

Proceedings

# Synthesis of carboxylated magnetite nanoparticles covalent conjugates with folic acid antibody FA-1 for lateral flow immunoassay<sup>†</sup>

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**Abstract:** Magnetite nanoparticles (MNPs) are quite preferable material for different bioassays because of their quite low toxicity both for cells and for mammals and big variety of their surface functionalization approaches. We have synthesized MNPs via simple and convenient co-precipitation method with preliminary filtration of FeCl<sub>2</sub> and FeCl<sub>3</sub> solution, under argon atmosphere and non-magnetic stirring. MNPs were citrate-stabilized and then modified stage-by-stage with tetraethoxysilane (TEOS), (3-Aminopropyl)triethoxysilane (APTES) and acylated with succinic anhydride resulting in carboxylated MNPs. Carboxylated MNPs were covalently bounded with folic acid antibody (FA-1) via 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). MNP-EDC-FA-1 were passed through test-stripe with the line consisting folic acid-gelatin conjugate. The conjugation of MNP-EDC-FA-1 with folic acid was observed visually, and the magnetic signal distribution was scanned through the test-stripe with magnetic particle quantification technique (MPQ) developed earlier. Visually, the line with folic acid-gelatin conjugate on the test-stripe has turned dark, with color intensity strongly depending on MNP-EDC-FA-1 concentration. MPQ has shown that the great majority of MNP-EDC-FA-1 was bounded with acid-gelatin conjugate. MPQ technique allowed quantifying down to 5 ng of MNP-EDC-FA-1 in this experiment with MNPs synthesized, with strong peak at the acid-gelatin conjugate line.

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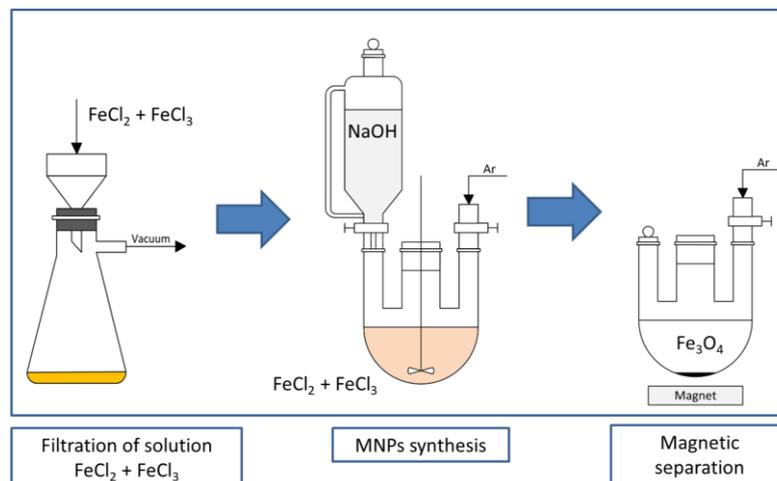
**Keywords:** Magnetite nanoparticles; folic acid; magnetic chemosensors; antibody conjugation; lateral flow assay

## 1. Introduction

Magnetite nanoparticles (MNPs) are quite preferable material for different bioassays[1] because of their quite low toxicity both for cells[2] and for mammals[3] and big variety of their surface functionalization approaches[4][5]. Superparamagnetic behavior provides an application of MNPs as magnetic labels both for cells[6] and for molecules[7]. Combination of MNPs optical properties at visible range with magnetic properties has been led into the fundament of magnetometric lateral flow immunoassay on test-stripes for rapid and sensitive qualitative and quantitative analysis of different biomolecules for which magnetic particle quantification (MPQ) technique has been de-

veloped earlier[8][9].

