Synthesis of Novel Iron-Oxide-Based Superparamagnetic Nanoparticles for MRI Contrasting Agent

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Abstract

Magnetite and maghemite demonstrate superparamagnetic behavior which makes them applicable for design of MRI contrast agents. Here we report synthesis of superparamagnetic iron magnetic nanoparticles (SPION) and coating them with galactaric and tartaric acids with following derivatization of those ligands on the surface of nanoparticles

Information

MRI imaging is one the most leading advances in medical field. However, the images are not as clear as they can be. Today, MRI contrasting agents are being used to clarify the details in the images.

SPION are known to shorten the spin-spin relaxation time for protons of water and can be used as so called negative contrast agents, T2, which darken the MRI image. Positive contrast agents, T1, shorten the spin-lattice relaxation for protons and brighten the MRI image in places of their accumulation. The current most widely used positive contrasting agent, gadolinium diethylenetriaminopentaacetic acid (Gd-DTPA), is considered to be highly toxic.

Iron oxide nanoparticles could be modified with covalently bonded biocompatible organic ligands to stabilize their aqueous colloids for the use as T1 MRI contrasting agents.
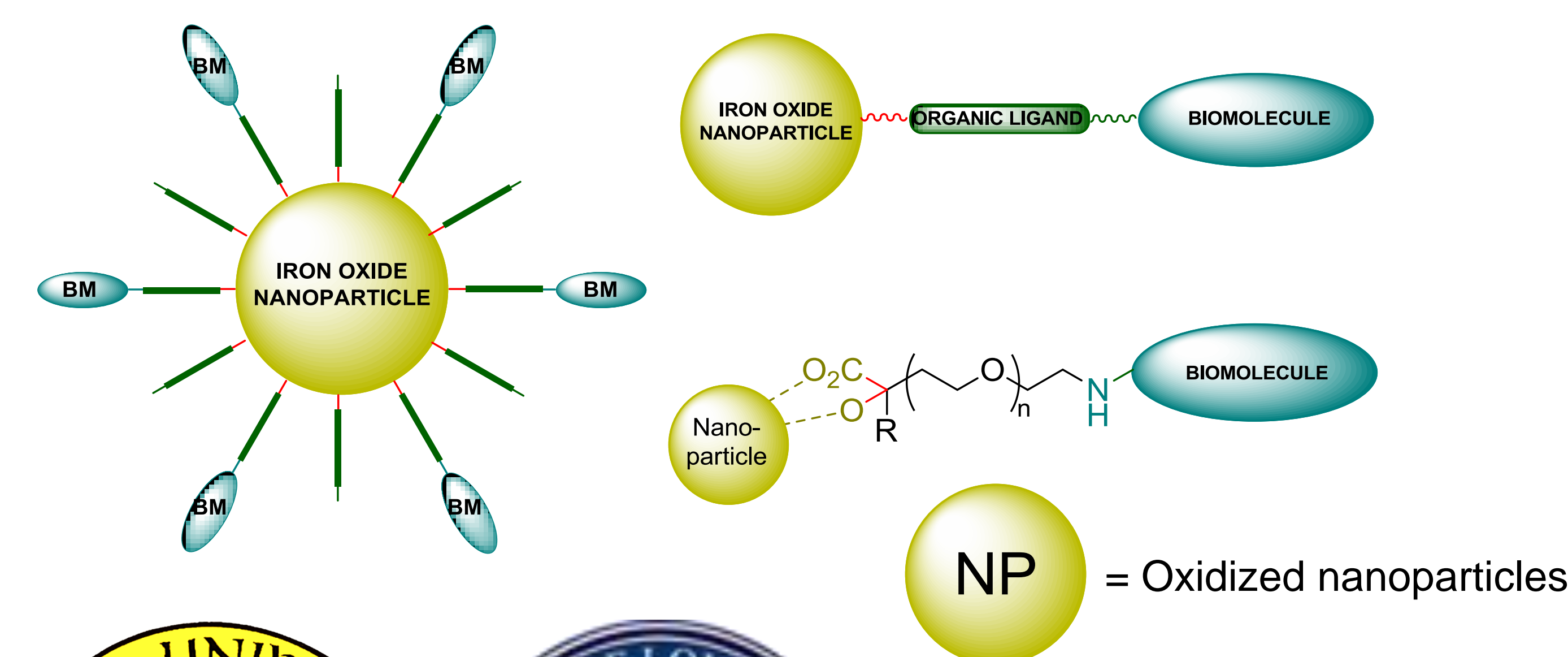
Materials and methods

