POLYION-SENSITIVE OPTODES AND THEIR

ANALYTICAL APPLICATIONS

by

NAZANGÜL ÜNAL

THESIS SUBMITTED TO

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

OF
THE ABANT IZZET BAYSAL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF
MASTER SCIENCE
IN
THE DEPARTMENT OF CHEMISTRY

SEPTEMBER 2010

POLYION-SENSITIVE OPTODES AND THEIR ANALYTICAL APPLICATIONS

NAZANGÜL ÜNAL

SEPTEMBER 2010

Approval of the Graduate School of Natural and Applied Science.

Prof. Dr. Nihat Çelebi
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master Science.

Prof. Dr. Çetin Bozkurt
Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality as a thesis for the degree of Master of Sciences.

Prof. Dr.Nedime Dürüst
Supervisor

Examining Committee Members

1.Prof. Dr. Nedime DURÜST .....

2. Prof. Dr.Ahmet ÜLGEN .....

3. Prof. Dr. Esma SEZER .....

ABSTRACT

POLYION-SENSITIVE OPTODES AND THEIR ANALYTICAL APPLICATIONS

Ünal, Nazangül

M.Sc., Department of Chemistry

Supervisor : Prof .Dr. Nedime DÜRÜST

September 2010, 67 pages

This work deals with the quantitative determination of DNA and thickening agents (Xanthan, Sodium Alginate and λ-Carrageenan). These four target polyions are quantitatively determined by spectrophotometric titration. These compound are widely used in food and pharmaceutical industries so the quantitative analysis of them is important. Overdose usage of these compounds may cause the gastrointestinal diseases.

The main aim of this study is to prove the compatibility of optical sensors for the quantitative determination of polyions. To manage this; two different kinds of dye and three different polycations (protamine, poly-L-lysine and poly-L- Arginine) have been employed.

By this method, the amount of polyions, which are present in the real food samples, was determined and the accuracy of the method was supported by spike experiments.

Keywords: Optic Sensors, DNA, Carrageenan, Xanthan and Sodium Alginate.

ÖZET

POLİİYON – DUYARLI OPTODLAR VE ANALİTİK UYGULAMALARI

Ünal, Nazangül

Yüksek Lisans, Kimya Bölümü

Danışman: Prof. Dr. Nedime DÜRÜST

Eylül 2010, 67 sayfa

Bu çalışma DNA ve kıvam arttırıcıların (ksantan, sodyum aljinat, λ-karragenan) kuantitatif tayini ile ilgilidir. Bu dört hedef poliiyon spektrofotometrik titrasyon ile kuantitatif olarak tayin edilmiştir. Bu maddeler çoğunlukla gıda ve ilaç endüstrilerinde kulanılırlar bu nedenle bunların kuantitatif analizleri önemlidir. Bu maddelerin aşırı dozda kullanımları, sindirim sistemi hastalıklarına neden olabilir.

Bu çalışmanın ana amacı; optik sensörlerin poliiyonların kantitatif analizlerine uygunluğunu ispat etmektir. Bunu gerçekleştirmek için iki farklı türde boya ve üç farklı polikatyon (protamin, poli-L-lisin, poli-L-arjinin) kullanılmıştır.

Bu metod ile, gerçek gıda numuneleri içerisindeki poiliyon miktarları bulunmuştur ve bu metodun doğruluğu geri kazanım deneyleriyle desteklenmiştir.

Anahtar kelimeler: Optik Sensör, DNA, Karragenan, Ksantan ve Sodyum Aljina

to my grandfather…

ACKNOWLEDGEMENTS

I am grateful to my advisor Prof.Dr. Nedime DURUST for her guidance and encouragement. I also thank Sibel NAÇ for her help, her friendship and for her patience about computer.

I also thank The Scientific and Technological Research Council of Turkey (TUBITAK), which has supported financially this work as a project TBAG-108T109. I am grateful to Prof. Dr. Yaşar DÜRÜST and his group members; Dr. Cevher ALTUĞ, Dr. Muhammet YILDIRIM, Akın SAĞIRLI, Hamza KARAKUŞ and Emine Özge GÖZLÜKAYA in the synthesis of some dye stuff.

I would like to thank my family; my father, my mother and my brother for their love, encouragement and their financial support. I also thanks my cousins; Seval BOYRAZ and Dilek ŞİMŞEK for their encoragement and for being my side everytime.

I also thank to my friends Nazmiye BABACAN, Çınar DÜZEN, Hatice DOĞAN for being my second family in Ankara and for their friendshipI also thank to Şükrüye ER and Feda GÜMÜŞBOĞA for their friendship and their patience to listen me during my trouble days.

I also would like to thank the following colleagues in the chemistry department; Dr. Dilek METE, Dr. Erol AKPINAR, Elif Berna Karaman OLUTAŞ, Günnur İPEK and Gülsün ÖZER for their cooperativity and friendship.

TABLE OF CONTENTS

TABLES

TABLE OF FIGURES

CHAPTER -1: INTRODUCTION

1.1.- SENSORS

A chemical sensor is a device which transforms chemical or biochemical information of a qualitative or quantitative type into an analytically useful signal.

Chemical sensors have been widely used in such applications as industrial hygiene, process control, product control, clinical diagnostics etc. (Hulanicki, 1991; Stetter, 2003).

In chemistry laboratories two types ofchemical sensors are widely used; one of them is electrochemical sensor and the other one is optical sensor.

1.1.1- -ELECTROCHEMICAL SENSORS

Electrochemical sensor is an important part of the chemical sensor. Electrochemical sensors are divided to three categories; potentiometric, amperometric, and impedance or admittance based devices. (Stetter, 2003).Ion-selective electrodes are used in the potentiometric based devices.

ISEs are used in a wide variety of applications for determining the concentrations of various ions in aqueous solutions. Some areas in which ISEs have been used; pollution monitoring, agriculture, food processing, detergent manufacture, biomedical laboratories etc. There are three different types of membrane which selectively interact with the ion(s) to be determined in ISEs;

1. Glass membrane
2. Solid-state membrane
    a) Homogeneous solid-state membrane
    b) Heteregeneous solid-state membrane
3. Liquid membrane
    a) Electrically charged ligand group (ion-exchangers as membrane components.
    b) Electrically neutral ligand groups as membrane components. (Simon, 1970).

ISEs consist of an ion selective membrane, an internal filling solution and an internal reference electrode or of an ion selective membrane and solid contact.(Kayansalan, 2005) Reference electrode is a half cell having a known electrode potential which remains constant (Skoog, 1996).

The potential of the cell;

$Ref_{(sample)}$ || $[A]_{sample}$ | $[A]_{int}$ || Ref_{int}

$E_{cell} = E_{Ref(int)} - E_{Ref(samp)} + E_{mem} + E_{lj}$

Where E_{mem} is potential across the membrane , E_{lj} is liquid junction potentials and E_{Ref} is reference electrode potentials.

Ion selective membrane in ISEs is used for determination of polyions (like heparin, protamine etc.) consists of an ionophone (ion-carrier) and a lipophilic salt as ion-exchanger (like TDMAC, DNNS). (Bakker, 1997). Ion carriers catalyze ion transport across hydrophobic membranes.

ISEs based upon the measurement of the interfacial potencial at an electrode surface caused by a selective ion exchange reaction. (Stetter, 2003). The membrane potential is given by Nernst-like equation.

$E = E^{o} - RT/zF. \ln [A]□/ [A]s$

[A]□ =concentration of analyte in the internal solution

[A]s = concentration of analyte in the sample

R = gas constant

T = the absolute temperature

F = faraday constant

E^{o}= is a constant which is characteristic of the particular ISE/reference pair.

z = the change of the Ion

E = the total potential developed between the sensing and reference electrodes.

Because of some advantages of ISEs, they are used in chemistry and clinical laboratories, some of these advantages are: they are relatively inexpensive, their usage is simple, they have an exremely wide range of application and have wide concentration range. Turbidity and color of the sample cannot affect them, and they are particularly useful in biological/medical applications because they measure the activity of the ions directly.

1.1.2- OPTICAL SENSORS

Since the absorbance is directly proportional to the concentration of absorbing species, UV-VIS spectroscopy is used for quantitative analysis in chemical and clinical laboratories in the world. The realtionship between absorbance and concentration of the absorbing species is expressed by Beer's Law;

$$A = a.\ b.\ c$$

where 'a' is termed as absorptivity, 'b' is the path length though the medium, 'c' is the concentration of the absorbing species and 'A' is absorbance.

In analytical chemistry laboratories, optical sensors are used to take the advantages of UV-VIS spectrophotometry. Optical sensors are the important part of the chemical sensors. The other name of optical sensors is bulk optodes and they were developed at the late of 1980s.

Optodes can select both small ions (K^+, Na^+, NH_4^+, Cl^-, NO_3^-, CO_3^2,…etc) and polyions (heparin, protamine, lysine…etc). Therefore ,optical sensors are created by various polymeric membranes and they are used in neutral-carrier pair ion-exchange sensing systems. (Wang, 1999; Bakker, 1997; Wang,1990; Seiler, 1991; Seiler,1989; Behringer, 1990; Hauser, 1990;Tan, 1991).

In this thesis two different types of pH indicators were studied ; one of them is a chromoionophore, DCFOE (2',7'-dichlorofluorescein octadecyl ester) and the other one is a chromophore, ETH-2412 (3-hydroxy-4-(4-nitrophenylazo)-phenylocta-decanote).

DCFOE is a polycation sensitive indicator and optical membrane doped with DCFOE refers to Film-1 and ETH-2412 with a lipophilic anion exchanger tridodecylmethyl ammonium chloride (TDMAC) is polyanion sensitive indicator and optical membrane doped with ETH-2412 refers to Film-2. The mechanism of optode is based upon the extraction of the target ions into the bulk of the organic membrane phase by mass transfer.

Advantages of optical sensors :

When we compare the advantages of ISEs and optic sensors, we favored working with optic sensors. Some advantages of optic sensors over ISEs are

- Optical sensors can be adopted to different formats, such as microtiter plate, optical fibers, UV-VIS spectrophotometer...etc.
- It does not need a reference electrode,

- It can be used at various thickness and shapes
- It has high selectivity, sensitivity and reversibility.
- In ISEs millivolt (mV) of sample is measured but with optical sensors absorbance (A) is measured.
- Microtiter plate format optodes (MPOs),optical sensors adopted to microtiter plate reader, provide quick and sensitive responses. By microtiter plate (MPR) high sample (96 samples) thoughput are provided and processed at the same time in few seconds.

1.1.2.1-Film -1

Organic polymeric films consists of a chromoionophore (DCFOE), PVC, PU and plasticizer (DOS). Chromoionophores are lipophilized hydrogen ion-selective indicators, their optical properties change by protonation/deprotonation of it. Some ionophores are also known as chromoionophores or fluoroionophores.

Today, two types of chromoionophores are designed. One of them is neutral in the basic form and positively charged when protonated (basic chromoionophores, neutral hydrogen carriers) and other one is neutral in the protonated form and negatively charged when deprotonated (acidic chromoionophores, charged hydrogen carriers).

The protonation degree and color of chromoionophores are affected by the activity of competing ions, H^+ and the the target ion in the measuring solution.

DCFOE dye into the organic polymeric films acts as a chromoionophore or pH indicator for neutral carrier pair ion-exchange systems. Lipophilic fluorescein chromoionophore DCFOE contains an acidic phenol group which becomes negatively change during deprotonation. (Bakker, 1997; Bakker, 1992; Lerchi,1992; Hauser, 1990)

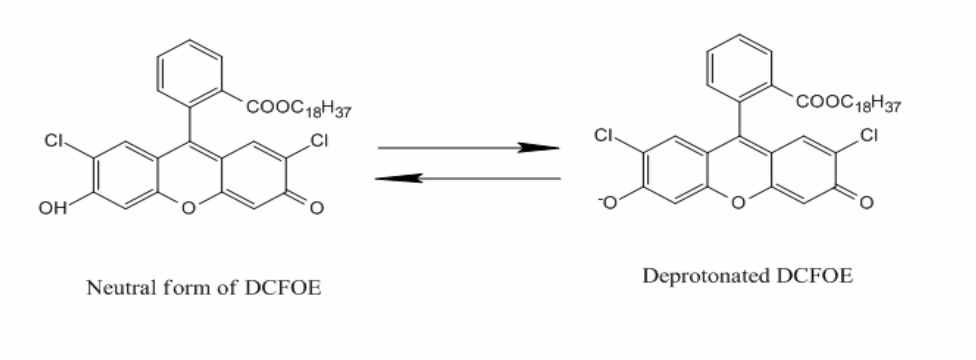


Figure 1: Forms of DCFOE

DCFOE in polimeric film forms a micelle-like complex with polycations and changes the absorbance of the membrane. DCFOE containing film have a maximal absorbance at 490 nm in the buffer (pH: 7,4) but when it exposed to a protamine solution its maximum absorbance shifts to 540 nm, because deprotonated DCFOE has sufficient association affinity to protamine so that protamine can be extracted from aqueous solution into the organic film.

DCFOE shows high selectivity for polycationic protamine but it can be used for other polycations (Poly-L-Lysine, PLA…etc) too. In clinical procedures protamine is widely used to reverse anticoagulant activity of heparin, at near neutral pH it is a polycation. It is obtained from fish. Average molecular weight of it is 4500 daltons. Protamine consists of amino acids and %67 amino acid content of it is arginine. (Yun, 1999; Byun, 1999).

The deprotonated DCFOE participates from the organic membrane phase and forms a third phase (colloid phase) with polycationic protamine by creating micelle-like complex. By the creating of colloid phase, a relatively pink-red color appeared in this phase and the intensity of the color changes with concentration of polycationic protamine.

In Buffer In solution of Protamine

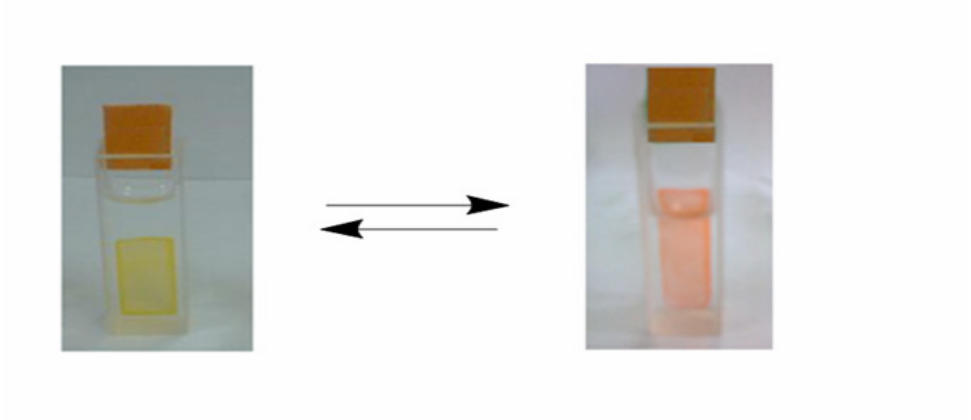


Figure 2: DCFOE doped films in buffer and in protamine solution

The amount of complex which is formed in third phase is directly proportional to the concentration of polycation in the sample.

A stable ion-pair occurs in the third phase between deprotonated DCFOE and protamine. This procedure is performed at pH 7.4 medium and the equilibrium between organic membrane phase and the aqueous solution is;

$$Prot^{z+}{}_{(aq)} + zHL_{org} \rightleftharpoons (Prot\text{-}Lz)_{org} + zH^{+}{}_{(aq)}$$

Where (Prot-Lz) shows the ion-pair complex which formed in third phase and ‘’org’’ and ‘’aq’’ refer to organic and aqueous phase respectively.

The equilibrium constant;

$$K_{eq} = \frac{[\mathrm{Prot} - \mathrm{L_z}][\mathrm{H^+}]^z}{[\mathrm{Prot^{z+}}][\mathrm{HL}]^z} = \frac{(\beta_{\mathrm{prot-L_z}})(k_{\mathrm{prot}})}{(\beta_{\mathrm{HL}} k_{\mathrm{H}})^z}$$

where $ß_{(Prot\text{-}Lz)}$ = complex formation constant, K_{Prot} is distribution coefficient of protamine, K_H is distribution coefficient of H^+ and β_{HL} is association constant of indicator.

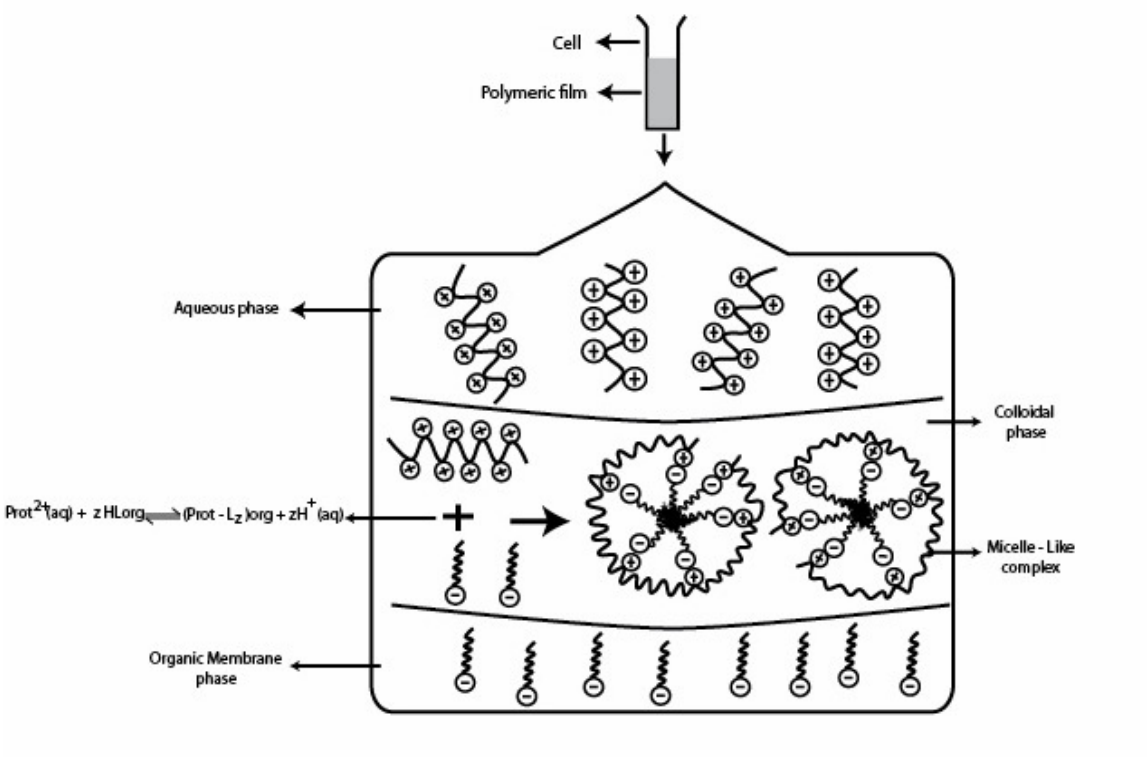


Figure 3: Interaction between DCFOE and Protamine

refers to protamine

refers to deprotonated DCFOE

In the bulk optodes, the analyte is extracted into the bulk and not only a surface layer of the membrane. (Bakker, 1997, Dai, 2000).

1.1.2.1.1- Factors that affect the response of membrane:

1- The concentration of components in organic membrane: The concentration of chromoionophores is too important in organic membrane. It affects the absorbance of optode. when DCFOE concentration increases the optical response of optode increase too, because the absorbance is based on the extraction of the deprotonated DCFOE from the membrane into aqueous solution and formation of micelle-like ion pairs. Also the ratio between PVC and DOS and the amount of plasticizer (DOS) affect the optical response of optode. The ratio between PVC and DOS optimized to obtain reproducible and rapid response. This ratio changes the optical response of the membrane by affecting the dissociation constant of DCFOE. The plasticizer (DOS) affects the dissociation constant of proton and DCFOE and dissociation coefficient of protonated DCFOE between organic phase and aqueous solution. Also the solvation of chromoionophores is supplied by DOS.

2 - Activity of target ion affects the response of optode.

3- Ion-pair interaction between DCFOE and polycations

4 - Buffer pH

5- Concentration of polycation: The response of the polymeric film is faster when the concentration of protamine in the buffer solution increases. At very low concentration of protamine the absorbance change is very little. (Bakker,

1997; Kim, 1998).

6- Type of polycations: Different polycations have different interactions with deprotonated DCFOE and forms different degree of micelle-like complex. Molecular weight, charge density and structure of polycations affect the optical response of the films. When molecular weight of polycation increases, the absorbance change at 540 nm also increases because every polycation has different binding constant. When the optical response of protamine compared with the optical response of Poly-L-Arginine, the results showed that the structure of polycation plays an important role, Poly-L-Arginine has more guanadinium groups than a protamine at similar polimer weight, so response of PLA is better than protamine's response in the same time and at the same mass concentrations. The larger molecules have a smaller diffusion coefficient. PLA has higher charge density which binds more chromophore sites and the resulting complexes are more stable. The molecular site of PLL (is a polycation at neutral pH medium and it is used as antagonist of heparin) is smaller than the molecular size of protamine and PLA, but it shows the smallest response at the same time period. The smaller molecular fractions showed slower response than the larger ones. (Wang, 1996) For PLA and protamine response time is 3 minutes, this time is enough to give optical response but for PLL this time is not enough because the extraction of PLL into the polymeric film is slow and less favorable than protamine and PLA, so the response time of PLL is 20 minutes.

