QUANTITATIVE DETERMINATION OF SOME POLYANIONS USING MICROTITER PLATE READER

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QUANTITATIVE DETERMINATION OF SOME POLYANIONS USING MICROTITER PLATE READER

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

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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.

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ABSTRACT

QUANTITATIVE DETERMINATION OF SOME POLYANIONS USING MICROTITER PLATE READER

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This scientific work concerning about determination of Pentosan Polysulfate (PPS), Sodium dodecylsulfate (NaDS), Sodium dodecyl benzenesulfonate (NaDBS), Potassium poly(vinyl sulfate) (PVSK), by some important titrants, Protamine, Poly-L-Arginine, Poly-L-Lysine, Zephiramine, Cetyltrimethylammonium bromide (CTAB), Cetyldimethylbenzylammonium chloride (CDB) with polymeric optical sensors including chromoionophore and ionophore but also establishing their stoichiometries interactions by using microtiter plate reader.

PPS is a heparin-like, highly sulfated polysaccharide and anti-arthritis drug which has several pharmaceutical properties. And also protamine, PLA and PLL are cationic polyaminoacids they have also many biological properties. In that case, determination of PPS with these polycations is so important.

Surfactants are extensively used in many areas such as agriculture, plastic industry, cosmetic etc. NaDS, NaDBS, PVSK are anionic surfactants. The determination of anionic surfactants in aqueous solutions is very important because of their toxicity to various organisms.

There are routine methods available determination of these target ions. However, they are very complicated, time-consuming and some poisoning solvents can be used. Instead of these classical methods a distinctive method “Microtiter Plate Optode’’ was used to determine these important ions. Microtiterplate optode is developed instead of classic bulk optodes due to its small size, easy sample handling, increased throughput, decreased sample preparation and small sample volume. Investigation of optical sensors comes from the hope of eliminating certain disadvantages of electrochemical sensors.

In conclusion, quantitative determination of target ions, stoichiometries of their interactions between macro and multiple charged ions and also their limit of detections were established by standard solutions by using microtiter plate reader. The amount of target ions in real samples were determined in μg/mL. Recovery studies support the reliability of the method.

Keywords: polyions, surfactants, bulk optodes, microplate reader, chromoionophore, ionophore

ÖZET

MİKROTİTRASYON PLAKA OKUYUCU KULLANARAK BAZI POLİANYONLARIN KANTİTATİF TAYİNLERİ

Naç, Sibel

Yüksek Lisans, Kimya Bölümü

Danışman: Prof. Dr. Nedime Dürüst

Eylül 2010, 70 sayfa

Bu bilimsel çalışma Pentosan Polisülfat (PPS), Sodyum dodecylsülfat (NaDS), Sodyum dodecylbenzensülfonat (NaDBS), Potasyum poli(vinyl sülfat) (PVSK), iyonlarının Protamin, Poli-l-arginin (PLA), Poli-l-lisin (PLL), Zephiramin, Cetiltrimetilamonyum bromür (CTAB), Cetildimetilbenzilamonyum klorür (CDB) gibi bazı önemli titrantlar ile kromoiyonofor ve iyonofor içeren polimerik bazlı optik sensörler ile tayinini kapsamaktadır. Aynı zamanda bu iyonların birbirleriyle reaksiyona girme oranları mikrotitrasyon plaka okuyucu kullanarak belirlenmiştir.

PPS, bir çok farmasötik özelliği olan, heparin benzeri, yüksekçe sülfatlanmış polisakkarit ve anti-romatizma ilacıdır. Protamin, PLA ve PLL ler katyonik poliaminoasitlerdir. Bu iyonlarında birçok biyolojik özelliği bulunmaktadır. Bu sebepten PPS' in bu polikatyonlarla tayini oldukça önemlidir.

Yüzey aktif maddeleri tarım, plastik endüstrisi, kozmetik vb. gibi birçok alanda yaygın olarak kullanılmaktadır. NaDS, NaDBS, PVSK iyonları anyonik yüzey aktif maddeleridir. Çeşitli organizmalara olan toksik özelliğinden dolayı anyonik yüzey aktif maddelerinin tayini çok önemlidir.

Hedef iyonların tayini için birçok alışılagelmiş metod bulunmaktadır. Fakat bu metodlar çok kompleks, zaman alıcı ve bazı zehirli çözücüler içermektedir. Bu sebepten klasik tayin metodları yerine farklı bir metod olan "Mikrotitrasyon Plaka Optod" hedef iyonların tayininde kullanıldı. Mikrotitre plaka optod küçük boyutu, kolay numune dağılımı, hızlı kullanımı, numune hazırlamasını süresini azaltması ve az hacimde numune içermesinden dolayı klasik optik sensörler yerine geliştirildi. Elektrokimyasal sensörlerin dezavantajlarını yok etmek ümidiyle optik sensörler geliştirilmiştir.

Sonuç olarak, hedef iyonların kantitatif tayini, makro ve çok yüklü iyonlarla olan reaksiyona girme oranları, ve ek olaran tayin sınırları standart çözeltiler kullanılarak mikrotitrasyon plaka okuyucuyla tayin edilmiştir. Gerçek numunelerdeki hedef iyonların miktarı µg/mL seviyesinde belirlenmiştir. Gerikazanım çalışmaları metodun güvenilirliğini desteklemektedir.

Anahtar Kelimeler: Poliiyonlar, yüzey aktif maddeleri, optik sensörler, mikroplaka okuyucu, kromoiyonofor, iyonofor.

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To My Family

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Chapter 1 .

INTRODUCTION

1.1. Chemical Sensors

The dramatic improvements in the technologies support the development of new analytical procedures year by year. Especially, the realization that the unique recognition and catalytic capabilities of chemical and biological systems could be coupled with electrochemical or optical transducers has initiated a branch of analytical science: sensors.

A chemical sensor can be defined as a device that responds to changes in its chemical/biological environment and converts this response into a signal that can be read. As for the basic characteristics of a useful sensor, its response should be predictable so that it scales with the magnitude of changes in the chemical environment, and the sensor should be sensitive and specific. In addition, the transduced signal can be electrical, magnetic, optical, etc.

Sensors are generally used quantitative but also qualitative analysis. A major aspect of on-line sensors is based on the fact that they can indicate trends in real time. This is an advantage in both medicine (for example during operations of the critical ill) and in process management (for instance in chemical or nuclear plants and in bioreactors) in that it enables undelayed counteractions to be performed in critical situations. [1]

There are two large groups of sensors which are electrochemical and optical sensors. This thesis focuses on optical sensors.

1.1.1. Electrochemical Sensors

In electrochemical sensors the response is derived from the interaction between chemistry and electricity. Thus potentiometric sensors are based on measurement of cell voltage, amperometric sensors on measurement of cell current, and conductometric sensors on measurement of cell admittance. [2]

The second large group of electrochemical sensors incorporates potentiometric transducers. Ion-selective electrodes (ISEs) has evolved to a well-established routine analytical technique. The essential part of carrier-based ISE is the ion-sensitive solvent polymeric membrane, physically a water-immiscible liquid of high viscosity that is commonly an ionophore (ion carrier) and lipophilic salt as ion exchanger. ISE membranes are typically investigated under zero-current conditions in galvanic cell. Meyerhoff and co-workers developed the first polymeric membrane electrode which is sensitive polyanion heparin [3].

1.1.2. Optical Sensors

The development of the optical hardware and the hope of eliminating certain disadvantages of electrochemical sensors [4, 5] accommodate the evolvement of chemical sensors with an optical transduction (optodes). Optical sensors are rely on optical detection of chemical species.

They can have several advantages over electrochemical sensors. Inherent safety, the lack of requirement reference electrode and the

relative novelty of the subject are the advantages of optodes. [6] The fundamental difference between optical and electrochemical sensor measurements lies in the fact that individual ions are counted in the former, and this count yields information about the concentration of ions in the light path. The quantitative relationship on which such sensors are based is the Beer-Lambert law or its equivalent. However, in potentiometric measurements, ions are partitioned between two phases and the macroscopic property of the entire phase (i.e., the Galvani potential difference) resulting from this equilibrium process is measured. The equilibrium itself is driven and described by the activities of the participating ions. The selective charge separation that occurs at the interphase between the two phases is then explicitly related to the potential difference by some form of the Nernst equation. Partitioning of electrically neutral species generally does not affect the electrochemical measurement. [7]

Very different types of chemical sensing layers have been developed and described. A ligand (ion carrier, ionophore, indicator, complexing agent) is either chemically bound or physically entrapped near the interface or in the bulk of the sensing layer, or it is directly immobilized on the surface of the optical element. The theoretical response behavior of optical sensors valid for electrically neutral analytes was discussed in 1984 by Seitz [3]. A large quantity of PVC based optical sensors were developed for many analytically relevant ions by Simon et al. at ETH Zurich. Optodes selective for some of the ions; $Na,^{+}$ [8] K^{+}, [9, 10]

Zn^{2+} ,[11] NH_4^+,[12] Pb^{2+} ,[13] Chiral ammonium ions [14, 15], NO_3^- [16], Cl^-, [17] etc.

Both hydrophilic membranes/surfaces and water immiscible hydrophobic films are used as matrices. While the former group often is based on poly(acrylamide) or other hydrogels and makes use of derivatives of classical water-soluble indicators, the latter, which is typically based on PVC or similar polymers and exploits the extremely high selectivity of the same lipophilic ionophores that are employed in ion-selective electrode membranes.

Some PVC films containing neutral ionophores have been shown to yield a useful optical response based on interfacial phenomena, e.g., with optical second harmonic generation [18] or by making use of potential sensitive dyes.[19] They are, however, not yet very well explored or accepted, partly because the theoretical framework of their response is difficult to establish.

There are two different types of sensing layers for charged species.

1.1.2.1. Optodes based on surface phenomena (surface optodes)

1.1.2.2. Bulk optodes

1.1.2.1.Optodes based on Surface Phenomena

The active components are immobilized near the interface or on the surface of the optical element or porous matrix, so that the active site is located in the sample solution. Very often, the recognition molecule is immobilized, either by a covalent binding, or by its physical adsorption on the surface, or it is part of a Langmuir-Blodgett film. The response of pH optodes based on conventional pH indicators immobilized on the surface

was thoroughly elucidated by Janata [5]. It influences both the activity coefficient of the charged form of the indicator and the surface potential, which is responsible for the relationship between the measured pH at the surface and the pH of the bulk.

1.1.2.2. Bulk Optodes

In the past few years, a novel optode principle has been introduced successfully with extra to classical indicator dyes, the idea of using ionophores designed for ion-selective electrodes which have been made part of many optical sensing layers [20, 21]. These optical sensors are called bulk optodes, the theoretical description of the response of them has been given firstly by Morf et al. in 1989 [22, 23].

Working mechanism of bulk optodes are based on extracting the sensed ionic components into a plasticized polymeric membrane by the mass transfer where the change of the optical signal which is generated upon interaction of the carrier with the analyte, whereupon the carrier itself or an additional compound (chromoionophore, fluoroionophore, indicator dye) changes its optical properties upon complexation with a different ion. That means the active components are uniformly entrapped in the bulk of homogeneous sensing layer. The optical signal is detected by means of absorption or fluorescence [24] shows the schematic differences between bulk optode and optodes based on surface phenomena.

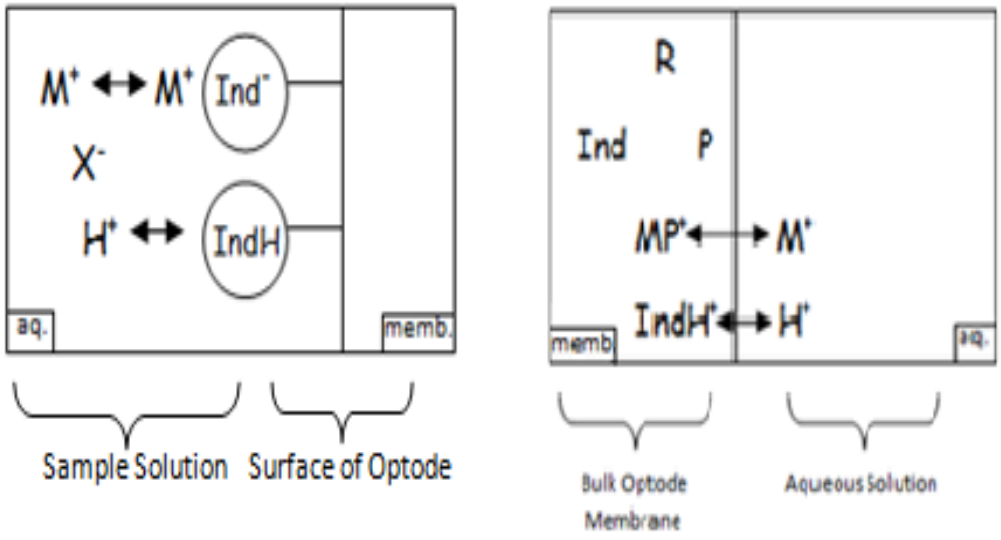


Figure 1. 1 Schematic representation of a pH optode based on an anionic indicator dye directly immobilized on the surface of the optical element. (**Figure 1. 1** left side) Schematic representation of Bulk optode membrane based on the ion-exchange mechanism and incorporating two electrically neutral ionophores and lipophilic anionic sites. (**Figure 1. 1** right side)

Morf et al. could give a straightforward and exact description of the selectivity of ions of the same charge and the same stoichiometry between the analyte and the ionophore [25].

Most conventional ionophores do not contain chromophoric group so that an additional, lipophilic chromoionophore, selective for a different ion, has to be combined into the same membrane phase. Generally, hydrogen-ion-selective chromoionophore have been used, and the activity of the hydrogen ion has generally been kept constant through the use of suitable pH buffers. In this way, the activity of the target ion can be calculated from the absorbance change measurements of the optode membrane. The selectivity of these systems is related to the distribution coefficients of the measured ions between the sample and the membrane, and by the respective complex formation constants in the membrane

phase. If the extraction of only one ion involves specific interaction with a carrier, the selectivity towards the second ion is mainly determined by their hydration enthalpies. If, however, the pH of the sample is required parameter, of course, the activities of the other cations or anions involved in the equilibrium must be estimated [26].

The thickness of the membranes turned out to be one of the central parameters of bulk optodes For this reason, the spin-on technique allowing the production of very homogeneous and reproducible PVC-based membranes is described.

1.1.2.2.1.Possible Bulk Optode Mechanisms

There are several types of optode membranes such as two different ionophores L and C, each selective for a particular ion which are dissolved in a polymeric membrane (such as PVC) with organic solvent (such as THF). Optode is equilibrium with the contacting probe, usually being an aqueous solution. The analyte ions are extracted by mass transfer into the bulk of the organic membrane phase.

Chromoionophore is an ionophore also typically lipophilized pH indicators [which changes optical signal intensely upon complexation with a specific ion. Chromoionophores are classified according to their electrical charge. The first class is neutral chromoionophores in the basic form and positively charged in their protonated form and neutral in their unprotonated form, (basic chromoionophores, neutral hydrogen carriers). The second class is charged chromoionophores are neutral in their protonated and negatively charged in their unprotonated form. (acidic chromoionophores, charged hydrogen carriers). With different charged

ionophores and chromoionophores, different membrane compositions are required.[27, 28] The response of ion-selective optodes is either a competitive ion-exchange or a carrier-mediated coextraction equilibrium, depending on the charge of the ions. Therefore, the response and selectivity of the ion sensing optodes relate to both the stability constant of the ionophore and pKa of the chromoionophore in the film.

In combination with neutral cation-or anion selective ionophores can be classified as;

1. Cation Sensitive Membranes:

   1.a) Ion-exchange System with Basic Chromoionophore

$$\alpha_{iV} + p\gamma_{L}[L] + v\gamma_{CH^+}[CH^+] \leftrightarrow \gamma_{ILp^{v+}}[IL_p^{v+}] + v\gamma_{C}[C] + v\gamma_{C}[C] + v\alpha_{H^+}$$

   1.b) Ion-exchange System with Acidic Chromoionophore

$$\alpha_{iV} + p\gamma_{L}[L] + v\gamma_{CH}[CH] \leftrightarrow \gamma_{ILp^{v+}}[IL_p^{v+}] + v\gamma_{C}[C] + v\gamma_{C^-}[C^-] + v\alpha_{H^+}$$

2. Anion-Sensitive Membranes

   2.a) Coextraction System with Acidic Chromoionophore

$$\alpha_{Xv^-} + p\gamma_{L}[L] + v\gamma_{C^-}[C^-] + v\alpha_{H^+} \leftrightarrow \gamma_{XLp^{v-}}[XL_p^{v-}] + v\gamma_{CH}[CH]$$

   2.b) Coextraction System with Basic Chromoionophore

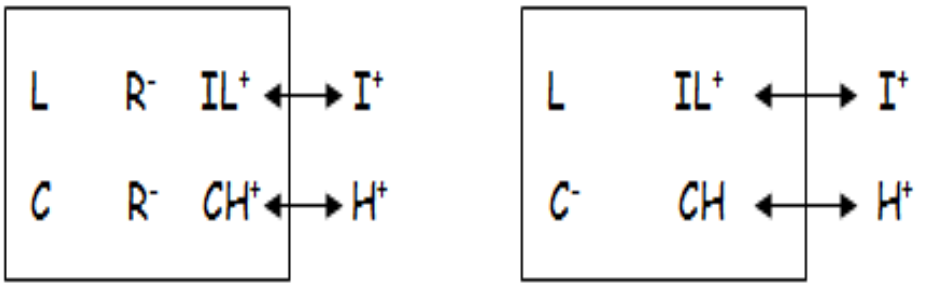
$$\alpha_{Xv^-} + p\gamma_{L}[L] + v\gamma_{C}[C] + v\alpha_{H^+} \leftrightarrow \gamma_{XLp^{v-}}[XL_p^{v-}] + v\gamma_{CH^+}[CH^+]$$

C is hydrogen ion selective chromoionophore which competing with the neutral cation or anion selective ionophore L, leading to the corresponding ion complexes CH, CH^+, IL_p^{v+}, or XL_p^{v-} within the membrane. α_i, α_H and α_X are the activities symbols and refers to species in the aqueous phase. Concentration symbols in the organic phase are given in square brackets. ϒ is the corresponding activity coefficients.

