930629

Essentials in bioresearch

Instrumentation, Chemistries, and Expertise for Biosynthesis and Separations

Poster Presentation

FASEB/ASBMB Symposium - April, 1991
Poster # 4790

New Ion Exchange Strategies for Protein Purification

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New Ion Exchange Strategies for Protein Purification

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Abstract

Combination of new ion exchange packings, membrane chromatography cartridges, and chromatography system advancements brings enhanced levels of resolution and speed to protein purification. New, rigid polymeric packings improve performance at every stage in a purification scheme - from the initialseparation of a crude extract to the final isolation of minute amounts of highly active protein. Chromatography cartridges based on a gigaporous stacked membrane structure provide very fast protein separations without compromising resolution. High speed separations performed on systems capable of automatically adjusting elution buffer pH, ionic strength, and gradient shape allow rapid optimization of chromatographic conditions. This strategy yields a higher degree of homogeneity, better yield, and a more reproducible purification process, as will be demonstrated by the isolation of active enzymes from crude biological extracts.

INTRODUCTION

Modern, high resolution chromatography of proteins facilitates the isolation of homogeneous, biologically active materials in high yield. This is a result of advances in column chemistry as well as in the use of instruments to reproducibly generate the separation conditions and to provide automation. It is now apparent that these parallel developments can interact synergistically

High resolution protein separation columns initially provided the sharp peaks associated with small particles of a narrow size distribution while utilizing the same functional groups that were prevalent with open columns. More recently, many new functional groups have appeared, each providing some unique advantages. In addition, the newest generation of columns is available in a range of particle sizes with consistent separation properties. This brings economic feasibility to the use of high resolution techniques early in the purification process for enhanced purity and yield. For the best separation of a particular protein in a specific sample type, it should be useful to compare some of the many different column types that are available, preferably undcer the best conditions for that material. The utility of this approach will be shown for the isolation of lactate dehydrogenase from mouse liver using four different columns. Two types of supporting materials, each with two functional groups will be tested. The DEAE columns represent weak anion exchangers while the Q materials are strong anion exchangers. The Protein-Pak™ HR family of packing materials (described fully in Posters #2529 and 2930) are modern, fully porous, particulate packings while the MemSep™ Chromatography Cartridges represent a very different physical structure based on membrane technology to provide an open network or gigaporous material. These devices, described fully in Poster #4788 ,give very good resolution and recovery with rapid separation times. The utility of each method will be assessed by measurement of enzyme specific activity as well as by determining electrophoretic heterogeneity using SDS and Native PAGE as well as CE.

Automation of high resolution separations has often been a simple matter of conveniently switching columns or regenerating between runs. It is, however, apparent that any manual process can be automated. For example, it is usually observed that almost all protein isolations involve multiple steps. These steps can be combined in a multi-dimensional chromatographic system by directing a portion of the effluent of one column onto a second column using a different separation mechanism. Such an approach will be particularly valuable when applying high resolution techniques early in a separation protocol with large particle packings. This approach will be demonstrated for the isolation of Egg White proteins. Automation can also facilitate the identification of the best column and gradient conditions so the Lə˙ isolation described above will be automated. The use of the Auto-Blend™ method is fundamental to both automation experiments

MATERIALS AND METHODS

Sample Preparation

Mouse Liver: Livers were harvested from 10 freshly sacrificed weanling mice, and homogenized in 20mM Tris-HCl, pH 7.5 (3ml/gm). The pooled homogenates were divided into 100µl aliquots and stored at -20°C. Immediately before use, an aliquot was thawed, diluted with 300µl of 20mM Tris-HCl, pH 7.5, and centrifuged at 14,000g for 10min at 4°C. The supernatant was used for three consecutive injections and discarded.

Egg White: Fresh egg white was diluted 1:20 with running buffer and filtered through a Millex HV 0.45µ membrane immediately before use.

Buffer Preparation

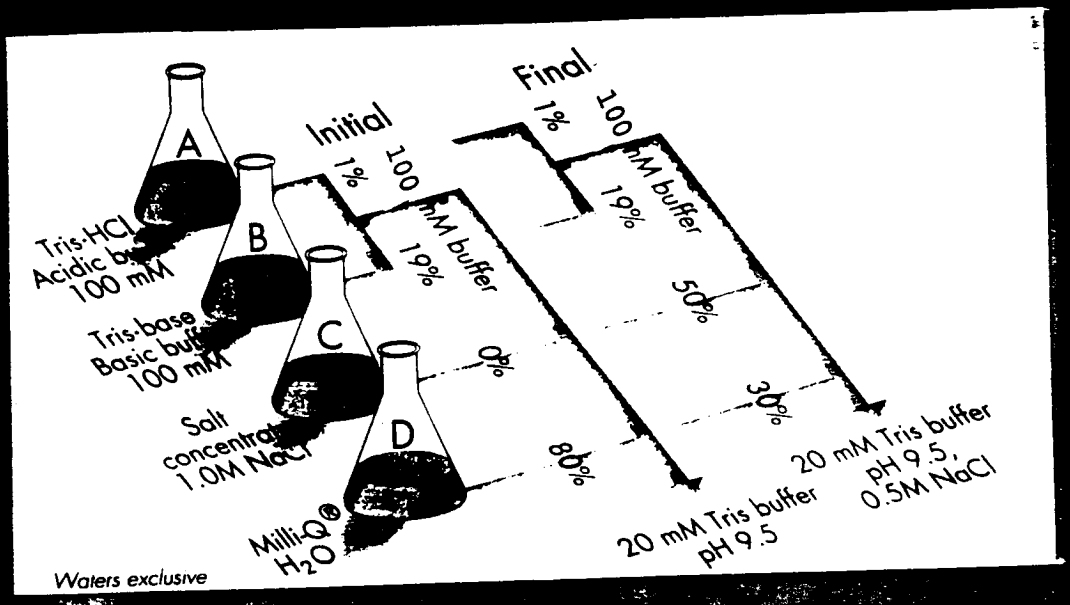
AccuPure™Z1-Methyl is a product of Waters. All other buffers were of the highest commercially available grade. The appropriate salts were dissolved in MilliQ water, and the pH was adjusted at near final volume after adding all components. All solutions were filtered through a Millex HV 0.45µ membrane and degassed under vacuum immediately before use.

Electrophoresis

All separations were performed in the Waters Quanta™ 4000 and monitored at 214nm or 185nm The capillaries were Waters AccuSep™ 50µ X 60cm. The capillaries were vacuum purged for 2min with running buffer prior to each hydrostatic injection. For more rigorous cleaning, the capillary was purged for three cycles of10min with 0.5M sodium hydroxide, 2min with running buffer, 10min with 1.0M phosphoric acid and 2min with running buffer.

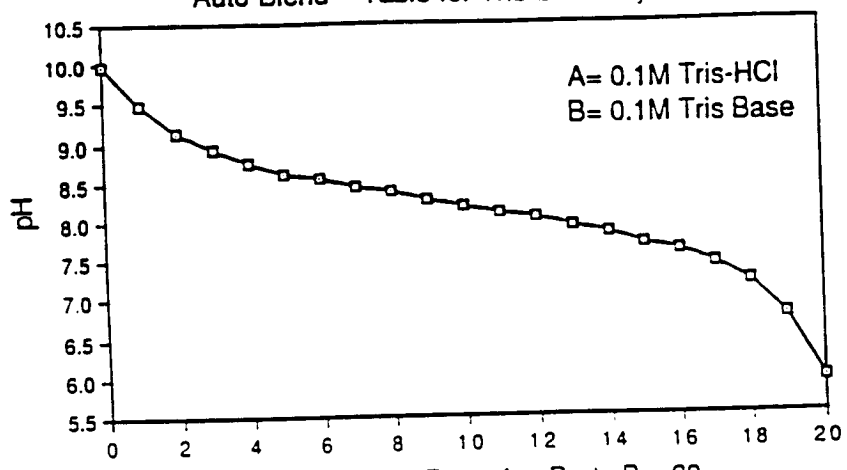
Ion Exchange Chromatography

The Waters 650 Advanced Protein Purification System equipped with appropriate PV series vaolves, a M484 Tunable UV Detector, and Waters Fraction Collector was used for all experiments. Columns and cartridges are products of Waters and Millipore. Chromatographic conditions are described in detail with appropriate figures.

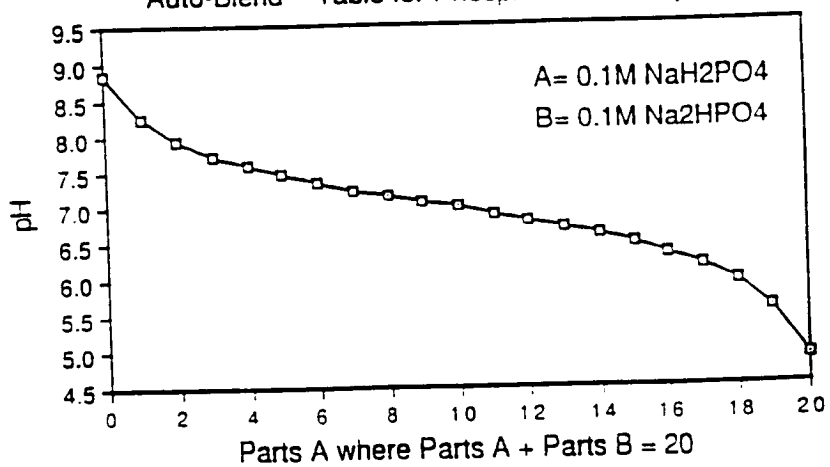


Waters Auto-Blend™ Tables For Tris and Phosphate

Auto-Blend™ Table for Tris Buffer System



Auto-Blend™ Table for Phosphate Buffer System



PLANNING THE EXPERIMENT

Isolation of Ovomucoid, Ovalbumin, Avidin, and Lysozyme

Major Proteins of Chicken Egg White

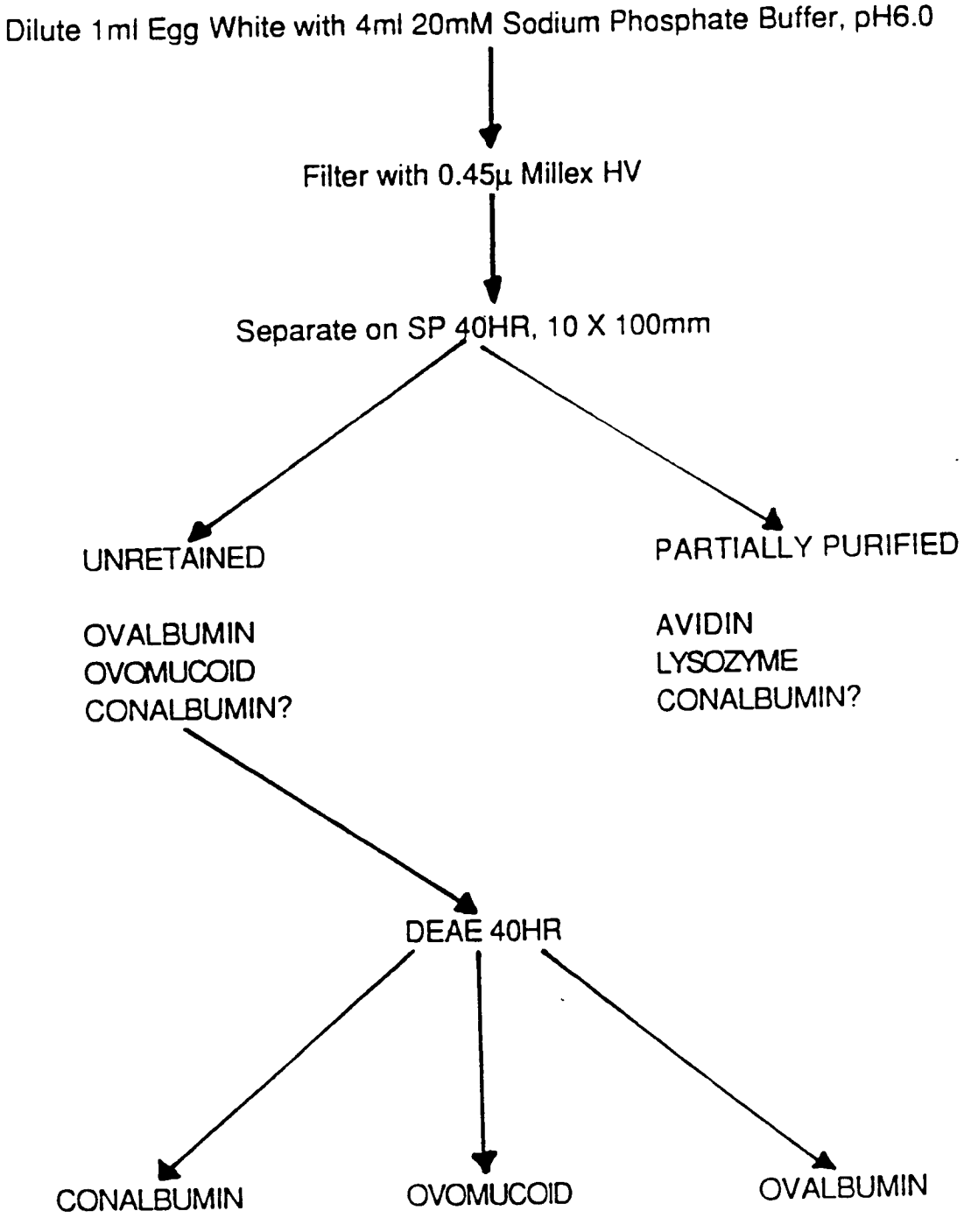
PROTEIN	MOLECULAR WEIGHT	pI	RELATIVE ABUNDANCE
Avidin	66,000	10.0	+
Conalbumin	77,000	6.8	++
Lysozyme	14,400	11.0	++
Ovalbumin	45,000	4.6	+++++
Ovomucoid	28,000	4.5	+++

Experimental Rationale

The proteins of interest can be divided into those that are relatively basic or relatively acidic. Therefore, both anion and cation exchange should be useful, and, in fact, both will probably be required. In selecting the chemistry to be used first, it should be remembered that proteins are more stable when the protein concentration is high. Since the basic proteins are also the least abundant, they could be absorbed from a large volume of sample onto a relatively small cation exchange column and separated to elute in a small, concentrated volume. The more acidic proteins, representing the bulk of the protein mass, would pass through the cation exchanger in a relatively concentrated and stable solution. They could then be separated on an anion exchange column that is larger in volume so as to hold the greater protein mass.

