No. 030

TOSOH

SEPARATION REPORT

新しい蛋白質分離用高速イオン交換カラムとその応用

——目　次——

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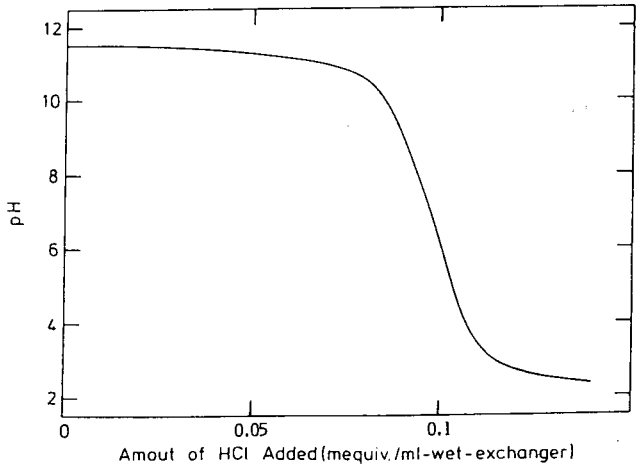
1. はじめに

イオン交換クロマトグラフィはセルロースイオン交換体の開発以来、蛋白質の分離精製手段として広く使用されてきた。さらに、数年前にポアーサイズの大きい多孔性シリカを基材とした高性能イオン交換体が開発され、短時間で高分離能が得られるようになった。しかし、基材のシリカが化学的に不安定であるため使用pH範囲に制限があり不便な点がある。この点を改良するため、親水性樹脂を基材とした高性能イオン交換体、TSKgel DEAE-5PW、TSKgel SP-5PWを開発した。ここでは、これら二種の高性能イオン交換体の基本的性質および蛋白質分離への応用例を紹介する。

2. TSKgel DEAE-5PW、TSKgel SP-5PWの基本的性質

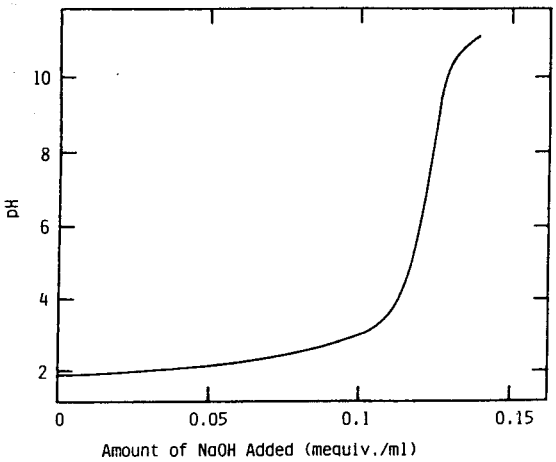
TSKgel DEAE-5PW、TSKgel SP-5PWは、それぞれ水系GPC用充填剤TSKgel G5000PWにジエチルアミノエチル基およびスルホプロピル基を導入したアニオン交換体およびカチオン交換体である。これらの基本的性質として、滴定曲線、蛋白質結合量、試料負荷量、蛋白質回収率、酵素活性回収率、化学的安定性に関する検討結果を図－1～図－6、表－1～表－7に示す。

図－1



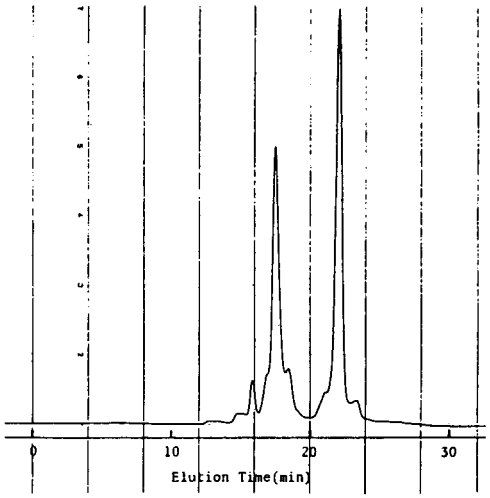
Titration curve of DEAE-5PW

図－2



Titration curve of TSKgel SP-5PW.

