## AMERICAN UNIVERSITY OF BEIRUT

# FUNCTIONALIZED NANOPARTICLES AS POTENTIAL PRECURSORS FOR SMART MATERIALS

## by ANTRANIK AGHOB JONDERIAN

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science to the Department of Chemistry of the Faculty of Arts and Sciences at the American University of Beirut

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# AMERICAN UNIVERSITY OF BEIRUT

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by ANTRANIK AGHOB JONDERIAN

Approved by:

Dr. Kamal Bouhadir, Professor Chemistry, AUB

2

Advisor

Member

For

Dr. Tarek Ghaddar, Professor Chemistry, AUB Dr. Rabih Sultan

Dr. Pierre Karam, Assistant Professor Chemistry, AUB

Member of Committee

Date of thesis defense: February 1, 2017

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## AN ABSTRACT OF THE THESIS OF

#### ANTRANIK AGHOB JONDERIAN for Master of Science Major: Chemistry

#### Title: Functionalized nanoparticles as potential precursors for smart materials

Nucleobase functionalized magnetic nanoparticles, which combine the magnetic properties of iron oxide and the hydrogen bonding property of nucleobases, were investigated in this research. The magnetic property can be manipulated by external constant magnetic field to collect the nanoparticles and drive them through a medium for drug delivery, catalyst magnetic collection for recycling, heating treatment for hyperthermia, among other applications. While the hydrogen bonding property allows the nanoparticles to behave as crosslinkers.

The magnetic nanoparticles were synthesized through two routes: the coprecipitation with hydrophilic surfaces and the thermal decomposition with hydrophobic surfaces. The surface functionalization was done by the "grafting to" and "grafting from" approaches. The anchoring groups that were investigated in the "grafting to" approach were catechol, hydroxamate, and silane. Whereas in the "grafting from" approach the Fe<sub>3</sub>O<sub>4</sub> NPs were first functionalized by isocyanate group. The size of the Fe<sub>3</sub>O<sub>4</sub> NPs was 35 nm for co-precipitation method and 8 nm for thermal decomposition method. Grafting densities increased from 7 uracil/nm<sup>2</sup> using "grafting to" approach to 18 uracil/nm<sup>2</sup> using "grafting from" approach. The success of the functionalization was confirmed by the proper analysis.

These functionalized magnetic nanoparticles will be used in future works as crosslinkers to prepare smart hydrogels.

In the second part of this thesis rhodamine B RhB loaded Poly Lactic-co-Glycolic Acid PLGA nanoparticles NPs were formulated by the single emulsion solvent evaporation method. The effect of the formulation parameters (PVA concentration, sonication time, and organic to aqueous volume ratio) on the size and encapsulation efficiency of the formulated PLGA NPs was studied. The optimized RhB loaded PLGA NPs had spherical morphology with 184 nm average diameter, 0.2 polydispersity index , -21 mV zeta potential, and 40% encapsulation efficiency. The *in vitro* rhodamine B release followed the Higuchi's model with Fickian diffusion mechanism and initial burst release.

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# **ABBREVIATIONS**

CA	Caffeic acid
COSY	Correlation spectroscopy
CP-MAS	Cross polarization magic angle spinning
DCM	Dichloromethane
DI	Deionized
DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide
DSC	Differential scanning calorimetry
DTG	Derivative thermogravimetric analysis
EDX	Energy dispersive X-ray
EGA	Evolved gas analysis
ESI	Electrospray ionization
EtOH	Ethanol
FT-IR	Fourier transform infrared spectroscopy
HCCOSW	Hydrogen carbon correlation spectrum
HMBC	Heteronuclear multiple bond correlation
HR-MAS	High resolution magic angle spinning
HSQC	heteronuclear single quantum correlation
IR	Infrared
MeOH	Methanol
Ms	Magnetic saturation
MS	Mass Spectrometry
NMR	Nuclear magnetic resonance
NP	Nanoparticle
OA	Oleic acid
PLGA	Poly(lactic-co-glycolic acid)
RhB	Rhodamine B
SEM	Scanning electron microscope
TEM	Transmission electron microscopy
TGA	Thermal gravimetric analysis
UA	Undecanoic acid
UV-Vis	Ultravio let-visib le
XPS	X-ray photoelectron spectroscopy
XRD	X-ray powder diffraction

## PART I

# FUNTIONALIZATION OF IRON OXIDE MAGNETIC NANOPARTICLES

# CHAPTER I

## INTRODUCTION

The aim of this research is to synthesize nucleobase coated iron oxide nanoparticles. The triethoxysilane functionalized nucleobase precursors were synthesized by coupling hydrazide with isocyanate. The core iron oxide nanoparticles were synthesized via two routes: the co-precipitation method producing hydrophilic nanoparticles and the thermal decomposition method producing hydrophobic nanoparticles. The functionalization of these iron oxide nanoparticles was achieved via chelation or covalent bonding. In the chelation bonding the hydroxamate functionalized nucleobase was introduced via biphasic ligand exchange method. On the other hand in the covalent bonding triethoxysilane anchor group was used. This covalent functionalization was achieved by the "grafting from" and "grafting to" methods. In the future work these coated nanoparticles will be used as crosslinkers when mixed with polymers functionalized with the complimentary nucleobases as shown in Fig. 1. The nanoparticles will bind to polymer via hydrogen bonding and self-assemble into a rigid structure. This rigidity can be destroyed by applying high alternating frequency magnetic field which induces heat, breaking the hydrogen bonding and softening the material. These magnetic responsive materials can be transformed from soft to hard and vice versa, thus can be implemented in many industrial and medical applications. For example, they can be used as smart molds for bone injuries where they can be shaped under the influence of the alternative magnet and then freezed into the final structure by removing the field.



**Fig. 1** Predicted hydrogen bonding between adenine functionalized polymer and thymine functionalized iron oxide nanoparticles.

Iron oxide nanoparticles Fe<sub>3</sub>O<sub>4</sub> NPs are interesting materials due to their high surface area and magnetic properties. They became the interest of many researchers in different fields such as industrial<sup>1</sup> and medical.<sup>2</sup> They have been used as MRI contrasting agents because of their high transverse relaxivity.<sup>3</sup> Moreover, they have been used to treat cancer by hyperthermia where the Fe<sub>3</sub>O<sub>4</sub> NPs are subjected to a high frequency alternating magnetic field to heat them up.<sup>4,5</sup> They are also used for drug delivery where they can be guided through the body and concentrated at the targeted area by the influence of an external magnetic field.<sup>6</sup> They are good candidates for catalyst support because of their high surface area and magnetic property.<sup>7</sup> The catalyst can be easily collected by an external magnet and be recycled.<sup>8</sup>

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#### A. Superparamagnetism

Fe<sub>3</sub>O<sub>4</sub> NPs with superparamagnetism are more attractive because they become un-magnetized when the external magnetic stress is released, so they don't agglomerate by the interparticles' magnetic attractions. For Fe<sub>3</sub>O<sub>4</sub> NPs to become superparamagnetic, they should be smaller than the magnetic domain limit which is approximately 20 nm. Magnetic saturation Ms is another property which researchers try to maximize so that the Fe<sub>3</sub>O<sub>4</sub> NPs will have a maximum response to the applied magnetic field. The magnetic saturation increases by increasing the Fe<sub>3</sub>O<sub>4</sub> NPs crystallinity and the type of coating.<sup>9,10</sup> For example in the case of Fe<sub>3</sub>O<sub>4</sub> NPs coated with different lengths PEG polymers it was shown that as the PEG length increased the Ms decreased.<sup>11</sup>

#### **B.** Stabilization modes

In general  $Fe_3O_4$  NPs tend to agglomerate because of their high surface to volume ratio. Agglomeration can be prevented by potential barriers (repulsion force) which are of two types: steric and electrostatic.

The steric stabilization can be accomplished by coating the Fe<sub>3</sub>O<sub>4</sub> NPs with long polymers or branched molecules such as dendrimers.<sup>12</sup> Adding charged functional groups on the surface of the Fe<sub>3</sub>O<sub>4</sub> NPs produces electrostatic stabilization. For example coating Fe<sub>3</sub>O<sub>4</sub> NPs with caffeic acid makes its surface negatively charged (carboxylate groups); on the other side, coating it with dopamine makes its surface positively charged (amonium groups).<sup>13</sup>

Steric stabilization increases the size of the nanoparticles which causes a decrease of transverse relaxivity. It can also cause lower grafting rate which leads to

3

vacancy causing agglomeration.<sup>14</sup> On the other hand, electrostatic stabilized NPs are prone to pH and ionic strength (external effects). For example reaching a pH below the  $pK_a$  of the Fe<sub>3</sub>O<sub>4</sub> NPs' surface carboxylate groups can protonate them and change the surface charge from negative to neutral. Moreover, charged NPs have some drawbacks; for example in biological applications, negatively charged NPs are uptaken by macrophages. Another example is the aggregation of positively charged NPs when proteins bind to them.<sup>15</sup>

#### C. Synthesis roots of Fe<sub>3</sub>O<sub>4</sub> NPs

The main two routes for preparation of  $Fe_3O_4$  NPs are the co-precipitation<sup>16</sup> and the thermal decomposition<sup>17</sup> methods.

#### 1. Co-precipitation

In the co-precipitation method an alkaline solution is added to a mixture of  $Fe^{3+}$ : $Fe^{2+}$  in a ratio of 2:1 (Eq. 1).<sup>16</sup> The sources of the  $Fe^{3+}$  and  $Fe^{2+}$  are usually their sulfate or chloride salts. This method yields poly-dispersed  $Fe_3O_4$  NPs because the nucleation and growth steps are not separated. The other drawback of this method is that the morphology of the NPs formed is random and not crystalline leading to low magnetic property specifically lower magnetic saturation. In this method the size is controlled by varying the concentrations of the salts, the heat of the reaction, the way of injection, and the amount and type of the base used (e.g. NaOH, NH<sub>3</sub> ...). They also have low colloidal dispersity in both organic and aqueous solution.<sup>18-20</sup>

$$Fe^{2+} + 2Fe^{3+} + 8OH^{-} \longrightarrow Fe_{3}O_{4} + 4H_{2}O$$
 (1)

#### 2. Thermal decomposition

In the thermal decomposition method, an  $Fe^{3+}$  complex (e.g.  $Fe(acac)_{3}$ ) is heated up to high temperatures (~250 °C) in high boiling organic solvents such as benzyl alcohol. Usually a surfactant (e.g. oleic acid) is added to the reaction (Scheme 1).<sup>17</sup> The thermal decomposition method forms mono-dispersed crystalline NPs which have a higher Ms compared to the co-precipitated ones. This mono-dispersity is due to the sufficient separation between nucleation and growth processes. The size of the Fe<sub>3</sub>O<sub>4</sub> NPs can be controlled by changing the heat rate, the time, the type of the complex used, and the type and amount of the surfactant. The main drawback of the thermal decomposition method is that the obtained Fe<sub>3</sub>O<sub>4</sub> NPs are only organic soluble and they need further processing to become water soluble.<sup>18,19</sup>



Scheme 1. Thermal decomposition route of synthesizing oleic acid coated Fe<sub>3</sub>O<sub>4</sub>NPs.

 $Fe_3O_4NPs$ ' surface is always modified in order to solubilize them in a specific solvent, stabilize them against aggregation, and protect them from acid and Oxygen which converts magnetite into the less magnetic maghemite form (Eq. 2).

$$Fe_3O_4 + 2H^+ \longrightarrow \gamma Fe_2O_3 + Fe^{2+} + H_2O$$
<sup>(2)</sup>

The modification of the iron oxide nanoparticles can be achieved in situ or post-synthesis. During in situ modification, the surfactant or the ligand is mixed with the synthesis mixture and it can intervene in the synthesis process. The ligand complexes to one face facilitating the growth in a specific direction leading to the formation of different shapes such as nano-needles or nano-cubes.<sup>21</sup> As for the postsynthesis modification, the ligand is added to the naked Fe<sub>3</sub>O<sub>4</sub> NPs or exchanged with existing surface ligands (ligand exchange method).<sup>22</sup> Ligand exchange method is only feasible when the new introduced ligand has a higher affinity than the original ligand; otherwise there will be partial exchange leading to a mixed shell. Usually, to ensure the exchange, the original ligand used is a carboxylate which chelates weakly to the surface of the Fe<sub>3</sub>O<sub>4</sub> NPs. Another factor that can enhance the successful exchange is adding an excess of the introduced ligand.<sup>22</sup> Ligand exchange can be single phased if both ligands dissolve in the same solvent, or biphasic if using two immiscible solvents (e.g. oleic coated Fe<sub>3</sub>O<sub>4</sub> NPs in hexane/OligoPEG-Dopa ligand in water) (Fig. 2).<sup>23</sup>



**Fig. 2** TEM images of Fe<sub>3</sub>O<sub>4</sub> NPs before (left) and after (right) biphasic ligand exchange with OligoPEG-Dopa. *Reprinted with permission from ref.* [23]. Copyright 2012 American Chemical Society.

#### D. Modification of Fe<sub>3</sub>O<sub>4</sub>NPs' surface

There are hydroxyl groups Fe-OH on the surface of the  $Fe_3O_4$  NPs through which they interact with the medium and the ligands. These hydroxyl groups are amphoteric; they are positively charged Fe-OH<sub>2</sub><sup>+</sup> when the pH is below the isoelectric point IEP and negatively charged Fe-O<sup>-</sup> when the pH is above the IEP. The ligands can interact with the surface electrostatically, through hydrogen bonding, or covalent bonding. The covalent bonding is the strongest and less susceptible to pH and ionic changes.<sup>24</sup>

#### Ligands

The ligand is divided into three parts: anchor, spacer, and terminal. The anchor is a functional group that has affinity to the surface of  $Fe_3O_4NPs$  (e.g. carboxylate, phosphonate ...) The spacer is the part between the anchor and the terminal groups which can be a short chain, polymer (e.g. PEG) or dendrimer. The terminal is a functional group that dictates the surface charge of the NPs and its reactivity towards further functionalization (e.g. terminal azide group can be easily coupled by and alkyne in CuSO<sub>4</sub> in a click chemistry reaction) (Fig. 3).<sup>25</sup> Further functionalization is needed for some applications such as to introduce antibodies, aptamers or fluorescence probes.



Fig. 3 Azide functonalized Fe<sub>3</sub>O<sub>4</sub> NPs for 'Click' Chemistry.

#### 1. Introduction of the ligands to the surface of $Fe_3O_4NPs$

Fe<sub>3</sub>O<sub>4</sub> NPs' surface can be modified by two paths: the "grafting from" and the "grafting to" routes (Fig. 4).

#### a. The "grafting from" route

In the "grafting from" route the anchor group is first bonded to the surface of the  $Fe_3O_4$  NPs, then the rest of the ligand is synthesized starting from the anchor on the surface of the  $Fe_3O_4$  NPs. "Grafting from" produces higher packing density compared to

the "grafting to" route but it's harder to control the thickness of the layer and its post functionalization.<sup>26,27</sup>

#### b. The "grafting to" route

On the other hand in the "grafting to" route the pre-synthesized ligand is added at once. In this route thickness and functionality is controlled because the ligand is engineered before addition but the packing density is lower than in the "grafting from" technique.<sup>28</sup>



Fig. 4 Presentation of two principal grafting methods: "grafting from" and "grafting to".<sup>29</sup>

#### 2. Anchors

Here are some of the functional groups that have been investigated by researchers as anchors and their characterization. The nature of their bonding is divided into chelation (carboxylate,<sup>30</sup> catechol,<sup>31</sup> phosphate,<sup>25</sup> and hydroxamate<sup>22</sup>) and covalent (siloxane<sup>32</sup>).

#### Chelation

#### a. Carboxylate anchor

The lone pairs on the oxygen of the carboxylate group can coordinate to the vacancy orbital of the  $Fe_3O_4NPs$ ' surface  $Fe^{3+}$  atoms. This coordination can be unidentate, bidentate or bridging as show in Fig. 5. The research done to investigate the modes of

chelation between carboxylate group and  $Fe_3O_4$  NPs reported a bidentate nature. This chelation is labile and can be broken at elevated temperatures. Moreover, because of their weak bonding nature, they can be exchanged by other carboxylate ligands or replaced by a higher affinity groups.



