# Supplementary Materials: The Effect of Synthesis Procedure on Hydrogen Peroxidase-Like Catalytic Activity of Iron Oxide Magnetic Particles

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## Experiment

The synthesized different magnetic nanoparticles (MNPs) were furthered characterised by using techniques as described.

An elemental analyzer SPECTRO XEPOS energy dispersive X-ray fluorescence (ED-XRF) spectrometer (SPECTRO Analytical Instruments GmbH, Kleve, Germany) equipped with a 10 mm$^2$ Si-Drift Detector with Peltier cooling and a 75 μm Be side window was employed. The instrument uses a Pd-target end window tube at a maximum power of 50 W and a maximum voltage of 50 kV. The spectral resolution of the instrument (FWHM) is < 170 eV for Mn K$\alpha$ (measured under input count rate 10,000 pulses). SPECTRO XEPOS was operated and data were evaluated through the software Spectro X-Lab Pro, Version 2.5. For light elements excitation (Mg-V, tube voltage 24.82 kV, tube current 1.00 mA, measurement duration 300 s, impulse rate ≤ 4717 cps, relative dead time ≤ 3.7%, peak time 2 μs, gain 12.5 eV channel$^{-1}$, zero peak rate 5000 cps) HOPG (Highly Oriented Pyrolytic Graphite) crystal target was used. For heavier elements determination Mo secondary target (Cr-Y, Hf-U, tube voltage 44.70 kV, tube current 0.55 mA, measurement duration 300 s, impulse rate ≤ 3120 cps, relative dead time ≤ 2.5%, peak time 2 μs, gain 25.0 eV channel$^{-1}$, zero peak rate 5000 cps) and $Al_2O_3$ polarisation target (Zr-Ce, tube voltage 49.16 kV, tube current 0.50 mA, measurement duration 300 s, impulse rate ≤ 1346 cps, relative dead time ≤ 1.3%, peak time 2 μs, gain 50.0 eV channel$^{-1}$, zero peak rate 5000 cps) were used. Synthesized MNPs were dried and measured directly in a sample cup (32 mm in diameter) on polypropylene X-Ray thin-film TF-240, 4 μm (FluXana, Bedburg-Hau, Germany) in vacuum using the so-called Turboquant method (fundamental parameters method). The MNPs were placed between two polypropylene foils and compressed to form a sample of sufficient area before each measurement. The measurement conditions were identical to those mentioned above.

FTIR-ATR spectra were collected using an INVENIO-R FTIR Spectrometer equipped with a single-reflection diamond ATR accessory—A225/Q Platinum ATR module (Bruker Optic Inc., Billerica, MA, USA). A small amount of different synthesized MNPs was put on crystal and a fixed load was applied to the sample to ensure full contact with the diamond ATR. A liquid sample (TEOS) was just simply dropped onto the crystal and analyzed directly. Before each measurement background spectra were collected. Spectra were recorded at 25 °C from 4000 to 400 cm$^{-1}$ at a resolution of 2 cm$^{-1}$. Each spectrum was acquired by merging 128 interferograms. Bruker OPUS software was used for IR spectra recording and JDXview v0.2 software and Microsoft® Office Excel 2013 was used for further spectra evaluation and presentation.

Raman spectra were recorded on dispersive inVia Reflex Raman microscope (Renishaw, UK) with integrated Leica microscope DM2700 (Leica, Germany) and controlled by Wire 5.2 software (Renishaw, UK). A diode laser with an excitation wavelength of 633 nm and 17 mW power was used. Spectra were collected over the approximate range of 100–3200 cm$^{-1}$ using microscope objective 50× and a power setting of 100%. Each spectrum was acquired by merging 32 scans.

The characterization by used MWCNT and synthesized different MNPs were further characterized by using transmission electron microscopy Tecnai F20 (FEI, Eindhoven, Netherlands).

