Characterization of Detergent Residues on Glass Surfaces

by

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ABSTRACT

The problem of white spots on dishwasher-washed dishes has been receiving significant attention with increased health awareness of consumers. There is a need for characterization of such white spots on dishes. In this thesis, we focused on identifying the chemical components in these white spots by considering penta-Sodium triphosphate (STPP, $Na_5P_3O_{10}$) and tri-Sodium citrate dihydrate (sodium citrate, $Na_3C_6H_5O_7$), the most widely used components in detergent formulations. Spectroscopic and diffraction measurements have been carried out to analyze the dishwasher washed glass sample surfaces both qualitatively and quantitatively. For qualitative analysis, surfaces of the dishware were analyzed by Fourier transform infrared spectroscopy in the attenuated total reflectance mode (ATR-FTIR), X-ray photoelectron spectroscopy (XPS), and laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS). For quantitative analysis, UV-VIS-NIR absorption spectroscopy, Fourier transform infrared spectroscopy in the attenuated total reflectance mode (ATR-FTIR), Raman spectroscopy, laser ablation-inductively coupled plasma mass spectroscopy (LA-ICP-MS) were utilized to obtain quantitative calibration models for a range of STPP and sodium citrate concentrations (1 to 8% w/w) precipitated on glass surfaces. Then using these calibration curves, STPP and sodium citrate residues on the dishwasher-washed dishes were determined quantitatively by ATR-FTIR. In addition to these, a simple colorimetric analysis was also developed to detect residues of STPP and sodium citrate on dishwasher washed glass surfaces. Moreover, the hydrophilicity of these

chemicals was measured by contact angle measurements and the toxicity assays were performed to elucidate the influence of STPP and sodium citrate on human kidney cells viability. Cell viability results showed a decreasing trend in the number of cells cultured with increasing concentrations and exposure time of sodium tripolyphosphate and sodium citrate in the medium. Results of this study provide opportunities for quantifying the detergent residues on dishwasher-washed dishes and assess their possible toxicity on live cells.

ÖZET

Tüketicilerin sağlık bilincinin giderek artması sebebiyle, bulaşık makinasında yıkama sonrası bulaşıkların yüzeylerinde görünen beyaz lekeler daha da dikkat çeker hale geldi. Bu lekelerin nitelendirilmesine yönelik bir ihtiyaç doğdu. Bu doğrultuda bu tezde, bahsi geçen beyaz lekelerin bulaşık makinası deterjanlarında sıklıkla kullanılan bileşenler olan sodyum trifosfat ve sodyum sitrat göz önünde bulundurularak tahlil edilmesi amaçlanmıştır. Yıkama sonra bulaşıkların hem nitel hem de nicel yüzey analizi için spektrometrik ve difraktometrik yöntemler kullanılmıştır. Nitel analiz için, bulaşık yüzeyleri Fourier Dönüşümlü Kızıl Ötesi Spektrometresi (FT-IR), X Işını Fotoelektron Spektroskopisi (XPS) ve Endüktif Eşleşmiş Plazma Kütle Spektrometresi (ICP-MS) kullanılarak analiz edilmiştir. Nicel analiz için ise, Uv-Vis spektrofotometresi (UV-Vis), Fourier Dönüşümlü Kızıl Ötesi Spektrometresi (FT-IR), Raman Spektrometresi ve Lazer Aşındırmalı Endüktif Eşleşmiş Plazma Kütle Spektrometresi (ICP-MS) kullanılmıştır. Bu analizlerden farklı olarak, bir de sadece kolorimetrik bir analize dayanan kalıntı tespit yöntemi geliştirdik. Değme açısı ölçümü yapılarak bahsi geçen iki komponent hidrofobisiteleri karşılaştırılmasına ek olarak, bu komoponentlerin insan böbrek hücreleri üzerindeki toksik etkisi hücre canlılık testleri ile belirlenmiştir. Hücrelerin maruz kaldığı sıvıların derişimi ve maruz kaldıkları süre artıkça hücrelerin canlılık faaliyetleri azalma yapılan canlılık testleri ile gözlemlenmiştir. Bu çalışmanın sonuçları, bulaşık makinasında yıkama sonrası deterjan kalıntı miktarının

belirlenmesi sağlamakla kalmayıp, kalıntıların canlı hücreler üzerindeki toksik etkilerini gözlemlemeye de olanak sağlamıştır.

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ABBREVIATIONS

FDA	Food and Drug Administration
BSA	Bovine serum albumin
HEK	Human endothelial kidney cell
UV	Ultraviolet
DMEM	Dulbecco's modified eagle medium
FBS	Fetal Bovine Serum
EDTA	Ethylenediaminetetraacetic acid
PBS	Phosphate buffered saline
STPP	penta-Sodium triphosphate
Na-C	tri-Sodium Citrate dihydrate
ABS	Alkyl benzene sulfonates
LAS	Linear alkyl benzene sulfonates
AE	Alcohol ethoxylate (AE),
APE	Alkylphenol ethoxylate
MEE	Methyl ester ethoxylate
APG	Alkyl polyglycoside
IDS	Iminodisuccinate
AE	Alcohol ethoxylates
NTA	Nitrilotriacetic acid
ODA	Disodium 3-oxapentanedioate
IDA	Iminodisuccinic acid

Chapter 1

INTRODUCTION

A detergent is an effective cleaning product containing many components. A dishwasher detergent consists six groups of substances: surfactants, builders, enzymes, bleaching agents, fillers and other minor additives, such as dispersing agents, fabric softening clay, dye-transfer inhibiting ingredient, and optical brighteners. Surfactants and builders are the most frequently used components in detergent compositions. While surfactants remove soil by lowering the surface tension to enable the cleaning solution to wet a surface, builders increase or maintain the cleaning efficiency of the surfactant by reducing water hardness (high amount of Ca^{2+} and Mg^{2+}). Penta-sodium triphosphate (STPP, $Na_5P_3O_{10}$) and tri-sodium citrate dihydrate (Na-C, $Na_3C_6H_5O_7$) are the most common types of builders. Sodium tripolyphosphate (STPP, $Na_5P_3O_{10}$), produced by the neutralization of phosphoric acid with sodium carbonate, is utilized commonly in a variety of cleaning products, foods, animal feeds, industrial cleaning processes, and ceramic industry.[1] From 1947 until late eighties, STPP was used almost exclusively as a complexing agent (builder) in detergents due to its multifunctional contribution to washing and cleaning processes. STPP provides lots of benefits, such as sequestration of hard water enabling surfactants to perform effectively, pH

buffering, soil emulsification, and prevention of deposition. Surfactants are deactivated by Mg^{2+} and Ca^{2+} in hard water. Highly charged chelating agent STPP binds to cations tightly and prevents them from interfering with surfactant's function. Today, the total consumption of STPP was approximately estimated as 300 000 tons in Western Europe in 2000.[2-4]

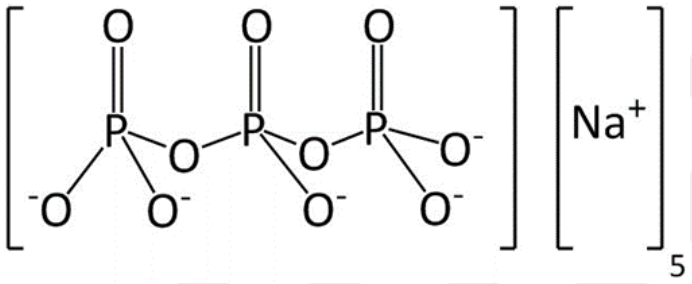


Figure 1.1. Skeletal model of penta-Sodium triphosphate

STPP in dishwasher and laundry detergent use contributes to about 50% of soluble (bioavailable) phosphorus in municipal wastewater. The use of phosphate based detergents have caused eutrophication, excessive plant, and algal growth due to the increased availability of one or more limiting growth factors needed for photosynthesis. Therefore, several countries including the USA, Japan, and some EU member states aim to reduce phosphorus loads, particularly from urban and industrial point sources. The use of STPP based detergents has been banned in EU via introduction of laws or voluntary agreements to change it to other builder types, such as zeolite A and tri-sodium citrate dehydrate (sodium citrate, $Na_3C_6H_5O_7$) for household dishwasher detergents.[5, 6] Among these alternatives, sodium citrate is a non-toxic, neutral tribasic salt of citric acid. It is produced by complete

neutralization of citric acid with high purity sodium hydroxide or carbonate and subsequent crystallization.[7] Like STPP, Trisodium citrate dihydrate (sodium citrate) is also widely used in foods, beverages, and various technical applications mainly as buffering, sequestering, or emulsifying agent. Sodium citrate is a non-toxic and neutral salt.[8] The detergent-building and rapid biodegradability characteristics of sodium citrate enable its use as an environmentally acceptable phosphate substitute in a large variety of household cleaning products.[9] However, consumer complaints were noted about the presence of residue and haze on dishes and inside the dishwasher, after polluting phosphates were banned from dishwasher detergents and replaced with sodium citrate a few years ago.[10] This report suggests that detergent builder could result in the appearance of white spots on dishwasher-washed dishes. Researchers have already studied the origin and content of these white spots. Most of them focused on glass surface damages induced by dishwashing detergents in long term dishwashing process.[11] In addition, very few research papers reported the residual characteristic of different detergent components by mimicking the dishwasher working mechanism. For instance, Pan *et al.* showed that alkylphenols, one of the component in Taiwan detergent formulations, was estimated between 1.71×10^{-5} to 2.13×10^{-3} (alkylphenols/detergent, mg/g) and they found that temperature was the only significant factor for residual characteristics.[12] Moreover, Tsai *et al.* analyzed a variety of detergents to determine the amount of anti-bacterial agent Tricolasan in Taiwan detergents. They also aimed to determine different factors that would affect residual characteristic of Tricolasan

on dishware.[13] These studies are especially important as the remnants of detergent on dishes can lead to the accumulation of a crucial level of these chemicals in consumer's body in the long run. Thus, these components remained on the surfaces of dishes are needed to be quantified to be able to improve the washing cycle to reduce the residue concentrations much below the acceptable levels. Moreover, such acceptable levels should also be determined. The amount of detergent builder residue on dishwasher washed dishes have not been determined quantitatively yet. In this thesis, we introduce two different methods which enable the measurement of residue on dishes after dishwasher. While one of these methods is mainly based on surface characterization measurements, the other one requires only colorimetric analysis. In the first method, qualitative analysis of dishwasher washed surfaces was performed by spectroscopic and diffraction measurements. Then, by designing calibration models of different characterization techniques, unknown detergent builder amount on the dishwasher washed dishes was determined. In a second method, simple new test kit was designed to detect detergent residue on glass surfaces by considering the chemical structure of builders in standard detergents and their colour change reactions.

In this thesis, Chapter 2 provides an overview historical development of main detergent components. This chapter includes recent advances in detergent formulations. Also, it presents studies about surface analysis of dishwasher washed dishes.

Chapter 3 presents materials and methods used in experimental part of this thesis. In the beginning of Chapter 3, preparation of experiment for qualitative and quantitative surface analysis methods were mentioned. Different characterization techniques were applied for both detergent analysis and surface analysis of dishwasher washed dishes. In the last part of this chapter, the procedure for simple colorimetric analysis was explained.

Following Chapter 4 with results and discussion, this thesis ends with Chapter 5 by summarizing conclusion.

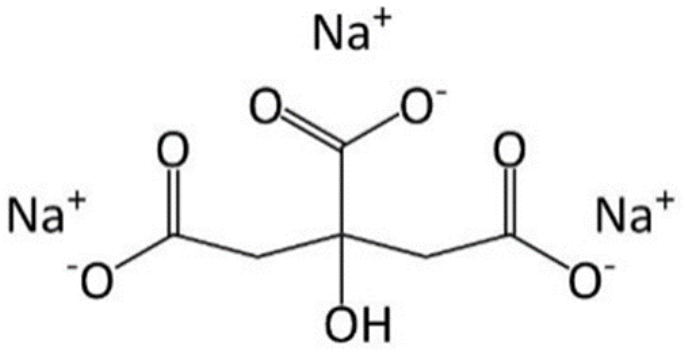


Figure 1.2. Skeletal model of sodium citrate

Chapter 2

LITERATURE REVIEW

2.1. Detergents

A detergent is an effective cleaning product containing many components. Because of their chemical structure, the surfactants used in detergents can be engineered to perform well under a variety of conditions. A dishwasher detergent consists six groups of substances: surfactants, builders, enzymes, bleaching agents, fillers and other minor additives, such as dispersing agents, fabric softening clay, dye-transfer inhibiting ingredient, and optical brighteners. Over half of surfactants is used in dishwasher and laundry detergents and, household and personal-care products. Therefore, the development of related chemical industry and chemical engineering which involve synthesis and production of surfactants and polymer builders become extremely important to satisfy the demand of the detergent industry.[14] This chapter will introduce the surfactants and builders frequently used in detergent compositions and discuss their development in the past, present, and future.

2.1.1. Surfactants

2.1.1.1 History of Surfactants

Surfactants play an essential role in our daily lives. By safely and effectively removing soils, they help us to stay healthy. The first surfactant was soap, a salt of a fatty acid, containing at least eight carbon atoms or a mixture of such salts.[15] Its origin dates back to prehistoric times. The excavation of ancient Babylon shows that soap making was known as early as 2800 B.C. They made soap by boiling fats with ashes. Soap got its name from an ancient Roman legend, Mount of Sapo. The chemistry of soap was a mystery until Michel Eugene Chevreul discovered the chemical nature and relationship of fats, glycerine and fatty acid. After this discovery, soap making becomes the fastest growing industry in America by 1850. Soap was the only choice of surfactant until the 20^{th} century. Detergent surfactants were developed in response to a shortage of animal and vegetable fats and oils during World War I and II. One of the main milestones in the development of detergents was the discovery of the first "built" detergent (containing a surfactant/builder combination) in 1946. Since those discoveries in detergent and builder chemistry, researchers and the industry have focused on developing cleaning products that are efficient and easy to use, as well as being safe for consumers and for the environment.[16]

2.1.1.2 Chemistry of Surfactants

Surfactant is an abbreviation for surface active agent, are organic chemicals that change the properties of water.[14] They contain both a hydrophilic group, and a hydrophobic group. Therefore, they can lower the surface tension to enable the cleaning solution to wet a surface. They also suspend oils and dirt, and so allow their removal. In working mechanism, hydrophilic (water loving) part stays in water and molecules of the water-insoluble material aggregate near the oil (Figure 2.1).

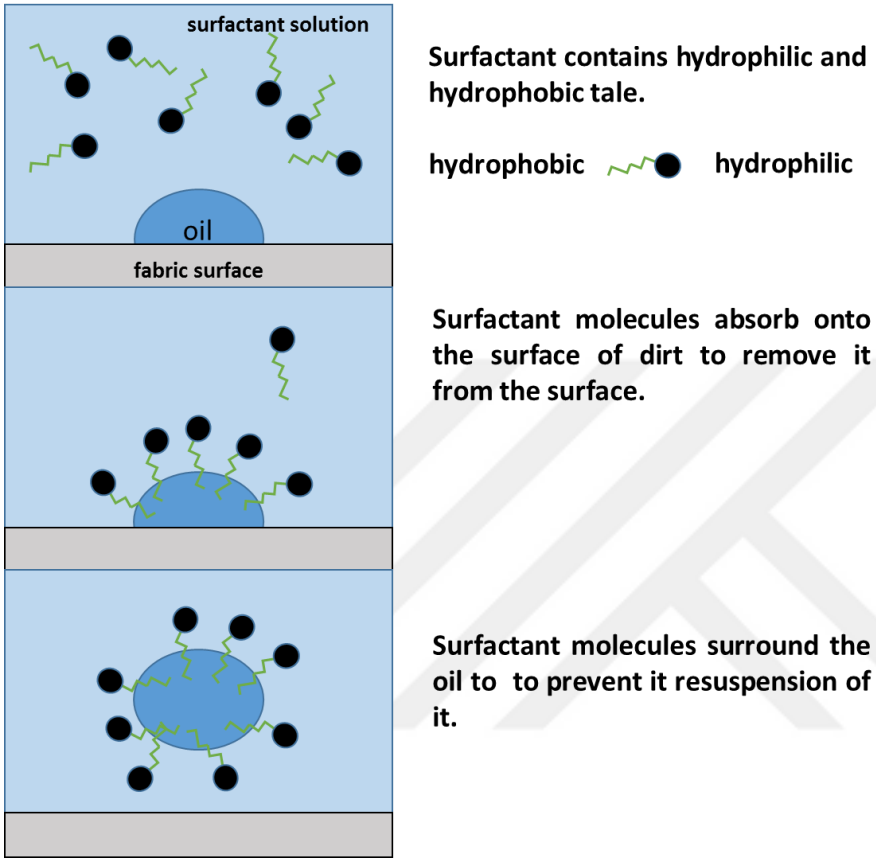


Figure 2.1. Action of a surfactant

Surfactants comprise from 15 to 40% of the total detergent formulation.[17] They can be classified into four groups according their polar heads: anionics, nonionics, cationics, and zwitterionics. It is illustrated in Figure 2.2. Nowadays, a certain combination of different types of surfactants is used in detergent formulation to increase their cleaning performance.

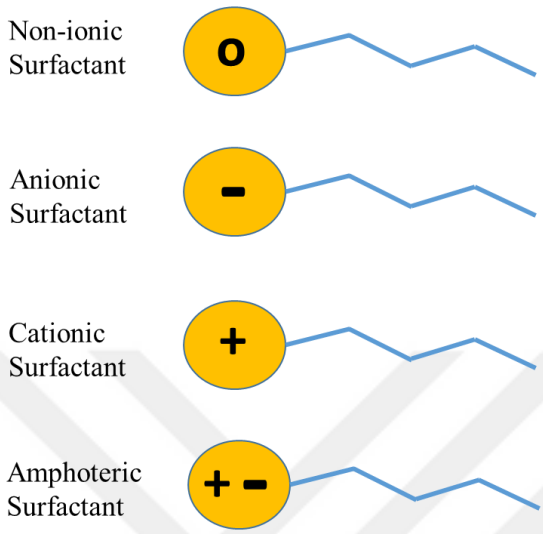


Figure 2.2. Types of surfactants

2.1.1.3 Types of Surfactant

2.1.1.3.1. Anionic Surfactants

Anionic surfactants are most widely used surfactant types due to their ease and low cost production.[17] They ionize (are converted to electrically charged particles) in solution, carry a negative properties. Linear alkyl benzene sulfonate, alcohol ethoxysulfates, alkyl sulfates, and soap are the most common anionic surfactants. Sodium, potassium, lithium, ammonium, and alkyl ammonium, especially sodium are preferred as counter ions of the anionic surfactants. Soap is an anionic surfactant and shows excellent performance under the appropriate conditions. However, it is sensitive to hard water and does not work well at lower temperatures, including cold water.[14]

Therefore, researchers have developed synthetic anionic surfactants to overcome these shortcomings. Natural alkyl sulfates were first introduced in laundry detergents around 1932. Then, instead of natural alkyl sulfates, branched-chain alkyl benzene sulfonates (ABS) started to be used in detergent compositions. However, microbes could not break down ABS and thus they left foam in river water. Therefore, linear alkyl benzene sulfonates (LAS) such as sodium dodecybenzene sulfonate and sodium xylenesulfonate were preferred instead of it due to its biodegradability. In today's market, LAS is widely used low-cost surfactant with combination alkyl sulfates (AS).[18]

2.1.1.3.2. Nonionic Surfactants

Nonionic surfactants have been extensively used in the area of the laundry detergents, automatic dishwasher detergent and personal-care formulations. They are low foaming and resistant to hard water since no precipitation occurs in the presence of divalent ions. Linear alcohol ethoxylates are the most common type of nonionic surfactant. They are derived from either petrochemical raw materials or natural resources. Alcohol ethoxylate (AE), alkylphenol ethoxylate (APE), methyl ester ethoxylate (MEE), ethoxylated amine, ethoxylated amide, alkyl polyglycoside (APG), polyethylene oxide-polyalkylene oxide diblock copolymer, *etc*. are the other examples of nonionic surfactants.[14] Although, APEs, such as nonylphenol ethoxylates deterge soils better than alcohol ethoxylates (AE), the metabolic products resulting from the degradation process do not readily degrade further and may have undesirable side effects on aquatic life.[19]

2.1.1.3.3. Cationic Surfactants

Cationic surfactants are used in fabric-softening laundry detergents. They are used as a common fabric softener and some of them are ingredient in some household cleaners to disinfect or sanitize. It works by reducing the friction between fibers, and between fibers and the skin.[20] They ionize in solution and have a positive charge. Quaternary ammonium compounds (quatz) are the principal cationics.[16] Other than water softener and disinfection

properties, the studies show that addition of ethoxylated quats to floor and stone cleaners enhances the spreading and drying abilities. They also increase the lime soap dispersing and emulsification properties. This made the cleaners more effective on slip resistant surfaces, leading to a reduced time consumption for washing those floors and stones.[21] Moreover, it has been also found that combination of quat salt and low levels of iminodisuccinate (IDS) in detergent formulation improve soil and stain removal in conjunction with decreased fading of dyes on colored fabrics.[22]

2.1.1.3.4. Amphoteric Surfactants

Amphoteric surfactants are mild, foaming and stable. Therefore, they are used in personal cleansing and household cleaning products.[16] They could be nonionic (no charge), anionic (negatively charged) or cationic (positively charged) in solution, depending on the pH of the solution. Sulfobetaines and betaines are the major amphoterics.[23]

Betaines are better than alcohol ethoxylate in detergency on oily soils and biodegradability. Their cleaning performance increases when they are used with nonionic surfactants. They can be used as a thickener in detergent formulation. They are excellent temperature and pH stable, and improve the mildness on the skin. Therefore, they are suitable for washing skin and hair. Furthermore, incorporation of betaine, sultaine or preferably their mixture to detergent containing anionic surfactant can be used as foam booster in the soap.[24]

In order to optimize the performance of detergents, various surfactants are used in combination. A combination surfactant system usually shows a better detergency performance than single-surfactant.[25]

2.1.2. Detergency Builders

Hard water reduces greatly the surfactant efficiency. Sometimes, surfactant does not exhibit efficient performance even in softer water.[14] Builders increase or maintain the cleaning efficiency of the surfactant by reducing water hardness (high amount of Ca^{2+} and Mg^{2+}). This is done either by sequestration or chelation (holding hardness minerals in solution), by precipitation (forming an insoluble substance), or by ion exchange (trading electrically charged particles).[16] Figure 2.3 represents the working mechanism of a builder. In addition, due to co-toxicity of surfactant, builders are used in detergent composition to decrease the amount of surfactant. They are also used for their sequestering ability, alkalinity, buffer capacity, bleach compatibility.