The highly pure SPION were synthesized in diethylene glycol (DEG) using the following reaction:
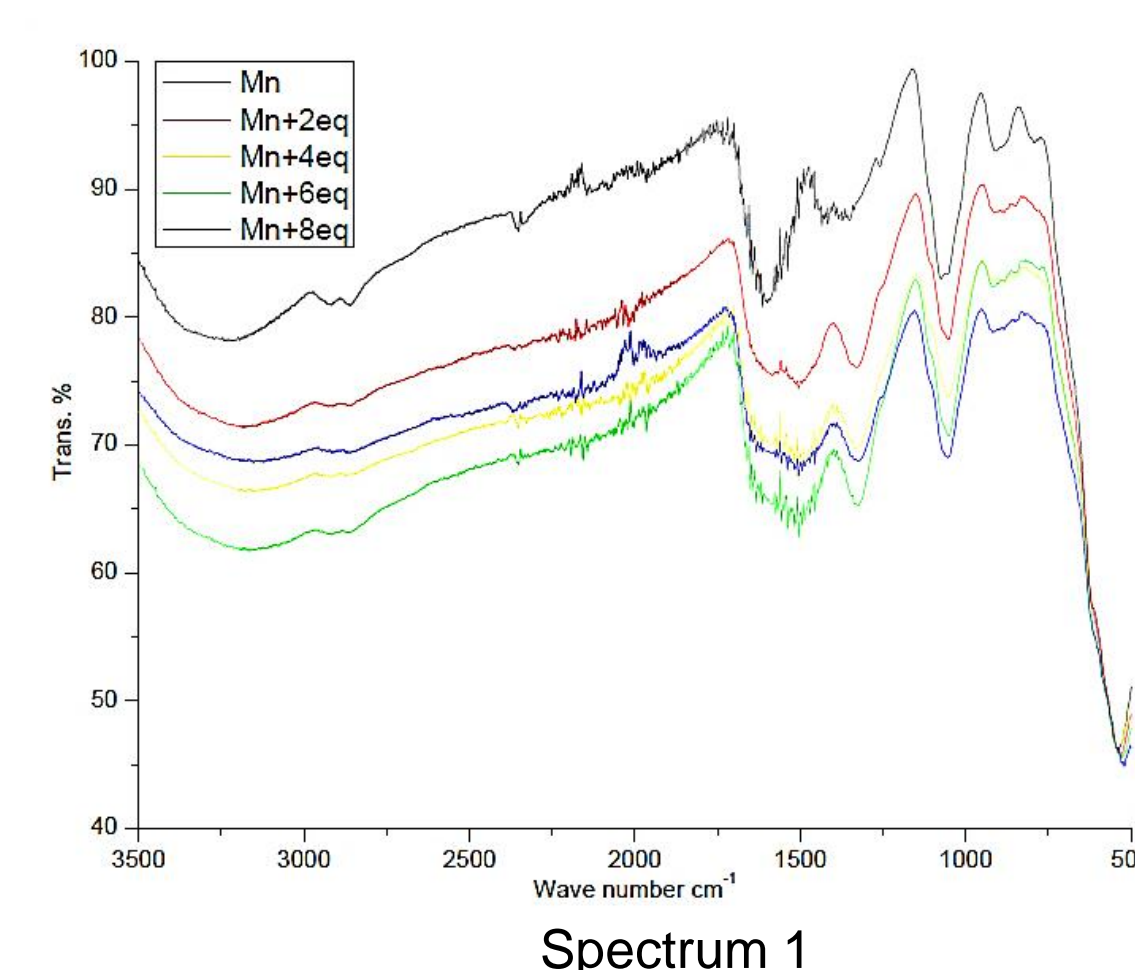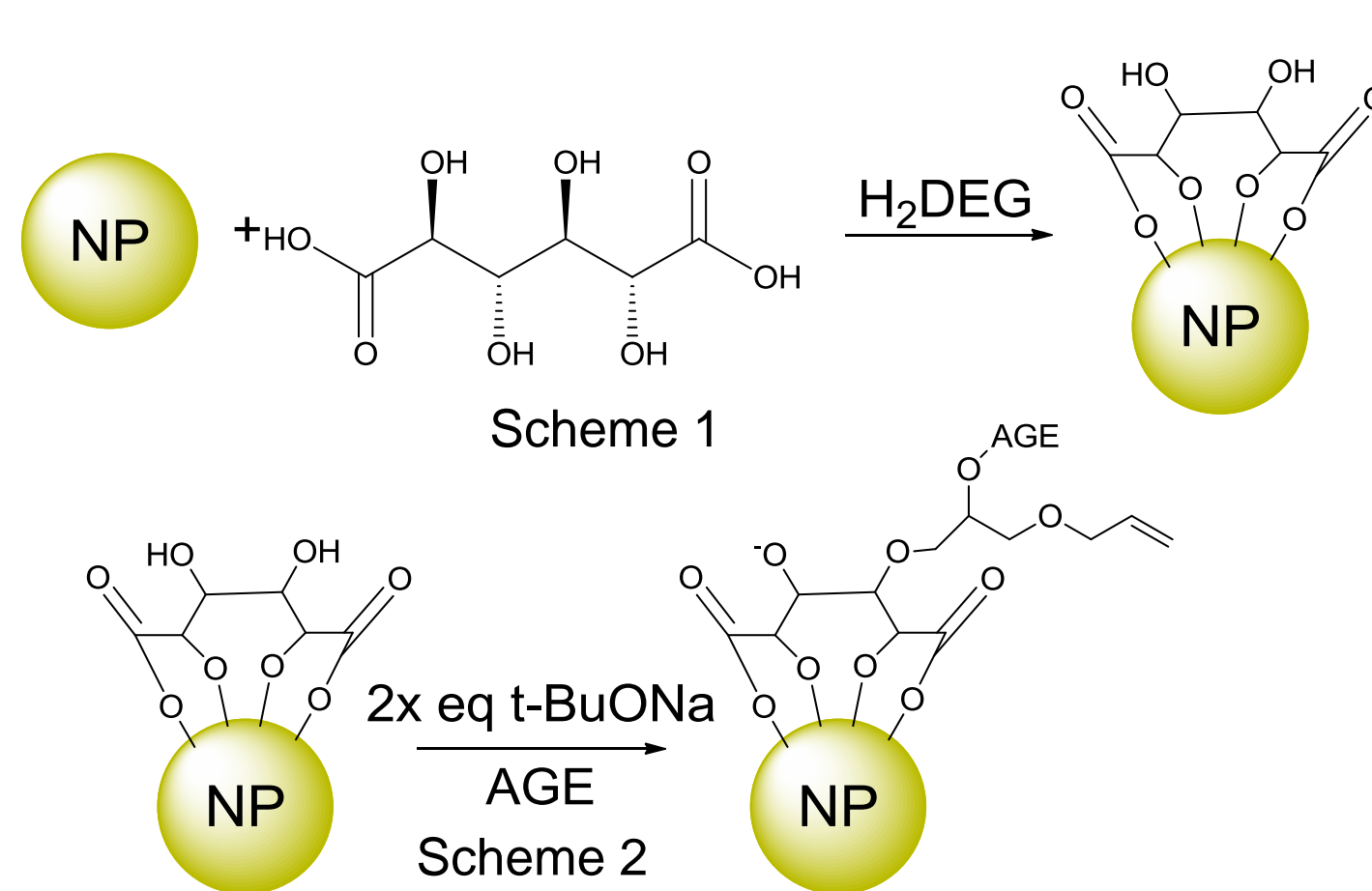
$$[Fe(HDEG)_2] + 2[Fe(HDEG)2Cl] + 2H_2O + 2OH^- \longrightarrow Fe_3O_4 + 5H_2DEG + 2Cl^-$$