1.1.2.2- Film -2

ETH-2412 is a chromophore. Chromophores are unsaturated organic functional groups that absorb in the UV or visible region. By covalent bonding and adsorption, chromophoric substance is immobilized on the sensor surface (Tan, 1989; Skoog, 1996).

The mechanism of optode which doped with ETH-2412 dye based on the extraction of polyanion into the bulk of the organic film. The target polyanion (i.e. λ- carrageenan and xanthan) in aqueous phase is extracted into the organic membrane phase by electrostatic interaction between tridodecylmethyl ammonium chloride (TDMAC) and polyanion, as a result, a micelle-like complex occured (Kim, 2006; Shortreed, 1996).

ETH-2412 doped optical membrane is highly sensitive for polyanion heparin which is a complex and highly negatively charged polysaccharide with an average molecular weight of 15,000. It is used in clinical procedure.

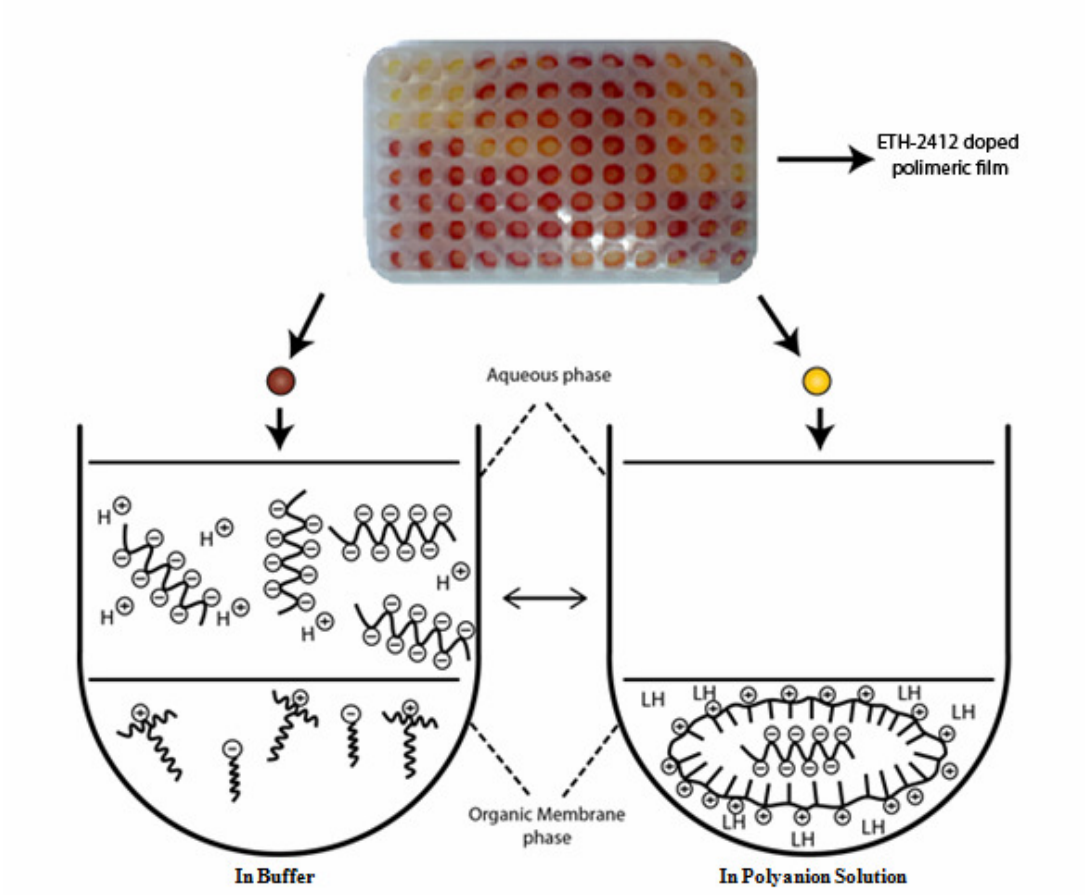
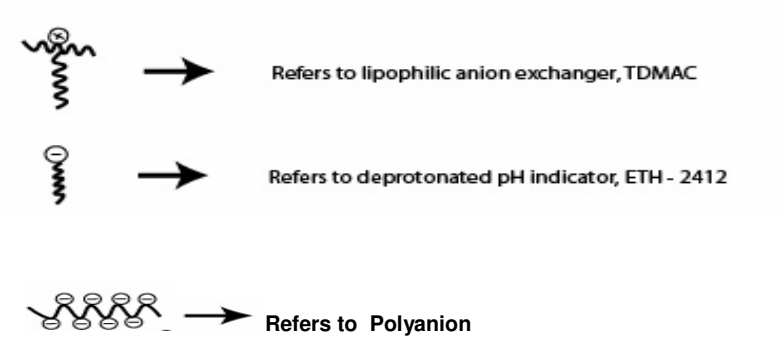


Figure 4: Interaction between TDMAC and Polyanions



the equilibrium between organic membrane phase and the aqueous solution is:

$$zT_{org}^{+} + zL_{org}^{-} + X^{z-}(aq) + zH^{+}(aq) \rightarrow T_zX_{org} + zLH_{org}$$

Where T^{+}, L^{-}, X^{-} and H^{+} refers to lipophilic anion exchanger (TDMAC), lipophilic pH indicator (ETH-2412), polyanion and proton respectively and

“aq” and “org” refer to aqueous phase and organic membrane phase respectively. (Wang, 1995).

The Equilibrium constant is:

$$Keq = \frac{[T_z X][LH]^z}{[T^+]^z.[L^-]^z[X^{z-}][H^+]^z}$$

The indicator ETH-2412 is neutral in its protonated form but it becomes negatively charged in its deprotonated form.

(a) (b)

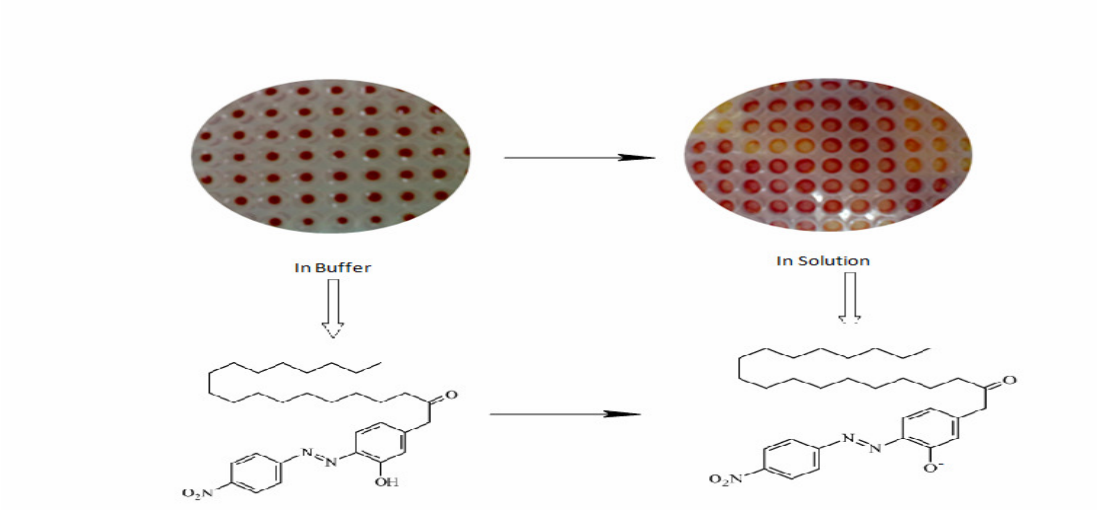


Figure 5: Forms of ETH-2412

(a) In the buffer solution (phosphate buffer, pH: 7,4) the lipophilic pH indicator (ETH-2412) in organic membrane is present in its deprotonated form.

(b) When a complex is formed between TDMAC and polyanion, proton is bound to lipophilic pH indicator (ETH-2412) and as a result the indicator is protonated and aborbance and color of the membrane change.

In buffer solution (phosphate buffer pH;7,4) the indicator in organic membrane is in deprotaned form, but when it exposed to solution of polyanion it protonated again.

Some factors affect the optical response of ETH-2412 doped polymeric film. Some of them are;

- The ratio between PVC and plasticizer (DOS) affects the performance, response time and sensitivity of MPO. This ratio was optimized to provide reproducible and rapid response.
- The molecular size of the polyanion analyte affects the response time of the film. For example this time is 120 min for xanthan and 60 min for carraageenan. Because xanthan is bigger than carrageenan.

CHAPTER-2 : POLYIONS

2.1- BIOLOGICALLY IMPORTANT POLYIONS:

2.1.1- DNA (DEOXIRIBONUCLEIC ACID):

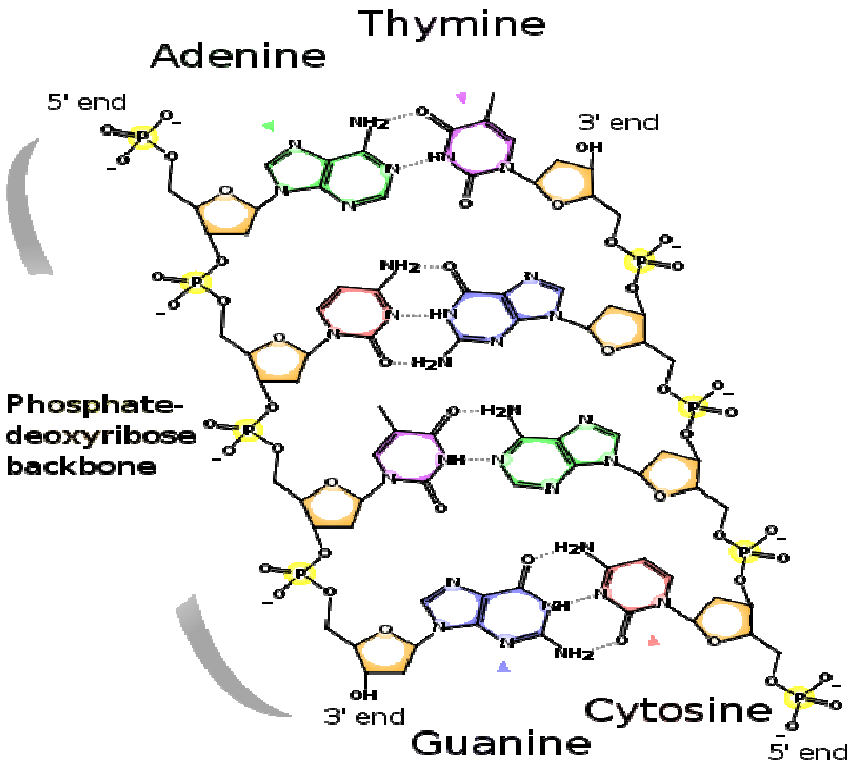


Figure 6: Structure of DNA

DNA is a molecule whch is the carrier of genetic İnformation. It is present in mitochondri. In 1953 James Watson and Francis Crick discovered the molecular structure of DNA. In 1962, they won Nobel Prize because of their work

DNA is a long polymer consists of repeating units, the nucleotides. The structure of DNA is studied in two parts; the external sugar-phosphate backbone and the internal bases.

The sugar in DNA is a pentose; deoxyribose. Bases found in a DNA molecule are classified into two groups; the purine bases and pyrimidine bases.(Acharya, 2006). Purines are Adenine (A) and Guanine (G), and pyrimidines are Cytosine (C) and Thymine (T).

Adenine (A) = $C_5H_5N_5$ = 6 - aminopurine

Guanin (G) = $C_5H_5N_5O$ = 6 - oxo - 2 - aminopurine

Cytosine (C) = $C_4H_5N_3O$ = 2 - oxo - 4 - aminopyrimidine

Thymine (T) = $C_5H_6N_2O_2$ = 2,4 - dioxo - 5 – methylpyrimidine

Two helical chains are present in DNA structure, each chain rounds the same axis, and each chain consists of phosphate diester groups joining 13-D–deoxyribofuranose residues with 3', 5' linkages. Two chains are held together by the purine and pyrimidine bases. A base of a single chain is banded to a base of the other single chain by hydrogen bonding.

One of the pair must be a purine and the other should be a pyrimidine to create hydrogen bonding. The phosphates are located on the outside and the bases are located on the inside of DNA molecule. (Watson, 1953). In a DNA molecule, phosphate groups are the charge carriers. Because of the phosphate groups, DNA is an anionic compound. (Kasyanenko, 1999). Reactive oxygen species such as hydroxyl radical, peroxyl radical, and hydrogen peroxide oxidize DNA bases. Free radicals such as hydroxyl radical reacts with pyrimidine and purine bases to give many oxidation products. Oxidative DNA damage is associated with cancer, aging, and genetic diseases. (Tsunoda, 2008).

The interaction of DNA with other molecules in solution has been investigated because it has fundamental importance in the life sciences, particularly in the replication and transcription of genetic infornmation in vivo. (Ge, 2007). Also this interaction is important for clarifying some illnesses and developing the treatment methods of illnesses.

In chemistry laboratories DNA is quantitatively determined by using different methods. Some of these methods are;

- Durust and Meyerhoff determined DNA guantitatively by potentiometric titrationand they also found the binding interaction of DNA with Protamine. (Durust & Meyerhoff, 2007).
- By the fluorescence – based method DNA is quantitatively determined, for this Hoeschst 33258 dye, which selectively binds to DNA, was used.

Either single - stranded or double - stranded DNA is quantitatively determined by Steel. He used an electrochemical method for this. Also Ding and Wong determined DNA quantitatively. Also cationic polystyrene (PS) nanoparticles are used as resonance light scattering (RLS) probe for the quantitattive analysis of DNA. (Chen,2010; Wang, 2005; Ding, 2005; Morozkin, 2003; Steel, 1998).

2.2- FOOD ADDITIVE POLYIONS

2.2.1- CARRAGEENAN

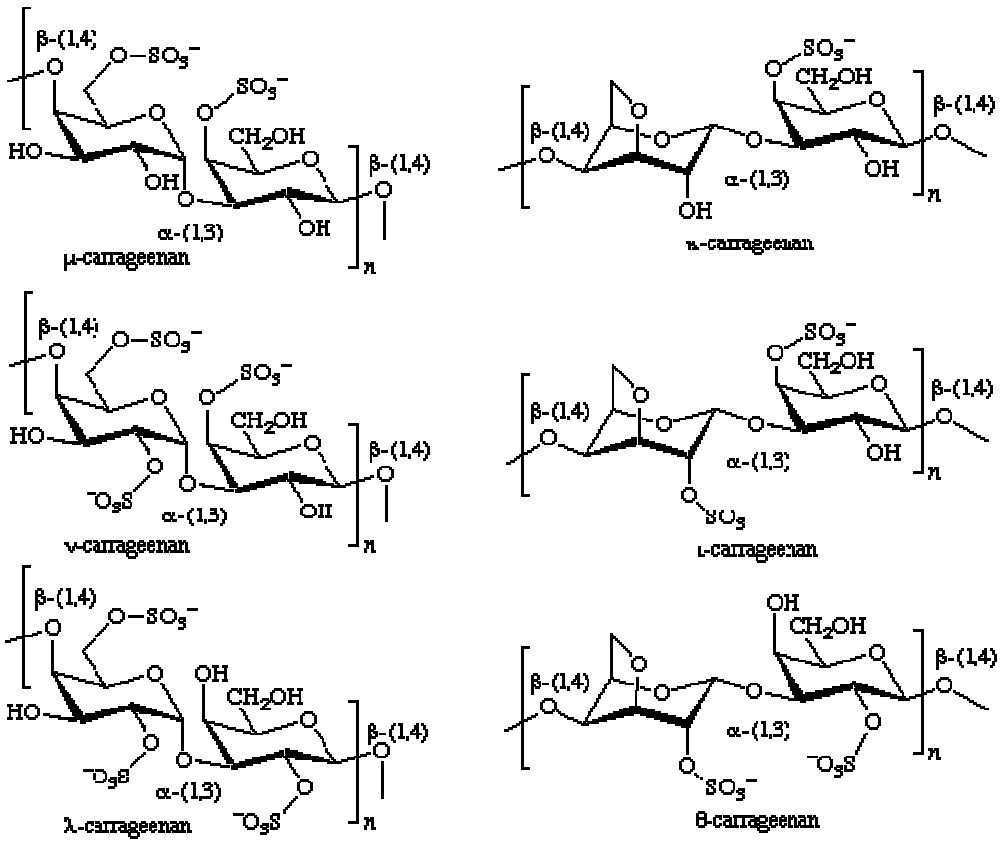


Figure 7: Structure of Carrageenan

Carrageenan are sulphated polysaccharides of D – galactose and 3,6 – anhydro – D – galactose. They are extracted from cell walls of the certain red seaweeds of the Rhodophyceae class. Red seaweeds are harvested around the coasts of the North atlantic, South America and the Far East. Different seaweed species produce different carrageenans.

The word carrageenan is derived from the colloquial Irish name for the red seaweed, carrageenan, which means 'little rock'. Carrageenans are used in pharmaceutical, cosmetic and food industries. In food industry they are used as thickening, gelling and protein suspending agents. In pharmaceutical industry they are used as excipient in pills and tablets. The food industry accounts for 70–80% of the total world production, estimated at about 45.000 metric tones per year, of which about 45% goes to dairy products and 30% to meat and meat derivates. (Imeson, 2010; Campo, 2009).

Carrageenans are different to each other depending on the presence of the 3,6 – anhydro bridge and the position of sulphate substituent. Because of their half ester sulphate moieties they are anionic polymers. Six types of carrageenans are present; iota (ι), kappa (κ), lambda (λ), Mu (μ), Nu (γ) and Theta (θ) carrageenans but most widely used carrageenans are iota (ι), kappa (κ) and lambda (λ) carrageenans. κ-carrageenan has one, ι–carrageenan has two and λ – carrageenan has three sulphate groups.

$\imath$ – carrageenan = carrageenase 2,4' – disulphate

κ – carrageenan = carrageenase 4' – sulphate

λ – carrageenan = carrageenase 2, 6, 2' – trisulphate

Sulphate content of λ – carrageenan is %41 w/w, κ – carrageenan is %20 w/w, and $\imath$ – carrageenan is %33 w/w. The average molecular mass of carrageenans is between 100 – 1000 kDa.

The thickening and gelling properties of each carrageenan is different to each other. Gel formation involves a conformational transition from random coil to helix form. κ – carrageenan forms thermally reversible firm, brittle gels, i – carrageenan gives soft, elastic gels, λ – carrageenan is non – gelling but thickens instant drinks and dairy deserts. κ – and i – carrageenans contain 3, 6 – anhydro bridges in the α (1 → 4) – linked D–galactopyranose residue, whereas λ–carrageenan does not have an anhydro bridge so, λ–carrageenan cannot form gel. Also i – and κ–carrageenans form helices in the presence of certain ions but λ–carrageenan does not appear to form helices and therefore does not form gels.

Variation in ester sulphate contents in carrageenans affects the gel strength, texture, solubility, setting and meltng temperatures, syneresis synergies and interctions with other hydrocolloids and ingredients.

All carrageenans are water soluble, but insoluble in organic solvents, oil or fats. The sulphate groups and associated cations in carrageenans affects the solubility of carrageenans in water because these parts are very hydrophilic. Also these groups affect the viscosity of carrageenan solutions.

The main ionizable cations founds in carrageenans are sodium, potassium, calcium and magnesium but other ions can also occur at low frequency.

Carrageenan solutions are viscous and show pseudoplasticity or shear thinning when pumped or stirred. The viscosity of carrageenans depends upon concentration, temperature, the presence of other solutes, the type of carrageenan and the molecular weight of carrageenan. If temperature of carrageenan solution increases, the viscosity of the solution decreases. The presence of salts can decrease the viscosity by reducing the electrostatic repulsion among the sulphate groups.

In our laboratory, λ–carrageenan was used. λ–carrageenan occurs naturally and obtained from different species of the Gigortina and Chondrus genera. The sporophytic plants of these seaweeds produce λ–carrageenan.

Carrageenans have some potential pharmaceutical properties including antitumor, immunmodulatory, antihyperlipidemic and anticoagulant activities. Carrageenans also have antiviral properties, inhibiting the replication of herpes and hepatitis A viruses. Therapeutic applications of low molecular weight carrageenans have been limited because of their gastrointestinal toxic side effect.

