SYNTHESIS AND CHARACTERIZATION OF POLYHEDRAL OLIGOMERIC SILSESQUIOXANE CONTAINING DYE AND ITS USE AS A DYE FOR CELL IMAGING

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
ATILIM UNIVERSITY

RIHAB FOUZI SALIH ALFALAH

A MASTER OF SCIENCE
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IN
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A THESIS SUBMITTED TO
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RIHAB FOUZI SALIH ALFALAH

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IN
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AUGUST 2020

Approval of the Graduate School of Natural and Applied Sciences, Atilim University.

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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science in Chemical Engineering and Applied Chemistry, Atilim University.

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This is to certify that we have read the thesis "Synthesis and characterization of polyhedral oligomericsilsesquioxane containing dye and it is used as a dye for cell imaging" submitted by " RihabFouziSalihAlfalah " and that in our opinion, it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF POLYHEDRAL OLIGOMERIC SILSESQUIOXANE CONTAINING DYE AND ITS USE AS A DYE FOR CELL IMAGING

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The importance of POSS containing polymers has increased, especially their optical properties, because of its use in many new applications in electronic and photonics devices. The presence of a POSS molecule enhances the mechanical, thermal and physical properties significantly, due to its unique hybrid structure consisting of two parts, one is organic alkyl groups, and the other is inorganic silsesquioxane cores.In this study, a conjugated poly(thiophene-pyrrole-thiophene) polymer was obtained by chemical polymerization of 1-poss-2,5 di (thiophen-2-yl) -^{1}H-pyrrole monomer. The synthesized dye,named as PSNS-POSS, then characterizedby^{1}H-NMR and FT-IR. The polymer is used for staining three different cells (MC3T3, L929, and MCF7).

Keywords: Conjugated polymer, POSS-polymer, POSS containing dyes, cell imaging.

ÖZ

POLİHEDRAL OLİGOMERİK SİLSESKUOKZANİÇEREN BOYA SENTEZİ VE KARAKTERİZASYONU VE HÜCRE GÖRÜNTÜLEME İÇİN BOYA OLARAK KULLANIMI

Alfalah Rihab

Yüksek Lisans, Kimya Mühendisliği ve Uygulamalı Kimya Bölümü
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Ağustos 2020, 54 sayfa

POSS içeren polimerlerin önemi elektronik ve fotonik cihazlarda ve birçok yeni uygulamada çokça kullanılması nedeniyle artmıştır. POSS biriminin varlığı, biri organik alkil grupları diğeri inorganik silseskuioksan çekirdekleri olmak üzere iki kısımdan oluşan benzersiz hibrid yapısından dolayı polimere ait mekanik, termal ve fiziksel özellikleri önemli ölçüde artırır. Bu çalışmada, 1-poss-2,5di(tiofen 2-il)-1 H-pirol monomerinin kimyasal polimerizasyonu ile yeni bir poli(tiyofen-pirol-tiyofen) konjuge polimer türevi sentezlendi. Monomer ve polimerin yapısı ^{1}H-NMR ve FT-IR ile karakterize edildi. Sentezlenen boya,PSNS-POSS olarak adlandırıldı ve üç farklı hücrede (MC3T3, L929, and MCF7) hücre boyama özelliği incelendi.

AnahtarKelimeler: Konjüge polimer, POSS-polimer, POSS içeren boyalar, hücre görüntüleme**.**

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CHAPTER 1

INTRODUCTION

The cell studies are one of the most important field in the medicine and biological science.Since they are the most crucial functional unit of any form of life the visualization of the cell is a critical issue to understand the structure of the cells. Therefore, scientists have been trying out various methods to make the cell visible, certainly not visible with the naked eye, but by using a microscope [1-4]. One of these methods is the staining of cells by using certain dyes in order to see the cell under microscope and this is called imaging technology. The main reasons for the synthesis of the polymer dye from conjugated polymers are because of their potential nature to enhance the optical properties, adjustable biocompatibility, good mechanical response, and chemical stability. Two decades ago, polyhedral oligomeric silsesquioxanes (POSS) have started to attract great attention due to their unique hybrid organic/inorganic structure which gives opportunities to mix the properties of the two groups, and also create composites with new and unique properties. The nanoscale sizes, interfacial and surface properties are acting an essential role in many POSS applications. The nano-structure POSS has been used to improve the properties of the conjugated polymers. The covalently bonded units showed improvements not only in mechanical, electrochemical, and optical properties of the polymers but also enhancements in the solubility of conjugated polymers which is vital for the easy applicantion of the polymers on electronic and photonic devices. This dissertation describes our work in the synthesis and characterization of PSNS-POSS polymer and its unique properties, especially their optical properties, and its use in staining of the different types of cells.

1.1 Conjugated polymers

Polymers were used commonly as structural components during the 20^{th} century, when the chemists produced few works in functional electronic and optical materials

fields besides organic small molecules. In the late 1970s, this has changed due to the discovery of conductance of polyacetylene. The polyacetylene ,which is an unsaturated polymer, conducts electricity along the backbone of the polymer when it is subjected to chemical doping. After this discovery, the groundwork for today understanding of conjugated polymers was laid by Hideki Shirakawa, AlanHeeger, and Alan MacDiarmid [5,6]. Conjugated polymers (CPs) are organic molecules have an alternating single and double bond along their backbone, and it is found out that these polymers have efficient light collection molecules and desirable properties of many applications such as light emitting diodes (LED) electrochromic devices and solar cells [7,8]. Conjugated polymers exhibit extraordinary electrical and optical properties because of interaction and molecular orbital overlap among *p* orbitals [9,10] Today the conjugated polymers can be used to transduce, detect, and amplify the biological, chemical, or physical information into electrical and/or optical signals [11,12] thismeans the chemists can design the polymer according to the application needed. Commonly used conjugated polymers can be named as polyaniline, polypyrrole, polyacetylene, poly(p-phenylene), polythiophene, poly (3,4-ethylene dioxythiophene). (Figure 1.1)

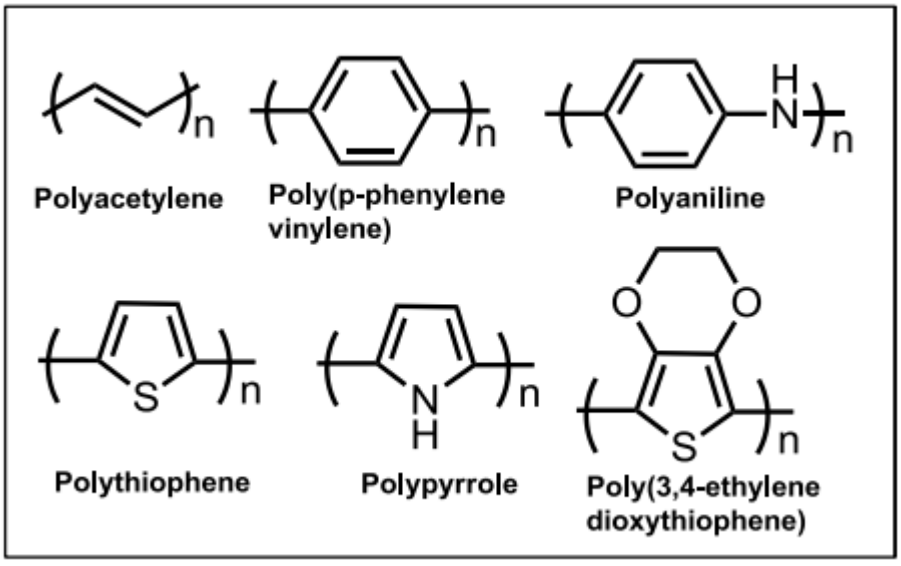


Figure 1.1. Commonly used conjugated polymers [13]

1.1.1 Conjugated polymer nanocomposites

We have been using composite materials in our daily life without even noticing. Scientist have been trying to enhance the different properties like thermal and mechanical to widen the usage of materials. The nanomaterials have been attracting the polymer scientists due to the promising outcomes that obtained from nanotechnological applications. It can be noted that polymer nanocomposites that consist of a polymer matrix and filler in nanoscale exhibited superior properties when they compared with analogous.Conjugated polymeric nanocomposites can be prepared by blending directly or by situ synthesis of the polymer. As shown in Figure 1.2 the synthesis of conjugated polymer nanocomposite includes monomer infiltration into material mesoporous, usage of surfactant to direct formation of the nanocomposite, the monomer partitioning and simultaneous incorporation, the emulsion encapsulation, and polymerization. The top-down nano-mechanism like ball milling are rarely employed. Mostly used nanofillers are graphene, fullerene, carbon nanotubes (single or multi-walled), metal or ceramic nanoparticles. It is also noted that after the addition of nano-fillers the conjugated polymeric nanocomposites attracted great attention because of their advantages like processability and good electroactivity [14,15]. It was demonstrated that the assembly of conjugated polymers in ordered nanoscale can control the interchain transfer of energy, add the mechanical strength, align chains, assist the processability, alsoit showed enhancement in the polymer functional properties such as conductivity, luminescence, energy excitation, and conformational changes control.All these improvements allow conjugated polymer nanocomposites to beusedin information storage devices, light-emitting diodes, optical signal processors, or as solar energy converters [16].

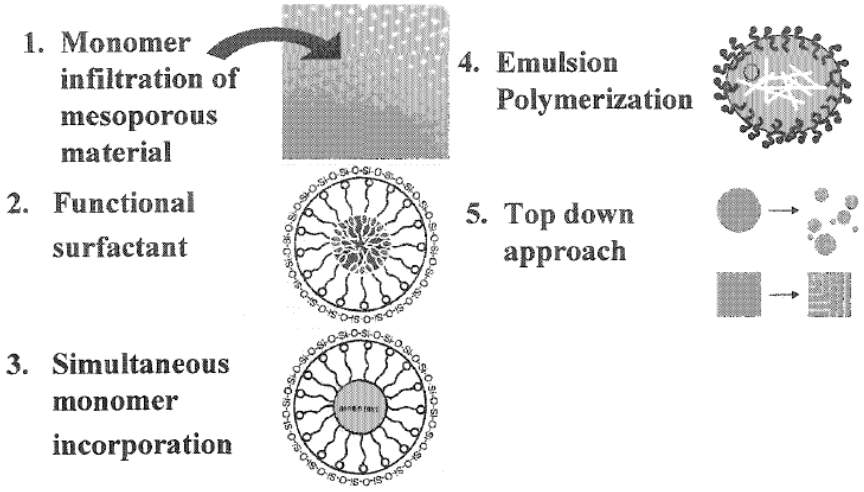


Figure 1.2. Conjugated polymers nanocomposite synthesis methods [16].

1.1.2 Polyhedral oligomeric silsesquioxane (poss) and its use in conjugated polymer nanocomposites

The name silsesquioxane is divided into three sections [sil - sesqui -oxane] where the term [sil-] expresses the presence of (silicon), [-sesqui-] indicates the ratio 1.5 between the oxygen and silicon atoms, and [-oxane] pointing to the presence of an oxygen atom. The term Silsesquioxanes refers to compound with formula $[R\_Si\_O_{1.5}]$ In which the hybrid structures consist of thermally stable and inert inorganic part [silicon and oxygen atoms] in the center, circulated by organic part (R group) that easily modified and reactive at the eight vertices [17,18] as shown in the figure 1.3. The R group may be hydrogen or various organic chains or functional groups such as alkylene, alkyl, arylene, aryl, or organo-functional derivatives as epoxy, amine, isocyanate, vinyl, acrylate, etc.

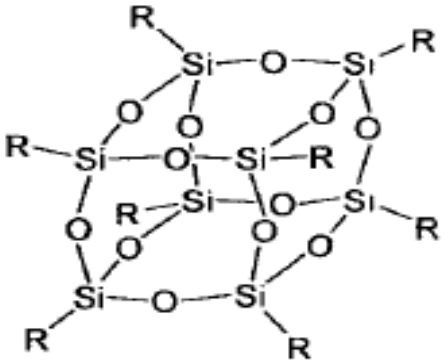
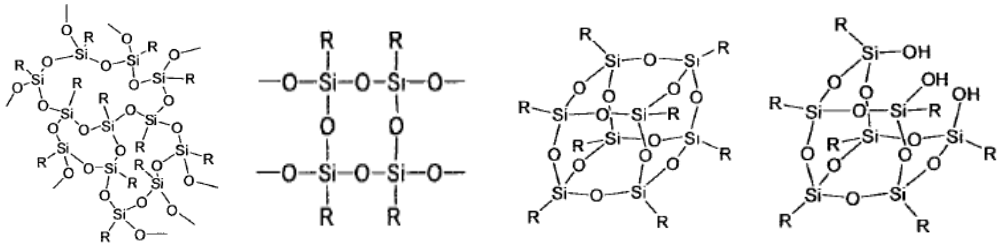


Figure 1.3. The structure of polyhedral oligomeric silsesquioxane (POSS). [19]

Based on the atomic arrangement, silsesquioxanes are categorized into two categories: non-caged silsesquioxanes containing random or ladder structures [20,21], and cage-shaped silsesquioxanes which are a fully or partially condense structure which is known as a polysesquioxane or polyhedral oligomeric silsesquioxane [POSS]. Figure 1.4 illustrates the different forms of silsesquioxanes.



(A) **(b)** **(c)** **(d)**

Figure1.4.Silsesquioxanes structure, a) Random structure with disorder structure POSS, b) ladder shape structure of POSS, c) Cage structure, d) Open cage structure of POSS. [19]

Silsesquioxane based material can be used in a set of applications, for example, as a compound in polymer nanocomposite catalysts [22-26], in silica surface models [27], low-k dielectrics [28,29], heterogenous catalysts [30], antimicrobial agent [31,32], in organic light-emitting diodes (OLEDs) as emitting layers [33], and in the coating [34]. POSS molecules can be easily combined due to their excellent compatibility with most monomers and polymers, biocompatibility is the primary characteristic as well as non-toxicity and cellular compatibility from its advantages as well. This makes it suitable for medical and biological applications [35,36]. The fully condensed cage-like structure contains regular 3-D shapes consisting of 8, 10, and 12 silicon atoms linked to an oxygen atom and the organic group's outer shell merged together and formed the 3D cage structure. There are different methods for manufacture partial cage-like POSS by controlling synthesis conditions like precursors stoichiometry, temperature, and catalysts [37] or split one corner of the full condensed cage [38].

1.1.2.1 Application Of POSS Polymer:

The studies showed that incorporation of POSS in the linear polymer as a pendant group, mostly increases glass-transition and degradation temperatures, and oxygen permeability, also reducing flammability. There are a lot of studies regarding POSS containing polymers in many applications with a wide variety of scopes starting from the catalyst, to coating, surface modifier, and membrane material.For example, these organic-inorganic hybrid structural polymers have engaging properties like rising the thermal stability and the elevated glass transition [T_g] Temperature, also it enhances the heat and flame resistance and increases the melting strengths [39], significantly enhances these properties, at low contents (<10 mol % of POSS).

i. Drug Delivery

In general, the clinical side effects of the drugs can be seen as the main reason for the study of drug delivery systems. Nowadays, the aim of drug delivery system is to

carry out the drug directly into a target to take the therapeutic action. Recently silicon hydrogels developed to use as a matrix transdermal drug delivery [40], also developed pH-controlled microspheres of silicon to deliver the drug in the gastrointestinal tract.To understand the silsesquioxane nanocomposite efficiency to be used as a drug delivery agent, McCusker et al, labelled fluorescent dye (BODIPY) with octaammonium Si_8O_{12}, by the subsequent substitution for the derivative of succinimidyl ester, and ammonium sites neutralization by trimethylamine.They foundout that a POSS unit is a key factor that allows POSS-BODIPY combination, also this combination did not effect the cellular morphology, furthermore, the viability assay showed that the cell with POSS-BODIPY has the same activity, and this indicates into the low toxicity. Moreover, it entered into a cell by diffusion not by endocytosis. The POSS cage was potential as a drug delivery system, it's directly conjugated to a drug molecule [41].

ii. Dental Nanocomposites

POSS containing materials can be used to enhance the properties of denture materials like wear resistance, shrinkage lack of strength, biocompatibility, the toxicity of polymeric material, elasticity coefficient. Also, the use of POSS nano-structure in hypersensitivity and inflammation reactions of denture materials were reported [30,32]. The nano in diameter and the ability to load a wide reactive group is the unique feature of POSS, furthermore increase in oxygen permeability and glass transition temperature, while reducing flammability observed when incorporated the POSS into the polymer material [42]. Now POSS nanomaterial considers as a potential solution with improvement in some or all these properties.

iii. Biosensors

POSS unit has the ability to combine with a wide range of organic compounds and this ability allows it to be used in probes for biomolecules detection, like proteins and DNA. The resonance light scattering [RLS] technique can be used in the

determination of DNA concentration. It is shown that after the addition of POSS unit in aqueous solution with DNA molecules, the intensity of [RLS] at 360 nm significantly improved, and it was found that it depends on the ionic strength and pH value. Due to these striking features, it can be concluded that POSS containing materials are useful as a probe to DNA concentration determination.Recently, many species that containing POSS has employed as reagents in [RLS] to DNA determination due to its advantages as good stability among a wide pH range, good water solubility, and high sensitivity [42].

iv. Cardiovascular Implants

A nanocomposite polymeric material was obtained by introducing the POSS unit as a pendant group onto poly (carbonate-urea) urethane [POSS-PCU] chain to meet the important requirements for this application as antithrombogenicity, cytocompatibility, and biostability [42,43]. Recently, development of heart valves, prostheses percutaneous heart valves, bypass grafts of the coronary artery, stent-grafts, and using of POSS-PCU nanocomposite as a coating of stents are under inquiry. For example on that bypass grafts of the coronary artery made from this material was implanted in sheep sample, and showed a patency rate 70% over two years [44]. POSS-PCU has been introduced generally in biomedical applications, and particularly in cardiovascular applications. POSS-PCU has been introduced generally in biomedical applications, and particularly in cardiovascular applications. The addition of POSS group enhanced the properties that needed for a biomedical application like mechanical properties, degradative resistance, biocompatibility and biostability. Also, this polymer used now in many biomedical device fabrication, including a microvascular bed of organs, including liver tissue, cartilage, muscle, breast implants [42], quantum dot coating material, and many other applications.

v. Breast Implants

The POSS derivatives could be an alternative to silicone in the breast implant materials. Recently, a study showed that, when compared with the use of silicon and usage of POSS-PCU in tissue implants it was found that the POSS-PCU managed to prevent the inflammations and material degradation.It's concluded in the study that the nanocomposite enhance interfacial biocompatibility and biological stability when compared with silicone, so its a safer in tissue implantation [42].

vi. Tissue Engineering

One of the most rapidly growing biological field is tissue engineering. It combines various sides of the medicine, cell and molecule biology. POSS polymer nanocomposites can be shown as one of the most commonly used materials for biomedical application and tissue engineering in order for the enhancement of people's quality of life and health. In 2007,Gupta et al. [45] conducted some studies on POSS-PCU and showed that the usage of this nanocomposite in the application of tissue engineering in the liver, small intestine, and cartilage is possible. The result of this study shows that the technique offers massive potential in the development of organs.

vii. Other application

A lot of applications on the POSS- derivative polymer has been attempted to other biomedical scopes. For example, it is used as a sun protection agent, cosmetics [43], and in greenhouse covers [46]. Actually the variety of POSS containing polymers have a wide range of applications that encourage prospects particularly in the biomedical area.