In order to make the sample stable in the open air the obtained colloid was oxidized in the flow of dry oxygen:

$$4Fe_3O_4(H_2DEG) + O_2 \longrightarrow 4Fe_3O_4(HDEG) + 2H_2O$$

The SPION were covered with the ligand by treatment of their colloid solution with calculated amount of tartaric or galactaric acid dissolved in H2DEG (Scheme 1). The coating with the ligand was proven by IR and TGA
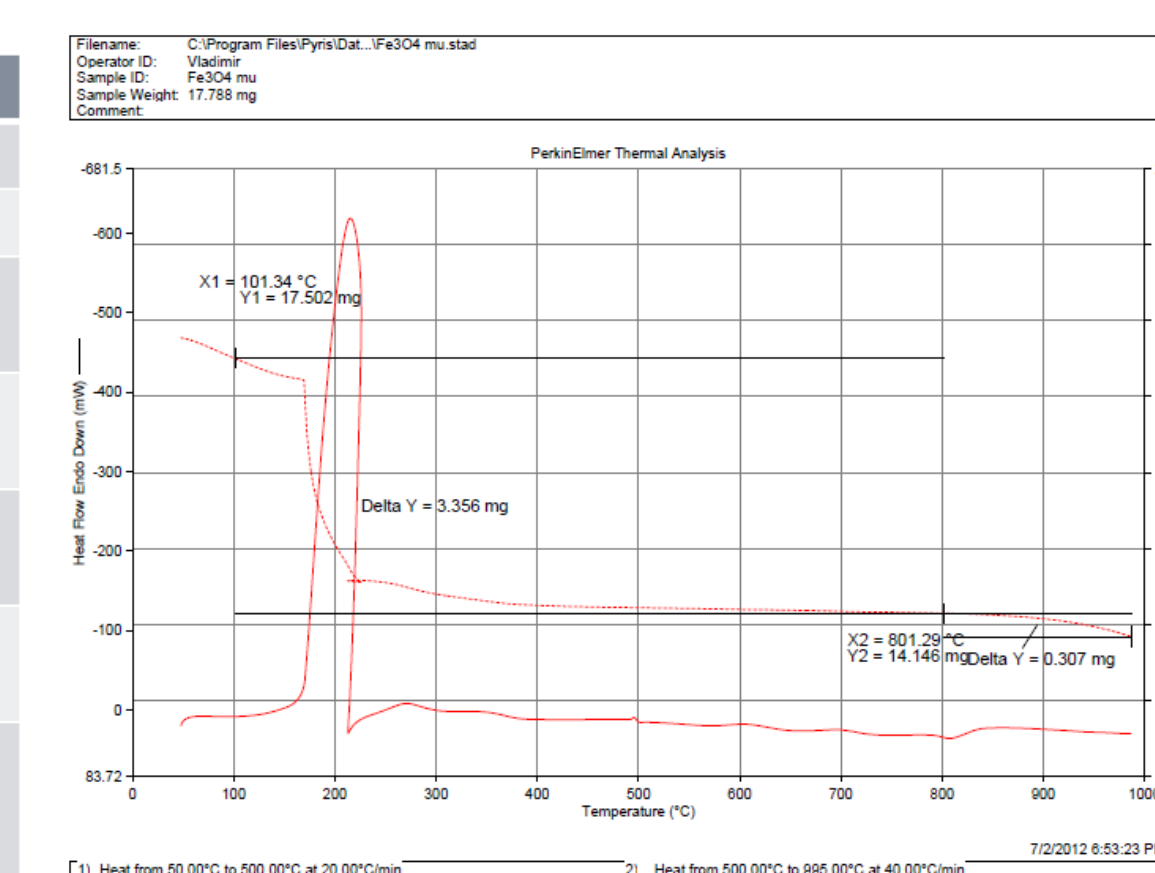