Schematic describtion of different types of bulk optode membranes:

<u>Optodes with Electrically Neutral Ionophores L</u>

For Cationic Analytes



For Anionic Analytes

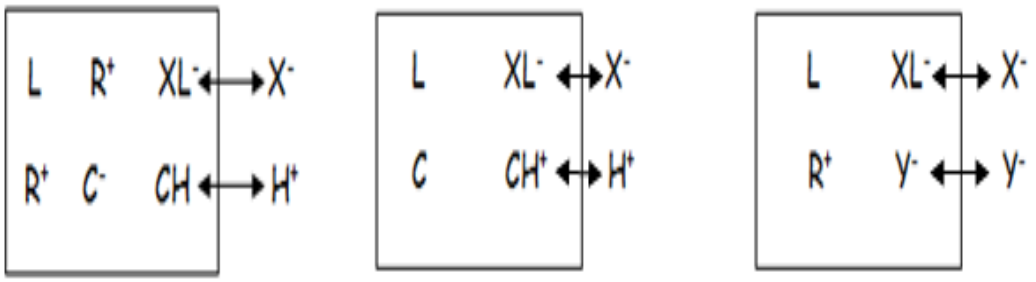
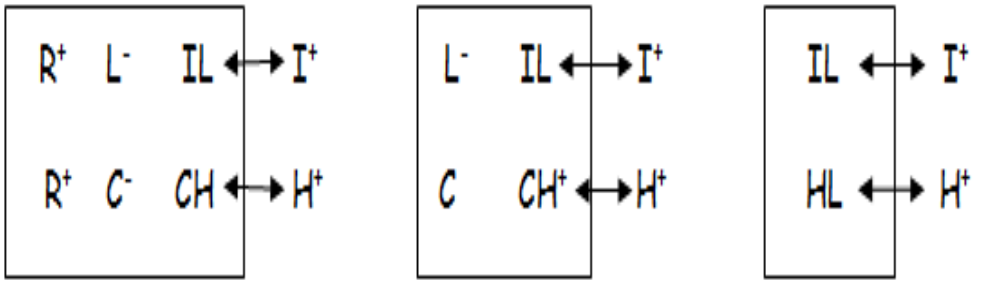


Figure 1. 2 Types of neutral-carrier-based optodes with neutral or charged chromoionophores (L is neutral carrier, C and C^- , neutral and charged H^+ - chromoionophores; R+ and R-, positively and negatively charged ionic sites). Squares indicate species in the organic bulk optode membrane phase.

Optodes with Electrically Charged Ionophore L^- or L^+

For Cationic Analytes



For Anionic Analytes

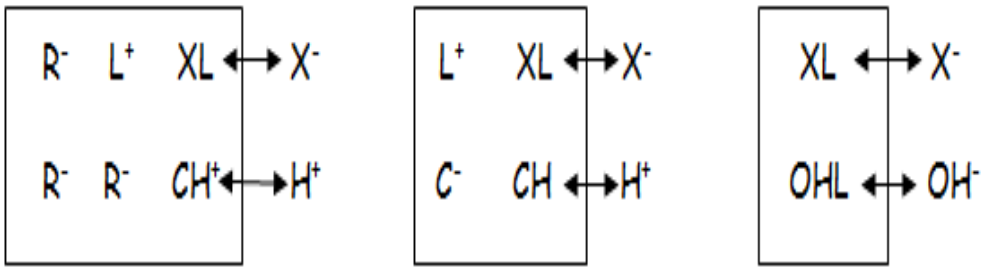
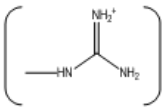


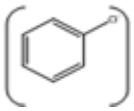
Figure 1. 3 Types of charged-carrier-based optodes with neutral or charged chromoionophores. (L^+ and L^- , charged carriers; C and C^-, neutral and charged H^+ chromoionophores; R^+ and R^-, positively and negatively charged ionic sites). Squares indicate species in organic phase.

For cation-exchange optodes based on two electrically neutral ionophores, one being a chromoionophore, the simultaneous presence of lipophilic anionic sites that give the membrane cation exchange properties is required (Figure 1. 2 first row, left side). If the H^+ selective chromoionophore is itself electrically charged (i.e., negatively charged when nonprotonated and neutral when protonated), no such trapped ionic sites are needed (Figure 1. 2 first row, right side). If, on the other hand, the ionophore is charged, ionic sites are needed with charged chromoionophores but not with neutral ones (Figure 1. 3 first row, first two

entries). Another type of optodes has been reported on the basis of compounds that act both as the ionophore and chromoionophore, i.e., the analyte ion and hydrogen ion can be selectively complexed by the same carrier, one of them inducing an absorbance change (Figure 1. 3 first row, right side). While such systems contain fewer components, they are less flexible since the chromoionophore and/or ion carrier content cannot be separately optimized (Figure 1. 3 the second row, right side) shows an equivalent system that is anion responsive). For anion sensing optodes based on coextraction equilibria, electrically neutral and/or charged carriers can be used, again with and without electrically charged trapped ionic sites, respectively. The second row in Figure 1. 2 shows, in the first two entries from the left, neutral-carrier-based optodes containing an electrically charged chromoionophore C- and cationic sites R+, and an electrically charged chromoionophore C without added sites. On the other hand, the second row of Figure 1. 3 shows, in the first two entries from the left, charged-carrier-based optodes containing an electrically neutral chromoionophore C and anionic sites R-, and a charged chromoionophore C- without added sites. On the other hand, films containing only a neutral H+-chromoionophore also function as anion optodes. However, they show a Hofmeister-type selectivity pattern, i.e., a preference for lipophilic anions, since the coextracted anions are not selectively complexed.

1.1.2.2.2. Working Mechanism of DCFOE doped Optode (Film 1)

Protamine is a low molecular weight (the average MW: 4,500) protein rich in the basic amino acid-arginine and is a polycation at near neutral pH (pH 7,4). The guanadinium groups of protamine 

can complex electrostatically with the sulfonate groups of PPS ($-SO_3^-$). Lipophilic fluorescein ester containing moderately acidic phenolate groups 

are capable of extracting protamine polycations from aqueous solution into organic membranes. Deprotanation of fluorescein esters due to cation exchange between the polycation and proton causes changes in membrane absorbance. Therefore, DCFOE can be used as protamine polycation carrier and chromophore at the same time (protamine chromoionophore). As describe herein, a polymer membrane film doped with a lipophilic 2',7'–dichlorofluorescein ester chromophore responds to micromolar levels of free protamine in solution, therefore it can be used as an indicator in the PPS-protamine titration.

DCFOE is a lipophilic fluorescein chromoionophore (HL), contains an acidic phenolate group (pK_a~4.9) which can become negatively charged upon deprotonation. The interaction of negatively charged chromoionophore (L^-) with multipositively charged protamine ($Prot^{z+}$) results in the extraction of protamine from aqueous solution and formation of a unique lyophobic colloidal phase at polymer film/test solution interface. Cation exchange occurs at suitable pH (7.4). Shematic describtion of this mechanism is shown on Figure 1. 4

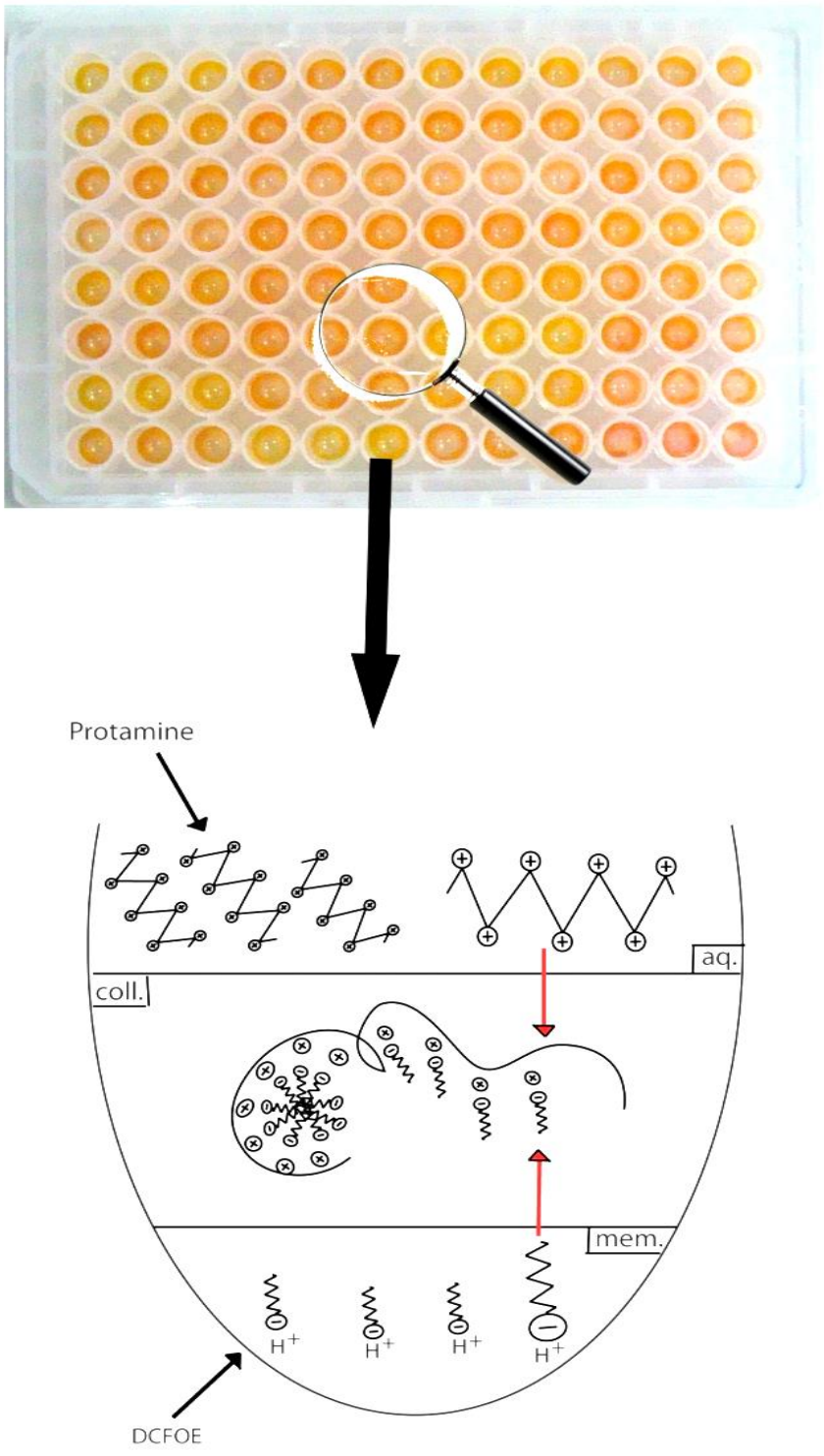


Figure 1. 4 Working Mechanism of DCFOE doped Optode (Film 1)

These equilibrium can be describe as :

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

$(Prot\text{-}L_z)$: ion-pair complex between protamine and chromoionophore.

''Org'' and ''aq'' refers to organic membrane of optode and aqueous phase of the test solution.

The equilibrium constant K_{eq} of this reaction can be expressed as :

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

According to this relationship K_{eq} depends on the complex formation constant ($\beta_{prot\text{-}Lz}$), the association constant of L^- (β_{HL}) in the membrane and the distribution coefficients of H^+ and $Prot^{z+}$ between the organic membrane phase and the aqueous solution (k_H and k_{prot}, respectively). [29, 30]

The membrane absorbance signal A, at the maximum absorbance wavelength of the complex $(Prot\text{-}L_z)$ correlates with the complex concentration according to

$A = k_z [Prot\text{-}L_z]$

k is related to membrane thickness and the molar absorptivity of the complexed dye in the film. The relative absorbance α is shown:

$$\alpha = \frac{z[Prot - L_z]}{L_T} = \frac{A - A_0}{A_1 - A_0}$$

$$\alpha \rightarrow 0\ ; \qquad \frac{L_T \alpha}{z\, L_T (1-\alpha)^z} = K_{eq} \frac{[Prot^{z+}]}{[H^+]}$$

The relative absorbance is defined as the ratio of the concentration of the micelle complexed form [Prot – L_z] over the total concentration of the dye L_T. A_0 and A_1 are limiting absorbance values for α=0 (fully protonated ligand HL) and α=1 (fully complexed ligand Prot-L_z). So that the difference between measured absorbance and the initial fully protonated absorbance of dye was measured at 540 nm. ($\Delta A = A_1 - A_0$)

1.1.2.2.3. Working Mechanism ETH 5294 doped Optode (Film 2)

Dinonylnaphthalene sulfonic acid (DNNS) is an anionic ionophore which is sensitive polycations and can form a strong ion-paring complex with polycations. But DNNS do not have a visible optical property. In that case, a second ionophore is needed which is a protonated pH indicator, can serve as chromophore.

Ion-exchange reaction between polycation and proton can change the absorbance of the film. The overall ion exchange reaction can be written as;

$zL_{org}^- + zCH_{org}^+ + PLL_{aq}^{z+} \leftrightarrow L_zPLL_{org} + zC_{org} + zH_{aq}^+$

L^- : Polycation sensititive ionophore (DNNS)

PLL_{aq} : Polycation in aqueous phase (such as Poly-L-Lysine)

CH_{org}^+ : Protonated ETH 5294 chromoionophore

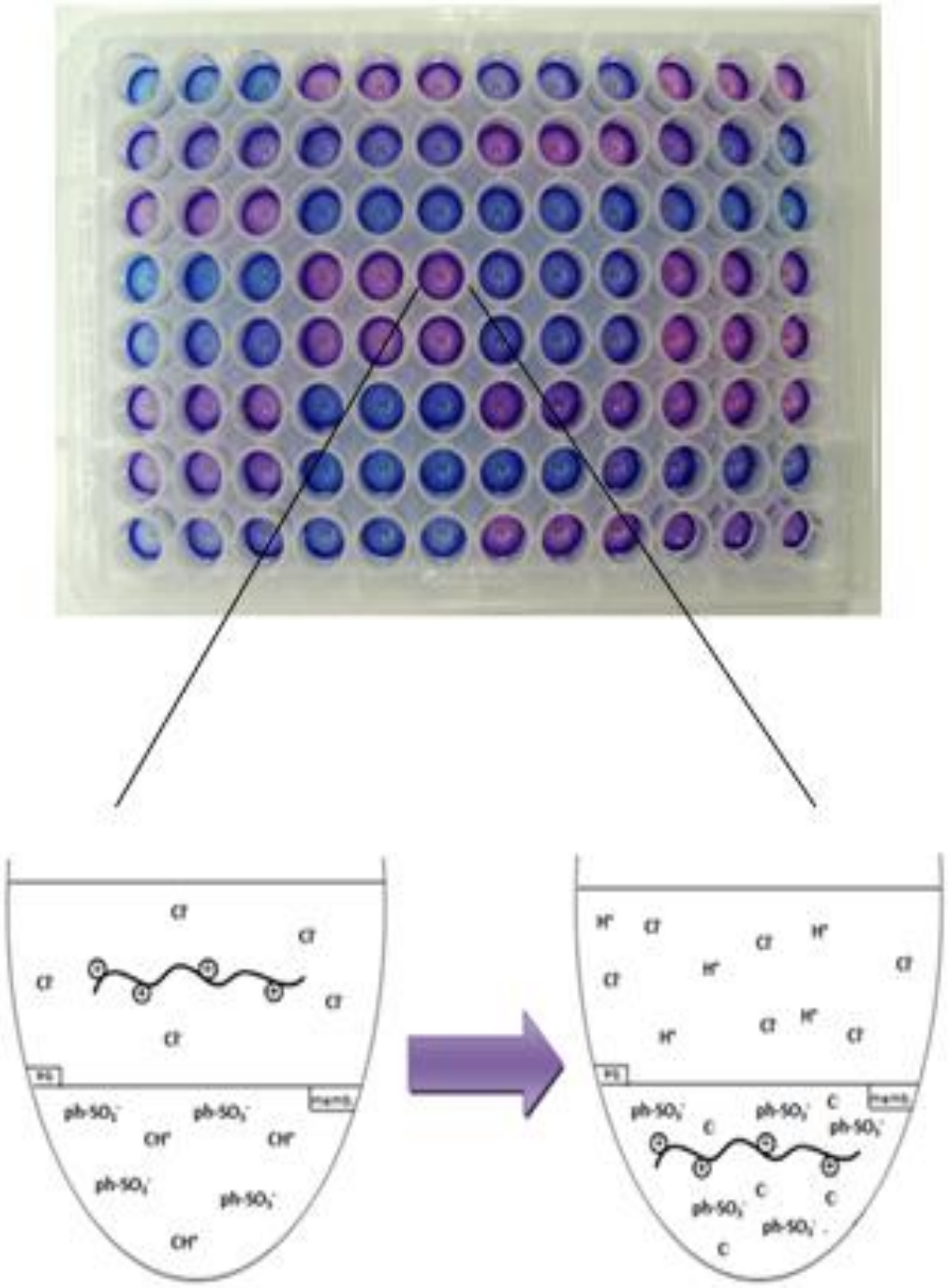


Figure 1. 5 Working Mechanism of ETH 5294 Doped Optode (Film 2)

1.2. Microtiter Plate Optode

According to the developments in optical sensor area, Kim and co-workers considered a new approach instead of classical analytical techniques [31].

The microtiter plate is an injection-molded rectangular plastic component with rows and columns of U-bottomed wells which was discovered in 1990. The microplate format has become increasingly popular in biological experimentation due to its small size, easy sample handling, increased throughput, decreased sample preparation and small sample volume. It is commonly used in biomedical laboratories to perform various types of immunoassays. Generally, microtiter plate reader is known as ELISA device. Employing a contemporary microtiter plate reader, multiple colorimetric changes occurring in 96 wells of a microtiter plate are simultaneously processed in few seconds.

In this dissertation work, we used U-bottomed polypropylene microtiter plate optode instead of polystyrene plate. Because polypropylene is resist to most organic solvents, as the optical substrate of bulk optode membranes. U-bottomed wells supply more uniform optode membrane preparation instead of flat-bottomed wells.

Chapter 2 .

POLYIONS

2.1. Pentosan Polysulfate

Pentosan Polysulfate (PPS) is a polyanionic, heparin-like, highly sulfated, polysaccharide, anti-arthritis drug which is a semisynthetic product. The hemicellulose is isolated from the wood of beech trees (*Fagus sylvatica*) and is then subjected to sulfate esterification using chlorosulfonic acid. A mixture of linear polymers of β-1→4 linked xylose, usually sulfated at the 2- and 3- positions and occasionally (approximately 1 in every 4 residues) substituted at the 2-position with 4-O-methyl-α-D-glucuronic acid 2,3-O-sulfate. The average molecular weight lies between 4000 and 6000 with a total molecular weight range of 1000 to 40 000. PPS is structurally related to glycosamionoglycans (GAGs) synthesized by cells. High sulfation rate and charge density enables PPS to complete more effectively than other polyanions with endogenous GAGs, such as heparin, heparan sulfate or dermatan sulfate [32].

