No. 074

TOSOH

SEPARATION REPORT

Separation of Monoclonal Antibodies Using TSKgel

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TOSOH CORPORATION

1. Introduction

Thanks to the recent development in biotechnology, use of monoclonal antibodies (Mab) has been advanced to application in diagnostic agents and therapeutic drugs. Because of such background, it is important to purify Mab rapidly and simply. Many reports have already been published as to Mab purification using high-performance liquid chromatography (HPLC), and features have been provided for each separation mode. This document reports on separation of Mab by HPLC using TSKgel with some applications. It also describes the applications on Mab purification using hydrophobic interaction chromatography and hydroxyapatite chromatography on which attention is focused currently.
There are also detailed description on antibody separation in Separation Reports No. 43, 62 and 67 for reference.

2. Separation Mode and Purification Method

Table-1 shows the HPLC separation modes used in Mab separation and purification and their features. IEC, HIC, HAC, and AFC (especially protein-A-support) have large sample load and high resolution, and they are often used in Mab purification. IEC is said to occupy about 75% of the Mab purifications as a method that follows ammonium sulfate fractionation (including low-speed chromatography). Though GFC (SEC) is inferior to the above separation mode in resolution or sample load, it is used as the final purification method and purity check on Mab preparation since it allows simple removal of high molecular weight Mab with antigenicity.

Table-1 Features of HPLC separation mode for monoclonal antibody purification

Separation mode	Separation mechanism	TSKgel	Feature
Gel filtration (size exclusion) chromatography (GFC, SEC)	Molecular size	(TSKgel G2000SW$_{XL}$) TSKgel G3000SW$_{XL}$ TSKgel G4000SW$_{XL}$	Though resolution and sample load are inferior to other separation modes, it allows simple removal of dimers and aggregates which cause the problem of Mab antigenic. It can also be used in final purification or purity check. Separation of Fab and IgM (TSK gel G4000SW$_{XL}$) is also possible.
Ion exchange chromatography (IEC)	Ionic interaction	TSKgel DEAE-5PW TSKgel SP, CM-5PW	Resolution is high and the sample load is large. Separation of Mab sub-class and impurities is favorable for anion exchanger. Cation exchanger allows simple purification by step-wise elution because many of the impurities are not retained in column.
		TSKgel DEAE-NPR TSKgel SP-NPR	NPR column can be used in rapid analysis and fractionation of trace amount Mab (μg to ng) and purity check that replaces electrophoresis on Mab fraction is also possible.
Hydrophobic interaction chromatography (HIC)	Hydrophobicity	TSKgel Ether-5PW TSKgel Phenyl-5PW TSKgel Butyl-NPR	Resolution is high and the sample load is large. Especially TSKgel Ether-5PW has high recovery of hydrophobic Mab. HIC allows simple purification by step-wise elution. TSKgel Butyl-NPR is for fractionation in trace amount analysis.
Affinity chromatography (AFC)	Bioaffinity	TSKgel Protein A-5PW (special order product)	Development of a new application in Mab separation.
		TSKgel Chelate-5PW	Activated affinity support.
		TSKgel Tresyl-5PW	Ligands such as antigen are immobilized to absorb and purify Mab.

In general, it is said that 1 general HPLC separation mode (except GFC) delivers Mab preparation of purity of about 95% for Mab separation and purification, thus high-purity preparation as diagnostic Mab can be obtained. However, higher purity (about 99% and higher) is required for Mab for medical treatment, and it is impossible for the general separation modes. While it is the unique method of AFC using protein A and antibody-antigen reaction which gives this purity as a single separation mode, 2 different separation modes must be used with general HPLC separation modes in order to obtain Mab preparation of this purity (for instance, IEC-HIC, or IEC-GFC).
Furthermore, high-purity Mab preparation (approximately 99.9% purity or higher) is required in some cases recently, so that 3 different types of separation modes are necessary in such cases (for instance, HIC-IEC-GFC or AFC-IEC-GFC). In addition, it is necessary to select proper elution method whether linear gradient elution or step-wise gradient elution with consideration of purity required in Mab preparation, time for purification, the cost for Mab preparation calculated from all processes, etc.
Table-2 shows the literature for Mab purification using various TSKgel products along with the outline on each. While majority uses IEC and GFC among HPLC methods, reports are being provided on application of HIC and AFC as well.