1.2 Cell imaging

Cancers are among all diseases a cause of increased mortality and morbidity rate around the world, especially in a developed country. For that for decades carried out enormous researches to overcome this disease. Cancer cell imaging by using a fluorescent probe becomes increasingly important in the diagnosis and treatment of cancer at the early stages.

1.2.1 Dye types

The dyes that using in cell staining are categorized according to chemical structure, their origin, or their properties. Whereas, the chemical structure determines the properties, colors, and uses of dyes, the most common dyes used in medical studies and researches are either natural or synthetic origin. The problems of natural dyes are its extinction from their natural sources, in addition, some environmental problems that happen during the production of these dyes, on the other hand, in the synthetic dyes the main disadvantage is their high price. Therefore, because of these disadvantages, the importance to develop and find a new dye had been increasing [47].

1.2.2 Cell culture

The cell culture is a tool that has been used over 100 years, and it was considered as the most important method used in cell biology, where it gives an excellent system to test the biochemistry and physiology of cells [48]. The first time in which this method used by Beebe and Ewing was in 1906 [49]. The cell culture has been defined as multicellular organism cells that can be kept alive in suitable environmental conditions like humidity, heat, and nutrients in special containers in a laboratory. These conditions changing according to the type of cell, but all cells are cultured at the suitable container that contains, firstly a medium or substrate for supply the cell by the essential nutrients (carbohydrates, minerals, amino acids, and

vitamins), and hormones, also contain growth factors, and gases (CO_2, O_2), and finally physicochemical environment regulation (osmotic pressure, pH, temperature) [50]. The cells for culture depending on the purpose of the study can be taken from an artificial or living environment or from any organ or tissue, then it can kept alive then multiply under suitable conditions. The cell culture method has been preferred in vaccine, and cancer study, also it has preferred for determining the drug cytotoxicity and drug development. The important step in these studies is determining the viability of cells that exposed to biological, chemical, and physical factors.

1.2.3 Cell line used in this study

In this study, three different cell lines were used, these are, MC3T3 cells, L929 cells, MCF7 cells, the description of these cells shown in table 1.1 as follows.

Table 1.1. The properties of cell lines used in the study

Cell line	**MC3T3**	**L929**	**MCF7**
Organism	Mus musculus (Mouse)	Mus musculus (Mouse)	Homo sapiens (human)
Tissue	Bone/calvaria	Connective tissue	Mammary gland, breast
Cell type	Preosteoblast	Fibroblast	Epithelial
Age	Newborn	-	69 years adult-female
Morphology	Fibroblast	-	Epithelial
Growth properties	Adherent	Adherent	Adherent
Storage condition	-130°C in Liquid nitrogen vapor	-130°C in Liquid nitrogen vapor	-130°C in Liquid nitrogen vapor
Disease	-	-	Adenocarcinoma

1.2.4 Use of fluorescence dye in cell imaging

Because of its advantages which are represented in specificity, high sensitivity, low detection limits, and quick response, the demand for Fluorescent imaging techniques accelerated in biological research and cell biology studies like antibody immunoassay, DNA sequencing, anti-cancer drug analysis, and disease diagnosis, for that a lot of fluorescent dye has been designed for detecting ions, labeling biomolecules, or in targeting specific regions in cells and organisms. The biologists have been used Fluorescent dyes for decades, they're also known as fluorophores or reactive dyes. Fluorescent dyes have brightness and photostability if compared with fluorescent proteins it also doesn't need for maturation time before use. We can use a lot of fluorescent dye in living cells, but in some cases still have limitations in their applicability.

i. Fluorescent dyes:

Optical polymers widely used in bioprobes for diagnosis, tracking, and treatment of disease [51], these polymers have a lot of superiorities compared with other fluorescent bioprobes like fluorescent proteins, organic dyes, and semiconductor quantum dots, the superiorities in optical polymers have excellent photostability, good biodegradability, low cytotoxicity, and large cross-section absorption. This makes them promising, useful tool to monitor the biological processes with excellent biocompatibility, and high sensitivity [52]

1.3 The study goals

The purpose of this study is the synthesis and characterization of fluorescent conjugated polymers. The cell staining properties and fluorescence behavior of these dyes were investigated. The dye is described as follows:

1-poss-2,5 di (thiophen-2-yl) -1H-pyrrole) (SNS-POSS)

Recently, the polyaromatics and polyenes polymers such as polypyrrole, polyaniline, poly (p-phenylene), and polythiophene, have great attention, because they are environment-friendly systems, but they are also infusible and insoluble. In order to solve these problems, a lot of substitution derivatives have been developed for these polymers [53]. As adding the POSS unit to the thiophene structure to improve its properties. It is expected to obtain soluble, fluorescent and non-toxic materials to be used in cell staining applications. Figure 1.5 shows the structure of our material.

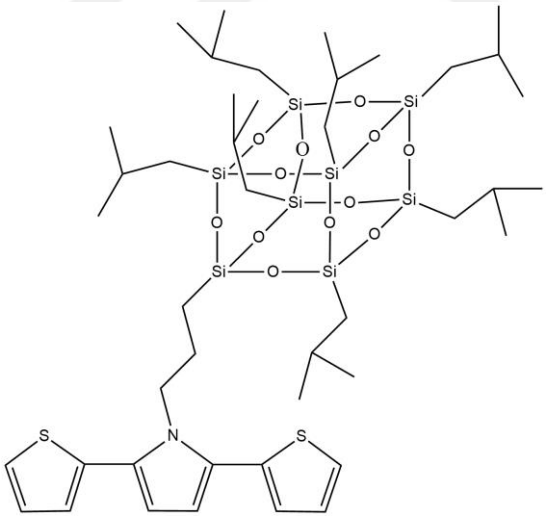


Figure 1.5. The Structure of SNS-POSS monomer

CHAPTER 2

MATERIALS AND METHODS

This chapter deals with synthesis of conjugated POSS polymers by combining the diketone with the unique POSS unit, then characterize the properties of resulting monomer and polymers.Optical activity, solubility, and biocompatibility studies are explained.

2.1Experimental techniques and syntheses:

2.1.1 Materials:

2.1.1.1 Polymer Synthesis Materials

$AlCl_3$ (Aldrich), Succinyl Chloride (Aldrich), Dichloromethane (Merck), $NaHCO_3$ (Aldrich), Hydrochloric Acid (Merck), Toluene (Sigma) which distilled before used, $MgSO_4$ (Aldrich), Thiophene (Aldrich), Chloroform (Merck), Hexan (Aldrich), Ethyl Acetate (Aldrich), Silica 35-70μm (Merck), Calcium Hydrate, aminoposs (Amino propylisobutulposs$C_{31}H_{71}NO_{12}Si_8$) from Hybrid Plastics, Paratoluensulfonic Acid (PTSA), Ethanol (Merck), $KMnO_4$, $FeCl_3$, nitromethane, methanol.

2.1.1.2 Cell Culture Materials

MC3T3 (preosteoblastic cell), L929 (fibroblastic cell), and MCF7 (breast cancer cell) cells line for cells study and cytotoxicity test, P3HB [Poly [(R)-3-hydroxy butyric acid] from Sigma-Aldrich, For poly-b-alanine (PBA) synthesis, chloroform from Merck and acetic acid (RDH Chemicals) that used as a solvent and porogen agent, L-glutamine, FBS, penicillin, streptomycin are obtained from Biowest, MEM, ethanol 70% used as washing and collecting solution from Colony Sugar Mills, D-PBS, MTT and, isopropanol solution are obtained from Sigma-Aldrich, PBS tablets from Sigma-Aldrich, D-PBS also is obtained from Biowest, glutaraldehyde solution that was used

for cell fixation, and HMDS for dehydration of cultured cells were obtained from the Sigma-Aldrich.

2.1.2 Synthesis of Monomer (SNS-POSS)

2.1.2.1 Synthesis Of 1,4-di (2- thienyl) - butane-1,4-dione (DTBD)

1,4-di (2- thienyl) - butane-1,4-dione was prepared by Friedel–Crafts acylation of thiophene with succinylchloride. To a suspension containing 1.6 g (0.012 mol) of$AlCl_3$ in 6ml of dichloromethane (DCM), a solution of thiophene (0.961 ml, 0.012mol) and succinylchloride (0.551 ml, 0.005 mol) in 6 ml of DCM were added dropwise at room temperature, and the mixture was stirred for 1day at the same temperature. The suspension was poured into a mixture of 50 g ice and 0.5ml hydrochloric acid and further stirred for 1hour.The organic layer was separated and the aqueous layer was extracted with dichloromethane. The organic layer and the extract were combined and washed with 150ml of water and aqueous $NaHCO_3$, then dried by $MgSO_4$.After evaporating the solvent a green colored material formation was observed. After dissolving in hot ethanol, it is left to recrystallize in refrigerator. To remove side products the material was subjected to column chromatography with 1:1 hexane: DCM eluent system. The product was recovered as greyish-white needle like solids. The synthetic path of 1,4-di (2- thienyl) - butane-1,4-dione is as shown in Figure 2.1.

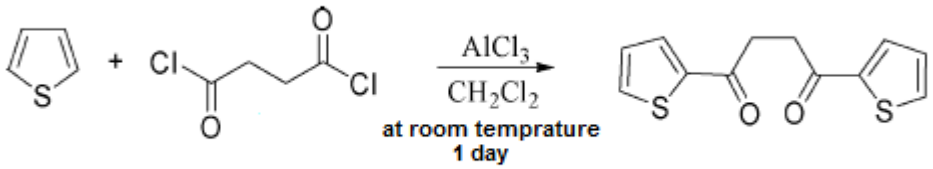


Figure 2.1. Synthesis route of 1,4-di (2- thienyl) – butane- 1,4-dione(DTBD)

^1H NMR (400 MHz, $CDCl_3$, δ(ppm)): 7.75 (d, 2H; thiopheα-H), 7.59 (d, 2H, thiopheneβ`-H), 7.08 (dd, 2H;thiopheneβ-H), 3.33 (s, 4H; CH_2), ^{13}C NMR (100 MHz, $CDCl_3$, δ(ppm)): 158.5, 142.6, 132.5, 130.6, 126.5, 32.1.

2.1.2.2 Synthesis of [SNS-POSS] Monomer

1.65 g (6.6×10^{-4}mol) of the 1,4-di(2-thienyl)- butane-1,4-dione (DTBD), 1.153g(1.32×10^{-3}mol) of the amino-poss (874.58 m.w), and 24 mg from paratoluen sulfonic acid (PTSA) were dissolved in 100 ml of distilled toluene. The mixture was stirred at 110^{o}C in oil container and left to reflux for 48 hours under argon atmosphere. Toluene was evaporated under vacuum and the product was separated by column chromatography on silica gel with hexane as eluent.The synthetic path of the monomer 1-poss-2,5 di (thiophen-2-yl) -1H-pyrrole (SNS-POSS) is a shown in figure 2.2.

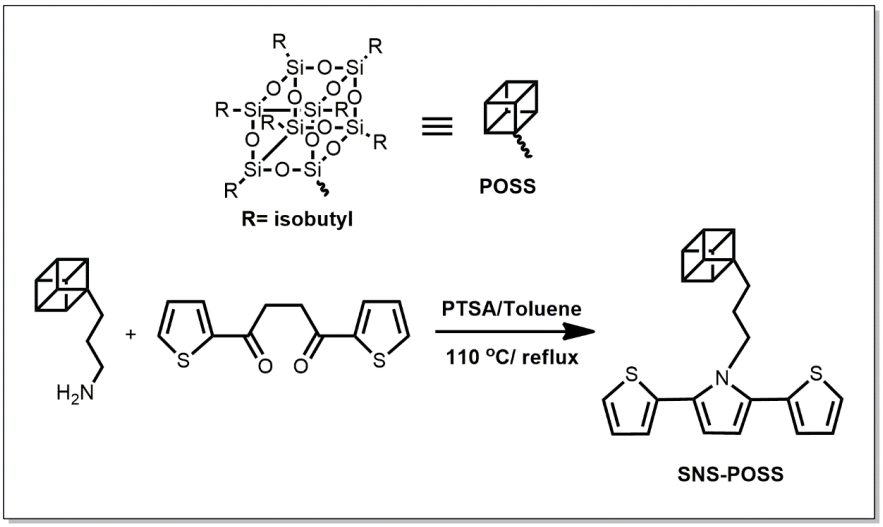


Figure 2.2. Synthesis of SNS-POSS monomer.

^{1}H NMR (400 MHz, $CDCl_3$, δ(ppm)): 7.35 (d, 2H; thiopheα-H), 7.10 (m, 4H, thiopheneβ and β`-H), 6.32 (s, 2H; pyrrole-H), 4.10 (t, 2H; CH_2 bonded to N), 1.80 (m, , 7H; CH_2), 1.80 (m, 2H; CH_2), 0.90 (d, J= 6.62 Hz, 42H; CH_2), 0.58 (t, 2H; CH_2), 0.58 (d, J= 7.02 Hz, 14H; CH_2).

^{13}C NMR (100 MHz, $CDCl_3$, δ(ppm)): 133.9, 128.2, 127.2, 126.1, 124.5, 109.1, 46.4, 32.3, 24.6, 22.8, 21.3, 8.5.

IR (KBr): *v* (cm^{-1}) = 3125 (s; *v*(=C-H in thiophene ring)), 2850-2950 (w; (alkyl units)), 1670 and 1330 (w; (C-N and C=N stretching), 1400-1500 (w; (C=C stretching)), 1230 (s; (Si-C)), 1080 (w; *v*(Si-O-Si and C-O-C), 836 and 680 (s; *v*(C-S in thiophene ring)), 742 (w; (Si-C)).