NP = Oxidized nanoparticles

TARTARIC ACID COMPLEX   GALACTARIC ACID COMPLEX

Based on its coordinating properties, it is believed that galactaric acid uses carboxyl and α-hydroxy groups to bind to the metal surface. This leaves the β-hydroxy groups available for functionalization (Scheme 1). Strong base such as sodium tert-butoxide (two equivalents) causes their deprotonation. For functionalization, allyl glycidyl ether (AGE) is used where its epoxide ring opens under basic condition (Scheme 2). The final product was shiny red/black crystals that were strongly magnetic.
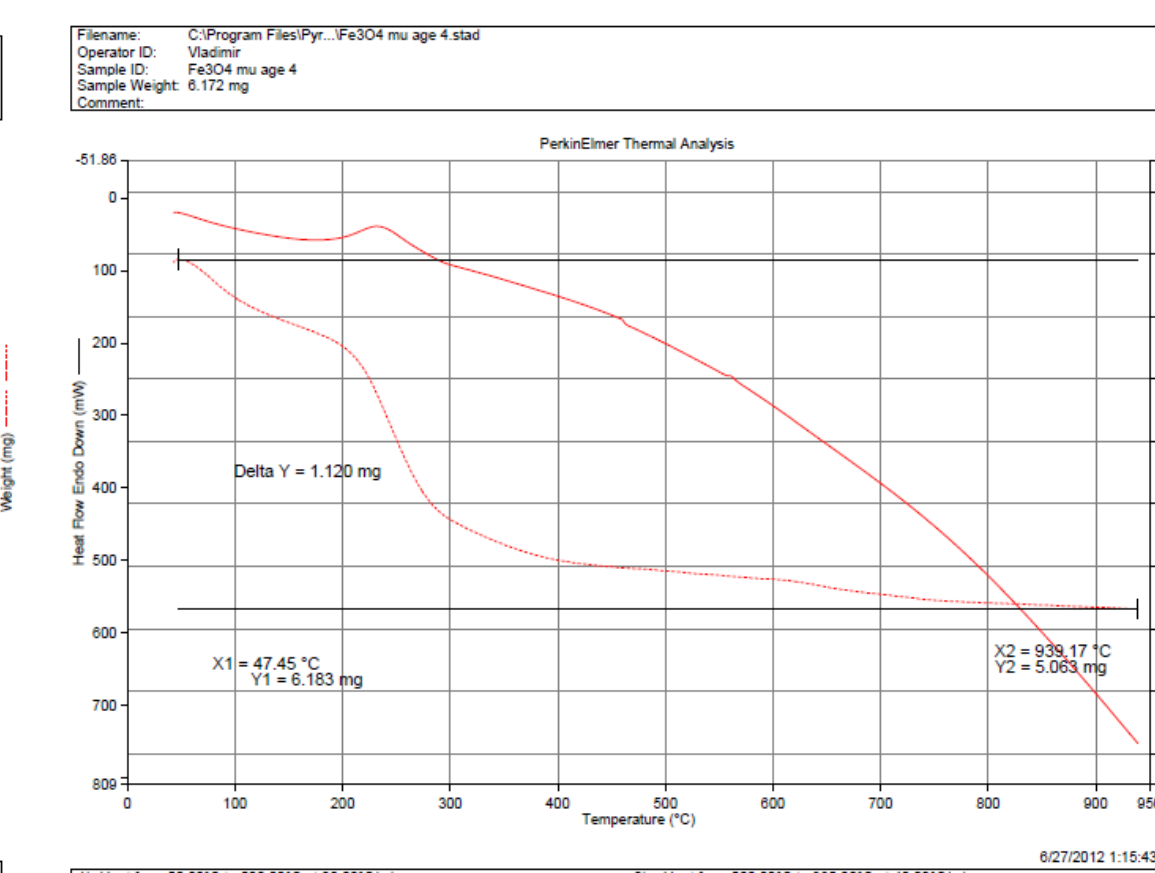

Scheme 1   Scheme 2   Spectrum 1   Table 1   Graph 1   Graph 2

Reaction	Size (nm)
Fe3O4+DEG	5.827
Fe3O4+DEG+ox	9.452
Fe3O4+DEG+ox+ Mu+250mmLAGE	8.401
Fe3O4+DEG+ox+ Mu+500mmLAGE	16.35
Fe3O4+DEG+Mu+ 750mmLAGE	11.57
Fe3O4+DEG+ox+ Mu+1mLAGE	8.533
Fe3O4+DEG+ox+ Mu+1mLAGE (final)	234.3