In food industry, usage of carrageenan over the limited value causes the gastrointestinal side effect. In the European Union, carrageenan is approved as a food additive and assigned the number of E 407. (Imeson, 2010; Quemener, 2000; Soedjak, 1994; Shtykova, 2000; Hambleton, 2008;

Lin, 1970; Santo, 2009; Schoeler, 2006; Tong, 1901; Campo, 2009; De Ruiter, 1997; Yamada, 1997; Caceres, 2000; Aguilan, 2006).

In chemistry laboratories carrageenans are quantitatively determined by using different methods, some of them are;

-Carrageenans are analyzed by HPLC. For determination of carrageenans several colorimetric methods are used. Also the carrageenans are found in complex food products such as yogurt, gelling milk can be determined by the methanalysis coupled to reverse HPLC, but this method is not applicable for the quantification of pure λ–carrageenan, it is applicable only for k– and i-carrageenans. The carrageenan is quantitatively determined by potentiometric titration. (Quemener, 2000; Soedjak, 1994; Campo, 2009; Kayansalan, 2010).

2.2.2- XANTHAN

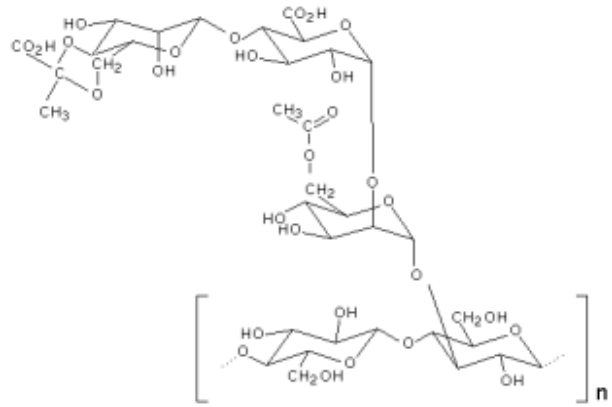


Figure 8: Structure of Xanthan

Xanthan gum is a natural and microbial polysaccharide, and produced by the bacterium Xanthomonas *campestris*. Xanthan gum discovered at 1959

at the Northern Regional Research Laboratories (NRRL) of the United States Department of Agriculture. Every year 20.000 tones of Xanthan produced industrially from X. *Campestris*. (Colegrove,1983; Ochoa, 2000).

Xanthan contains a pentasaccharide repeating units which formed by two glucose, two mannose and one glucuronic acid units in the molar ratio 2.8 : 2.0 : 2.0. The primary structure of xanthan consists of β – (1,4) linked D– glucose units. The chemical structure of the main chain is identical to that of cellulose. Xanthan may be chemically considered as an anionic polyelectrolyte, because of its structure. (Ochoa, 2000; Imeson, 2010; Rodd, 2000).

Molecular weight of xanthan changes by its contents of glucose, glucuronic acid, mannose, pyruvate, acetate and galactose into the molecule. The molecular weight of xanthan is about 2.10^6 daltons.

Xanthan is used in industries of food, oil, pharmaceutical, cosmetic, paper, paint and textile. Some properties of xanthan makes it available for industrial usage. Some of them are;

- It is soluble in both cold and hot water.
- It has high viscosity even at low concentration.
- Its solution is pseudoplastic.
- Its production is not expensive.
- It is found in ordered conformation at moderate temperature.
- It has excellent solubility and stability in an acid system.
- Unique rheological properties that provide high viscosity under low shear and low viscosity under high shear.

- It has excellent thermal stability.
- Excellent suspending properties owing to a high yield value.
- Xanthan gum is compatible with the other thickeners, like carrageenan, alginate, pectin, gelatin... etc.

In industries; xanthan gum is used as thickening, stabilizing, gelling and emulsifying agent. (Casas, 1999; Becker, 1998**;** El-Sayed; 2002; Imeson, 2010; Ochoa, 2000).

Viscosity of xanthan at moderate temperature is highly viscous. The viscosity of xanthan solution changes by temperature because the conformation of xanthan changes. At low temperature ordered conformation is found and at high temperature disordered conformation is observed. The other factors which affect the viscosity of xanthan solutions are the polymer concentration, concentration of salts and pH. (Ochoa, 2000; Casas, 1999). If concentration of polymer increases, viscosity increases too. Adding of salt on the xanthan solution affects the viscosity because molecular interaction is affected .(Ochoa, 2000) Viscosity of xanthan solution is not affected by pH which changes between pH : 1 to pH : 13. Only pH conditions pH (11 – 12) and pH (1 -2) affect viscosity. Viscosity of xanthan decreases when the pH of the solution is below pH : 3.

Sometimes xanthan can used with other gums such as locust bear gum and guar gum in industry. The interaction between xanthan gum with other gums increases the viscosity of solution. (Imeson, 2010; Lachke,2004).

Xanthan is not a toxic compound, it does not cause irradiation of skin or eye. United States Food and Drug Administration approved xanthan for use

as a food additive without any specific quantity limitations, and in 1980 xanthan is added to list of Europian Economic Community as a food emulsifier/stabilizer (E415).

Xanthan gum concentration can be determined by dry weight of xanthan isolated by precipitation but also as a function of broth apparent viscosity. Xanthan is quantitatively analyzed by methanolysis coupled to reverse HPLC. (Ochoa, 2000; Quemener, 2000). Also xanthan is determined quantitatively by potentiometric titration.(Kayansalan, 2005).

2.2.3- SODIUM ALGINATE:

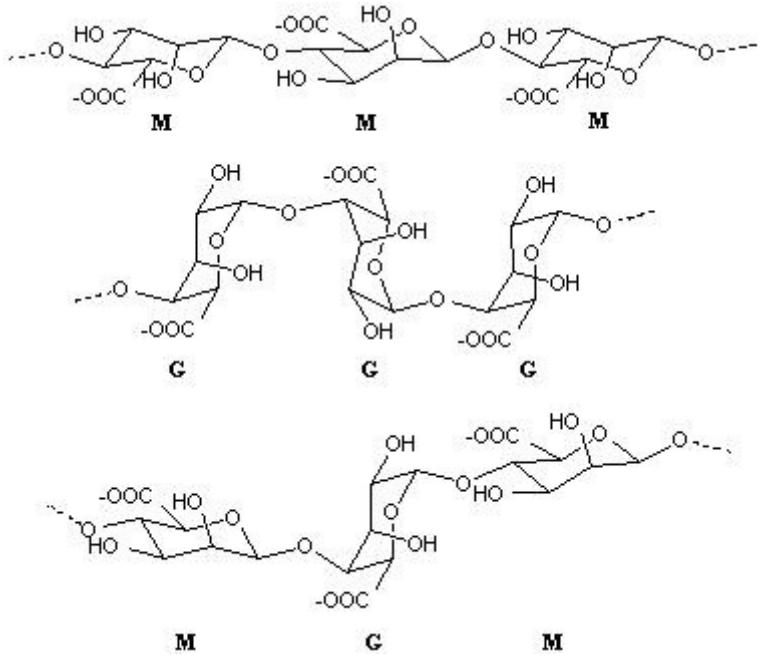


Figure 9 : Structure of Sodium Alginate

Alginates are negatively charged polymers produced from various species of brown seaweeds. Brown seaweeds are found the coasts of the North Atlantic, South America and Asia.

Stanford (1881) described alginate, and the name of alginate is now used as the general term for the alginic acid salts. The alginic acid is transformed to the alginate salts by the incorporation of different inorganic salts. The predominantly used salt of alginic acid is Sodium Alginate. Sodium alginate is a water soluble polysaccharide.

In Europe, alginic acid, sodium, potassium, calcium and ammonium alginate salts and propylene glycol alginate (PGA) are listed as a range of E-numbers from E400 to E405. E number of Sodium Alginate is 401. Also FAO/ WHO Joint Expert Committe on Food Additives (JECFA) listed them as food additives.

The molecular weights of alginates are approximately 10-600 kDa. Alginate is unbranched binary copolymers of (1 →4) –linked b-D-mannuronic acid (M) and a-L-gulunoric acid (G) residues as monomers.

These monomers are located in three different ways; homopolymeric MM blocks, homopolymeric GG blocks and heteropolymeric MG blocks.

Three types of glycosidic linkages are found in the block structures, because the M and G residues are in the $^{4}C_{1}$ and $^{1}C_{4}$ conformation respectively. These glycosidic linkages are including diequatorial (MM), diaxial (GG), and equatorial – axial (MG).

The charge and bulk of the carboxylic acid groups give these different conformations, different degrees of extention and flexibility. M-G is the most flexible conformation and G-G is the most rigit arrangement. (Flexibility MG > MM > GG).

Alginates can form gels. Alginate gels are used in pharmaceutical industry as wound dressing, control release drugs and immobilization matrices for cells or other materials. The dissolution of alginate is depend on the particle size, and viscosities of alginate solutions depends upon the composition of the aqueous solution. (Imeson, 2010**;** Davidovich, 2010; Doume'che, 2007; Schroeder & Racicot, 1941; Toti, 2004; Funami, 2009).

CHAPTER – 3: EXPERIMENTAL:

Optodes can be adopted to different formats. They can be used either in microtiter plate or UV-VIS spectrophotometer. In UV-VIS spectrophotometer, usage of optodes is difficult every sample should be worked alone, and you have a possibility of making error, during the casting of membrane cocktail on the slides, it cannot be homogeneous. Because of U-shapes of microtiter plate (MPR) you cast the membrane cocktail into the each well homogeneously, and usage of MPR is more simple than the usage of UV-VIS spectrophotometer.

Microtiter plate provide high samples (96-samples) throughput and these samples can be processed at the same time in few seconds. Microtiter plate is an injection- molded rectangular plastic component with rows and columns of honeycomb-like wells, and it can be used for determination of polyions. (Kim, 1998; Kim, 2001). In our laboratory polypropylene-based microtiterplate is used because it shows resistance to most of the organic solvents like THF.

3.1-REAGENTS:

Protamine Sulfate salt, HEPES Sodium salt, Poly-L-Arginine, Poly-L-Lysine and 2',7'-dichlorofluorescein were purchased from Sigma. Carrageenan, Xanthan, 3-Hydroxy-4-(4-nitrophenylazo) phenyloctadecanoate (ETH-2412), Polyuratane (PU) and high molecular weight poly(vinyl chloride) PVC, dioctylsebacate (DOS)were obtained from Fluka. TRIS (hydroxymethyl) aminomethane, 99.9 + % ultrapure grade, iodooctadecane (IOD) were obtained from Aldrich. Polypropylene –based U-bottomed microtiter plate (96 well –greiner bio-one) is obtained from Teknis.

All other solvents and chemicals were analytical grade. Standard solutions and buffers were prepared with deionized water (0.055 µS). TRIS Buffer contains 0.12 M NaCI. Concentrations of TRIS Buffer and HEPES Buffer are is 0.05 M.

Apparatus: Hitachi U-2900 Spectrophotometer, and Versa Max Microplate Reader.

3.2 - Experiments of Film-1

3.2.1- Synthesis of 2',7'-dichlorofuorescein octadecylester (DCFOE):

A mixture of 1-iodooctadecane (1 mmol, 380 mg), 2',7'-dichlorofluorescein (430 mg, 1,07 mmol), K_2CO_3 (290 mg, 2,1 mmol) and 5 mL dimethylsulfoxide was stirred in an oil bath at 65 ºC for 20 hours. When reaction is completed, 10 mL of saturated NaCl was added to reaction mixture to form red precipitate. This precipitate was filtered by vacuum and washed with deionized water, then redissolved in a mixture of ethylacetate and 1 M HCl solution (1:1). Organic phase was separated (this separation procedure repeated three times with Ethyl acetate and 1 M HCl solution mixture)and washed with phosphate buffer (pH: 7,4) and deionized water. The organic phase was separated again and evaporated to dryness by evaporator under pressure. Finally the obtained product was recrystallized twice with acetone.

3.2.2-Preparation of Film-1

The dye cocktail contains %15 high molecular weight Poly(vinyl chloride)(PVC), %15 Polyurethane (PU), %69 Bis (2-ethylhexyl) sebacate (DOS) and %1 polycation sensitive chromoionophore 2', 7'-dichlorofluorescein octadecyl ester (DCFOE) and the 200 mg mixture of dye components are dissolved in freshly distilled 2 ml of tetrahydrofuran (THF). This coctail is mixed well to obtain homogeneous mixture.

The dye cocktail is uniformly dispensed (10 µL) into polypropylene based U-bottomed microwells, and air dried in a dust free area for 1 day.

Also this coctail can cast on the slides (30 µL) and after this casting process slides were allowed to dye in dryness medium for 20 min and then same procedures with MPO was applied. These slides were used in UV-VIS Doble beam Spectrophotometer.

3.2.3-Titration:

Polyanions (DNA, λ -Carrageenan, Xanthan, Na-Alginate) are titrated with different polycations (Poly-L-Lysine, Poly-L-Arginine and Protamine). Different polycations show different interaction with deprotonated DCFOE, thus; optical response of them different.

3.2.3.1-Titration of Polyanions with Protamine and poly-L-Arginine by using DCFOE dye;

Polyanions (DNA, λ -Carrageenan, Xanthan, Na-Alginate) are titrated with polycations protamine and poly-L-Arginine. Firstly, absorbances of each DCFOE dye containing optodes are pre-readed at 540-620 nm after stabilizing of their absorbances in TRIS Buffer (pH: 7,4) or HEPES Buffer (pH: 7,4) for 20 mins. Buffered solutions of polycations (Protamine and poly-L-Arginine) were prepared at different levels.

300 µL of these solutions are filled in the polymeric film coating wells and they were waited in the wells for 3 mins, after 3 mins solutions are recovered from wells immediately to replace with buffer solution and absorbance of each polymeric films are recorded at 540-620 nm, blank's curve were plotted by the obtained absorbance values. Then, different compositions of polyanions (DNA, λ -Carrageenan, Xanthan, Na-Alginate)

and polycations (protamine, poly-L-Arginine) mixtures were prepared. After absorbances of each optodes were pre-readed with 300 µL of TRIS Buffer (pH: 7,4) or HEPES Buffer (pH: 7,4) for 20 mins. 300 µL of different levels of polyanion (DNA, λ -Carrageenan, Xanthan, Na-Alginate) and polycation (protamine, poly-L-Arginine) mixtures were filled in the wells immediately and these solutions were waited in the wells for 3 mins, after 3 mins solutions are recovered from wells to replace with 300 µL of buffer solution, and the absorbance of each optodes are measured at 540-620 nm by the microplate reader. Finally, absorbance versus concentration of Polycation (Protamine, Poly-L-Arginine (PLA)) graph were plotted. Stochiometries between polyanions and polycations were found from these graphs and by the food product samples recovery experiments our method was checked.

3.2.3.2-Titration of Polyanions with Poly-L-Lysine by using DCFOE dye;

In this part; polyanions (DNA, λ -Carrageenan, Xanthan, Na-Alginate) are titrated with polycationic Poly-L-Lysine. Firstly, absorbance of DCFOE dye containing polymeric films were stabilized by 300 µL of HEPES Buffer (pH: 7,4) for 20 mins, then absorbances of each optodes were prereaded at 540-620nm. Buffered solutions which contain different levels of Poly-L-Lysine (PLL) are prepared. Each 300 µL of these solutions were filled in the microwells immediately and they were waited in the wells for 20 mins, after 20 mins solutions were recovered from wells and 300 µL of HEPES Buffer (pH: 7,4) were filled instead of them and absorbance of each wells were recorded at 540-620 nm, and by the obtained values blank's curve were plotted. For the titrations of polyanions (DNA, λ -Carrageenan, Xanthan, Na-

Alginate) with titrant Poly-L-Lysine, absorbance of different compositions of polyanions (DNA, λ -Carrageenan, Xanthan, Na-Alginate) and Poly-L-Lysine solutions were determined. Different levels of polyanions were titrated with Poly-Lysine to obtained a graph. At first, the absorbance of polymeric films were stabilized by 300 µL of HEPES Buffer (pH: 7,4) for 20 mins and pre-readed at 540-620 nm with microplate reader. Solutions with different compositions of polyanion (DNA, PPS, λ -Carrageenan, Xanthan, Na-Alginate) – PLL mixtures were filled instead of HEPES buffer (pH:7,4) after pre-read and these solutions were waited in the wells for 20 mins and after this time solutions into the microwells were recovered immediately from wells and 300 µL of HEPES Buffer (pH: 7,4) was filled into the wells. Finally, absorbance of optodes were recorded at 540-620 nm and absorbance versus concentration of PLL (µg/mL) graphes were plotted, from these graphes stochiometries between polyanions and PLL were found.

3.2.3.3-Determination of Thickening Agents in Food Products:

Known weights of the samples of food products containing thickening agents (1 g- 10 g) were dissolved in pure water and these dissolved samples were filtered by vacuum and volume of the sample solutions were completed to 100 mL with ultra pure water . At first absorbances of each optodes which containing DCFOE dye were pre-readed at 540-620 nm with 300 µL of HEPES Buffer (pH : 7,4) or TRIS Buffer (pH : 7,4) (for polycation Poly-L-Lysine only HEPES Buffer were used) for 20 mins. Buffered solutions of polycations (Protamine, Poly-L-Arginine, Poly-L-Lysine) were prepared at different levels. 300 µL of these solutions were filled in the microwells and these solutions were waited in wells for 3 mins for polycations protamine and Poly-L-Arginine and 20 mins for Poly-L-Lysine and then, solutions were recovered from microwells and buffer solution were filled instead of them. Absorbance of each optodes were recorded at 540-620 nm with the microplate reader. Blank's curve were plotted by the obtained absorbance values. Selected concentration of sample solutions were titrated with standart polycation solutions (protamine, PLA , PLL). These prepared solutions were filled into the microwells and waited in wells for 3 mins for titrants protamine and PLA, and 20 mins for titrant PLL and then solutions were recovered from microwells and 300 µL buffer solution (pH : 7.4) were filled into the wells. Absorbance of each optodes were measured at 540-620 nm by the microplate reader .

Finally absorbance versus polycation concentration (µg/ mL) graphs were plotted for each sample and from these graphs amount of thickening agent in the sample solution were found and then amount of thickening agent were calculated in 100 g of food product.

3.2.3.4- Experiment for the validity of method:

Our analyzing method were checked by recovery experiments of the food products. For these experiments, in the first part a preferred concentration of any food product were titrated with the polycation (Protamine, PLA, PLL) standart solutions. In the second part, the same amount of sample with excess amount of thickening agents (10, 20,40 µg/mL Carrageenan, Xanthan or Na-Alginate), which is the same polyanion in food product, were titrated with polycation standard solutions. By this way a set graph was created. These recovery experiments were repeated and the graphs were plotted with the average values.

3.3 - Experiments of Film-2:

3.3.1-Titration of Polycations with Polyanions (Carrageenan and Xanthan) by using ETH-2412 dye:

In the previous parts of the experiments, polyanions (DNA, λ -Carrageenan, Xanthan, Na-Alginate) were titrated with different polycations (Protamine, PLL and PLA) and polycation sensitive DCFOE dye were used for these titrations, but in this part; polycationic protamine were titrated with different polyanions (λ -Carrageenan and Xanthan). In this part polyanion responsible optode were used, because dye cocktail which used in these part contains a lipophilic anion exchanger TDMAC.

3.3.2-Preparation of ETH-2412 Dye Cocktail:

Polyanion sensitive optode contains %20.0 PVC, %77,3 DOS, %1,7 ETH-2412 and %1.0 TDMAC. 200 mg of the mixture of the dye coctail components were dissolved in 700 µL of freshly distilled THF. After dissolving of all components in THF homogenously, dye cocktail were uniformly dispensed with micropipette in each microwell (5 µL/ well) for a U-bottomed microplate well, and air dried in a dust free medium for 1 day.

3.3.3-Titration:

Before titration procedures, the response time of polyanions was found, ETH-2412 doped optodes were exposed to 500 µg/mL carrageenan and 500 µg/mL xanthan solutions and at the different time intervals the optical response of these optodes were recorded. Response time of each polyanions with TDMAC is different to each other, this time is 60 min for carrageenan and 120 min for Xanthan. When molecule get bigger response time of polyanions increases.

Polycation protamine were titrated with polyanions Carrageenan and Xanthan standart solutions. Before the titrations, pH indicator ETH-2412 deprotonated with phosphate Buffer (pH: 7,4). Once the color of the optode was yellow but after deprotonation, color is changed from yellow to brown. Buffer were waited into the microwells of microplate until the colour of the optode changed completely. In the first part, protamine was titrated with carrageenan, the concentration of protamine in carrageenan-protamine titrations were 10 µg/mL and the concentration of titrant carrageenan changed between 0-15 µg/mL. The solutions were waited into the wells for 60 mins. After this time absorbances of each well was recorded at 540-750 nm with microplate reader and ΔA versus [Protamine]/[Carrageenan] graph was plotted, from this graph, stochiometries between protamine and carrageenan were found directly.