Table-2 Major literature on separation of monoclonal antibody using TSKgel

Literature No.	Column	Sample	Antibody type	Outline
1	TSKgel G3000SW	Ascites, cultured supernatant	IgG_1, $F_{(ab)2}$	Review article on Mab against class I, MHC antigen
2	TSKgel G3000SW	Ascites	IgG_1, $F_{(ab')2}$	Separation of IgG_1 and $F_{(ab')2}$
3	TSKgel G3000SW	Ascites	IgG_1, IgG_{2b}, IgG_3	Final purification of Mab purified by IEC Recovery is 85% and higher
4	TSKgel DEAE-5PW TSKgel G3000SW	Ascites, cultured supernatant	IgG_1, IgG_{2a} to the total of 8 species	1 step IEC purification of Mab by sophisticated eluent Recovery is 95%
5	TSKgel G3000SW	Ascites	IgG_1, IgG_{2a}, IgG_{2b}	Purity check on Mab purified by IEC
6	TSKgel DEAE-5PW	Ascites	IgG_1, IgG_{2a}, IgG_{2b}	1 step IEC purification of Mab by sophisticated eluent Recovery is 92% and higher
7	TSKgel Phenyl-5PW	Ascites, cultured supernatant	IgG	Examination of separation by IEC, HIC and HAC IEC and HIC were found to be good separation
8	TSKgel DEAE-TOYOPEARL TSKgel CM-TOYOPEARL	Cultured supernatant	IgG_{2b}	Rapid separation of sample, simple removal of indicator phenol red Recovery of activity is 90% and higher
9	TSKgel SP-5PW TSKgel G4000SW	Ascites	IgG_1, IgM	Simple purification by IEC, automatic control by IEC-GFC connection Recovery of activity is 80% and higher
10	TSKgel SP-5PW	Cultured supernatant	IgG	Step-wise gradient elution of pretreated sample (100mL) Purity 99%
11	TSKgel Chelate-5PW	Ascites	IgG	Application of IMAC, purity increased to 10-fold in 1 step
12	TSKgel DEAE-5PW TSKgel G3000SW	Ascites	IgG_1, IgG_{2a}, IgG_{2b}, IgM	Mab purification on preparative column by sophisticated eluent Recovery is 89% and higher
13	TSKgel DEAE-5PW TSKgel G3000SW	Ascites	IgG_1	Comparative examination of purification by IEC, HAC and AFC on preparative columns Purity 99.2% and recovery of activity 72%
14	TSKgel DEAE-5PW	Ascites	IgM (2 species)	Purification of IgM to high purity in 1 step using simultaneous gradient of salt and pH

3. Applications

1) Gel Filtration (Size Exclusion) Chromatography (GFC, SEC)

GFC is inferior to other separation modes in terms of resolution and sample load. Since, mouse ascites, etc. contain large amount of albumin as an impurity in the sample, separation of Mab (IgG) and albumin may be difficult with this mode. Thus GFC is often used in separation of partially purified samples instead of direct Mab purification from crude samples. Figure-1 shows an example of Mab (IgG_1) separation from mouse ascites. It is found that separation of IgG and albumin could be improved somewhat by changing the eluent pH and salt species (pH 5.0, 0.1mol/L Na_2SO_4) (for detailed examination, see Separation Report No.62). In IgM separation, TSKgel G4000SW_{XL} would be suited since it has large pore size which enables separation of larger molecules.

2) Ion-Exchange Chromatography (IEC)

IEC uses anion exchange type packing materials with DEAE group as the functional group and cation exchange type packing materials with SP or CM group as the functional group. In general, anion exchanger with DEAE group is used for precise separation of Mab or separation among subclasses by linear gradient method. Separation seems favorable when a relatively high pH of 8 or higher is used for the eluent. Figures-2 and -3 show Mab (IgG_1) separation from mouse ascites and culture supernatant on TSKgel DEAE-5PW. In separation of mouse ascites in Figure-2, IgG_1 elutes in about 15 minutes, and it is well separated from the impurities that elute before or after IgG_1 such as transferrin (about 11 minutes) and albumin (about 22 minutes). However, when the culture supernatant was applied as shown in Figure-3, Mab was not able to be detected as a peak because of its trace amount.

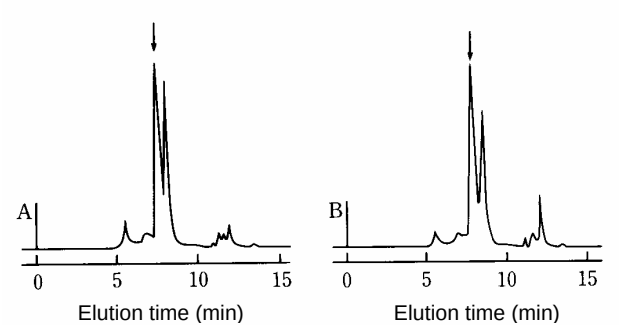

Figure-1 Separation of monoclonal antibody by GFC

Column:	TSKgel G3000SW_{XL} 7.8mmI.D. × 30cm
Eluent:	A: 50mmol/L sodium phosphate buffer (pH 6.7) + 0.3mol/L NaCl
	B: 50mmol/L sodium phosphate buffer (pH 5.0) + 0.1mol/L Na2SO4
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	UV (280nm)
Sample:	Anti-human proline hydroxylase (IgG_1), diluted solution of mouse ascites (50μl)

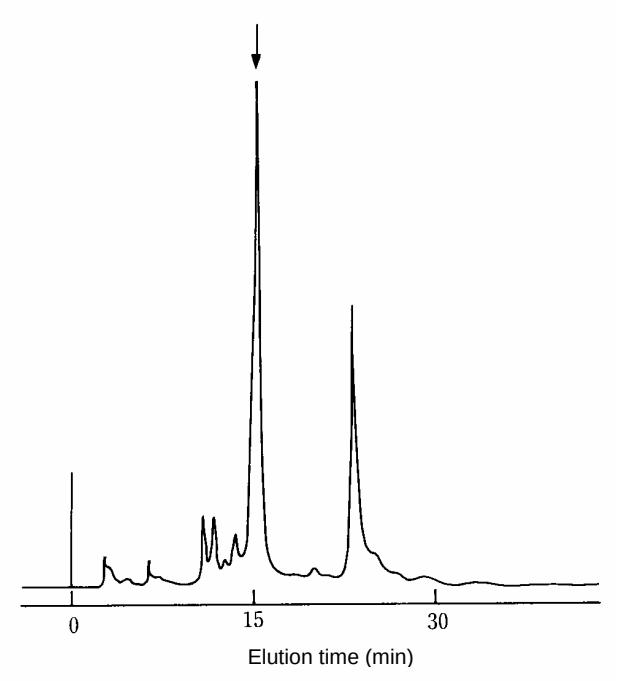

Figure-2 Separation of monoclonal antibody by IEC

Column:	TSKgel DEAE-5PW 7.5mmI.D. × 7.5cm
Eluent:	A: 20mmol/L Tris-HCl (pH 8.5)
	B: A + 0.5mol/L NaCl
	A → B linear gradient (60 min.)
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	UV (280nm)
Sample:	Anti-human albumin (IgG_1)
	Diluted solution of mouse ascites (168μg in 40μl)