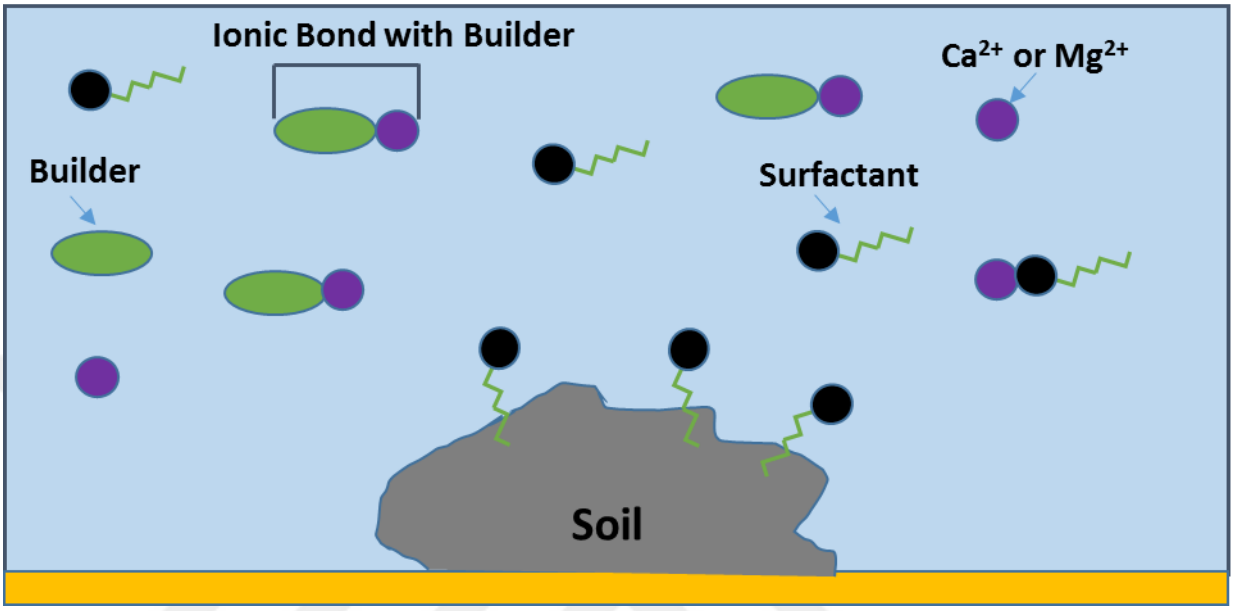


Figure 2.3. Action of a builder

2.1.2.1. Inorganic Builders

2.1.2.1.1 penta-Sodium triphosphate (STPP)

Sodium tripolyphosphate (STPP) was the most widely used builder in the past due to its excellent performance and multifunction property as a builder. It primarily remove hard ions like the Ca^{2+} and Mg^{2+} and it facilitates dissolution of detergents, maintains alkalinity during washing, soil emulsification, prevention of deposition. STPP can perform efficiently under all washing conditions.[14] From 1947 until the late eighties STPP was used almost exclusively as a complexing agent ("builder") in detergents due to its multifunctional

contribution to washing and cleaning processes. Today, the total consumption of STPP was estimated to be approximately 300 000 tones in Western Europe in 2000. [4]

However, phosphates cause excessive plant, algae, bacteria, and other flora growth in rivers, lakes, and oceans due to the increased availability of one or more limiting growth factors needed for photosynthesis. This exhausts the oxygen supply both in the surface and in the bottom layers of water bodies, and killing fish. This phenomenon is called eutrophication.[6]

STPP in household detergent contributes up to 50% of soluble (bioavailable) phosphorus in municipal wastewater. Although sewage treatment plants could eliminate 80 to 95% of all phosphorus, the process is very costly to immediate action and general application.[26] To overcome this problem, USA, Japan, and some EU member countries limit or ban the use of STPP based detergents in the EU via introduction of laws or voluntary agreements to change it to other builder's types. As a consequence, sodium carbonate, sodium silicate, and ethylenediaminetetraacetic acid (EDTA) were started to be used as a substitute for STPP.

2.1.2.1.2 Borates

Borates are another group of common constituent of many types of detergents. Although it is originally used to bleach hydrogen peroxide, it is also included in detergent formulation as a builder. Researches compared the performances of borate with STPP and sodium carbonate and they showed that it has similar performance with STPP and sodium carbonate

except the Ca^{2+} capturing capacity. Ca^{2+} sequestration performance of borate is weaker than the other common builder types.[26] Borax solution has a pH of approximately 9.13 at 313 K which is in the optimum range for detergency. The experiment also demonstrated that borate exhibits better detergency performance of pigment and oily soils in hard water than carbonate did.

2.1.2.1.3 Zeolites

Zeolites are started to be used in phosphate-free detergents, necessarily in conjunction with other builders such as polycarboxylates or 41itrile triacetic acid, EDTA, and sodium carbonate. The studies show that zeolites are nontoxic and have no adverse effects on the environment like eutrophication. Therefore, they have been used as a substitute for STPP to satisfy growing public sensitivity to environmental issues.[27] However, the use of zeolites increases suspended solids and may cause fouling of pipeline. Therefore, it makes the disposal of sludge more difficult. In addition, it sometimes trapped the surfactant and this causes a time delay in diffusion of surfactant into the wash liquor. Incorporation of other builders like sodium carbonate or sodium citrate is a possible solution for this problem.

2.1.2.2. Organic Builders

Due to adverse environmental effect of some inorganic builders, organic builders become potential alternative to overcome this problem. Sodium citrate (Na-C), nitrilotriacetic acid

(NTA), disodium 3-oxapentanedioate (ODA), iminodisuccinic acid (IDA), and, ethylenediaminetetraacetic acid (EDTA) are several examples of organic builders.

2.1.2.2.1. tri-Sodium Citrate Dihydrate

Sodium citrate has been used as a builder in some commercial P-free detergents.[14] It is fully biodegradable and has no adverse effect on environment, its cost is high and its sequestering power is not well. As sodium citrate is generally recognized as safe, there is no limit on dosage of intake.[28] However, it is still recommended consumers to consult professionals before using large quantity of sodium citrate for long period, because the possible side effects of high amount usage of sodium citrate are not known. It could have some adverse effects on human health. For instance, US. National Library of Medicine reported that excess amount of exposure to sodium citrate may cause tetany or depress the heart by decreasing the ionized calcium level of the blood.

2.1.2.2.2 Other Organic Builders

Toxicity level of nitrilotriacetic acid (NTA) are 10^5 times higher than exposure levels to NTA. Therefore, it does not cause skin or eye irritation. However, in production of NTA, high toxic chemicals are used and so heavy metal transmission can be occurred. Therefore, it is banned in USA and Switzerland.[29] EDTA has similar adverse effect and it is not well biodegradable. Therefore, both of them are not listed in detergents eligible for the European Union Eco-label.

2.1.3. Other Detergent Ingredients

Detergent formulations composed of many ingredient with different functions other than surfactants and builder. Here are the several ingredients in the detergent composition;

Abrasives: They supply smoothing, scrubbing, and polishing action.

Acids: They balance the alkalinity.

Anti-microbial agents: They inhibit the growth of bacteria and other microorganisms.

Bleaches: They help to remove soils and they whiten and brighten.

Colorants: They provide special color to products.

Enzymes: They help to degrade the soils containing protein.

Foam Control Agent: They optimize the foaming.

Surfactants and builders are two main ingredients in detergent formulations. Studies have focused on the development of surfactants and builders to improve the cleaning efficiency and sequestering capacity without causing adverse effects on human health and environment. The chemical industry technology will continue to develop low-cost and highly efficient surfactants and builders for use in detergents.[16]

In order to address the importance of detergent formulations in dishwasher usage, Pan and Tsai were determined concentrations of alkyphenols in dishwasher detergents. They also applied Taguchi experimental design to assess the possible factors that might affect the residual characteristic of alkylphenols on dishwasher washed dishes. The results indicated that while cleaning temperature was found as only significant factor for 4-tert-octyphenol residue on dishware, detergent concentration has an effect on 4-nonyphenols, technical nonyphenols residue amount. [12]

In addition to this study, Tsai et al. determined anti-bacterial agent (triclosan) concentrations of Taiwan dishwasher detergents. They also evaluate the factors, that including the concentrations of detergents used, the temperature and immersion time for water before the cleaning processes, the temperatures of water used for the cleaning processes, and the materials of dishware made of, might affect the residual characteristic of triclosan on dishware. Contrary to their previous study, the material type was found as significant factor that affects the triclosan residue on dishware.[13] Other than this study, to determine the Na_4EDTA concentrations of detergent solutions, Suarez et al. designed calibration and prediction models by utilizing ATR-FT-IR. Moreover, they compared the results of HPLC and UV absorbance with ATR-FT-IR. The results show that ATR-FT-IR can be used as a rapid and simple method to quantify Na_4EDTA in detergent solutions as an alternative to HPLC and UV absorbance. [30]

Glass scientists have also investigated the milky effects occurred on dishes washed in dishwasher. Petruskova et al. presents the results of controlled dishwashing conditions in studying the iridescence and milky effect created on the glass products. Eventually, foreign components were detected on the outer side of washed damaged glass surface. [11]

All of these previous studies demonstrated that altered techniques might be applied to determine the components of dishwasher detergents and dishwasher washing process can cause accumulation of detergent components on dishwasher washed dishes. In this thesis, the results of comprehensive characterization of dishwasher-washed dishes' surfaces by surface characterization methods and simple colorimetric analysis for both STPP and sodium citrate were explained. Characterization and quantification of remnants on surfaces of dishes would be significant to improve dish-washer cycles and to reduce the amount of residues below acceptable levels. Moreover, the acceptable levels are not known precisely and they should also be determined. Here, we introduce a simple method that allows for the measurement of such residues on surfaces and their quantification for the first time in the literature.

Chapter 3

EXPERIMENTAL PART

3.1 Materials

STPP and sodium citrate were purchased from Merck Chemical. Ethanol (99.8%) were purchased from Sigma–Aldrich. Standard detergents (Europe B, Europe D, Australia, and America) were provided by Arçelik. Phosphate-buffered saline (PBS) tablets were purchased from Amresco (Solon, Ohio). Inc. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS, heat inactivated) were obtained from Gibco. L-glutamine (200 mM), penicillin-streptomycin, (stabilized, 10,000 units penicillin and 10 mg streptomycin/mL, penn-strep) and trypsin-EDTA (25%) were purchased from Sigma. The CellTiter-Glo Luminescent Cell Viability Assay was obtained from Promega and slide-a-lyzer dialysis cassettes (MWCO 3500) were purchased from ThermoScientific. 96 well plates and tissue culture dishes were purchased from Greiner Bio-one.

3.2. Methods

3.2.1. Sample Preparation

3.2.1.1. Preparation of Hand-Washed Samples

To demonstrate the effect of increasing rinsing cycle on residue characteristics of detergent, an experimental procedure was designed to mimic the dishwasher washing process. It is presented in Figure 3.1.

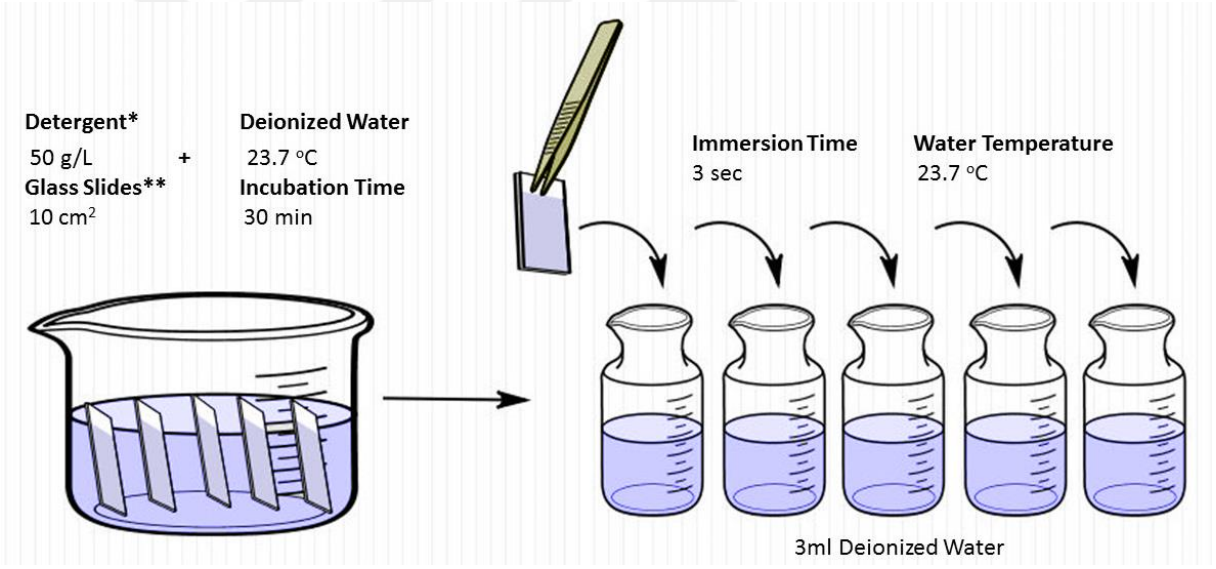


Figure 3.1. Preparation of hand-washed samples by mimicking dishwasher washing process. (*: detergent sample diluted to 50 g/l with deionized water; **: cutted microscope slides

Figure 3.1. illustrates that detergent solution with a concentration of 50 g/l was prepared. Glass samples were incubated in this solution for 30 min. Then the glass samples were rinsed for three seconds in 5 ml distilled water.

3.2.1.2. Preparation of Dishwasher-Washed Sample

Glass samples (cutted microscope slides) were washed in Arçelik dishwasher in economic program at 50 ˚C. The glass samples were placed ten different points in dishwasher. The time was 165 min for total cycle of washing and drying. Dishwasher washed glass samples were prepared by washing them with five different detergents in a dishwasher including commercial detergent, American, Australian and European Type B and Type D.

Table 3.1. shows the detergent formulations containing variety of different components. Due to difficulty in detecting all the component on the surface, the builders were selected as indicator compounds in detection of detergent residue. In other words, if a builder residue will be observed in a spectra, this will be assumed as detergent residue.

Table 3.1. Ingredient list of standard detergents

Europe Detergent Ingredients IEC- B		
Chemical Component	**W%**	
Sodium Citrate Dihydrate	30	Builder
Maleic Acid/ Acrylic Acid Copolymer Na	NA	
Sodium Perborate Monohydrate	5	
Tetraacetyl Ethylenediamine	2	
Sodium Disilicate	25	Builder
ILnear Fatty Acid Ethoxylate	2	
Protease	0.5	
Amylase	0.5	
Sodium Carbonate	NA	

Europe Detergent Ingredients IEC- D		
Chemical Component	**W%**	
Sodium Citrate Dihydrate	30	Builder
Maleic Acid/ Acrylic Acid Copolymer Na	12	
Sodium Percarbonate	7	
Tetraacetyl Ethylenediamine	2	
SodiumDisilicate	10	Builder
Linear fatty acid ethoxylate	2	
Protease	1	
Amylase	0.5	
sodium carbonate	35.5	

Australian Standard Detergent Ingredients		
Chemical Component	**W%**	
Sodium Tripolyphosphate	50	Builder
Sodium Metasilicate	40	Builder
Sodium Sulphate	5.8	Builder
Sodium Dichloroisocyanurate-dihydrate	2.2	low toxicity
Teric 164	2	

American Standard Detergent Ingredients		
Chemical Component	**W%**	
sodium silicate	Builder	
sodium sulphate	Builder	
Sodium carbonate	Builder	
NA		
NA		

3.2.1.3. Preparation of Sample for Designing Calibration Models

After qualitative surface analysis of dishwasher washed samples was performed, an experimental procedure including the design of different calibration curves were prepared to determine the amount of detergent residue precipitated upon dishwashing.

As shown in Figure 3.2., STTP and sodium citrate solutions were prepared from 1 to 8 wt% firstly. Then all solutions were mixed for 45 s by a vortex mixer. Prepared solutions were dropped on a clean glass surface and dried in the oven according to measurement method. Measurements were carried out with a variety of characterization equipment.

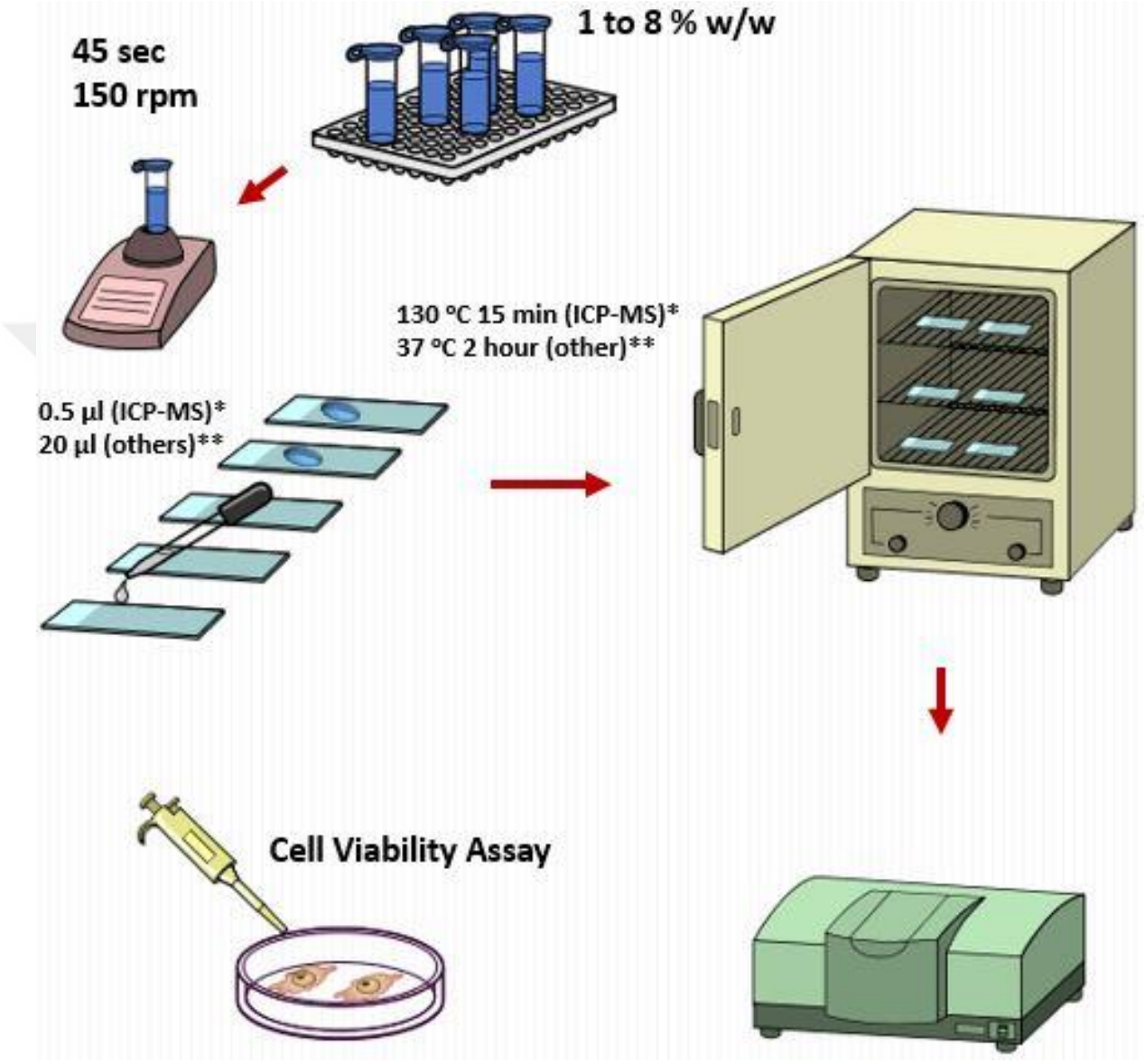


Figure 3.2. Schematic illustration of sample preparation to obtain calibration curves

3.2.1.4 Simple Method Development for Surface Analysis

This part of the study was performed to meet the expectations of Arçelik. They demand to determine residue on dishwasher washed surface by designing a simple colorimetric method. Colorimetric method was developed by considering the builders of standard detergents (Europe B, Europe D, Australia and America). According to this method, dishwasher washed samples were dipped into a deionized water cap after dishwashing process, then this water was further used for colorimetric analysis. In other words, the residue on dishwasher washed glass surface was aimed to be solved in deionized water and new solution containing residue of detergent was analyzed by colorimetric method. The color change and its intensity show the concentrations of residue in solution. Standard detergents and methods recommended or designed are listed below.

