Product Information

Introduction and Evaluation of TSK-Gel IEX-500 Series Columns for High Speed Ion-Exchange Chromatography

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New ion-exchangers for high speed ion-exchange chromatography have been developed. They are additional modifications of TSK-Gel Type SW which is employed for the separation of proteins and water-soluble polymers by gel filtration chromatography. It is found that these new packing materials can be successfully used for the separation of amino acids, peptides, nucleic acid derivatives and their metabolites, proteins and other electrolytes by high speed ion-exchange chromatography.

Characteristics of the ion-exchangers and their packed columns, evaluation results for the separation efficiency of proteins, and application data of each grade column are described.

1. INTRODUCTION

The remarkable success of ion-exchange chromatography in the separation of low molecules such as amino acids, nucleic acid components, their metabolites, and inorganic electrolytes have been obtained by ion-exchangers based on polystyrene or other synthetic polymer and pellicular ion-exchangers.

As these ion-exchangers are rigid or semi-rigid, they can be employed for high speed liquid chromatography.

On the other hand, the carbohydrates-based ion exchangers[1~3] have been employed to the separation of macro-molecules like proteins, to which the polystyrene-based ion-exchangers can not be applied due to denaturation caused by strong hydrophobic interaction.

Since the carbohydrates-based ion-exchangers are too soft to be used for high speed liquid chromatography, the following difficulties are often encountered in the ion-exchange chromatography using them:

(b) Time consuming both in the column preparation and elution.
(b) Poor reproducibility due to unstable gel bed.
(c) Poor resolution due to the large particle size.

Toyo Soda Mfg. Co., Ltd. has succeeded in the development of gel filtration chromatography (GFC) supports.

Particularly TSK-GEL Type SW was employed in the separation of proteins by GFC with the successful results[4~6].

Further more the molecular weights of protein polypeptide chains in 6M guanidine hydrochloride[7] or SDS-solution[8] were estimated accurately by high speed GFC in much shorter time than on the soft gels such as crosslinked dextran and polyacrylamidegel. We have developed by the addition of ionic functional groups of controlled capacity to Type SW, in order to separate the polymer of electrolytes such as proteins and peptides by high speed ion-exchange chromatography.

In this paper the grades and basic properties of them, some evaluation data and applications of the individual grades are introduced.

2. FEATURES OF IEX-500 SERIES ION-EXCHANGERS AND COLUMNS

(a) IEX-500 series ion-exchangers are rigid, hydrophilic, spherical and porous gel like TSK-GEL Type SW.

(b) IEX-500 series columns provide measurement at high flow rates with high efficiencies.

(c) IEX-500 series columns are recommended for use in analytical and semi-preparative separation of synthetic electrolytes and biochemical substances, such as amino acids, proteins, nucleic acid derivatives and their metabolites.

(d) Scince the surfaces of supports are covered with hydroxyl groups and controlled ionic groups, it is possible to obtain high recoveries of labile solutes without denaturation.

(e) IEX-500 series columns are compatible with eluents of various salt concentration and/or containing polar organic solvents.

3. GRADES OF TSK-GEL IEX-500 SERIES

They are derivatives of TSK-GEL Type SW, consisting of totally six grades of different pore sizes and different functional groups, as shown in **Table 1.**

Table 1 IEX 500 series column list

Grade (Nick name)	Particle size μm	Functional group	Characteristic	Ion-exchange capacity meq./g drygel	Column size	Paired ion	Solvent
IEX 510 SP (SP 2000 SW)	5	$-SO_3^{\ominus}$	Strong cation exchanger	>0.3	4mmID×30cm	$Na^{\oplus}$	Methanol
IEX 520 QAE (QAE 2000 SW)	5	$-\overset{\oplus}{N}Et_2Me$	Strong anion exchanger	>0.3	4mmID×30cm	$Cl^{\ominus}$	Methanol
IEX 530 CM (CM 2000 SW)	5	$-CO_2^{\ominus}$	Weak cation exchanger	>0.3	4mmID×30cm	$Na^{\oplus}$	Methanol
IEX 540 DEAE (DEAE 2000 SW)	5	$-\overset{\oplus}{N}HEt_2$	Weak anion exchanger	>0.3	4mmID×30cm	$Cl^{\ominus}$	Methanol
IEX 535 CM (CM3000 SW)	10	$-CO_2^{\ominus}$	Weak cation exchanger	>0.3	6mmID×15cm	$Na^{\oplus}$	Methanol
IEX 545 DEAE (DEAE 3000 SW)	10	$-\overset{\oplus}{N}HEt_2$	Weak anion exchanger	>0.3	6mmID×15cm	$Cl^{\ominus}$	Methanol

Four grades of them (510SP, 520QAE, 530CM and 540DEAE) are derivatives of TSK-GEL G2000SW. They employ microparticulate silica with a particle diameter of ca. 5 μm. Other two grades (535CM 545DEAE) are derivatives of TSK-GEL G3000SW. These two grades employ silica of a slightly larger particle diameter of ca. 10 μm. All of IEX 500 series are supplied as prepacked column.

4. BASIC PROPERTIES

[1] Ion-exchange capacity and titration curve

Total ion-exchange capacities are shown in **Table 1.**

The capacities of cation-exchangers were determined by the titration in 0.5 M NaCl. On the other hand, because of the chemical instability of the base material, the capacities of anion-exchangers were estimated by elemental analysis of nitrogen. The titration curves of typical supports of the cation-exchangers and the back-titration curves of typical supports of the anion-exchangers from pH 2.5 to 8.0 are shown in **Fig. 1** and **2.**

The approximate bulk volumes are shown as follows.

IEX-510, 520, 530, 540 : 2.4 ± 0.3 cm^3/g

IEX-535, 545 : 3.3 ± 0.3 cm^3/g

[2] Adsorption capacity of standard protein

The adsorption capacities of the cation-exchangers and the anion-exchangers were

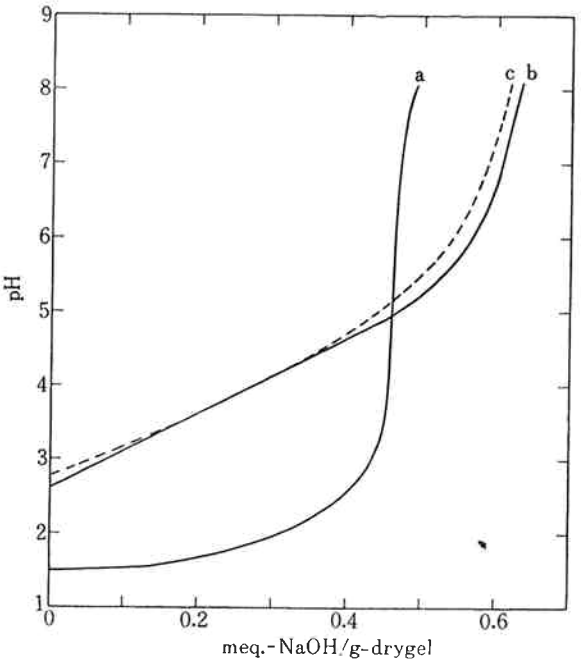

Fig. 1 Titration curves of cation-exchangers
a, IEX510SP(Lot. No. 800624-715M) ; b, IEX530CM (Lot. No. 800902) ; c, IEX535CM(Lot. No. 800909). Curves obtained in 0.5 M NaCl.

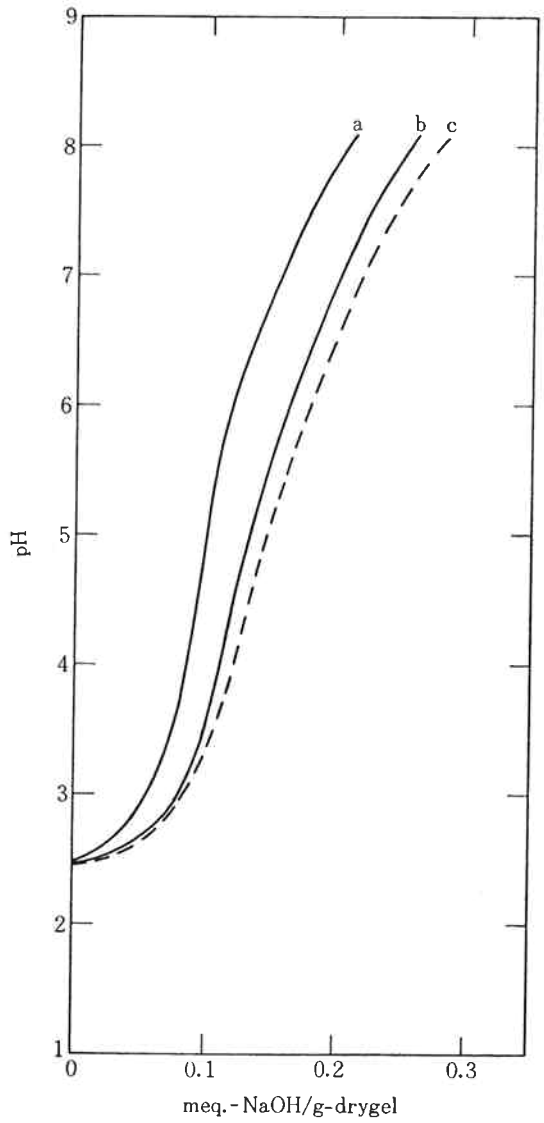

Fig. 2 Titration curves of anion-exchangers
a, IEX520QAE(Lot. No. 801331) ; b, IEX540 DEAE (Lot. No. 800711) ; c, IEX545DEAE(Lot. No. 801120 -1217 M). Curves obtained in 0.5 M NaCl.