The coordination mode between the carboxylate group and the metal can be assigned by evaluating the IR spectrum. The difference between asymmetric and symmetrical C-O stretching ( $\Delta v = v_{as} - v_s$ ) shows the mode of chelation. A  $\Delta v$  less than 110 cm<sup>-1</sup> is indicative to a bidentate mode. On the other hand it is a bridging mode when the  $\Delta v$  is between 140-200 and a unidentate at  $\Delta v$  more than 200. For example, in the FT-IR spectrum of Fe<sub>3</sub>O<sub>4</sub> NPs coated with undecanoic acid (UA), the C=O stretch at 1710 cm<sup>-1</sup> disappeared which indicates that the carboxylic group is in its deprotonated form (carboxylate form) and the  $\Delta v$  is equal to 86 indicating a bidentate chelation (Fig. 6).<sup>33</sup>



Fig. 6 FT-IR spectra of UA (a),  $Fe_3O_4$  coated with UA (b), and  $Fe_3O_4$  (c).<sup>33</sup>

Other than IR, Solid NMR (CP-MAS or HR-MAS) have been used to

characterize the surface of the  $Fe_3O_4$  NPs. For example the <sup>1</sup>H NMR spectrum of oleic acid coated  $Fe_3O_4$  NPs shows all the oleic acid OA assigned protons peaks along with other minor peaks which might be contaminants from the  $Fe_3O_4$  NPs synthesis step (Fig. 7).<sup>34</sup>



**Fig. 7** Comparison between the <sup>1</sup>H NMR spectrum of oleic acid in solution (upper spectrum) and the <sup>1</sup>H HR-MAS NMR spectrum of the ligand OA bound to MNPs (lower spectrum) performed in DMSO- $d_6$  at 5 kHz. *Reprinted with permission from ref.* [34]. Copyright 2008 American Chemical Society.

Fig. 8 shows an example where the weak carboxylate group chelation is enforced by thiol groups like the cysteine anchor. The thiol groups chelate to the surface of  $Fe_3O_4NPs$  and crosslink with each other by oxidation to disulfide bonds increasing the  $Fe_3O_4NPs$  stability (Fig. 8).<sup>35</sup>



Fig. 8 Surface of Fe<sub>3</sub>O<sub>4</sub> NPs modified with LCP-PEG-Cys for lung cancer targeting.<sup>35</sup>

#### b. Catechol



#### Fig. 9 Catechol chelation to Fe<sub>3</sub>O<sub>4</sub> NPs.

Enediol's oxygens coordinate to the Fe<sub>3</sub>O<sub>4</sub>NPs' surface Fe<sup>3+</sup> atoms though five membered ring as shown in Fig. 9 which makes its binding stronger than carboxylate.<sup>36</sup> The drawback of the catechol anchor is that it can reduce Fe<sup>3+</sup> which causes the etching the Fe<sub>3</sub>O<sub>4</sub>NPs. As shown in Scheme 2, one electron is transferred from the catechol to Fe<sup>3+</sup> reducing it to Fe<sup>2+</sup> and oxidizing catechol to semiquinone. In acidic and aerobic medium the Fe<sup>2+</sup> precipitates as rust and the semiquinone further oxidizes to quinone. Semiquinones are toxic which makes Fe<sub>3</sub>O<sub>4</sub>NPs coated with catechol not suitable for biological application. This process is called redox etching and it can be stopped by preventing electron transfer from the catechol to the Fe<sup>3+</sup>. Introduction of a nitro group (which is electron withdrawing) to the catechol makes the aromatic ring less electron rich increasing the oxidation potential of the diol which prevents the electron transfer to the Fe<sup>3+</sup> atom.<sup>37</sup>



**Scheme 2.** Dopamine-induced etching of Fe<sub>3</sub>O<sub>4</sub> nanoparticle surfaces and precipitation of Fe(OH)<sub>2</sub>. *Reprinted with permission from ref. [38]. Copyright 2007 American Chemical Society.* 

Mimosine is a catechol like molecule with high affinity and strong binding to the surface of  $Fe_3O_4$  NPs, however, those properties can cause the etching of  $Fe_3O_4$  NPs' surface. Therefore groups with intermediate affinity are needed to obtain a good binding without causing the surface etching. An example of these groups is nitrocatechol.<sup>28</sup>

The FT-IR spectrum of Fe<sub>3</sub>O<sub>4</sub> NPs coated with dopamine which is summarized in Table 1 shows that the C-C (ring) and C-O vibrations shifted to lower wavenumbers compared to the FT-IR of the free dopamine. On the other hand, the FT-IR spectrum of the Fe<sub>3</sub>O<sub>4</sub> NPs coated with nitrodopamine shows that the C-C (ring) vibrations didn't shift significantly but the C-O vibrations shifted to lower wavenumbers, the N-O symmetric vibration shifted to higher wavenumbers, and the N-O asymmetric vibrations shifted to lower wavenumbers. These shifts in the FT-IR bands indicate the chelation of the catechol and nitrocatechol to the Fe<sub>3</sub>O<sub>4</sub> NPs' surface.<sup>28</sup>

Table 1. FT-IR peaks' wavenumbers of Fe<sub>3</sub>O<sub>4</sub>@dopamine and Fe<sub>3</sub>O<sub>4</sub>@nitrodopamine.<sup>28</sup>

Wavenumber (cm <sup>-1</sup> )	C-C	C-0	NO <sub>2</sub> asymmetric	NO <sub>2</sub> symmetric	OH
dopamine	1499	1287			1320
Fe <sub>3</sub> O <sub>4</sub> @dopamine	1484	1265			
nitrodopamine	1493	1294	1541	1351	1340
Fe <sub>3</sub> O <sub>4</sub> @nitrodopamine	1494	1280	1551	1319	

c. Phosphonate

Fig. 10 Phosphonate ligand chelation mode to Fe<sub>3</sub>O<sub>4</sub> NPs.

Phosphonate group complexes with  $Fe^{3+}$  atoms of the  $Fe_3O_4$  NPs' surface forming a bond which is stable at high temperature (Fig. 10). It has higher affinity to  $Fe_3O_4$  than carboxylate which facilitates higher packing density. The Fe-O-P bond's stability in DI and PBS buffer makes it a good anchor candidate for biological applications. Moreover, it was found that phosphonate based ligands enhances the magnetism of the Fe<sub>3</sub>O<sub>4</sub>NPs increasing their Ms.<sup>39</sup>

In the FT-IR spectrum of  $Fe_2O_3$  NPs coated with hexadecylphosphonic acid HDPA, the phosphonate bands appeared at 1088 and 1027 cm<sup>-1</sup> which were absent from the FT-IR spectrum of the free HDPA. On the other hand, the free phosphonic acid bands appeared at 1217, 1075, 997, and 950 cm<sup>-1</sup> (Fig. 11).<sup>39</sup>



**Fig. 11** FT-IR spectra of free HDPA (a), and HDPA coated Fe<sub>2</sub>O<sub>3</sub> NPs (b). *Reprinted with permission from ref.* [39]. *Copyright 2011 American Chemical Society.* 

In addition to IR, XPS was used to investigate the bonding between phosphonate and Fe<sub>3</sub>O<sub>4</sub> NPs. Fig. 12 shows the O1s XPS spectrum of free HDPA and Fe<sub>2</sub>O<sub>3</sub>@HDPA. The O1s peak at  $531.5 \pm 0.1$  eV corresponds to P=O and P-O-Fe bonds while the peak at  $533.1 \pm 0.1$  eV corresponds to the P-OH bond. The ratio of P=O and P-O-Fe to P-OH for the Fe<sub>2</sub>O<sub>4</sub>@HDPA was 1:1 but for the free HDPA was 1:2 which indicated that some of the P-OH converted to P-O-Fe bond.<sup>39</sup>



**Fig. 12** XPS O1s signal of free HDPA (a) and Fe<sub>2</sub>O<sub>3</sub>@HDPA NPs (b). *Reprinted with permission from ref.* [39]. *Copyright 2011 American Chemical Society.* 

#### d. Hydroxamate

Hydroxamate complexes to the  $Fe^{3+}$  atoms of the  $Fe_3O_4$  NPs' surface by a bidentate chelation as shown in Fig. 13. It has more stability than carboxylate because it forms a five membered chelation ring like the catechols. The disappearance of C=O stretching vibration at 1635 cm<sup>-1</sup> from the FT-IR spectrum of the hydroxamate based NPs' coating suggested that the chelation mode is as shown in Fig. 13 (b).<sup>40</sup>



Fig. 13 Hydroxamate chelation modes to Fe<sub>3</sub>O<sub>4</sub> NPs.<sup>40</sup>

All the anchors presented above bind to the surface of the Fe<sub>3</sub>O<sub>4</sub> NPs through chelation. The grafting pH is very crucial for successful anchoring. The pH should be below the IEP of IONP and above the pK<sub>a</sub> of the anchor so that the surface of the Fe<sub>3</sub>O<sub>4</sub> NPs will be positively charged and the anchor negatively charged. An example is the grafting of the ligand 1 shown in Fig. 14. It was grafted at pH below the IEP (5.4) of iron oxide nanoparticles and above the pK<sub>a</sub> of the phosphonic acid (3.1). A more complicated ligand is the dendron 2 shown in Fig. 14, which has a terminal carboxylate group (pK<sub>a</sub>  $\approx$  4). In this case the grafting was conducted at pH 3.5 to avoid competitive grafting.<sup>41</sup>



**Fig. 14** Phosphonate anchor dendron with no terminal (1) and carboxylate terminal (2). *Reprinted with permission from ref. [41]. Copyright 2012 Royal Society of Chemistry.* 

#### Covalent (siloxane)

Silane based ligands bond covalently to the surface of Fe<sub>3</sub>O<sub>4</sub>NPs by the heterocondensation with the surface hydroxyl groups Fe-OH. Trialkoxy silane based ligands have the general formula RSi(OR')<sub>3</sub> where R' group is alkane. First step of the silanization is the hydrolysis of the alkoxy groups through acidic or basic catalysis. The mechanism of alkoxy silane hydrolysis to alcohol group via acidic or basic catalysis is shown in Scheme 3. Smaller alkoxy groups increase the hydrolysis and condensation rates.<sup>42</sup> After the hydrolysis of trialkoxy silane to triol silane, they form hydrogen bonds with the hydroxyl groups on the surface of the metal oxide. Upon heat appliance or pH adjustment the silanol groups hetero-condense with the surface hydroxyl groups and homo-condense (crosslink) with each other forming siloxane shell (Scheme 4). The hydrolysis and condensation rates of TEOS at different pH are shown in Fig. 15, which reveals that the hydrolysis rate is minimal at pH 7 while the condensation rate is minimal at pH 4. Hydrolysis and condensation pHs are dictated by the nature of the ligand (R and R' groups). The disadvantages of siloxane based anchors are the formation of no core silica NPs and the crosslink aggregation which both yield from the homo-condensation. Another drawback is that siloxanes hydrolyze in basic media.43

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Acid Catalysis

$$\begin{array}{cccc} H & & RO & & + \\ O & + & & RO & Si - OR \\ H & & & RO & \end{array}^{+} & + & \left[ \begin{array}{c} H_{\delta +} OR_{\delta +} H \\ O - Si - O \\ H & & RO & \end{array} \right] \xrightarrow{OR} HO - Si_{OR} + ROH + H^{+} \\ OR & & OR & OR \end{array}$$

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**Base Catalysis** 

$$HO^{-} \xrightarrow{RO}_{RO^{+}SI^{-}OR} \xrightarrow{OR}_{HO^{-}SI^{-}OR} \xrightarrow{OR}_{HO^{-}SI^{-}OR} \xrightarrow{OR}_{HO^{-}SI^{-}OR} \xrightarrow{OR}_{HO^{-}SI^{-}OR} \xrightarrow{OR}_{OR} \xrightarrow{OR}_{HO^{-}SI^{-}OR} \xrightarrow{OR}_{OR} \xrightarrow{OR}_{HO^{-}SI^{-}OR} \xrightarrow{OR}_{OR} \xrightarrow{OR}_{HO^{-}SI^{-}OR} \xrightarrow{OR}_{HO^{-}SI^{-}OR} \xrightarrow{OR}_{OR} \xrightarrow{OR}_{HO^{-}SI^{-}OR} \xrightarrow{OR}_{HO^{-}OR} \xrightarrow{OR}_{HO^{$$

Scheme 3. Acidic and basic hydrolysis mechanism of alkoxy silane.<sup>44</sup>

Scheme 4. Representation of silane condensation with the surface hydroxyl groups of  $Fe_3O_4$  NPs.



Fig. 15 Rate of hydrolysis and condensation of alkoxy silane vs pH (TEOS).<sup>45</sup>

The FT-IR spectrum of 11-(trimethoxysilyl)undecane nitrile CS coated  $Fe_3O_4$ NPs shown in Fig. 16 reveals that the peak at 585 cm<sup>-1</sup> corresponding to Fe-O vibration shifts and broadens because of the formation of Fe-O-Si bond and a new broad band between 1000 and 1150 cm<sup>-1</sup> appears which is characteristic to Si-O-Si vibrations.<sup>19</sup>



**Fig. 16** FT-IR spectra of the Fe<sub>3</sub>O<sub>4</sub> NPs oleic acid coated (a) and cyano-silane coated (b). *Reprinted with permission from ref.* [19]. *Copyright 2007 American Chemical Society*.

#### E. Characterization of Fe<sub>3</sub>O<sub>4</sub> NPs

Fe<sub>3</sub>O<sub>4</sub> NPs are characterized by their size, morphology and surface charge. Dynamic light scattering DLS determines the hydrodynamic radius and distribution of the NPs in their suspension. Scanning electron microscopy SEM gives information about the size and the morphology of the NPs but it lacks accuracy in providing the size distribution. The average size obtained by SEM is usually smaller than the size characterized by DLS. Transmission electron microscopy TEM gives same data as SEM but it can be also used to characterize the surface of the NPs (core-shell). The surface charge characterization is very important because it dictates the colloidal stability of the NPs. The zeta potential is an indirect measurement of the surface charge. Moreover zeta potential can reveal information about the surface functionality. For the surface functionality characterization FT-IR, XPS, EDX, and CP-MAS are usually used. XRD is used to confirm the crystal structure and determine the crystallite size (Scherrer equation). The surface coverage of the modified NPs can be characterized by the thermogravimetric analysis TGA. TGA gives information about the quantity of organic components of the Fe<sub>3</sub>O<sub>4</sub> NPs. TGA is also used to study thermal stability of the coated Fe<sub>3</sub>O<sub>4</sub> NPs. In addition, atomic absorption AA is used to quantify the amount of the inorganic components of the Fe<sub>3</sub>O<sub>4</sub> NPs.

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### CHAPTER II

## **RESULTS AND DISCUSSION**

#### Synthesis of Fe<sub>3</sub>O<sub>4</sub> NPs

The Fe<sub>3</sub>O<sub>4</sub> NPs were prepared via two routes which are the co-precipitation method and the thermal decomposition<sup>16,17</sup>. Briefly, in the co-precipitation method Fe<sup>2+</sup> and Fe<sup>3+</sup> salts in a ratio of 2:1 were dissolved in deionized water with continuous mechanical stirring then ammonia was added (Eq. 4)<sup>16</sup>. The Fe<sub>3</sub>O<sub>4</sub> NPs **1** formed as a black precipitate were collected by external magnetic field and washed. The hydrodynamic diameter of the Fe<sub>3</sub>O<sub>4</sub> NPs synthesized by this method was 35 nm with 0.2 PDI and zeta potential of -16 mV  $\pm$  1 at pH 7. The hydrophobic Fe<sub>3</sub>O<sub>4</sub> @OA NPs **2** were prepared by the thermal decomposition method. Briefly, Fe(acac)<sub>3</sub> was dissolved in diphenyl ether in the presence of 1, 2-dodecadiol, oleylamine, oleic acid then it was refluxed (Scheme 5)<sup>17</sup>. The prepared Fe<sub>3</sub>O<sub>4</sub>@OA NPs were precipitated by addition of ethanol and collected by external magnetic field and washed. The hydrodynamic diameter of Fe<sub>3</sub>O<sub>4</sub>@OA NPs suspended in hexane was 8 nm with 0.3 PDI. In the thermal decomposition method, we got smaller MNPs compared to the co-precipitation method.

$$2FeCl_3 + FeCl_2 + 8NH_3 + 4H_2O \rightarrow Fe_3O_4 + 8NH_4Cl \quad (4)$$



Scheme 5. Synthesis of Fe<sub>3</sub>O<sub>4</sub>@OA via thermal decomposition method.