Meanwhile, it is possible to process a large amount of sample at once with cation exchanger with SP or CM group because albumin, etc. that are contained in large amount pass through the column instead of being absorbed under the normal eluent pH. It is also possible to purify Mab simply by step-wise gradient elution. Figure-4 shows the chromatogram in which Mab (IgG_1) is separated from culture supernatant on TSKgel SP-5PW. In this case, it is clear that the elution pattern varies for citrate buffer and acetate buffer used as the eluents, and the selectivity for separation varies by the type of eluent.
Though non-porous packing materials cannot be applicable to purification of Mab in large volumes, it is possible to conduct a quick purity check on purified Mab preparation using its feature of rapid purification in trace amount.

Figure-5 shows the results of purity check on Mab fraction. For separation of mouse ascites samples, resolution nearly equivalent to that of TSKgel DEAE-5PW has been obtained (see Figure-2). In addition, it is evident that the amount of impurities is considerably small in partially purified Mab as well. If TSKgel DEAE-NPR which is a non-porous packing material is used in this fashion, purity check or monitoring on sample can be made in a matter of several minutes and this method can be extremely useful. Furthermore, use of non-porous packing material also allows micro fraction of nanograms of Mab in a sample (see Separation Report No.65 for details).

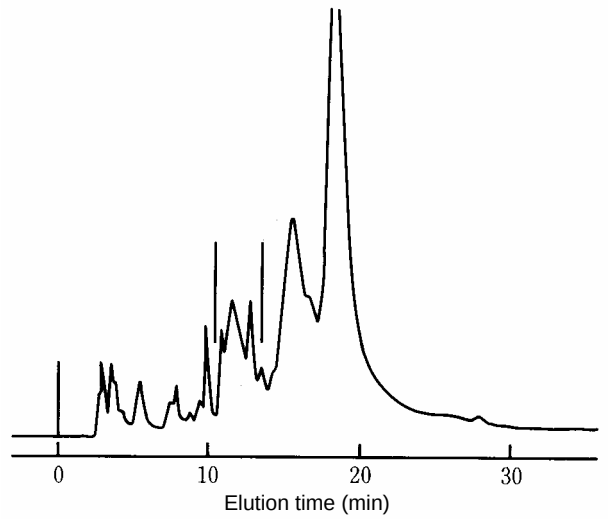

Figure-3 Separation of monoclonal antibody by IEC

Column:	TSKgel DEAE-5PW 7.5mmI.D. × 7.5cm
Eluent:	A: 20mmol/L Tris-HCl (pH 8.8) B: A + 0.5mol/L NaCl A → B linear gradient (30 min.)
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	UV (280nm)
Sample:	Anti-HLA-A, B, C (IgG_1), NS-1 culture supernatant

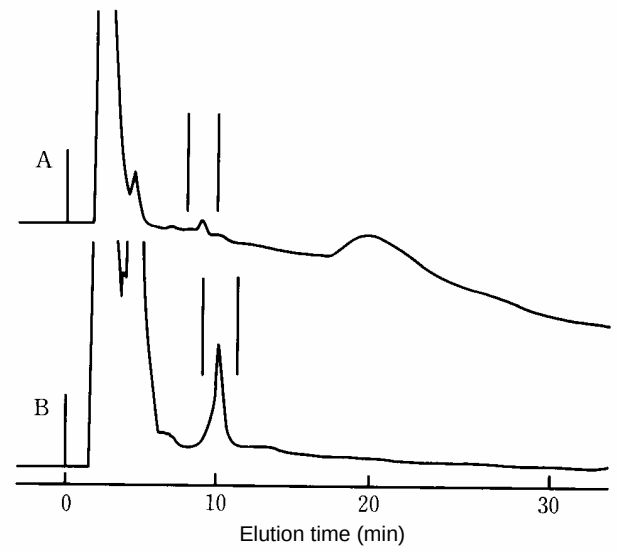

Figure-4 Separation of monoclonal antibody on IEC

Column:	TSKgel SP-5PW 7.5mmI.D. × 7.5cm
Eluent:	A: a: 20mmol/L citrate buffer (pH 5.7) b: a + 0.5mol/L NaCl a → b linear gradient (30 min.) B: c: 20mmol/L acetate buffer (pH 5.7) d: c + 0.5mol/L NaCl c → d linear gradient (30 min.)
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	UV (280nm)
Sample:	Anti-HLA-A, B, C (IgG_1), NS-1 culture supernatant (500μg in 500μl)

3) Hydrophobic Interaction Chromatography (HIC)

HIC is widely used as a method of protein purification as well as IEC, and it begins to be applied to Mab purification. Conventionally, TSKgel Phenyl-5PW is widely applied to separation of water-soluble proteins by HIC. However, good results have been obtained for separation and recovery of strongly hydrophobic proteins (membrane proteins and proteins with molecular weights of 100 thousand or higher) with TSKgel Ether-5PW in which hydrophobicity of packing materials is reduced somewhat (for details, please see Separation Report No.43).

