Essentials in biore

930631

Poster Presentation

FASEB/ASBMB Symposium - April, 1991
Poster # 2529

Protein Purification Using Cation Exchange Chromatography in Analytical and Preparative Glass Columns

D. Phillips*, D. Dion*, B. San Souci*, & H. Hodgdon†
***Millipore Corporation, Waters Chromatography Division**
Milford, MA. 01757 U.S.A.
†Worcester Polytechnic Institute, Worcester, MA. U.S.A.

MILLIPORE

Waters Chromatography Division
34 Maple Street
Milford, MA 01757
508 478-2000

ABSTRACT

Waters new family of porous resins for ion exchange chromatography was expanded to include a weak cation exchanger. The addition of Protein-Pak™ CM HR series to the DEAE and SP packings allows scientists to select the material most appropriate for the isoelectric point and pH limitations of their protein. Three particle sizes (8, 15 and 40 micron) are available either in Advance Purification (AP) glass columns or as bulk packings, allowing one to purify on either an analytical or preparative scale. Using the Protein-Pak CM HR series columns with the Waters 650 Advanced Protein Purification non-metallic chromatography system facilitates the transition from process to laboratory scale.

A protein mixture was well resolved with the Protein-Pak CM 40HR packing in the AP Minicolumn (5 mm x 100 mm) and in the AP-1 column (10 mm x 100 mm). A mixture of five standard proteins was baseline resolved in each case and competitive with other commercial weak cation exchangers. The protein binding capacity is relatively stable between pH 5 and 7, which is the pH range where most proteins retain their biological activity. Mass recovery was greater than 80% for a set of 10 proteins varying in size and isoelectric point. Lysozyme, which was isolated from crude egg white on the new cation exchanger, maintained its biological activity.

INTRODUCTION

The Protein-Pak™ CM HR packings are members of the family of ion exchangers designed to deliver high resolution, excellent recovery and full scalability in the chromatographic separation of biomolecules such as proteins, peptides and nucleic acids.

Four Chemistries

Protein-Pak SP and CM HR cation exchange packings with covalently bonded propyl sulfonic acid and carboxymethyl functionalities, respectively, on hydrophilic supports.

Protein-Pak DEAE and Q HR anion exchange materials with covalently bonded diethylaminoethyl and quaternary methylamine functionalities, respectively, on hydrophilic supports

Three Particle Sizes

The packing materials are made from a rigid spherical, fully porous polymeric gel with nominal particle diameters of 8, 15 or 40 microns.

Different Column Sizes for Scale-up

The packing materials are available in scalable pre-packed glass columns (8 and 15 microns) or in bulk (15 and 40 microns) to meet all analytical or preparative needs. The glass column diameters are 5, 10, 20, and 50 mm with lengths ranging from 50 mm to 600 mm.

PROTEIN-BINDING CAPACITY OF THE PROTEIN-PAK™ HR SERIES PACKINGS

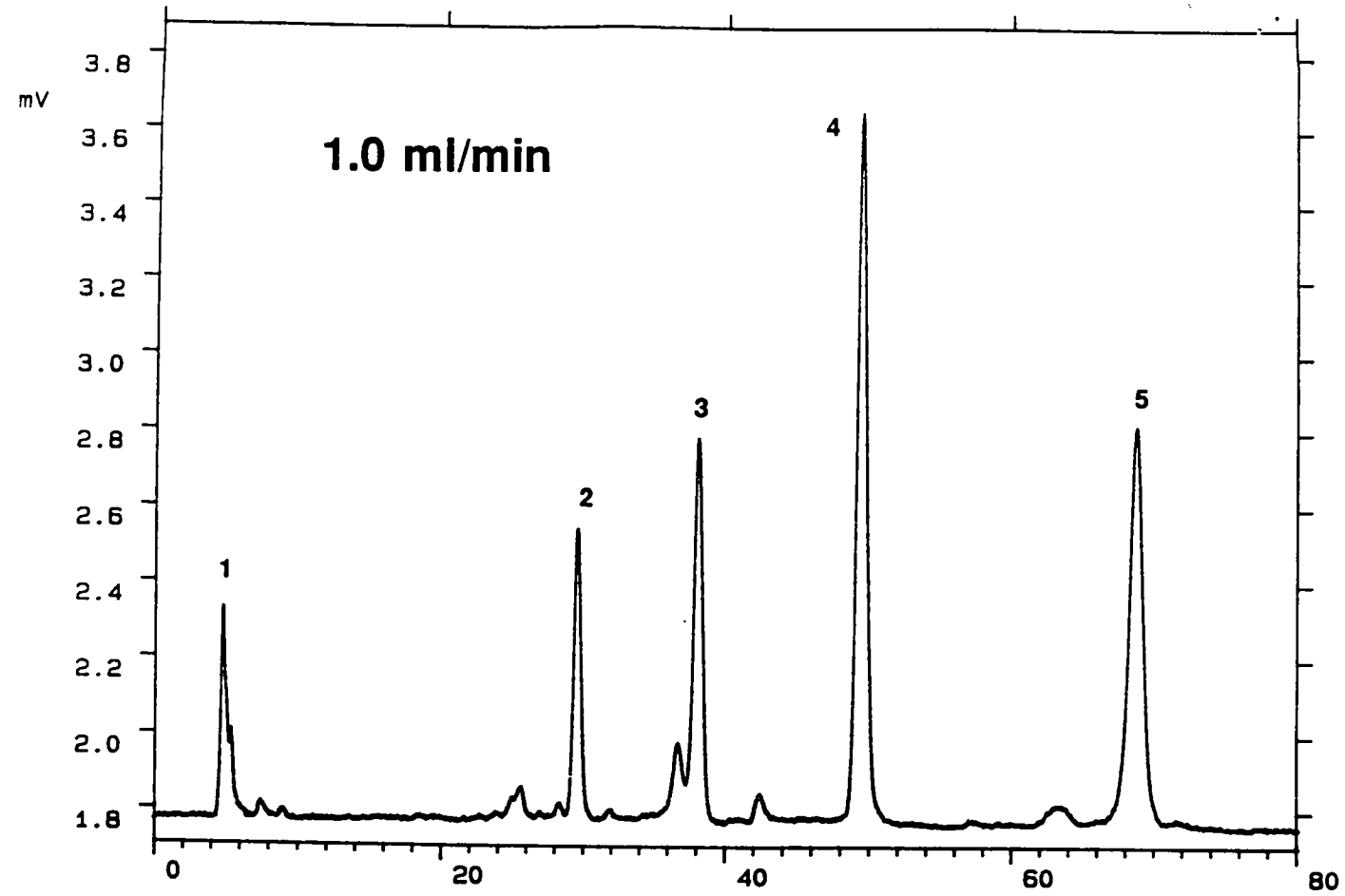
	mg BSA/ ml	mg BSA/ column	mg cyto-chrome c/ml	mg cytochrome c/column
Anion Exchangers				
DEAE 8HR	45	353		
DEAE 15HR	40	314		
DEAE 40HR	40	314		
Q 8HR	65	510		
Q 15HR	75	590		
Q 40HR	74	581		
Cation Exchangers				
SP 8HR			40	314
SP 15HR			40	314
SP 40HR			22	173
CM 8HR			23	181
CM 15HR			25	196
CM 40HR			32	250

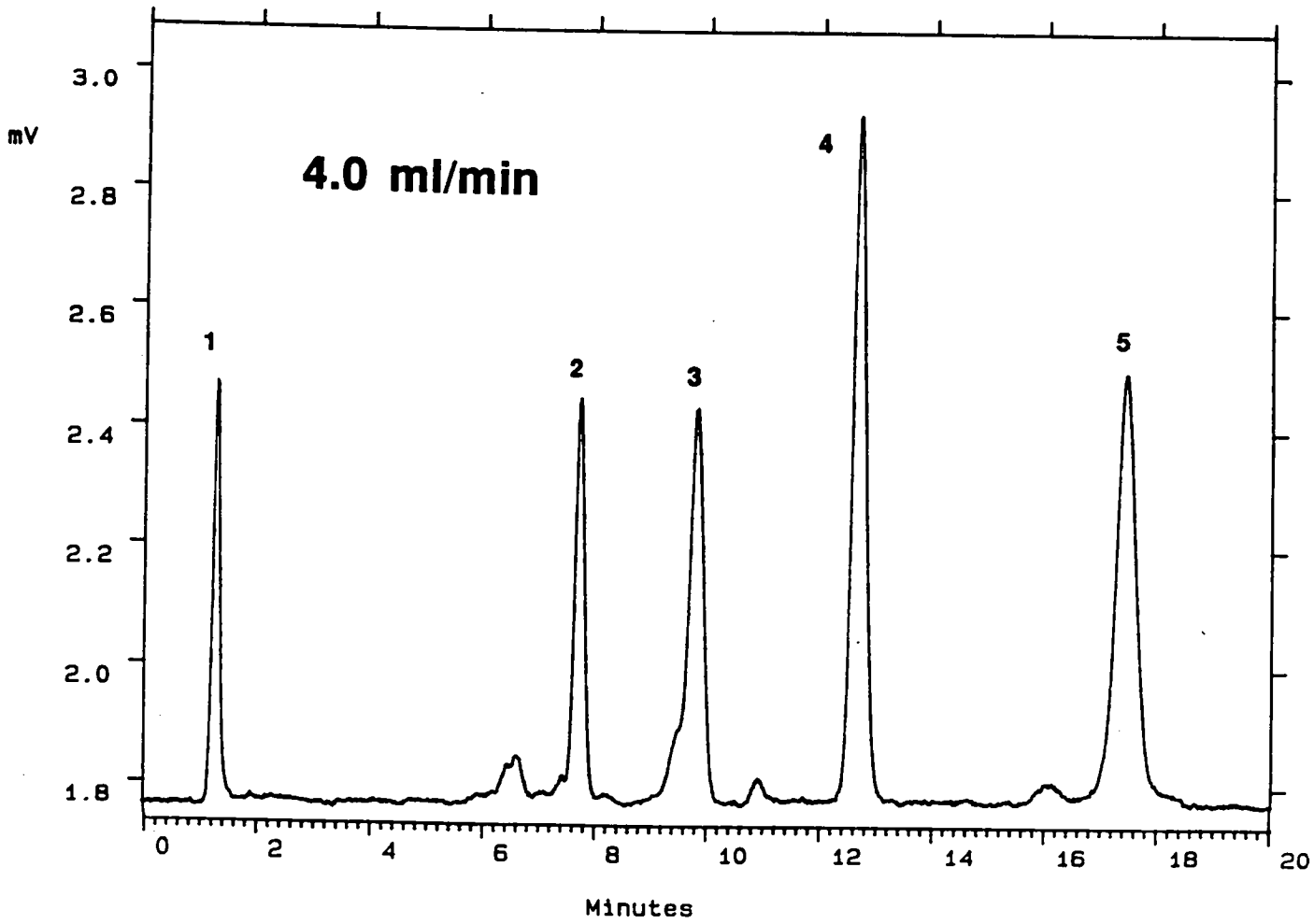
Conditions: Anion: 20 mM Tris-HCl, pH 8.2; Sample: bovine serum albumin (BSA).
Cation: 25 mM MES, pH 5.0; Sample: cytochrome c. Column: AP1 (10 mm x 100 mm) glass column.
Packings: 8HR and 15HR are sold as pre-packed columns and the15HR and 40HR as bulk materials.