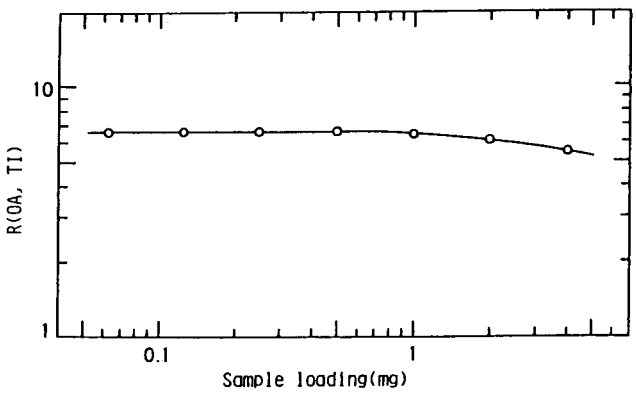
図－3



Chromatogram of a mixture of ovalbumin(first peak) and trypsin inhibitor (second peak) obtained on DEAE-5PW with 40 min linear gradient elution from 0.02 M tris-HCl buffer of pH 8.0 to 0.02 M tris-HCl buffer of pH 8.0 containing 0.5 M NaCl at a flow rate of 1 ml/min.

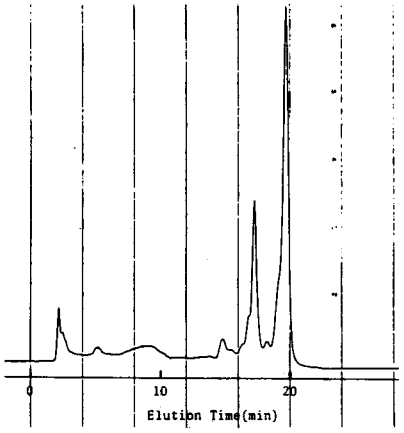
$$R(OA, TI) = 2(V_{TI} - V_{OA})/(W_{TI} + W_{OA})$$

図－4



Sample loading dependence of resolution between ovalbumin and trypsin inhibitor on DEAE-5PW.

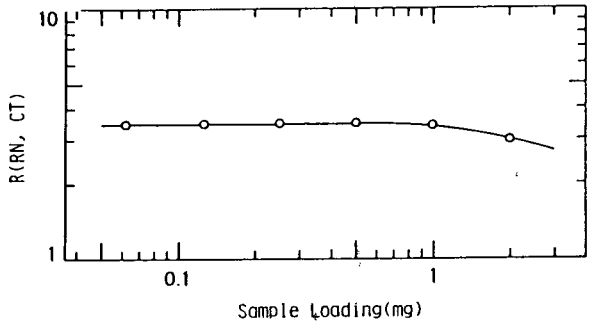
図－5



Chromatogram of a mixture of ribonuclease A(first peak) and α-chymotrypsinogen (second peak) obtained on SP-5PW with 40 min linear gradient elution from 0.02 M phosphate buffer of pH 7.0 to 0.02 M phosphate buffer of pH 7.0 containing 0.5 M NaCl at a flow rate of 1 ml/min.

$$R(RN, CT) = 2(V_{CT} - V_{RN})/(W_{CT} + W_{RN})$$

図－6



Sample loading dependence of resolution between ribonuclease A and α-chymotrypsinogen A on SP-5PW.