HRMS (ESI) *m/z*: calcd for $C_{43}H_{77}NO_{12}S_2Si_8$, 1088.88; found 1088.32. (Appendix C)

2.1.2.3 Chemical polymerization of SNS-POSS monomer

The chemical polymerization has been done by direct oxidation of SNS-POSS monomer by $FeCl_3$ [54,55]. By adding three different equivalent molar concentrations (1:2, 1:3, 1:4 monomer to catalyst molar ratio) of pre-dissolved $FeCl_3$solution in nitromethane and chloroform dropwise to 0.1M of SNS-POSS monomer dissolved also in chloroform at 0 ^oC. Then, the mixture was left to stir 24h at room temperature under inert atmosphere.After that the solution was concentrated to a couple of milliliters and 5 ml methanol poured on it to obtain precipitated polymer. The solution was left in the refrigerator overnight. The precipitate was filtrated and washed with methanol, then the obtained product was left to wash at least 12h by Soxhlet equipment with methanol. The chemically obtained polymer was recovered as reddish-brown solid. (Polymers are named as follows: P1 is 1:2 ratio polymer, P2 is 1:3 ratio polymer and P3 1:4 ratio polymer) (Figure 2.3). The Gel Permeation Chromatography results show that there is no result for P1 and the molecular weights of P2 and P3 are found to be 51750 and 128592 respectively. (See Appendix B)

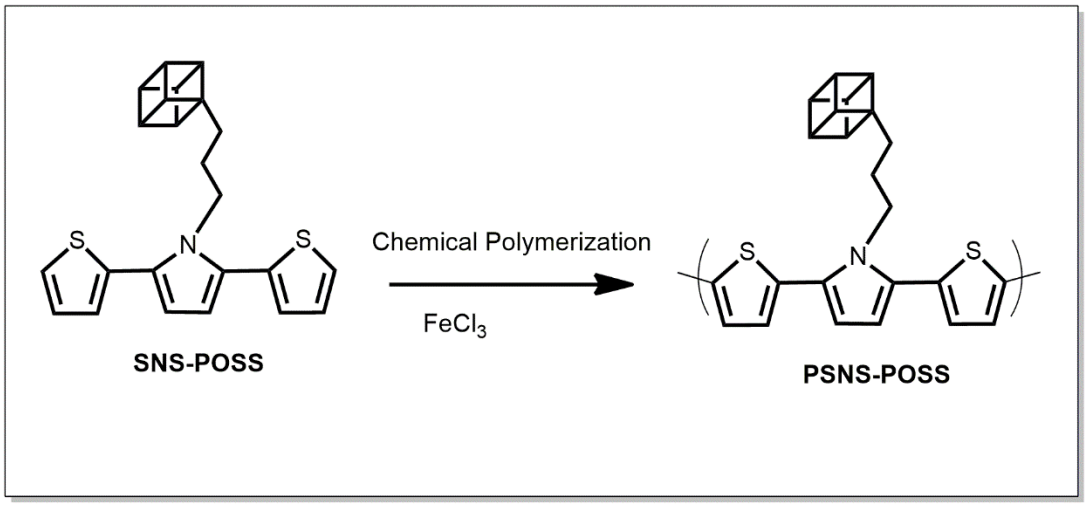


Figure 2.3. Chemicalpolymerization of SNS-POSS monomer

2.2 Cell Culture

2.2.1 Cell culture studies

Cell culture studies were carried out with MC3T3 mouse pre-osteoblastic cell line, L929 mouse fibroblast cell line, MCF7 human epithelial adenocarcinoma cell line. L-L-glutamine (1% v/v), FBS (10% v/v), and penicillin, streptomycin mix (1% v/v) containing α -MEM, DMEM High Glucose and RPMI 1640 cell media were used as growth media for these cell lines, respectively (Figure 2.4). Cells were seeded with density $2x10^4$ cells/well and added 1ml of growth medium upon the cells after 1h. The cells at 37°C and 5% CO_2 atmosphere in (Heraus Instruments, Germany) incubator were cultured for 21 days and the media was refreshed every 3 days. Mitochondrial activities and liabilities of MC3T3, L929, and MCF7 cells have been performed by MTT test on the cell lines at 1st, 4th, then 7th, 14th, and at 21st days from the culture days. The medium has been aspirated from the surface of the cells that are attached to the cell culture plates, then to each well 600UL culture mediumwithout FBS (fetal bovine serum) and 60 UL from MTT ((3-[4,5-dimethyltiazol-2-IL] -diphenyltetrazolium bromide)) solution (2.5 mg/ml from MTT in PBS)wereadded.After incubation for 3h at 37°C, formazan crystals were dissolved by adding 400 UL from 0.04 M HCl that contains isopropanol solution and was measured optical density of 200 uL of the supernatant at 570 nm with reference wavelength 690 nm, and with (Asys UVM 340, Austria) the microplate reader, by SEM analysis the proliferation and attachment of cells were observed morphologically. The medium removed and the samples were rinsed twice with sterile D-PBS (Dulbecco's phosphate buffer solution) at 1^{st}, 7^{th}, then at14^{th} and 21^{st} days of culture day, then the cells were fixed for 30 min with 2.5% (v/v)glutaraldehyde solution, after that the cells were rinsed again with PBS twice and the cells were stored at 4°C in PBS. After series dehydration of the sample with different ethanol dilutionsolutions (30%, 50%, 70%, 90%, and 100%) respectively, HMDS (hexamethyldisilazane) was added to the samples for 5 min before the samples were dried overnight. Before the SEM analysis, coated the sample by gold-palladium.

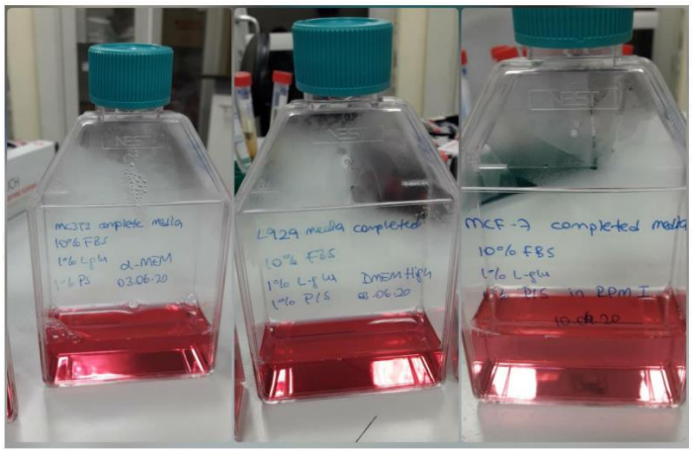


Figure 2.4.Growth media of MC3T2, L929,and MCF7 cells

2.2.2 Manual Cell Viability (Dye-Exclution) Analysis

By viability analysis, separation of live cells from the dead one, the cells from cell culture flask taken onto a hemocytometer (Neubauer chamber) glass lam. By adding trypan blue dye, only the dead cells absorb the dye while live-cell can not. So allocation the live cell from dead cells.

2.3 Cell Counting

Stained/non-stained cells with Trypan Blue dye was analyzed under microscope for cell count. The cells by the dye are enumerated under microscope by a hemocytometer. As shown in figure 2.5.

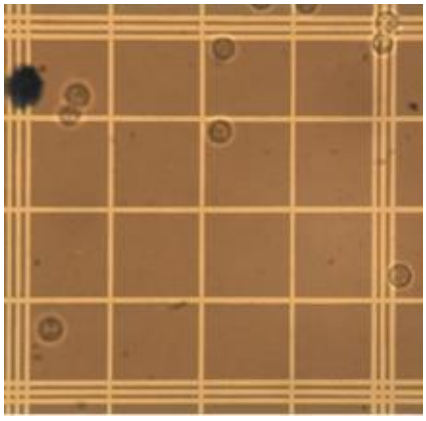


Figure2.5.Cell Counting Procedure

The formula used in the calculation of cell concentration is:

$$\text{Total cell number} = \frac{\text{counted cell}}{\text{number of counted area on hemocytometer}} \times 10^4 \times \text{dilution factor.}$$

2.4 Cell Staining

The cell staining was carried out by (Olympus CKX53 with fluorescent attachment) inverted microscope (Figure 2.6)by using a suitable emission fluorescent filter. The cells that were stained by the fluorescent polymer dye had been imaged by using the microscope. The cells were stained by the polymer just by addition of the polymer dye on to the cells in the 12 well cell culture plates and stored the plates for 24h at the CO_2 Incubator. The cells were seeded in the wells before they were stained.

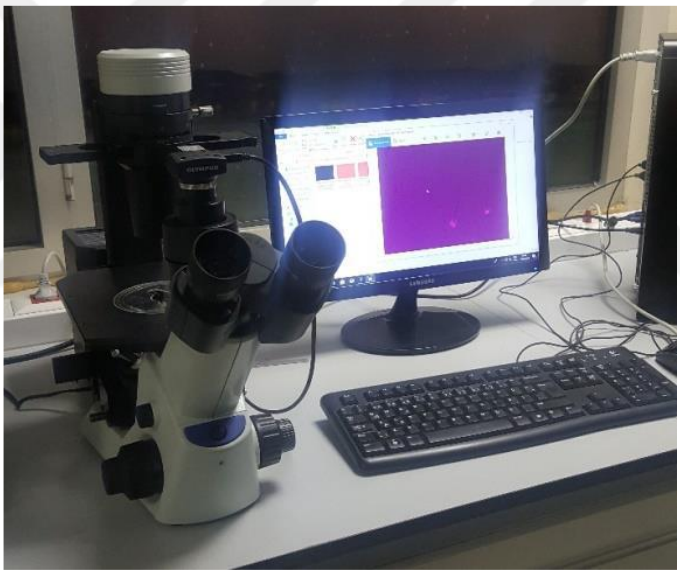


Figure 2.6. Inverted Microscope (Olympus CKX53)

2.5 Cytotoxicity Studies by MTT Assay

MTT method is one of the most widely used methods to analyze cell proliferation and viability. The MTT assay is a colorimetric assay used to determine cell metabolic activity. The assay depends on the conversion of tetrazolium dye MTT to its insoluble purple color formazan by means of nicotinamide adenine dinucleotide

phosphate (NADPH)-dependent cellular oxido-reductase enzymes. This assay therefore measures cell viability by means of enzymatic reduction of tetrazolium compound to to water insoluble formazan crystals which is catalyzed by dehydrogenases occurring in the mitochondria of living cells(Lu et al., 2012; Stockert et al., 2012). In the MTT dimethyl sulfoxide is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution is used for quantification by measuring the wavelength (usually between 500 and 600 nm) by a spectrophotometer.

2.6. Statistical Analysis

Three similar experiments conducted in triplicate and all analysis data were symbolized as mean ± standard deviation (SD). Statistical analysis was carried out by One Way Anova and Student's t-test of GraphPadInstat software. The significant differences among groups were found, shown in related figures, and $p<0.05$ values were accepted as significant.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Characterization of The Polymer

3.1.1 FTIR (Fourier Transform Infrared) Spectra Results of Compounds:

FTIR spectroscopy is a useful way to clarify the POSS cages presence in the backbone of the polymer. In order to do that, it is necessary to look for Si-O-Si stretching band located around 1080 cm^{-1}. As it can be seen in the spectra of both monomer and polymer this band appears very strongly. Also, the signal around 1230 cm^{-1} refers to C-Si bond and this confirms the presence of POSS unit in both monomer and polymer. The peak located around 3125 cm^{-1} is responsible for C-H vibration in thiophene ring and when compared with the spectra of monomer and polymer, it can be concluded that the disappearance of this small peak is the result of polymerization through alpha hydrogen of thiophene unit. The peak located at 1670 cm^{-1}which can be seen in both monomer and polymer represents the C-N stretching and it can be clearly said that the intensity of the peak increases with polymerization. The peaks responsible from alkyl groups bonded to POSS unit is located around 2850-2950 cm^{-1}. In addition to all these, the peaks resulting from C-S bond can be seen clearly around 836 and 680 cm^{-1}.

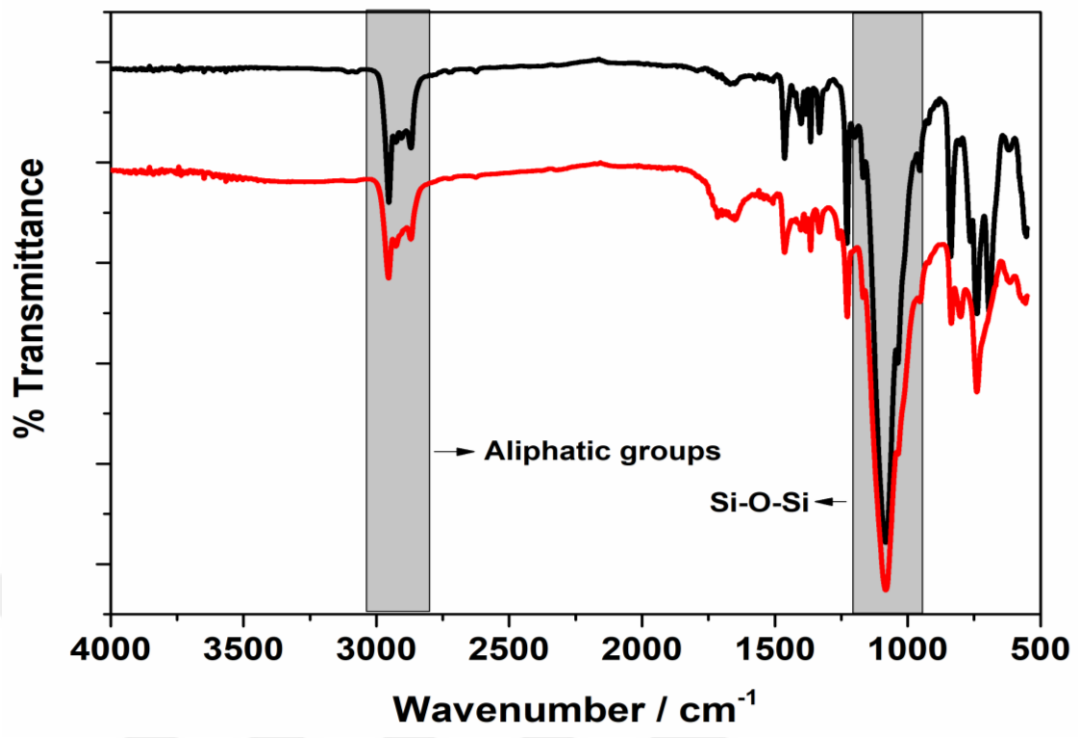


Figure 3.1. FTIR Spectra Results of SNS-POSS

3.1.2 Optical Behavior of Polymers

Optical behaviors of the monomer and polymers were investigated by utilizing UV-Vis and Fluorescence spectrometers. Figure 3.2 gives the comparison of the UV-Vis spectra of the monomer and polymers. Absorbance spectrum of the SNS-POSS monomer shows that there is a peak centered at 285 nm. Due to solvent cut off the peak located below 230 nm can not be determined. After the monomer was polymerized in the presence of $FeCl_3$ the formation of a shoulder has started to appear. It is expected that the increase in the concentration of oxidant used during polymerization yields higher molecular weights for the corresponding polymers. It is also known that the fine structure of corresponding UV-Visspectra is influenced strongly with the chain length. When the absorbance spectrum of the polymers were inspected it can be clearly seen that increase in the molecular weight of the polymer caused a bathochromic shift in absorption spectra of corresponding polymers. In the

absorption spectra of P3 the shoulder starting to appear on P1 and P2 becomes a clear peak located at 345 nm.

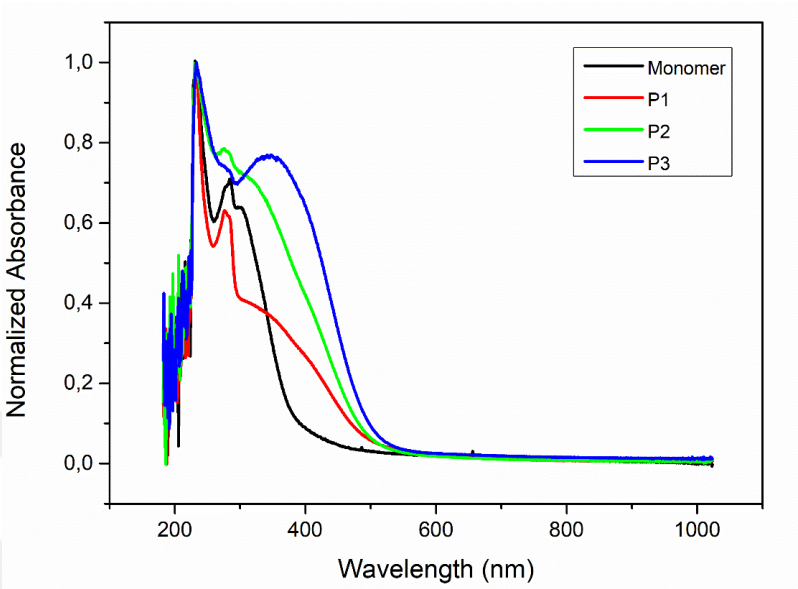


Figure 3.2. Comparison of absorbance spectrum of SNS-POSS, P1, P2 and P3. Monomer and all the polymers have fluorescent behavior under UV light

The emission behaviors of the monomer and the polymers are given in the following figures. While the monomer has an absorption peak at around 285 nm, it showed an blueish emission with a 410 nm wavelength. Luckly all the polymers were recorded as soluble in most of the organic solvents and have fluorescent behavior under UV light. P1 polymer has an absorption maxima at around 280 nm and a shoulder located around 330 nm and it has an emission centered at 540 nm with a yellow color. On the other hand, in the absorption spectra of P2 the shoulder becomes more apperant and the peak at 280 nm is still the dominant one. P2 also showed a yellow emission with 540 nm wavelength. Finally, P3 showed a clear absorption peak with a 345 nm maxima while the peak at 280 nm starts to become invisible. In addition to that, P3 has a yellow emission with a 547 nm wavelength. It can be stated that all the polymers have very bright emission under UV light as it can be seen in the corresponding figures.

