CYCLODEXTRIN BASED MICROCAPSULES AND GELS

by

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To My Family

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ABSTRACT

CYCLODEXTRIN BASED MICROCAPSULES AND GELS

Microcapsules and hydrogels are two classes of functional materials that have recently arouse great interest. The former can be utilized as containers as an alternative to micelles and vesicles whereas the latter have been used for many biomedical applications such as drug delivery devices and tissue engineering. In this study, based on the host-guest complexation ability of cyclodextrins, fabrication of new microcapsules and functionalizable hydrogels was examined.

In the first part, adamantane and cyclodextrin functionalized gold nanoparticles were synthesized for making microcapsules by supramolecular interactions. As a result of specific non-covalent interactions between the adamantyl and cyclodextrin ligands on the nanoparticle surfaces, crosslinking occurs at the interface of water/toluene emulsion. Resulting microcapsules were characterized using a variety of techniques such as optical, fluorescence and transmission electron microscopy. Furthermore, it was shown that the microcapsules were transferrable into aqueous phase.

In the second part, by using click chemistry a new cyclodextrin based hydrogel was synthesized. The hydrogel can be functionalized both non-covalently by specific hydrophobic interactions and covalently by click chemistry. Besides, the hydrogel was successfully patterned on silicon and glass substrates by micromolding in capillaries technique.

ÖZET

SİKLODEKSTRİN BAZLI MİKROKAPSÜLLER VE JELLER

Mikrokapsüller ve hidrojeller yakın zamanda büyük ilgi uyandıran iki işlevsel malzeme sınıfıdırlar. Mikrokapsüller misel ve vesikellere alternatif taşıma araçları olarak kulanılabilirken, hidrojellerden ilaç-ulaştırma ve doku mühendisliği gibi biyomedikal uygulamalarda faydalanılmıştır. Bu çalışmada siklodekstrinlerin konak-konuk kompleksleşme yeteneğine dayanılarak yeni mikrokapsüllerin ve işlevselleştirilebilien hidrojellerin üretimi incelenmiştir.

İlk kısımda, süpramoleküler etkileşimlerle mikrokapsül üretimi yapmak için adamantan ve siklodekstrin kaplı altın nanoparçacıklar sentezlenmiştir. Nanoparçacıkların yüzeyündeki adamantil ve siklodekstrin ligantların spesifik kovalent-olmayan etkileşimi sonucunda su/toluen emülsiyonun arayüzünde çapraz bağlanma gerçekleşmektedir. Bu şekilde oluşan mikrokapsüller optik, floresan ve tünelleme elekton mikroskopileriyle gözlemlenmiştir. Tünelleme elektron mikroskobu görüntüleri sıkı istiflenmiş örgüyü doğrulamıştır. Ayrıca mikrokapsüllerin suya transfer edilebileceği gösterilmiştir.

İkinci kısımda, klik kimyası kullanılarak siklodekstrin bazlı yeni bir hidrojel yapılmıştır. Bu hidrojel spesifik hidrofobik etkileşimlerlerle işlevselleştirilebilmektedir. Bunun yanında kılcal kanallarda kalıplama yöntemi kullanılarak, hidrojellerin mikrokalıplanması başarıyla sağlanmıştır.

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
AD	Adamantane
CD	Cyclodextrin
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMF	Dimethyl Formamide
DMSO	Dimethyl Sulfoxide
FM	Fluorescence Microscopy
FTIR	Fourier Transform Infrared
MHz	Mega Hertz
NMR	Nuclear Magnetic Resonance
NP	Nanoparticle
PDMS	Polydimethylsiloxane
PEG	Polyethylene Glycol
SAM	Self Assembled Monolayer
SEM	Scanning Electron Microscopy
TEM	Tunneling Electron Microscopy
TEG	Tetraethylene Glycol
THF	Tetrahydrofuran
UV	Ultraviolet

1. INTRODUCTION

1.1. Microcapsules

1.1.1. Control at the Nanometer Scale

In spite of the fact that a great amount of the complexity in the biosphere is based on the objects of nanometer size, such objects are very far from being extensively exploited in the technologies brought by chemistry. The problem arises from the lack of control at that size. Organic chemists have been able to synthesize molecules as complicated as taxol, on the other hand by current photolithographic methods devices with components which are close to 100 nm in size can be fabricated. Nanoscience is the study of objects between the limits of these two approaches, that is, between a few nanometers to 100 nanometers [1].

Chemists are already familiar with some nanometer sized objects like polymers, colloids and micelles for a long time. Recently, new members such as dendrimers [2], quantum dots [3], fullerenes [4], carbon nanotubes [5] and silicon nanorods [6] are added to this class of materials. Along with the difficulty in preparation and characterization of these small objects, a challenging task in nanoscience is to control their spatial arrangement in order to get functional materials.

1.1.2. Self-Assembly

Self-assembly which is defined as “the autonomous organization of components into patterns or structures without human intervention” [7], can be employed for addressing the issue of making assemblies of desired architectures from smaller building blocks. Typically, interactions among the building blocks or the surroundings are exploited to bring order from disorder. Such interactions for the nano-scale self-assembly are listed in Table 1.1.

Table 1.1. Nanoscale Interactions [8]

Type Of Interaction	Strength (kJ mol^{-1})
Coulomb	250
hydrogen bond	10-65
ion-dipole	50-200
dipole-dipole	5-50
cation-π	5-80
π - π	0-50
van der Waals forces	<5
hydrophobic effects	difficult to assess
metal-ligand	0-400
covalent bond	100-400

When a target made of several different building blocks is considered or when the orientation of the building blocks is important, undesired interactions must be opted out. This can be achieved by molecular recognition in which complementary shapes or complementary forces stronger than the non-specific competitors are employed. DNA strands and enzymes are examples that demonstrate the level of molecular recognition in nature.

When two building blocks are attached together by multiple binding sites, a synergetic enhancement of the complex formation constant is observed because the attachment of one or more binding sites leads to a tremendous increase in the effective concentration of the remaining binding sites on the same building block. This is called “multivalency”.

1.1.3. Gold Nanoparticles

Gold nanoparticles are colloids of metallic gold that are smaller than 100 nm and they have been extensively studied with the emergence of nanoscience. Due to pronounced quantum mechanical effects at their range of size, their electronic, magnetic

and optical properties are different from those of bulk metal. These properties depend on size, interparticle distance, ligands and the shape of the particles [9].

There are two common methods for the synthesis of gold nanoparticles. In the "citrate reduction" method introduced by Turkevitch [10], $HAuCl_4$ is reduced by trisodium citrate in aqueous media whereas in Brust-Schiffrin [11] method (Figure 1.1) thiol stabilized gold nanoparticles are obtained by reduction of $HAuCl_4$ by $NaBH_4$ in a biphasic system of toluene and water. Size control can be achieved by tuning the stabilizer to gold ratio in both methods.

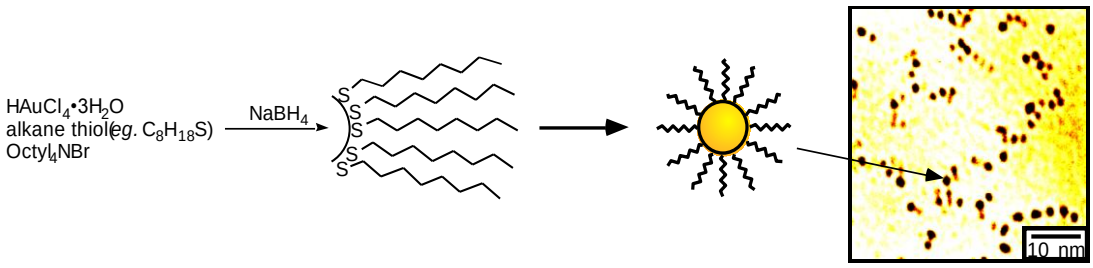

Figure 1.1 Synthesis of Au NPs via Brust-Schiffrin method

Protecting ligands are required for preventing the nanoparticles from aggregation. Ligands that have affinity to the gold nanoparticles surfaces are thiols, phosphines, phosphine oxides, amines and carboxylates, among which thiols are most commonly used owing to stability of their self assembled monolayers (SAMs) on gold. In addition to stabilization, choice of ligand is essential for tailoring the surface functionality of the nanoparticles. SAMs of desired ligands can be prepared either during the synthesis of nanoparticles by Brust-Schiffrin method or by ligand exchange reactions.

1.1.4. Microcapsules Made of Nanoparticle Networks

Ordered networks of nanoparticles have been made by crystallization [12], by directed supramolecular interactions [13] and by templating at the liquid-liquid interfaces [14]. In the latter method, highly regular arrays can be obtained owing to the error correction mechanism provided by the dynamic nature of the system [15] .

When interfaces of emulsions are used for templating, spherical shells of nanoparticle networks are obtained. These shells are called “microcapsules”. For applications such as drug delivery devices microcapsules can be an alternative to vesicles and micelles.

There are two approaches for templating the nanoparticles at liquid-liquid interfaces. In one approach, hydrophobicity of the nanoparticles is rendered so that they are best stabilized at oil-water interfaces (and they stabilize the interface). By this method a monolayer of the nanoparticles can be obtained. Pickering emulsions, in which particles larger than one micron stabilize the liquid-liquid interfaces, have been known for over a century [16]. Binks et al. developed a theory by which energy of interface stabilization can be predicted by the following equation [17]:

$$\Delta E = -\frac{\pi r^2}{\gamma_{O/W}} \times \left[\gamma_{O/W} - \left(\gamma_{P/O} - \gamma_{P/W}\right)\right]^2 \qquad (1.1)$$

Where; r is the effective nanoparticle radius, $\gamma_{O/W}$ is the oil/water interfacial tension, $\gamma_{P/O}$ is the particle/oil interfacial tension and $\gamma_{P/W}$ is the particle/water interfacial tension. The equation implies that interface stabilization increases, when particles are larger and when the particle/oil and particle/water interfacial tensions are close.

An example is the stabilization of the toluene/water interface with 2.5 nm Au nanoparticles by Glogowski et al [18]. Compared to the particles used in Pickering emulsions (> one micron) these nanoparticles are very small, so the extent of interfacial assembly is highly dependent on the particle/oil and particle/water interfacial tensions, that is, on the interactions of the SAMs with the two phases. Initially, when dedacanethiol coated Au nanoparticles were used, nanoparticles were dispersed in the toluene phase and no interfacial assembly was observed. After that, ligand exchange reactions were performed in order to get mixed monolayer coverages of 11-mercapto-1-undecanol and dedacanethiol, and the ratio of ligands in the resulting nanoparticles was followed by ^{1}H-NMR spectroscopy. It was found that when the surface coverage of undecanol ligand was less than 40 %, mole nanoparticles dispersed in toluene, whereas

when it was more than 50 %, they did not disperse in either solvent. However when the undecanol-to-dodecane ratio was approximately 1-to-1.1, stable microcapsules that are transferrable to methanol were obtained (Figure 1.2).

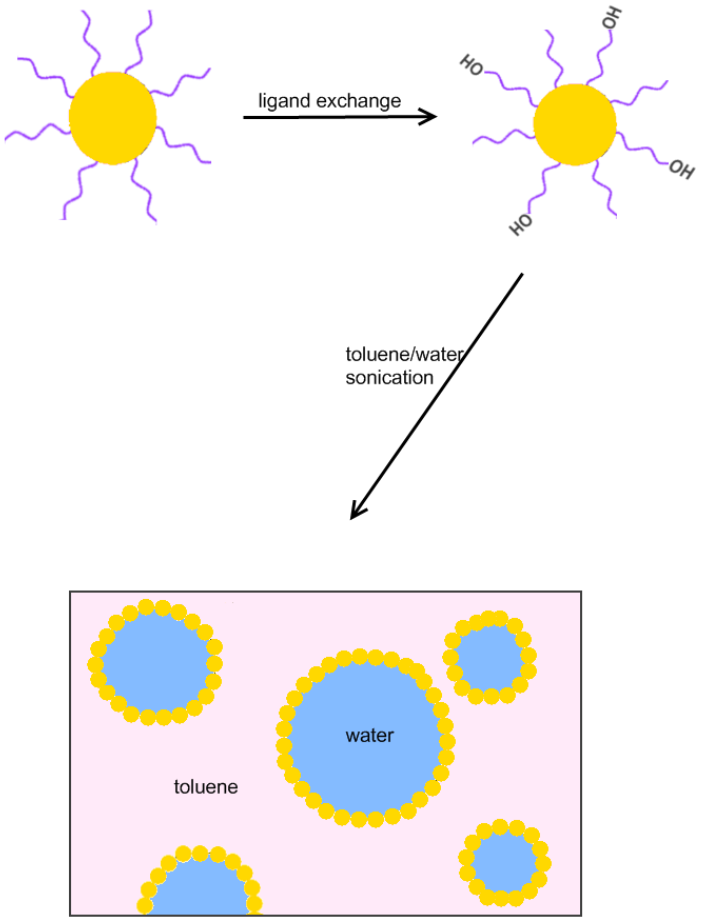

Figure 1.2. Microcapsule fabrication interfacial stabilization of Au NPs

In the second approach nanoparticles are crosslinked by covalent or non-covalent interactions. The nanoparticles may not necessarily stabilize the interface because instead of the nanoparticle–solvent interactions, nanoparticle-nanoparticle interactions are exploited in this case. Once the nanoparticles with the SAMs of the desired ligands

are made, a biphasic system is prepared in which the reaction/interaction conditions are only met at the interfaces.

By following this strategy, Arumugam et al. crosslinked FePt and Au nanoparticles by metal–ligand coordination [19]. In their system, toluene phase had the dispersed terpyridine coated nanoparticles and the water phase contained Fe(II) tetrafluoroborate hexahydrate as a crosslinking agent. Stable microcapsules were obtained when the mixture was shaken for a few seconds (Figure 1.3). It was also shown that microcapsule size could be tuned by Fe(II) concentration.