Fig. 17 FT-IR spectra of  $Fe_3O_4$  (a) and  $Fe_3O_4$  @OA (b).

The FT-IR spectrum of the Fe<sub>3</sub>O<sub>4</sub> NPs prepared by the co-precipitation method shown in Fig. 17(a) shows two characteristic bands. The Fe-O stretching vibration appeared at 584 cm<sup>-1</sup>, and the surface O-H groups' vibrations appeared at 1624 cm<sup>-1</sup> (bending) and 3381 cm<sup>-1</sup> (stretching). On the other hand, the FT-IR of Fe<sub>3</sub>O<sub>4</sub>@OA NPs synthesized by the thermal decomposition shown in Fig. 17(b) has main four characteristic bands at 602, 1024, 1628, 2924 cm<sup>-1</sup> which can be assigned to the Fe-O, C-O, C=O, and C-H stretching vibrations respectively. The wavenumber of the Fe-O stretching vibration shifted to higher value which could be due to the formation of Fe-O-C bond between the oleic acid and the Fe<sub>3</sub>O<sub>4</sub> NPs.



**Fig. 18** TGA thermograms of  $Fe_3O_4$  (a) and  $Fe_3O_4$  @OA (b). The analysis were carried out under nitrogen flow at a heating rate of 10°C/min.

The TGA thermogram of Fe<sub>3</sub>O<sub>4</sub> NPs synthesized by co-precipitation method and Fe<sub>3</sub>O<sub>4</sub>@OA NPs synthesized by thermal decomposition are shown in Fig. 18. The TGA thermogram of Fe<sub>3</sub>O<sub>4</sub> NPs shows only 5% weight loss over a temperature range of 100-1000 °C. In contrast, the TGA thermogram of Fe<sub>3</sub>O<sub>4</sub>@OA NPs is divided into four stages. In the first stage, there is a weight loss of 6 wt% in the range of 100-266 °C (DTGmax = 196 °C) which is attributed to the adsorbed water. In the second, third and fourth stages there are weight losses of 7, 5, and 4 wt% in the ranges of 266-481 °C (DTGmax = 353 °C), 481-692 °C (DTGmax = 645 °C), and 692-100 °C (DTGmax = 750 °C) respectively. These stages can be attributed to the decomposition of the oleic acid.

The  $Fe_3O_4@SiO_2 NPs 3$  were prepared following Stober's method.<sup>46</sup> In brief, the  $Fe_3O_4 NPs$  prepared by co-precipitation method were suspended in ethanol/water/ammonia solution, then TEOS was added dropwise. The  $Fe_3O_4@SiO_2$ NPs were collected by external magnetic field and washed.



Fig. 19 XRD patterns of simulated Fe<sub>3</sub>O<sub>4</sub>(a), Fe<sub>3</sub>O<sub>4</sub>(b), and Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub>(c).

Fig. 19 shows the XRD patterns for (a) simulated  $Fe_3O_4$ , (b)  $Fe_3O_4$ , and (c)  $Fe_3O_4@SiO_2$ . The X-ray diffractograms of  $Fe_3O_4$  and  $Fe_3O_4@SiO_2$  show diffractions that can be indexed to (220), (311), (400), (422), (511), and (440) planes which agree with the face-centered cubic structure of magnetite. The broad peak at low diffraction angles  $20^{\circ}$  to  $30^{\circ}$  of the  $Fe_3O_4@SiO_2$  XRD pattern can be attributed to the amorphous SiO\_2 shell surrounding the  $Fe_3O_4$  NPs.

The hydrodynamic diameter of  $Fe_3O_4@SiO_2$  synthesized by Stober's method was 82 nm with 0.2 PDI and zeta potential of -7 mV at pH 7. The size of the  $Fe_3O_4@SiO_2$  is bigger than the bare  $Fe_3O_4$  NPs by 47 nm which indicates that most of the  $Fe_3O_4@SiO_2$  NPs contain more than one core.



Fig. 20 FT-IR spectra of  $Fe_3O_4$  (a) and  $Fe_3O_4$ @SiO<sub>2</sub> (b).

The FT-IR spectrum of  $Fe_3O_4@SiO_2$  NPs shown in Fig. 20(b) has two main characteristic bands at 586, and 1072 cm<sup>-1</sup> which can be assigned to Fe-O, Si-O stretching vibrations respectively. Furthermore, the Si-O band at 1072 cm<sup>-1</sup> confirms the formation of SiO<sub>2</sub> shell.



Fig. 21 TGA thermograms of  $Fe_3O_4$  (a) and  $Fe_3O_4@SiO_2$  (b). The analysis were carried out under nitrogen flow at a heating rate of  $10^{\circ}C/min$ .

The TGA thermograms of Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> are shown in Fig. 21 TGA thermograms of Fe<sub>3</sub>O<sub>4</sub> (a) and Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> (b). The analysis were carried out under nitrogen flow at a heating rate of 10°C/min.. The TGA thermogram of Fe<sub>3</sub>O<sub>4</sub> shows only 5% weight loss over temperature range of 100-1000 °C. The TGA thermogram of Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> shows a weight loss of 6% in the range of 100-1000 °C which can be due to the removal of the adsorbed water. As shown both Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> NPs and Fe<sub>3</sub>O<sub>4</sub> NPs have a similar weight loss profiles which confirms the complete condensation of the TEOS' ethoxy groups.



Fig. 22 SEM (a), STEM (b), and EDX (c) images of  $Fe_3O_4@SiO_2$ .

The presence of SiO<sub>2</sub> coating of the synthesized  $Fe_3O_4@SiO_2 NPs$  was confirmed by the elemental analysis carried out using energy dispersive X-ray spectroscopy (EDX). The EDX spectrum of  $Fe_3O_4@SiO_2$  shown in Fig. 22(c) reveals
that the sample is composed of iron and silicon. The presence of Si element confirms the presence of  $SiO_2$  shell.

The morphology of the  $Fe_3O_4@SiO_2 NPs$  was characterized using scanning electron microscopy (SEM) as shown in Fig. 22(a). It reveals that the  $Fe_3O_4@SiO_2 NPs$  have approximately the same size with an average diameter 42 nm with 0.3 PDI (calculated using ImageJ). The STEM images  $Fe_3O_4@SiO_2$  is shown in Fig. 22(c).

# A. Functionalization of $Fe_3O_4$ NPs through chelation bonding (catechol, hydroxamate)

### 1. Preparation of Fe<sub>3</sub>O<sub>4</sub>@caffeic acid 4

The Fe<sub>3</sub>O<sub>4</sub>@CANPs were prepared by anchoring the caffeic acid (CA) through the chelation of its catechol unit with the Fe<sub>3</sub>O<sub>4</sub> NPs' surface iron atoms (Scheme 6). Different amounts of CA were added to an ethanol Fe<sub>3</sub>O<sub>4</sub> NPs suspension. The suspensions were sonicated then collected by external magnetic field and washed three times with ethanol. The combined washes' supernatants were analyzed by UV-Vis spectrometry to quantify the unbounded CA. The amount of the bounded CA which was deduced from the unbounded CA was used to calculate the grafting density using Eq. 3. The maximum grafting density of CA on Fe<sub>3</sub>O<sub>4</sub> NPs was 3 CA/nm<sup>2</sup> at 0.03 CA:Fe<sub>3</sub>O<sub>4</sub> weight ratio (Fig. 23).

$$\sigma = \frac{\frac{Mass_{shell}}{Mass_{core}} p_{core} \frac{4}{3} \pi r_{core}^3 N_A}{MW4\pi r_{core}^2}$$
(3)

The hydrodynamic diameter of  $Fe_3O_4@CANPs$  was 173 nm with 0.2 PDI and zeta potential of -22 mV at pH 7. The zeta potential of the  $Fe_3O_4@CANPs$  is lower than of the  $Fe_3O_4NPs$  which is due to the pendant carboxylic groups.



Scheme 6. Preparation of Fe<sub>3</sub>O<sub>4</sub>@CANPs through catechol-Fe chelation.



Fig. 23 Grafting density of CA vs the amount of CA used.



Fig. 24 FT-IR spectra of  $Fe_3O_4(a)$ ,  $Fe_3O_4@CA(b)$ , and CA(c).

The FT-IR spectrum of CA shown in Fig. 24(c) has four main characteristic bands at 1279, 1450, 1645, 3234 and 3431 cm<sup>-1</sup> which can be assigned to the C-O, C-H, C=O, and O-H vibrations respectively. On the other hand, the FT-IR spectrum of the Fe<sub>3</sub>O<sub>4</sub>@CA in Fig. 24(b) shows three characteristic bands at 584, 1278, and 1628 cm<sup>-1</sup> for the Fe-O, C-O, and C=O stretching vibrations respectively. The characteristic functional groups that appeared in the FT-IR of the Fe<sub>3</sub>O<sub>4</sub>@CA confirm the presence of the CA coating.



**Fig. 25** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of Caffeic acid. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.



**Fig. 26** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of Fe<sub>3</sub>O<sub>4</sub>@CA. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.



Fig. 27 TGA thermograms of  $Fe_3O_4$  (a),  $Fe_3O_4$  @CA (b), and CA (c). The analyses were carried out under nitrogen flow at a heating rate of  $10^{\circ}C/min$ .

The TGA thermograms of the Fe<sub>3</sub>O<sub>4</sub>, Fe<sub>3</sub>O<sub>4</sub>@CA, and CA are shown in Fig. 27. The TGA thermogram of Fe<sub>3</sub>O<sub>4</sub> shows only a 5 wt% weight loss over a temperature range of 100-1000 °C. On the other hand, the TGA thermogram of Fe<sub>3</sub>O<sub>4</sub>@CA shows four weight loss' steps (Fig. 26). The first weight loss is 2 wt% in the range of 100-210 °C (DTGmax = 155 °C) which is due to the removal of adsorbed water. The weight losses in the second, third and fourth steps are 5, 2, and 7 wt% in the ranges of 210-378 °C (DTGmax = 254 °C), 378-463 °C (DTGmax = 414 °C), and 463-1100 °C (DTGmax = 655 °C) respectively. These three stages can be attributed to the decomposition of caffeic acid. In comparison, the TGA thermogram of the Caffeic acid has two main decomposition stages (Fig. 25). In the first stage there is a 31 wt% weight loss in the range of 158-229 °C (DTGmax = 210 °C) and in the second stage a 55 wt% weight loss in the range of 229-1100 °C (DTGmax = 292 °C). The decomposition temperature of Fe<sub>3</sub>O<sub>4</sub>@CA is shifted to higher temperature compared to the CA which indicates that the Fe<sub>3</sub>O<sub>4</sub> WPs increased the thermal stability of CA.

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#### 2. Preparation of Fe<sub>3</sub>O<sub>4</sub>@adenine 8 through hydroxamate anchor

The Fe<sub>3</sub>O<sub>4</sub>@adenine NPs were prepared by the ligand exchange method. The Fe<sub>3</sub>O<sub>4</sub>@OA NPs' surface oleic acid anchored through the carboxylate groups were exchanged with the adenine hydroxamate **7**. In brief, an aqueous solution of adenine hydroxamic acid was added to a hexane suspension of Fe<sub>3</sub>O<sub>4</sub>@OA NPs prepared by the thermal decomposition method. The two phases were shaken vigorously to facilitate the ligands exchange (Scheme 7). After 12 hours of shaking, the Fe<sub>3</sub>O<sub>4</sub> NPs moved to the aqueous phase which indicates that the oleic acid was successfully replaced by the adenine hydroxamate. The aqueous layer was separated and the adenine coated Fe<sub>3</sub>O<sub>4</sub> NPs were collected by centrifugation and washed with hexane, ethanol, and acetone.



Scheme 7. Synthesis route of  $Fe_3O_4@$  adnenine NPs through hydroxamate chelation by the ligand exchange method.

The synthesis route of ligand 3-(6-amino-9H-purin-9-yl)-*N*hydroxypropanamide **7** is outlined in Scheme 8. The intermediate **6** was synthesized by Michael addition reaction between adenine **5** and ethyl acrylate in ethanol using a catalytic amount of sodium under reflux. Finally, the ester **6** was converted to hydroxamic acid **7** by treating it with hydroxylamine and potassium hydroxide in methanol.



Scheme 8. Synthesis of coumpound 7

The structure of the intermediate **6** was verified by <sup>1</sup>H NMR, IR and MS. The <sup>1</sup>H NMR spectrum of the intermediate **6** revealed four new signals which can be assigned to 4.02 ppm for the O-CH<sub>2</sub>- as a quartet, 1.1 ppm for the -CH<sub>3</sub> of the ethoxy group as a triplet, 2.93 ppm for the -CH<sub>2</sub>- of the carbonyl group as a triplet, and 4.37 ppm for the -CH<sub>2</sub>- connected to the amine as a triplet. The FT-IR spectrum of the intermediate **6** shows a strong absorption band at 1727 cm<sup>-1</sup> for the carbonyl group of the ester.

## Table 2. <sup>1</sup>H (500 MHz, DMSO-*d*<sub>6</sub>) NMR data for compound 6

Structure of compound 6



0		
	No	δ <sub>H</sub> (ppm)
	4	8.13
	9	8.1
	14	7.24
	10	4.37
	16	4.02
	11	2.93
	17	1.1

The structure of compound 7 was verified by <sup>1</sup>H NMR, <sup>13</sup>C NMR, IR and MS. The <sup>1</sup>HNMR spectrum of the compound **7** revealed the disappearance of two signals at 4.37 and 1.1 ppm which is attributed to the loss of the ethoxy group. On the other hand, two new signals appeared at 10.47, and 8.83 ppm which can be assigned to the -NHand -OH protons of the hydroxamic group. The -CH<sub>2</sub>- signal of the carbonyl group shifted upfield from 2.93 to 2.57 ppm which confirms the conversion of the ester to hydroxamic acid. The IR spectrum of the compound 7 showed strong absorption band for the C=O stretching vibration at 1657 cm<sup>-1</sup>. The shift of the C=O stretching vibration band to lower wavenumber from 1727 cm<sup>-1</sup> to 1657 cm<sup>-1</sup> confirms the formation of hydroxamic acid.

Table 3. <sup>1</sup>H (500 MHz, DMSO- $d_6$ ) and <sup>13</sup>C (126 MHz, DMSO- $d_6$ ) NMR data for compound 7

Structure of compound 7



No	δ <sub>H</sub> (ppm)	No	$\delta_{\rm C}$ (ppm)
16	10.47	12	166.32
15	8.83	6	155.92
4	8.14	4	152.37
9	7.99	2	149.4
14	7.21	9	140.96
10	4.35	7	118.72
11	2.57	10	39.19
		11	32.19



Fig. 28 FT-IR spectra of Fe<sub>3</sub>O<sub>4</sub> (a), Fe<sub>3</sub>O<sub>4</sub>@adenine (b), and adenine hydroxamic (c).

The FT-IR spectrum of adenine-hydroxamic shown in

Fig. 28(c) has two main characteristic bands at 1655, and 2939 cm<sup>-1</sup> which are attributed to C=O, C-H stretching vibrations respectively. On the other hand, the FT-IR spectrum of Fe<sub>3</sub>O<sub>4</sub>@adenine shown in

**Fig. 28**(b) shows three characteristic bands at 594, 1655, and 2924 cm<sup>-1</sup> for the Fe-O, C=O, and C-H stretching vibrations respectively. The wavenumber of the Fe-O stretching vibration shifted to higher value from 548 to 594 cm<sup>-1</sup>, which can be due to the formation of Fe-O-N bonds on the surface of the Fe<sub>3</sub>O<sub>4</sub> NPs.