FLOW RATE STUDY ON PROTEIN-PAK™ AP-1 GLASS COLUMN

COLUMN:	AP-1 (10mm x 100mm) GLASS COLUMN;
BUFFER A:	20mM SODIUM PHOSPHATE, pH 7.0;
BUFFER B:	BUFFER A + 1M SODIUM CHLORIDE;
DETECTOR:	280nm
SAMPLE:	100µl (500µg) OF MIXTURE WITH 1. MYOGLOBIN, 2. RIBONUCLEASE A, 3. α-CHYMOTRYPSINOGEN A, 4. CYTOCHROME C, 5. LYSOZYME
FLOW RATE 1:	1.0 ml/min;
FLOW RATE 2:	4.0 ml/min;
GRADIENT 1:	0-50% BUFFER B IN 125 min;
GRADIENT 2:	0-50% BUFFER B IN 32 min

8HR AP-1 GLASS COLUMN

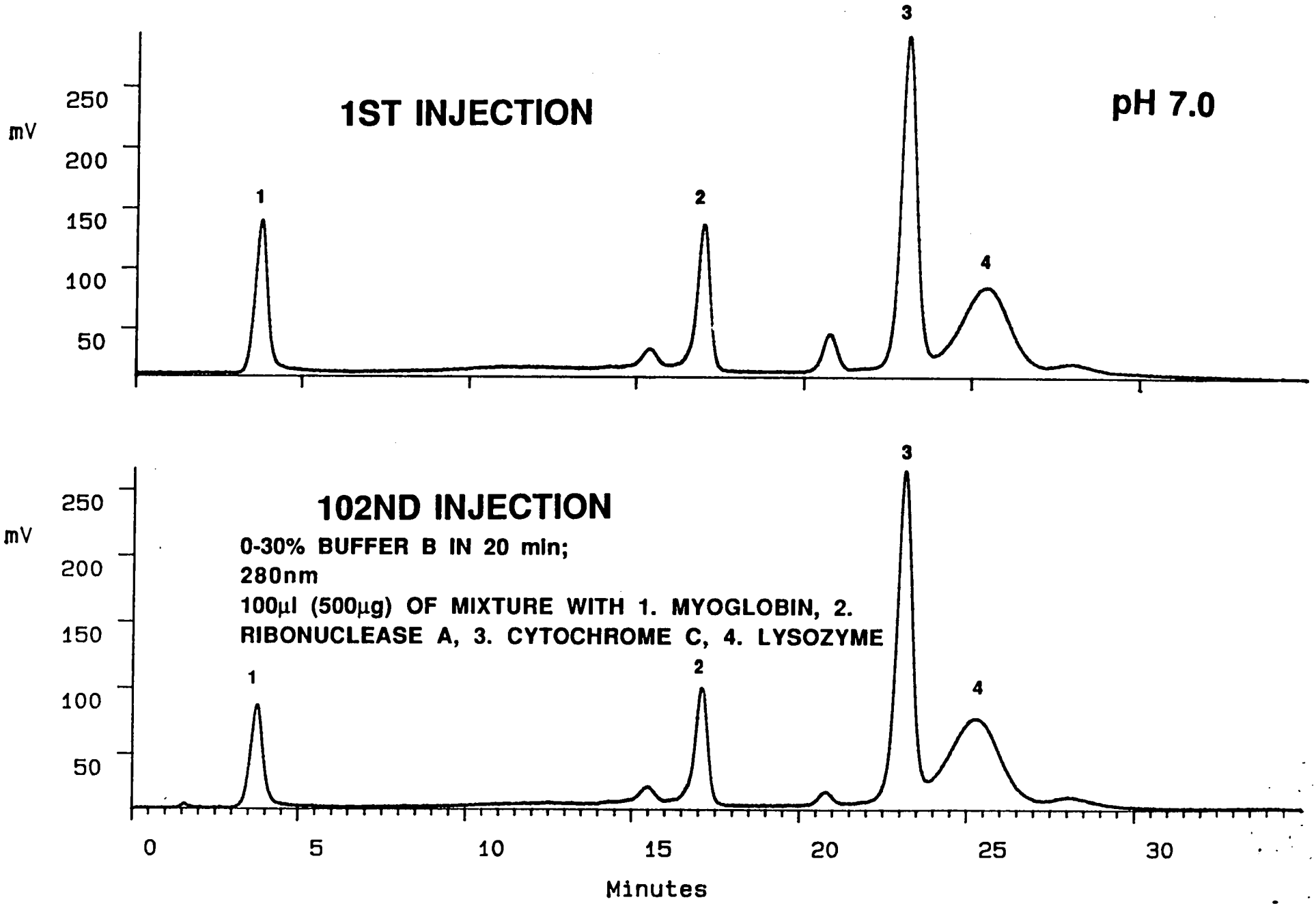




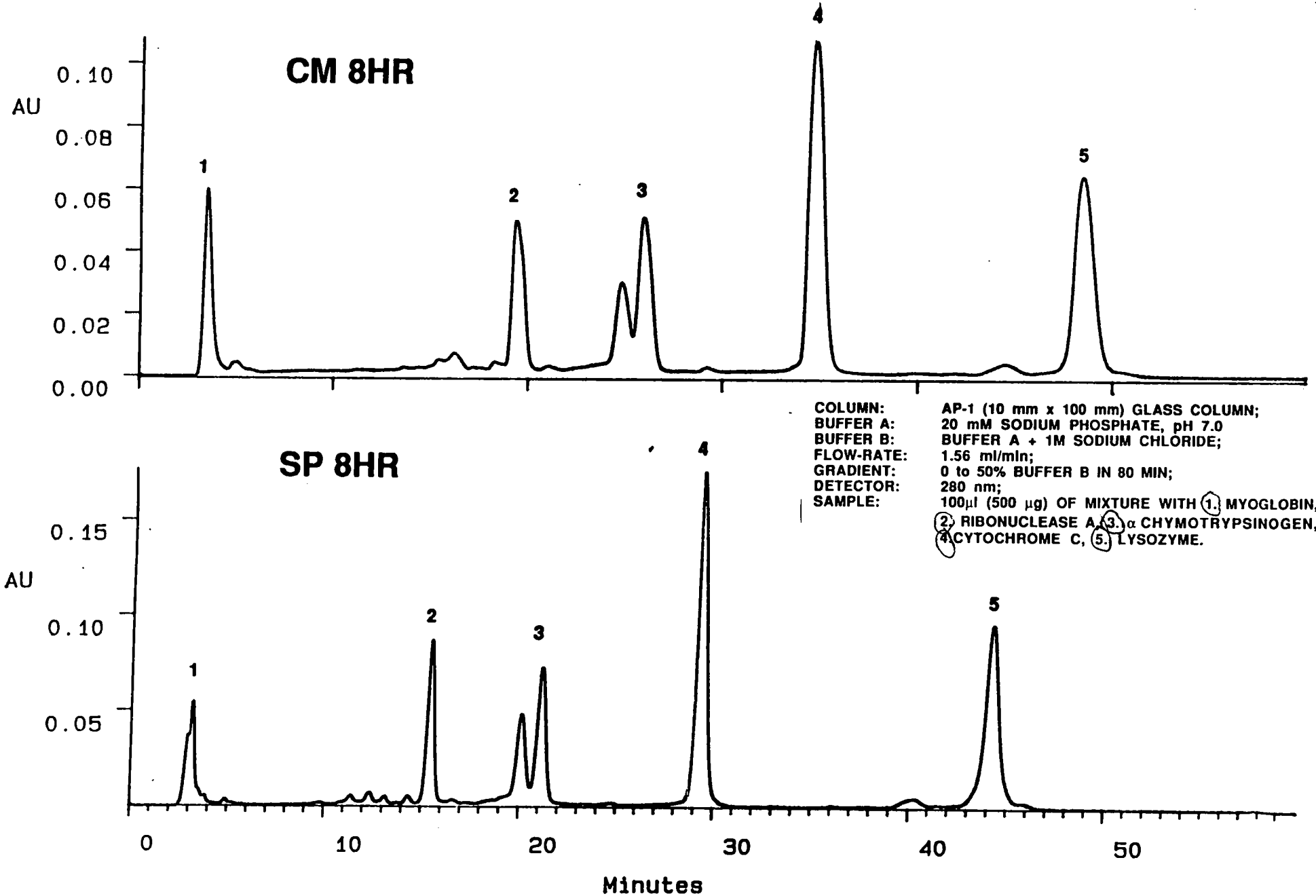
PROTEIN-PAK™ CM 8HR AP-1 GLASS COLUMN: MASS RECOVERY