In the second part, protamine was titrated with Xanthan. The concentration of protamine in xanthan-protamine titration was 25 µg/mL and the concentration of titrant xanthan changed between 0-100 µg/mL. Solutions of Xanthan were waited into the wells for 120 mins. After this time absorbances of each wells were recorded at 540-750 nm with microplate reader and ∆A versus [Protamine]/[Xanthan] graph were plotted, from this graph, stochiometries between protamine and xanthan were found directly.

CHAPTER – 4: RESULTS

4.1. RESULTS OF DCFOE

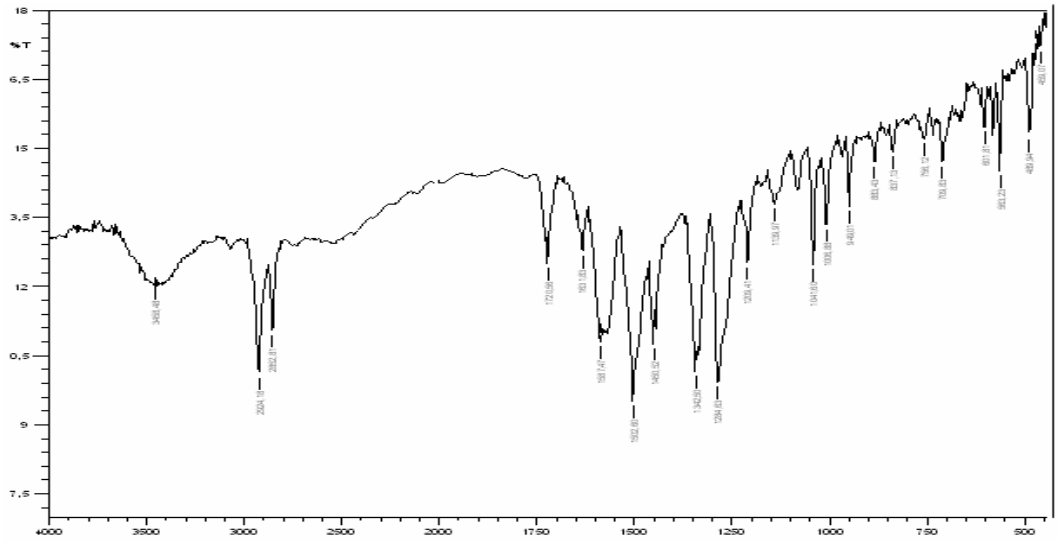


Figure 10 : IR result of DCFOE

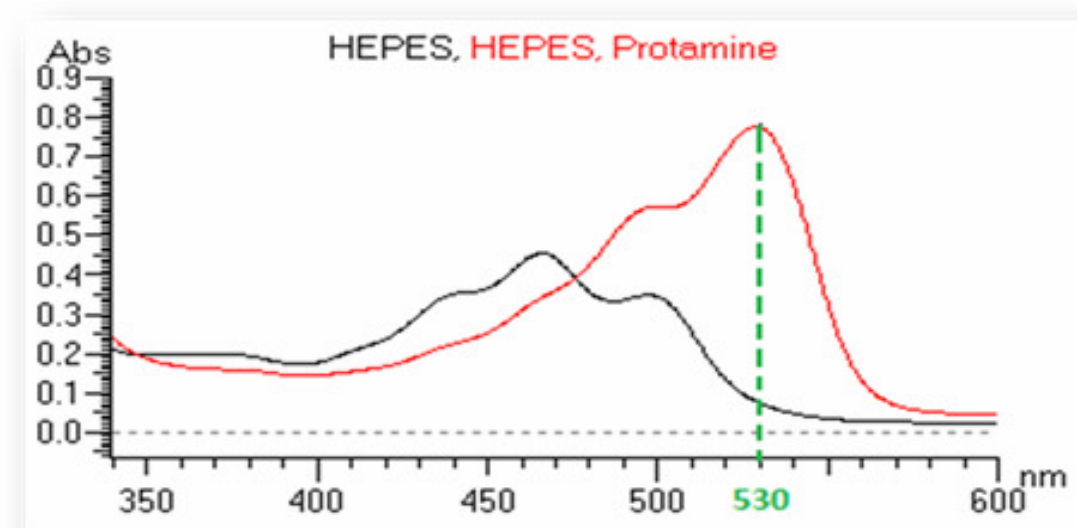


Figure 11: Wavelength scan of DCFOE doped polymeric film: black line refers to absorbance change of optode in buffer (pH : 7,4) and the red line refers to absorbance change of optode which exposed to protamine solution.

4.1.1-TITRATION OF POLYIONS

By Spectrophotometric titration detection limit of polyions were determined and also for these target polyanions; set graphs were created. The obtained results are;