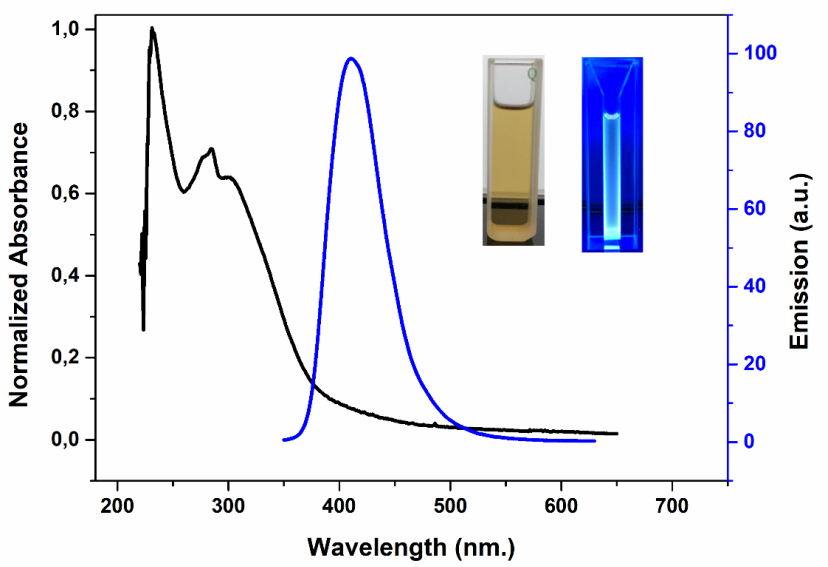


Figure 3.3. Absorbance (black) and emission (blue) spectra of SNS-POSS in DCM. Inset: Colors of the SNS-POSS in DCM under ambient light (left) and hand held UV lamp (right) at 365 nm.

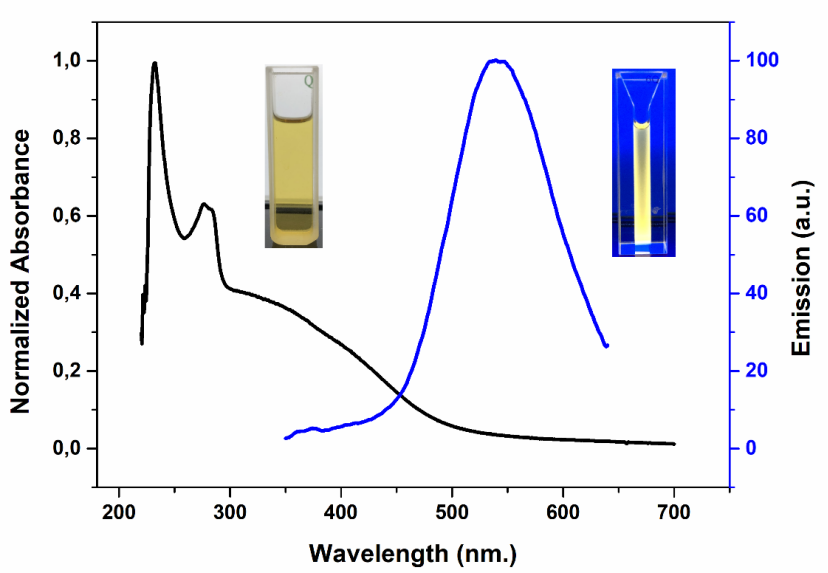


Figure 3.4. Absorbance (black) and emission (blue) spectra of SNS-POSS in DCM. Inset: Colors of the PSNS-POSS-1 (P1) in DCM under ambient light (left) and hand held UV lamp (right) at 365 nm.

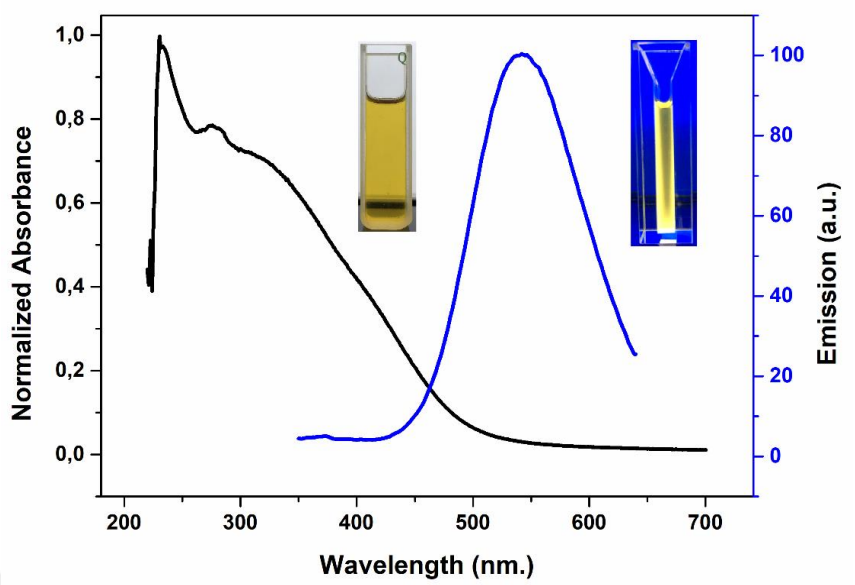


Figure 3.5. Absorbance (black) and emission (blue) spectra of SNS-POSS in DCM. Inset: Colors of the PSNS-POSS-2 (P2) in DCM under ambient light (left) and hand held UV lamp (right) at 365 nm.

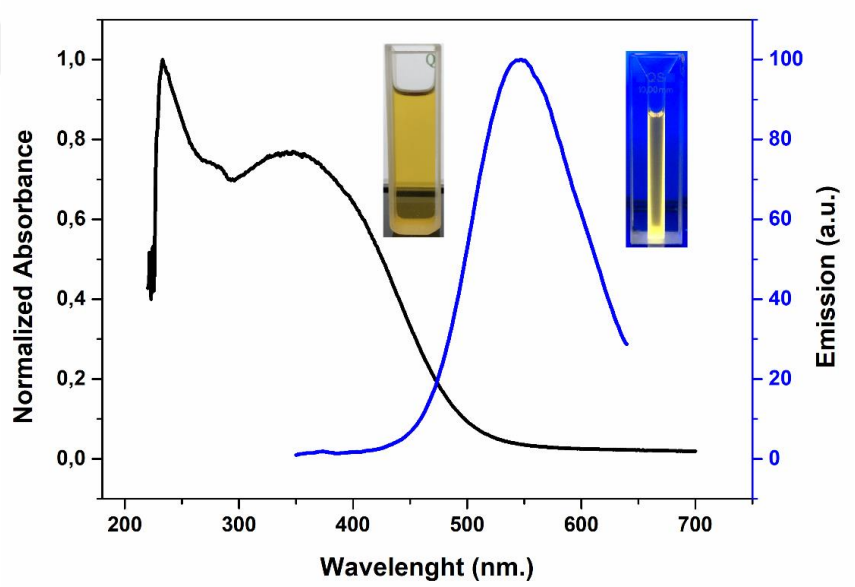


Figure 3.6. Absorbance (black) and emission (blue) spectra of SNS-POSS in DCM. Inset: Colors of the PSNS-POSS-3 (P3) in DCM under ambient light (left) and hand held UV lamp (right) at 365 nm.

Due to the fluorescent behaviors of the monomer and the polymers it is necessary to determine the fluorescence equantum yields (QY). QY can be determined by the following method with Quinine as a reference (0,1 M H_2SO_4, QY = 0.54) as standard:

$$QY_S = QY_R\ (F_S/F_R)(A_R/A_S)(n_S/n_R)^2$$

n: refractive index of the solvent

A: the optical density at the excitation wave length

F: the measured integrated emission intensity

Where the subscripts"R" and "S" stands for the reference and the sample. The solvent used to dissolve the monomers and the polymers during the measurements was THF solvent was used for the measurement of the QY of the polymer. The QY of the monomer was calculated as 0.130. The QY values fort he polymers P1, P2 and P3 are 0.020, 0.036 and 0.040 respectively. The following figures hows the related absorbance and fluorescence parameters to calculate the QY of the materials and the reference. (The absorbance and fluorescence spectrum of the materials were provided in Appendix B.)

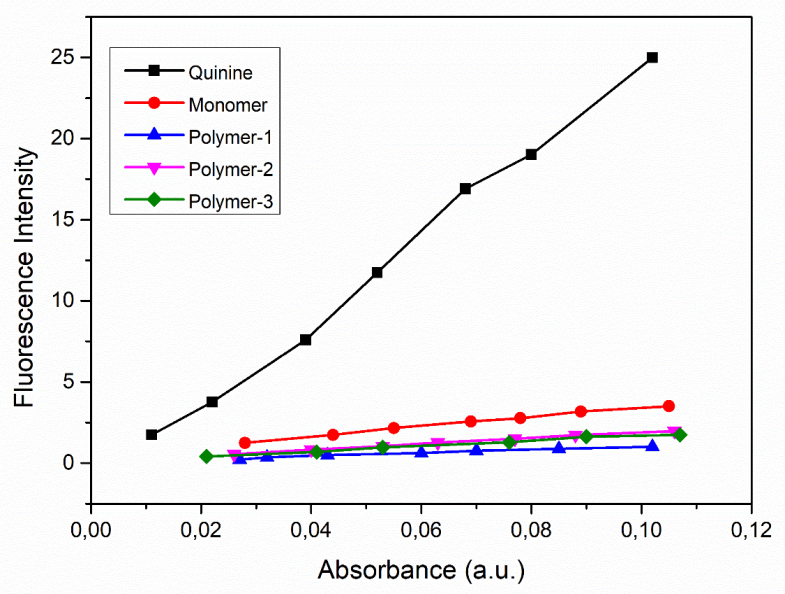


Figure 3.7. Fluorescence and absorbance diagram of Quinine,monomer, P1, P2, and P3 in Toluene.

The change in the optical behavior of the polymer (P3) was recorded by using UV-Vis spectrometer. The chemically obtained polymer was dissolved in toluene and dilute $SbCl_5$ solution was added onto polymer solution to dope the polymer chemically. The peak at around 340 nm has started to decrease with the addition of the oxidant solution. Also, with the further addition the formation of bipolarons can be followed by the emerging of the new absorption band after 460 nm.The color changes were recorded simultaneously and pictures of the neutral and oxidized polymer solutions were given in the inset of Figure 3.8.

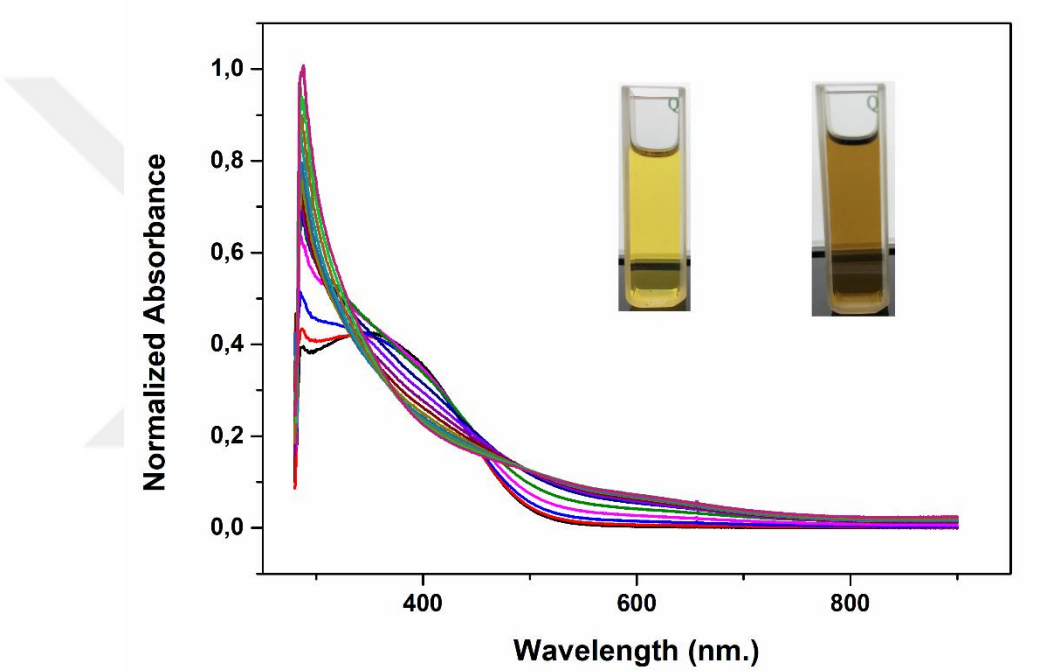


Figure 3.8. Absorption spectra of chemical oxidation of chemically obtained polymer P3 with $1x10^{-4}$ M $SbCl^5$ solution in toluene. (Inset:Neutral and oxidized polymer solutions.)

3.2. Cell imaging

To stain the cells, monomer and all the polymers were used and the results are recorded by confocal microscopy (METU central research laboratory) with excitation wavelength of 471 nm.

3.2.1 Imaging of SNS-POSS dye withMC3T3 cells

For staining of MC3T3 cells,1mg/ml of different molecular weight polymers,P1,P2, P3 were used together with the monomer.Different concentrations of the monomer and polymers were tested. Concluding that there was no noticeable change with the change of volume or concentration on cell imaging (data not shown), the rest of the studies were conducted by using1mg/ml of the sample product.The experiments with MC3T3cells showed that: 1 mg/ml SNS-POSS monomer seems to stain the cells (Figure 3.9) but comparing the staining results with 3 different polymers showed that P3is the best. Although polymer P2seems more efficient (Figure 3.11) than P3 (Figure 3.12) it is concluded that the better staining is the result of more solvent ratio in the polymer P3 which dissolves the polypropylene tissue culture plates.

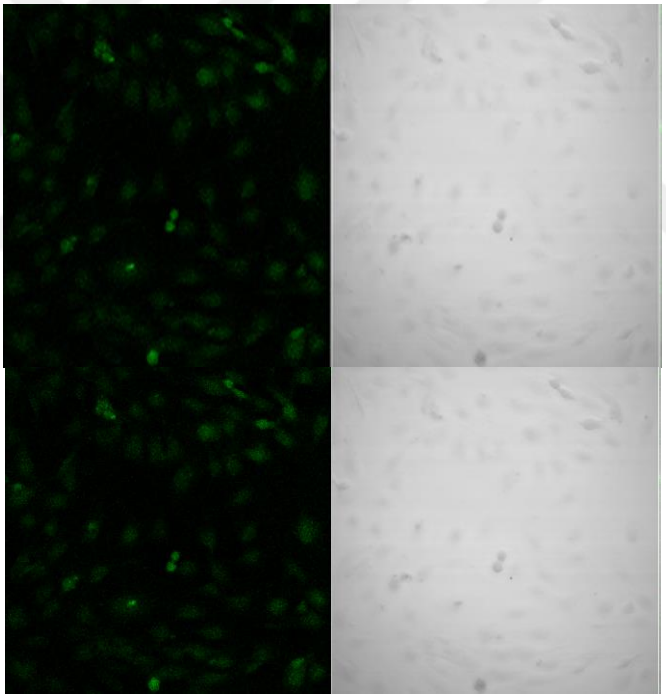


Figure 3.9. Imaging of 1mg/ml SNS-POSS monomer with MC3T3 cells

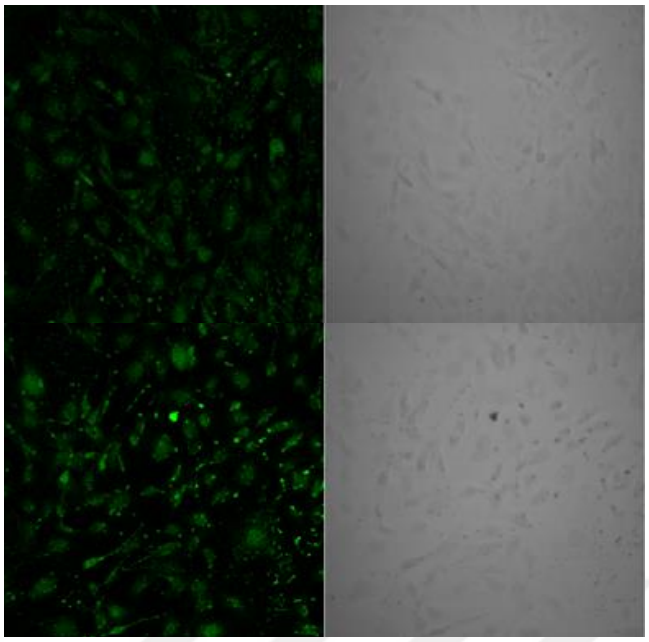


Figure 3.10. Imaging of 1mg/ml of SNS-POSS dye (P1) with MC3T3

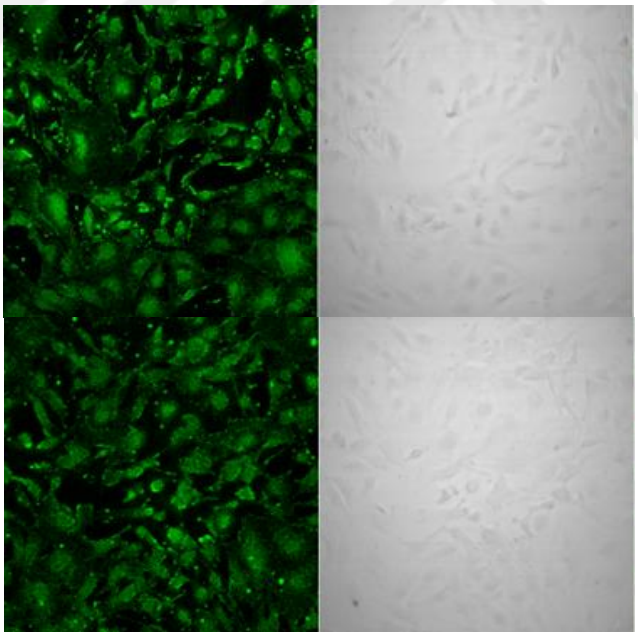


Figure 3.11. Imaging of 1mg/ml of SNS-POSS dye (P2) with MC3T3 cells

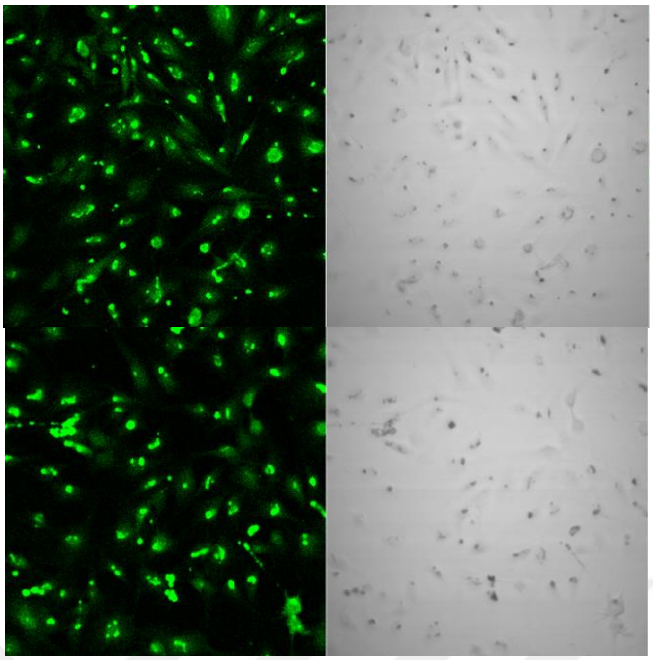


Figure 3.12. Imaging of 1ml of SNS-POSS polymer (P3) with 1mL of MC3T3 cells

3.2.2 Imaging of SNS-POSS dye with MCF7 cells

The SNS-POSS dye staining results with MCF7 showed that P1 stained the cells better (Figure 3.14) meaning that more brilliant and the cell integrity were maintained than the rest of the polymers.

