Poster Presentation

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Adsorptive Protein and Peptide Sequence Analysis on Chemically-Modified Polyvinylidine Difluoride Membranes

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ABSTRACT:

Polyvinylidene difluoride (PVDF) membranes are commonly utilized for adsorptive sequence analysis of polypeptides. Sequence analysis of short and/or hydrophobic peptides is improved by the addition of polymeric cationic carriers such as polybrene™ or polyethyleneimine. Unfortunately, these carriers require time consuming precycling before sample application and contribute to increased background peaks in the HPLC analysis. We have utilized a chemically-modified-membrane which does not require precycling and facilitates the sequence analysis of small peptides. Also improved initial step yield and repetitive sequencing yields from protein or peptide samples applied or blotted to PVDF are obtained when a chemically-modified-membrane is added to the ProSequencer™ reaction chamber as a trapping membrane.

INTRODUCTION:

In an adsorptive sequencing mode glass supports often have polymeric cationic carriers such as polybrene™ (Tarr *et al.*, 1978) added to improve the retention of small peptides on these supports (Hewick *et al.*, 1981). Glass-fiber sheets have also been modified to allow the covalent attachment of polypeptide chains (Abersold *et al.*, 1986). There has been a shift in the last few years from glass-based solid supports to PVDF membranes (Matsudaira 1987). Membrane supports offer the advantages of mechanical and chemical stability to a wide variety of conditions and they are easily merged with other analytical techniques such as sodium dodecylsulfate-polyacrylamide gel electrophoresis. Functionalized PVDF based supports have also been developed to allow the covalent attachment of proteins and peptides (Coull *et al.*, 1989; Coull *et al.*, 1991).

We report here the use of an quarternary amine modified PVDF membrane (Immobilon-N™) as an effective support for the adsorptive sequence analysis of small peptides.

MATERIALS & METHODS:

The Immobilon-N and -P membranes were obtained from Millipore in 25 x 25 cm sheets. Batches of 8 mm disks were prepared from the sheets and stored at -20 °C. A 6625 ProSequencer™ as described in poster T63 was used to evaluate the various membrane supports.

Two test peptides were synthesized at the 0.1 mmol scale on a methylbenzhydrylamine polystyrene-based support PAC™ using an Excell™ peptide synthesizer. Peptides were synthesized using 9-fluorenyl-methoxycarbonyl (Fmoc) alpha-amino protected amino acids and benzotriazolyloxy-trisdimethyl-aminophosphonium hexafluorophosphate (BOP)/1-hydroxybenzotriazole (HOBt) activation. The acylation times used for each amino acid addition were determined by an expert system. All of the peptide synthesis and protein sequencing chemicals were supplied by MilliGen/Biosearch.

Stock solutions of the test peptides were prepared in 25% (v/v) acetonitrile/ Milli-Q water (Fluka Chemical Corporation, Ronkonkoma, NY) . An aliquot of 10 uL (200 pmol) was applied to the center of the sample disks and allowed to air dry before use. The horse heart myoglobin sample was collected from a prototype capillary electrophoresis system.

RESULTS & DISCUSSION:

The membrane supports were evaluated in terms of the amount and number of background peaks observed on each type of blank support (Figures 1-3). The Immobilon-P supports provided chromatograms with the smallest number of interfering background peaks and at lower levels than the Immobilon-N membranes (Figure 4). There was a slight advantage in using the modified Immobilon-N membrane over the Immobilon-N, as lower initial background peaks were observed.

Small peptides less than 10 residues could be sequenced on either of the Immobilon-N membranes to the C-terminal amino acid (Figure 5). Useful sequence information was obtain on Immobilon-P, although the C-terminal residue was not identified. Factors such as the hydrophobicity of the sample and composition of the peptide will affect the peptide removal rate from the membrane during the sequencing process.

The addition of a backing or trapping membrane of Immobilon-N resulted in the determination of the C-terminal amino acid from peptide 1 applied to an Immobilon-P support (Figure 6). This is significant as polypeptides are easily detected on Immobilon-P membranes by various visualization techniques. The sample could then be retained by simply placing a Immobilon-N membrane underneath the Immobilon-P support. The low background levels of the Immobilon-P membrane aided the sequence analysis of low-picomole amounts of sample (Figure 7).

CONCLUSIONS:

- Immobilon-P membranes yield a cleaner background than the Immobilon-N based supports.
- Immobilon-N or the modified version retains small peptides better than Immobilon-P.
- Immobilon-N can be used as a backing membrane to retain sample which is washed off the primary membrane.
- Immobilon-N and -P can be used for adsorptive sequence analysis without precycling the membrane.

ACKNOWLEDGMENTS:

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REFERENCES:

Aebersold, R.H., Teplow, D.B., Hood, L.E., & Kent, S.B.H. (1986) Electroblotting onto activated glass; high efficiency preparation of proteins from analytical sodium dodecylsulfate-polyacrylamide gels for direct sequence analysis. J. Biol. Chem. **261:**4229-4238.

