomputer for data processing. This system had a time resolution of 1.4 nsec, and was used for determination of trace amounts of polynuclear aromatic hydrocarbons.

Various detection systems have been employed with HPLC for qualitative and quantitative measurements of separated compounds. A HPLC–ESR (electron spin resonance) system was described above[27]. This instrument is very useful for free-radical chemistry, and many kinds of new radicals produced upon γ-irradiation of amino acids, peptides, nucleic bases, nucleosides and nucleotides in aqueous solution were discovered and characterized by Hatano and co-workers[58–61]. An extremely highly-sensitive infra-red spectrometer for HPLC was reported by Mori *et al.*[62]. The sensitivity of the detection system was at least 40 times greater than that of conventional instruments, for acrylonitrile–styrene and styrene–methyl methacrylate copolymers. Inductively coupled argon-plasma-emission spectrometric detection for HPLC was developed by Morita *et al.*[63], and inductively coupled plasma-atomic-emission spectrometric detection for micro-HPLC by Jinno and Tsuchida[39]. A Raman spectroscopic detector for HPLC was reported by Koizumi *et al.*[64] and by Iriyama *et al.*[65]. A capillary flow cell and argon-ion laser were used for the Raman spectroscopic detection of 2,4-dinitrophenylhydrazone derivatives of aliphatic aldehydes. The detection limit was about 100 ng for formaldehyde derivatives. A cold vapour atomic absorption spectrometer was used with success for HPLC determination of mercuric compounds by Fujita and Takabatake[66]. Recent excellent work on LC spectrometry was performed with the buffer-memory[67,68]. A vacuum nebulizing interface for HPLC–mass spectrometry was used successfully by Tsuge[69].

These developments in column packing materials and detection techniques have made significant contributions to separation procedures and to their applications in various fields of chemical and biological sciences.

REFERENCES

1 S. Egashira, *Japan Analyst*, 10 (1961) 693*.
2 H. Hatano, *Hitachi Sci. Instr. News*, 5 (1962) 141*.
3 H. Hatano, *Automatic Amino Acid Analysis*, Kagaku-dojin Co., Kyoto, 1959*.
4 H. Hatano, S. Egashira, K. Ozawa and S. Ganno, *Hitachi Hyoron*, 44 (1962) 2068*.
5 H. Hatano (Editor), *Liquid Chromatography*, Vol. 1, Nankodo Co., Tokyo, 1964*.
6 H. Hatano (Editor), *Liquid Chromatography*, Vol. 2, Nankodo Co., Tokyo, 1965*.
7 H. Hatano (Editor), *New Liquid Chromatography*, Nankodo Co., Tokyo, 1969*.
8 H. Hatano (Editor), *High Speed Liquid Chromatography*, Nankodo Co., Tokyo, 1973*.
9 H. Hatano (Editor), *Applied Liquid Chromatography*, Nankodo Co., Tokyo, 1976*.
10 H. Hatano (Editor), *New High Speed Liquid Chromatography*,Nankodo Co., Tokyo, 1977*.
11 H. Hatano (Editor), *High Resolution Chromatography*, Nankodo Co., Tokyo, 1983*.
12 H. Hatano (Editor), *Data Books of High Performance Liquid Chromatography*, Vols. 1–25, International Publishing and Consulting Co., Tokyo, 1978–1983*.
13 G. Muto and Y. Takata, *Anal. Chem.*, 45 (1973) 1864.
14 H. Hatano, Y. Yamamoto, M. Saito, E. Mochida and S. Watanabe, *J. Chromatogr.*, 83 (1973) 373.
15 H. Hatano, *Res./Dev.*, 24 (4) (1973) 28.
16 H. Hatano, *Res./Dev.*, 26 (5) (1975) 46.
17 A. P. Graffeo and N. H. C. Coode, *J. Chromatogr. Sci.*, 17 (1979) 202.
18 M. Novotny and D. Ishii (Editors), *Microcolumn Separation Methods*, Elsevier, Amsterdam, 1985.
19 A. Zlatkis (Editor), *Advances in Chromatography*, University of Houston, Houston, 1982.
20 E. Heftmann (Editor), *International Symposium on High-Performance Liquid Chromatography, Kyoto, 1985, J. Chromatogr.*, Vol. 332 (1985).
21 H. Hatano, M. Hori, S. Rokushida and F. Murakami, *Liquid Chromatography and Application*, Kodansha, Tokyo, 1974*.
22 H. Hatano and T. Hanai, *Experimental High Performance Liquid Chromatography*, Kagaku-dojin, Kyoto, 1977*.
23 S. Egashira, *Liquid Chromatography*, Sankyo Publ. Co., Tokyo, 1977*.
24 T. Hashimoto (Editor), *Packing Material (Stationary Phase)*, Musashino Book Co., Tokyo, 1978*.
25 S. Hara and A. Tsuji (Editors), *Modern Liquid Chromatography*, Nanzando, Tokyo, 1978*.
26 T. Yamabe (Editor), *Introduction to High Performance Liquid Chromatography*, Saiwai Book Co., Tokyo, 1978*.
27 S. Rokushika, H. Taniguchi and H. Hatano, *Anal. Lett.*, 8 (1975) 205.
28 H. Hatano, in A. J. C. Nicholson (Editor), *Detectors and Chromatography*, Australian Science and Industry Association, Melbourne, 1983.
29 Y. Hanaoka, T. Murayama, S. Muramoto, T. Matsuura and A. Nanba, *J. Chromatogr.*, 239 (1982) 537.
30 D. Ishii, K. Asai, K. Hibi, T. Jonokuchi and M. Nagaya, *J. Chromatogr.*, 144 (1977) 157.
31 D. Ishii, K. Hibi, K. Asai and M. Nagaya, *J. Chromatogr.*, 151 (1978) 341.
32 D. Ishii, A. Hirose, K. Hibi and Y. Iwasaki, *J. Chromatogr.*, 157 (1978) 43.
33 M. Goto, E. Sakurai and D. Ishii, *J. Chromatogr.*, 238 (1982) 357.
34 T. Tsuda and M. Novotny, *Anal. Chem.*, 50 (1978) 271.
35 S. Rokushika, Z. Y. Qui and H. Hatano, *J. Chromatogr.*, 260 (1983) 81.
36 T. Takeuchi, Y. Hirata and Y. Okuyama, *Anal. Chem.*, 50 (1978) 659.
37 S. Tsuge, Y. Hirata and T. Takeuchi, *Anal. Chem.*, 51 (1979) 166.
38 T. Imato, A. Jyo and N. Ishibashi, *Anal. Chem.*, 52 (1980) 1893.
39 K. Jinno and H. Tsuchida, *Anal. Lett.*, 15 (1982) 427.
40 T. Yamabe and S. Takai, *Seisan Kenkyu*, 22 (1970) 485*.
41 T. Yamabe and S. Takai, *Hitachi Sci. Instr. News*, 15 (1972) 10*.
42 S. Takai and T. Yamabe, *Bunseki Kiki*, 10 (1972) 153*.
43 T. Yamabe, N. Takai and H. Nakamura, *J. Chromatogr.*, 104 (1975) 359.
44 S. Rokushika, T. Ohkawa and H. Hatano, *J. Chromatogr.*, 176 (1979) 456.
45 H. Hatano, in F. Bruner (Editor), *The Science of Chromatography (J. Chrom. Library, Vol. 32)*, Elsevier, Amsterdam, Oxford, New York, Tokyo, 1985, pp. 165–178.