Figure-6 shows separation of mouse ascites on TSKgel Phenyl-5PW and TSKgel Ether-5PW, respectively. With TSKgel Phenyl-5PW,) the Mab (IgG_1) peak is not separated from the albumin peak (approximately 47 minutes) by overlapping. However, the Mab is well separated from the albumin peak (approximately 13 minutes) on TSKgel Ether-5PW. In addition, Mab elutes faster on TSKgel Ether-5PW than TSKgel Phenyl-5PW under the same separation conditions (concentration of ammonium sulfate: approximately 0.5mol/L) due to the difference in hydrophobicity of packing materials. As you can see, it is evident that TSKgel Ether-5PW is better in Mab separation by HIC.

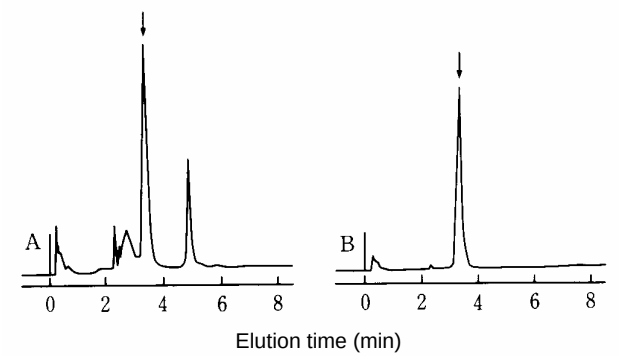

Figure-5 Purity check on monoclonal antibody fraction by IEC

Column:	TSKgel DEAE-NPR 4.6mmI.D. × 3.5cm
Eluent:	a: 50mmol/L Tris-HCl (pH 8.5)
	b: a + 0.5mol/L NaCl
	a → b linear gradient (10 min.)
Flow rate:	1.5mL/min
Temperature:	25°C
Detection:	UV (280nm)
Sample:	Anti-fowl 14K lectin (IgG_1)
	A: Mouse ascites (× 4, 5μl)
	B: TSKgel HA-1000, Mab fraction (100μl)

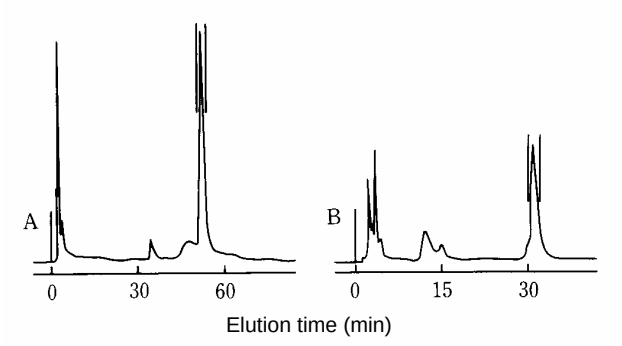

Figure-6 Separation of monoclonal antibody by HIC

Column:	A: TSKgel Phenyl-5PW
	7.5mmI.D. × 7.5cm
	B: TSKgel Ether-5PW
	7.5mmI.D. × 7.5cm
Eluent:	a: 0.1mol/L sodium phosphate buffer
	(pH 7.0) + 1.5mol/L ammonium sulfate
	b: 0.1mol/L sodium phosphate buffer
	(pH 7.0)
	a → b linear gradient (60 min.)
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	UV (280nm)
Sample:	Anti-fowl 14K lectin (IgG_1)
	Mouse ascites (1.5mg in 100μl)

Figure-7 shows an example of separating a large volume of sample on TSKgel Ether-5PW. 3mL of mouse ascites (2-fold dilution, 1.0mol/L ammonium sulfate concentration) was directly injected and eluted with linear gradient and step-wise gradient elution. A chromatogram nearly identical to Figure-6 has been obtained for linear gradient elution. In addition, it was possible with step-wise gradient elution to let most of the impurities with weaker absorption than Mab pass through the column so that only Mab is absorbed by setting the initial ammonium sulfate concentration to 1.0mol/L. The absorbed Mab could be eluted with 0.5mol/L ammonium sulfate. As you can see, it is possible to easily separate a large volume of sample with step-wise gradient elution.

Moreover, if one wishes to purify a large amount of Mab, it is also possible to employ packing materials for medium speed chromatography (TOYOPEARL) instead of packing materials for HPLC. TSKgel Ether-TOYOPEARL 650M has a larger particle size for packing materials compared to TSKgel Ether-5PW with nearly identical absorption properties, and delivers similar selectivity though resolution deteriorates somewhat compared to TSKgel Ether-5PW. For relationship between HPLC and TOYOPEARL, please see Separation Report No.52.

There are also non-porous packing materials for HIC, and Mab can be separated rapidly. Figure-8 shows separation of mouse ascites on TSKgel Butyl-NPR. Separation completes in about 3 minutes and it is clear that Mab is well separated from impurities.

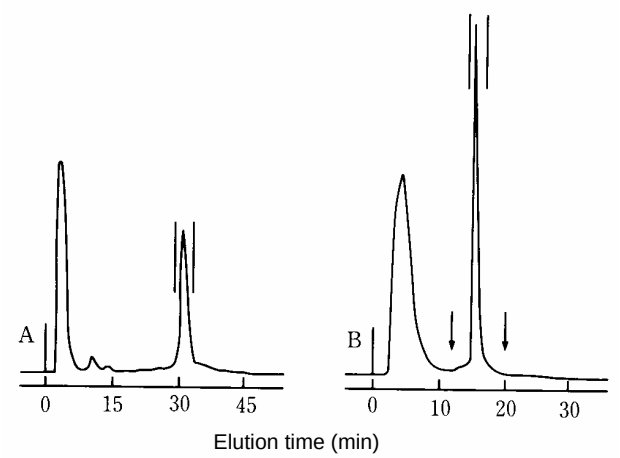

Figure-7 Separation of large amount of monoclonal antibody by HIC

Column: TSKgel Ether-5PW 7.5mmI.D. × 7.5cm
Eluent: A: Same as Figure-6
B: a: 0.1mol/L sodium phosphate buffer (pH 7.0) + 1.0mol/L ammonium sulfate
b: 0.1mol/L sodium phosphate buffer (pH 7.0)
a → a/b (50/50) (12 min.) → b (20 min.) step-wise gradient
Flow rate: 1.0mL/min
Temperature: 25°C
Detection: UV (280nm)
Sample: Anti-fowl 14K lectin (IgG_1)
Mouse ascites (90mg in 3.0mL)