Table 2 An example of the adsorption capacity of protein

Grade (Gel Lot. No.)	Capacity mg·protein/mℓgel
IEX510SP (800624-715M)	74 (Hb)
IEX530CM (800902)	108 (Hb)
IEX535CM (800909)	118 (Hb)
IEX520QAE (801031)	98 (BSA)
IEX540DEAE (54001)	112 (BSA)
IEX545DEAE (800718M)	135 (BSA)

Symbols as shown in **Table 3.**

measured with hemoglobin (human) and albumin (bovin serum) respectively and shown in **Table 2.**

〔3〕 Pore size

The calibration curves of IEX500 series columns were measured by standard polyethyleneoxides (PEO) in 0.1 M NaCl.

The pore size is dependent on that of the base materials, but independent on the functional groups of the supports.

The calibration curves of IEX530CM and IEX535CM by PEO are shown in **Fig. 3**, and the relationship between molecular weight of PEO and globular proteins for TSK-Type SW columns by GFC is shown in **Fig. 4.**

〔4〕 Flow property

The relationship between the flow rate and pressure drop depends on the particle size, its distribution and hardness of the support and viscosity of mobile phase. It should be noted there are some differences between the columns of TSK-G2000SW derivatives and TSK-G3000SW derivatives, for example, the particle size and hardness of the supports and the column size. The relationship between the flow rate and pressure drop of IEX540 column and IEX545 column are shown in **Fig. 5.**

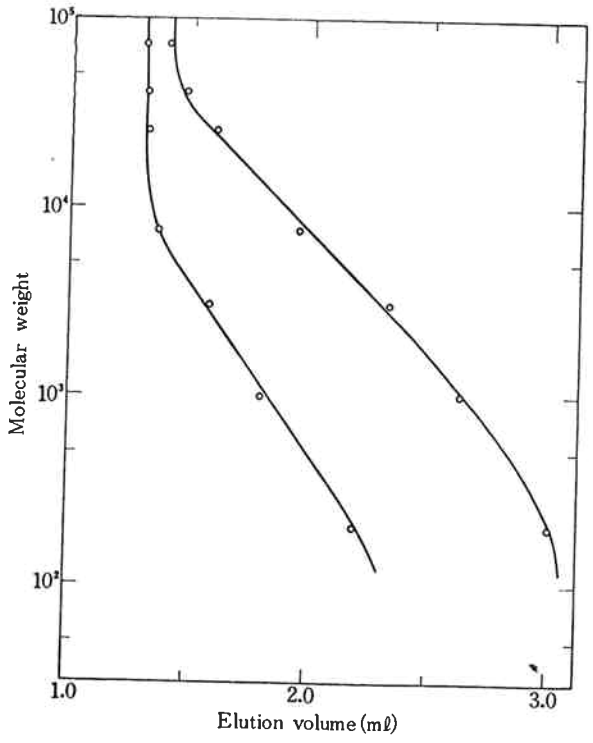

Fig. 3 Calibration curves of IEX500 columns, measured by standard polyethyleneoxides (PEO) in 0.1 M NaCl
a, IEX530CM column (4 mm ID × 30 cm) ; b, IEX535CM (6 mmID × 15 cm).

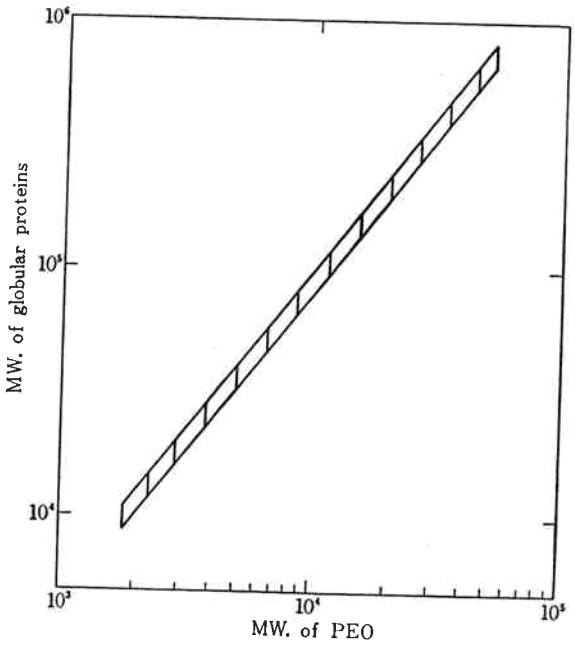

Fig. 4 The relationship between molecular weight of standard polyethyleneoxides (PEO) and globular proteins for TSK Type SW columns

That is linear to more than the allowed range of flow rate and pressure drop for a short period.

The allowed range of flow rate and pressure drop is shown as follows.

	column size	flow rate	pressure drop
A	4 mmID × 30 cm	less than 1.0 mℓ/min	less than 150 kg/cm²
B	6 mmID × 15 cm	less than 1.0 mℓ/min	less than 25 kg/cm²

A : The columns of TSK-G2000SW derivatives (510, 520, 530, 540)

B : The columns of TSK-G3000SW derivatives (535, 545)

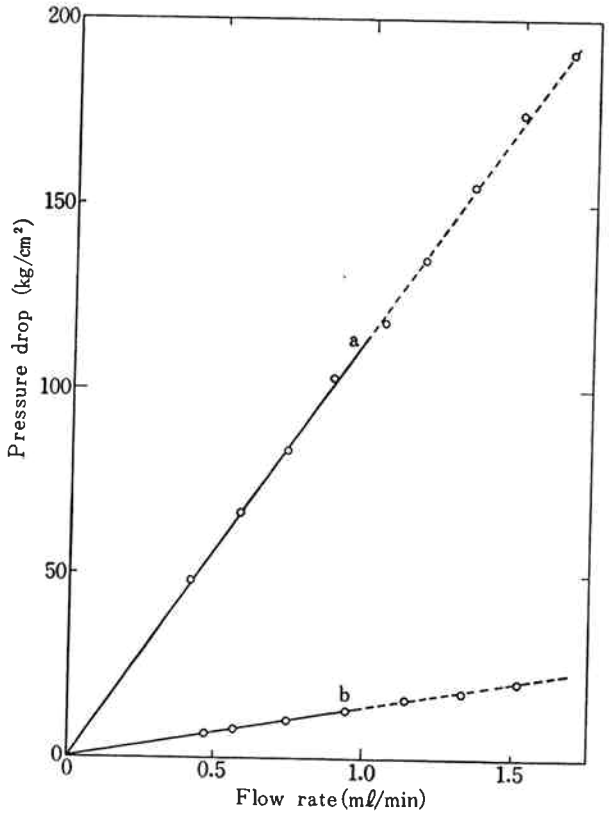

Fig. 5 Dependence of pressure drop on flow rate a, IEX540 DEAE column (4 mmID × 30 cm) ; b, IEX545DEAE column (6 mmID × 15 cm). Mobile phase : Distilled water Temperature : 25°C

〔5〕 Stability

pH range

The eluents must be kept within the pH range from 2.0 to 8.0. The supports are particularly unstable in the basic solutions, because they are silica-based.

Temperature

The columns must be kept at relatively constant temprature between 0°C and 45°C. They should not be frozen.

5. SEPARATION OF PROTEINS BY ION-EXCHANGE CHROMATOGROPHY ON IEX-535CM COLUMN AND IEX-545DEAE COLUMN

〔1〕 The recovery of proteins

In the purification of proteins, the recovery yields of proteins from IEX-500 series columns depend on the conditions such as eluents, temperature and others. In this test the recovery yields of commercially available proteins are listed in **Table 3** under the following experimental condition.