表－1

PROTEIN ADSORPTION CAPACITY OF TSKgel DEAE-5PW

Protein	Adsorption capacity(mg/ml)
Thyroglobulin	35
Ferritin	32
Bovine serum albumin	43

in 0.05 M Tris-HCl buffer(pH 8.3)

表－2

PROTEIN ADSORPTION CAPACITY OF TSKgel SP-5PW

Protein	Adsorption capacity(mg/ml)
γ-Globulin	40
Hemoglobin	43
Lysozyme	54

in 0.02 M phosphate buffer(pH 6.0)

表－3

RECOVERY OF PROTEINS FROM TSKgel DEAE-5PW

Protein	Recovery (%)
Thyroglobulin	98
Ferritin	99
γ-Globulin	100
Bovine serum albumin	102
Hemoglobin	96
Ovalbumin	104
β-Lactoglobulin	103
Trypsin inhibitor	104
Myoglobin	103

Each protein of 0.4 mg was applied to DEAE-5PW column(75 x 7.5 mm I.D.) in 0.02 M tris-HCl buffer(pH 8.5) and the adsorbed protein was desorbed in 0.02 M tris-HCl buffer(pH 8.5) containing 0.5 M NaCl.

表－4

RECOVERY OF PROTEINS FROM TSKgel SP-5PW

Protein	Recovery (%)
γ-Globulin	98
Hemoglobin	96
Trypsinogen	101
α-Chymotrypsinogen A	98
α-Chymotrypsin	104
Myoglobin	88
Lysozyme	95
Ribonuclease A	100
Cytochrome C	103

Each protein of 0.4 mg was applied to SP-5PW column(75 x 7.5 mm I.D.) in 0.02 M phosphate buffer(pH 6.0) and the adsorbed protein was desorbed in 0.02 M phosphate buffer(pH 6.0) containing 0.5 M NaCl.

表－5

RECOVERY OF ENZYMATIC ACTIVITY FROM TSKgel DEAE-5PW

Enzyme	Recovery (%)
Catalase	80
Lipoxidase	95
α-Chymotrypsin	93

表－6

RECOVERY OF ENZYMATIC ACTIVITY FROM TSKgel SP-5PW

Enzyme	Recovery(%)
Lipoxidase	84
α-Chymotrypsin	97

表－7

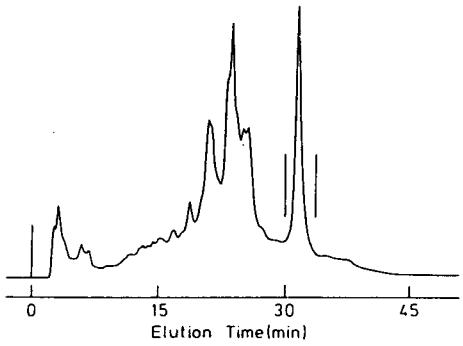
Change in Ion-Exchange Capacity of DEAE-5PW and SP-5PW during the Exposure in Alkaline or Acid Solution for 10 Days at 25°C.

Ion-exchanger	Solution	Ion-exchange capacity(meq/ml)	
		Before exposure	After exposure
DEAE-5PW	0.5 N NaOH	0.09	0.09
DEAE-5PW	0.5 N HCl	0.09	0.09
DEAE-5PW	20 % CH_3COOH	0.09	0.08
SP-5PW	0.5 N NaOH	0.13	0.13
SP-5PW	0.5 N HCl	0.13	0.13
SP-5PW	20 % CH_3COOH	0.13	0.13