Dynamic light scattering (DLS) measurements were carried out to find the size distribution of the synthesized particles. The Zetasizer-Nano ZS (Malvern, UK) was utilized which is equipped with a red laser.
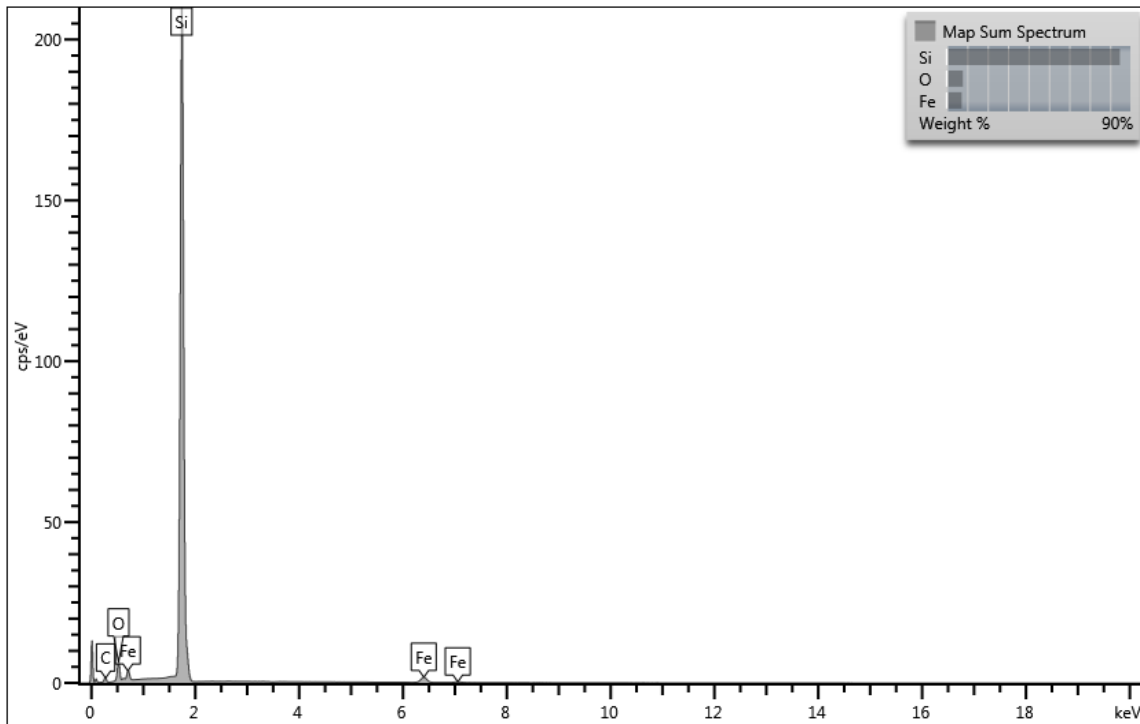
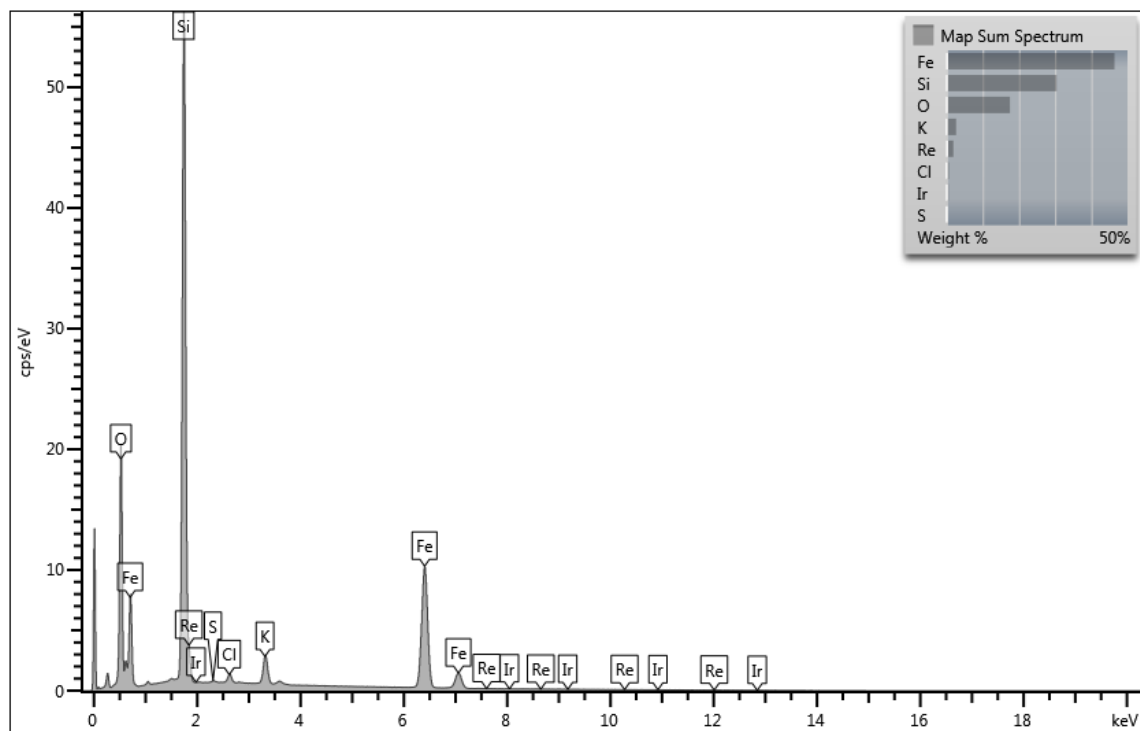**Figure S1.** EDX-SEM element composition for synthesized UN-MNPs.

