Protocol	#1.1
Title	**BOMB magnetic core nanoparticles synthesis**
Keywords	magnetic nanoparticles, Fe_3O_4, magnetic separation
Authors	Oberacker P*, Stepper P*, Bond DM*, Höhn S, Focken J, Meyer V, Schelle L, Sugrue VJ, Jeunen GJ, Moser T, Hore SR, von Meyenn F, Hipp K, Hore TA# and Jurkowski TP#
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Summary

We provide a simple co-precipitation protocol for preparation of magnetic nanoparticles (MNP). It relies on forming Fe_3O_4 magnetic particles during co-precipitation of $FeCl_2$ and $FeCl_3$ in alkali solutions [1]. The formed MNPs have a diameter of ~5-20 nm. Oxygen is known to interfere with the synthesis, therefore the reactions can be either flushed with N_2 for at least 30 minutes, performed immersed in argon or degassed and preheated to 80 °C right before the synthesis.

Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
Iron (II) chloride 4-hydrate puriss ≥99% ($FeCl_2 \cdot 4\ H_2O$)	Honeywell/Fluka	44939	198.81	 Danger	H: 290-302-318 P: 280, 301+330+331, 305+351+338, 308+313
Iron (III) chloride, anhydrous, pure ($FeCl_3$)	Roth Chemicals	5192.3	162.2	 Danger	H: 290-302-315-317-318
Sodium hydroxide, ≥99% (NaOH)	Roth Chemicals	6771.1	40.0	 Danger	H: 290-314
37% HCl fuming	Roth Chemicals	4625.1	36.46	 Danger	H: 290-314-335
Ammonia solution (NH_4OH, 25%)	EMD Millipore	1.05432	n.a.	 Corrosive Danger	H:290+314+335+400 P:273+280+301+330+331+305+351+338+308+310

Please consult appropriate MSDS information before working with these chemicals! Use lab coat, gloves and eye protection at all times! The chemicals are available from other providers as well. No preference is given to the indicated vendors.

Buffers and solutions

2 M NaOH (prepare 0.5 L) –can be stored at RT for at least 12 months

0.1 M HCl (prepare 0.5 L) – can be stored at RT for at least 12 months

Fe2/3 solution (prepare freshly 60 ml)

0.333 M $FeCl_3$ (3.24 g $FeCl_3$)
0.167 M $FeCl_2$ (2 g $FeCl_2 \cdot 4\ H_2O$)

Dissolve with magnetic stirring in 60 ml of 0.1 M HCl solution

Equipment

Fume hood

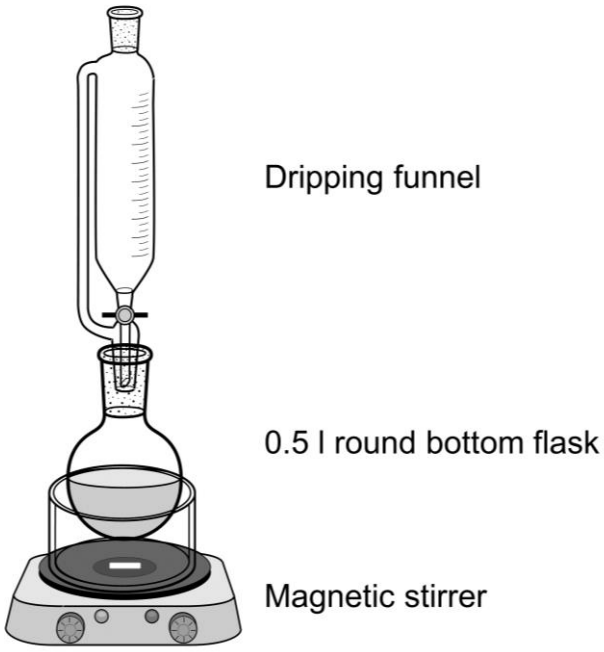
Magnetic stirrer with heating (e.g. IKAMAG REO)

Strong neodymium permanent magnet (e.g. Neodymium disc magnet 80x10 mm grade N45 adhesive force ~300 kg – be extremely careful when using such strong magnets!)

Sterile plastic 0.5 L bottle (e.g. Corning® Costar®, Nunc® T175 uncoated PS flask)

Protective clothing (lab coat, eye protection, hand gloves)

Experimental setup



The **Fe2/3 solution** is added drop wise from a dripping funnel to a collection flask containing the 2 M NaOH solution that is magnetically stirred. Alternatively, as the collection flask one can use a standard 0.5 L bottle and add the Fe2/3 solution drop wise manually with a pipette.