Experimental conditions

	IEX-535CM	IEX-545DEAE
First eluent	: 0.05 M Phosphate buffer (pH 6.0)	0.05 M Tris-HCl buffer (pH 7.5)
Second eluent	: 0.05 M Phosphate buffer (pH 7.0) +0.5 M NaCl	0.05 M Tris-HCl buffer (pH 7.5) +0.5 M NaCl
Elution	: A sample was injected onto the column with the first buffer which was replaced by the second buffer after one minute.	
Sample size	: 4.0～4.8 mg/mℓ, 0.50 mℓ.	
Flow rate	: 1.0 mℓ/min	
Temperature	: 25°C	
Detection	: UV. 280 nm	

Preparation of sample solution: Sample solutions were prepared by dissolving the proteins

Table 3 Recovery of commercial proteins from each column of IEX-535CM and IEX-545 DEAE

IEX-535CM			IEX-545DEAE		
Protein	Abbreviation	Recovery (%)	Protein	Abbreviation	Recovery (%)
γ-Globurin Fr. II (Human serum)	G G	98	Trypsininhibitor (Soy bean)	T I	99
Hemoglobin (Human)	H b	100	Thyrogloburin (Bovine)	T Y	92
Myoglobin (Whale skeletal mustle)	M Y	98	Ovalbumin (Egg white)	O A	99
α-Chymotrypsinogen A (Bovine pancreas)	A C	99	Albumin (Bovine serum)	B S A	100
Ribonuclease (Bovine pancreas)	R N	99	β-Lactogloburin (Milk)	B L	100
Cytochrome C (Horse heart)	C Y	99	γ-Globurin Fr. II (Human serum)	G G	98
Lysozyme (Egg white)	L Y	99	Hemoglobin (Human)	H b	95

(lyophilized) with the first buffer.

The solution of α-chymotrypsihogen A was injected after the filtration (0.45-μm Millipore®), because it contained an insoluble component.

Measurement of recovery yields: 10 mℓ of the effluent from the column was collected and its absorbance at UV 280 nm was measured and compared with the original solution.

〔2〕 Effect of some factors for the resoluton of proteins in the linear gradient elution of salt concentration

The gradient elution of salt concentration is commonly employed in the separation of proteins by ion-exchange chromatography. The influences of the salt gradient slope, flow rate and weight of sample loading were examined in the separation of proteins by ion-exchange chromatography on IEX-535CM column and IEX-545DEAE column at constant temperature.

Apparatus: The flow diagram of apparatus for this measurement is shown in **Fig. 6.**

The linear gradient slope obtained by this apparatus is constant as a function of time and inversely proportional to the flow rate as a function of volume in different flow rates.

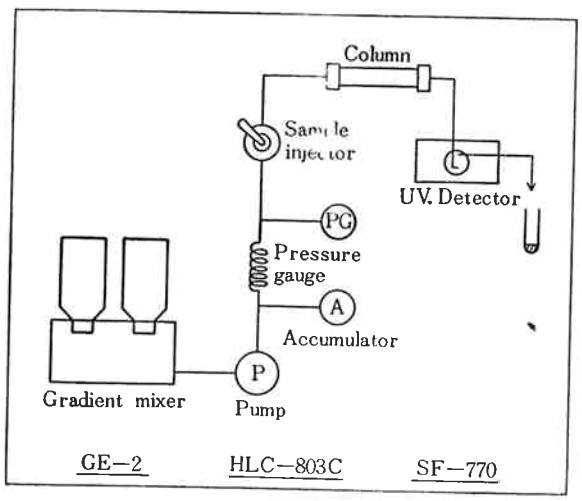

Fig. 6 Flow diagram of apparatus for the measurements

Calculation; $R=2(V_{i+1}-V_i)/(W_{i+1}+W_i)$

R: Resolution, W_i, W_{i+1}: Peak width, V_i, V_{i+1}: Elution volume.

(1) Study of protein resolution on IEX-545 DEAE column

Ovalbumin (Seikagaku Co., Tokyo, Japan), 5 times recrystallized, was used for this study. That was separated into only two components by GFC on TSK-GEL G3000SW(7.5 mmID × 60 cm).

The amount of the miner component, molecular weight ca. 90,000, was estimated only less than 2.0% of the main component by UV280 absorbance.

On the other hand the ovalbumin could be separated into several components by ion-exchange chromatography on IEX-545DEAE (**Fig. 9**). In this test, the ovalbumin was slowly eluted with the first buffer, 0.1 M tris-HCl buffer pH 7.5, followed by the linear salt gradient of relatively gentle slope.

The effects of some factors for resolution of proteins, contained in the ovalbumin, were examined in these condition. Experimental condition: Described in **Fig. 7.**

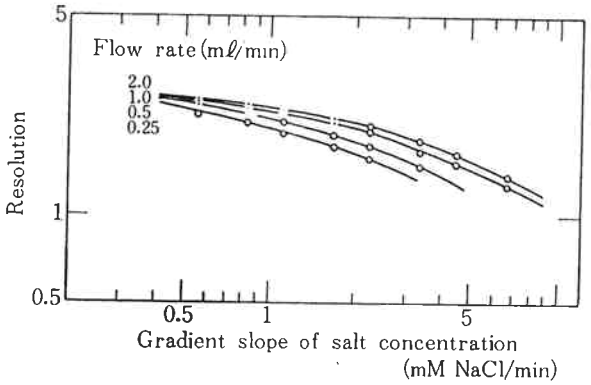

Fig. 7 Dependence of resolution for the main peak A (in **Fig. 9**) and the following peak B (in **Fig. 9**) of ovalbumin on gradient slope of salt concentration

Test conditions

Column	: IEX-545 DEAE (6 mmID × 15 cm)
Sample	: Ovalbumin, 2mg/mℓ 0.21 mℓ
Eluent	: Linear gradient from A to B.
	A ; 0.1 M tris-HClbuffer (pH 7.5)
	B ; 0.1 M tris-HCl buffer (pH7.5) containing 0.2 M NaCl
Detection	: UV 280 nm
Temperature	: 25°C

Results: Dependence of resolution for the main peak A and the following peak B on the gradient slope of salt concentration and flow rate and dependence of peak width of the main peak on sample loading are shown in **Fig. 7, 8** and **10**, respectively.

The elution profiles of ovalbumin on IEX-545DEAE at the different flow rates are shown

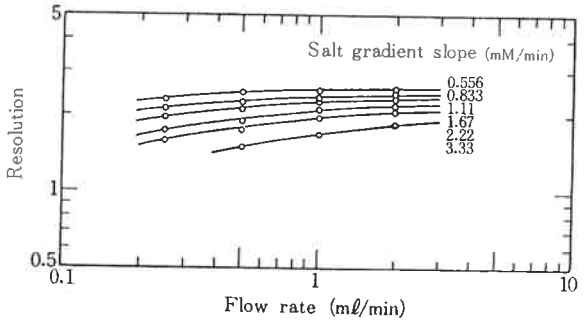

Fig. 8 Dependnce of resolution for the main peak A (in **Fig. 9**) and the following peak B (in **Fig. 9**) of ovalbumin on flow rate
Test conditions are the same as in **Fig. 7.**

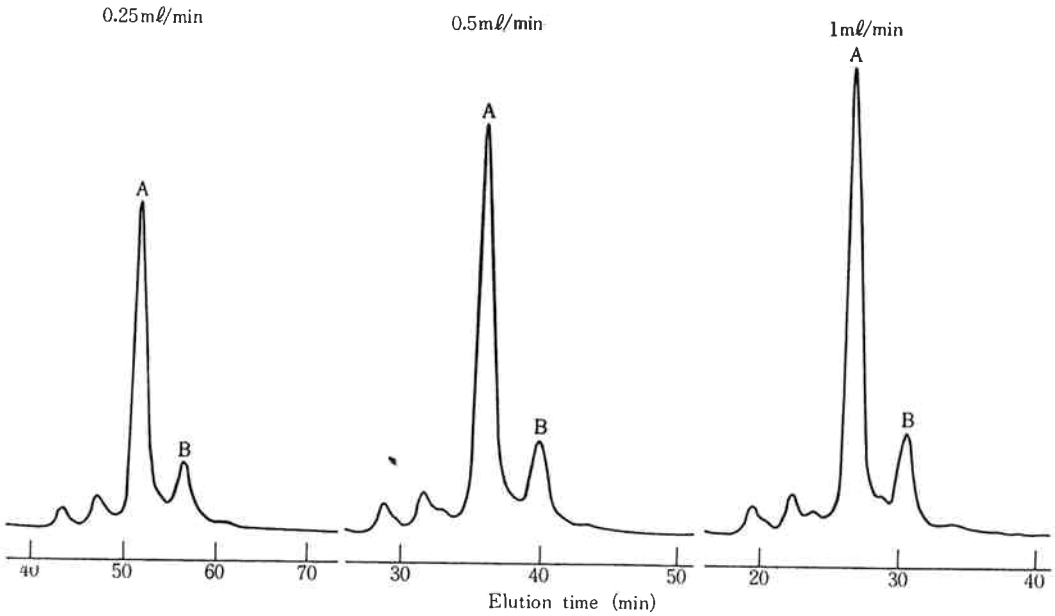

Fig. 9 Elution profiles of ovalbumin at different flow rate by use of IEX-545 DEAE column Test conditions are the same as in **Fig. 7** (Gradient slope : 2.22 mM NaCl/min)