STARTING MATERIAL: Egg White; Dilute with Buffer and Filter Through Millex™HV, 0.45µ

SEPARATION PROTOCOL



At Injection

Protein-Pak™ SP 40HR, 10 X 100 mm Equilibrated in 20mMSodium Phosphate, pH 6.0
Protein-Pak™ DEAE 40HR, 50 X 100mm Equilibrated in 20mM Tris-HCl, pH 8.0
UltraLoop™ Equilibrated in 20mM Tris-HCl, pH 8.0

650: Flow at 1.5 ml/min; Composition at 18%A: 2%B:0%C:80%D
At Injection, Gradient and Event Tables Start

Switch and Valve Positions
S1 = Off Valve PV-D3W in Position A
S2= Off Valve PV-6B A and B in "Ready" Position 1
A Line Drawing From Monobasic Phosphate [Valve PV-6B A in Position 1]
B Line Drawing From Dibasic Phosphate [Valve PV-6B B in Position 1]
S3= Off Valve PV-6C in "Ready" Port 1
S4= Off Valve PV-2C in Position A

Fraction Collector in "Ready"; At Injection, 12.5min "Wait" Window Begins

Flow Path:
650>Manual Injector(Inject)> Port 3 of PV-2C>Port 2 of PV-2C> Common of PV-6CA> Port 2 of PV-6C-A> SP 40HR, 10X100mm> Port 2 of PV-6C-B> Common of PV-6C-B> Detector>Port 5 of PV-D3W>Port 4 of PV-D3W> Fract.Coll. Div.Valve>Waste

Trapping Unretained Proteins in UltraLoop™ 1.5 - 12min

650 Gradient and Event Tables Continue

Switch and Valve Events

1.5min

S1= On PV-D3W Switches to Position B [Sends Detector Out Put to Fill UltraLoop]

Flow Path:
650>Manual Injector(Inject)> Port 3 of PV-2C>Port 2 of PV-2C> Common of PV-6CA> Port 2 of PV-6C-A> SP 40HR, 10X100mm> Port 2 of PV-6C-B> Common of PV-6C-B> Detector>Port 5 of PV-D3W>Port 6 of PV-D3W> Port 3 of PV-D3W>Port 2 of PV-D3W>Port 6 of PV-2C> Port 1of PV-2C> Top of UltraLoop>Bottom of UltraLoop>Port4 of PV-2C>Port 5 of PV-2C>Waste

12min

S1= Off PV-D3W Switches to Position A [Sends Detector Out Put to Fraction Collector]

Fraction Collector Begins 12min Collection Window

650>Manual Injector(Inject)> Port 3 of PV-2C>Port 2 of PV-2C> Common of PV-6CA> Port 2 of PV-6C-A> SP 40HR, 10X100mm> Port 2 of PV-6C-B> Common of PV-6C-B> Detector>Port 5 of PV-D3W>Port 4 of PV-D3W> Fract.Coll. Div.Valve>Collection

12 - 24min

650 Gradient and Event Tables Continue

Fraction Collector Collects Fractions at 30sec Intervals

● Initiating Anion Exchange Step 27-32min

At 27.5min

650 Gradient and Event Tables Continue

Fraction Collector ibegins 26min "Wait" Window

Switch and Valve Events

At 27.5min

S3=On PV-6C Indexes to Position 2 [ByPass]
S2=On PV-6B A.and B Index to Position 2[Pump Lines A and B Drawing From Tris]

At 27.6min
S3=Off PV-6C "Ready" for Next Signal
S2=Off PV-6B A.and B "Ready" for Next Signal

650>Manual Injector(Inject)> Port 3 of PV-2C>Port 2 of PV-2C> Common of PV-6CA> Port 3of PV-6C-A> Bypass Tube> Port 3of PV-6C-B> Commjon of PV-6C-B> Detector>Port 5 of PV-D3W>Port 4 of PV-D3W> Fract.Coll. Div.Valve>Waste

27.6-32min

650 Gradient Table Continues
Flow Goes to 20ml/min, 50%A:50%Bto flush IPhosphate From A and B Pump Lines
Then to 37.5ml/min 12%A:8%B:0%C:80%D to System with Initial Conditions for DEAE

32min

S3=On PV-6C Indexes to Position 4 [DEAE 40HR, 50 X 100mm]

32.1min

S3=Off PV-6C "Ready" for Next Signal

650>Manual Injector(Inject)> Port 3 of PV-2C>Port 2 of PV-2C> Common of PV-6CA> Port4of PV-6C-A> DEAE 40HR, 50 X 100mm>Port 4of PV-6C-B> Common of PV-6C-B> Detector>Port 5 of PV-D3W>Port 4 of PV-D3W> Fract.Coll. Div.Valve>Waste

UltraLoop™ Injection onto DEAE Column 34-36min

34.0min

650 Gradient and Event Tables Continue

S4=On PV-2C Moves to Position B (UltraLoop™ Inject)

650>Manual Injector(Inject)> Port 3 of PV-2C>Port 4of PV-2C>Bottom (Buffer Side) of UltraLoop>:Top (Sample Side) of Ultra Loop>Port 1 of PV-2C >Port 2 of PV-2C> Common of PV-6CA> Port 4 of PV-6C-A> DEAE 40HR, 50 X 100mm> Port 4 of PV-6C-B> Common of PV-6C-B> Detector>Port 5 of PV-D3W>Port 4 of PV-D3W> Fract.Coll. Div.Valve>Waste

36.0min

S4=Off PV-2C Moves to Position A (UltraLoop™ Bypass)

650>Manual Injector(Inject)> Port 3 of PV-2C>Port 2 of PV-2C> Common of PV-6CA> Port 4 of PV-6C-A> DEAE 40HR, 50 X 100mm> Port 4 of PV-6C-B> Common of PV-6C-B> Detector>Port 5 of PV-D3W>Port 4 of PV-D3W> Fract.Coll. Div.Valve>Waste

53.0min

650 Gradient and Event Tables Continue

Fraction Collector ends Wait 2 and Begins Collection Window 2 (30min. 0.5 min Fractions)

650>Manual Injector(Inject)> Port 3 of PV-2C>Port 2 of PV-2C> Common of PV-6CA> Port 4 of PV-6C-A>DEAE 40HR, 10 X 100mm> Port 4 of PV-6C-B> Common of PV-6C-B> Detector>Port 5 of PV-D3W>Port 4 of PV-D3W> Fract.Coll. Div.Valve>Collection

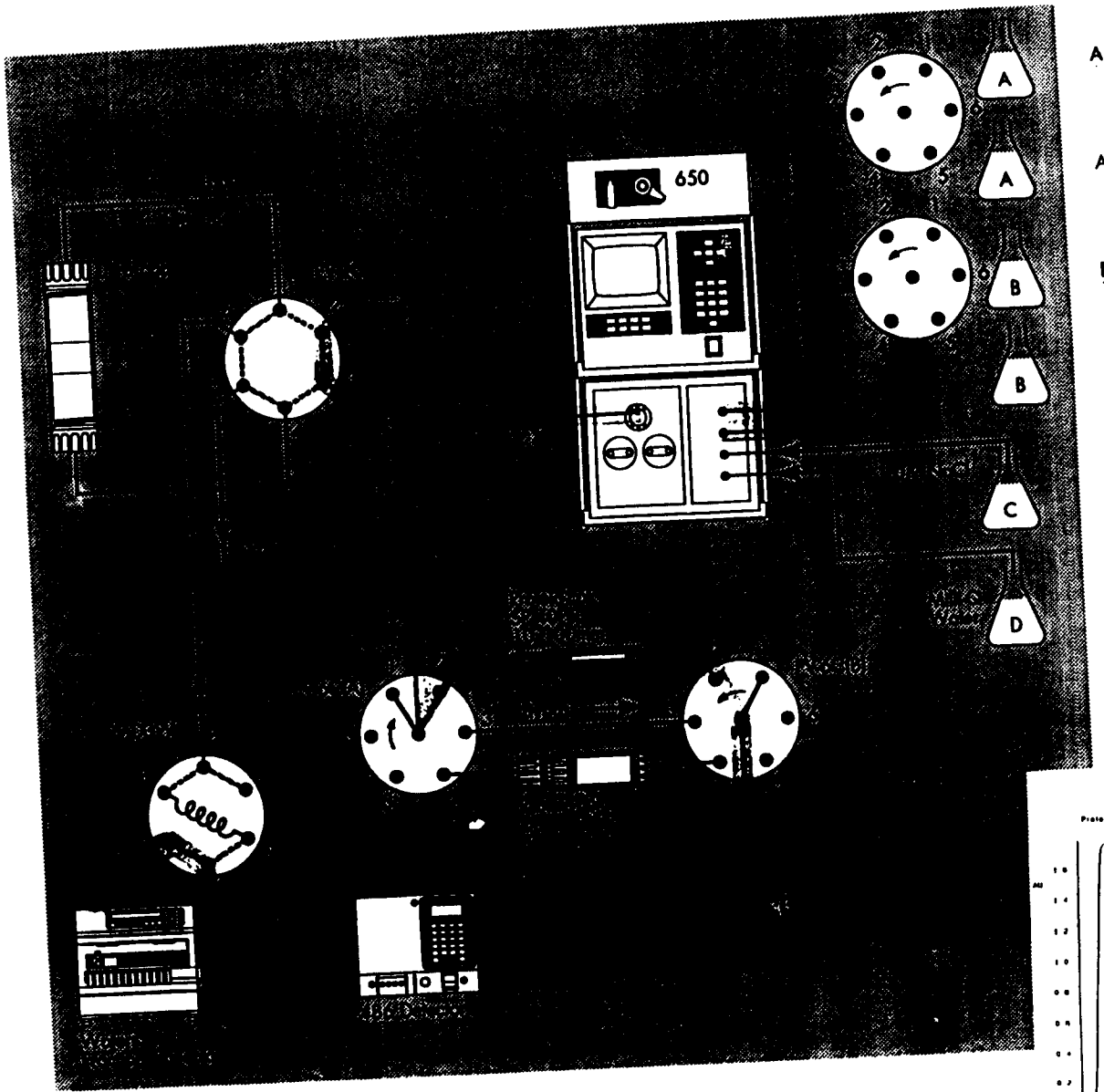
650 Gradient Table

Time	Flow	%A	%B	%C	%D	Curve
INIT	1.5	18	2	0	80	*
15.0	1.5	18	2	50	30	6
24.5	1.5	18	2	50	30	6
25.0	20.0	50	50	0	0	6
27.0	37.5	12	8	0	80	6
37.0	37.5	12	8	0	80	6
80.0	37.5	12	8	20	60	6
100.0	5.0	12	8	50	30	11

650 Event Table

Time	Event	Action
INIT	Alarm	Pulse
1.50	S1	On
12.00	S1	Off
27.50	S3	On
27.51	S2	On
27.60	S3	Off
27.61	S2	Off
32.00	S3	On