PROTEINS	% RECOVERY	MOLECULAR WEIGHT	ISOELECTRIC POINT
CYTOCHROME C ✓	97.8	12400	9.4
α CHYMOTRYPSIN	93.5	21600	8.8
MYOGLOBIN ✓	88.7	17500	7
RIBONUCLEASE A ✓	91.5	13500	8.8
OVALBUMIN	88.2	44500	4.7
HEMOGLOBIN	72.6	64500	7
LYSOZYME ✓	92.2	14400	11
SB. TRYPSIN INHIBITOR	91.2	21500	4.5
ß-LACTOGLOBULIN A	88.7	35000	5.1
α CHYMOTRYPSINOGEN A ✓	91.7	25000	9
IMMUNOGLOBULIN G	92.2	155000	6.0-7.0

PROTEIN-PAK™ CM 15HR AP1 GLASS COLUMN: STABILITY TEST

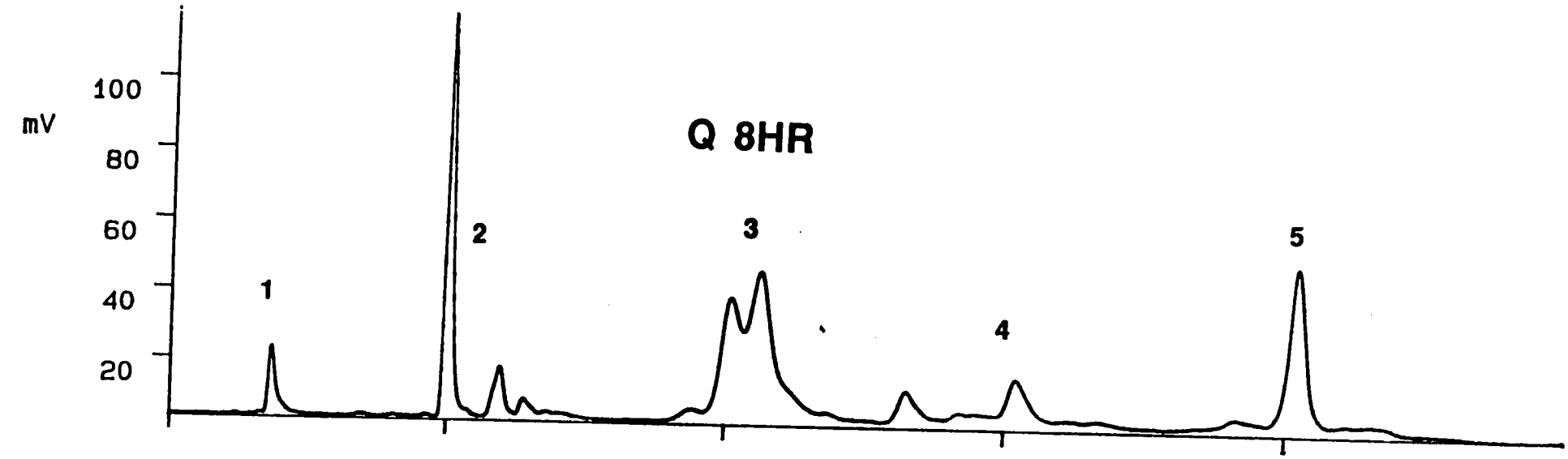


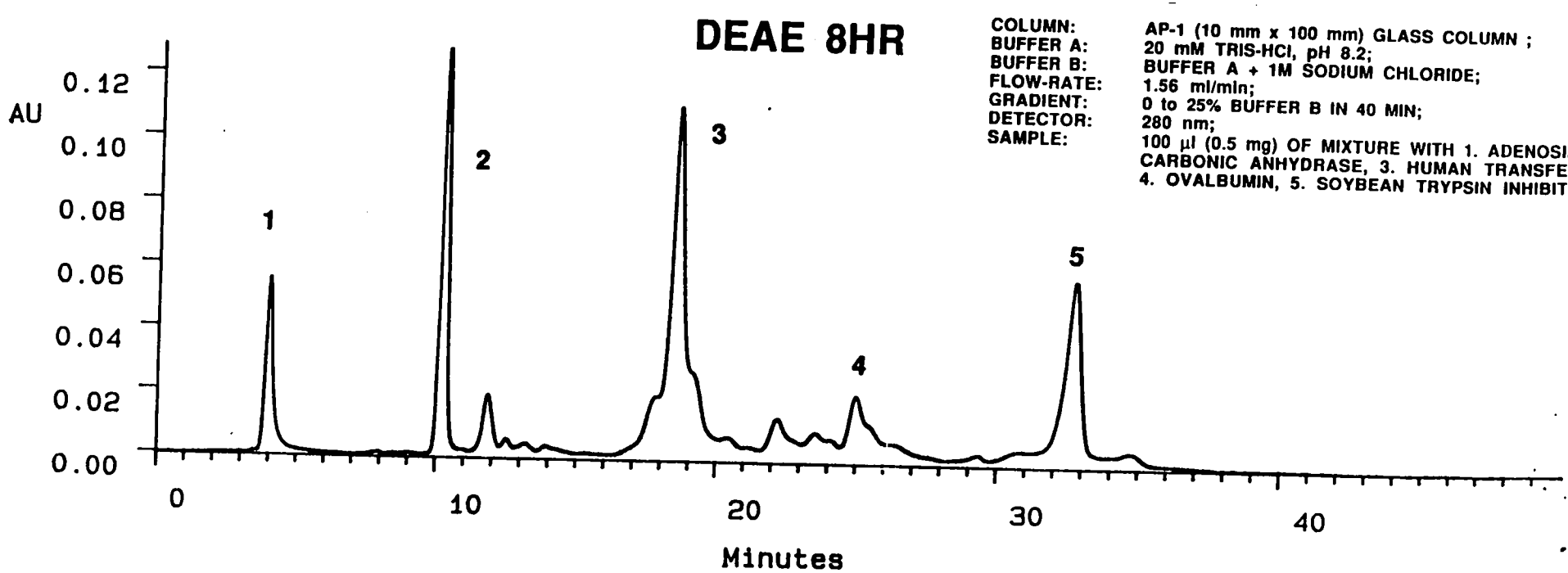
SEPARATION OF PROTEIN MIXTURE: PROTEIN-PAK™ CATION EXCHANGERS



COLUMN: AP-1 (10 mm x 100 mm) GLASS COLUMN;
BUFFER A: 20 mM SODIUM PHOSPHATE, pH 7.0
BUFFER B: BUFFER A + 1M SODIUM CHLORIDE;
FLOW-RATE: 1.56 ml/min;
GRADIENT: 0 to 50% BUFFER B IN 80 MIN;
DETECTOR: 280 nm;
SAMPLE: 100µl (500 µg) OF MIXTURE WITH 1. MYOGLOBIN, 2. RIBONUCLEASE A, 3. α CHYMOTRYPSINOGEN, 4. CYTOCHROME C, 5. LYSOZYME.