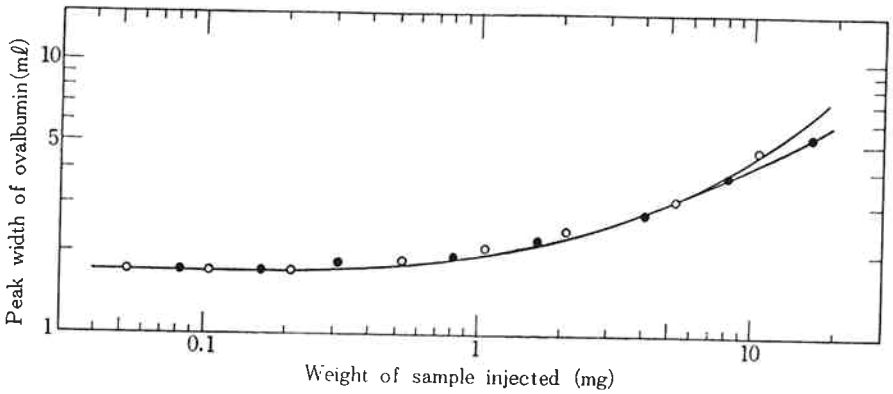

Fig. 10 Dependence of peak width on weight of sample injected
Test conditions
Sample volume : (○) ; 0.21 mℓ, (●) ; 1.64 mℓ
Eluent : Linear gradient elution from A to B in 90 min
Flow rate : 1.0 mℓ/min
Other conditions are the same as in **Fig. 7**

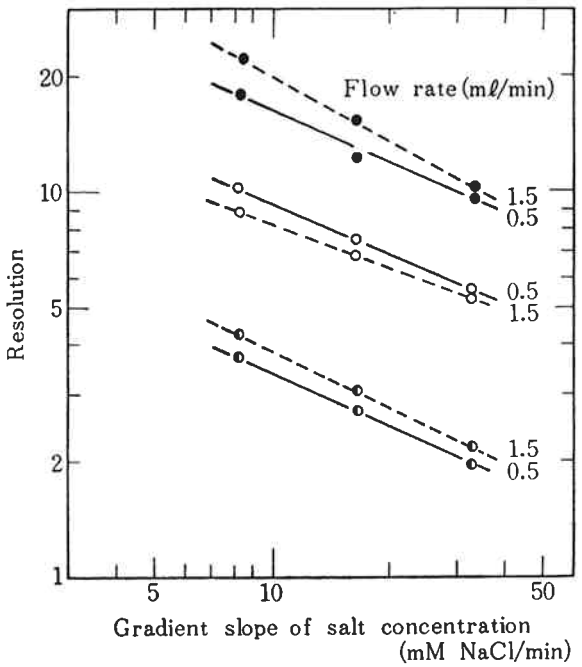

Fig. 11 Dependence of resolution for some proteins on gradient slope of salt concentration
Resolution :
● for myoglobin and α-chymotrypsinogen A
◑ for α-chymotrypsinogen A and cytochrome C
○ for cytochrome C ano lysozyme
Test condition
Sample : myoglobin, α-chymotrypsinogen A, cytochrome C and lysozyme
Sample size : My and LY, 0.4 mg/mℓ / AC and CY, 0.5 mg/mℓ } 0.20 mℓ
Eluent : Linear gradient elution from A to B.
A ; 25 mM Na_2HPO_4 pH 6.4 (adjusted with H_3PO_4)
B ; A+0.5 M NaCl
Flow rate : 0.5 and 1.5 mℓ/min as shown in the insert
Detection : UV 280 nm
Temperaturs : 25°C

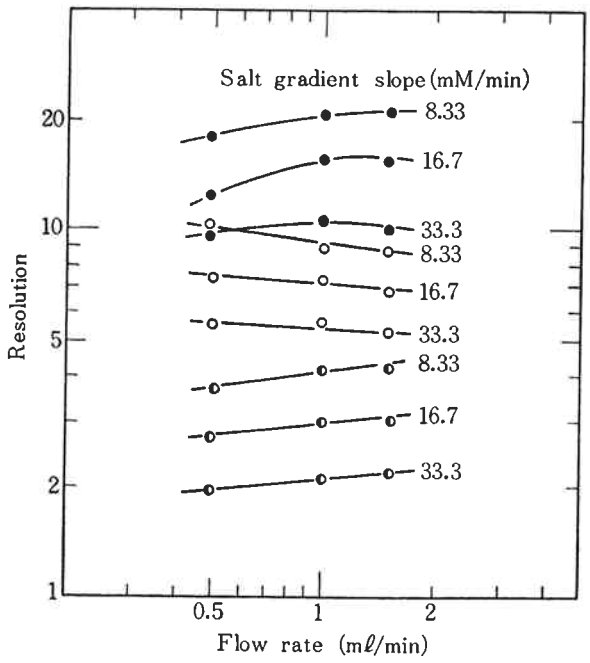

Fig. 12 Dependence of resolution for some proteins on flow rate
Test conditions
Gradient slope of salt concentration
8.33, 16.7 and 33.3 mM NaCl/min as shown in the insert.
For other conditions and symbols see **Fig. 11**

in **Fig. 9.**

(2) Separation of proteins on IEX-535 CM column

The proteins whose isoelectric points (pI) were different in the wide range were selected as the sample.

The effects of some factors for resolution of the proteins were observed in the condition, where all of the proteins were eluted with comparatively steep gradient of salt-concentration in short elution time.

Conditions: Described in **Fig. 11.**

Results: Dependence of resolution for the proteins on gradient slope of salt concentration, flow rate and weight of sample injected are shown in **Fig. 11, 12** and **14**, respectively.

The elution profiles of some proteins on IEX535CM at the different gradient of salt concentration are shown in **Fig. 13.**

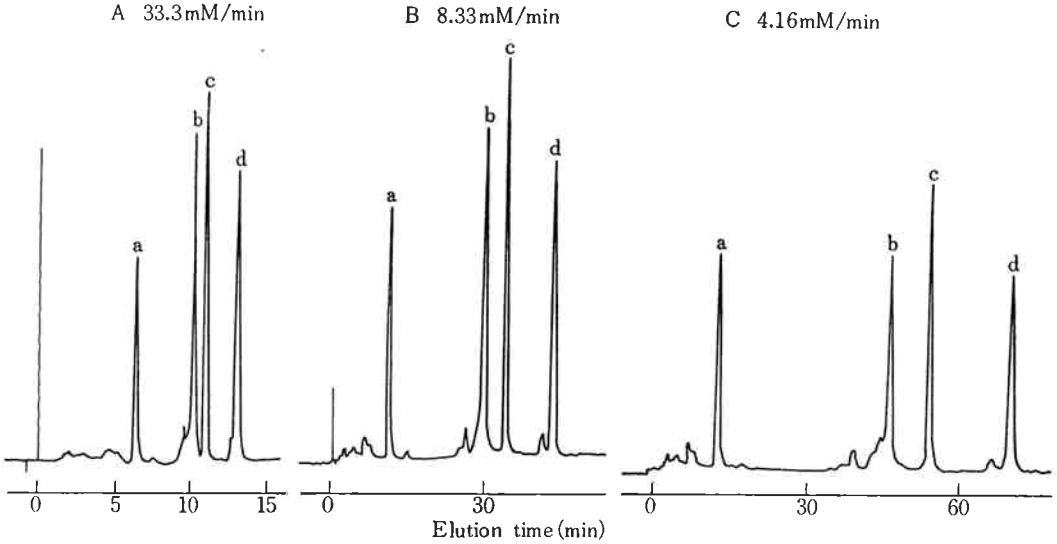

Fig. 13 Elution profiles of some proteins at different gradient slope of salt concentrationd by use of IEX-535CM column
a, myoglobin ; b, α-chymotrypsinogen A ; c, cytochrome C ; d, lysozyme. Flow rate : 1.0 mℓ/min
Other conditions are the same as in **Fig. 11**

(3) Discussion

1) Gradient slope of salt concentration

The more the gradient slope of salt concentration is derceased, the more both resolutions of the proteins both on IEX-545DEAE column and IEX-535CM column are improved as shown in **Fig. 7** and **11.**

Therefore, when the suitable eluent is selected, isocratic elution will be most effective for the separation of proteins which are eluted in the near retention time.

However, if each component in the sample can sufficiently be separated, the time required in the separation is shortened by the gradient elution.

The gradient slope of salt concentration is the main factor that determines the resolution of the proteins and the separation time.