Table 1
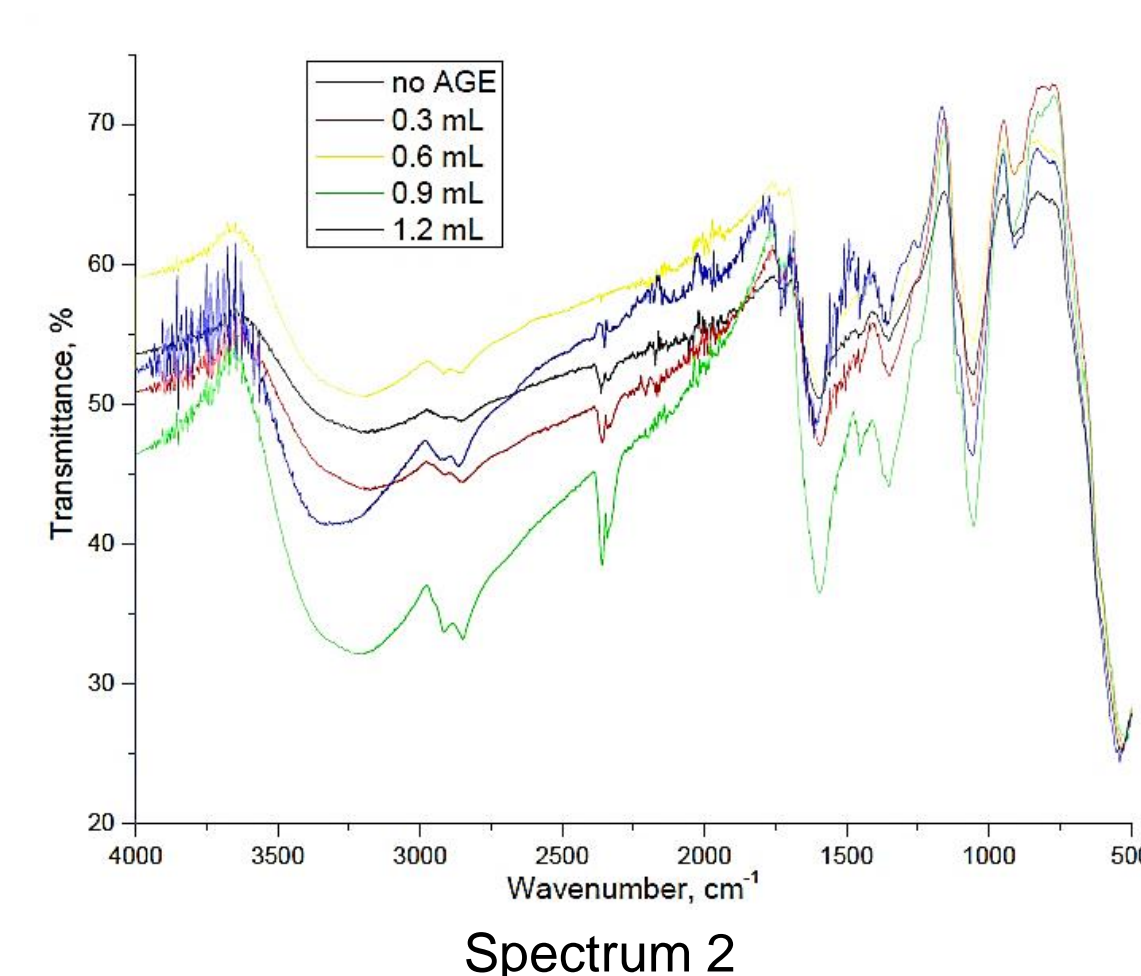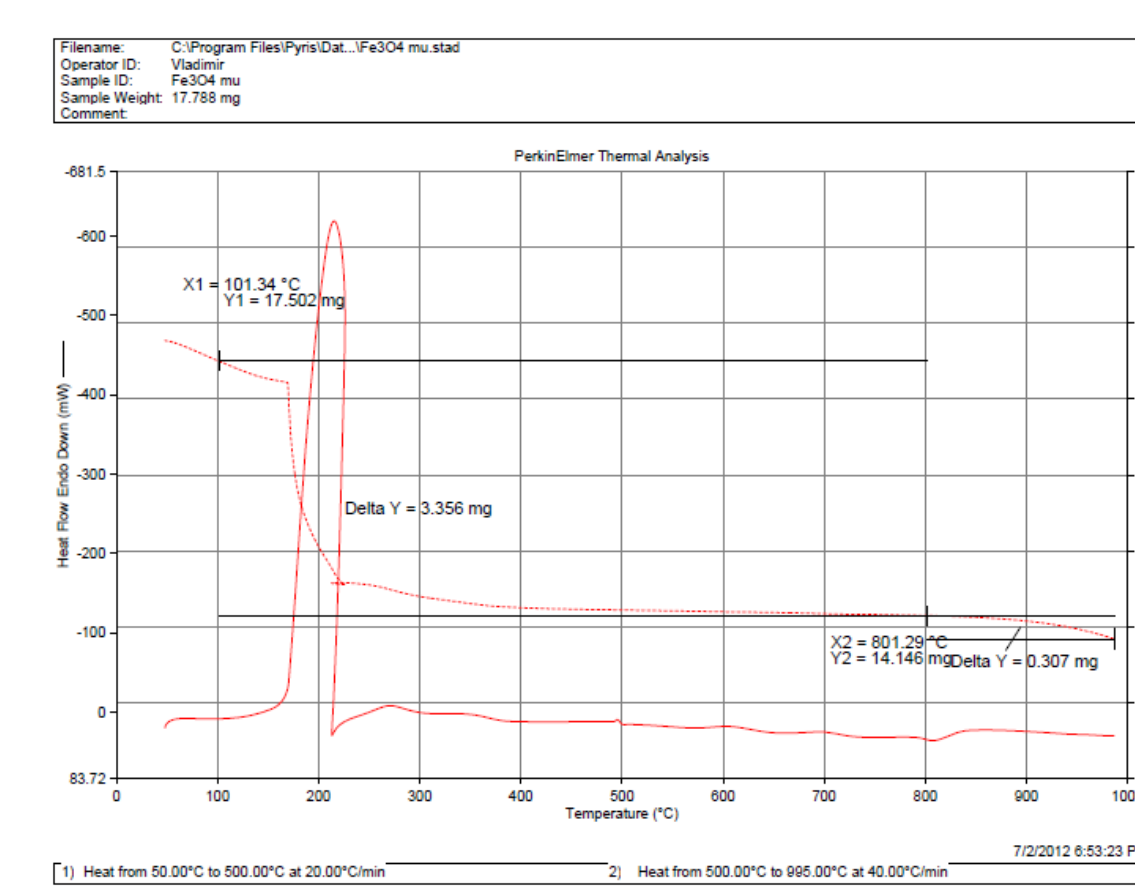
The IR spectrum above (Spectrum 1) shows increase in relative intensity of the C=C bond and O-H bond stretching frequency (~1500 cm-1 and ~3200cm-1). The ligand growth from AGE addition was also monitored by DLS measurements (Table 1). The results show the overall growth of the nanoparticle size, which constitutes to the idea of the ligand growth with addition of more AGE. The TGA results were obtained for the galactarate-covered