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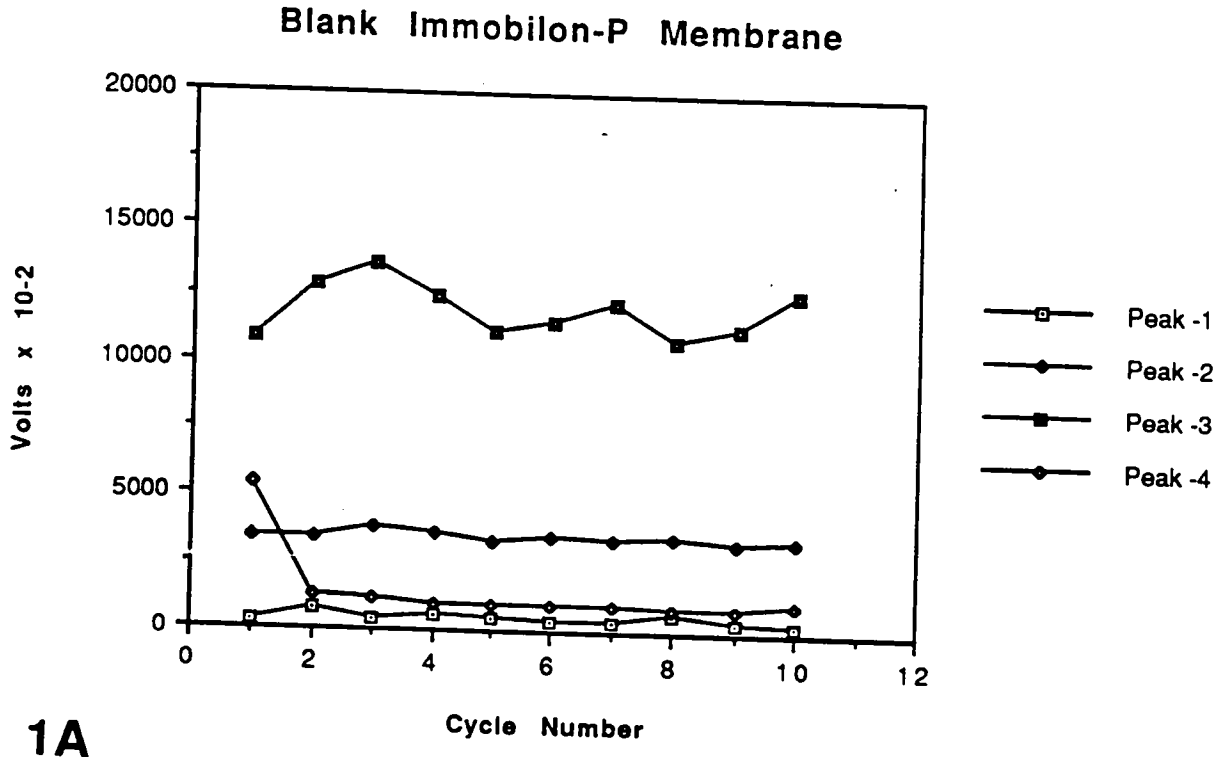
Coull, J.M., Pappin, D.J.C., Mark, J., Aebersold, R., & Köster, H. (1991) Functionalized membrane supports for covalent protein microsequence analysis. Anal. Biochem. **194:**110-120.

Hewick, R.M., Hunkapiller, M.W., Hood, L.E., & Dreyer, W.J. (1981) A gas-liquid-solid-phase peptide and protein sequenator. J. Biol. Chem. **256:**7990-7997.

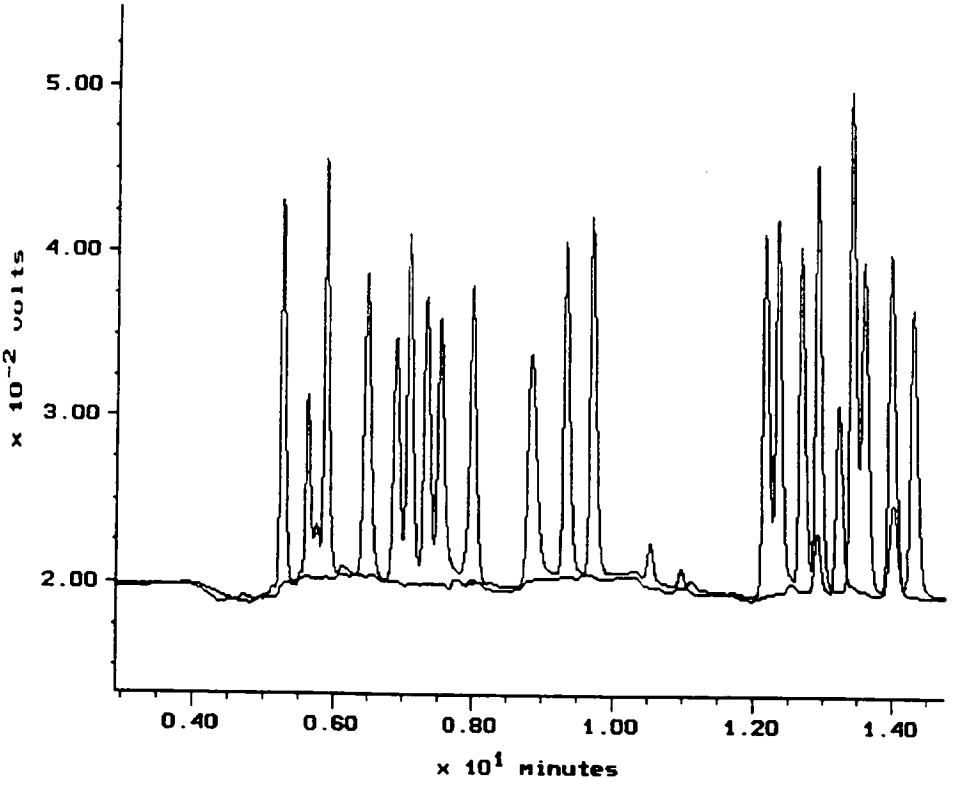
Matsudaira, P. (1987) Sequence from picomole-quantities of proteins electroblotted onto polyvinylidene difuoride membranes. J. Biol. Chem. **262:**10035-10038.

Tarr, G.E., Beecher, J.F., Bell, M., & McKean, D.J. (1978) Polyquarternary amines prevent peptide loss from sequencers. Anal. Biochem. **84:**622-627.