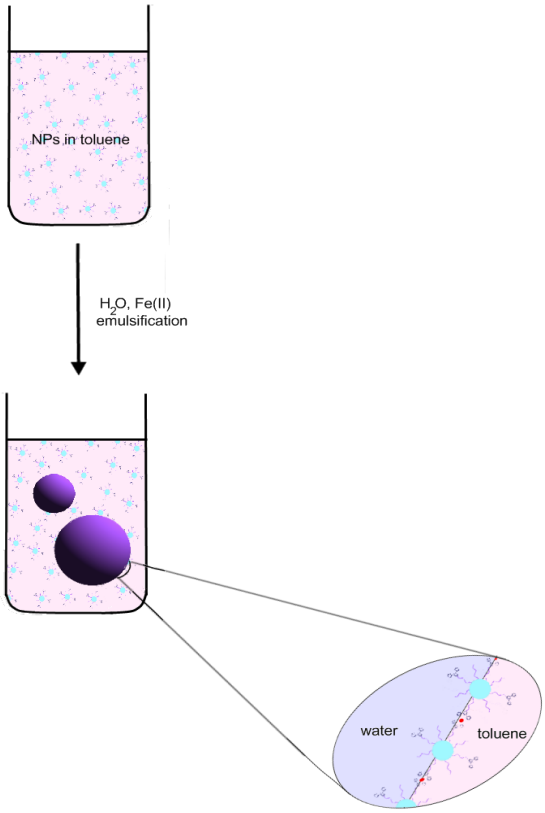

Figure 1.3. Microcapsule fabrication by crosslinking terpyridine coated NPs

In another study by the same group, copper catalyzed alkyne-azide cycloadditon was utilized for microcapsule fabrication. Azide and alkyne coated iron oxide nanoparticles were dispersed in the toluene and an aqueous solution of copper sulfate and sodium ascorbate was added which lead to the crosslinking of the nanoparticles at the interface. Resulting microcapsules were transferrable to water. It was shown that a

high molecular weight dextran did not diffuse from the microcapsule whereas a lower molecular weight polymer demonstrated a slow release profile.

1.2. Hydrogels

Hydrogels are networks of crosslinked polymer chains that absorb water upon absorption of water [20]. Due to their use in biomedical applications like drug delivery systems, contact lenses and corneal implants, there has been a great interest in hydrogels since the early 1960s.

Among the precursor polymers for synthesizing hydrogels are natural polymers such as collagen, chitosan and hyaluronic acid as well as biocompatible synthetic polymers such as poly(hydroxyethyl methacrylate), poly(ethylene glycol) and poly(vinyl alcohol). When crosslinking is achieved by covalent bonds these gels are called “chemical gels”, whereas hydrogels that are crosslinked by non-covalent interactions and entanglements are called “physical gels”.

Depending on the crosslink density of the network and the nature of the hydrophilic domains, the amount of water that can be absorbed by different hydrogels ranges from 10% to thousands of their dry weights. Diffusion rate of solute molecules inside swollen hydrogels depends on several parameters like the size and shape of the solute, pore size, temperature and the amount of free water that is not bound to the polymer network.

1.2.1. Functionalizable Hydrogels

In the last two decades there has been acceleration in the hydrogel research due to the emergence of tissue engineering which aims producing biological substitutes for damaged tissues and organs [21]. The role of hydrogels in the prospective tissue substitutes is to mimic the extracellular matrix (ECM).

Presence of biomolecules such as growth factors and cell adhesion peptides in the ECM are essential for the development and survival of tissues. Hence, these biomolecules must be incorporated into hydrogels that will be used for tissue engineering purposes. This can be achieved either by diffusion or by covalent or non-covalent immobilization. Diffusion is limited by the lack of control over rate and uniformity; in addition, it is not applicable to incorporation of cell adhesion peptides. Thus, hydrogels bearing immobilized biomolecules have a great potential of use in tissue engineering.

For example, DeLong et al. made a hydrogel scaffold with covalently immobilized gradients of bFGF. It was observed that cells seeded on this hydrogel were aligned in the direction of increasing bFGF concentration within 24 hours.

For bioimmobilization on hydrogels, usually a monomer of the desired biomolecule is synthesized. An alternative and more convenient strategy is post–functionalization. However, number of methods for making functionalizable hydrogels is limited due to lack of functional group tolerance with the convential free radical polymerization methods.

1.2.2. Cyclodextrin Hydrogels

Cyclodextrins are cyclic oligomers of glucose units joined by α-1,4-glucosidic linkages. Most common cyclodextrins are α, β, and γ cyclodextrins which have 6, 7 and 8 repeating units respectively. Due to the hydrophobicity of their cavities they can make inclusion complexes with hydrophobic guest molecules in water. They are useful for many pharmaceutical applications because they increase the bioavailability of hydrophobic drugs [22].

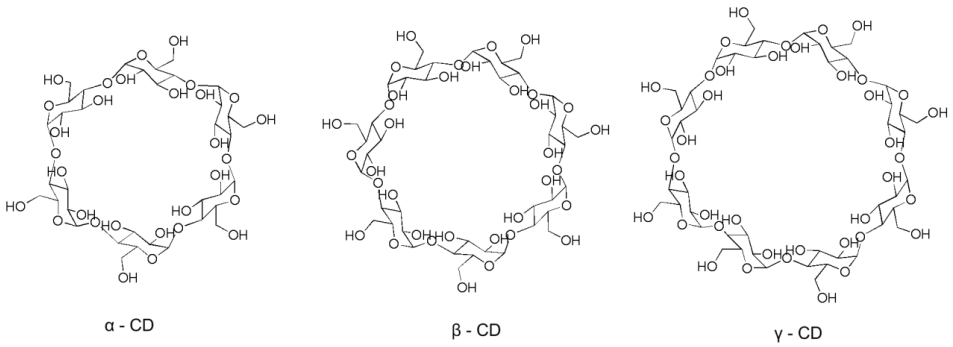

Figure 1.4. Cyclodextrins

Hydrogels containing β-cyclodextrin have been synthesized for their slow release behavior. In early examples, hydrogels with free β-cyclodextrin were investigated. An improvement was observed due to the slower diffusion of the bulky host-guest complex compared to the free guest molecules [23]. In subsequent studies, β-cyclodextrins were incorporated into the hydrogel network.

For example Liu et al [24] synthesized a monomer by reacting β-cyclodextrin by maleic anhydride and compolymerized it with *N*-isopropylacrylamide (NIPAM) by free radical polymerization. The resulting hydrogel was temperature and pH sensitive because of the presence of NIPAM and the acid groups on the β-cyclodextrin, respectively.

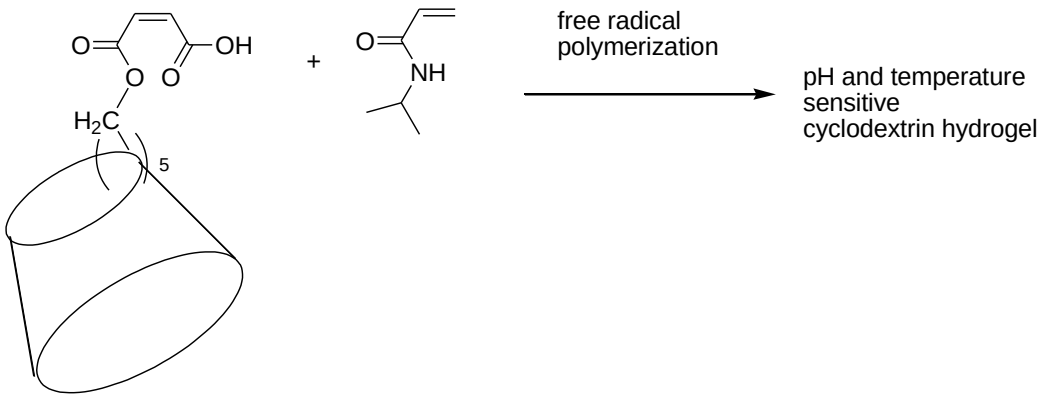

Figure 1.5. Random thermoresponsive CD hydrogel

In another study, the same authors synthesized a β-cyclodextrin containing hydrogel by copolymerization of 2-hydroxyethyl acrylate and a monovinyl cyclodextrin monomer. It was found that compared to the hydrogel without β-cyclodextrin, β-cyclodextrin containing hydrogel demonstrated a sustained release of the drug *N*-acety-5-methoxytryptamine due to the inclusion complex formation.

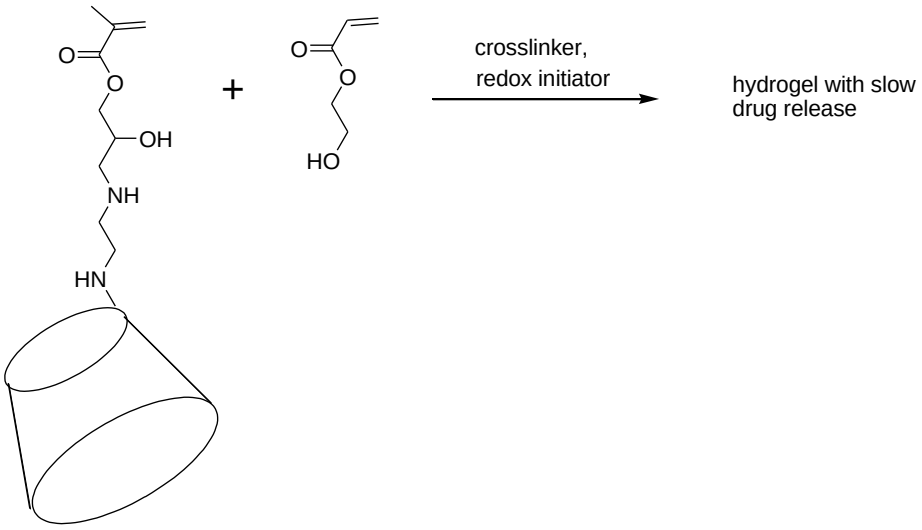

Figure 1.6. CD based hydrogel with slow release activity

1.2.3. Hydrogels by Click Chemistry

Copper catalyzed alkyne-azide cycloadditon click reaction has been of great interest in macromolecular research [25]. Its high yield at mild conditions, tunable kinetics as well as functional group tolerance, are very desirable characteristics for hydrogel synthesis. In addition, compared to commonly used free-radical polymerization techniques, hydrogels made by click reaction are more homogeneous and have a well-defined topology.

Malkoch et al. synthesized a PEG based hydrogel by the click reaction between PEG di alkyne and a PEG tetra azide [26]. It was found that obtained gels had highly improved mechanical properties compared to the hydrogels made by photochemical methods.

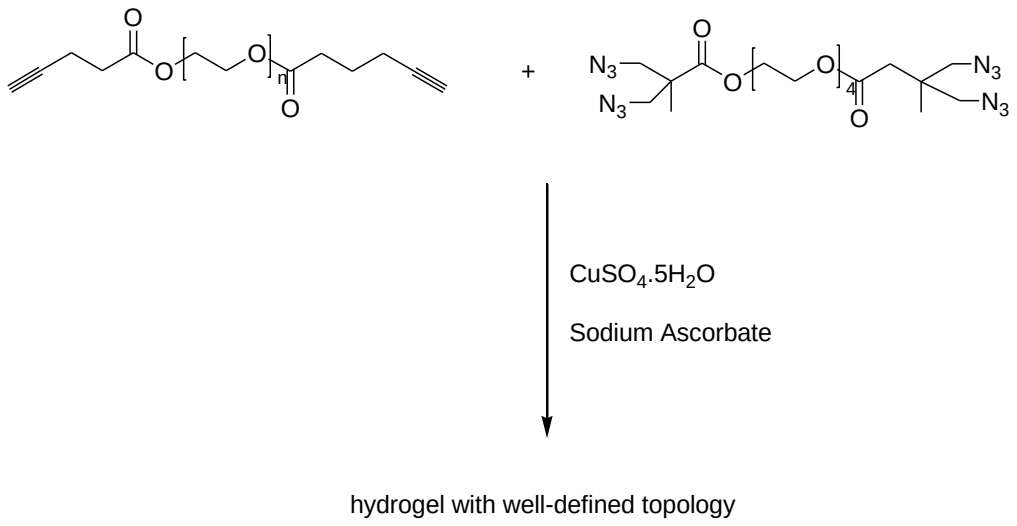

Figure 1.7. Precisely tailored hydrogels via Click chemistry

For surveying residual acetylene/azide functionalities, the hydrogels were modified by azide and alkyne functionalized chromophores. Fluorescence analyses revealed that the hydrogel contained less than 0.2 percent unreacted acetylene/azide groups which proves the efficiency of the click reaction for making hydrogels.

Ossipov et. al made hydrogel by crosslinking linear PVA copolymers bearing azide and alkyne pendant groups by click chemistry (Figure 1.8.). The hydrogels made by these precursors had higher gelation ratios compared to hydrogels made by a linear PVA copolymer bearing alkyne pendant groups and a bis-azide functionalized PEG crosslinker.

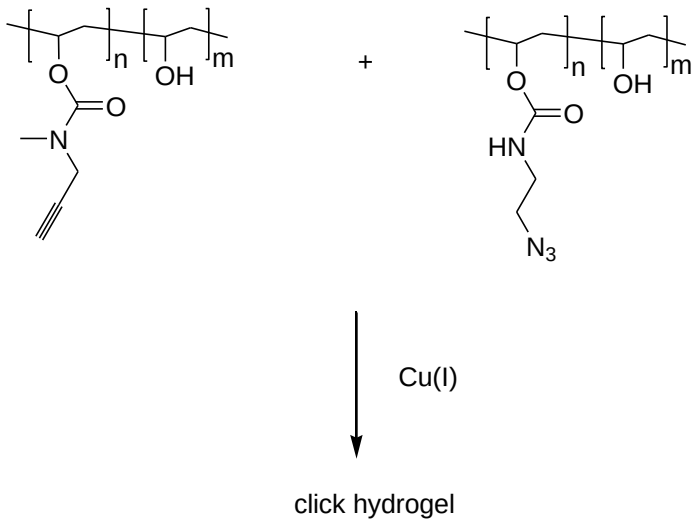

Figure 1.8. PVA based click hydrogel

Another example is the hydrogel made by Crescenzi et al. [27] via the click reaction between hyaluronans containing azide and alkyne side chains. Control on doxorubicin release was achieved by tuning the crosslink density of the hydrogels. Besides, when hydrogels were synthesized in the presence of yeast cells, 60% of the entrapped cells proliferated after 24 hours. Polizzotti et al. used an azide terminated multiarm PEG and an alkyne functionalized polypeptide sequence to make a thiolene functionalizable click hydrogel [28]. Double bonds of the Alloc groups at the side chains were unaffected by the gelation process owing to the functional group tolerance of the click chemistry. Three dimensional biochemical patterning of the resulting hydrogel was demonstrated by thiol-ene photopolymerization.

