

**POLYELECTROLYTE REACTIONS IN AQUEOUS
SOLUTIONS**

**M.Sc. Thesis by
Ülkem ZORLU, M.Sc.**

Department: Chemistry

Programme: Chemistry

Supervisor : Assoc. Prof. Dr. Tülay TULUN

JANUARY 2008

**POLYELECTROLYTE REACTIONS IN AQUEOUS
SOLUTIONS**

**M.Sc. Thesis by
Ülkem ZORLU, M.Sc.**

(50904120)

**Date of submission : 24 December 2007
Date of defence examination: 29 January 2008**

**Supervisor (Chairman): Assoc. Prof. Dr. Tülay TULUN
Members of the Examining Committee Prof.Dr. Süleyman AKMAN (I.T.U.)
Prof.Dr. Sıdıka SUNGUR (Y.T.U.)**

JANUARY 2008

**POLİELEKTROLİTLERİN SULU ÇÖZELTİDEKİ
REAKSİYONLARI**

**YÜKSEK LİSANS TEZİ
Ülkem ZORLU
(509041220)**

**Tezin Enstitüye Verildiği Tarih : 24 Aralık 2007
Tezin Savunulduğu Tarih : 29 Ocak 2008**

**Tez Danışmanı : Prof.Dr. Tülay TULUN
Diğer Jüri Üyeleri Prof.Dr. Süleyman AKMAN (İ.T.Ü.)
Prof.Dr. Sıdika SUNGUR (Y.T.Ü.)**

OCAK 2008

ACKNOWLEDGEMENT

It is acknowledged to Institute of Science and Technology of Istanbul Technical University for the financial support in this work which was carried out in the department of Chemistry, Faculty of Science and Letters.

I am very much grateful to Associate Prof.Tülay TULUN who brought this interesting subject to my attention and for her deep interest, fruitful help and constructive discussions through out my research.

I am appreciated to Nejla ÇİNİ for her valuable discussions in this work. I really appreciate my mother İclal, my father Nizamettin and my precious daughter İclal because of their understanding, tolerant and supportiveness. I dedicated my study to my parents and also my daughter.

January, 2008

Ülkem ZORLU

CONTENTS

ABBREVIATIONS	vi
TABLE LIST	vii
FIGURE LIST	viii
SUMMARY	xi
ÖZET.....	xiii
1. INTRODUCTION.....	1
1.1 Reviews of Literature.....	2
2. THEORETICAL PART	2
2.1. Polyelectrolytes	5
2.1.1. Polyelectrolyte Charge	5
2.1.2. Polyelectrolyte Conformation	6
2.1.3. The Conductance of Polyelectrolytes	7
2.1.4. Polyelectrolyte Applications	7
2.1.5. Polyphosphates as Polyelectrolytes.....	8
2.2. Amino Acids.....	9
2.2.1. Amino Acid Reactions	13
3. EXPERIMENTAL PART	17
3.1. Chemicals.....	17
3.2. Methods.....	17
3.3. Solutions.....	17
3.4. Equipments.....	20
3.5. Experiments.....	20
3.5.1. Molecular Weight Determination of Polyions	20
3.5.1.1. Molecular Weight Determination of Polysodium Phosphate by End Group Titration.....	20
3.5.1.2. Molecular Weight Determination of Polysodiumphosphate by Viscosimetry	22
3.5.1.3. Molecular Weight Determination of Poliallylaminehydrochloride by Viscosimetry.....	24
3.5.2. Determination of Equivalent Weight of Polyions	25
3.5.2.1 Determination of Equivalent Weight of Polysodiumphosphate	25
3.5.2.2 Determination of Equivalent Weight of Poliallylaminehydrochloride	25
3.5.3. Stoichiometric Determinations.....	25
3.5.3.1. Conductometric Titration Method.....	26

3.5.3.2. Mol Ratio by Conductometry.....	30
3.5.4. Determination of Complex Stability Constants	31
3.5.5. IR Spectrum of the Complexes	35
4. RESULTS AND DISCUSSION	35
REFERENCES.....	38
APPENDIX	40
CURRICULUM VITAE.....	73

ABBREVIATIONS

PA	: Polyanion
PC	: Polycation
PAACl	: Polyallylamine hydrochloride
PAAOH	: Polyallylamine hydroxide
PSP	: Polysodium phosphate
NaPABA	: Paraaminobenzoic acid sodium salt
PABACl	: Paraaminobenzoic acid hydrochloride
PABSA	: Paraaminobenzene sulfonic acid
NaPABSA	: Paraaminobenzene sulfonic acid sodium salt

TABLE LIST

	<u>Page Number</u>
Table 3.1 The Results of End Group Titration	22
Table 3.2 Viscosity Values for Polisodiumphosphate ($K=69 \times 10^{-5}$ dl/g, $a=0,61$).....	23
Table 3.3 Viscosity Values for Poliallilyaminehydrochloride ($K=7,19 \times 10^{-5}$ dl/g, $a=0,794$).....	24
Table 3.4 Results of Conductometric Titration Polyanion as Titrant.....	27
Table 3.5 Results of Reverse Titration	29
Table 3.6 Results of Conductometric Titration Polycation as Titrant.....	29
Table 3.7 Mol Ratio of Amino Acids to Polyanion and Polycation.....	31
Table 3.8 Stability Constant of Amino Acid and Polyanion	33
Table 3.9 Stability Constant of Amino Acid and Polycation	34

FIGURE LIST

	<u>Page Number</u>
Figure 2.1 : Common Structure of Amino Acids.....	10
Figure 2.2 : The Studied Amino Acids	11
Figure 2.3 : The Hydrolysis of Amino Acids	14
Figure 2.4 : Titration Curve of Alanine	15
Figure 3.1 : Titration curve of $(\text{NaPO}_3)_n$ Before Hydrolysis	21
Figure 3.2 : Titration curve of $(\text{NaPO}_3)_n$ After Hydrolysis.....	21
Figure 3.3 : Viscosity Curve for Polysodiumphosphate	23
Figure 3.4 : Viscosity Curve for PAA-HCl.....	24
Figure A.1 : 10^{-4}M Glysine with 10^{-4}M $(\text{NaPO}_3)_n$ in $(I=10^{-3})$	40
Figure A.2 : 10^{-2}M GlysineCl with 10^{-2}M $(\text{NaPO}_3)_n$ in salt free solution.....	40
Figure A.3 : 10^{-2}M Glutamic Acid with 10^{-2}M $(\text{NaPO}_3)_n$ in salt free solution.....	41
Figure A.4 : 10^{-2}M Glutamic AcidNa with 10^{-2}M $(\text{NaPO}_3)_n$ in salt free solution.	41
Figure A.5 : 10^{-2}M Glutamic AcidCl with 10^{-2}M $(\text{NaPO}_3)_n$ in salt free solution..	41
Figure A.6 : 10^{-2}M Lysine with 10^{-2}M $(\text{HPO}_3)_n$ in salt free solution	42
Figure A.7 : 10^{-2}M LysineNa with 10^{-2}M $(\text{HPO}_3)_n$ in salt free solution	42
Figure A.8 : 10^{-2}M LysineCl with 10^{-2}M $(\text{NaPO}_3)_n$ in salt free solution.....	42
Figure A.9 : 10^{-2}M PhenylalanineNa with 10^{-2}M $(\text{HPO}_3)_n$ in salt free solution....	43
Figure A.10 : 10^{-4}M PABA with 10^{-4}M $(\text{NaPO}_3)_n$ in salt free solution.....	43
Figure A.11 : 10^{-2}M NaPABA with 10^{-2}M $(\text{HPO}_3)_n$ in salt free solution.....	43
Figure A.12 : 10^{-2}M PABSA with 10^{-2}M $(\text{NaPO}_3)_n$ in salt free solution	44
Figure B.1 : 10^{-2}M GlycineNa with 10^{-2}M PAACl in salt free solution.....	45
Figure B.2 : 10^{-3}M GlycineNa with 10^{-3}M PAACl in $I=10^{-2}$	45
Figure B.3 : 10^{-2}M Glutamic Acid with 10^{-3}M PAA-OH in salt free solution.....	45
Figure B.4 : 10^{-3}M Glutamic AcidNa with 10^{-3}M PAACl in $I=10^{-2}$	46
Figure B.5 : 10^{-3}M Lysine with 10^{-3}M PAACl in $I=10^{-2}$	46
Figure B.6 : 10^{-3}M LysineNa with 10^{-3}M PAACl in $I=10^{-2}$	46
Figure B.7 : 10^{-2}M LysineCl with 10^{-2}M PAA-OH in salt free solution.....	47
Figure B.8 : 10^{-4}M PhenylalanineNa with 10^{-2}M PAACl in $I=10^{-3}$	47
Figure B.9 : 10^{-2}M PhenylalanineNa with 10^{-2}M PAACl in salt free solution.....	47
Figure B.10 : 10^{-2}M PhenylalanineCl with 10^{-2}M PAA-OH in salt free solution	48
Figure B.11 : 10^{-3}M PABACl with 10^{-3}M PAA-OH in $I=10^{-2}$	48
Figure B.12 : 10^{-2}M PABSA with 10^{-2}M PAA-OH in salt free solution.....	48
Figure C.1 : 1.10^{-2}M GlycineNa and 1.10^{-2}M $(\text{HPO}_3)_n$ mixture in salt free solution	49
Figure C.2 : 1.10^{-2}M Glutamic Acid and 1.10^{-2}M $(\text{NaPO}_3)_n$ mixture in salt free solution	49
Figure C.3 : 1.10^{-2}M LysineNa and 1.10^{-2}M $(\text{HPO}_3)_n$ mixture in salt free solution	49
Figure C.4 : 1.10^{-2}M PhenylalanineNa and 1.10^{-2}M $(\text{HPO}_3)_n$ mixture in salt free solution	50
Figure C.5 : 1.10^{-2}M NaPABA and 1.10^{-2}M $(\text{HPO}_3)_n$ mixture in salt free solution	50

Figure C.6 :	1.10 ⁻² M NaPABSA and 1.10 ⁻² M (HPO ₃) _n mixture in salt free solution	50
Figure C.7 :	1.10 ⁻² M Glycine and 1.10 ⁻² Na-PAACl mixture in salt free solution.	51
Figure C.8 :	1.10 ⁻² M Glutamic Acid and 1.10 ⁻² M PAAOH mixture in salt free solution	51
Figure C.9 :	1.10 ⁻² M LysineNa and 1.10 ⁻² M PAACl mixture in salt free solution	51
Figure C.10:	1.10 ⁻² M Phen.AlanineCl and 1.10 ⁻² M PAAOH mixture in salt free solution	52
Figure C.11:	1.10 ⁻² M PABACl and 1.10 ⁻² M PAAOH mixture in salt free solution	52
Figure C.12:	1.10 ⁻² M PABSACl and 1.10 ⁻² M PAAOH mixture in salt free solution	52
Figure D.1 :	pH Curve of GlycineNa – (NaPO ₃) _n Complex.....	53
Figure D.2 :	Degree of Linkage for GlycineNa – (NaPO ₃) _n Complex	53
Figure D.3 :	Stability Constant of GlycineNa – (NaPO ₃) _n Complex.....	53
Figure D.4 :	pH Curve of Glutamic Acid – (NaPO ₃) _n Complex	54
Figure D.5 :	Degree of Linkage for Glutamic Acid – (NaPO ₃) _n Complex.....	54
Figure D.6 :	Stability Constant of Glutamic Acid – (NaPO ₃) _n Complex	54
Figure D.7 :	pH Curve of LysineNa – (NaPO ₃) _n Complex	55
Figure D.8 :	Degree of Linkage for LysineNa – (NaPO ₃) _n Complex.....	55
Figure D.9 :	Stability Constant of LysineNa – (NaPO ₃) _n Complex	55
Figure D.10:	pH Curve of PhenylalanineNa – (NaPO ₃) _n Complex.....	56
Figure D.11:	Degree of Linkage of PhenylalanineNa – (NaPO ₃) _n Complex	56
Figure D.12:	Stability Constant of PhenylalanineNa – (NaPO ₃) _n Complex.....	56
Figure D.13:	pH Curve of NaPABA – (NaPO ₃) _n Complex.....	57
Figure D.14:	Degree of Linkage for NaPABA – (NaPO ₃) _n Complex	57
Figure D.15:	Stability Constant of NaPABA – (NaPO ₃) _n Complex.....	57
Figure D.16:	pH Curve of PABSA – (NaPO ₃) _n Complex	58
Figure D.17:	Degree of Linkage for PABSA – (NaPO ₃) _n Complex	58
Figure D.18:	Stability Constant of PABSA – (NaPO ₃) _n Complex	58
Figure D.19:	pH Curve of NaPABSA – (NaPO ₃) _n Complex	59
Figure D.20:	Degree of Linkage of NaPABSA – (NaPO ₃) _n Complex	59
Figure D.21:	Stability Constant of NaPABSA – (NaPO ₃) _n Complex	59
Figure D.22:	pH Curve of GlycineNa – PAACl Complex	60
Figure D.23:	Degree of Linkage for GlycineNa – PAACl Complex.....	60
Figure D.24:	Stability Constant of GlycineNa – PAACl Complex	60
Figure D.25:	pH Curve of Glutamic Acid - PAA-OH Complex	61
Figure D.26:	Degree of Linkage for Glutamic Acid - PAA-OH Complex.....	61
Figure D.27:	Stability Constant of Glutamic Acid - PAA-OH Complex	61
Figure D.28:	pH Curve of Lysine – PAACl Complex.....	62
Figure D.29:	Degree of Linkage for Lysine – PAACl Complex	62
Figure D.30:	Stability Constant of Lysine – PAACl Complex.....	62
Figure D.31:	pH Curve of Phenylalanine – PAACl Complex	63
Figure D.32:	Degree of Linkage for Phenylalanine – PAACl Complex	63
Figure D.33:	Stability Constant of Phenylalanine – PAACl Complex.....	63
Figure D.34:	pH Curve of NaPABA – PAACl Complex	64
Figure D.35:	Degree of Linkage for NaPABA – PAACl Complex.....	64
Figure D.36:	Stability Formation Constant of NaPABA – PAACl Complex.....	64
Figure D.37:	pH Curve of PABSACl – PAA-OH Complex.....	65
Figure D.38:	Degree of Linkage for PABSACl – PAA-OH Complex	65
Figure D.39:	Stability Constant of PABSACl – PAA-OH Complex.....	65

Figure E.1 :	10^{-2} M Glutamic Acid- 10^{-2} M (NaPO ₃) _n	66
Figure E.2 :	7.2 (NaPO ₃) _n	66
Figure E.3 :	10^{-2} M Lysine - 10^{-2} M (HPO ₃) _n	66
Figure E.4 :	Lysine	67
Figure E.5 :	10^{-4} M PABA - 10^{-4} M (NaPO ₃) _n	67
Figure E.6 :	PABA	67
Figure E.7 :	10^{-2} M PABSA - 10^{-2} M (NaPO ₃) _n	68
Figure E.8 :	PABSA	68
Figure E.9 :	10^{-2} M (HPO ₃) _n	69
Figure E.10:	10^{-2} M GlycineNa- 10^{-2} M PAACl	69
Figure E.11:	Glycine	69
Figure E.12:	10^{-2} M Glutamic Acid - 10^{-2} M PAAOH.....	69
Figure E.13:	Glutamic Acid	70
Figure E.14:	10^{-3} M Lysine - 10^{-3} M PAACl(I= 10^{-2}).....	70
Figure E.15:	Lysine	70
Figure E.16:	10^{-4} M Ph.Al.Na- PAACl(I= 10^{-3}).....	71
Figure E.17:	10^{-3} M NaPABA- 10^{-3} M PAACl (I= 10^{-2}).....	71
Figure E.18:	NaPABA	71
Figure E.19:	10^{-2} M PABSACl- 10^{-2} M PAAOH.....	71
Figure E.20:	PABSACl	72
Figure E.21:	10^{-3} M PAACl(I= 10^2)	72

POLYELECTROLYTE REACTIONS IN AQUEOUS SOLUTIONS

SUMMARY

Polyelectrolytes are polymers whose repeating units bear an electrolyte group. These groups will dissociate in aqueous solutions, making the polymers charged. Charged molecular chains, commonly present in soft matter systems, play a fundamental role in determining structure, stability and the interactions of various molecular assemblies.

The unique properties of polyelectrolytes are being exploited in a wide range of technological and industrial fields. One of their major roles, are in the field of biology and biochemistry. Many biological molecules are polyelectrolytes. For instance, polypeptides (thus all proteins) and DNA are polyelectrolytes. Both natural and synthetic polyelectrolytes are used in a variety of industries.

Polyelectrolyte complexes which are formed by interaction of polyelectrolytes with polyaminoacids causes distinctive properties of proteins so that enzymes bound to biological polymer can possess stability. These enzymes are used in enzyme industry in recent years and improvement of strong vaccine systems. Polyelectrolyte complexes formed between polyamino acids and polyelectrolyte are of increasing importance in the field of gene delivery since they offer safe use and versatile application.

In the present study, studies on the interaction of synthetic polyelectrolytes which are poly(allylaminehydrochloride) (polycation) and poly(sodiumphosphate) (polyanion) with a group of aliphatic and aromatic amino acids (glycine, glutamic acid, lysine and phenylalanine, 4-aminobenzoic acid, 4-aminobenzenesulfanilic acid) were carried out. The methods chosen for this study were conductometry mainly. Besides, some additional methods such as wet chemistry, viscometry and potentiometry were used.

In the experimental part reaction stoichiometry of polycation and polyanion with different amino acids were determined depending on the polyions concentration, ionic strength, added salt concentration and the order of titrant addition. In the experimental part of study; molecular and equivalent weight determination of polyions, and the stoichiometry of interaction between polyions and amino acids were carried out, and the results are given in Appendix A(Figure A.1 - A.12), Appendix B(Figure B.1 – B.12), Appendix C(Figure C.1 – C.12) and Tables 3.4, 3.5, 3.6. Besides, stability constants for the interaction of polyions and amino acids were determined (Appendix D (Figure D.1 – D.39) and Tables 3.8, 3.9.) and IR spectrum of the complexes were given in Appendix E (Figure E.1 – E.21).

It was concluded that the stoichiometry ratio of polycation to amino acids and polyanion to aminoacids, in general, were 1:1 in salt free and salt solution. Results were obtained only in a narrow concentration range, and studies in the constant ionic

strength did not give the expected results. Besides the order of titrant both polycation and polyanion, addition gave 1:1 ratio with a reasonable deviation from the unity. The complex stability constants determined at different temperatures were in the reasonable magnitude and altered between particular temperature range possessing relatively high stability values. IR studies confirmed the binding between polyions and amino acids.

POLİELEKTROLİTLERİN SULU ÇÖZELTİDEKİ REASİYONLARI

ÖZET

Polimer zincirinde iyonik gruplar içeren ve elektrolit özellikleri taşıyan makromoleküllere “polielektrolit” denir. Polielektrolitler polar çözücülerde çözünürler. Orta derecede zayıf asidik ve bazik özelliklere sahip poliiyonların seyreltik sulu çözeltideki reaksiyonlarının incelenmesi doğal ve sentetik polimerlerin yapısal karakteristiklerinin öğrenilmesinden başka, biyolojik sistemlerdeki makromoleküller ile proteinlerin reaksiyon mekanizmalarının anlaşılmasına olanak verecektir.

Bu çalışmada, canlı organizmalardaki sulu elektrolit dengeleri dikkate alınarak suda çözünen polielektrolitler seçilmiştir. Polianyon olarak seçilen poli(sodyumfosfat)’ın canlılardaki biyolojik fonksiyonlarının önemi dikkat çeken önemli bir faktördür. Polielektrolitlerin birbirleriyle ve düşük molekül ağırlıklı molekül ve iyonik bileşiklerle verdikleri reaksiyonlar, genel olarak, başlıca iletkenlik, viskometri, turbidimetri, potansiyometri ve kinetik sedimentasyon yöntemleri ile incelenmektedir. Ancak, polielektrolit reaksiyonları ile ilgili kuramlar henüz tam olarak geliştirilememiştir. Çalışmada, poli(allilaminhidroklorür) (polikasyon) ve poli(sodyumfosfat) (polianyon)’un alifatik(glisin, glutamik asit, lizin) ve aromatic(4-aminobenzoik asit, 4-aminobenzenesülfonik asit, fenilalanin)’in amino asitlerle verdikleri reaksiyonlar, poliiyon konsantrasyonları, iyonik şiddet, ilave tuz konsantrasyonu, titrant ilave sırası parametreleri dikkate alınarak incelenmiştir. Sonuç olarak, bu çalışmayla canlı organizmadaki doğal poliiyonların mikro iyon ve nötral moleküllemlerle etkileşim mekanizmalarının yapısal özelliklerinin anlaşılması için bir model oluşturmakve biyotıpta yeni materyallerin uygulanmasına olanak veren bilgilerin kazanılması amaçlanmıştır.

Bu çalışmada; başlıca, kondüktometrik, viskometrik ve potansiyometrik yöntemler kullanılmıştır. Yapılan tayinler aşağıdaki bölümlerde kısaca özetlenmiştir.

Poli(sodyumfosfat)’ın molekül ağırlığı viskometrik yöntem kullanılarak tayin edilmiştir. Ayrıca polisodyumfosfatın molekül ağırlığı son grup titrasyon yöntemi ile de belirlenmiştir. (Tablo 3.1, Tablo 3.2)

Poli(alilaminhidroklorür)’ün molekül ağırlığı viskometrik yöntem kullanılarak tayin edilmiştir. (Tablo 3.3)

Poli(sodyumfosfat)’ın eşdeğer ağırlığı, $(\text{NaPO}_3)_n$ ’ın iyon değıştirici Amberlite IR-120 (kuvvetli asit) kolonu ile tamamen $(\text{HPO}_3)_n$ ’e dönüştürölmesinden sonra alkalimetri ile tayin edilmiştir.

Poli(alilaminhidroklorür)'ün eşdeğer ağırlığı arjentimetrik olarak 0,05 M AgNO_3 çözeltisi ile titre edilerek olarak bulundu.

Farklı konsantrasyonlarda poli(sodyumfosfat) – amino asit ve $(\text{HPO}_3)_n$ – aminoasit çözeltilerinin tuzlu ortamda ve tuzsuz ortamda ve iyonik şiddeti 0,1;0,01 ve 0,001'e ayarlandıktan sonra sulu çözeltide poli(sodyumfosfat) ya da $(\text{HPO}_3)_n$ titrant olarak kullanılarak konduktometrik titrasyonu yapılmış, amino asit-polianyon kompleks stokiyometrisi belirlenmeye çalışılmıştır. (Tablo 3.4, Ek A (Şekil A.1 - A.12))

Ayrıca bu titrasyonlar sonucunda belirlenen optimum koşullarda amino asit-polianyonların ters titrasyonları da yapılmıştır. (Tablo 3.5)

Farklı konsantrasyonlarda poli(alilaminhidroklorür) – amino asit ve poli(alilaminhidroksit) – aminoasit çözeltilerinin tuzlu, tuzsuz ortam ve farklı iyonik şiddetlerde poli(alilaminhidroklorür) ya da poli(alilaminhidroksit) titrant olarak kullanılarak sulu çözeltideki konduktometrik titrasyonları yapılmış, amino asit-polikasyon kompleks stokiyometrisi belirlenmeye çalışılmıştır. (Tablo 3.6, Ek B (Şekil B.1 – B.12))

Farklı mol oranlarında karıştırılan amino asit- polianyon ve aminoasit-polikasyon çözeltilerinin farklı mol oranındaki karışımlarının tuzsuz ortamda direkt iletkenlik ölçümleri yapılarak amino asit- polianyon ve aminoasit-polikasyon kompleks stokiyometrisi belirlenmeye çalışılmıştır. (Tablo 3.7, Ek C (Şekil C.1 – C.12))

Amino asit-poli(sodyumfosfat)(polianyon), amino asit-poli(alilaminhidroklorür)(polikasyon) ve amino asit- poli(alilaminhidroksit)(polikasyon) çözeltilerinin farklı sıcaklıklardaki pH ölçümleri yapılarak, bağlanma derecesi θ tayin edilmiş ve komplekslerin stabilite sabitleri hesaplanmıştır. (Tablo 3.8, 3.9, Ek D (Şekil D.1-D.39)).

Amino asit ve amino asit-polielektrolit komplekslerinin spektrumları alınarak, bağlanmanın gerçekleştiği saptanmıştır. (Ek E (Şekil E.1 – E.21))

Tuzlu ve tuzsuz ortamda polikasyon ve polianyonun aminoasitle stokiyometri oranı genel olarak 1:1 bulunmuştur. Deneyler, yalnızca dar bir konsantrasyon aralığında sonuç vermiştir ve sabit iyonik şiddette yapılan çalışmalara umulan sonuçlara ulaşamamıştır. Bununla birlikte, sırasıyla polikasyon ve polianyonun titrant olarak kullanıldığı ters titrasyonların 1:1 stokiyometrik oranı verdiği sonucuna ulaşılmıştır. Farklı sıcaklık değerlerinde kompleks stabilite katsayısının belirlendiği deneylerde sonuçlar, makul değerlerde değişmekte ve sıcaklık aralıklarında göreceli olarak oldukça yüksek stabilite değerleri vermiştir.

1. INTRODUCTION

The interaction of synthetic polyelectrolytes with polyaminoacids in aqueous solution according to different parameters will provide a model for understanding of structural properties and reactions of natural polyions in living organisms.

Polyelectrolyte complexes which are formed by interaction of polyelectrolytes with polyaminoacids causes distinctive properties of proteins so that enzymes bound to biological polymer can possess stability. These enzymes are used in enzyme industry in recent years and improvement of strong vaccine systems. Polyelectrolyte complexes formed between polyaminoacids and polyelectrolyte are of increasing importance in the field of gene delivery since they offer safe use and versatile application.

In the last two decades intensive studies on polyelectrolyte complexes and on the interaction between polyelectrolytes, low molecular salts and polyaminoacids has been carried out and numerous results have been given to explain the main specific feature of cooperative interactions, particularly, in solid state. However studies on the aqueous solution involving polyelectrolytes and various amino acids are less compared to the interaction of polyaminoacids and polyelectrolytes.

Amino acids are the main elements of proteins. The present study attempts to explain general features of chemical and structural interaction between a group of aliphatic and aromatic amino acids and particular polyelectrolytes such as poly(sodiumphosphate) and poly(allylaminehydrochloride).

The results expected from this studies enabling to be the models for the unexplained phenomena occurring in biological systems, and to provide fundamental knowledge on the applications of biotechnology and medicine (drug delivery systems, protein purification, artificial immunogens, vaccines enzyme immobilization, biocompatible materials, etc...) and the environmental control technology (water and industrial waste treatment, stabilization and flocculation colloid dispersion, etc...).

It is hoped that complexation between the chosen polyelectrolytes and amino acids might provide a simple model system for the complicated biopolymer –neutral molecules and ionic salts reactions.

1.1 Reviews of Literature

Literature studies revealed that studies on the synthetic polyelectrolytes and amino acids interactions less than the exhaustive researches on the interaction between polyelectrolytes, proteins and surfactants. It may be drawn attention in this work that studies on the interaction between particular polyelectrolytes and a group of aliphatic and aromatic amino acids were carried out. The main original part of this study is the chosen anionic polyelectrolyte, poly(sodiumphosphate) which has received little attention though, it is rather interesting due to its being one of the few anionic polyelectrolyte of integral type [1,2,3].

In a part of M.Sc.Thesis [4] entitled “Studies on Polyelectrolyte Properties of Polysphosphate” prepared by Hülya Gün on the interaction between two aromatic amino acids and poly(sodiumphosphate) as polyelectrolyte. Results show that final complex was 1:1 stoichiometry with respect to the ratio of poly(phosphate) to amino acid.

In the study given by Tülay Tulun “Investigation of interaction between poly(sodium phosphate) and p-aminobenzoic acid hydrochloride”, the interaction between poly(sodium phosphate) and p-aminobenzoic acid, hydrochloride was investigated by conductometry, viscometry, spectrometry and gravimetry. It was found that the complex stoichiometry deviates from unity salt and salt free aqueous solution [5].

In the Carole L. Cramer and Rowland H. Davis’ paper, the vacuoles of *Neuropora crassa*, grown in minimal medium, contain a 1:1 ratio of basic amino acids and phosphate, the latter in the form of long-chain, inorganic polyphosphate-P. Vacuoles isolated from cells depleted of polyphosphate retain basic amino acids despite the absence of over 90% of their polyphosphate [6].

In another study “Interaction of Aromatic Amino Acids with Neutral Polyadenylic Acid”, the aromatic amino acids tryptophan, phenylalanine, and histidine interact with single-stranded polyadenylic acid [poly(A)] as observed by proton magnetic resonance spectroscopy. The relative magnitude of this interaction is tryptophan phenylalanine histidine [7].