3. TSKgel DEAE-5PW、TSKgel SP-5PW による蛋白質の分離

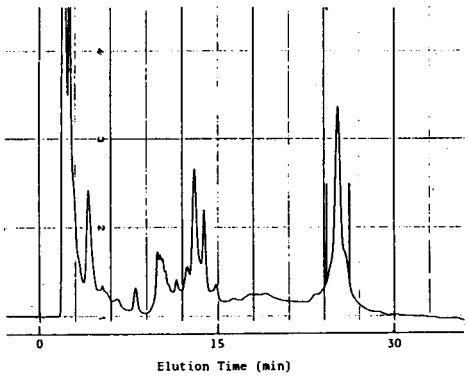
市販の各種蛋白質を分離した結果を図－7～図－13に示す。

図－7



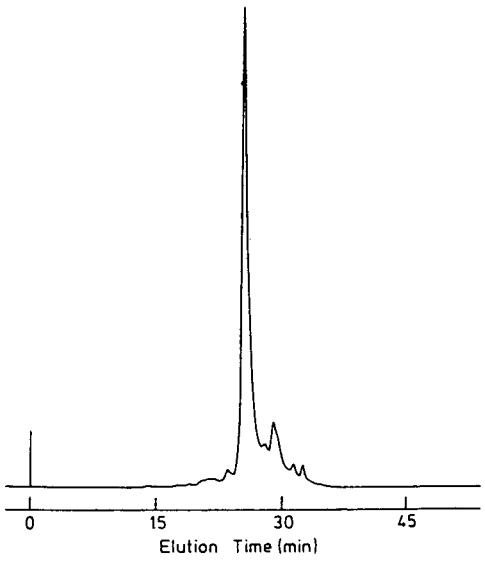
Separation of commercial lipoxidase by high-performance ion-exchange chromatography
Column: DEAE-5PW Sample loading: 1 mg in 0.1 ml
Elution: 60 min linear gradient from 0.02 M ethanolamine-HCl buffer of pH 9.0 to 0.02 M ethanolamine-HCl buffer of pH 9.0 containing 0.5 M NaCl
Flow rate: 1 ml/min Temperature: 25°C Detection: UV absorbance at 280 nm
Recovery of enzymatic activity was 95 %.

図－8



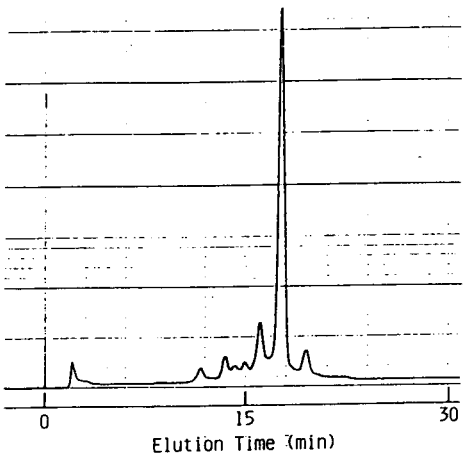
Separation of commercial lopoxidase by high-performance ion-exchange chromatography.
Column: SP-5PW Sample loading: 1 mg in 0.1 ml
Elution: 60 min linear gradient from 0.02 M acetate buffer of pH 4.5 to 0.02 M acetate buffer of pH 4.5 containing 0.5 M Na_2SO_4
Flow rate: 1 ml/min Temperature: 25°C Detection: UV absorbance at 280 nm
Recovery of enzymatic activity was 84 %.

図－9



Separation of commercial trypsinogen by high-performance ion-exchange chromatography
Column: DEAE-5PW Sample loading: 0.3 mg in 0.1 ml
Elution: 60 min linear gradient from 0.02 M 1,3-diaminopropane-HCl buffer of pH 9.8 to 0.02 M 1,3-diaminopropane-HCl buffer of pH 9.8 containing 0.5 M NaCl
Flow rate: 1 ml/min Temperature: 25°C Detection: UV absorbance at 280 nm