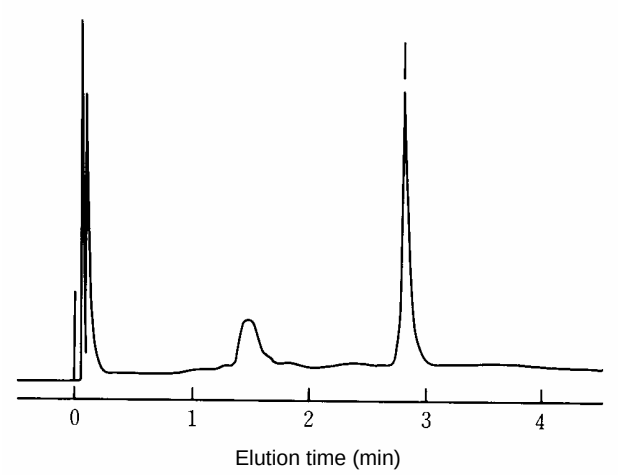

Figure-8 Separation of monoclonal antibody by HIC

Column: TSKgel Butyl-NPR 4.6mmI.D. × 3.5cm
Eluent: A: 0.1mol/L sodium phosphate buffer (pH 7.0) + 1.5mol/L ammonium sulfate
B: 0.1mol/L sodium phosphate buffer (pH 7.0)
A → B linear gradient (10 min.)
Flow rate: 1.0mL/min
Temperature: 25°C
Detection: UV (280nm)
Sample: Anti-fowl 14K lectin (IgG_1)
Mouse ascites (38μg in 2.5μl)

4) Affinity Chromatography (AFC)

In AFC, ProteinA is the most popular ligand in Mab purification. Figure-12 shows Mab (IgG_1) separation from mouse ascites on TSKgel Protein A-5PW (special order product). In order to strengthen Mab absorption, high-concentration solution (1.5mol/L glycine) is used in Mab absorption solution. With AFC using ProteinA, most of the impurities pass through the column instead of being absorbed. It is possible to elute IgG_1 by decreasing the eluent pH to 5.0 (eluent of pH 2 or 3 is required in order to elute other IgG subclasses). The obtained Mab fraction indicated no impurity even in GFC or SDS-PAGE, and it indicates that Mab can be purified to a high degree (approximately 99%) in 1 step using AFC with ProteinA.

Besides AFC using Protein A, it is also possible to separate Mab using Immobilized Metal Ion Affinity Chromatography (IMAC).

Though ligand leak is a problem for AFC, Protein A leak from the packing materials of TSKgel Protein A-5PW (special order product) is only about a few ng/mL in eluent.

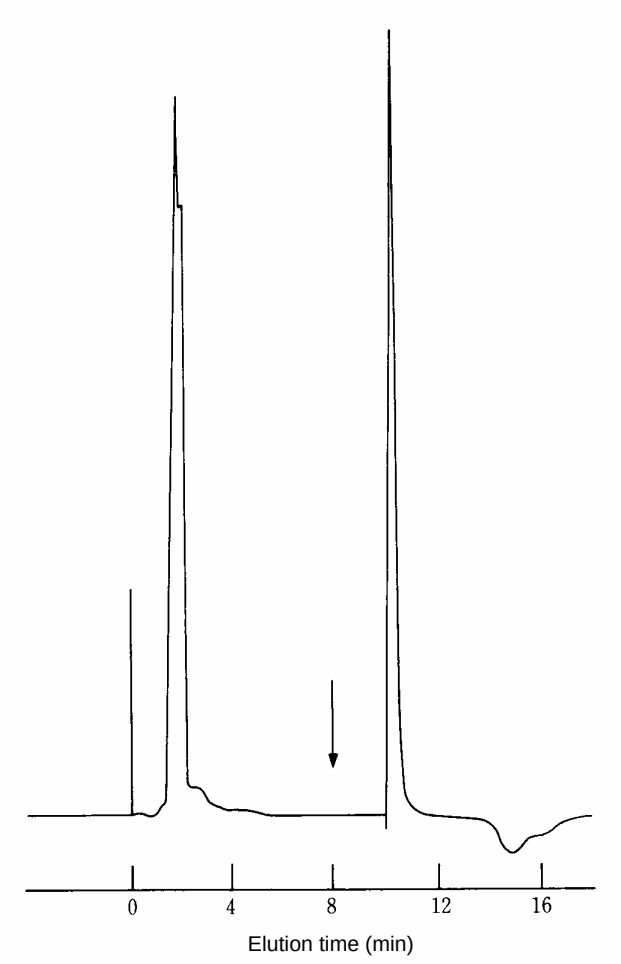

Figure-12 Separation of monoclonal antibody by AFC

Column:	TSKgel Protein A-5PW Glass (Special order product) 8mmI.D. × 4cm
Eluent:	A: 1.5mol/L glycine-NaOH (pH 8.9) B: 0.1mol/L citrate-NaOH (pH 5.0) A → B step-wise gradient (8 min.)
Flow rate:	0.8mL/min
Temperature:	25°C
Detection:	UV (280nm)
Sample:	Anti-human proline hydroxylase (IgG_1) Mouse ascites (210μg in 100μl)