American Standard Detergent

As shown in the figure 4.1, sodium sulfate and sodium carbonate are the builders of American Standard Detergent. To detect sodium sulfate at low concentrations, the color change reactions of sodium sulfate were investigated and a sulfate test kit was recommended to analyze the residue of them on glass surface after dishwashing.

Australian Standard Detergent

STPP is the builder of Australian Standard Detergent (Figure 4.1.). Therefore, it was selected as indicator compound for colorimetric detergent residue analysis. Like in the

sodium sulfate residue analysis, the color change reaction was found in the literature and a phosphate test kit available in market was recommended as a simple colorimetric analysis.[31]

European B and D Standard Detergents

European B and European D standard detergents contain common builder type, sodium citrate as listed in Figure 4.1. The color change reactions of sodium citrate was investigated at first. However, no reaction was found in the literature. In contrast to other two type builders, a citrate test kit is not available in the literature. Therefore, there is a need for developing new test kit for sodium citrate. After a deep literature review, it was found that citrate ions could react with iron ions, which will change the color of solution. Accordingly, the reaction between sodium citrate and iron (III) chloride hexahydrate was performed for different concentrations of sodium citrate. Finally, observable results was supported by designed UV-Vis and FT-IR calibration curves.

3.2.2. Characterization Techniques

The analysis of detergent itself was the first step. By analyzing detergent, the ingredient list of different detergent types were obtained and this makes an important contribution in detecting the residues of detergent on surfaces. In detergent analysis, five different detergent types including commercial, Europe B, Europe D, Australia, and America were characterized. To analyze detergent, three different methods were performed including ATR-

FTIR, XRF and XRD. After detergent analysis, the glass surface samples were analyzed qualitatively by ATR-FT-IR, XPS and ICP-MS. To analyze the glass surfaces washed in dishwasher, calibration models were obtained by UV-vis spectroscopy, Raman spectroscopy, ATR-FTIR spectroscopy and ICP-MS. In addition, for simple colorimetric analysis, UV-vis and ATR-FTIR spectroscopy were utilized.

3.2.2.1 ATR-FTIR Spectroscopy

IR absorbance of different detergents were compared by using ATR-FTIR spectroscopy. Fourier transform infrared spectroscopy in the attenuated total reflectance mode (ATR-FTIR) spectra were recorded using a Thermo Scientific Nicolet iS50 spectrometer. OMNIC 8.1 (Thermo FisherScientific Inc.) software analytical procedures were used to convert the spectra from ATR to absorbance and to perform additional analysis such as peak resolution. The spectrum was collected as an average of 64 scan, each at 4 cm^{-1} resolution between 400 cm^{-1} to 4000 cm^{-1} wavenumber. Infrared (IR) spectroscopy is substantially based on the infrared light absorbed by the examined material. Absorption occurs through sending the necessary amount of wave energy to the vibration and rotation of the bond in molecules in the infrared region of the electromagnetic spectrum. Infrared light is solely absorbed by molecules with dipole moment (for example, equivalent two atoms as N_2, 0_2 showed any result in FTIR, however HCI may be taken by FTIR spectrum). In short, functional groups of a sample can be obtained by IR spectroscopy. Depending on the type of sample, the

chemical formula of a sample can be found out by using only IR spectroscopy. However, it sometimes should be used with combination of other characterization techniques to obtain more accurate results.

The IR spectrum of commercial detergent was obtained. Then its spectrum was divided into a certain wavelength. All the peak positions and intensities were determined by de-convoluting through Origin Pro8 software. Finally, each separated peak was matched a component of commercial detergent.

In addition to detergent analysis, clean glass samples were washed in a dishwasher by using five different detergents. IR spectra of these samples were collected after washing. Then, IR spectrum of a blank glass sample and dishwasher washed glass samples were compared to observe the differences between them. Increasing rinsing cycle effect was not observed by this method.

To design calibration curves for quantitative surface analysis, the aperture was selected as 0.082 mm. FTIR experiments were performed with 0.5 µl solutions dried on the glass surface for 15 min at 130 ^{o}C. Dishwasher washed glass samples were measured by ATR-FTIR. Then the residue amount of STPP and sodium citrate was determined by using FTIR calibration curves.

In simple colorimetric analysis, IR absorbance of different solutions were compared by using ATR-FTIR spectroscopy.

3.2.2.2. X-Ray Fluorescence Spectrometer (XRF)

In X-Ray fluorescence spectrometer, photons from the X-Ray source is sent through the sample to analyze it elementally. As shown in the Figure 3.3, when photons interact with the atoms, it displaces an electron from the inner shell of the atom and brings atoms into a high energy level than the ground state. Energy is released to fill this gap by an electron from the upper shell and atom returns to its ground state again. Released energy is characteristic fluorescence radiation. Wavelengths of characteristic lights are constant and they belong to specific element. This allows to perform chemical analysis. In this study, XRF was performed to make an elemental analysis of different detergent types. The chemical composition of the samples were determined using a sequential spectrometer of X-ray fluorescence per wavelength XRF – BRUKER TIGER S8; the powdered samples were pressed to disc shape under a pressure of 10 ton, using boric acid as support (base).

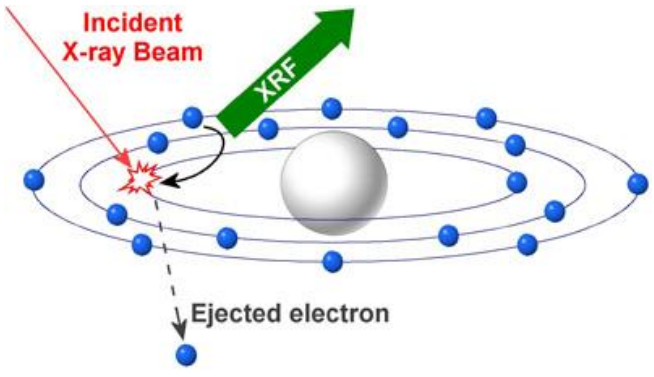


Figure 3.3. X-Ray florescence spectroscopy.[32]

3.2.2.3. X-Ray Diffraction (XRD)

X Ray Diffraction (XRD) is based on that each crystalline phases depending on the specific atomic packing diffracts X Rays in a characteristic pattern (order). This diffraction profiles are kind of fingerprint for each crystalline phase. XRD does not destroy the sample during the analysis and requires small amount of the sample. Qualitative and quantitative analysis of rocks, crystalline materials, thin films and polymers can be examined by X-Ray diffraction. In short, XRD method is perfectly suitable for chemical analysis of crystalline structures. Figure 3.4 illustrates the working mechanism of XRD.

A Bruker D8 Advance was used with a Cu Kα1 radiation source employing a wavelength of 1.5418 Å. 40 kV and 40 mA was the power rating of the X-ray generator, a slit size of 1 mm was determined and a Vantec-1 was used as a detector. The measurement was performed between 2Θ values of 2 and 90°. For the sample preparation, approximately 20 mg of sample was placed on double-sided tape attached on a glass slide. The glass slide was attached on a gum and leveled with the sample holder using a smooth piece of glass. The sample was spread thoroughly to cover the double-sided tape to prevent amorphous signals coming from the tape. In measurements where a slight background is observed due to the tape, background subtraction was performed.

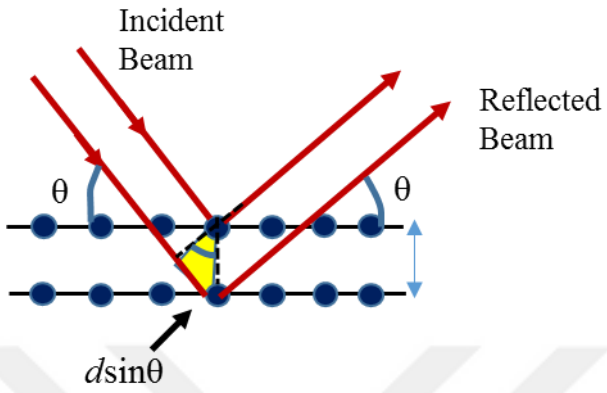


Figure 3.4. X-Ray Diffraction (XRD)

3.2.2.4. X-Ray Photoelectron Spectroscopy

X-Ray Photoelectron Spectroscopy (XPS) is a numerical analysis technique providing atomic and molecular information regarding the surface of the material. XPS spectra are obtained by irradiating a solid surface with a beam of X-rays. The kinetic energy simultaneously is being measured. The top 1-10 nm of the material is analyzed. Ejected electrons over a range of electron kinetic energies is counted to obtain a photoelectron spectrum. Peaks appear in the spectrum from atoms emitting electrons of a particular characteristic energy. The energies and intensities of the photoelectron peaks enable identification and quantification of all surface elements (except hydrogen).[33] The measurements were recorded in the fixed analyzer transmission mode at an instrument base pressure of below 5×10^{-9} mbar. The angle of emission was 45°. The surfaces were analyzed

with a XPS thermos K-alpha. Survey scans from 0 to 1000 eV binding energy were performed to determine the elemental composition of the surfaces. High resolution scans for carbon (C1s), oxygen (O1s), and phosphorus (P1s) (20 eV window) were also recorded. Charge compensation was implemented during spectral acquisition using an electron flood gun operated at 5 eV. The residual pressure in the analysis chamber was on the order of 10-9 Torr or lower during spectral acquisition. The spectral envelopes were resolved into Gaussian peaks to fit the spectra, and the hydrocarbon C1s peak was referenced to 285 eV. Besides analyzing the surface of dishwasher washed glass samples with 5 different detergent, increased rinsing cycle effect was examined by this method.

3.2.2.5. Laser Ablation Inductively Coupled Plasma Mass Spectroscopy

ICP-MS is an analytical technique used for high precision elemental analysis. An ICP-MS includes a high-temperature ICP (Inductively Coupled Plasma) source and a mass spectrometer. The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the mass spectrometer.[34] Laser ablation created fine particles from the surface of glass sample, which are transported to the secondary excitation source of the ICP-MS instrument for digestion and ionization of the sampled mass. The excited ions in the plasma torch are subsequently introduced to a mass spectrometer detector for both elemental and isotopic analysis.[35] ICP-MS-Laser ablation Agilent CY is used for the direct surface analysis of glass samples. In this method, dishwasher washed

glass surface with commercial detergent was analyzed. Moreover, the effect of rinsing cycle was also investigated by this method to support the results of XPS measurements.

Laser ablation was performed with a solid state laser at a wavelength of 213 nm (NWR 213) to obtain calibration curves for surface analysis of dishwasher washed glass sample. An optical sample map of glass was generated prior to the measurement. The laser beam path was equipped with a square-shaped laser spot table ensuring a constant delivery of energy onto the moving sample throughout the entire diameter of the laser beam. The output laser energy was tuned separately for every sample in order to ensure complete ablation of the sample material. Ablation was performed with parallel line scans, a frequency of 20 Hz, a spot size of 10 μm and a scan speed of 10 μm s^{-1}.The ablated sample material was transferred to the ICP-MS instrument with helium (quality 5.0) at a flow rate of 400 mL min^{-1}. Laser ablation data were recorded with a Agilent 7700x ICP-MS instrument. Argon was used as plasma gas (15 L min^{-1}) and as carrier gas with a flow rate of ~1.1 L min^{-1}.

Laser ablation techniques were utilized for analysis of dishwasher washed sample to show the rinsing cycle effect on residue characteristic and design of calibration models.

Laser ablation created fine particles from the surface of glass sample, which are transported to the secondary excitation source of the ICP-MS instrument for digestion and ionization of the sampled mass. Samples for designed calibration models of STPP and

sodium citrate were prepared with 0.5 µl solutions dried on the glass surface for 15 min at 130 °C. The UV beam diameter was selected as 200 µm.

3.2.2.6 Contact Angle Measurement

Contact angle measurement was performed to compare the hydrophobicity of two different types of builders.

20 µl solutions were dried on the glass surface for 2 h at 37 °C. Then water contact angle was measured with 10 µl water dropped onto surface of glass sample by Krüss G10 goniometer.

3.2.2.7 UV-VIS-NIR Spectroscopy

UV–Vis measurements were carried out with on a UV-VIS-NIR Shimadzu UV-3600 spectrophotometer equipped on a 1-cm path length cell. UV–vis spectroscopy was used to monitor the absorbance change in the spectra for different concentration of STPP and sodium citrate solutions dried on the surface. 20 µl solutions were dried on the quartz cuvettes with 10 mm path length. Sampling interval was 1 nm and scan speed was medium. Slit width was chosen as 8 nm. The spectra were run between 200-500 nm. White spots' areas on quarts and their absorbance values were recorded. UV–vis spectroscopy was also utilized the simple colorimetric analysis. Altered concentrations of iron (III) chloride hexahydrate and sodium citrate mixture were analyzed by UV–vis spectroscopy.

3.2.2.8 Raman Spectroscopy

Raman spectroscopy was used to obtain calibration curves in quantitative surface analysis.

Raman shifts of prepared samples were measured by using a Raman microscope (Renishaw Invia). Raman spectra of glass samples were collected using a spectrometer equipped with a 10% power laser-diode emitting at 532 nm (2400 grating). 20 µl solutions were dried on the glass surface for 2 hours at 37 ^{o}C. The laser was focused on single point on the surface. The data was collected from three different points for statistical analysis.

3.2.3. Cell Viability Experiments

To investigate the cell viability of cells seeded in medium with different concentrations of STPP and sodium citrate, a human kidney cell line (passage number: 6) was used. Cells were cultured at 37 °C with 5% CO_2 in an incubator and maintained in DMEM supplemented with 1% penicillin/streptomycin, 2 mM L-glutamine and 10% FBS. The hybrid hydrogels were immersed in the complete culture medium for 24 h at 37 °C before seeding. For static seeding of the cells, were added to the wells of a 96-well plate and 100 µl cell suspensions (5000 cells) were seeded. After 15 min, 1 ml of compete culture medium was added to each well. The cells were placed in the incubator for 24 h, and the CellTiter-Glo luminescent cell viability assay (Promega) was used on days 1 and 2 to characterize the metabolic activity of cells. A standard curve was generated using ATP solutions at specific concentrations (1, 0.1

and 0.01 μM) prepared in the cell culture medium. Next, 100 μl from each medium was taken and placed in the same amount of CellTiter-Glo Reagent. Both ATP solutions and samples were incubated at 25 °C for 15 min and the luminescence was measured using a plate reader.

Chapter 4

RESULTS AND DISCUSSION

4.1. Qualitative Surface Analysis

4.1.1. Detergent Analysis

To analyze the as-received standard detergents, three different characterization techniques (ATR-FT-IR, XRF, and XRD) were combined. For this purpose, one commercial detergent, four standard detergent (Europe B, Europe D, Australia, and America) were examined.

4.1.1.1 ATR-FTIR Spectroscopy

IR spectrum of commercial detergent is demonstrated in Figure 4.1.

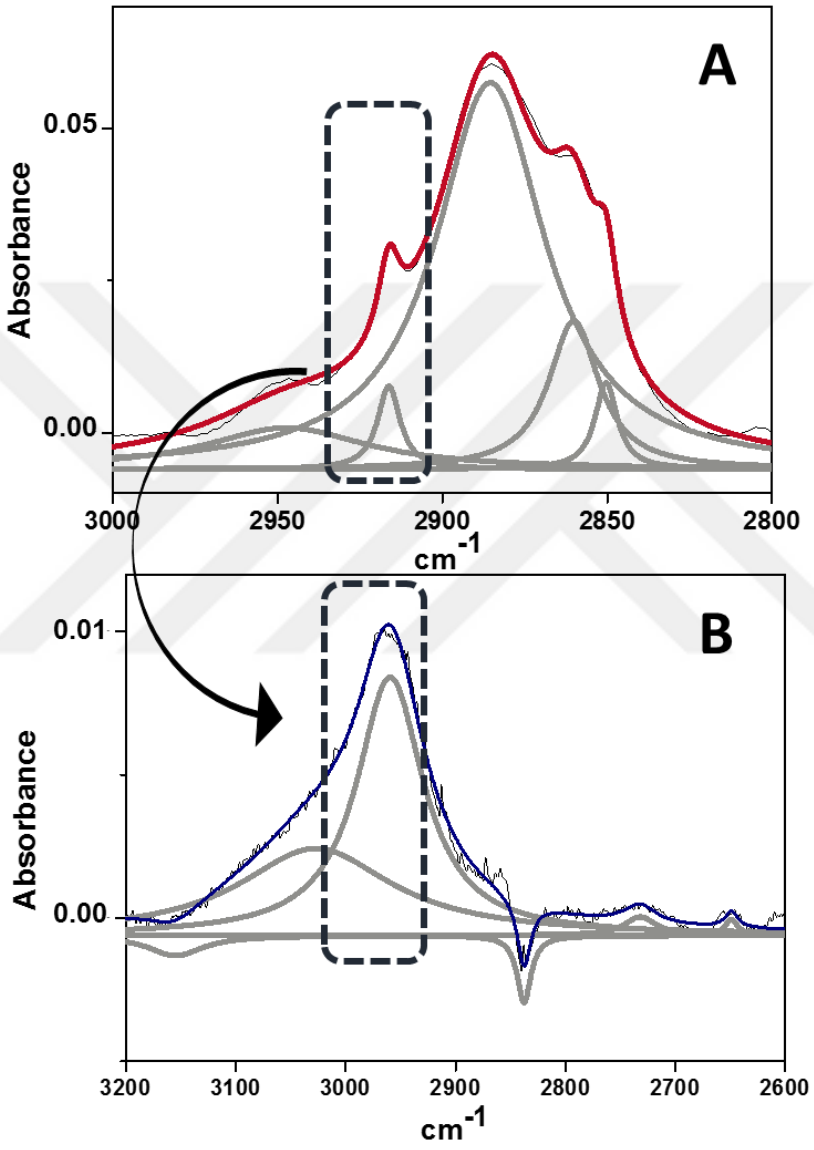


Figure 4.1. A) IR spectrum of commercial detergent between 2800 to 3000 cm^{-1}. B) IR spectrum of sodium carbonate between 2800 and 3000 cm^{-1}.

In figure 4.1., IR spectrum of sodium carbonate and commercial detergent were represented. Sodium carbonate is one of the builder of a commercial detergent. As it could be seen from Figure 4.1, commercial detergent peaks between 2800 cm^{-1} to 3000 cm^{-1} wavenumber were matched with the peaks of sodium carbonate. This shows that commercial detergent consists of sodium carbonate in it. This system was applied for the whole spectrum of commercial detergent and its peaks were matched with the components in its ingredient list.

4.1.1.2. X-Ray Fluorescence Spectrometer (XRF)

The XRF results of commercial tablet detergent is demonstrated in Table 4.1.

Table 4.1. XRF result of commercial detergent

Sample	Calibration Method	Sum (%)	Matrix (%)	Na (%)	S (%)	Ca (%)	Si (%)	K (%)	P (%)
red	Elements	7.70		6.46	0.25	0.16	0.04	0.03	0.02
red	Organics	100	84.58	14.92	0.12	0.05	0.03	0.01	0.01
white	Elements	17.90		9.07	0.55	0.02	0.13	0.33	7.74
white	Organics	100	74.90	18.84	0.31		0.12	0.16	5.59
blue	Elements	12.90		7.41	0.48	0.04	0.07	0.27	4.10
blue	Organics	100	80.28	16.21	0.25	0.02	0.06	0.12	2.82

Tablet commercial detergent has three different part and each possesses different colors. Table 4.1 shows that each part of tablet detergent contain different amount of element. For instance, white part of tablet detergent contains more Na and P elements which present in penta-sodium tri phosphate (STPP, $Na_5P_3O_{10}$). This means that white part mainly involves builders of detergent formulations.

4.1.1.3. X-Ray Diffraction (XRD)

The corresponding five different detergents were also analyzed by XRD. In Figure 4.2, XRD results of commercial detergent is demonstrated.