A limitation of click chemistry for hydrogel synthesis is the use of copper salts that are usually only water soluble. Since good solubility of the all reactants are required for obtaining homogeneous hydrogels, either all the precursors must be water soluble or an inhomogeneous gel will be obtained.

2. AIM OF THE STUDY

2.1. Microcapsule Fabrication via Supramolecular Interactions of Nanoparticles

Fabrication of nanoparticle based microcapsules using the oil-water microemulsions have generally relied on either the tendency of amphiphilic nanoparticles to assemble at the interphase, or chemical crosslinking between nanoparticles induced at the phase boundary. As a novel alternative, we envisioned that supramolecular interactions between ligands on water soluble and organo soluble nanoparticles could lead to crosslinking of nanoparticles at the interphase to afford stable microcapsules. Water soluble cyclodextrin functionalized gold nanoparticles when mixed with toluene soluble adamantane functionalized gold nanoparticles as a micro-emulsion would result in the formation of microcapsules due to crosslinking via specific non-covalent ‘host-guest’ chemistry.

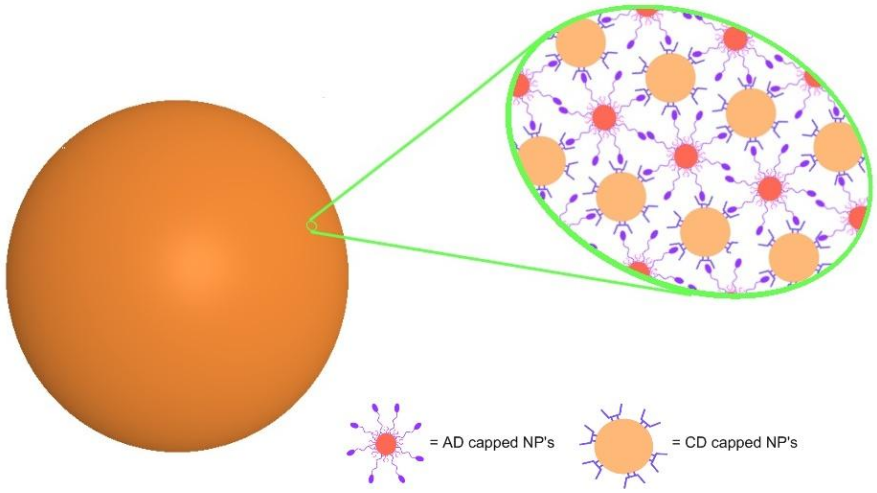

Figure 2.1. Microcapsules made of AD and CD functionalized Au NPs.

2.2. Synthesis And Micropatterning Of Cyclodextrin Based Hydrogels

Traditionally, hydrogels have been functionalized using non-specific absorption or encapsulation of guests such as drugs, biomolecules and cells. Selective and controlled incorporation of guest molecules within the hydrogel matrix via specific 'host-guest' chemistry and/or efficient covalent functionalization will broaden utility of these scaffolds. We proposed that a β-cyclodextrin containing hydrogel with a well-defined topology can be synthesized by the click reaction of per azido β-cyclodextrin and bis alkyne functionalized PEG using the copper catalyzed Huisgen-type 'click' cycloaddition reaction. Such a hydrogel can be functionalized non-covalently by making host-guest inclusion complexes with functional guest molecules bearing adamantane moieties. In addition to the non-covalent functionalization, covalent functionalization by click chemistry can be achieved by tuning the stoichiometry of the precursors to adjust residual alkyne or azide functional groups.

3. EXPERIMENTAL

3.1. Methods and Metarials

All chemicals were used as received from manufacturer (Merck, Aldrich, Alfa Aesar, Riedel de Haen). Dry solvents (CH_2Cl_2, THF, toluene) was obtained from ScimatCo Purification System, other dry solvents were dried of molecular sieves. Column chromatography was performed using silicagel-60 (43-60 nm). Thin layer chromatography was performed using silica gel plates (Kiesel gel 60 F254, 0.2mm, Merck). Plates were viewed under 254nm UV lamp otherwise plates are developed either by $KMnO_4$ stain or $EtOH/H_2SO_4$ (95:5). Infrared spectroscopy was carried out on Thermo Scientific Nicolet 380 FT-IR spectrophotometer. Removing water from cyclodextrins was accomplished with LabConco lyophilizer. ^1H NMR (operating at 400 MHz) and ^{13}C NMR (operating at 100 MHz) were recorded on Varian Mercury-MX in either D_2O or $CDCl_3$ as solvent at the Advanced Technologies Research and Development Center at Boğaziçi University.

3.2. Microcapsules

3.2.1. Synthesis of Adamantane Tetraethylene Glycol

Adamantane tetraethylene glycol was synthesized according to a lituretature procedure [29]. Adamantyl bromide (0.5 g, 2.32 mmol) was dissolved in tetraethylene glycol (TEG) **(**10 mL) and triethylamine (TEA) (98 µL, 6.52 mmol) added. The mixture was allowed to react for 48 h at 120 ^oC. The reaction mixture was diluted with 25 mL CH_2Cl_2 and extracted with 1M HCl (2 x 25 mL) and then H_2O (3 x 25 mL). The residue was dried over Na_2SO_4, filtered and volatiles evaporated under vacuum. The crude product was purified by column chromatography with ethyl acetate:hexane (80:20) to yield adamantane tetraethylene glycol (AD-TEG) as yellow oil. ^1H NMR spectrum was in agreement with the literature data.

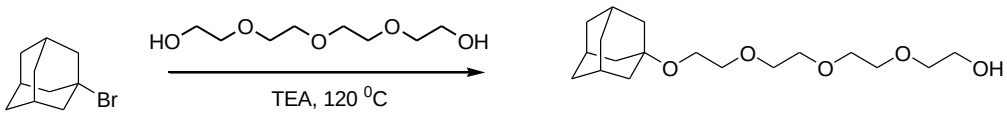

Figure 3.1. Synthesis of AD-TEG

3.2.2. Synthesis of Adamantyl Ligand

To a solution of tetraethylene glycol mono-adamantyl ether (205 mg, 0.63 mmol) and thioctic acid (103 mg, 0.50 mmol) in dry CH_2Cl_2 (5 mL), were added EDCI (105 mg, 0.55 mmol) and DMAP (24 mg, 0.20 mmol). The reaction was allowed to stir under nitrogen overnight. The solvent was concentrated under reduced pressure and the residue was purified by column chromatography (40% EtOAc in hexane) to afford the ADA ligand as a yellow oil (196 mg, yield 76 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm) = 4.20 (2H, t), 3.67 (2H, t), 3.63 (8H, s), 3.56 (5H, m), 3.12 (2H, m), 2.43 (1H, m), 2.33 (2H, t), 2.11 (3H, s), 1.88 (1H, m), 1.72 (6H, s), 1.63 (10H, m), 1.44 (2H, m). ^{13}C NMR(400 MHz, $CDCl_3$) δ(ppm): 173.43, 72.23, 71.27, 70.63, 70.58, 70.57, 70.55, 69.15, 63.46, 59.23, 56.30, 41.45, 40.19, 38.46, 36.44, 34.57, 33.92, 30.48, 28.71, 24.59. Anal. Calcd for $C_{26}H_{44}O_6S_2$: C, 60.43; H, 8.58; S, 12.41. Found: C, 60.61; H, 8.62; S, 12.37.

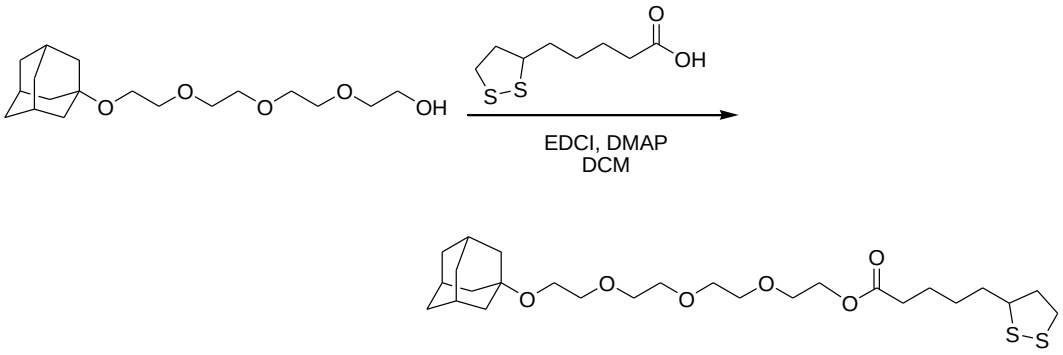

Figure 3.2. Synthesis of AD Ligand

3.2.3. Synthesis of ADA functionalized Au Nanoparticles

$HAuCl_4$ (255 mg, 0.75 mmol) was dissolved in 75 mL of deionized H_2O in a 250 mL round-bottom flask to give a golden yellow solution. A 75 mL of toluene solution of tetra-*n*-octylammonium bromide (615 mg, 1.13 mmol) was then added and the mixture was stirred. After 15 min, the organic layer had become dark red and the aqueous phase colorless. A 5 ml solution containing octanethiol (192 mg, 1.32 mmol) and the ADA ligand (96 mg, 0.186 mmol) in toluene was then added, causing the organic phase to become pale yellow. $NaBH_4$ (570 mg, 15 mmol) was then added all at once as a solution in 5 mL of H_2O, causing an immediate color change to dark brown and the evolution of gas. After 2h, the organic phase was collected and the solvent removed. The resulting residue was dissolved in 2 mL of CH_2Cl_2 and precipitated in 200 mL of ACN. When precipitation was complete, the product was collected by centrifugation and washed multiple times with ACN to fully remove unreacted ligands. ^1H NMR (400 MHz, $CDCl_3$) δ(ppm) = 4.2 (broad signal), 3.60 (broad signal), 2.13 (broad signal), 1.72 (broad signal), 1.58 (broad signal), 0.8-1.4 (broad signals).

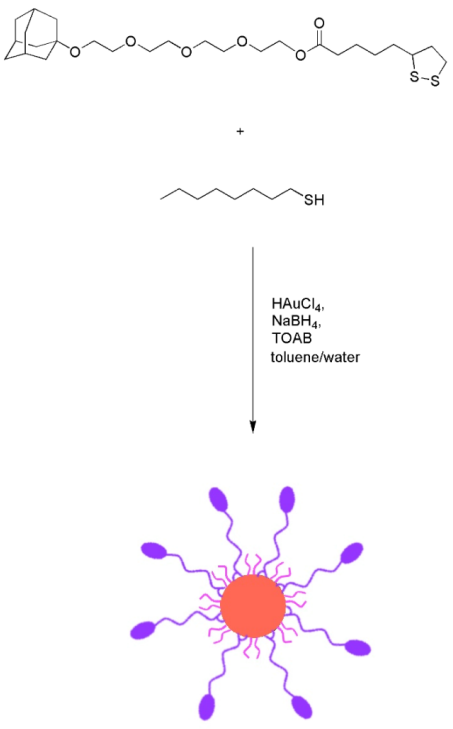

Figure 3.3. Synthesis of ADA functionalized Au NPs

3.2.4. Synthesis of Per iodo β cyclodextrin:

Per iodo β cyclodextrin was synthesized according to a literature procedure[30]. To a solution of Ph_3P (6.91 g, 26.4 mmol) in dry DMF (30 mL), I_2 (6.98 g, 27.6 mmol) was added over 15 mins. Heat evolved during this addition and the solution increased in temperature from RT to approximately 50 °C. Dry D-cyclodextrin (2.00 g, 10.2 mmol) was then added to this dark brown solution and the temperature was raised to 70 °C using an oil bath. The solution was stirred at this temperature for 20 h. The heat was removed, and the solution was concentrated to 1/3 of its original volume. To this residue, NaOMe in MeOH (3.00 M, 10.3 mL) was added where the reaction was cooled in an ice/salt slurry and the mixture was stirred for 30 mins. The reaction mixture was then poured into MeOH (150 mL) to form a precipitate, which was then washed with MeOH 6-7 times by successive dispersions and centrifugations. ^{1}H NMR spectrum was in agreement with the literature data.

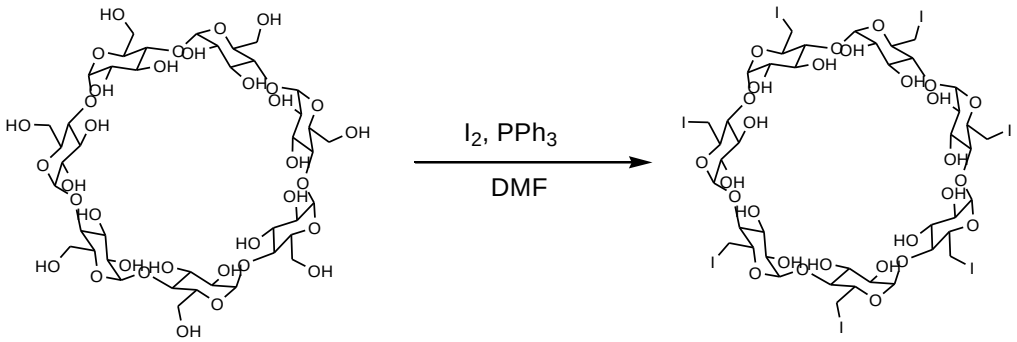

Figure 3.4. Synthesis of Per iodo β CD

3.2.5. Synthesis of Per thio β cyclodextrin:

Per thio β cyclodextrin was synthesized according to a literature procedure[31]. Per iodo cyclodextrin (0.500 g, 0.26 mmol) was dissolved in DMF (6 mL); thiourea (0.156 g, 2.05 mmol) was then added and the reaction mixture heated to 70 ^{o}C under a nitrogen atmosphere. After 19 h, the DMF was removed under reduced pressure to give a yellow oil, which was dissolved in water (25 mL). Sodium hydroxide (0.135 g, 3.37 mmol) was added and the reaction mixture heated to a gentle reflux under a nitrogen

atmosphere. After 1 h, the resulting suspension was acidified with aqueous $KHSO_4$ and the precipitate filtered off, washed thoroughly with distilled water, and dried. To remove the last traces of DMF, the product was suspended in water (25 mL) and the minimum amount of potassium hydroxide added to give a clear solution; the product was then reprecipitated by acidifying with aqueous $KHSO_4$. The resulting fine precipitate was carefully filtered off and dried in the freeze-drier. ^{1}H NMR spectrum was in agreement with the literature data.