In the paper of “The enthalpies of interaction of polyvinyl benzyltrimethylammonium hydroxide with amino acids in aqueous solutions”, the enthalpies of interaction of polyvinylbenzyltrimethylammonium (PVTa) hydroxide with glycine, glutamic acid, and tyrosine anions were determined calorimetrically. The interaction of PVTa with amino acid anions was shown to be an exothermic process. The enthalpy of interaction depended on the nature and concentration of the amino acid. The reaction became less exothermic and the time of the establishment of equilibrium increased as the concentration of amino acids grew [8].

Studies of Yoshiko Moriyama, Kunio Takeda have clarified that a strong binding of surfactant with a polyelectrolyte is promoted by the electrostatic force between the charged groups and the hydrophobic interaction between bound surfactants. On the other hand, various studies have been made on conformational changes of homopolypeptides such as a poly-L-lysine (PLL) upon their interactions with ionic surfactants [9].

In another paper, the interaction of amino acid residues with polyribonucleotides was characterized by measurements of melting temperatures (t_m) for poly(A) poly(U) and poly(I)poly(C) as functions of the concentrations of various amino acid amides. [10]

In the study of “Charge Determination of Proteins with Polyelectrolyte Titration”, photometric version of polyelectrolyte titration was applied for the determination of the number of charged residues on globular proteins. Based on the observation that oppositely charged polyelectrolytes form, in general, stoichiometric polyelectrolyte complexes, the protein solutions were incubated in excess with an oppositely charged polyelectrolyte, and the residual amount was back-titrated using otoluidine blue for end point detection. It is concluded that polyelectrolyte titration offers an easy access to the determination of the surface charge of proteins and other biopolymers. The data further support the notion of the importance of electrostatic cooperative interactions in biological systems [11].

In the paper of B. David and R. Briggs, some observations on metaphosphoric acid and its protein combination complex which are of interest in relation to this apparently stoichiometric reaction of metaphosphoric acid with proteins are recorded [12].

In another study, interaction between the conjugated polyelectrolyte poly{1,4-phenylene[9,9-bis(4-phenoxybutylsulfonate)]fluorene-2,7-diyl}copolymer and the lecithin mimic 1-O-(L-Arginyl)-2,3-O-dilauroyl-sn-glycerol has been studied in aqueous solution by electronic spectroscopy and small-angle neutron scattering. [13]

Paul Ander and Mahmoud Kardan have been determined the sodium ion interaction with polyelectrolytes of varying charge densities from tracer diffusion measurements [14].

The report of Lixiao Wang and Ryuk Yu has a simple objective. It is to show that a linear synthetic polyelectrolyte responds to solvent ionic conditions in the context of theories of de Gennes et al, of Skolnick and Fixman, of Odijk, and Odijk and Houwaart while a globular protein and rodlike fragment of DNA remain impervious to such [15].

In the study "Preparation and characterization of biocompatible polyelectrolyte complex multilayer of hyaluronic acid and poly-L-lysine, a novel biocompatible polyelectrolyte complex multilayer (PECML) was successfully prepared using hyaluronic acid (HA) and poly-L-lysine (PLL). The formation of PECML through the electrostatic interaction of HA as a polyanion and PLL as a polycation was confirmed by contact angle measurement [16].

2. THEORETICAL PART

2.1 Polyelectrolytes

Polyelectrolytes are polymers whose repeating units bear an electrolyte group. These groups will dissociate in aqueous solutions (water), making the polymers charged. Polyelectrolyte properties are thus similar to both electrolytes (salts) and polymers (high molecular weight compounds), and are sometimes called polysalts. Like salts, their solutions are electrically conductive. Like polymers, their solutions are often viscous. Charged molecular chains, commonly present in soft matter systems, play a fundamental role in determining structure, stability and the interactions of various molecular assemblies. Theoretical approaches to describing their statistical properties differ profoundly from those of their electrically neutral counterparts, while their unique properties are being exploited in a wide range of technological and industrial fields. One of their major roles, however, seems to be the one played in biology and biochemistry. Many biological molecules are polyelectrolytes. For instance, polypeptides (thus all proteins) and DNA are polyelectrolytes. Both natural and synthetic polyelectrolytes are used in a variety of industries.

2.1.1 Polyelectrolyte Charge

Acids are classified as either weak or strong (and bases similarly may be either weak or strong). Similarly, polyelectrolytes can be divided into 'weak' and 'strong' types. A 'strong' polyelectrolyte is one which dissociates completely in solution for most reasonable pH values. A 'weak' polyelectrolyte, by contrast, has a dissociation constant (pK_a or pK_b) in the range of ~ 2 to ~ 10 , meaning that it will be partially dissociated at intermediate pH. Thus, weak polyelectrolytes are not fully charged in solution, and moreover their fractional charge can be modified by changing the solution pH, counterion concentration, or ionic strength. The physical properties of polyelectrolyte solutions are usually strongly affected by this degree of charging. Since the polyelectrolyte dissociation releases counter-ions, this necessarily affects the solution's ionic strength. This in turn affects other properties, such as electrical conductivity.

The characteristic reactions of polyelectrolytes are namely electrolytic dissociation and electrostatic association depending on the polyelectrolyte concentration.

Most of the polyelectrolytes give reaction only in a narrow concentration range. Debye-Hückel theory can not be valid for the polyelectrolyte solution due to the high electrical charge on polyions. For very rare polyelectrolytes in limited concentration range $F=1/2 (C_i^2 Z_i^2)$

When solutions of two oppositely charged polymers (that is, a solution of polycation and one of polyanion) are mixed, a bulk complex (precipitate) is usually formed. This occurs because the oppositely-charged polymers attract one another and irreversibly bind together.

2.1.2 Polyelectrolyte Conformation

The conformation of any polymer is affected by a number of factors: notably the polymer architecture and the solvent affinity. In the case of polyelectrolytes, charge also has an effect. Whereas an uncharged linear polymer chain is usually found in a random conformation in solution (closely approximating a self-avoiding three-dimensional random walk), the charges on a linear polyelectrolyte chain will repel each other (Coulomb repulsion), which causes the chain to adopt a more expanded, rigid-rod-like conformation. If the solution contains a great deal of added salt, the charges will be screened and consequently the polyelectrolyte chain will collapse to a more conventional conformation (essentially identical to a neutral chain in good solvent).

Polymer conformation of course affects many bulk properties (such as viscosity, turbidity, etc.). Although the statistical conformation of polyelectrolytes can be captured using variants of conventional polymer theory, it is in general quite computationally intensive to properly model polyelectrolyte chains, owing to the long-range nature of the Coulomb interaction.

Polyelectrolytes which bear both cationic and anionic repeat groups are called polyampholytes. The competition between the acid-base equilibria of these groups leads to additional complications in their physical behavior. These polymers usually only dissolve when there is sufficient added salt, which screens the interactions between oppositely charged segments. Many proteins are polyampholytes, as some amino acids tend to be acidic while others are basic.

2.1.3 The Conductance of Polyelectrolytes

Polyelectrolyte solutions show large conductance. Conductance measurements give further information on the nature of polyelectrolyte in solution. Conductance of polyelectrolytes increases when dielectric constant of the medium increases. Because the energy removal of a mobile ion from electrostatic field of molecule decreases as the dielectric constant increased and the number of free ions and the net charge on the polymeric ion should increase so that the conductance increases.

Conductance or specific and equivalent conductivity are directly reflect the electric transport of micro ions (low molecular mass), but this phenomenon do not truly reveal the transport of polyions and counter ions released from the polyions through the solution. Many difficulties arise because of the asymmetry between the large, highly charged macroions, and counter and coions of which have few charges so that these small ions influence the flexibility of polyion chains. Although it seems that phenomena encountered in polyelectrolyte solution are in conflict with the conductivity treatment, conductivity is one of the valid method to study of polyelectrolyte solution.

2.1.4 Polyelectrolyte Applications

Polyelectrolytes have many applications, mostly related to modifying flow and stability properties of aqueous solutions and gels. For instance, they can be used to either stabilize colloidal suspensions, or to initiate flocculation (precipitation). They can also be used to impart a surface charge to neutral particles, enabling them to be dispersed in aqueous solution. They are thus often used as thickeners, emulsifiers, conditioners, flocculents, and even drag reducers. They are used in water treatment and for oil recovery. Many soaps, shampoos, and cosmetics incorporate polyelectrolytes. Additionally, they are added to many foods. Some of the polyelectrolytes that appear on food labels are pectin, carrageenan, alginates, polyvinylpyrrolidone and carboxymethyl cellulose. All but the last two are of natural origin. Finally, they are used in a variety of materials, including cement.

Because some of them are water-soluble, they are also investigated for biochemical and medical applications. There is currently much research in using biocompatible polyelectrolytes for implant coatings, for controlled drug release, and other applications.

Recently, polyelectrolytes have been utilized in the formation of new types of materials known as polyelectrolyte multilayers (PEMs). These thin films are constructed using a layer-by-layer (LbL) deposition technique. During LbL deposition, a suitable growth substrate (usually charged) is dipped back and forth between dilute baths of positively and negatively charged polyelectrolyte solutions. During each dip a small amount of polyelectrolyte is adsorbed and the surface charge is reversed, allowing the gradual and controlled build-up of electrostatically cross-linked films of polycation-polyanion layers. Scientists have demonstrated thickness control of such films down to the single-nanometer scale. LbL films can also be constructed by substituting charged species such as nanoparticles or clay platelets in place of or in addition to one of the polyelectrolytes. LbL deposition has also been accomplished using hydrogen bonding instead of electrostatics.

The main benefits to PEM coatings are the ability to conformably coat objects (that is, the technique is not limited to coating flat objects), the environmental benefits of using water-based processes, reasonable costs, and the utilization of the particular chemical properties of the film for further modification, such as the synthesis of metal or semiconductor nanoparticles, or porosity phase transitions to create anti-reflective coatings, optical shutters, and superhydrophobic coatings.

If polyelectrolyte chains are added to a system of charged macroions, an interesting phenomenon called the polyelectrolyte bridging might occur. The term bridging interactions is usually applied to the situation where a single polyelectrolyte chain can adsorb (see adsorption) to two (or more) oppositely charged macroions (e.g. DNA molecule) and via its connectivity mediate attractive interactions between them. Due to its connectivity the behavior of the polyelectrolyte chain bears almost no resemblance to the case of confined unconnected ions.

2.1.5 Poly(phosphates) as Polyelectrolytes

For several reasons, the polyphosphates are ideally suited for a study of polyelectrolyte behavior. First, they are easily prepared to a degree of purity. Second, molecular weight distribution function has been determined both theoretically and experimentally. Third, viscosity behavior is uncomplicated because polyphosphate chains are unbranched. In addition phosphorous element imparts particular property to the synthetic polymer depending on phosphorous concentration.

Polyphosphates are phosphate polymers linked between hydroxyl groups and hydrogen atoms. The polymerization that takes place is known as a condensation reaction. Phosphate chemical bonds are typically high-energy covalent bonds, which mean that energy is available upon breaking such bonds in spontaneous or enzyme catalyzed reactions. Adenosine triphosphate (ATP) is an example of a phosphate trimer, a polymer with three phosphate groups.

When sodium phosphate is heated above 650°C is polymerized by condensation process and water soluble glasses are obtained. These products are known under various names such as Graham's Salt and poly(sodiumphosphate). The structural differences of phosphorous containing polymer, e.g., whether phosphorous is linked to the main chain or to side chain or whether it is incorporated homogeneously or inhomogeneously exhibit important properties such as inflammability, increased water absorption, polarity and adhesion to glass, ceramic materials and metals etc. Graham's salts contain long chain phosphates and it is good precipitating agent. In general there are two types of precipitates formed with Graham's salt. One of them is viscous oils and jellies. It also precipitates with high molecular weight cations such as quarternary amines. Linear polyphosphates are stable in neutral or alkaline solutions at room temperature. The hydrolysis of polyphosphates are very slow so that the half life of P-O-P bond at 25°C is in the magnitude of years. They can not be converted to orthophosphate unless they are boiled with acid catalyst.

2.2 Amino Acids

The amino acids are the monomeric units from which proteins are derived, they are the main structural elements of protein. The word protein is derived from the (Greek) word proteins which means principal or prime. Proteins are, in fact, principal components of biochemical systems. They serve in a structural capacity, they are utilized as a source of energy, and most enzymes that catalyze the reactions occurring in living organisms are proteins. The characteristics of the monomers, the amino acids, are important with respect to the functions of the polymers, the proteins. The formation of a protein from amino acids entails a polymerization process involving the amino group of one amino acid and the carboxyl group of another.

Amino acids are usually characterized on the basis of the fourth substituent (i.e., that in addition to the amino group, the carboxyl group, and the hydrogen) that is

bonded at C (2). The trivial name of the amino acid is followed by its abbreviation in parentheses. Next, the systematic name of the amino acid is given. Common structure of amino acids is given in Figure 2.1.

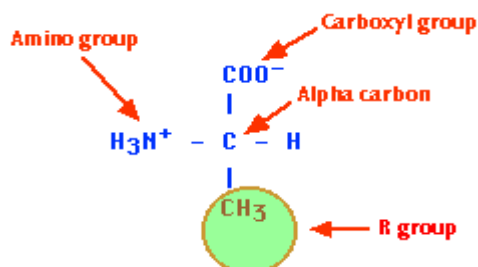
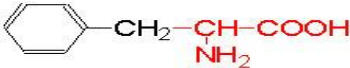
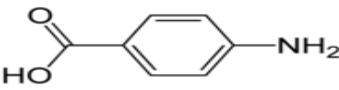
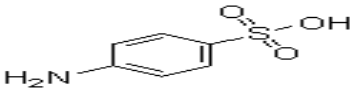


Figure 2.1: Common Structure of Amino Acids

R--CH--COOH In this structure the -COOH is carbon #1 and is attached to a --CH-- which is called alpha carbon or carbon atom #2. The amino group NH₂ attached to alpha carbon is known as alpha amino group. R group attached to alpha carbon is known as side chain. The fourth substituent bonded at C (2) (called the side chain) is not involved in forming a protein, and thus it is available to participate in the reactions and processes in which the protein is involved. For example, such a substituent may form hydrogen bonds, electrostatic and hydrophobic interactions and disulfide bonds. Some side chains may undergo covalent modification (phosphorylation, methylation, adenylation) that alters the chemical or physical characteristics of the protein, or it may function as a proton donor or a proton acceptor in the reaction mechanism when the protein is an enzyme, or it may influence the conformation of a structural element and, thereby, alter the nature of its contribution to the structural characteristics of the molecule. The studied amino acids are given in Table 2.1.

Table 2.1: The Studied Amino Acids

Amino Acid	Symbol	Structure
Amino Acids with Aliphatic R-Groups		
Neutral Amino Acid		
Glycine	Gly - G	$\begin{array}{c} \text{H}-\text{CH}-\text{COOH} \\ \\ \text{NH}_2 \end{array}$
Acidic Amino Acid		
Glutamic Acid	Glu - E	$\text{HOOC}-\text{CH}_2-\text{CH}_2-\text{CH}-\text{COOH} \\ \\ \text{NH}_2$
Basic Amino Acid		
Lysine	Lys - K	$\text{H}_2\text{N}-(\text{CH}_2)_4-\text{CH}-\text{COOH} \\ \\ \text{NH}_2$
Amino Acids with Aromatic Rings		
Phenylalanine	Phe - F	
Paraaminobenzoic Acid	PABA	
Paraaminobenzene sulfonic acid	4-PABSA	

Glycine (abbreviated as Gly or G) is one of the 20 amino acids commonly found in proteins, coded by codons GGU, GGC, GGA and GGG. Because of its structural simplicity, this compact amino acid tends to be evolutionarily conserved in, for example, cytochrome c, myoglobin, and hemoglobin. Glycine is the unique amino acid that is not optically active. Most proteins contain only small quantities of glycine. A notable exception is collagen, which contains about 35% glycine.

Glutamic acid (abbreviated as Glu or E; Glx or Z represents either glutamic acid or glutamine), is one of the 20 proteinogenic amino acids. It is not among the human essential amino acids. Its codons are GAA and GAG. The carboxylate anion of glutamic acid is known as glutamate. As its name indicates, glutamic acid has a carboxylic acid component to its side chain. At typical pH's, the amino group is protonated and one or both of the carboxylic groups will be ionized. At neutral pH all

three groups are ionized, and the species has a charge of -1. The pK_a value for glutamic acid is 4.1, which means that below this pH, the carboxylic acid groups are not ionized in more than half of the molecules.

Lysine (abbreviated as Lys or K) is an α -amino acid. This amino acid is an essential amino acid, which means that humans cannot synthesize it. Its codons are AAA and AAG.

Lysine is a base, as are arginine and histidine. The ϵ -amino group often participates in hydrogen bonding and as a general base in catalysis. Common post translational modifications include methylation of the ϵ -amino group, giving methyl-, dimethyl-, and trimethyllysine. The latter occurs in calmodulin. Other posttranslational modifications include acetylation. Collagen contains hydroxylysine which is derived from lysine by lysyl hydroxylase. O-Glycosylation of lysine residues in the endoplasmic reticulum or Golgi apparatus is used to mark certain proteins for secretion from the cell.

Phenylalanine (abbreviated as Phe or F) is an α -amino acid is classified as nonpolar because of the hydrophobic nature of the benzyl side chain. The codons for L-phenylalanine are UUU and UUC. It is a white, powdery solid. L-Phenylalanine (LPA) is an electrically-neutral amino acid, one of the twenty common amino acids used to biochemically form proteins, coded for by DNA.

4-Aminobenzoic acid (also known as *para*-aminobenzoic acid or PABA) is an organic white crystalline substance that is only slightly soluble in water. It consists of a benzene ring substituted with an amino group and a carboxylic acid. PABA is an essential nutrient for some bacteria and is sometimes called Vitamin B_x. However, PABA is not essential to human health, and is therefore not officially classified as a vitamin. Although humans lack the ability to synthesize folate from PABA, it is sometimes marketed as an essential nutrient under the premise that it can stimulate intestinal bacteria. PABA is an intermediate in bacterial synthesis of folate. Sulfonamides are chemically similar to PABA, and their antibacterial activity is due to their ability to interfere with conversion of PABA to folate, and subsequent utilization, by bacteria.

In the past, PABA has been widely used as a UV filter in sunscreen formulations. However, it has been determined that it increases the formation of a particular DNA

defect in human cells, thus increasing the risk of skin cancer in people who lack the mechanisms to repair these cellular defects. Currently, safer and more effective derivatives of PABA, such as octyl dimethyl PABA, are more commonly used. The potassium salt is used as a drug against fibrotic skin disorders under the trade name POTABA. PABA is also occasionally used in pill form by sufferers of Irritable bowel syndrome to treat the associated gastrointestinal symptoms. PABA also finds use in the manufacture of esters, folic acid, and azo dyes.

Sulfanilic acid (4-aminobenzene sulfonic acid) is a colourless crystalline solid produced from sulfonation of aniline. It readily forms diazo compounds and is used to make dyes and sulpha drugs. It is a zwitterion with an unusual high melting point.

2.2.1 Amino Acid Reactions

An amino acid carries simultaneously :

a carboxylic acid function -COOH , which is a weak acid ($2 < \text{p}K_a < 2.5$)

an amine function -NH_2 , which is a weak base ($9 < \text{p}K_a < 9.5$).

In solution as well as in the solid state, the proton of the carboxylic acid group is transferred onto the amine to give a neutral entity, called zwitterion. A zwitterion (from German "*Zwitter*" — "hybrid," "hermaphrodite") is a chemical compound that is electrically neutral but carries formal positive and negative charges on different atoms. Zwitterions are polar and usually have a high solubility in water and a poor solubility in most organic solvents.

Any compound which has a net zero charge is called a zwitter ion. Among amino acids, neutral amino acids have net zero charge at pH 7 because their structure at this pH is the positive and negative charges neutralize each other.

Under these form, amino acids can be considered as being salts of the weak acid -COO^- and the weak base -NH_3^+ and therefore they behave as amphoteric particles. The hydrolysis of an amino acid is given in Figure 2.3.

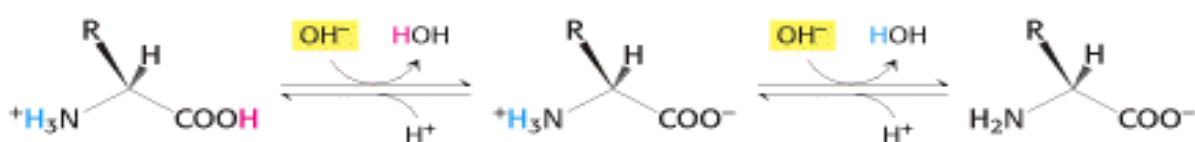


Figure 2.2: The Hydrolysis of Amino Acids

Amino acids, under their zwitterionic form behave as amphoteric particles; the pH of their solutions is given by :

$$\text{pH} = \frac{1}{2} \left(\text{p}K_{a_1} + \text{p}K_{a_2} \right) \quad (2.1)$$

This pH is called isoelectric pH because the zwitterion is overall neutral. It is noted pH_i .

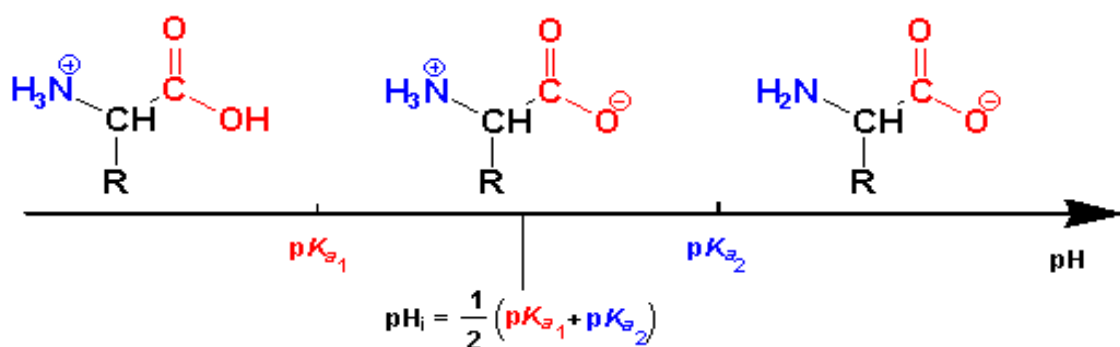
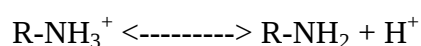
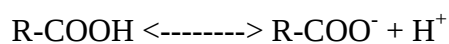


Figure 2.3: Unprotonated and Zwitterionic Form of Amino Acids

The α -COOH and α -NH₂ groups in amino acids are capable of ionizing (as are the acidic and basic R-groups of the amino acids). As a result of their ionizability the following ionic equilibrium reactions may be written:



The equilibrium reactions, as written, demonstrate that amino acids contain at least two weakly acidic groups. However, the carboxyl group is a far stronger acid than the amino group. At physiological pH (around 7.4) the carboxyl group will be unprotonated and the amino group will be protonated. An amino acid with no ionizable R-group would be electrically neutral at this pH. This species is termed a zwitterion.

Like typical organic acids, the acidic strength of the carboxyl, amino and ionizable R-groups in amino acids can be defined by the dissociation constant, K_a or more commonly the negative logarithm of K_a , the pK_a . The net charge (the algebraic sum of all the charged groups present) of any amino acid, peptide or protein, will depend upon the pH of the surrounding aqueous environment. This phenomenon can be observed during the titration of any amino acid or protein. When the net charge of an amino acid or protein is zero the pH will be equivalent to the isoelectric point: pI. Titration curve of alanine is given in Figure 2.4.

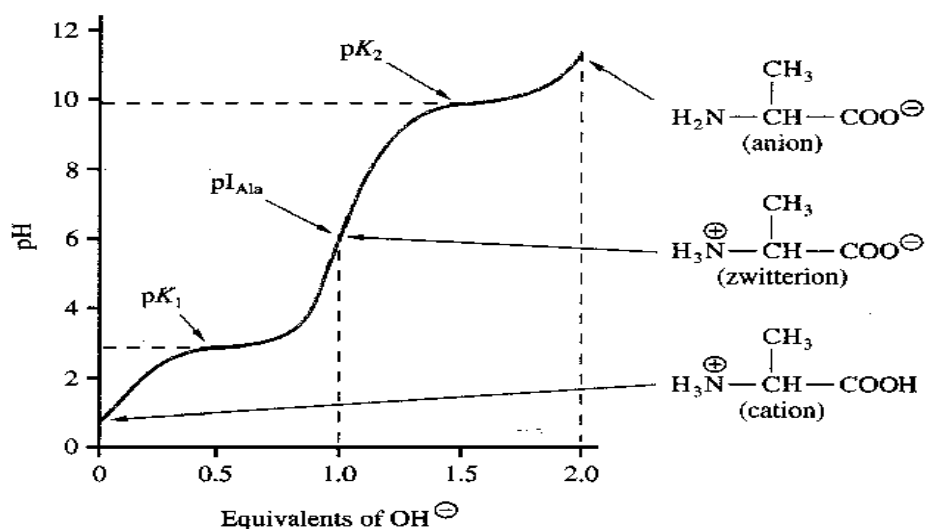


Figure 2.4: Titration Curve of Alanine

Amino acids form polymers through a nucleophilic attack by the amino group of an amino acid at the electrophilic carbonyl carbon of the carboxyl group of another amino acid. The carboxyl group of the amino acid must first be activated to provide a better leaving group than OH^- .

The resulting link between the amino acids is an amide link which biochemists call a peptide bond. In this reaction, water is released. In a reverse reaction, the peptide bond can be cleaved by water (hydrolysis).

3. EXPERIMENTAL PART

3.1 Chemicals

Commercially available poly(sodiumphosphate)(Merck) was utilized as polyanion. Glycine (Merck), Sulfanilic acid (Sigma-Aldrich), DL-Glutamic acid monohydrate (Fluka), L-Phenyl alanine (Sigma-Adrich), L-Lysine (Fluka), Poly(allylaminehydrochloride) (Alfa Aesar), 4-Aminobenzoic acid (Merck), Sodium hydroxide (Fluca), hydrochloric acid (Merck), exchanger 1W (Merck) (Strong base) and AmberliteIR 120 (Merck) (Strong acid) were used as ion exchange resins in the necessary reactions.

3.2 Methods

The methods chosen for this study were mainly conductometry. The stoichiometry of complexes were determined by conductometric titration methods. In case of the necessity ion-exchange method was used to obtain the particular form of polyions and amino acids. Molecular weight determinations of polyions were carried out using viscometry and wet chemistry methods. Formation constants of the complexes were determined by potentiometric method.

3.3 Solutions

All solutions were freshly prepared for a weak period.

10^{-1} M (NaPO₃)_n

1 .02 g of (NaPO₃)_n is dissolved in 100 mL of distilled water (Stock solution pH, 4.36).

10^{-2} M (NaPO₃)_n (I= 10^{-1})

0.25 g g of (NaPO₃)_n is dissolved in enough amount of 0.1 M HClO₄ solution, then diluted to 250 mL with 0.1 M HClO₄ solution.

10^{-2} M PABACl

0.14 g of PABA is dissolved in 1 mL of 0.1 M HCl, then diluted to 100 mL with distilled water.

10^{-1} M NaPABA

1.4 g of PABA is dissolved in 10 mL of 1 M NaOH, then diluted to 100 mL with distilled water.

10^{-2} M NaPABA ($I=10^{-1}$)

0.14 g NaPABA is dissolved in 1 mL of 1 M NaOH, then diluted to 100 mL with 0.1 M HClO_4 solution.

10^{-2} M PAAHCl ($I=10^{-1}$)

0.23 g PAAHCl is dissolved in 0.1 M HClO_4 solution then the volume of solution completed to 250mL with 0.1 M HClO_4 solution.

10^{-1} M Glycine

1.86 g glycine is diluted to 250mL with distilled water.

10^{-2} M GlycineCl

0.375 g of glycine is dissolved in 5mL of 1N HCl and then the solution is diluted to 500mL with distilled water.

10^{-1} M GlycineNa

0.375 g of glycine and 0.2 g of NaOH pellets are dissolved in enough water and then the solution is diluted to 50mL with distilled water.

10^{-1} M Lysine

1.46 g of lysine is diluted to 100mL with distilled water.

10^{-1} M LysineCl

- a. 1.46 g of lysine is dissolved in 100mL of 0.1 M HCl solution and then the solution is diluted to 100mL with distilled water.
- b. 1.46 g of lysine is dissolved in 100mL of 0.2 M HCl solution and then the solution is diluted to 100mL with distilled water.

10^{-1} M LysineNa

- a. 1.46 g of lysine and 0.4 g of NaOH pellets are dissolved in enough water and then the solution is diluted to 100mL with distilled water.
- b. 1.46 g of lysine and 0.8 g of NaOH pellets are dissolved in enough water and then the solution is diluted to 100mL with distilled water.