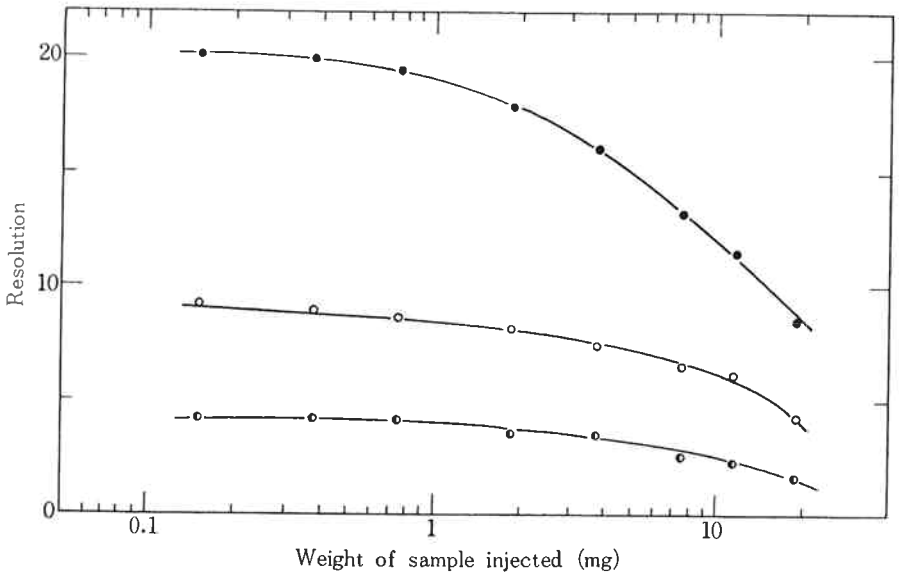

Fig. 14 Dependence of resolution for some proteins on weight of sample injected
Test conditions
Eluent : Linear gradient elution from A to B in 60 min
Flow rate : 1.0 mℓ/min
Other conditions and symbols are the same as in **Fig. 11**

2) Flow rate

From results on IEX 545 DEAE column.

There is the tendency that increase of the flow rate gradually improves the resolution, because the salt gradient slope as a function of volume decreases in proportion to the increase of the flow rate.

Therefore, as the salt gradient slope decreases, the elution becomes similar to the isocratic elution and the resolution of the proteins becomes almost equal in the different flow rates. (**Fig. 7** and **8**)

The results (not shown here) indicate that the resolution of proteins in the isocratic elution becomes to be improved according to the decrease of flow rate in more than 0.25 mℓ/min.

From results on IEX-535CM colum.

The tendency similar to the results on IEX-545DEAE column was observed for the resolution of AC. and MY. (see **Table 3**), which were eluted with the first eluent, on IEX-535CM column.

However, it seems that the resolutions of the other proteins are independent on the flow rate, because those may be offsetting each other by both effects that the peak width of each protein broadens and the difference between the elution volume of each peak increases according to the increase of the flow rate.

3) Sample loading capacity

It was found that the resolution of the proteins gradually deteriorates according to the increase of sample loading above 0.5 mg of each component. Consequently it is desirable to inject a sample below 0.5 mg in view of analytical separation. However, since no serious overloading is observed up to ca. 10 mg, it is possible to load a sample of ca. 10 mg for the purpose of

preparative separation so far as resolution is still satisfactory.

6. THE INTERACTION BETWEEN THE SMALL MOLECULES AND IEX-510SP

[1] The nonionic interaction between p-hydroxybenzoates and strong cation-exchangers

The comparison between IEX-510SP and TSK-GEL IEX-210SC, the polystyrene-based cation-exchangers with sulfonic group, is shown in **Fig. 15.**

In proportion to the length of the alkyl chains of p-hydroxyalkylbenzoates, the elution volume increased on IEX-210SC column, while it was kept almost constant on IEX-510SP column. This means that the nonionic interaction between IEX-510SP and a little polar organic substances is very weak in comparison with that on IEX-210SC.

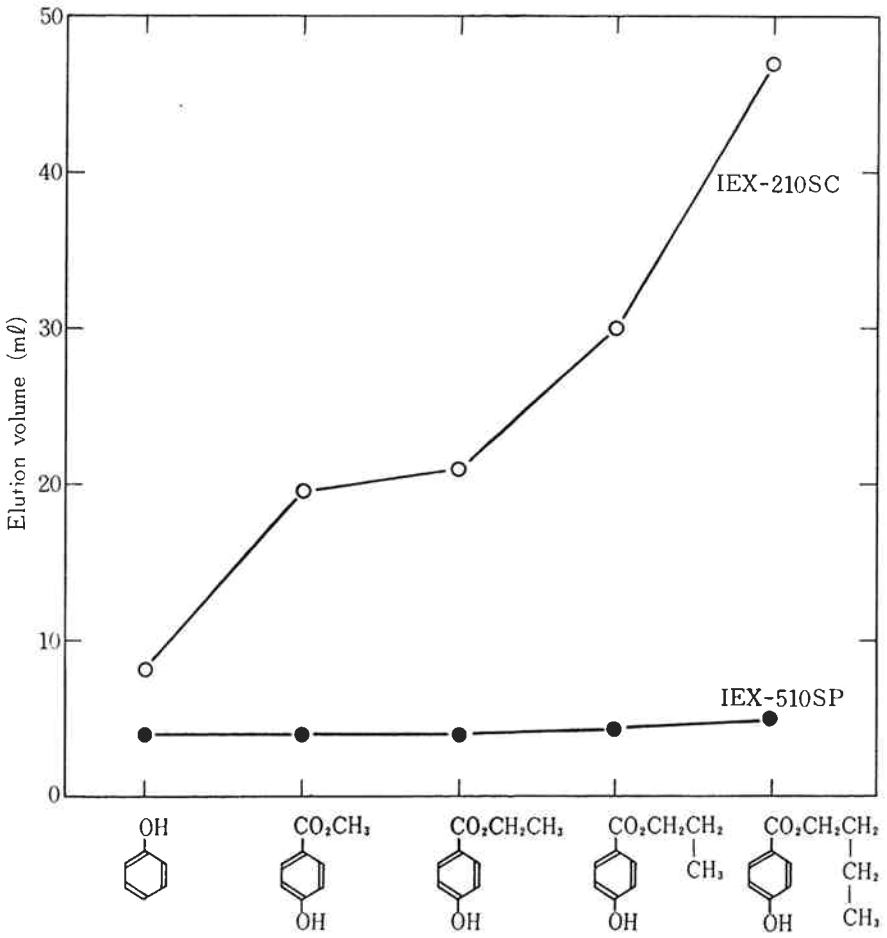

Fig. 15 Relationship between elution volume and alkyl chain length of p-hydroxyalkylbenzoates
Test condition. Eluent : 20v% CH_3CN+80v% 0.1 M phosphate buffer (pH 7.0), column : —○—IEX210SC, —●—IEX510SP.

[2] Separation of amino acids by IEX-510 SP column

The elution volumes of amino acids as the typical sample in small molecular electrolytes were examined by cation-exchange chromatography on IEX-510SP column. The neutral and acidic amino acids were eluted as shown in **Fig. 16A** probably due to the difference of the

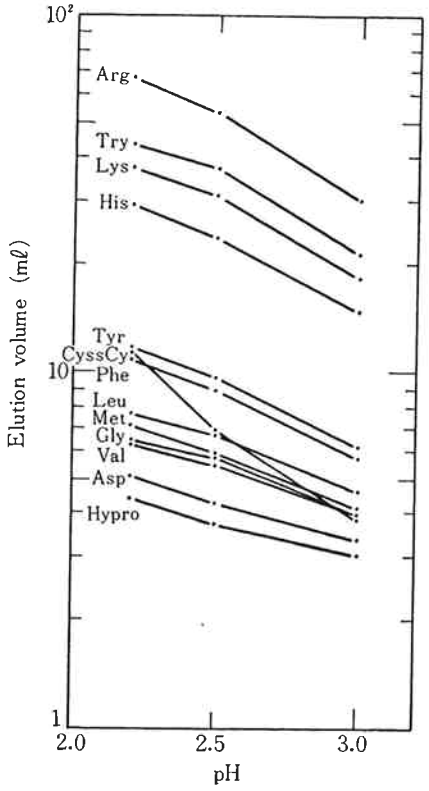

Fig. 16A Dependence of elution volume of amino acids on pH
Test condition Column : IEX 510SP 4mmID × 30 cm, eluent : 50 mM Na_2HPO_4 pH adjusted with H_3PO_4, temperature : 25°C

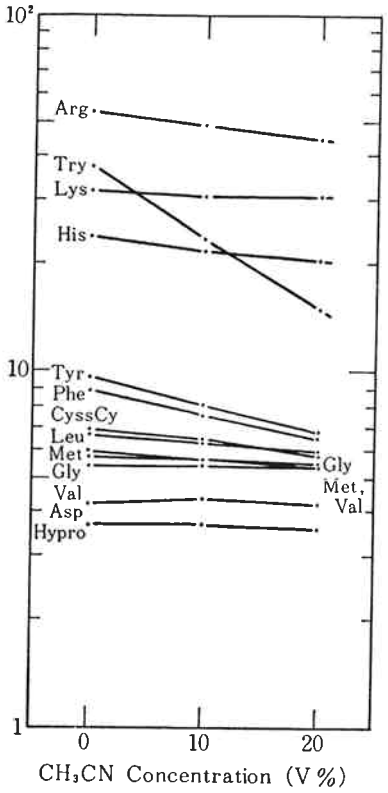

Fig. 16B Dependence of elution volume of amino acids on CH_3CN concentration
Test condition. Eluent : 50 mM Na_2HPO_4 pH 2.5 (adjusted with H_3PO_4) + CH_3CN. Other conditions are as in **Fig. 16A**

polarity and hydrophobicity of their functional groups at individual pH.