PPS has already been shown to exhibit numerous pharmacological activities. PPS can be used as a potent anti-osteoarthritis drug because it attenuates the events responsible for loss of components in the cartilage extracellular matrix in osteoarthritis joints and, due to its concomitant anti

coagulant activity, may also increase blood flow in the capillaries of osteoarthritic sites Sodium salt (NaPPS) has been used in Europe as an anti-thrombotic/anti-lipidemic agent. Elmiron is the commercial name of the pentosan polysulfate sodium which is given orally in a dose of 100 mg three times daily for the management of interstitial cytitis. Calcium derivative (CaPPS) is a new compound that shows many in vitro pharmacological similarities to NaPPS. On the other hand CaPPS exhibits some distict in vivo activities, including higher oral bioavailability than NaPPS. PPS can also be relevant in the control of brain endothelial functions. Migration of endothelial cells in vitro and re-growth of endothelium after balloon catheter injury is induced by PPS. Furthermore, PPS can inhibit protein kinases A and C, tyrosine protein kinase, serine proteases and matrix metalloproteinases, in different cultured cells and tissues. It also stimulates the release of tissue-type plasminogen activator and superoxide dismutase from peripheral endothelium. It has been reported that PPS is an effective inhibitor of heparin-binding growth factors released from tumor cells and can actually inhibit the growth of tumors in animals.[33 , 34] PPS is used in clinical practice as an anticoagulant and as a treatment for interstitial cystitis. PPS can favorably regulate BBB phenotype in brain endothelial cells, and protect them in models of prion peptide treatment. PPS did not change BBB parameters TEER, permeability or fluid phase endocytosis [35]. It also have hypolipidaemic and anti-inflammatory effects. It is used in thromboembolic disorders, although its anticoagulant effect is less than heparin.

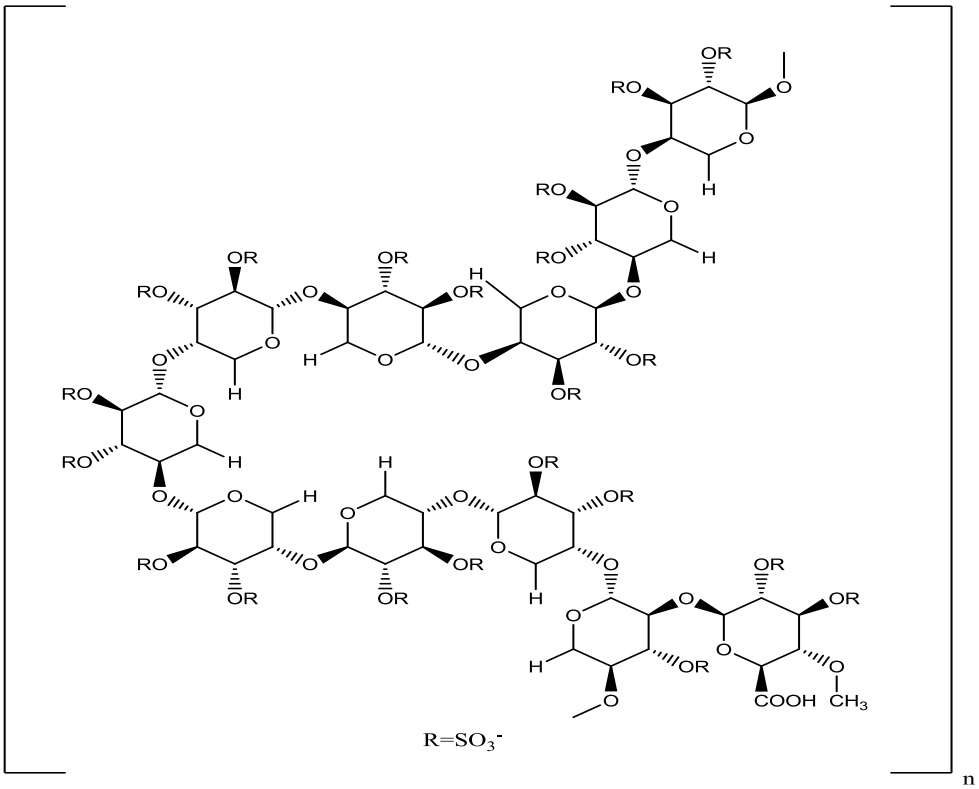


Figure 2. 1 The Chemical Structure of PPS

Due to these pharmacological effects of PPS, determination of it is so important. Determination of PPS using polyion-sensitive membrane electrodes (TDMAC) was reported by Durust et al. [36] with potentiometric titration. However optic sensor is more practically than potentiometric. In order that, this dissertation work we detected the mass stoichiometry ratios between PPS and some biologically important polycations protamine, poly-L-Lysine and poly-L-Arginine with PVC based microtiter bulk optode. Pre-studies of this work also published. [37]

2.2. Protamine

Protamine is a basic protein, a mixture of the sulfates of basic peptides which is prepared from the sperm or roe of suitable species of fish, usually from the families Clupeidae or Salmonidae.

Protamine is a biological important polycation which has several pharmacological effects. For pharmaceutical use the protamine or its salts are obtained from salmon sperm. Besides it is used to neutralize the anticoagulant action of heparin in the treatment of haemorrhage resulting from severe heparin or low molecular weight heparin overdosage protamine became in the last few years a promising agent in gene therapy for enhancing the transfection of genes with lipoplexes and the delivery of antisense oligonucleotides using nanoparticles. It is also used to neutralize the effect of heparin given before surgery and during extracorporeal circulation as in dialysis or cardial surgery.

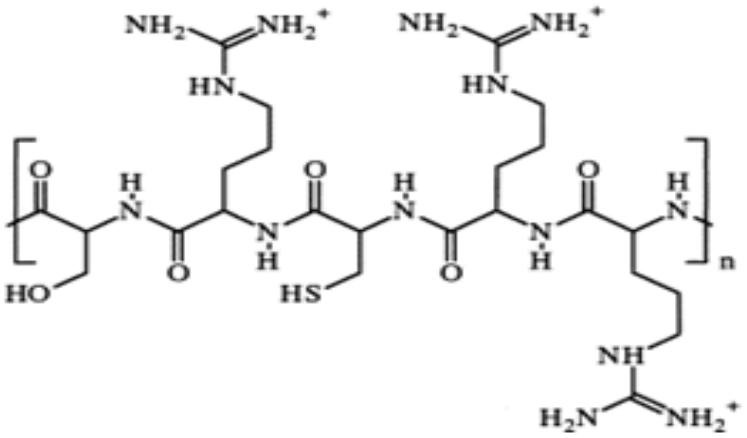


Figure 2. 2 Chemical Structure of Protamine

2.3. Poly-L-Arginine

Poly-L-Arginine (PLA) is a cationic, synthetic, polyamino acid. Its molecular weight ranges between 14.0-141.4 kDa. PLA has attracted considerable attentions due to its nontoxicity, excellent biocompatibility, nutritional function and pharmacological efficacy. For example, it can

inhibit tumor growth; enhance the drug delivery across biological membranes and tissues, and posses antimicrobial activity. It is not produced by human body and is essential for infant growth and also used dietary supplement. PLA has been used as a membrane-forming material to prepare microcapsules due to non-toxicity, excellent biocompatibility, nutritional function and pharmacological efficacy. [38] PLA motivates the release of growth hormone by the pituitary gland and may be used instead of, or in addition to, other tests such as insulin-induced hypoglycaemia, for the evaluation of growth disorders; false-positive and false-negative results are relatively common and evaluation therefore should not made on the basis of a single arginine test. Arginine hydrochloride has also been used as an acidifying agent [39]. It is commercially sales in markets.

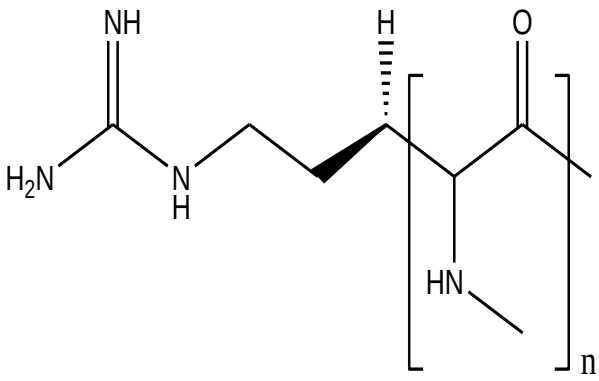


Figure 2. 3 The Chemical Structure of Poly-L-Arginine

2.4. Poly-L-Lysine

Poly-L-Lysine (PLL) is a kind of polycationic amino acid which consist of only one type of amino acid (lysine) linked by amide bonds. It forms an important class of biodegradable biopolymers which is being

investigated for a variety of pharmaceutical applications. PLL has two different structures, one that is naturally occurring and the other is chemically synthesized. PLL is naturally occurring cationic polymer made up of L-lysine residues connected between ε-amino and α-carboxyl groups. It is water soluble, biodegradable and non-toxic towards human and the environment. PLL shows a wide range of antimicrobial and antiphage activities which is used in the food industry as a food preservative [40].

L-Lysine-HCl (L- lysine monohydrochloride) is the major commercial form of PLL. It is mainly used as a feed additive in the animal feed industry, mixed with various common livestock such as cereals which do not contain sufficient levels of PLL and as a supplement for humans, improving the feed quality by increasing the absorbtion of other amino acids. PLL can be produced either by a chemical or a biochemical methods [41].

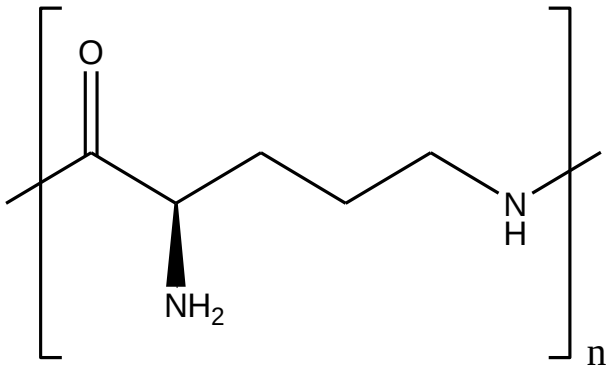


Figure 2. 4 The Chemical Structure of Poly-L-Lysine

2.5. Surfactants

Surfactants (or surface active agents) are organic compounds with at least one lyophilic (solvent-loving) group and one lyophobic (solvent-fearing) group in the molecule. If the solvent in which the surfactant is to be used is water or an aqueous solution, then the respective terms “hydrophilic” and “hydrophobic” are used. In the simplest terms, a surfactant contains at least one non-polar group and one polar (or ionic) group and is represented in

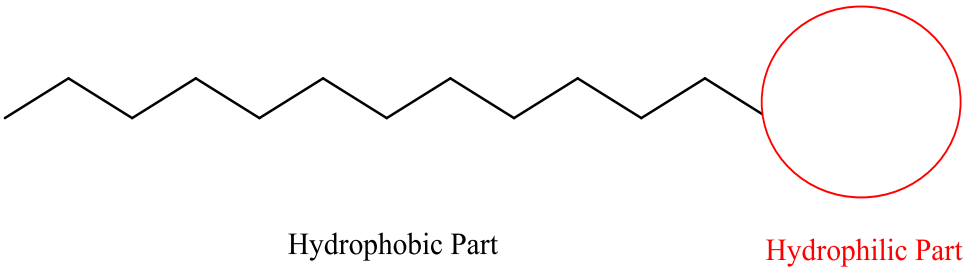


Figure 2. 5 General Structure of a Surfactant Molecule

Two phenomena result from these opposing forces within the same molecule: adsorption and aggregation.

For example, in aqueous media, surfactant molecules will migrate to air/water and solid/water interfaces and orientate in such a fashion as to minimize, as much as possible, the contact between their hydrophobic groups and the water. This process is referred to as “adsorption” and results in a change in the properties at the interface.

Likewise, an alternative way of limiting the contact between the hydrophobic groups and the water is for the surfactant molecules to aggregate in the bulk solution with the hydrophilic “head group” orientated

towards the aqueous phase. These aggregates of surfactant molecules vary in shape depending on concentration and range in shape from spherical to cylindrical to lamellar (sheets/layers). The aggregation process is called “micellisation” and the aggregates are known as “micelles”. Micelles begin to form at a distinct and frequently very low concentration known as the “critical micelle concentration” or “CMC”.

In simple terms, in aqueous media, micelles result in hydrophobic domains within the solution whereby the surfactant may solubilise or emulsify particular solutes. Hence, surfactants will modify solution properties both within the bulk of the solution and at interfaces [42].

Surfactants are classified according to the nature of their hydrophilic parts;

Anionic Surfactant: a surfactant in which the hydrophilic part carries a negative charge. Examples: soaps, $RCOO^-$; alkyl sulphates, $ROSO_3^-$

Nonionic Surfactant: a surfactant in which the hydrophilic part is uncharged. Examples : acyl diethanolamides, $RCON(C_2H_4OH)_2$; ethoxylated fatty alcohols, $R(OC_2H_4)OH$.

Cationic Surfactant: a surfactant in which the hydrophilic part carries a positive charge. Examples: alkytrimethylammonium salts, $RN^+(CH_3)_3$; alkyldimethylbenzylammonium salts, $RN^+(CH_3)_2CH_2C_6H_5$.

Amphoteric Surfactant: a surfactant in which the hydrophilic part contains both positive and negative charges. Examples : alkylaminopropionates, $RNH_2^+(CH_2)_2COO^-$; alkylbetaines, $RN^+(CH_3)_2CH_2COO^-$. It is possible for amphoteric surfactants to have

more than one charge of either sign, or to lose charhe by addition or removal of a proton [43].

Surfactant exists in both natural and synthetic forms. Due to this characteristic structure properties which are extensively applied in agriculture, pharmaceutical, biotechnology, nanotechnology, cosmetic, detergent, printing, recording, microelectronics, petroleum, mining, in capillary chromatography and other industries. Therefore considerable effort has been assigned over many years to development of new techniques for the measurement of surfactants [44].

The science and the technology of surfactants have possibly suffered a double blow from the functional divergence of academic and applied research. Academic interest in surfactants, while increasing, has generally concentrated on highly purified, homogeneous materials [quite often limited to a few materials such as sodium dodecylsulfate (NaDS), or cetyltrimethylammonium bromide (CTAB)] and elegant analytical techniques. While providing a wealth of useful information related to the particular system under investigation, the application of such information to more complex practical materials and processes is often less than obvious, and is sometimes misleading. The sad fact of life is that real surfactant systems are almost always composed of mixed chemical isomers, contaminants, and added materials that can dramatically alter the effects of a given surfactant on a system. High purity is necessary for the interpretation of delicate laboratory experiments, but requires the use of techniques that may be impractical at the industrial level [45]. A large number of ISEs were reported for analysis of surfactants [46, 47].

2.5.1. Anionic Surfactants

The largest class of surface-active materials in general use today fall in the anionic classification, constituting 70–75% of total worldwide surfactant consumption. The major subgroups of this class are the alkali carboxylates or soaps, sulfates, sulfonates, and to a lesser degree, phosphates. The variety of anionic materials available arises primarily from the many types of hydrophobic groups that can be modified

The determination of anionic surfactants in aqueous solutions is very important in the process control of anionic surfactants and the monitoring of environmental water, and so on, because of their toxicity to various organisms. Solvent extraction spectrophotometric methods based on the formation of an ion-pair of the anionic surfactant with a cationic dye, such as Methylene Blue, and extraction of the resulting ion-pair into a suitable organic solvent, such as chloroform, have been widely used for the determination of anionic surfactants. However, the procedures are very complicated and time-consuming. Furthermore, much poisonous organic solvent was often used in this method. A simpler method for the determination of anionic surfactants without using a poisonous organic solvent is required. Recently, optical sensors have been become one of very useful analytical tools because the optical sensors have the advantages of simple, safe and rapid [48]. Hence, optical sensors have become very useful analytical tools.

Several reports on optical sensors for the determination of surfactants have been published so far. An optical detection of cationic surfactants using an environment-sensitive fluorophore, 5-[(2-

aminoethyl)amino]naphthalene-1-sulfonate (ETH 5294), has been reported by Seitz et al. [49] Also specific determination of anionic surfactants with the same chromophore reported by Chan et al. [50] However ETH 5294 is not a suitable chromophore because it is expensive. In addition, synthesis of ETH 5294 is not easy. A cationic surfactant biosensor reported by Lundgren et al. Masadome et al. Reported lots of article about determination of surfactants. Optical detection of cationic surfactants using optical sensing membrane based on tetrabromophenolphthalein ethyl ester reported by Masadome et al. [51] By the way, determination of anionic surfactants using lactone-form of rhodamine B membrane also reported by Masodome et al. [52] Wang et al. [53] Reported that polymeric films doped only with chromophores such as DCFOE or ETH 5350 were found to be selective and useful for determination of nonionic surfactants, Triton X-100 and Brij 35, in the range of $5*10^{-6}$ M to $3*10^{-4}$ M using 0,1-0,01 M potassium or barium ions as background. However determination of anionic surfactant by using DCFOE sensor have not reported yet.

Surfactant part of this thesis related to titration of anionic surfactants by using cationic surfactants and polymer based optical sensor with microtiter plate reader.

Sodium Dodecyl Sulfate (NaDS)

Synonims: Natrium Lauryl Sulphuricum; Sodium Dodecyl Sulphate; Sodium Lauryl Sulfate; Sodium Lauryl Sulphate.

A mixture of sodium alky sulfates, consisting mainly of sodium dedecyl sulfate ($C_{12}H_{25}NaO_4S$ = 288.4). It contains not less than 85% of sodium alkyl sulfates and not more than a total of 8% of sodium chloride and sodium sulfate. A white or pale yellow powder or crystals with a slight, characteristic odor. Freely soluble in water giving an opalescent solution ; partly soluble in alcohol.

NaDS is an anionic emulsifying agent. It is a detergent and wetting agent, effective in both acid and alkaline solution and in hard water. It is used in medicated shampoos and as a skin cleanser and in toothpastes. It is used in the preparation of Emulsifying Wax.