1A

Figure 1. Adsorptive mode blank cycles from Immobilon-P membrane. The amount of three interfering background peaks and diphenylthiourea (DPTU) are shown in Panel A. The values are compared in terms of peak height. Approximately 400 units is equivalent to 1 pmol of phenylthiohydantoin (PTH) amino acid. The chromatograms from the first and tenth cycles are shown in Panel B. The PTH standard is at the 50 pmol level and the first cycle below the standard is traced in yellow.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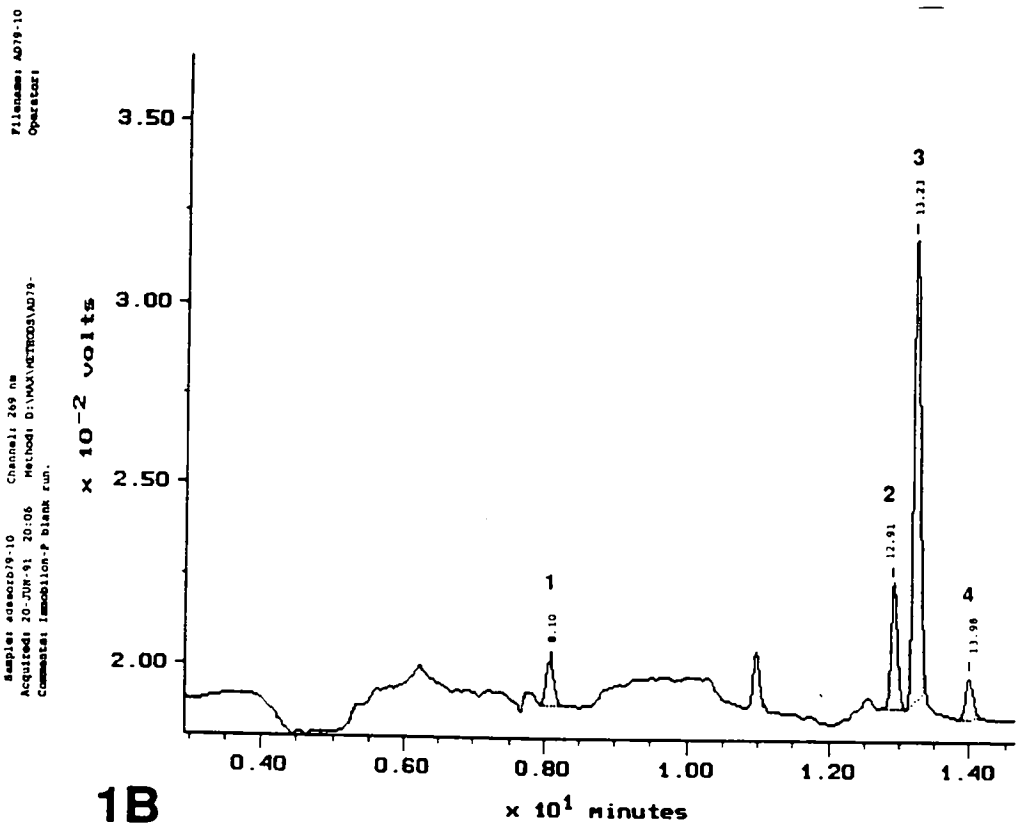
4.00
3.00
2.00
x 10-2 volts
0.40
0.60
0.80
1.00
1.20
1.40
x 10¹ minutes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Operator:
Sample: asesorb79-10
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Acquired: 20-JUN-91 20:06
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Comments: Immobilon-P blank run.
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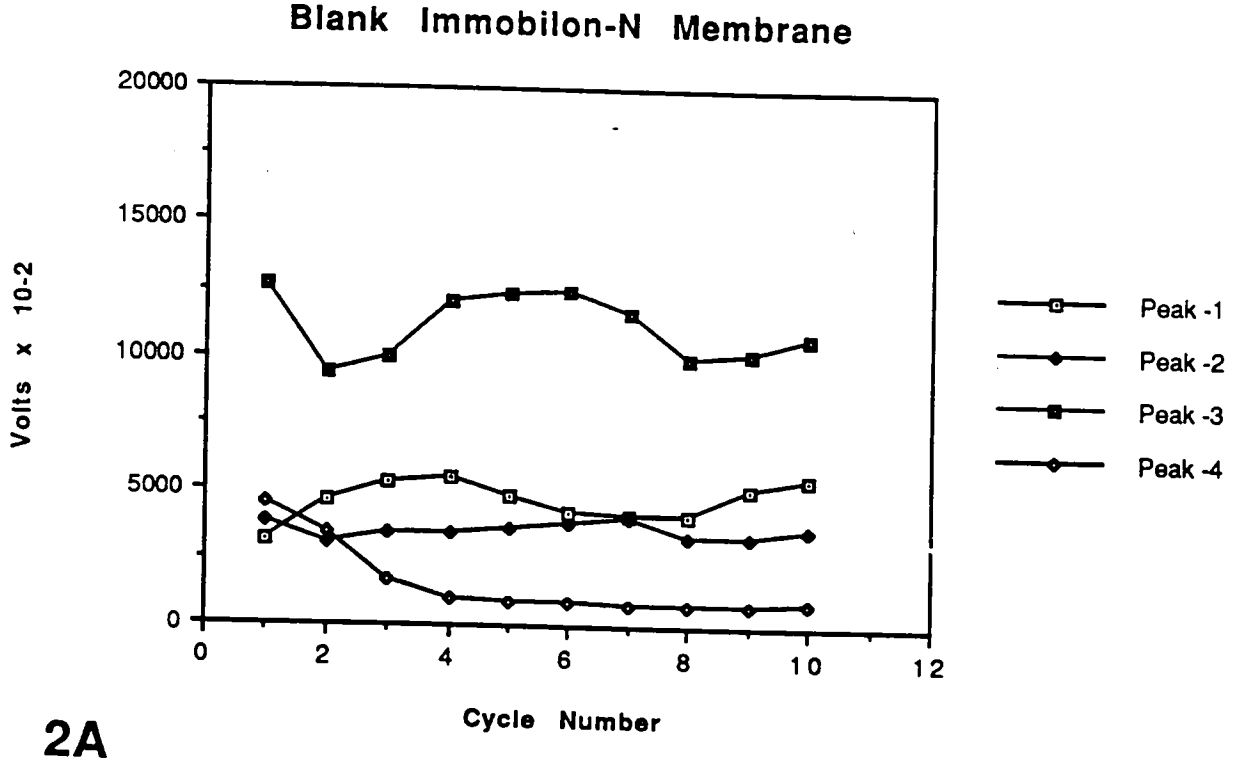
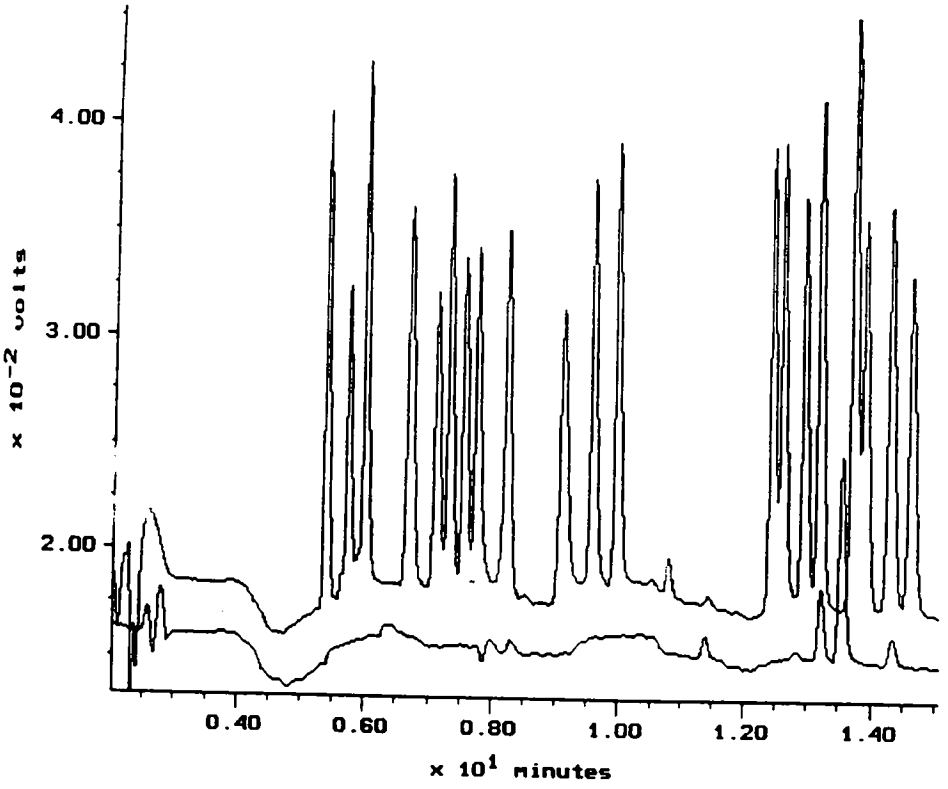
3.00
2.50
2.00
x 10-2 volts
1
8.10
2
12.91
3
13.23
4
13.96
0.40
0.60
0.80
1.00
1.20
1.40
x 10¹ minutes