10^{-1} M Phenylalanine

0.41 g of phenylalanine is diluted to 25mL with distilled water.

10^{-1} M PhenylalanineNa

0.935 g of phenylalanine and 0.2 g of NaOH pellets are dissolved in enough water and then the solution is diluted to 50mL with distilled water.

10^{-1} M PABSA

0.865 g of PABSA is diluted to 50mL with distilled water.

10^{-1} M NaPABSA

0.865 g of PABSA and 0.2g of NaOH pellets are dissolved in enough water and then the solution is diluted to 50mL with distilled water.

10^{-1} M PABSACl

0.865 g PABSA is dissolved in 5mL of 1N HCl solution and then the solution is diluted to 50mL with distilled water.

10^{-1} M Glutamic Acid

0.327 g of glutamic acid is dissolved in enough amount of 3N HCl solution and then the solution is diluted to 25mL with distilled water.

10^{-1} M Glutamic AcidNa

- a. 0.327 g of glutamic acid and 0.1g of NaOH pellets are dissolved in enough amount of 3N HCl solution and then the solution is diluted to 25mL with distilled water.
- b. 0.327 g of glutamic acid and 0.2g of NaOH pellets are dissolved in enough amount of 3N HCl solution and then the solution is diluted to 25mL with distilled water.

3.4 Equipments

The following instruments were used in measurements.

Conductometer, WTW

pH meter, Jenway 3040 Ion Analyser

Viscosimeter, Ostwald

TERMO, Nicolet 380 FT-IR, ATR

Thermostat, P Selecta

Analytical Balance, GECAVERY

Stirrer, IKAMAG

3.5 Experiments

3.5.1 Molecular Weight Determination of Polyions

Molecular weight of Poly(allylaminehydrochloride) was carried out viscosimetry. In addition, molecular weight of poly(sodiumphosphate) was also determined by end group titration method.

3.5.1.1 Molecular Weight Determination of Poly(sodiumphosphate) by End Group Titration

0.5 g of polysodium phosphate was dissolved in 100 mL of water and pH was lowered to about 3 using HCl (1 M) and then titrated potentiometrically with standart NaOH (0.05 M) solution until the pH value of 4,5 and number of mL equivalents of base, A mL, consumed until the first end point was determined.

The total phosphorus amount of the phosphate was determined in another poly sodium phosphate sample of the same weight after a complete hydrolysis procedure.

The complete reversion of the polyphosphate to the orthophosphate form can be achieved by gently boiling the sample in a HNO_3 (1 M) solution for three hours under reflux then, pH was lowered to about 3 and the solution was titrated potentiometrically with 0.05M standart NaOH solution until the pH value of 4,5 and the procedure was continued beyond the second end point at about pH, 9 and the number of mL equivalents of base, A_h mL, consumed between two end points were determined.

Titration curves of $(\text{NaPO}_3)_n$ before and after hydrolysis are given in in Figure 3.1.and 3.2.

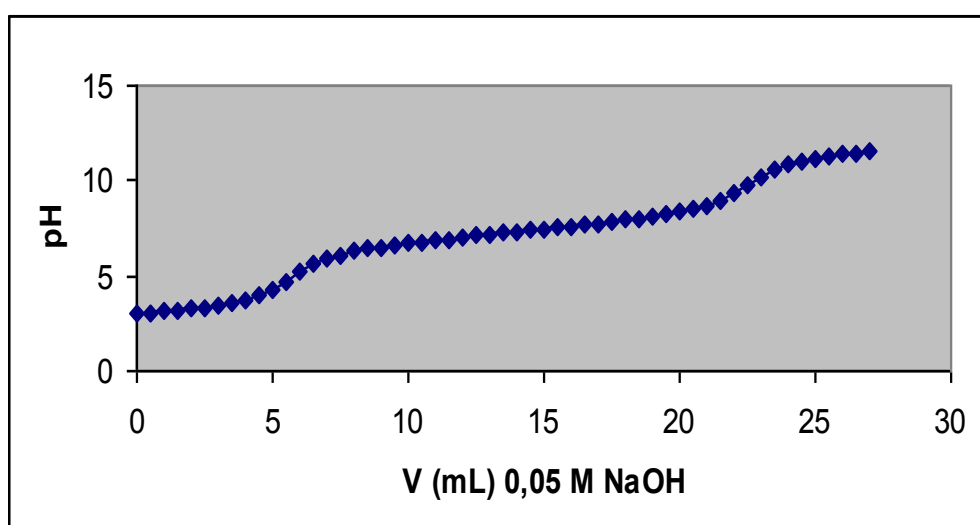


Figure 3.1: Titration Curve of $(\text{NaPO}_3)_n$ Before Hydrolysis

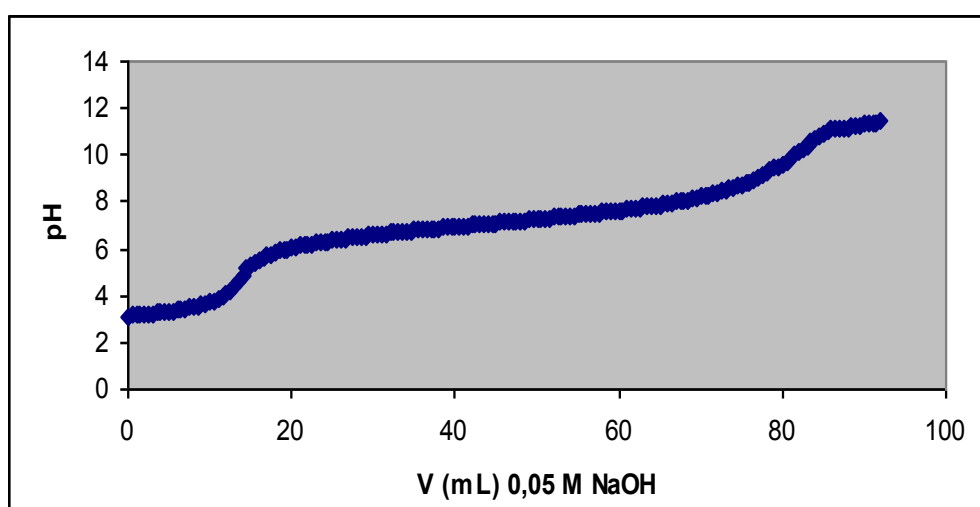


Figure 3.2: Titration Curve of $(\text{NaPO}_3)_n$ After Hydrolysis

Finally P₂O₅ % (End Group) and P₂O₅ (Total) were calculated. (Table 3.1.).

$$\%P_2O_5(\text{endgroup}) = \frac{A}{Ah} 100 \quad (3.1)$$

$$\frac{P_2O_5(\text{total})}{P_2O_5(\text{end})} = \frac{n}{2} = \frac{Ah}{A} \quad (3.2)$$

2 indicates two end groups, n indicates length of chain or polymerization degree.

Table 3.1: The Results of End Group Titration

Titration Procedure	mL of NaOH added (V mL)	%P ₂ O ₅
For End Group (Before hydrolysis)	5.5	8.33
For Total Phosphorus (After hydrolysis)	66.0	99.6
n =24		
Molecular Weight= 2446.8 g/mol		

3.5.1.2 Molecular Weight Determination of Poly(sodiumphosphate) by Viscometry

Solution viscosity is basically a measure of the size of polymer molecules and it is empirically related to molecular weight of polymers. Thus, viscosity measurement constitutes an extremely valuable tool for the molecular characterization of polymer. Dilute solution viscosity is usually measured in capillary viscometer of the Ostwald or Ubbelohde type and concentration, c, is expressed in grams per deciliter (g/dL, g/100 mL). Measurements of solution viscosity are usually made by comparing the efflux time, t, required for a specific volume of polymer solution to flow through a capillary tube with the corresponding efflux time, t₀, for the solvent. From t₀, and the solute concentration the following equations are derived.

$$\text{Relative viscosity} \quad \eta_r = t/t_0 \quad (3.3)$$

$$\text{Specific viscosity} \quad \eta_{sp} = \eta_r - 1 \quad (3.4)$$

$$\text{Reduced viscosity} \quad \eta_{red} = \eta_{sp} / C \quad (3.5)$$

$$\text{Intrinsic viscosity} \quad [\eta] = (\eta_{sp}/C)C_0 \quad (3.6)$$

The molecular weights of linear polyions were calculated from the intrinsic viscosity of the solution by the following Mark Houwink equation:

$$[\eta] = K M^a \quad (3.7)$$

In this equation a and K are constants dependent to the temperature, type of solvent and polymer and M represents the molecular weight.

In these measurements Ostwald type viscometry is used. Viscosity measurements of polysodiumphosphate were carried out in 0,035 M NaBr solution at 25,5°C. Results are given in Table 3.2. and Figure 3.3.

Table 3.2: Viscosity Values for Poly(sodiumphosphate)($K=69 \times 10^{-5}$ dL/g, $a=0.61$)

Concentration of (NaPO_3) _n C (g/100mL=g/dL)	t (dak)	t (sn)	η_r	η_{sp}	$\eta_{red} = \eta_{sp} / C$
0.3087	1.257	75.4	1.016	0.016	0.052
0.3704	1.255	75.3	1.015	0.015	0.039
0.4630	1.251	75.1	1.011	0.011	0.024
0.6173	1.245	74.7	1.006	0.006	0.010
0.9260	1.245	74.7	1.006	0.006	0.007
NaBr	1.237	74.2			

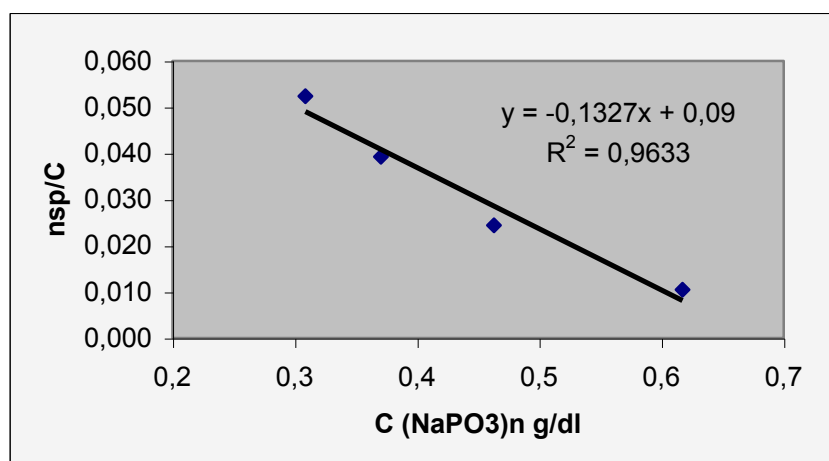


Figure 3.3: Viscosity Curve for Poly(sodiumphosphate)

The molecular weight of $(\text{NaPO}_3)_n$ was found as 2936.69 g/mol by using the equation 3.7.

3.5.1.3 Molecular Weight Determination of Poly(allylaminehydrochloride) by Viscometry

Viscosity measurements of Poly(allylaminehydrochloride) are done in 0,5 M NaCl solution at 25°C. Results are given in Table 3.3. and Figure 3.4.

Table 3.3: Viscosity Values for Poly(allylaminehydrochloride)
($K=7,19 \times 10^{-5}$ dL/g, $a=0,794$)

Concentration of PAAHCl C (g/100mL=g/dl)	t (dak)	t (sn)	η_r	η_{sp}	$\eta_{red} = \eta_{sp} / C$
0.33	1.449	86.94	1,15	0,15	0,45
0.40	1.493	89.58	1,18	0,18	0,46
0.50	1.559	93.54	1,24	0,24	0,47
0.67	2.074	12.44	1,64	0,64	0,96
1.00	2.336	14.16	1,85	0,85	0,85
NaCl	1.262	75.72			

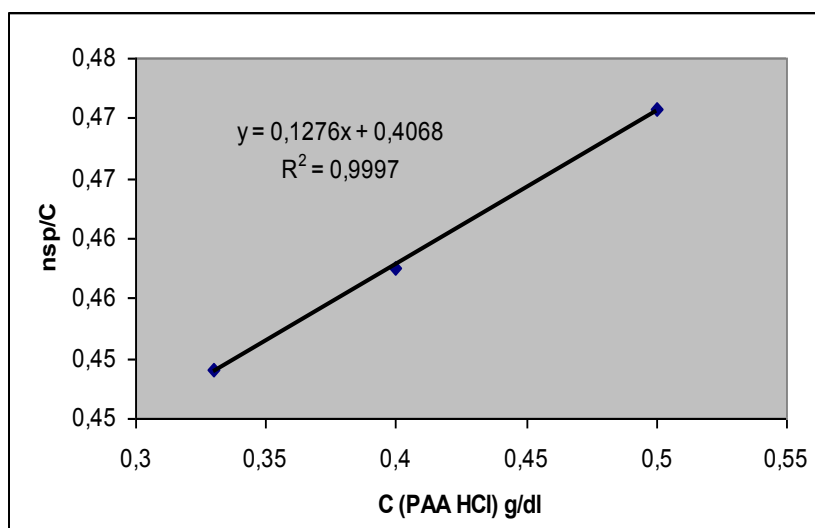


Figure 3.4: Viscosity Curve for PAAHCl

The molecular weight of PAAHCl was found as 52242.94 g/mol by using the equation 3.7.

3.5.2 Determination of Equivalent Weight of Polyions

3.5.2.1 Determination of Equivalent Weight of Poly(sodiumphosphate)

Equivalent weight of poly(sodiumphosphate)((NaPO₃)_n) was determined by alkalimetry after poly(sodiumphosphate) has been converted to its acid form (HPO₃)_n by ion exchange method using Amberlite IR-120 (strong acid cation exchanger). Ion Exchange Column is prepared as explained below.

15g of resin was weighed and covered with distilled water and let to stand overnight. After pouring off water, 3M HCl was added. And then it was shaken and filtered. Acid treatment was repeated for three times and washed with large portions of water by shaking. It was stand for three hours in 1M HCl for cation exchange column and in 0.5M NaOH for anion exchange column. And then it washed several times with water. The activated anion exchanger resin (2/3 of column lenght) was transferred to column with an excess of alkali and the activated cation exchanger resin (2/3 of column lenght) was transferred to another column with an excess of acid was removed by treatments of distilled water. Column lenght: 17 cm, diameter: 1cm, flow rate:1.5mL/min, for anion and cation exchange column efficiencies are 98.9% and 98.5% respectively.

The eluate (HPO₃)_n was titrated with standard 0.05M NaOH volumetrically by using methyl orange indicator. The calculated equivalent weight of Polysodiumphosphate is 100.46 g eqw.

3.5.2.2 Determination of Equivalent Weight of Poly(allyaminehydrochloride)

Equivalent Weight of PAAHCl was determined by argentimetry and found as 94.50 g eqw.

3.5.3 Stoichiometric Determinations

3.5.3.1 Conductometric Titration Method

Solutions of electrolytes conduct an electric current by the migration of ions under the influence of an electric field. Like a metallic conductor, they obey Ohm's law. Exceptions to this law occur only under abnormal conditions—for example, very

high voltages or high-frequency currents. Thus, for an applied electromotive force E , maintained constant but at a value which exceeds the decomposition voltage of the electrolyte, the current i flowing between the electrodes immersed in the electrolyte will vary inversely with the resistance of the electrolyte solution, R . The reciprocal of the resistance, $1/R$, is called the conductance, L , and is expressed in reciprocal ohms, or mhos.

The standard unit of conductance is specific conductance, χ , which is the conductance in mhos of one cubic centimeter of solution between two electrodes one centimeter square and one centimeter apart. The observed conductance L of a solution depends inversely on the distance l between the electrodes and directly upon their area S , cm^2 ,

$$\text{Specific Resistance, } r = R \text{ ohm} \times S \text{ cm}^2 / 1 \text{ cm} \quad (3.8)$$

$$1 / r = \chi \text{ specific conductance} = \text{conductivity} \quad (3.9)$$

$$\chi = (1 \text{ cm} / S \text{ cm}^2) / (1 / R \text{ ohm}^{-1}) \quad (3.10)$$

$$\chi \text{ ohm}^{-1} \times \text{cm}^{-1} = K \text{ cm}^{-1} \times L \text{ ohm}^{-1} \quad (3.11)$$

where $K \text{ cm}^{-1}$ is the coefficient related with the instrument and $\chi \text{ ohm}^{-1} \times \text{cm}^{-1} = \text{siemens}$. The electrical conductance of a solution is a summation of contributions from all the ions present. It depends upon the number of ions per unit volume of the solution and upon the velocities with which these ions move under the influence of the applied electromotive force. As a solution of an electrolyte is diluted, the specific conductance, χ will decrease. Fewer ions to carry the electric current are present in each cubic centimeter of solution. However, in order to express the ability of individual ions to conduct, a function called the equivalent conductance is needed. If C is the concentration of the solution in gram equivalents per liter, then the volume of solution in cubic centimeters per equivalent is equal to $1000/C$, so that,

$$\Lambda = 1000 \chi / C \quad (3.12)$$

Conductometric titration of Aminoacid-Poly(sodiumphosphate) and Aminoacid-(HPO_3)_n and also reverse titration for optimum conditions were carried out for different polyanion and amino acids' concentrations in salt free and salt solutions. Titrations also repeated in the certain ionic strength in the possible concentration range. Results are given in Table 3.4, Table 3.5 and Appendix A(Figure A.1 – A.12)

Table 3.4: Results of Conductometric Titration Polyanion as Titrant

Titrant	Solution	Ionic Strenght	Salt Free Solution	NaCl	NaCl	Na ₂ SO ₄	Na ₂ SO ₄
(mol/L)	(mol/L)			1M	2M	1M	2M
Na(PO ₃) _n	Glycine						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	1:0.7				
1x10 ⁻⁴	1x10 ⁻⁴		—				
(HPO ₃) _n	GlycineNa						
1x10 ⁻²	1x10 ⁻²		1:0.65	1:0.9	1:1	1:0.8	1:1
Na(PO ₃) _n	GlycineCl						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	1:1.7				
1x10 ⁻²	1x10 ⁻²		1:1				
(NaPO ₃) _n	Glut.Acid						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		1:0.9				
(HPO ₃) _n	Glut.AcidNa						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		1:0.9	1:0.8	1:0.9	1:1	1:1
(NaPO ₃) _n	Glut. AcidCl						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		1:0.9				
(HPO ₃) _n	Lycine						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻³	1x10 ⁻³		1:0.8				
1x10 ⁻²	1x10 ⁻²		1:1				
1x10 ⁻²	1x10 ⁻²			1:0.8	1:0.9	1:0.8	
(HPO ₃) _n	LycineNa						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		1:1				
1x10 ⁻²	1x10 ⁻²			1:0.9	1:0.8	1:1	1:0.7
Na(PO ₃) _n	LycineCl						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				

1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		1:1				
1x10 ⁻³	1x10 ⁻³		—				
1x10 ⁻⁴	1x10 ⁻⁴		—				
Na(PO ₃) _n	Phenylalanine						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		—				
1x10 ⁻⁴	1x10 ⁻⁴		—				
(HPO ₃) _n	PhAlanineNa						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		1:0.7	1:1	1:0.8	1:1	
Na(PO ₃) _n	PABA						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		—				
1x10 ⁻⁴	1x10 ⁻⁴		1:1				
(HPO ₃) _n	NaPABA						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		1:0.8	1:1	1:0.7	1:0.8	1:1
Na(PO ₃) _n	PABSA						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		1:1.2				
1x10 ⁻⁴	1x10 ⁻⁴		1:1				
(HPO ₃) _n	NaPABSA						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		1:1.4	1:0.9	1:0.8	1:0.9	1:1

Table 3.5: Results of Reverse Titration

			Mole Ratio of AA to PC
Titration Mode	Reagent	Solution	
	1x10 ⁻²	1x10 ⁻²	
	LycineNa	(HPO ₃) _n	1:1.4
	NaPABSA	(HPO ₃) _n	1:0.7
	Phen.Al.Na	(HPO ₃) _n	1:0.4
	NaPABA	(HPO ₃) _n	1:1
	NaGL	(HPO ₃) _n	1:0.7
	NaGlut.Ac.	(HPO ₃) _n	1:1

Aminoacid-poly(allylamine hydrochloride)(PAAHCl) and aminoacid-poly(allylaminehydroxide) (PAAOH) stoichiometry were determined by conductometry. The same procedures given in last part were repeated for amino acid and polycation interactions. In this case, two forms of polycation (PAACl and PAAOH) were used as titrant. Results are given in Table 3.6 and Appendix B(Figures B.1 – B.12)

Table 3.6: Results of Conductometric Titration Polycation as Titrant

Titrant	Solution	Ionic Strenght	Salt Free Solution	NaCl	NaCl	Na ₂ SO ₄	Na ₂ SO ₄
(mol/L)	(mol/L)			1M	2M	1M	2M
PAA-OH	Glycine						
1x10 ⁻²	1x10 ⁻²			1:1.3	1:0.9	1:1.3	1:0.9
PAA	GlycineNa						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹					
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	1:1.3				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	1:1				
1x10 ⁻²	1x10 ⁻²						
PAA-OH	Glut.Acid						
1x10 ⁻²	1x10 ⁻²		1:1	1:1	1:1.2	1:1	1:1
PAA	Glut.AcidNa						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	1:1.2				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	1:1.6				
1x10 ⁻²	1x10 ⁻²		1:1.3				
PAA-OH	Glut.AcidCl						
1x10 ⁻²	1x10 ⁻²		1:1	1:1	1:1.2	1:1	1:1
PAACl	Lysine						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	1:1				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	1:1.3				
1x10 ⁻²	1x10 ⁻²		—				
PAACl	LysineNa						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	1:1				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	—				
1x10 ⁻²	1x10 ⁻²		1:0.8				
PAA-OH	LycineCl						
1x10 ⁻²	1x10 ⁻²		1:1	1:1	1:1.2	1:1	1:1
PAA-OH	Phenylalanine						
1x10 ⁻²	1x10 ⁻²		—				
PAACl	Ph.AlanineNa						
1x10 ⁻²	1x10 ⁻²	1x10 ⁻¹	—				
1x10 ⁻³	1x10 ⁻³	1x10 ⁻²	—				
1x10 ⁻⁴	1x10 ⁻⁴	1x10 ⁻³	1:1				
1x10 ⁻²	1x10 ⁻²		1:1.6				
PAA-OH	PhAlanineCl						
1x10 ⁻²	1x10 ⁻²		1:1.7	1:1	1:1	1:1	1:0.9

PAACl	NaPABA						
1×10^{-1}	1×10^{-1}	—					
1×10^{-2}	1×10^{-2}	—					
1×10^{-3}	1×10^{-3}	—					
1×10^{-4}	1×10^{-4}	1:0.23					
1×10^{-5}	1×10^{-5}	—					
1×10^{-2}	1×10^{-2}	1×10^{-1}	—				
1×10^{-3}	1×10^{-3}	1×10^{-2}	1:1				
1×10^{-4}	1×10^{-4}	1×10^{-3}	—				
PAA-OH	PABACl						
1×10^{-2}	1×10^{-2}		1:0.9	1:1	1:1	1:1	1:0.9
PAA-OH	PABSA						
1×10^{-2}	1×10^{-2}		1:1.2				
PAA-OH	PABSACl						
1×10^{-2}	1×10^{-2}		—				

3.5.3.2 Mol Ratio by Conductometry

The complex stoichiometry of amino acid and PA(PC) solutions with different mol ratios in the salt free solution were studied by conductometry.

Results are given in Table 3.7. and Appendix C (Figure C.1- C.12).

Table 3.7: Mol Ratio of Amino Acids to Polyanion and Polycation

Titrant	Solution	Mole Ratio of AA to PA	Figure
1×10^{-2}	1×10^{-2}		
GlycineNa	(HPO ₃)n	1: 0.9	C.1
Glutamic Acid	(NaPO ₃)n	1:1	C.2
Lysine Na	(HPO ₃)n	1:1	C.3
Phen.Al.Na	(HPO ₃)n	1:1.2	C.4
NaPABA	(HPO ₃)n	1:1.2	C.5
NaPABSA	(HPO ₃)n	1:1.2	C.6
Titrant	Solution	Mole Ratio of AA to PC	Figure
1×10^{-2}	1×10^{-2}		
Glycine	PAACl	1:1	C.7
Glutamic Acid	PAAOH	1:1.2	C.8
LysineNa	PAACl	1:0.8	C.9
Phen.Al.Na	PAAOH	1:1	C.10
PABACl	PAAOH	1:1.3	C.11
PABSACl	PAAOH	1:1	C.12

3.5.4. Determination of Complex Stability Constants

Complex stability constants of amino acid-polyanion (polysodium phosphate and $(\text{HPO}_3)_n$) and amino acid-polycation (poly(allylaminehydrochloride) and PAAOH) for the optimum stoichiometry which was determined by potentiometry as the function of temperature and pH parameters. In the case of diluted solution, this relationship is considered to be valid for amino acid both in the absence and presence of polycation and polyanion.

The experimental procedure is to measure pH of the amino acid in the presence and absence of PSP also PAAHCl is measured at different temperatures, and then the proton concentration is calculated corresponding to definite temperatures.

For weak acids the relation between the degree of dissociation (α), dissociation constant (K_α) and concentration C can be expressed as

$$K_\alpha = \left(\frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} \right) = \frac{(C\alpha)^2}{C(1-\alpha)} = \frac{C\alpha^2}{(1-\alpha)} \quad (3.10)$$

$$\alpha = \left(\frac{K_\alpha}{C} \right)^{1/2} \quad (3.11)$$

Degree of dissociation can be approximated for weak acids (where $1 - \alpha \approx 1$) as

$$\alpha = \frac{[\text{H}^+]}{C} \quad (3.12)$$

gives

$$[\text{H}^+] = \sqrt{K_\alpha \times C} \quad (3.13)$$

Finally the degree of linkage (θ) is determined from the equation

$$\theta = \frac{(C_o - C)}{C_o} = 1 - \frac{C}{C_o}$$

$$= 1 - \left(\frac{[H^+]}{[H^+]_o} \right)^2 \quad (3.14)$$

$[H^+]_o$: the proton concentration in absence of PSP

$[H^+]$: the proton concentration in absence of PSP

C_o : concentration of aminoacid

C : concentration of aminoacid-PSP complex

And then complex stability constants of amino acid-polysodiumphosphate is calculated from the following equation

$$K = \frac{(\text{komplex})}{(\text{Polysodium phosphate})(\text{Amino acid})} = \frac{C_o \theta}{(C_o(1 - \theta))^2} \quad (3.15)$$

The plots of pH, degree of linkage and $\ln K$ values as function of temperature were obtained.

The results are given in Table 3.8, Table 3.9 and Appendix D(Figure D.1 – D.39).