**Fig. 29** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of denine-hydroxamic. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.

Table 4. Thermal decomposition stages of adenine-hydroxamic.

Stage	1	2
Temperature range (°C)	100-259	259-1100
DTGmax (°C)	235	322
% Weight loss	21	68



**Fig. 30** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of Fe<sub>3</sub>O<sub>4</sub>@adenine. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.

Table 5. Thermal decomposition stages of Fe<sub>3</sub>O<sub>4</sub>@adenine.

Stage	1	2	3	4
Temperature range (°C)	100-211	211-399	330-450	450-1000
DTGmax (°C)	202	232	401	512
% Weight loss	5	8	4	27



**Fig. 31** TGA thermograms of bare  $Fe_3O_4$  (a),  $Fe_3O_4$  @ adenine (b), and adeninehydroxamic (c). The analyses were carried out under nitrogen flow at a heating rate of  $10^{\circ}C/min$ .

The TGA thermogram of adenine-hydroxamic shown in Fig. 29 shows two decomposition stages summarized in Table 4. The two stages are at DTGmax 235 and 322 °C. The gases evolved in these two stages as shown in Fig. 29 (b) are CO<sub>2</sub>, NH<sub>3</sub>, and C=O at stage one (100-259 °C) and mainly NH<sub>3</sub> at stage two (259-1100 °C). On the other hand, the TGA thermogram of Fe<sub>3</sub>O<sub>4</sub>@adenine shown in Fig. 30(a) shows four decomposition stages summarized in Table 5. The main two stages are at DTGmax 202 and 512 C°. The gases evolved in these two stages as shown in Fig. 30(b) are CO<sub>2</sub>, NH<sub>3</sub>, and C=O at stage one (100-211 °C), and CO<sub>2</sub> and CO at stage three (399-1000 °C). We can observe that the Fe<sub>3</sub>O<sub>4</sub>@adenine and the adenine-hydroxamic have close decomposition profiles but with shifted DTGmax. Fig. 31 reveals that the Fe<sub>3</sub>O<sub>4</sub>@adenine layer.



Fig. 32 SEM image of Fe<sub>3</sub>O<sub>4</sub>@adenine NPs.

In Fig. 32 the SEM image of the  $Fe_3O_4$ @adenine NPs is shown. The hydrodynamic diameter of the  $Fe_3O_4$ @adenine NPs anchored by hydroxamate group was 18 nm with 0.2 PDI and grafting density of 13 adenine/nm<sup>2</sup>.

## B. Functionalization of Fe<sub>3</sub>O<sub>4</sub> NPs through covalent bonding (siloxane)

## 1. Preparation of Fe<sub>3</sub>O<sub>4</sub>@nucleobase (Route 1)

#### a. Fe<sub>3</sub>O<sub>4</sub>@Si-adenine 12

The Fe<sub>3</sub>O<sub>4</sub> NPs coated with adenine through siloxane anchor were prepared by the condensation of triethoxy-silane group of  $(EtO)_3$ Si-adenine **10** with the surface hydroxyl groups of the Fe<sub>3</sub>O<sub>4</sub> NPs. This siloxane covalent bonding makes the ligand binding more resistant to the pH changes compared to the chelation. In brief,  $(EtO)_3$ Siadenine was pre-hydrolyzed in basic medium to  $(HO)_3$ Si-adenine. Then, dried Fe<sub>3</sub>O<sub>4</sub> NPs prepared by co-precipitation were added and the suspension was sonicated. After that, the pH was adjusted (pH: 10) to trigger the condensation (Scheme 9).



Scheme 9. Synthesis route of Fe<sub>3</sub>O<sub>4</sub>@Si-adenine NPs through siloxane bonding via condensation of pre-synthesized ligand.

The synthesis route of ligand 2-(3-(6-amino-9H-purin-9-yl)propanoyl)-*N*-(3-(triethoxysilyl)propyl)hydrazine-1-carboxamide **10** is outlined in Scheme 10. Briefly, the intermediate ester **6** was synthesized by Michael addition reaction between adenine **5** and ethyl acrylate in ethanol using catalytic amount of sodium under reflux. Next, the ester **6** was converted to hydrazide by treating it with hydrazine hydrate in ethanol under reflux. Finally, the intermediate **9** was reacted with 3-(triethoxysilyl)propyl isocyanate in dry DMSO under anhydrous condition to yield ligand **10**.



Scheme 10. Synthesis of compound 10

The structure of the intermediate **9** was verified by <sup>1</sup>H NMR and MS. <sup>1</sup>H NMR spectrum of the intermediate **9** revealed the disappearance of two signals at 4.37 and 1.1 ppm which is attributed to the loss of the ethoxy group. On the other hand, two new signals appeared at 9.05 and 4.19 ppm which can be assigned to the -NH- and NH<sub>2</sub> of the hydrazide group respectively. The -CH<sub>2</sub>- signal of the carbonyl group shifted upfield from 2.93 ppm to 2.61 ppm which confirms the conversion of the ester to hydrazide.

Table 6. <sup>1</sup>H (500 MHz, DMSO- $d_6$ ) NMR data for compound 9

Structure	of compound	9
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	No	$\delta_{\rm H}$ (ppm)
NH <sub>2</sub> 14	15	9.05
	4	8.13
	9	7.99
3 1 10-11	14	7.19
12~ NH	10	4.34
o 15 13 <sub>NH2</sub>	16	4.19
16 <sup>2</sup>	11	2.61

The structure of the compound 10 was verified by <sup>1</sup>H NMR, <sup>13</sup>C NMR, HSQC, COSY, IR and MS. The <sup>1</sup>H NMR spectrum of the compound **10** shows that the -NH<sub>2</sub> signal shifted downfield from 4.19 ppm to 7.68 ppm which indicates the formation of an amide. The protons of the triethoxy groups were present: the O-CH<sub>2</sub>- signal appeared as a quadruplet at 3.73 ppm and the -CH<sub>3</sub> signal as a triplet at 1.14 ppm. Moreover, the presence of upfield signal at 0.5 ppm which is attributed to the -CH<sub>2</sub>-Si confirms the presence of the silane group. The <sup>13</sup>C NMR spectrum displayed a peak at 57.74 ppm corresponding to the O-CH<sub>2</sub>- carbon attached to the oxygen atom. The assignments of the <sup>1</sup>H and <sup>13</sup>C spectra signals were further confirmed by the HSQC and COSY data which are fully analyzed in the supplementary section. The IR spectrum of the compound 10 shows a new characteristic band at 1075 cm<sup>-1</sup> which can be attributed to Si-O-C bond. The band at 2975 cm<sup>-1</sup> can be assigned to the stretching vibration of -CH<sub>2</sub>groups. The stretching vibration of C=O which appeared at 1667 cm<sup>-1</sup> with the bending and stretching vibrations of N-H which appeared at 1555 and 3283 cm<sup>-1</sup> respectively, confirm the presence of amide group. Moreover, the characteristic band of -N=C=O at 2270 cm<sup>-1</sup> disappeared indicating the success of the coupling.

**Table 7.** <sup>1</sup>H (500 MHz, DMSO- $d_6$ ) and <sup>13</sup>C (126 MHz, DMSO- $d_6$ ) NMR data for compound **10** 

Structure of compound 10



No	δ <sub>H</sub> (ppm)
15	9.56
4	8.14
9	8.04
16	7.68
14	7.21
19	6.37
10	4.35
25,28,31	3.73
28	3.73
31	3.73
20	2.95
11	2.71
21	1.41
26,29,32	1.14
22	0.5

No	δ <sub>C</sub> (ppm)
12	169.63
17	157.99
6	155.97
4	152.4
2	149.42
9	141.02
7	118.71
25,28,31	57.74
20	41.97
10	39.26
11	33.19
21	23.45
26,29,32	18.28
22	7.19



Fig. 33 FT-IR spectra of Fe<sub>3</sub>O<sub>4</sub> (a), Fe<sub>3</sub>O<sub>4</sub>@Si-adenine (b), and (EtO)<sub>3</sub>Si-adenine (c).

The FT-IR of the (EtO)<sub>3</sub>Si-adenine shown in Fig. 33(c) has main three characteristic bands at 1074, 1668, 2976 cm<sup>-1</sup> for the Si-O, C=O, and C-H stretching vibrations respectively. On the other hand, the FT-IR spectrum of the Fe<sub>3</sub>O<sub>4</sub>@Si-adenine NPs in Fig. 33(b) shows four characteristic bands at 588, 1034 and 1126, 1649

 $cm^{-1}$  which can be assigned to the Fe-O, Si-O-Si. C=O, C-H stretching vibrations respectively. These bands confirm the presence of adenine on the surface of the Fe<sub>3</sub>O<sub>4</sub> NPs.



Fig. 34 TGA thermogram of Fe<sub>3</sub>O<sub>4</sub>. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.



**Fig. 35** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of (EtO)<sub>3</sub>Si-adenine. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.

Table 8. Thermal	decomposition	stages	of (EtO) <sub>3</sub> Si-adenine.
	-	<u> </u>	. ,

Stage	1	2	3	4	5
Temperature range (°C)	100-158	158-219	219-271	271-389	389-1100
DTGmax (°C)	143	193	252	303	467
% Weight loss	3	9	11	21	20



**Fig. 36** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of Fe<sub>3</sub>O<sub>4</sub>@Si-adenine. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.

Table 9. Thermal decomposition stages of Fe<sub>3</sub>O<sub>4</sub>@Si-adenine.

Stage	1	2	3	4	5	6	7
Temperature range (°C)	100-183	183-256	156-344	344-511	511-719	719-777	777-1100
DTGmax (°C)	146	234	284	444	539	653	865
% Weight loss	2	7	12	14	7	7	5



**Fig. 37** TGA thermograms of bare  $Fe_3O_4$  (a),  $Fe_3O_4$  @Si-adenine (b), and  $(EtO)_3Si-adenine$  (c). The analyses were carried out under nitrogen flow at a heating rate of  $10^{\circ}C/min$ .

The TGA thermogram of (EtO)<sub>3</sub>Si-adenine shown in Fig. 35(a) shows five decomposition stages summarized in Table 8. The two main stages are at DTGmax 303, and 467 °C. The gases evolved in these two stages as shown in Fig. 35(b) are CO<sub>2</sub> and NH<sub>3</sub> at stage four (271-389 °C) and CH<sub>4</sub>, NH<sub>3</sub> at stage five (389-1100 °C). On the other hand, the TGA thermogram of the Fe<sub>3</sub>O<sub>4</sub>@Si-adenine NPs shown in Fig. 36(a) shows seven decomposition stages summarized in Table 9. The two main stages are at DTGmax 284, and 444 °C. The gases evolved in these two stages as shown in Fig. 36(b) are CO<sub>2</sub> and NH<sub>3</sub> at stage three (156-344 °C) and HCN, CH<sub>4</sub>, NH<sub>3</sub> at stage four (344-511 °C). We can deduce that the Fe<sub>3</sub>O<sub>4</sub>@Si-adenine and (EtO)<sub>3</sub>Si-adenine have close decomposition profiles with shifted DTGmax. Moreover, the (EtO)<sub>3</sub>Si-adenine shows in its first two stages of decomposition as shown in Fig. 35(b) the evolution of ethanol gas which came from the ethoxy groups. In contrast, the evolution of ethanol was absent in the decomposition profile of the Fe<sub>3</sub>O<sub>4</sub>@Si-adenine NPs, which confirms the complete condensation. The thermograms shown in Fig. 37 reveals that the Fe<sub>3</sub>O<sub>4</sub>@Si-adenine

NPs has more weight loss than the  $Fe_3O_4$  NPs. This difference can be attributed to the adenine layer.



**Fig. 38** DSC thermograms of  $Fe_3O_4@Si$ -adenine (a), (EtO)<sub>3</sub>Si-adenine (b), and  $Fe_3O_4$  (c). The analyses were carried out under nitrogen flow at a heating rate of  $10^{\circ}C/min$ .

The DSC thermograms of (a)  $Fe_3O_4@Si$ -adenine, (b) (EtO)<sub>3</sub>Si-adenine, and (c)  $Fe_3O_4$  are shown in Fig. 38. The DCS thermogram of the  $Fe_3O_4$  NPs shown in Fig. 38(c) shows broad exothermic peak between 150 and 400 °C. Meanwhile, the DSC of the ligand (EtO)<sub>3</sub>Si-adenine shown in Fig. 38(b) shows three exothermic peaks at 179, 249, and 320 °C which can be assigned as exothermic decompositions when complemented with the TGA data. On the other hand, the DCS thermogram of  $Fe_3O_4@Si$ -adenine shown in Fig. 38(a) shows two exothermic peaks one broad 150-240 °C and the other at 311 °C which also can be assigned as decompositions of (EtO)<sub>3</sub>Si-adenine are absent in the DSC profile of  $Fe_3O_4@Si$ -adenine NPs which are attributed to the decomposition of the ethoxy groups as ethanol vapor.



Fig. 39 SEM image of Fe<sub>3</sub>O<sub>4</sub>@Si-adenine NPs.

In Fig. 39 the SEM image of the  $Fe_3O_4@$  adenine NPs is shown. The hydrodynamic diameter of the  $Fe_3O_4@$ Si-adenine NPs synthesized by the 'grafting to' approach was 74 nm with 0.3 PDI and grafting density of 53 adenine/nm<sup>2</sup>.

## b. Fe<sub>3</sub>O<sub>4</sub>@Si-uracil 15

The Fe<sub>3</sub>O<sub>4</sub> NPs coated with uracil through siloxane bonding was prepared by the condensation of triethoxy-silane groups of the (EtO)<sub>3</sub>Si-uracil with the surface hydroxyl groups of the bare Fe<sub>3</sub>O<sub>4</sub> NPs. This solixane covalent bonding makes the ligand binding more resistant to pH changes compared to the chelation. In brief, (EtO)<sub>3</sub>Si-uracil was pre-hydrolyzed in basic media to (HO)<sub>3</sub>Si-uracil (silanol). Then dried Fe<sub>3</sub>O<sub>4</sub> NPs prepared by co-precipitation was added and bath sonicated. After, the pH was adjusted to trigger the condensation (Scheme 11).



Scheme 11. Synthesis route of  $Fe_3O_4@Si$ -uracil NPs through siloxane bonding via the condensation of pre-synthesized ligand.

The synthesis route of ligand 2-(3-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)yl)propanoyl)-*N*-(3-(triethoxysilyl)propyl)hydrazine-1-carboxamide **16** is outlined in Scheme 12.Briefly, the intermediate ester **14** was synthesized by Michael addition reaction between uracil **13** and ethyl acrylate in ethanol using a catalytic amount of sodium under reflux. After, the ester **14** was converted to hydrazide **15** by treating it with hydrazine hydrate in ethanol under reflux. Finally, the intermediate **15** was reacted with 3-(triethoxysilyl)propyl isocyanate in dry DMSO under anhydrous condition to yield ligand **16**.



Scheme 12. Synthesis of compound 16

The structure of the intermediate **14** was verified by <sup>1</sup>H, <sup>13</sup>C, IR, and MS. The <sup>1</sup>H NMR spectrum of the intermediate **14** revealed four new signals which can be assigned to the O-CH<sub>2</sub>- as a quartet at 4.05 ppm, -CH<sub>3</sub> of the ethoxy group as triplet at 1.16 ppm, -CH<sub>2</sub>- of the carbonyl group as triplet at 2.93 ppm , and the -CH<sub>2</sub>- connected to the amine as triplet at 4.37 ppm. The IR spectrum of intermediate **14** showed strong absorption band at 1703 cm<sup>-1</sup> for the carbonyl group of the ester.