1B



2A

Figure 2. Adsorptive mode blank cycles from Immobilon-N membrane. The amount of three interfering background peaks and DPTU are shown in Panel A. The values are compared in terms of peak height. Approximately 400 units is equivalent to 1 pmol of PTH amino acid. The chromatograms from the first and tenth cycles are shown in Panel B. The PTH standard is at the 50 pmol level and the first cycle below the standard is traced in yellow.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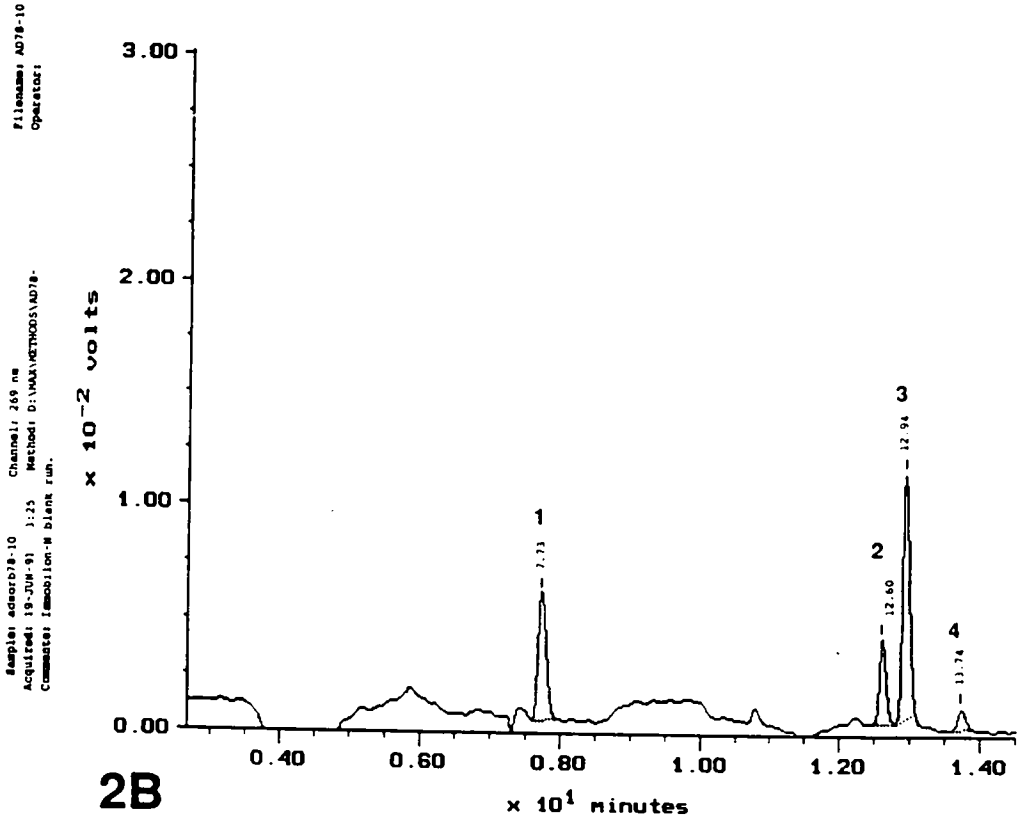
x 10⁻² volts
0.40
0.60
0.80
1.00
1.20
1.40
x 10¹ minutes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Channel: 269 nm
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Comments: Immobilon-N blank run.
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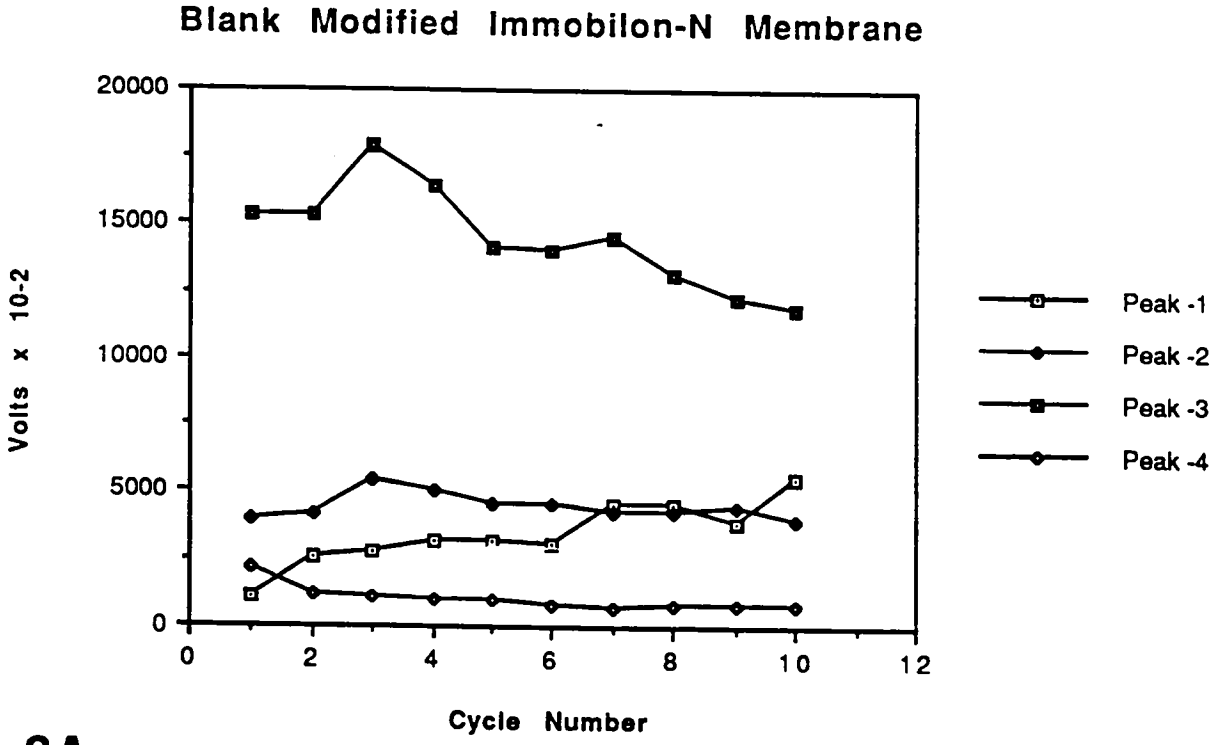
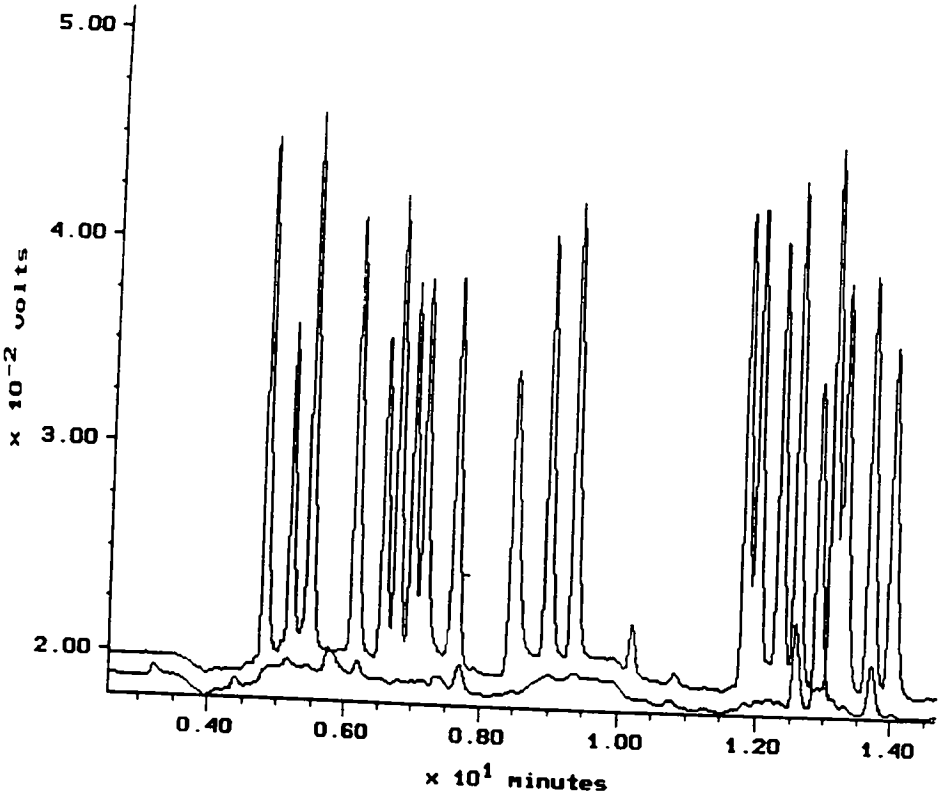
2.00
1.00
0.00
x 10⁻² volts
1
2
3
4
12.60
12.94
13.74
0.40
0.60
0.80
1.00
1.20
1.40
x 10¹ minutes