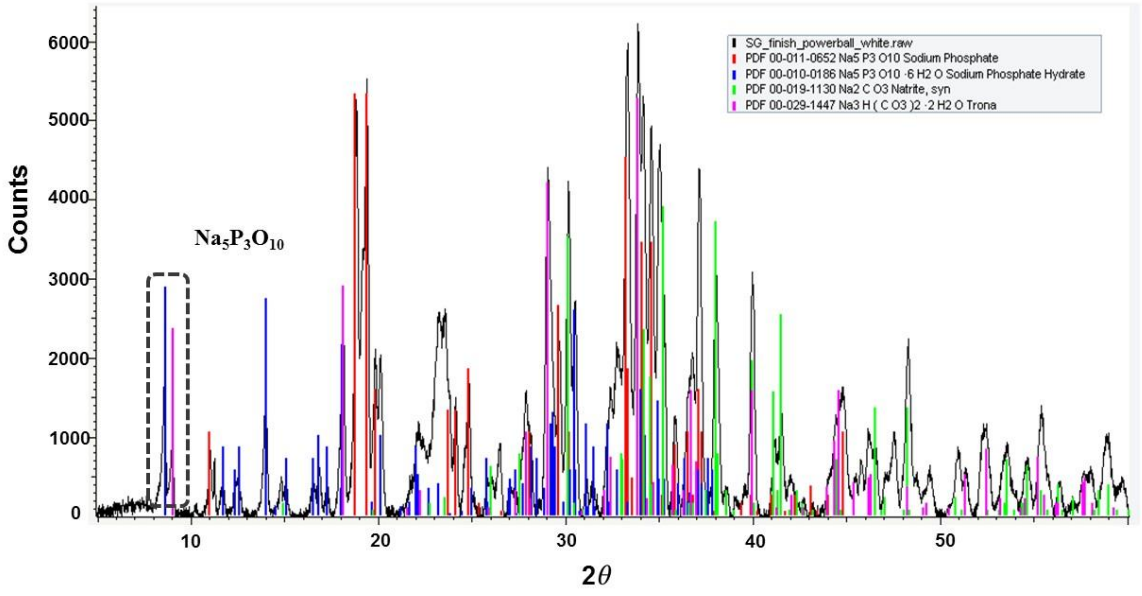


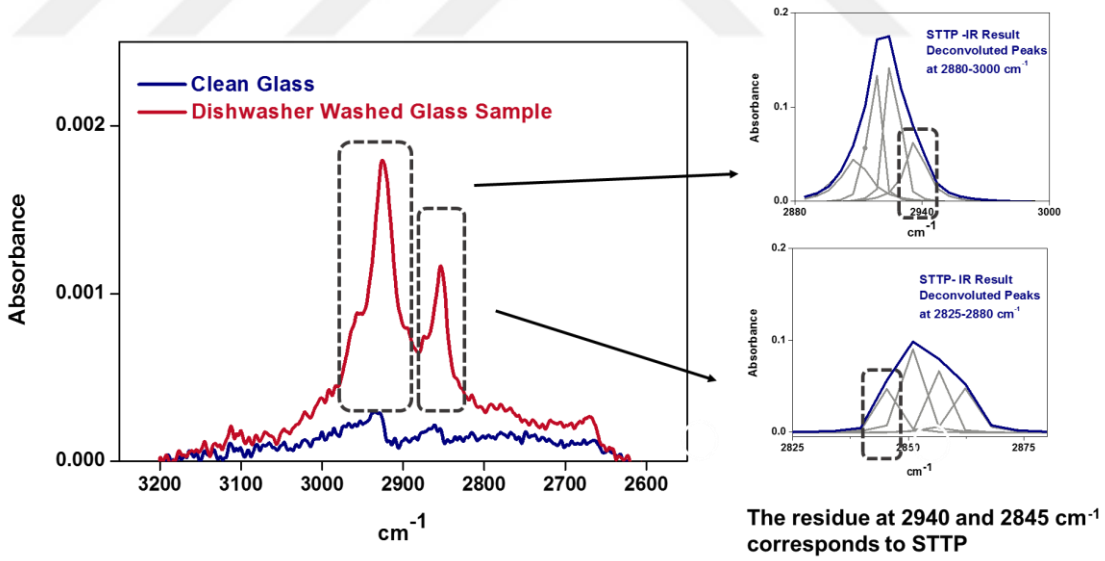
Figure 4.2. XRD result of commercial detergent

Figure 4.2 illustrates that major ingredients of commercial detergent were matched by XRD. XRD is the most suitable and conventional method for detergent analysis, because many components of detergent possess crystalline structure.

4.1.2. Surface Analysis Results

Qualitative analysis of dishwasher washed glass samples was performed by three different surface characterization methods: (ATR-FTIR), X-ray photoelectron spectroscopy (XPS) and laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS).

4.1.2.1 ATR-FTIR Spectroscopy



The residue at 2940 and 2845 cm^{-1} corresponds to STTP

Figure 4.3. The red line shows the IR spectrum of dishwasher washed glass sample. The peaks in dishwasher washed glass was matched with the peaks of STPP at the right side. The blue line shows the clean glass (blank) IR spectrum.

In Figure 4.3, IR results of dishwasher washed sample and clean glass samples were compared. As it could be seen, dishwasher washed samples have extra peaks between 2800 to 3000 cm^{-1}. These peaks are coming from residue of STPP after dishwashing process. To support IR results, we also applied XPS and ICP-MS for surface characterization.

4.1.2.2 X-Ray Photoelectron Spectroscopy

By performing XPS experiments, both dishwasher samples surface and hand-washed sample surfaces were analyzed. Figure.4.4 shows the XPS results of dishwasher washed and hand-washed samples.

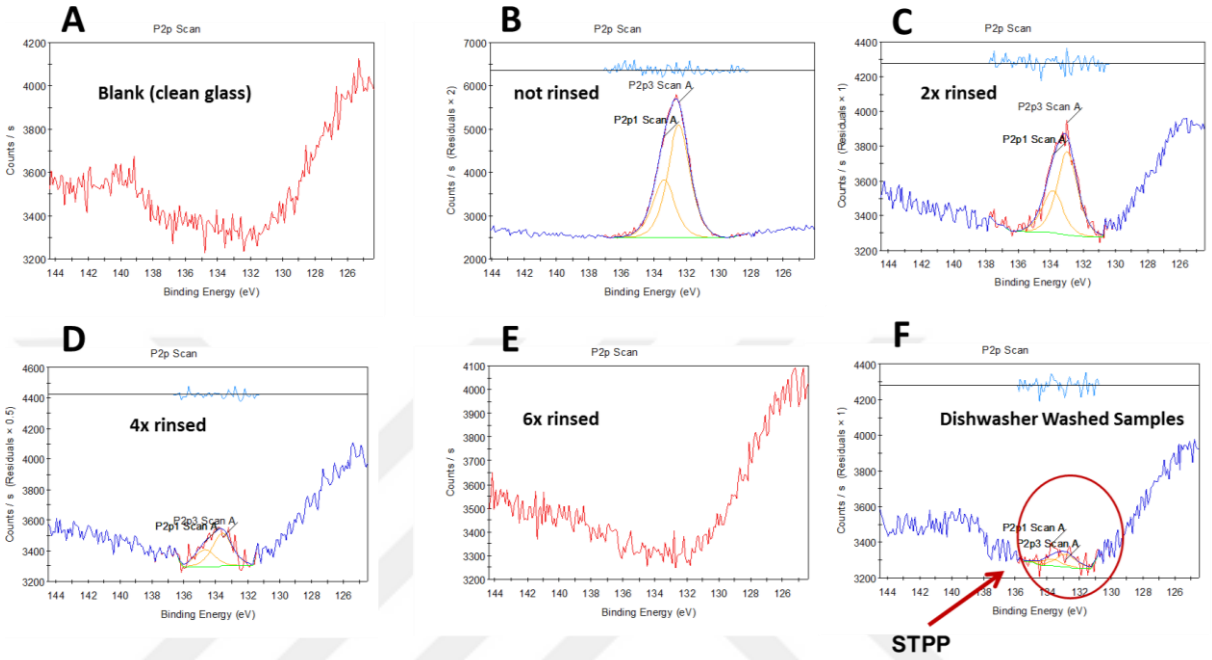


Figure 4.4. XPS results of A) Clean Glass (Blank);B) Not rinsed hand-washed sample; C) 2 times rinsed hand-washed sample; D) 4 times rinsed hand-washed sample. E) 6 times hand-washed sample; F) XPS results of dishwasher washed sample with commercial detergent.

As it could be seen from Figure 4.4., increasing rinsing cycle causes the decrease in intensity of STPP peak. After six rinsing cycle, the surface of glass sample is free from STPP. Dishwasher washed sample contains STPP as a residue on it. The XPS analysis of dishwasher washed samples with standard detergents are represented in Figure 4.5.

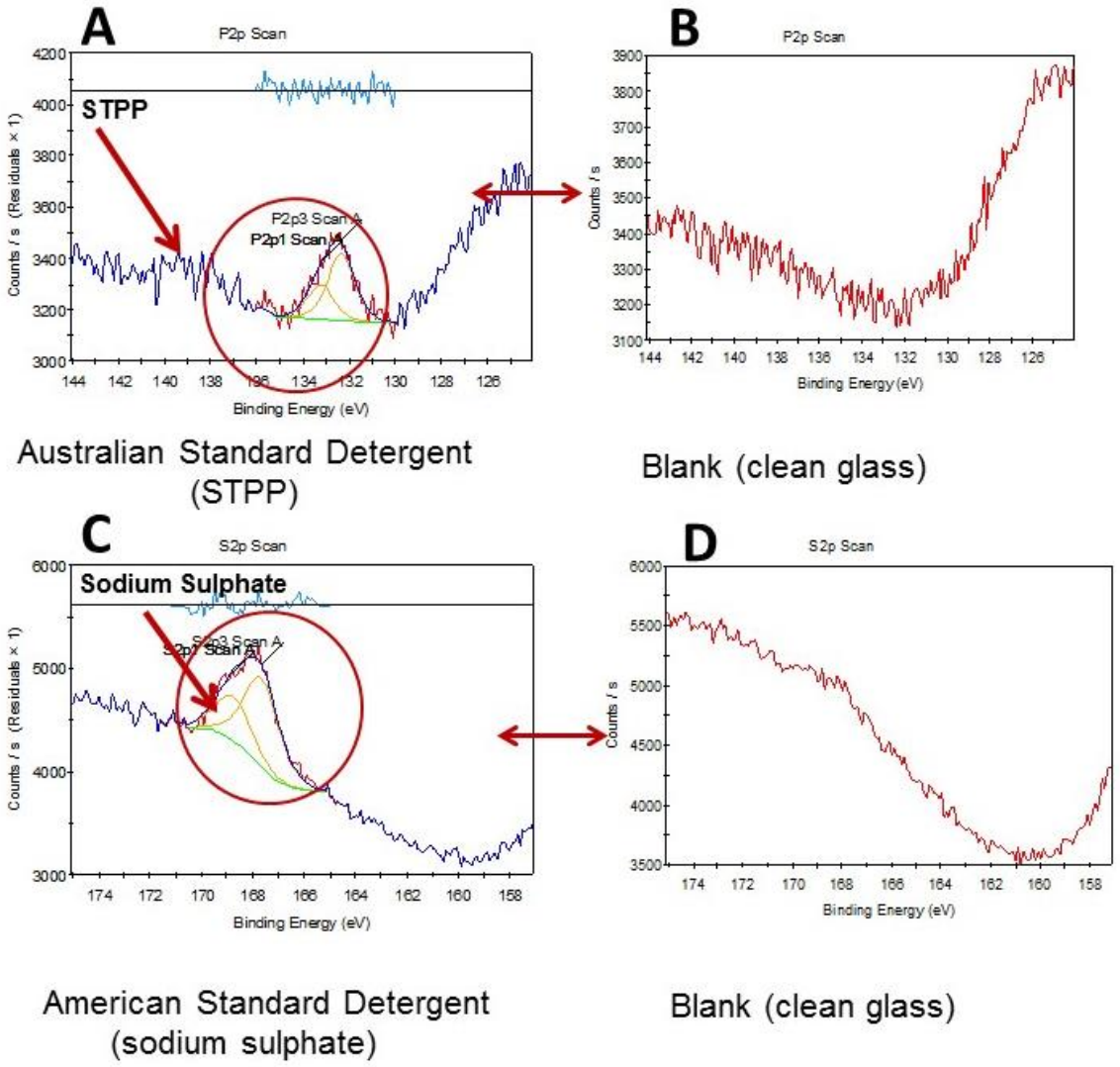


Figure 4.5. A) XPS analysis of dishwasher washed glass samples, washed using A) Australian standard detergent; B) Clean glass (Blank); C) XPS analysis of dishwasher washed glass samples washed using American standard detergent; D) Clean glass (Blank).

The samples washed in dishwasher washed using standard detergents (Australian and American) have residues of their builders on them.

The samples washed with European standard detergents in dishwasher were also analyzed by XPS and their results are shown in Figure 4.6.

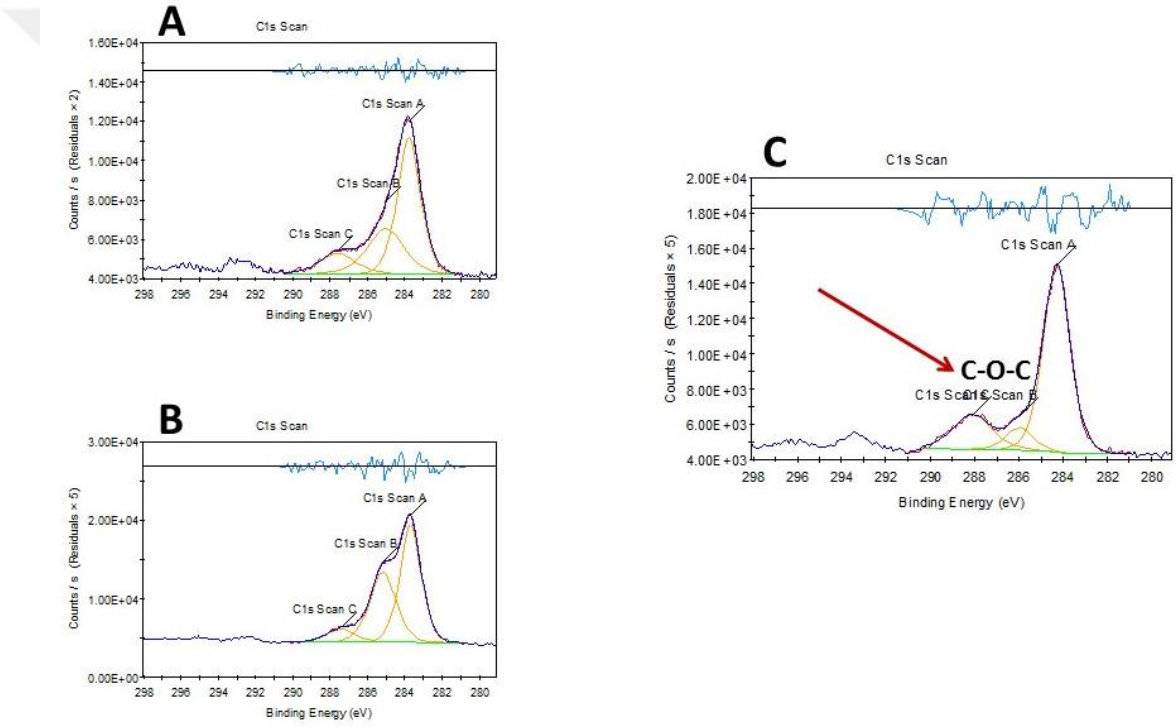


Figure 4.6. XPS results of A) dishwasher washed glass samples washed using European standard detergent B; B) dishwasher washed glass samples washed using European standard detergent D. C) Clean glass (Blank).

C-O-C peaks of samples washed with European standard detergents in dishwasher has higher intensity with respect to clean glass (Blank). C-O-C vibrations corresponds to sodium citrate which is the builder of European standard detergents. This reveals that dishwasher washed samples with European standard detergent has also detergent residue on them after dishwashing.

4.1.1.3. Laser Ablation Inductively Coupled Plasma Mass Spectroscopy

To support the results of XPS, LA-ICP-MS was performed. The results of LA-ICP-MS is shown in Figure 4.7.

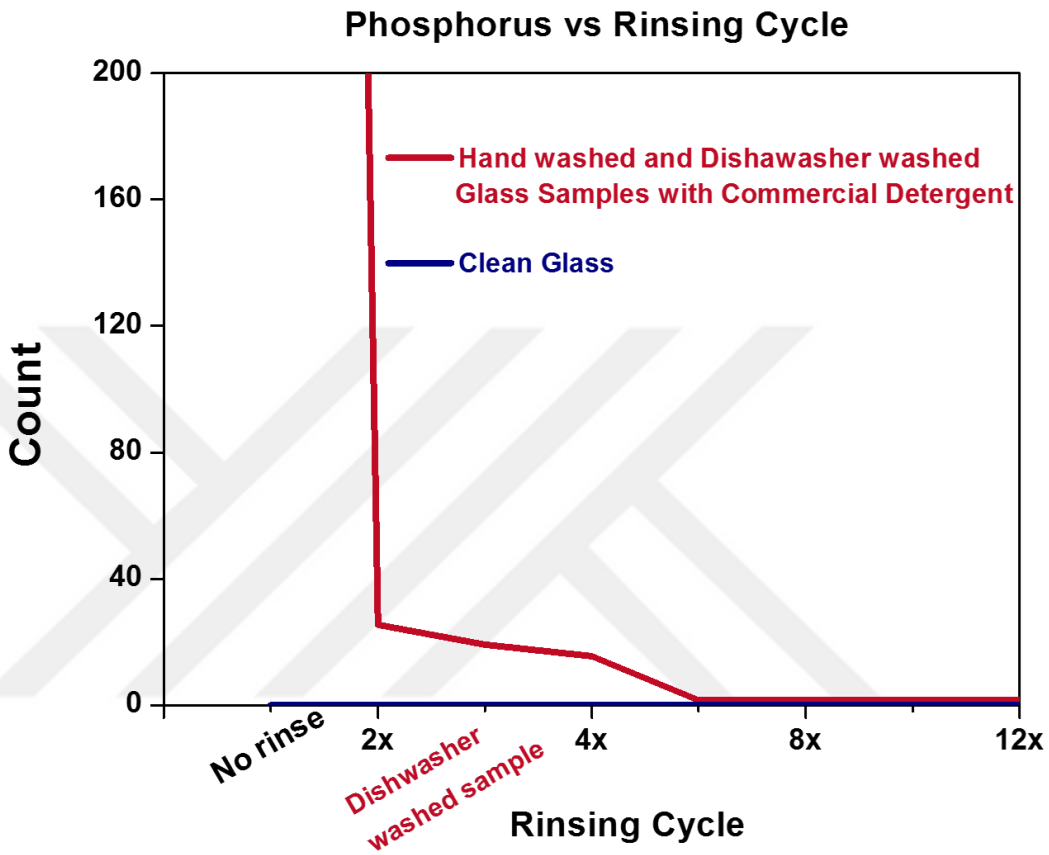


Figure 4.7. The red line shows LA-ICP-MS results of hand-washed and dishwasher washed samples. The blue line represents LA-ICP-MS results of clean glass (blank) sample.

Like in the case of XPS results, increasing number of rinsing cycle decreases the amount of STPP residue on surfaces. STPP precipitated as a residue on the surface of dishwasher washed samples. Surface characterization results indicate that builders of detergent leaves residue upon dishwasher washing process. Moreover, increasing rinsing cycle decreases the

amount of residue on surfaces and it can be a solution for residue problem in dishwasher washing process.

4.2. Quantitative Surface Analysis

4.2.1 Contact Angle Measurement

STPP and sodium citrate solutions with different concentrations were prepared and their corresponding contact angles were measured on surfaces. Data showed that 0.05 g/ml STPP solution dried on glass surface had a contact angle of 11.7°, where the corresponding value measured for sodium citrate was 21.2°. Before the surface was treated with any solution, contact angle was measured as 30.7°. These results indicate that both builder types slightly promote the hydrophilicity of the glass surface (Figure 4.8).