**Table 10.** <sup>1</sup>H (500 MHz, DMSO- $d_6$ ) and <sup>13</sup>C (126 MHz, DMSO- $d_6$ ) NMR data for compound 14

- -

Structure	of compound	14		
			No	

	No	δ <sub>H</sub> (ppm)	No	δ <sub>C</sub> (ppm)
0	3	11.27	9	170.83
12	6	7.62	4	163.88
$\mathbb{H}^{\mathbb{N}}$ 5 3	5	5.53	2	150.94
0 <sup>11</sup> ,1	14	4.05	6	146.16
7_8	7	3.87	5	100.71
0 0	8	2.67	14	60.3
10 13 14	15	1.16	7	44.1
15			8	32.77
			15	14.07

The structure of the intermediate **15** was verified by <sup>1</sup>H, <sup>13</sup>C, IR and MS. <sup>1</sup>H NMR spectrum of the intermediate **15** revealed the disappearance of two signals at 4.37, and 1.1 ppm which attributes to the loss of the ethoxy group's -CH<sub>2</sub>- and -CH<sub>3</sub> respectively. On the other hand, two new signals appeared at 9.05, and 4.19 ppm which can be assigned to the -NH- and NH<sub>2</sub> of the hydrazide group respectively. The signal of the -CH<sub>2</sub>- of the carbonyl group shifted upfield from 2.93 ppm to 2.61 ppm which confirms the conversion of the ester to hydrazide. The FT-IR spectrum of the intermediate **15** revealed that the strong absorption band assigned to C=O stretching vibration shifted to a lower frequency from 1703 to 1678 cm<sup>-1</sup> which confirms the success of ester conversion to hydrazide.

**Table 11.** <sup>1</sup>H (500 MHz, DMSO- $d_6$ ) and <sup>13</sup>C (126 MHz, DMSO- $d_6$ ) NMR data for compound **15** 

Structure of compound 15

0	No	δ <sub>H</sub> (ppm)	No	δ <sub>C</sub> (ppm)
12	3	10.75	9	168.96
	13	9.09	4	163.91
0 <sup>1</sup> / <sub>N</sub> <sup>0</sup> 11 1	6	7.51	2	150.84
7_8	5	5.5	6	146.24
2 2 2	14	4.2	5	100.59
0 NH 10 13	7	3.85	7	44.73
14	8	2.39	8	32.41

The structure of the compound 16 was verified by <sup>1</sup>H NMR, 13C NMR, HMBC, COSY and MS. The <sup>1</sup>H NMR spectrum of the compound 16 shows that the -NH<sub>2</sub> signal shifted downfield from 4.2 ppm to 7.67 ppm which indicates the formation of amide. The protons of the triethoxy groups were present. The O-CH<sub>2</sub>- signal appeared at 3.74 ppm as a quartet and the -CH<sub>3</sub> signal at 1.15 ppm as a triplet. Moreover, the presence of upfield signal at 0.5 ppm which attributes to the -CH<sub>2</sub>-Si proton signal confirms the presence of the silane group. The <sup>13</sup>C NMR spectrum displayed a peak at 57.72 ppm corresponding to the O-CH<sub>2</sub>- carbon attached to the oxygen atom. The assignments of the <sup>1</sup>H and <sup>13</sup>C spectra signals were further confirmed by the HMBC and COSY data which are fully analyzed in the supplementary section. The HMBC spectrum revealed that the -NH- signal from the hydrazide part at 7.67 ppm and the -CH<sub>2</sub>- signal from the 3-(triethoxysilyl)propyl isocyanate part at 2.95 ppm both showed correlations to same carbon at 158 ppm (C=O) confirming the connectivity of the two parts. The FT-IR spectrum of the compound 16 shows a new characteristic band at 1104 cm<sup>-1</sup> which can be attributed to Si-O-C bond. The band at 2975 cm<sup>-1</sup> is assigned to the stretching vibration of -CH<sub>2</sub>- groups. The strong C=O absorption stretching band at 1646 cm<sup>-1</sup> with the bending and stretching vibrations of N-H at 1583, 3280 cm<sup>-1</sup>

respectively, confirms the presence of amide group. Additionally, the characteristic band of -N=C=O at 2270 cm<sup>-1</sup> disappeared indicating the success of the coupling.

**Table 12.** <sup>1</sup>H (500 MHz, DMSO- $d_6$ ) and <sup>13</sup>C (126 MHz, DMSO- $d_6$ ) NMR data for compound **16** 

Structure of coumpound 16



No	δ <sub>H</sub> (ppm)	No	$\delta_{\rm C}$ (ppm)
3	11.27	9	169.74
13	9.56	4	163.84
14	7.67	15	158
6	7.56	2	150.9
17	6.4	6	146.29
5	5.5	5	100.6
7	3.86	23,26,29	57.72
23,26,29	3.74	7	44.4
18	2.95	18	41.95
8	2.49	8	32.24
19	1.43	19	23.44
27,24,30	1.15	24,27,30	18.28
20	0.5	20	7.19



Fig. 40 FT-IR spectra of Fe<sub>3</sub>O<sub>4</sub> (a), Fe<sub>3</sub>O<sub>4</sub>@Si-uracil (b), and (EtO)<sub>3</sub>Si-uracil (c).

The FT-IR spectrum of the  $(EtO)_3$ Si-uracil shown in Fig. 40(c) has four main characteristic bands 1080 and 1105, 1649, 2974, 3279 and 3318 cm<sup>-1</sup> which are

attributed to Si-O, C=O, C-H, and N-H vibrations respectively. On the other hand, the FT-IR spectrum of the Fe<sub>3</sub>O<sub>4</sub>@Si-uracil NPs shown in Fig. 40(b) shows four characteristic bands at 588, 1051 and 1012, 1670, 2926 cm<sup>-1</sup> for the Fe-O, Si-O-Si. C=O, C-H stretching vibrations respectively. The Fe-O stretching vibration wavenumber shifted to a higher value which can be due to the formation of Fe-O-Si bonds on the surface of the Fe<sub>3</sub>O<sub>4</sub> NPs.



**Fig. 41** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of (EtO)<sub>3</sub>Si-uracil. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.

Stage	1	2	3	4
Temperature range (°C)	100-177	177-240	240-367	367-1100
DTGmax (°C)	133	212	272	462
% Weight loss	2	15	22	19

Table 13. Thermal decomposition stages of (EtO)<sub>3</sub>Si-uracil.



**Fig. 42** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of Fe<sub>3</sub>O<sub>4</sub>@Si-uracil. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.

Stage	1	2	3
Temperature range (°C)	100-431	431-518	518-1100
DTGmax (°C)	266	462	611
% Weight loss	8	1	3

Table 14. Thermal decomposition stages of Fe<sub>3</sub>O<sub>4</sub>@Si-uracil.



**Fig. 43** TGA thermograms of  $Fe_3O_4$  (a),  $Fe_3O_4$  @Si-uracil (b), and (EtO)\_3Si-uracil (c). The analyses were carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.

The TGA thermogram of the (EtO)<sub>3</sub>Si-uracil shown in Fig. 41(a) shows four decomposition stages summarized in Table 13. The main two stages are at DTGmax 272 and 462 °C. The gases evolved in these two stages as shown in Fig. 41(b) are CO<sub>2</sub> and NH<sub>3</sub> at stage three (240-367 °C), and CH<sub>4</sub>, NH<sub>3</sub>, HCN at stage four (367-1100 °C). On the other hand, the TGA thermogram of the Fe<sub>3</sub>O<sub>4</sub>@Si-uracil NPs shown in Fig. 42(a) shows three decomposition stages summarized in Table 14. The main two stages are at DTGmax 266 and 611 °C. The gases evolved in these two stages as shown in Fig. 42(b) are CO<sub>2</sub> and NH<sub>3</sub> at stage one (100-431 °C) and CO<sub>2</sub> at stage four (518-1100 °C). Moreover, the TGA thermogram of the (EtO)<sub>3</sub>Si-uracil shows in its second stage as shown in Fig. 41(b) the evolution of ethanol vapor which came from the ethoxy groups. In contrast, the ethanol vapors were absent in the decomposition profile of Fe<sub>3</sub>O<sub>4</sub>@Si-

uracil NPs. From Fig. 43 we can notice that  $Fe_3O_4@Si$ -uracil NPs has more weight loss than  $Fe_3O_4$  NPs which can be attributed to the uracil layer.



Fig. 44 SEM image of Fe<sub>3</sub>O<sub>4</sub>@Si-uracil NPs

In Fig. 44 the SEM image of  $Fe_3O_4@Si$ -uracil is shown. The hydrodynamic diameter of  $Fe_3O_4@Si$ -uracil NPs synthesized by the "grafting to" approach was 75 nm with 0.3 PDI and grafting density of 5 (O) uracil/nm<sup>2</sup>.

#### 2. Preparation of Fe<sub>3</sub>O<sub>4</sub>@nucleobase (Route 2)

#### a. Fe<sub>3</sub>O<sub>4</sub>@Si-uracil NPs 20

The Fe<sub>3</sub>O<sub>4</sub> NPs coated with uracil through siloxane bonding via "grafting from" method was prepared by first functionalizing the Fe<sub>3</sub>O<sub>4</sub> NPs with isocyanate functionality and then reacting it with the uracil hydrazide. In brief, dried Fe<sub>3</sub>O<sub>4</sub> NPs prepared by the co-precipitation method were suspended in dry toluene and (EtO)<sub>3</sub>Si-NCO was added and refluxed. Then the isocyanate functionalized Fe<sub>3</sub>O<sub>4</sub> NPs were suspended in dry DMF. After, uracil hydrazide was added and refluxed under continuous mechanical stirring (Scheme 13).



Scheme 13. Synthesis route of  $Fe_3O_4@Si$ -uracil NPs through siloxane bonding via "grafting from" method.



Fig. 45 FT-IR spectra of Fe<sub>3</sub>O<sub>4</sub> (a), Fe<sub>3</sub>O<sub>4</sub>@Si-uracil (b), and uracil-hydrazide (c).

The FT-IR spectrum of uracil-hydrazide shown in Fig. 45(c) has main three characteristic bands at 1680, 3028, 3313 and 3356 cm<sup>-1</sup> corresponding to the C=O, C-H, and N-H stretching vibrations respectively. On the other hand, Fe<sub>3</sub>O<sub>4</sub>@Si-uracil in Fig. 45(b) shows four characteristic bands at 571, 1001, 1635, 2875 cm<sup>-1</sup> for the Fe-O, Si-O, C=O, and C-H stretching vibrations respectively. The existence of these characteristic bands confirms the presence of the uracil coating.



**Fig. 46** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of uracil-hydrazide. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.

Stage	1	2
Temperature range (°C)	100-394	394-1000
DTGmax (°C)	297	427
% Weight loss	77	12

Table 15. Thermal decomposition stages of uracil-hydrazide



**Fig. 47** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of Fe<sub>3</sub>O<sub>4</sub>@Si-uracil. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.
Stage	1	2	3	4
Temperature range (°C)	100-383	383-557	557-707	707-1100
DTGmax (°C)	268	426	685	767
% Weight loss	12	4	4	7

Table 16. Thermal decomposition stages of Fe<sub>3</sub>O<sub>4</sub>@Si-uracil.



**Fig. 48** TGA thermograms of  $Fe_3O_4$  (a),  $Fe_3O_4$  @Si-uracil (b), and (EtO)\_3Si-uracil (c). The analyses were carried out under nitrogen flow at a heating rate of  $10^{\circ}C/min$ .

The TGA thermogram of uracil-hydrazide shown in Fig. 46(a) shows two main decomposition stages summarized in Table 15 with DTGmax 297 and 427 °C. The gases evolved in these two stages as shown in Fig. 46(b) are NH<sub>3</sub>, C=O at stage one (100-394 °C) and CO<sub>2</sub>, NH<sub>3</sub> at stage two (394-1100 °C). On the other hand, the TGA thermogram of Fe<sub>3</sub>O<sub>4</sub>@Si-uracil NPs shown in Fig. 47(a) shows four decomposition stages summarized in Table 16. The two main stages are at DTGmax 268 and 767 °C. The gases evolved in these two stages as shown in Fig. 47(b) are CO<sub>2</sub>, NH<sub>3</sub>, CH<sub>4</sub> at stage one (100-383 °C) and CO<sub>2</sub>, CO at stage four (707-1100 °C). Moreover, the absence of ethanol vapor in the EGA profile of Fe<sub>3</sub>O<sub>4</sub>@Si-uracil NPs, indirectly proves that the ethoxy groups of 3-(triethoxysilyl)propyl isocyanate are absent. The TGA

profiles shown in Fig. 48 reveals that  $Fe_3O_4@Si$ -uracil NPs has more weight loss than  $Fe_3O_4$  NPs which confirms the presence of uracil coating.



Fig. 49 SEM image of Fe<sub>3</sub>O<sub>4</sub>@Si-uracil NPs.

In **Fig. 49** the SEM image of Fe<sub>3</sub>O<sub>4</sub>@Si-uracil NPs is shown. The hydrodynamic diameter of Fe<sub>3</sub>O<sub>4</sub>@Si-uracil NPs synthesized by the "grafting from" approach was 240 nm with 0.5 PDI and zeta potential of  $\pm 0.1 \text{ mV} \pm 0.2$  at pH 7. The change of the zeta potential of the magnetic nanoparticle from  $\pm 16 \text{ mV}$  for the bare Fe<sub>3</sub>O<sub>4</sub> NPs to  $\pm 0.1 \text{ mV}$  for the coated confirms the present of the uracil group. The grafting density calculated from the TGA's weight loss was 19 uracil/nm<sup>2</sup>.

#### b. Fe<sub>3</sub>O<sub>4</sub>@Si-thymine NPs 25

The Fe<sub>3</sub>O<sub>4</sub> NPs coated with thymine though siloxane bonding via "grafting from" method was prepared by first functionalizing the Fe<sub>3</sub>O<sub>4</sub> NPs with isocyanate functionality then reacting it with the thymine hydrazide. In brief, dried Fe<sub>3</sub>O<sub>4</sub> NPs prepared by the co-precipitation method were suspended in dry toluene. After, (EtO)<sub>3</sub>Si-NCO was added and refluxed. The isocyanate functionalized Fe<sub>3</sub>O<sub>4</sub> NPs were suspended in dry DMF. After, thymine hydrazide was added and refluxed under continuous mechanical stirring (Scheme 14).



Scheme 14. Synthesis route of  $Fe_3O_4@Si$ -thymine NPs through siloxane bonding via "grafting from" method.

The synthesis route of ligand 3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propanehydrazide **23** is outlined in Scheme 15. Briefly, the intermediate ester **22** was synthesized by Michael addition reaction between thymine **21** and ethyl acrylate in ethanol using a catalytic amount of sodium under reflux. After, the ester **22** was converted to hydrazide **23** by treating it with hydrazine hydrate in ethanol under reflux.



Scheme 15. Synthesis of compound 23

The Structure of intermediate **22** was verified by <sup>1</sup>H, <sup>13</sup>C, IR and MS. The <sup>1</sup>H NMR spectrum of the intermediate **22** revealed four new signals which can be assigned to the O-CH<sub>2</sub>- protons' signal which appeared at 4.05 ppm as a quartet, and the -CH<sub>3</sub> at 1.15 ppm as a triplet which attribute to the ethoxy group. The other two signals corresponded to the -CH<sub>2</sub>- of the carbonyl group which appeared at 2.66 ppm as a triplet, and the -CH<sub>2</sub>- connected to the amine at 3.84 ppm as a triplet. The FT-IR spectrum of intermediate **14** showed a strong absorption band at 1693 cm<sup>-1</sup> for the carbonyl group of the ester.