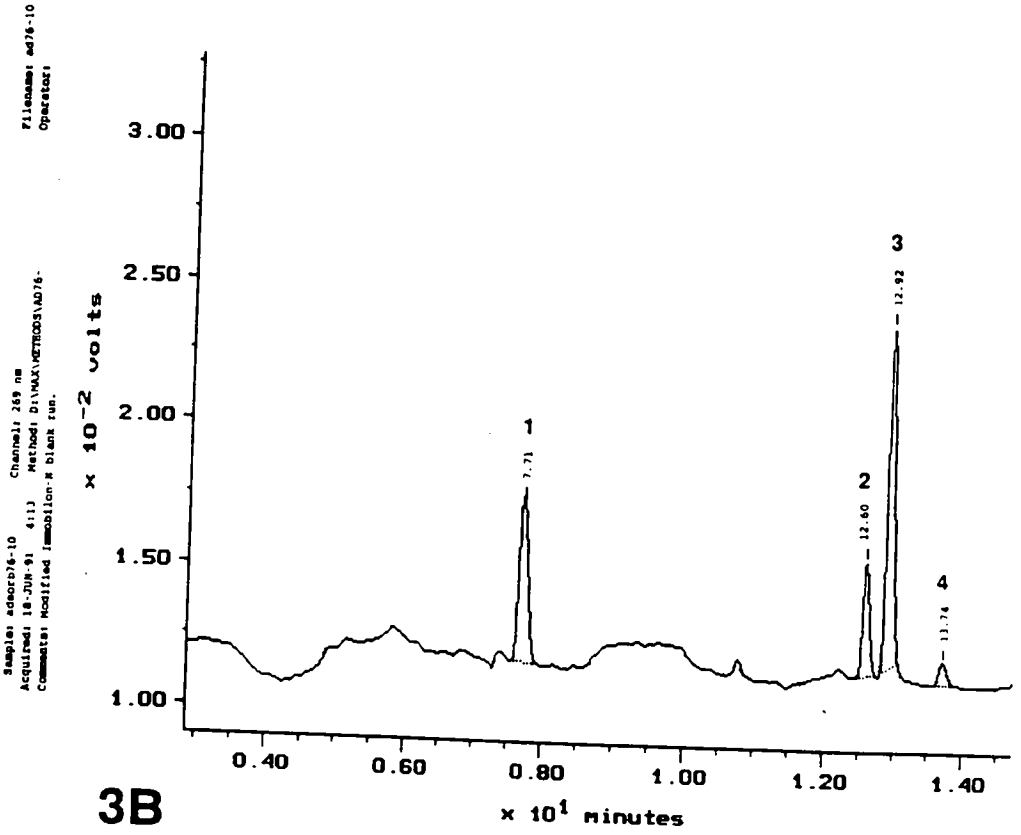
2B



3A

Figure 3. Adsorptive mode blank cycles from a modified Immobilon-N membrane. The amount of three interfering background peaks and DPTU are shown in Panel A. The values are compared in terms of peak height. Approximately 400 units is equivalent to 1 pmol of PTH amino acid (Panel A). The chromatograms from the first and tenth cycles are shown in Panel B. The PTH standard is at the 50 pmol level and the first cycle below the standard is traced in yellow.


5.00
4.00
3.00
2.00
x 10-2 volts
0.40
0.60
0.80
1.00
1.20
1.40
x 10¹ minutes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Sample: adsorb76-10
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Method: D:\MAX\METHODS\AD76-
Comment: Modified Immobilon-N blank run.
3.00
2.50
2.00
1.50
1.00
x 10-2 volts
1
7.71
2
12.60
3
12.92
4
13.74
0.40
0.60
0.80
1.00
1.20
1.40
x 10¹ minutes