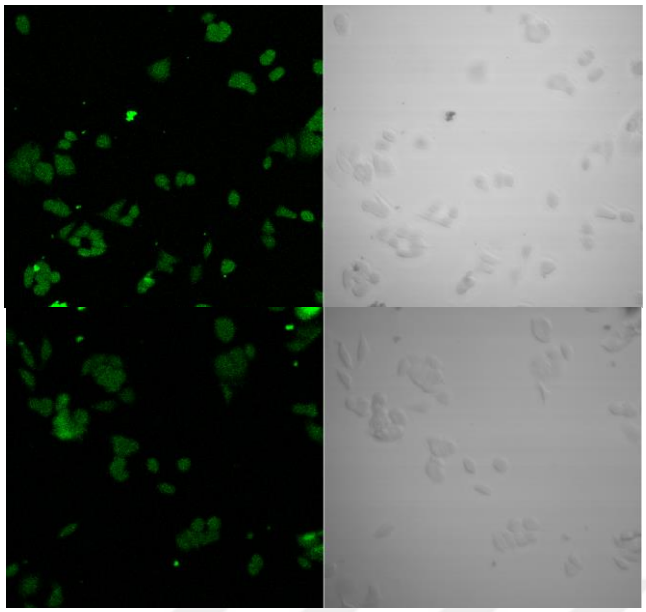


Figure 3.13. Imaging of 1mg/ml SNS-POSS monomer with MCF7 cells

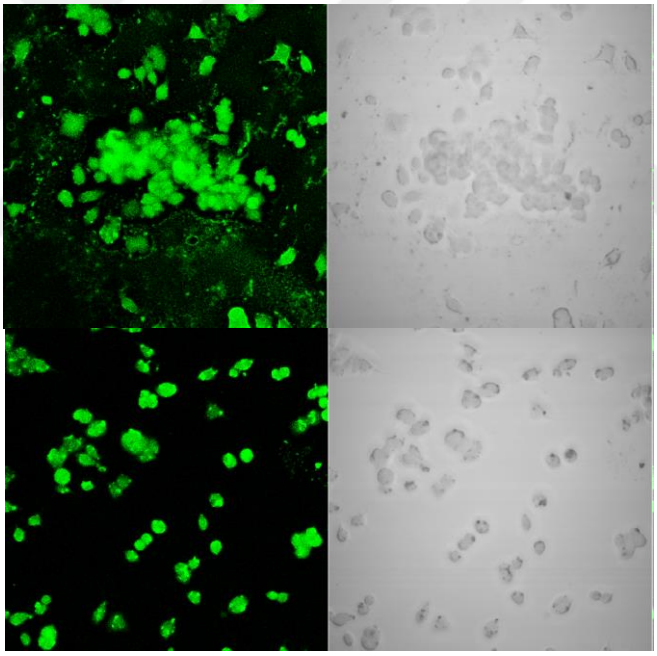


Figure 3.14. Imaging of 1mg/ml of SNS-POSS dye (P1) with MCF7cells

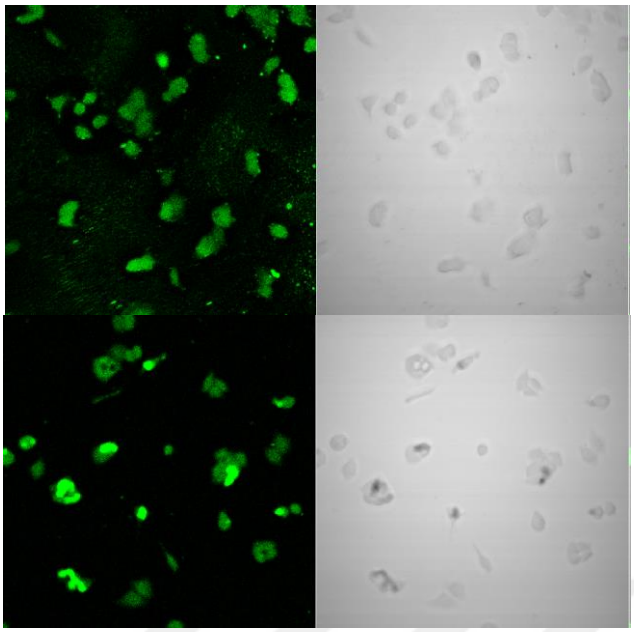


Figure 3.15. Imaging of 1mg/ml of SNS-POSS dye (P2) with MCF7cells

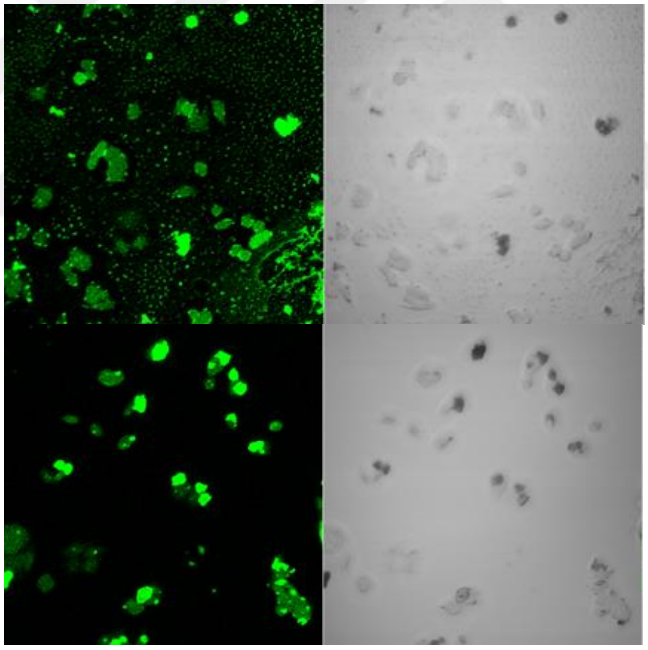


Figure 3.16. Imaging of 1mg/ml of SNS-POSS dye (P3) with MCF7 cells

3.2.3 Imaging of SNS-POSS dye with L929 cells

Three SNS-POSS polymer with L929 cell line resulted that the cell integrity were maintained much more than the other cell lines with L929. When the results were

compared within L929 cells P1 seems better on the basis of either cell integrity or brightness of the dye (Figure 3.18).

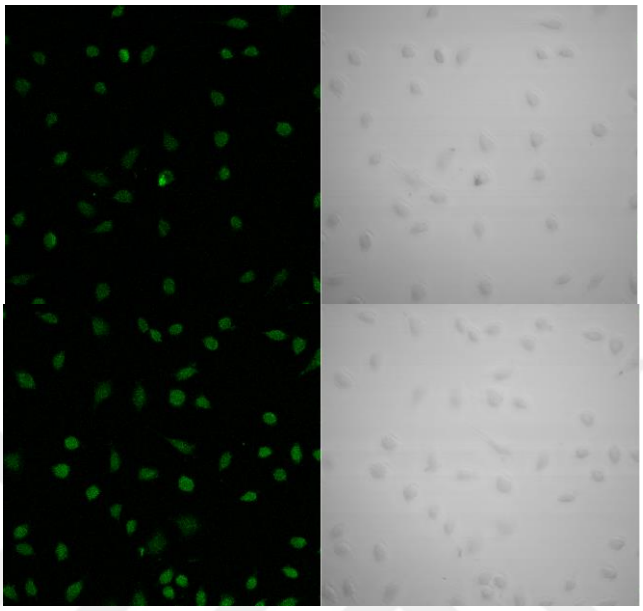


Figure 3.17. Imaging of 1mg/ml SNS-POSS monomer with L929 cells

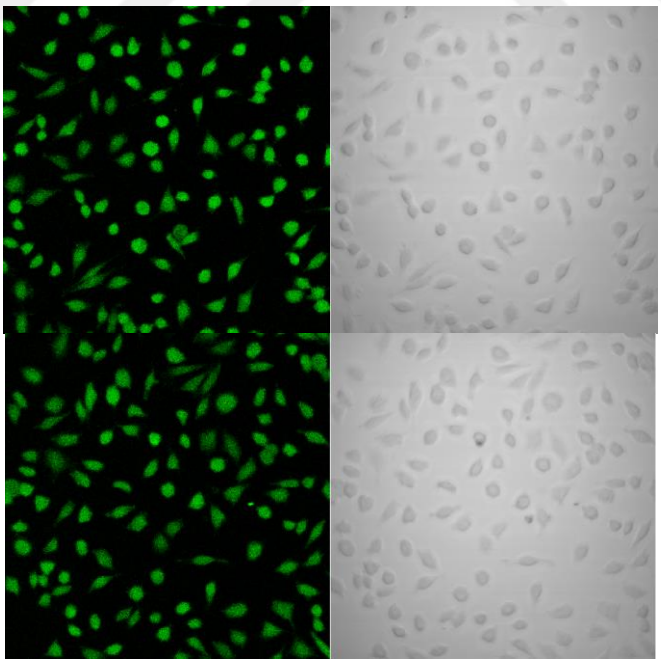


Figure 3.18. Imaging of 1mg/ml SNS-POSS dye (P1) with L929 cells

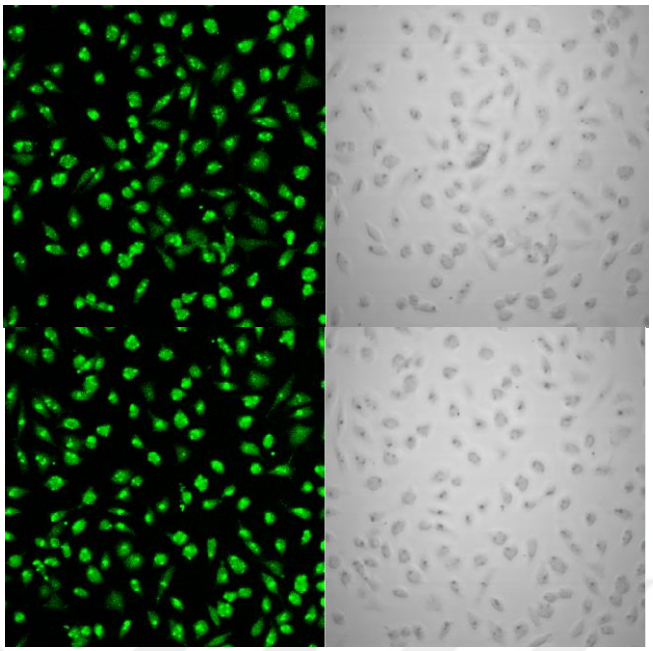


Figure 3.19. Imaging of 1mg/ml SNS-POSS dye (P2) with L929 cells

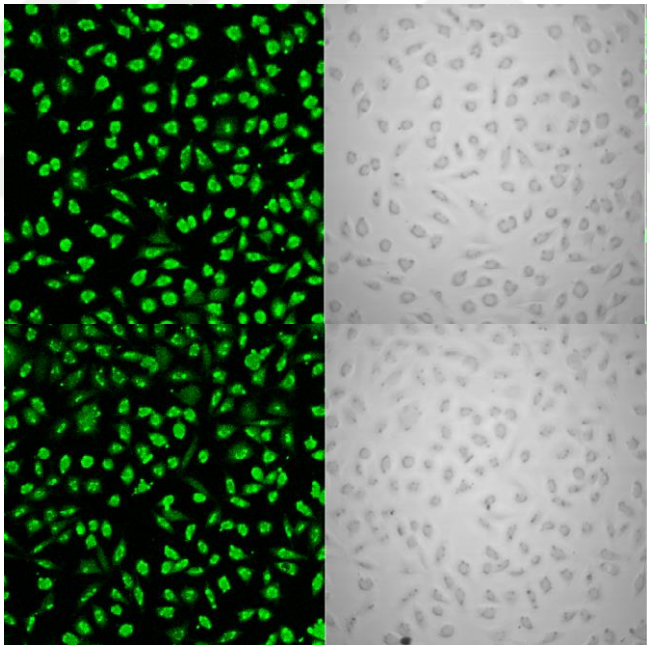


Figure 3.20. Imaging of 1mg/ml SNS-POSS dye (P3) with L929 cells

The cell staining studies of SNS-POSS polymer by using three different cell lines showed that, MC3T3 cells seemed to stained well with P3 but P2 and P1 also stained the cells. P3 seems better because from the images (Figure 3.12) it was concluded

that the polymer (P3) seems to stain organelle. However, in order to be sure whether the nucleus or mitochondria were stained further analysis required.

Staining of MCF7 cells with SNS-POSS showed that only P1stained the cells well with maintained cell integrity and brilliant cell images (Figure 3.14).

L929 cell line with P1, P2 and P3 showed that P1 is the best because the cell integrity was maintained (Figure 3.18) and the cells were more visible than the monomer (Figure 3.17). With L929 cells P2 (Figure 3.19) and P3 (Figure 3.20) polymers seemed to stain the organelles like the results obtained from MC3T3 cells. But this comment requires further analysis.

3.3 Cytotoxicity assay result

3.3.1. Cytotoxicity Test for Samples

MTT analysis is used to investigate cytotoxic effect of different materials and/or material extracts on cells based on mitochondrial activities of cells. In this study, L929 cell line was used as a standard cell line for direct and/or indirect cytotoxicity studies. The optical microscope images were represented in the above figures and the morphological changes were clearly observed. The rounded cell morphology in Hexane-Media (1:1) mixture and Hexane samples indicates that the cell viability was decreased at first 2 h of incubation. According to the MTT analysis result (Figure 3.21), it is clearly seen there is a dramatic decrease when the media replaced even by 1:1 mixture of Hexane and Media.