The aromatic amino acids (Phe. Tyr and Try) were particularly retarded and by the addition of acetonitrile to the eluent the remarkable decrease of their volumes was observed compared with aliphatic amino acids (**Fig. 16B**). The result, shown in **Fig. 15**, indicates that IEX-510SP ion-exchanger is hydrophilic. However, it is found that IEX-510SP has also some interaction with aromatic functional groups in aqueous solutions.

It may be suitable that IEX-510SP column is employed to the separation of amino acids when they must be eluted with a solution of low ionic strength.

[3] Separation of catecholamines by IEX-510 SP columns

Catecholamines and their metabolic pathway have been known because of the importance of their functions.

The condition in the separation of catecholamines and their derivatives was researched in acidic pH region, wherein they are stable (**Fig. 17A**) The effect of the addition of acetonitrile is shown in **Fig. 17B**. The elution profile of catecholamines and their derivatives in the typical condition is shown in **Fig. 18.**

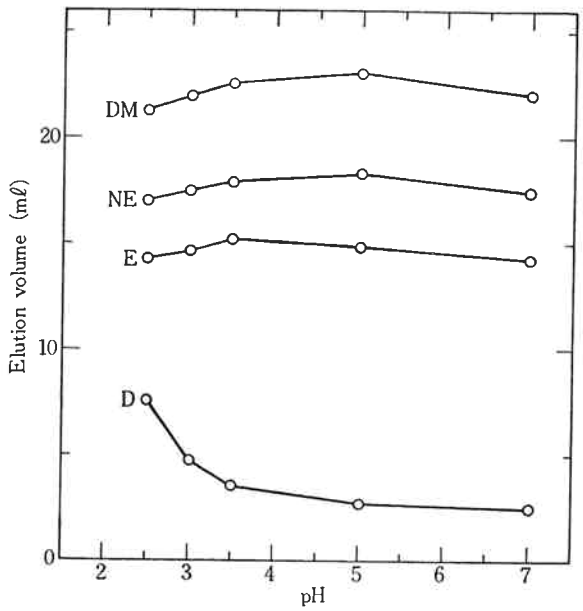

Fig. 17A Dependence of elution volume of catecholamines on pH
Test condition. Column : IEX510SP (4 mmID × 30 cm), eluent : 20% CH_3CN+0.1M Na_2HPO_4 pH adjusted with H_3PO_4 : Sample, D : DOPA, E : epinephrine, NE : norepinephrine, DM : dopamine.

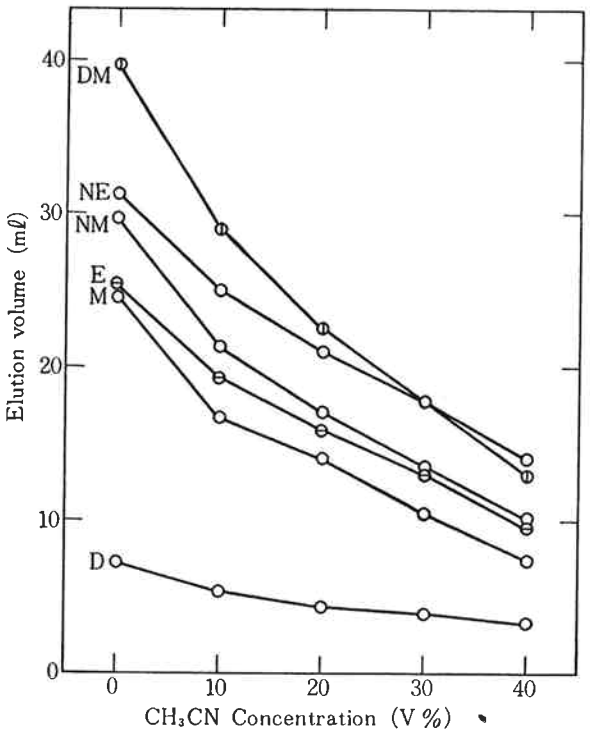

Fig. 17B Dependence of elution volume of catecholamines on CH_3CN concentration
Test condition. Column : IEX510SP (4 mmID × 30 cm), eluent : 50 mM Na_2HPO_4 pH 3.0 (adjusted with H_3PO_4)+CH_3CN. Sample, D : DOPA, M : methanephrine E : epinephrine, NM : normethanephrine, NE : norepinephrine, DM : dopamine

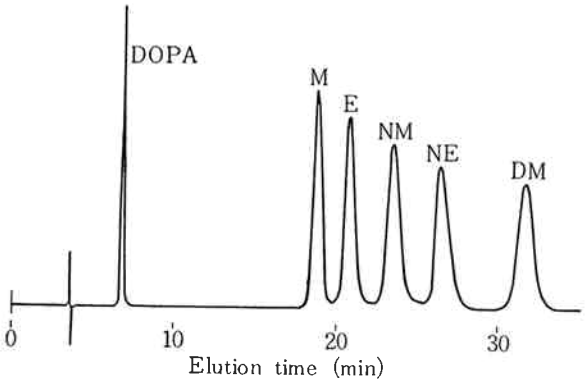

Fig. 18 Separation of catecholamines by IEX510SP
Condition
Column : IEX510SP (4 mmID × 30 cm)
Elvent : 8.6 v% CH_3CN+91.4v% 50 mM Na_2HPO_4 pH 3.0 (adjusted with H_3PO_4)
Flow rate : 1.0 mℓ/min
Detection : UV 280 nm
Temperature : Ambient
Sample : Symbols as in **Fig. 17B**.

7. THE SEPARATION OF PEPTIDES ON IEX-510SP OR IEX-530CM

Recently the separations of peptides by high speed liquid chromatography have mostly been studied by means of reversed-phase[9,10] and nomal-phase liquid chromatography. If the separetion based on ionic interaction by use of IEX-500 series columns, is developed, the analysis and preparation of them may be achieved at the higher level. Even if a sample contains slightly soluble peptides in water, it is possible to separate them with the eluent containing a polar organic solvent by ion-exchange chromatography on IEX-500 series columns.

Scince the gel beds of IEX-500 series column little swell or shrink according to the exchanges of eluents, the search of the separating condition can speedily be carried out.

[1] Separation of biologically active peptides by IEX-510 SP column

The dependence of the elution volumes of some peptides on pH and the proportions of added acetonitrile were examined and the results are shown in **Fig. 19A** and **19B**,

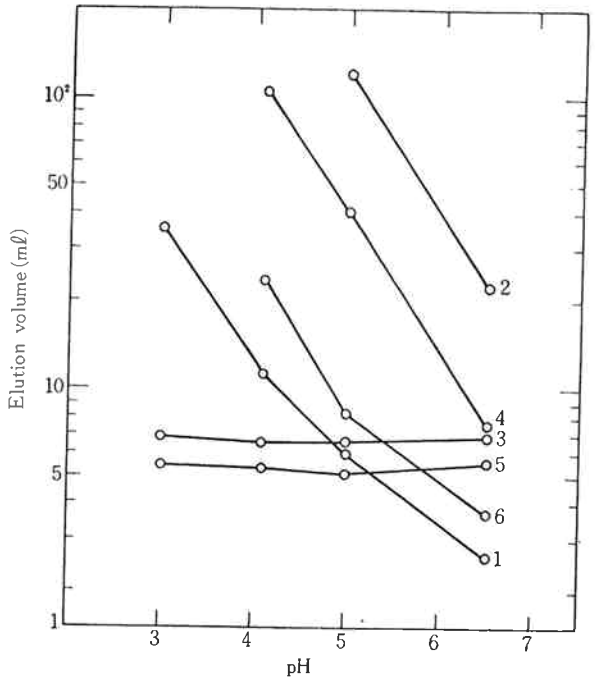

Fig. 19A Dependence of elution volume of peptides on pH

Test condition. Column : IEX510SP (4 mm ID×30 cm), eluent : 20 v% CH_3CN+80 v% 50 mM Na_2HPO_4 pH adjusted with H_3PO_4.