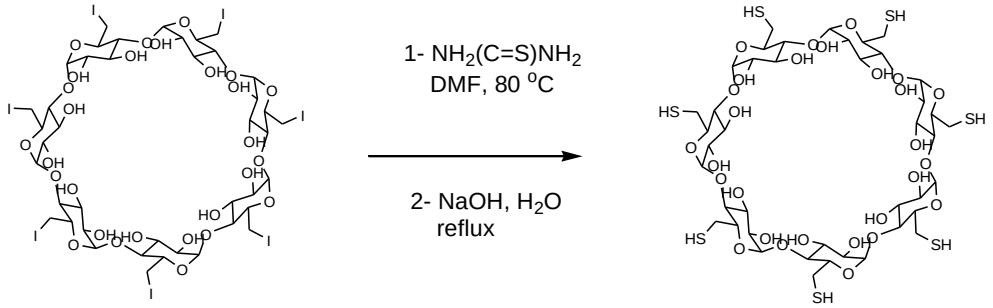

Figure 3.5. Synthesis of Per thio β CD

3.2.6. Synthesis of Cyclodextrin Functionalized Au Nanoparticles

CD-functionalized Au nanoparticles were synthesized according to a literature procedure [32]. $HAuCl_4$ (90 mg, 0.27 mmol) was dissolved in DMSO (10 mL). This solution was mixed quickly with a solution of $NaBH_4$ (123 mg, 3.25 mmol) and per thio cyclodextrin (100 mg, 0.08 mmol) in DMSO (10 mL). The reaction mixture turned deep-brown immediately, and was allowed to continue for 24 h. At this point, CH_3CN (20 mL) was added to precipitate the nanoparticles, which were collected and purified by centrifugation, followed by washing with CH_3CN:DMSO (1:1 v/v, 30 mL) and ethanol (30 mL), dissolution in water, and freeze-drying. ^{1}H NMR spectrum was in agreement with the literature data.

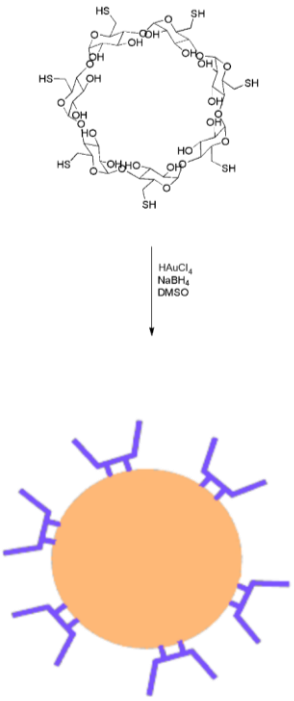

Figure 3.6. Synthesis of CD Functionalized Au NPs

3.3. Hydrogels

3.3.1. Synthesis of Per-6-azido-cyclodextrin

Per-6-azido-cyclodextrin was synthesized according to a procedure[30].

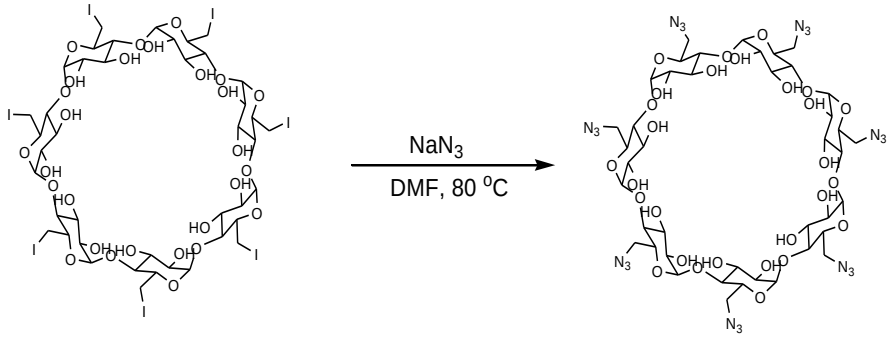

Figure 3.7. Synthesis of per azido CD

To a solution of per-6-iodo-β-cyclodextrin (3.2 g, 1.68 mmol) in dry DMF (25 mL), NaN_3 (1.09 g, 16.8 mmol) was added and the resulting suspension was stirred at

80 °C for 24h. The suspension was concentrated under reduced pressure to 1/3 of the starting volume. The residue was then treated with water (~ 50 mL). The precipitate was filtered, washed with water, and dried under high vacuum to yield a white powder. ^{1}H NMR spectrum was in agreement with the literature data.

3.3.2. Synthesis of PEG Dialkyne:

Prior to use, PEG (MW 6000) was dried via azeotropic distillation with toluene twice. Then, 3 g polymer was treated with NaH (120 mg, 5 mmol) in THF (35 mL). Propargyl bromide (0.86 mL, 10 mmol) and a catalytic amount of KI were then added to the solution and the resulting mixture was heated to reflux for 2 h. Water (1 mL) was then added and the solvent was removed under vacuum. To the residue was added CH_2Cl_2 (25 mL) and the organic layer was separated, dried over anhydrous Na_2SO_4, and the volume was reduced to approximately 2 mL. This CH_2Cl_2 solution was added to diethyl ether (150 mL) drop-wise. The resulting precipitate was collected, washed with several portions of cold diethyl ether, and dried to afford PEG dialkyne.
The same procedure was followed for making PEG 3000 dialkyne and PEG 10000 dialkyne.

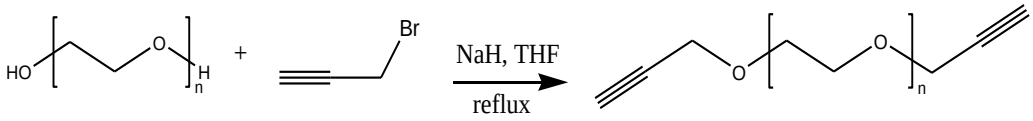

Figure 3.8. Synthesis of PEG dialkyne

3.3.3. Synthesis of Hydrogels

Per-azido-β-cyclodextrin(4 mg, 3 x 10^{-3} mmol) was dissolved in an aqueous solution (0.75 ml) of tetraethylene glycol mono adamantyl ether (100 mg) by sonication for 15 minutes. To this solution was added PEG dialkyne (MW 6080, 65 mg, 1.1 x 10^{-2} mmol), sodium ascorbate (4.3 mg, 2.1 x 10^{-3} mmol) and aqueous $CuSO_4.5H_2O$ (1.1 x 10^{-3} mmol, 55 µl, 20mg/ml). The solution was allowed to react for 30 min.

Hydrogel obtained was washed with deionized water (50 ml, 1h, x 2) and ethanol (50 ml, 1h, x 2). Finally it was allowed to reach maximum absorption in deionized water and freeze-dried.

The same procedure was followed for making hydrogels with PEG 3000 dialkyne and PEG 10000 dialkyne as well as with PEG 6000 dialkyne where the azide:alkyne ratio was 2:1 and 1:2.

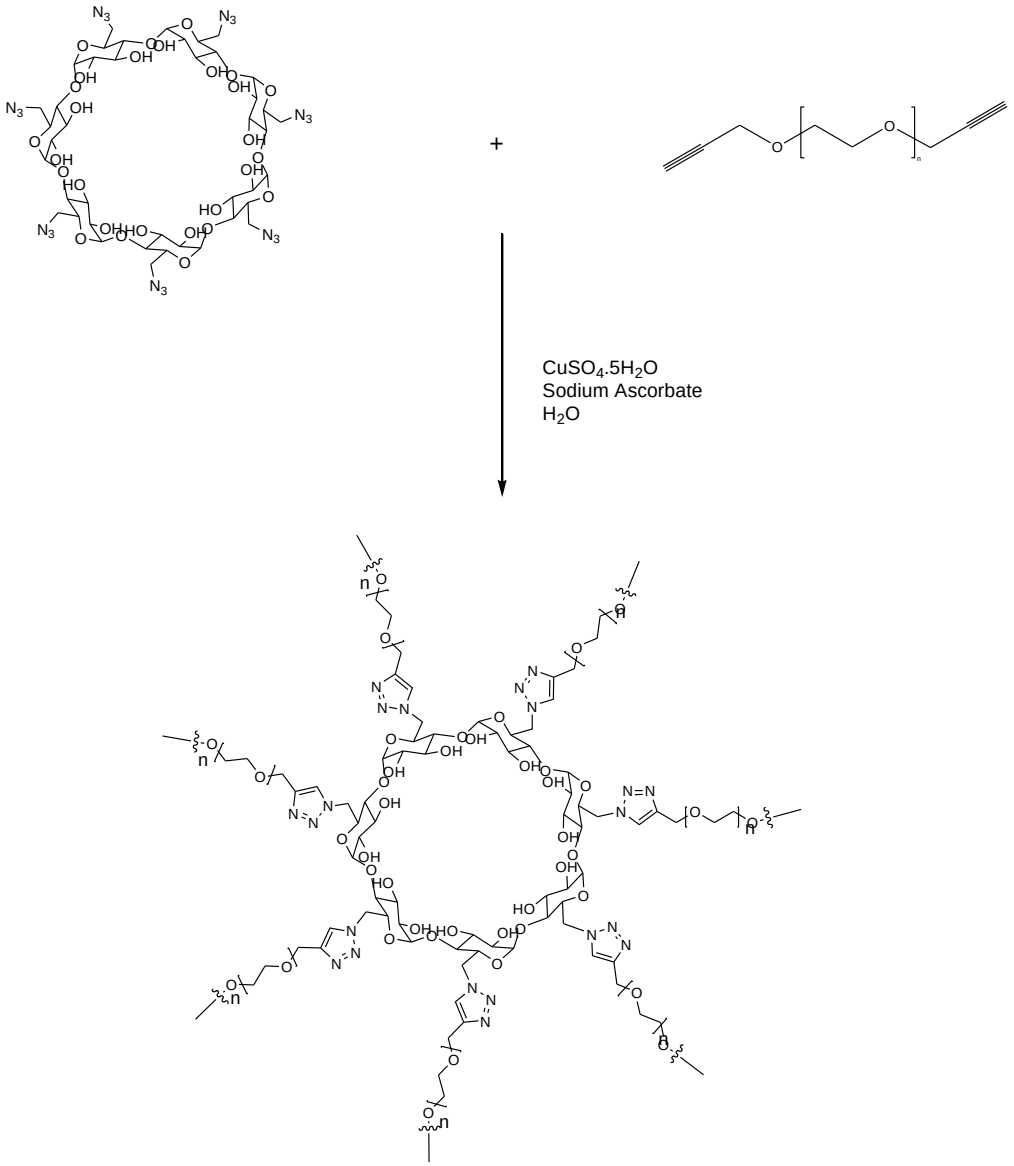

Figure 3.9. Synthesis of hydrogel by per azido CD and PEG dialkyne

3.3.4. Synthesis of AD-Lissamine

Lissamine-tetraethylene glycol mono adamantyl ether was synthesized according to a literature procedure [33].

A solution of tetraethylene glycol mono-adamantyl ether (41 mg, 0.125 mmol) and 0.25 ml triethylamine in 5 mL acetonitrile was cooled down to 0–5 °C. Then lissamine sulfonyl chloride (108 mg, 0.19 mmol) was added and the mixture was stirred overnight. The solvent was evaporated at reduced pressure and the residue was purified by column chromatography (15:85 MeOH:EtOAc) . Product was obtained as a purple solid. ^{1}H NMR spectrum was in agreement with the literature data.

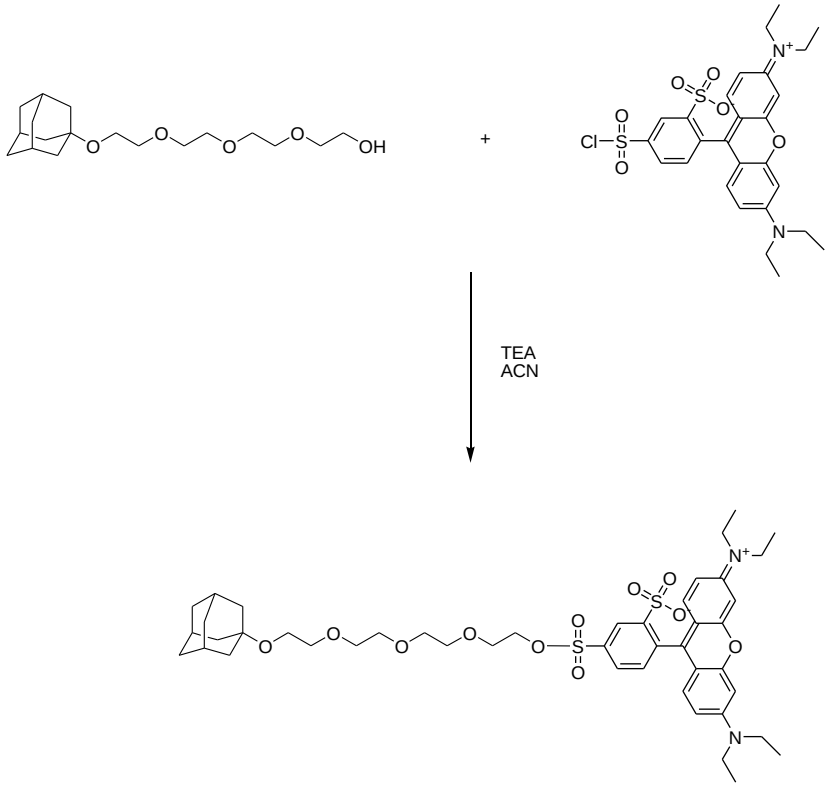

Figure 3.10. Synthesis of AD-Lissamine

4. RESULTS

4.1. Microcapsules

4.1.1. Synthesis of the AD and CD functionalized Au nanoparticles

Water soluble cyclodextrin (CD) functionalized gold nanoparticles have been utilized for making disordered supramolecular networks for more than a decade [34]. For this study literature protocols were followed to obtained the required CD coated nanoparticles.