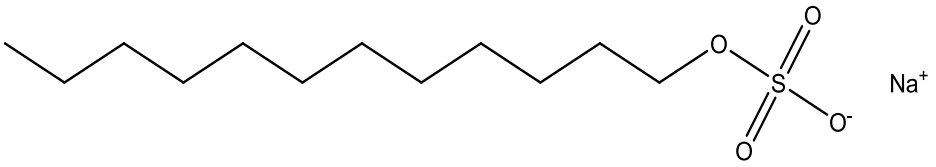


Figure 2. 6 The Structure of Sodium Dodecyl Sulfate (NaDS)

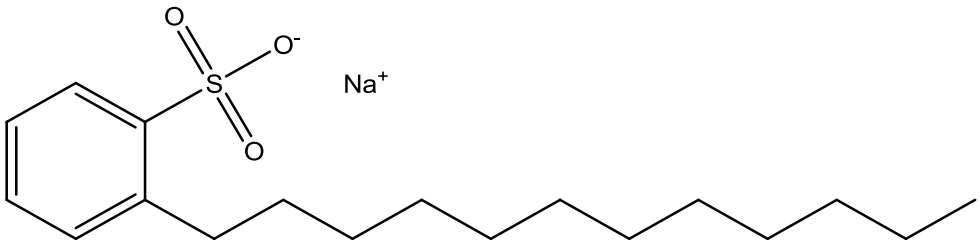


Figure 2. 7 The Structure of Sodium Dodecylbenzenesulfonate (NaDBS)

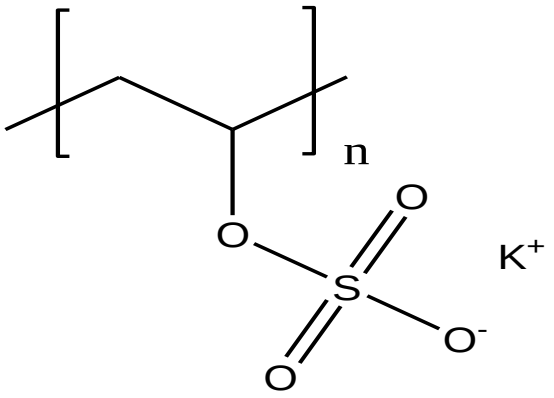


Figure 2. 8 The Structure of Potassium Poly(vinyl Sulfate) (PVSK)

2.5.2. Cationic Surfactants

Cationic surfactants first became important when the commercial potential of their bacteriostatic properties was recognized in 1938. Currently, cationic surfactants are widely used in industry as emulsify agents, as antirots, and as additives in laundry detergents [54]. Many new applications for cationic surfactants have been developed since World War II, so that these compounds can no longer be considered to be specialty chemicals; rather, they truly fall into the category of bulk industrial surfactant products.

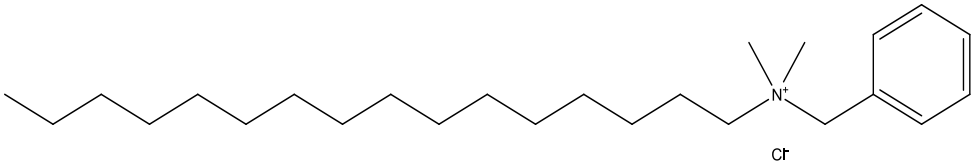


Figure 2. 9 The Structure of Cetyldimethylbenzylammonium Chloride (CDB)

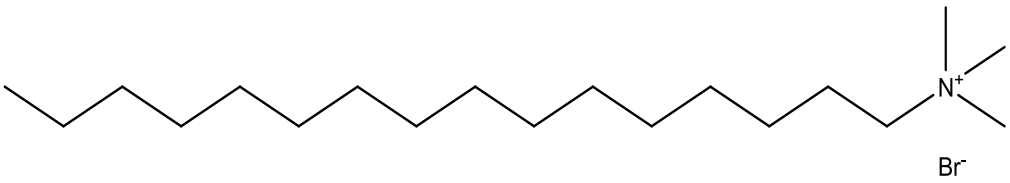


Figure 2. 10 The Structure of Cetyltrimethylammonium bromide (CTAB)

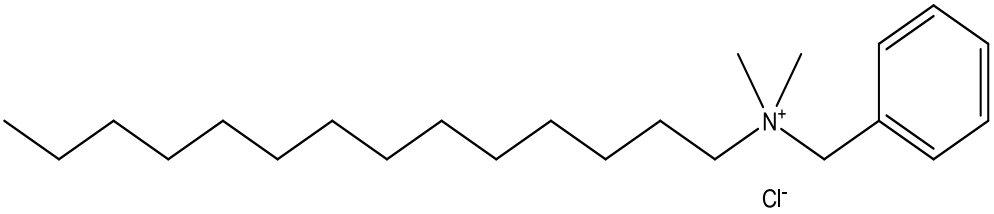


Figure 2. 11 The Structure of Tetradecyldimethylbenzylammonium chloride (Zephiramine)

Chapter 3 .

EXPERIMENTAL

3.1. Reagents

Bis(2-ethylhexyl) sebacate (DOS), high molecular weight poly(vinyl chloride) (PVC), 2',7'-dichlorofluorescein, 1-iodooctadecane, tetrahydrofuran (THF) (HPLC Grade), Tecoflex polyurethane (PU, SG-80A), 1,2-benzo-7-(diethylamino)-3-(octadecanoylimino) phenoxazine (ETH 5294), protamine sulfate salt (from herring, grade III), poly-L-arginine) hydrochloride (MW 11,800) poly-L-lysine hydrochloride, sodium dodecyl benzene sulfonate (NaDBS), sodium dodecyl sulfate (NaDS), potassium poly(vinyl sulfate) (PVSK), cetyldimethylbenzyl ammonium chloride (CDB), benzyldimethylteradecyl ammonium chloride (Zephiramine), Tris (hydroxymethyl) aminomethane, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES-Na). They were purchased from Sigma-Aldrich Chemical Co., samples of PPS (sodium salt form) and dinonylnapthalene sulfonate (DNNS) were kindly provided by Prof. Dr. Mark E. Meyerhoff. All other reagents were analytical grade. Solutions were prepared with $5.5*10^{-2}$ μS/cm deionize water. 50 mM Tris–HCl (pH 7.4, containing 120 mM NaCl)

3.2. Materials

Molecular Devices (Versa max) Microplate reader was used to absorbance measurements of microtiter plate optodes. 96 well, U-bottomed, polypropylene plate Greiner-Bio One (Cat 650201) Eppendorf Multipette plus and research pipettes were used. pH adjusment of buffers were made by Thermo Scientific Orion 4 Star pH/ISE meter.

3.3. Synthesis of 2',7'-Dichlorofluorescein Octadecyl Ester(DCFOE)

Synthesis of DCFOE-based optode membrane firstly developed by Wang et al. 2', 7'-dichlorofluorescein (1.07 mmol, 430 mg) , 1-iodooctadecane (1 mmol, 380 mg) and K_2CO_3 (2.1 mmol, 290 mg) were mixed in 5 mL dimethylsulfoxide (DMSO) at 65^oC 20 hours in an oil bath. The red precipitate was formed upon addition of 10 mL saturated NaCl was filtered. The filter paper was washed with 1 M HCl and ethyl acetate mixture. The orange organic phase was seperated, washed with phosphate buffer (pH 7.4) and ultrapure water by using seperatory funnel. Then which was evaporated to dryness under reduced pressure by evaporator. The product was recrystallized twice with acetone to yield pure DCFOE. IR results of product and reactants are shown on Figure 3.2 – Figure 3.3.

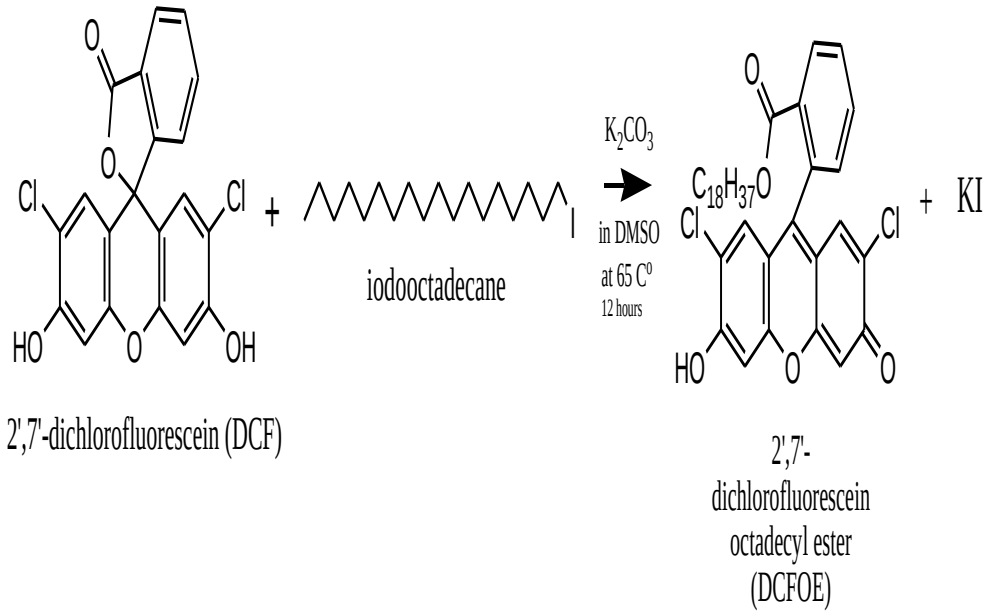


Figure 3. 1 Shematic Representation of DCFOE Synthesis

3.4. Preparation of Microtiter Plate Optode

3.4.1. Preparation of Film 1 Type Microtiter Plate Optode

Polycation sensitive DCFOE optic sensor contained 15 wt % PVC , 15 wt % PU, 69 wt % DOS, 1 wt % DCFOE.

3.4.2. Preparation of Film 2 Type Microtiter Plate Optode

ETH 5294 based polycation sensitive optic sensor cocktail contains 28.7 wt % PVC, 68.7 wt DOS , 1 wt % DNNS, 1.6 wt % ETH 5294.

Totally 200 mg of these components of each optode were shaked in 2 mL freshly distilled THF to dissolve approximately one day. 10 µL of this solution was uniformly dispensed in each well in 96-well round bottomed polypropylene plate by using multipette. The plates were air-dried in a dust free vessel for 1 day prior to use.

3.5. Titration Study of Film 1 Type Optode

Before the titration study, all the wells including optodes were pre-soaked Tris-HCl buffer (300 µL) or HEPES (pH: 7.4) for PPS 0,12 M NaCl solution for surfactants until a stable absorbance value happen (app. 20 min.). Polycation standard solutions (0, 10,20... µg/mL) were prepared by diluting with buffer. The same concentrate polyanion standard solutions were added in each polycation solutions. To find true stoichiometry between analyte and titrant, they were mixed rigorously. When the concentration of polycations were increased, the color of DCFOE was changed orange to pink. Analyte-Titrant mixtures (300 µL) were waited in each wells (3 min.) rapidly removed from wells then rapidly filled with buffer again. Absorbance measurements were made in buffer. But there is an important point, response time of polycation test solutions changeable. 3 minutes are enough for protamine and poly-l-arginine, zephiramine, CTAB, CDB on the other hand not sufficient for poly-l-lysine. So that to observe clear absorbance and color change, optodes were waited 20 min. in poly-l-lysine test solutions. All the other things are the same all the absorbance change measurements were made in buffer at 540 nm and to eliminate some errors comes from small ions or other things measurements also made at 620 nm (dye does not give absorbance at 620 nm). The results are at least the average measurements of 3 wells to reduce standard deviations NaCl is added in Tris-HCl Buffer. All the measurements were carried out under ambient conditions.

3.6. Titration Study of Film 2 Type Optode

There are not enormous difference between Film 1 and Film 2 type microtiter plate optode studies. Waiting time of optodes in buffer to calibrate 15 min. The color of the optode change purple to blue. Response time in polycation test solutions 10 min. Increasing the polycation concentration color turned back. Absorbance measurements made at 650 nm and 800 nm. All the other parameters as the same as film 1 type optode.

3.7. Determination of Total Surfactants in Domestic Samples

Known weights of the domestic samples were weighed (100 mg-500 mg) and dissolved in 10 mL ultrapure water and titrated with Zephiramine, CTAB and CDB by using film 1. The determined amounts of surfactants in domestic samples were presented in Table 4. 3

Chapter 4 .

RESULTS

4.1. Proportional Titration Curves of Polyions with Film 1 Type Optode

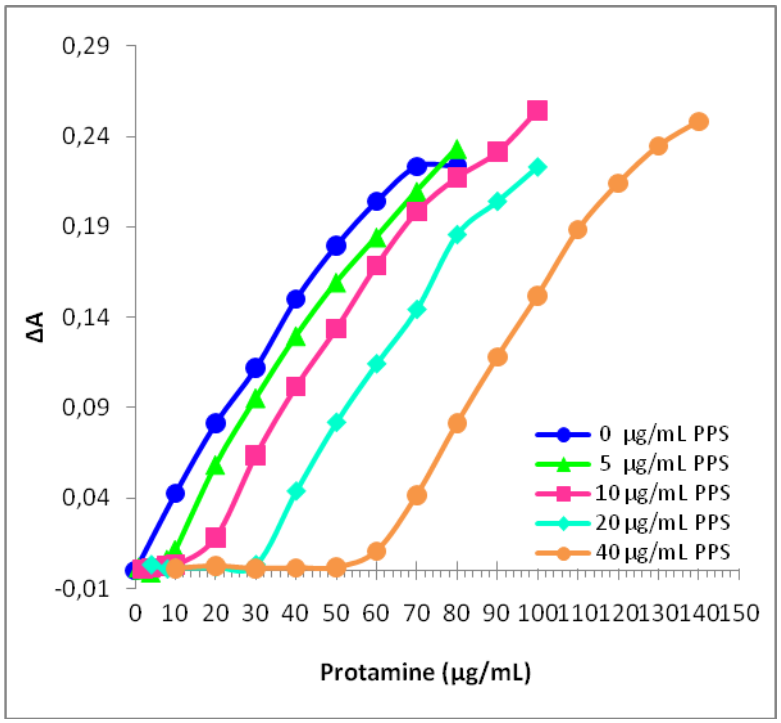


Figure 4. 1 Proportional Titration Curves of PPS by Protamine

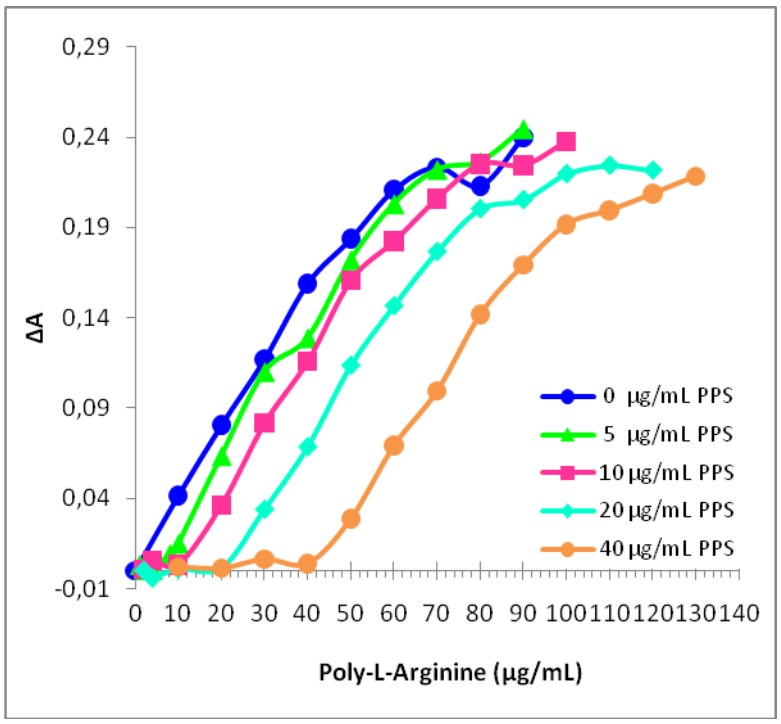


Figure 4. 2 Proportional Titration Curves of PPS by Poly-L-Arginine

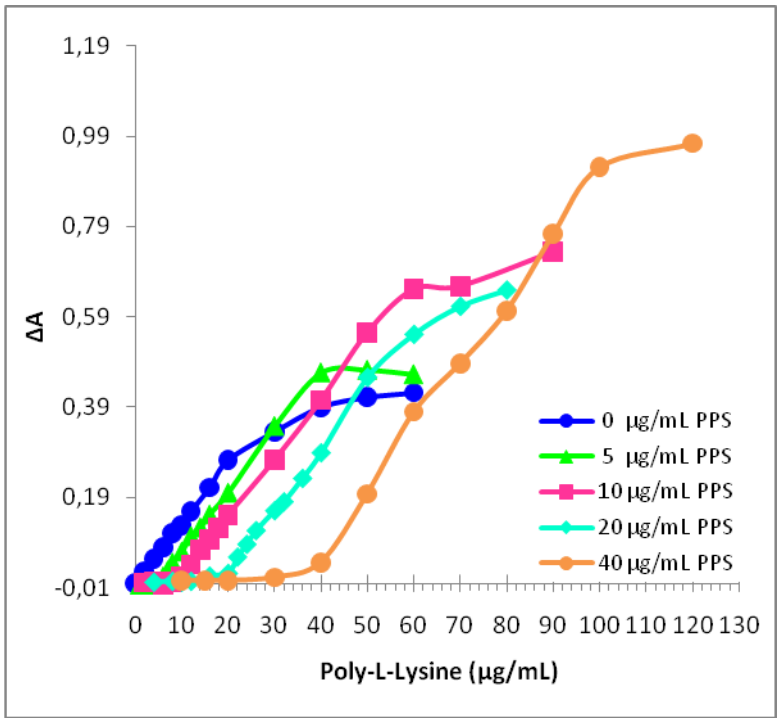
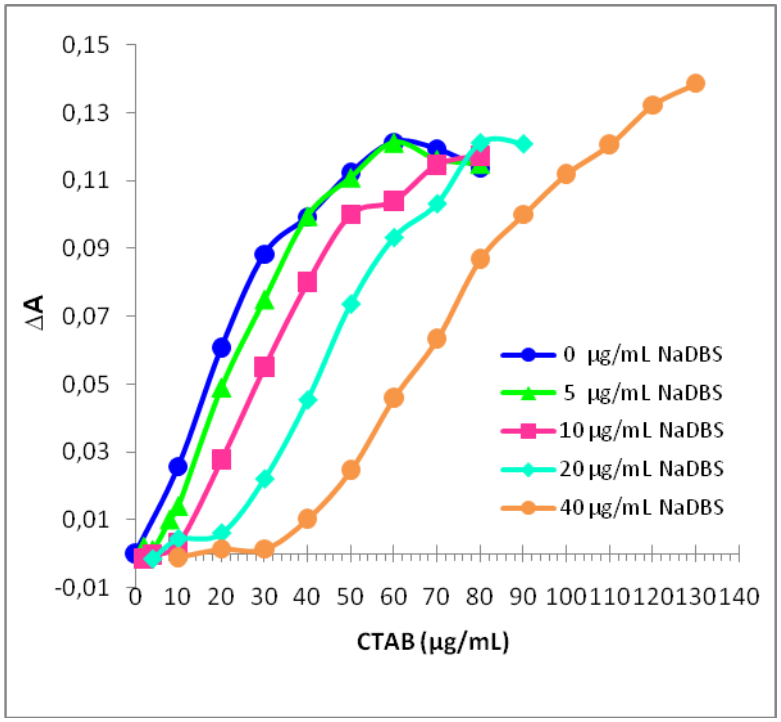


Figure 4. 3 Proportional Titration Curves of PPS by Poly-L-Lysine

Table 4. 1 The Stoichiometry Ratios of PPS with Polycations by Microtiter Plate Optode

Titrant/Analyte	Mass Ratio
Protamine/PPS	1.50 (± 0.08)
Poly-L-Arginine/PPS	1.10 (± 0.08)
Poly-L-Lysine/PPS	0.90 (± 0.04)

50 mM Tris-HCl including 0.12 M NaCl was used.
Average of at least three measurements.