At Injection

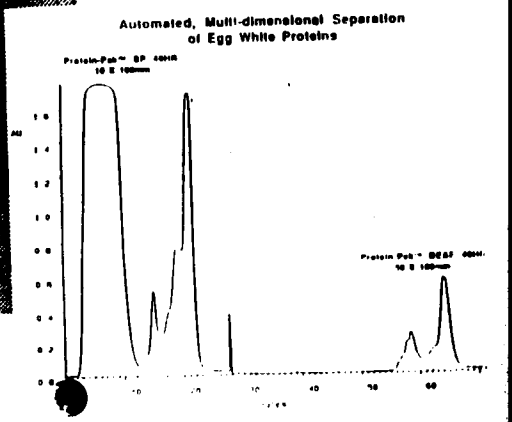


A1 100mM NaH_2PO_4

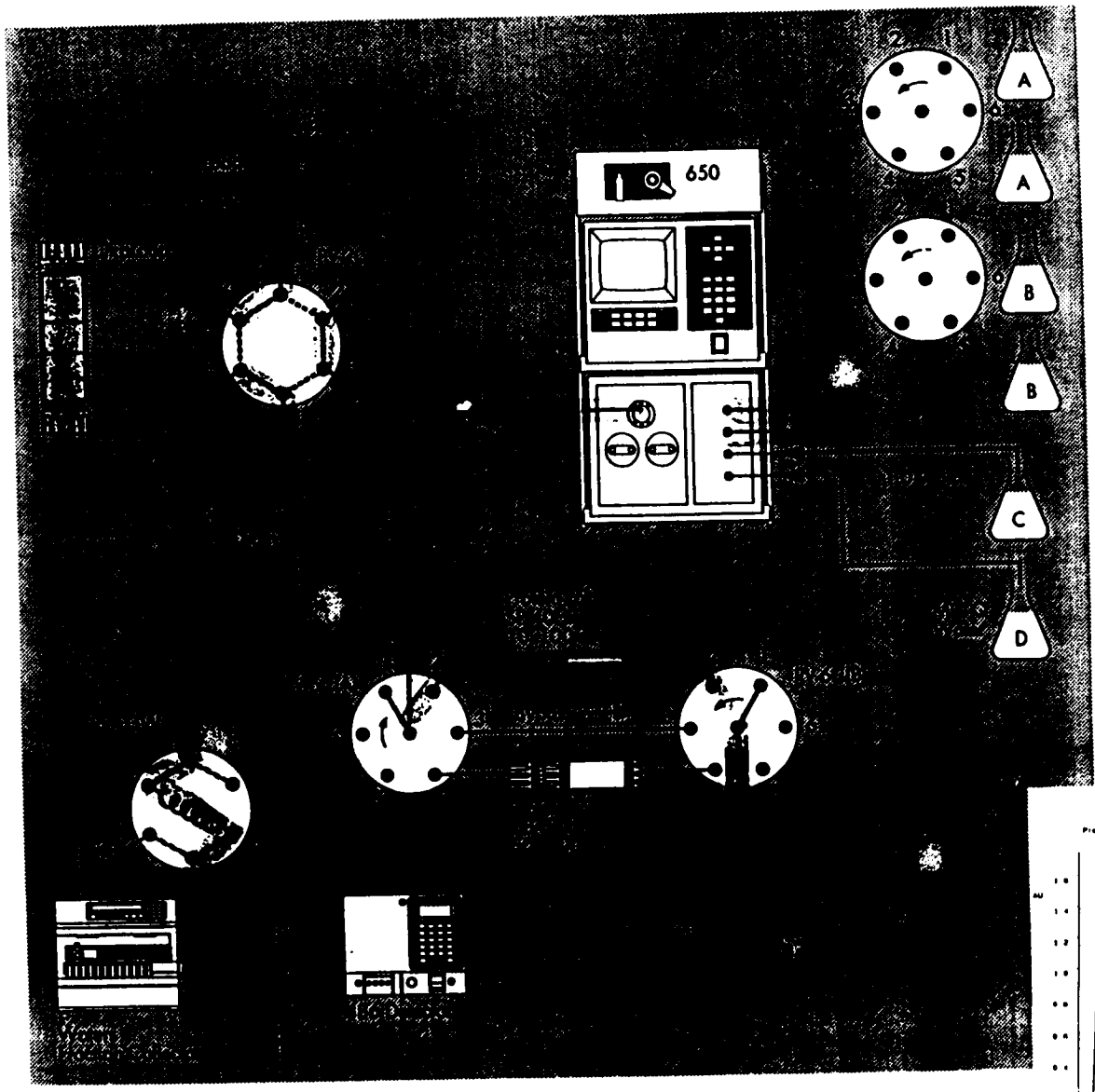
A2 100mM Tris - HCl

B1 100mM Na_2HPO_4

B2 100mM Tris - Base



Trapping Unretained Proteins in UltraLoop™

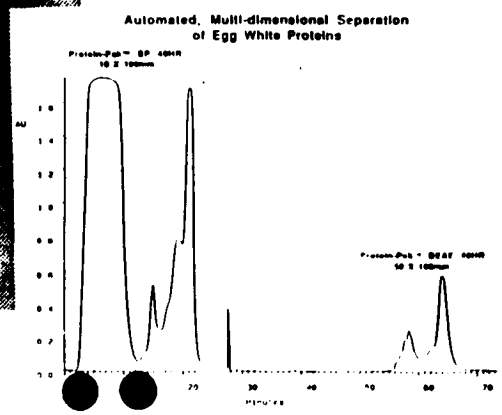


A1 100mM NaH_2PO_4

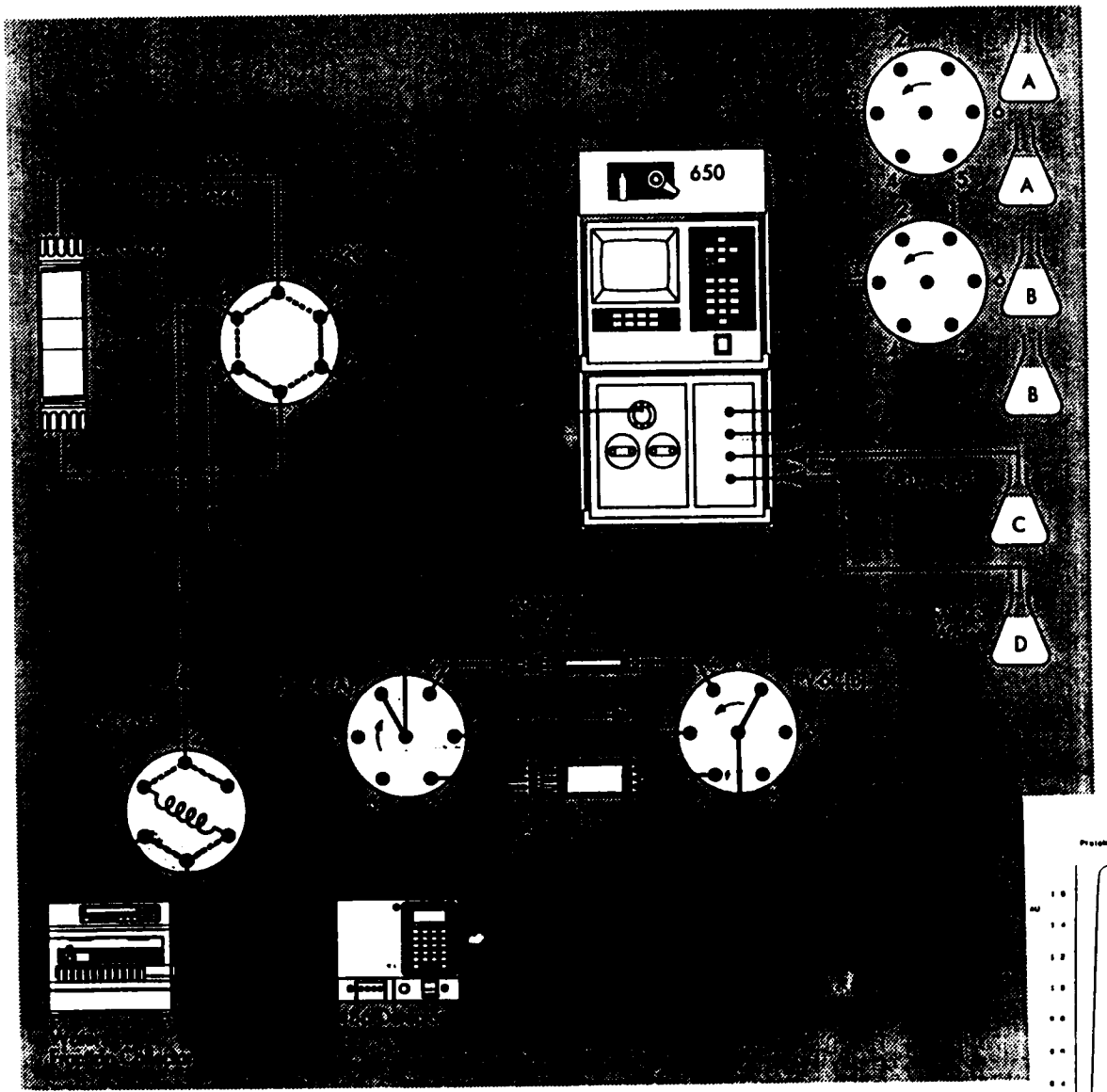
A2 100mM Tris - HCl

B1 100mM Na_2HPO_4

B2 100mM Tris - Base



Initiating Anion Exchange Step

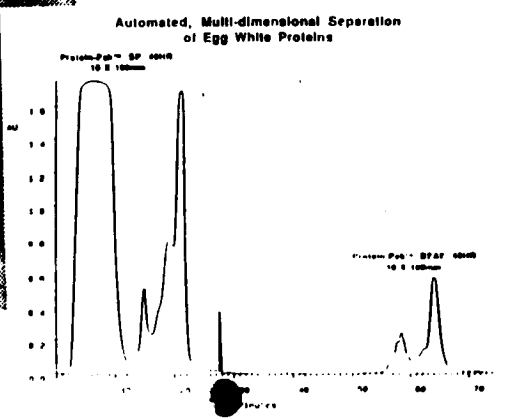


A1 100mM NaH_2PO_4

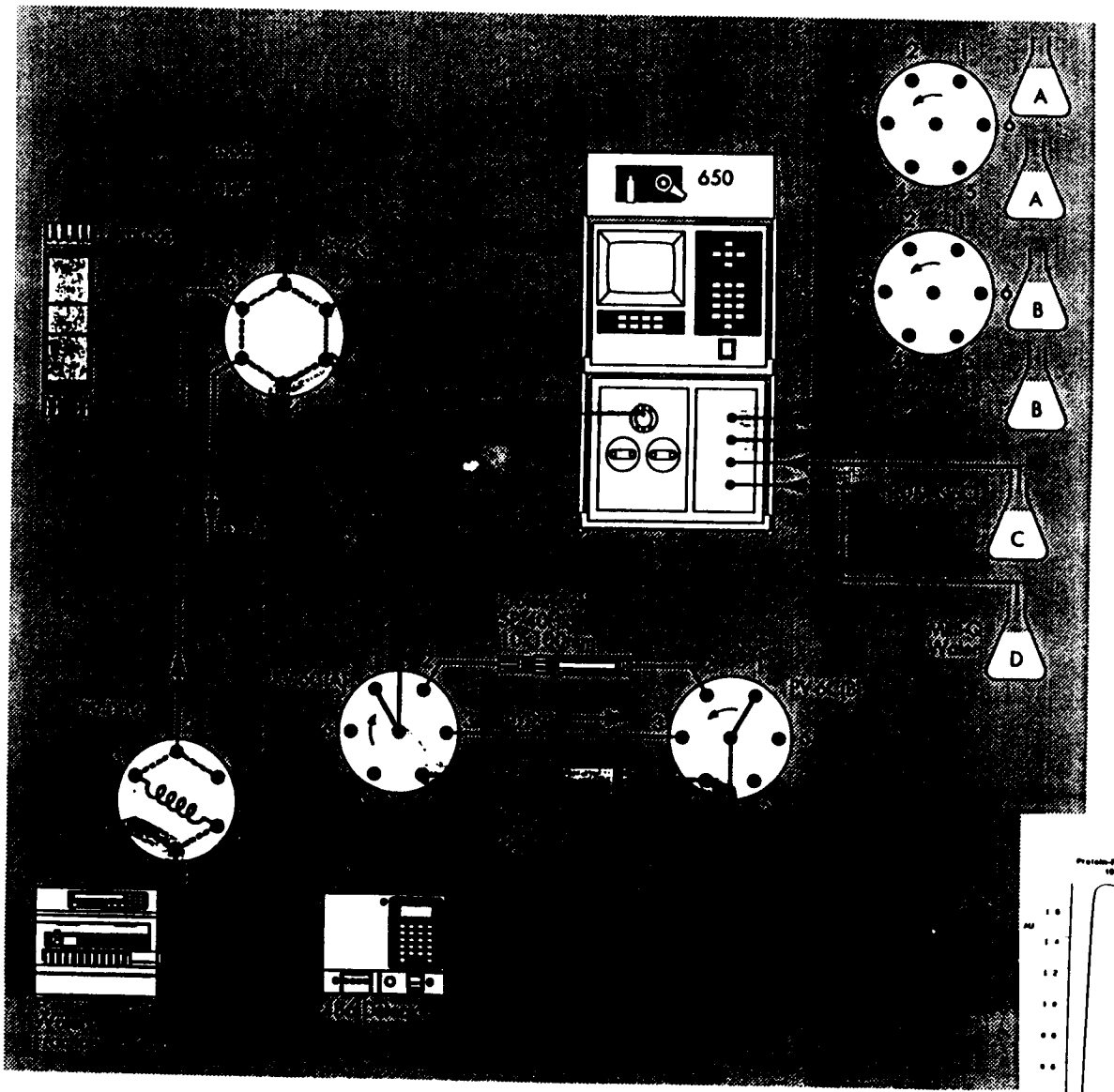
A2 100mM Tris - HCl

B1 100mM Na_2HPO_4

B2 100mM Tris - Base



UltraLoop™ Injection onto DEAE Column

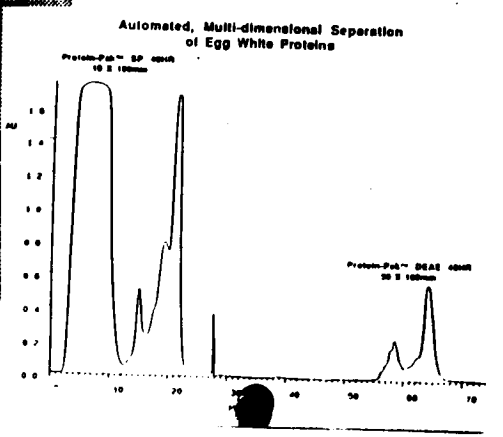


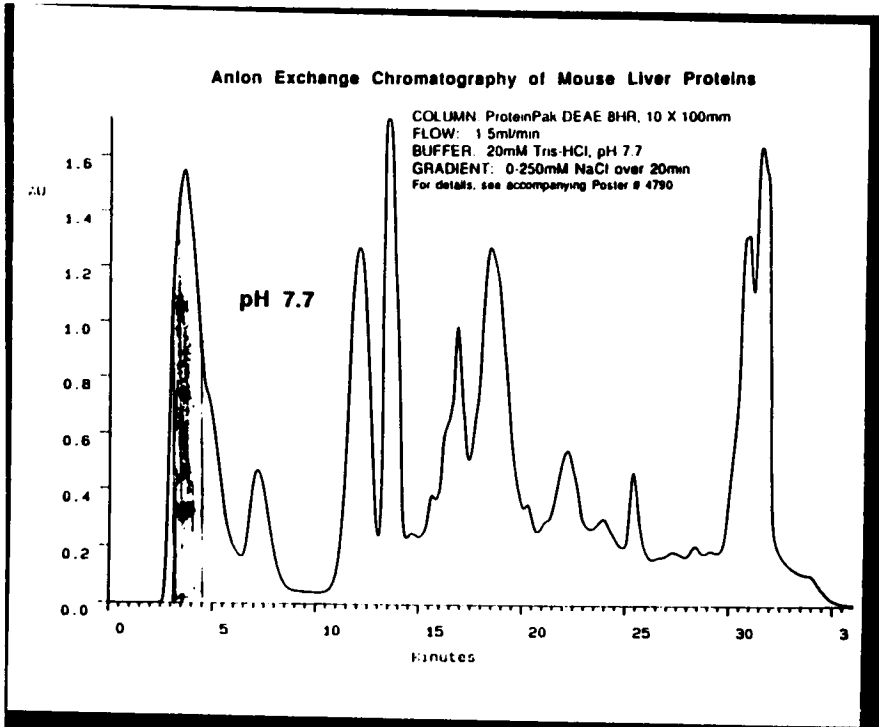
A1 100mM NaH_2PO_4

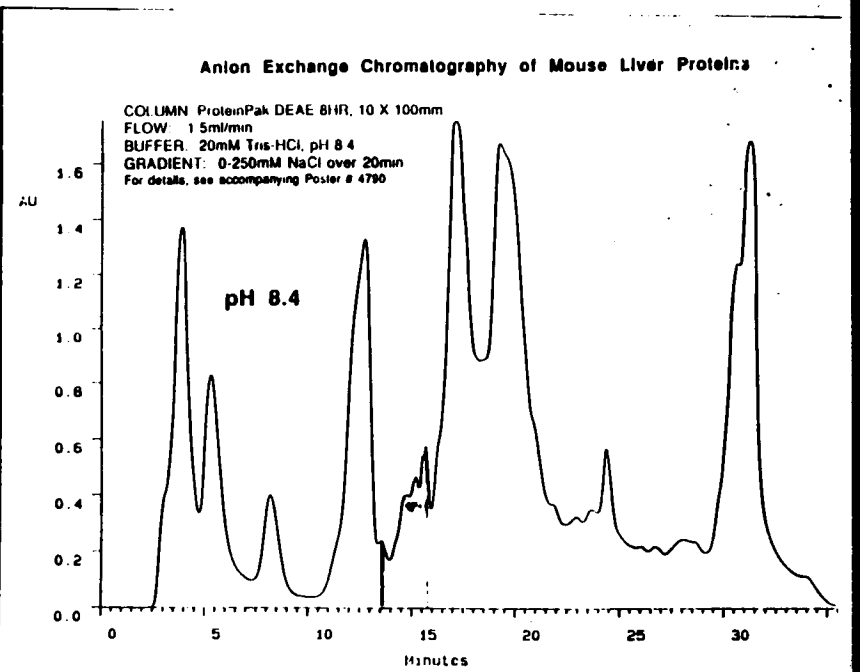
A2 100mM Tris - HCl

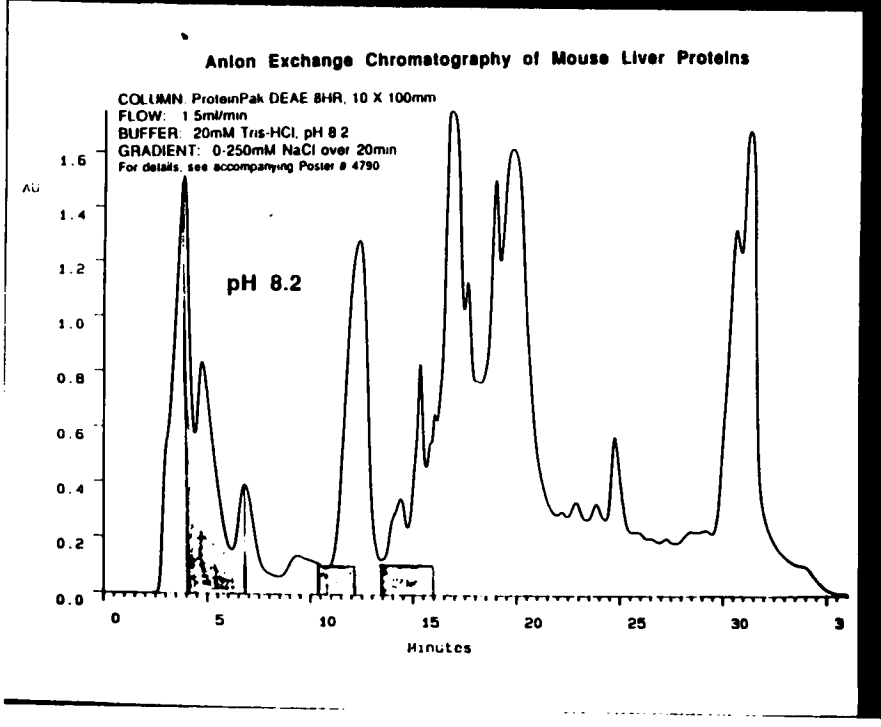
B1 100mM Na_2HPO_4

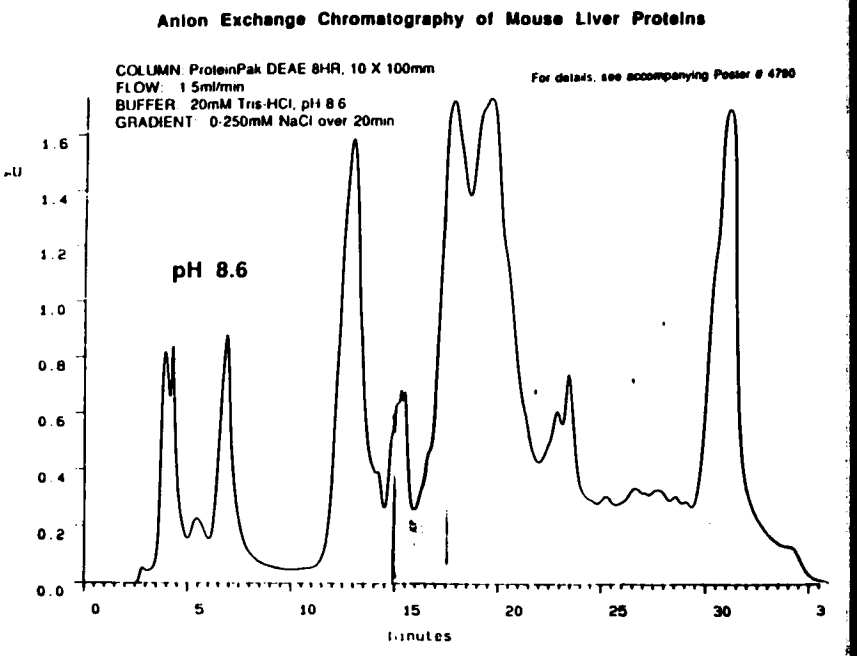
B2 100mM Tris - Base

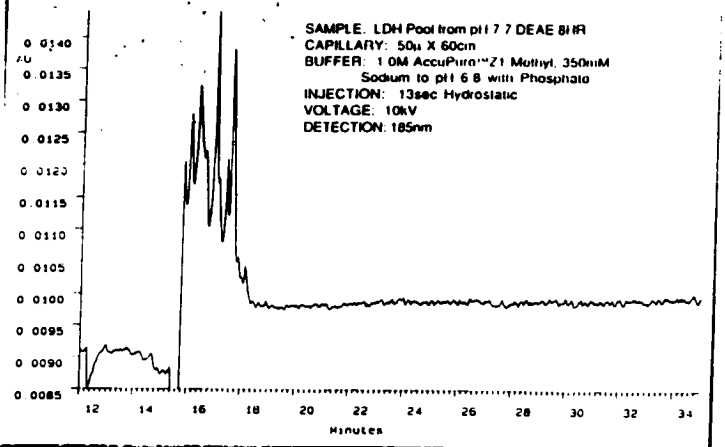


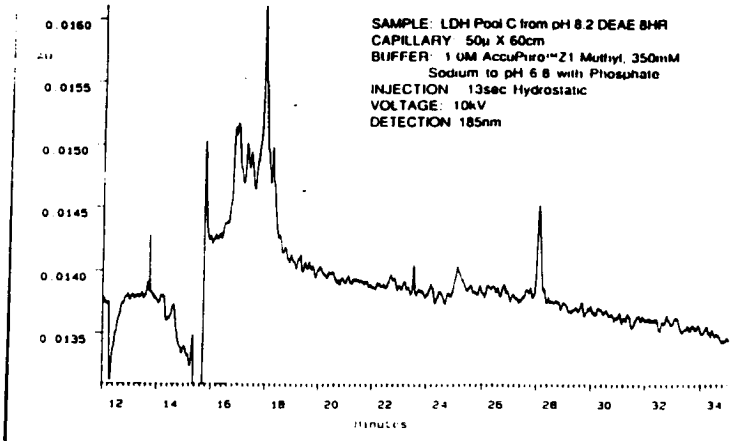

Anion Exchange Chromatography of Mouse Liver Proteins
COLUMN: ProteinPak DEAE 8HR, 10 X 100mm
FLOW: 1.5ml/min
BUFFER: 20mM Tris-HCl, pH 7.7
GRADIENT: 0-250mM NaCl over 20min
For details, see accompanying Poster # 4790
pH 7.7
AU
Minutes