nanoparticles (Graph 1), and for the galactarate-covered NP subsequently treated with AGE (Graph 2). Our calculations based on mass of the residue remaining after combustion, however, do not support the idea that AGE chain grows on galactarate. We are currently repeating the experiment to analyze it by TGA again.
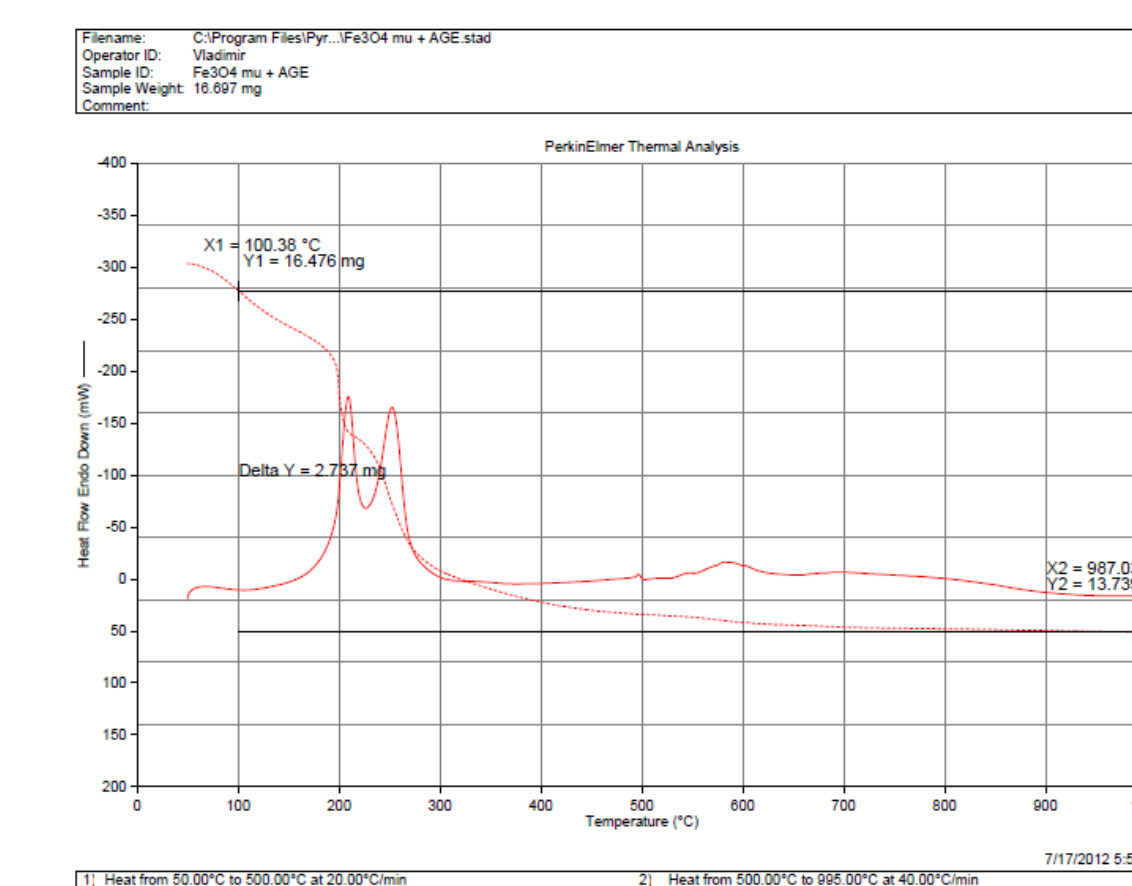
The following results are for the galactarate-covered SPION treated with 1 equivalent of t-BuONa. This synthesis procedure was the same as in the previous synthesis. The final