Sample. 1 : calcitonin, 2 : angiotensin III, 3 : deamino-dicarba-arginine-vasopressin, 4 : angiotensin I, 5 : deamino-dicaba-arginine-vasotosin, 6 : angiotensin II

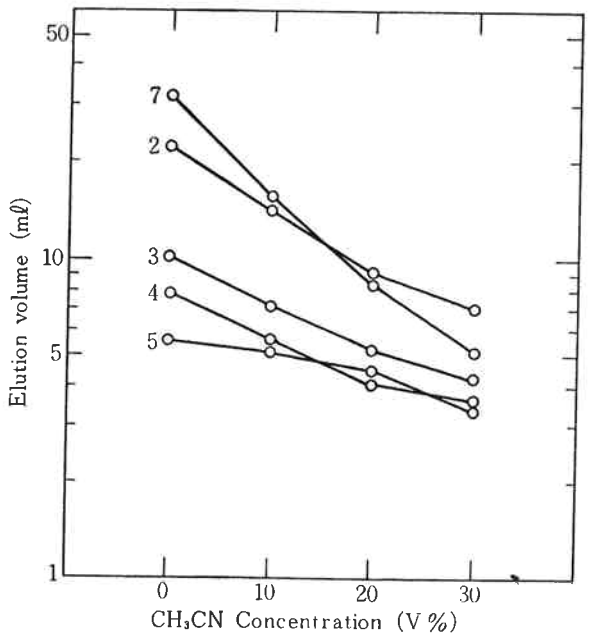

Fig. 19B Dependence of elution volume of peptides on CH_3CN concentration

Test condition. Column : IEX510SP (4 mmID×30 cm), eluent : CH_3CN+0.1 M Na_2HPO_4 pH6.5 Sample. Symbol 2~5 as in **Fig. 19A**. 7 : LH-RH.

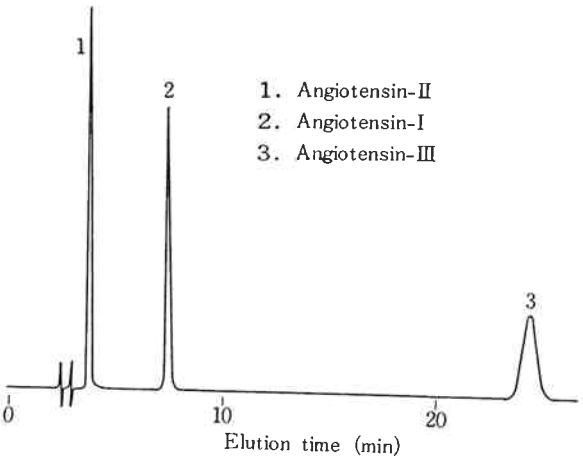

Fig. 20 Separation of angiotensins by IEX-510SP

Condition

Column : IEX510SP (4 mmID×30 cm)

Eluent : 20 v% CH_3CN+80 v% 50 mM Na_2HPO_4 pH 6.5 (adjusted with H_3PO_4)

Flow rate : 1.1 mℓ/min

Detection : UV 210 nm

Temperature : Ambient

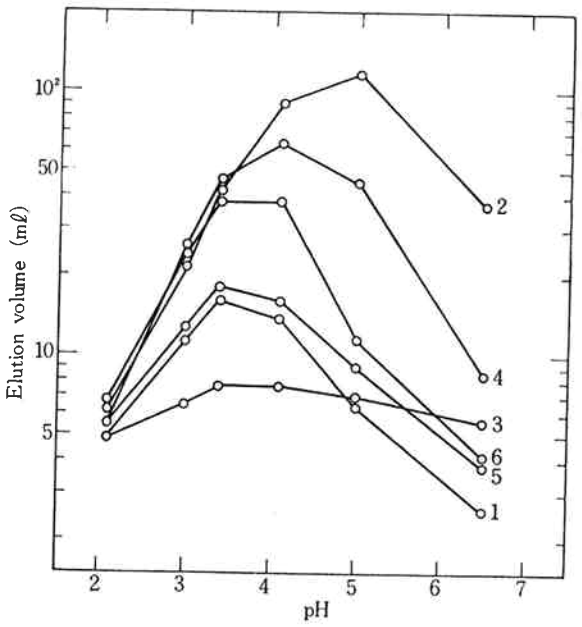

Fig. 21 Dependence of elution volume of peptides on pH

Test condition. Column : IEX530CM (4 mmID×30 cm), eluent : 10v% CH_3CN+90 v% 50 mM Na_2HPO_4 pH adjusted with H_3PO_4.

Sample. 1 : calcitonin, 2 : angiotensin III, 3 : deamino-dicarba-aiginine-vasopressin, 4 : angiotensin I, 5 : angiotensinII, 6 : neurotensin.

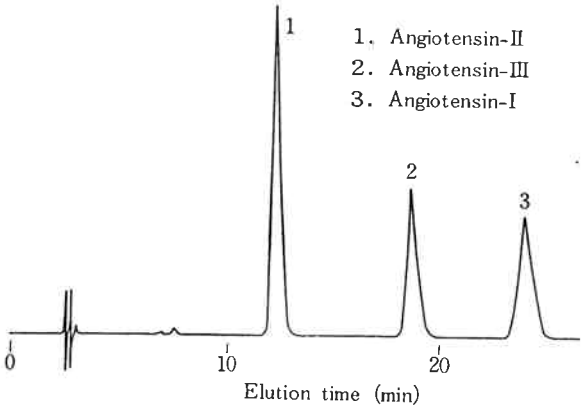

Fig. 22 Separation of angiotensins by IEX-530CM
Condition
Column : IEX530CM (4 mmID × 30 cm)
Eluent : 10 v% CH_3CN+90 v% 50 mM Na_2HPO_4 pH 3.0 (adjusted with H_3PO_4)
Flow rate : 1.1 mℓ/min
Detection : UV 210 nm
Temperature : Ambient

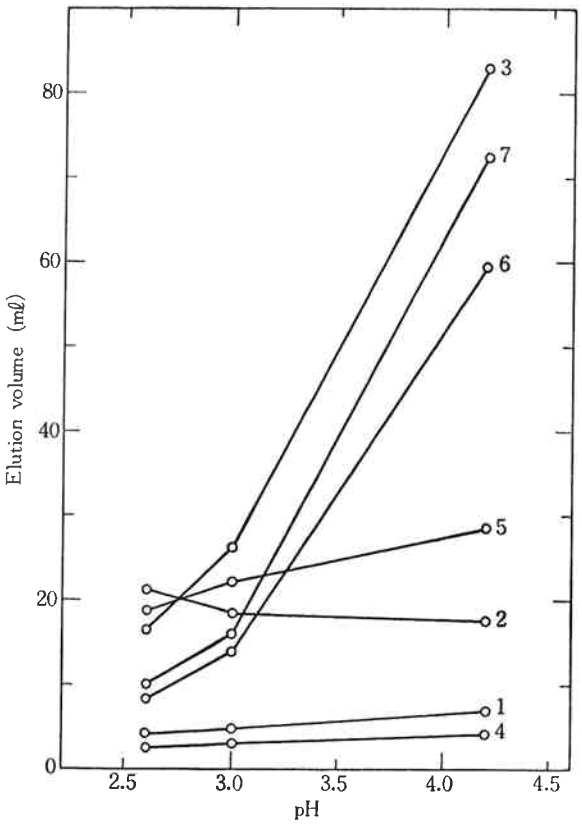

Fig. 23A Dependence of elution volume of organic acids on pH
Test condition. Column : IEX520QAE (4 mmID × 30 cm), eluent : 30 v%CH_3CN+70 v% 50 mM Na_2HPO_4 pH edjusted with H_3PO_4, sample, 1 : benzoic acid, 2 : maleic acid, 3 : fumaric acid, 4 : adipic acid, 5 : o-phthalic acid, 6 : m-phtalic acid, 7 : p-phthalic acid.

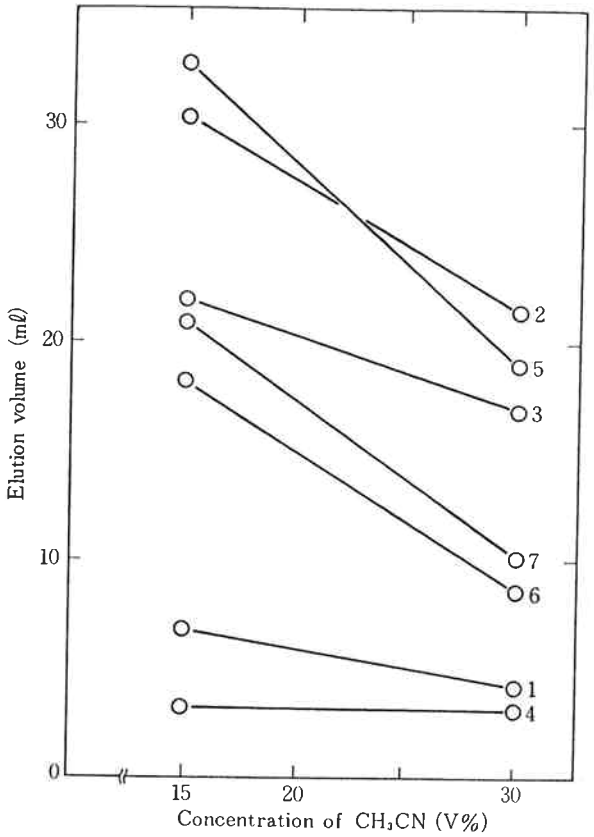

Fig. 23B Dependence of elution volume of organic acids on CH_3CN concentration
Test condition. Column : IEX520QAE (4 mmID × 30 cm), eluent CH_3CN+50mM Na_2HPO_4 pH 2.75 (adjusted with H_3PO_4).
Sample : Symbols as in **Fig. 20A**

respectively.