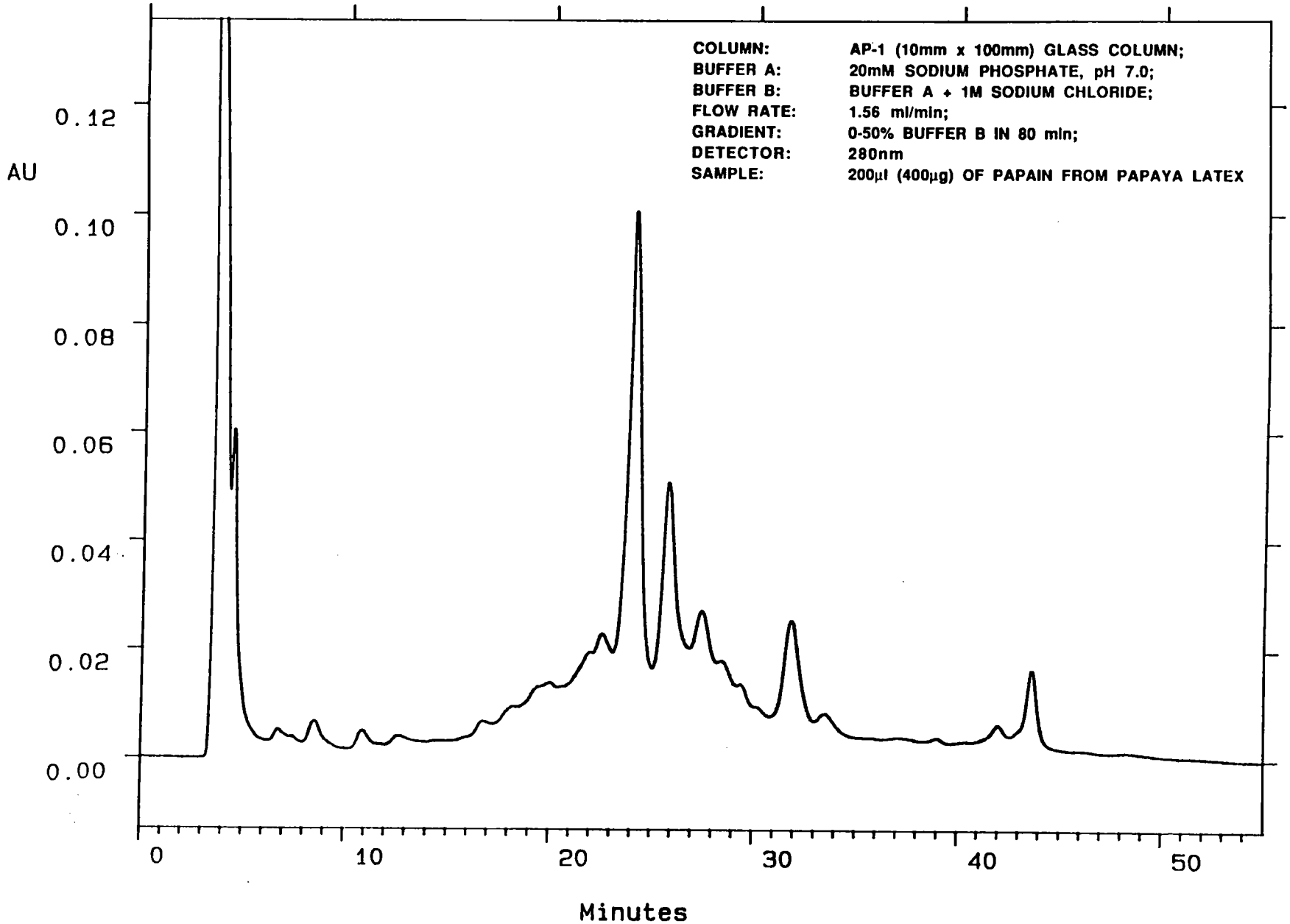
RESOLUTION OF PROTEIN MIXTURE ON PROTEIN-PAK™ ANION EXCHANGERS



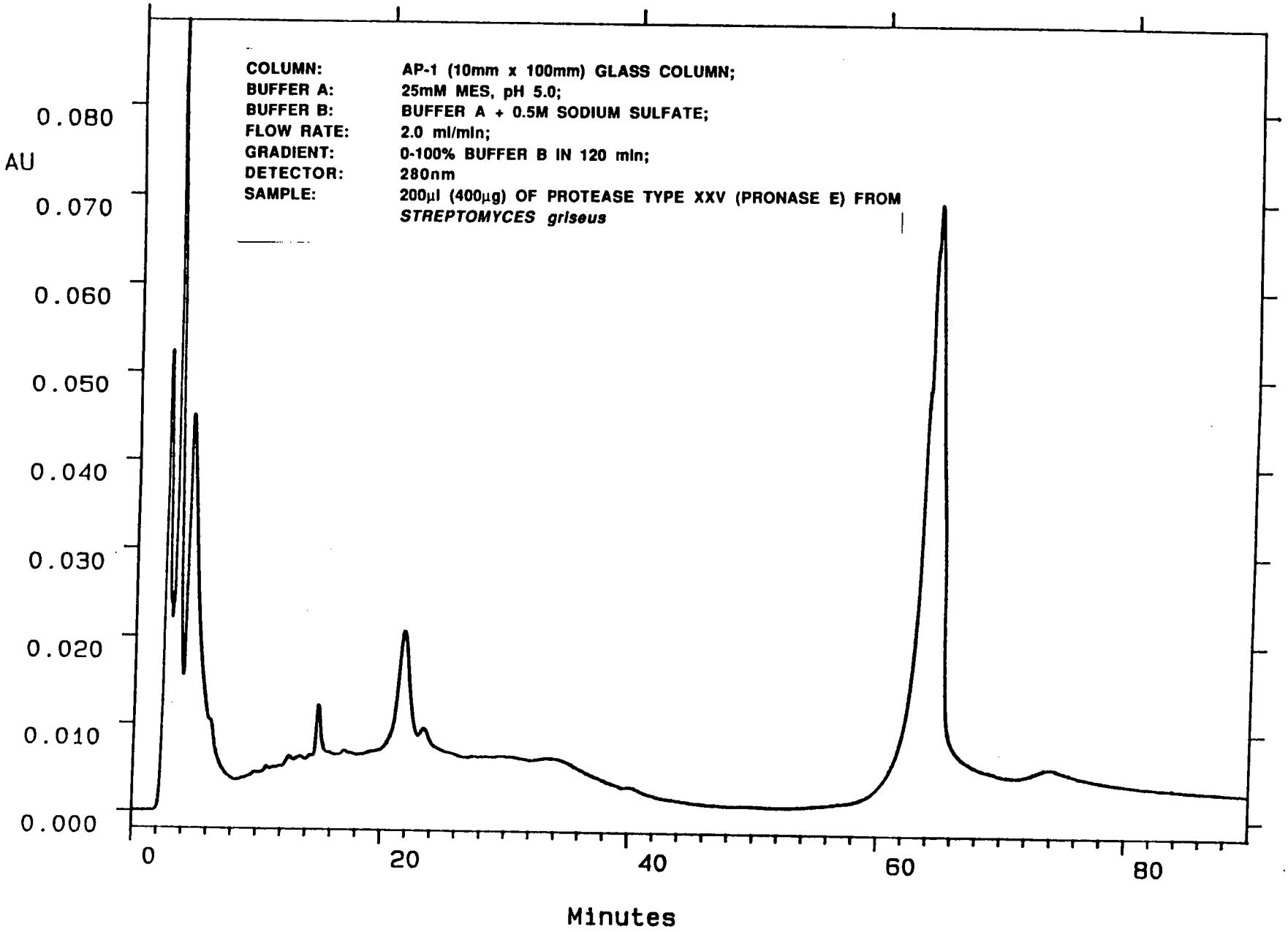


COLUMN: AP-1 (10 mm x 100 mm) GLASS COLUMN ;
BUFFER A: 20 mM TRIS-HCl, pH 8.2;
BUFFER B: BUFFER A + 1M SODIUM CHLORIDE;
FLOW-RATE: 1.56 ml/min;
GRADIENT: 0 to 25% BUFFER B IN 40 MIN;
DETECTOR: 280 nm;
SAMPLE: 100 µl (0.5 mg) OF MIXTURE WITH 1. ADENOSINE, 2. CARBONIC ANHYDRASE, 3. HUMAN TRANSFERRIN, 4. OVALBUMIN, 5. SOYBEAN TRYPSIN INHIBITOR.