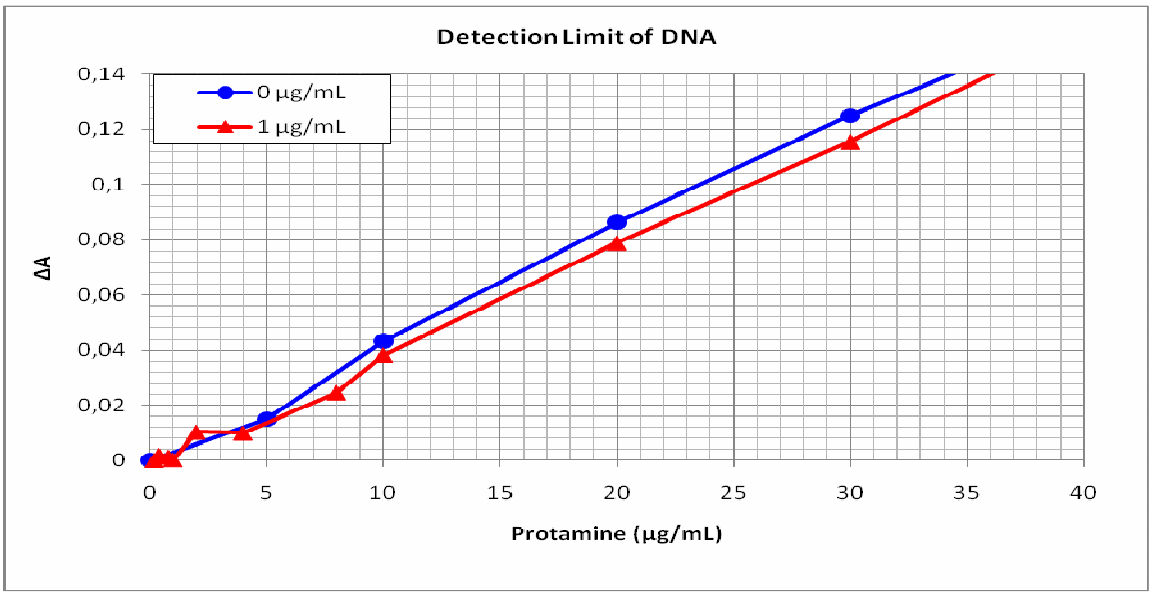


Figure 12: Detection Limit of DNA with Protamine

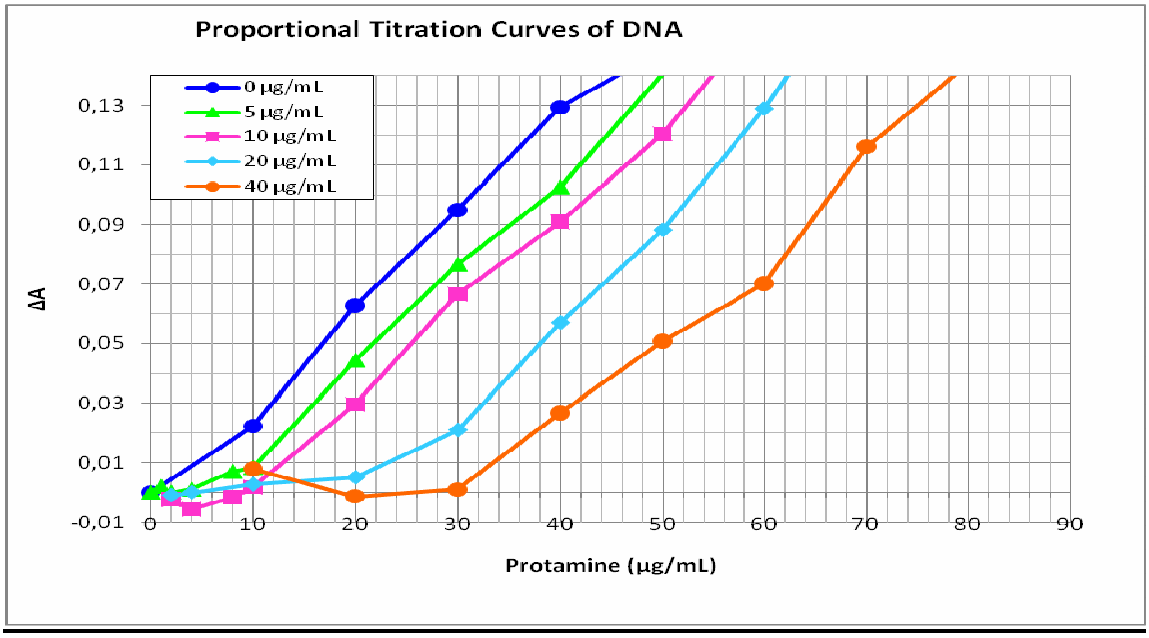


Figure 13: Proportional Titration Curves of DNA with Protamine

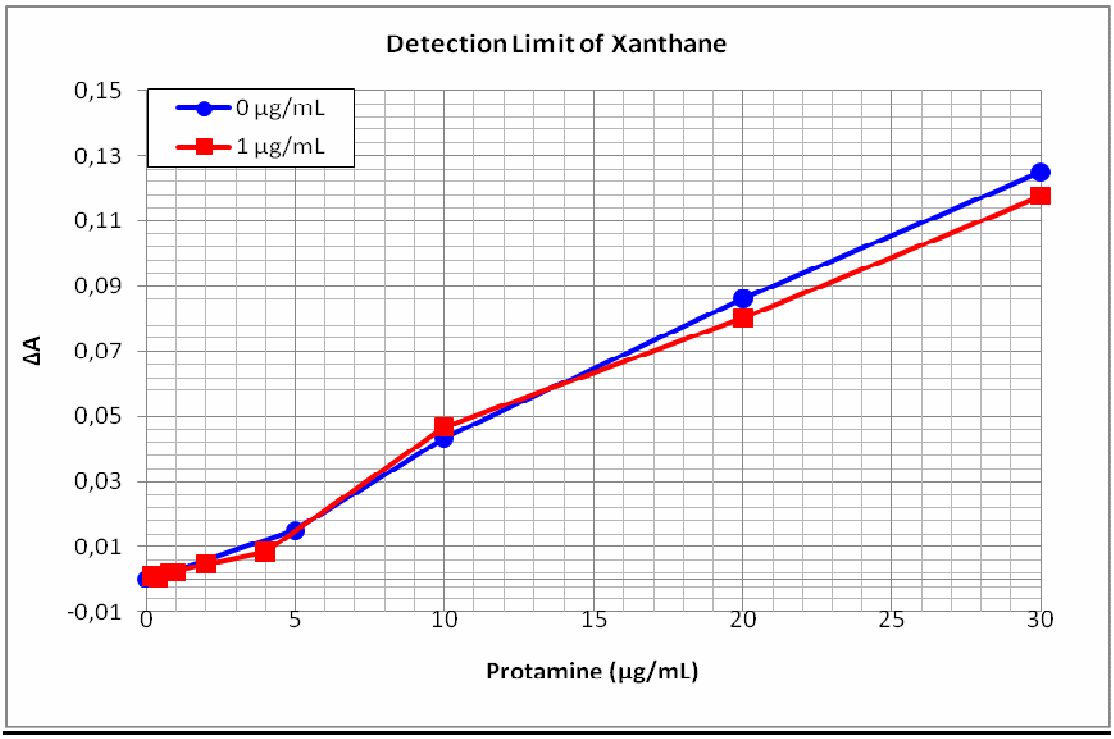


Figure 14: Detection Limit of Xanthan with Protamine

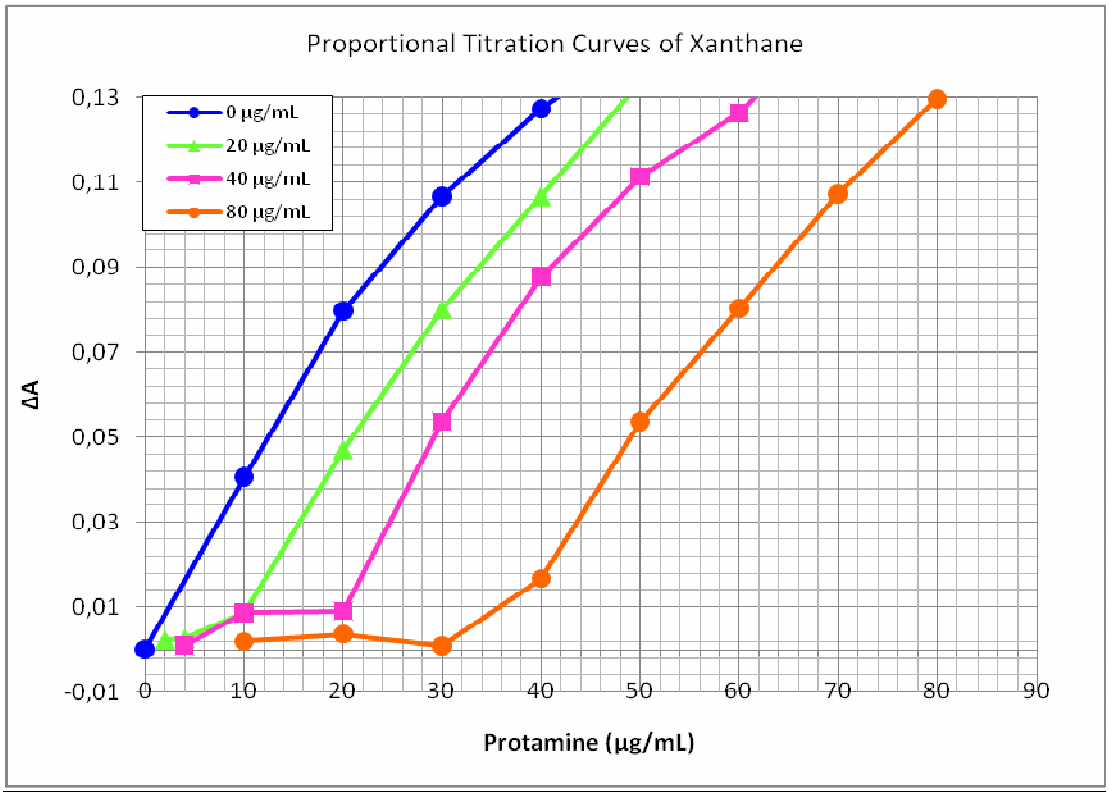


Figure 15: Proportional Titration Curves of Xanthan with Protamine

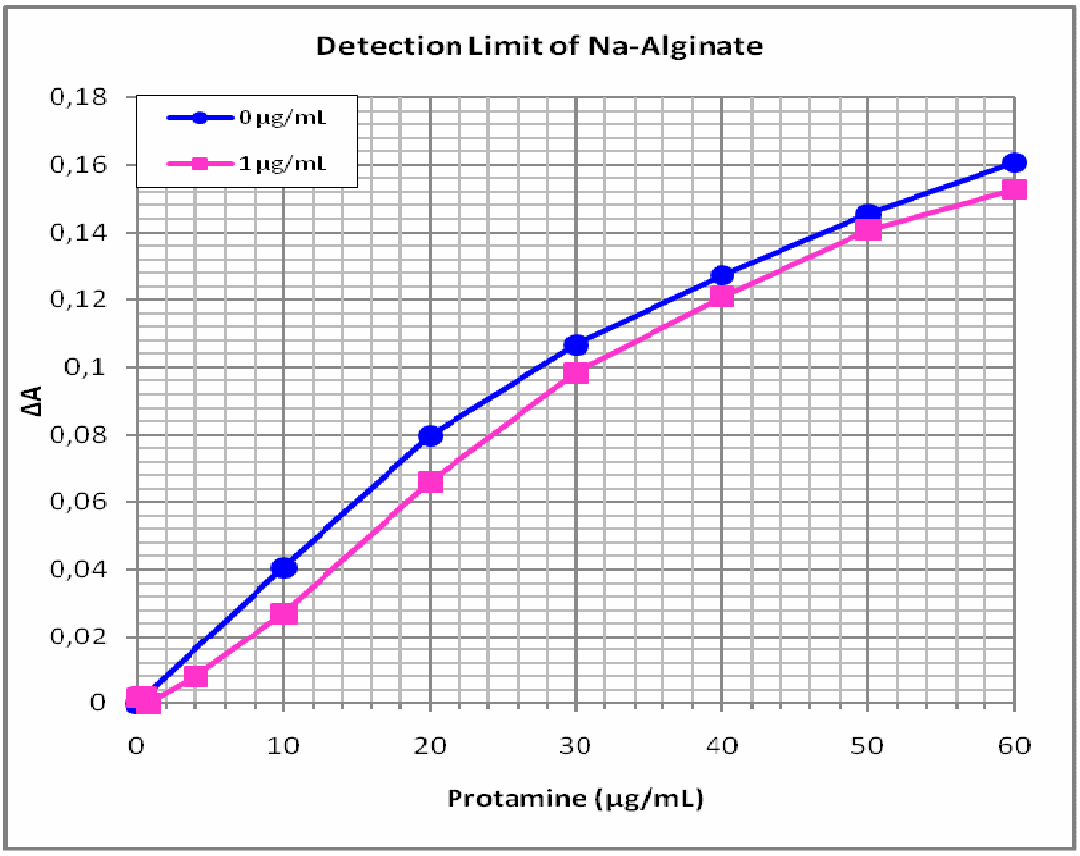


Figure 16: Detection Limit of Na-Alginate with Prtamine

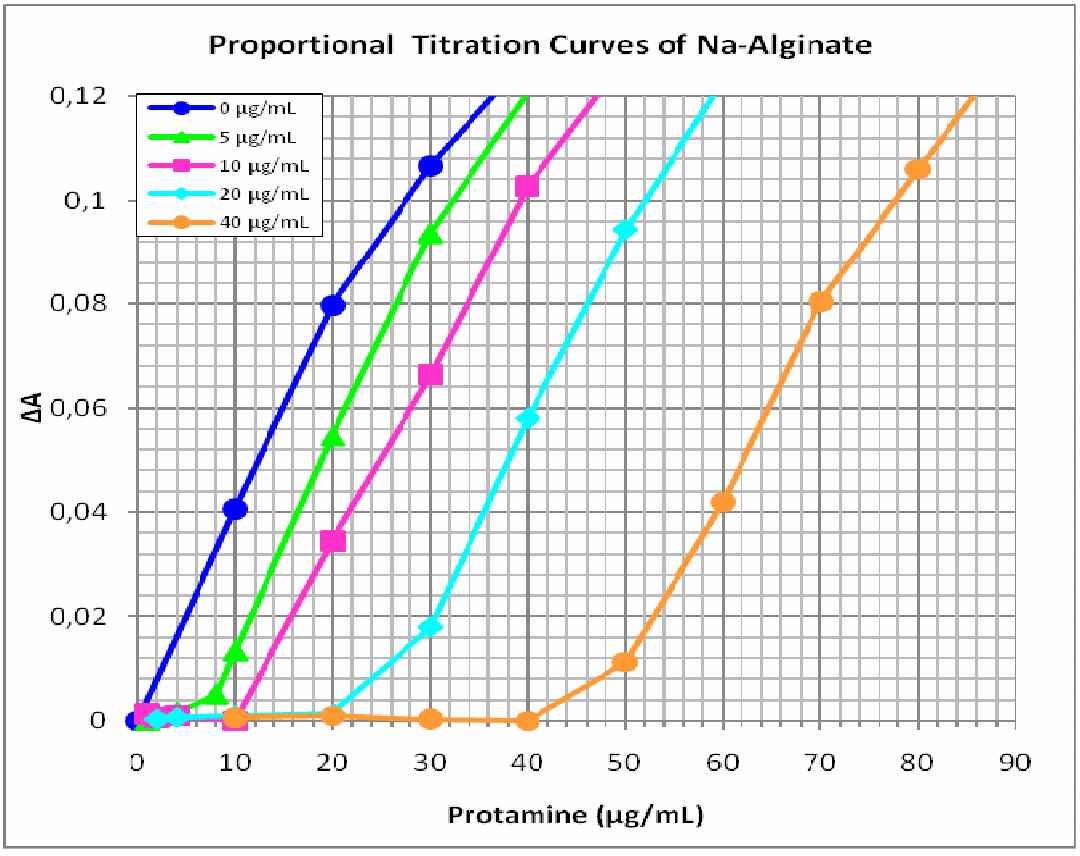


Figure 17: Proportional Titration Curves of Na-Alginate with Protamine

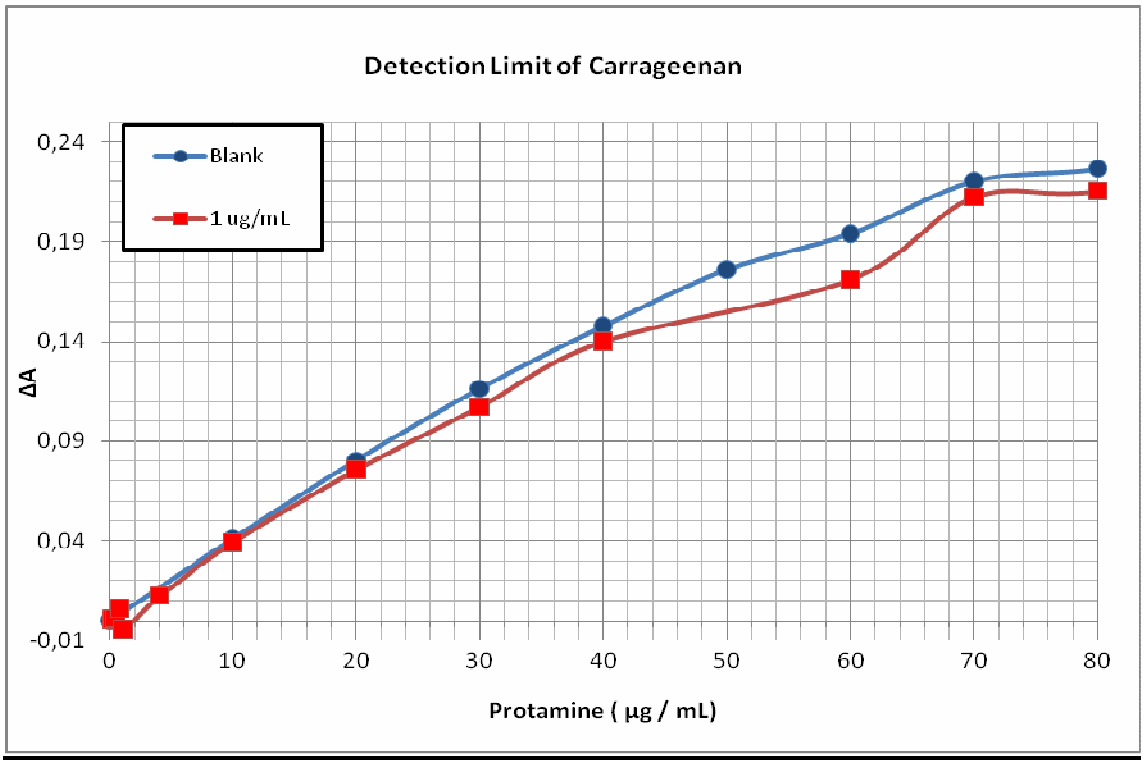


Figure 18: Detection Limit of Carrageenan with Protamine

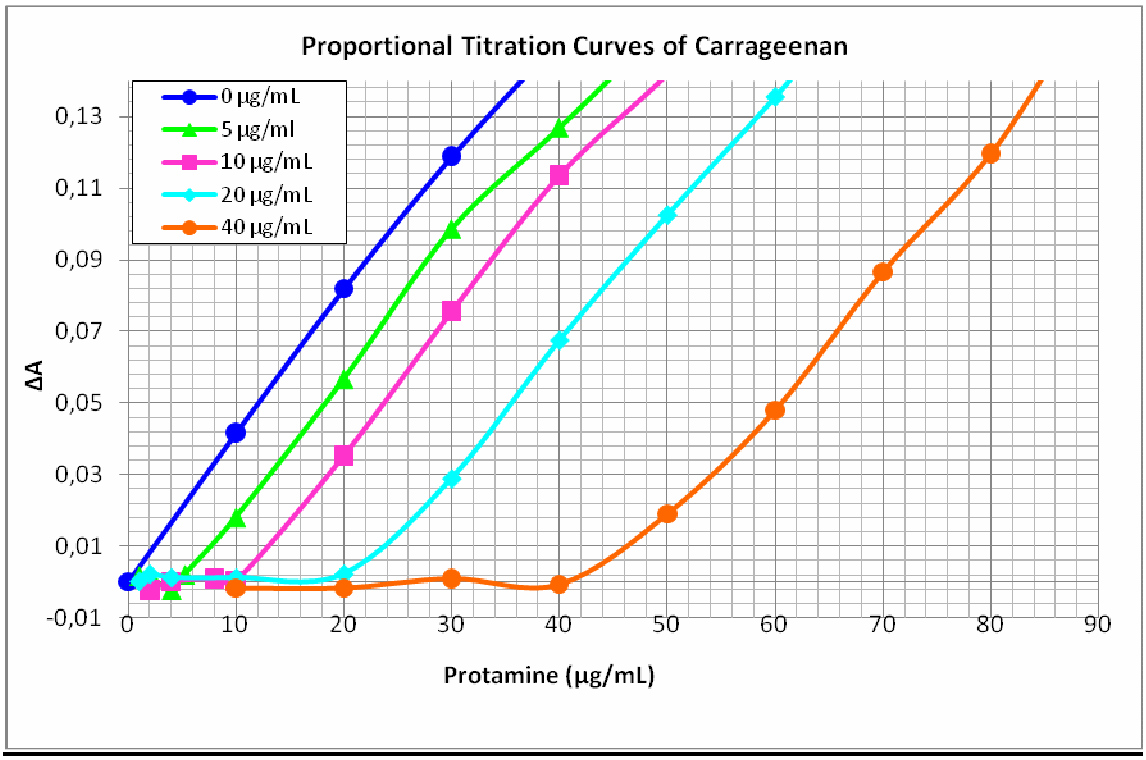


Figure 19: Proportional Titration Curves of Carrageenan with Protamine

DNA, Carrageenan, Na-Alginate and Xanthan titrated with PLL and PLA;