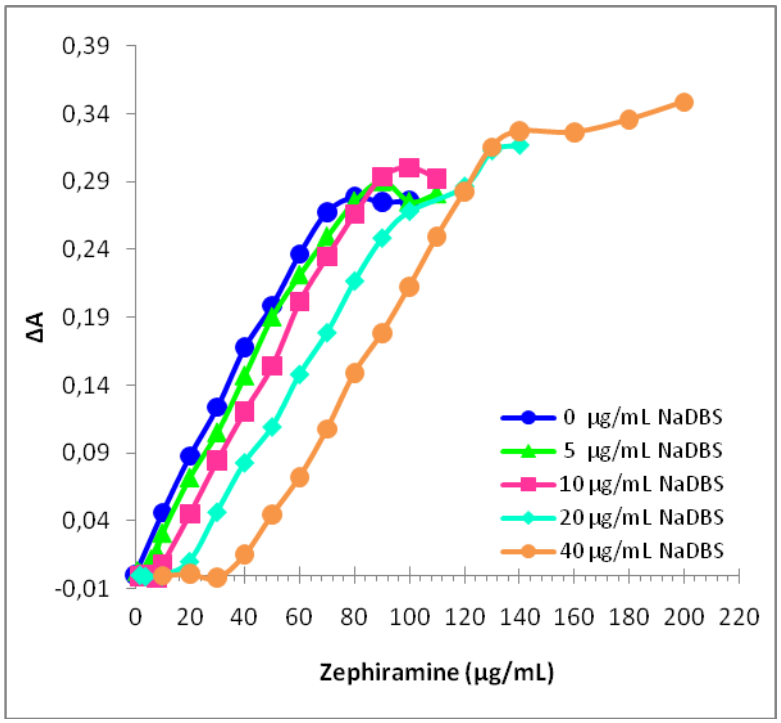
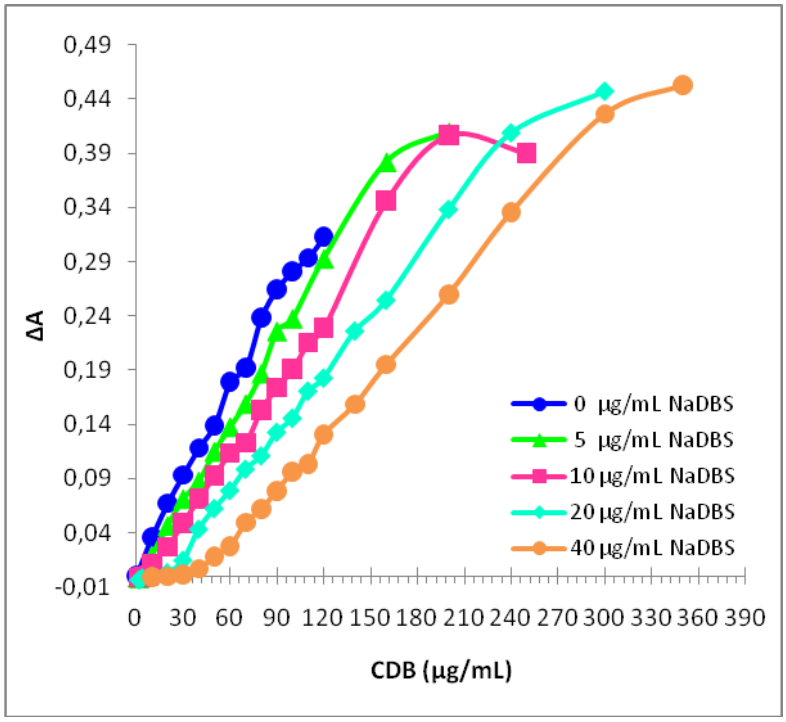


Figure 4. 4 Proportional Titration Curves of NaDBS by CTAB

Figure 4. 5 Proportional Titration Curves of NaDBS by Zephiramine



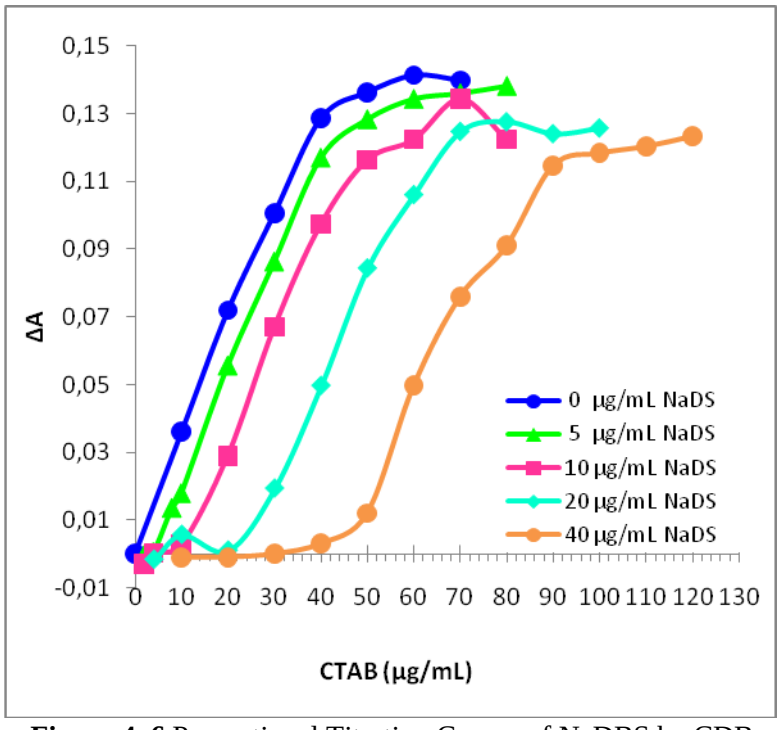
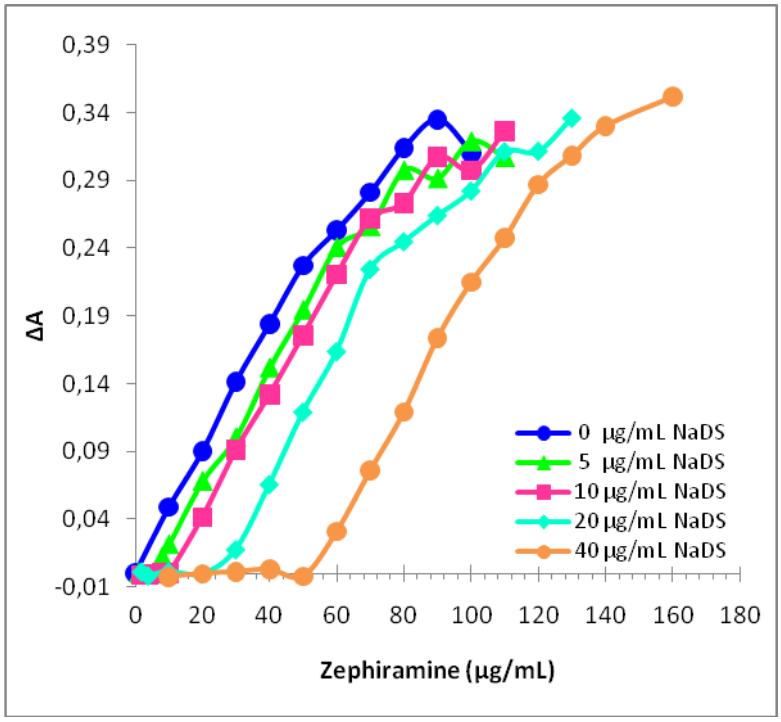


Figure 4. 6 Proportional Titration Curves of NaDBS by CDB

Figure 4. 7 Proportional Titration Curves of NaDS by CTAB



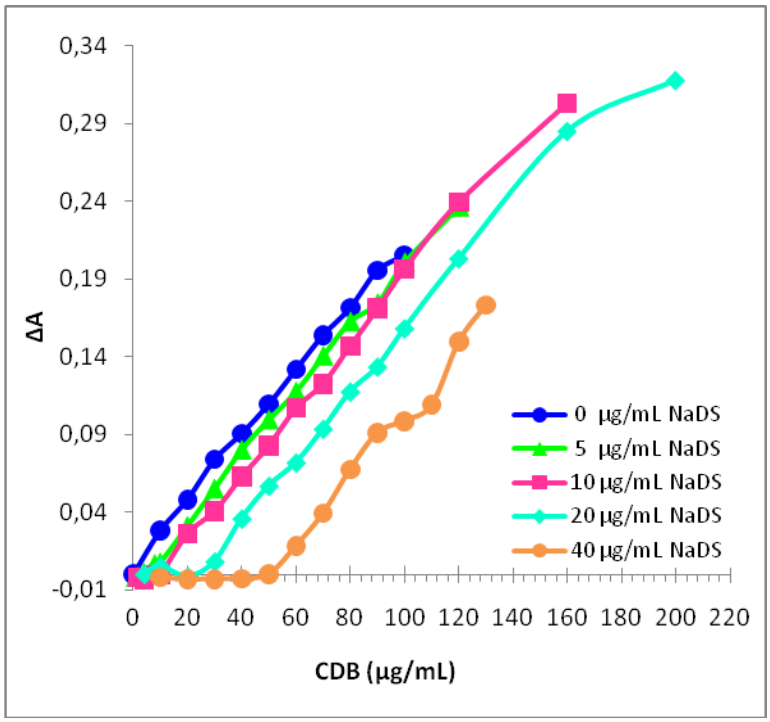


Figure 4. 8 Proportional Titration Curves of NaDS by Zephiramine

Figure 4. 9 Proportional Titration Curves of NaDS by CDB

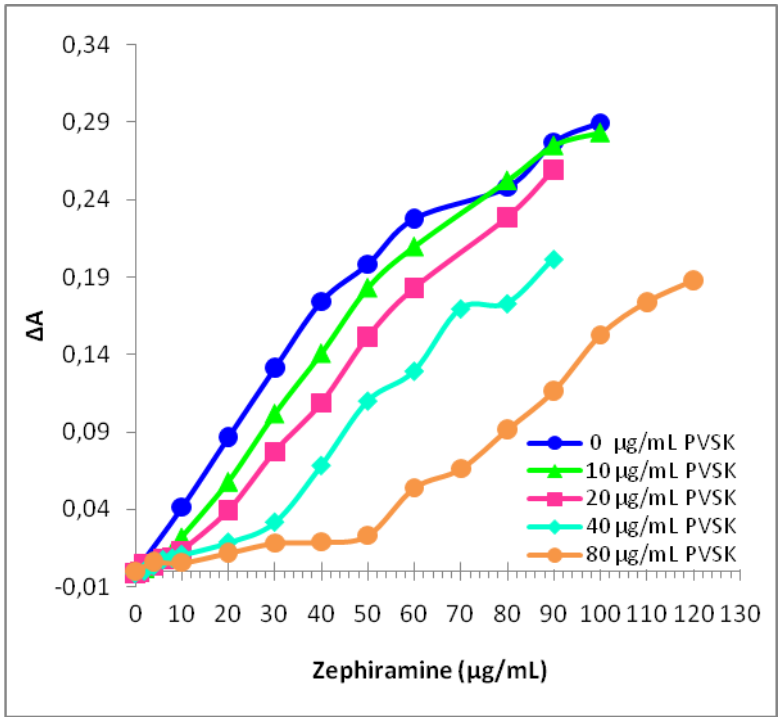


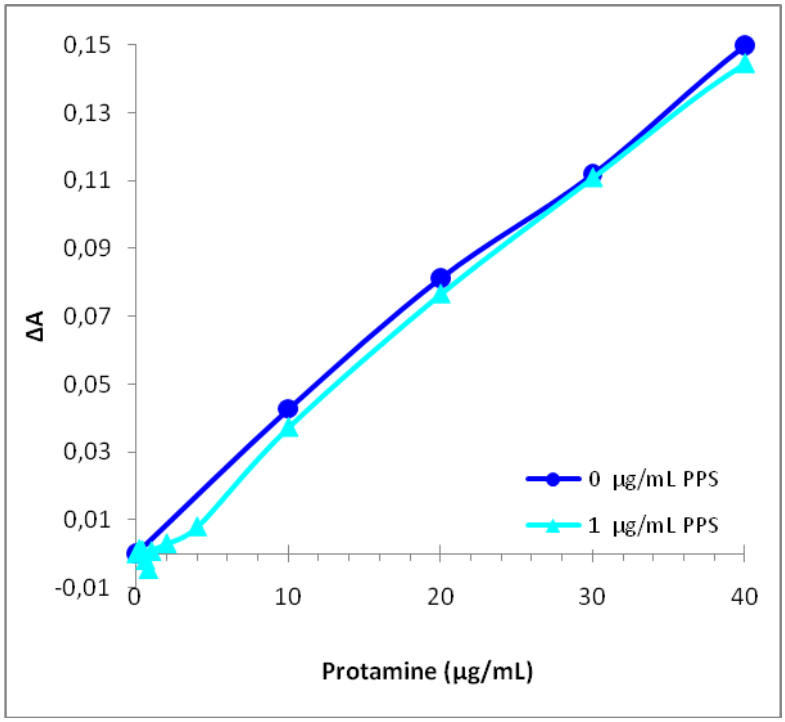
Figure 4. 10 Proportional Titration Curves of PVSK by Zephiramine

Table 4. 2 The Stoichiometry Ratios of Cationic Surfactants with Anionic Surfactants

Titrant/Analyte	Mass Ratio
Zephiramine/NaDBS	0.83 (±0.04)
Zephiramine/NaDS	1.24 (±0.08)
Zephiramine/PVSK	1.29 (±0,09)
CTAB/NaDBS	0.91 (±0.08)
CTAB/NaDS	1.23 (±0.08)
CDB/NaDS	1.21 (± 0.09)
CDB/NaDBS	0.81 (±0.09)

0,12 M NaCl was used.
Average of at least three measurements.

4.2. Limit of Detections Polyions with Film 1 Type Optode



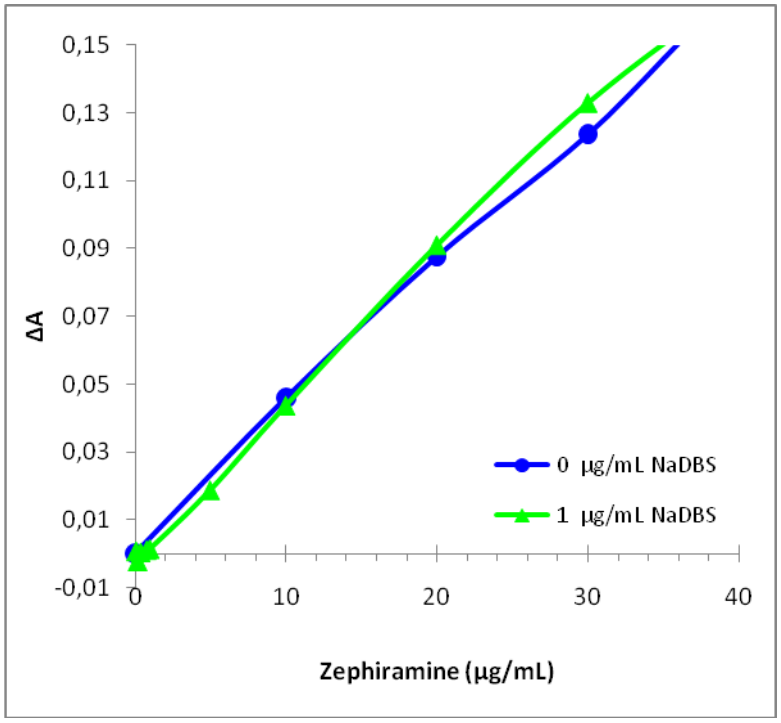
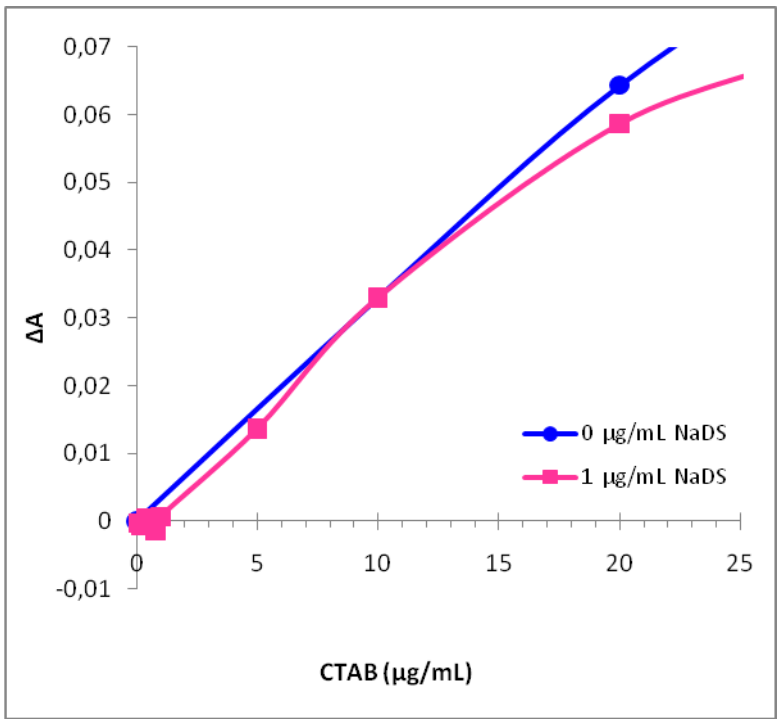