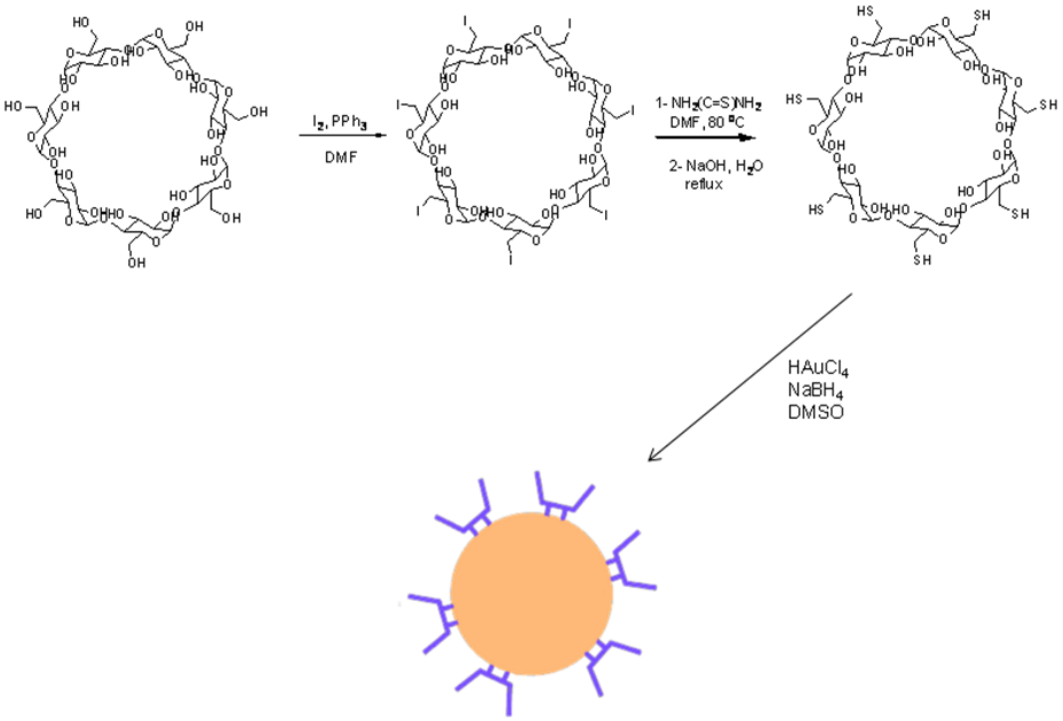

Figure 4.1 Synthesis route to CD functionalized Au NPs.

According to TEM images (Figure 4.2.) average particle size was found to be about 4 nm.

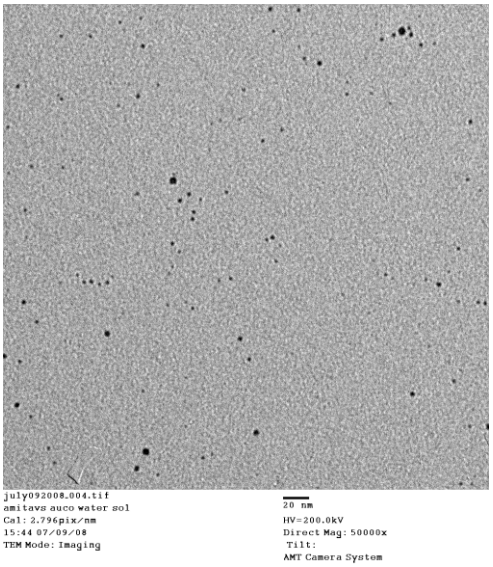

Figure 4.2. TEM image of CD functionalized Au NPs

Synthesis of the AD functionalized gold nanoparticles was accomplished using a modified Brust-Schiffrin method in which adamantane disulfide based ligands were used.

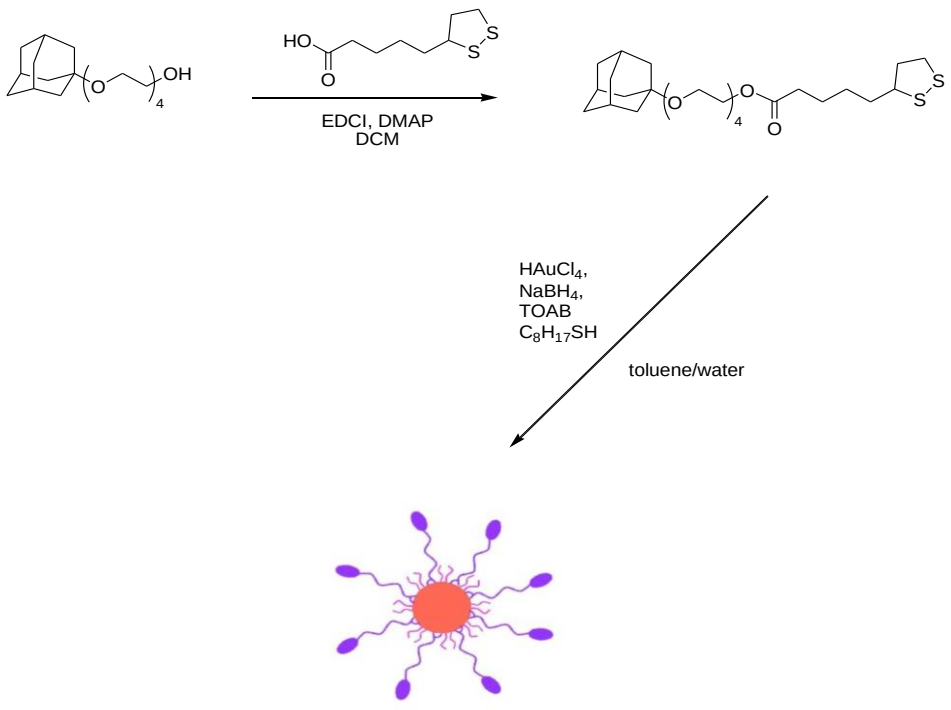

Figure 4.3. Synthesis route to AD functionalized Au NPs.

4.1.2. Synthesis of the Microcapsules

15 µl of aqueous CD functionalized Au NP solution (2% w.) was added to a 0.3 ml solution of AD functionalized Au NPs (0.4% w.) in toluene. The mixture was emulsified by shaking vigorously for a few seconds. Consequently, toluene was removed by a pipette as much as possible and fresh toluene was added. Last step was repeated until the toluene phase became colorless.

Microcapsules obtained were monitored by optical microscopy:

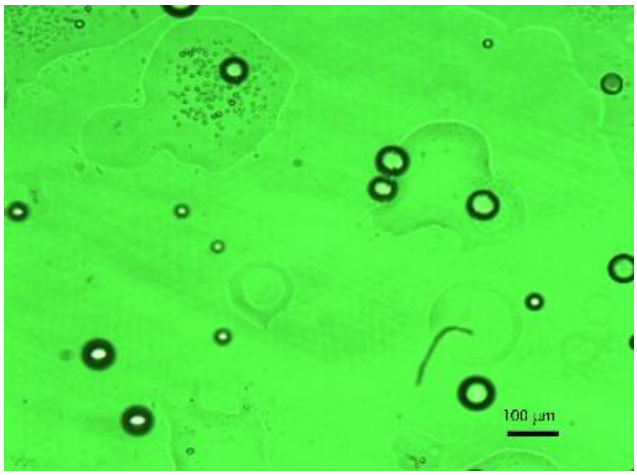

Figure 4.4. Microcapsules in toluene

Upon gradual evaporation of the toluene, microcapsules began wrinkling:

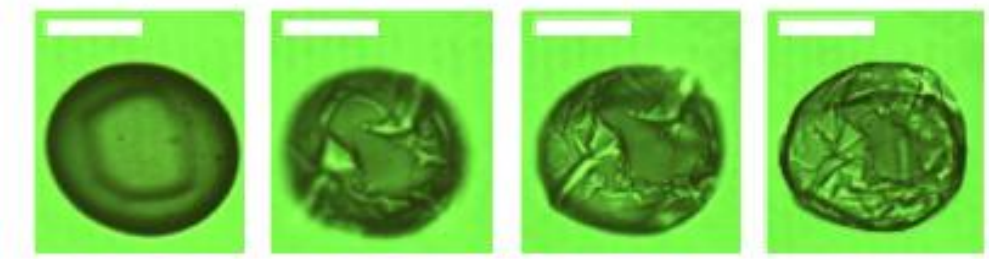

Figure 4.5. Wrinkiling microcapsules in toluene. (Scalebars are 50 µm.)

In the TEM image of the microcapsules, close packed assembly of the nanoparticles is visible.

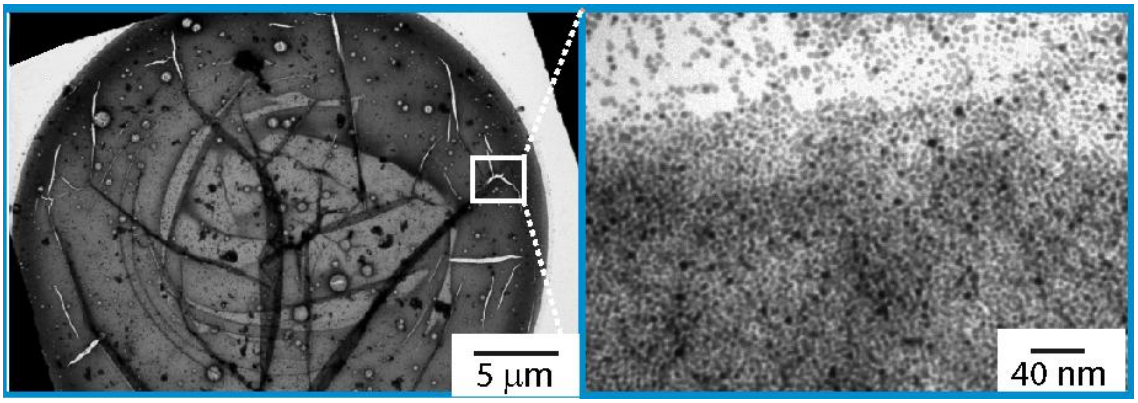

Figure 4.6. TEM image of the microcapsules

4.1.3. Transfer of the Microcapsules into Water

Most of the toluene was removed and methanol was added slowly. This usually leads to the dispersion of the microspheres. When the microspheres came to rest at the bottom (10 min), most of the methanol was removed and fresh methanol was added. This step was repeated a few times to remove toluene completely. Finally most of the methanol was removed and water was added. This gave a white turbid mixture.

Optical microscopy images show that the microcapsules were successfully transferred into water.

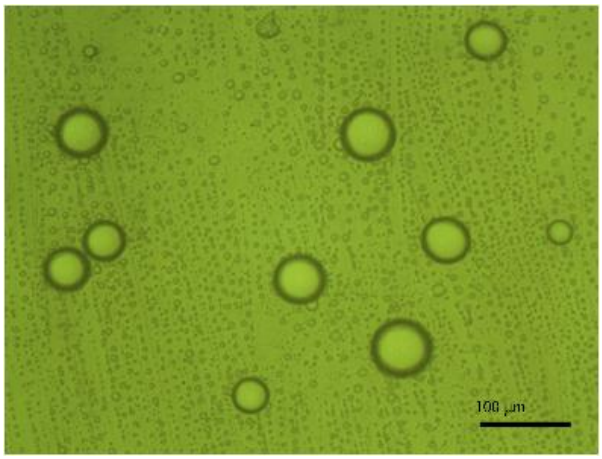

Figure 4.7. Microcapsules in water

Figure 4.8. shows the wrinkiling microcapsules upon gradual evaporation of water.

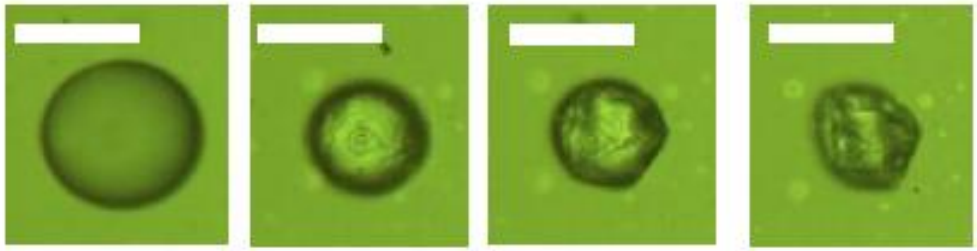

Figure 4.8. Wrinkiling microcapsules in toluene.(Scalebars are 50 μm)

4.1.4. Membrane Formation

When the nanoparticle concentrations are low (about 0.03 % and 0.08 % for AD and CD functionalized NPs respectively) and the volumes of the two solvents are close, in stead of the formation of microcapsules, a membrane is observed at the interface of two liquids upon shaking. Besides, color of the less concentrated layer disappears (Figure 4.9). It was observed that membrane was destroyed upon addition of AD-TEG.

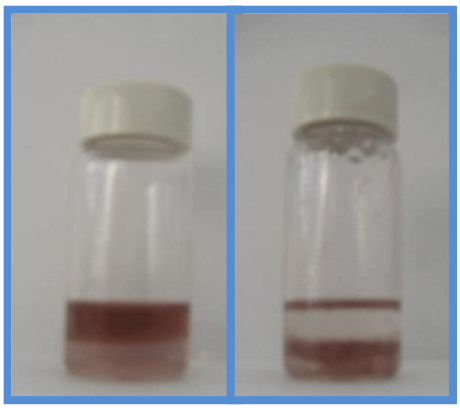

Figure 4.9. Biphasic nanoparticle mixture before and after shaking

4.2. Hydrogels

4.2.1. Synthesis of Hydrogels:

For the synthesis of the hydrogel we chose water as the solvent because copper salts are only water soluble. In addition, for some tissue engineering purposes especially when gelation occurs in the presence of cell cultures, physiological conditions are more desirable. Among our precursors, per azido cyclodextrin was water insoluble. In order to solubilize it we exploited its ability to make inclusion complexes. We synthesized a guest molecule in which adamantane is attached to a tetraethylene glycol tail. Later we dissolved the per azido cyclodextrin in a concentrated solution of it (Figure 4.10). Hydrophilic tail provides the water solubility of the resulting complex. Since host-guest complex formation is reversible, guest molecules can be removed from the cavities by washing after gelation. Figure 4.11. shows the obtained gel before any treatment.