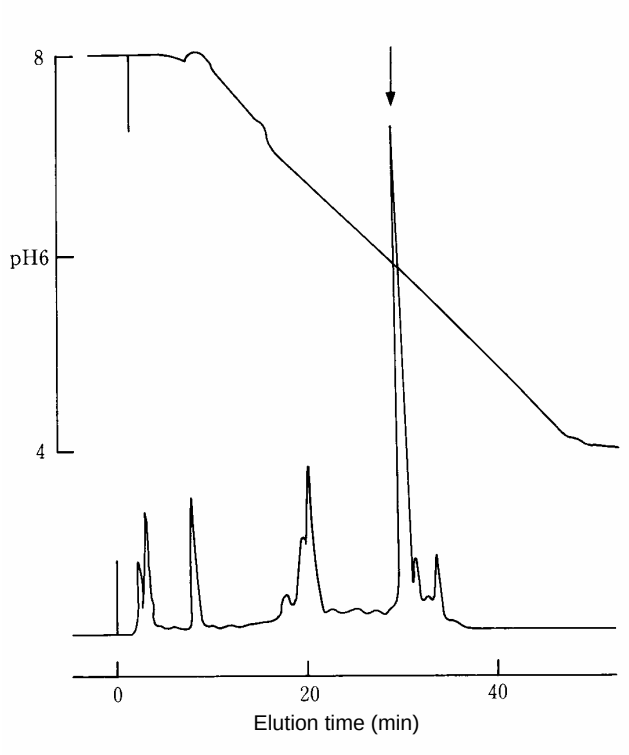

Figure-13 Separation of monoclonal antibody by AFC

Column:	TSKgel Chelate-5PW Glass (Zn^{2+}) 8mmI.D. × 7.5cm
Eluent:	A: 20mmol/L HEPES-MES-acetate buffer (pH 8.0) + 0.5mol/L NaCl B: 20mmol/L HEPES-MES-acetate buffer (pH 4.0) + 0.5mol/L NaCl A → B linear gradient (40 min.)
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	UV (280nm)
Sample:	Anti-fowl 14K lectin (IgG_1) Mouse ascites (50μl)

Figures-13 and -14 show the examples of separation of Mab from mouse ascites and culture supernatant on TSKgel Chelate-5PW with Zn^{2+} as the metal ion. Figure-13 shows that Mab (IgG_1) is eluted in about 32 minutes by pH gradient elution, and is well separated from other impurities. As you can see, IMAC is considered a new method applicable to Mab separation. For details about instruction on TSKgel Chelate-5PW, please see Separation Reports No.45 and No.70.

4. Application Examples

Combination between HAC and HIC

Though this document has discussed on Mab separation by only one separation mode, this section examines separation with a purpose of obtaining Mab preparation at higher purity by combining 2 different separation modes. While IEC-HIC, IEC-GFC, etc. have been reported as combinations of separation modes as shown in Table-2, this section describes the quick and simple Mab purification method with combination of HAC and HIC.

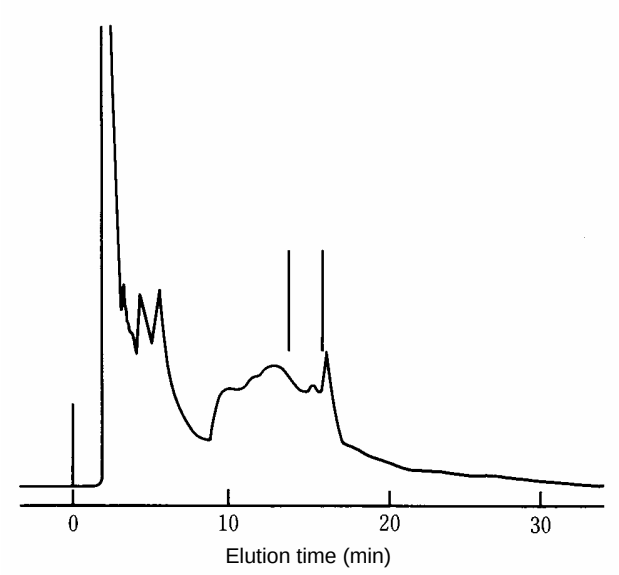

Figure-14 Separation of monoclonal antibody by AFC

Column:	TSKgel Chelate-5PW (Zn^{2+}) 7.5mmI.D. × 7.5cm
Eluent:	A: 20mmol/L Tris-HCl (pH 8.0) + 0.5mol/L NaCl B: A + 200mmol/L glycine A → B linear gradient (30 min.)
Flow rate:	1.0mL/min
Temperature:	25°C
Sample:	Anti-HLA-A, B, C (IgG_1) NS-1 cultured supernatant