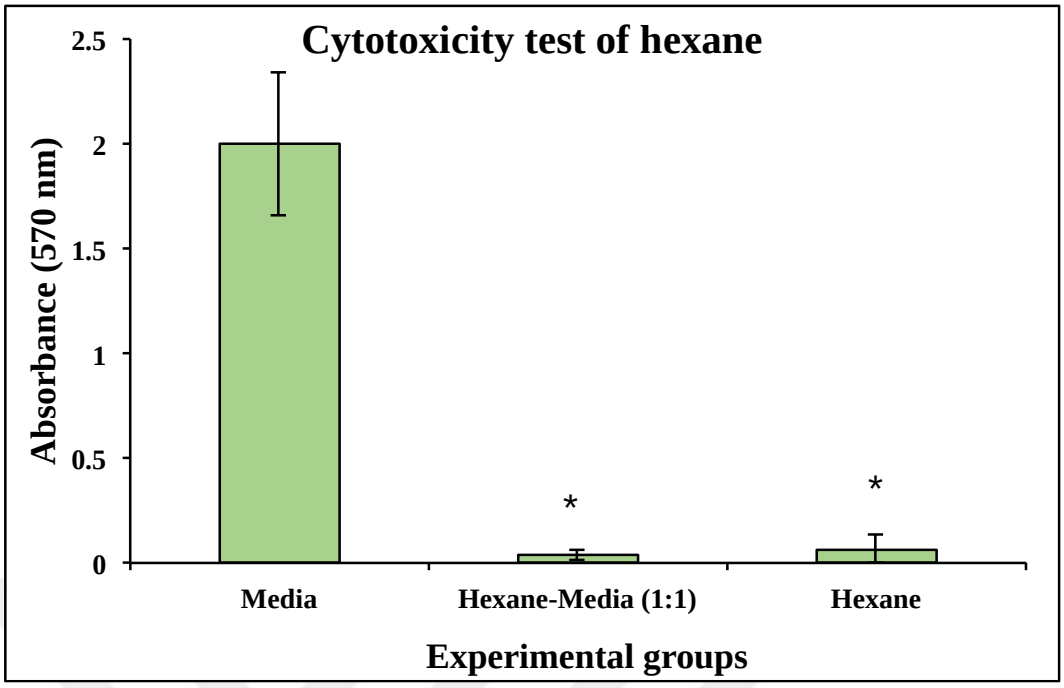


Figure 3.21. MTT analysis results of L929 cells after hexane treatment (*p<0.05 when groups compared with cells in media)

When the cells treated with Hexane-Media (1:1) mixture and hexane, statistical analysis demonstrated that optical densities of formazan crystals of L929 cells are smaller then incubation with cell culture media for 2 h (*p<0.05). However, the optical density values of Hexane-Media (1:1) and Hexane alone treatments have no statistical difference when they are compared (*p>0.05).Since hexane is highly toxic to cells, the new solvent was required in this study. Dimethylsulfoxide-Dichloromethane (DMSO-DCM mix) solvent mixture was used. However, the solubility of polymeric samples relatively low in DMSO-DCM mix when it compared to hexane. The most important aim of cell staining studies is usage of minimum amount of dye solution to minimize both material consumption and costs. Therefore, it was decided that minimum polymer solution concentration (1mg/mL) is adequate for staining of all cell lines. For this purpose, 10 mg of each sample was dissolved in 1 ml DMSO-DCM mixture and it was diluted to 1mg/ml concentration with cell culture media. The monomer among all the samples has the smallest molecule structures and P3 polymer has the longest chain among polymeric

materials. Thus, the cytotoxic comparison was performed between smallest and largest molecules.

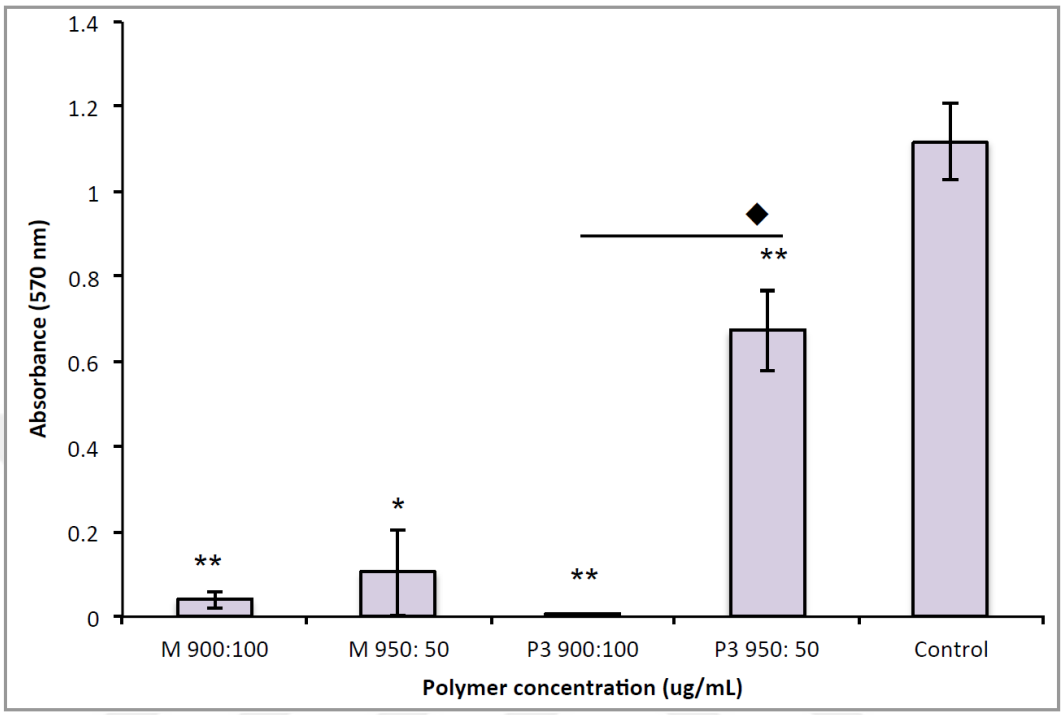


Figure 3.22. MTT analysis results of L929 cells after DMSO-DCMsolvent mixture treatment(**p<0.01 when groups compared with cells in media, ♦p<0.05 when the lower concentration of polymer solution compared with higher concentration).

The 10 mg/ml solutions of monomer and P3 samples were diluted with 900:100 and 950:50 dilution ratios in cell culture media, respectively. The cytotoxicity study results show that both higher and lower dilution of monomer solution is relatively toxic when it compared with control samples (*p<0.05). This outcome indicates that smaller molecules can permeate cell membrane easier than larger molecules and create a cytotoxic effect as a result of chemical stress on cells. In addition to that it can be concluded that the toxicity of of the monomer could be higher than that of polymers due to the higher active sites on the thiophenes on the monomer structure when compared to lower number of active sites on the polymers' structure. Also, the

lower dilution of P3 sample (P2 950:50) has the smallest cytotoxicity on cells when it compared both control samples (**p<0.01) and higher dilution (♦p<0.05). This result demonstrates that even the higher concentration of polymeric samples dissolved in DMSO-DCM mixture, the 950:50 dilution ratio with media is useable for cell culture studies without cytotoxic effect on cell lines.

CHAPTER 4

CONCLUSION

The synthesis and characterization of the monomer and its polymer were successfully achieved. The monomer and the materials synthesized in intermediate steps were characterized by NMR spectroscopy. After the oxidative polymerization, FT-IR spectroscopy was utilized to understand the pathway of the polymerization. All the polymers were recorded as soluble in most organic solvents and can be oxidized chemically. Optical characteristics of the monomer and the corresponding polymers were investigated by UV-Vis spectroscopy and Fluorescence spectrophotometer. It is found that not only the monomer but also the polymers have fluorescent behavior under UV light. Due to these properties it was expected to be used as a fluorescent dye in cell staining application.

It is concluded that the synthesized polymer dyes were found to be successful to stain three cell linesused in this study, namely MCF7, L929, MC3T3.To compare the staining success of the polymers P1, P2, and P3 for three cell lines used in this study, P1 was found to be better dye compared to the result obtained with the monomer. Because the cell integrity was maintained for each of the cell line studied with.

Under the light of previous staining studies, it has shown that increasing neither the concentration, nor the volume of polymer solution increases staining efficiency. So it is concluded that itis cost effective the dye used to stain cells here were not concentrated and also they were not consumed with higher volume, meaning that the staining cell with the mentioned polymer and also national.

REFERENCES

[1] W. Xu, Z. Zeng, J.H. Jiang, Y.T. Chang, L. Yuan, Discerning the chemistry in individual organelles with small molecule fluorescent probes, Angew. Chem. Int. Ed. 55 (2016) 13658e13699.

[2] D.G. Colak, I. Cianga, D.O. Demirkol, O. Kozgus, E.I. Medine, S. Sakarya, P. Unak, S. Timur, Y. Yagci, A the synthesis and targeting of PPP-type copolymers to breast cancer cells: multifunctional platforms for imaging and diagnosis, J. Mater. Chem. 22 (2012) 9293e9300.

[3] H. Li, X. Zhang, X. Zhang, K. Wang, Q. Zhang, Y. Wei, Fluorescent polymeric nanoparticles with ultra-low CMC for cell imaging, J. Mater. Chem. B 3 (2015) 1193e1197.

[4] J. Mei, Y. Hong, J.W. Lam, A. Qin, Y. Tang, B.Z. Tang, Aggregation induced emission: the whole is more brilliant than the parts, Adv. Mater 26 (2014) 5429e5479.

[5] Ito, T; Shirakawa, H.; Ikeda, S. "Thermal Cis - Trans Isomerization and Decompostiono Polydiacetylene," Journal of Polymer Science Polymer Chemistry Edition, 1974,12, 11.

[6] Chiang, C. K.; Fincher, C. R., Jr.; Park, Y. W.; Heeger, A. J.; Shirakawa, H.; Louis, E. J. Gau, S. C.; MacDiarmid, A. G. "Electrical conductivity in doped polyacetylene," Physical Review Letters, 1977, 39, 1098-101.

[7] Gaylord, B. S; Heeger, A. J.; Bazan, G. C. DNA detection using water-soluble conjugated polymers and peptide nucleic acid probes. Proc. Natl. Acad. Sci. USA. 2002, 99, 10954.

[8] Gaylord, B. S; Heeger, A. J.; Bazan, G. C. DNA hybridization detection with water-soluble conjugated polymers and chromophores labeled single stranded DNA. J. Am. Chem. Soc. 2003.

[9] Kaur, A.; Cazeca, M. J.; Chittibabu, K. G.; Kumar, J.; Triphany, S. K. "Mechanism of electroluminescence in dye doped thiophene based conjugated polymer,"Journalo f Applied Physics, 2001, 89, 3250-3255.

[10] Yamamoto, T.; Takagi, M.; Kizu, K.; Maruyama, T.; Kubota, K.; Kanbara, H.; Kurihara, T.; Kaino, T. "Preparation and optical properties of soluble .piconjugated poly(aryleneethynylene) type polymers," Journal of the Chemical Society, Chemical Communications, 1993, 797-8.

[11] Bronich, T. K.; Nguyen, H. K.; Eisenberg, A.; Kabanov, A. V. Recognition of DNA topology in reactions between plasmid DNA and cationic copolymers. J. Am. Chem. Soc. 2000, 122, 8339.

[12] Principles of fluorescence spectroscopy. (Lakowicz, J R. Ed.) Kluwer academic, Plenum Publishers, New York, 1999).

[13] Ertan, Salih. Synthesis and characterization of conjugated polymers with polyhedral oligomeric silsesquioxane pendant groups. Diss. MIDDLE EAST TECHNICAL UNIVERSITY, 2017.

[14] Gangopadhyay R., De A., Chem. Mater., 2000, 12, 608

[15] Zhan C., Yu G., Wang L., Wujcik E., Wei S., J. Mater. Chem. C, 2017, 5, 1569-1585,

[16] McCaughey, Byron. Self-assembly of ordered, conjugated polymer nanocomposites. Diss. Tulane University, 2005.

[17] G. Li, L.Wang, H. Ni, C.U. Pittman, Polyhedral oligomeric silsesquioxane (POSS) polymers and copolymers: a review, Inorg. J. Organomet. Polym. 11 (2001) 123–154.

[18] J. Wu, P.T. Mather, POSS polymers: physical properties and biomaterials applications, J. Macromol. Sci. Polym. Rev. 49 (2009) 25–63.

[19] Lickiss, P.D.; Rataboul, F. "Fully Condensed Polyhedral Oligosilsesquioxanes (POSS): From Synthesis to Application." Adv. Organomet. Chem. 2008, 57, 1-116.

[20] R.Y. Kannan, H.J. Salacinski, M.J. Edirisinghe, G. Hamilton, A.M. Seifalian, Polyhedral oligomeric silsequioxane–polyurethane Nanocomposite microvessels for an artificial capillary bed, Biomaterials 27 (2006) 4618–4626.

[21] R.H. Baney, M. Itoh, A. Sakakibara, T. Suzuki, Silsesquioxanes, Chem. Rev. 95 (1995) 1409–1430.

[22] Zhang, C; Babonneau, F.; Bonhomme, C; Laine, R.M.; Soles, C.L.; Hristov, H.A.; Yee, A.F. "Highly Porous Polyhedral Silsesquioxane Polymers. Synthesis and Characterization." J. Am. Chem. Soc. 1998,120, 8380-8391.

[23] Sellinger, A.; Laine, R.M. "Silsesquioxanes as Synthetic Platforms. 3. Photocurable, Liquid Epoxides as Inorganic/Organic Hybrid Precursors." Chem. Mater. 1996, 8, 1592-1593.

[24] Choi, J.; Kim, S.G.; Laine, R.M. "Organic/Inorganic Hybrid Epoxy Nanocomposites from Aminophenylsilsesquioxanes." Macromol. 2004, 37, 99-109.

[25] Asuncion, M.Z.; Laine, R.M. "Silsesquioxane Barrier Materials." Macromol. 2007, 40, 555-562.

[26] Feher, F.J.; Budzichowski, T.A. "Silasesquioxanes as Ligands in Inorganic and Organometallic Chemistry." Polyhedron 1995,14, 3239-3253.

[27] Feher, F.J.; Newman, D.A.; Walzer, J.F. "Silsesquioxanes as Models for Silica Surfaces." J. Am. Chem. Soc. 1989, 111, 1741-1748.

[28] Leu, C. M.; Reddy, M.; Wei, K-H; Shu, C.-F. "Synthesis and Dielectric Properties of Polyimide-Chain-End Tethered Polyhedral Oligomeric Silsesquioxane Nanocomposites." Chem. Mater. 2003,15, 2261-2265.

[29] Liu, Y.-L.; Lee, H.-C. "Preparation and Properties of Polyhedral Oligosilsesquioxane Tethered Aromatic Polyamide Nanocomposites through Michael Addition between Maleimide-Containing Polyamides and an Amino-Functionalized Polyhedral Oligosilsesquioxane." J. Polym. Scl, Part A: Polym. Chem., 2006, 44, 4632-4643.

[30] Maschmeyer, T.; Klunduk, M.C.; Martin, CM.; Shephard, D.S.; Thomas, J.M.; Johnson, B.F.G. "Modeling the Active Sites of Heterogeneous Titanium-Centred Epoxidation Catalysts with Soluble Silsesquioxane Analogues." Chem. Comm. 1997, 1847-1848.

[31] Chojnowski, J; Fortuniak, W; Rosciszewski, P; Werel, W.; Lukasiak, J.; Kamysz, W.; Halasa, R. "Polysilsesquioxanes and Oligosilsesquioxanes Substituted by Alkylammonium Salts as Antibacterial Biocides." J. Inorg. Organomet. Polym. Mater. 2006,16, 219-230.

[32] Majumdar, P.; Lee, E.; Gubbins, N.; Stafslien, S.J.; Daniels, J.; Thorson, C.J.; Chisolm, B.J. "Synthesis and Antimicrobial Activity of Quaternary Ammonium-Functionalized POSS (Q-POSS) and Polysiloxane Coatings Containing Q-POSS." Polymer 2009, 50, 1124-1133.

[33] Chan, K.L.; Sonar, P.; Sellinger, A. "Cubic Silsesquioxanes for Use in Solution Processable Organic Light Emitting Diodes (OLED)." J. Mater. Chem. 2009, 19, 9103-9120.

[34] Gromilov, S.A.; Basova, T.V.; Emel'yanov, D.Y.; Kuzmin, A.V.; Prokhorova, S.A. "Layer Arrangement in the Structure of Octakis (trimethylsiloxy) octasilsesquioxane and Dodecakis (trimethylsiloxy) octasilsesquioxane." J. Struct. Chem. (Engl. Trans.) 2004, 45, 471-475.

[35] S.K. Kim, S.J. Heo, J.Y. Koak, J.H. Lee, Y.M. Lee, D.J. Chung, A biocompatibility study of a reinforced acrylic-based hybrid denture composite resin with polyhedral oligosilsesquioxane, J. Oral Rehabil. 34 (5) (2007) 389–395.

[36] G. Punshon, D.S. Vara, K.M. Sales, A.G. Kidane, H.J. Salacinski, A.M. Seifalian, Interactions between endothelial cells and a poly(carbonate-silsesquioxane-bridgeurea) urethane, Biomaterials 26 (32) (2005) 6271–6279.

[37] S. Kuo, F. Chang, POSS related polymer nanocomposites, Prog. Polym. Sci. 36 (2011) 1649–1696.

[38] F. Carniato, E. Boccaleri, L. Marchese, A versatile route to bifunctionalized silsesquioxane (POSS): synthesis and characterization of Ti-containing aminopropylisobutyl-POSS, Dalton Trans. 1 (2008) 36–39.

[39] Shockey EG, Bolf AG, Jones PF, Schwab JJ, Chaffee KP, Haddad TS, Lichtenhan JD (1999) ApplOrganometChem 13:311.

[40] Jang J, Bae J, Kang D (2001) J ApplPolymSci 82(9):2310-2318.

[41] Wu X, Sun Y, Xie W, Liu Y, Song X (2010) Dental Mater 26(5):456-462.

[42] Wheeler PA, Fu BX, Lichtenhan JD, Jia WT, Mathias LJ (2006) J ApplPolymSci 102(3):2856 2862.

[43] Kannan RY, Salacinski HJ, Edirisinghe MJ, Hamilton G, Seifalian AM (2006) Biomaterials 27(26):4618-4626.

[44] Kidane AG, Burriesci G, Edirisinghe M, Ghanbari H, Bonhoeffer P,

Seifalian AM (2009) ActaBiomater 5 (7): 2409-2417.

[45] Gupta A, Seifalian AM, Ahmad Z, Edirisinghe MJ, Winslet MC (2007)

J Bioactive CompatPolym 22(3):265-280.