**Figure S2** EDX-SEM element composition graph for the synthesized MNPs-CH.
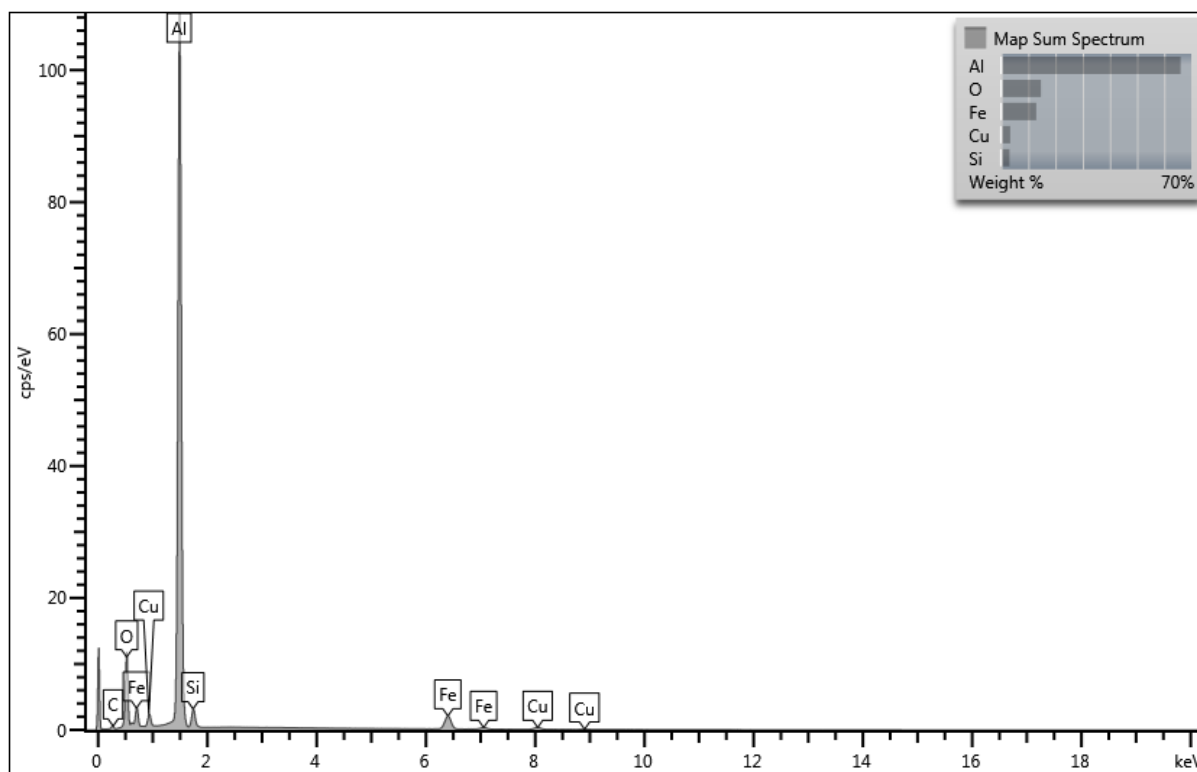
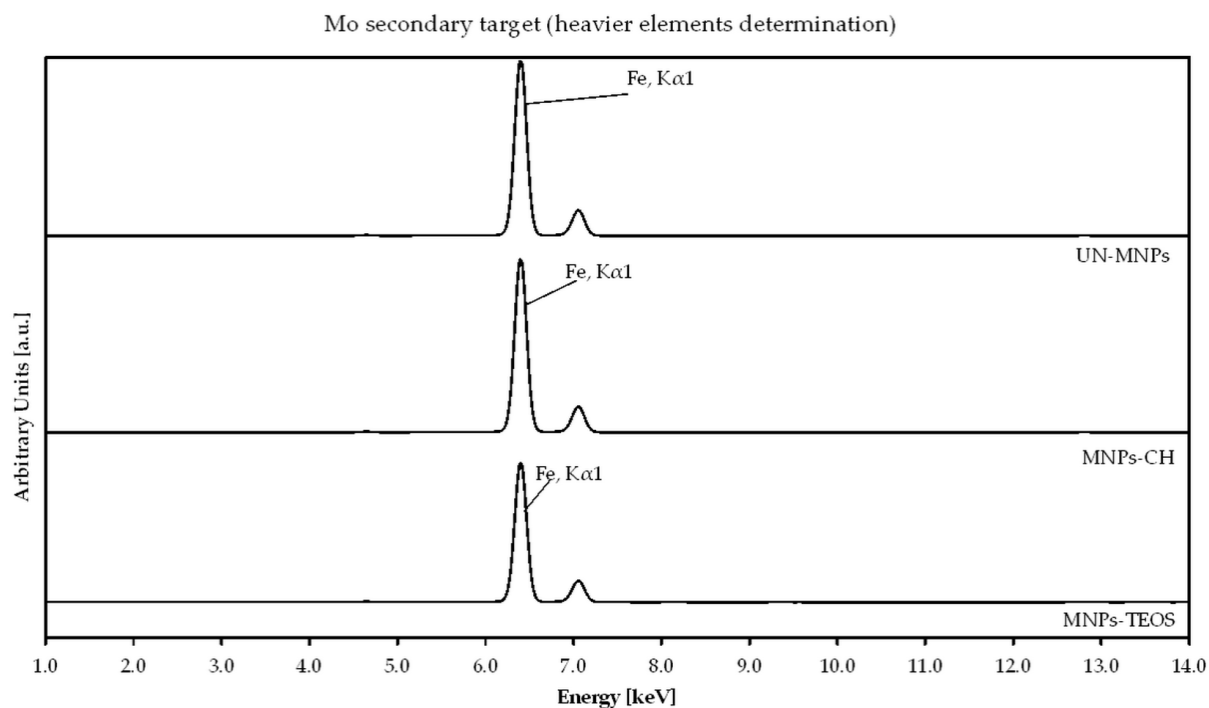**Figure S3** EDX-SEM element composition graph for the synthesized MNPs-TEOS