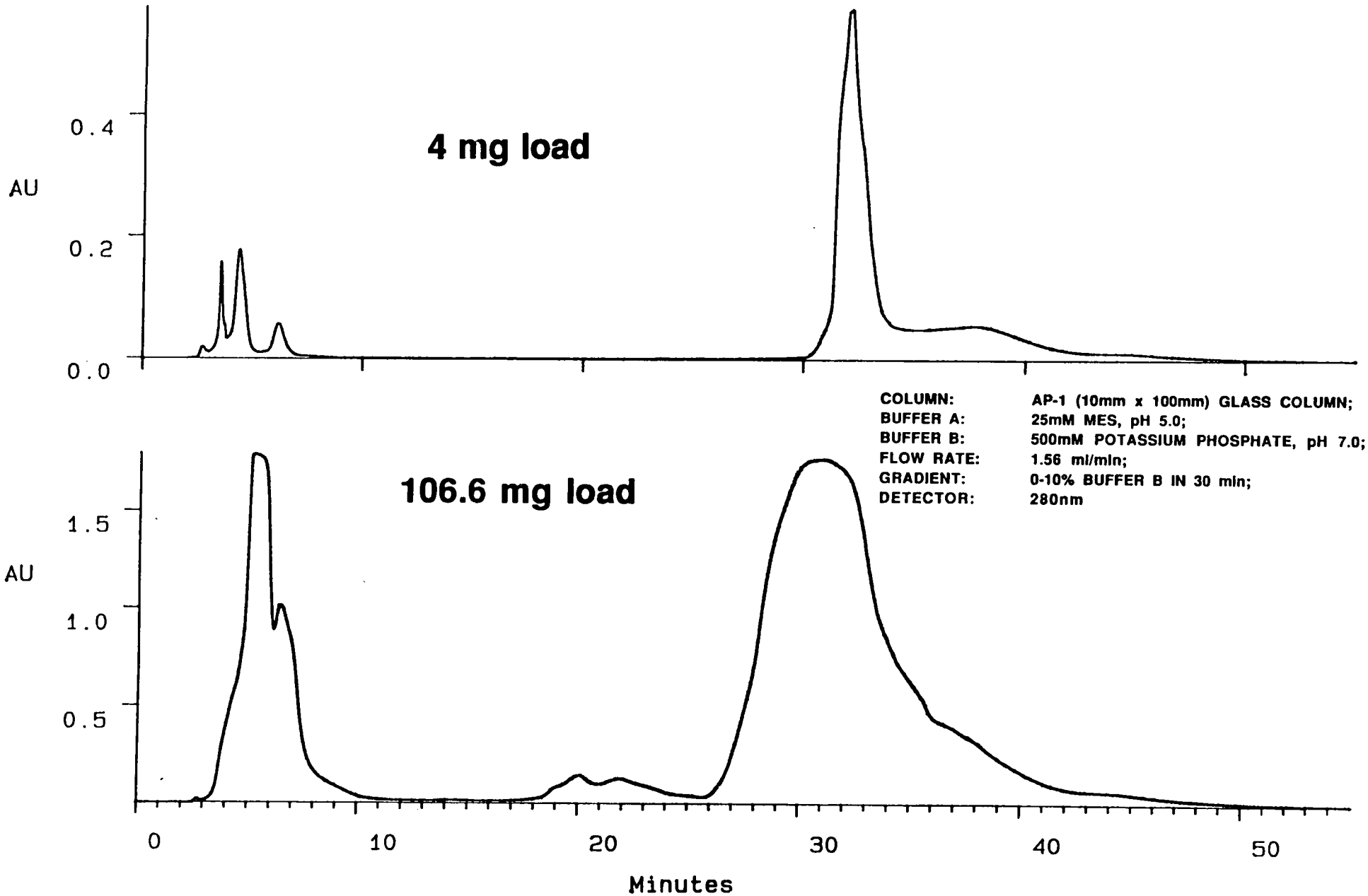
PROTEIN-PAK™ CM 8HR AP-1 GLASS COLUMN: SEPARATION OF PAPAIN



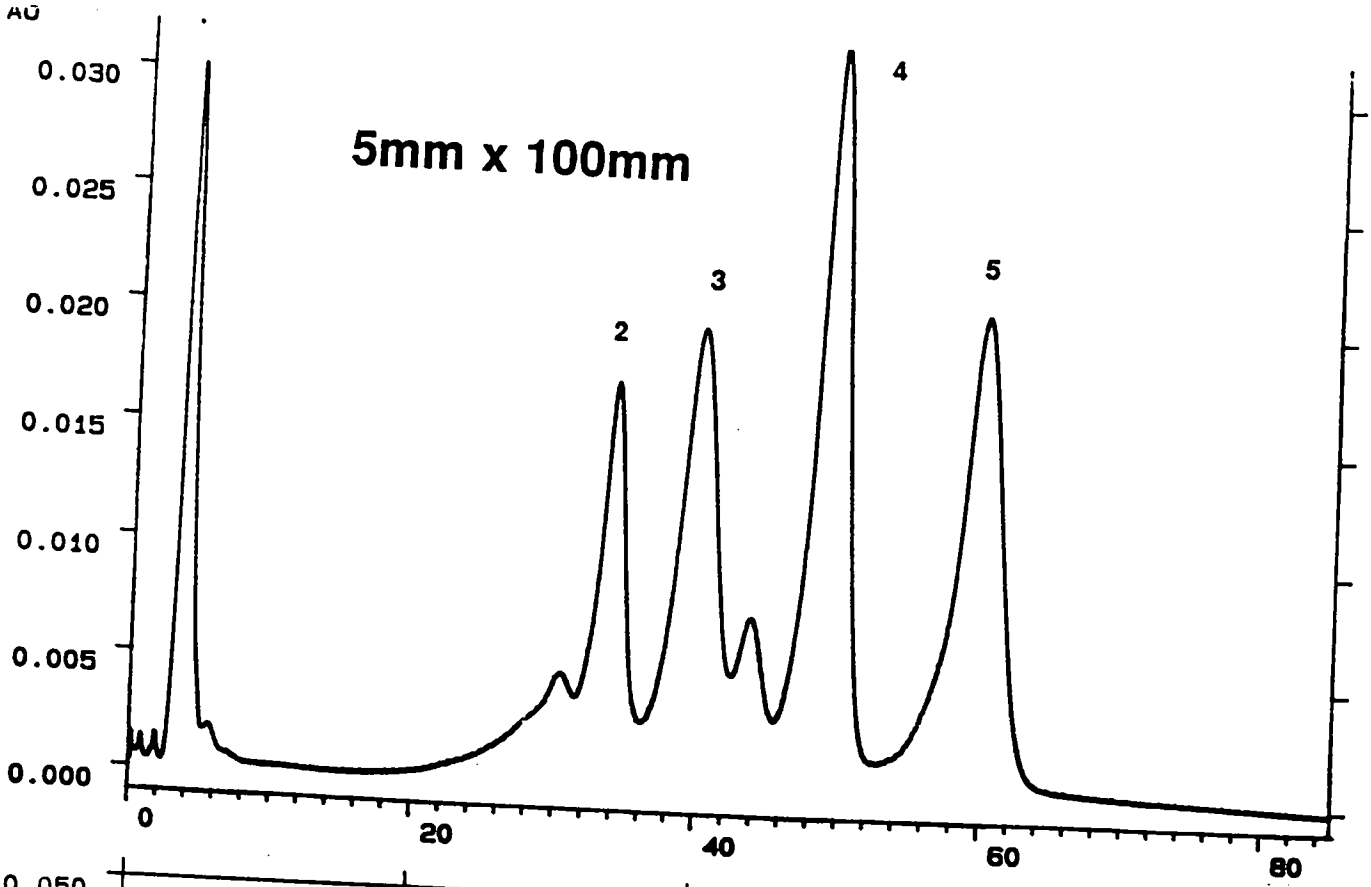
PROTEIN-PAK™ CM 8HR AP-1 GLASS COLUMN: SEPARATION OF PROTEASE