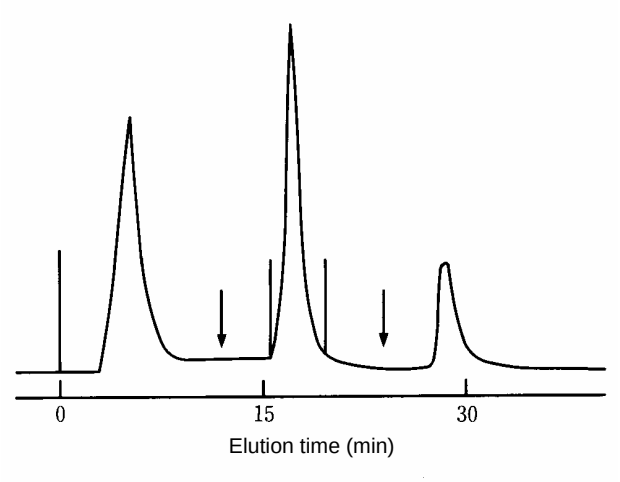

Figure-15 Separation of monoclonal antibody with combination of separation modes (1)

— Separation by HAC using TSKgel HA-1000 —

Column:	TSKgel HA-1000 7.5mmI.D. × 7.5cm
Eluent:	A: 10mmol/L sodium phosphate buffer (pH 5.8) + 0.1mmol/L $CaCl_2$ B: 300mmol/L sodium phosphate buffer (pH 5.8) + 0.1mmol/L $CaCl_2$ A/B (80/20) → A/B (50/50) (12 min.) → B (24 min.) step-wise gradient
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	UV (280nm)
Sample:	Anti-fowl 14K lectin (IgG_1) Mouse ascites (20.8mg in 1.6mL)

Step-wise gradient elution similar to 3) and 4) was used for HAC and HIC. Figure-15 shows Mab elution by HAC using TSKgel HA-1000 as the first step. With the initial phosphate concentration for elution (60mmol/L), most of the impurities elute without being absorbed to the column. Then the target Mab is eluted at phosphate concentration of 150mmol/L. The peak is seen when the phosphate concentration is raised even further, but this peak turned out to be a fraction of high molecule substances such as nucleic acids and different antibodies as a result of examination by GFC, etc. Then this Mab fraction was processed by HIC with TSKgel Ether-5PW (Figure-16).

Sample was diluted to 2-fold with 2mol/L ammonium sulfate solution (final concentration 1.0mol/L) to be injected directly in sample loop (3.0mL × 2). Similar to HAC, impurities also eluted without being absorbed to the column for HIC. Then it was possible to elute Mab at ammonium sulfate concentration of 0.5mol/L.

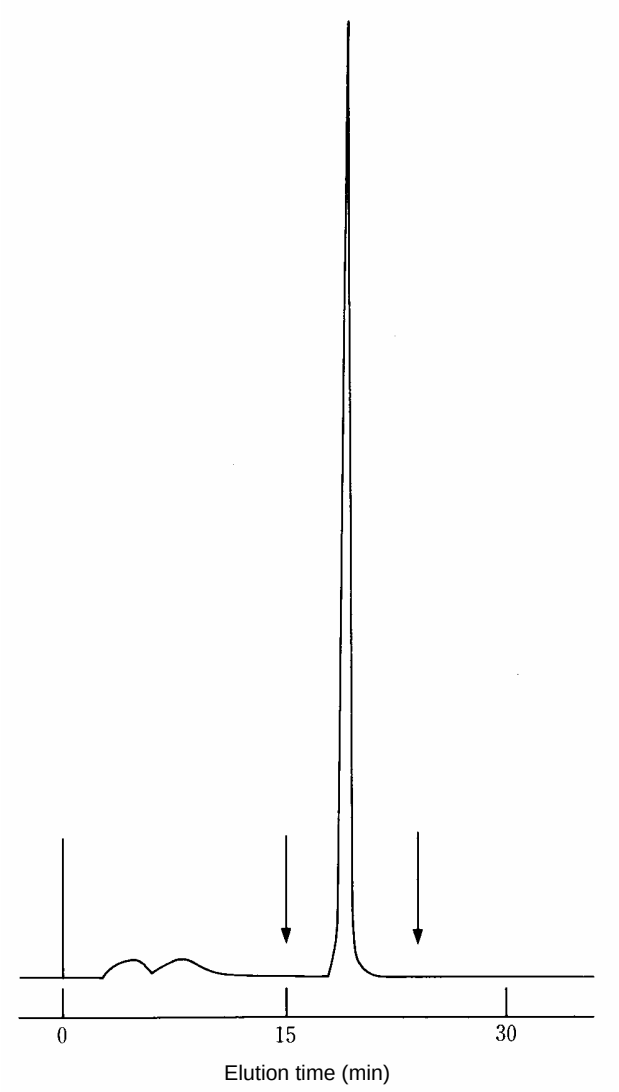

Figure-16 Separation of monoclonal antibody with combination of separation modes (2)

— Separation by HIC using TSKgel Ether-5PW —

Column:	TSKgel Ether-5PW 7.5mmI.D. × 7.5cm
Eluent:	A: 0.1mol/L sodium phosphate buffer (pH 7.0) + 1.0mol/L ammonium sulfate B: 0.1mol/L sodium phosphate buffer (pH 7.0) A → A/B (50/50) (15 min.) → B (24 min.) step-wise gradient
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	UV (280nm)
Sample:	Mab fraction obtained in Figure-15 (2-fold dilution 6.0mL)

Purity was checked on the Mab fraction obtained by the above method.
Figure-17 shows separation of Mab fraction by GFC using TSKgel G3000SW$_{XL}$. While Mab elutes at approximately 9 minutes, Mab fraction after HAC has been removed with impurities compared to mouse ascites though some are still mixed such as albumin eluting at 11 minutes.