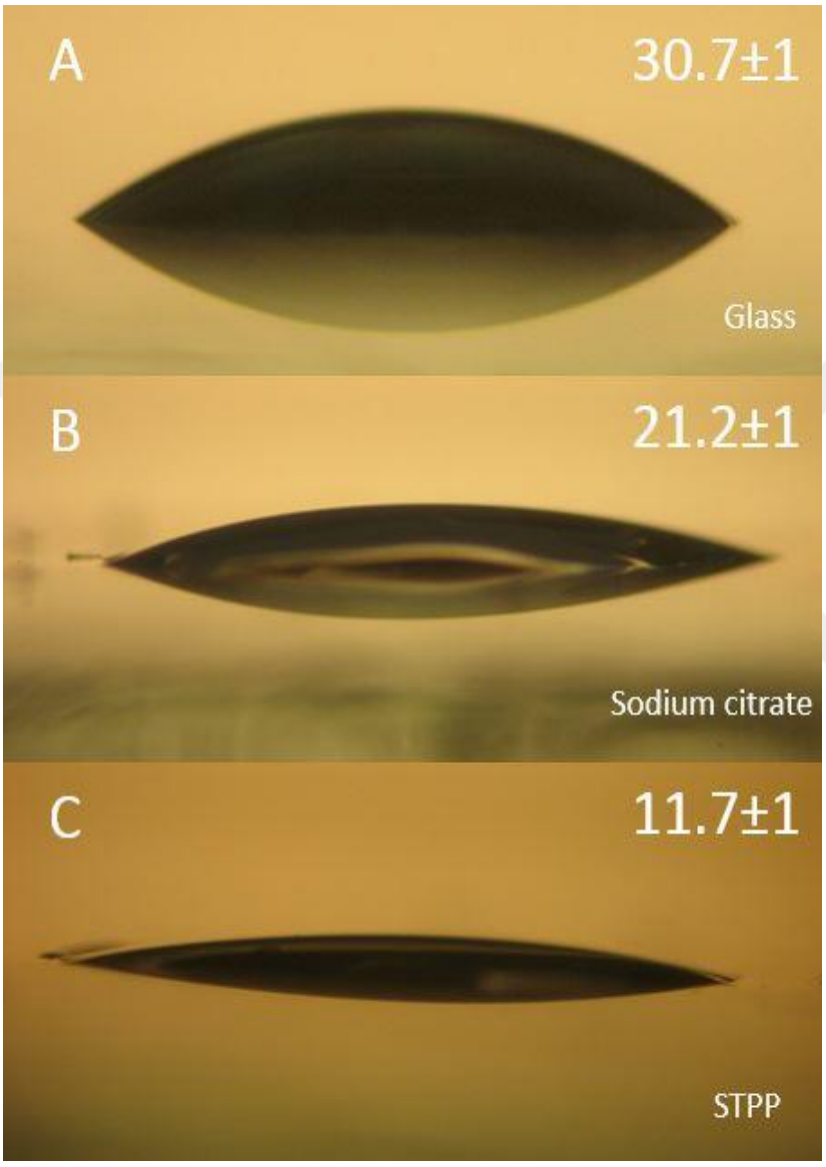


Figure 4.8. (A) Contact angle of clean glass sample; (B) Contact angle glass surface treated with STPP (C) Contact angle glass surface treated with sodium citrate

4.2.2. UV-Vis Spectroscopy

Absorbance values of white spots obtained for varying concentrations of STPP or sodium citrate solutions on quartz cuvette were also quantified by UV-vis spectroscopy. Figure 4.9.A compares the absorbance profiles of STPP solutions with varying concentrations precipitated on the side surface of quartz cuvette. With increasing concentration, the peaks shift towards UV region (Figure 4.9.B). Interestingly, the dependence of the absorbance with the sodium citrate concentration had an opposite trend for sodium citrate, where the spectra shifted towards visible light region with increasing sodium citrate amount in the solution (Figure 4.9.C). UV/vis spectroscopy is another conventional method that can be utilized to analyze the concentration of STPP or sodium citrate on surfaces. Figure 3.2 demonstrates the steps of sample preparation experiments to obtain calibration curve. To convert concentration/area to mole/area, specific volume of the solutions were dropped on glass surfaces and after drying, the dimensions of the white spots due to STPP and sodium citrate were recorded. Uniform distribution of molecules on glass surfaces after drying was assumed. Because the concentrations of solutions were kept constant, the number of moles of molecules (STPP or sodium citrate) in the solution was calculated according to dropped volume and it was assumed that no particle loss occurred by evaporation after drying. Finally, the mole number was divided by white spots area to obtain mole/area (moles/cm^2) was determined for different solutions dried on glass surfaces.

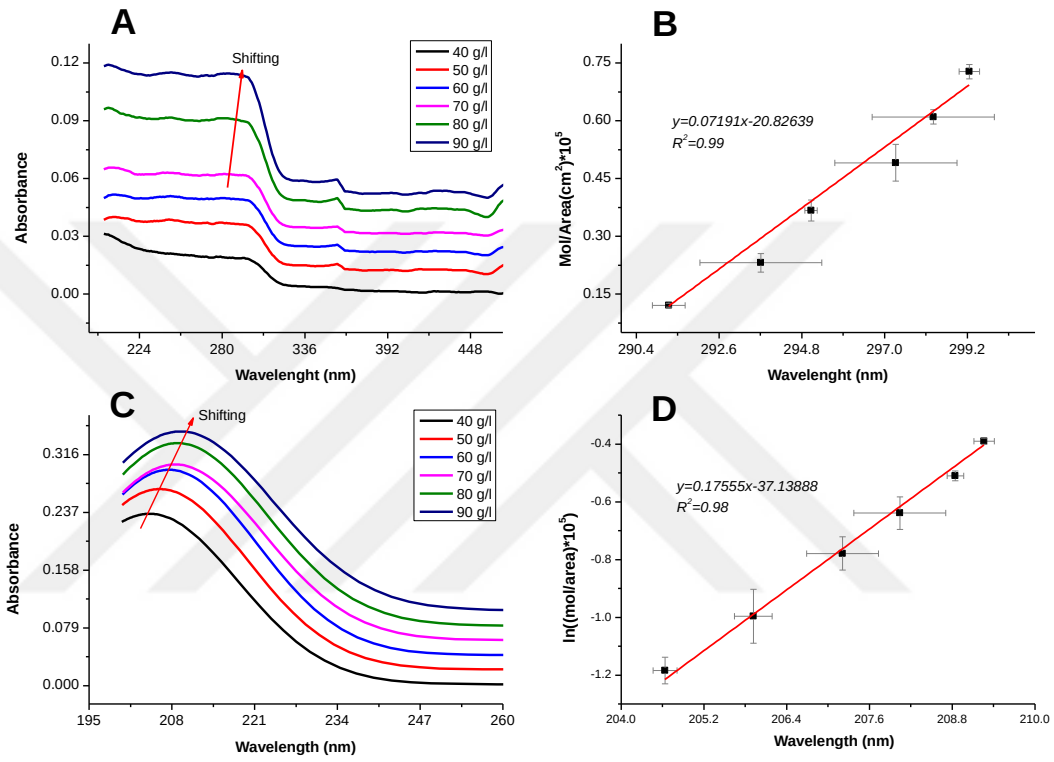


Figure 4.9. (A) UV absorbance of STPP dried on quartz cuvette (B) calibration curve of STPP solutions dried on quartz surface (C) UV absorbance of sodium citrate dried on quartz cuvette (D) Calibration curve of sodium citrate solutions dried on quartz surface.

4.2.3. Raman Spectroscopy

Glass surfaces treated with STPP and sodium citrate were also characterized by Raman spectroscopy. The characteristic absorption band of P=O stretching can be observed at about 1102 cm^{-1} in STPP treated surfaces. As in the case of ATR-FT-IR, Figure 4.10.A shows that peak intensity increases with increasing concentration of STPP. A correlation between the intensity and concentration can be observed in Figure 4.10.B. with an R^2 value of 0.98. Results of surfaces with dried sodium citrate were illustrated in Figure 4.10.C, where point at 1420 cm^{-1} corresponded to C=O stretching. Increases in sodium citrate concentration lead to increases in the intensity of C=O stretching, where R^2 value was calculated as 0.97 (Figure 4.10.D.). The results of two calibration curves obtained from different characterization techniques will allow for determination of Raman spectra for an unknown sample by measuring FT-IR without the need of performing Raman spectroscopy.

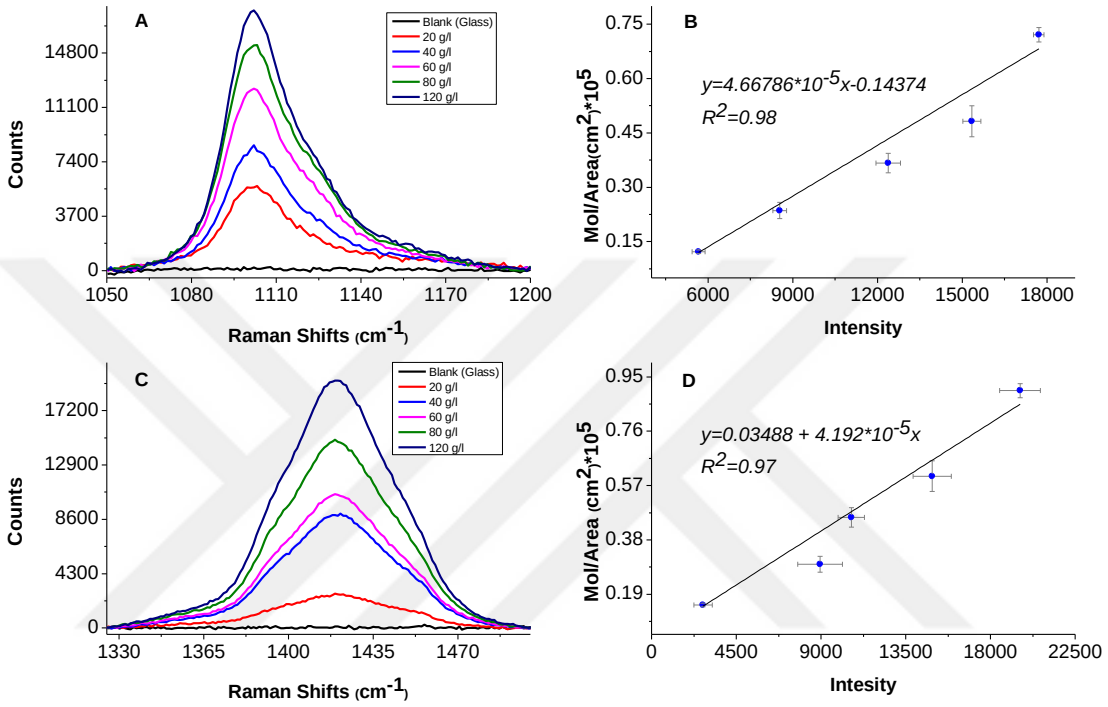


Figure 4.10. (A) Raman spectra of STPP dried on glass surface (B) calibration curve of STPP solutions dried on glass surface (C) Raman spectra of sodium citrate dried on glass surface (D) Calibration curve of sodium citrate solutions dried on glass surface

4.2.4. Laser Ablation-Inductively Coupled Plasma Mass Spectroscopy (LA-ICP-MS)

For characterization of samples via LA-ICP-MS two different calibration steps were used. The first calibration technique used for standardization with P, Si, Na, and C solutions as internal standard. The experiments in the second step included STPP and sodium carbonate calibration solutions dried on the glass surface, in which the concentrations of the elements were known. After obtaining second calibration curves for both STPP and sodium citrate containing surfaces, the unknown sample could be measured by using these two calibration models. Figure 4.11.A. and Figure 4.11.B. demonstrates the calibration curves obtained for STPP and sodium citrate, respectively. These calibration curves by LA-ICP-MS are useful for determination of the amount of unknown sample surface by using ATR-FT-IR data. This approach will allow for the use of a conventional method to estimate the corresponding values by highly precise instrument.

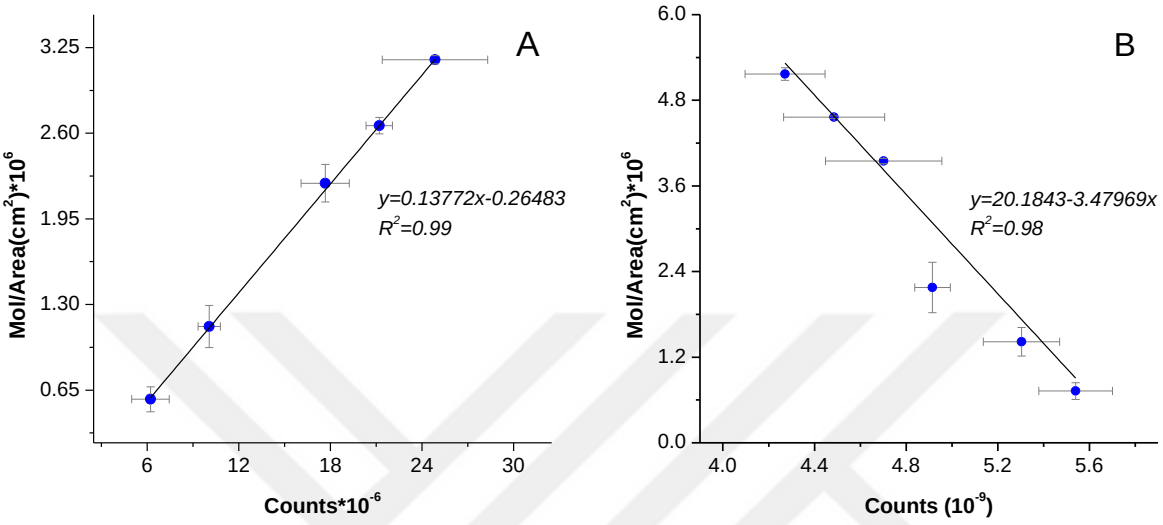


Figure 4.11. (A) Phosphorous elemental analysis by LA-ICP-MS on the glass surface treated with STPP (B) Silica element analysis by LA-ICP-MS on the glass surface treated with sodium citrate.

4.2.5. Fourier Transform Infrared Spectroscopy in the Attenuated Total Reflectance Mode (ATR-FTIR)

IR spectra of for altered concentrations of STPP and sodium citrate solutions dried on glass surfaces were obtained by ATR-FT-IR. Both STPP and sodium citrate have several specific absorption bands due to the presence of different functional groups. For STPP, the peak around at 1100-1200 cm^{-1} refers to PO_3^{2-} group in phosphate. The peak at 895 cm^{-1} could be attributed to P-O-P vibration of phosphate on glass surface. For sodium citrate,

peak at 1646 cm^{-1} may be assigned to symmetric stretching of C=O from COOH group, suggesting the presence of a citric acid radical on the glass surface. The next strong FT-IR band observed around 1429 cm^{-1} can be assigned to the asymmetric stretching of CO from citrate.

Figure 4.12.A. shows IR spectra of various glass surfaces treated with altered concentrations of STPP Data show that the intensity of the corresponding bands increase with increasing chemical concentration. Based on these results a calibration curve was obtained to determine unknown amount of STPP on dishwasher washed samples (Figure 4.12.B.). Similarly, for sodium citrate, as shown in Figure 4.12.C. C=O stretching intensity increases with increasing concentration of sodium citrate dried on glass surface. Relative calibration curve was prepared to quantify sodium citrate on dishwasher washed glass surfaces (Figure 4.12. D.).

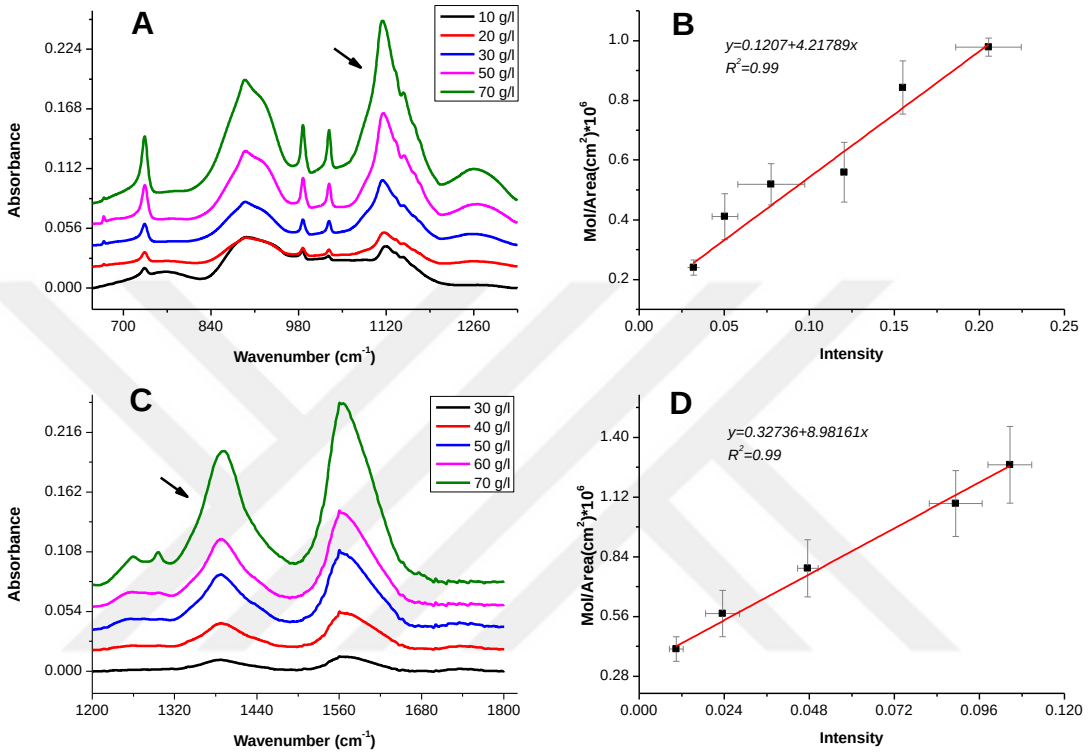


Figure 4.12. (A) IR spectra of different concentrations of STPP dried on glass surface (B) ATR-FT-IR calibration curve of STPP solutions dried on surface (C) IR spectra of different concentrations of sodium citrate dried on glass surface (D) ATR-FT-IR calibration curve of sodium citrate solutions dried on glass surface.

In Figure 4.13, the calibration curves obtained for STPP and sodium citrate on three different surface characterization methods were combined. For instance, Fig. 4.13A illustrates IR intensity vs UV/vis and Raman intensities for samples containing STPP and sodium citrate residues.

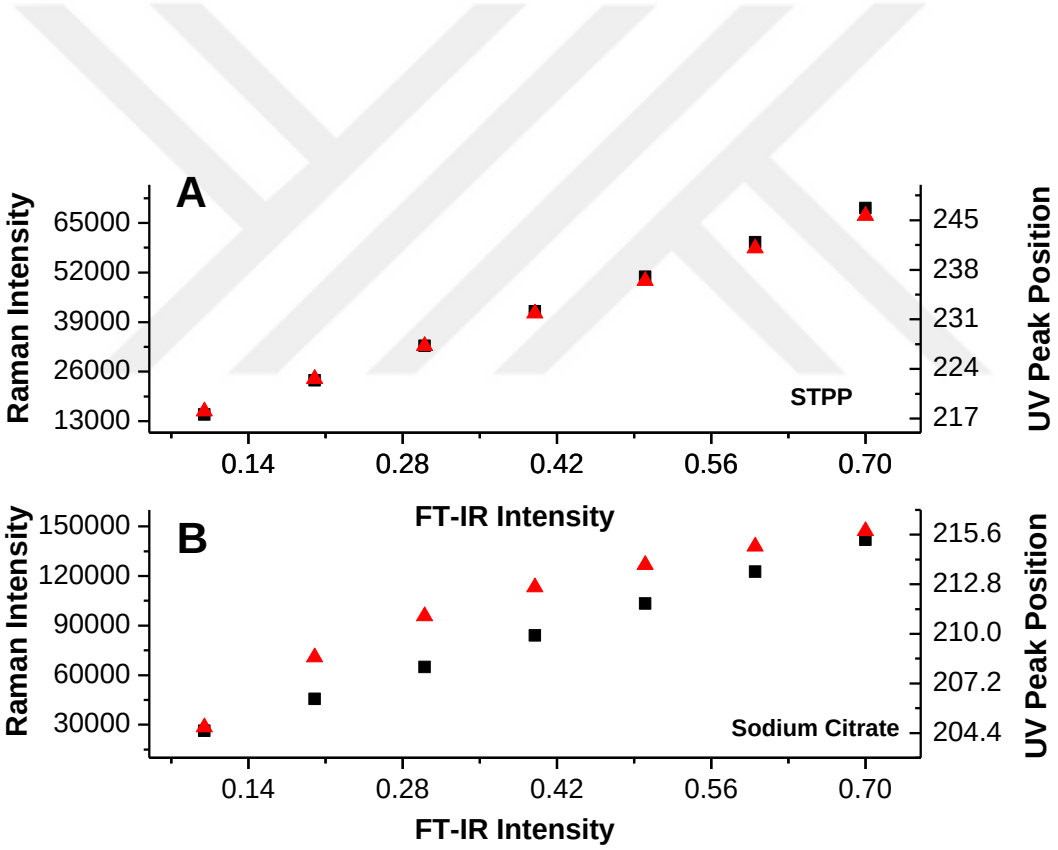


Figure. 4.13. (A) Combination of FT-IR, Raman, UV for STPP dried glass surface (B) Combination of FT-IR, Raman, UV for sodium citrate dried glass surface.

As in the case of STPP, the collaborated plot for three different method was also designed for sodium citrate in Figure 4.13B. We infer that these calibration models can be utilized for analysing surface quantitatively and combining three different characterization methods. One can easily estimate STPP and sodium citrate characteristic peaks' intensities of Raman or UV-vis spectra by performing FT-IR experiment only. In other words, this method enables the measurement of detergent residues directly by performing one of three methods available in a laboratory. In addition, they can also help to verify the results obtained from different techniques.