**Figure S4.** XRF spectra confirming elemental compositions of synthesized MNPs and characterization by using Molybendum oxygen secondary target specifically responsible for the presence of heavier elements determination.
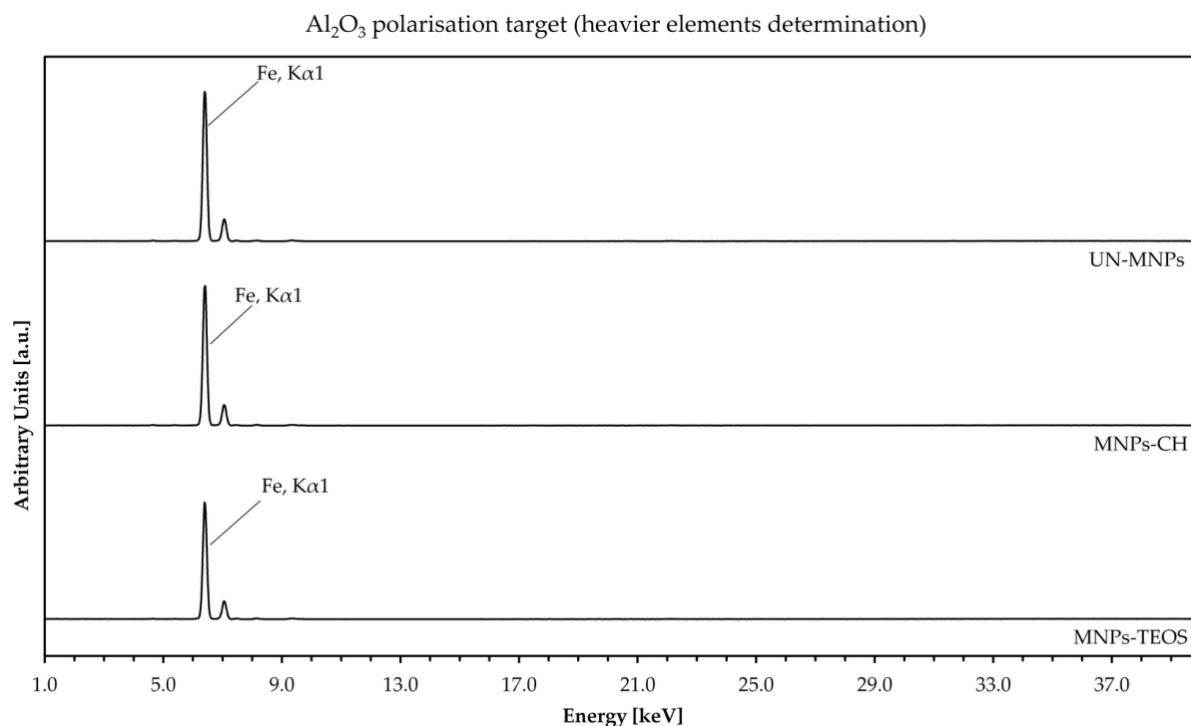
Al$_2$O$_3$ polarisation target (heavier elements determination)

**Figure S5.** XRF spectra confirming elemental compositions of synthesized MNPs and characterization by using Aluminium oxide secondary target specifically responsible for the detection of the higher element. All the MIONs exhibits the presence of Fe in different synthesized particles.
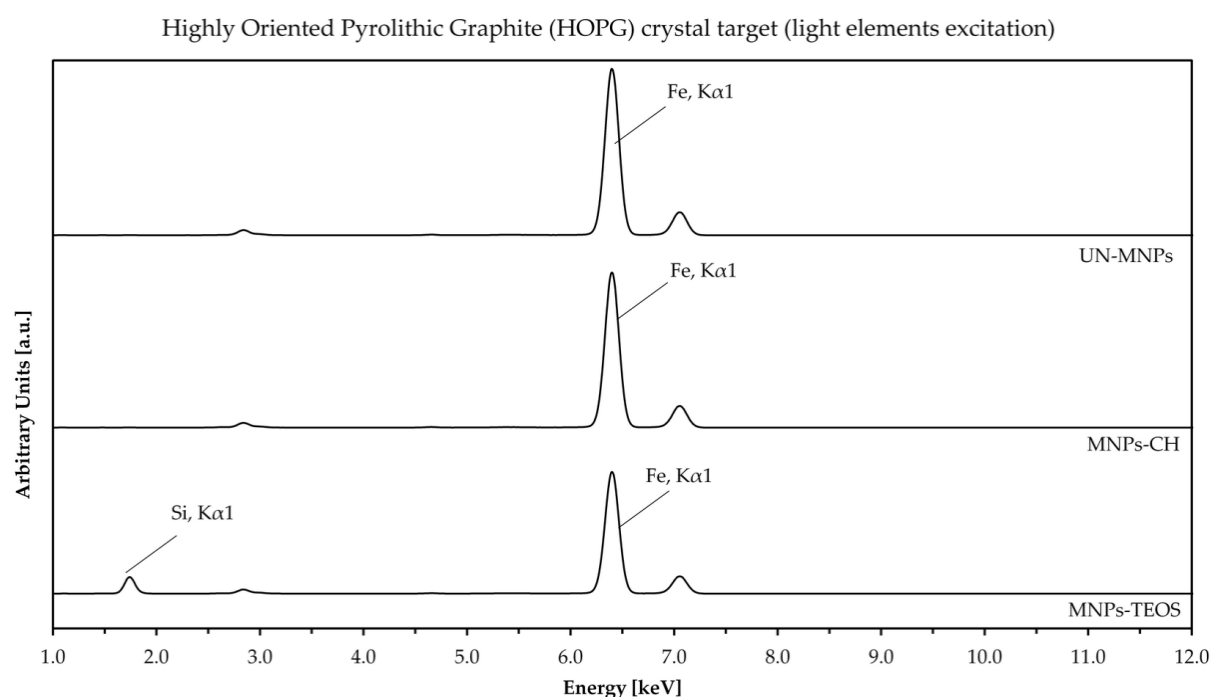
Highly Oriented Pyrolithic Graphite (HOPG) crystal target (light elements excitation)