For HIC fraction after HAC, nearly no peak is seen other than Mab (mixture of high molecule substances was found though in extremely small amount), and it was found that Mab preparation of purity approximately 99% was obtained calculating from the peak area.
Figure-18 shows the results of SDS-polyacrylamide gel electrophoresis on these Mab fractions. Nearly no bands other than the H-chain and L-chain of Mab are seen in Mab preparation after HIC.
As shown above, it is possible to obtain high-purity Mab preparation by combining 2 different separation modes called HAC and HIC with simple step-wise gradient elution method. Furthermore, it is possible to further combine GFC after HAC-HIC as shown in Figure-18 to collect the Mab faction when further purity is desired in Mab preparation.

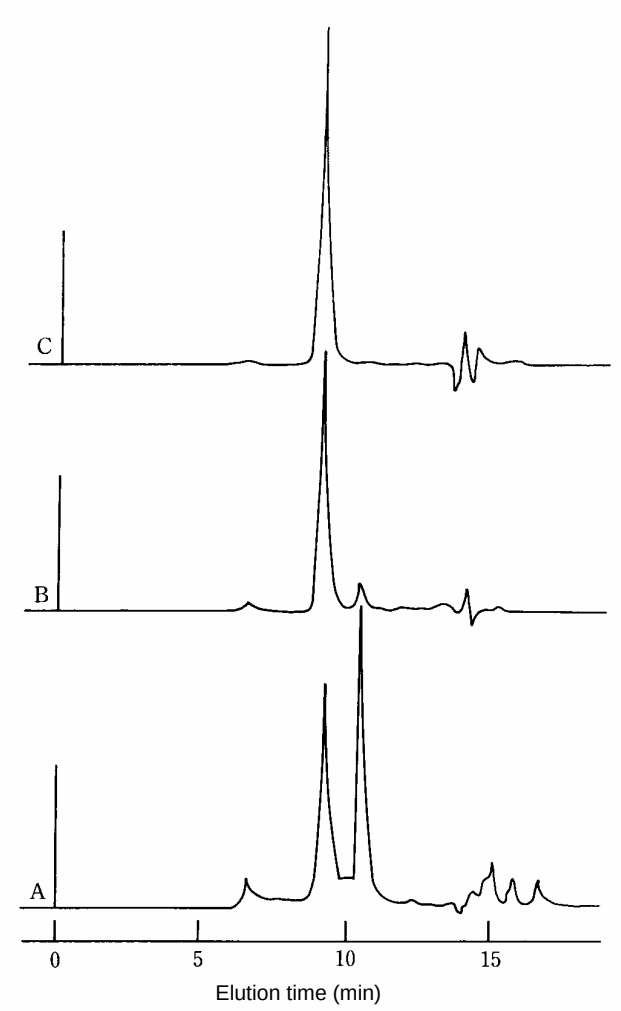

Figure-17 Purity check on purified monoclonal antibody by GFC on TSKgel G3000SW$_{XL}$

Column: TSKgel G3000SW$_{XL}$ 7.8mmI.D. × 30cm
Eluent: 50mmol/L sodium phosphate buffer (pH 6.7) + 0.3mol/L NaCl
Flow rate: 0.8mL/min
Temperature: 25°C
Detection: UV (280nm)
Sample: Anti-fowl 14K lectin (IgG1)
A: Mouse ascites (× 4, 2.5μl)
B: Mab fraction obtained in Figure-15 (10μl)
C: Mab fraction obtained in Figure-16 (10μl)

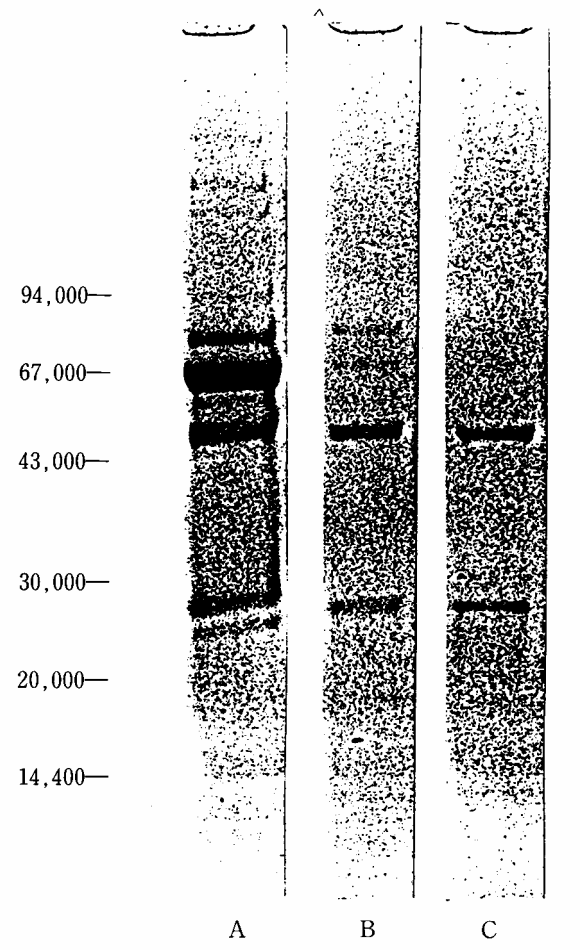

Figure-18 Purity check on purified monoclonal antibody by SDS-polyacrylamide gel electrophoresis

SDS-PAGE 4 to 20% gradient gel
Sample: Same as Figure-17

5. Conclusion

As discussed in this document, there are several different separation modes for purification of monoclonal antibody, and each separation mode has its advantages. Therefore, it seems important in purification of monoclonal antibody to select the most appropriate separation mode and column with consideration of various factors for purification (resolution, sample load, operability, cost, purity, etc.).

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