Quantification of detergent residues

To determine the amount of detergent residues on dishwasher washed samples, ATR-FT-IR measurements were performed. The spectra of eight different glass samples washed with Australian and European B standard detergents in a dishwasher were obtained. The results were collected from three different points on dishwasher-washed glass surface. Figure 4.14A and Figure 4.14B show IR results of samples washed with STPP together with the spectra of a blank glass. As it could be observed from Figure 4.14A and Figure 4.14B, the sample washed with STPP in a dishwasher has an extra peak associated with P=O stretching. The P=O peak intensity was determined by the deconvolution of the peak at 1100 cm^{-1}. The peak intensity was obtained as 0.046. This intensity corresponds to a chemical amount of $3.15x10^{-7}$ mole/area (cm^2) according to the calibration curve given in Figure 4.12B. Similarly, the

characteristic peaks of sodium citrate at 1429 and 1646 cm^{-1} could be observed on the samples washed with sodium citrate as illustrated in Figure 4.12C. The corresponding intensity of the band at 1429 was recorded as 0.00452. Similar to the case with STTP, the residue amount for the sodium citrate was found as $3.68x10^{-7}$ mole/area (cm^2). According to Human & Environmental Risk Assessment on ingredients of European household cleaning products, oral exposure to detergent residue via food intake was estimated as 0.0082 mg/kg of body/day.[36] HERA recommended a method to estimate the daily exposure to STPP via dishwasher-washed dishware by assuming worst case scenario:

Exposes of STPP = F1 x C x Ta x Sa x F" x F / BW

F1= STPP weight fraction in typical detergent (60 %)

C= Concentration of dishwasher detergent solutions (2.78 mg/ml) [37]

Ta= Amount of water (3 ml) left on dishwasher washed surface on 5400 cm^2 i.e. 0.00055 ml/cm^2. With a factor 10 for rinsing, the amount of water left on dinnerware is 0.000055 ml/cm^2.

Sa= Total surface area of dishes for a day (5400 cm^2)

F"= Weight fraction of ingested STPP (worst case 100%)

F = Weight fraction of absorbed STPP (100%)

BW= 60 kg of body

Exposes of STPP= [(60/100) x (2.77 mg/ml) x (0.000055 ml/cm2) x (5400 cm2) x 1 x 1] / 60

EXP= 0.0082 mg/kg/day

This STPP residue amount was estimated by collecting the liquid accumulating on the surface of dishes washed in dishwasher (0.3 ml with a rinsing factor). We used a similar detergent residue containing area and we calculated the human oral exposure for a day from detergent residue as 0.0226 mg/kg of body/day and 0.024 mg/kg of body/day for STPP and sodium citrate, respectively (assuming a body weight of 60 kg). Our values of STPP and sodium citrate that we detected on dishwasher washed glass samples are significantly higher than the corresponding value reported in Human & Environmental Risk Assessment (0.0082 mg/kg of body/day).[36] HERA assumed that the detergents remain as a liquid on dishes. Therefore, they ignored the dried areas containing detergent residues. This might be the main reason of this difference. When maximum tolerable daily intake amount (70 mg/kg of body/day) of STPP is considered, these results indicate that STPP residue on surfaces could have adverse effect on human body in the long term.[38]

In addition to case with LA-ICP-MS, UV-vis and ATR-FT-IR calibration curves were combined (Fig. 4.13A and 4.13B). This establishes the relationship between two different instrument responses and sample concentrations. In other words, UV-vis calibration curve was utilized to estimate the corresponding values obtained by ATR-FT-IR. As a

consequence, obtaining two different characterization techniques values by performing one of them brings flexibility to researches in determination residue amount of STPP and sodium citrate. Moreover, the combinations of Raman-UV-vis and ATR-FT-IR were illustrated in Figure 4.13A and 4.13B. Because Raman spectroscopy is a laborious and sophisticated technique to analyse surface of samples, combined ATR-FT-IR and Raman calibration models provide the opportunity of obtaining corresponding data of Raman spectroscopy easily without performing it. In short, Raman intensity of glass surface covered by STPP or sodium citrate was found by IR measurement by combining calibration models of these characterization techniques.

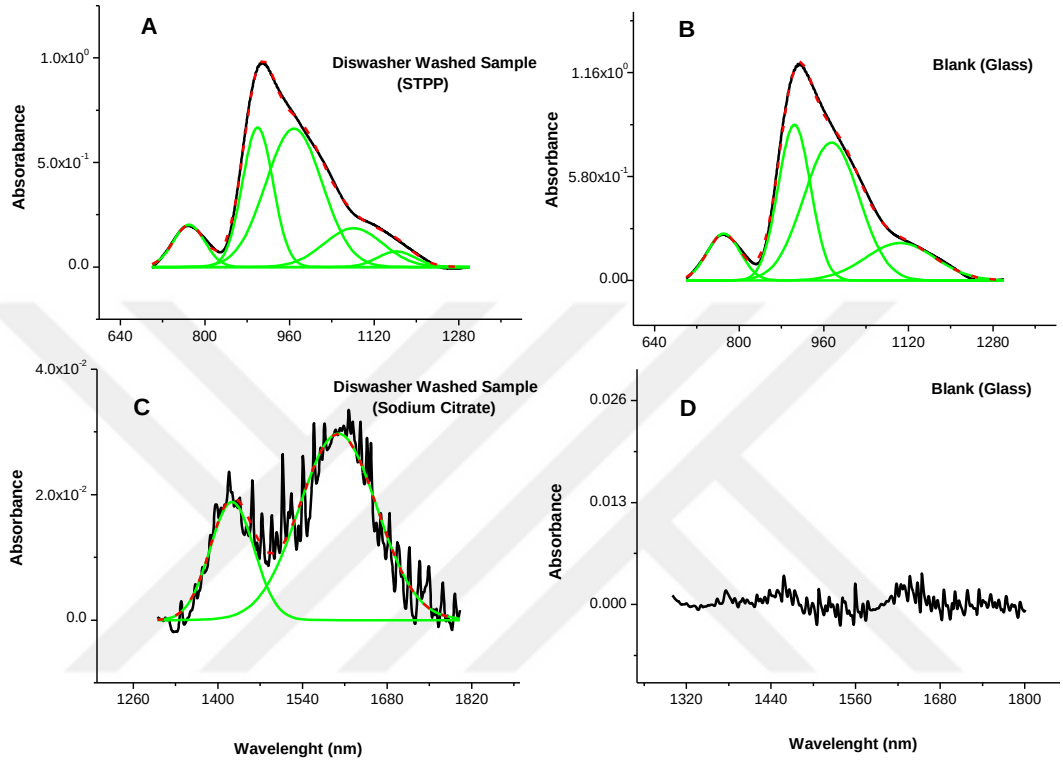


Figure 4.14. (A) IR spectra of dishwasher washed glass sample with STPP (B) IR spectra of clean glass as a blank between 700 and 1300 cm^{-1} (C) IR spectra of dishwasher washed glass sample with sodium citrate (D) IR spectra of clean glass as a blank between 1300 and 1800 cm^{-1}.

4.2. Simple Colorimetric Analysis of Surfaces

As it was stated in Chapter 3.2.3, phosphate and sulfate test kits are available in the market and the surface of dishes washed in dishwasher could be analyzed directly by these test kits. Since they have ppm level detection and analysis could be made by eye observation. However, there is none citrate test kit available in market, it might be the because of the fact that its color change reactions are not well known. Therefore, there was a need for the development of a test kit to assess the sodium citrate concentrations quickly by a simple calorimetric method.

4.3.1. tri-Sodium Citrate Dihydrate Analysis by a Colorimetric Method

4.3.1.1. Color Change Reaction of Sodium Citrate

As shown in Figure 4.15, yellow iron (III) chloride hexahydrate solution has changed its color to green by sodium citrate addition.

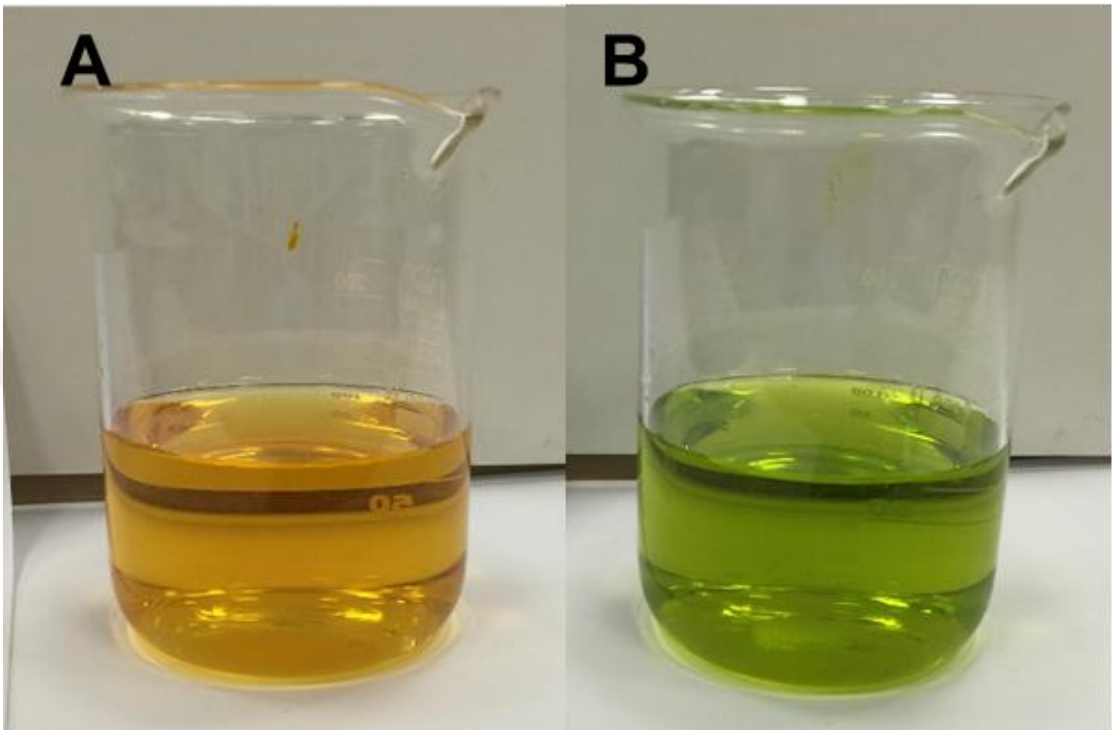


Figure 4.15. Picture of samples prepared by addition of iron (III) chloride hexahydrate into sodium citrate solution to show the color change reaction between iron (III) chloride hexahydrate and sodium citrate. A) 0.05 M iron (III) chloride hexahydrate solution. B) The mixture of 0.05 M iron (III) chloride hexahydrate and 0. 2 M sodium citrate solution.

The result shows that $Fe^{+2/3}$ ions and citrate could make a complex and can react with each other leading to a color change.

4.3.1.2. Determination of Sodium Citrate Residue on Dishwasher Washed Sample by a Simple Colorimetric Method

After finding the above mentioned color change reaction of sodium citrate, it was investigated that whether this method can work at low concentrations and the color intensity of solution would change with respect to sodium citrate concentration.

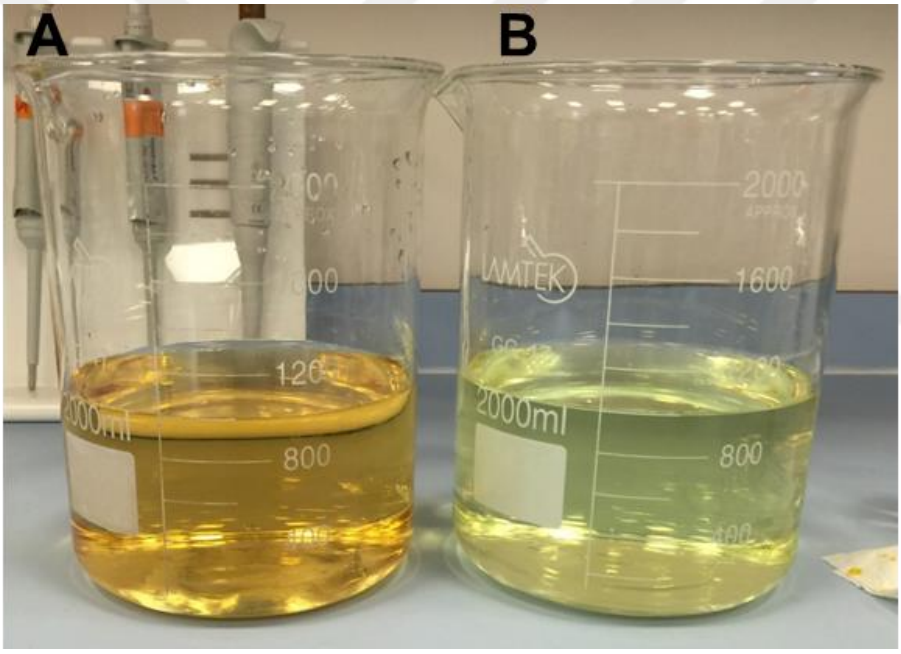


Figure 4.16. Picture of samples prepared by addition of iron (III) chloride hexahydrate into dilute concentrations of sodium citrate solution to show the color change reaction between iron (III) chloride hexahydrate and sodium citrate. A) 0.001 M iron (III) chloride hexahydrate solution. B) The mixture of 0.001 M iron (III) chloride hexahydrate and 0.0005 M sodium citrate solution.

Figure 4.16 shows that even at ppm levels, this method is applicable for sodium citrate analysis on surfaces washed in dishwasher containing sodium citrate as a builder in it. After this result, the real case was performed. In other words, the samples washed in dishwasher with European detergents were put in distilled water. Then, this water was analyzed by adding iron (III) chloride hexahydrate on it.

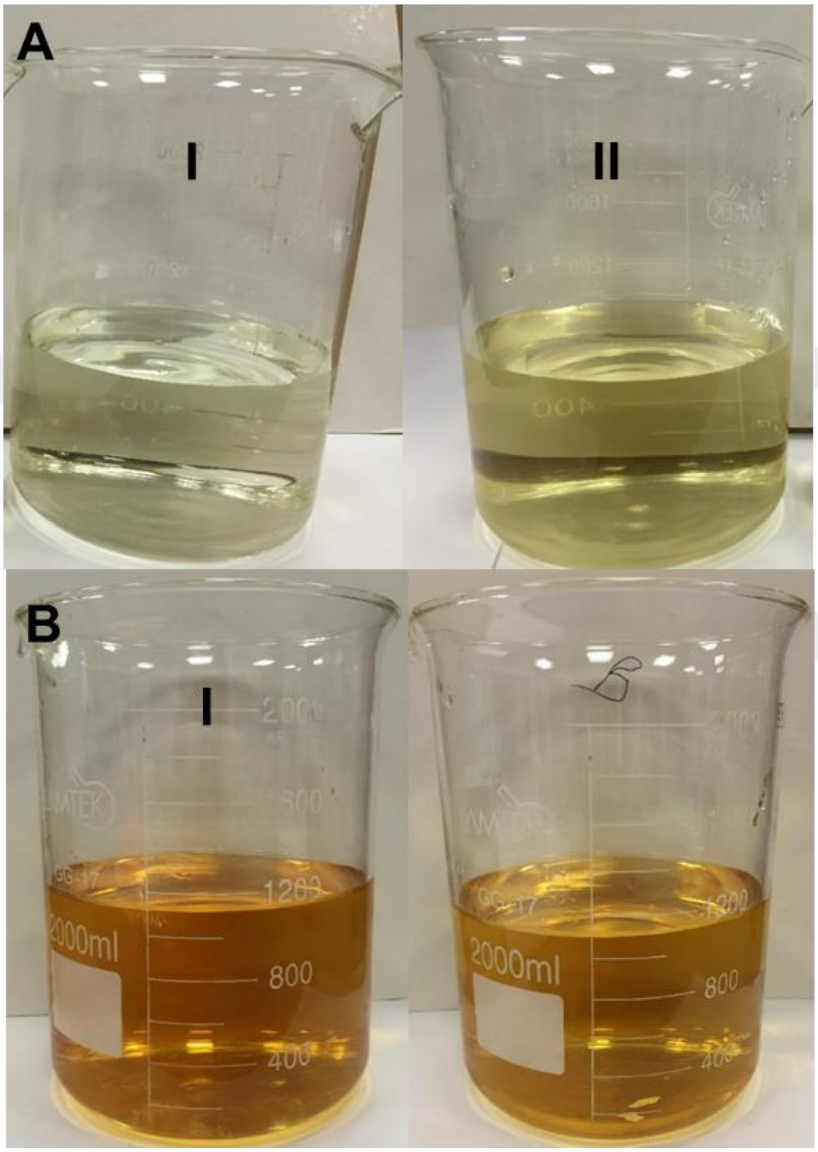


Figure 4.17. Picture of samples prepared by addition of iron (III) chloride hexahydrate to distilled water containing dishwasher washed glass samples to show the color change due to detergent residue on dishes A) I) Control Sample- only 0.001 M iron (III) chloride

hexahydrate solution (Just after the reaction had started). II) The solution of **5**0 glass samples washed in dishwasher with European standard detergent dipped in 0.001 M iron (III) chloride hexahydrate (Just after the reaction had started). B) I) Control Sample- 0.001 M iron (III) chloride hexahydrate solution (60 min after the reaction had started). II) The solution of **5**0 glass samples washed in dishwasher with European standard detergent dipped in 0.001 M iron (III) chloride hexahydrate (60 min after the reaction had started).

As it could be seen from Figure 4.17, dishwasher washed glasses washed using European standard detergent caused color change in hexahydrate solution. This means that sodium citrate leaves residues after dishwasher washing process and it was determined by simple colorimetric test.

4.3.1.3. Determination Residue Amount of Sodium Citrate after Dishwasher Washing Process by Simple Colorimetric Method (Sodium Citrate Test-Kit Development)

4.3.1.3.1. Standardization of iron (III) chloride hexahydrate Concentration

The concentration of iron (III) chloride hexahydrate is so significant that effects the color intensity of final solution directly. In order to standardize the concentration, different concentrations of iron (III) chloride hexahydrate solutions were prepared. They were observed by both eyes and UV/vis spectroscopy.

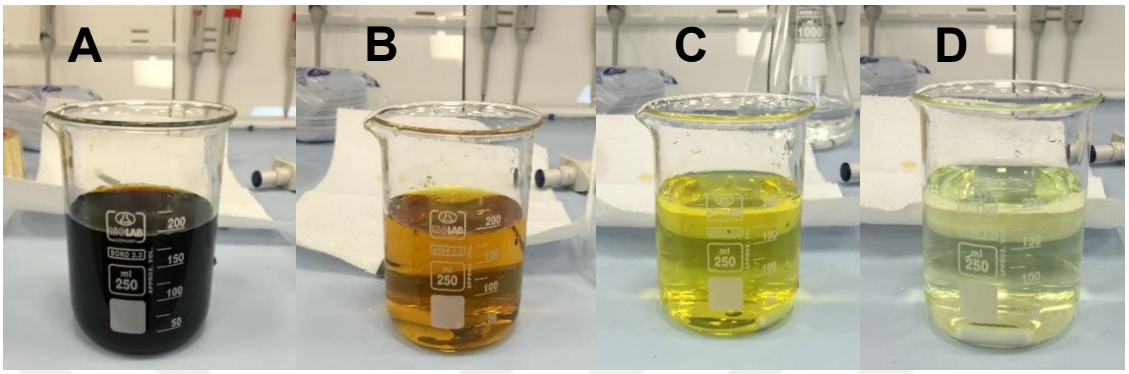


Figure 4.18. Picture of samples prepared by addition of varying concentration of iron (III) chloride hexahydrate into altered concentration of sodium citrate solution to show the color intensity relation with decreasing iron (III) chloride hexahydrate concentrations. A) The mixture of 1 M iron (III) chloride hexahydrate and 1 M sodium citrate solution. B) The mixture of 0.1 M iron (III) chloride hexahydrate and 0.1 M sodium citrate solution. C) The mixture of 0.01 M iron (III) chloride hexahydrate and 0.01 M sodium citrate solution. D) The mixture of 0.001 M iron (III) chloride hexahydrate and 0.001 M sodium citrate solution.

Figure 4.18 demonstrates that although same ratio of iron (III) chloride hexahydrate and sodium citrate was used, the colors of solutions have changed drastically. This color change indicates that selecting proper concentration for iron (III) chloride hexahydrate is crucial.