Table 3.8: Stability Constant of Amino Acid and Polyanion

Amino Acid-PA	T °C	pH (complex)	pH (PA)	K	Amino Acid-PA	T °C	pH (complex)	pH (PA)	K
GlycineNa - (NaPO3)n					NaPABA- (NaPO3)n				
	28	6,27	2,76	1,10E+17		28	5,69	2,76	5,40E+14
	32	5,32	3,04	1,35E+12		32	5,39	3,04	2,56E+12
	35	6,02	2,89	3,38E+15		35	5,69	2,89	1,62E+14
	40	5,34	2,86	8,49E+12		40	5,63	2,86	1,23E+14
	45	6	2,85	4,06E+15		45	5,25	2,85	4,06E+12
	50	6,25	2,7	1,62E+17		50	5,14	2,7	5,87E+12
	55	6,26	2,61	4,06E+17		55	5,2	2,61	2,34E+13
	60	5,79	2,46	2,13E+16		60	5,57	2,46	2,81E+15
Glutamic Acid-(NaPO3)n					PABSA - (NaPO3)n				
	28	3,04	2,14	4,00E+06		28	3,04	2,43	2,60E+05
	32	2,95	2,09	2,76E+06		32	2,95	2,36	2,18E+05
	35	2,85	2,19	4,24E+05		35	2,85	2,38	6,85E+04
	40	2,63	2,13	9,18E+04		40	2,63	2,27	2,27E+04
	45	2,36	2,05	1,35E+04		45	2,36	2,07	1,09E+04
	50	2,48	2,01	6,85E+04		50	2,48	1,97	1,01E+05
	55	2,55	2,05	9,18E+04		55	2,55	1,99	1,64E+05
	60	2,44	1,99	5,63E+04		60	2,44	1,85	2,18E+05
LycineNa - (NaPO3)n					NaPABSA - (NaPO3)n				
	28	7,72	2,76	7,10E+22		28	3,93	2,76	4,90E+07
	32	7,73	3,04	5,87E+21		32	3,89	3,04	2,51E+06
	35	7,81	2,89	4,88E+22		35	3,73	2,89	2,29E+06
	40	7,32	2,86	7,06E+20		40	3,69	2,86	2,09E+06
	45	7,28	2,85	5,36E+20		45	3,65	2,85	1,58E+06
	50	7,55	2,7	2,56E+22		50	3,63	2,7	5,28E+06
	55	7,89	2,61	1,35E+24		55	3,5	2,61	3,64E+06
	60	7,86	2,46	4,06E+24		60	3,42	2,46	6,97E+06
Ph.AlanineNa - (NaPO3)n									
	28	6,48	2,76	7,70E+17					
	32	6,35	3,04	1,77E+16					
	35	6,32	2,89	5,36E+16					
	40	6,22	2,86	2,81E+16					
	45	5,97	2,85	3,08E+15					
	50	6,33	2,7	3,38E+17					
	55	6,37	2,61	1,12E+18					
	60	6,15	2,46	5,87E+17					

Table 3.9 Stability Constant of Amino Acid and Polycation

Amino Acid-PC	T °C	pH (complex)	pH (PC)	K	Amino Acid-PC	T °C	pH (complex)	pH (PC)	K
GlycineNa - PAACl					NaPABA- PAACl				
	28	6,27	2,86	4,50E+16		28	5,79	2,86	5,40E+14
	32	6,02	3,21	1,77E+14		32	5,43	3,21	7,74E+11
	35	5,37	2,94	5,36E+12		35	5,49	2,94	1,62E+13
	40	5,28	2,79	9,31E+12		40	5,72	2,79	5,36E+14
	45	6,34	2,83	1,12E+17		45	5,42	2,83	2,34E+13
	50	6,41	2,72	5,87E+17		50	5,35	2,72	3,38E+13
	55	6,18	2,89	1,47E+16		55	5,45	2,89	1,77E+13
	60	5,92	2,35	1,94E+17		60	5,7	2,35	2,56E+16
Glutamic Acid-PAAOH					PABSACl - PAAOH				
	28	3,12	2,14	8,40E+06		28	4,04	2,45	2,30E+09
	32	3,07	2,09	8,39E+06		32	3,95	2,56	3,70E+08
	35	3,15	2,19	6,97E+06		35	3,85	2,38	7,73E+08
	40	3,15	2,13	1,22E+07		40	3,69	2,4	1,47E+08
	45	3,12	2,05	1,93E+07		45	3,12	2,39	8,19E+05
	50	2,98	2,01	7,65E+06		50	3,24	2,29	6,36E+06
	55	3,09	2,05	1,46E+07		55	3,34	2,05	1,47E+08
	60	2,87	1,99	3,32E+06		60	3,45	1,99	7,05E+08
Lycine - PAACl									
	28	7,11	2,86	1,00E+20					
	32	7,21	3,21	1,02E+19					
	35	7,32	2,94	3,38E+20					
	40	7,3	2,79	1,12E+21					
	45	7,28	2,83	6,44E+20					
	50	7,3	2,72	2,13E+21					
	55	7,33	2,89	5,87E+20					
	60	7,42	2,35	1,94E+23					
PhenylalanineCl-PAAOH									
	28	6,36	2,86	1,00E+17					
	32	6,56	3,21	2,56E+16					
	35	6,54	2,94	2,56E+17					
	40	6,42	2,79	3,38E+17					
	45	6,49	2,83	4,45E+17					
	50	6,33	2,72	2,81E+17					
	55	6,45	2,89	1,77E+17					
	60	6,15	2,35	1,62E+18					

3.5.5 IR Spectrum of the Complexes

IR spectra of amino acids and amino acid-polyelectrolyte complexes are given in Appendix E (E.1 – E.21)

4. RESULTS AND DISCUSSION

Molecular and equivalent weights of polyions: molecular weight of poly(allylamine hydrochloride) (polycation) was found to be 52242.94 g/mol by viscometric method.

Molecular weight of poly(sodium phosphate) (polyanion) was found to be 2447.8 g/mol by end group titration methods.

Equivalent weights of polycation and polyanion were 94.50 g.eqw and 100.46 g.eqw respectively.

Results of conductometric titration:

The stoichiometry of complexes varies as a function of both polyions and aminoacids concentration in salt and salt free solution at the given specific concentrations which were considered as a narrow concentration range.

It is reasonable to conclude from the results (Table 3.4, 3.5, 3.6) that the stoichiometric ratio between polycation to aminoacids and polyanion to aminoacids, in general, were 1:1 in the possible concentration range. Studies on the constant ionic strength showed that only the some case responds to determine a ratio between the interacted species. This result is quite reasonable according to the literature data that theories[19] given for polyelectrolyte phenomena are still lacking in respect of ionic strength. As shown in the titration curves (Appendix A, Figures A.1 – A.12), in most cases, specific conductivity decreases during the titration until the end point and then remains constant. The decreased part of the curve shows the binding of polyions to aminoacids with the release of counter ions. Due to the increase in the effective charges of the domain of polyions, reaction between the polyions and aminoacids slow down and conductivity remains unchanged. Besides, the added salts also avoid the active groups to come mutual position in order to react with each other.

The results of specific conductivity as a function of mol ratio also confirmed the stoichiometry obtained by conductometric titrations (Appendix C, Figures C.1 – C.12).

The results of Stability Constants of Complexes are shown in (Table 6.1, 6.2 and Appendix D, Figures D.1 – D.39).

The fundamental principles of the method in order to determine the stability constant is to measure changes in pH depending on the temperature.

For GlycineNa – PAACl complex, between the temperature of 32⁰C – 35⁰C and 40⁰C – 50⁰C, as the temperature increases, Ink increases and between the temperatures of 55⁰C – 60⁰C, 35⁰C – 40⁰C and 28⁰C – 32⁰C as the temperature increases, Ink decreases.

For Glutamic Acid – PAOH complex, between the temperatures of 50⁰C – 55⁰C and 35⁰C – 45⁰C, as the temperature increases, In K increases and between 55⁰C – 60⁰C and 45⁰ – 50⁰C, as the temperature increases, In K decreases and In K stays constant between 28⁰C – 35⁰C.

For Lysine – PAACl complex, In K increases, as the temperature increases between the temperatures of 55⁰C – 60⁰C and 32⁰C – 35⁰C In K stays constant between 35⁰C – 55⁰C.

For Phenylalanine – PAACl complex, In K increases as the temperature increases between 55⁰C – 60⁰C and 32⁰C – 35⁰C. Between the temperatures of 35⁰C – 50⁰C, In K stays constant.

For NaPABA – PAACl complex, In K stays constant between 45⁰C – 55⁰C, Between the temperatures of 55⁰C – 60⁰C and 32⁰C – 40⁰C In K increases between the ranges of 40⁰C – 45⁰C and 28⁰C – 32⁰C, In K decreases and stays constant between 45⁰C – 55⁰C temperature range.

For PABSACl – PAAOH complex, In K increases with increasing temperature between 45⁰C – 60⁰C and stays constant between the temperature range of 28⁰C – 40⁰C. Between the temperatures of 40⁰C – 45⁰C In K decreases with increasing temperature.

For Glycine Na - (NaPO₃)_n complex, between 55⁰C – 40⁰C and 35⁰C – 32⁰C, as the temperature increases, In K increases and between the temperatures of 40⁰C – 35⁰C and 32⁰C – 28⁰C, as the temperature increases, In K decreases.

For glutamic acid -(NaPO₃)_n complex, between 55⁰C – 45⁰C, as the temperature increases In K decreases and between 45⁰C – 32⁰C as the temperature increases, In K decreases. Between 28⁰-32⁰C In K stays constant.

For LysineNa – (NaPO₃)_n complex between the temperatures of 60⁰C – 45⁰C and 35⁰C – 40⁰C, as the temperature increases, InK increases. Between 32⁰C – 28⁰C, as the temperature increases, In K decreases.

For PhenylalanineNa – (NaPO₃)_n complex, between the temperatures of 55⁰C – 45⁰C and 32⁰C – 35⁰C as the temperature increases, In K increases. Between the temperatures of 32⁰C – 28⁰C and 35⁰C – 45⁰C, as the temperature increases, In K decreases.

For NaPABA – (NaPO₃)_n complex, between 60⁰C – 45⁰C and 35⁰C – 32⁰C, as the temperature increases, In K increases and between the temperatures of 35⁰C - 45⁰C and 28⁰C-32⁰C, as the temperature increases, In K decreases.

For PABSA – (NaPO₃)_n complex, between 55⁰C – 60⁰C and 45⁰C – 50⁰C as the temperature increases, lnK increases and between the temperatures of 50⁰C – 55⁰C and 28⁰C - 32⁰C, as the temperature increases, lnK decreases. lnK stays constant between 32⁰C - 45⁰C.

The linear parts of the curves of $\ln K = f(1/TK)$ which corresponds the result of increases in temperature and lnK provides the calculation of activation energy of the reaction between polyions and aminoacids. The result obtained from the literature is related with activation energy of this kind of interactions are considered relatively reasonable. The calculated stability constants shown in the Table 3.8 and 3.9 can also be accepted in a reasonable strength in connection with the activation energy of the reaction.

IR spectra of the interaction between amino acids and polycation – polyonion was given in Appendix E Figure E.1 – E.21. The results confirmed the binding phenomena between the interacted species.

REFERENCES

- [1] **Acar, N. and Tulun, T.**, 1996. Studies on the Interaction of Poly (4-vinylpyridiniumchloride) with Poly (sodiumphosphate) in an Aqueous Solution by Conductometry, *Journal of Polymer Science: Part A: Polymer Chemistry*, **34**, 1251-1256.
- [2] **Acar, N., Huglin M.B. and Tulun, T.**, 1999. Complex Formation Between Poly (Sodium phosphate) and Poly (4-vinylpyridiniumchloride) in Aqueous Solution, *Polymer*, **40**, 6429-6435.
- [3] **Acar, N., Tulun, T.**, 2001. Interaction of polymer-small molecule complex with cupric (II)ions in aqueous solution, *European Polymer Journal* **37**, 1599-1607.
- [4] **Gün, H.**, 1990. Studies on Polyelectrolyte Properties of Polyphosphates, *M. Sc. Thesis*, İ.T.Ü. Fen Bilimleri Enstitüsü, İstanbul.
- [5] **Acar, N. and Tulun, T.**, 2001. Investigation of interaction between poly (sodium phosphate) and p-aminobenzoic acid, hydrochloride, *European Polymer Journal*, **37**, 651-657.
- [6] **Cramer, C.L. and Davis, R.H.**, 1984. Polyphosphate-Cation Interaction in the Amino Acid-containing Vacuole of *Neurospora crassa*, *The Journal of Biological Chemistry*, **259**, 5152-5157.
- [7] **Raszka, M. and Mandel, M.**, 1971. Interaction of Aromatic Amino Acids with Neutral *Polyadenylic Acid*, *Proc. Nat. Acad. Scie. USI*, **68**, 1190-1191.
- [8] **Kopylova, V., Peregudov, Y. and Astapov, A.**, 1975. The enthalpies of interaction of polyvinylbenzyltrimethylammonium hydroxide with amino acids in aqueous solutions, *Russian Journal of Physical Chemistry A*, **81**, 731-734.
- [9] **Moriyama, Y. and Takeda, K.**, 2006. Ionic Surfactant Interactions with Polyelectrolytes, Poly L-Amino Acids, and Dextran Sulfates, *Encyclopedia of Surface and Colloid Science*.
- [10] Differential effect of amino acid residues on the stability of double helices formed from polyribonucleotides and its possible relation to the evolution of the genetic code, *Journal of Molecular Evolution*, **21**, February 1985.
- [11] **Horn, D. and Heuck, C.C.**, 1983. Charge Determination of Proteins with Polyelectrolyte Titration, *The Journal of Biological Chemistry*, USA, **258** (3), 1665-1670.
- [12] **Briggs, D.R.**, 1940. The Metaphosphoric Acid-Protein Reaction, *The Journal of Biological Chemistry*, The Division of Agricultural Biochemistry, University of Minnesota, 261-272.

- [13] **Tapia, M., Burrows, H.D., Knaapila, M., Monkman, A.P., Arroyo, A., Pradhan, S., Scherf, U., Pinazo, A., Pèrez, L. and Moran, C., 2006.** Interaction between the Conjugated Polyelectrolyte Poly (1, 4-phenylene [9,9-bis(4-phenoxybutylsulfonate)] fluorine-2,7-diyl] Copolymer and the Lecithin Mimic 1-O-(L-Arginyl)-2, 3-O-dilauroyl-sn-glycerol in Aqueous Solution, *Acs Publications*, **22**(24), 10170-10174.
- [14] **Ander, P. and Kardan, M., 1984.** Interactions of Sodium Ions with Polyelectrolytes of Varying Charge Density, *Macromolecules*, **17**, 2431-2436.
- [15] **Wang, L. and Yu, H., 1988.** Chain Conformation of Linear Polyelectrolyte in Salt Solutions: Sodium Poly (styrene sulfonate) in Potassium Chloride and Sodium Chloride Solutions, *Macromolecules*, **21**, 3498-3501.
- [16] **Hahn, S.K. and Hoffman, A.S., 2005.** Preparation and characterization of biocompatible polyelectrolyte complex multilayer of hyaluronic acid and poly-L-lysine, *International Journal of Biological Macromolecules*, **37**, 227-231.
- [17] **Möller, M. and Nordmeier, E., 2001.** Polyelectrolyte complexes formed by poly (diallyl-N, N-dimethylammoniumchloride) and oligo (dextran sulfate), *European Polymer Journal*, Germany , 445-450.
- [18] **Yamanaka, J., Matsuoka, H., Kitano, H., Hasegawa, M. and Ise, N., 1990.** Revisit to the Intrinsic Viscosity-Molecular Weight Relationship of Ionic Polymers. 2. Viscosity Behavior of Salt-Free Aqueous Solutions of Sodium Poly (styrenesulfonates), *J. Am. Chem. Soc.*, **112**, 587-591.
- [19] **Hara, M., 1993.** Polyelectrolytes: Science and Technology, Dekker, New York.

APPENDIX A

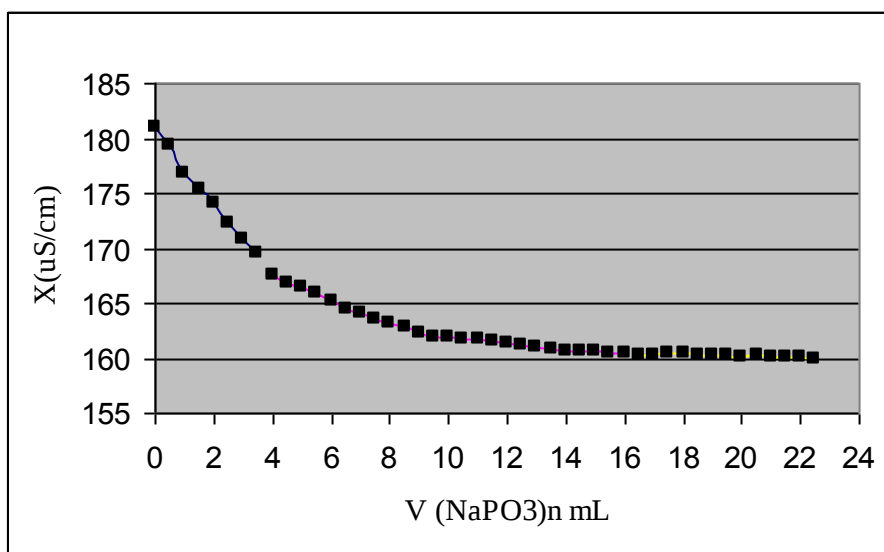


Figure A.1: 10^{-4}M Glycine with 10^{-4}M $(\text{NaPO}_3)_n$ in $(I=10^{-3})$

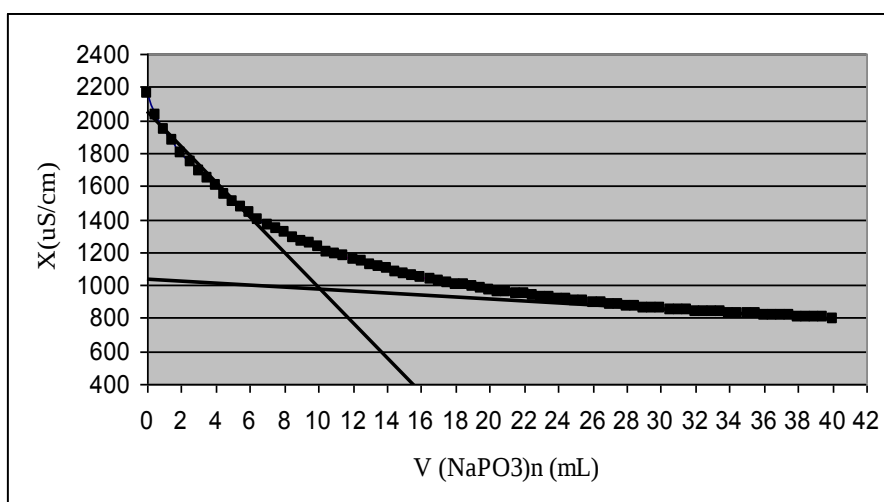


Figure A.2: 10^{-2}M GlycineCl with 10^{-2}M $(\text{NaPO}_3)_n$ in salt free solution

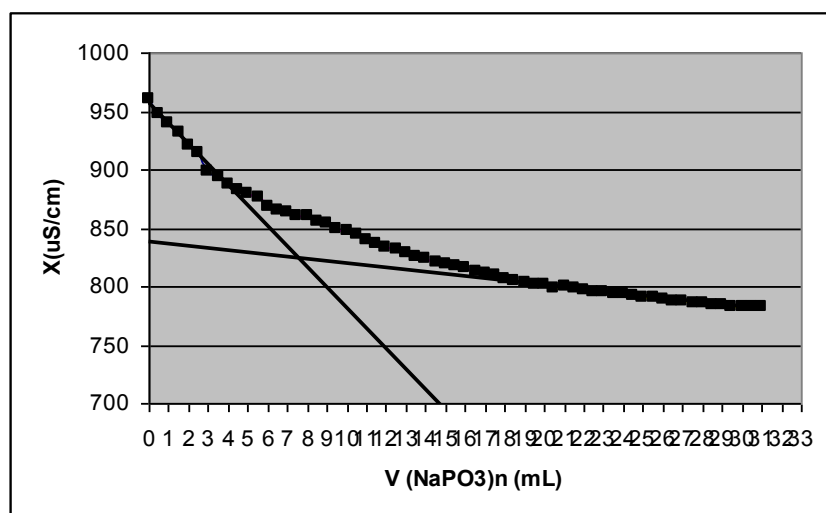


Figure A.3: 10^{-2} M Glutamic Acid with 10^{-2} M (NaPO₃)_n in salt free solution

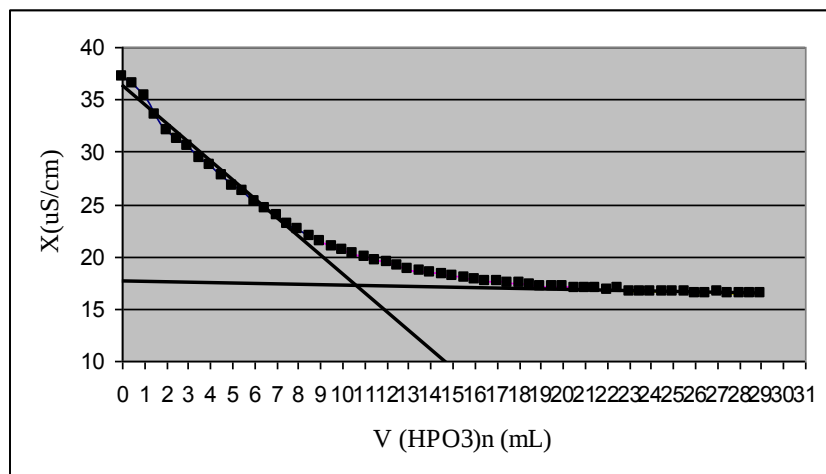


Figure A.4: 10^{-2} M Glutamic AcidNa with 10^{-2} M (NaPO₃)_n in salt free solution

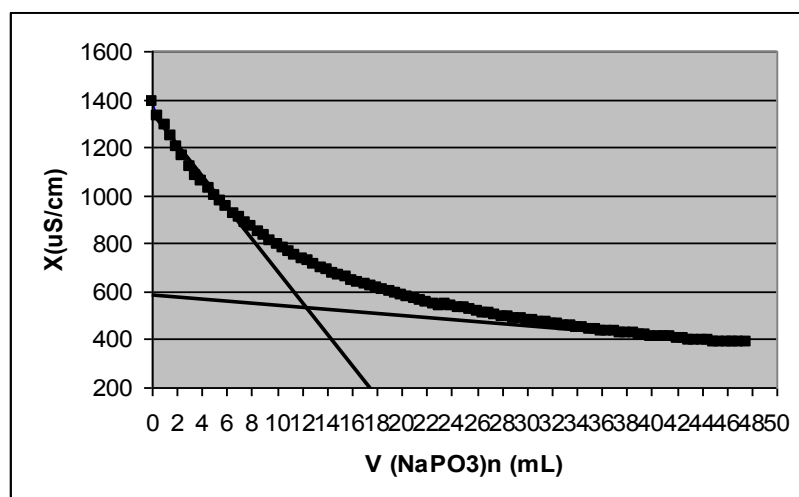


Figure A.5: 10^{-2} M Glutamic AcidCl with 10^{-2} M (NaPO₃)_n in salt free solution

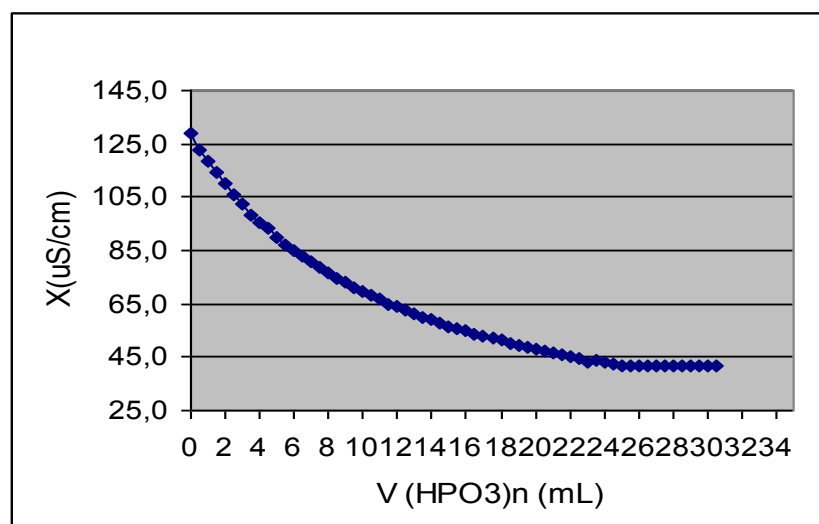


Figure A.6: 10^{-2} M Lysine with 10^{-2} M (HPO3)n in salt free solution

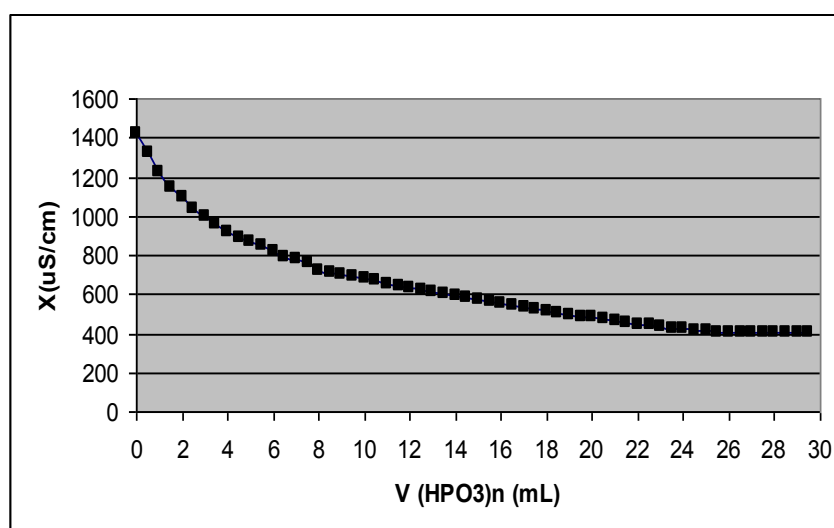


Figure A.7: 10^{-2} M LysineNa with 10^{-2} M (HPO3)n in salt free solution

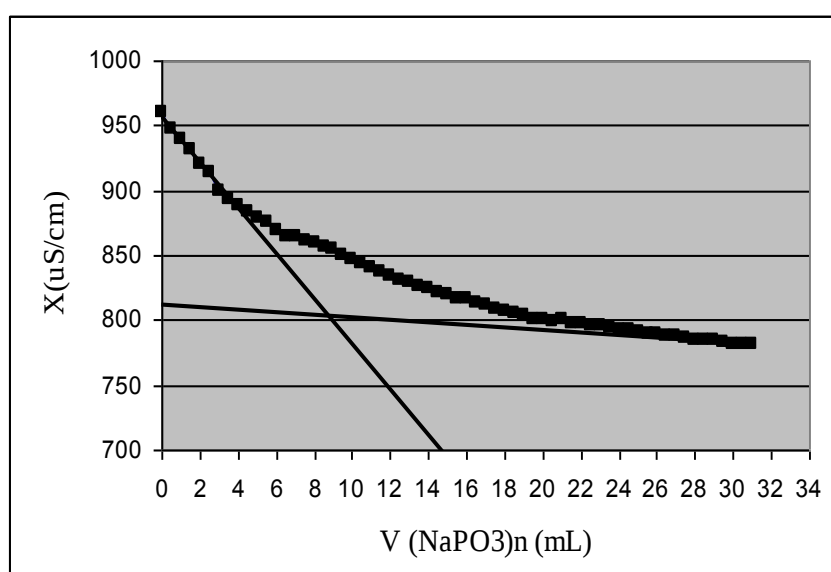


Figure A.8: 10^{-2} M LysineCl with 10^{-2} M (NaPO3)n in salt free solution

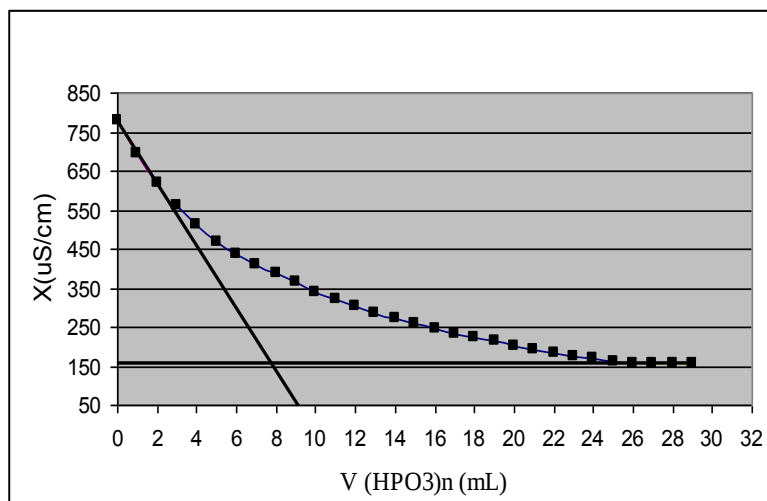


Figure A.9: 10^{-2} M PhenylalanineNa with 10^{-2} M (HPO3)n in salt free solution