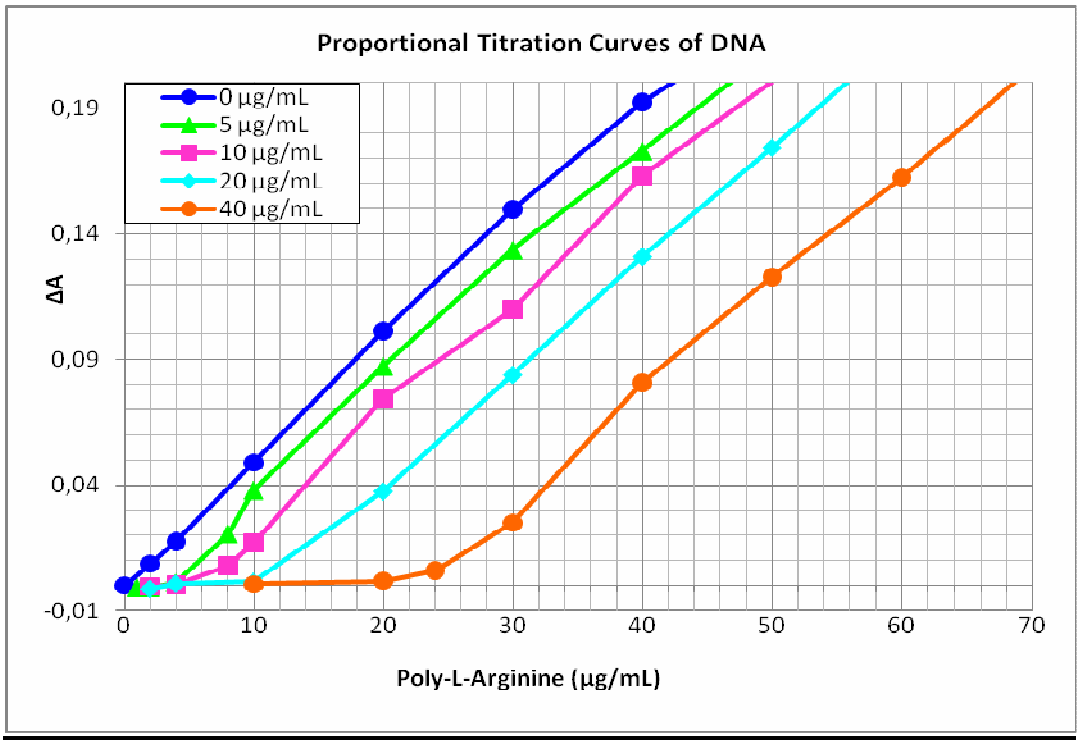


Figure 20: Proportional Titration Curves of DNA with PLA

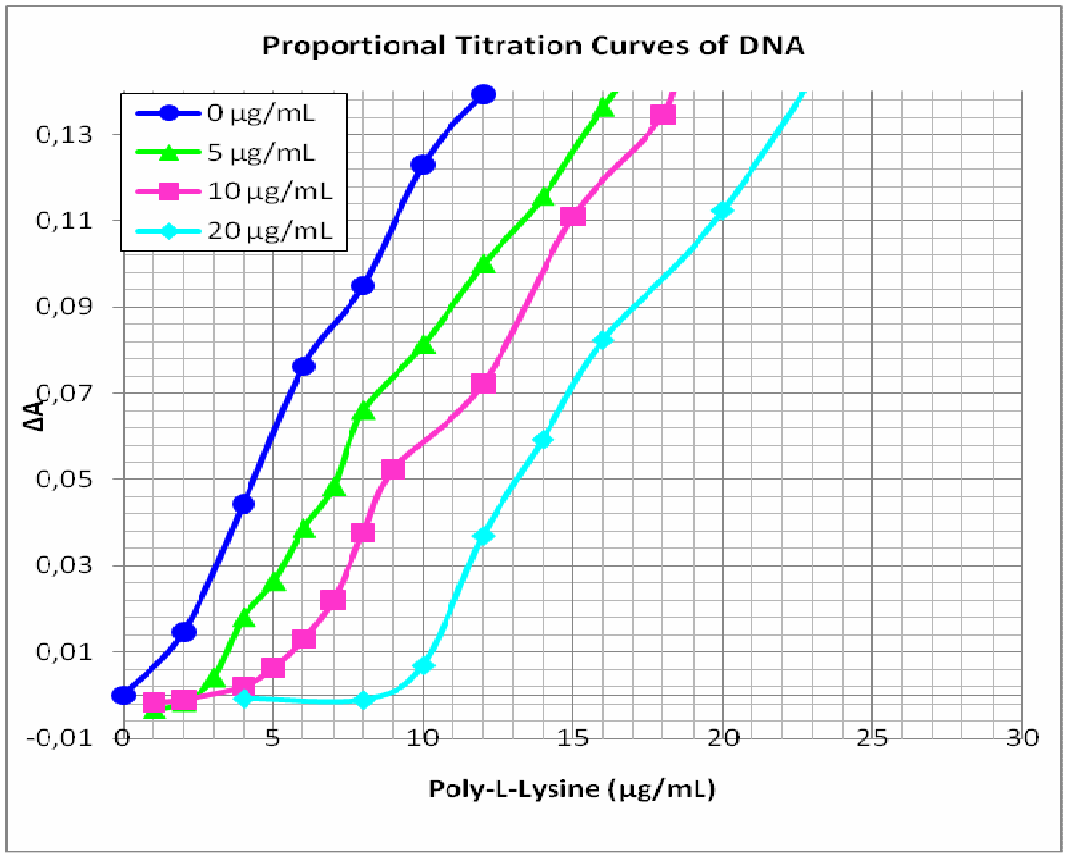


Figure 21: Proportional Titration Curves of DNA with PLL

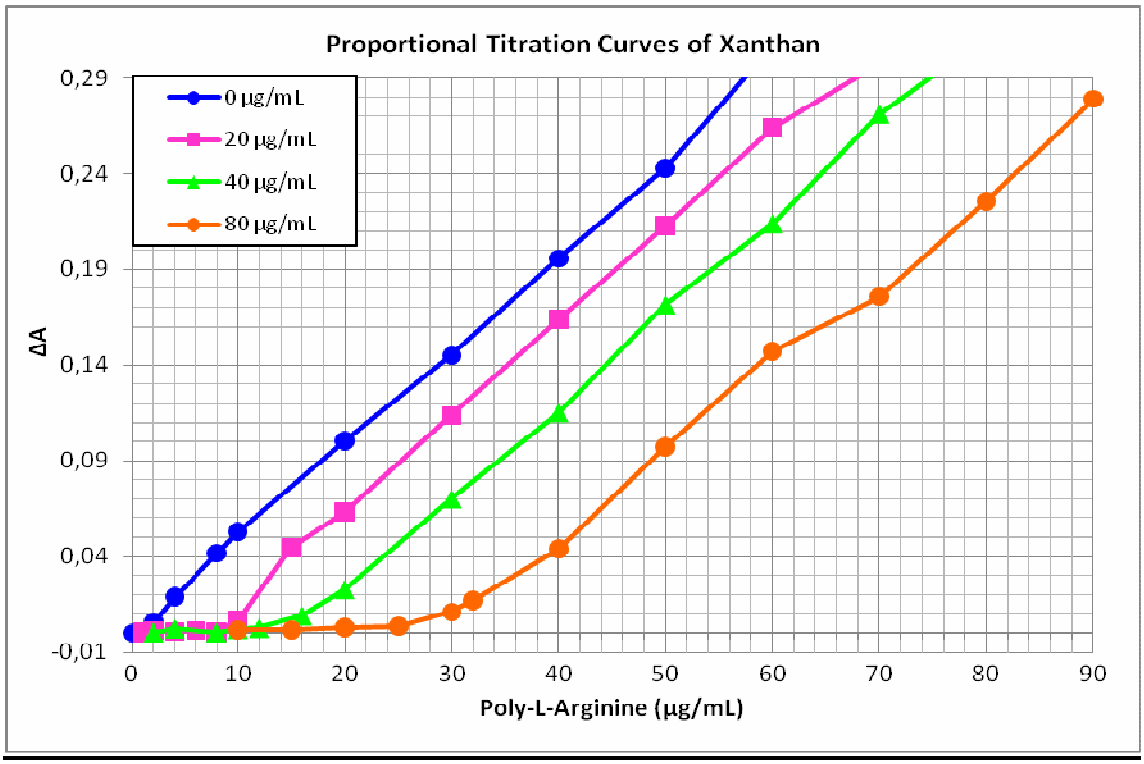


Figure 22: Proportional Titration Curves of Xanthan with PLA

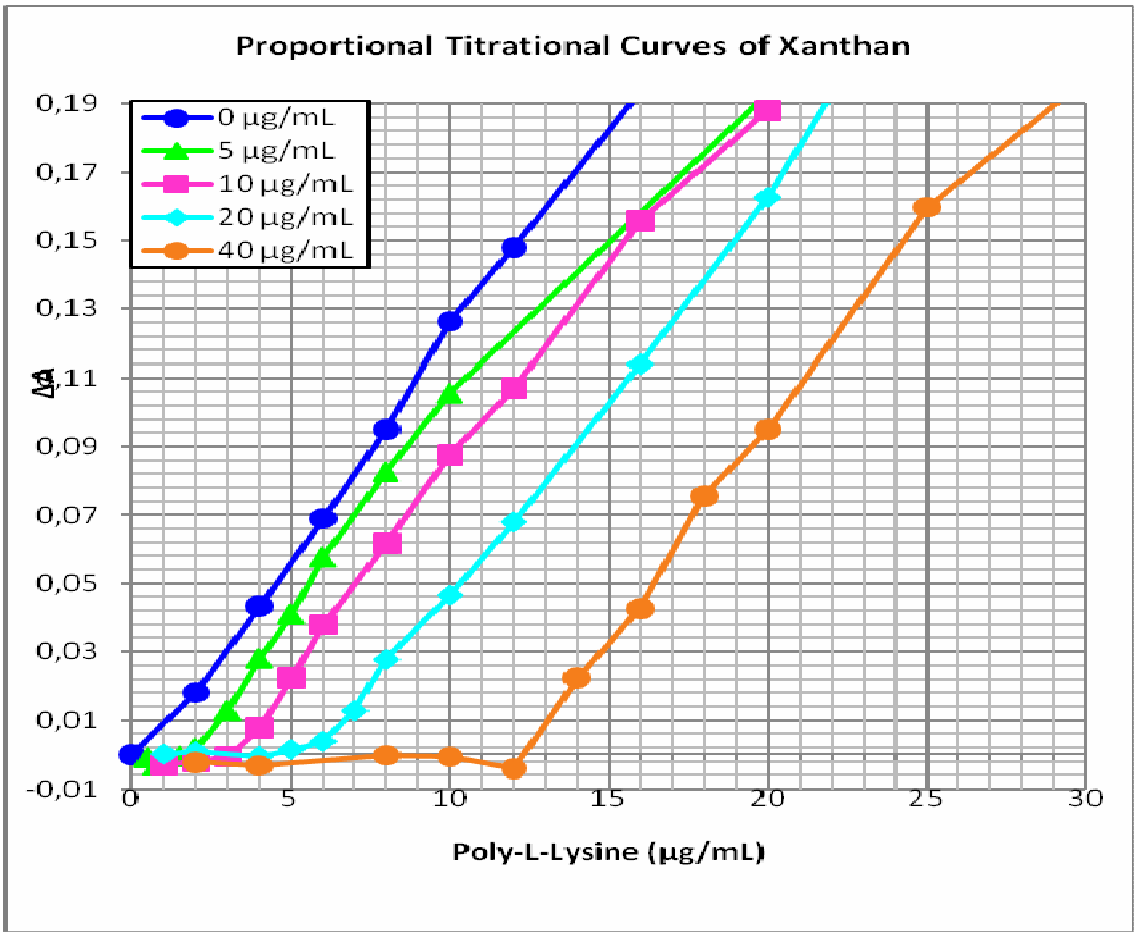


Figure 23: Proportional Titration Curves of Xanthan with PLL

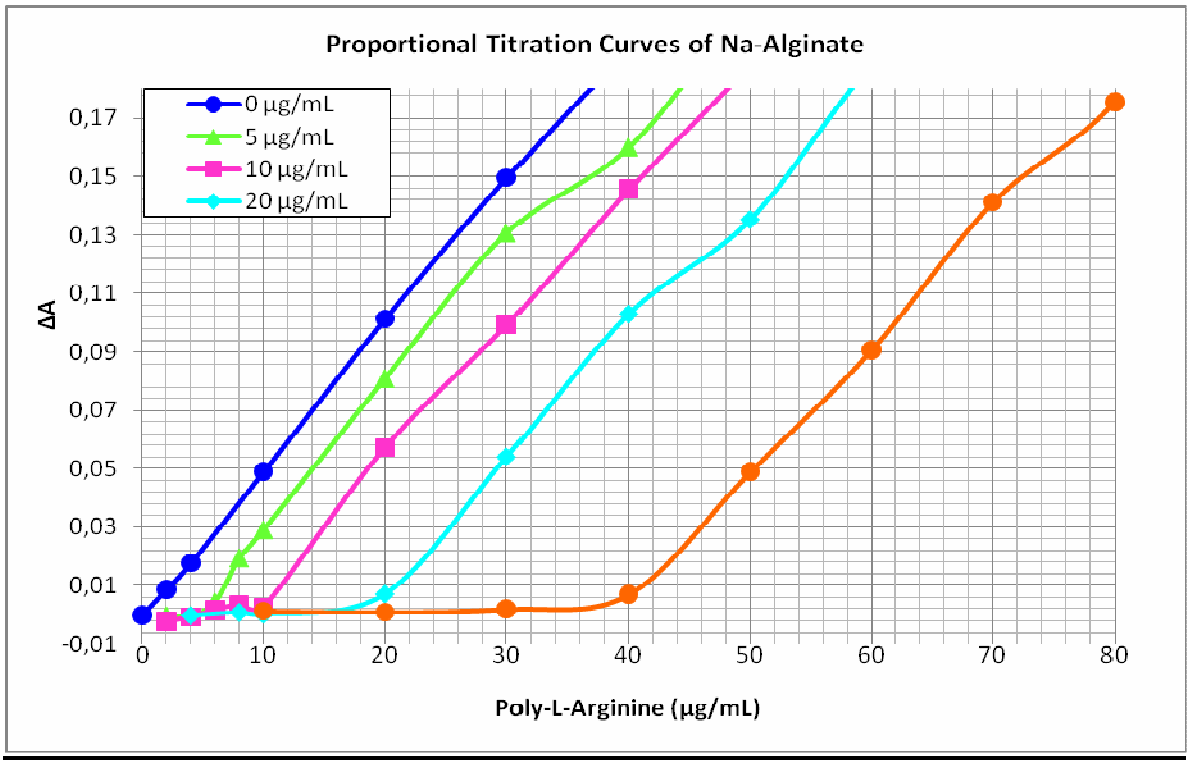


Figure 24: Proportional Titration Curves of Na-Alginate with PLA

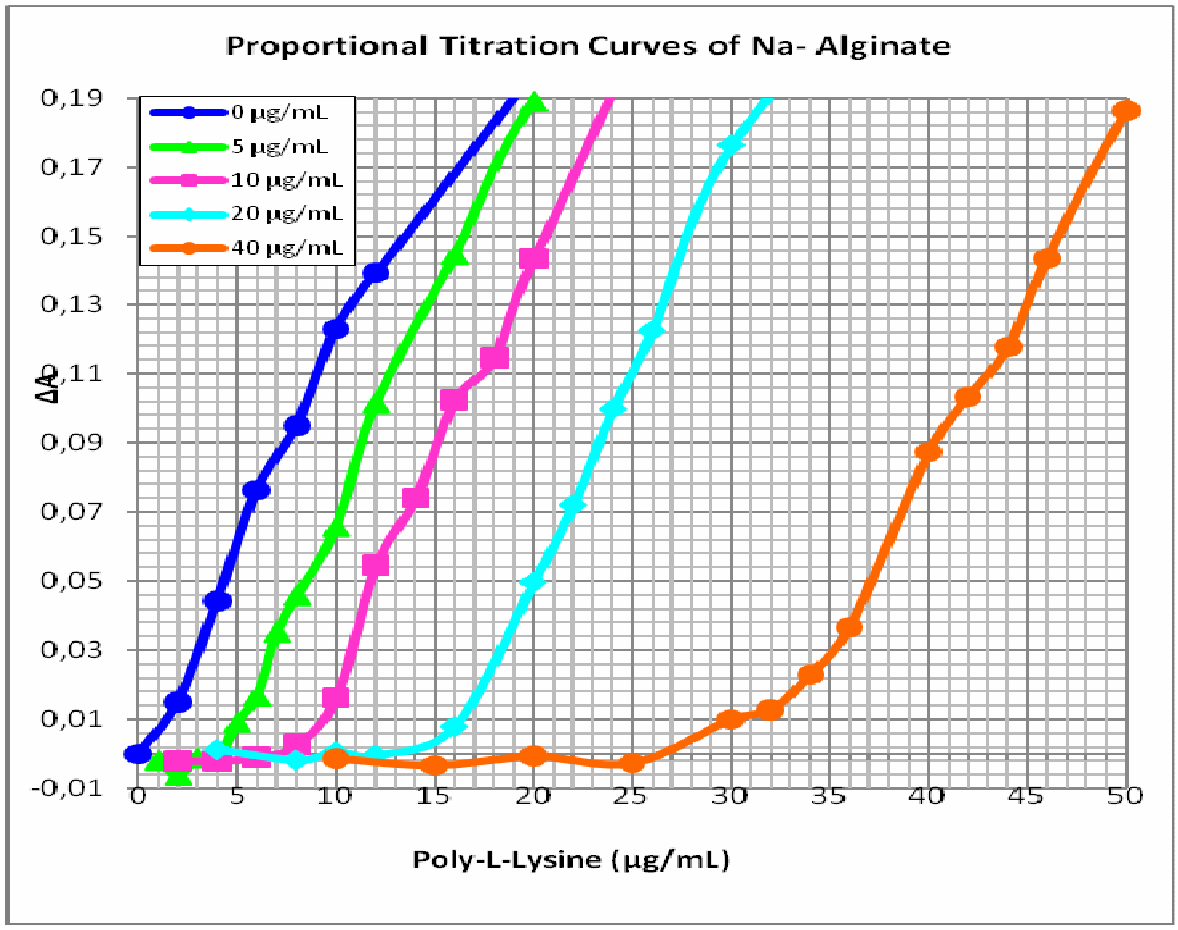


Figure 25: Proportional Titration Curves of Na-Alginate with PLL

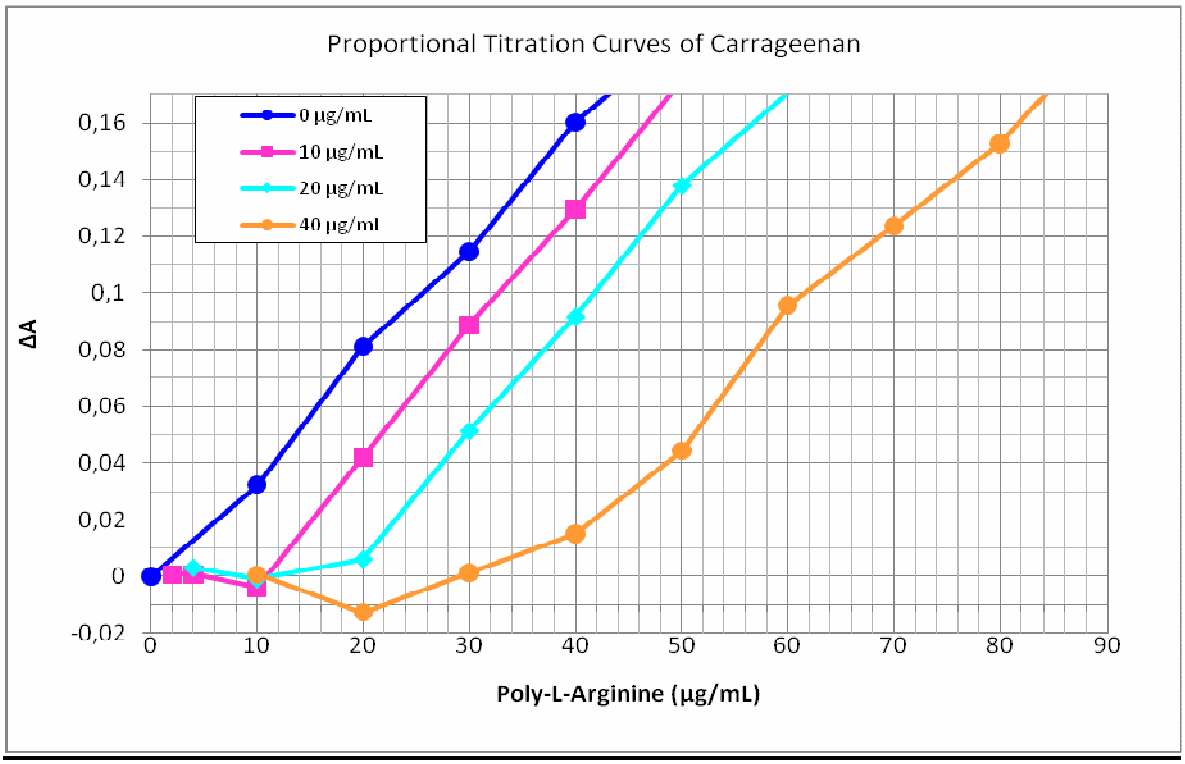


Figure 26: Proportional Titration Curves of Carrageenan with PLA

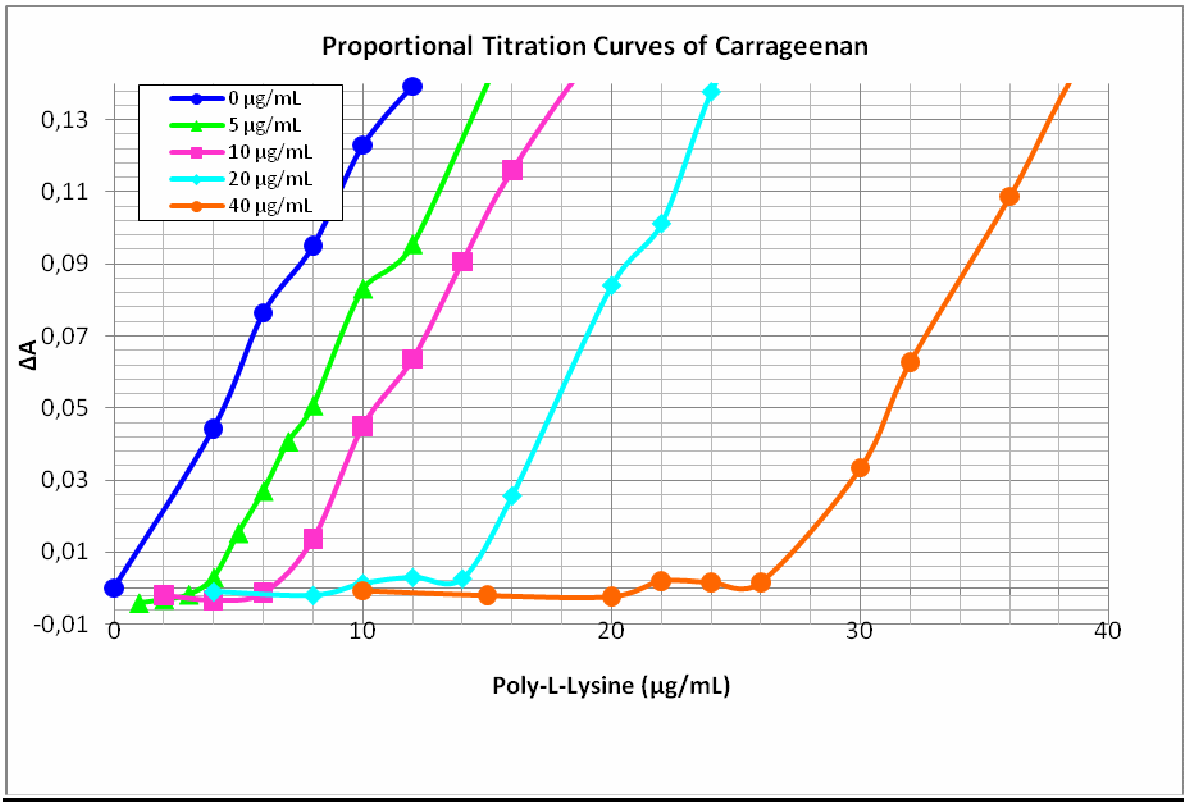


Figure 27: Proportional Titration Curves of Carrageenan with PLL

4.1.2-TITRATION RESULTS FOR FOOD PRODUCTS:

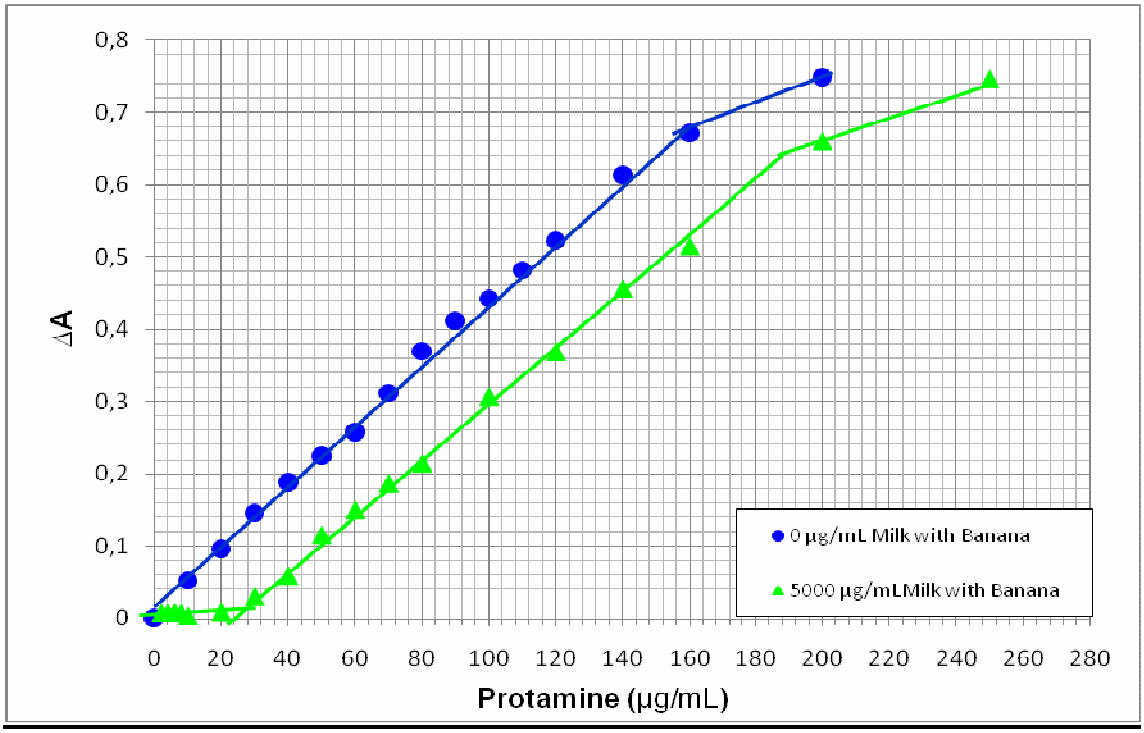


Figure 28: Pınar Kido Milk with Banana: (Carrageenan)

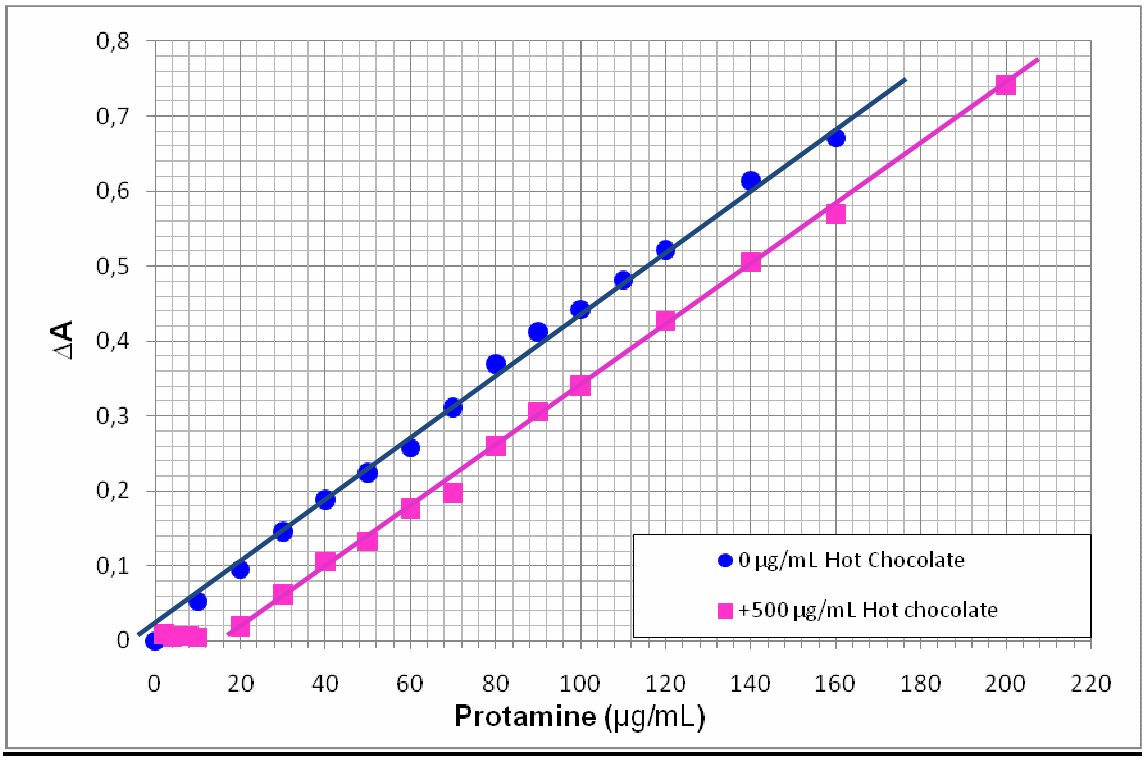


Figure 29: Dr. Oetker Hot Chocolate: (Carrageenan)

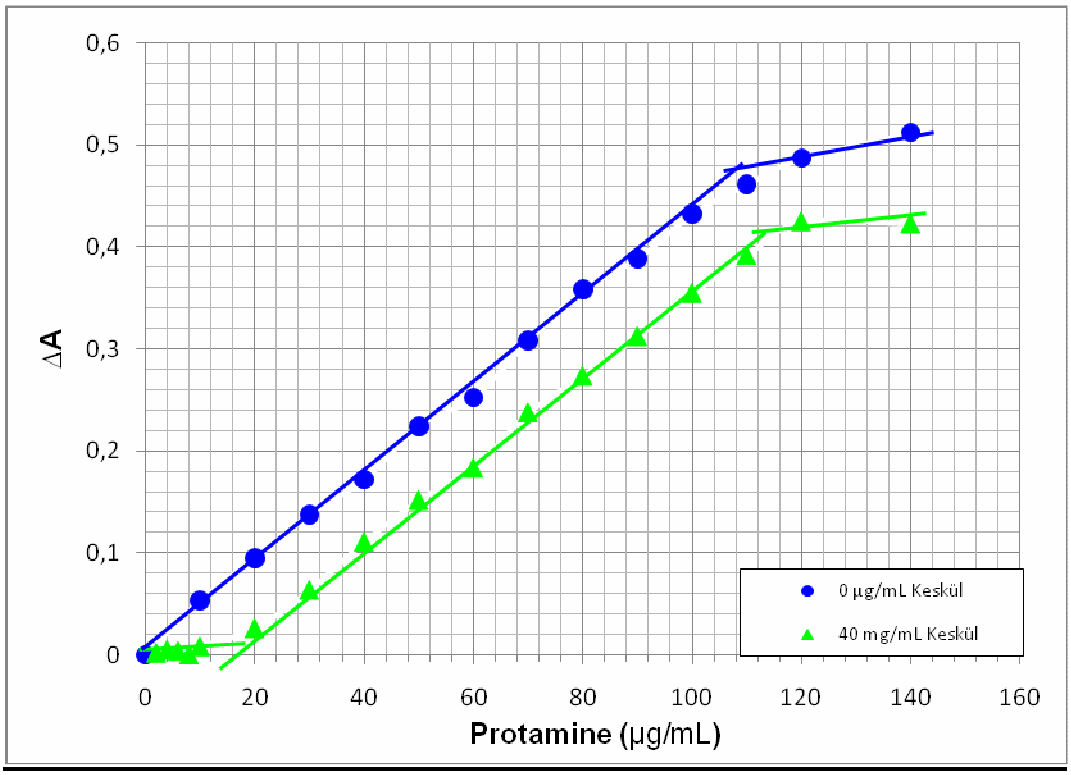


Figure 30: Dr. Oetker Keskül: (Xanthan)

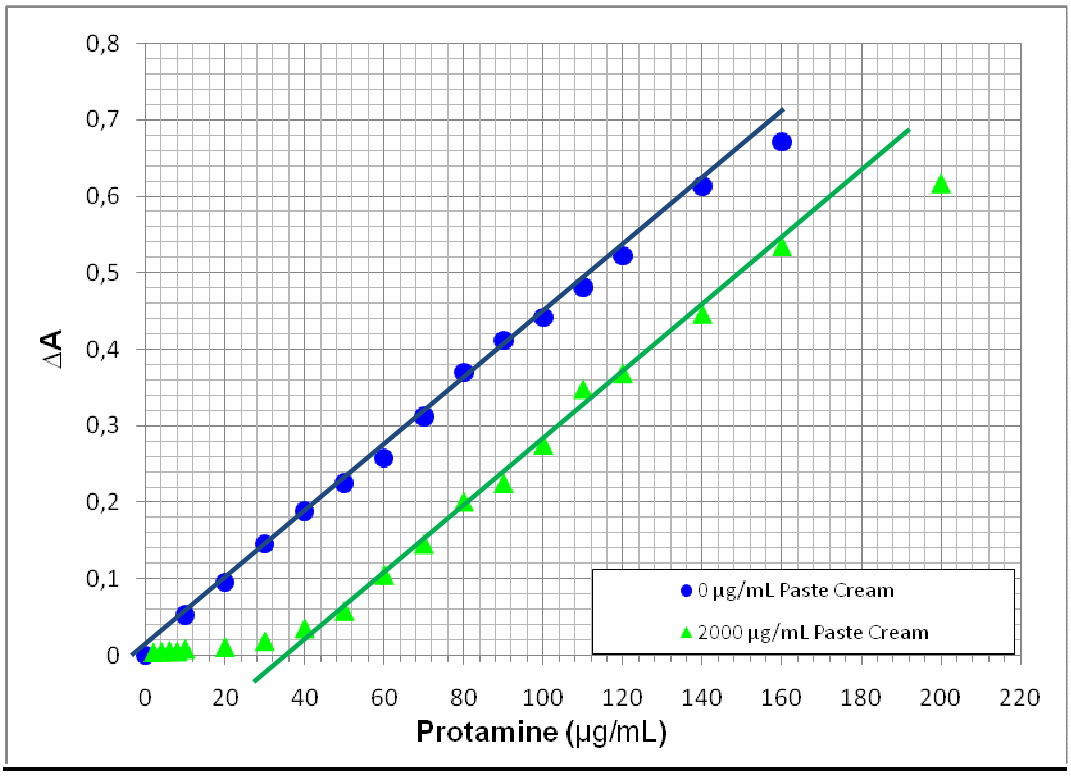


Figure 31: Dr. Oetker Paste Cream with Cacao: (Na-Alginate)