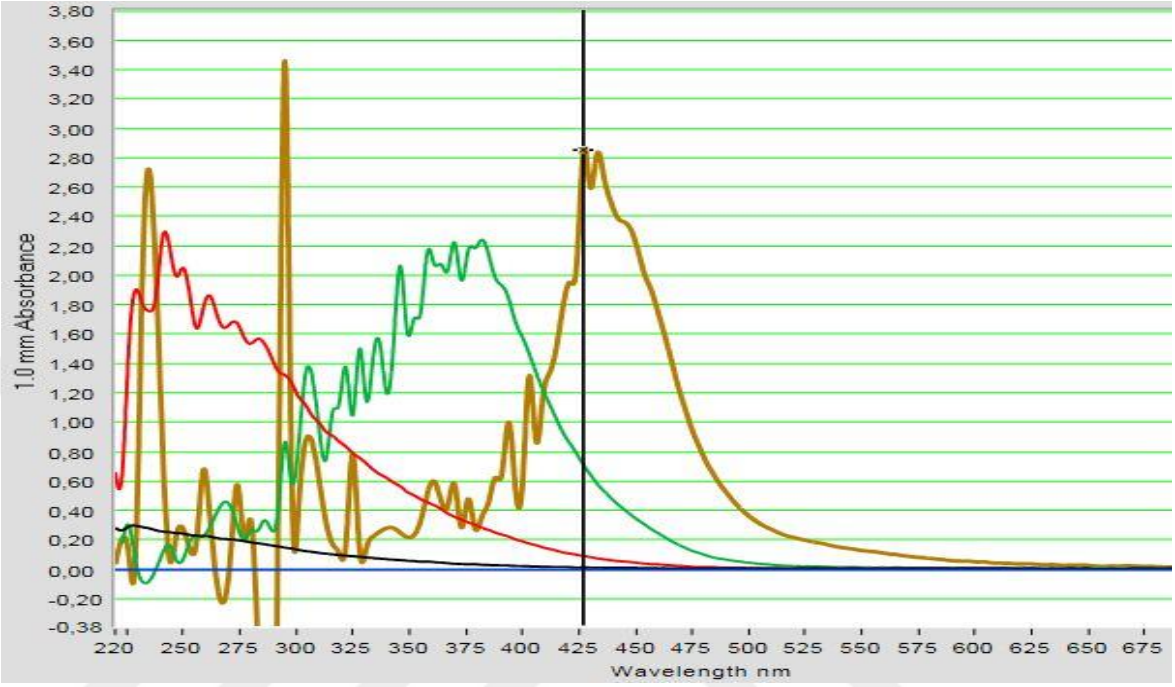


Figure 4.19. Graph of samples prepared by addition of varying concentration of iron (III) chloride hexahydrate into altered concentration of sodium citrate solution to show the color intensity relation with decreasing iron (III) chloride hexahydrate concentrations. The yellow line represents the absorbance of mixture of 1 M iron (III) chloride hexahydrate and 1 M sodium citrate solution. The green line represents the absorbance of mixture of 0.1 M iron (III) chloride hexahydrate and 0.1 M sodium citrate solution. The red line represents the absorbance mixture of 0.01 M iron (III) chloride hexahydrate and 0.01 M sodium citrate solution. The black line represents the absorbance mixture of 0.001 M iron (III) chloride hexahydrate and 0.001 M sodium citrate solution.

As shown in Figure 4.19, the features in the UV/vis spectra shifts with increasing solution concentration as expected. However, highest concentration solution is dark and it is the only concentration that visible light was absorbed. At this concentration, low level of sodium citrate will not be determined. In other words, the residue analysis cannot be done at this concentration. On the other hand, at the lowest concentration considered, the absorbance peak shifts through the X-Ray region. This could cause a problem during analysis of detergent residue. In order to overcome this problem, the calibration curves of different concentrations of solutions were prepared by using both 0.001 M iron (III) chloride hexahydrate and 0.01 M iron (III) chloride hexahydrate separately.

4.3.1.3.2. Standardization of Measurement Time

In Figure 4.19., the color of solution changed in one hour. Therefore, time of measurement should be determined effectively. Prepared solutions at same concentration were analyzed by UV-Vis spectroscopy after 1, 2, 3, 4, 5, and 6 hours respectively. As a result, absorbance intensities of solutions were not changed significantly after 1 hour. Hence, 1 hour was selected as the optimum time for measurement.

4.3.1.3.3. Developing Calibration Curves for Colorimetric Analysis

To prepare calibration curves, two different concentrations of 0.01 M iron (III) chloride hexahydrate and 0.001 M iron (III) chloride hexahydrate was prepared. For two different

concentrations, six different concentrations of sodium citrate were prepared. The measurements were performed by both ATR-FT-IR and UV/vis spectroscopy.

4.3.1.3.3.1 UV/vis Calibration Curve with 0.001 M iron (III) chloride hexahydrate

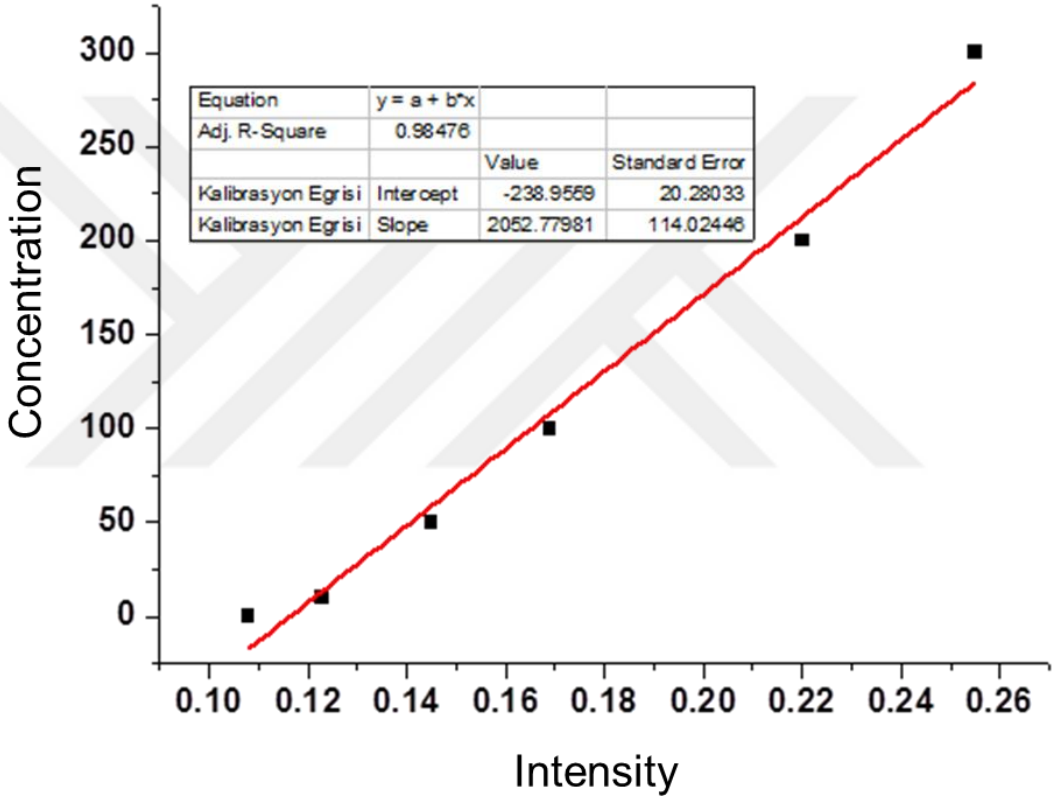


Figure 4.20. Absorbance change of 0.001M solution of iron (III) chloride hexahydrate and different concentrations of sodium citrate (100 ppb, 10 ppm, 50 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, 1000 ppm).

At 294 nm, the absorbance of different concentrations of sodium citrate with constant iron (III) chloride hexahydrate concentration were measured by UV/vis spectroscopy. Above

300 ppm, the intensity of absorption was no change significantly and the spectra began to shift through visible light. Therefore, the determination of proper concentration for iron (III) chloride hexahydrate is so important that we prepared another calibration curve for 0.01M iron (III) chloride hexahydrate.

4.3.1.3.3.2 UV-Vis Calibration Curve with 0.01 M iron (III) chloride hexahydrate

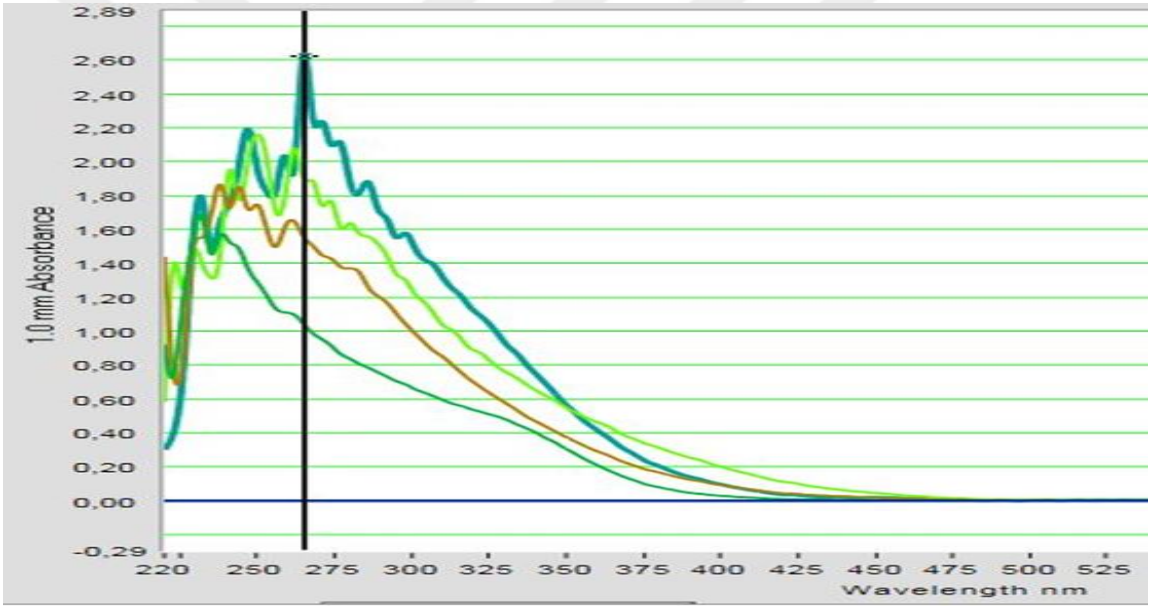


Figure 4.21. Graph of samples prepared by addition of varying dilute concentration of iron (III) chloride hexahydrate into altered concentration of sodium citrate solution to show the color intensity relation with decreasing iron (III) chloride hexahydrate concentrations. The green line represents the absorbance of mixture of 0.01 M iron (III) chloride hexahydrate and 0.001 M sodium citrate solution. The brown line represents the absorbance of mixture

of 0.01 M iron (III) chloride hexahydrate and 0.005 M sodium citrate solution. The light green line represents the absorbance mixture of 0.01 M iron (III) chloride hexahydrate and 0.01 M sodium citrate solution. The blue line represents the absorbance mixture of 0.01 M iron (III) chloride hexahydrate and 0.02 M sodium citrate solution.

Figure 4.21 indicates that increasing concentration of sodium citrate causes shifting in the features in UV/vis absorbance graph. Therefore, the calibration curve was designed to base on wavelength instead of intensity.

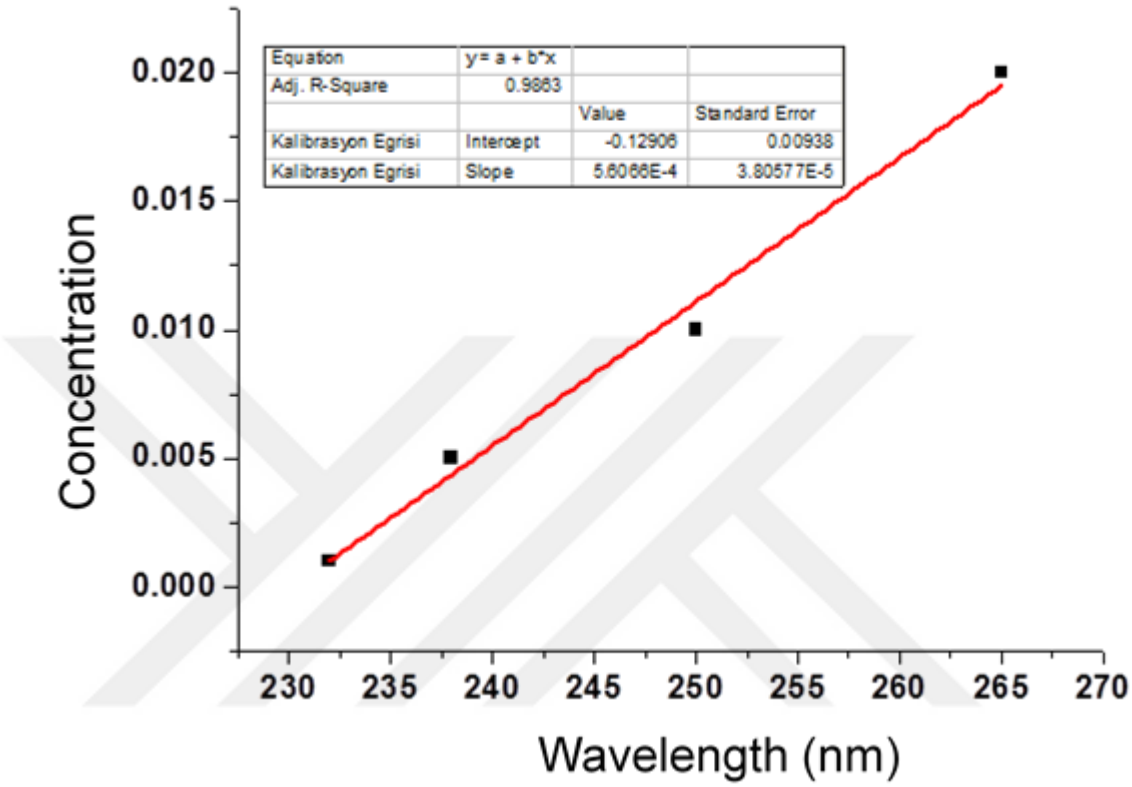


Figure 4.22. Absorbance change of 0.01 M solution of iron (III) chloride hexahydrate and different concentrations of sodium citrate (0.001 M, 0.005 M, 0.01 M, 0.02 M)

In Figure 4.22., calibration curve of 0.01 M iron (III) chloride hexahydrate reacted with different concentrations of sodium citrate was shown. Figure 4.21. and 4.23. enable to measure long range of sodium citrate concentration. According unknown residue concentration, one of these graph will be used to measure the amount of residue on dishes.

4.3.1.3.3.3 ATR-FT-IR Calibration Curve with 0.01 M iron (III) chloride Hexahydrate

To support the results of UV/vis spectroscopy calibration curves, IR calibration curve was also obtained. Different concentrations of sodium citrate was mixed with 0.01M iron (III) chloride hexahydrate and their IR spectrum was collected.

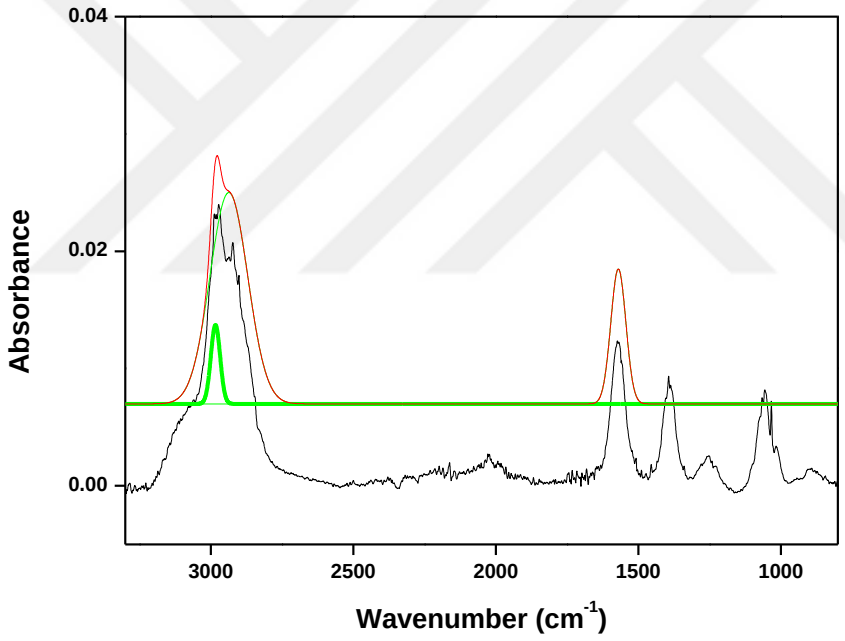


Figure 4.23. IR spectrum of 0.01 M iron (III) chloride hexahydrate and 0.1 M sodium citrate mixture. Red line represents the spectra obtained by fitted peaks. Green line illustrates the fitted peaks.

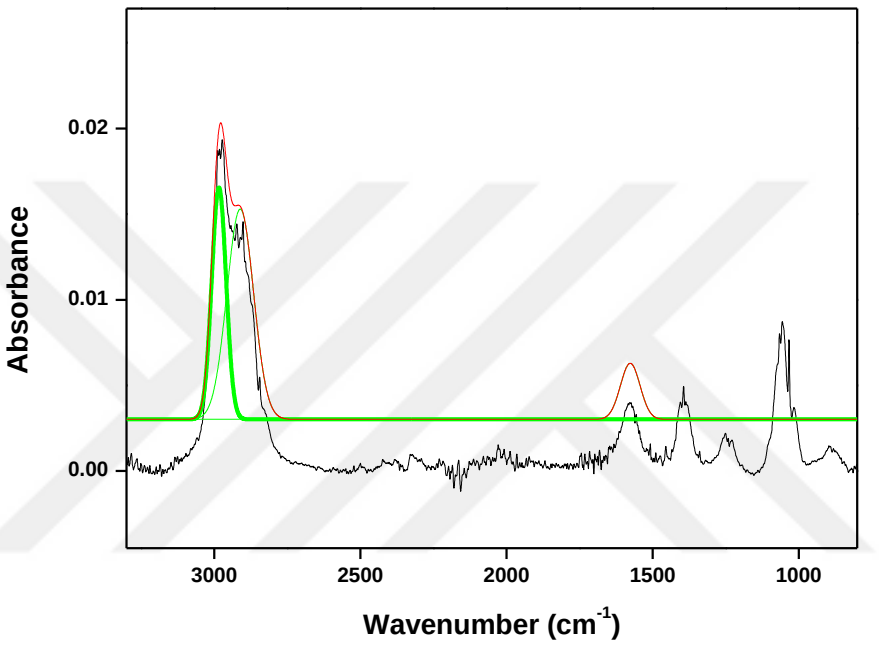


Figure 4.24. IR spectrum of 0.01 M iron (III) chloride hexahydrate and 0.04 M sodium citrate mixture. Red line represents the spectra obtained by fitted peaks. Green line illustrates the fitted peaks.

As shown in Figure 4.23 and 4.24, the peak intensity of the band at 1600 cm^{-1} increases with increasing concentration of sodium citrate. To obtain a calibration curve, this peak height was normalized to peak at 2950 cm^{-1}, because intensity of at 2950 cm^{-1} was constant for all spectra.

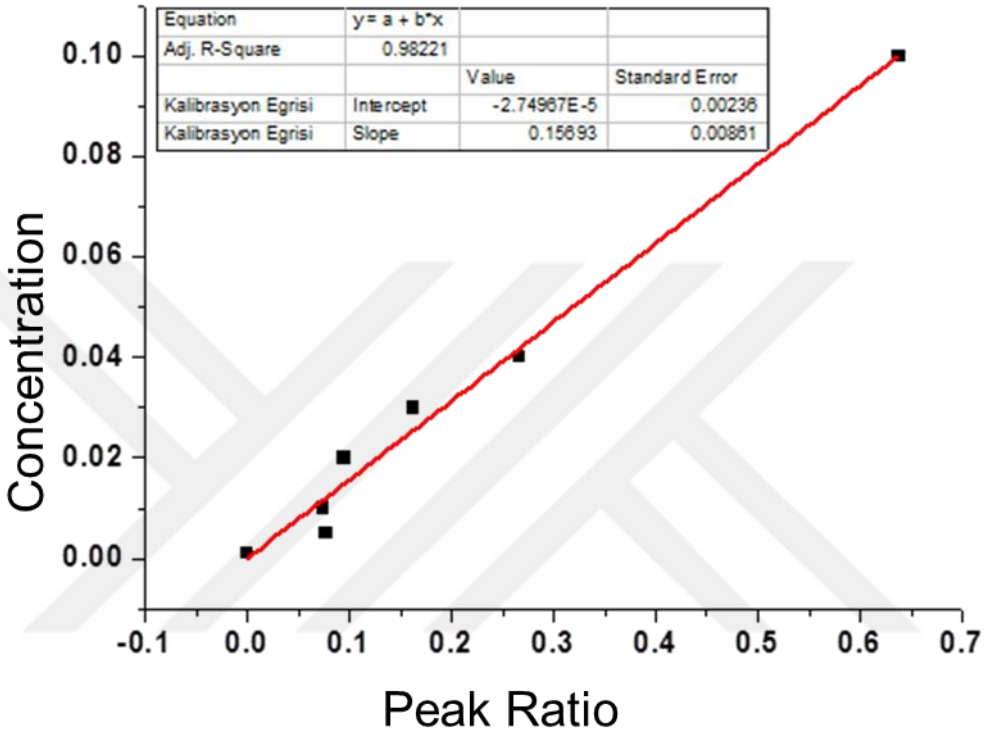


Figure 4.25. IR calibration curve of the mixture of 0.01 M iron (III) chloride hexahydrate and different sodium citrate concentrations.

As it could be seen from Figure 4.26, a good fit was obtained by IR results of solutions. Beside determination of the concentrations by just color difference, the unknown residue amount of sodium citrate after dishwashing could also be easily determined by both UV/vis and FT-IR calibration curves. With those results, we obtained our own test kit for quick determination of sodium citrate.