BOMB Synthesis of MNPs

Step	Task	Time	☑
⚠	*All procedures can be performed under inert argon atmosphere (alternatively purging the system with high purity N_2 for 30 minutes should be sufficient. We generally perform the reaction at atmospheric oxygen levels yet degas and heat up the solutions before use)*		
1	Assemble the reaction bottle and the dripping funnel and place on a magnetic stirrer, add 100 ml of degassed **2 M NaOH** to the collection flask on the bottom and the **Fe2/3 solution** to the dripping funnel. Heat up the flask containing the 2 M NaOH solution to 80-85 °C.	10 min	☐
2	Add drop wise the 60 ml **Fe2/3 solution** into 100 ml of preheated (80-85 °C) **2 M NaOH** solution under vigorous stirring (>400 rpm)	20 min	☐
👁	*Black precipitate of Fe_3O_4 ($FeO{\cdot}Fe_2O_3$) is formed*		
3	After the complete **Fe2/3 solution** was added, add 10 ml of 35% ammoniac to the reaction mixture and stir for a further 30 minutes and then cool to RT	30 min	☐
4	Transfer the solution to a sterile plastic container and magnetically pellet the black precipitate (MNPs), discard the supernatant	5 min	☐
5	Resuspend in 200 ml of ddH_2O, pellet magnetically and discard supernatant. Repeat for a total of 5 washes (or until the pH of the wash reaches ~7)	1 h	☐☐ ☐☐
6	After the last wash resuspend the MNPs in 30-40 ml of ddH_2O and transfer to a fresh 50 ml tube		☐
End	To measure the synthesis yield, magnetically pellet the MNPs, remove the water and weigh the product wet-mass, alternatively flash freeze the pelleted MNPs in liquid nitrogen and lyophilise and measure the dry mass	**~2 h** (~1 h hands-on)	
🗄	*Lyophilised MNPs under N_2 or argon atmosphere can be stored in a closed container at RT for a longer time, the wet MNPs slowly oxidise over time, therefore we functionalise them further within a week*		

Modifications

Different protocols provide MNPs with different properties. This protocol was selected and optimized for robustness and because it yields relatively small sized magnetic particles (~5-20 nm in diameter as judged by the TEM images). However, a plethora of other methods can be employed for the synthesis of suitable MNPs as reviewed in [2].

Troubleshooting

Problem	Solution
No precipitate formed	Use freshly made alkali solutions, correct iron salts at indicated concentrations; Preheat the alkali solution to at least 70 °C or degas before adding the Fe2/3 solution
Precipitate not magnetic	Make sure you are using freshly made Fe2/3 solution and that you have used correct iron salts for the mixture

Exemplary Results

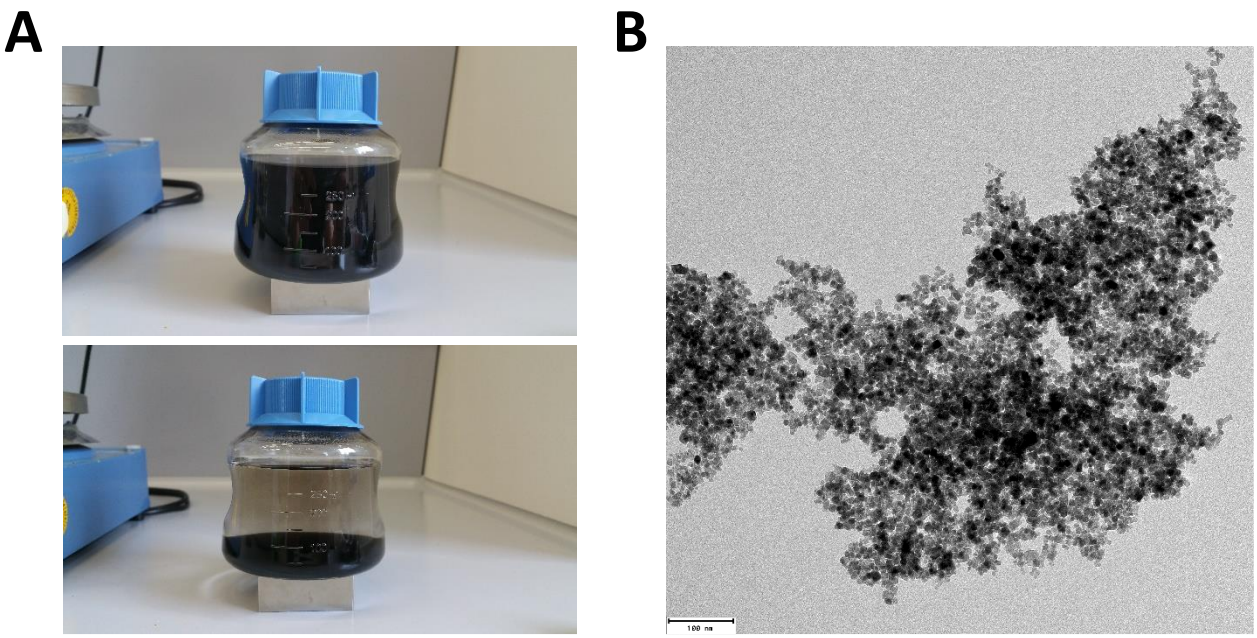


Fig 1: Synthetized Fe_3O_4 MNPs. *(A) Exemplary picture of the MNPs before and after magnetic decantation. (B) TEM picture of the synthetized MNPs.*

References

1. Choi J, Kim JC, Lee YB, Kim IS, Park YK, Hur NH. Fabrication of silica-coated magnetic nanoparticles with highly photoluminescent lanthanide probes. Chem Commun. 2007; 1644–1646. doi:10.1039/b617608a

2. Wu W, Wu Z, Yu T, Jiang C, Kim WS. Recent progress on magnetic iron oxide nanoparticles: synthesis, surface functional strategies and biomedical applications. Sci Technol Adv Mater. 2015;16: 023501. doi:10.1088/1468-6996/16/2/023501