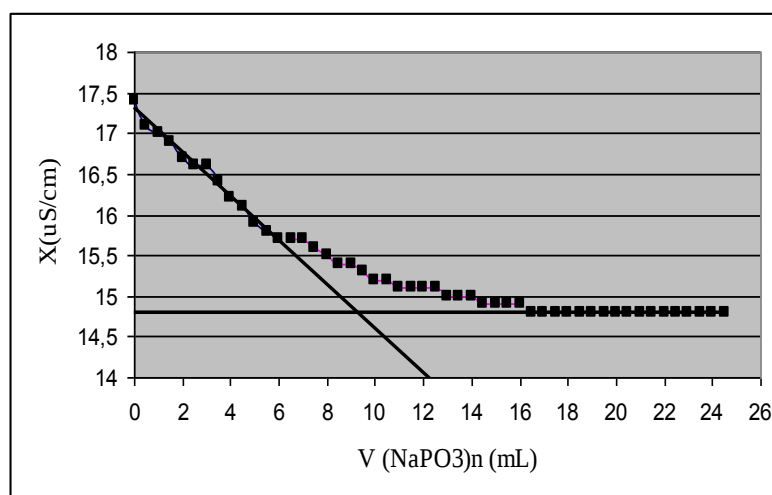


Figure A.10: 10^{-4} M PABA with 10^{-4} M (NaPO3)n in salt free solution

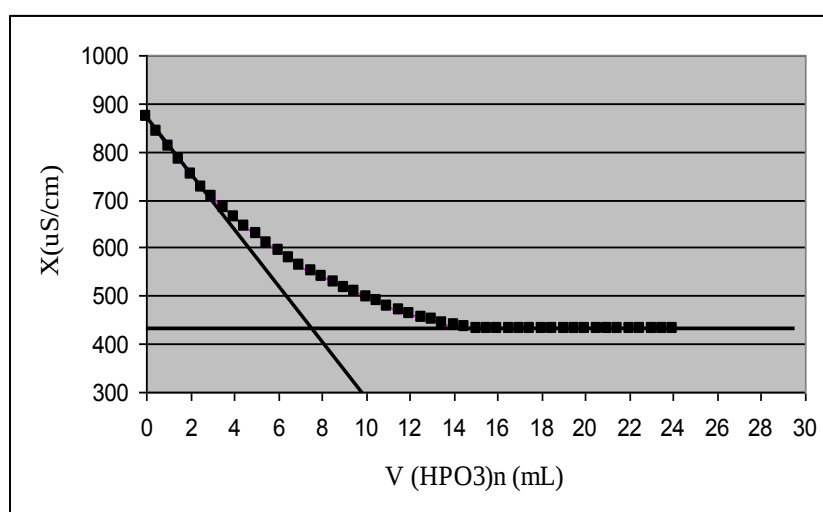


Figure A.11: 10^{-2} M NaPABA with 10^{-2} M (HPO3)n in salt free solution

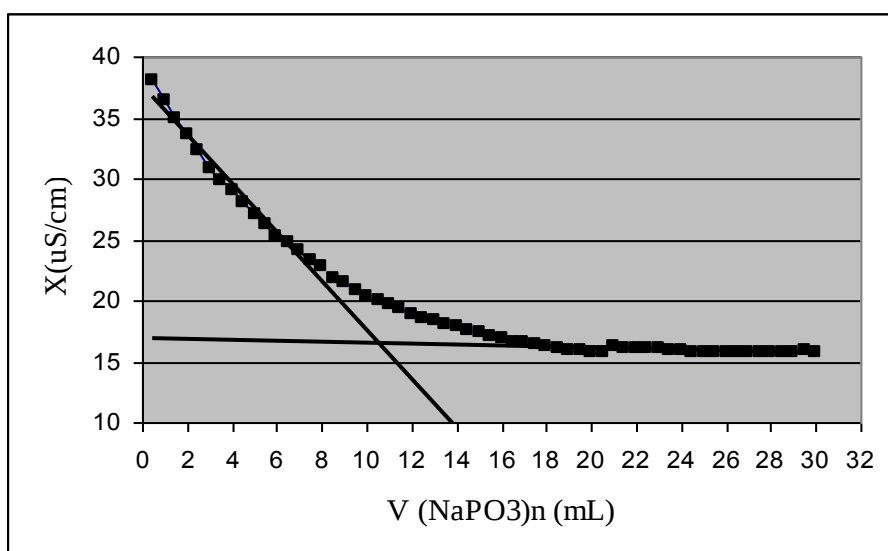


Figure A.12: 10^{-2} MPABSA with 10^{-2} M (NaPO₃)_n in salt free solution

APPENDIX B

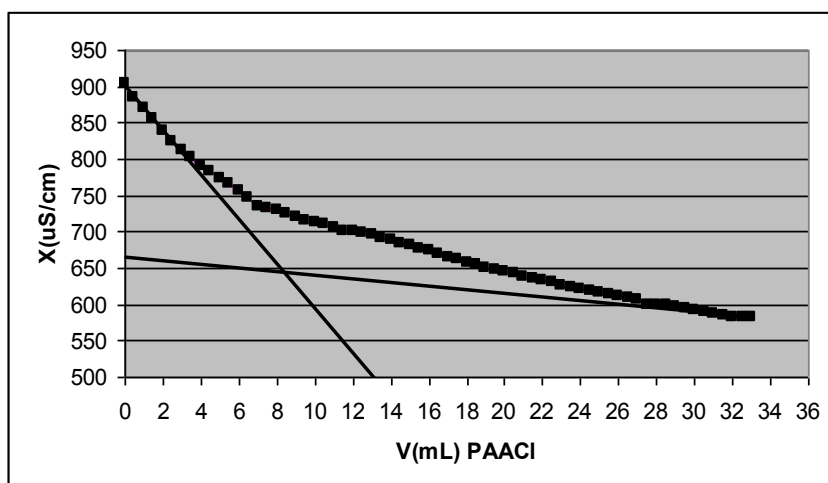


Figure B.1: 10^{-2} M GlycineNa with 10^{-2} M PAAHCl in salt free solution

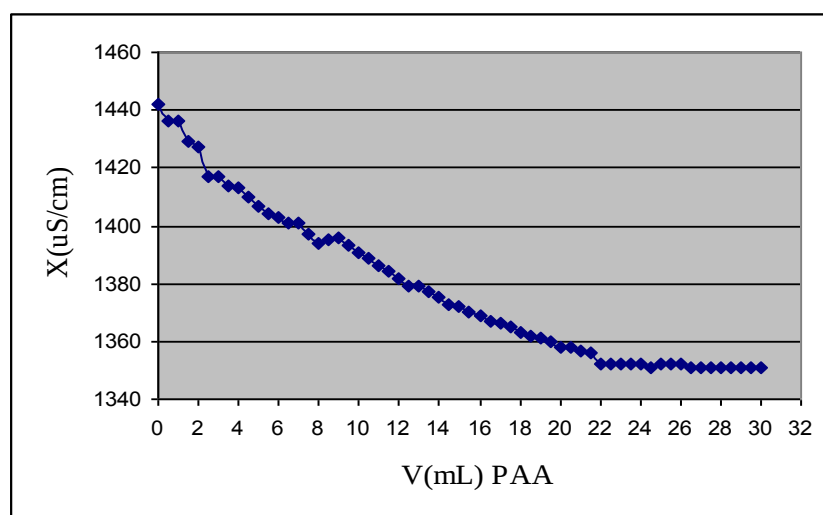


Figure B.2: 10^{-3} M GlycineNa with 10^{-3} M PAAHCl in $I=10^{-2}$

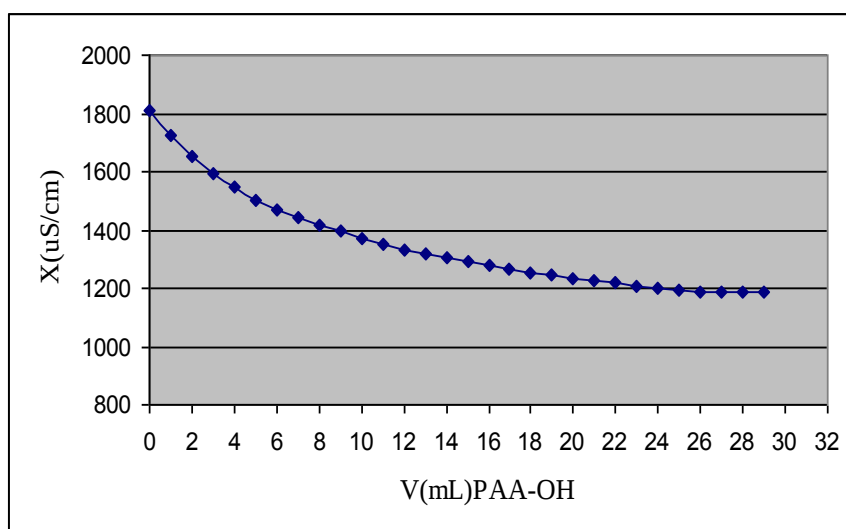


Figure B.3: 10^{-2} M Glutamic Acid with 10^{-3} M PAAOH in salt free solution

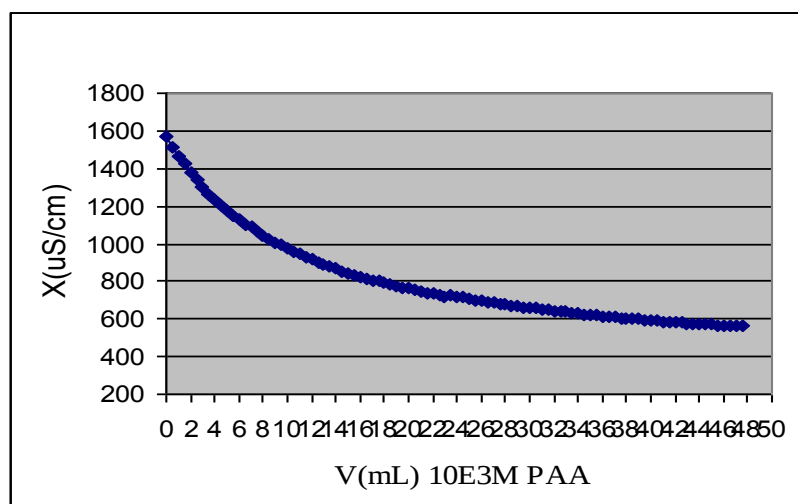


Figure B.4: 10^{-3} M Glutamic AcidNa with 10^{-3} M PAAHCl in $I=10^{-2}$

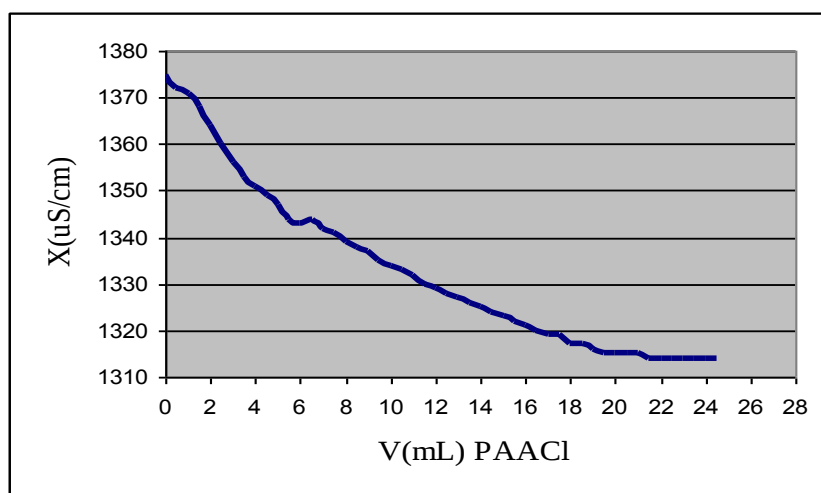


Figure B.5: 10^{-3} M Lysine with 10^{-3} M PAAHCl in $I=10^{-2}$

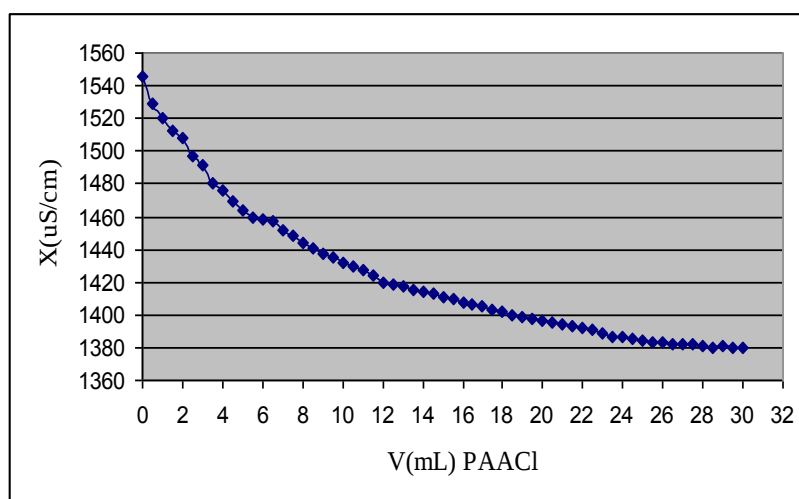


Figure B.6: 10^{-3} M LysineNa with 10^{-3} M PAAHCl in $I=10^{-2}$

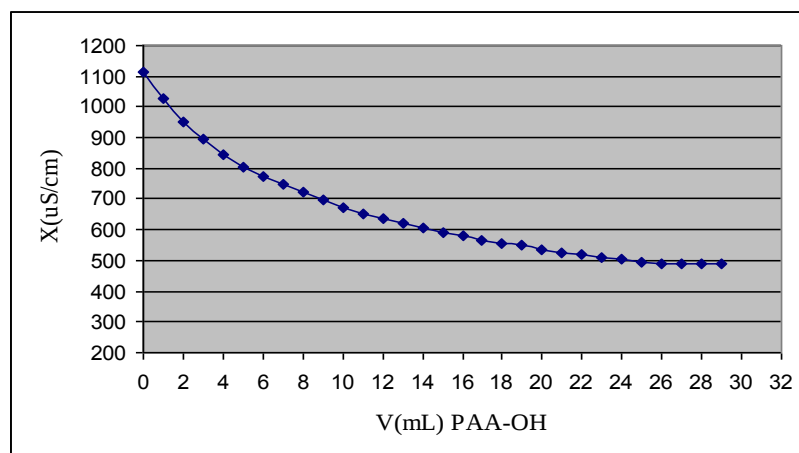


Figure B.7: 10^{-2} M LysineCl with 10^{-2} M PAAOH in salt free solution

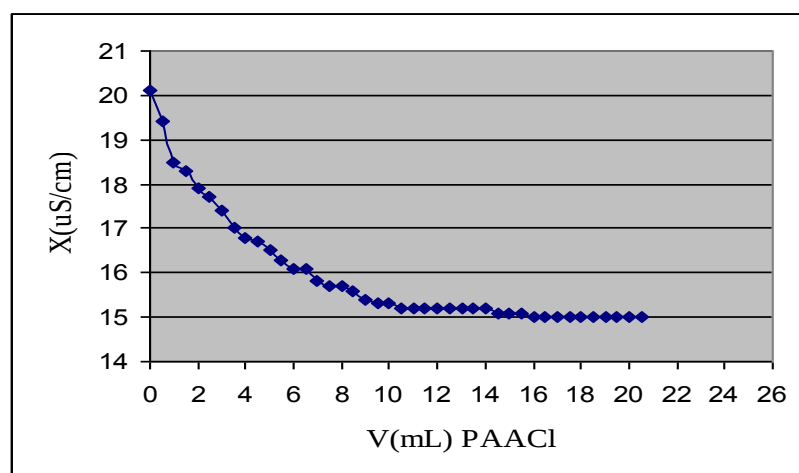


Figure B.8: 10^{-4} M PhenylalanineNa with 10^{-2} M PAAHCl in $I=10^{-3}$

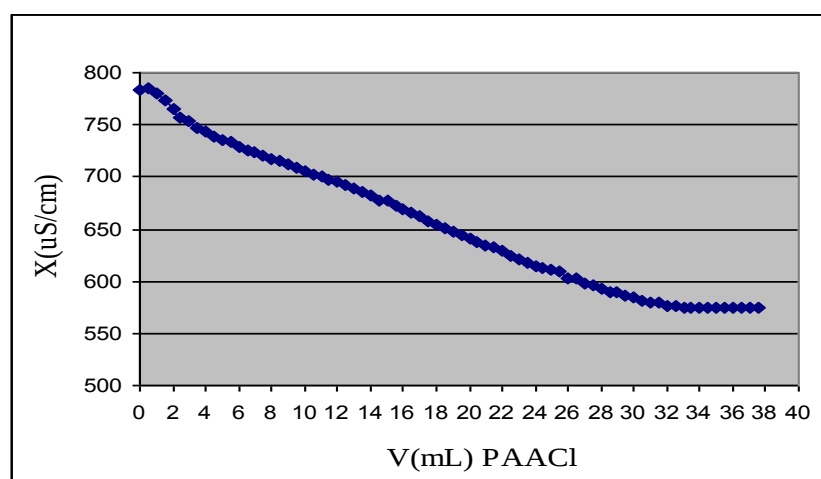


Figure B.9: 10^{-2} M PhenylalanineNa with 10^{-2} M PAAHCl in salt free solution

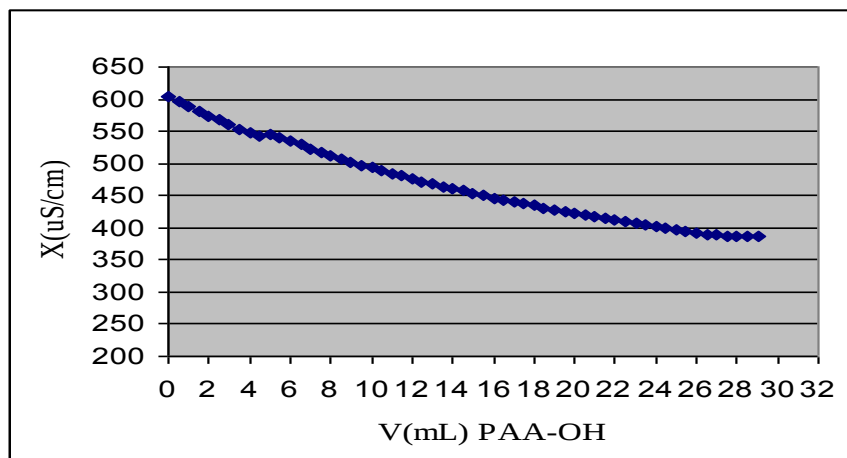


Figure B.10: 10^{-2} M PhenylalanineCl with 10^{-2} M PAAOH in salt free solution

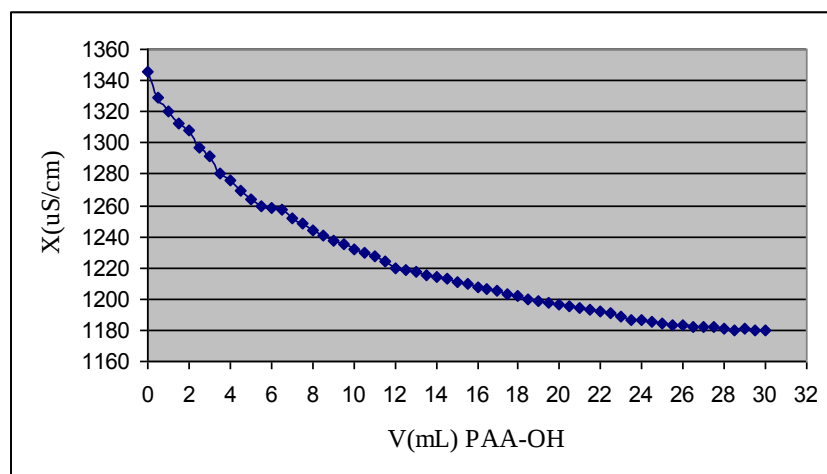


Figure B.11: 10^{-3} MPABAClwith 10^{-3} M PAA-OH in $I=10^{-2}$

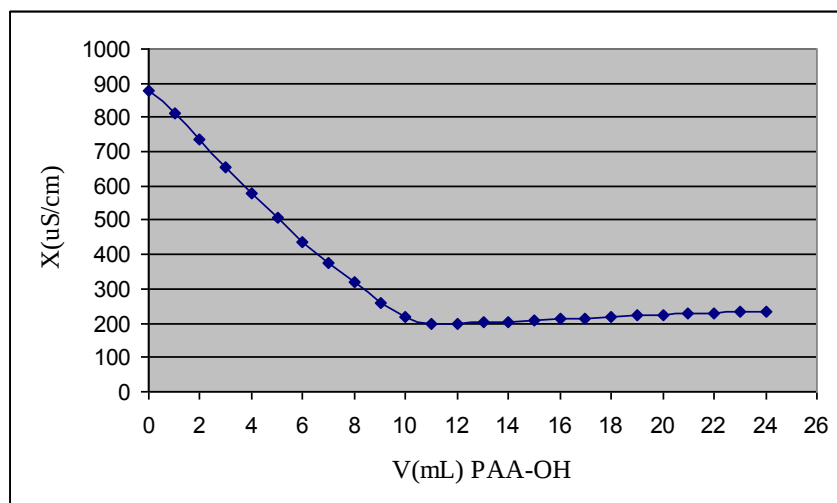


Figure B.12: 10^{-2} M PABSA with 10^{-2} M PAAOH in salt free solution

APPENDIX C

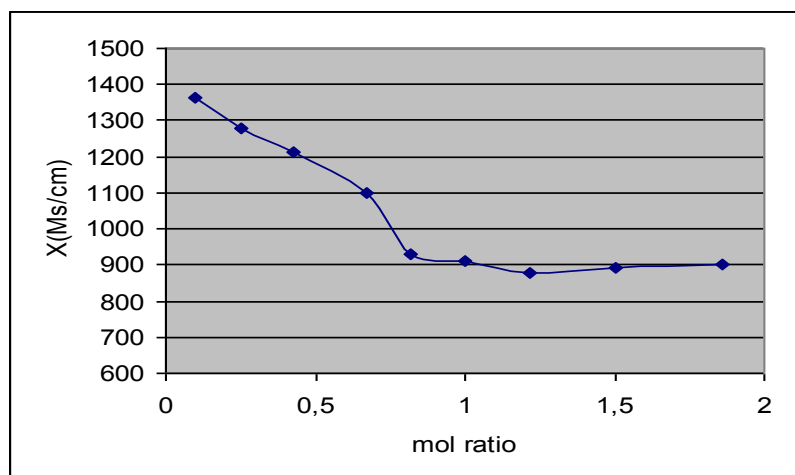


Figure C.1: 1.10^{-2} M GlycineNa and 1.10^{-2} M $(\text{HPO}_3)_n$ mixture in salt free solution

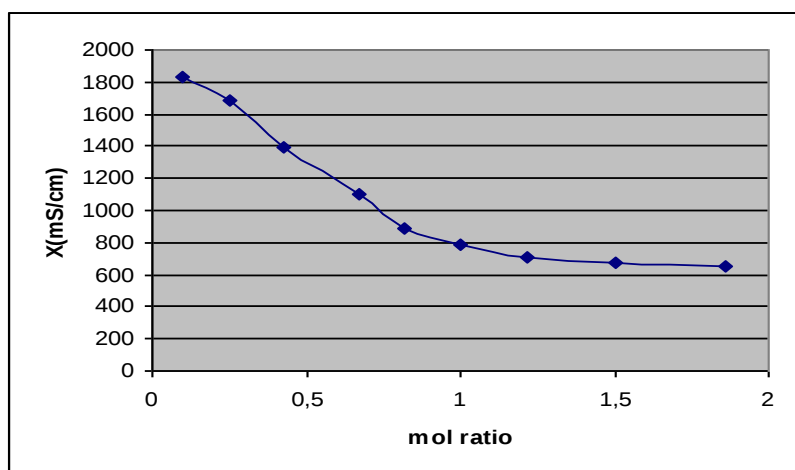


Figure C.2: 1.10^{-2} M Glutamic Acid and 1.10^{-2} M $(\text{NaPO}_3)_n$ mixture in salt free solution

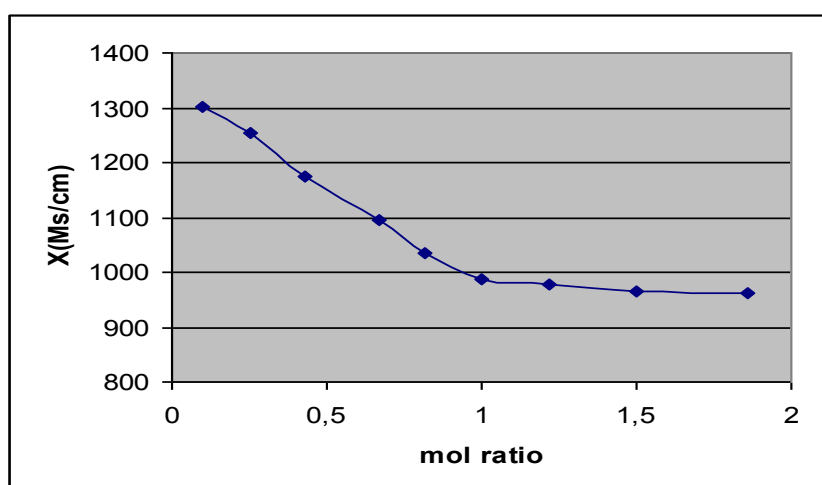


Figure C.3: 1.10^{-2} M LysineNa and 1.10^{-2} M $(\text{HPO}_3)_n$ mixture in salt free solution

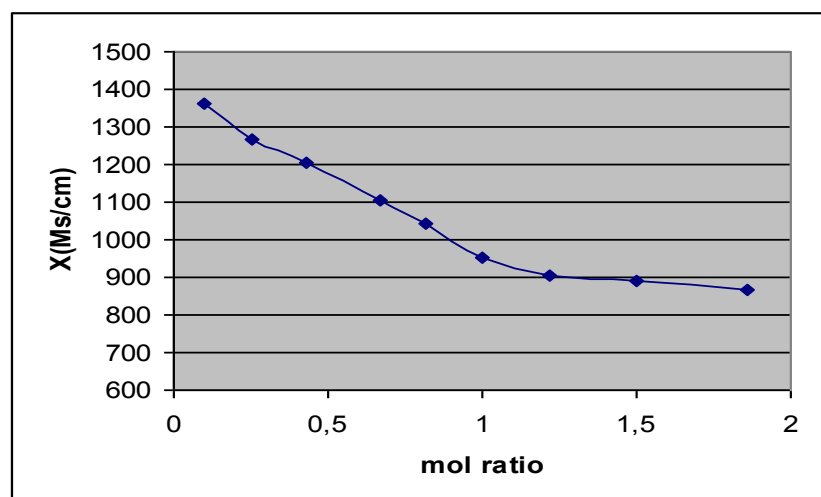


Figure C.4: 1.10^{-2} M PhenylalanineNa and 1.10^{-2} M $(\text{HPO}_3)_n$ mixture in salt free solution

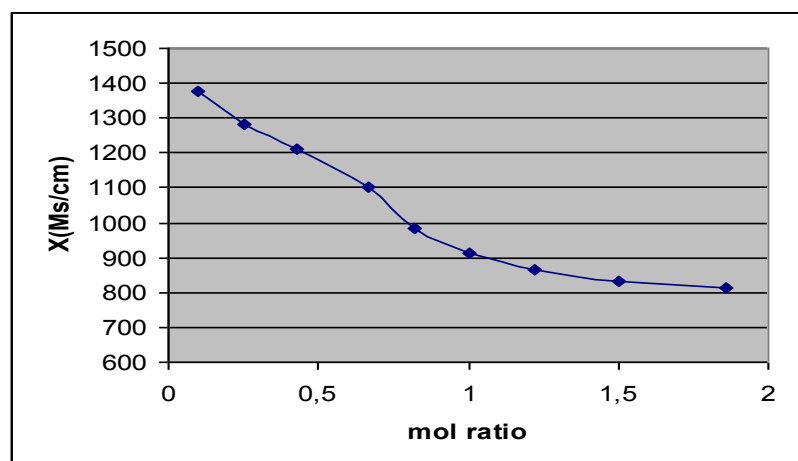


Figure C.5: 1.10^{-2} M NaPABA and 1.10^{-2} M $(\text{HPO}_3)_n$ mixture in salt free solution

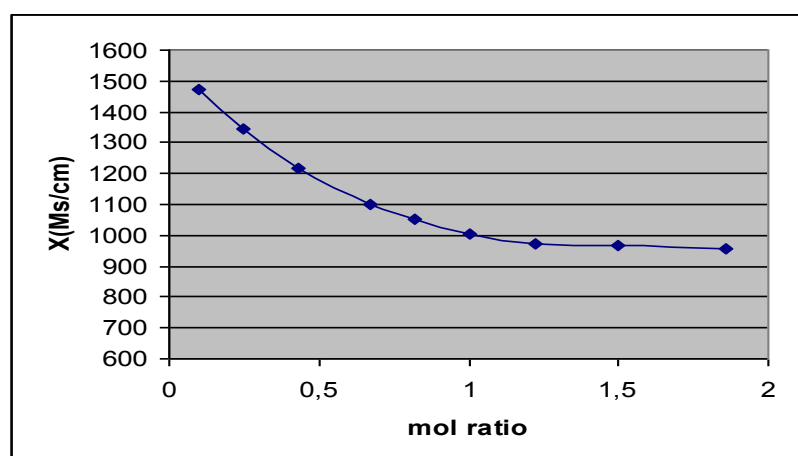


Figure C.6: 1.10^{-2} M NaPABSA and 1.10^{-2} M $(\text{HPO}_3)_n$ mixture in salt free solution

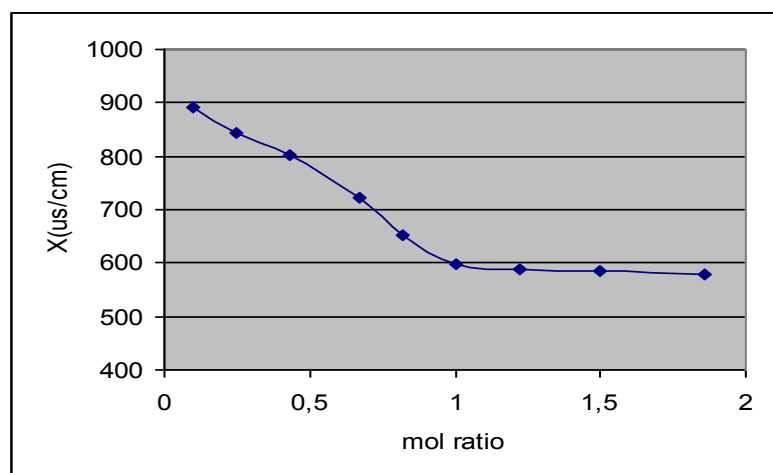


Figure C.7: 1.10^{-2} M Glycine and 1.10^{-2} M Na-PAAHCl mixture in salt free solution