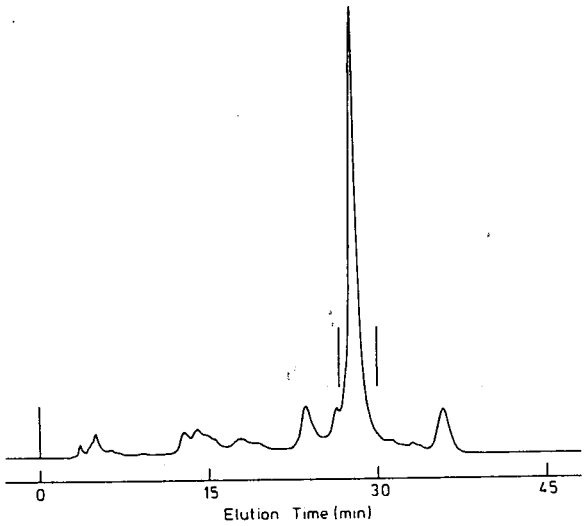
図－10



Separation of trypsinogen by high-performance ion-exchange chromatography

Column: SP-5PW　　Sample loading: 0.2 mg
Elution: 90 min linear gradient from 0.02 M phosphate buffer(pH 7.0)
to 0.02 M phosphate buffer containing 0.5 M NaCl(pH 7.0)
Flow rate: 1 ml/min　　Temperature: 25°C
Detection: UV absorbance at 280 nm

図－11

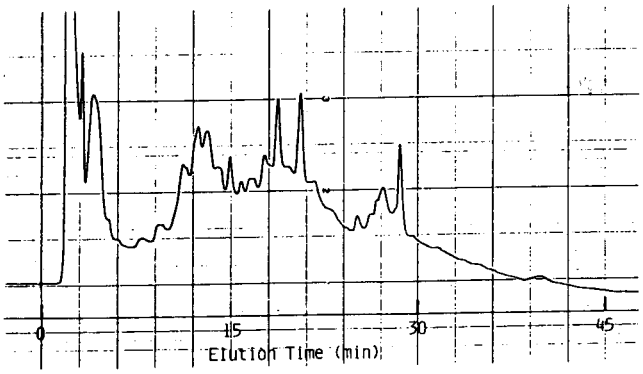


Separation of commercial α-chymotrypsin by high-performance ion-exchange chromatography

Column: DEAE-5PW　　Sample loading: 0.2 mg in 0.1 ml
Elution: 60 min linear gradient from 0.02 M 1,3-diaminopropane-HCl buffer of pH 9.8
to 0.02 M 1,3-diaminopropane-HCl buffer of pH 9.8 containing 0.5 M NaCl
Flow rate: 1 ml/min　　Temperature: 25°C　　Detection: UV absorbance at 280 nm

Recovery of enzymatic activity was 93 %.

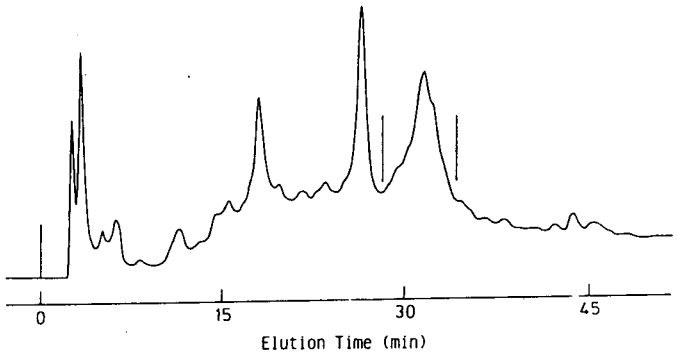
図－12



Separation of commercial urease by high-performance ion-exchange chromatography

Column: DEAE-5PW　　Sample loading: 1 mg in 0.1 ml
Elution: 60 min linear gradient from 0.02 M tris-HCl buffer of pH 8.0
to 0.02 M tris-HCl buffer of pH 8.0 containing 0.5 M NaCl
Flow rate: 1 ml/min　　Temperature: 25°C　　Detection: UV absorbance at 280 nm

図－13



Separation of commercial catalase by high-performance ion-exchange chromatography

Column: DEAE-5PW　　Sample loading: 1 mg in 0.1 ml
Elution: 60 min linear gradient from 0.02 M ethanolamine-HCl buffer of pH 9.0
to 0.02 M ethanolamine-HCl buffer of pH 9.0 containing 0.5 M NaCl
Flow rate: 1 ml/min　　Temperature: 25°C　　Detection: UV absorbance at 280 nm

Recovery of enzymatic activity was 80 %.