**Figure S6.** XRF spectra confirming elemental compositions of synthesized MNPs and characterization by using HOPG mode specifically responsible for the detection of light element. All the MNPs exhibits the presence of Fe as well as the presence of light material Silicon (Si) in the MNPs-TEOS.
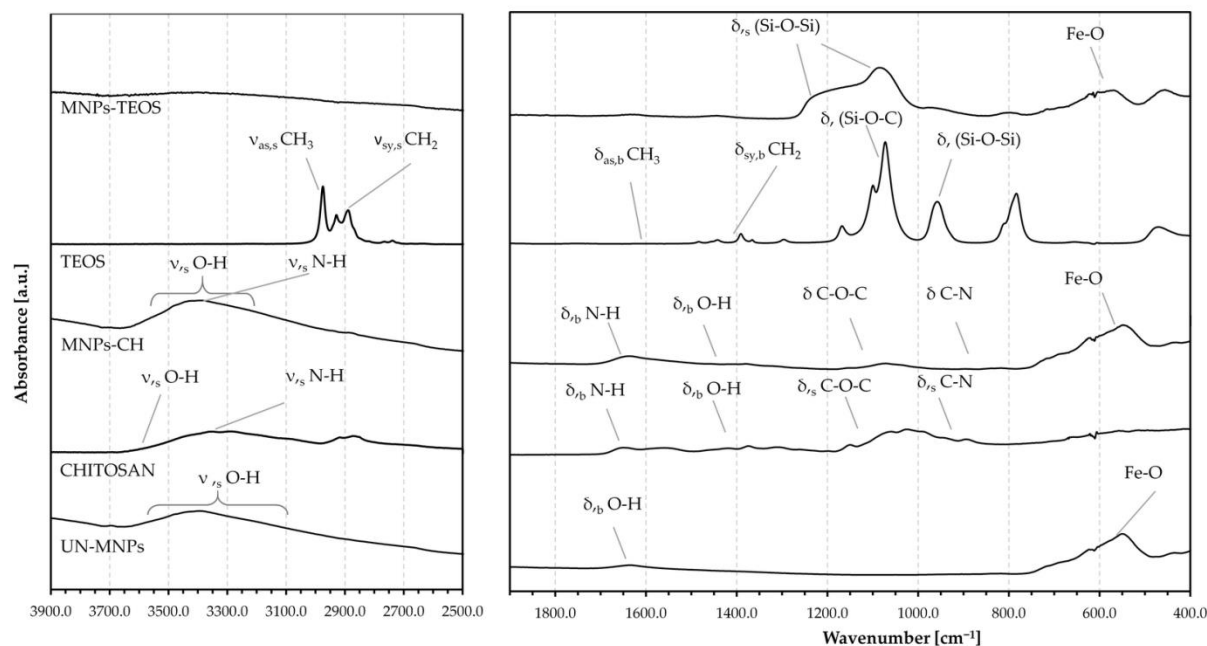
**Figure S7.** FTIR-ATR spectra of different MNPs and other composites like MNPs-TEOS, TEOS, MNPs-CH, CHITOSAN, UN-MNPs. Visible spectra of Fe-O and respective modification of MIONs and precursor elements were observed
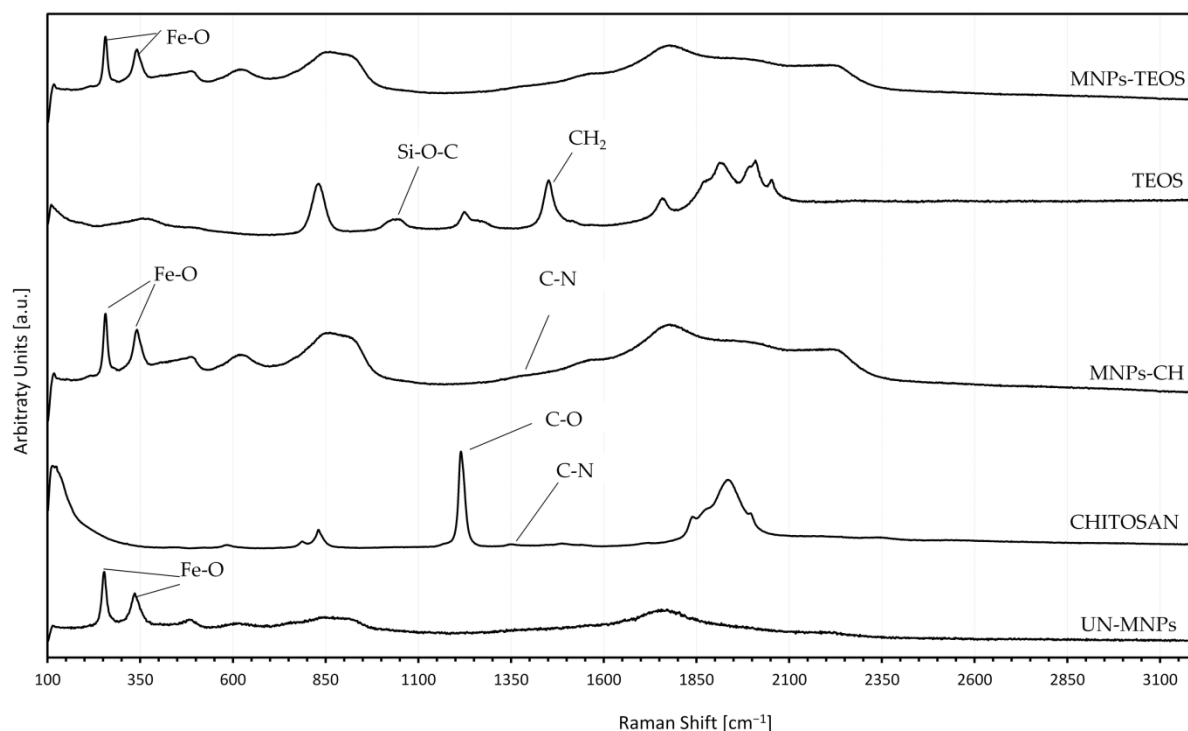