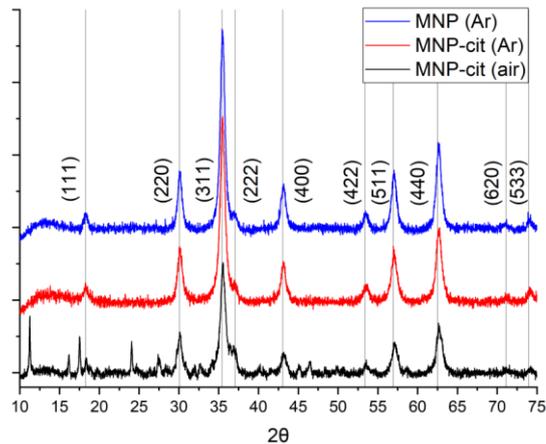
## 2. Methods



**Figure 1.** Synthesis of MNP via co-precipitation method.

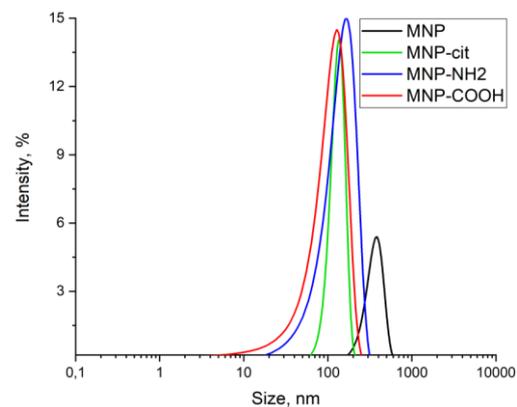
We have synthesized MNPs via simple and convenient co-precipitation method (Figure 1). Briefly,  $\text{FeCl}_2$  and  $\text{FeCl}_3$  were dissolved in degassed water in stoichiometric rate and filtered in order to exclude hydroxy- and oxychlorides that may act as undesirable and big crystallization centers due to their low solubility. The synthesis of MNPs was carried out by adding  $\text{NaOH}$  in degassed water solution to the mixture of  $\text{FeCl}_2$  and  $\text{FeCl}_3$  and stirred non-magnetically in order to minimize the formation of non-spherical structures, under argon atmosphere to prevent the MNPs from oxidation. MNPs were washed and stabilized with sodium citrate. Citrate-stabilized MNPs (MNP-cit) were modified stage-by-stage with tetraethoxysilane (TEOS), (3-Aminopropyl)triethoxysilane (APTES) resulting in aminated MNPs (MNP- $\text{NH}_2$ ) and acylated with succinic anhydride resulting in carboxylated MNPs (MNP- $\text{COOH}$ ). Carboxylated MNPs were covalently bounded with folic acid antibody (FA-1) via 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) by incubation of carboxylated MNPs with EDC, washing them and consequent incubation with FA-1. MNP-EDC-FA-1 suspension was mixed with albumin buffer solution in order to block the unreacted EDC and to simulate blood serum media. Porous test-strips with folic acid-gelatin conjugates were put into MNP-EDC-FA-1 suspension and left for 15 min. Then, the test-strips were scanned with MPQ-scanner in order to measure magnetic signal distribution along the test-strips.