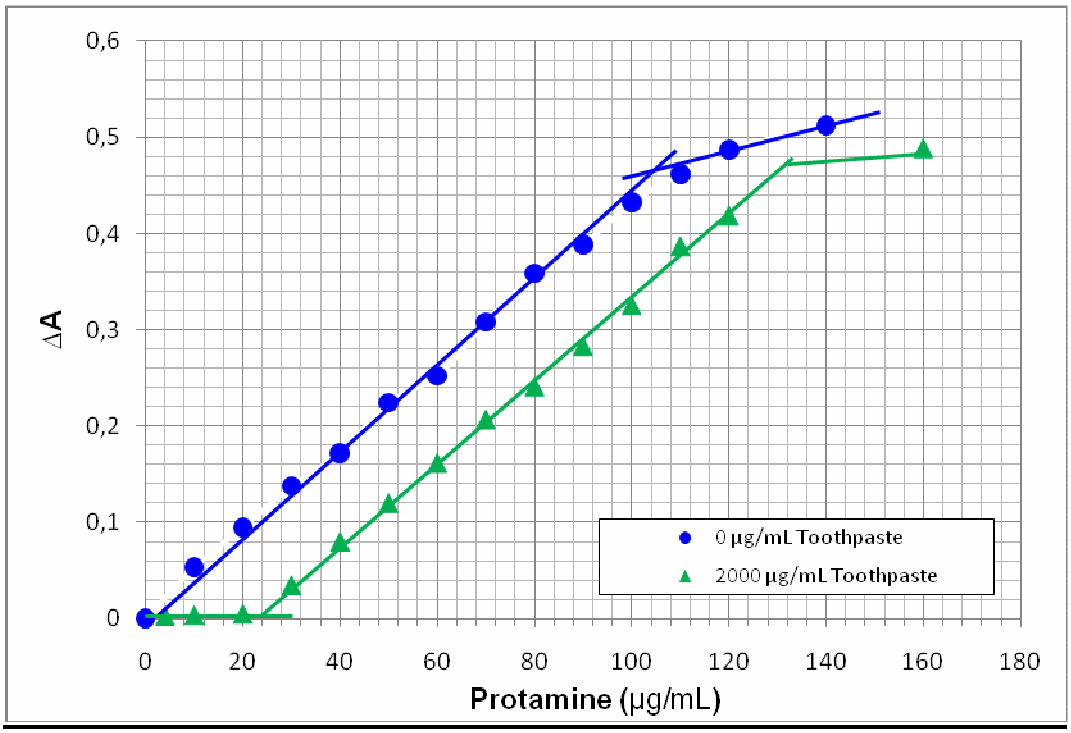


Figure 32: Theramed Toothpaste: (Mixture)

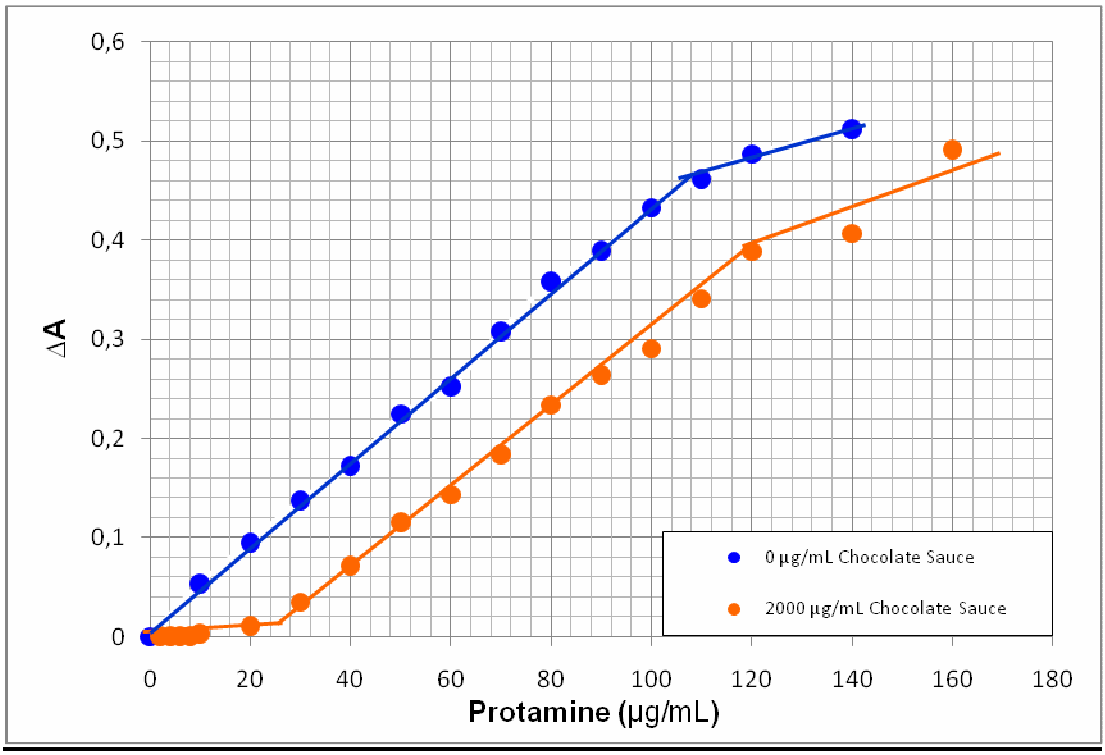


Figure 33: Dr.Oetker Chocolate Sauce: (Xanthan)

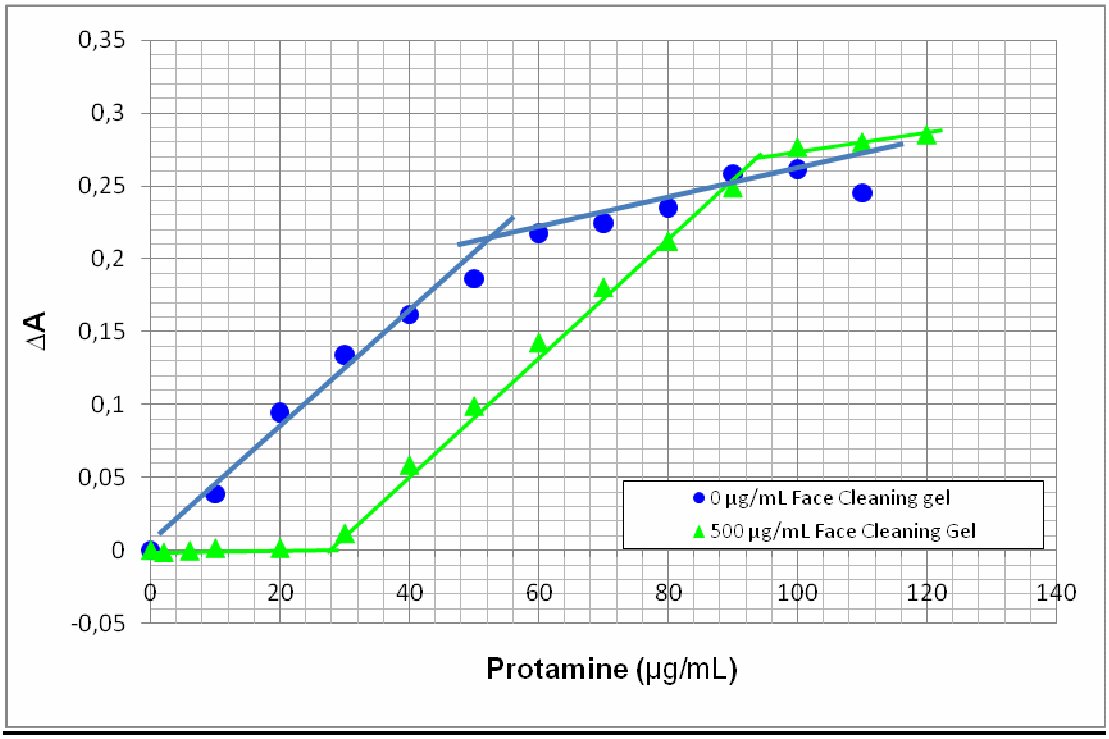


Figure 34: Nivea Face Cleaning Gel: (Mixture)

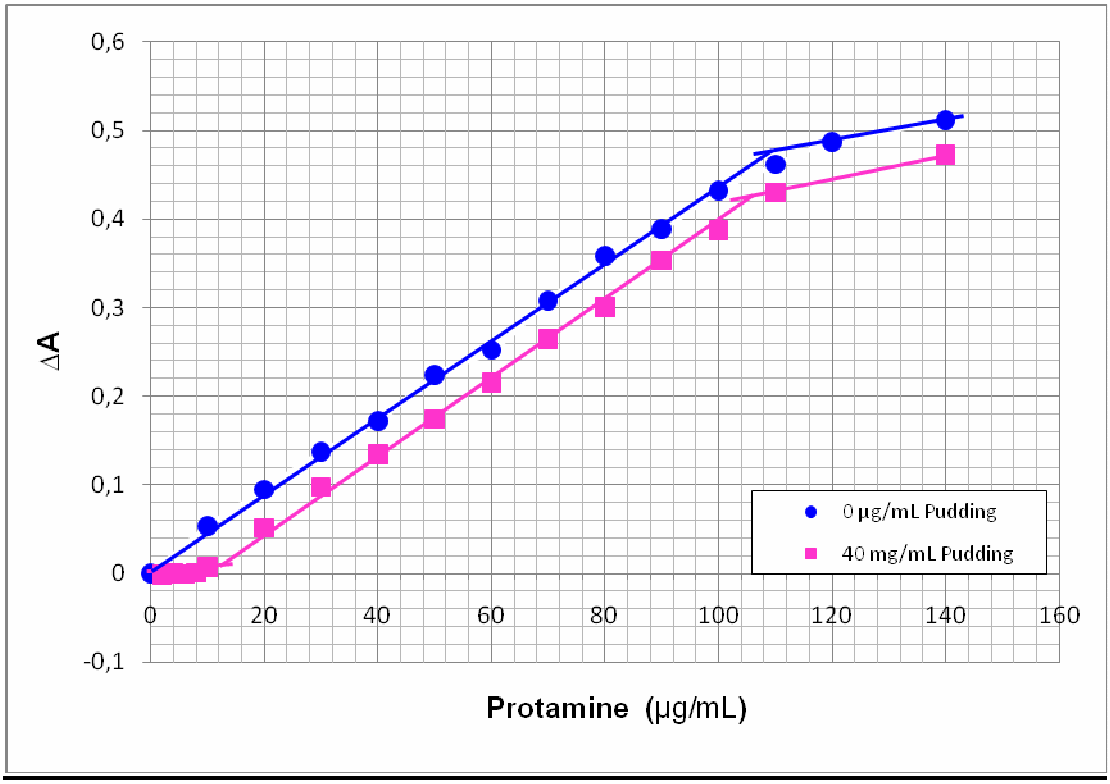


Figure 35: Şok Pudding with Banana: (Carrageenan)

4.1.3-Results for Recovery Experiments

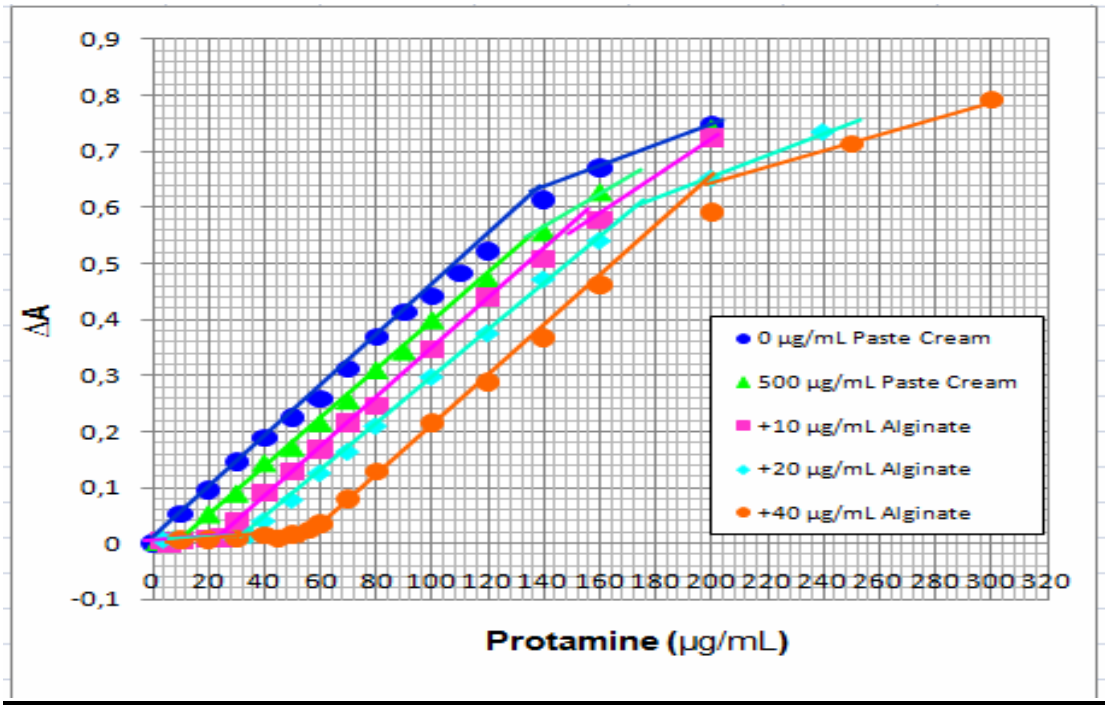


Figure 36: Katsan Paste Cream: (Na-Alginate) with Protamine

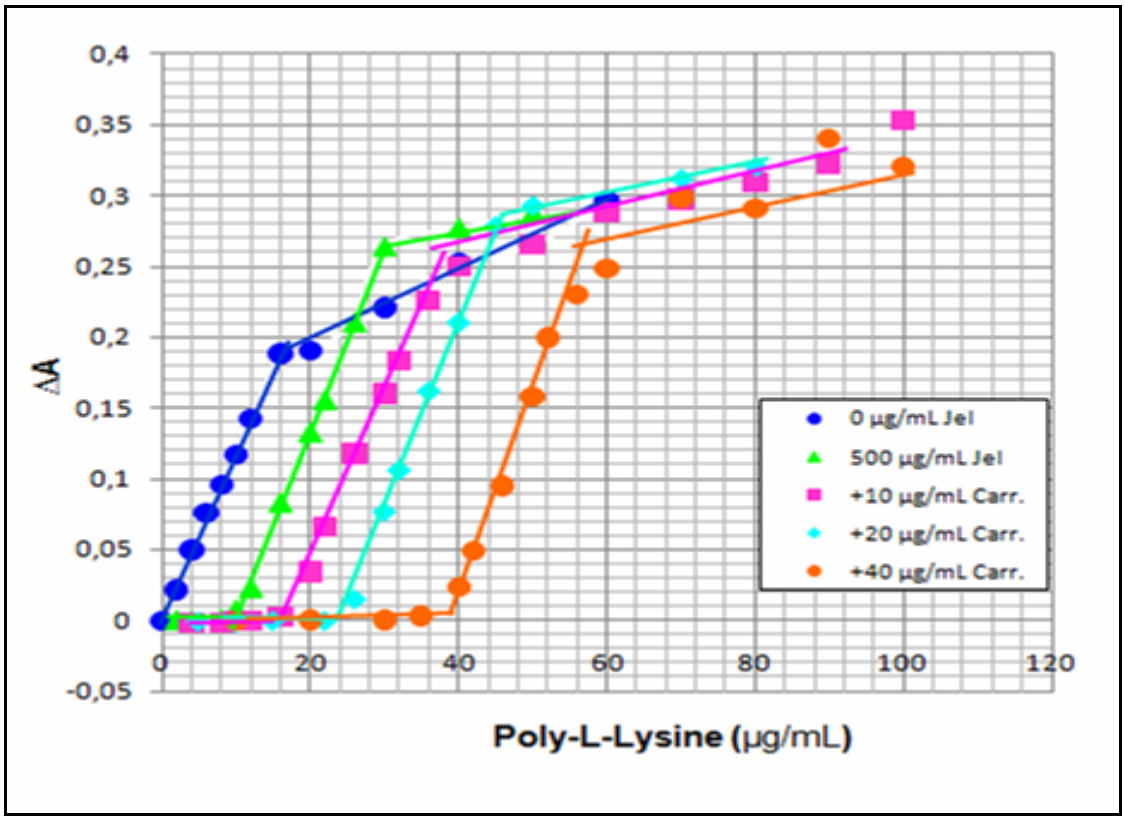


Figure 37: Dr. Oetker Jel with Berry: (Carrageenan) with PLL

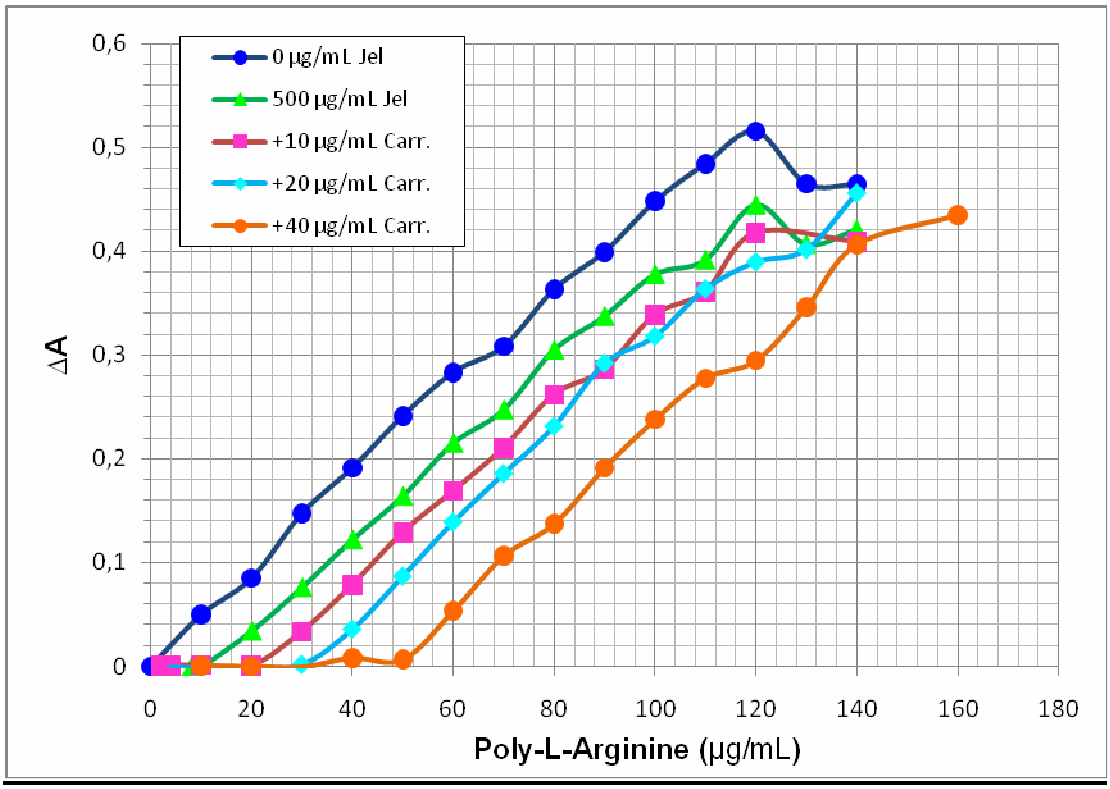


Figure 38: Dr. Oetker Jel with Berry: (Carrageenan) with PLA

Stochiometries between polycations and polyanions are given in the following tables;

Table 1 : Stochiometries between polycations and polyanions

Polycation (s)	[Polycation] / [Polyanion]			
	DNA	Carrageenan	Na-Alginate	Xanthan
Protamine	1.25 ± 0.12	1.10 ±0.03	1.30 ± 0.09	0.40 ± 0.06
Poly-L-Arginine	0.62 ± 0.04	0.92 ± 0.04	1.05 ± 0.01	0.35 ± 0.01
Poly-L-Lysine	0.45 ± 0.03	0.74 ± 0.03	0.82 ± 0.01	0.3 ± 0.05

Results for Food Products:

% Analyte (w/w)			
Food Sample	**Alginate**	**Carrageenan**	**Xanthan**
Theramed Toothpaste	———	———	1,71 ± 0,11
Dr.Oetker Keşkül	———	———	0,060 ± 0,001
Dr.Oetker Hot Chocolate	———	3,43 ± 0,19	———
Dr. Oetker Chocolate Sauce	———	———	1,86 ± 0,11
Dr. Oetker Paste Cream with Cacao	1,28 ± 0,07	———	———
Pınar Kido Milk with Banana	———	0,46 ± 0,06	———
Şok Pudding with Banana	———	0,020 ± 0,002	———
Katsan Paste Cream	1,42 ± 0,16	———	———
Nivea Face Cleaning Gel	———	———	7,71 ± 0,16
Dr. Oetker Gel with Berry	———	3,00 ± 0,13	———

Table 2: Results for Food Products. The amounts of polyanions in 100 g of real food products supplied from markets in Turkey

4.2-RESULTS FOR ETH-2412 :