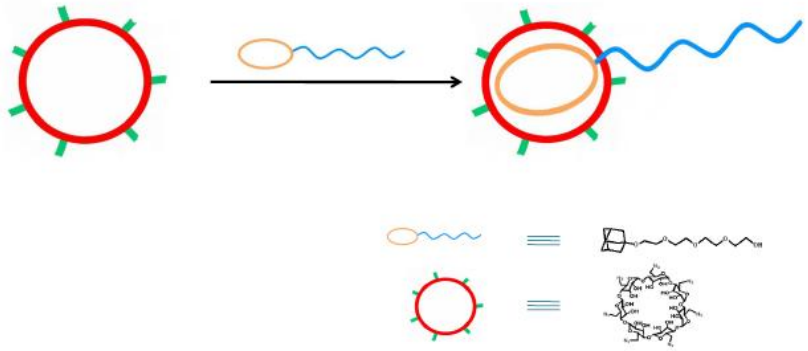

Figure 4.10. Schematic illustration of the method for solubilizing per azido CD



Figure 4.11. Hydrogel without any treatment

From the IR spectrum of the hydrogel, it can be seen that no azide peak at 2100 cm^{-1} was left as expected (Figure 4.12.).

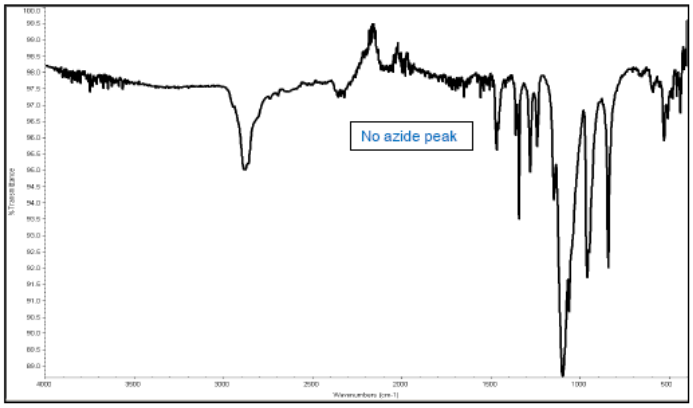

Figure 4.12. FT-IR spectrum of the hydrogel made by PEG 6000 dialkyne with an azide:alkyne ratio of 1:1.

Figure 4.13. shows the IR spectra of the hydrogels with 2:1 and 1:2 azide:alkyne ratio. An azide peak is visible for the former due to the unreacted azide groups left.

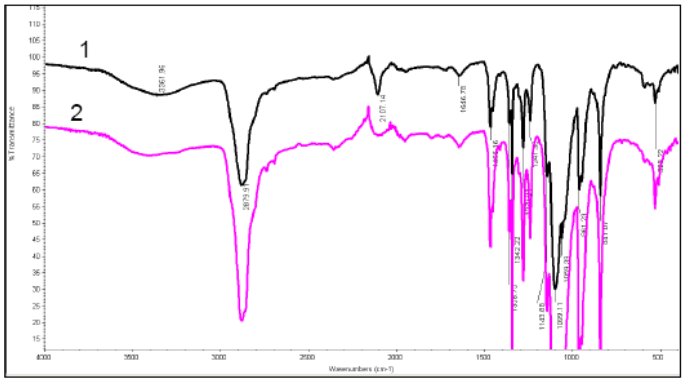

Figure 4.13 FT-IR spectrum of the hydrogels made by PEG 6000 dialkyne with the azide:alkyne ratio 2:1 (1), and 1:2 (2)

Highly porous nature of the freeze-dried hydrogel can be seen in the SEM image (Figure 4.14.)

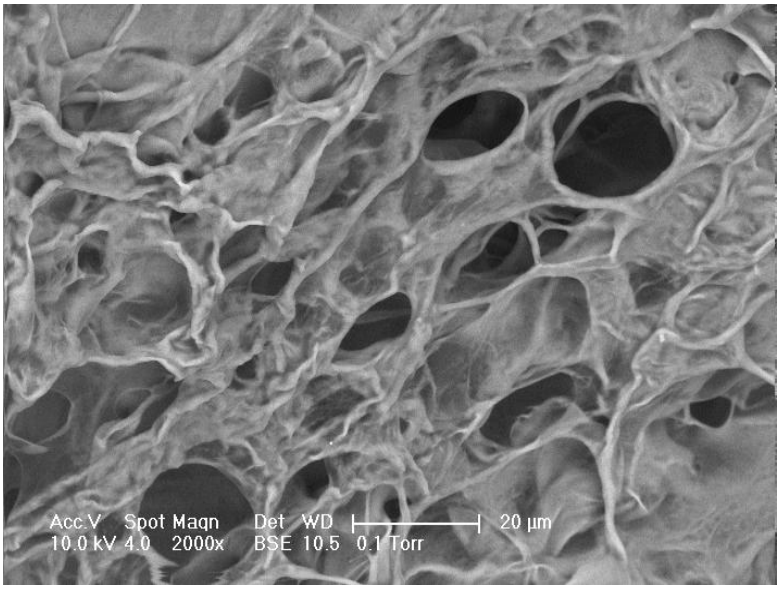

Figure 4.14. SEM image of the freeze-dried hydrogel made by PEG 6000 dialkyne.

4.2.2. Swelling Measurements

Dry hydrogel samples were immersed into 50 ml water and were weighed after wiping the water with paper towel at designated time intervals. The amount of absorbed

water was calculated by the ratio of the increase in the weight of the gel to the weight of the dry gel: $(W-W_0)/ W_0$.

Figure 4.15 shows the water uptake plots of the hydrogels with different PEG chain lengths. As expected, amount of water uptake increases with increasing PEG chain length.

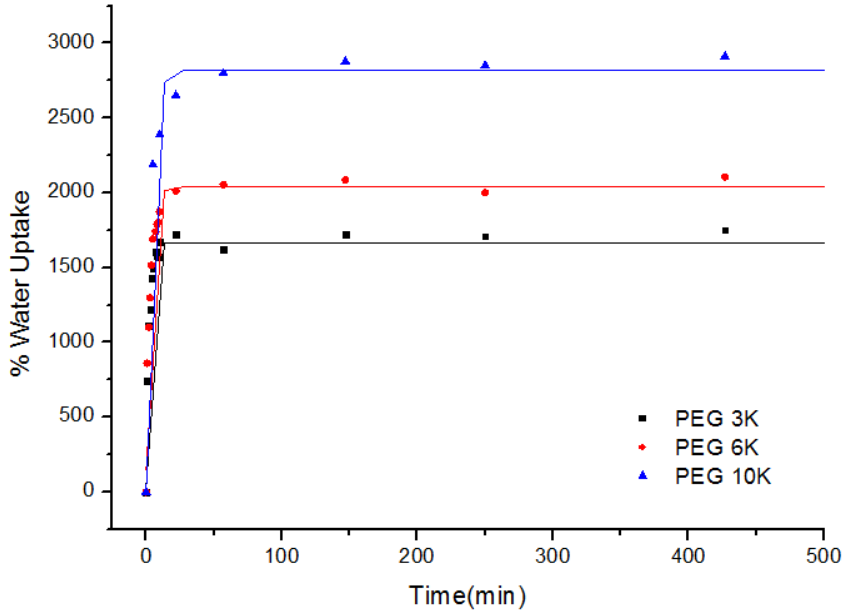

Figure 4.15. Water uptake plots of the hydrogels with different PEG chain lengths.

Figure 4.16. shows the water uptake plots of hydrogels with different azide:alkyne ratios. It is observed increasing the PEG content increases the water uptake.

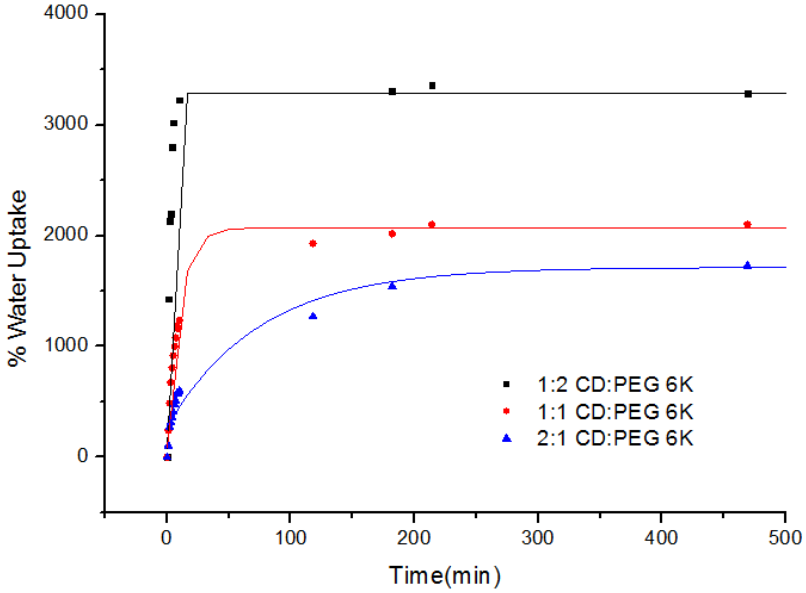

Figure 4.16. Water uptake plots of hydrogels with different azide:alkyne ratios.

4.2.3. Availability of Cyclodextrin binding sites

Availability of cyclodextrin's hydrophobic cavity can be tested by phenolphthalein probe. At basic media phenolphthalein has a pink color but in the presence of cyclodextrin, it enters inside the hydrophobic cavity to make an inclusion complex which leads to the disappearance of the color. A 2 ml phenolphthalein solution (30 μM, 1 eq.) was prepared with pH 10.5 Na_2CO_3-$NaHCO_3$ buffer. Freeze-dried hydrogels containing about 4 mg of cyclodextrin (60 eq.) were immersed into this solution and phenol phthalein uptake was followed by the disappearance of the pink color.

Since during the hydrogel, for solubilizing per azido cyclodextrin a large excess of adamantyl tetra ethylene glycol guest is used, whether this guest is completely removed from the hydrogel after treatment remains a question. Since hydrophobic interactions work only in water, it is expected that washing with ethanol will take all the guest molecules, whereas washing with water will leave bound guest molecules inside the hydrogel.

Result of the uptake test can be seen in Figure 4.17. Control hydrogel that was washed with water only (A) retains the color of phenolphthalein which means cyclodextrin

cavities are full with adamantyl tetraethylene glycol. Hydrogel washed with ethanol (B) changes the pink color to colorless, which means all adamantyl tetraethylene glycol is removed and the cavities are empty.

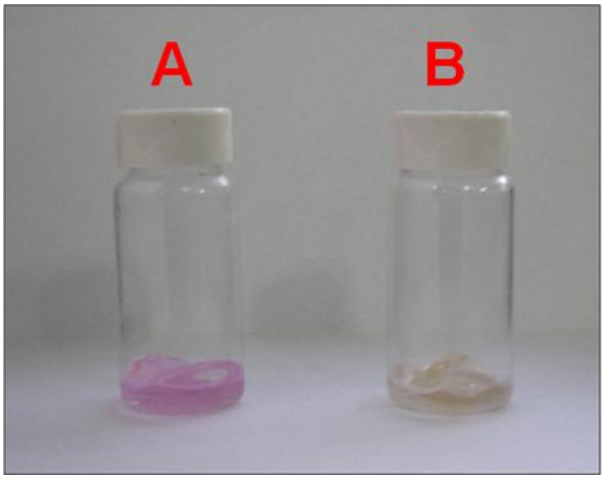

Figure 4.17. Result of the phenolphthalein uptake test. Vial A: hydrogel washed with water, Vial B: hydrogel washed with ethanol.

4.2.4. Specific Functionalization

Since one of the aims of our project is achieving functionalization of the hydrogels via specific non-covalent interactions, we synthesized a fluorescent dye molecule attached to adamantyl tetraethylene glycol as a model functional guest.

In order to prove the specific binding, a solution of the AD-Lissamine (0.9 mg/ml, 1 eq.) was prepared. Dry gels containing 4 mg CD (3 eq.) was added into this solution and the specific functionalization was followed by the UV visible spectra of the non-absorbed solution.

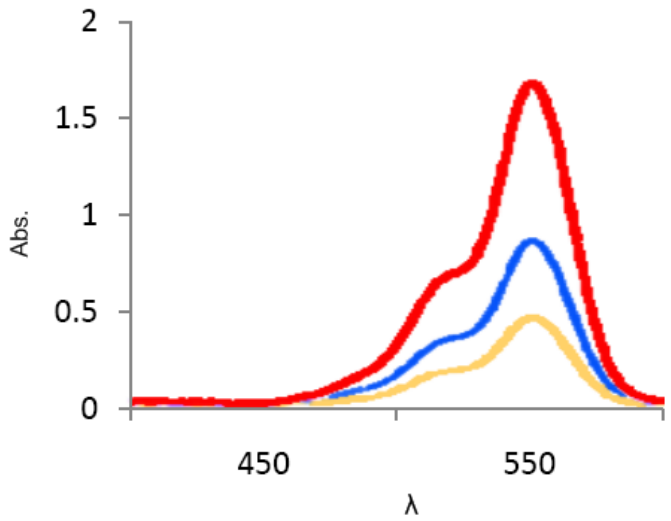

Figure 4.18 UV-vis spectra of the AD lissamine solution without (red curve) hydrogel and with (blue curve: RT, yellow curve:4 ^{o}C) hydrogel.

It can be observed that UV absorption decreases to half the initial value after the addition of hydrogel. Since hydrophobic interactions are enhanced in lower temperature, at 4 ^{o}C absorption decreases to ¼ of the initial value. Optimization of parameters is in progress for increasing the guest uptake of the hydrogel.

4.2.5. Hydrogels Patterned on Silicon and Glass

In tissue engineering applications, microengineered hydrogels are used for fixing the issues such as introduction of oxygen and nutrients as well as monitoring the interactions among the cells.

We followed the “micromolding in capillaries” (MIMIC) strategy for in situ patterning of our hydrogels. In this method a PDMS stamp is put onto a substrate. When precursor solution is placed next to the stamp, it enters inside the channels by capillary action. When the stamps are removed microstructures are obtained.