**Figure S8.** RAMAN spectra of different MNPs and other composites like MNPs-TEOS, TEOS, MNPs-CH, CHITOSAN, UN-MNPs. Visible strong spectra for lower Raman shift (252 and 338 cm$^{-1}$) values correspond to vibration modes of Fe-O bond of UN-MNPs. MNPs-CH, and MNPs-TEOS[1].

[1]Mishra, A.K., and Ramaprabhu, S., 2011. Nano magnetite decorated multiwalled carbon nanotubes: a robust nanomaterial for enhanced carbon dioxide adsorption. *Energy Environ. Sci.* **2011**, *4*, 889–895. DOI: 10.1039/C0EE00076K
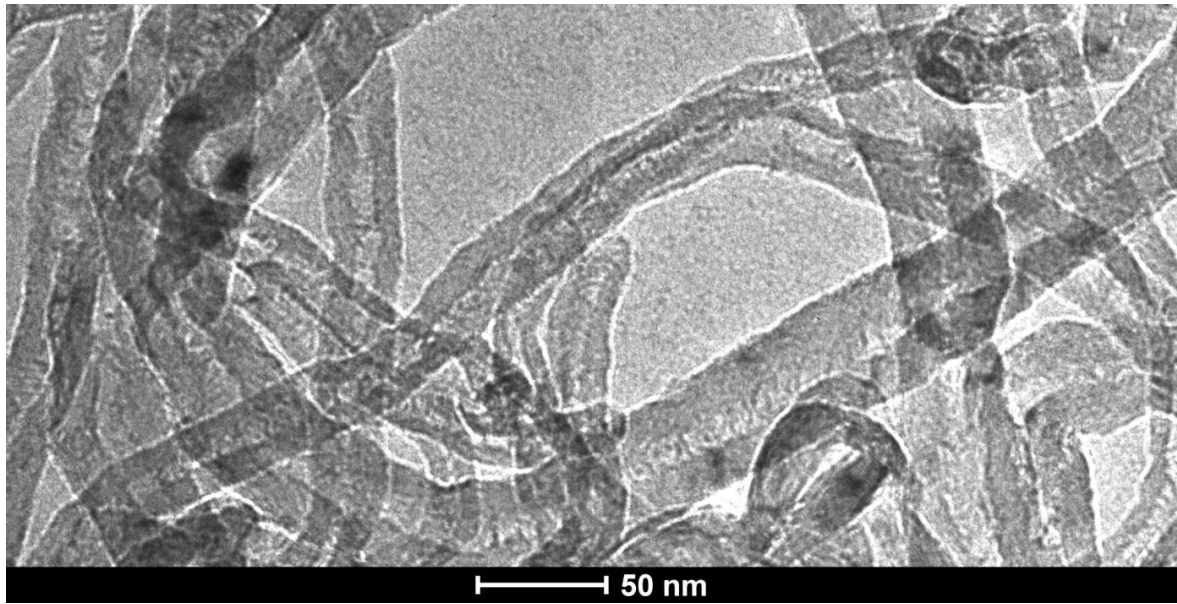
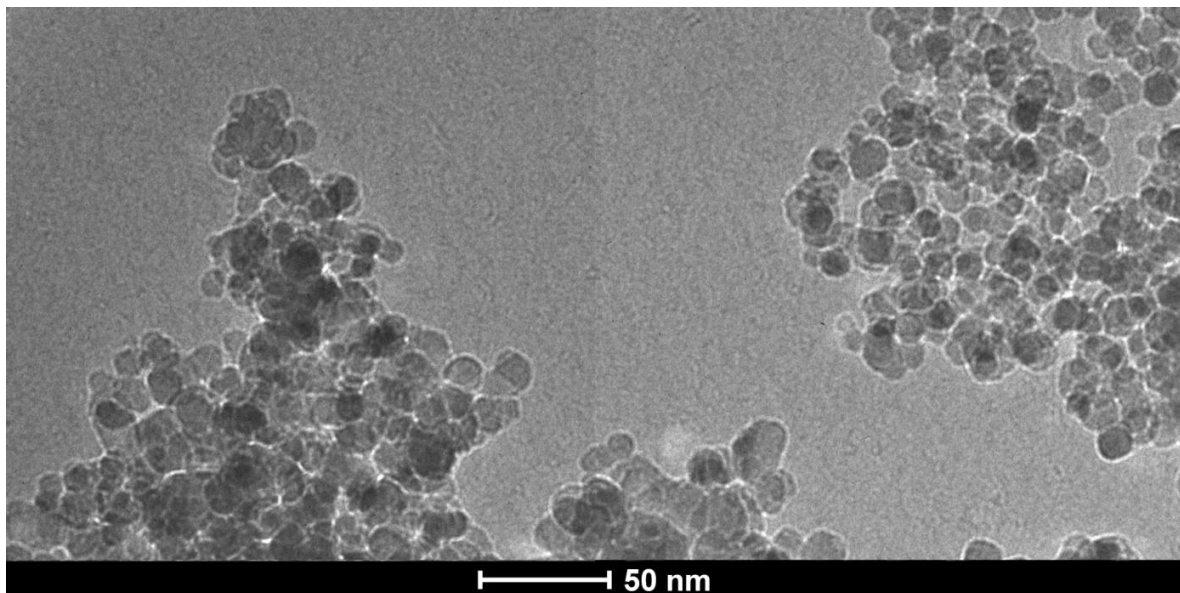**Figure S9:** Characterisation of the MWCNT by TEM