[46] Hu L, Chen X, Wang D, Liu Y (2009) Polym Mater SciEng 100:271- 272.

[47] M. KÖSTEL, M.A. SULEIMAN, (Investıgatıon Of Bioindicator Properties of Poss Containing Conducting Polymer), 2019.P1.

[48] Roy, S. Kumar, V., Barman, D., Kumar, A. Paul, L., Datta M. Cell Culture & Its Application. http://aquafind.com/articles/Cell_Culture.php.

[49] BEEBE, S. P., and EWING, J. A Study of the Biology of Tumor Cells. Brit. Med. J., ii: 1559-60,1906.

[50] J. PAUL The Cancer Cell in Vitro: A Review, Cancer Research,1962.

[51] A. M. Breul, M. D. Hager and U. S. Schubert, Chem. Soc. Rev., 2013, 42, 5366–5407.

[52] X. Gao, Y. Cui, R. M. Levenson, L. W. Chung and S. Nie, Nat. Biotechnol., 2004, 22, 969–976.

[53] D. Kumar and R. C. Sharma, Eur. Polym. J. 34, 1053 (1998).

[54] Jen K. Y., Miller G. G. And Elsembaumer, R. L. J., Chem. Soc., Chem. Commun., 1986, 1346.

[55] Osterholm J-E., Laakso J., Nyholm P., Isotalo H., Stubb H., Inganas O. And Salaneck W. R., Synth. Met., 1989, 28, C435.

APPENDIX A

NMR Results

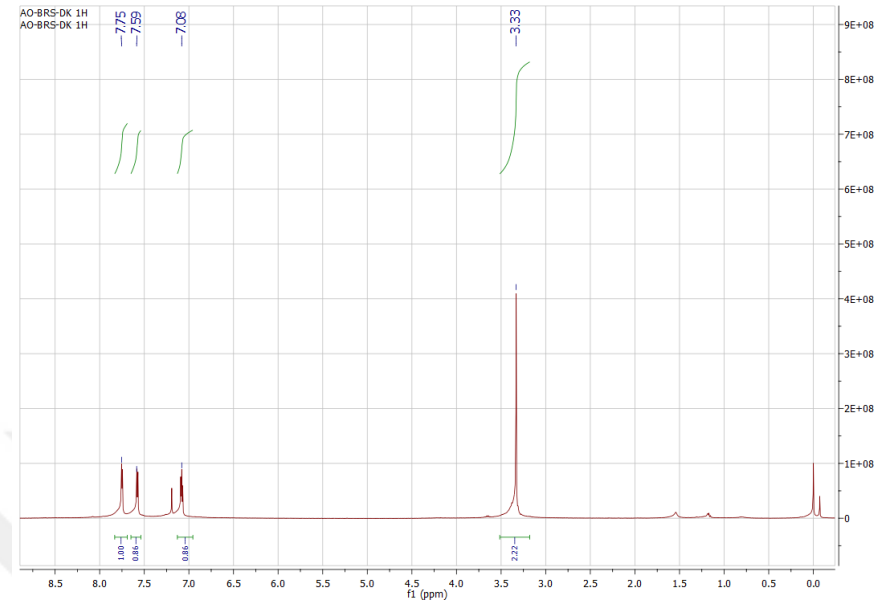


Figure A.1.^{1}H NMR spectrum of Diketone in d-$CHCl_3$.

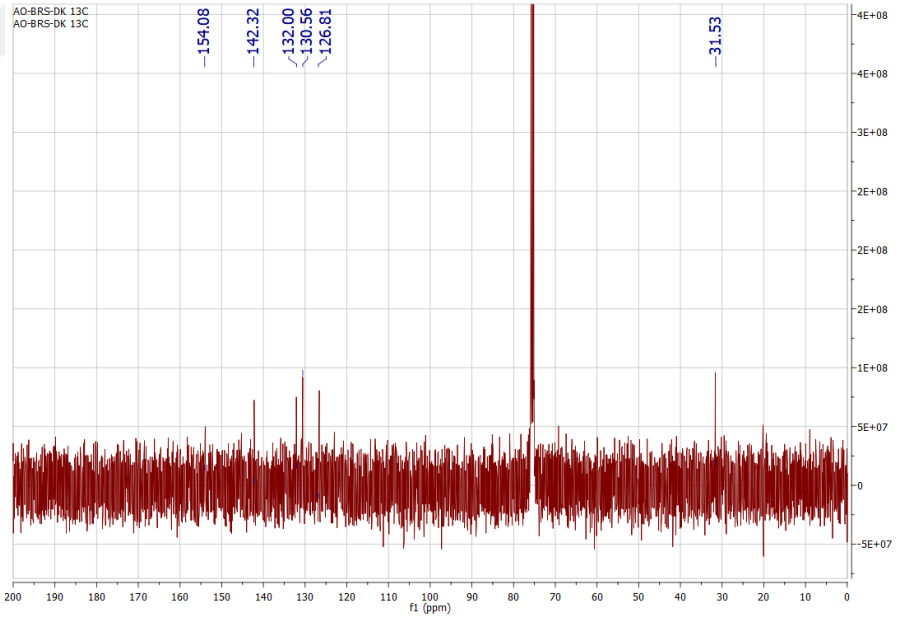


Figure A.2.^{13}C NMR spectrum of Diketone in d-$CHCl_3$.

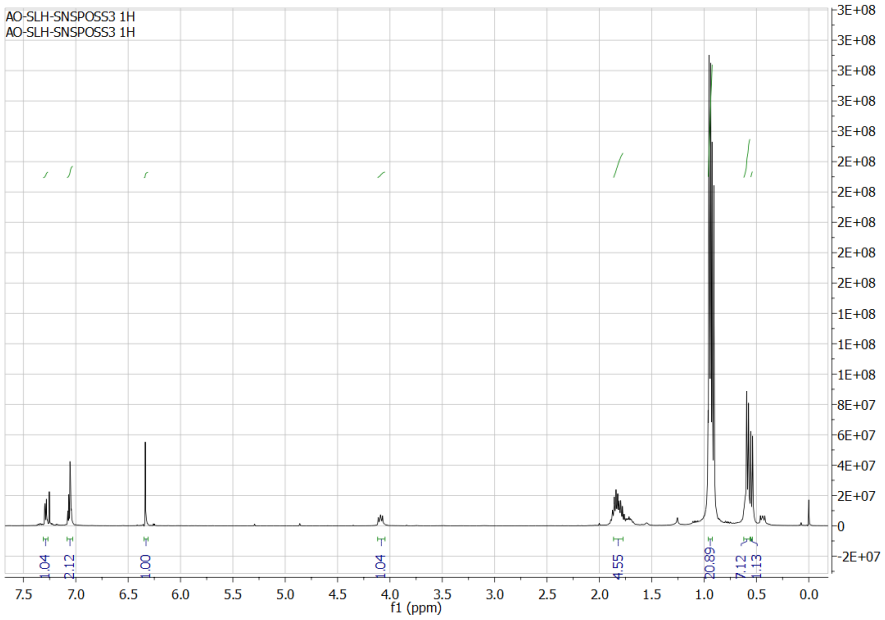


Figure A.3.^{1}H NMR spectrum of SNS-POSS in d-$CHCl_3$.

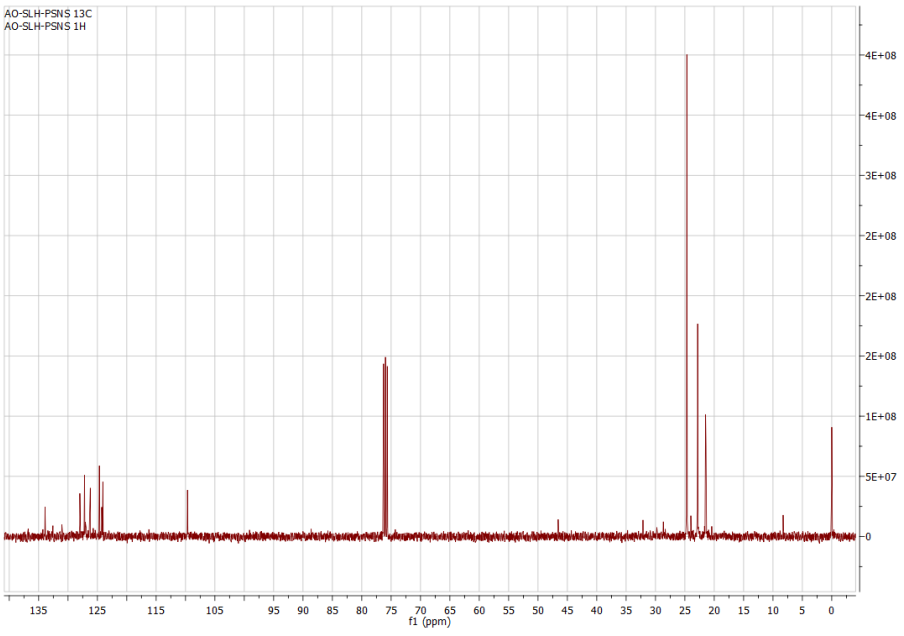


Figure A.4.^{13}C NMR spectrum of SNS-POSS in d-$CHCl_3$.

APPENDIX B

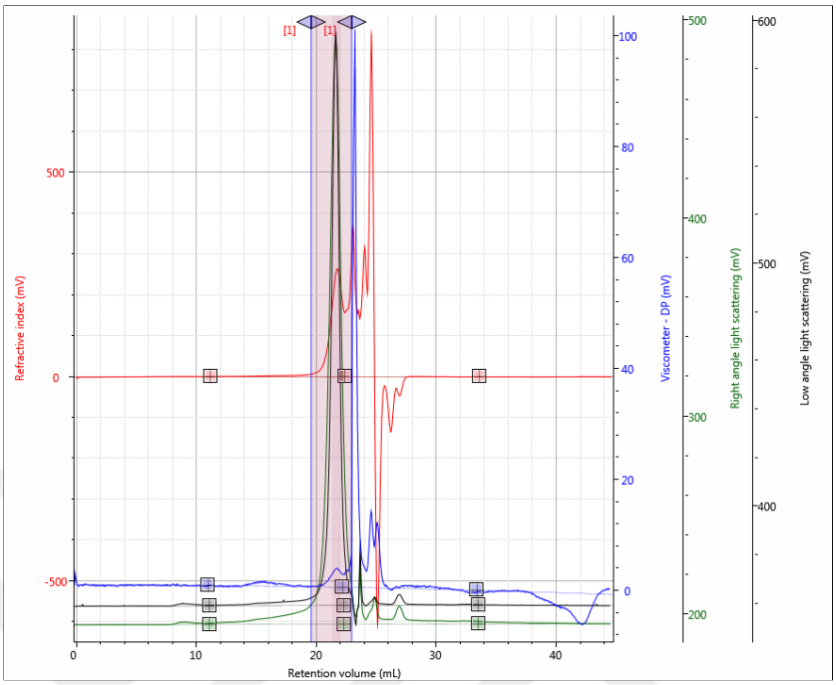


Figure B.1. Gel permeation Chromatogram of P2.

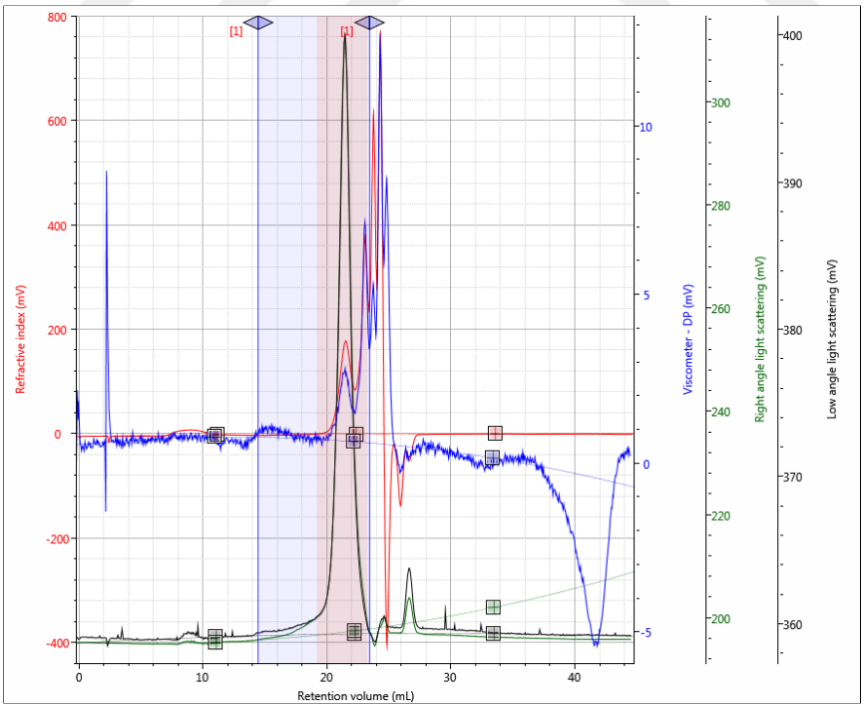


Figure B.2. Gel permeation Chromatogram of P3.

APPENDIX C
Absorbance and Fluorescence Data

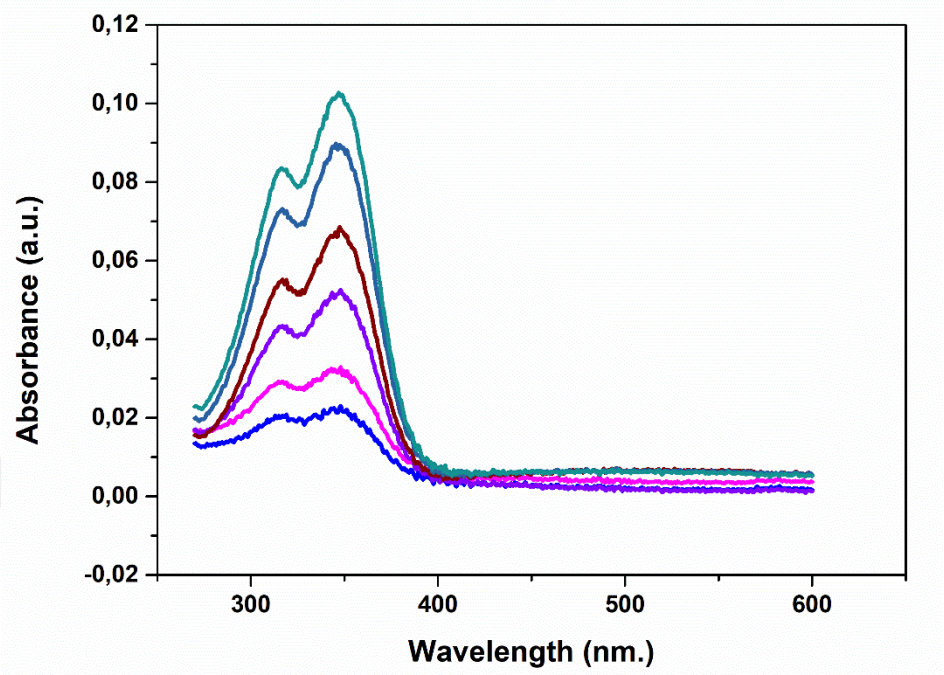


Figure C.1. Absorbance spectra of Quinine sulphate.