4.3.1.4. Possible Interruptions for Colorimetric Analysis of Sodium Citrate

As it is stated before, sodium citrate test kit was obtained for quick residue analysis. However, in real case, polisher or food residues could interrupt to observe desired color change. To determine their effect on quick colorimetric residue analysis, several foods and polisher were added to mixture of iron (III) chloride hexahydrate and sodium citrate.

4.3.1.4.1 Effect of Rinse Aid (Shinier)

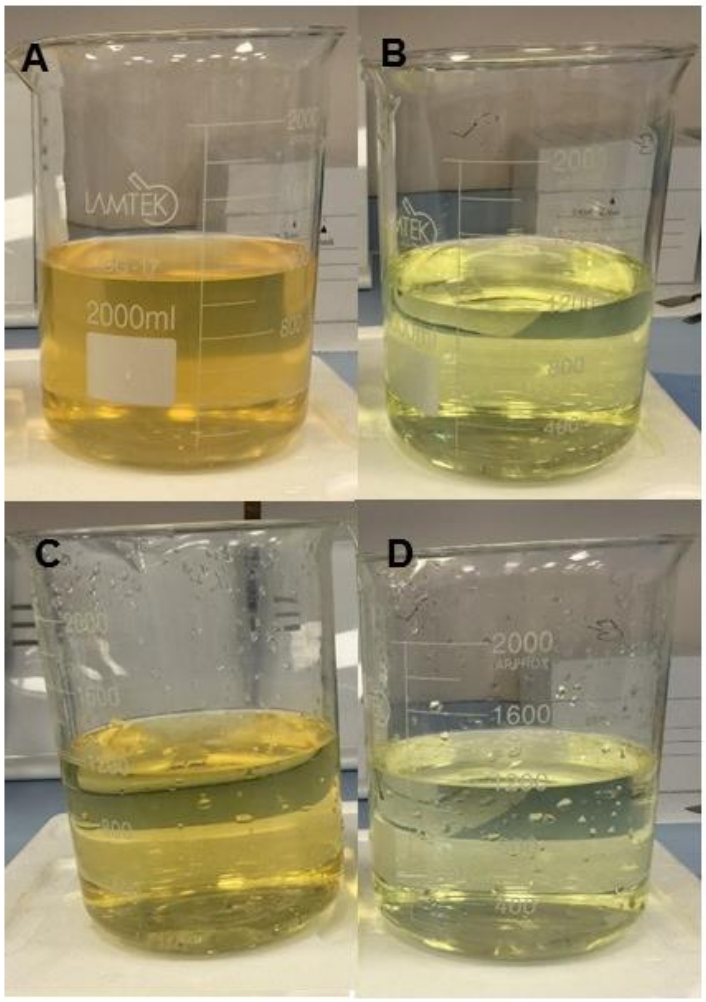


Figure 4.26. Picture of samples prepared by addition of iron (III) chloride hexahydrate to distilled water containing dishwasher washed glass samples and rinse aid (shinier) to show the effect of rinse aid in color change reaction between iron (III) chloride hexahydrate and detergent residues. A) Control Sample- 0.001 M iron (III) chloride hexahydrate solution; B)

The solution of 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate; C) Control Sample- 1 ml rinse aid (shinier) added 0.001 M iron (III) chloride hexahydrate solution; D) 1 ml rinse aid (shinier) added 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate solution.

Figure 4.26 demonstrates that the rinse aid (shinier) effects the color slightly. However, the amount of polisher was too much in this experiment with respect to real case. That is, it is though that it will not affect the color of solution for detection residue of sodium citrate in real case.

4.3.1.4.2 Effect of Food Residues

Food residues could also interrupt to color change analysis for residue detection of sodium citrate after dishwashing. To investigate the effect of food residues, the color change was observed by using several food added to the solution of 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate.

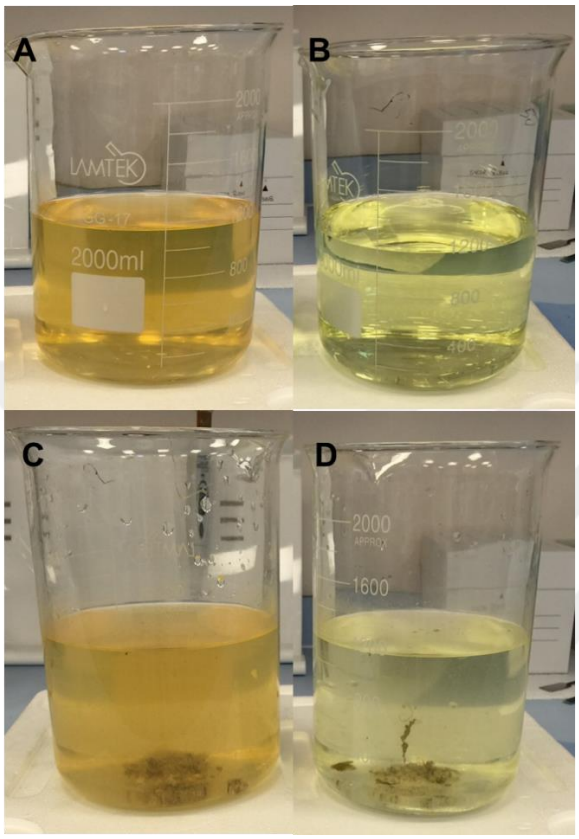


Figure 4.27. Picture of samples prepared by addition of iron (III) chloride hexahydrate to distilled water containing dishwasher washed glass samples and spinach to show the effect of spinach in color change reaction between iron (III) chloride hexahydrate and detergent residues. A) Control Sample- 0.001 M iron (III) chloride hexahydrate solution; B) The solution of 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate; C) Control Sample- Spinach added 0.001 M iron (III) chloride hexahydrate solution; D) Spinach added 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate solution.

Spinach affects only the clarity of solution. It does not cause any color change.

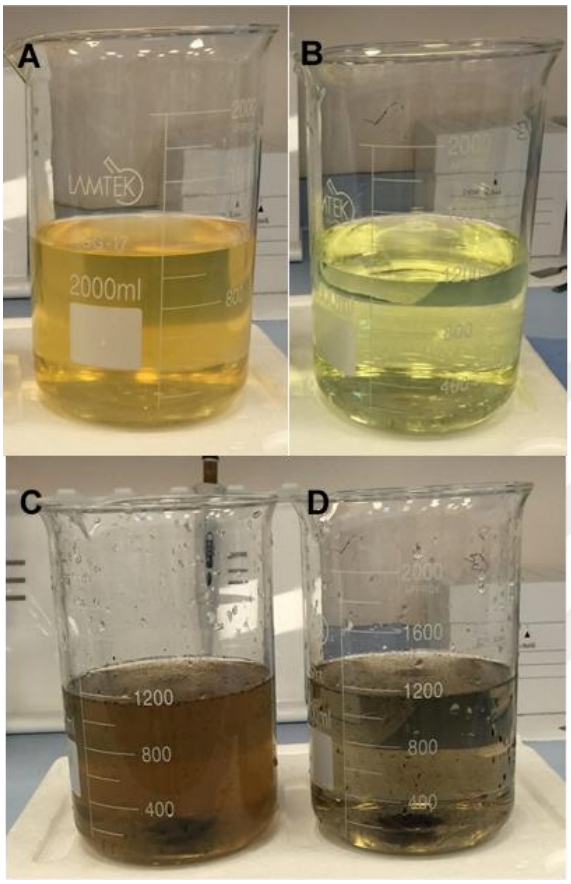


Figure 4.28. Picture of samples prepared by addition of iron (III) chloride hexahydrate to distilled water containing dishwasher washed glass samples and tea to show the effect of tea in color change reaction between iron (III) chloride hexahydrate and detergent residues. A) Control Sample- 0.001 M iron (III) chloride hexahydrate solution; B) The solution of 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate; C) Control Sample- Tea added 0.001 M iron (III) chloride hexahydrate solution; D) Tea added 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate solution.

Tea causes a change in color of solution. However, the difference between two solutions is still obvious. Therefore, it could be said that tea residue will not be a problem in colorimetric detection of sodium citrate.

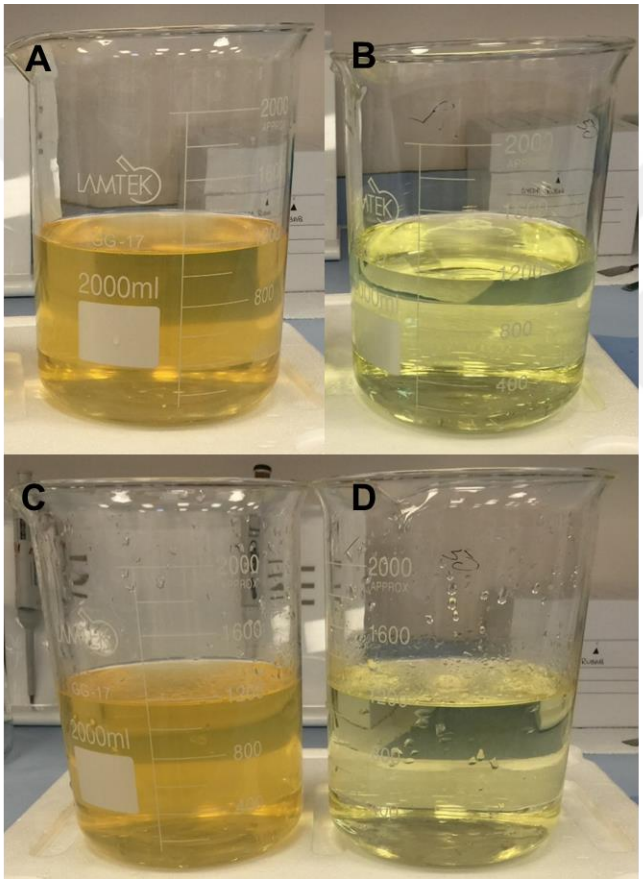


Figure 4.29. Picture of samples prepared by addition of iron (III) chloride hexahydrate to distilled water containing dishwasher washed glass samples and oil to show the effect of oil in color change reaction between iron (III) chloride hexahydrate and detergent residues. A) Control Sample- 0.001 M iron (III) chloride hexahydrate solution; B) The solution of

0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate; C) Control Sample- Oil added 0.001 M iron (III) chloride hexahydrate solution; D) Oil added 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate solution.

Oil affects only the clarity of solution. It does not cause any color change.

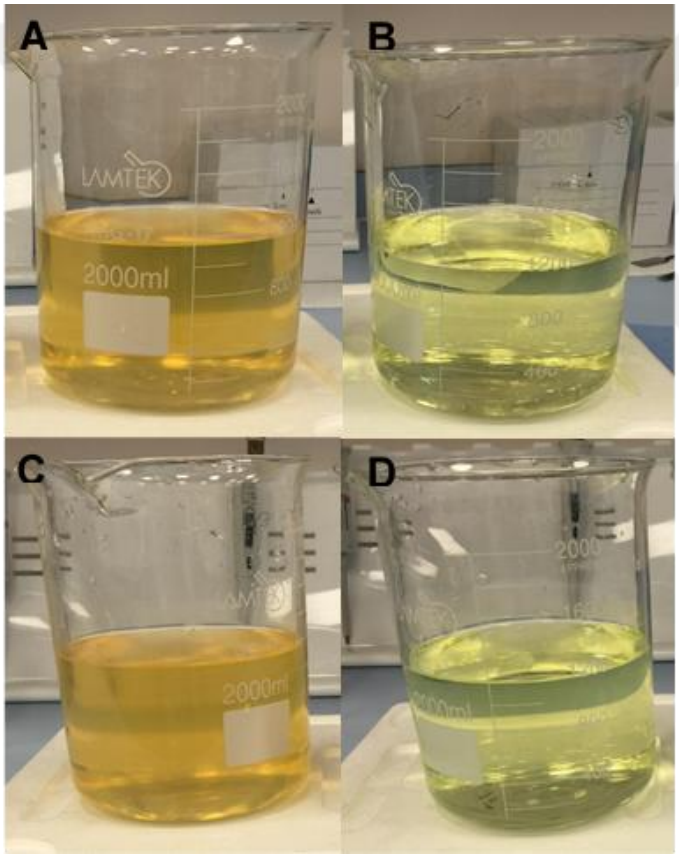


Figure 4.30. Picture of samples prepared by addition of iron (III) chloride hexahydrate to distilled water containing dishwasher washed glass samples and milk to show the effect of milk in color change reaction between iron (III) chloride hexahydrate and detergent

residues. A) Control Sample- 0.001 M iron (III) chloride hexahydrate solution; B) The solution of 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate; C) Control Sample- Milk added 0.001 M iron (III) chloride hexahydrate solution; D) Milk added 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate solution.

Milk does not cause any color change.

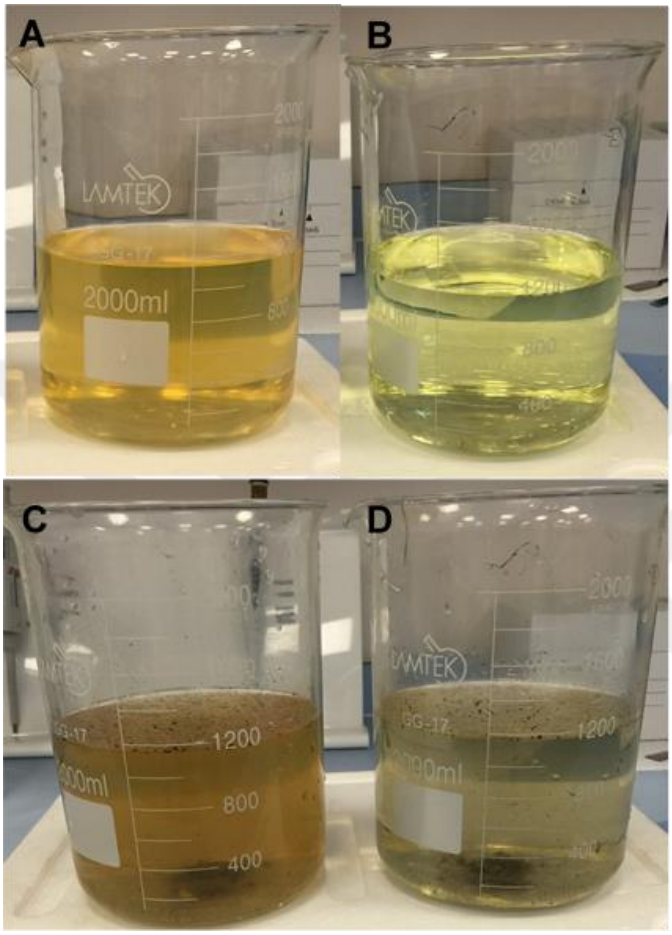


Figure 4.31. Picture of samples prepared by addition of iron (III) chloride hexahydrate to distilled water containing dishwasher washed glass samples and combination of all types of food to show the effect of combination of all types of food in color change reaction between iron (III) chloride hexahydrate and detergent residues. A) Control Sample- 0.001 M iron (III) chloride hexahydrate solution. B) The solution of 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate C) Control Sample- Combination of all types of food added

0.001 M iron (III) chloride hexahydrate solution. D) Combination of all types of food added 0.001 M sodium citrate and 0.001 M iron (III) chloride hexahydrate solution.

Combination of foods causes a change in color of solution. However, the difference between two solutions is still obvious. Therefore, it could be said that combination of food residues will not be a problem in colorimetric detection of sodium citrate.

The results show that polisher and food residues dos not affect the color change reaction of odium citrate and iron (III) chloride hexahydrate. Therefore, it can be said that this colorimetric analysis method is also suitable for real case.

4.4. Viability of Hek 293 (Kidney Cells) Incubated in Detergent Builders Containing Medium

Figure 4.32. demonstrates the cell viability results of human kidney cells seeded in STPP and sodium citrate containing medium. While, 0.0233 mg corresponds to the amount of residues calculated for human daily exposure to these substances via food intake, 70 mg/kg of body/day demonstrates maximum tolerable daily intake amount of STPP. The lowest level that produced nephrocalcinosis in the rat (1% P in the diet) is used as a basis of evaluation daily intake amount of phosphate. Here, the consistent results with the maximum daily intake amount of STPP were obtained by performing cell viability experiments. Data given in Figure 4.32. shows that the cells continue to proliferate in a medium containing

0.014 mg STPP corresponding to 0.84 mg/kg of body/day. 0.014 mg STPP was exposed to 5000 cells. This value was converted 0.84 mg/kg of body/day by considering the cell amount in 60 kg of human body weight and it is significantly higher than the residue amount that we calculated. (0.0233 mg/kg of body/day). Thus, we infer that the amounts of STPP and sodium citrate residue that were detected in our measurements are considered as safe. However, with increasing concentration of STPP, cell viability decreased significantly. Cell survival was minimum on day four when cells were exposed to 14 mg of STPP, this much amount corresponds to 84 mg/kg of body/day. This result supports the results of evaluated daily intake amount of phosphate (70 mg/kg of body/day). Similarly, cells could proliferate in low amount of sodium citrate containing medium. Increasing amount of sodium citrate in the medium compromised cell survival on day four. The results indicated that 84 mg/kg of body/day of sodium citrate decreased viability critically for both STPP and sodium citrate. Although, cells seeded in citrate show higher viability than cells incubated in 84 mg STPP containing medium, the cell proliferation decreases in low concentrations of sodium citrate (0.84 mg/kg of body/day) containing medium on day four. Viability results reveal that daily residue amounts of STPP and sodium citrate are in the safe zone for human health. However, their long term effects are still not known.

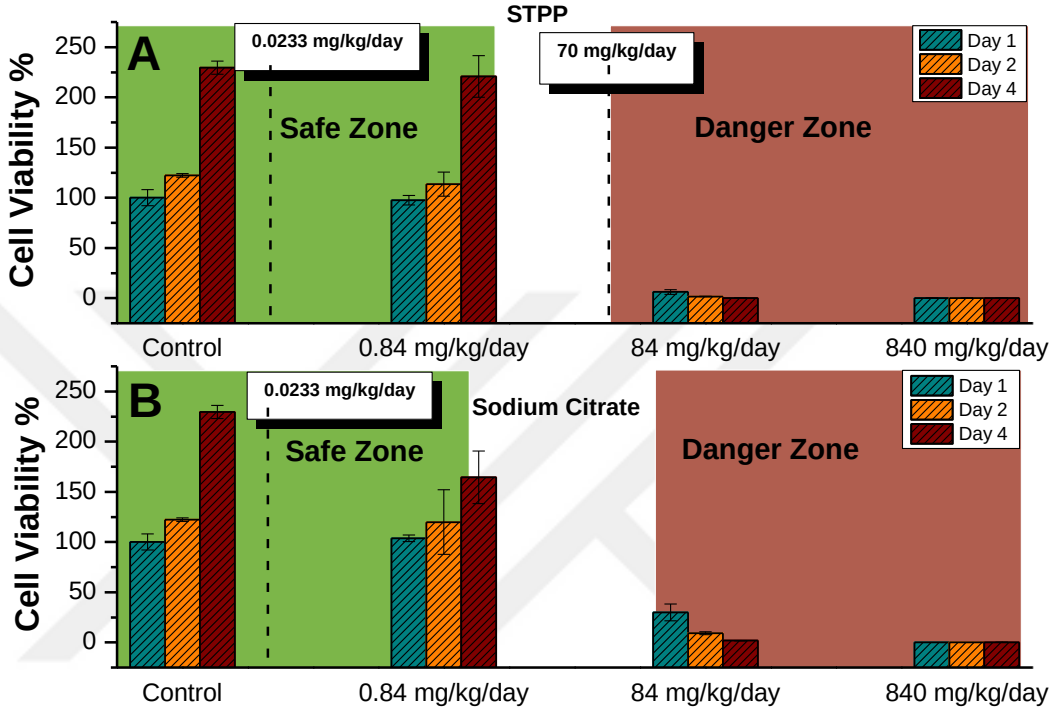


Figure 4.32. Cell viability results of human kidney cells seeded in STPP and sodium citrate containing medium.

Chapter 5

CONCLUSION

In this study, methods for the determination of detergent residues on dishes have been developed by combining different characterization techniques. First, dishwasher washed glass surfaces were analyzed qualitatively to detect whether detergent residue presents or not on dishwasher washed glass surface. Spectroscopic and diffraction methods were performed for surface analysis. Then, in order to calculate the residue amount on glass surface washed in dishwasher, calibration models for a range of STPP and sodium citrate concentrations (1 to 8 wt%) precipitated on glass surfaces was successfully developed for different characterization techniques. The amount of STPP and sodium citrate residue on the surface of dishwasher-washed dishes was determined by using ATR-FTIR calibration curve. Other than spectroscopic techniques, we also developed a method enabling quick colorimetric analysis of dishwasher washed samples. To analyze the surface of dishes washed with European standard detergent, we designed a test kit to determine the amount of sodium citrate. Results from these techniques show that the detergents leaves residues upon dishwashing process in dishwasher. Therefore, cell viability assay was performed to observe the effects of these residues. Cell viability results suggested higher cell cytotoxicity with

increasing STPP and sodium citrate concentrations. This study is significant for the comparison of two equivalent commercial builders in terms of their residue characteristic, so that the amount of builders could be optimized through careful consideration of environmental and health effects.

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VITA

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