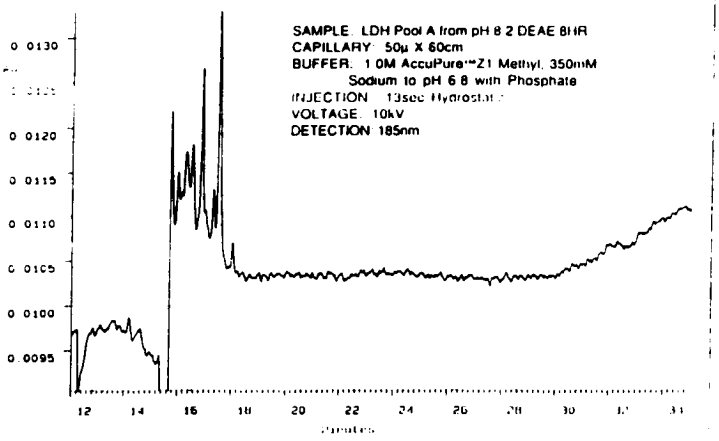

Anion Exchange Chromatography of Mouse Liver Proteins
COLUMN: ProteinPak DEAE 8HR, 10 X 100mm
FLOW: 1.5ml/min
BUFFER: 20mM Tris-HCl, pH 8.4
GRADIENT: 0-250mM NaCl over 20min
For details, see accompanying Poster # 4790
pH 8.4
AU
Minutes

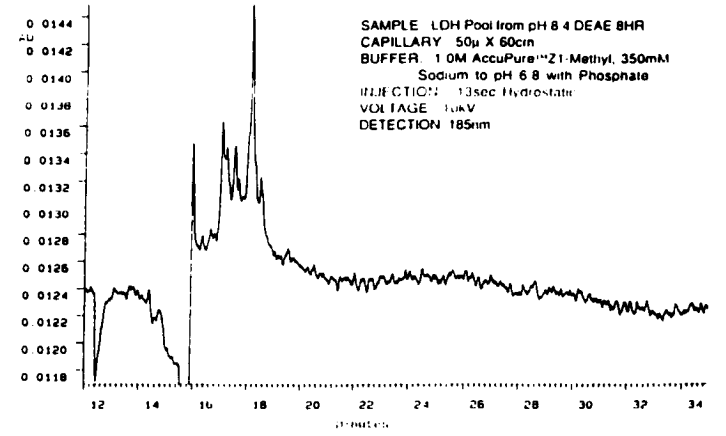

Anion Exchange Chromatography of Mouse Liver Proteins
COLUMN: ProteinPak DEAE 8HR, 10 X 100mm
FLOW: 1.5ml/min
BUFFER: 20mM Tris-HCl, pH 8.2
GRADIENT: 0-250mM NaCl over 20min
For details, see accompanying Poster # 4790
pH 8.2
AU
Minutes

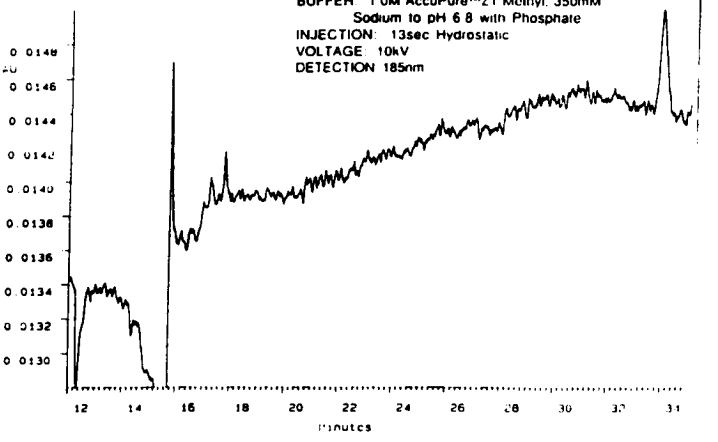

Anion Exchange Chromatography of Mouse Liver Proteins
COLUMN: ProteinPak DEAE 8HR, 10 X 100mm
FLOW: 1.5ml/min
BUFFER: 20mM Tris-HCl, pH 8.6
GRADIENT: 0-250mM NaCl over 20min
For details, see accompanying Poster # 4790
pH 8.6
AU
Minutes

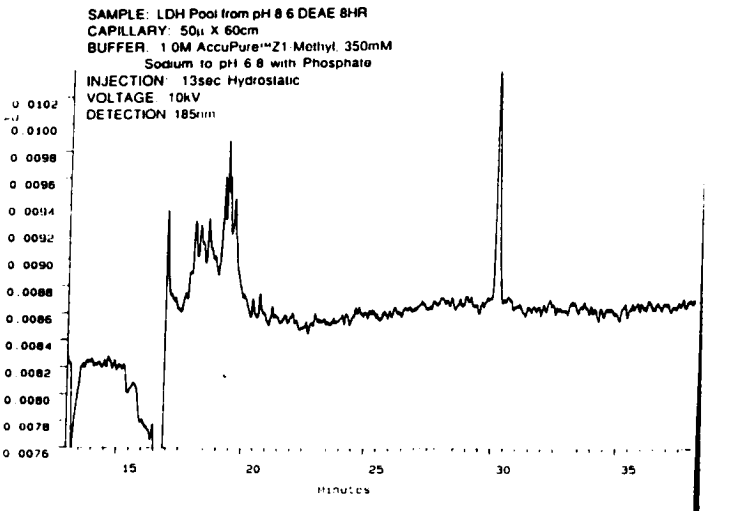

SAMPLE: LDH Pool from pH 7 7 DEAE 8HR
CAPILLARY: 50µ X 60cm
BUFFER: 1 0M AccuPure™Z1 Methyl, 350mM
Sodium to pH 6 8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
Minutes


SAMPLE: LDH Pool C from pH 8.2 DEAE 8HR
CAPILLARY: 50µ X 60cm
BUFFER: 1 0M AccuPure™Z1 Methyl, 350mM
Sodium to pH 6 8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
Minutes


SAMPLE: LDH Pool A from pH 8 2 DEAE 8HR
CAPILLARY: 50µ X 60cm
BUFFER: 1 0M AccuPure™Z1 Methyl, 350mM
Sodium to pH 6 8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm


SAMPLE: LDH Pool from pH 8 4 DEAE 8HR
CAPILLARY: 50µ X 60cm
BUFFER: 1 0M AccuPure™Z1 Methyl, 350mM
Sodium to pH 6 8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm

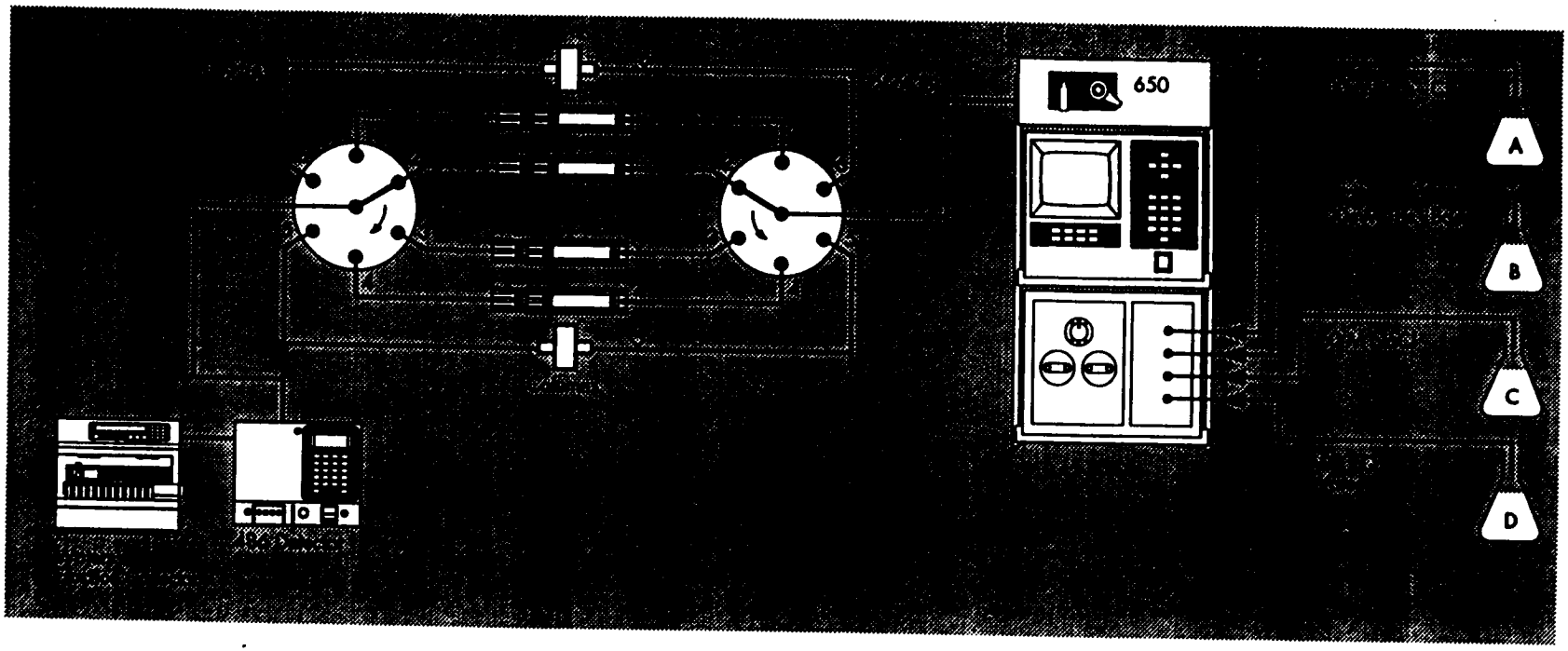

SAMPLE: LDH Pool B from pH 8 2 DEAE 8HR
CAPILLARY: 50µ X 60cm
BUFFER: 1 0M AccuPure™Z1 Methyl, 350mM
Sodium to pH 6 8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm


SAMPLE: LDH Pool from pH 8 6 DEAE 8HR
CAPILLARY: 50µ X 60cm
BUFFER: 1 0M AccuPure™Z1 Methyl, 350mM
Sodium to pH 6 8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
Minutes

Pooled Active Fractions
Specific Activity (I.U./mg)

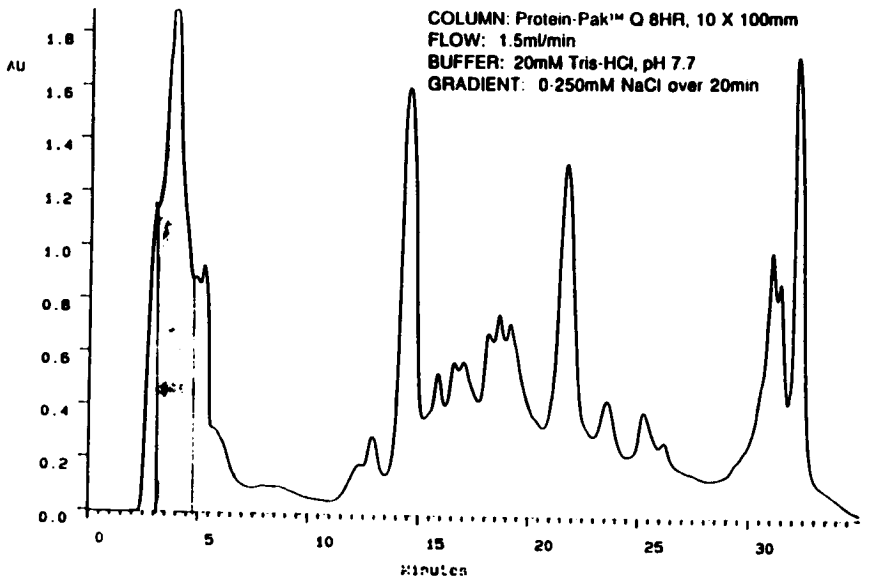
pH	DEAE 8HR	DEAE MemSep	Q 8HR	QMA MemSep
7.7	0.71	1.48	0.78	1.03
8.2	0.44	1.23	0.81	1.16
	1.10			
	1.14			
8.4	3.68	0.96	0.77	1.16
8.6	3.00	0.25	0.88	1.22
		1.60		
		0.15		

Mouse Liver Extract: S.A.= 0.16 I.U./mg

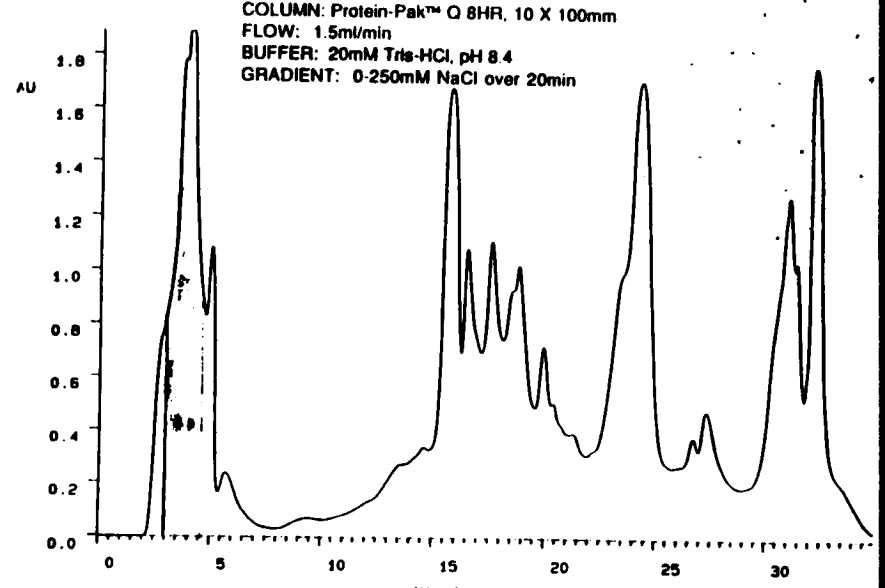


Automatic Selection of
Best Column and Gradient Conditions
for Sample

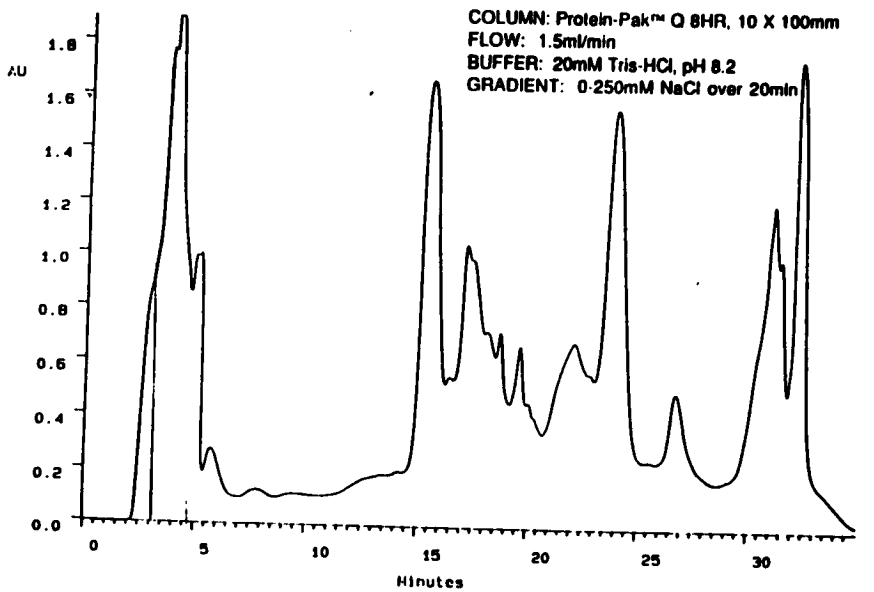
pH 7.7 **Anion Exchange Chromatography of Mouse Liver Proteins**