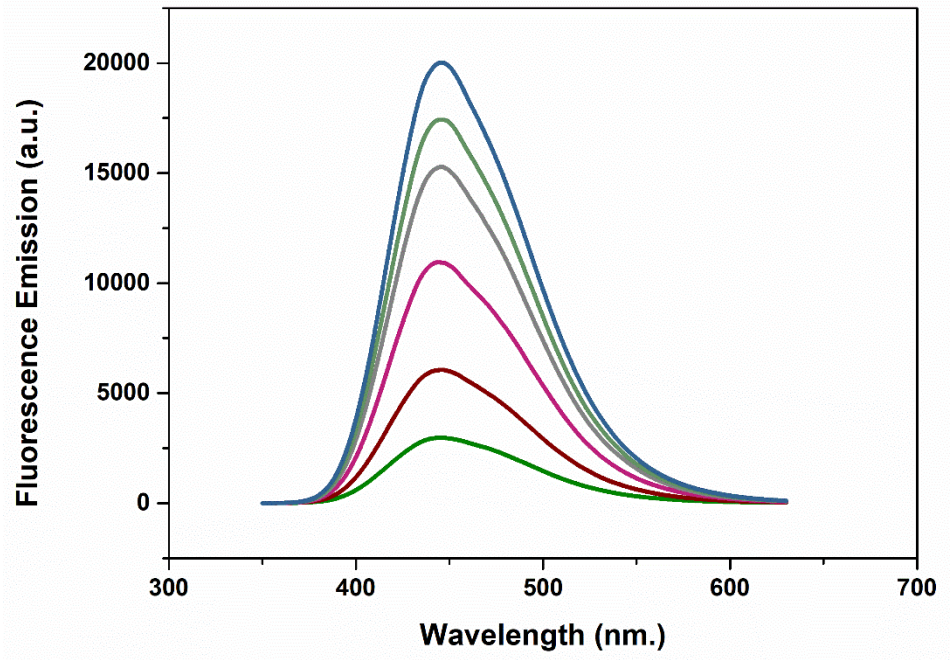


Figure C.2. Fluorescence spectra of Quinine sulphate

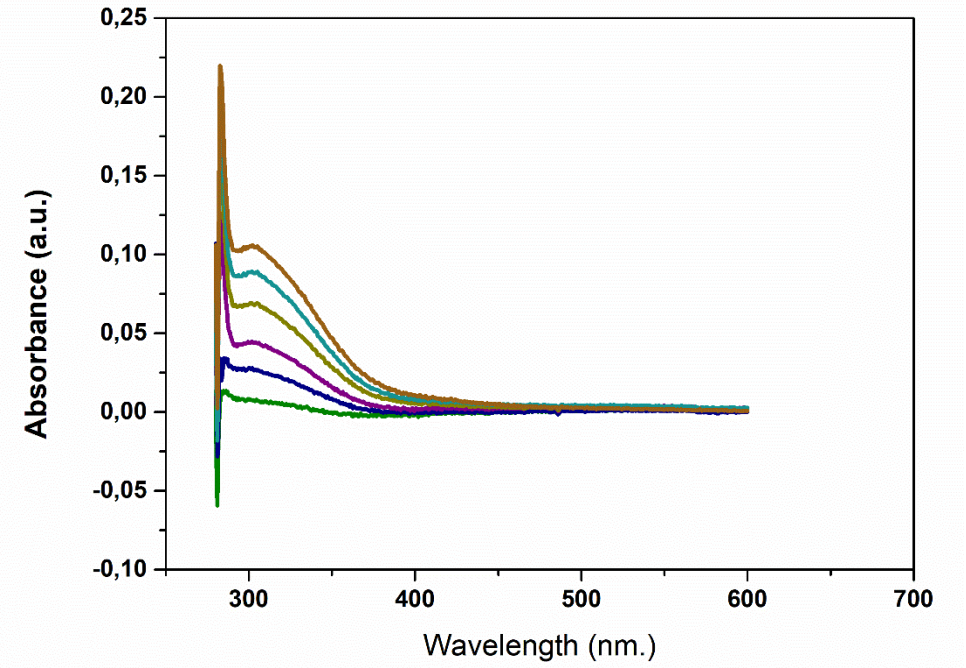


Figure C.3. Absorbance spectra of SNS-POSS monomer.

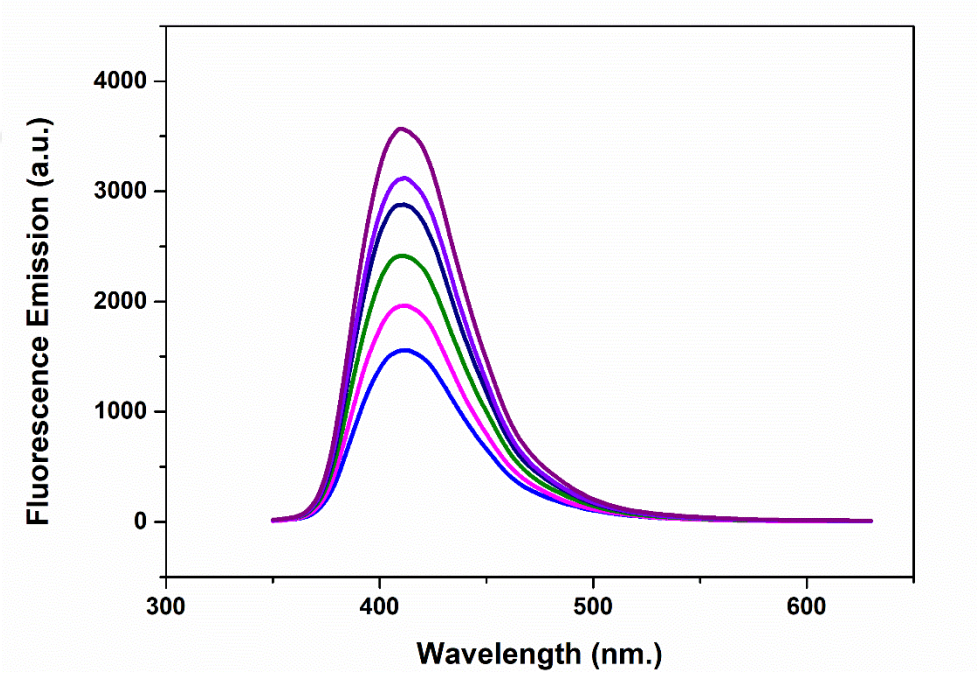


Figure. C.4. Fluorescence spectra of SNS-POSS monomer.

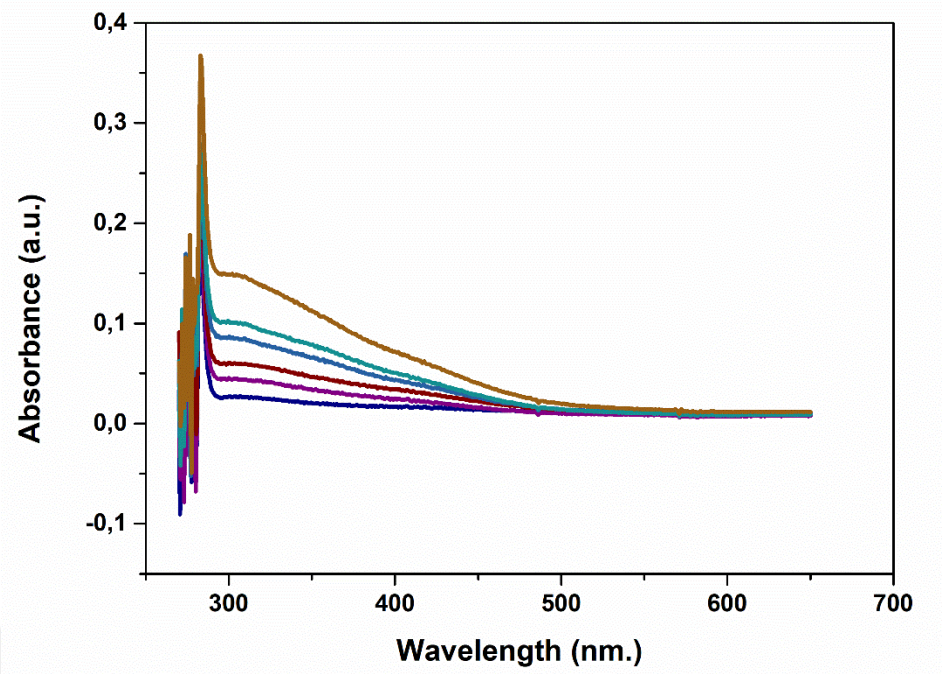


Figure C.5. Absorbance spectra of PSNS-POSS-1 (P1).

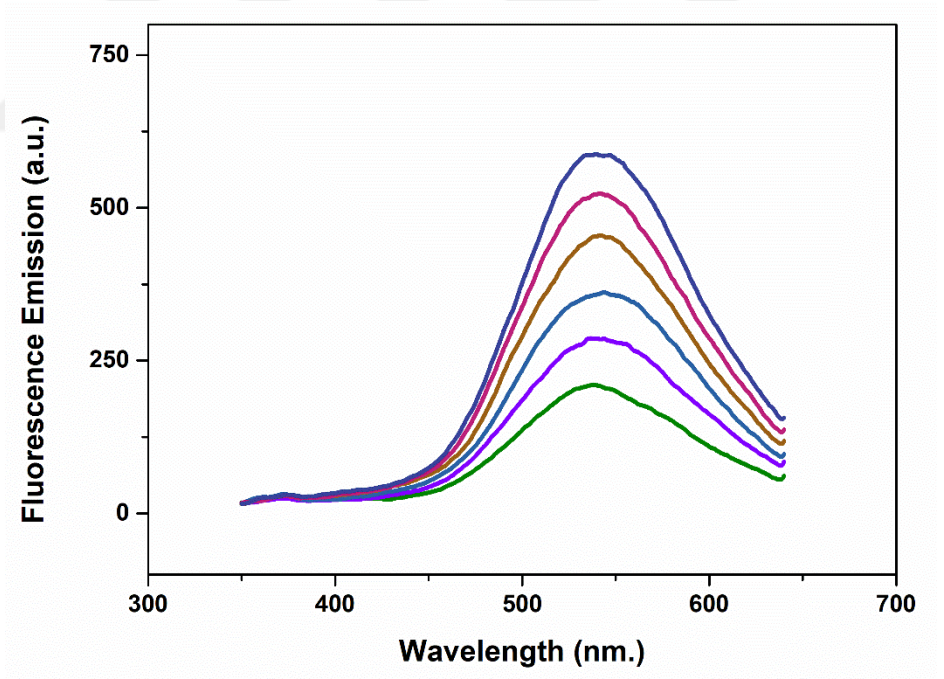


Figure C.6. Fluorescence spectra of PSNS-POSS-1 (P1).

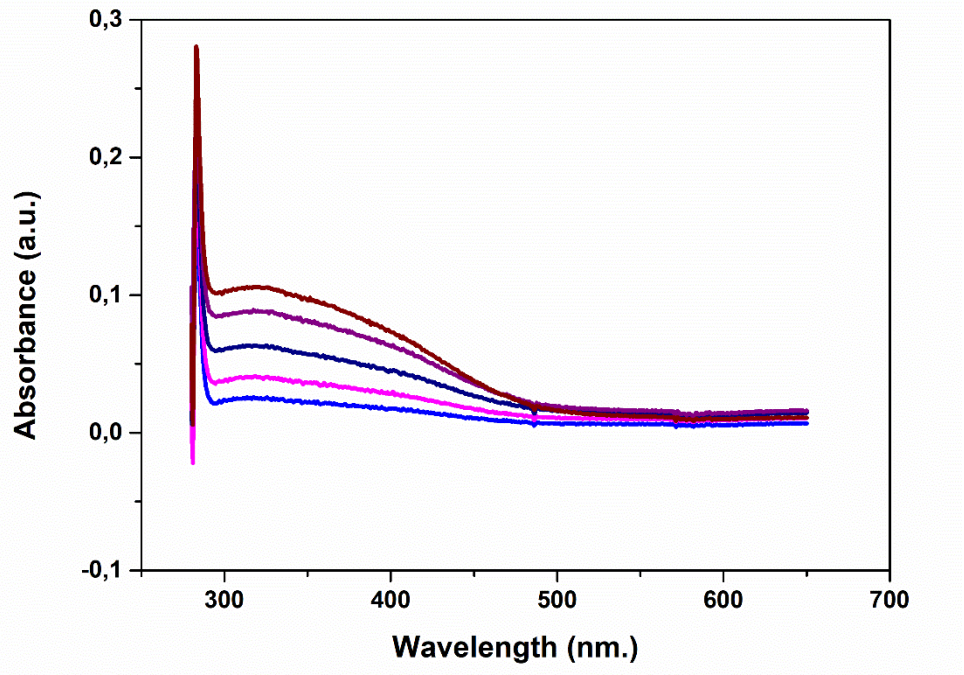


Figure C.7. Absorbance spectra of PSNS-POSS-2 (P2).

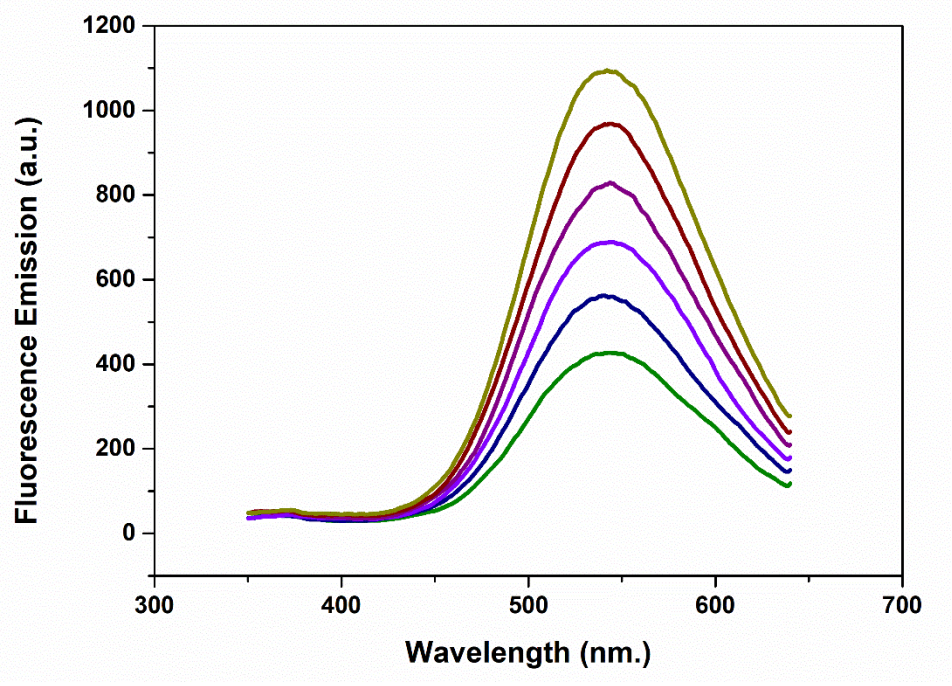


Figure C.8. Fluorescence spectra of PSNS-POSS-2 (P2).

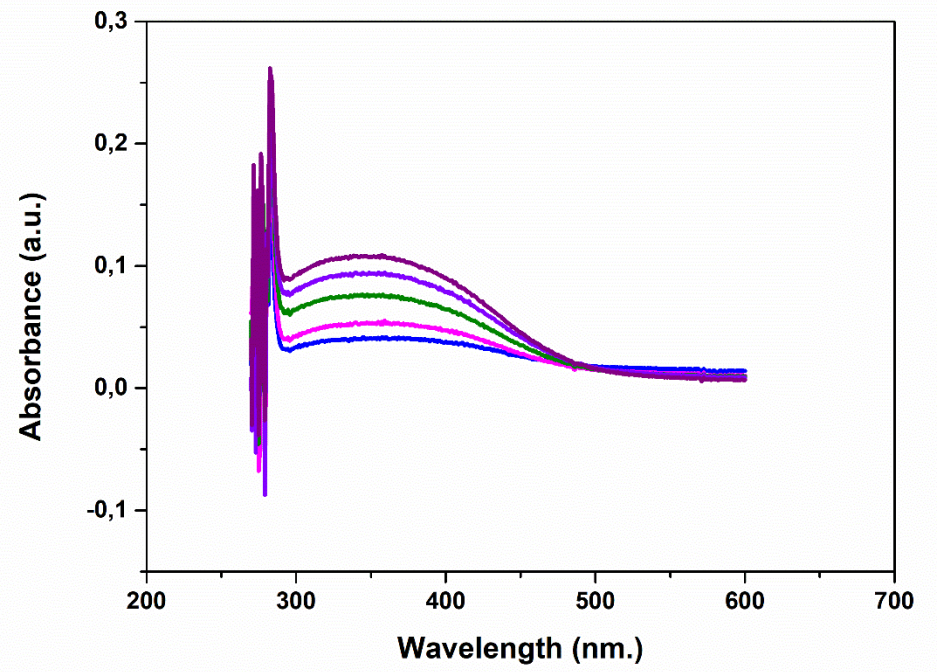


Figure C.9. Absorbance spectra of PSNS-POSS-3 (P3).

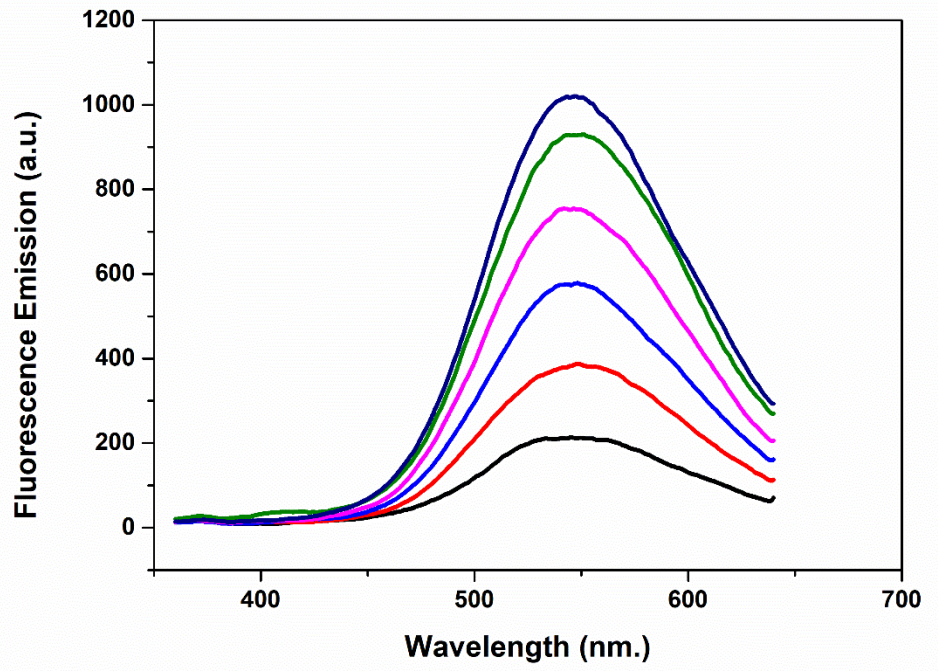


Figure C.10. Fluorescence spectra of PSNS-POSS-3 (P3).