* In Japanese.

46 H. Hatano, *Bunseki*, 10 (1984) 721*.
47 Y. Kato and T. Hashimoto, *Bunseki*, 10 (1984) 725*.
48 H. Hatano (Editor), *Report of the Physical and Chemical Property Data, Liquid Chromatography Data*, Vol. 5, Promotion Bureau, Science & Technology Agency, 1984*.
49 K. Makino and H. Hatano, in P. L. Dubin (Editor), *Size Exclusion Chromatography*, Elsevier, Amsterdam, 1985, in press.
50 H. Hatano, H. Wada, K. Makino, T. Takeuchi, K. Noguchi and Y. Yanagihara, *Abstracts of 9th International Symposium on Column Liquid Chromatography, Edinburgh, 1985*, p. 163.
51 F. Yamamoto and H. Hatano, *Protein, Nucleic Acid, Enzyme* (Tanpakushitsu, Kakusan, Kohso), Kyoritsu Publ. Co., Tokyo, 1985, in press*.
52 M. Ozaki, H. Itakura, K. Shinaishi and I. Hara, *Clin. Chem.*, 29 (1983) 768.
53 K. Makino, H. Wada, H. Ozaki, T. Takeuchi and H. Hatano, *Proceedings of 9th International Symposium on Column Liquid Chromatography, Edinburgh, 1985*, p. 297.
54 O. Hiroshima, S. Ikenoya, T. Naito, K. Kusube, M. Omae, K. Kawabe, S. Ishikawa, H. Hoshida and T. Kurahashi, *Chem. Pharm. Bull.*, 31 (1983) 3571.
55 M. Goto, E. Sakurai and D. Ishii, *J. Chromatogr.*, 238 (1982) 357.
56 S. Terabe, K. Yamamoto and T. Ando, *J. Chromatogr.*, 239 (1982) 515.
57 T. Imasaka, K. Ishibashi and N. Ishibashi, *Anal. Chim. Acta*, 142 (1982) 1.
58 F. Moriya, K. Makino, N. Suzuki, S. Rokushika and H. Hatano, *J. Phys. Chem.*, 84 (1980) 3085.
59 F. Moriya, K. Makino, N. Suzuki, S. Rokushika and H. Hatano, *J. Phys. Chem.*, 84 (1980) 3614.
60 S. Kominami, S. Rokushika and H. Hatano, *Int. J. Radiat. Res.*, 30 (1976) 525.
61 S. Kominami, S. Rokushika and H. Hatano, *Radiat. Res.*, 72 (1977) 89.
62 S. Mori, A. Wada, F. Kaneuchi, A. Ikeda, M. Watanabe and K. Mochizuki, *J. Chromatogr.*, 246 (1982) 215.
63 M. Morita, T. Uehiro and K. Fuwa, *Anal. Chem.*, 52 (1980) 351.
64 H. Koizumi, H. Kojima and Y. Suzuki, *Japan Analyst*, 30 (1981) 99.
65 K. Iriyama, Y. Ozaki, K. Hibi and T. Ikeda, *J. Chromatogr.*, 254 (1983) 285.
66 M. Fujita and E. Takabatake, *Anal. Chem.*, 55 (1983) 454.
67 K. Jinno, C. Fujimoto and D. Ishii, *J. Chromatogr.*, 239 (1982) 625.
68 C. Fujimoto, K. Jinno and Y. Hirata, *J. Chromatogr.*, 258 (1983) 81.
69 S. Tsuge, *Fresenius' Z. Anal. Chem.*, 311 (1982) 674.