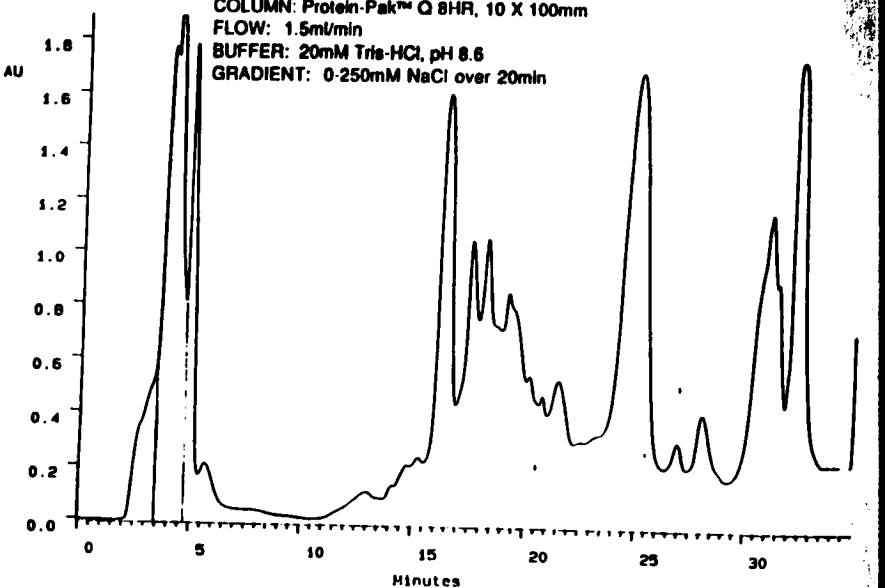
pH 8.4 **Anion Exchange Chromatography of Mouse Liver Proteins**



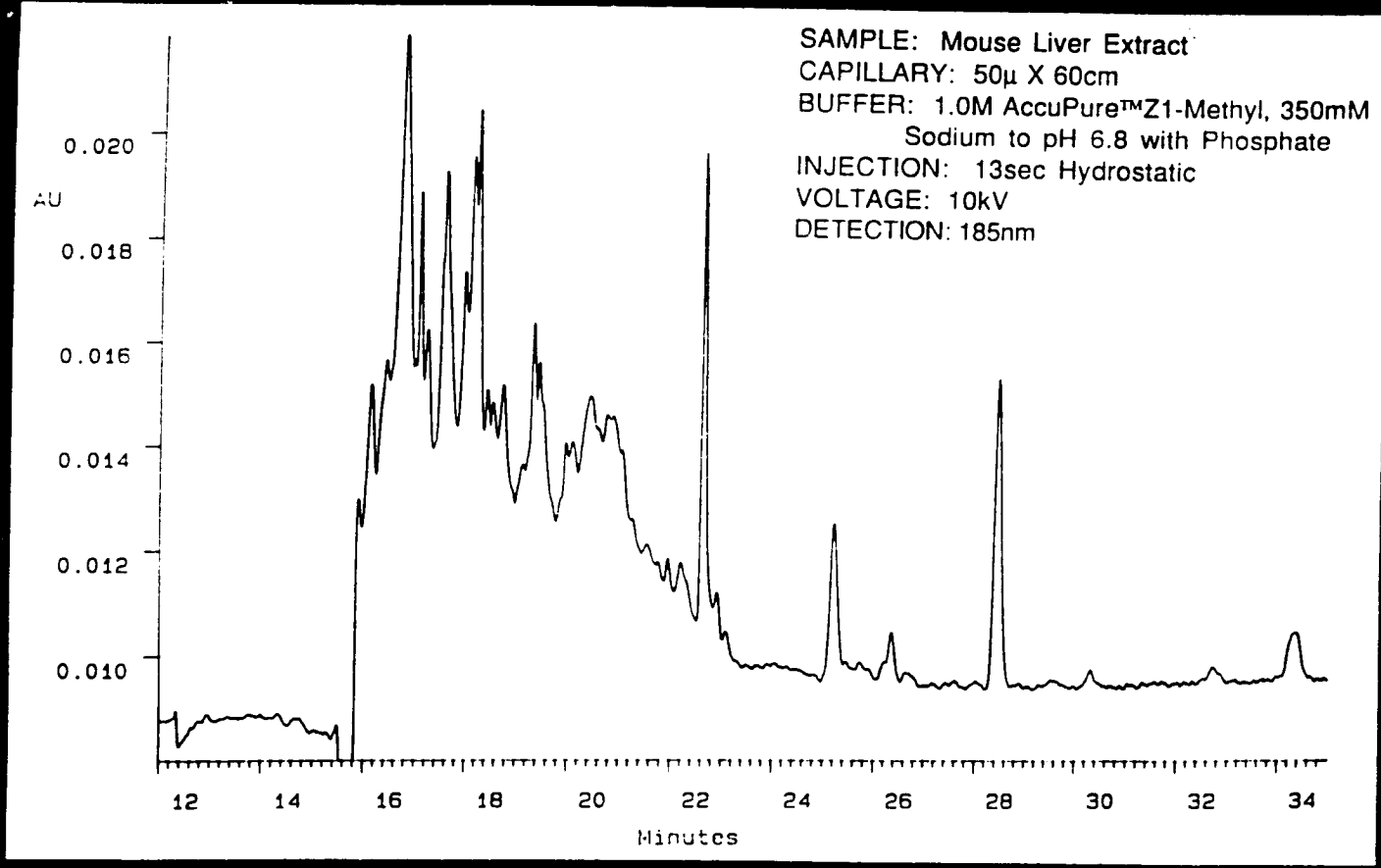
pH 8.2 **Anion Exchange Chromatography of Mouse Liver Proteins**



pH 8.6 **Anion Exchange Chromatography of Mouse Liver Proteins**

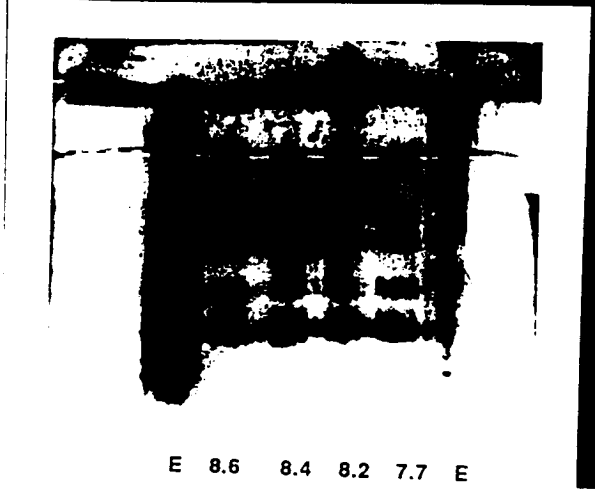


CE Separation of Mouse Liver Extract

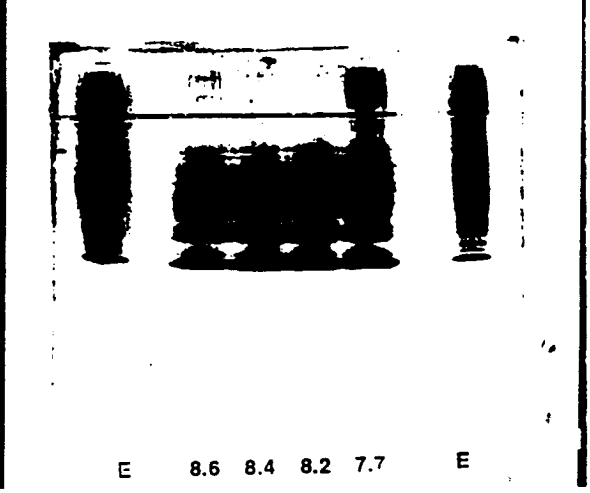


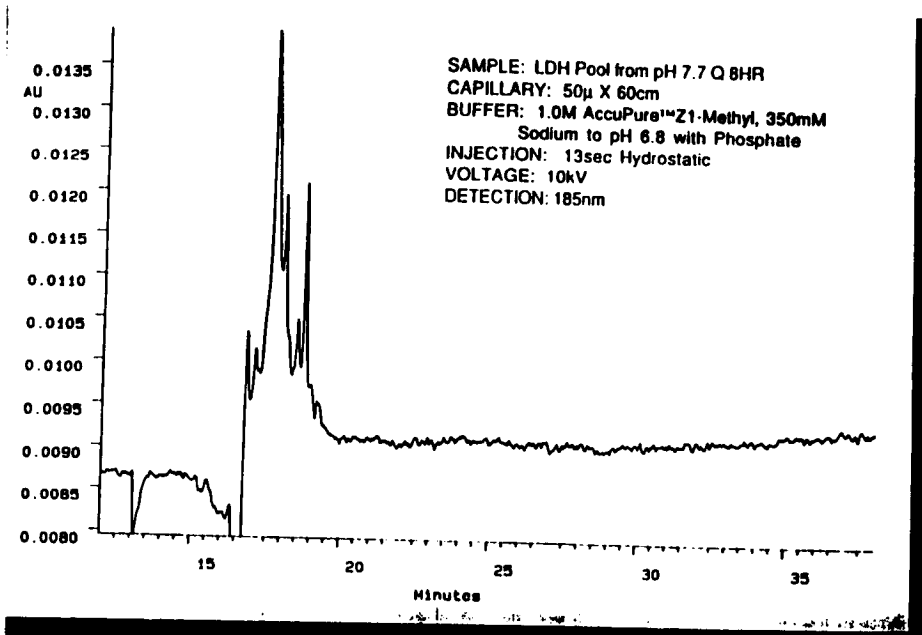
Analysis of Anion Exchange Fractions

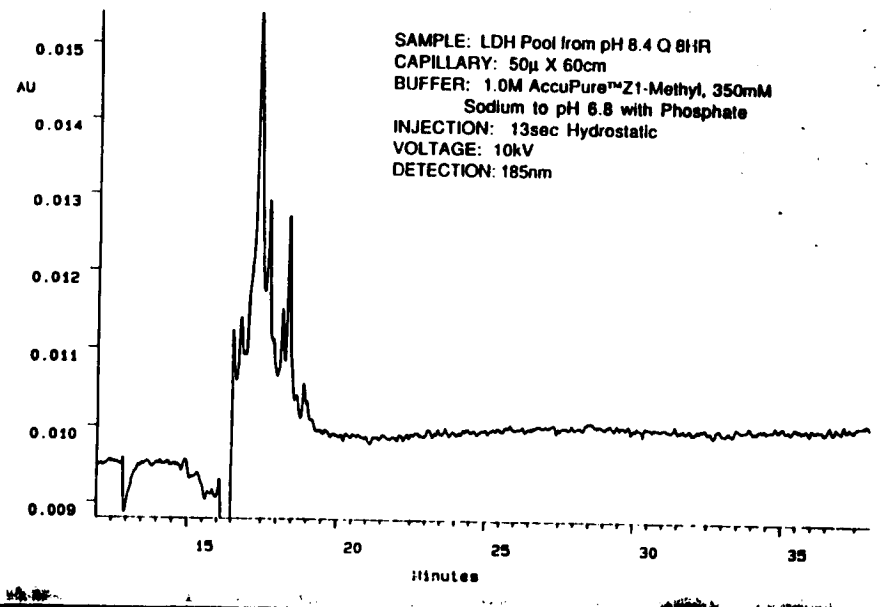
Native Gel - Q 8HR Fractions

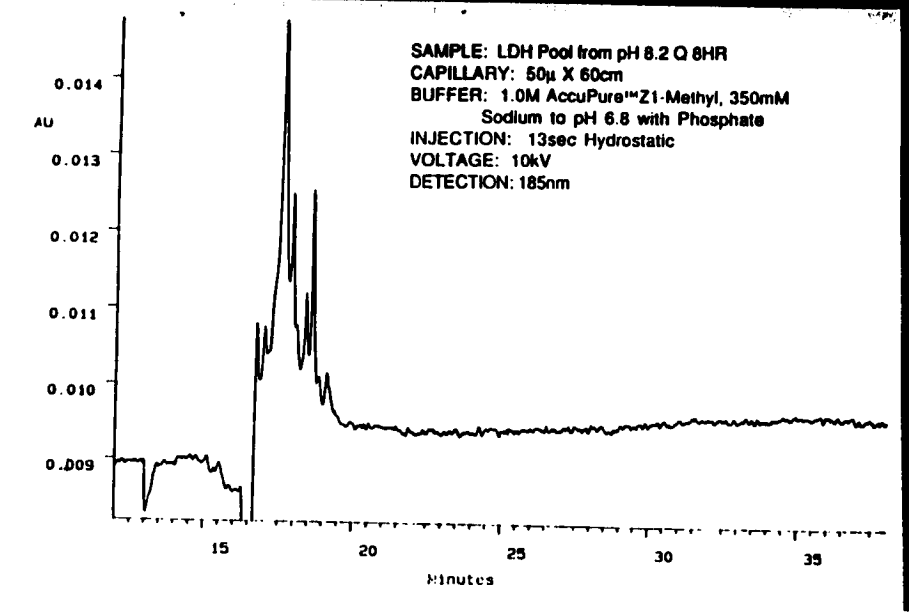


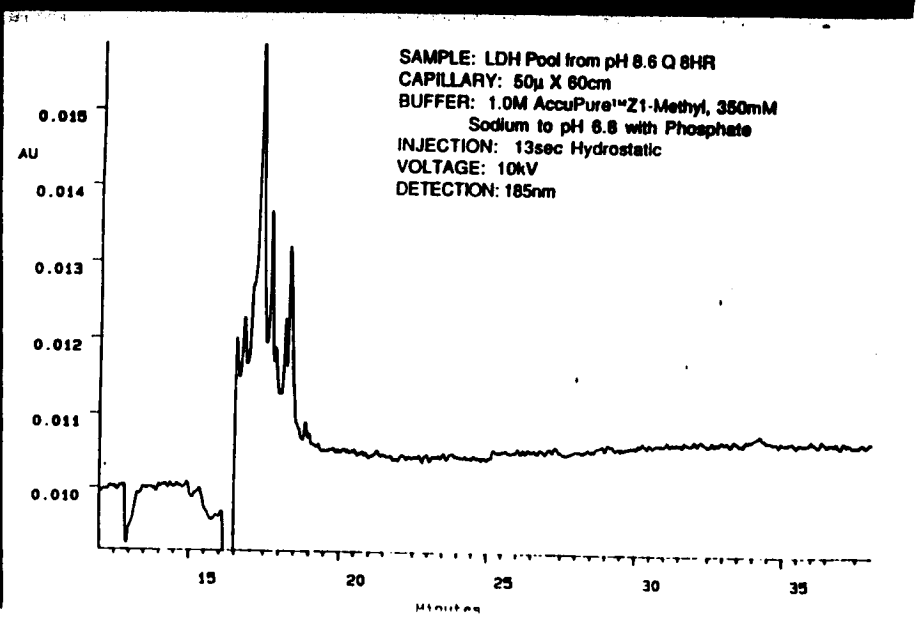
SDS Gel - Q 8HR Fractions



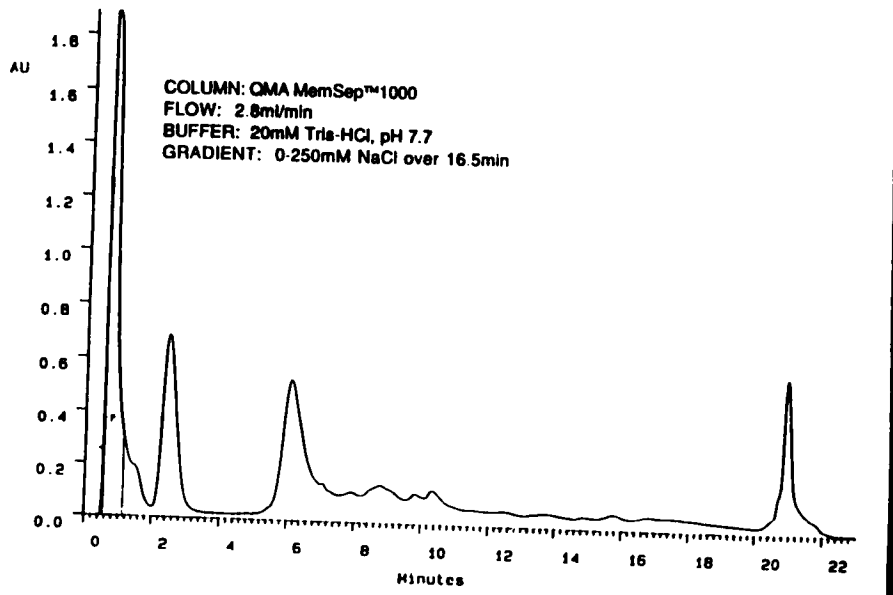

SAMPLE: LDH Pool from pH 7.7 Q 8HR
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0135
0.0130
0.0125
0.0120
0.0115
0.0110
0.0105
0.0100
0.0095
0.0090
0.0085
0.0080
15
20
25
30
35
Minutes