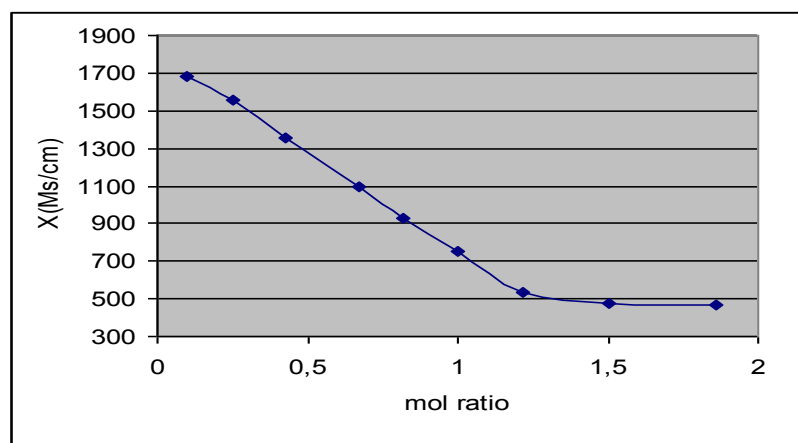


Figure C.8: 1.10^{-2} M Glutamic Acid and 1.10^{-2} M PAAOH mixture in salt free solution

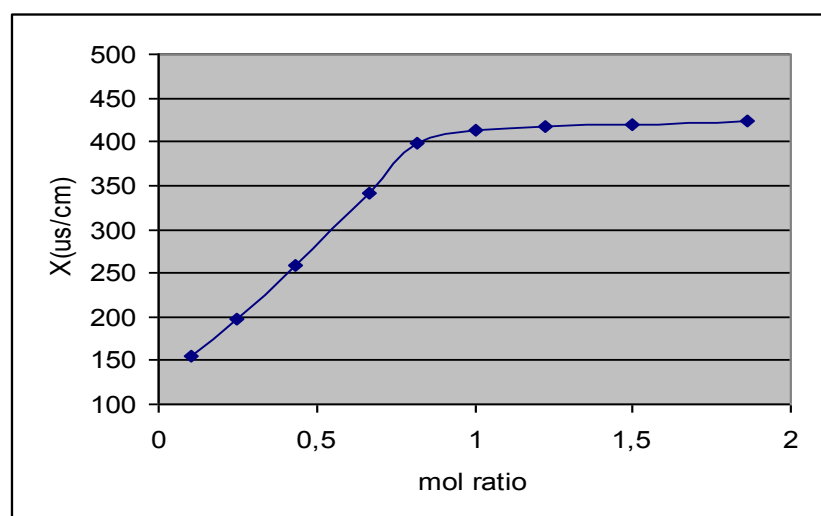


Figure C.9: 1.10^{-2} M LysineNa and 1.10^{-2} M PAAHCl mixture in salt free solution

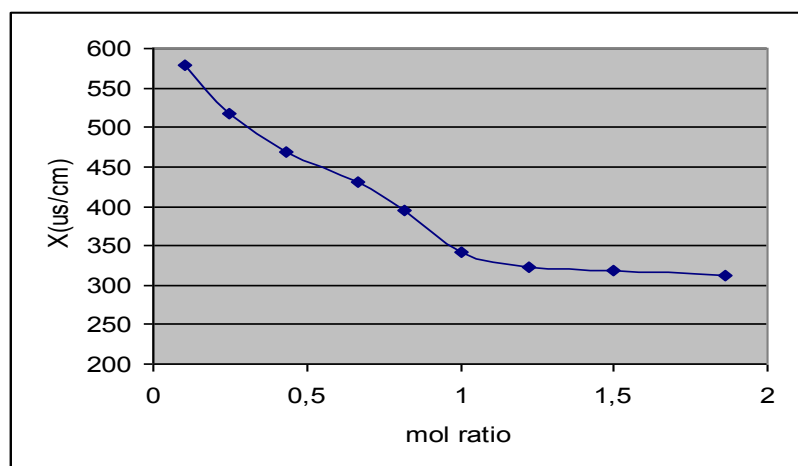


Figure C.10: 1.10^{-2}M PhenylalanineCl and 1.10^{-2}M PAAOH mixture in salt free solution

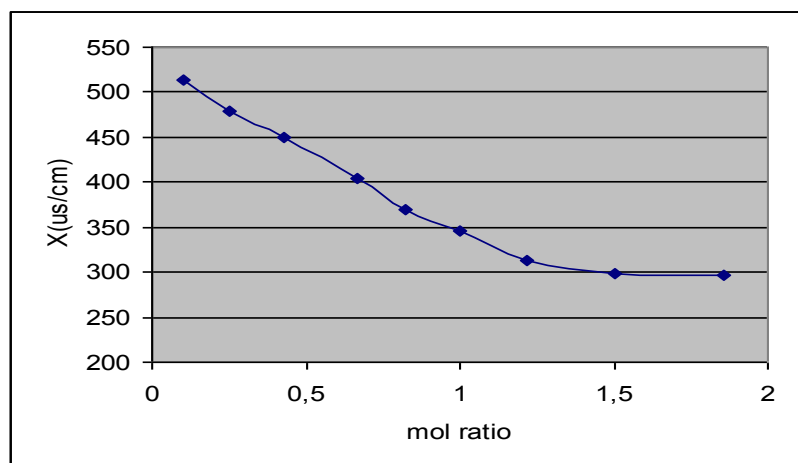


Figure C.11: 1.10^{-2}M PABACl and 1.10^{-2}M PAAOH mixture in salt free solution

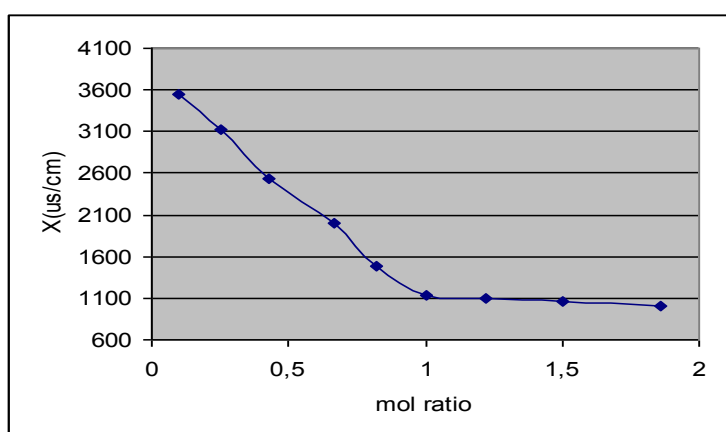


Figure C.12: 1.10^{-2}M PABSACl and 1.10^{-2}M PAAOH mixture in salt free solution