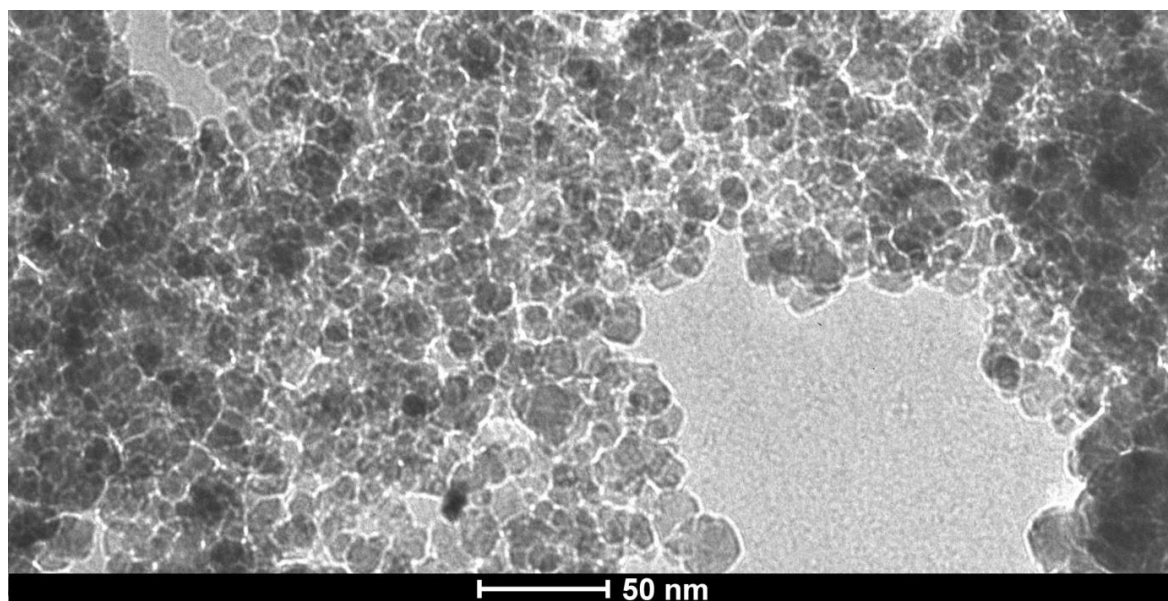**Figure S10:** Characterisation of the synthesized UN-MNP by TEM

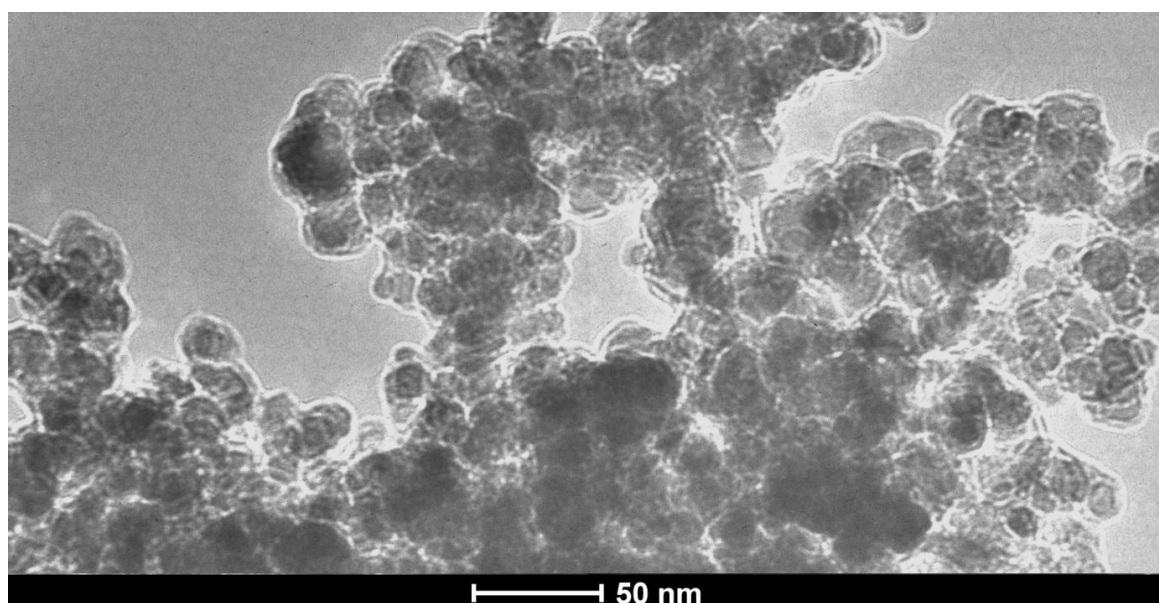**Figure S11:** Characterisation of the synthesized MNP-CH by TEM