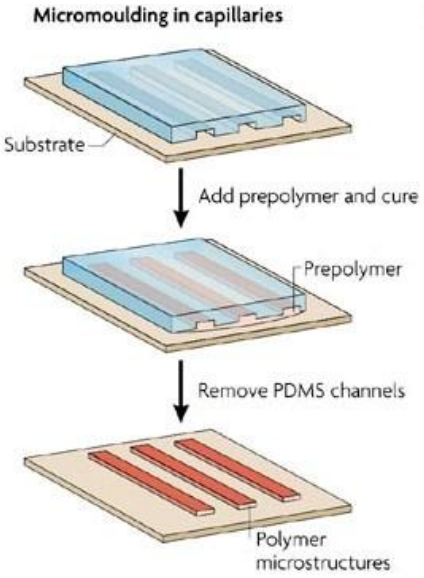

Figure 4.19. Schematic representation of MIMIC[35]

We chose silicon and silica as the substrate due to the strong adhesion of PEG on these surfaces by hydrogen bonding. We placed the precursor solution in front of the channels, and before peeling the stamp we left the system for a day to make sure hydrogels are dry. Patterned hydrogels were washed with water and ethanol to remove the catalysts and AD-TEG. Resulting patterns are durable at least for several weeks.

Patterns made by PDMS stamps containing 65-25 μm, 35-25 μm and 5-5 μm microchannels are shown in figures 4.20, 4.21 and 4.22.



Figure 4.20. Patterns made by 65-25 μm PDMS stamps

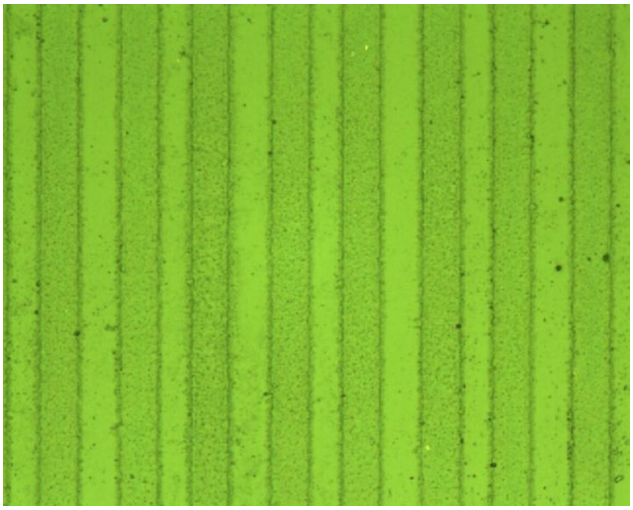

Figure 4.21. Patterns made by 35-25 μm PDMS stamps

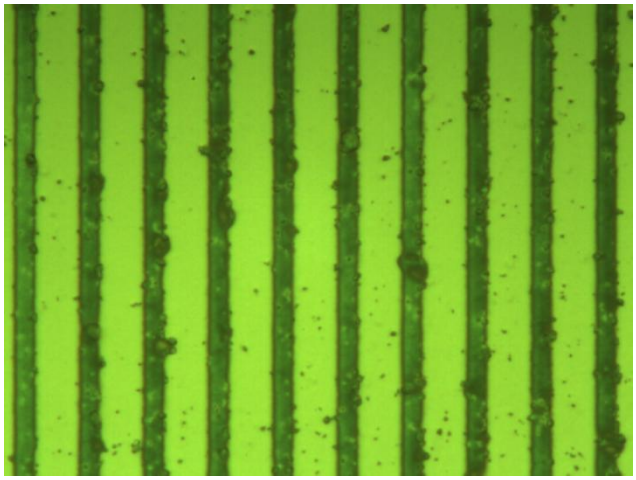

Figure 4.22. Patterns made by 5-5 μm PDMS stamps

Figure 4.23 shows the SEM image of the pattern made by the PDMS stamps containing 65-25 um channels.

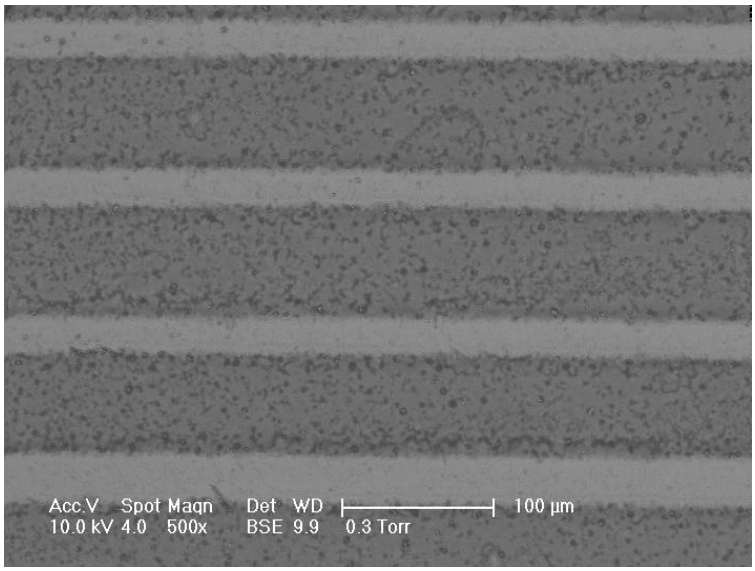

Figure 4.23. SEM image of the pattern made by the 65-25 μm PDMS

4.2.6. Functionalization of the Patterned Hydrogel

Noncovalent functionalization of the patterned hydrogel was demonstrated by AD-Lissamine guest binding. After the treatment of the hydrogel patterns by AD-Lissamine

solution (1 mg/ml), the patterns were washed and left in water for 45 minutes. Figure 4.24 shows the fluorescence microscopy image of the functionalized pattern. Release of the guest was achieved when this pattern was left in 50 ml AD-TEG (5 mg/ml) solution.

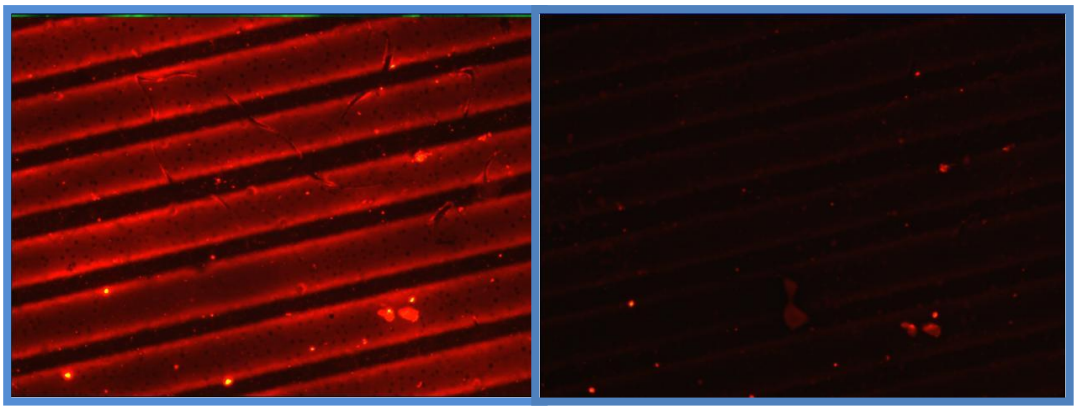

Figure 4.24. Fluorescence microscopy images of the hydrogel patterns functionalized with AD-Lissamine before and after AD-TEG treatment

4.2.7. Future Studies

Studies are in progress for enzyme immobilization by utilization of appropriately functionalized guest molecules.

5. CONCLUSIONS

In the first part of the study, new microcapsules were prepared by crosslinking water soluble cyclodextrin functionalized gold nanoparticles and toluene soluble adamantane functionalized gold nanoparticles. Close-packed network of the nanoparticles on the microcapsules were confirmed by TEM. Besides, these microcapsules were successfully transferred into water. Furthermore, a membrane film was obtained when the crosslinking experiment was run at low concentrations of the nanoparticles.

In the second part, novel cyclodextrin-PEG based hydrogels were synthesized via click chemistry. To circumvent the insolubility problem of per-azido cylodextrin guest molecule in aqueous media, complex with adamantane based tetraethylene glycol ligand was added into the precursor solution. Specific non-covalent functionalization of the hydrogel was demonstrated by binding a fluorescent guest molecule. Patterned hydrogels were obtained by in situ micromolding in capillaries. These patterns were also functionalized by the model fluorescent dye using the specific ‘host-guest’ chemistry.

APPENDIX A: SPECTROSCOPY DATA

^{1}H and ^{13}C NMR spectroscopy data for the newly synthesized compounds are given.