product obtained after multiple washes with different solvents was magnetic . It was isolated as red/black crystals. IR analysis shows the peak intensity increase for alkyl groups as more AGE is added (Spectrum 2). The TGA analysis (Graph 3 and 4) also approves the growth of the coating on the nanoparticle. DLS measurements (Table 2) show evidence of growth, but there is an overall decrease in the NP size.
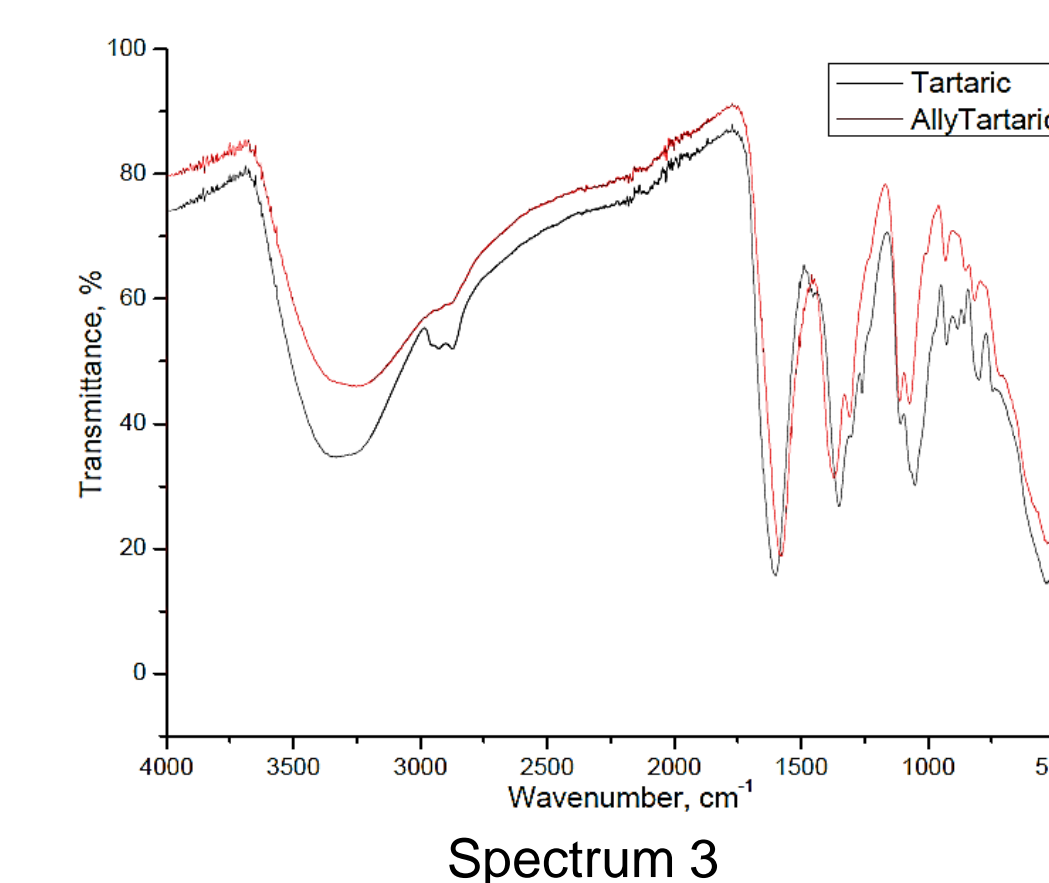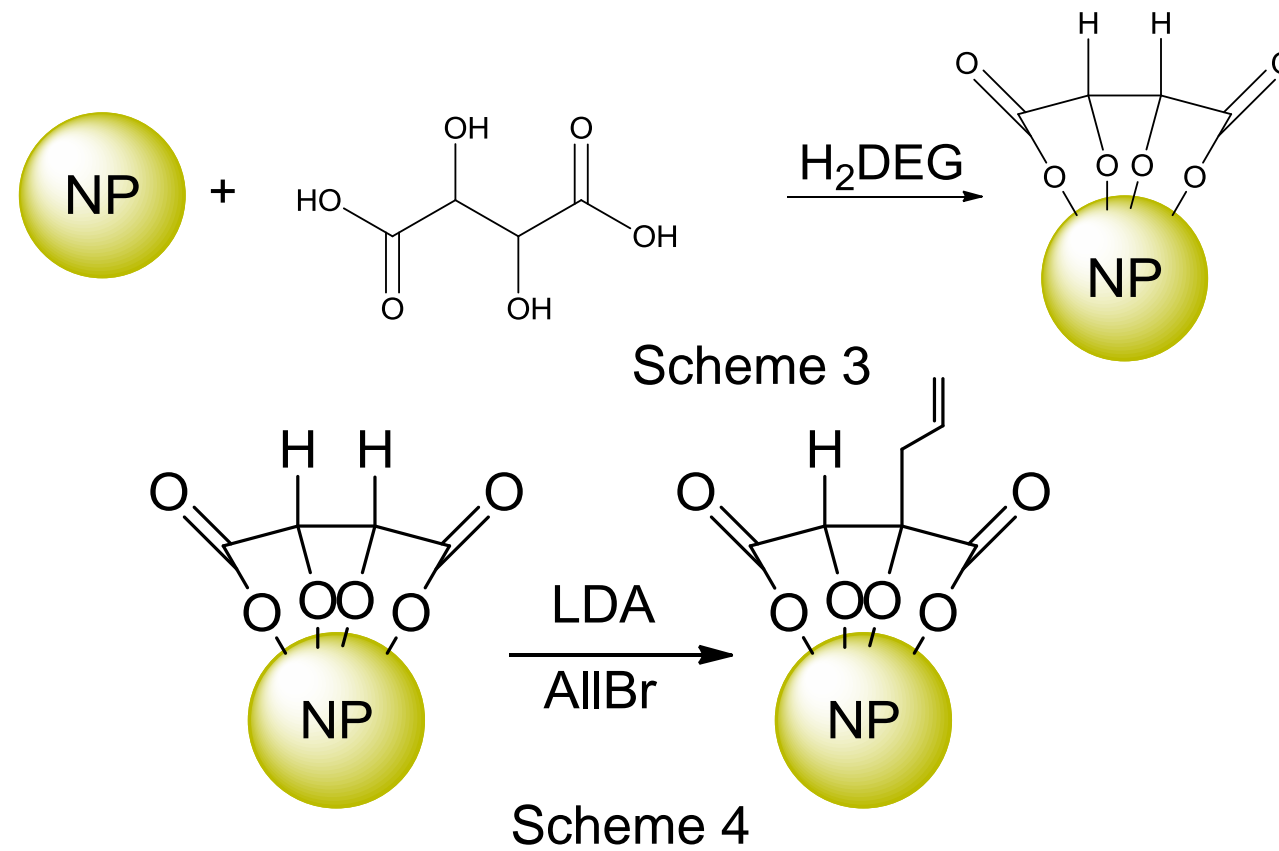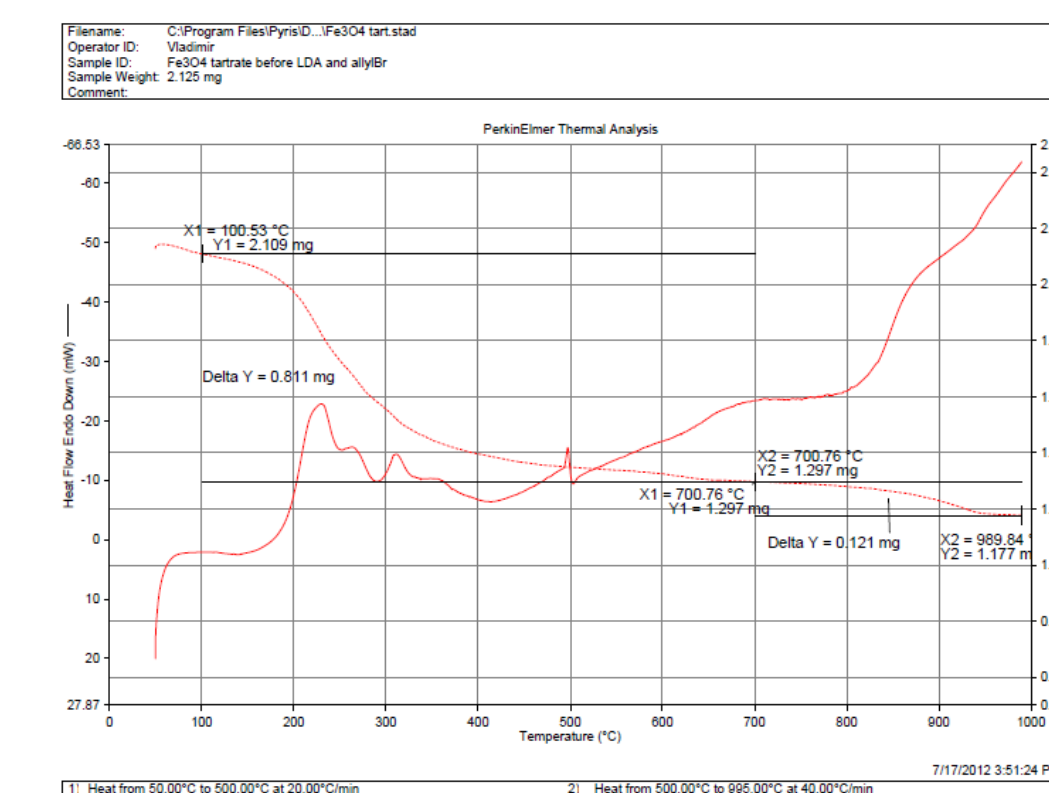