SAMPLE: LDH Pool from pH 8.4 Q 8HR
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.015
0.014
0.013
0.012
0.011
0.010
0.009
15
20
25
30
35
Minutes

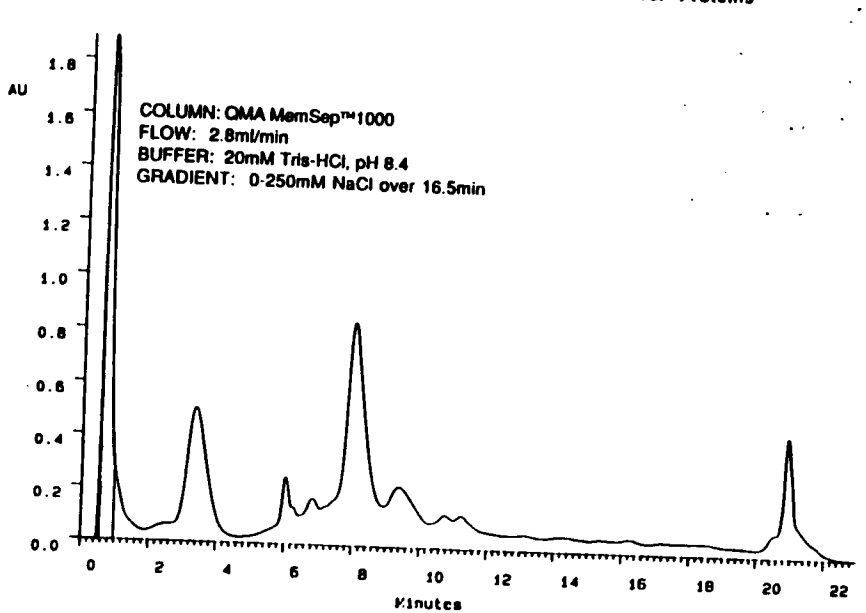

SAMPLE: LDH Pool from pH 8.2 Q 8HR
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.014
0.013
0.012
0.011
0.010
0.009
15
20
25
30
35
Minutes


SAMPLE: LDH Pool from pH 8.6 Q 8HR
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.015
0.014
0.013
0.012
0.011
0.010
15
20
25
30
35
Minutes

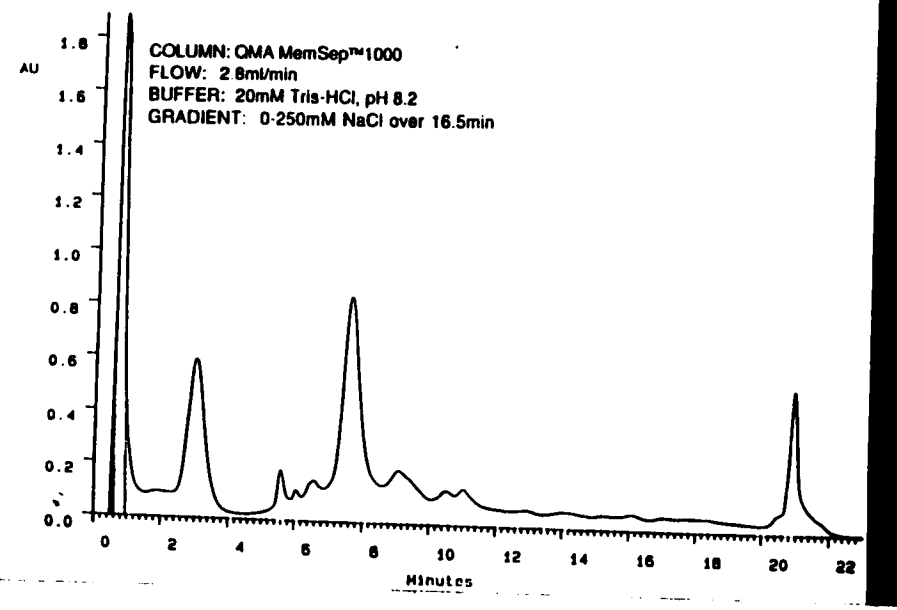
pH 7.7 **Anion Exchange Chromatography of Mouse Liver Proteins**



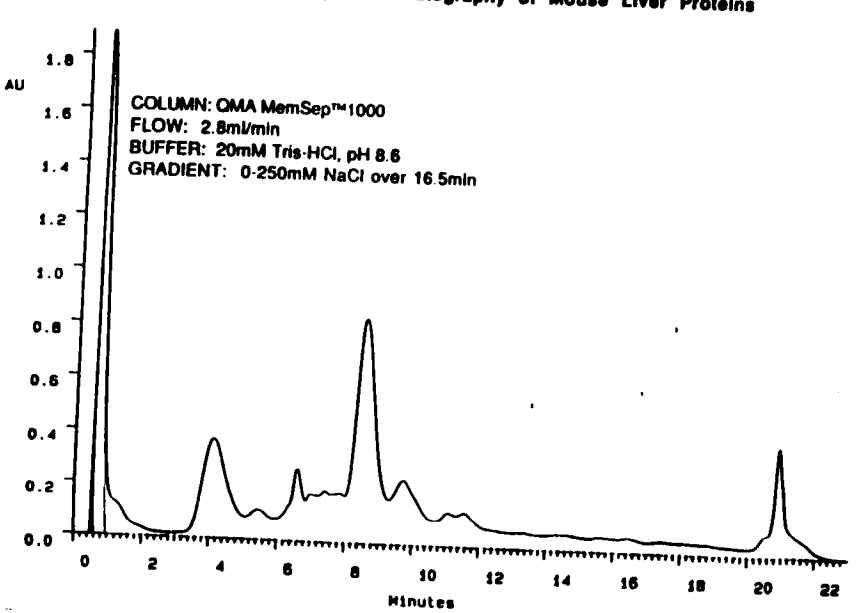
pH 8.4 **Anion Exchange Chromatography of Mouse Liver Proteins**



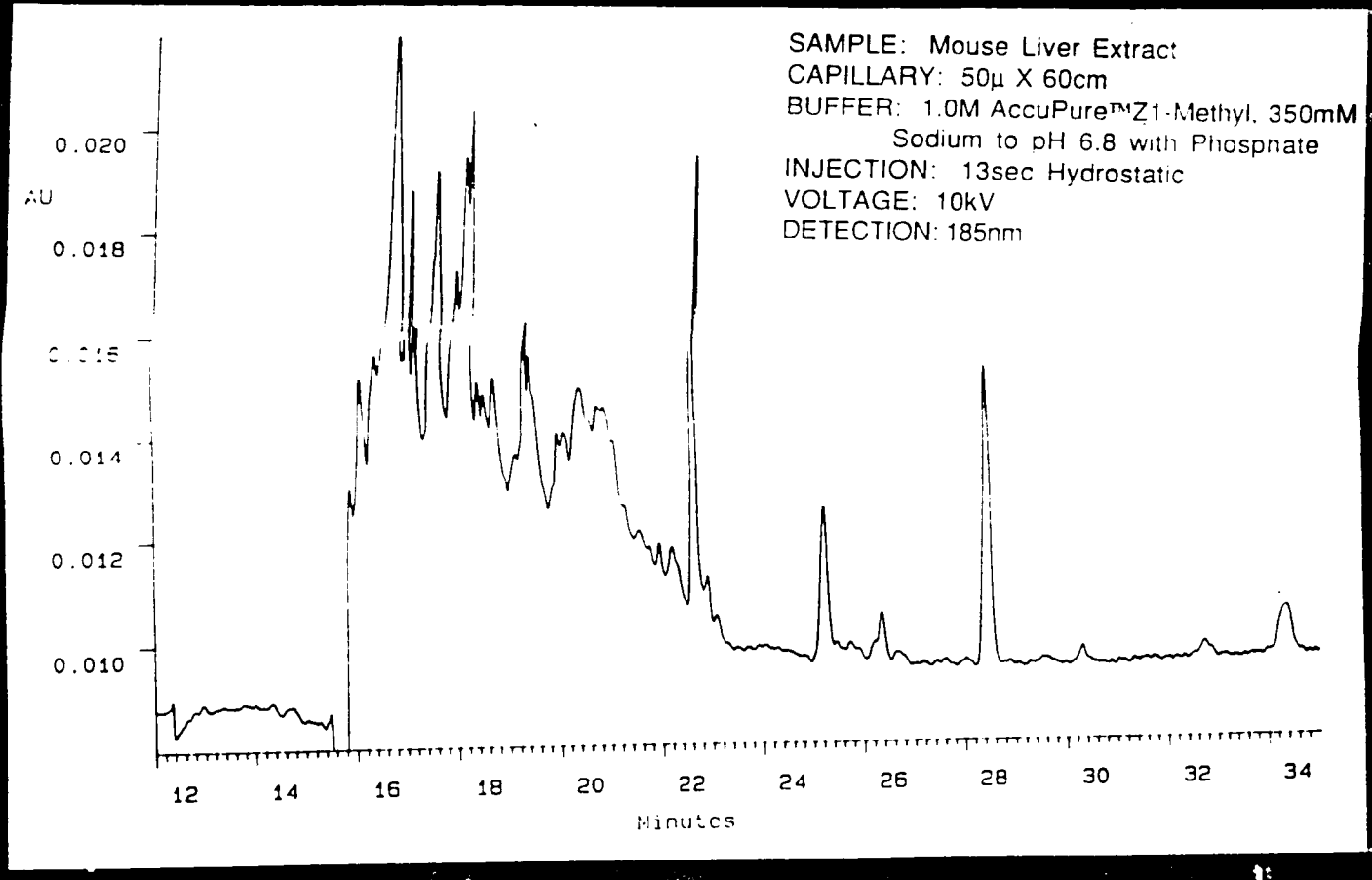
pH 8.2 **Anion Exchange Chromatography of Mouse Liver Proteins**