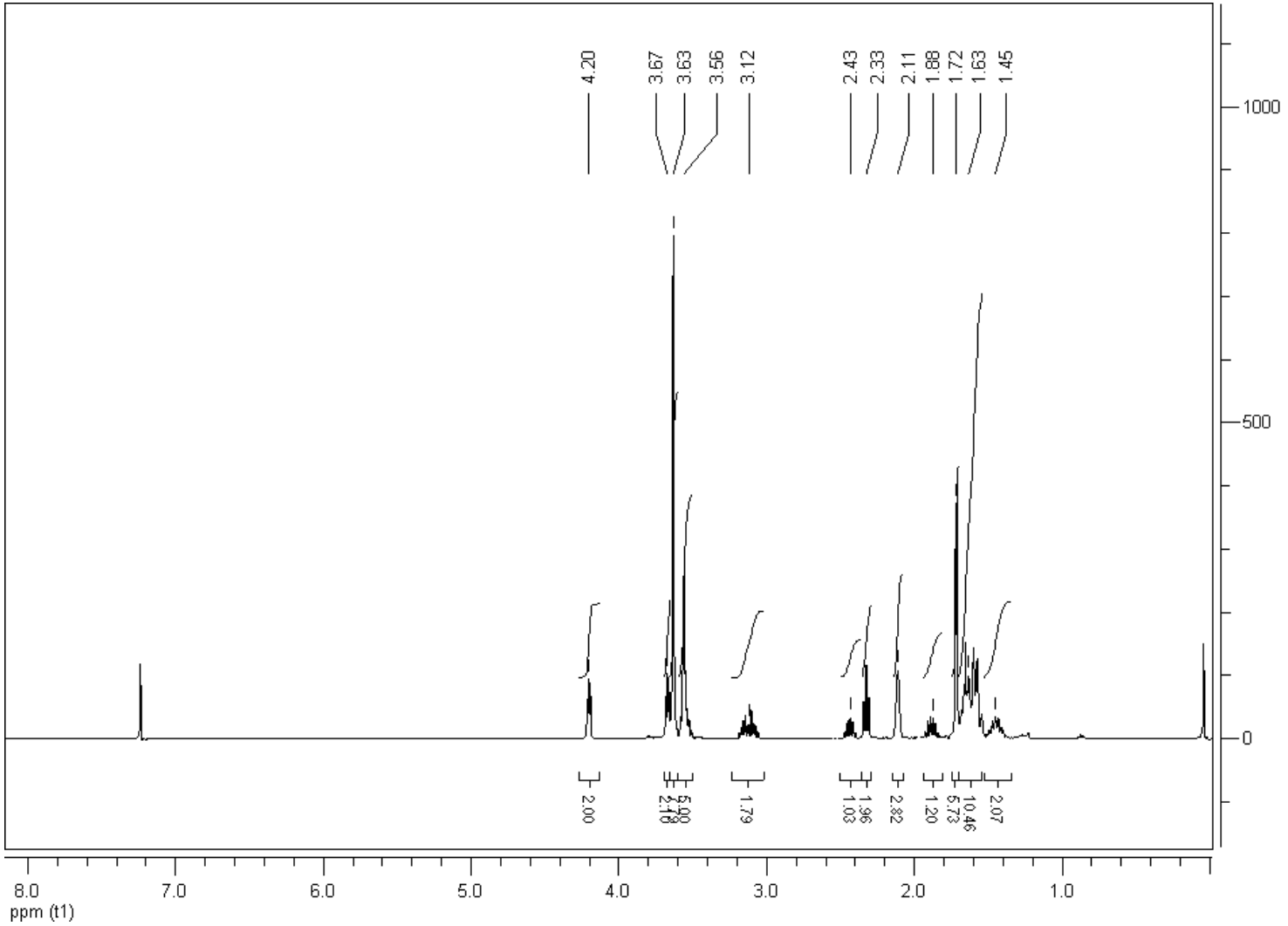

Figure 0.1. ^{1}H NMR spectrum of AD-Ligand

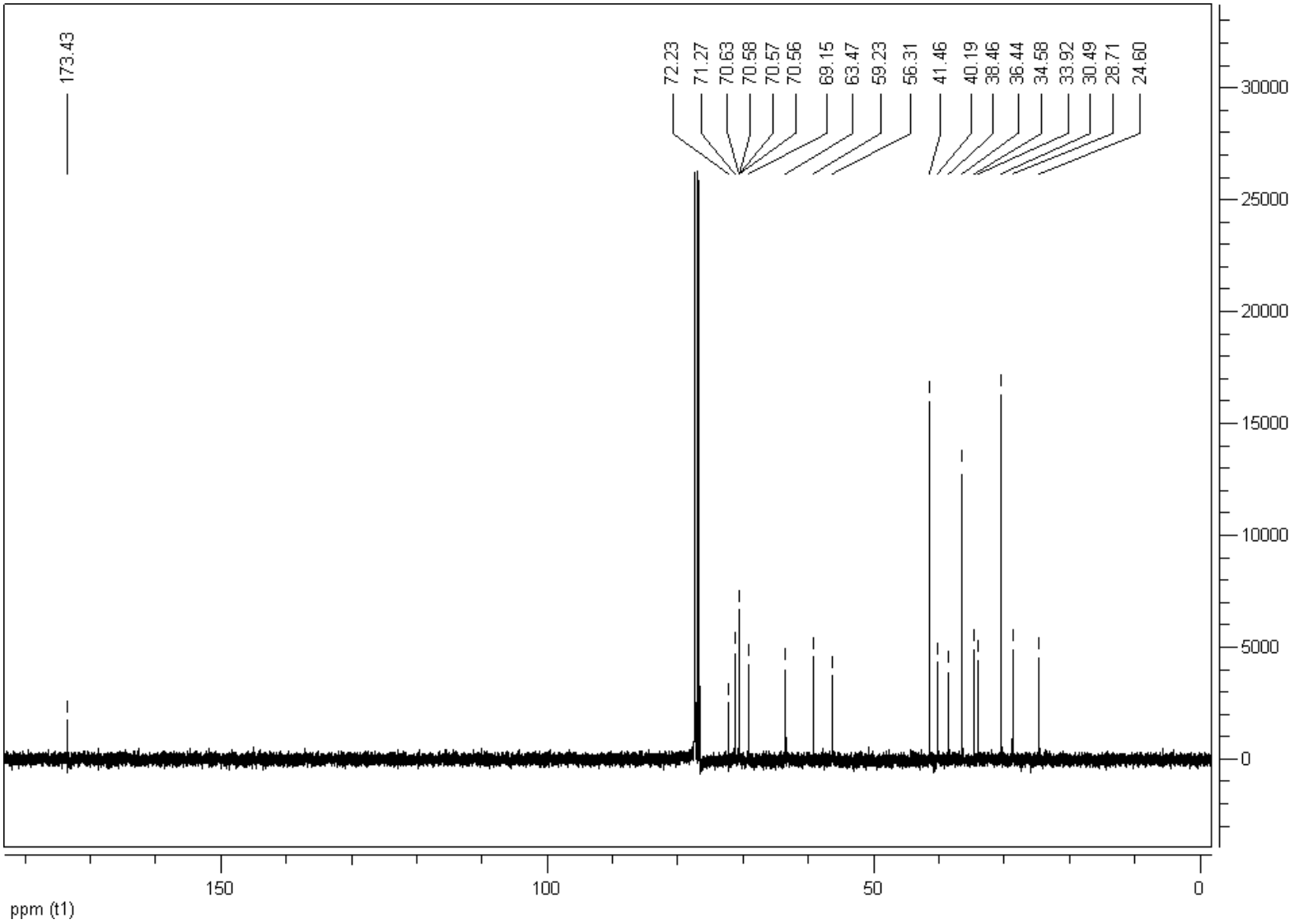

Figure 0.2. ^{13}C NMR spectrum of AD-Ligand

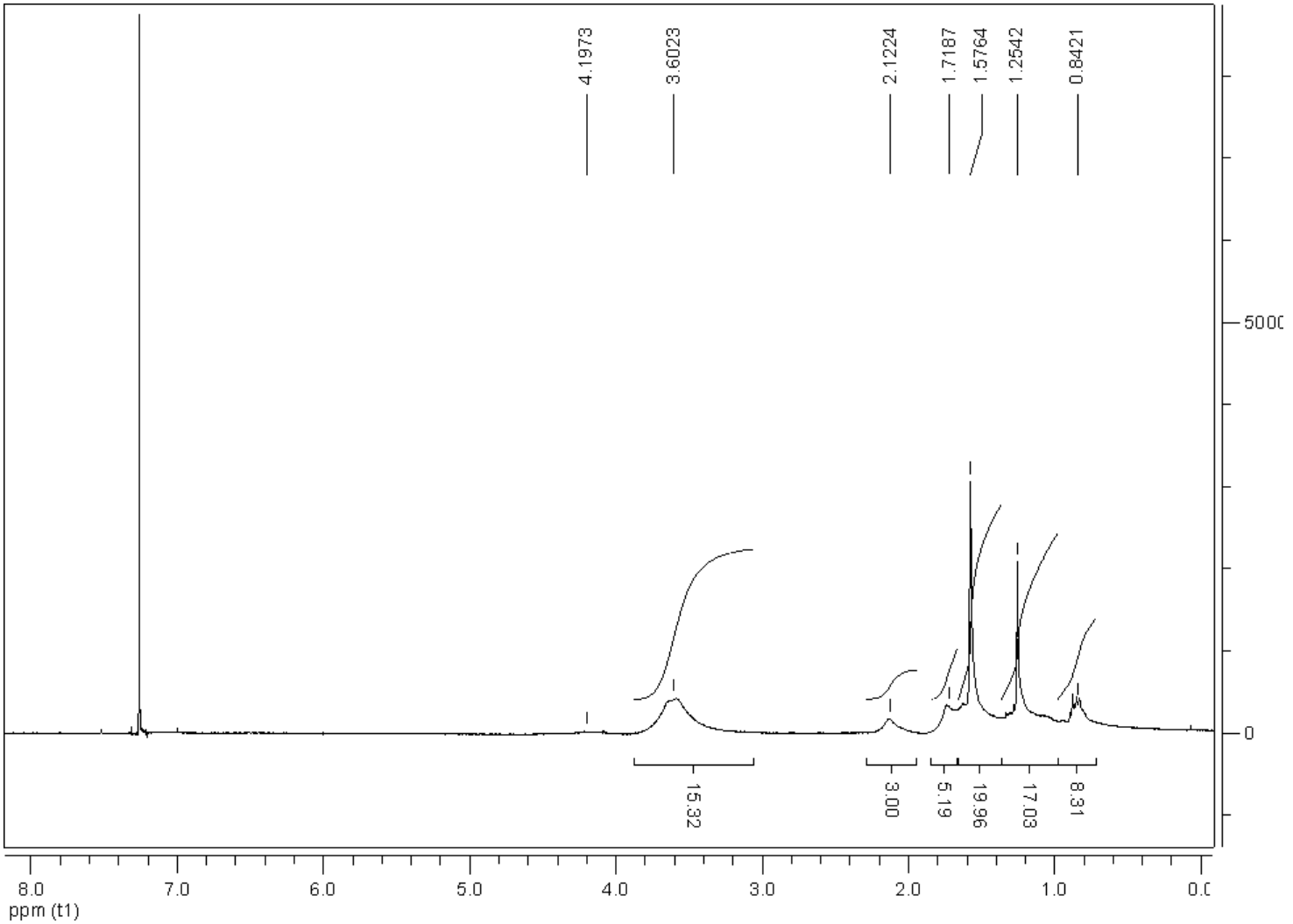

Figure A.3. ^{1}H NMR spectrum of AD functionalized Au NPs

REFERENCES

1. Whitesides, G., "Nanoscience, nanotechnology, and chemistry.," *Small* , vol. 1, pp. 172-179, 2005.

2. Bosman,A., H. Janssen, and E. Meijer, "About dendrimers: Structure, physical properties, and applications," *Chemical Reviews*, vol. 99, pp. 1665-1688, 1999.

3. Michalet,X., F. Pinaud, L. Bentolila, J. Tsay, S. Doose, J. Li, G. Sundaresan, A. Wu, S. Gambhir, and S. Weiss, "Quantum dots for live cells, in vivo imaging, and diagnostics," *Science*, vol. 307, pp. 538-544, 2005.

4. Prato, M. "Fullerene chemistry for materials science applications," *Journal Of Materials Chemistry*, vol. 7, pp. 1097-1109, 1997.

5. Baughman,R., A. Zakhidov, and W. de Heer, "Carbon nanotubes - the route toward applications," *Science*, vol. 297, pp. 787-792, 2002.

6. Rao,C., F. Deepak, G. Gundiah, and A. Govindaraj, "Inorganic nanowires," *Progress In Solid State Chemistry*, vol. 31, pp. 5-147, 2003.

7. Whitesides, G.M. and B. Grzybowski, "Self-Assembly at All Scales," *Science*, vol. 295, pp. 2418-2421, 2002.

8. Steed, J. and J. Atwood, *Supramolecular chemistry*, Wiley, New York, 2000.

9. Daniel, M and D. Astruc, "Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology," *Chemical Reviews*, vol. 104, pp. 293-346, 2004.

10. Turkevitch,,J., P. Stevenson, and J. Hillier, "Nucleation and growth process in the synthesis of colloidal gold," *Discussions Faraday Society,* vol. 11, pp. 55-75, 1951.

11. Brust, M., M. Walker, D. Bethell, D. Schiffrin, and R. Whyman, "Synthesis of thiol-derivatised gold nanoparticles in a two-phase liquid–liquid system," *Journal of the Chemical Society, Chemical Communications,* issue 7, pp. 801-802, 1994.

12. Murray, C., C. Kagan, and M. Bawendi, "Synthesis and characterization of monodisperse nanocrystals and close-packed nanocrystal assemblies," *Annual Review Of Materials Science,* vol. 30, pp. 545-610, 2000.

13. Rosi, N., C. Thaxton, and C. Mirkin, "Control of nanoparticle assembly by using DNA-modified diatom templates," *Angewandte Chemie-International Edition,* vol. 43, pp. 5500-5503, 2004.

14. Reincke, F., S.G. Hickey, W.K. Kegel, and D. Vanmaekelbergh, "Spontaneous Assembly of a Monolayer of Charged Gold Nanocrystals at the Water/Oil Interface," *Angewandte Chemie International Edition,* vol. 43, pp. 458-462, 2004.

15. Binder, W., "Supramolecular assembly of nanoparticles at liquid-liquid interfaces.," *Angewandte Chemie International Edition*, vol. 44, p. 5172, 2005.

16. Aveyard, R., B. Binks, and J. Clint, "Emulsions stabilised solely by colloidal particles," *Advances in Colloid and Interface Science,* vol. 100, pp. 503-546, 2003.
17. Binks , B., J. Clint, "Solid wettability from surface energy components: Relevance to Pickering emulsions," *Langmuir,* vol. 18, pp. 1270-1273, 2002.

18. Glogowski,E., J. He, T.P. Russell, and T. Emrick, "Mixed monolayer coverage on gold nanoparticles for interfacial stabilization of immiscible fluids," *Chemical Communications,* pp. 4050-4052, 2005.

19. Arumugam,,P., D. Patra, B. Samanta, S.S. Agasti, C. Subramani, and V.M. Rotello, "Self-Assembly and Cross-linking of FePt Nanoparticles at Planar and Colloidal

Liquid−Liquid Interfaces," *Journal of the American Chemical Society*, vol. 130, pp. 10046-10047, 2008.

20. Hoffman, A., "Hydrogels for biomedical applications," *Advanced Drug Delivery Reviews*, vol. 54, pp. 3-12, 2002.

21. Drury, J. and D. Mooney, "Hydrogels for tissue engineering: scaffold design variables and applications," *Biomaterials*, vol. 24, pp. 4337-4351, 2003.

22. Davis , M.E. and M.E. Brewster, "Cyclodextrin-based pharmaceutics: past, present and future," *Nature Reviews in Drug Discovery*, vol. 3, pp. 1023-1035, 2004.

23. Quaglia, F., G. Varricchio, A. Miro, M. Immacolata La Rotonda, D. Larobina, and G. Mensitieri, "Modulation of drug release from hydrogels by using cyclodextrins: the case of nicardipine/[beta]-cyclodextrin system in crosslinked polyethylenglycol," *Journal of Controlled Release*, vol. 71, pp. 329-337, 2001.

24. Liu, Y. and X. Fan, "Synthesis and characterization of pH- and temperature-sensitive hydrogel of N-isopropylacrylamide/cyclodextrin based copolymer," *Polymer*, vol. 43, pp. 4997-5003, 2002.

25. Johnson, A., M.G. Finn, J.T. Koberstein, and N.J. Turro, "Construction of Linear Polymers, Dendrimers, Networks, and Other Polymeric Architectures by Copper-Catalyzed Azide-Alkyne Cycloaddition Click Chemistry," *Macromolecular Rapid Communications*, vol. 29, pp. 1052-1072, 2008.

26. Malkoch, M., R. Vestberg, N. Gupta, L. Mespouille, P. Dubois, A.F. Mason, J.L. Hedrick, Q. Liao, C.W. Frank, K. Kingsbury, and C.J. Hawker, "Synthesis of well-defined hydrogel networks using Click chemistry," *Chemical Communications*, pp. 2774-2776, 2006.

27. Crescenzi, V., L. Cornelio, C. Di Meo, S. Nardecchia, and R. Lamanna, "Novel Hydrogels via Click Chemistry: Synthesis and Potential Biomedical Applications," *Biomacromolecules*, vol. 8, pp. 1844-1850, 2007.

28. Polizzotti, B., B. Fairbanks, and K. Anseth, "Three-dimensional biochemical patterning of click-based composite hydrogels via thiolene photopolymerization," *Biomacromolecules*, vol. 9, pp. 1084-1087, 2008.

29. Mulder, A., T. Auletta, A. Sartori, S. Del Ciotto, A. Casnati, R. Ungaro, J. Huskens, and D.N. Reinhoudt, "Divalent Binding of a Bis(adamantyl)-Functionalized Calix[4]arene to β-cyclodextrin-based Hosts: An Experimental and Theoretical Study on Multivalent Binding in Solution and at Self-Assembled Monolayers," *Journal of the American Chemical Society*, vol. 126, pp. 6627-6636, 2004.

30. Ashton, P.R., R. Koniger, J.F. Stoddart, D. Alker, and V.D. Harding, "Amino Acid Derivatives of β-Cyclodextrin," *The Journal of Organic Chemistry*, vol. 61, pp. 903-908, 1996.

31. Rojas, M.T., R. Koeniger, J.F. Stoddart, and A.E. Kaifer, "Supported Monolayers Containing Preformed Binding Sites. Synthesis and Interfacial Binding Properties of a Thiolated beta-Cyclodextrin Derivative," *Journal of the American Chemical Society*, vol. 117, pp. 336-343, 1995.

32. Liu, J., W. Ong, E. Roman, M.J. Lynn, and A.E. Kaifer, "Cyclodextrin-Modified Gold Nanospheres," *Langmuir*, vol. 16, pp. 3000-3002, 2000.

33. Sadhu, V., A. Perl, X. Duan, D. Reinhoudt, and J. Huskens, "Supramolecular microcontact printing with receptor-functionalized PDMS stamps," *Soft Matter*, vol. 5, pp. 1198-1204, 2009.

34. Liu, J., S. Mendoza, E. Roman, M.J. Lynn, R. Xu, and A.E. Kaifer, "Cyclodextrin-Modified Gold Nanospheres. Host−Guest Interactions at Work to Control Colloidal

Properties," *Journal of the American Chemical Society*, vol. 121, pp. 4304-4305, 1999.

35. Weibel, D.B., W.R. DiLuzio, and G.M. Whitesides, "Microfabrication meets microbiology," *Nature Reviews in Microbiology*, vol. 5, pp. 209-218, 2007.