**Table 17.** <sup>1</sup>H (500 MHz, DMSO- $d_6$ ) and <sup>13</sup>C (126 MHz, DMSO- $d_6$ ) NMR data for compound **22** 

Structure of compound 22

	No	δ <sub>H</sub> (ppm)		No	$\delta_{\rm C}$ (ppm)
	3	11.26		9	170.82
0 12	6	7.5		4	164.41
HN 4 5 13	15	4.05		2	150.88
0 <sup>2</sup> N <sup>6</sup>	7	3.84		6	141.89
11 <u>1</u> 7	8	2.66		5	108.25
8   ~ 9.	13	1.73		15	60.28
0 10 14	16	1.15		7	43.86
15 CH <sub>3</sub> 16				8	32.86
				16	14.05
			ĺ	13	12.02

The structure of the intermediate **23** was verified by <sup>1</sup>H, <sup>13</sup>C, IR and MS. The <sup>1</sup>H NMR spectrum of the intermediate **23** revealed the disappearance of two signals at 4.05, and 1.15 ppm which attributes to the  $-CH_2$ - and  $-CH_3$  protons of the ethoxy group respectively. On the other hand, two new signals appeared at 11.23, and 4.21 ppm which can be assigned to the -NH- and  $NH_2$  of the hydrazide group respectively. The signal of the  $-CH_2$ - of the carbonyl group shifted upfield from 2.66 ppm to 2.38 ppm

which confirms the conversion of the ester to hydrazide. The FT-IR spectrum of the intermediate **22** revealed that the C=O strong absorption band was shifted to a lower frequency from 1693 to 1667 cm<sup>-1</sup> confirming the success of ester conversion to hydrazide.

**Table 18.** <sup>1</sup>H (500 MHz, DMSO- $d_6$ ) and <sup>13</sup>C (126 MHz, DMSO- $d_6$ ) NMR data for compound **23** 

Structure of compound 23					
	No	δ <sub>H</sub> (ppm)	Γ	No	δ <sub>C</sub> (ppm)
0 12	14	11.23		9	169.03
HN 4 5 13	3	9.08		4	164.48
	6	7.4		2	150.82
11 <u>1</u> 7	15	4.21		6	142.01
8	7	3.81		5	108.15
0 <sup>9</sup> NH 10 14	8	2.38		7	44.54
NH <sub>2</sub> 15	13	1.71		8	32.52
			· [	13	12 07



Fig. 50 FT-IR spectra of Fe<sub>3</sub>O<sub>4</sub> (a), Fe<sub>3</sub>O<sub>4</sub>@Si-thymine (b), and thymine hydrazide (c).

The FT-IR of the thymine-hydrazide shown in Fig. 50(c) has main three characteristic bands at 1670, 3022, 3348 cm<sup>-1</sup> for the C=O, C-H, and N-H stretching

vibrations respectively. On the other hand, the FT-IR spectrum of the Fe<sub>3</sub>O<sub>4</sub>@Sithymine NPs in Fig. 50(b) shows five characteristic bands at 582, 1012, 1635, 2926, 3346 cm<sup>-1</sup> which can be assigned to the Fe-O, Si-O, C=O, C-H, and N-H stretching vibrations respectively. The appearance of these bands confirms the presence of thymine coating.



**Fig. 51** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of thymine-hydrazide. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.



Table 19 Thermal decomposition stages of thymine-hydrazide.

**Fig. 52** Thermal gravimetric analysis (TGA) ((a) black solid line), and  $1^{st}$  derivative DTG ((a) red solid line), with evolved gas analysis (EGA) (b) of Fe<sub>3</sub>O<sub>4</sub>@Si-thymine. The analysis was carried out under nitrogen flow at a heating rate of  $10^{\circ}$ C/min.

Stage	1	2	3	4
Temperature range (°C)	100-382	382-546	713-880	880-1100
DTGmax (°C)	269	420	682	753
% Weight loss	11	4	5	5

Table 20. Thermal decomposition stages of Fe<sub>3</sub>O<sub>4</sub>@Si-thymine.



**Fig. 53** TGA thermograms of  $Fe_3O_4$  (a),  $Fe_3O_4$  @Si-thymine (b), and thymine hydrazide (c). The analyses were carried out under nitrogen flow at a heating rate of  $10^{\circ}C/min$ .

The TGA thermogram of thymine-hydrazide shown in Fig. 51(a) shows two decomposition stages summarized in Table 19 which are at DTGmax 290 and 401 °C. In these two stages the decomposition started with the evolution of NH<sub>3</sub>, C=O gases shown in Fig. 51(b) which can be attributed to the hydrazide part then the sublimation of thymine. On the other hand, the TGA thermogram of Fe<sub>3</sub>O<sub>4</sub>@Si-thymine shown in Fig. 52(a) shows four decomposition stages summarized in Table 20. The gases evolved in stage one (100-382 °C) as shown in Fig. 52 are CO<sub>2</sub>, NH<sub>3</sub> and in stages two, three (382-880 °C) are HCN, CH<sub>4</sub> and for the last stage (880-110 °C) is C=O. The absence of ethanol vapor from the evolved gases of the Fe<sub>3</sub>O<sub>4</sub>@Si-thymine decomposition profile confirm the absent of ethoxy groups of 3-(triethoxysilyl)propyl isocyanate. As shown

in Fig. 53 the TGA profiles in Fig. 53,  $Fe_3O_4@Si$ -thymine NPs has more weight loss than  $Fe_3O_4$  which can be due to the thymine coating.



Fig. 54 SEM image of Fe<sub>3</sub>O<sub>4</sub>@Si-thymine NPs

In **Fig. 54** the SEM image of Fe<sub>3</sub>O<sub>4</sub>@Si-thymine NPs is shown. The hydrodynamic diameter of Fe<sub>3</sub>O<sub>4</sub>@Si-thymine produced by the "grafting from" approach was 48 nm with 0.2 PDI and zeta potential of +16 mV  $\pm$  2 at pH 7. The change of the surface charge of the Fe<sub>3</sub>O<sub>4</sub> NPs from -16 for bare Fe<sub>3</sub>O<sub>4</sub> to +16 for coated Fe<sub>3</sub>O<sub>4</sub> confirms the present of the thymine group. The grafting density of thymine coated Fe<sub>3</sub>O<sub>4</sub> NPs by grafting from was 16 thymine/nm<sup>2</sup>.

#### Conclusion

Iron oxide nanoparticles were functionalized by nucleobases through two covalent routes. The coatings were characterized by FT-IR spectroscopy and TGA. The grafting densities were calculated for each route from the TGA weight loss, and was found that it increased from 7 thymine/nm<sup>2</sup> for the "grafting to" method to 18 thymine/nm<sup>2</sup> for "grafting from". Other chelation based functionalization was also investigated using "ligand exchange" method, where the carboxylate groups were successfully exchange by the hydroxamate groups on the surface of the Fe<sub>3</sub>O<sub>4</sub> NPs. Moreover the structures of the ligands used for the coating were confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR, and MS.

### CHAPTER III

## EXPERIMENTALS

Iron(II) chloride tetrahydrate (99%), Iron(III) chloride hexahydrate (98%),Iron(III) acetylacetonate (97%), Ammonium hydroxide solution 25% in H<sub>2</sub>O, Tetraethyl orthosilicate (99%), Sodium hydroxide, Hydrochloric acid (37%), Caffeic acid (97%), Adenine (99%), Uracil (99%), Thymine (99%), Ethyl acrylate (99%), Sodium, Hydrazine hydrate (60%), Hydroxylamine hydrochloride (96%), Potassium hydroxide, 3-(Triethoxysilyl)propyl isocyanate (95), Toluene, Dimethylformamide, Methanol, Ethanol, Acetonewere purchased from Sigma-Aldrich.

Absorption spectra were recorded using a SCOV-570 UV-Vis Spectrophotometer. The hydrodynamic diameter of the particles were characterized using Brookhaven 90 Plus Nanoparticle Size Analyzer and zeta potential using Malvern Zetasizer Nano ZS. The morphology of the NPs were investigated using scanning electron microscope (SEM), Tescan, Vega 3 LMU with Oxford EDX detector. SEM samples were prepared by mounting a drop of our suspension on carbon tape and let it dry. STEM samples were also performed on Tescan Vega 3 LMU but using TE detector at 30 keV. STEM samples were prepared by adding a drop our sample on a cupper mesh previously coated by carbon layer. XRD diffractions were collected using a Bruker D8 advance X-ray diffractometer at 40 kV, 40 mA using Cu anode. The X-ray diffractions were carried out from 20 and 70 20 (degree). FT-IR spectra were collected using Thermo Scientific Nicolet iS5 FT-IR Spectrophotometer. The samples for FT-IR were prepared as KBr pellets. Thermogravimetric analysis was done on NETZSCH libra and

was coupled to Thermo Scientific Nicolas FT-IR for evolved gases analysis. Melting points were measured on a DigiMelt apparatus. NMR spectra were obtained on a Bruker Avance III HD 500 NMR spectrometer. LCMS instrument (AGILENT 1100 series with a quaternary pump HPLC and Agilent LC/MSD Trap XCP mass spectrometry detector).

Fe<sub>3</sub>O<sub>4</sub> NPs **1**: The hydrophilic Fe<sub>3</sub>O<sub>4</sub> NPs were synthesized via the coprecipitation route following modified reported procedure.<sup>16</sup> A solution of (5.00 g, 18.5 mmol) FeCl<sub>3</sub>.6H<sub>2</sub>O and (1.84 g, 9.25 mmol) of FeCl<sub>2</sub>.4H<sub>2</sub>O molar ratio (Fe<sup>3+</sup>:Fe<sup>2+</sup> 2:1) were prepared in DI water (200 mL) with nitrogen purging. While vigorously stirring 15 mL of 25% ammonia was injected. A black precipitated was formed instantly. After 1 hour, the Fe<sub>3</sub>O<sub>4</sub> NPs were collected by an external magnet and were washed three times with deionized water. The Fe<sub>3</sub>O<sub>4</sub> NPs were dried under reduced pressure at 60 °C to remove residual solvent and stored in a nitrogen atmosphere to eliminate oxidation of Fe<sub>3</sub>O<sub>4</sub> to Fe<sub>2</sub>O<sub>3</sub>.

Fe<sub>3</sub>O<sub>4</sub>@OA NPs **2**: The hydrophobic Fe<sub>3</sub>O<sub>4</sub> NPs were synthesized via the thermal decomposition route following modified reported procedure.<sup>17</sup> A solution containing (1.4 g, 4 mmol) Fe(acac)<sub>3</sub>, (4.5 g, 20 mmol) 1,2-Dodecanediol, (4.6 g, 12 mmol) Oleylamine, (3.4 g, 12 mmol) Oleic acid was prepared in phenyl ether (40 mL). The solution was purged with nitrogen for 10min than refluxed for two hours under nitrogen atmosphere<sup>17</sup>. After, the solution was left to cool to room temperature. The Fe<sub>3</sub>O<sub>4</sub>@OA NPs formed were precipitated by adding ethanol and collected by external magnetic field. The Fe<sub>3</sub>O<sub>4</sub>@OA NPs were washed with ethanol three times and then dried under reduced pressure at 60 °C to remove any residual solvents. The synthesized Fe<sub>3</sub>O<sub>4</sub>@OA NPs were stored under nitrogen.

Synthesis of Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> **3**: The synthesis of Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> NPs was done by following Stober's method.<sup>46</sup> 100 mg of previously synthesized dried Fe<sub>3</sub>O<sub>4</sub> NPs prepared by co-precipitation method was dispersed in a mixture of ethanol (80 mL) and deionized water (20 mL) using probe sonicator. After sonicating for 10 min, 2.5 mL of 25% ammonia was added. TEOS (2 mL, 13 mmol) was added dropwise using syringe pump (1 mL/h) and then was left under mechanical stirring for 4 h. Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> NPs were collected by external magnetic field and were washed with deionized water, and ethanol then dried under reduced pressure at 60 °C.

Fe<sub>3</sub>O<sub>4</sub>@Caffeic acid **4**: Fe<sub>3</sub>O<sub>4</sub>@CA was prepared by the chelation of caffeic acid's catechol group with the surface of the Fe<sub>3</sub>O<sub>4</sub> NPs. Dry Fe<sub>3</sub>O<sub>4</sub> NPs prepared by coprecipitation method (80 mg) was suspended in ethanol (10 mL) and sonicated for 15 min. Different amounts of caffeic acid (1, 2, 3, 4 mg) was added to the suspension before and probe sonicated for 1 min. Fe<sub>3</sub>O<sub>4</sub>@CA NPs were collected by external magnet and washed three times with ethanol. The supernatant was pipetted out to indirectly quantify the amount of coating. UV-Vis analysis was utilized to indirectly determine the bound molecules by measuring the free molecules in solution. The Fe<sub>3</sub>O<sub>4</sub>@CA NPs were magnetically collected and the supernatant transferred to a clean flask. The Fe<sub>3</sub>O<sub>4</sub>@CA NPs were combined with the supernatant. The amount of free caffeic acid in the combined solution was estimated from the calibration curve. Calibration was performed before the quantification of the unbounded CA in the supernatant at absorbance 396 nm. The R<sup>2</sup> of the calibrations were between 99.63-99.95.

Ethyl 3-(6-amino-9H-purin-9-yl)propanoate 6:<sup>47</sup> In a 500 mL round bottom flask adenine 5 (10 g, 74 mmol) was dissolved in ethanol (200 mL), and sodium (170

mg, 7.4 mmol) was added. After the complete disappearance of the sodium ethyl acrylate (24 mL, 220 mmol) was added. The reaction was refluxed overnight. After, the reaction was let to cool down and was concentrated under reduced pressure. The precipitate was filtered and washed twice by ethanol by slurring-filtration cycles. The washed precipitate was filtered and dried to yield pure ester (13 g, 75%).<sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>)  $\delta$  8.13 (s, 1H), 8.10 (s, 1H), 7.24 (s, 2H), 4.37 (t, J = 6.8 Hz, 2H), 4.02 (q, J = 7.1 Hz, 2H), 2.93 (t, J = 6.8 Hz, 2H), 1.10 (t, J = 7.1 Hz, 3H); FTIR-KBr (cm<sup>-1</sup>): 3299, 3139, 1726, 1717, 1678, 1609, 1419, 1328, 1305, 1197, 1015, 794, 642

3-(6-amino-9H-purin-9-yl)-*N*-hydroxypropanamide **7**:<sup>48</sup> In a 250 mL round bottom flask, compound **6** (2 g, 8.5 mmol) was dissolved in MeOH (17 mL). HONH<sub>2</sub>.HCl (4.3 g, 61.3 mmol) was dissolved in MeOH (40 mL). KOH (5.4 g, 97.0 mmol) was dissolved in MeOH (30 mL). The KOH solution was poured into the HONH<sub>2</sub>.HCl solution and the resulting mixture was cooled to 0 °C for 1 h. the KOH/HONH<sub>2</sub>.HCl solution was then filtered into the compound solution, and the reaction mixture was stirred at room temperature overnight. The product was collected by suction filtration and was washed with cold methanol (0.5 g, 90%). 1H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  10.47 (s, 1H), 8.83 (s, 1H), 8.14 (s, 1H), 7.99 (s, 1H), 7.21 (s, 2H), 4.35 (t, J = 6.6 Hz, 2H), 2.57 (t, J = 6.7 Hz, 2H). <sup>13</sup>C NMR (126 MHz, DMSO)  $\delta$ 166.32, 155.92, 152.37, 149.40, 140.96, 118.72, 39.19, 32.19; FTIR-KBr (cnr<sup>-1</sup>): 3383, 3195, 2790, 1654, 1610, 1487, 1417, 1306, 1080, 593. MS(ESI) calcd for C<sub>8</sub>H<sub>10</sub>N<sub>6</sub>O<sub>2</sub> 222.09; Found 220.7 (M-H)<sup>-</sup>, 245.1 (M+Na)<sup>+</sup>, 223.2 (M+H)<sup>+</sup>

Fe<sub>3</sub>O<sub>4</sub>@adenine 8: Adenine coated Fe<sub>3</sub>O<sub>4</sub> NPs via hydroxamate chelation was done by exchanging Fe<sub>3</sub>O<sub>4</sub>@OA NPs' 2 surface oleic acid by adnenine-hydroxamic acid 7. Adenine-hydroxamic 7 (0.02 g, 0.09 mmol) was dissolved in DI water (4 mL) then was added to 4 mL of hexane Fe<sub>3</sub>O<sub>4</sub> NPs suspension and sonicated for 30 min by bath sonicator then it was shaken for 24 hrs. The Fe<sub>3</sub>O<sub>4</sub>@OANPs were ligand exchanged and transferred to the aqueous layer. The aqueous phase which contains the Fe<sub>3</sub>O<sub>4</sub>@adenine NPs were separated by the separatory funnel and then was collected by centrifugation 4000 rpm for 30 min. The Fe<sub>3</sub>O<sub>4</sub>@AD were washed three times with hexane, ethanol, and acetone and dried under reduced pressure at 60 °C to remove residual solvents.