The elution profile of angiotensins in the typical condition on IEX-510SP column is shown in **Fig. 20.**

[2] Separation of biologically active peptides by IEX-530 CM column

The dependence of the elution volumes of some peptides on pH by use of IEX-530CM column was examined and shown in **Fig. 21.** It is found that the elution volumes of them decrease against increasing cationic charges of components below pH 4, because anions, which are prepared on the supports by the ionization of carboxyl groups, gradually decrease in proportion to decreasing pH value in less than pH 6, (see **Fig. 1** and compare with **Fig. 19A.**)

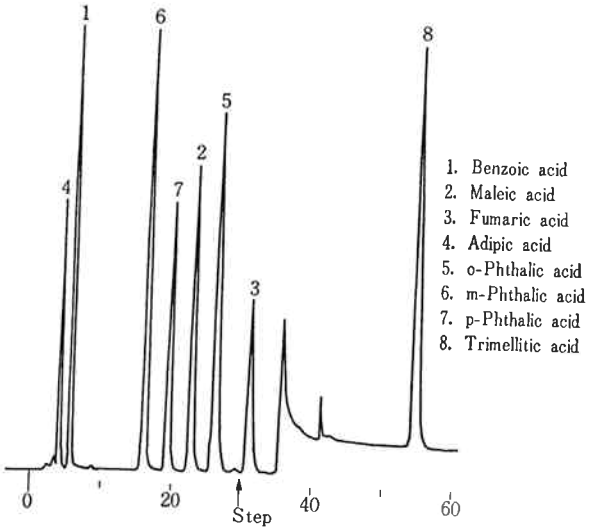

Fig. 24 Separation of organic acids
Condition
Column : IEX520QAE (4 mmID × 30 cm)
Elvent : Stepwise gradient elution from A to B. A : 30 v% CH_3CN in 50 mM Na_2HPO_4 pH 3.0, B : 40 v% CH_3CN in 50 mM Na_2HPO_4 pH 2.2.
Flow rate : 0.92 mℓ/min
Detection : UV 210 nm
Temperature : Ambient

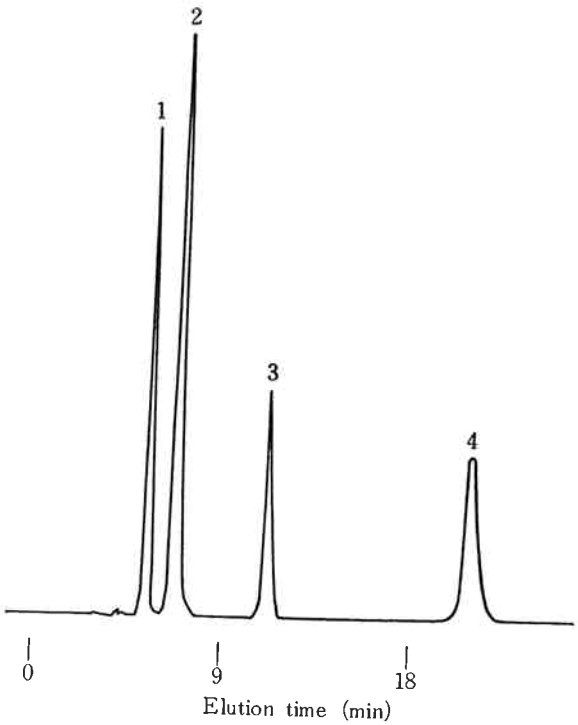

Fig. 25 Chromatogram of uric acid, xanthine, hypoxantine and orotic acid by IEX540DEAE
Condition
Sample : 1 : hypoxanthine, 2 : xanthine, 3 : orotic acid, 4 : uric acid.
Column : IEX540DEAE (4 mmID × 30 cm)
Eluent : 1/15 M phosphate buffer (pH 6.4)
Flow rate : 0.6 mℓ/min
Deteotion : UV 254 nn
Temperature : Ambient

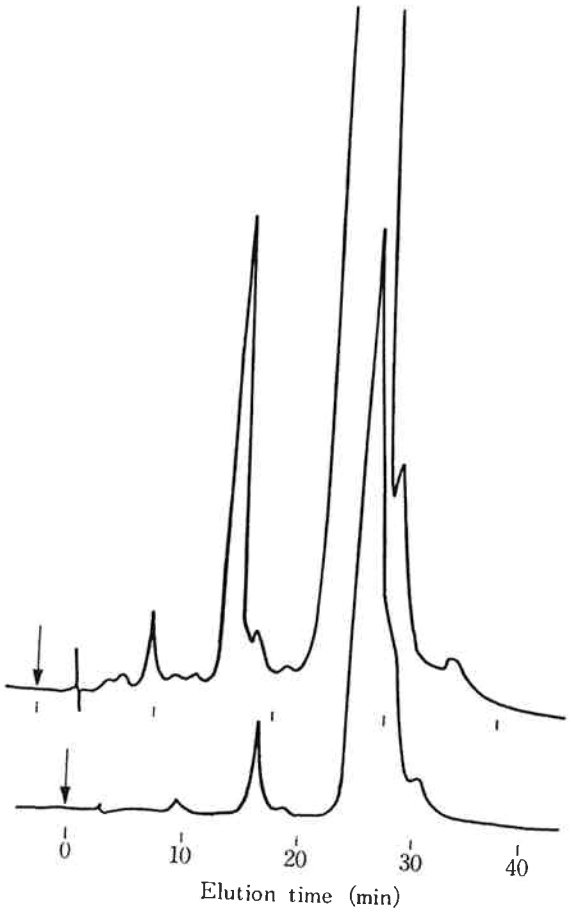

Fig. 26 Elution profile of human hemolysate on IEX-535CM
Test conditions
Column : IEX-535CM (6 mmID × 15 cm)
Eluent : Linear gradient elution from A to B in 30 min.
A ; 50mM Na_2HPO_4 pH 5.8 (adjusted with H_3PO_4)
B ; A + 0.1 M NaCl
Flow rate : 1.0 mℓ/min
Detection : VIS 415 nm
Temperature : 20°C
Sample : hemolysate diluted with five-fold distilled water (10 μℓ)

The elution profile of angiotensins in the typical condition on IEX-530CM column is shown in **Fig. 22.**

8. APPLICATION DATA BY ANION-EXCHANGER COLUMNS

〔1〕 Separation of organic acids containing some isomers by IEX-520 QAE column

The dependence of the elution volumes of organic acids on pH and the proportions of added acetonitrile was examined and the results are shown in **Fig. 23A** and **23B**, respectively.

The elution profile of organic acids in the typical condition on IEX-520QAE column is shown in **Fig. 24.**

〔2〕 Separation of nucleic acid derivatives by IEX-540 DEAE column

IEX-540DEAE column is employed to the separation of organic acids as IEX-520QAE column and effectively used to that of nucleic acid derivatives. Herein chromatogram of bases and metabolites is shown in **Fig. 25.**

9. SEPARATION OF HEMOGLOBIN (HUMAN) BY IEX-535CM COLUMN

A clinical interest in the analysis of hemoglobin components has been increasing since the relation between diabetes mellitus and glycosylated hemoglobins was shown[11,12].

Upto the present, analytical methods by means of electrophoresis[13~15] and ion-exchange chromatography[12,15,16] were investigated for purposes of the analysis of them and have been employed to the clinical test.

Now, the analytical column kits, in which cation-exchanger is packed, have been commonly employed for the clinical test. However, some problems such as poor resolution of hemoglobin components, necessity of solution containing cyanate and poor reproducibility have appeared.

Sample preparation

Erythrocytes were separated by centrifugation from human blood and washed three times with twofold volume of isotonic saline solution.

After fivefold volume of distilled water was added to erythrocytes for hemolysis of them, a half volume of toluene was added and vigorously mixed in order to remove the slightly water-soluble substances.

The water layer was analysed after filtration by 0.45-μm Millipore®.

The elution profile of the hemolysate by linear gradient elution on IEX-535CM column is shown in **Fig. 26.**

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