APPENDIX D

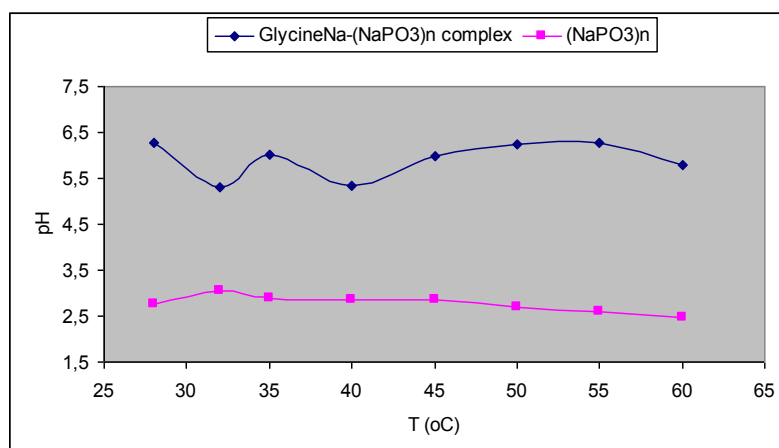


Figure D.1: pH Curve of GlycineNa – (NaPO3)_n Complex

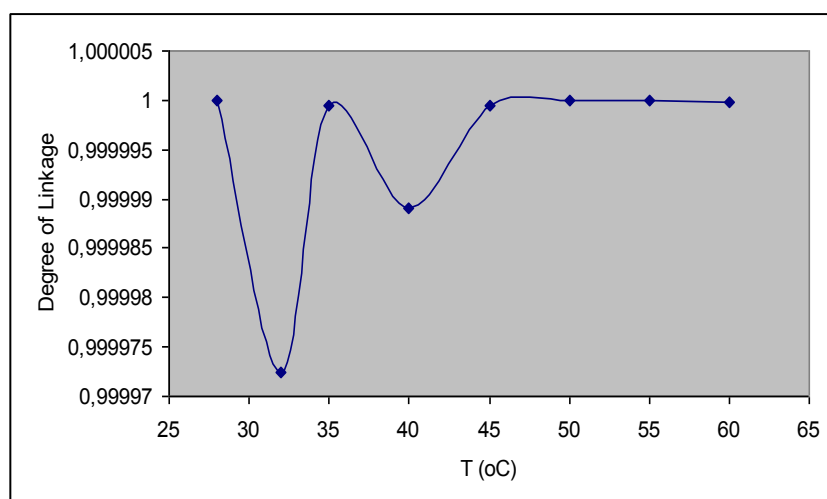


Figure D.2: Degree of Linkage for GlycineNa – (NaPO3)_n Complex

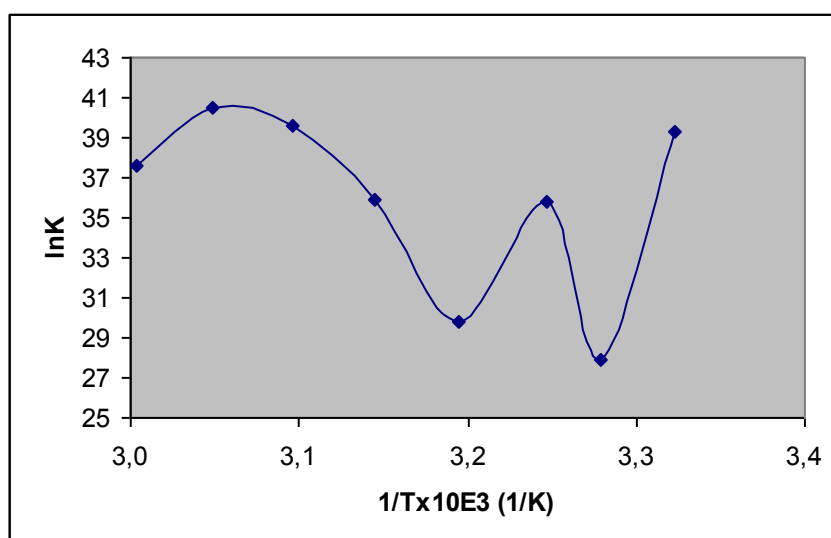


Figure D.3: Stability Constant of GlycineNa – (NaPO3)_n Complex

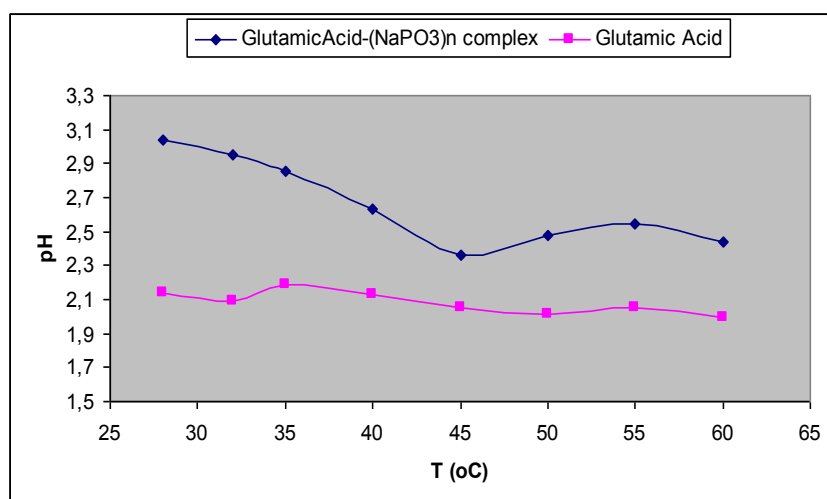


Figure D.4: pH Curve of Glutamic Acid – (NaPO3)_n Complex

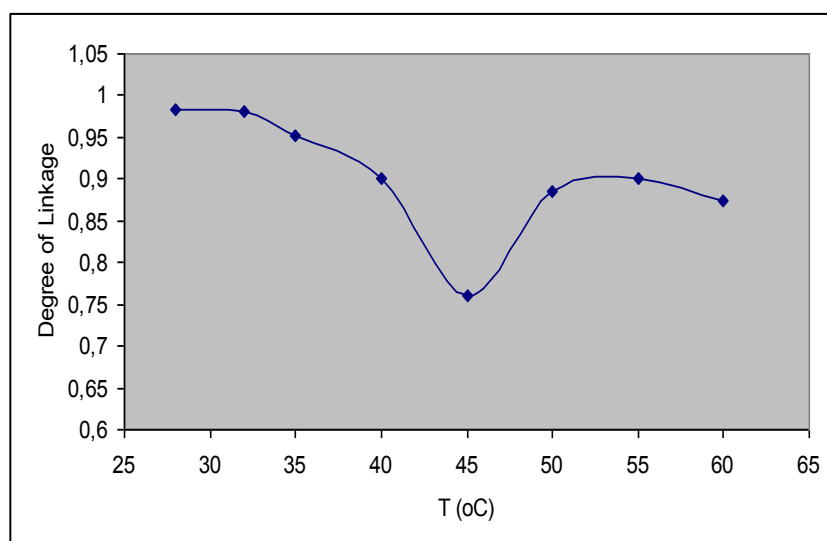


Figure D.5: Degree of Linkage for Glutamic Acid – (NaPO3)_n Complex

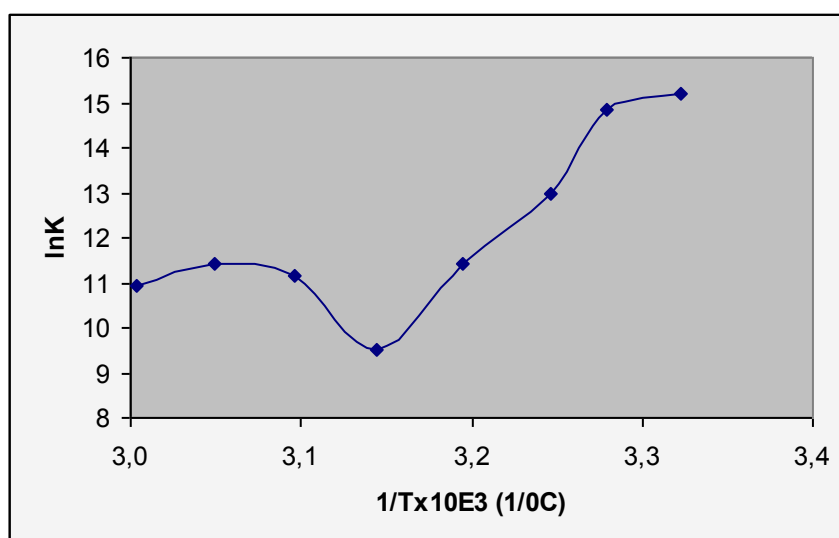


Figure D.6: Stability Constant of Glutamic Acid – (NaPO3)_n Complex

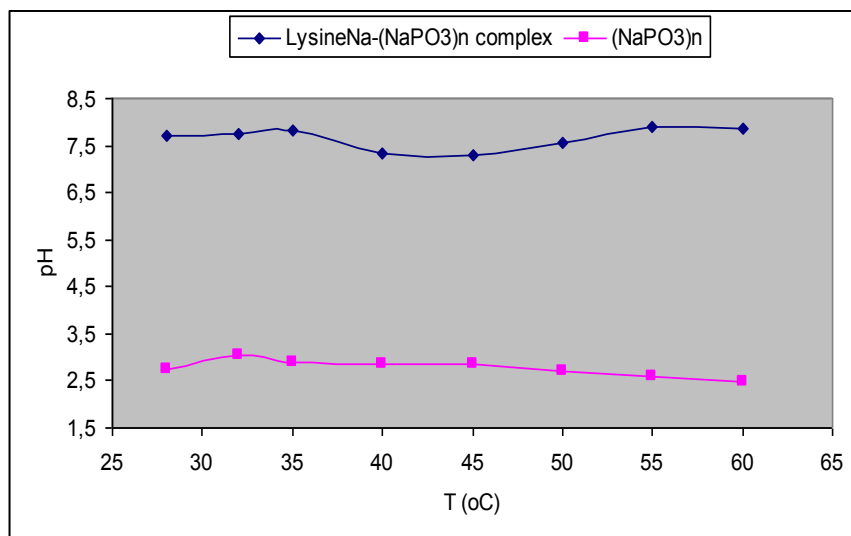


Figure D.7: pH Curve of LysineNa – (NaPO3)_n Complex

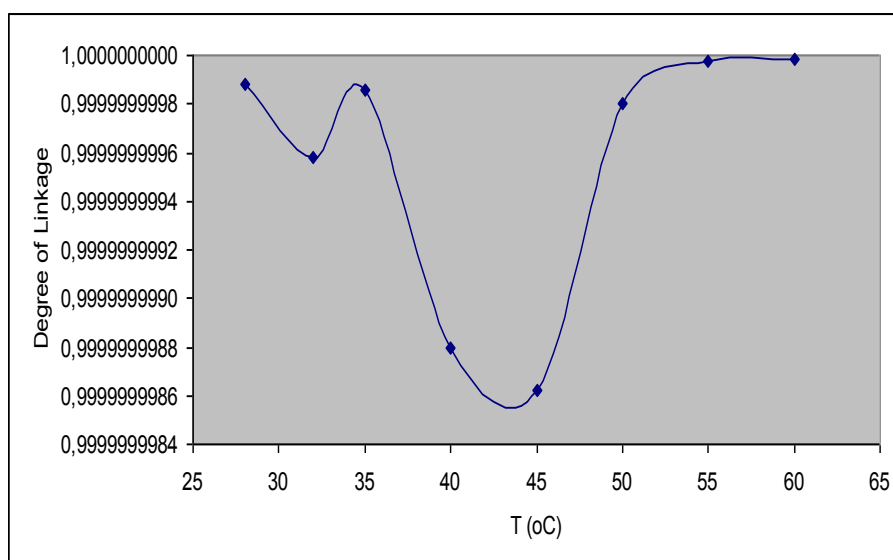


Figure D.8: Degree of Linkage for LysineNa – (NaPO3)_n Complex

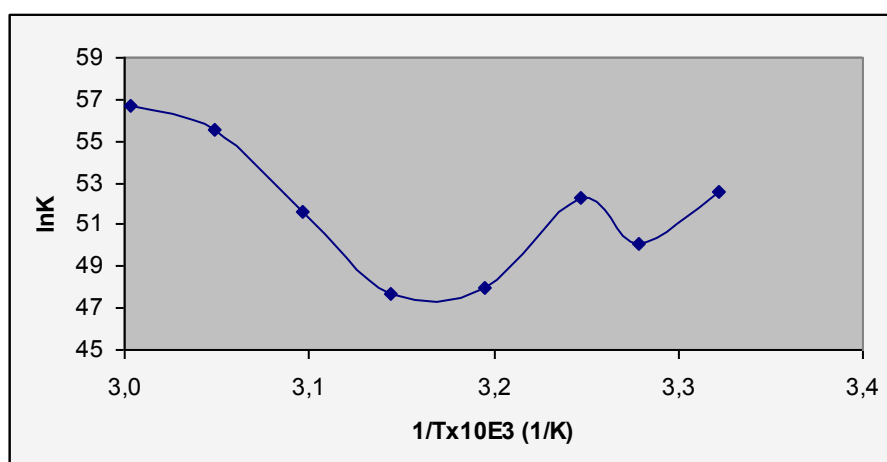


Figure D.9: Stability Constant of LysineNa – (NaPO3)_n Complex

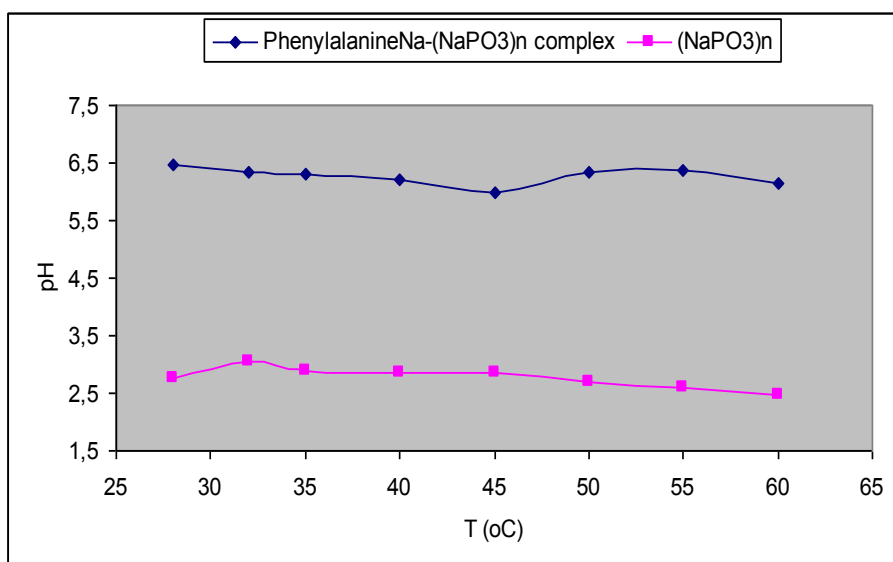


Figure D.10: pH Curve of PhenylalanineNa – (NaPO3)n Complex

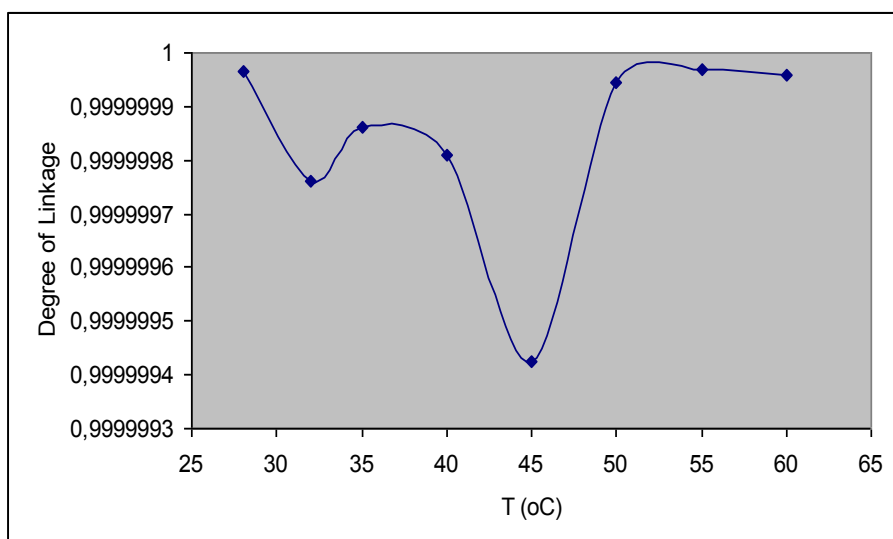


Figure D.11: Degree of Linkage of PhenylalanineNa – (NaPO3)n Complex

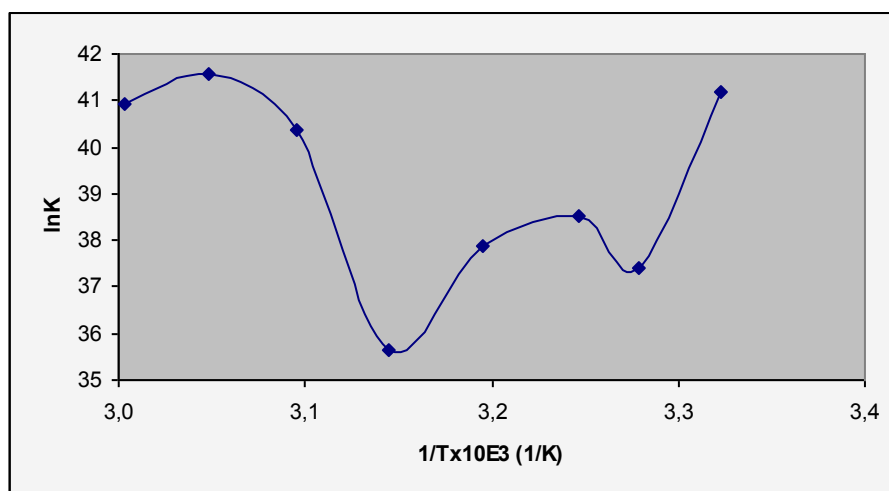


Figure D.12: Stability Constant of PhenylalanineNa – (NaPO3)n Complex

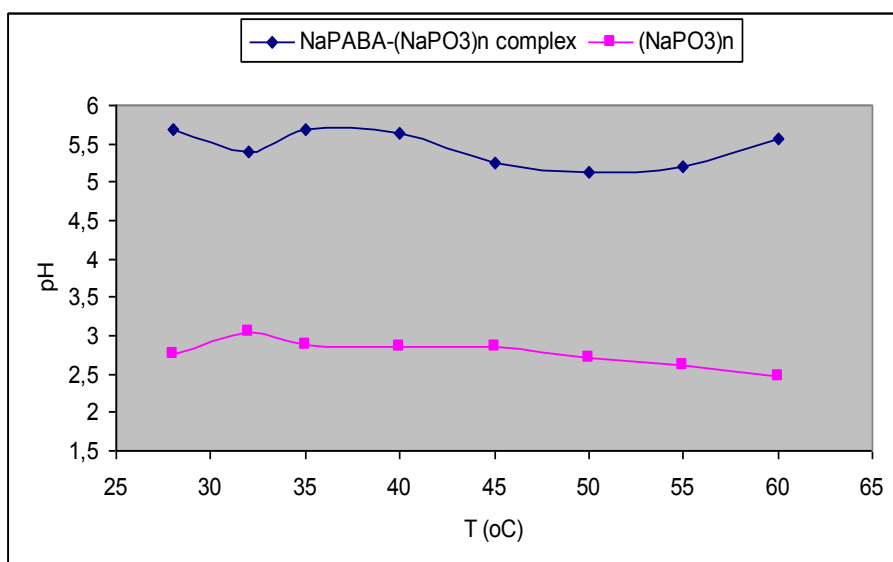


Figure D.13: pH Curve of NaPABA – (NaPO3)n Complex

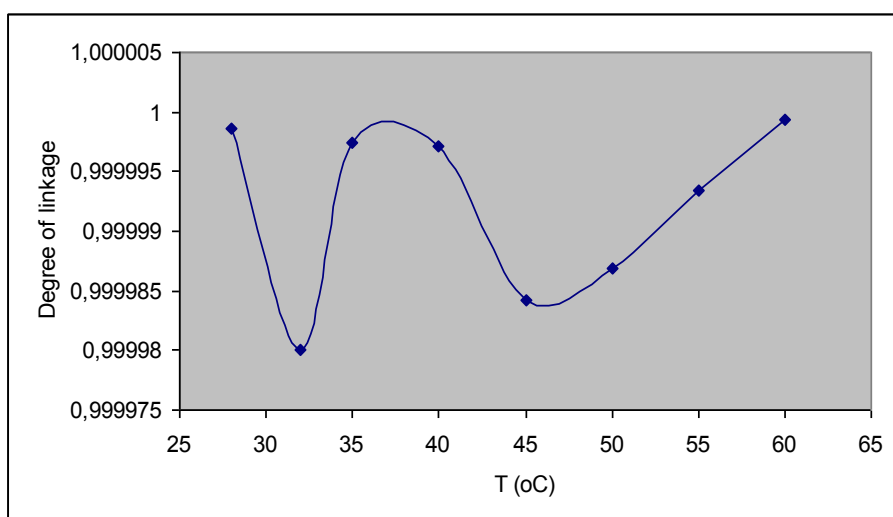


Figure D.14: Degree of Linkage for NaPABA – (NaPO3)n Complex

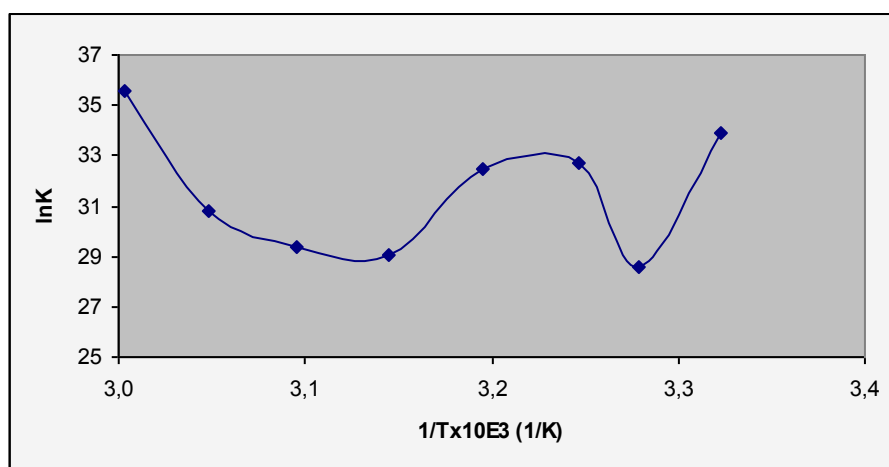


Figure D.15: Stability Constant of NaPABA – (NaPO3)n Complex

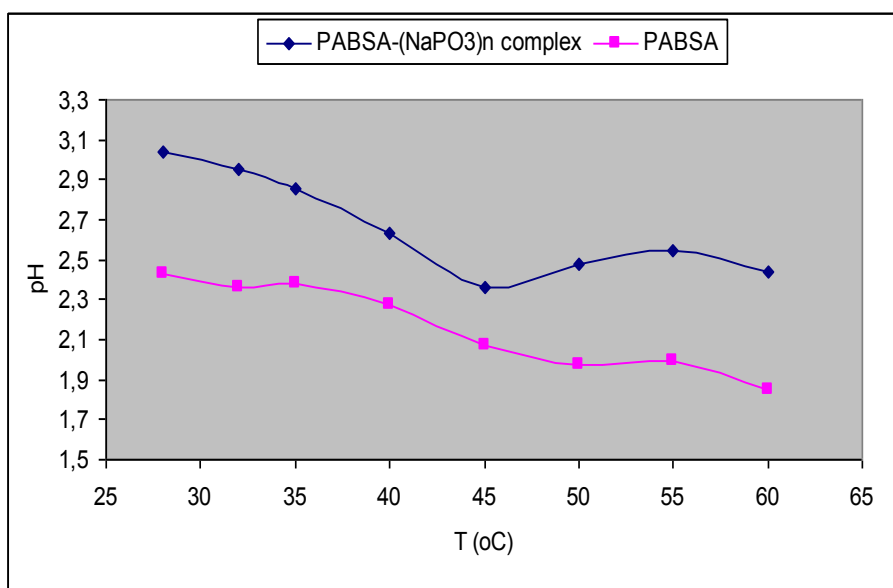


Figure D.16: pH Curve of PABSA – (NaPO3)_n Complex

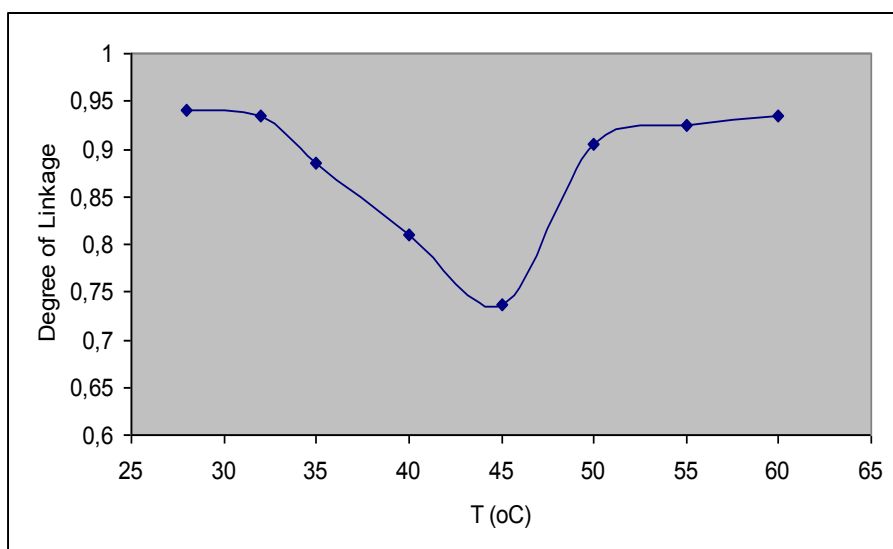


Figure D.17: Degree of Linkage for PABSA – (NaPO3)_n Complex

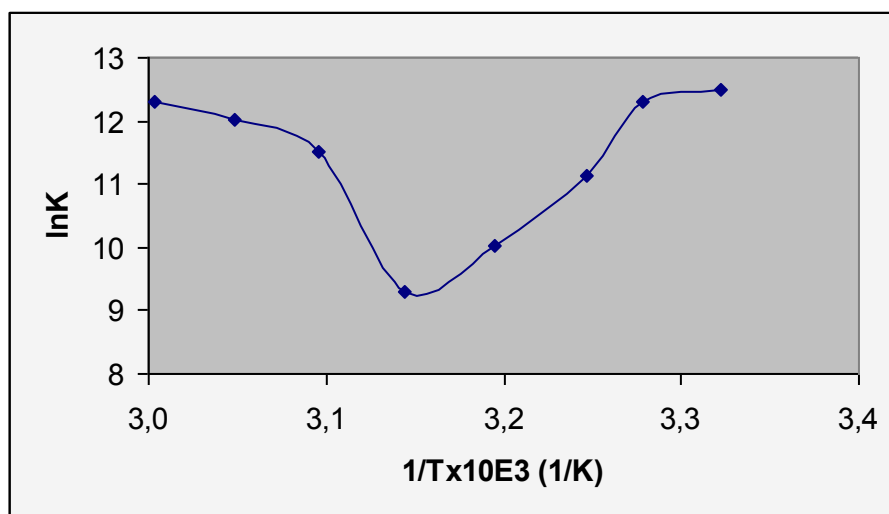


Figure D.18: Stability Constant of PABSA – (NaPO3)_n Complex

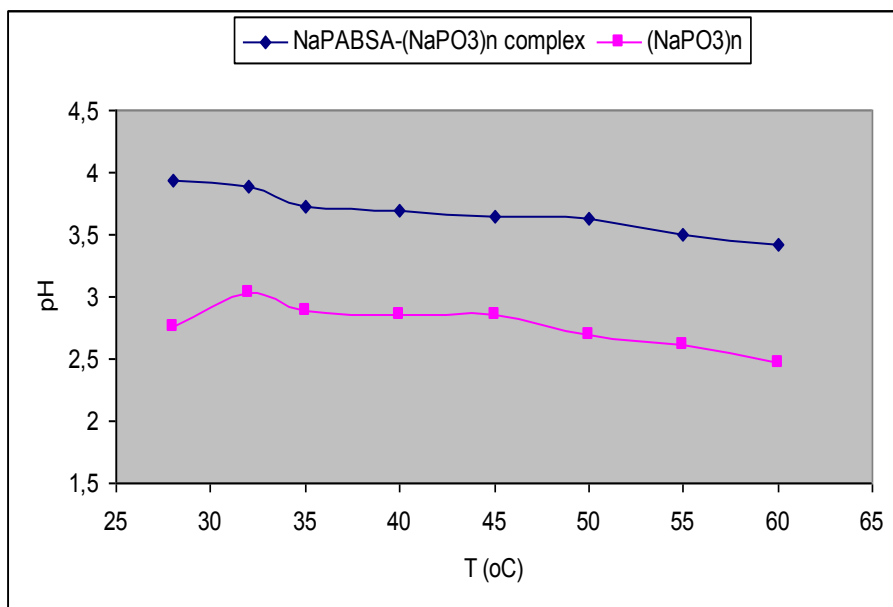


Figure D.19: pH Curve of NaPABSA – (NaPO3)_n Complex

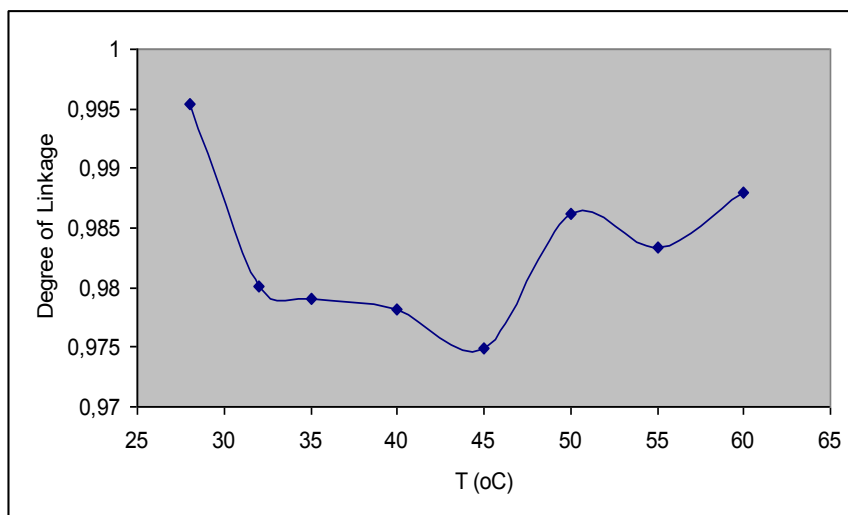


Figure D.20: Degree of Linkage of NaPABSA – (NaPO3)_n Complex

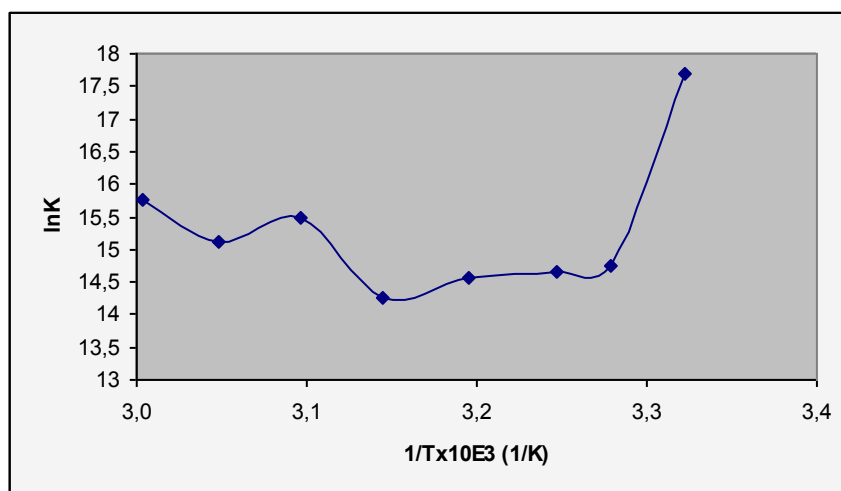


Figure D.21: Stability Constant of NaPABSA – (NaPO3)_n Complex

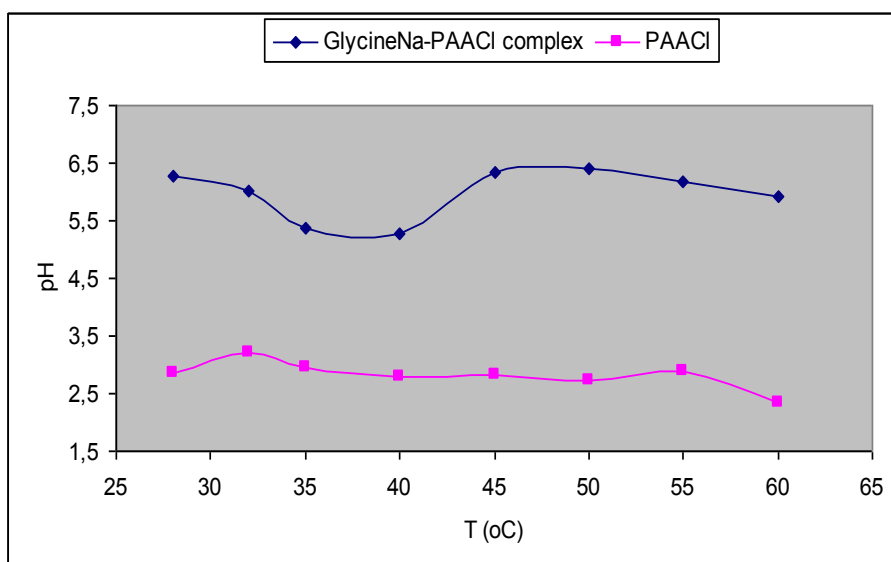


Figure D.22: pH Curve of GlycineNa – PAAHCl Complex

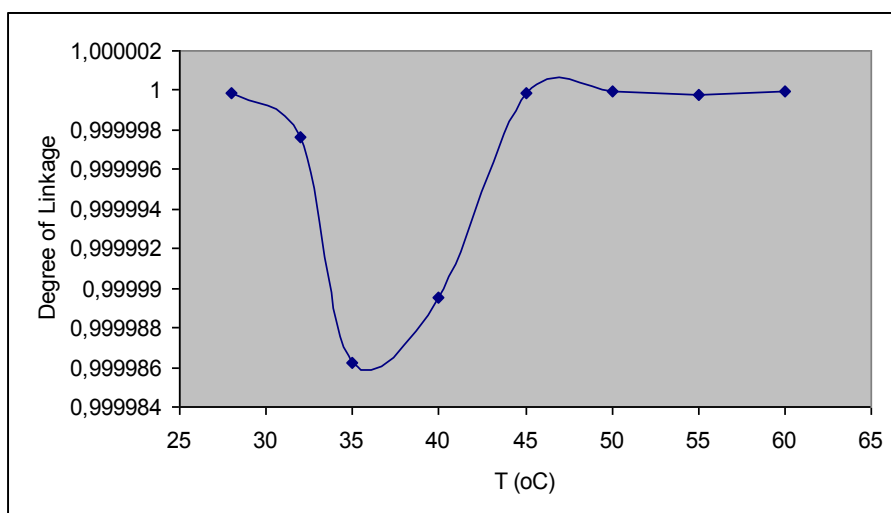


Figure D.23: Degree of Linkage for GlycineNa – PAAHCl Complex

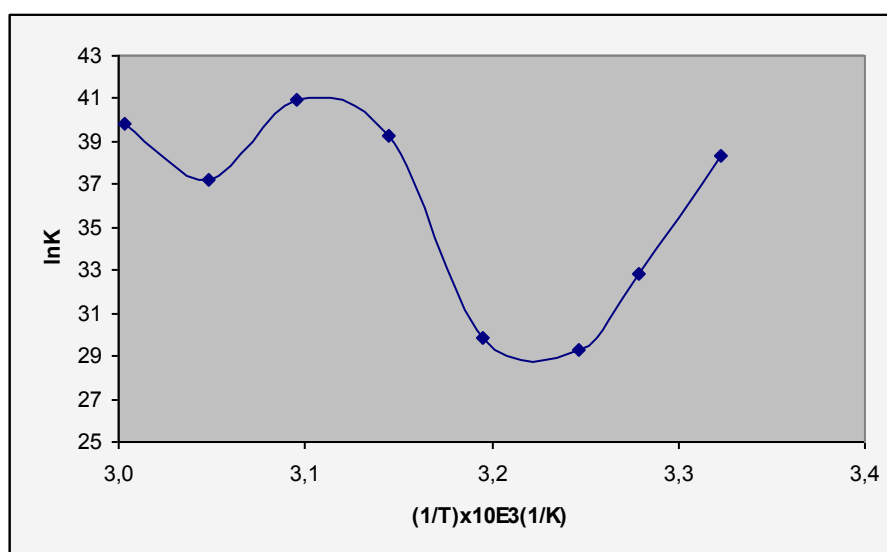


Figure D.24: Stability Constant of GlycineNa – PAAHCl Complex

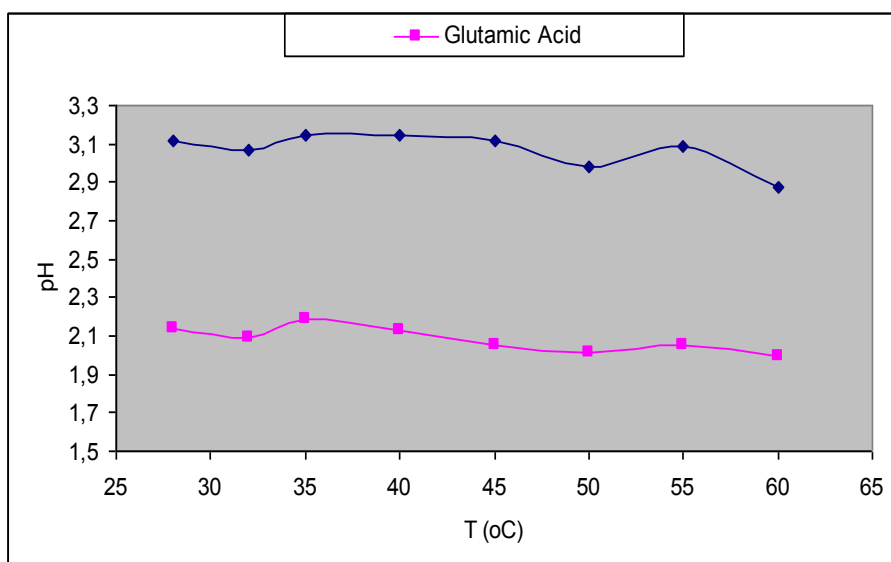


Figure D.25: pH Curve of Glutamic Acid - PAAOH Complex

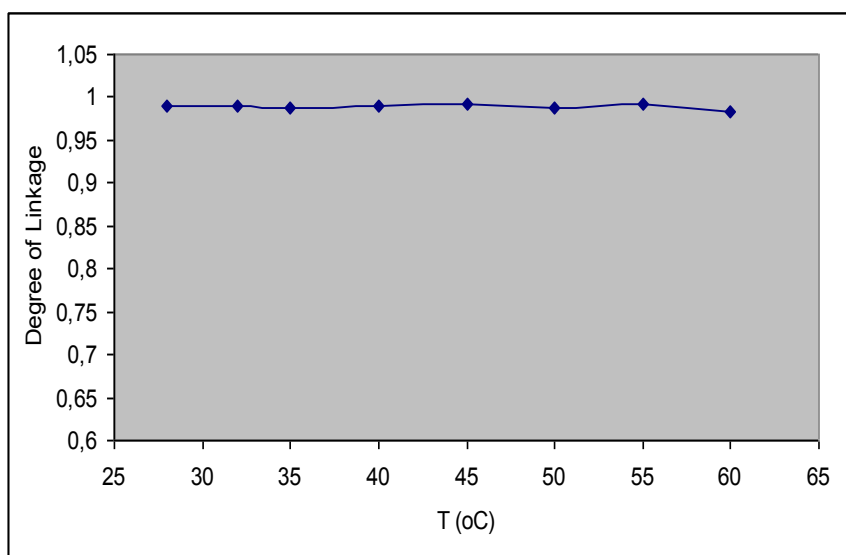


Figure D.26: Degree of Linkage for Glutamic Acid - PAAOH Complex

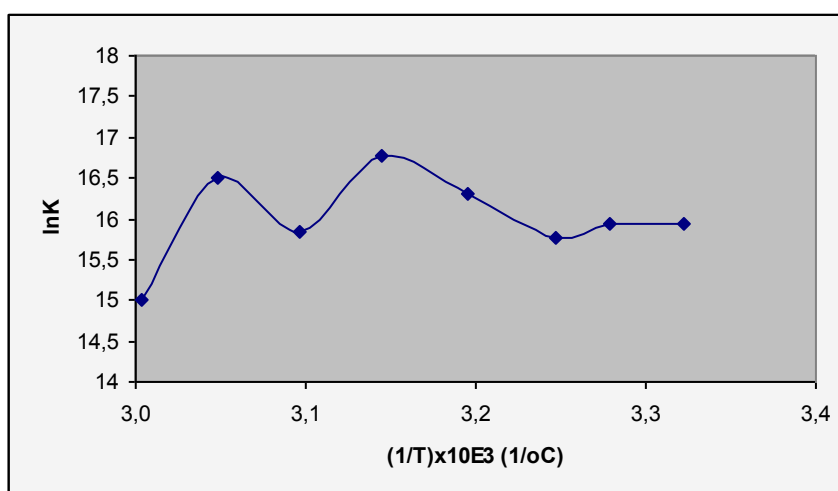


Figure D.27: Stability Constant of Glutamic Acid - PAAOH Complex

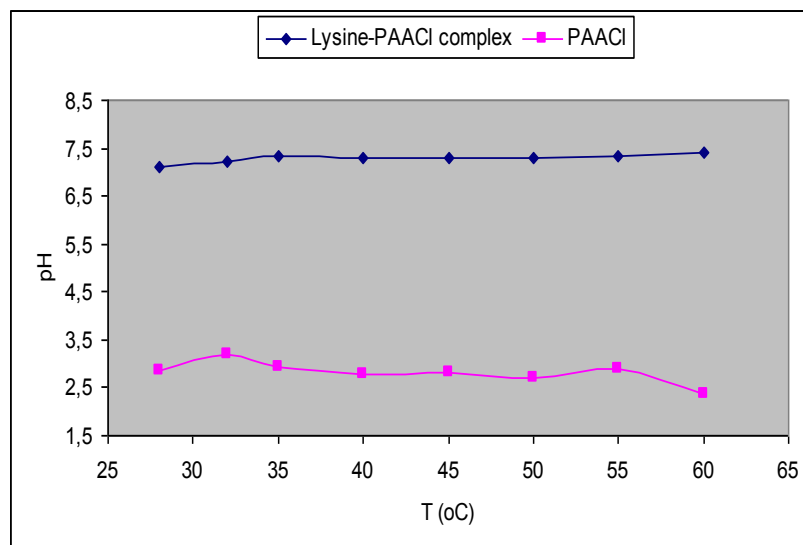


Figure D.28: pH Curve of Lysine – PAAHCl Complex

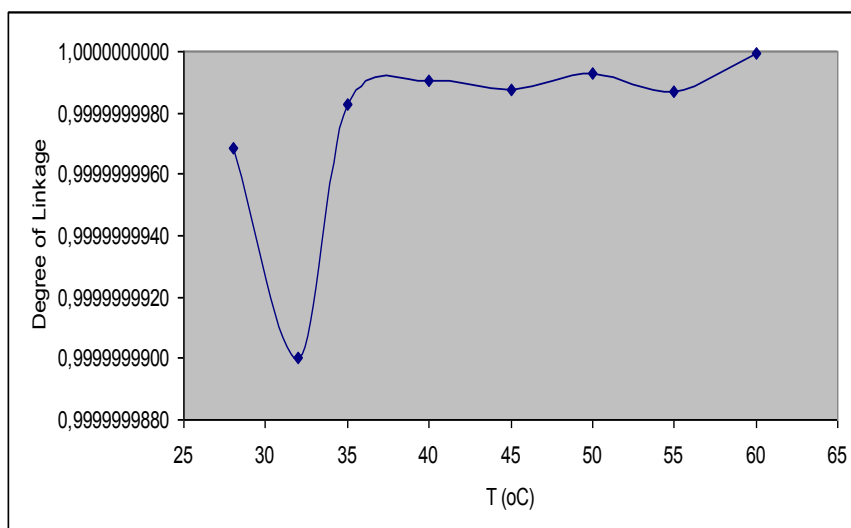


Figure D.29: Degree of Linkage for Lysine – PAAHCl Complex

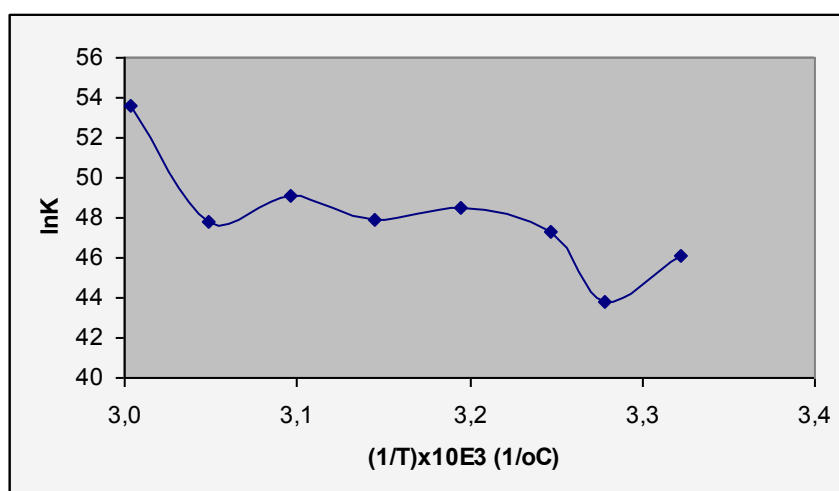


Figure D.30: Stability Constant of Lysine – PAAHCl Complex

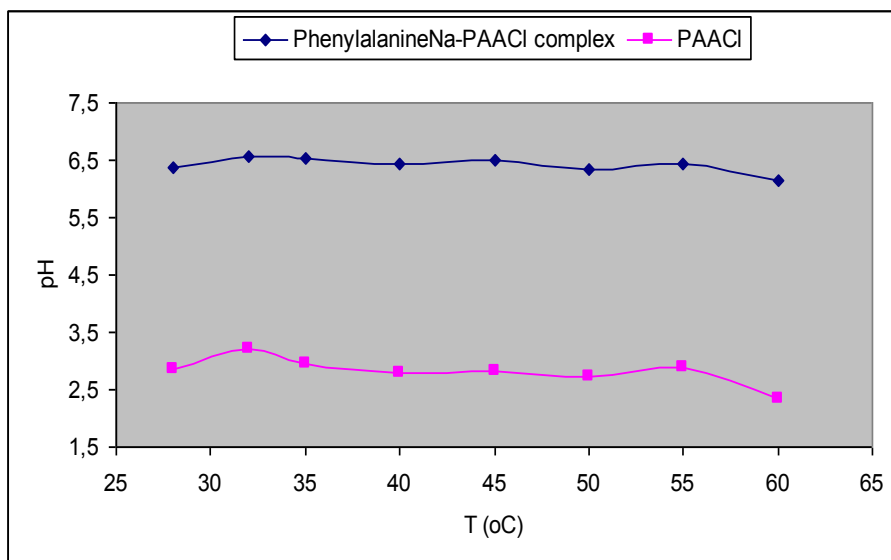


Figure D.31: pH Curve of Phenylalanine – PAAHCl Complex

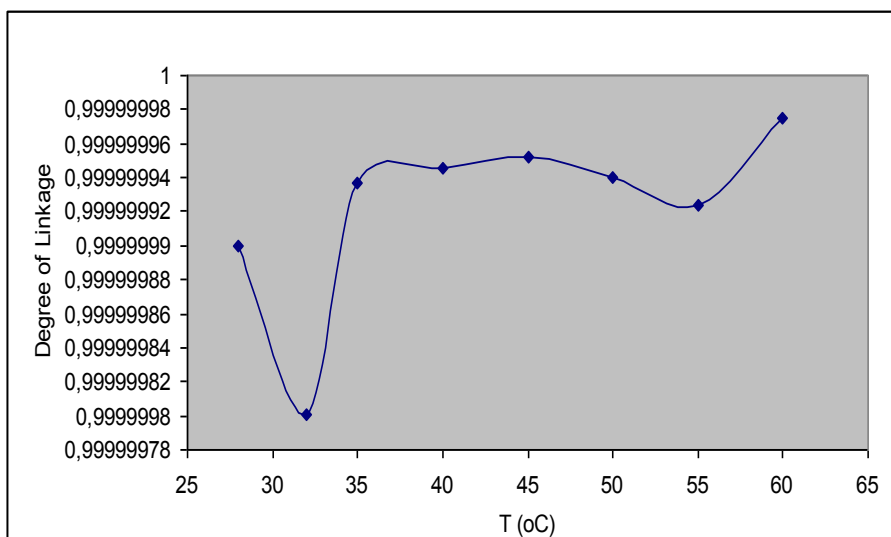