3-(6-amino-9H-purin-9-yl)propanehydrazide 9.<sup>49</sup> In a 100 mL round bottom flask compound **6** (5 g, 21.3 mmol) was dissolve in EtOH (40 mL), and hydrazine hydrate (4.8 mL, 63.8 mmol) was added. The reaction was left under reflux overnight. After, the reaction was left to cool to room temperature. The precipitate formed was filtered and washed with ethanol to afford pure hydrazide (3.5 g, 75%).<sup>1</sup>H NMR (500 MHz, DMSO- $d_6$ )  $\delta$  9.05 (s, 1H), 8.13 (s, 1H), 7.99 (s, 1H), 7.19 (s, 2H), 4.34 (t, J = 6.8 Hz, 2H), 4.19 (s, 2H), 2.61 (t, J = 6.8 Hz, 2H).

2-(3-(6-amino-9H-purin-9-yl)propanoyl)-N-(3-

(triethoxysilyl)propyl)hydrazine-1-carboxamide **10**:<sup>50</sup> In a flame dried 50 mL round bottom flask compound **9** (1 g, 4.5 mmol) was dissolved in anhydrous DMSO (23 mL) with heating. Then 3-(triethoxysilyl)propyl isocyanate (1.2 mL, 5 mmol) was dissolved in anhydrous DMSO (5 mL) and added dropwise to the compound solution at room temperature under nitrogen with continuous stirring. The reaction was left at room temperature for 2 days. After removal of DMSO under reduced pressure, the white residue was washed with methanol, filtered then dried under reduced pressure. Compound was obtained in (1.3 g, 61%). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  9.56 (s, 1H), 8.14 (s, 1H), 8.04 (s, 1H), 7.68 (s, 1H), 7.21 (s, 2H), 6.37 (s, 1H), 4.35 (t, J = 6.8

Hz, 2H), 3.73 (q, J = 7.0 Hz, 6H), 2.95 (q, J = 6.7 Hz, 2H), 2.71 (t, J = 6.8 Hz, 2H), 1.40 (p, J = 8.1 Hz, 2H), 1.14 (t, J = 7.0 Hz, 9H), 0.50 (t, 2H); <sup>13</sup>C NMR (126 MHz, DMSO*d*<sub>6</sub>)  $\delta$  169.63 , 157.99 , 155.97 , 152.40 , 149.42 , 141.02 , 118.71 , 57.74 , 41.97 , 39.26 , 33.19 , 23.45 , 18..28, 7.19; FTIR-KBr (cm<sup>-1</sup>): 3283, 3189, 2975, 2928, 2890, 1667, 1555, 1417, 1260, 1075, 639. MS(ESI) calcd for C<sub>18</sub>H<sub>32</sub>N<sub>8</sub>O<sub>5</sub>Si468.23; Found 467.0 (M-H)<sup>-</sup>, 491.3 (M+Na)<sup>+</sup>, 469.3 (M+H)<sup>+</sup>

Fe<sub>3</sub>O<sub>4</sub>@Si-adenine **12**: The preparation of adenine coated Fe<sub>3</sub>O<sub>4</sub> NPs was done through siloxane covalent bonding. Briefly (EtO)<sub>3</sub>Si-adenine (0.40 g, 0.89 mmol) was dissolved in DI water (20 mL). The pH of the solution was increased by adding 1 M NaOH solution while stirring until it reached pH 11. At pH 11 the (EtO)<sub>3</sub>Si- was prehydrolyzed to (HO)<sub>3</sub>Si- **11** for later condensation. To the solution above Fe<sub>3</sub>O<sub>4</sub> NPs (40 mg) were added and suspended by 10 min sonication. While stirring the pH of the solution was decreased while stirring by adding 1 M HCl until it reached pH 10 to facilitate the condensation. The solution was stirred for 12 hours. The adenine functionalized Fe<sub>3</sub>O<sub>4</sub> NPs were collected by external magnetic field and were washed by methanol, ethanol, and acetone then dried under reduced pressure at 60 °C.

Ethyl 3-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propanoate 14:<sup>47</sup> In a 500 mL round bottom flask uracil 13 (10 g, 89 mmol) was dissolved in ethanol (270 mL) and sodium (205 mg, 8.9 mmol) was added. After the complete disappearance of the sodium ethyl acrylate (30 mL, 280 mmol) was added. The reaction was refluxed overnight. After the reaction was let to cool down and concentrated under reduced pressure. The precipitate was filtered and washed twice with ethanol by slurring-filtration cycles. The washed precipitate was filtered and dried to yield pure ester (12.5 g, 67%), mp 99.8-102°.<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.27 (s, 1H), 7.62 (d, J = 7.9

Hz, 1H), 5.53 (d, J = 7.9 Hz, 1H), 4.05 (q, J = 7.1 Hz, 2H), 3.87 (t, J = 6.7 Hz, 2H), 2.67 (t, J = 6.7 Hz, 2H), 1.16 (t, J = 7.1 Hz, 3H). <sup>13</sup>C NMR (126 MHz, DMSO) δ 170.83, 163.88, 150.94, 146.16, 100.71, 60.30, 44.10, 32.77, 14.07; FTIR-KBr (cm<sup>-1</sup>): 3182, 3053, 3000, 2963, 2935, 2909, 1703, 1453, 1414, 1356, 1326, 1211, 1183, 1017, 864, 813, 793, 513

3-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propanehydrazide **15**.<sup>49</sup> In a 100 mL round bottom flask compound **14** (5 g, 18.9 mmol) was dissolve in ethanol (40 mL) and hydrazine hydrate (4.3 mL, 56.6 mmol) was added. The reaction was left under reflux overnight. After the reaction was left to cool to room temperature. The precipitate formed was filtered and washed with ethanol to afford pure hydrazide (2.6 g, 69%), mp 184-186.2°.<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  10.75 (s, 1H), 9.09 (s, 1H), 7.51 (d, J = 7.9 Hz, 1H), 5.49 (d, J = 7.8 Hz, 1H), 4.19 (s, 2H), 3.85 (t, J = 6.7 Hz, 2H), 2.39 (t, J = 6.7 Hz, 2H). <sup>13</sup>C NMR (126 MHz, DMSO)  $\delta$  168.96, 163.91, 150.84, 146.24, 100.59, 44.73, 32.41; FTIR-KBr (cm<sup>-1</sup>): 3355, 3312, 3289, 3161, 3027, 2800, 1678, 1616, 1527, 1463, 1392, 1358, 1253, 1227, 821, 767, 549, 478

2-(3-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propanoyl)-N-(3-(triethoxysilyl)propyl)hydrazine-1-carboxamide **16**.<sup>50</sup> In a flame dried 50 mL round bottom flask Compound **15** (1 g, 5.0 mmol) was dissolved in anhydrous DMSO (25 mL). Then 3-(triethoxysilyl)propyl isocyanate (1.4 mL, 2.5 mmol) was dissolved in anhydrous DMSO (5 mL) and added dropwise to the compound solution at room temperature under nitrogen with continuous stirring. The reaction was left at room temperature for 2 days. After removal of DMSO under reduced pressure, the white residue was washed with methanol, filtered then dried under reduced pressure. Compound was obtained in (1.5 g, 67%). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.26 (s,

1H), 9.55 (s, 1H), 7.66 (s, 1H), 7.55 (dd, J = 7.8, 2.8 Hz, 1H), 6.39 (s, 1H), 5.63 – 5.42 (s, 1H), 3.86 (t, J = 6.6 Hz, 2H), 3.73 (q, J = 7.0 Hz, 2H), 3.06 – 2.88 (m, 2H), 1.42 (dt, J = 15.6, 7.5 Hz, 2H), 1.14 (t, J = 7.0 Hz, 3H), 0.53 – 0.46 (m, 2H). <sup>13</sup>C NMR (126 MHz, DMSO)  $\delta$  169.74, 163.84, 158.00, 150.90, 146.29, 100.60, 57.72, 57.23, 44.40, 41.95, 32.24, 23.44, 18.61, 18.36, 18.28, 7.19; FTIR-KBr (cm<sup>-1</sup>): 3280, 3040, 2975, 2933, 2886, 1646, 1583, 1419, 1261, 1199, 1104, 765. MS(ESI) calcd for C<sub>17</sub>H<sub>31</sub>N<sub>5</sub>O<sub>7</sub>Si 445.20; Found 444.1 (M-H)<sup>-</sup>, 468.2 (M+Na)<sup>+</sup>

Fe<sub>3</sub>O<sub>4</sub>@Si-uracil **18**: The preparation of uracil coated Fe<sub>3</sub>O<sub>4</sub> NPs was done through siloxane covalent bonding. Briefly (EtO)<sub>3</sub>Si-uracil (0.20 g, 0.42 mmol) was dissolved in DI water (20 mL). The pH of the solution was increased by adding 1 M NaOH solution while stirring until it reached pH 11. At pH 11 the (EtO)<sub>3</sub>Si- was prehydrolyzed to (HO)<sub>3</sub>Si- **17** for later condensation. To the solution above Fe<sub>3</sub>O<sub>4</sub> NPs (40 mg) were added and suspended by 10 min sonication. While stirring the pH of the solution was decreased while stirring by adding 1 M HCl until it reached pH 10 to facilitate the condensation. The solution was stirred for 12 hours. The uracil functionalized Fe<sub>3</sub>O<sub>4</sub> NPs were collected by external magnetic field and were washed by methanol, ethanol, and acetone then dried under reduced pressure at 60 °C.

Fe<sub>3</sub>O<sub>4</sub>@Si-uracil NPs **20**<sup>51</sup> The synthesis of uracil coated Fe<sub>3</sub>O<sub>4</sub> NPs was via "grafting from" method. First, the Fe<sub>3</sub>O<sub>4</sub> NPs, were functionalized with isocyanate, then it was further reacted with uracil hydrazide. Dry Fe<sub>3</sub>O<sub>4</sub> NPs prepared by co-precipitation method (0.1 g) were suspended in dry toluene (5 mL) by 10 min sonication. 200  $\mu$ l of 3-(triethoxysilyl)propyl isocyanate (200  $\mu$ l, 0.8  $\mu$ mol) were added to the suspended before and sonicated for 10 min. The solution was refluxed for 12 hours under mechanical stirring for total condensation. The isocyanate functionalized Fe<sub>3</sub>O<sub>4</sub> NPs **19** were

collected by external magnetic field and were washed 3 times with dry toluene. After washing further with dry DMF the Fe<sub>3</sub>O<sub>4</sub>@NCO NPs were suspended in dry DMF and were sonicated for 10 min. Uracil-hydrazide (0.2 g, 1 mmol) was added to the suspended MNPs and were sonicated for 10 min and later shaken for 24 hours. The uracil functionalized MNPs were collected by external magnetic field and were washed three times with DMF then methanol, and acetone. The washed Fe<sub>3</sub>O<sub>4</sub>@Si-uracil were dried under reduced pressure for later characterization.

Ethyl 3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propanoate **22**.<sup>47</sup> In a 500 mL round bottom flask thymine **21** (10 g, 79.4 mmol) was dissolved in ethanol (240 mL) and sodium (182 mg, 7.9 mmol) was added. After the complete disappearance of the sodium ethyl acrylate (26 mL, 238.1 mmol) was added. The reaction was refluxed overnight. After, the reaction was let to cool down and was concentrated under reduced pressure. The precipitate was filtered and washed twice by ethanol by slurring-filtration cycles. The washed precipitate was filtered and dried to yield pure ester (12.5 g, 70%), 153-155°.<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.26 (s, 1H), 7.50 (s, 1H), 4.05 (q, J = 7.1 Hz, 2H), 3.84 (t, J = 6.8 Hz, 2H), 2.66 (t, J = 6.8 Hz, 2H), 1.73 (s, 3H), 1.15 (t, J = 7.1 Hz, 3H). <sup>13</sup>C NMR (126 MHz, DMSO)  $\delta$  170.82, 164.41, 150.88, 141.89, 108.25, 60.28, 43.86, 32.86, 14.05, 12.02; FTIR-KBr (cm<sup>-1</sup>): 3168, 3039, 2835, 1693, 1464, 1350, 1323, 1201, 1013, 878, 797, 759, 431

3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propanehydrazide (23):<sup>49</sup> In a 100 mL round bottom flask compound 23 (6 g, 26.6 mmol) was dissolve in ethanol (50 mL), and hydrazine hydrate (6 mL, 79.6 mmol) was added. The reaction was left under reflux overnight. After, the reaction was left to cool to room temperature. The precipitate formed was filtered and washed with ethanol to afford pure hydrazide

(2.4 g, 60%), mp 194.8-196.<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 11.23 (s, 1H), 9.08 (s, 1H), 7.40 (s, 1H), 4.21 (s, 2H), 3.81 (t, J = 6.8 Hz, 2H), 2.38 (t, J = 6.7 Hz, 2H), 1.71 (s, 3H). <sup>13</sup>C NMR (126 MHz, DMSO) δ 169.03, 164.48, 150.82, 142.01, 108.15, 44.54, 32.52, 12.07; FTIR-KBr (cm<sup>-1</sup>): 3348, 3156, 3103, 3021, 2825, 1704, 1674, 1667, 1643, 1486, 1425, 1347, 1225, 452

Fe<sub>3</sub>O<sub>4</sub>@Si-thymine NPs (**25**).<sup>51</sup> The synthesis of thymine coated Fe<sub>3</sub>O<sub>4</sub> NPs was via "grafting from" method. Fist the Fe<sub>3</sub>O<sub>4</sub> NPs were functionalized with isocyanate, then it was further reacted with thymine hydrazide. Dry Fe<sub>3</sub>O<sub>4</sub> NPs prepared by co-precipitation method (0.1 g) were suspended in dry toluene (5 mL) by 10 min sonication. 3-(triethoxysilyl)propyl isocyanate (200 µl, 0.8 µmol) were added to the suspended before and sonicated for 10 min. The solution was refluxed for 12 hours under mechanical stirring for total condensation. The isocyanate functionalized Fe<sub>3</sub>O<sub>4</sub> NPs **19** were collected by external magnetic field and were washed 3 times with dry toluene. After washing further with dry DMF, the Fe<sub>3</sub>O<sub>4</sub>@NCO NPs were suspended in dry DMF and were sonicated for 10 min. thymine-hydrazide (0.2 g, 0.9 mmol) was added to the suspended MNPs and were sonicated for 10 min and later shaken for 24 hours. The thymine functionalized MNPs were collected by external magnetic field and were washed three times with DMF then methanol, and acetone. The washed Fe<sub>3</sub>O<sub>4</sub>@Si-thymine were dried under reduced pressure for later characterization.

# PART II

# FORMULATION OF RHODAMINE B LOADED PLGA NANOPARTICLES

# CHAPTER IV INTRODUCTION

Polymer-based NPs have been recently the drug delivery field's research interest. They have been good candidates for drug delivery because of their biocompatibility. Moreover, their surface can be functionalized easily to target specific cells.<sup>52</sup> Different surface charges can be accomplished to target specific cellular paths. Their formulation parameters can be adjusted to control the drug release profile. All of these properties are attracting more research to be conducted in this field. They have been investigated in drug, RNA, and DNA delivery.<sup>53</sup> Polymeric nanoparticles can be formulated from the dispersion of their preformed polymer or the polymerization of their monomers.<sup>54,55</sup>

The methods used in the dispersion of preformed polymer NPs' formulation are solvent evaporation<sup>54</sup>, nanoprecipitation<sup>56</sup>, emulsion/solvent diffusion<sup>57</sup>, salting out<sup>58</sup>, dialysis<sup>59</sup>, and solvent displacement<sup>60</sup>. The method that we will elaborate on is the solvent evaporation method. In brief, the polymer is dissolved in an organic solvent that is immiscible with water then added dropwise to the aqueous phase which usually contains a surfactant to form an emulsion.<sup>54</sup> The drug can be dissolved in the aqueous or organic layer depending on its relative solubility. The emulsion is formed by exerting shear power which source can be homogenization, mechanical stirring, or sonication.