Figure 4. 11 Limit of Detection PPS (1 µg/mL) by Protamine

Figure 4. 12 Limit of Detection NaDBS (1 µg/mL) by Zephiramine



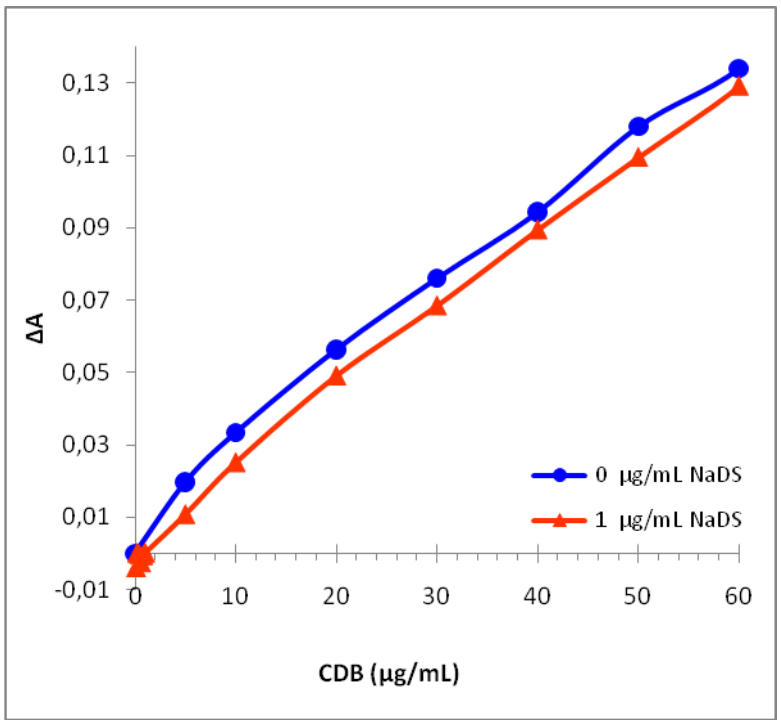


Figure 4. 13 Limit of Detection NaDS (1 µg/mL) by CTAB

Figure 4. 14 Limit of Detection NaDS (1 µg/mL) by CDB

4.3. Determination of Total Surfactants in Real Samples with Film 1 Type Optode

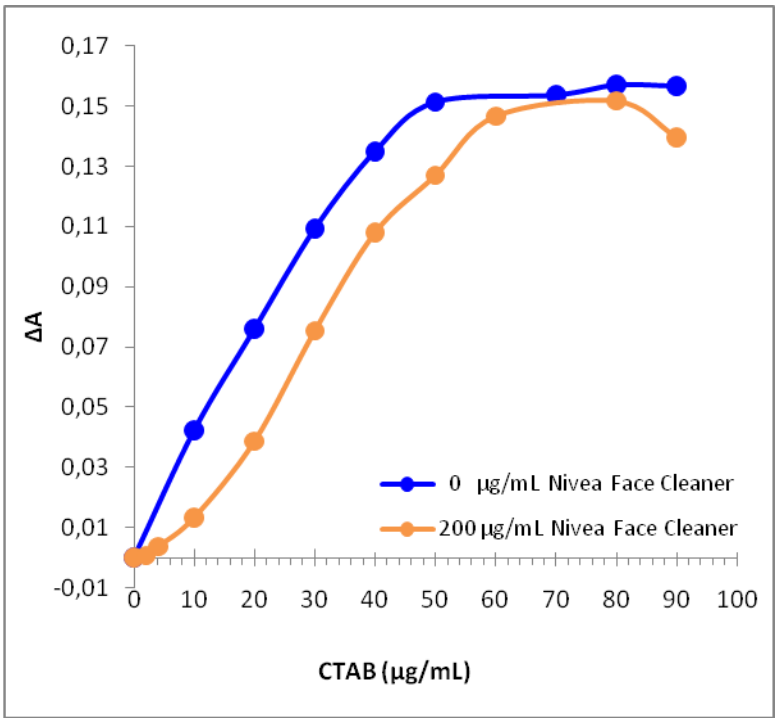


Figure 4. 15 Titration Curve of Nivea Face Cleaner Jel (200 µg/mL)

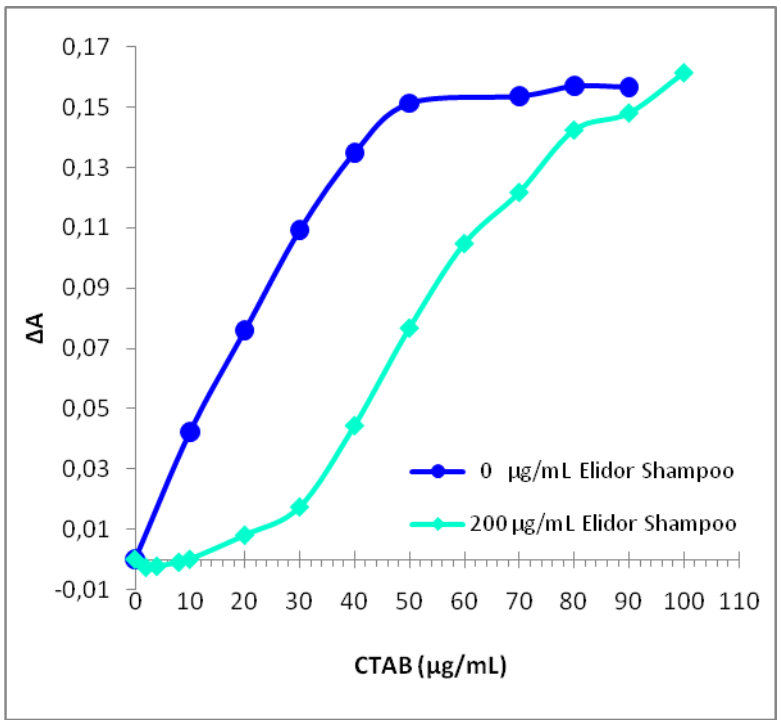


Figure 4. 16 Titration Curve of Elidor Shampoo (200 µg/mL)

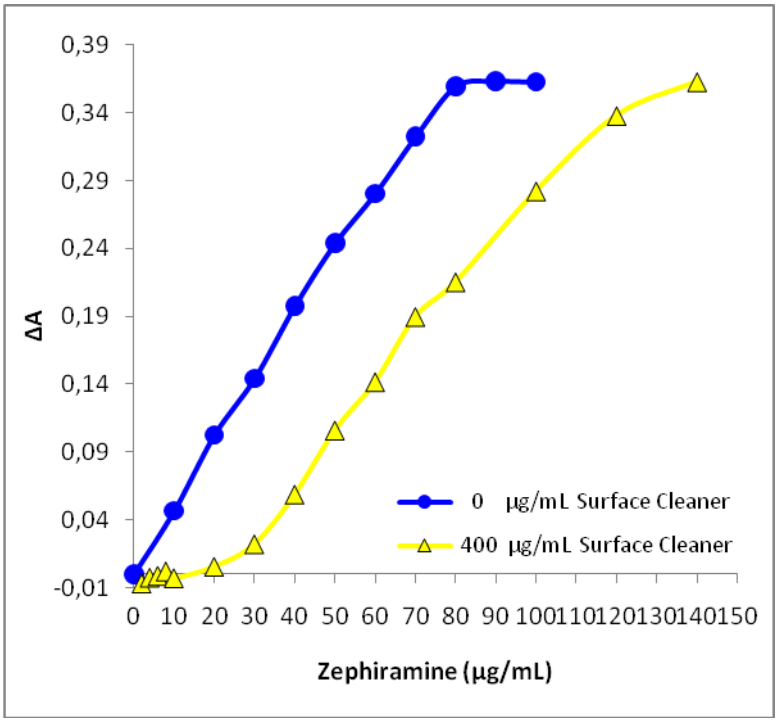


Figure 4. 17 Titration Curve of Super Markus Surface Cleaner (400 µg/mL)

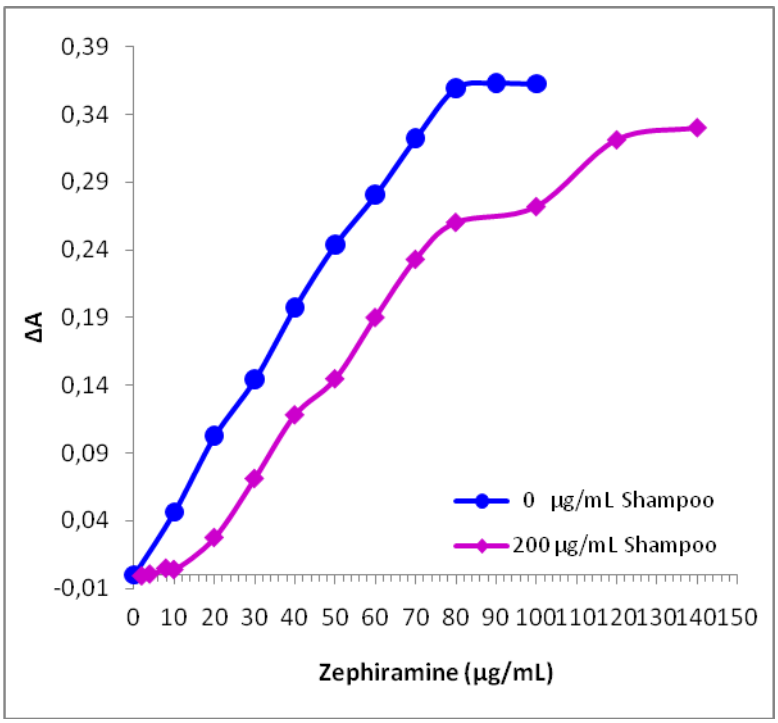
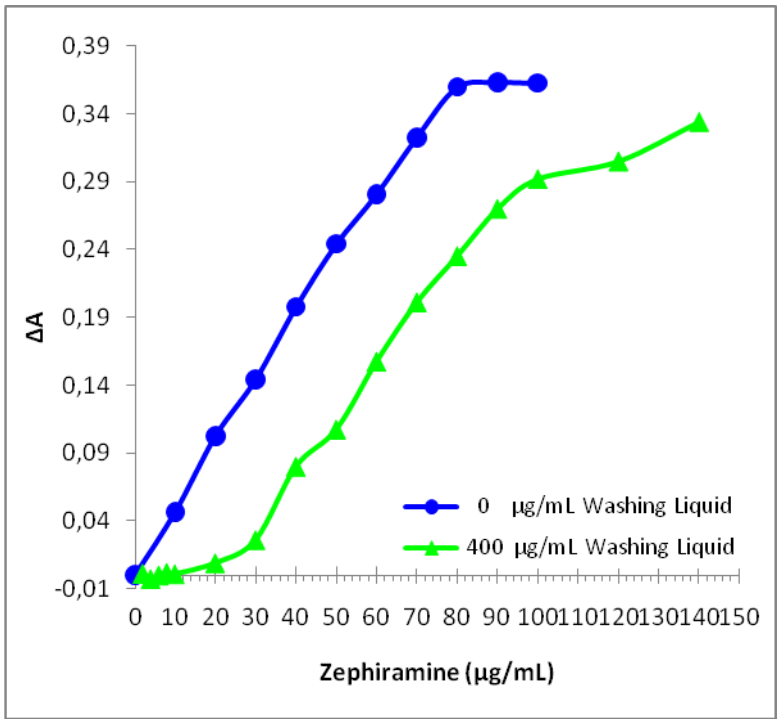


Figure 4. 18 Titration Curve of İpek Shampoo (200 µg/mL)



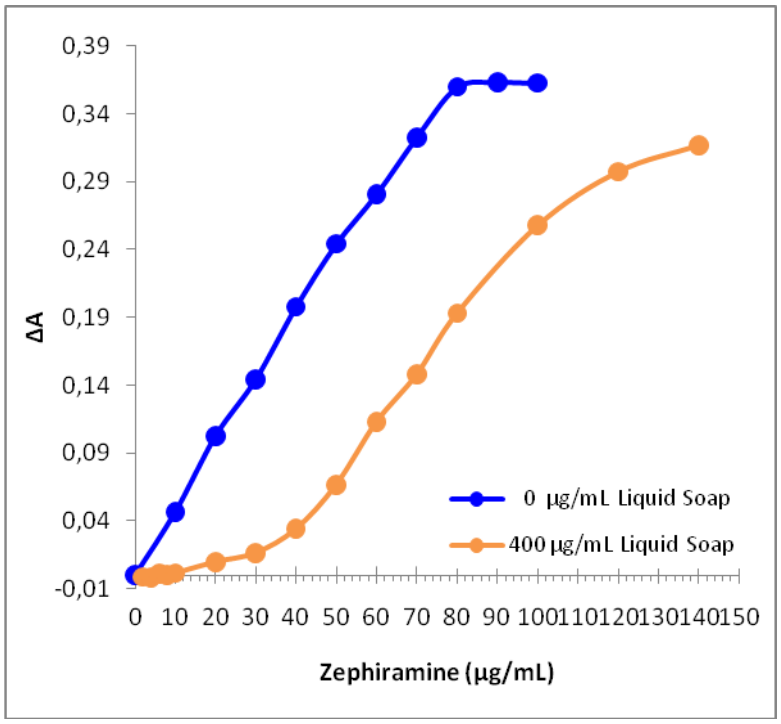


Figure 4. 19 Titration Curve of Şok Washing Liquid (400 µ/mL)

Figure 4. 20 Titration Curve of Berry Liquid Soap (400 µg/mL)

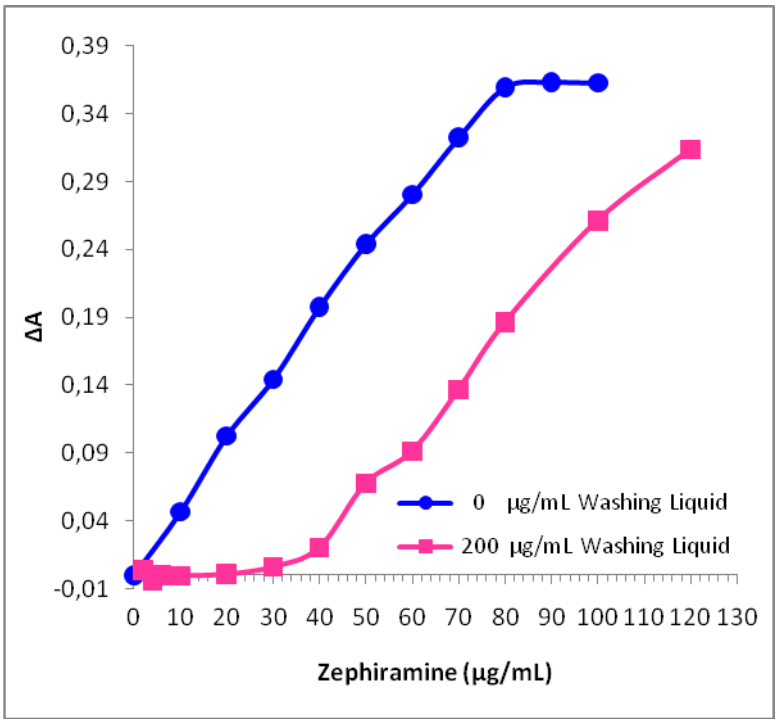


Figure 4. 21 Titration Curve of Cif Washing Liquid (200 µg/mL)

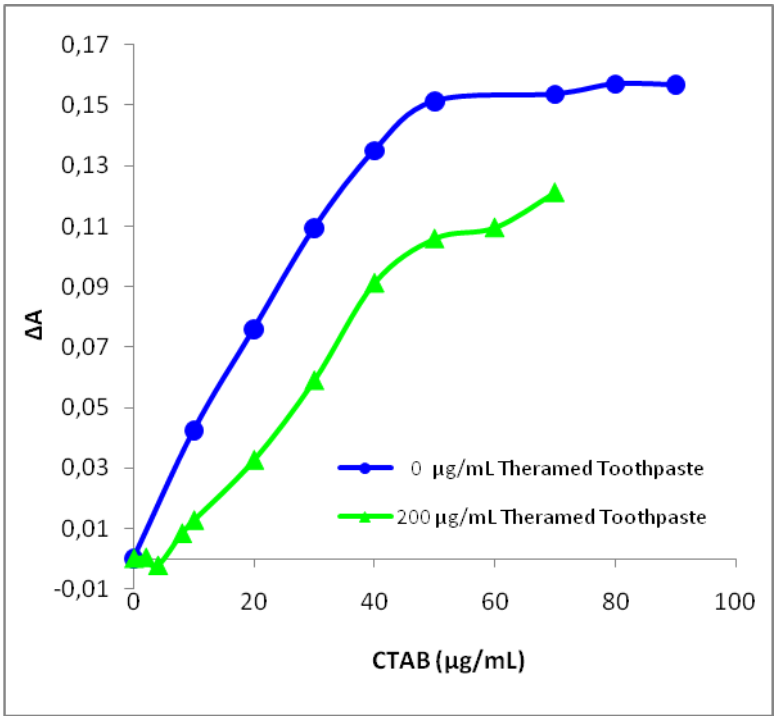


Figure 4. 22 Titration Curve of Theramed Toothpaste (200 µg/mL)

4.4. Recovery Studies with Domestic Samples

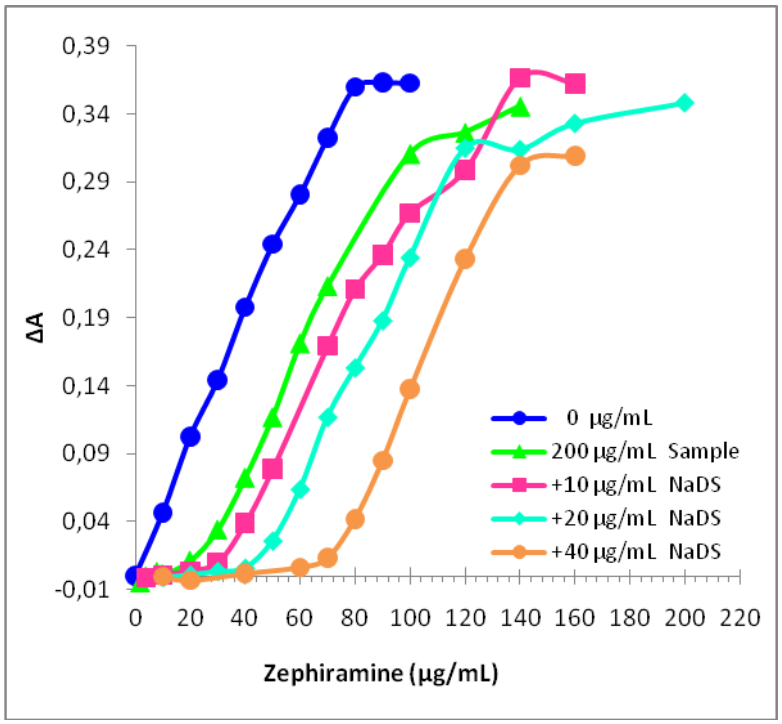


Figure 4. 23 Titration Curve of Duru Shower Jel (200 µg/mL)

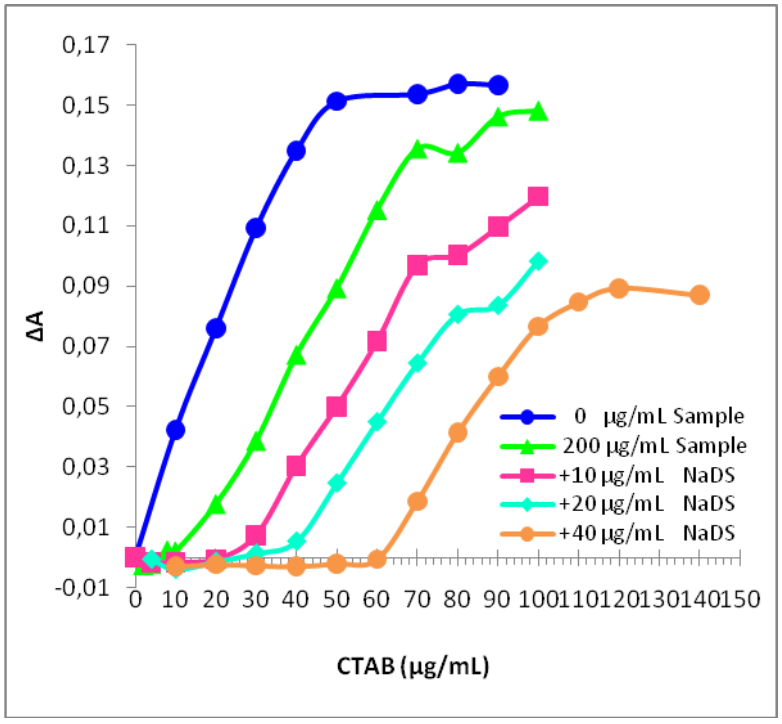


Figure 4. 24 Titration Curve of Lux Shower Jel (200 µg/mL)

Table 4. 3 Determination of Total Surfactant Amounts in Real Samples with Film 1 Type Optode

Sample	Total Surfactant %
İpek Shampoo	5,66 (±0,15)
Berry Liquid Soap	7,69 (±0,05)
Cif Surface Cleaner	14,03 (±0,15)
Elidor Shampoo	10,41 (1,6)
Duru Shower Jel	8,75 (±2,16)
Cif Washing Liquid	14,03 (±0,15)
Nivea Face Cleaner	3,47 (±1,01)
Super Markus Surface Cleaner	5,42 (±0,58)
Theramed Toothpaste	1,76 (±0,58)
Lux Shower Jel	4,92 (±0,87)

The amount of analyte (g) in 100 g sample
0.12 M NaCl was used.
Average of at least three measurements.