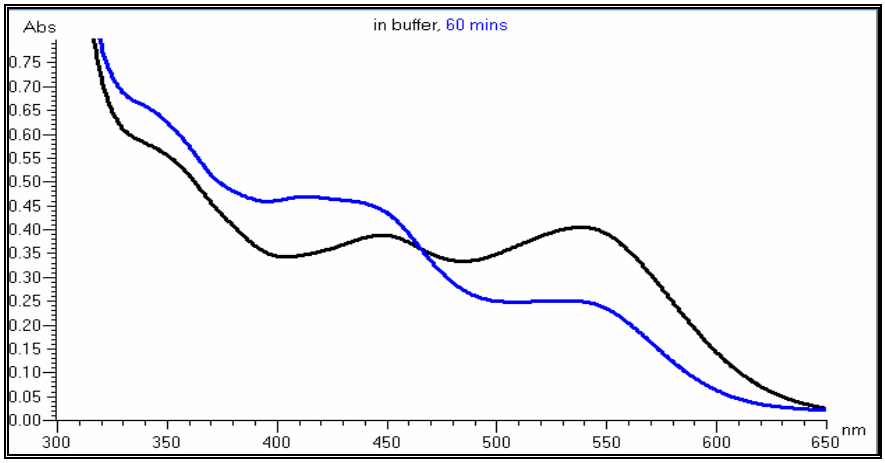


Figure 39 : Wavelength scan for Carrageenan

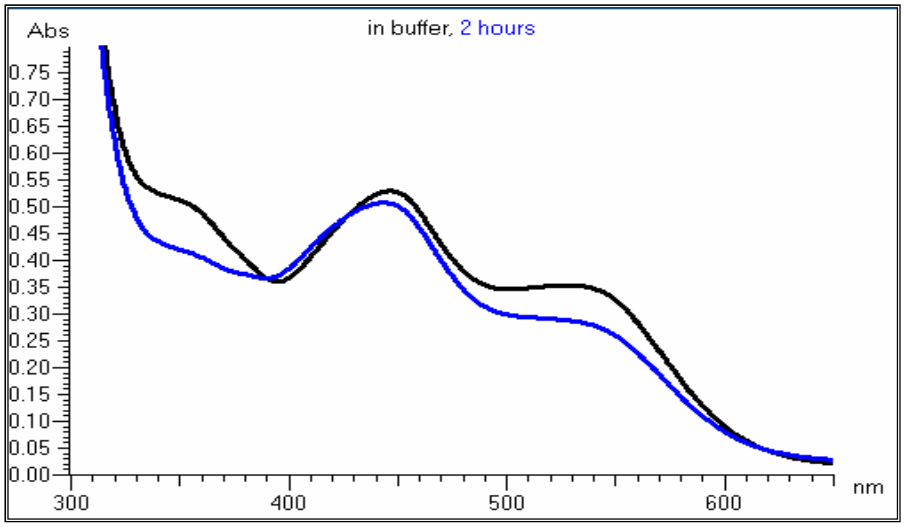


Figure 40: Wavelength scan for Xanthan

Titration Results for dye ETH-2412

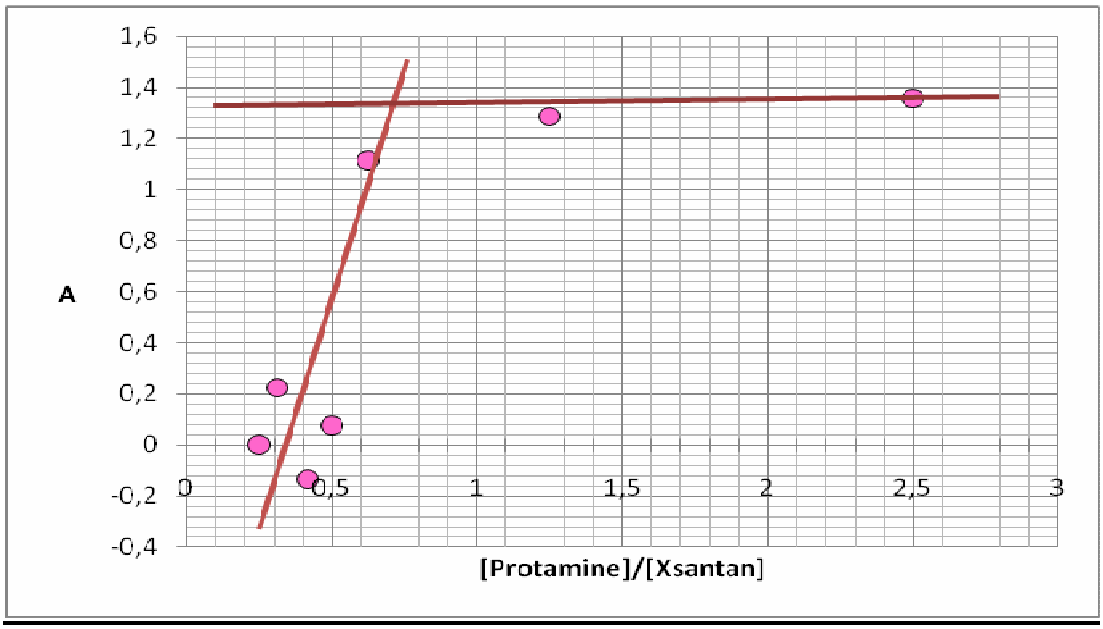


Figure 41: Titration Curve of Xanthan for ETH-2412

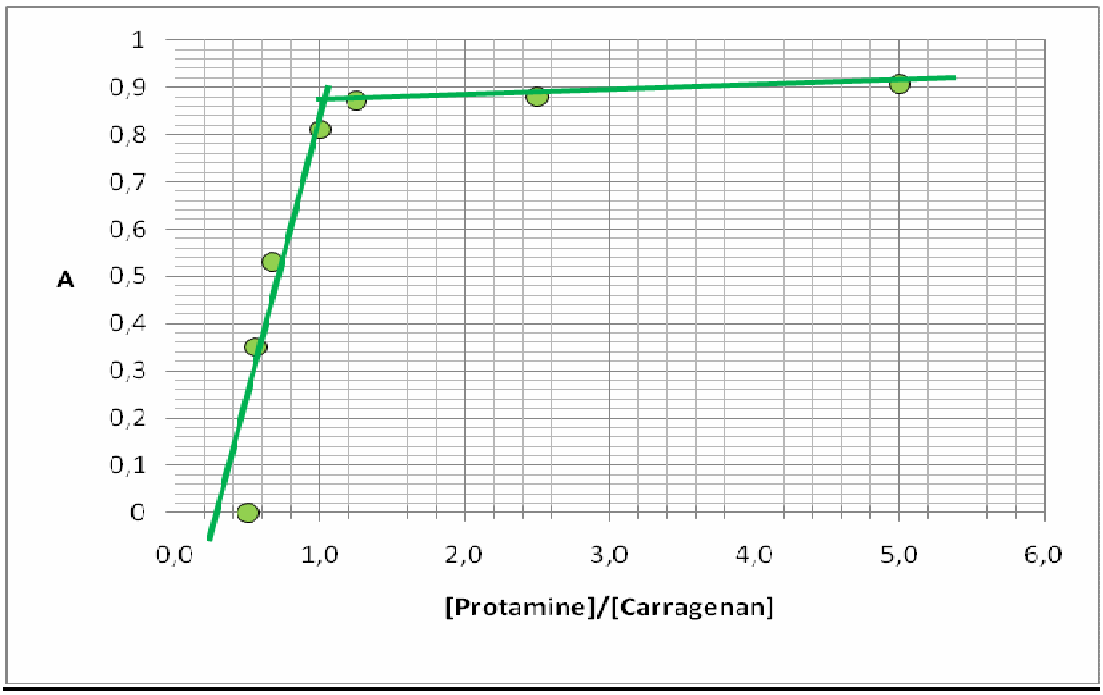


Figure 42: Titration Curve of Carrageenan for ETH-2412

CHAPTER – 5: DISCUSSION:

Polyion sensitive electrodes are quite common in the literature. Recent years, much attention has been drawn to the optical sensors on the fact that some specified disadvantages of the electrochemical sensors would be get rid of. Among the polyions planned to be determined in this work are primarily DNA which is known as the key code of the liveness and the food additives; Xanthan, Carrageenan and Alginate. These macromolecules are currently being added to the foods such as dairy products, pudding, ice-cream, sauces, soup, jam and sossage. In addition, these substances, as an example, are frequently used in tooth paste, in drugs and in cosmetics as filling, homogenising, gel forming and strenthening material. In the case of the excess addition of these substances, as it was known that can be harmful to the digestive system, overdose was prohibited and in this regard the determination of the above-mentioned polyions are important.

The optical sensors proposed in this work for the quantitative determination of these four polyions mentioned above and selected as targets are important not only for analytical chemistry but also for the clinical and food chemistry applications.

Current study is not only focused on the quantitative determination of the analyte polyions but also focused on the determination of the reaction stoichiometries between these polyions and macro and multiple charged ions. The stoichiometries between the complexes formed give valuable information about the polyions studied. As an example, the interaction studies between DNA and some molecules can provide useful information in designing new and effective drugs so that, in this work, the quantitative determination of protamine, poly-L-arginine, poly-L-lysine and DNA was performed optically and reaction stoichiometries were determined.

Very good results were obtained from the one of the two types of film sensors used in the work. Basically, it was planned to work within one unique type sensor, herewith, we proposed another sensor type which allows the determination of the two polyions among the selected four target polyions possible. While the film-1 type sensor can be obtained from a solution of the mixture of the defined ratios of a chromoionophore, one plastisizer, and polyvinyl chloride in THF; the film-2 type sensor is obtained from a solution of the mixture of the defined ratios of a polyion exchanger and an indicator; and one plastisizer, and polyvinyl chloride in THF. It was shown that the film sensor containing chromoionophore was sensitive to all the four polyanions; but only the two anions were determined by means of the film sensor containing a colorless ion-exchanger and indicator instead of chromoionophore. Therefore we can say that the first type film sensor may be used widely.

Especially, film-1 type sensor of the ones proposed for the quantitative determination of the target polyions was found to have been best one, and this was confirmed by the rational titration curves which clearly exhibit how the minimum determination limits can be achieved (several ppm) (Figures 12, 14, 16 and 18).

The rational titration curves are especially important in terms of supporting the repeatable results. The titrations of each concentrations of the analytes have been repeated at least three times, but, moreover, different concentrations (as increasing by ratio) were also titrated and in this way, checked if the repeatable results would be obtained through the entire work. From the titration graphs of the analyte polyion concentrations with the increasing values of 0, 5, 10, 20, 40 ppm, it has been performed that the end-points showed also same rational increments and the stoichiometric mass ratios between the titrant and analyte polyions remained constant (Table-1 and Table-2).

After having been determined the repeatable results for each target polyion in the buffered solutions, it was assayed the determinability of these target polyions in the real samples. For this purpose ten food samples were selected and the analytes were determined. The measured amounts were presented in Table-3. In some selected samples, in order to investigate whether the sample matrix would have an effect on the determination , by applying spike process (Figures 36, 37 and 38) in this way it was searched if we can obtain the same results and found that sample content has no effect on the determination values.

After the vast number of indicators and chromoionophores has been studied spectrophotometrically in order to determine whether they are sensitive or not to the polyions targeted to analyze through this work, the appropriate chromoionophore was decided. Furthermore, much attempt was spent to synthesize dye materials which may exhibit chromoionophore property and in this regard, two dye compound were synthesized. But, these compounds have been found not sensitive to the analytes under investigation. Instead, it was found that these compounds could be sensitive to the remarkably small size ions and this would be the scope of another research.

As a final remark related to this work, it was shown that these four polyanionic analytes targeted can be quantitatively determined spectrophometrically in ppm levels by the two different type of polymer-based film sensor (four analytes with type-1 sensor; two analytes, carragenean and xanthate with type-2 sensor) having different mechanisms. First type optical sensor proposed for the optical determination of the four target polyanion (DNA, Carragenaen, Xanthate, Alginate) in this work can be considered to have potential for the usage as optode in the production of the small sized instruments recently designed for the various analytical purposes or other appliances.

REFERENCES:

Some Aspects of Physicochemical Properties of DNA and RNA, Acharya, S., ***Acta Universitatis Upsaliensis***, 2006, Uppsala, Sweden.

Aguilan, J.T., Dayrit, F.M., Zhang, J., Ninonuevo, M.R., Lebrilla, C.B. ***J.Am. Soc. Mass Spectrom***., 2006, 17, 96-103.

Bakker, E. and Simon, W. ***Anal. Chem.,*** 1992. 64, 1805-1812.

Bakker, E., Bhlmann, P., Pretsch, E. ***Chem. Rev.,*** 1997, 97(8), 3083-3132.

Becker, A., Katzen, F., Pühler, A, Ielpi, L. ***Appl. Microbiol. Biot.,*** 1998, 50, 145-152.

Behringer, C.; Lehmann, B.; Haug, J.P.; Seiler, K.; Morf, W.E.; Hartmann, K.; Simon, W. ***Anal. Chim. Acta.,*** 1990, 233, 41-47.

Byun, Y.; Singh V.K.; Yang, V.C. ***Thromb. Res***., 1999, 94, 53-61.

Caceres, P.J., Carlucci, M.J., Damonte, E.B., Matsuhiro, B., Zuniga, E.A.***Phytochemistry.,*** 2000, 53, 81-86.

Campo, V.L., Kawano, D.F. ***Carbohyd. Polym.,*** 2009, 77, 167-180.

Casas, J.A. and Ochoa, F.G. ***F. Sci. Food Agric.,*** 1999, 79, 25-31.

Chen, H., Zou, Q.C., Yu, H., Peng, M., Song, G.W., Zhang, J.Z., Chai, S.G., Zhang, Y., Yan, C. ***Microchim. Acta***., 2010, 168, 331-340.

Colegrove, GT. ***Ind. Eng. Chem. Prod. Res. Dev.,*** 1983, 22(3), 456-460.

Dai, S., Ye, Q., Wang, E., Meyerhoff, M.E. ***Anal. Chem.,*** 2000, 72(14), 3142-3149.

Davidovich-Pinhas, M., Bianco-Peled, H. ***Carbohyd. Polym.,*** 2010, 79, 1020-1027.

De Ruiter, G.A. and Rudolph, B. ***Trends Food Sci. Tech.,*** 1997, 8, 389-395.

Determination of some food additives using polyion–sensitive membrane sensors, Kayansalan, A., Ms.Thesis, ***Abant Izzet Baysal University***, 2005, Bolu, Turkey.

Ding, X., Li, J., Hu, J., Li, Q. ***Anal. Biochem***., 2005, 339, 46-53.

Doume'che, B., Picard, J., Larreta-Garde, V. ***Biomacromolecules.,*** 2007, 8, 3613- 361.

Durust, N. and Meyerhoff. ***J. Electroanal. Chem.,*** 2007, 602, 138- 141.

El-Sayed, E.M., El-Gawad, I.A.A., Murad, H.A., Salah, S.H. ***Eur. Food Res. Technol.,*** 2002, 215, 298-304.

Funami, T., Fang, Y., Noda, S., Ishihara, S., Nakauma, M., Draget, K., Nishinari, K., Philips, G.O. ***Food Hydrocolloids.,*** 2009, 23, 1746-1755.

Fundamentals of Analytical Chemistry, Skoog, D.A., West, D.M., Holler, F.J., ***Saunders College Publishing***, 7th Ed., 1996, Fluorida, USA.

Ge, B.; Huang, Y-C.; Sen, D.; Yu, H-Z. ***J. Electroanal. Chem.,*** 2007, 602, 156 – 162.

Hambleton, A., Debeaufort, F., Beney, L., Karbowiak, T., Voilley, A. ***Biomacromolecules.,*** 2008, 9, 1058-1063.

Hauser, P.C., Perisset, P.M.J., Tan, S.S.S., Simon, W. ***Anal. Chem.***, 1990, 62, 1919-1923.

Hulanicki, A., Glab, S., Ingman, F. ***Pure Appl. Chem.,*** 1991, 63 (9), 1247-1250.

Food Stabilisers, Thickeners and Gelling Agents, Imeson, A., **Wiley-Blackwell**, 2010, Oxford, United Kingdom.

Kim, S.B., Kang, T.Y., Cho, H. C., Choi, M. H., Cha, G.S., Nam, H. ***Anal. Chim. Acta.,*** 2001, 439, 47-53.

Kim, S.B., Kang, T.Y., Cha, G.S., Nam, H. ***Anal. Chim. Acta***., 2006, 557, 117-122.

Kim, S.B., Cho, H.C., Cha, G.S., Nam, H. ***Anal. Chem.,*** 1998, 70(22), 4860-4863.

Lachke, A. ***Resonance.,*** 2004, 10, 25-33.

Lerchi, M., Bakker, E., Rusterholz, B., Simon, W. ***Anal. Chem.,*** 1992, 64, 1534-1540.

Lin, C.F. and Hansen P.M.T. ***Macromolecules.,*** 1970, 3(3), 269-274.

Morozkin, E. S., Laktionov, P.P., Rykova, E.Y., Vlassov, V.V. ***Anal. Biochem.,*** 2003, 322, 48-50.

Ochoa, F.G., Santos, V.E., Casas, J.A. ***Biotechnol. Adv.,*** 2000, 18, 549-579.

Ochoa, F.G., Santos, V.E., Casas, J.A., Gomez, E. ***Biotechnol. Adv.,*** 2000, 18, 549-579.

Quemener, B., Marot, C., Mouillet, L., Da Riz, V., Diris, J. ***Food Hydrocolloids.,*** 2000, 14, 9-17.

Quemener, B., Marot, C., Mouillet, L., Da Riz, V., Diris, J. ***Food Hydrocolloids***., 2000, 14, 19-28.

Rodd, A.B., Dunstan, D.E., Boger, D.V. ***Carbohyd. Polym.,*** 2000, 42, 159-174.

Santo, V.E., Frias, A.M., Carida, M., Cancedda, R., Gomes, M.E., Mano, J.F., Reis, R.L. ***Biomacromolecules.,*** 2009, 10, 1392-1401.

Seiler, K.; Wang, K.; Bakker, E.; Morf, W.E.; Rusterholz, B.; Spichiger, U.E.; Simon, W. ***Clin. Chem.,*** 1991, 37, 1350-1355.

Seiler, K.; Morf, W.E.; Rusterholz, B.; Simon, W. ***Anal. Sci.,*** 1989, 5, 557-561.

Schroeder, C. W. and Racicot P. A. ***Ind. Eng. Chem. Anal.,*** 1941, 13(3), 165 – 166.

Shortreed, M.R., Barker, S.L.R., Kopelman, R. ***Sensor Actuat.,*** 1996, B 35-36, 217-221.

Shtykova, E. and Dembo, A. ***Langmuir.,*** 2000, 16, 5284-5288.

Soedjak, H.S. ***Anal. Chem.,*** 1994, 66, 4514-4518.

Steel, A.B., Herne, T.M., Tarlov, M.J. ***Anal. Chem.,*** 1998, 70, 4670-4677.

Stetter, J.R., Penrose, W.R.,Yao, S. ***J. Electrochem. Soc.,*** 2003, 150 (2), S11-S16.

Tan, S.S.S., Hauser, P.C., Chaniotakis, N. A., Suter, G., Simon, W. ***Chimia.,*** 1989, 43, 257-261.

Tan, S.S.S.; Hauser, P.C.; Wang, K.; Fluri, K.; Seiler, K.; Rusterholz, B.; Kruttli, M.; Spichiger, U.E.; Simon, W. ***Anal. Chim. Acta*.,** 1991, 255, 35-44.

Tong, C.B.S. and Hicks, K.B. ***J. Agr. Food Chem.,*** 1901, 39, 1719-1722.

Toti, U.S. and Aminabhavi, T.M. ***J. Membrane Sci*.,** 2004, 228, 199- 208.

Tsunoda, H.; Ohkubo, A.; Taguchi, H.; Seio, K.; Sekine, M. ***J. Org. Chem.,*** 2008, 73 (4), 1217– 1224.

Wang, E., Wang, G., Ma, L., Stivanello, C.M., Lam, S., Patcl, H. ***Anal. Chim. Acta***., 1996, 334, 139-147.

Wang, E., Meyerhoff, M.E., Yang, V.C. ***Anal. Chem.,*** 1995, 67(3), 522-527.

Wang, L.R., Wang, R.X., Yang, L.Z., Lu, G.H. ***J. Electroanal. Chem.***, 2005, 585, 214-219.

Watson, J.D. and Crick, F.H.C. ***Nature.***, 1953, 171 (4356), 737-738.

Yamada, T., Ogamo, A., Saito, T., Watanabe, J., Uchiyama, H., Nakagawa, Y. ***Carbohyd. Polym***., 1997, 32, 51-55.

Yun, J.H., Han, I.S., Chang, L.C., Ramamurthy, N., Meyerhoff, M.E., Yang, V.C. ***PSTT.,*** 1999, 2(3), 102-110.

Wang, K.; Seiler, K.; Morf, W.E.; Spichiger, U.E.; Simon, W.; Lindner, E.; Pungor, E. ***Anal. Sci.,*** 1990, 6, 715-72