**Figure S12:** Characterisation of the synthesized MNP-TEOS by TEM
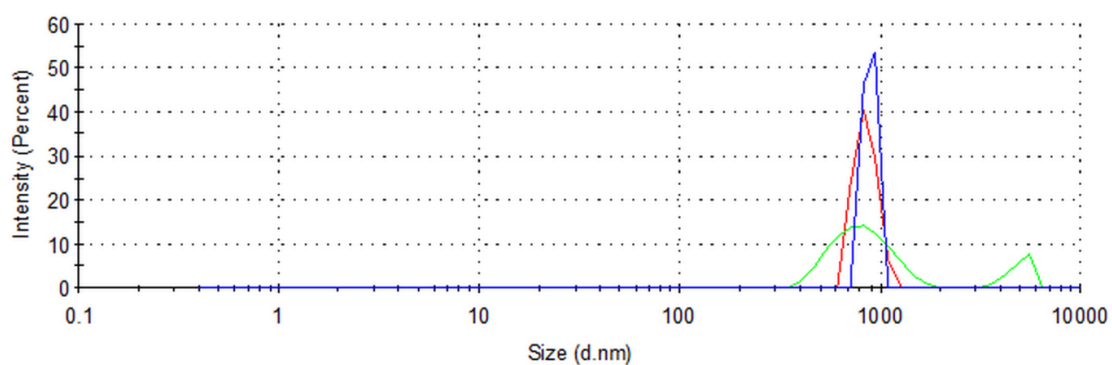


**Figure S13.** The size distribution of the synthesized MNPs by dynamic line scattering (DLS), **red**: UN-MNPS, **blue**: MNPs-CH, **green**: MNPs-TEOS.

**S14 The synthesized different MNPs were studied by using AAS are presented in Table (ST-1):-:**

<div align="center">ST-1</div>

| | |
|---|---|
| UN-MNP= | 648.6 mg/L |
| MNP-CH= | 569.8 mg/L |
| MNP-TEOS= | 4493 mg/L |

### S15. Determination of total Fe in NPs samples

The samples were digested with a mixture of acids using the microwave digestion unit Ultra Wave (Milestone). Quadrupole ICP-MS Agilent 7700x.was used for the detection of Fe. The calibration range for Fe was tested by the ICP-MS analysis of 8 working standards at concentration levels of 0; 50; 100; 250; 500; 1000; 2500; 5000 µg/L (Figure S16). For quality control of ICP-MS measurement, the QC sample (at a concentration level of 500 µg/L) was repeatedly analyzed at the beginning and the end measurement. Obtained results are summarized in ST 2.
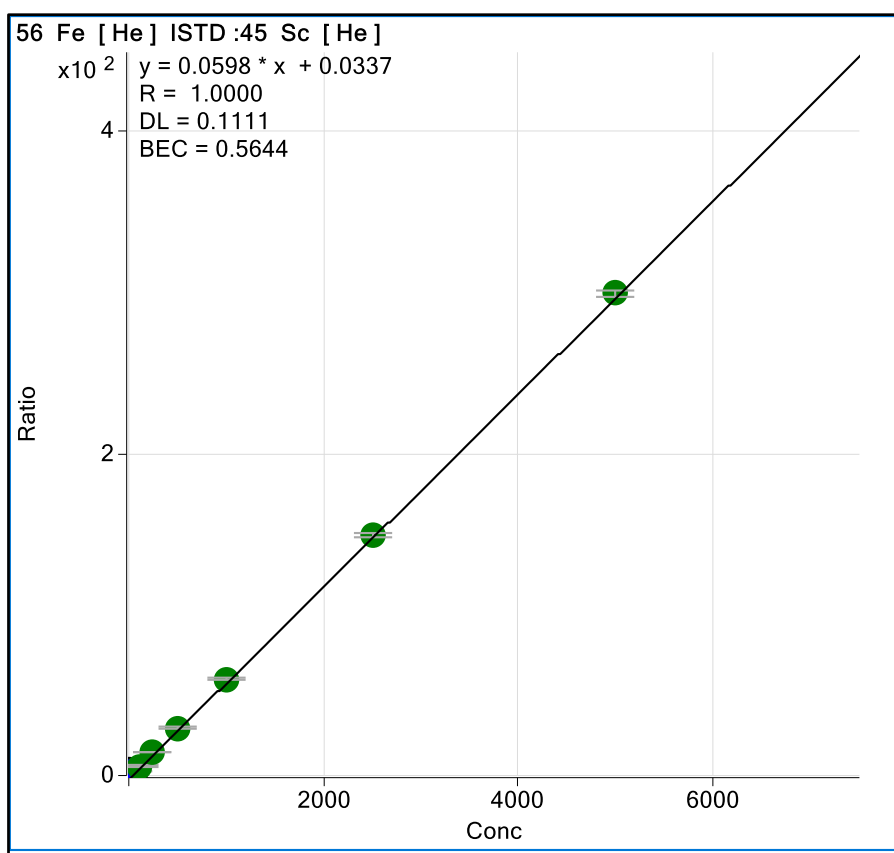


**Figure S16**. The calibration graph for iron.

**ST–2. Results of total Fe determination in NPs samples.**

| Sample | Fe concentration (mg/L) |
|---|---|
| UN-MNP | 705 |
| MNP-CH | 2482 |
| MNP-TEOS | 4922 |

QC sample:

Recovery of the QC sample (at a concentration level of 500 µg/L) was 100% (n = 2).