4.4. The Wavelength Scan of Polyions with Film 1 Type Optode

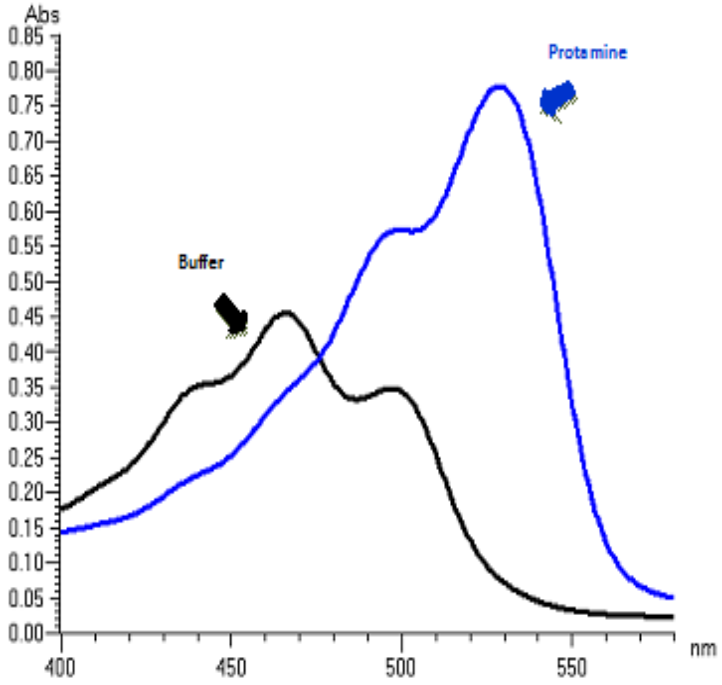


Figure 4. 25 The Wavelength Scan of Protamine (500 µg/mL)

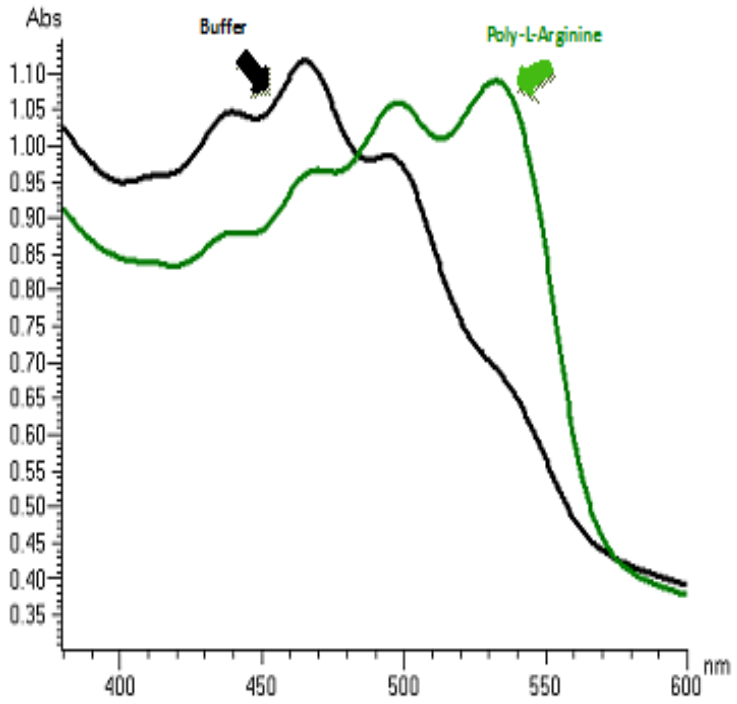


Figure 4. 26 The Wavelength Scan of Poly-L-Arginine (500 µg/mL)

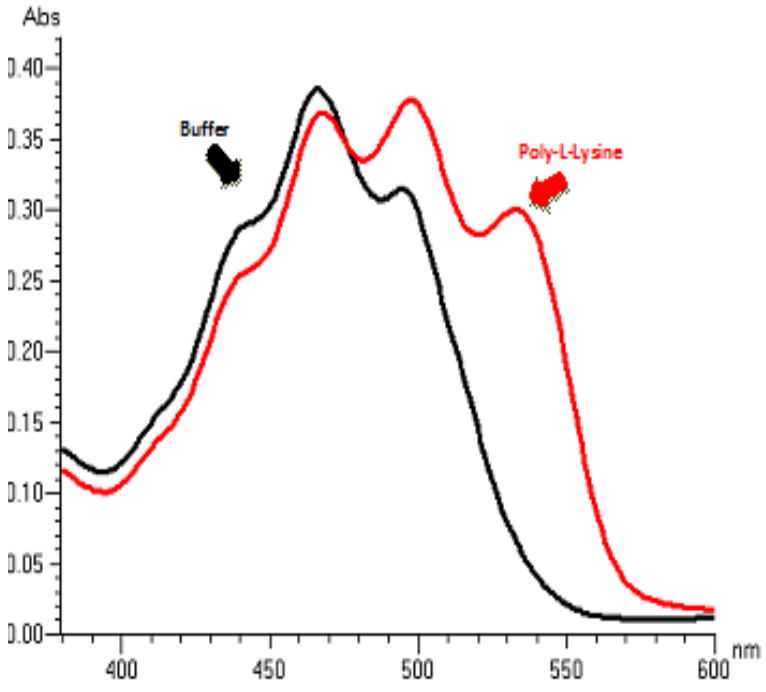


Figure 4. 27 The Wavelength Scan of Poly-L-Lysine (100 µg/mL)

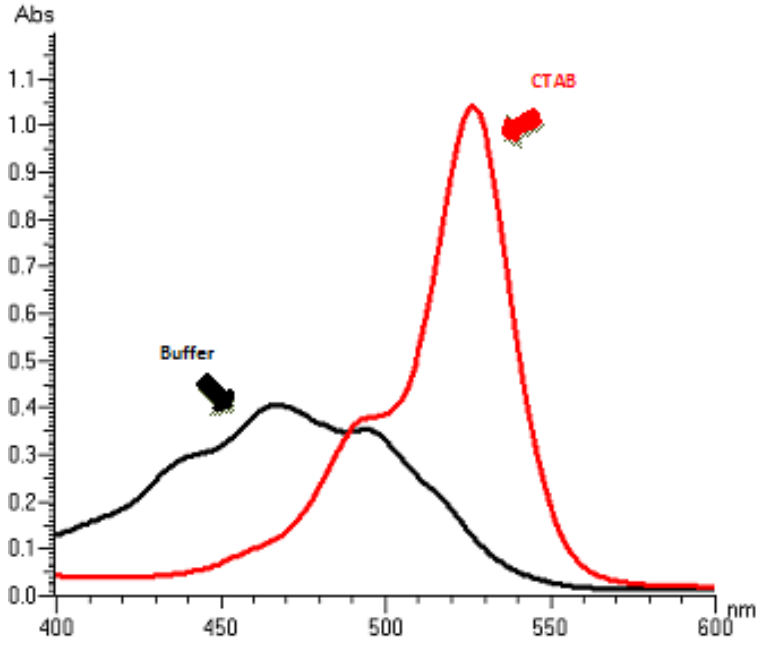


Figure 4. 28 The Wavelength Scan of CTAB (500 µg/mL)

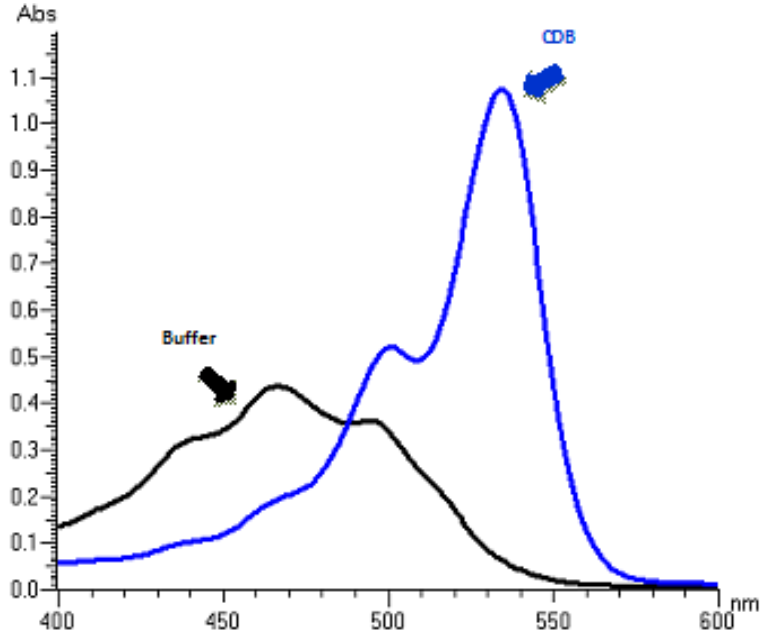


Figure 4. 29 The Wavelength Scan of CDB (500 µg/mL)

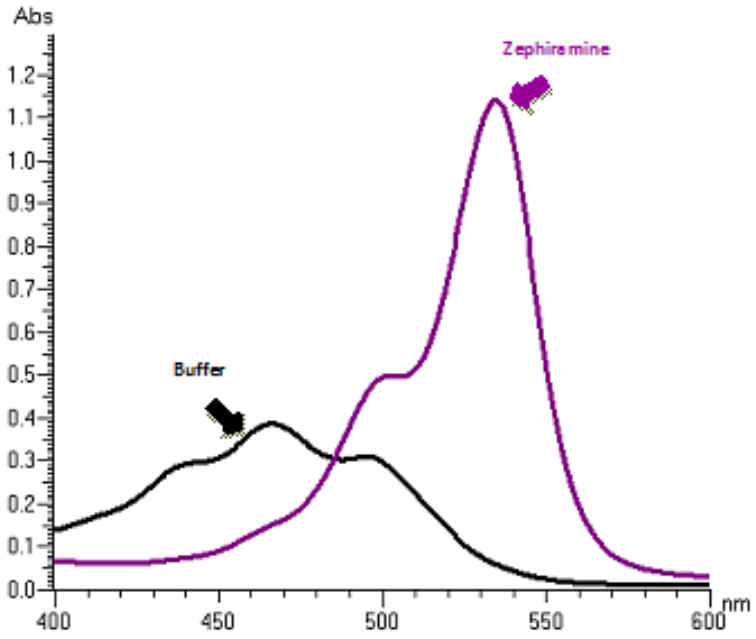


Figure 4. 30 The Wavelength Scan of Zephiramine (500 µg/mL)

4.5. Time-Absorbance Graphs of Polycations with Film 1 Type Optode

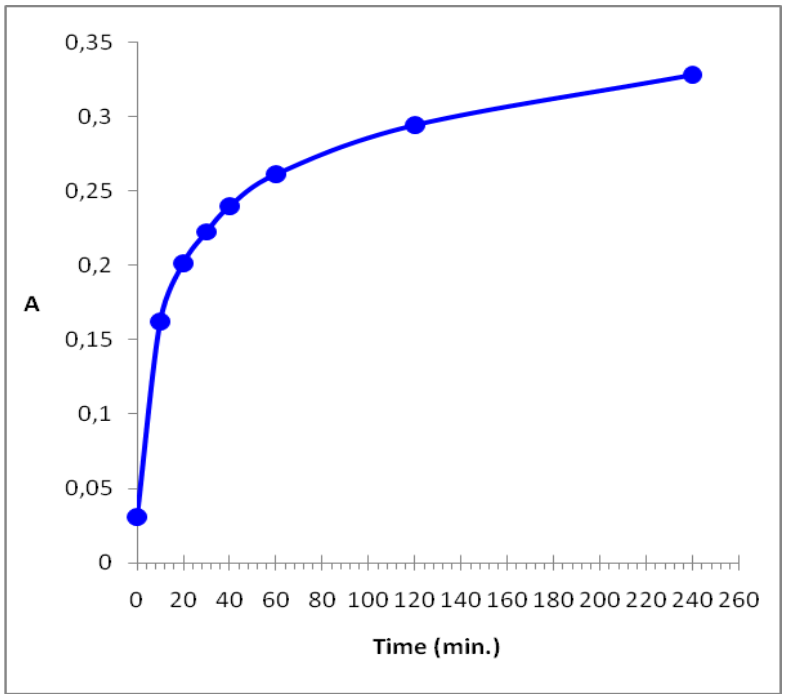
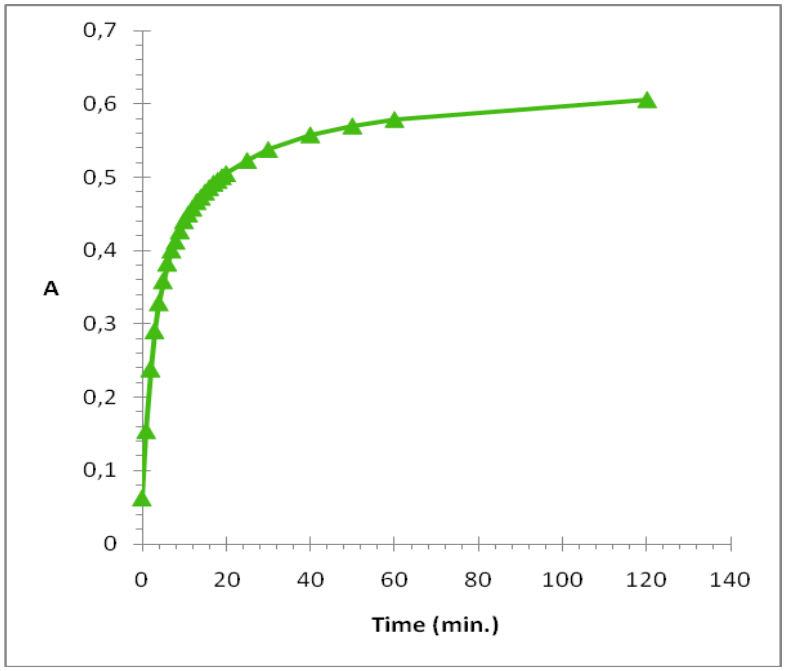


Figure 4. 31 Time-Absorbance Graph of Protamine (500 µg/mL)



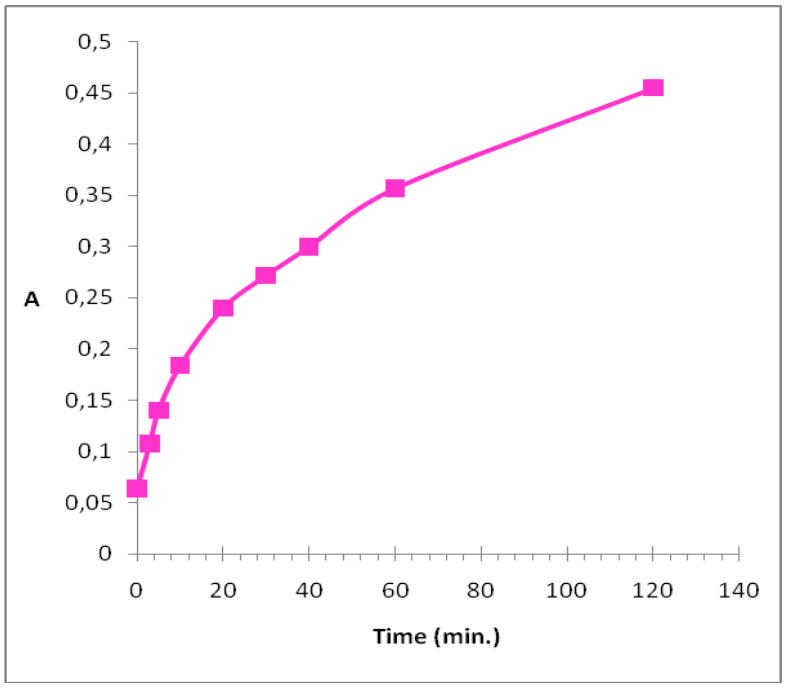


Figure 4. 32 Time-Absorbance Graph of Poly-L-Arginine (100 µg/mL)