Figure D.32: Degree of Linkage for Phenylalanine – PAAHCl Complex

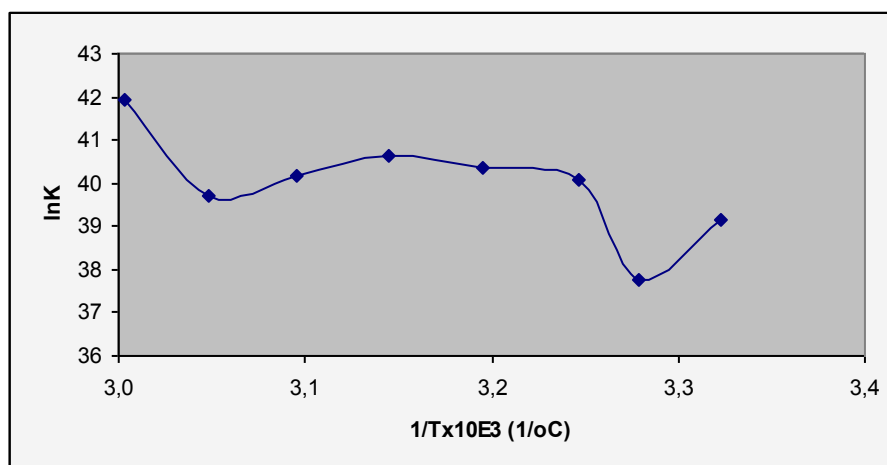


Figure D.33: Stability Constant of Phenylalanine – PAAHCl Complex

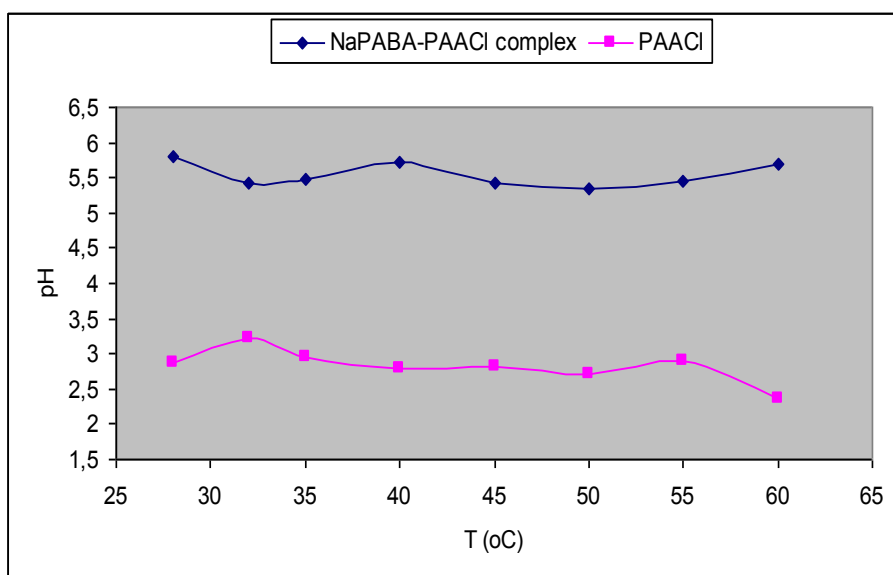


Figure D.34: pH Curve of NaPABA – PAAHCl Complex

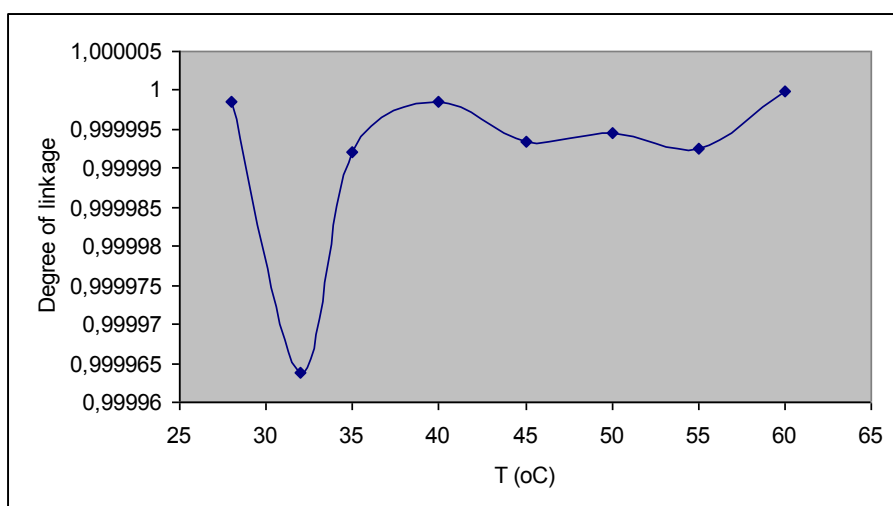


Figure D.35: Degree of Linkage for NaPABA – PAAHCl Complex

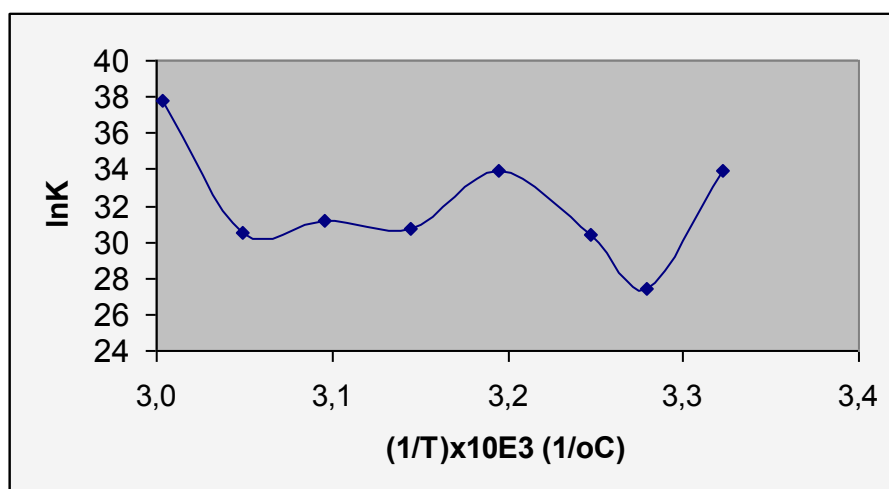


Figure D.36: Stability Formation Constant of NaPABA – PAAHCl Complex

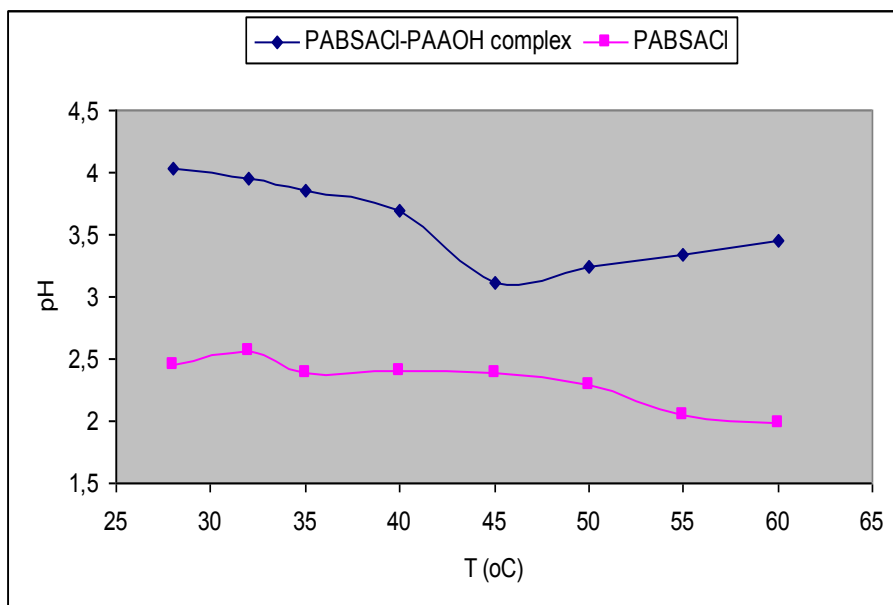


Figure D.37: pH Curve of PABSACl – PAAOH Complex

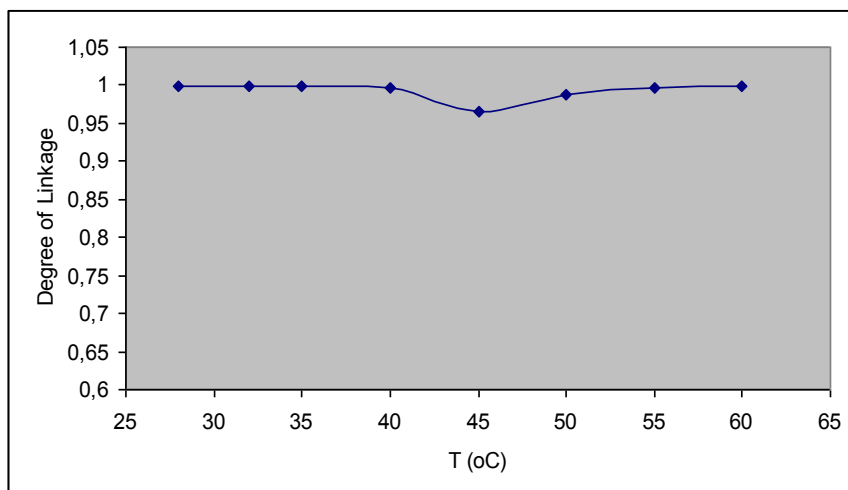


Figure D.38: Degree of Linkage for PABSACl – PAAOH Complex

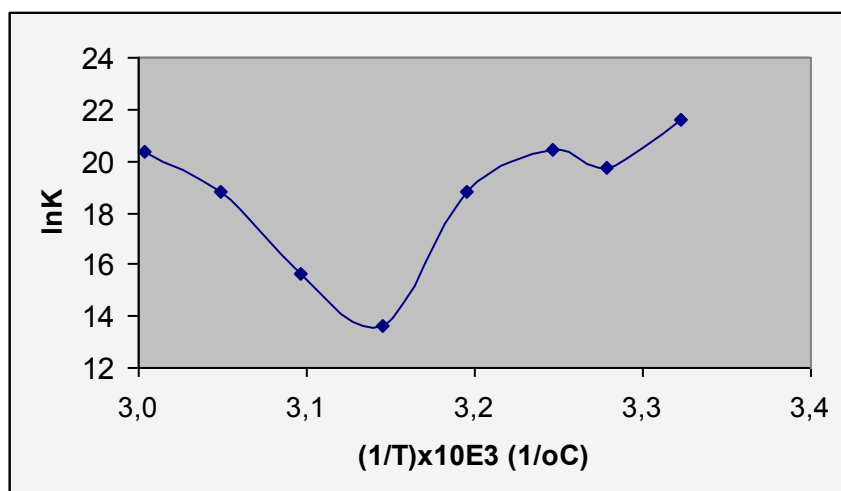


Figure D.39: Stability Constant of PABSACl – PAAOH Complex

APPENDIX E

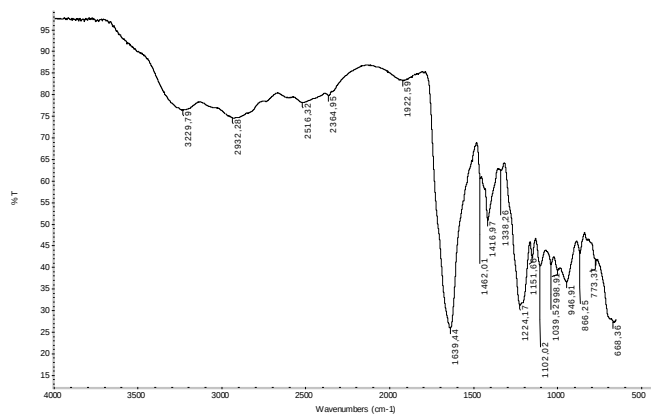


Figure E.1: IR Spectrum of 10^{-2}M Glutamic Acid- 10^{-2}M $(\text{NaPO}_3)_n$ Complex

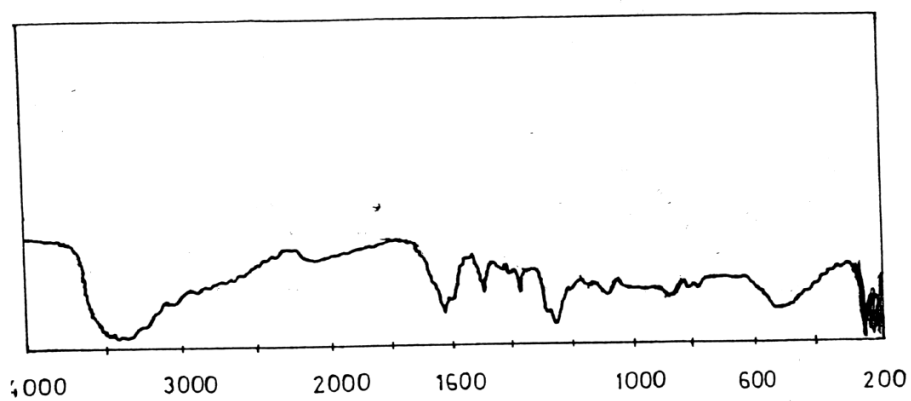


Figure E.2 : IR Spectrum of $(\text{NaPO}_3)_n$

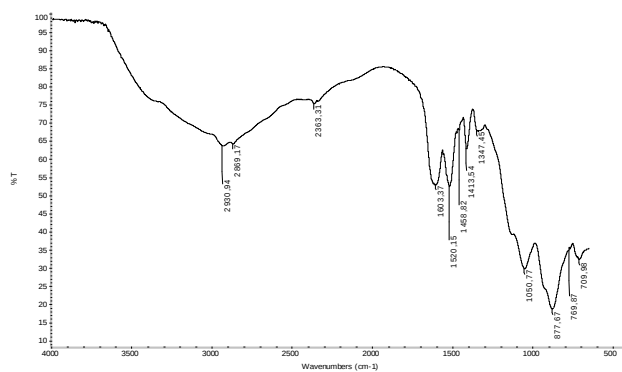


Figure E.3 : IR Spectrum of 10^{-2}M Lysine - 10^{-2}M $(\text{HPO}_3)_n$ Complex

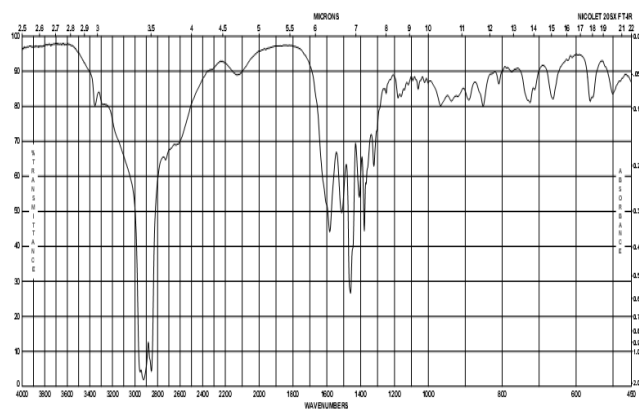


Figure E.4 : IR Spectrum of Lysine

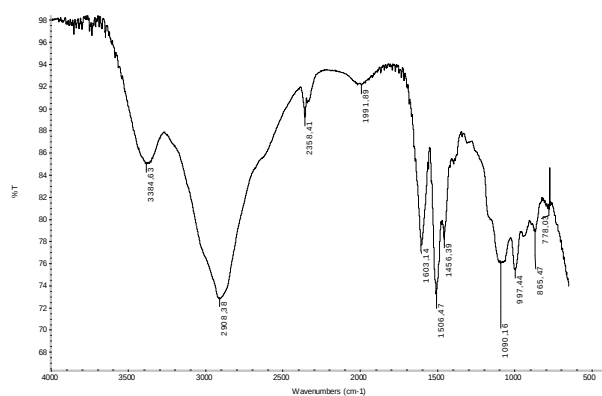


Figure E.5 : IR Spectrum of 10^{-4} M PABA - 10^{-4} M (NaPO₃)_n Complex

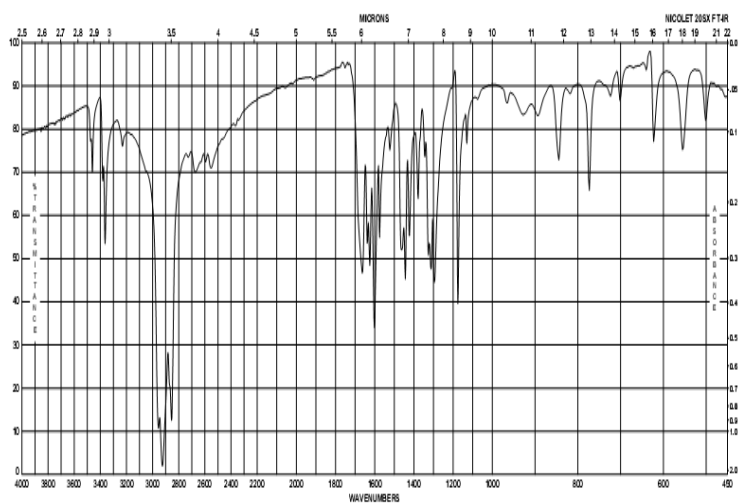


Figure E.6 : IR Spectrum of PABA

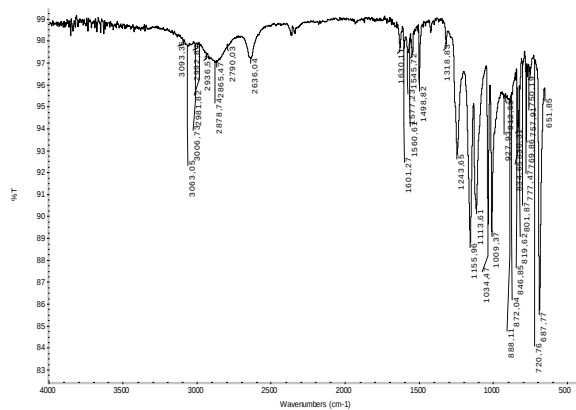


Figure E.7 : IR Spectrum of 10^{-2}M PABSA - 10^{-2}M $(\text{NaPO}_3)_n$ Complex

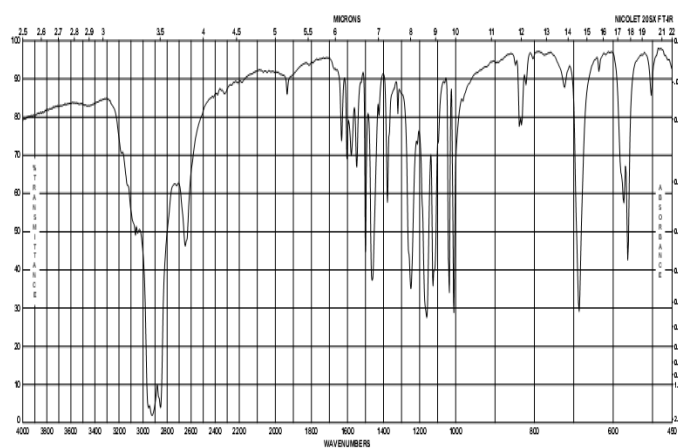


Figure E.8 : IR Spectrum of PABSA

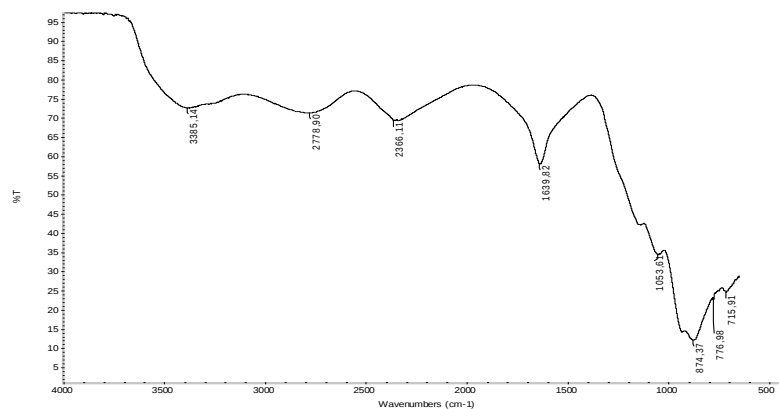


Figure E.9 : IR Spectrum of 10^{-2}M $(\text{HPO}_3)_n$

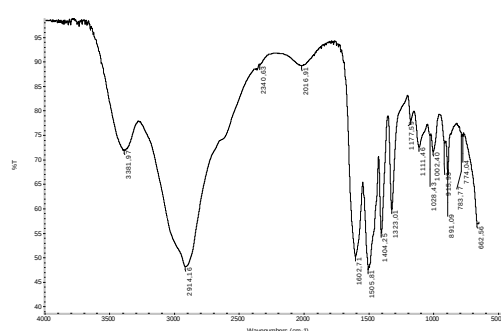


Figure E.10 : IR Spectrum of 10^{-2}M GlycineNa- 10^{-2}M PAAHCl Complex

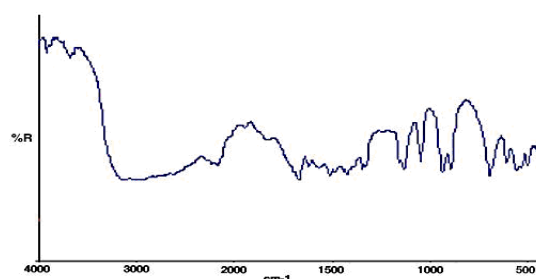


Figure E.11 : IR Spectrum of Glycine

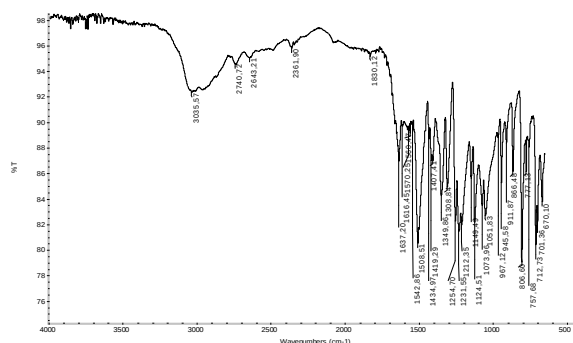


Figure E.12: IR Spectrum of 10^{-2}M Glutamic Acid - 10^{-2}M PAAOH Complex

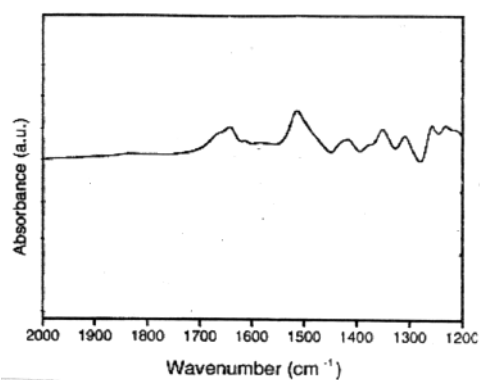


Figure E.13: IR Spectrum of Glutamic Acid

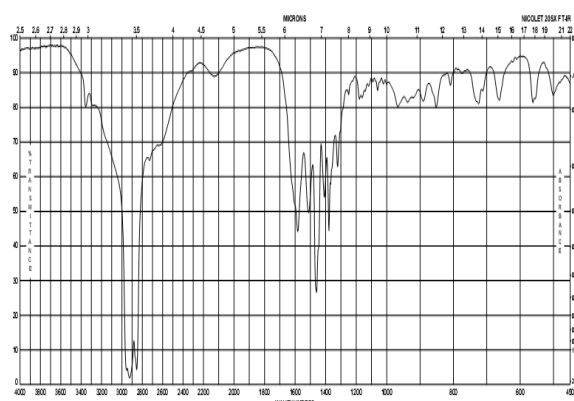


Figure E.14: IR Spectrum of 10^{-3} M Lysine - 10^{-3} M PAAHCl($I=10^{-2}$) Complex

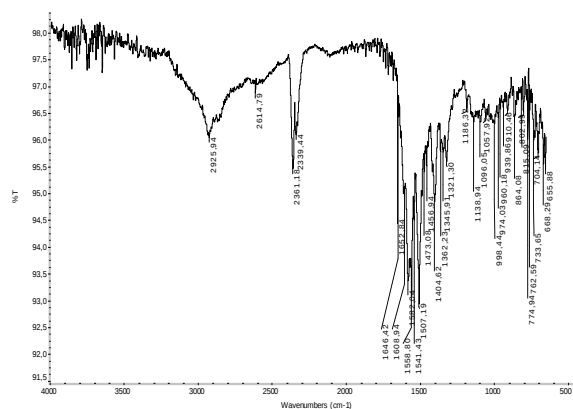


Figure E.15: IR Spectrum of Lysine

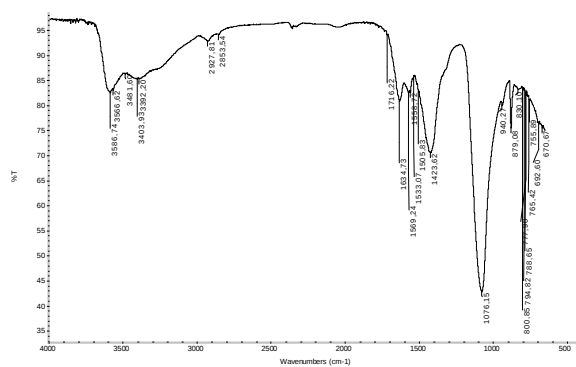


Figure E.16: IR Spectrum of 10^{-4} M Ph.Al.Na- PAAHCl($I=10^{-3}$) Complex

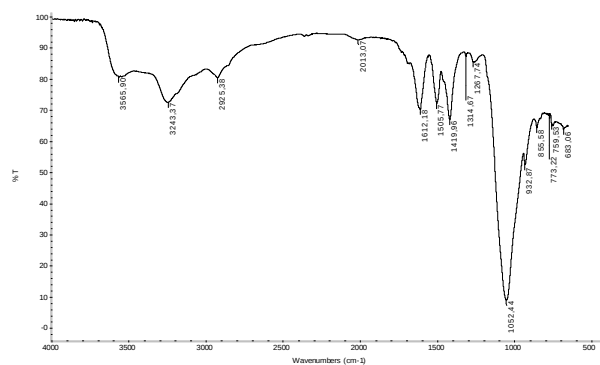


Figure E.17: IR Spectrum of 10^{-3} M NaPABA- 10^{-3} M PAAHCl ($I=10^{-2}$) Complex

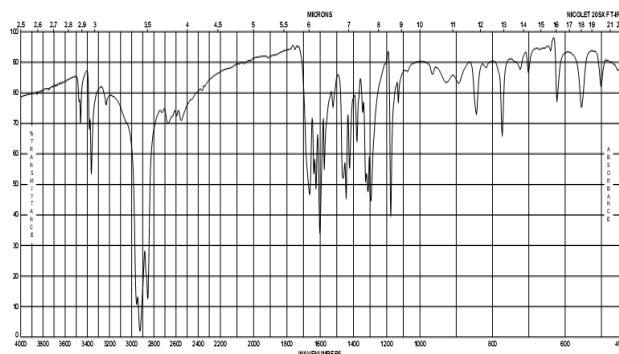


Figure E.18: IR Spectrum of NaPABA

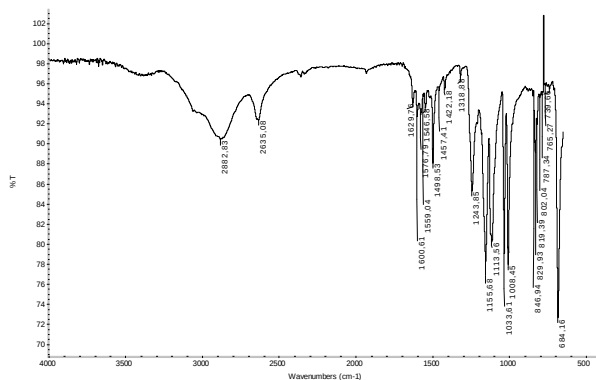


Figure E.19 : IR Spectrum of 10^{-2} M PABSACl- 10^{-2} M PAAOH Complex

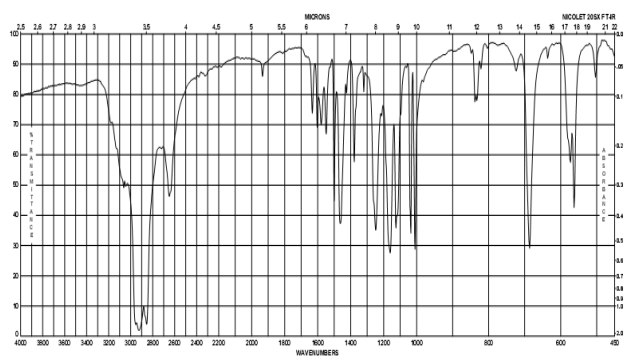


Figure E.20 : IR Spectrum of PABSACl

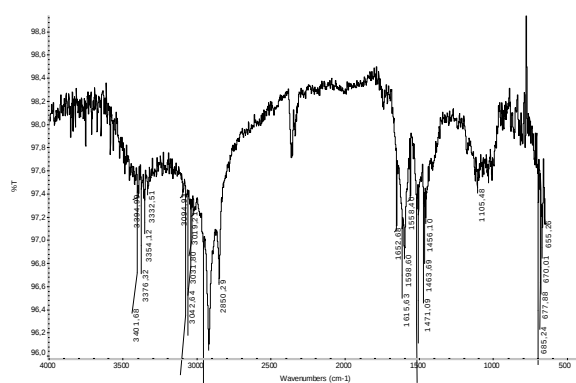


Figure E.21: IR Spectrum of 10^{-3} M PAAHCl(I=10E2)

CURRICULUM VITAE

She was born in Balıkesir, Gönen 1975, graduated from Gönen Ömer Seyfettin Lisesi in 1992, and Atatürk Eğitim Faculty of Marmara University as a chemistry teacher in 1997.

Since 1997, she has been working as a chemistry teacher in different private high schools.