3B

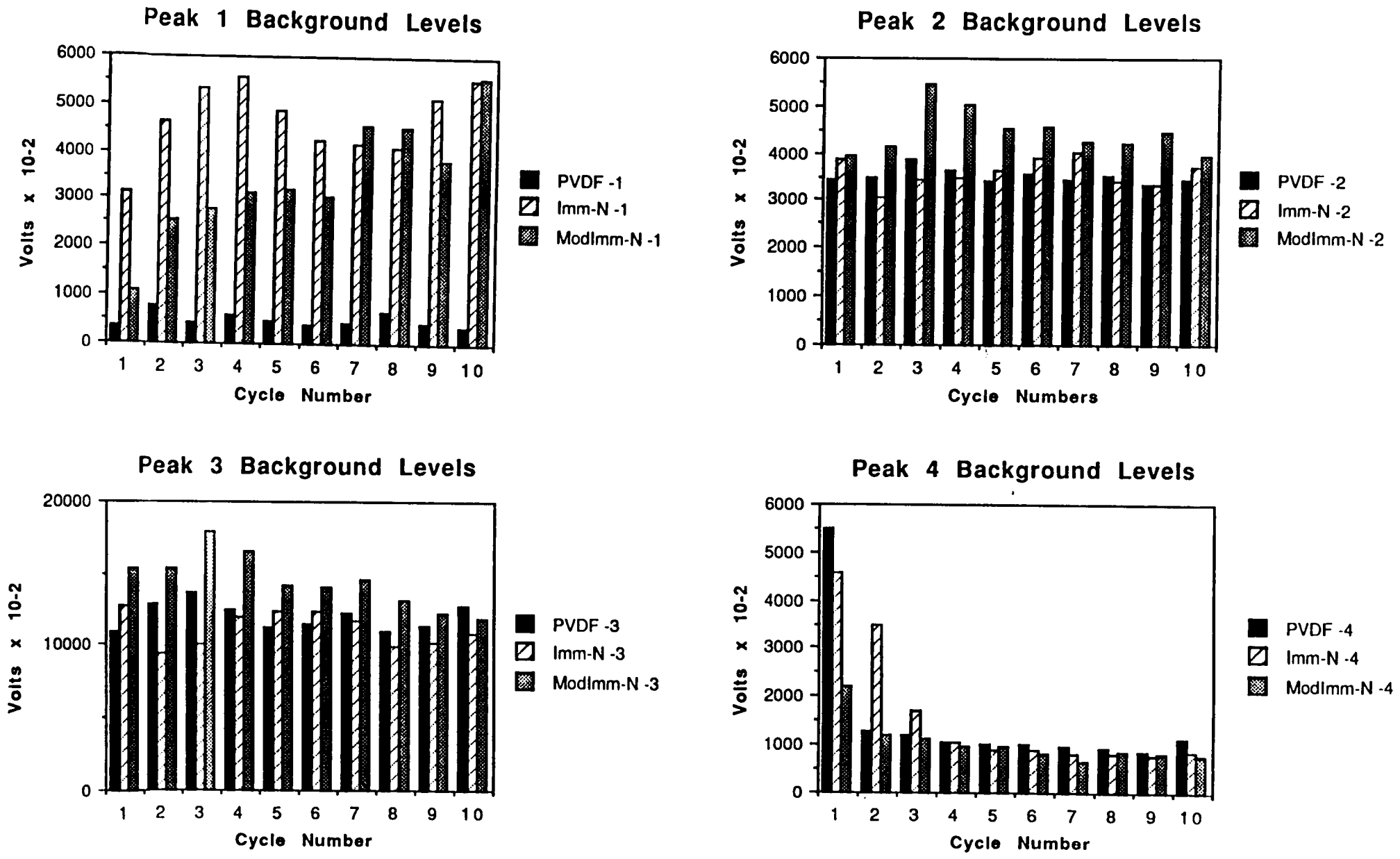
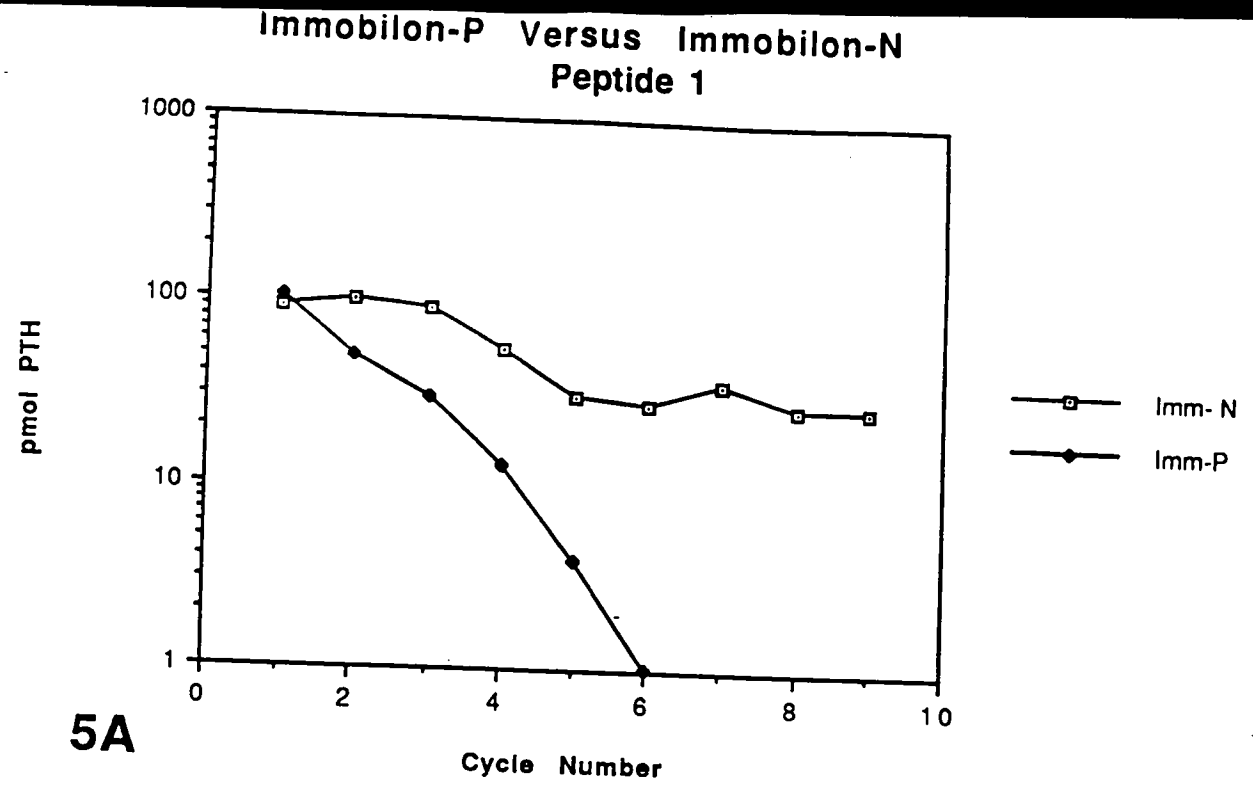
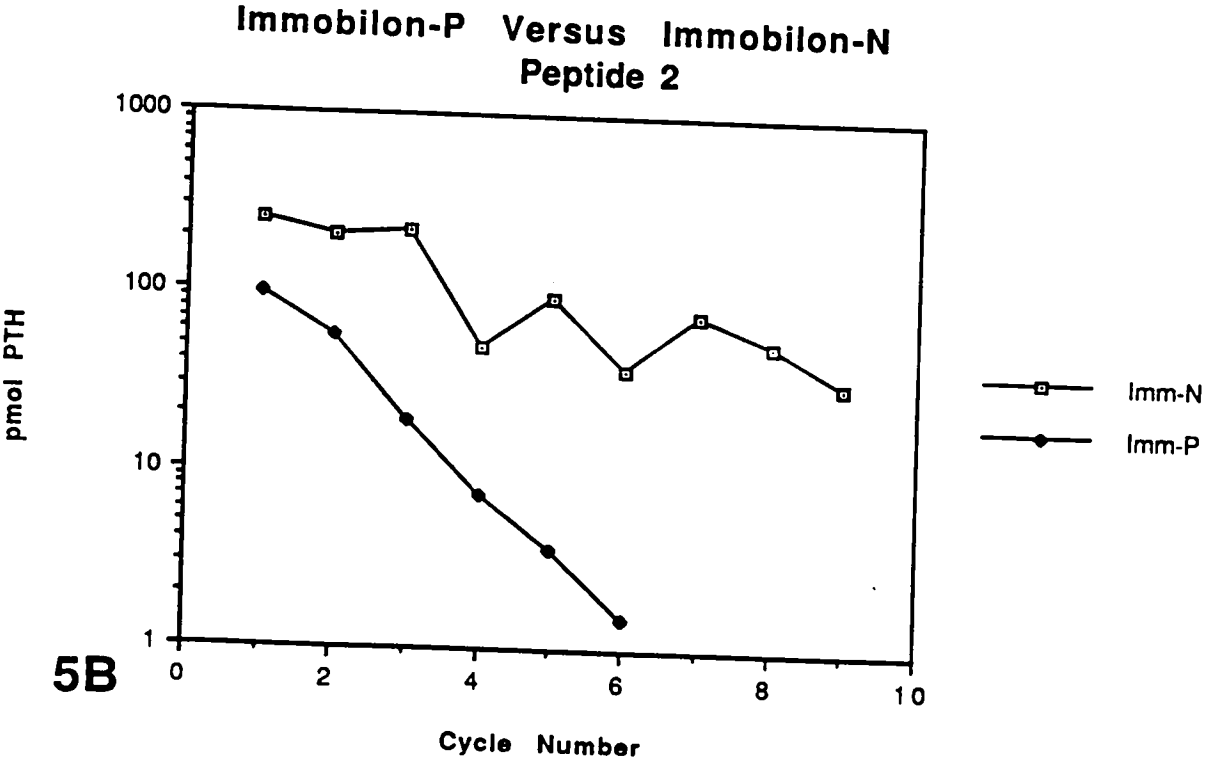


Figure 4. Summary of background peaks from the three types of membranes. Where peak 1, aniline (co-elutes with PTH-Gly); peak 2, co-elutes with PTH-Trp; peak 3, DPTU; and peak 4, co-elutes with PTH-Ile



(1) $^{+}NH_3$-Asn Ser Ala Gln Arg Thr Gly Asn Glu COO-



(2) $^{+}NH_3$-Tyr Met Lys Cys Val Pro Phe Tyr Glu COO-

Figure 5. Adsorptive mode sequence analyses of test peptides 1 (Panel A) and 2 (Panel B) from Immobilon-P and -N membranes.