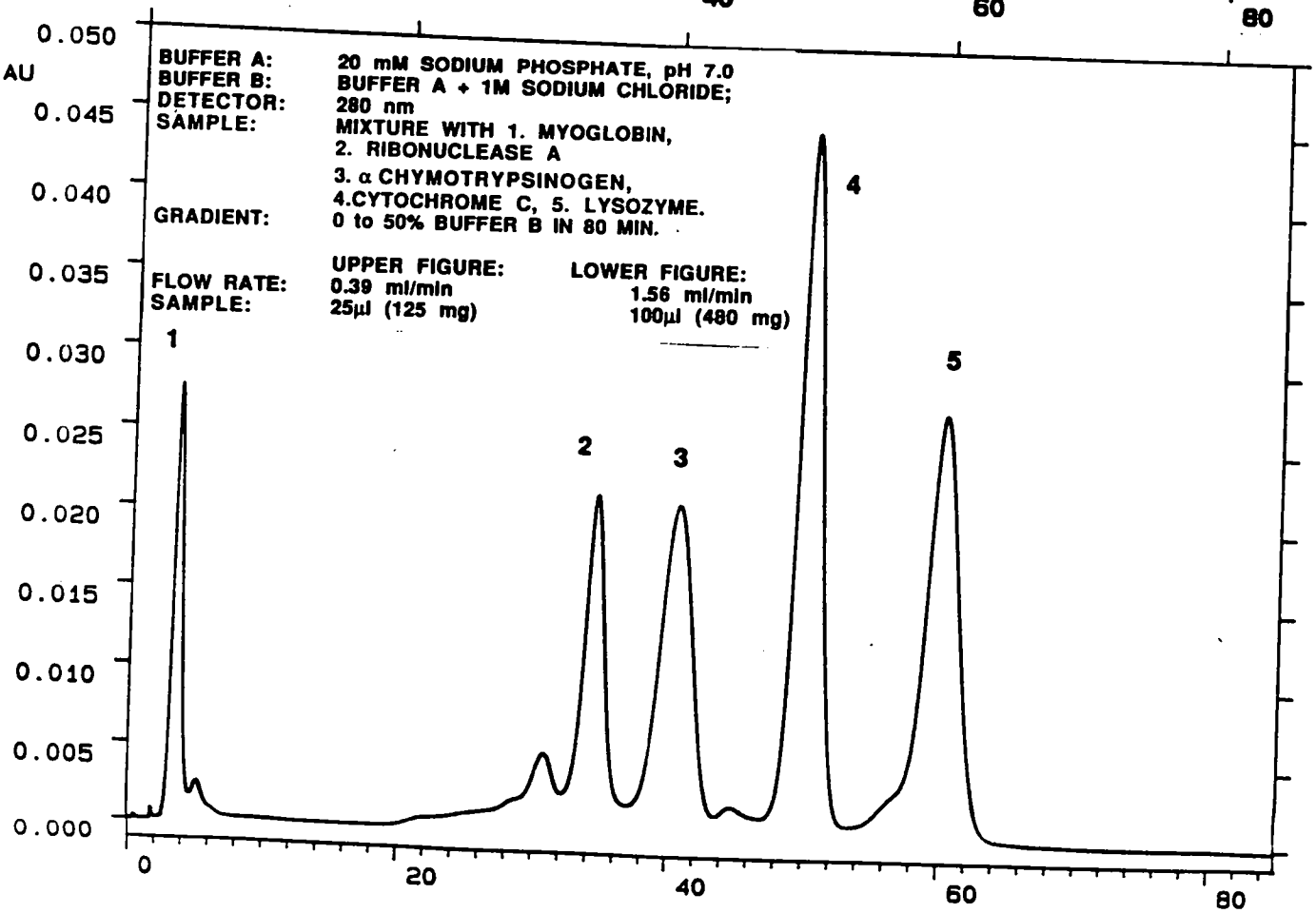


PROTEIN-PAK™ CM 8HR AP-1 GLASS COLUMN: SEPARATION OF MOUSE SERUM

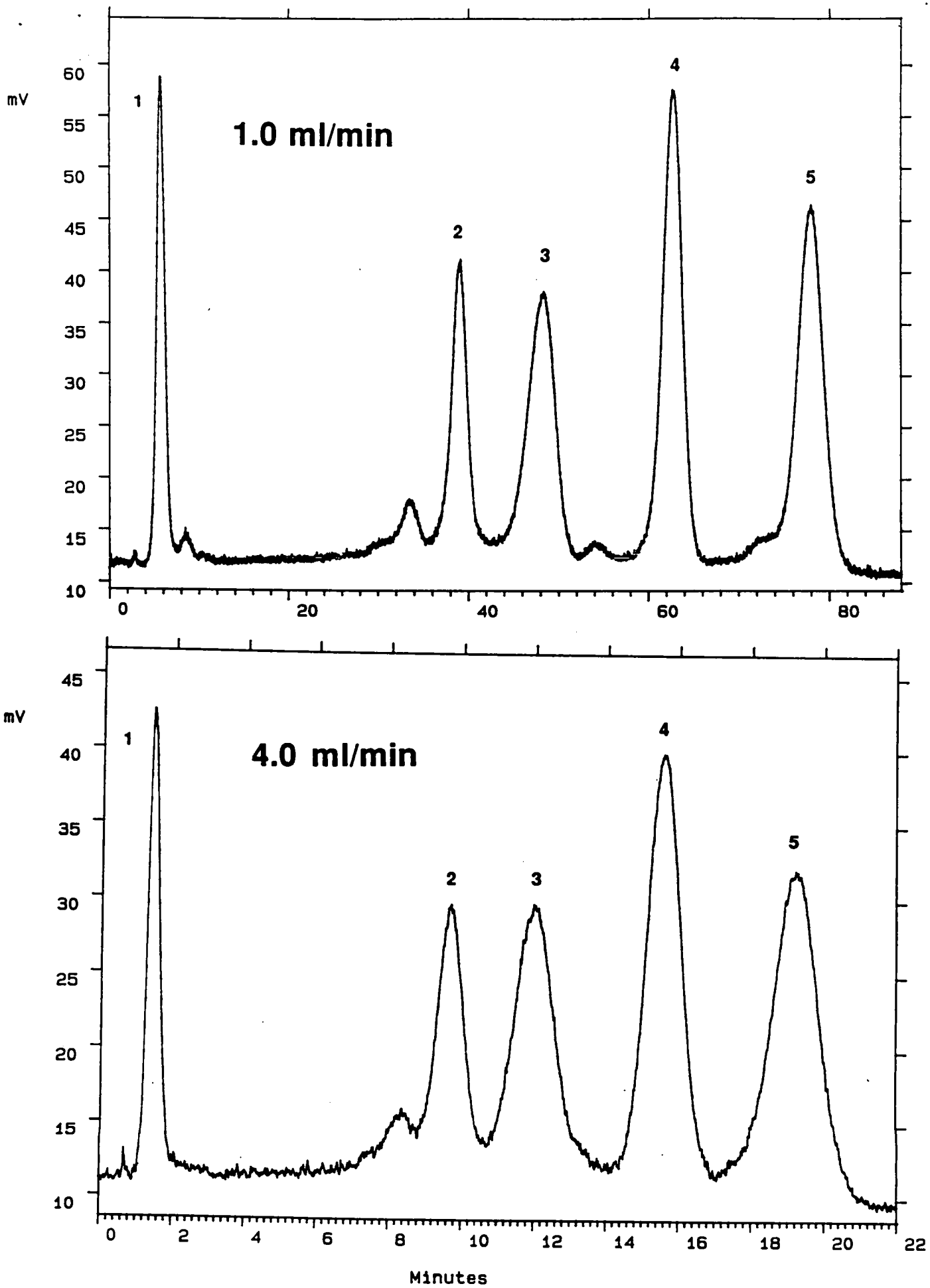


PROTEIN-PAK™ CM 40HR: GLASS AP MINICOLUMN (5mm x 100mm) VS. GLASS AP-1 COLUMN (10mm x 100mm)