## 3. Results and discussion



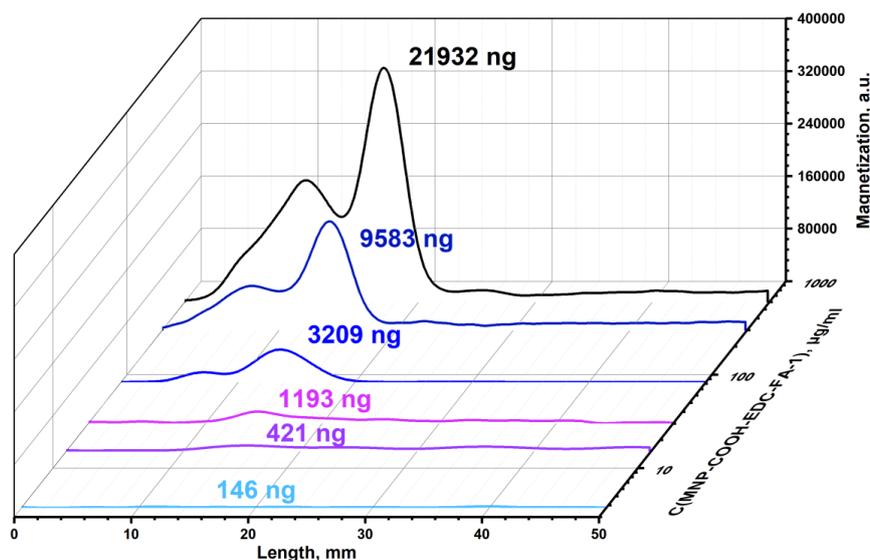
**Figure 1.** XRD of MNPs synthesized: MNP (Ar) – pristine MNPs synthesized in Ar atmosphere, MNP-cit (Ar) – citrate-stabilized MNPs synthesized in Ar atmosphere, MNP-cit (air) – citrate-stabilized MNPs synthesized in air atmosphere.

The suspension of synthesized nanoparticles was of dark black color. The magnetic response was strong. XRD (Figure 1) has shown that synthesized nanoparticles consisted of pure magnetite, with crystallite size about 12 nm, according to the Scherrer equation. Sodium citrate dihydrate peaks are observed on the diffractogram of MNP-cit synthesized in air atmosphere that may indicate its excess on the MNP surface due to the insufficient washing of MNPs. Peaks corresponding to the magnetite are better pronounced when MNPs are synthesized in Ar atmosphere.



**Figure 2.** Hydrodynamic radii of particles in MNP suspensions: MNP – pristine MNPs, MNP-cit – citrate-stabilized MNPs, MNP-NH<sub>2</sub> – aminated MNPs, MNP-COOH – carboxylated MNPs.

Hydrodynamic radii (Figure 2) of pristine MNPs agglomerates were about 380 nm and decreased to 136 nm after modification with sodium citrate. Carboxylation caused no sufficient resultant change in MNPs agglomerates size.  $\zeta$ -potential has changed from neutral to  $-48 \pm 7$  mV after modification with sodium citrate that among with size decrease indicate stabilization of the suspension; turned to  $+25 \pm 7$  mV after amination with APTES and to  $-25 \pm 10$  mV after acylation indicating that carboxylation was successful.



**Figure 3.** Magnetograms of test stripes with MNP-COOH-EDC-FA-1 against FA-gelatin conjugates.

The conjugation of MNP-EDC-FA-1 with folic acid was observed visually, and the magnetic signal was scanned through the test-stripe by MPQ-magnetometer (Figure 3). Visually, the line with folic acid-gelatin conjugate on the test-stripe has turned dark, with color intensity strongly depending on MNP-EDC-FA-1 concentration. MPQ has shown that the great majority of MNP-EDC-FA-1 was bounded with acid-gelatin conjugate. MPQ technique allowed quantifying down to 5 ng of MNP-EDC-FA-1 in this experiment with MNPs synthesized, with strong peak at the acid-gelatin conjugate line.

#### 4. Conclusions

Synthesis of MNPs in Ar atmosphere allows obtaining MNPs without any other phases but magnetite. MNPs may serve both as a platform for immobilization of antibodies and a magnetic label for them. MPQ may be quite a precise tool for detection and quantitative analysis of MNPs, so the magnetometric lateral flow immunoassay is possible with use of them, for example, to quantify the interaction antibody-antigen.

**Author Contributions:** conceptualization, Artem V. Sinolits, Sergey L. Znoyko; investigation, Maria O. Zolotova; methodology, Artem V. Sinolits, Sergey L. Znoyko; project administration, Alexey V. Orlov, Petr I. Nikitin; resources, Petr I. Nikitin; supervision, Artem V. Sinolits; writing, Maria O. Zolotova, Artem V. Sinolits; writing – review & editing, Maria O. Zolotova, Artem V. Sinolits. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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