pH 8.6 **Anion Exchange Chromatography of Mouse Liver Proteins**

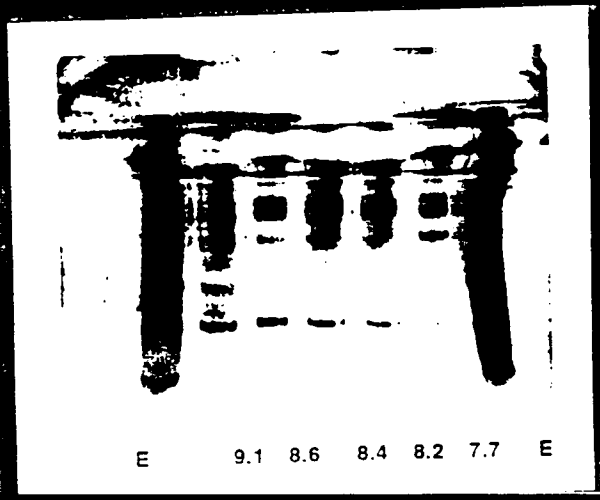


CE Separation of Mouse Liver Extract

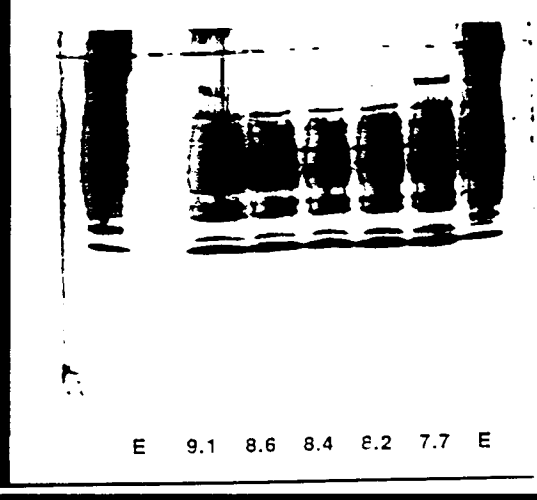


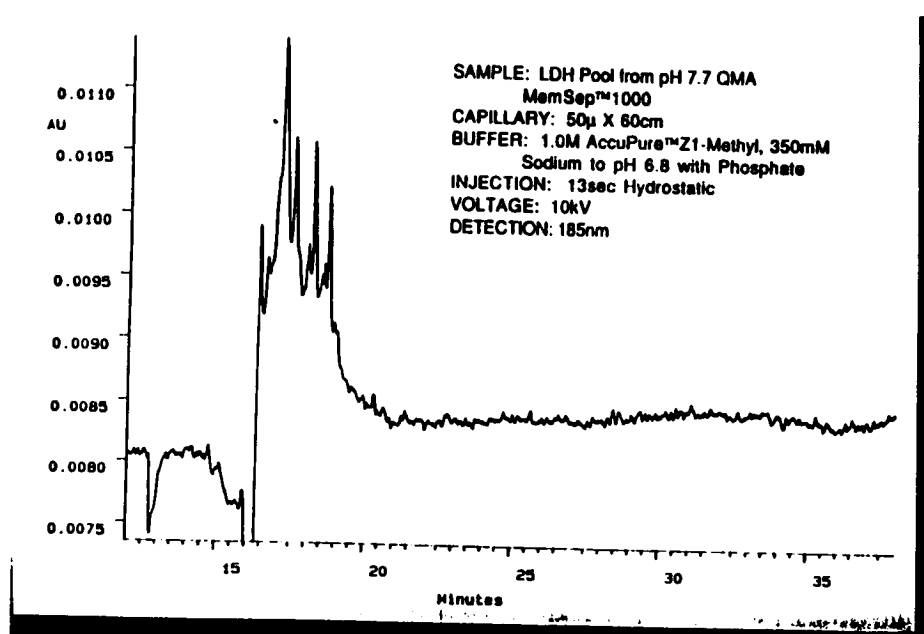
Analysis of Anion Exchange Fractions

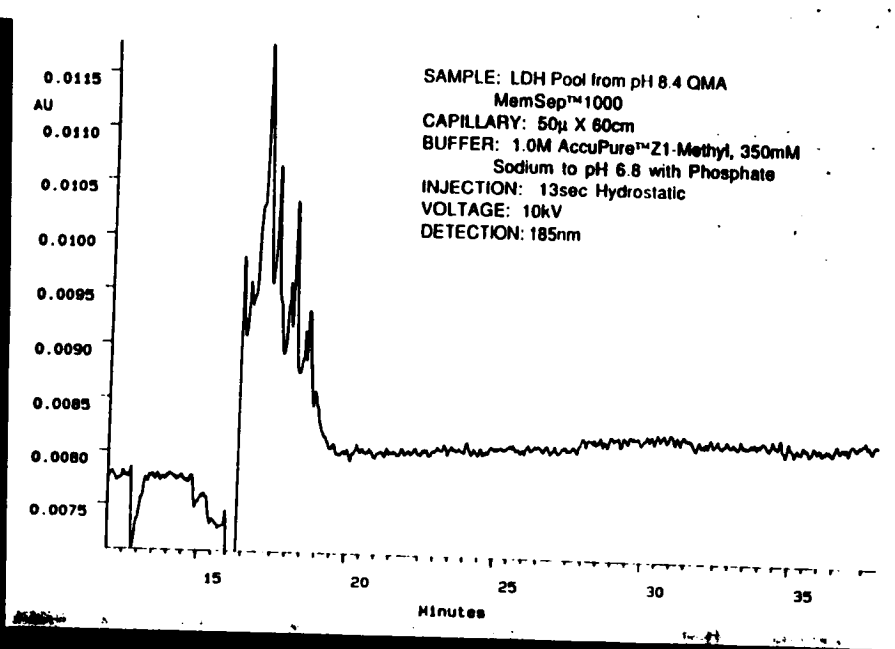
Native Gel - QMA MemSep Fractions

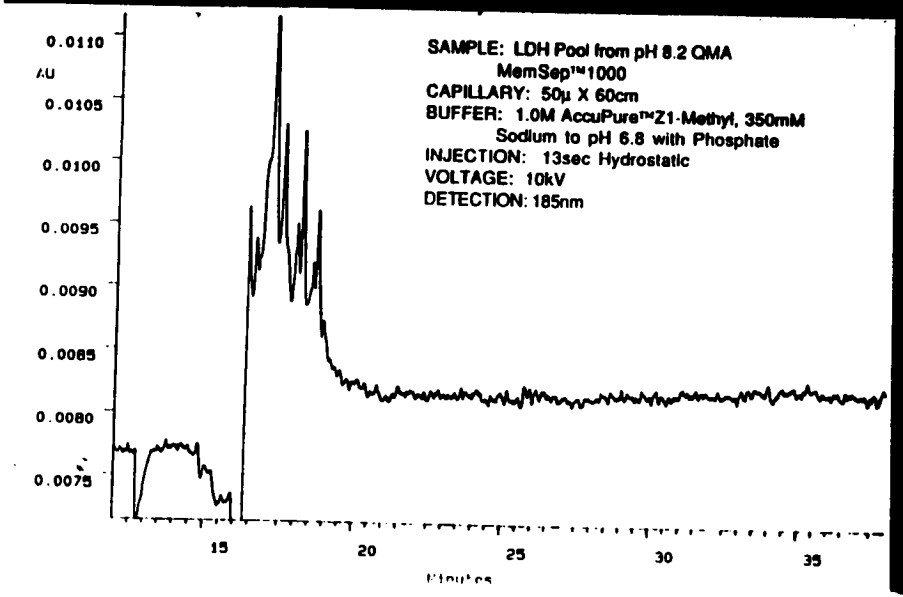


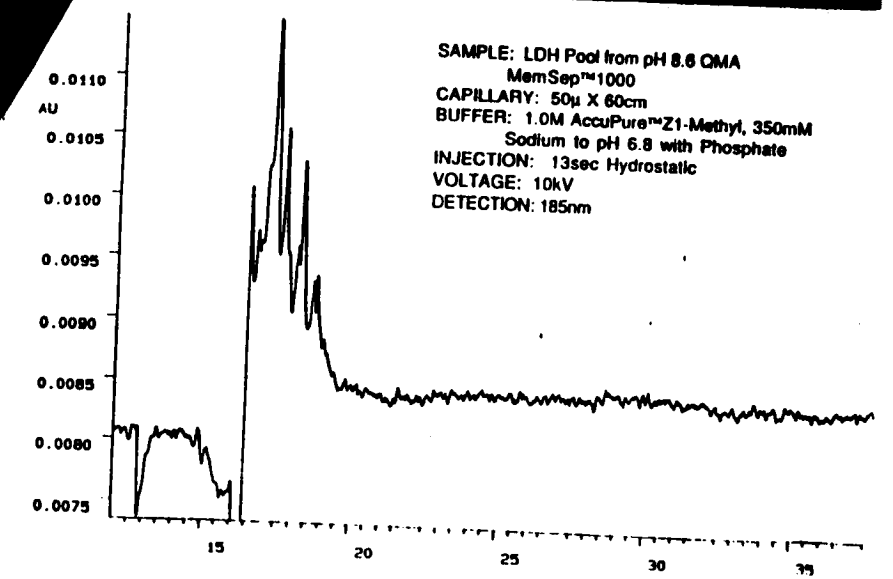
SDS Gel - QMA MemSep Fractio



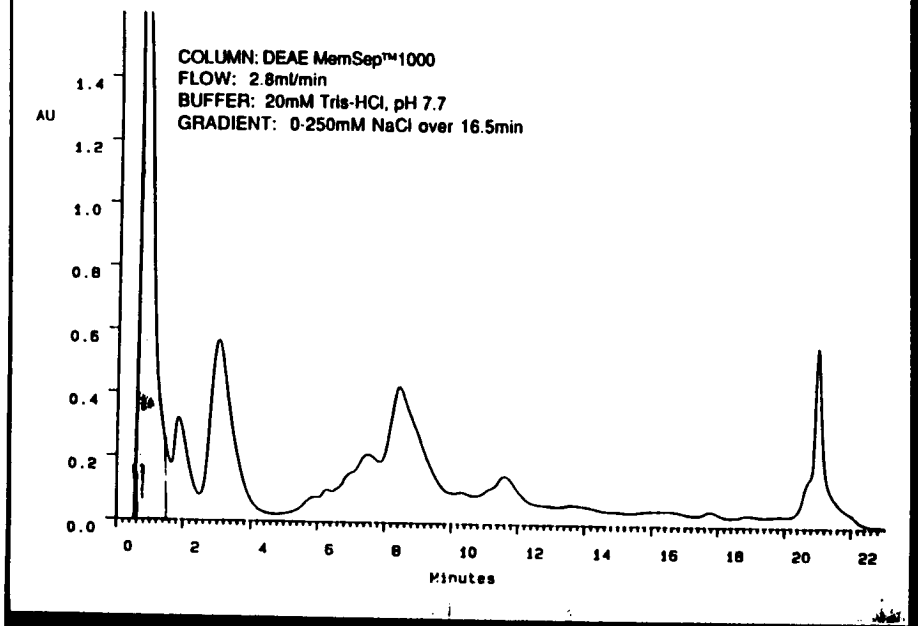

SAMPLE: LDH Pool from pH 7.7 QMA
MemSep™1000
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0110
0.0105
0.0100
0.0095
0.0090
0.0085
0.0080
0.0075
15
20
25
30
35
Minutes


SAMPLE: LDH Pool from pH 8.4 QMA
MemSep™1000
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0115
0.0110
0.0105
0.0100
0.0095
0.0090
0.0085
0.0080
0.0075
15
20
25
30
35
Minutes

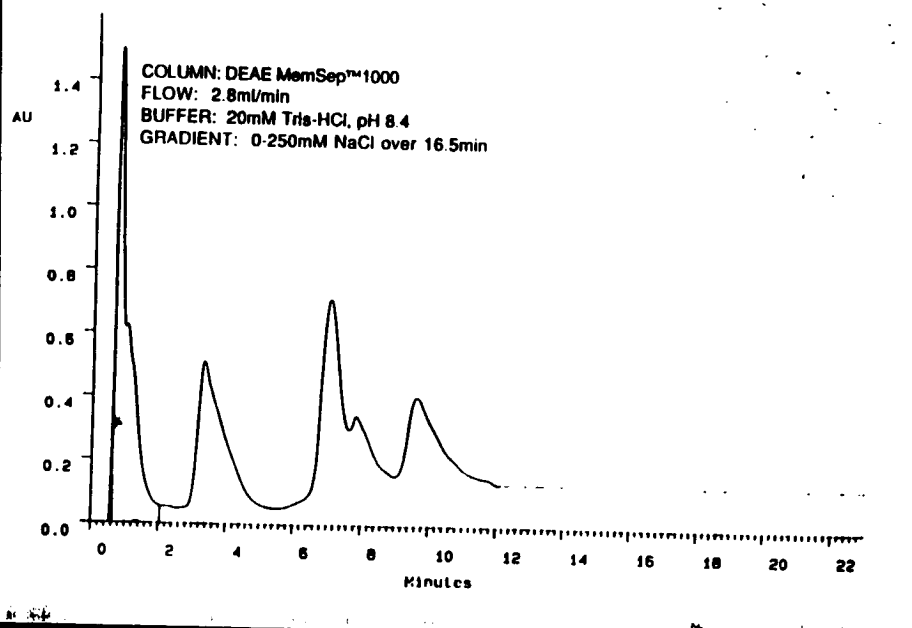

SAMPLE: LDH Pool from pH 8.2 QMA
MemSep™1000
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0110
0.0105
0.0100
0.0095
0.0090
0.0085
0.0080
0.0075
15
20
25
30
35


SAMPLE: LDH Pool from pH 8.6 QMA
MemSep™1000
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0110
0.0105
0.0100
0.0095
0.0090
0.0085
0.0080
0.0075
15
20
25
30

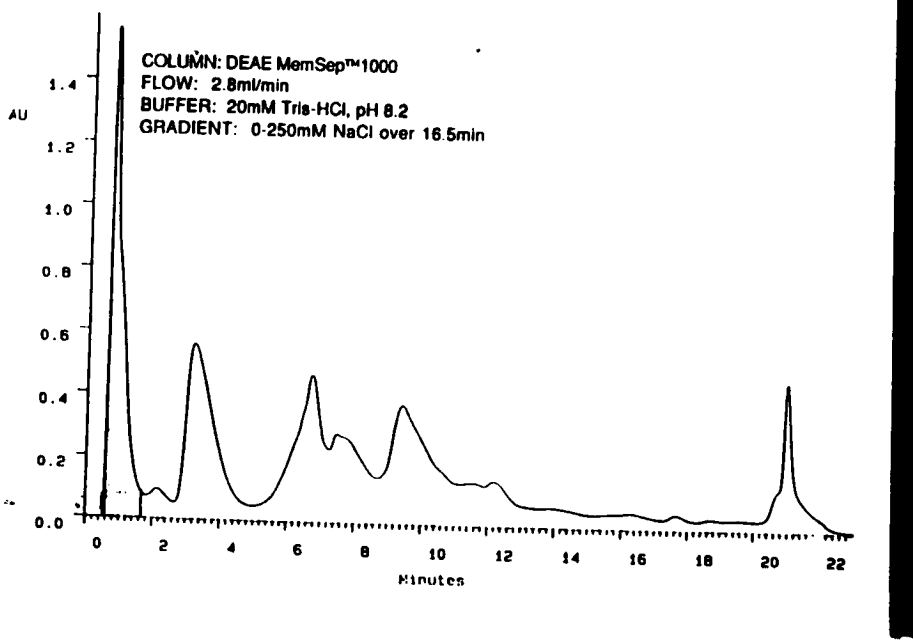
pH 7.7 **Anion Exchange Chromatography of Mouse Liver Proteins**



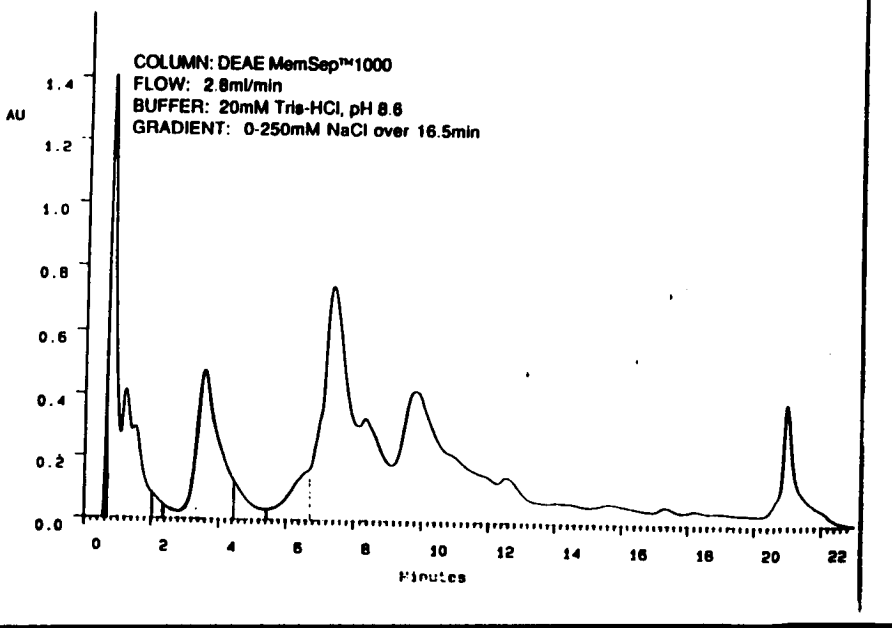
pH 8.4 **Anion Exchange Chromatography of Mouse Liver Proteins**



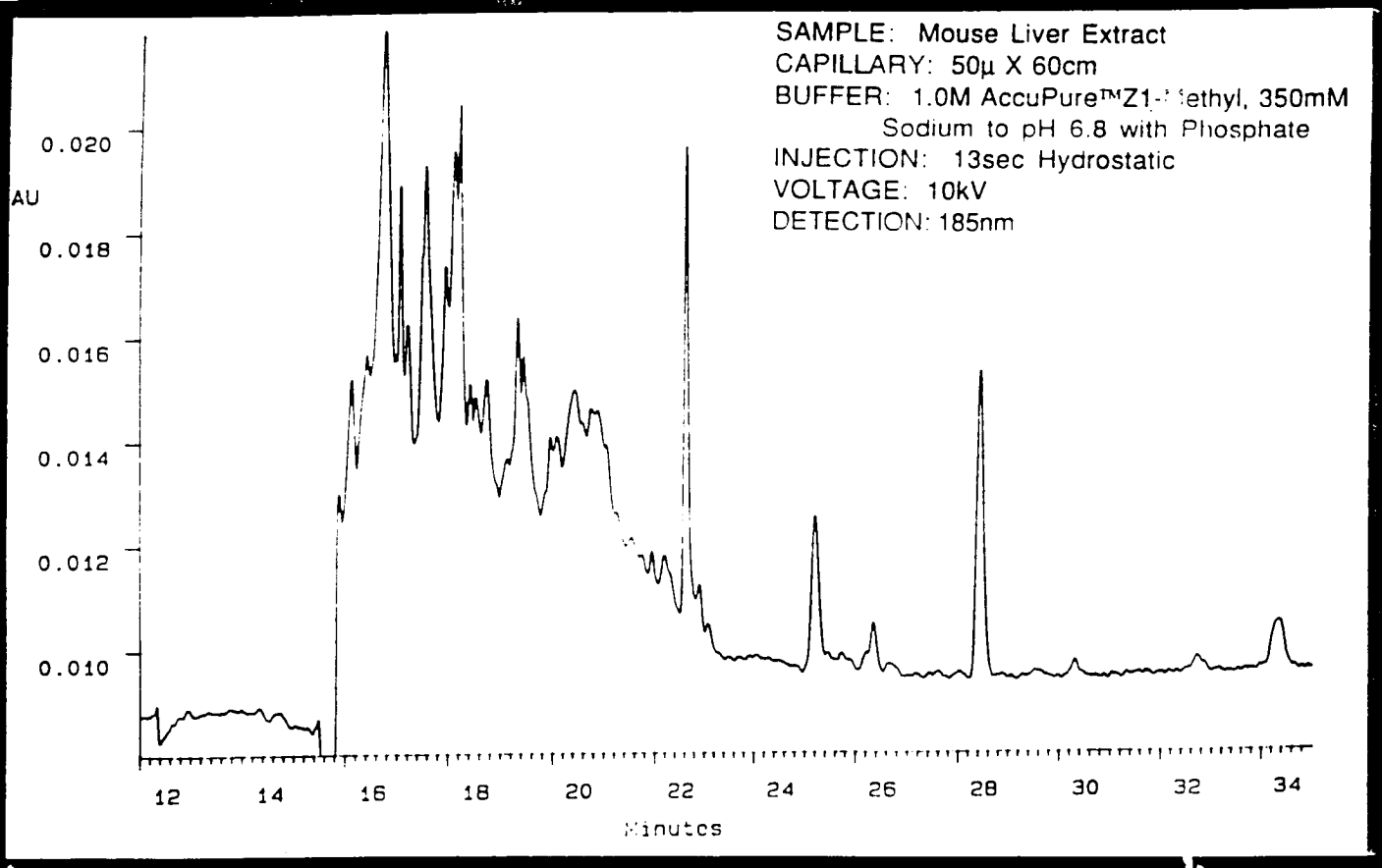
pH 8.2 **Anion Exchange Chromatography of Mouse Liver Proteins**