Figure 4. 33 Time-Absorbance Graph of Poly-L-Lysine (100 µg/mL)

4.6. Titration Curves of PPS with Film 2 Type Optode

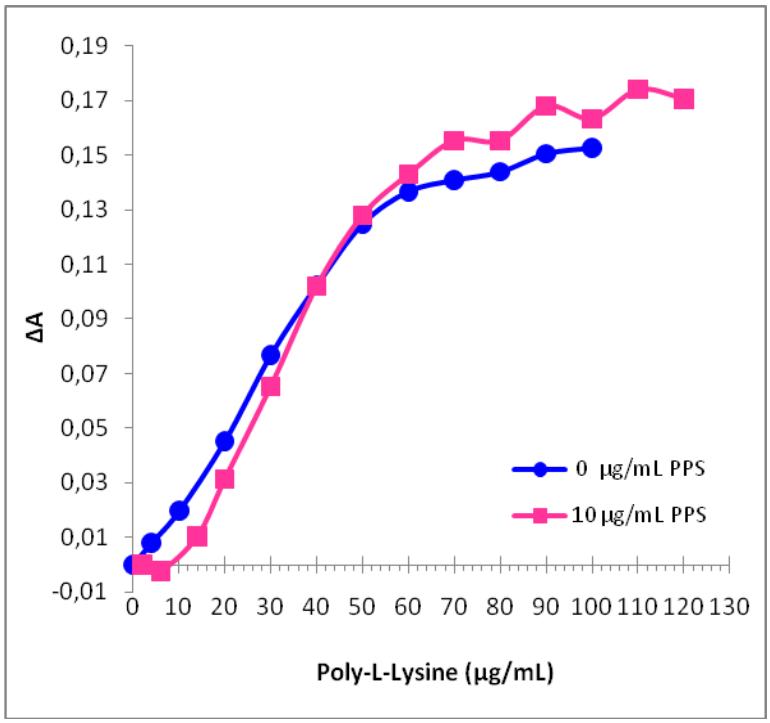


Figure 4. 34 Titration Curve of PPS by Poly-L-Lysine (Film 2)

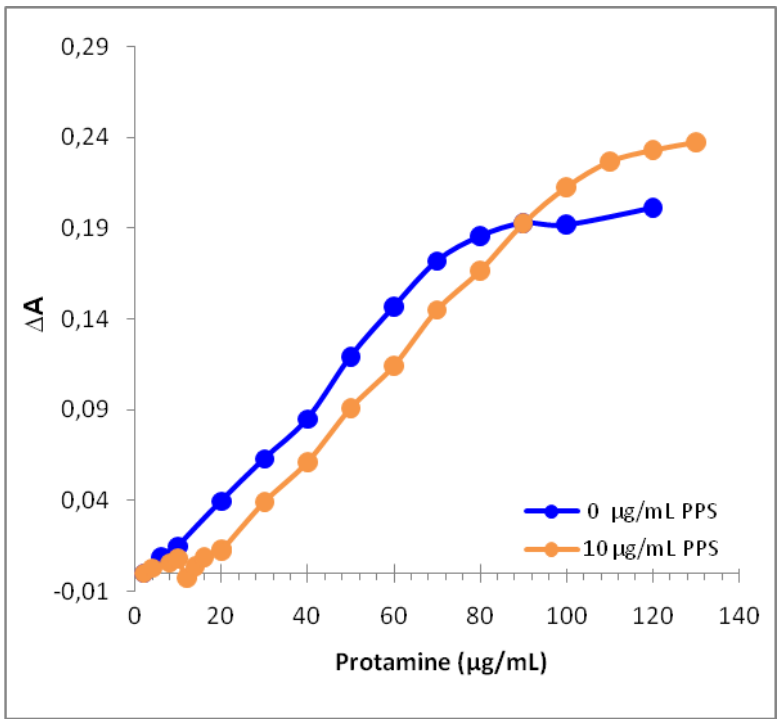


Figure 4. 35 Titration Curve of PPS by Protamine (Film 2)

4.7. Wavelength Scan of Protamine with Film 2 Type Optode

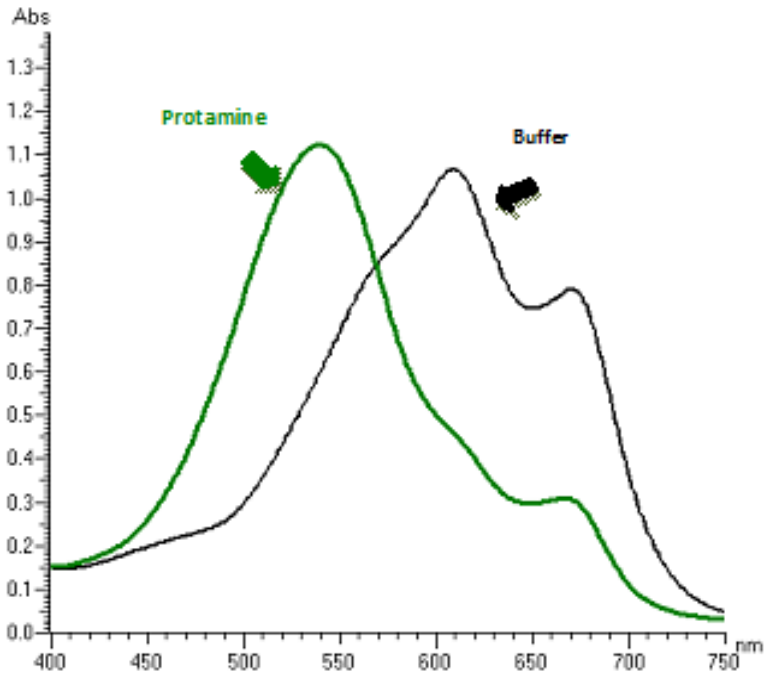


Figure 4. 36 The Wavelength Scan of Protamine (500 µg/mL)

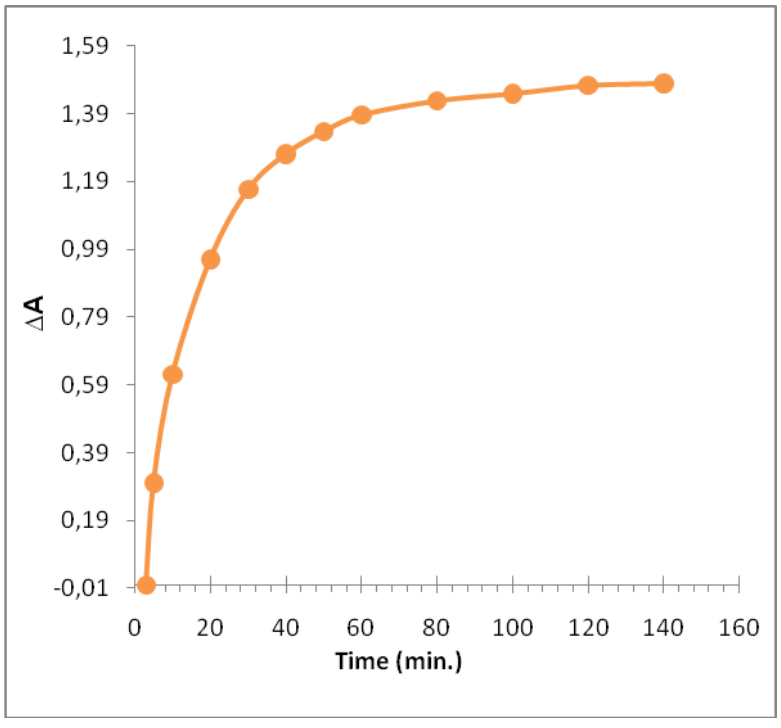


Figure 4. 37 Time-Absorbance Graph of Protamine (500 µg/mL)

Chapter 5 .

DISCUSSION

Multiple polyionic species can be frequently found at the various areas and a great number of those have meaningful roles. Pentosan polysulfate (PPS) ; one of the polyions which was selected as target in this work and is being used as drug in contemporary medicine is also a multiple charged polyion. Other polyions selected as target are multiple charged surfactants ; NaDS (sodium dodecyl sulfate), NaDBS (sodium dodecyl benzene sulfonate), PVSK (potassium polyvinyl sulfate), CDB (cetyldimethylbenzyl ammonium chloride), Zephiramine (Benzyldimethylteradecyl ammonium chloride) and CTAB (Cetyltrimethyl ammonium chloride).

Polymer-based micro optical sensors proposed in this study for the quantitative determination of the above-mentioned polyions and selected as targets are important analytical equipments which can find applications in the diverse areas such as clinical chemistry, biology, agriculture and environmental science. Polymeric materials recently draw attention progressively in the production of sensors as the imitative analogy of the natural sense organs. By using polymers instead of the classical sensor materials such as semi-conductive metaloxides, solid electrolytes, organic semi-conductors, the sensors

which can allow the more rapid measurements with better selectivity are being manufactured.

The coverage of this work is to prepare the films containing ionophore and/or chromoionophore which are carrying the properties of plasticized polymer-based optical sensors and sensitive to the targeted polyions and modify these films into the microplate formate and finally to determine the polyions selected by microtitration plate reader instrument in a simple, rapid and precise manner. After the determinations of standards in buffered solutions have been successfully managed, application was performed with some real substances.

This work not only involves in the determination of the quantitative determination of the analyte polyions but also in establishing the stoichiometries of their interactions between macro and multiple charged ions. The stoichiometric ratios between the complexes generated *in situ* during the reaction give valuable information about the polyions mentioned. For example, determination of some surfactants was also among our planned purposes as they (active gradients of detergents) were known environmental pollutants. In this regard, it can be considered that the determination of the reaction stoichiometries between these substances and counter-charged polyions can serve important hints

to the researchers dealing with the discarding of the surfactants from the environment, especially from the aquatic media, safely.

We are proposing two types of film sensor in this work. While the film-1 type sensor is obtained from a solution of the mixture of the defined ratios of a chromoionophore, one plasticizer, 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 plasticizer, and polyvinyl chloride in THF. This work is essentially consisted of the determinations carried out by film-1 type sensor, but a representative experiment has been performed to show the applicability of the film-2 type sensor for the determination of the one of the polyions (Figure 4. 34 - Figure 4. 35).

The titrations of each concentration 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. For example, 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 (Figure 4. 1 - Figure 4. 10). In addition, it can clearly be followed by the rational titration curves to what extent the minimum determination limits be reached (several ppm) (.Figure 4. 11 - Figure 4. 14)

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 4. 3 In some selected samples, in order to investigate whether the sample matrix would have an effect on the determination, by applying spike process (Figure 4. 23, Figure 4. 24).

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 a 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 compounds which may act as chromoionophore and in this regard, two dye compounds 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, in this work, it was shown that these four polyanionic analytes targeted can be quantitatively determined spectrophometrically in µg/mL levels by the two different type of polymer-based film sensor having different mechanisms. These film sensors proposed for the determination of the four polyanions

(PPS,NaDS, NaDBS, and PVSK) by microplate reader 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 medical appliances. Besides their widely known properties, polyionic surface active ingradients are the substances which find applications in textile, petroleum industry also in medicine. Therefore, to present the methods at which the determination of these type of substances by a reliable and rapid way will make an important contribution to the analytical chemistry literature.

The determination performed by adapting the film sensor into the microplate formate has advantages over the classical spectrophotometric methods. Taking account of being 96 wells of each microplate, in a very short time regardless, it is obviously clear that it is important advantage to carry out determinations such a short time. Furthermore, another advantage of this method, as the volumes of the wells in microplate are in microliter level, it will diminishes the cost of determination.

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APPENDICES

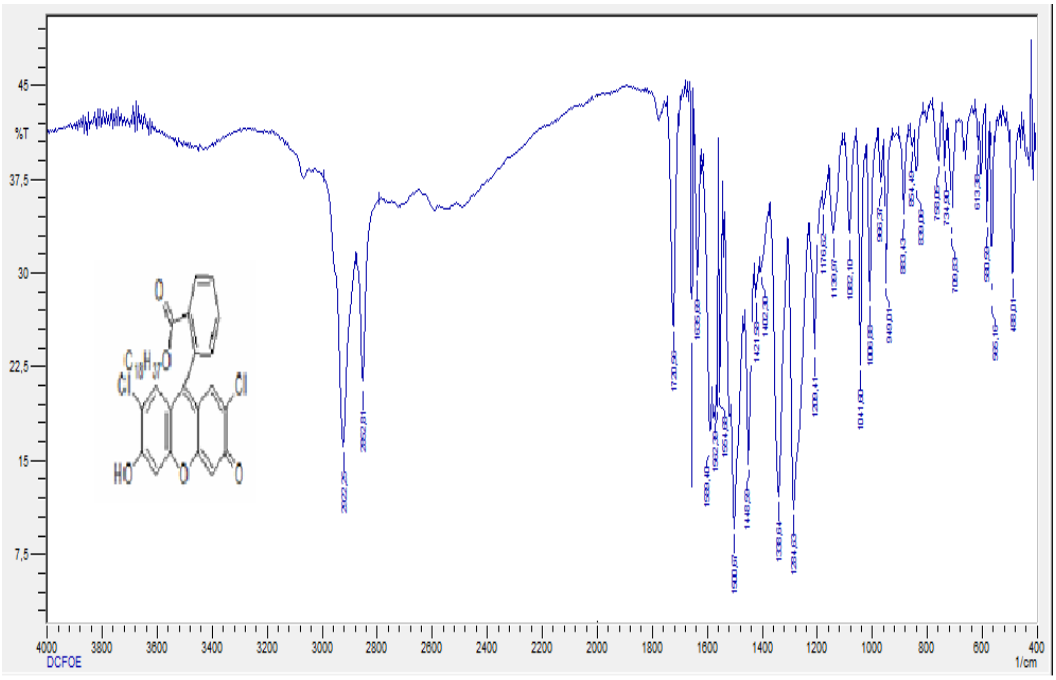

Figure 3. 2 IR Spectrum of 2', 7'-dichlorofluorescein octadecyl ester (DCFOE)

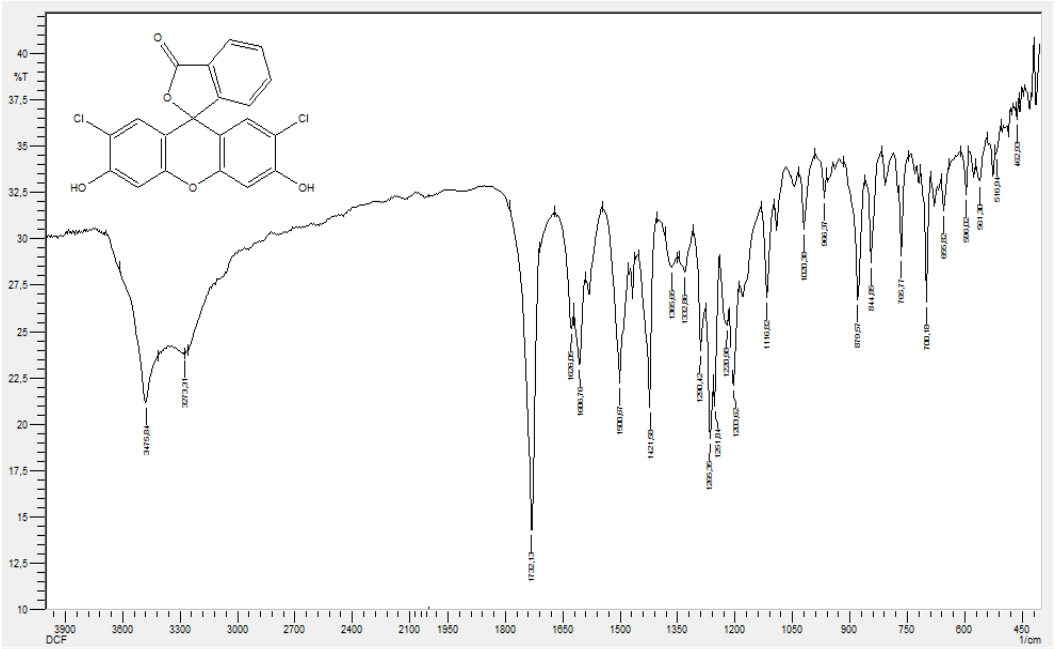

Figure 3. 3 IR Spectrum of 2', 7'-dichlorofluorescein

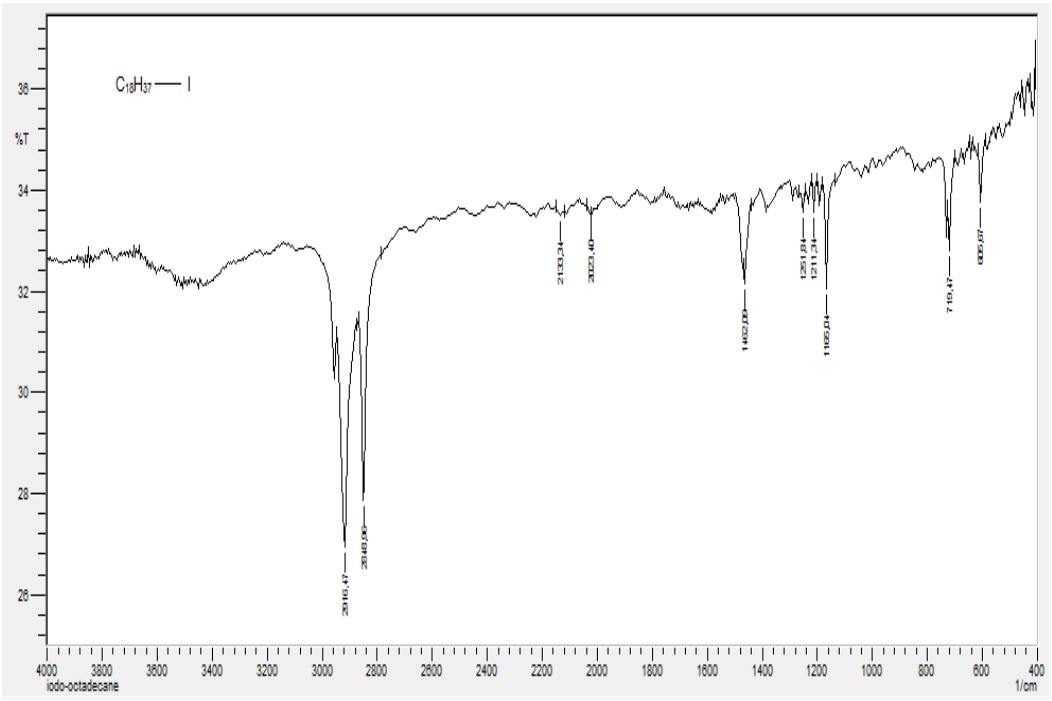

Figure 3. 4 IR Spectrum of iodooctadecane

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