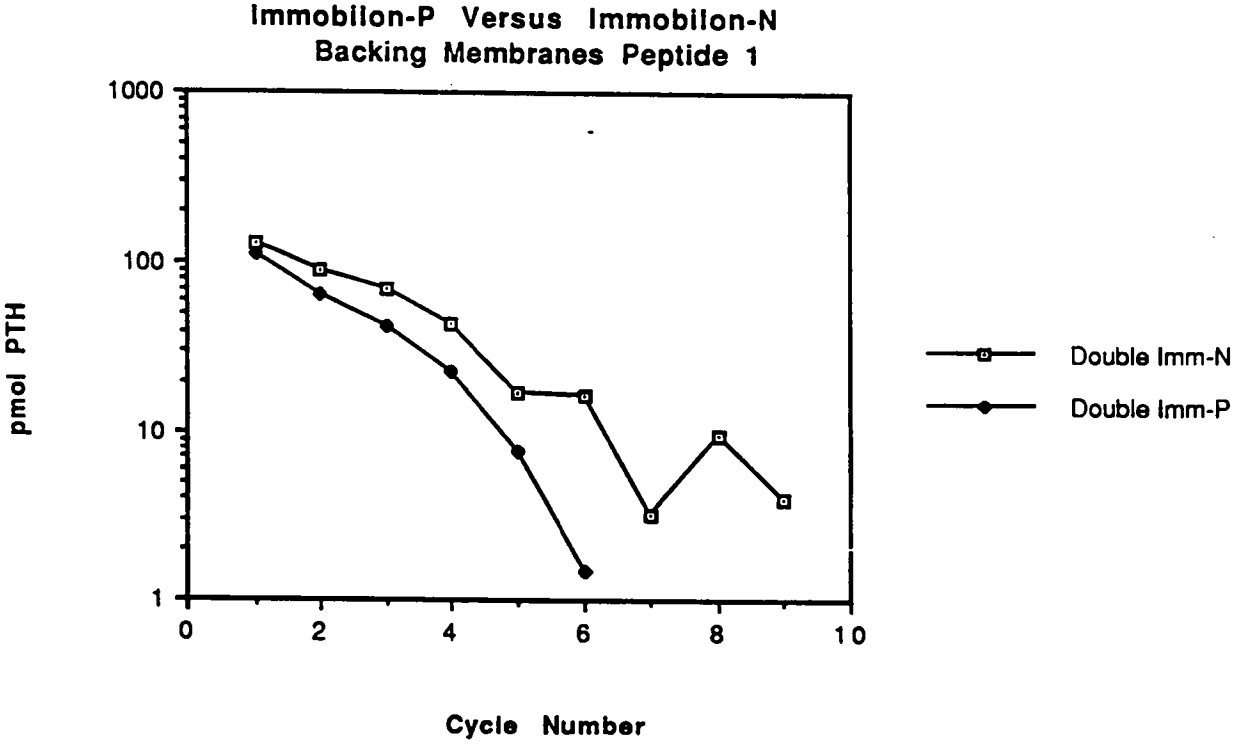
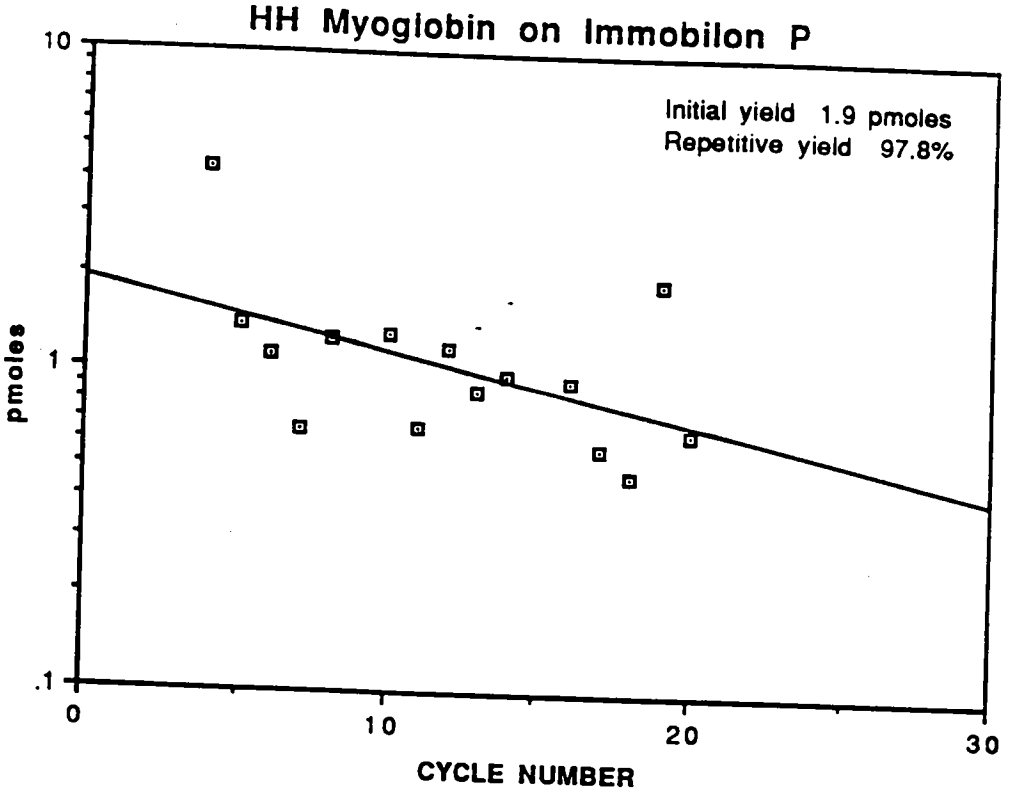


Figure 6. Adsorptive mode sequence analyses of test peptide 1 from Immobilon-P with backing membranes of Immobilon-P or -N.



HORSE HEART MYOGLOBIN MW 16,950 Daltons

1 10 20

G L S D G E W Q Q V L N V W G K V E A D

Figure 7. Horse heart myoglobin sequenced from Immobilon-P membrane.