pH 8.6 **Anion Exchange Chromatography of Mouse Liver Proteins**

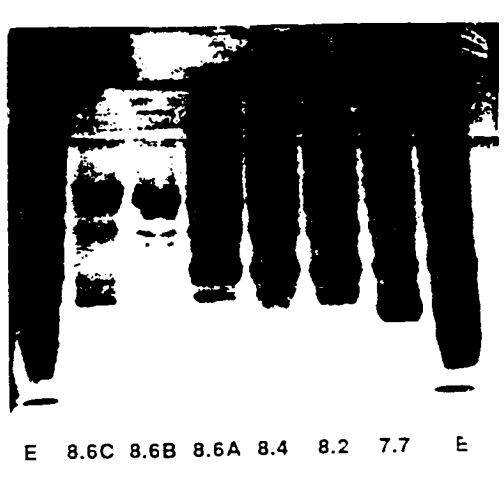


CE Separation of Mouse Liver Extract

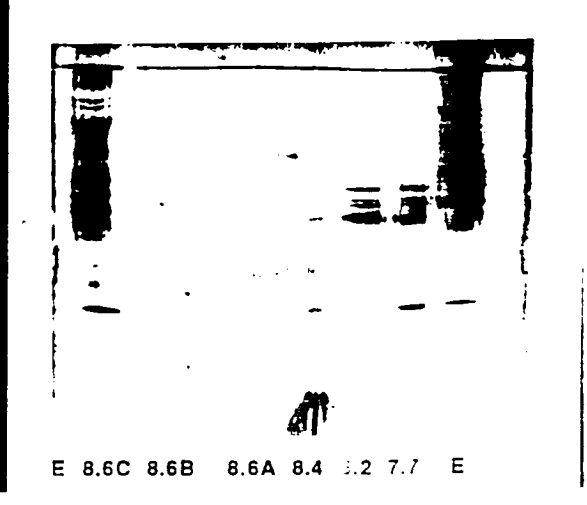


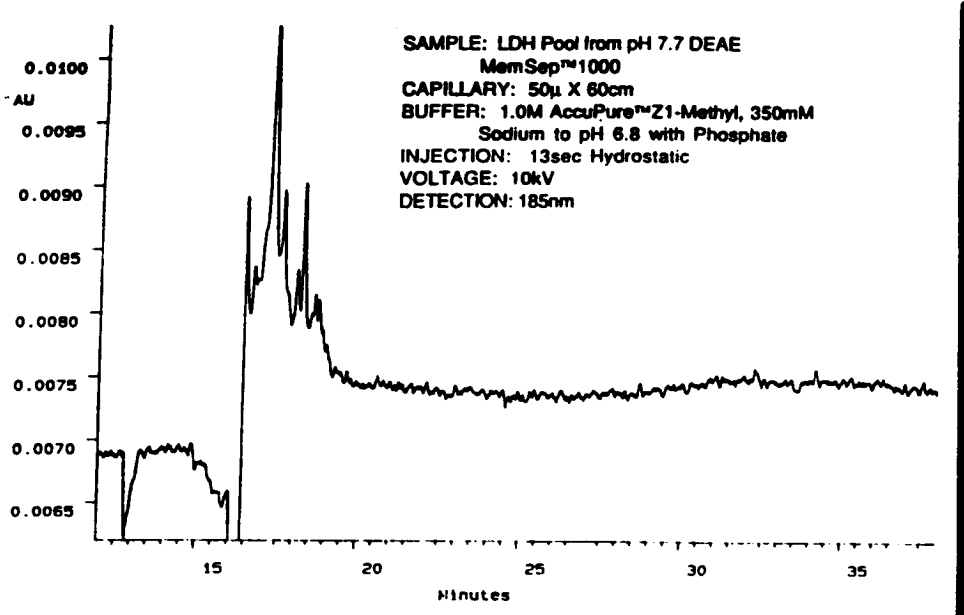
Analysis of Anion Exchange Fractions

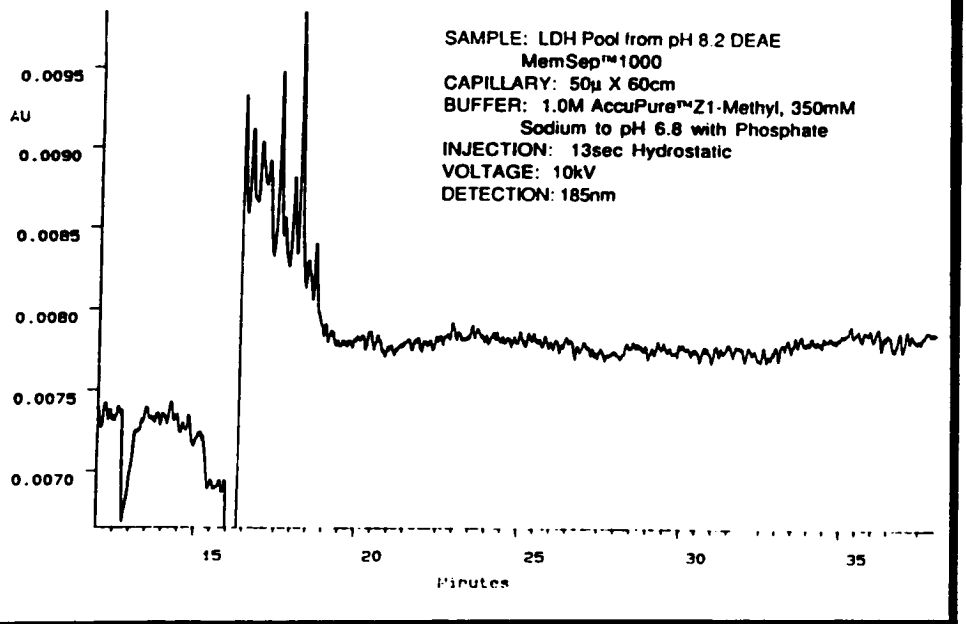
Native Gel - DEAE MemSep Fractions

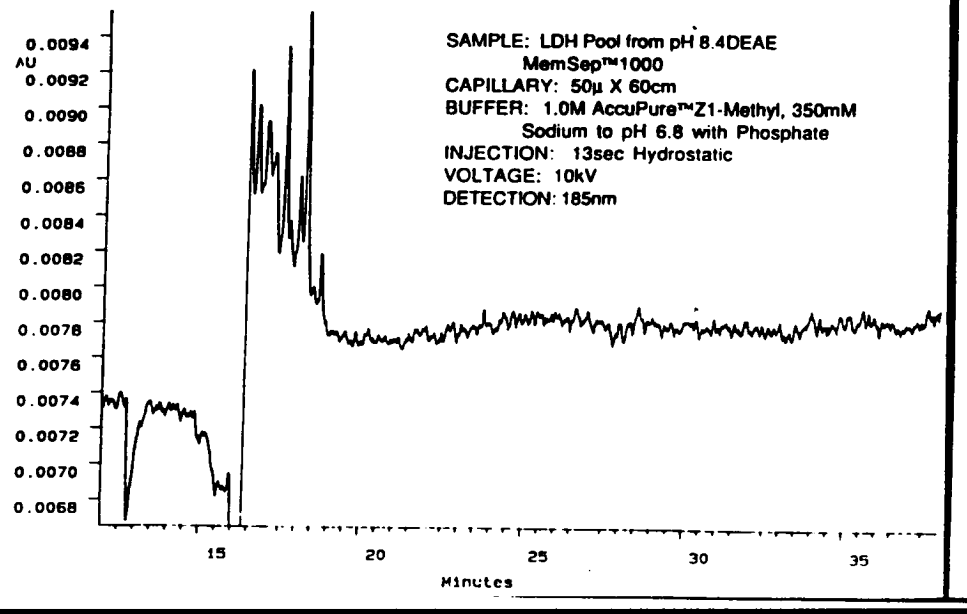


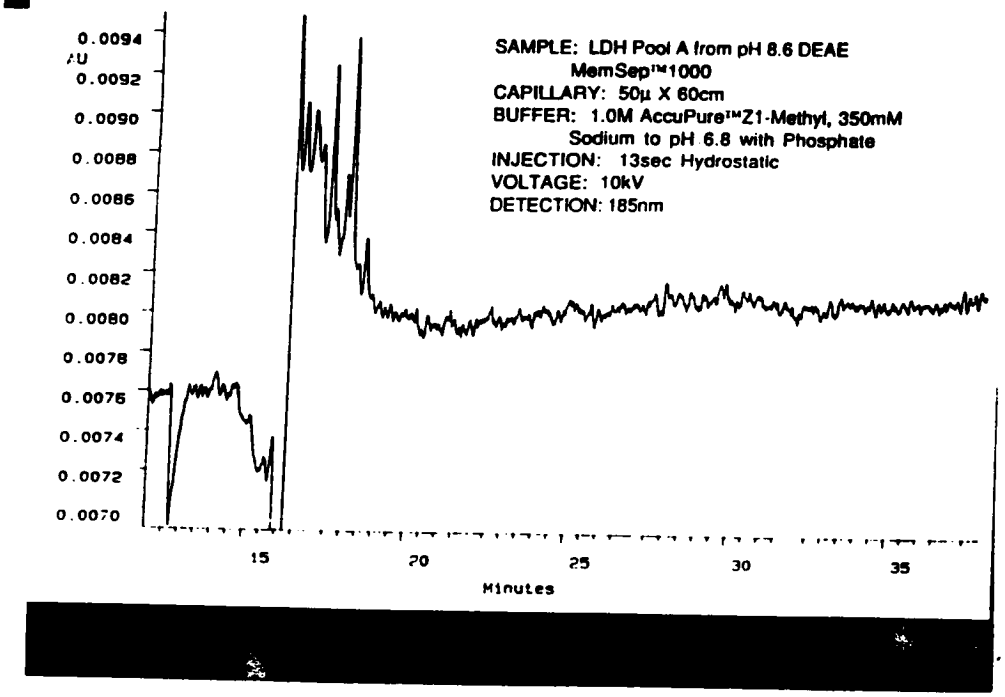
SDS Gel - DEAE MemSep Fractions

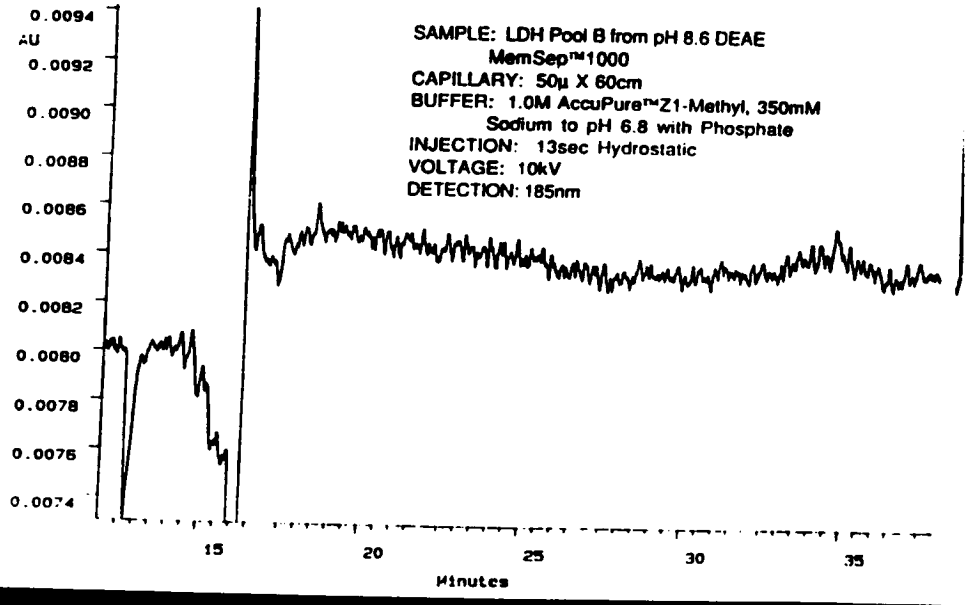


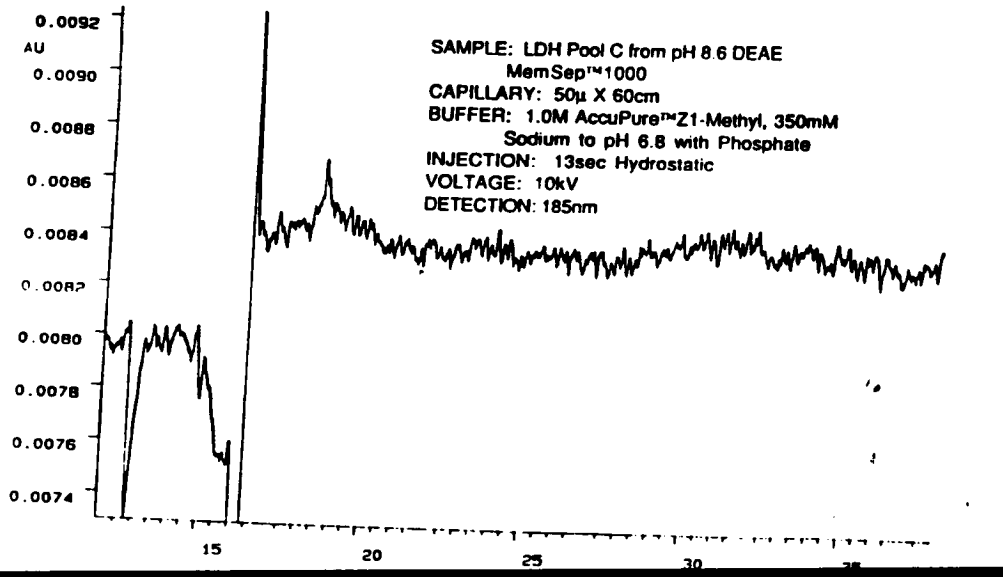

SAMPLE: LDH Pool from pH 7.7 DEAE
MemSep™1000
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0100
0.0095
0.0090
0.0085
0.0080
0.0075
0.0070
0.0065
15
20
25
30
35
Minutes


SAMPLE: LDH Pool from pH 8.2 DEAE
MemSep™1000
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0095
0.0090
0.0085
0.0080
0.0075
0.0070
15
20
25
30
35
Minutes


SAMPLE: LDH Pool from pH 8.4DEAE
MemSep™1000
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0094
0.0092
0.0090
0.0088
0.0086
0.0084
0.0082
0.0080
0.0078
0.0076
0.0074
0.0072
0.0070
0.0068
15
20
25
30
35
Minutes


SAMPLE: LDH Pool A from pH 8.6 DEAE
MemSep™1000
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0094
0.0092
0.0090
0.0088
0.0086
0.0084
0.0082
0.0080
0.0078
0.0076
0.0074
0.0072
0.0070
15
20
25
30
35
Minutes


SAMPLE: LDH Pool B from pH 8.6 DEAE
MemSep™1000
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0094
0.0092
0.0090
0.0088
0.0086
0.0084
0.0082
0.0080
0.0078
0.0076
0.0074
15
20
25
30
35
Minutes


SAMPLE: LDH Pool C from pH 8.6 DEAE
MemSep™1000
CAPILLARY: 50µ X 60cm
BUFFER: 1.0M AccuPure™Z1-Methyl, 350mM
Sodium to pH 6.8 with Phosphate
INJECTION: 13sec Hydrostatic
VOLTAGE: 10kV
DETECTION: 185nm
AU
0.0092
0.0090
0.0088
0.0086
0.0084
0.0082
0.0080
0.0078
0.0076
0.0074
15
20
25
30

Conclusions

1. The automation of multidimensional chromatography is straightforward and reliable with modern instruments and accessories.

2. The use of economical, large particle packings brings the benefits of modern high resolution protein chromatography to every stage of the purification.

3. The Auto-Blend™ method is valuable in such automated, multidimensional isolations since the use of concetrated buffer stocks reduces the labor and inherent errors associated with the preparation of large volumes of dilute buffers that require careful pH adjustment.

4. The selection of the best column and gradient for the isolation of a particular protein can also be readily automated.

5. The use of the Auto-Blend™ Method for automated gradient optimization ensures the highest yield and purity from a given column. Relatively small changes in pH can significantly improve the product.

6. Comparison of different columns reveals useful differences in the separations. It is clear with a sample of the complexity of the liver extract that the different columns perform differently for different proteins and that while one may be best for one enzyme, another column will be better suited for another protein In addition, electrophoretic assays show that preparations of similar specific activity have a different spectrum of contaminating proteins. This suggests that superficially similar column chemistries can be profitably used in series in a sequential separation protocol.

7. The synergistic combination of modern high resolution columns and instrumental methods amenable to automation can facilitate obtaining higher yields of pure proteins from crude biological extracts.