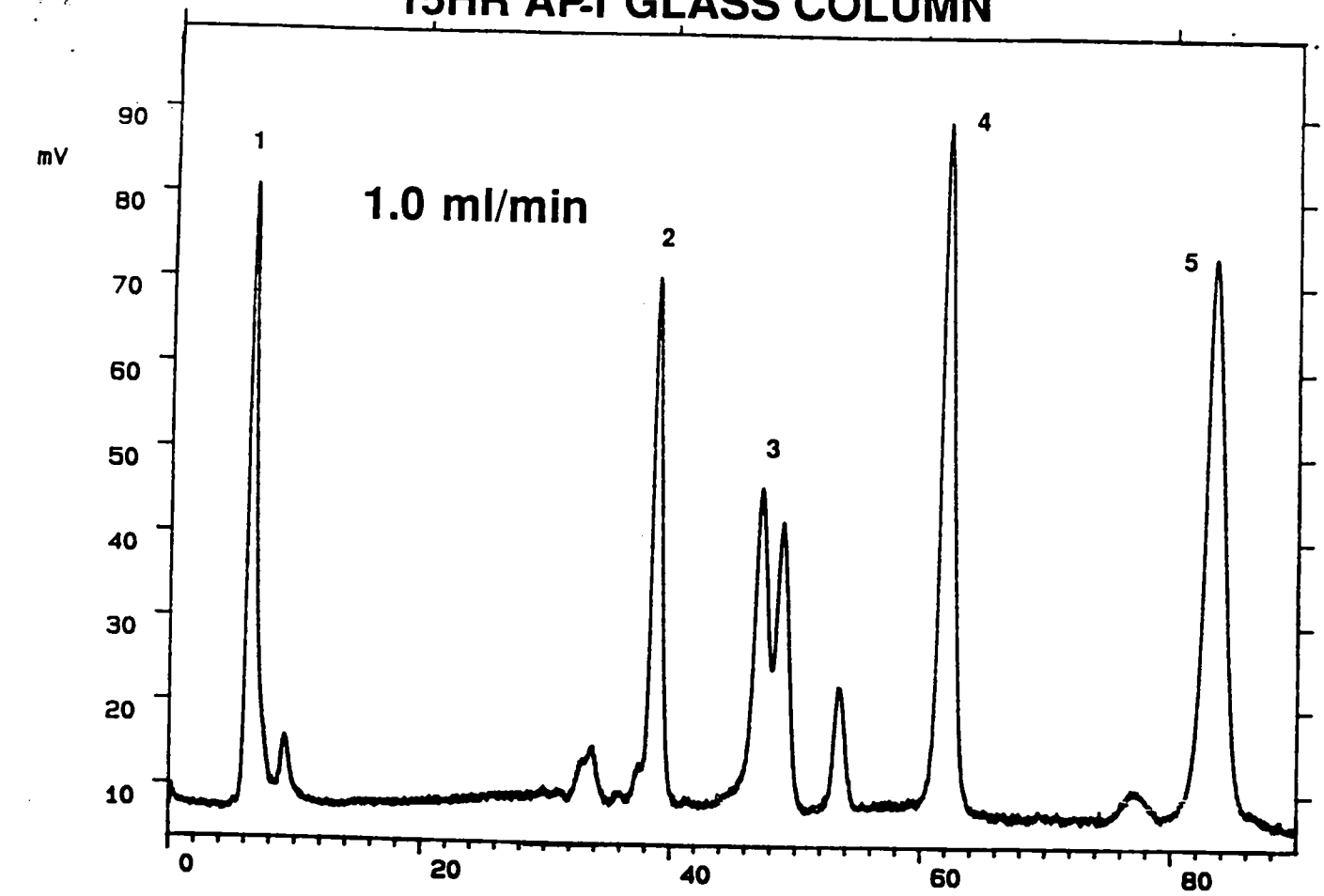


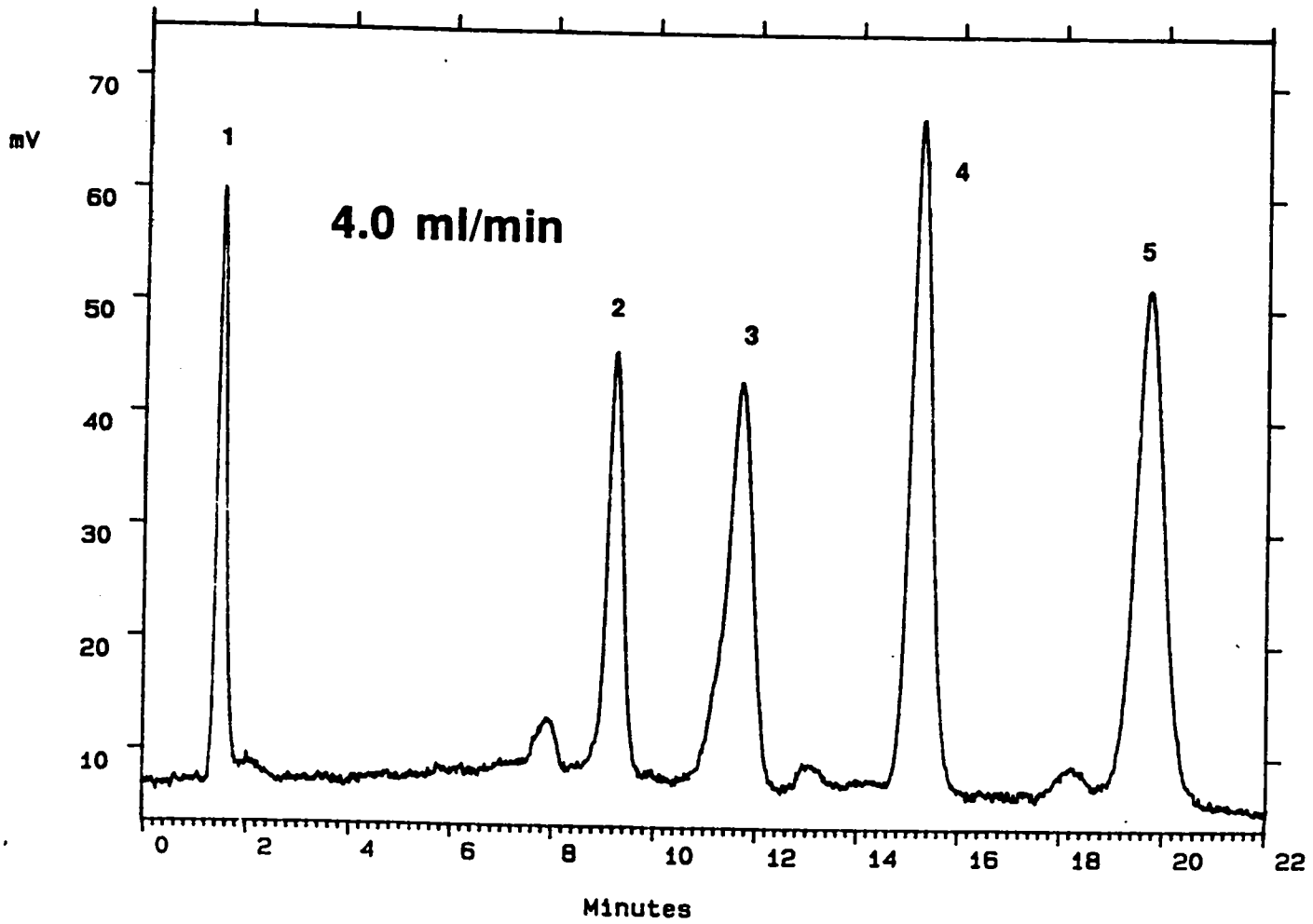


40HR AP-1 GLASS COLUMN

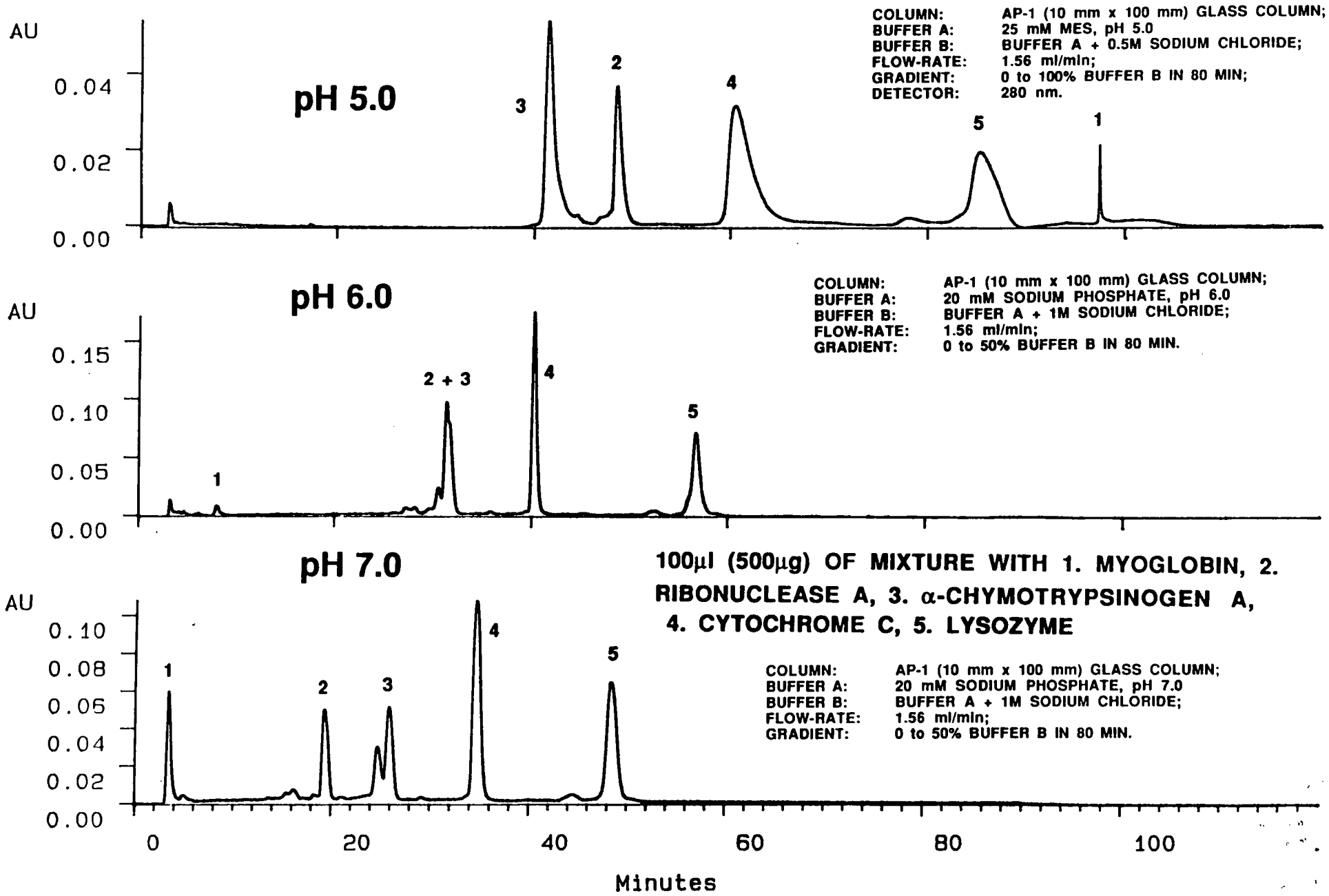


15HR AP-1 GLASS COLUMN

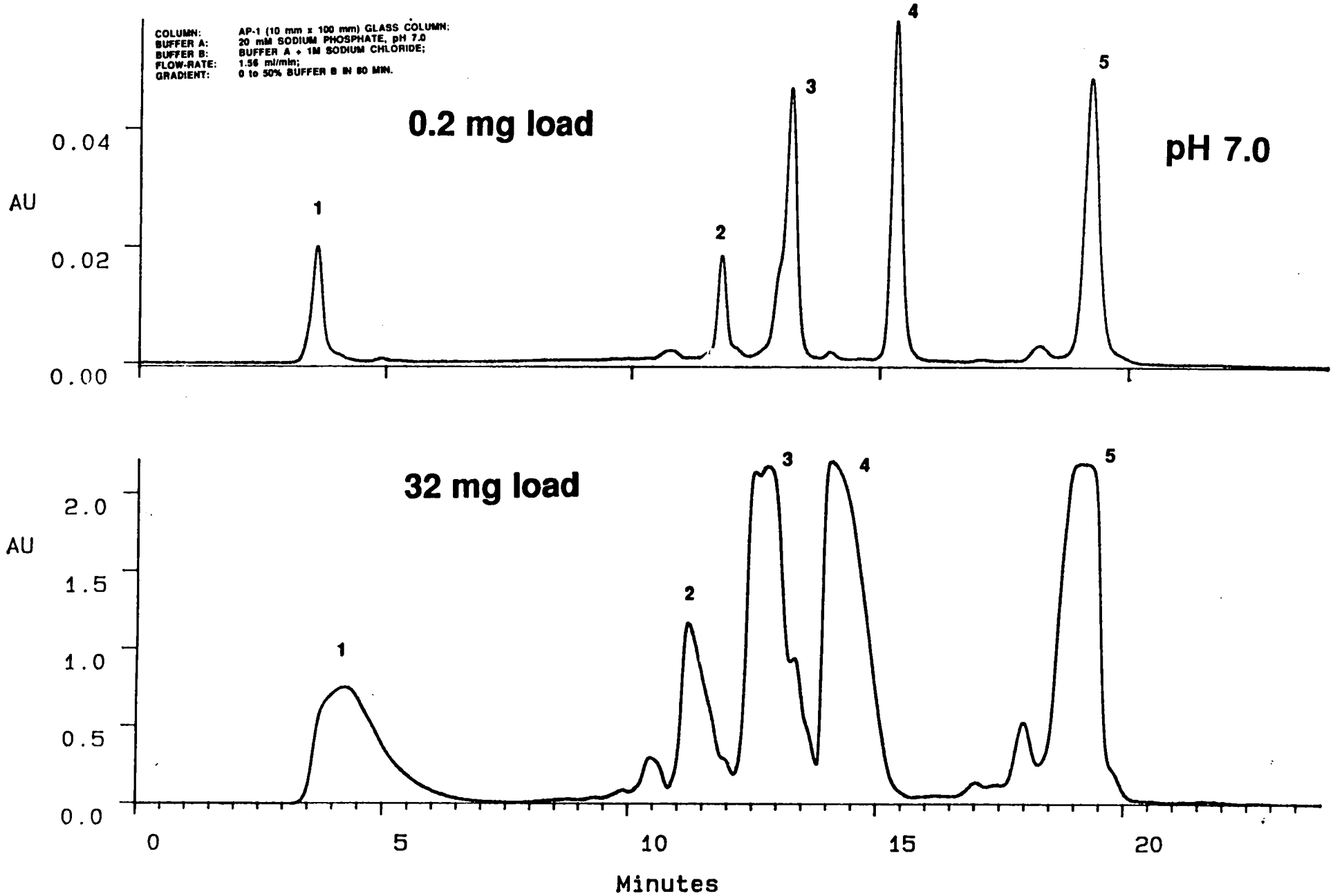




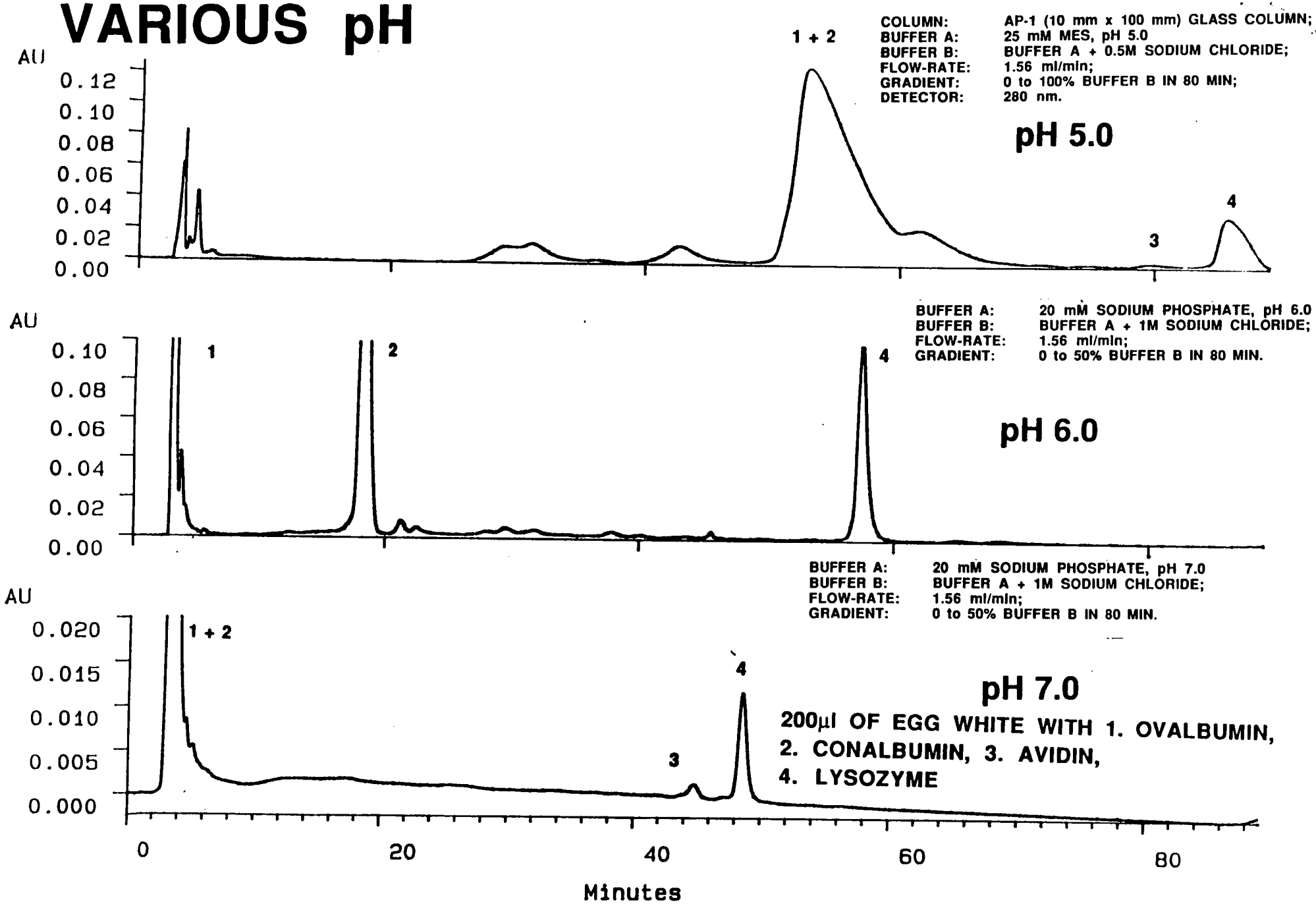
PROTEIN-PAK™ CM 8HR AP-1 GLASS COLUMN: RESOLUTION VS. pH



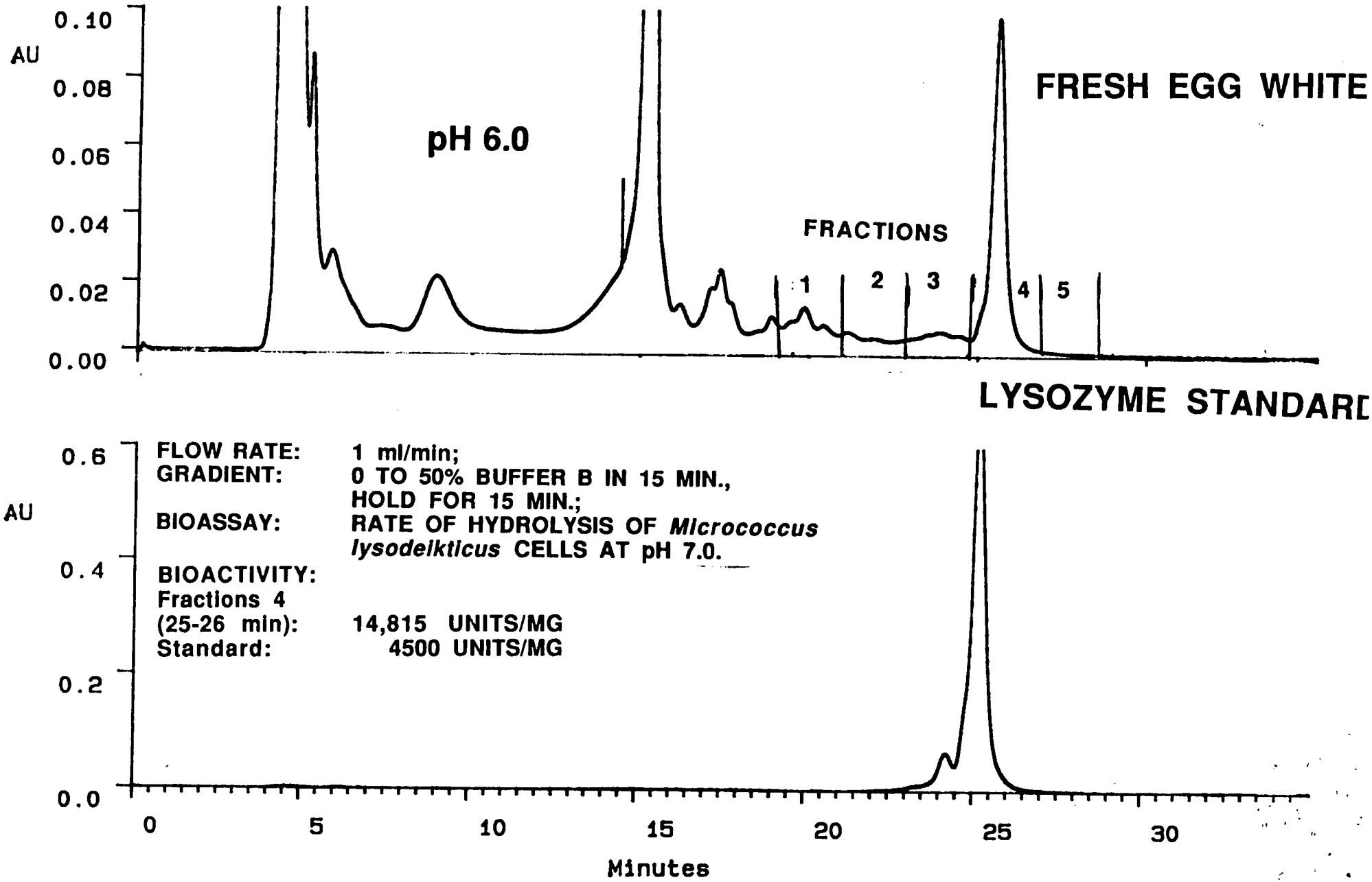
PROTEIN-PAK™ CM 8HR AP-1 GLASS COLUMN: LOADING STUDY



PROTEIN-PAK™ CM 8HR AP-1 GLASS COLUMN: SEPARATION OF EGG WHITE AT VARIOUS pH



PROTEIN-PAK™ CM 8HR: RECOVERY OF BIOLOGICALLY ACTIVE ENZYME



CONCLUSIONS

Ion exchange chromatography is used extensively for protein purification. The proteins isolated from natural mixtures differ in physical and chemical properties. A scientist will select the ion exchanger with the correct functional group (DEAE, Q, SP, and CM) for his/her application. The data shown here demonstrates the ability of Protein-Pak™ anion and cation exchange packings to separate proteins. The proteins in several different natural samples were separated by the cation exchange packing.

There is increasing demand for the isolation of proteins with maximum recovery of product and activity. The Protein-Pak™ CM 8HR column meets this demand as shown by the recovery of biologically active lysozyme from hen egg white.

Scalability is an important factor in any purification which requires the isolation of milligram to gram quantities of protein. The scale-up example shown here demonstrates that the new Protein-Pak™ CM HR series successfully employs the same particle size and chemistry at the analytical and preparative scales.