Spectrum 2   Graph 3   Graph 4   Table 2

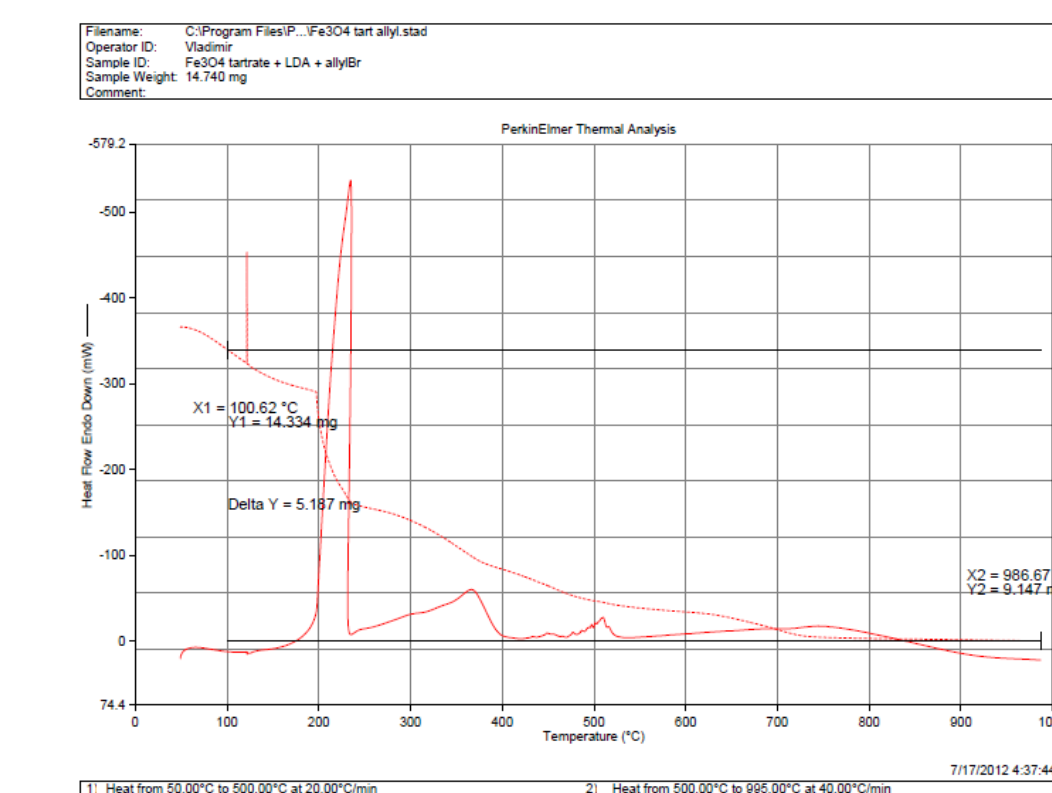| Reaction	Size (nm)
Fe3O4+DEG	5.827
Fe3O4+DEG+ox	9.452
Fe3O4+DEG+ox+ Mu+300mmLAGE	9.798
Fe3O4+DEG+ox+ Mu+600mmLAGE	7.608
Fe3O4+DEG+Mu+ 900mmLAGE	8.272
Fe3O4+DEG+ox+ Mu+1200mmLAGE	13.42
Fe3O4+DEG+ox+ Mu+1200mmLAGE (final)	6.172

Table 2

The tartaric acid was reacted with the nanoparticle in H2DEG using the same procedure as for galactaric acid (Scheme 3). The coordination is believed to be similar to the coordination of galactaric acid. Both, carboxyl and α-hydroxyl groups, bind to the metal surface. The coated NP were treated with LDA to deprotonate the methyne hydrogens followed by addition of allyl bromide (Scheme 4). The final product was isolated as brown crystals that were strongly attracted to magnet. The modification of the tartaric acid coated nanoparticle was checked with IR (Graph 4), DLS (Graph 5), and TGA (Graph 6). The IR shows that there was actual decrease in the intensity of C=C bonding and O-H stretching vibration frequency (~1500 cm^{-1} and ~3200cm^{-1}). The TGA graphs (Graph 5 and 6) and their calculations also support the decrease of the size. This could be caused by minimal amount of the pre-LDA sample. The DLS values also show a minor decrease in the size (Table 3).

Scheme 3   Scheme 4   Spectrum 3   Graph 5   Graph 6   Table 3

Reaction	Size (nm)
Fe3O4+Tar	8.845
Fe3O4+Tar+ LDA/AllBr	8.409

Table 3

Summary

5 nm iron oxide nanoparticles were obtained as colloids in diethylene glycol. In the post-synthesis step their surface was covered with galactaric and tartaric acids. Based on IR and DLS measurements it is likely that b-hydroxyl groups of the galactaric acid can be functionalized with allyl glycidyl ether after deprotonation with strong base.

Acknowledgements