Smaller and more uniform suspension droplets yield smaller and more monodispersed NPs. After the formation of the emulsion, the organic solvent is evaporated by stirring over a period of time or by rotary evaporation<sup>61</sup>. The rate of evaporation plays a major role in dictating the properties of the final NPs formed.<sup>62</sup> The synthesized polymeric NPs are collected by centrifugation, washed, and lyophilized. The amount of the drug encapsulated can be determined by two methods: the first (destructive) method is by dissolving the PLGA NPs in an appropriate solvent like DMSO and quantifying the amount of drug by spectrometry<sup>63</sup>. The second method is by collecting the supernatant and calculating the amount of encapsulated drug.<sup>64</sup>

Poly(lactic-co-glycolic acid) PLGA is a synthetic polyester which is a copolymer of lactic and glycolic acid (Fig. 55).PLGA is a good candidate for the nanoscale delivery system because it is biodegradable. Moreover, PLGA is approved for drug delivery use in humans by U.S. Food and Drug Administration (FDA) and European Medicine Agency (EMA).<sup>65</sup> The advantages of PLGA NPs over other drug carriers are: they can encapsulate hydrophobic as well as hydrophilic drugs; their drug release rate can be modified easily; their interior cargo volume is large; and their surface can be modified easily to target specific cells. PLGA degrades into the biocompatible products lactic and glycolic acid in aqueous media as shown in Fig. 55. They can be processed by the body through the Krebs cycle and be eliminated as carbon dioxide and water. The degradation of PLGA is through the hydrolysis of the ester bonds. In aqueous media, it starts with the hydration of the pores and then autocatalytic deesterification.<sup>66</sup> PLGA with higher amount on lactic acid increases the encapsulation efficiency of hydrophobic drugs because the methyl group of the lactic acid increases the hydrophobicity of the formulated NPs. This increase in hydrophobic ity also makes

high lactic acid content PLGA NPs interact less with water, therefore, delaying their hydrolysis and slowing the drug release profile.<sup>67</sup> The other property of PLGA that plays a role in its degradation is the molecular weight. It was shown that as the molecular weight increased the degradation rates decreased slowing down the drug release.<sup>68</sup> The surface zeta potential of PLGA NPs is negative due to the carboxylic end groups. This charge can be changed to positive by introducing new functional groups or adding other polymers like chitosan.<sup>69</sup> PLGA NPs with different surface charges have different advantages in cell targeting.

#### Single emulsion evaporation method

The single emulsion evaporation method is one of the most used methods for the formulation of PLGA NPs. The PLGA polymer is dissolved in an organic solvent (e.g. DCM) which is immiscible with water. The organic phase containing PLGA is then added dropwise to an aqueous solution containing a surfactant (e.g. PVA). The mixture is emulsified using a homogenizer or a sonicator then the organic solvent is evaporated by stirring for a period of time or by rotary evaporator under reduced pressure.<sup>54</sup> The different parameters that dictate the final properties of the PLGA NPs are: organic:aqueous volume ratio, surfactant concentration, PLGA concertation, and sonication time.

#### A. Effect of organic:aqueous volume ratio

The organic:aqueous volume ratio affects the final size of the PLGA NPs. As the organic:aqueous volume ratio increases the size of the nanoparticles increases because the emulsion viscosity increases which resists the emulsification process by the sonicator.<sup>70</sup> Higher organic volume leads to slower solvent evaporation which lets some of the drug diffuse to the surface. The drugs close to the surface cause a burst in the release profile. On the other hand as the organic:aqueous volume ratio decreases the nanoparticles size decreases, but when the amount of aqueous volume increases to a certain extent, the size of the nanoparticles starts to increase because of the high volume which disturbs the even distribution of the ultra-sonication power. In contrast to the high organic:aqueous volume ratio, the increase of the aqueous volume leads to increase the rate of the organic layer evaporation. This causes the surface of the nanoparticles to be less porous.<sup>71</sup> Less porosity makes the drug release profile slower. Furthermore high aqueous volume dissolves more of the drug which makes the encapsulation efficiency lower compared with the high O:A volume ratio.<sup>72</sup>

#### B. Effect of the surfactant concentration

The surfactant plays a major role in the process of emulsion formation. It reduces the interfacial tension between the organic and the aqueous phase which helps to form a stable emulsion with small suspended organic droplets. Therefore, at low concentrations of surfactant, the organic and the aqueous phase tend to separate as two layers. As the concentration of the surfactant increases better emulsion is formed but when the concentration exceeds a certain amount the NPs' size increases again because of the missile or gel formed by the surfactant.<sup>73</sup> In addition, the increase of the

surfactant concentration makes the encapsulation efficiency decreases especially in case of the hydrophobic drugs because the surfactant increases their solubilization in the aqueous phase.

#### C. Effect of the PLGA concentration

The concentration of the PLGA changes the viscosity of the organic phase. As the concentration of the PLGA increases, the viscosity of the organic phase increases. The increase of the viscosity resists the formation of emulsion droplets and also the diffusion of the drug that leads to bigger nanoparticles with higher encapsulation efficiency.<sup>74</sup> It was found that the encapsulation efficiency was greater at higher PLGA concentrations because the particles did not exceed their encapsulation saturation.<sup>75</sup> Moreover, the increase of PLGA NPs' size was accompanied by slower release profile due to the diffusion of the drug from the core to the surface of the nanoparticles.<sup>76</sup>

#### D. Effect of the sonication time

The longer the emulsion is subjected to sonication, it forms smaller and more uniform organic suspended droplets.<sup>77</sup> The release profile is dictated by the size of the particles as it becomes slower with bigger particles.

### CHAPTER V

# **RESULTS AND DISCUSSION**

#### **RhB encapsulated PLGA NPs formulation**

The PLGA NPs were synthesized using the single emulsion evaporation method. In brief, PLGA and rhodamine B were dissolved in DCM then were added dropwise to a PVA solution under continuous sonication. The emulsion was left under sonication for about 10 minutes and then DCM was evaporated by rotary evaporator under reduced pressure.<sup>54</sup> The effect of PVA concentration, organic to aqueous volume ratio, and sonication time on the size and encapsulation efficiency of PLGA NPs were investigated.

#### A. Effect of PVA concentration

The effect of PVA concentration on PLGA NPs' size and encapsulation efficiency is shown in Fig. 56. As the PVA concentration increased from 0.5% to 2.5% the size of the PLGA NPs decreased from 229 to 184 nm accompanied by a decline in the PDI, but the encapsulation efficiency decreased from 72% to 40%. Size and PDI decrease can be attributed to the increase of the emulsion stability as the surfactant molecules lower the interfacial tension between the two phases. On the other hand, the decrease of encapsulation efficiency can be due to the solubilization of RhB molecules by the surfactant molecules.



**Fig. 56** Effect of PVA concentration on PLGA NPs' Z-average diameter and PDI (a) and encapsulation efficiency (b) (n = 3).

#### **B.** Effect of sonication time

The effect of sonication time on PLGA NPs' size and encapsulation efficiency is shown in Fig. 57. As the sonication time increased, the formed PLGA NPs were smaller, and their encapsulation efficiency was higher. The longer the emulsion is left under sonication smaller and more uniform droplets suspension are formed thus formulating a smaller PLGA NPs.



Fig. 57 Effect of sonication time on PLGA NPs' Z-average diameter and PDI (a) and encapsulation efficiency (b) (n = 3).

#### C. Effect of organic:aqueous volume ratio

The effect of organic:aqueous volume ratio on PLGA NPs' size and encapsulation efficiency is shown in Fig. 58. As the O:A volume ratio decreased the size of the PLGA NPs increased which could be due to the non-uniform distribution of shear stress of the sonicator. On the other hand as the O:A volume ratio increased the size of the PLGA NPs increased because of the higher emulsion viscosity which is harder to break by shear stress to smaller droplets. In conclusion, the primal parameters that we found were 2.5% PVA, 10 min sonication, and 1:10 O:A volume ratio. The zeta potential of the synthesized PLGA NPs was  $-21 \pm 5$  mV due to the surface carboxylate groups. The SEM image of the optimized PLGA NPs shown in Fig. 59 shows that they have spherical morphology with close diameters  $72 \pm 10$  nm. The bulk diameter is smaller than the hydrodynamic diameter due to the double layer.



**Fig. 58** Effect of O:A volume ratio on PLGA NPs' Z-average diameter and PDI (a) and encapsulation efficiency (b) (n = 3).



Fig. 59 SEM micrograph of RhB-loaded PLGA NPs prepared by the optimized single emulsion method.

#### In vitro release

#### A. Dialysis diffusion method

RhB release profile of the optimized PLGA NPs was studied using dialysis diffusion method. The release was done using 1000 Da dialysis bag using PBS buffer at biological pH 7.2 under continuous stirring and constant temperature 37 °C. The release profile of RhB from PLGA NPs is shown in Fig. 60. It shows two stages, initial burst within the first 8 hours then slow release. This burst could be due to adsorbed or close to surface encapsulated RhB molecules. The release profile data was fitted into release kinetic models zero order, first order, Higuchi, Hixson Crowell and Korsemeyer Peppas. The values of the fittings are shown in Table 21. The release profile had highest R<sup>2</sup> value for the Higuchi model which means that the release is diffusion controlled.



Fig. 60 Release profile of RhB loaded PLGA NPs using dialysis diffusion method (n = 3).

 Table 21. In vitro release kinetics assessment: correlation coefficients of the kinetics models.

Model	k	R <sup>2</sup>	n
Zero order	4.5	0.96	-
First order	0.02	0.97	-
Higuchi	22.8	0.98	-
Hixson-Crowell	0.08	0.97	-
Korsmeyer's	-	0.96	1.3

#### **B.** Flowcytometry method

RhB release profile of the optimized PLGA NPs was also studied using a new trial method by flowcytometry that needs further validation. The release profile of RhB from PLGA NPs using flowcytometry is shown in Fig. 61. It showed two stages very similar to the dialysis diffusion method with an initial burst followed by a slow release. The release profile data were fitted into release kinetic models like zero order, first order, Higuchi, Hixson Crowell and Korsemeyer Peppas. The values of the fitting are shown in Table 22. The release profile had highest R2 value for the Higuchi model which means that the release is diffusion controlled.



Fig. 61 Release profile of RhB loaded PLGA NPs using flowcytometry method (n = 3).

Model	k	<b>R</b> <sup>2</sup>	n
Zero order	1.1	0.94	I
First order	0.01	0.95	I
Higuchi	7.83	0.98	-
Hixson-Crowell	0.02	0.95	-
Korsmeyer's	-	0.96	0.68

 Table 22. In vitro release kinetics assessment: correlation coefficients of the kinetics models.

#### Conclusion

RhB loaded PLGA NPs with 184 nm diameter and 0.2 polydispersity were obtained after optimizations. The effect of the PVA concentration, sonication time, and organic to aqueous volume ratio on the size and encapsulation efficiency was investigated. The sizes of the NPs were characterized by DLS and their encapsulation efficiency by fluorescence microscopy. The *in vitro* RhB release followed the Higuchi's model with Fickian diffusion mechanism and initial burst release. A new method for studying the release profile using flowcytometry was explored.

# CHAPTER VI

# **EXPERIMENTALS**

Poly(D,L-lactide-co-glycolide) lactide:glycolide (50:50), mol wt 30,000-60,000, poly(vinyl alcohol) Mw 13,000-23,000, 87-89% hydrolyzed, rhodamine  $B \ge 95\%$ (HPLC), dichloromethane and acetonitrile HPLC grade. Fluorescence spectra were recorded using Jobin-Yvon-Horiba Fluorolog III fluorometer. The source of excitation was a 100 W Xenon lamp, and the detector used was R-928 operating at 950 V. LC-FLD instrument (AGILENT 1100 series with a quaternary pump HPLC and FLD detector).

#### Formulation of RhB encapsulated PLGA NPs

The PLGA NPs were formulated using solvent evaporation single emulsion method.<sup>54</sup> PLGA (50 mg) was dissolved in DCM (1 mL) then added to 10 ml of 2.5% PVA solution dropwise while sonicating using probe sonicator (50 W). The emulsion was left under sonication for 10 min and then the DCM was evaporated using rotary evaporator for 30 min. The formulated PLGA NPs were collected by centrifugation at 20 000 rpm and were washed three times by DI water then lyophilized. The supernatants were collected and free rhodamine was measured by fluorescence spectrometry ( $\lambda ex = 553$  nm,  $\lambda em = 574$  nm). Encapsulation efficiency was calculated using the equation below.

$$EE(\%) = \frac{\text{Weight of feeding RhB} - \text{Weight of unencapsulated RhB}}{\text{Weight of feeding RhB}} \times 100$$

The effect of each parameter on the PLGA NPs' formulation was investigated by varying it and leaving the other parameters constant. The optimal parameter from each parameter optimization was used for the following experiment.

#### In vitro release profile

#### Dialysis diffusion method

The RhB release profile was investigated using dialysis diffusion method. PLGA NPs (5 mg) were suspended in (0.5  $\mu$ L) PBS buffer and transferred to a dialysis bag (cut-off 1000 Da). This bag was soaked in a beaker contains PBS buffer (35 mL, pH 7.2). At different time intervals under continuous stirring and constant temperature 37 °C, 0.5 mL of the outer phase was pipetted and were replaced by the same amount of PBS buffer. The amount of RhB released at each time point was measured by HPLC-FLD ( $\lambda ex = 539$  nm,  $\lambda em = 573$  nm).

#### Flowcytometry method

The lyophilized PLGA NPs (0.5 mg) were suspended in 3.5 ml. Blank PLGA NPs (without rhodamine B) were also prepared as reference. The samples were analyzed by flow cytometry taking 10,000 events/sample. The PE fluorochrome settings were used (excitation blue laser 488 nm and emission 578 nm). The samples were analyzed at different time intervals and were compared to their zero time emission. The fluorescence intensity collected by the flowcytometry measures the amount of rhodamine B inside the PLGA NPs. Therefore, the decrease in intensity with time reflects the amount of rhodamine B that has been released.

# CHAPTER VII

# SUPPORTING DATA



<sup>1</sup>H (500 MHz, DMSO- $d_6$ ) NMR spectrum of compound **6** 



FT-IR (KBr) spectrum of compound 6




FT-IR (KBr) spectrum of compound 7



HPLC/ESI-MS(-) of compound 7 [methanol/water (50:50)]



HPLC/ESI-MS(+) of compound 7 [methanol/water (50:50)]







HPLC/ESI-MS(+) of compound 7 [acetonitrile/water (50:50)].









HMBC (DMSO- $d_6$ ) NMR of compound 10







HPLC/ESI-MS(-) of compound 10 [methanol/water (50:50)]



HPLC/ESI-MS(+) of compound 10 [methanol/water (50:50)]







HPLC/ESI-MS(+) of compound 10 [acetonitrile/water (50:50)].







FTIR (KBr) spectrum of compound 14





 $^{13}$ C (126 MHz, DMSO- $d_6$ ) NMR spectrum of compound 15



FT-IR (KBr) spectrum of compound  $\ 15$ 









COSY (DMSO-d<sub>6</sub>) NMR of compound 16







HPLC/ESI-MS(-) of compound 16 [methanol/water (50:50)].



HPLC/ESI-MS(+) of compound 16 [methanol/water (50:50)].



HPLC/ESI-MS(-) of compound 16 [acetonitrile/water (50:50)].



HPLC/ESI-MS(+) of compound 16 [acetonitrile/water (50:50)].





 $^{13}$ C (126 MHz, DMSO- $d_6$ ) NMR spectrum of compound 22



FT-IR (KBr) spectrum of compound 22







FTIR (KBr) spectrum of compound 23

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