Evaluation of the effect of magnetic nanoparticles as additives on qPCR

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Introduction

Nowadays, magnetic nanoparticles (MNPs) play an important role in different technological areas such as electronics, energy and biomedicine [1]. Magnetic iron oxide NPs have interesting advantages such as controllable sizes, and the possibility of being manipulated by an external magnetic gradient which make their use for bioanalytical applications attractive. The development of faster, more sensitive and cheaper analytical methods can benefit from the use of these nanomaterials, however their use for PCR based applications should be investigated in detail to evaluate the effect of such NPs on the reaction efficiency, and therefore on the reliability of the results.

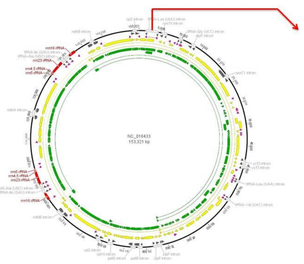
On the other side, the use of NPs to improve and/or better control both end-point and quantitative PCR (qPCR) has shown an increased interest by the scientific community [2]. Different nanomaterials, such as carbon nanotubes, quantum dots, and gold, have been combined with PCR with interesting results [3]. In some cases, they have demonstrated to improve PCR specificity and efficiency. However, other groups have found negative effects, such as inhibition of PCR reaction, fluorescent quenching on SYBR green I based detection systems, or DNA duplex destabilization[4]. Therefore a case by case evaluation of the effect of different NPs on PCR must be performed.

Purpose

The objective of this study was to evaluate the effect of 20-nm magnetite, Fe_3O_4, MNPs functionalized with oleate ligand (OL) and stabilized in aqueous solution by tetramethylammonium hydroxide (TMAOH) surfactant on a previously designed and optimized qPCR method for the detection and quantification of sesame DNA.

Methodology

Sesame seed is considered within allergenic foods explicitly mentioned in the European Food Labeling Directive (Directive 2003/89/EC), therefore its presence in food products should be indicated. Reliable detection methods for allergenic ingredients in food are necessary to ensure compliance with this directive and to improve consumer protection.



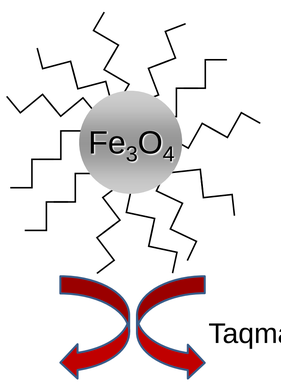
NC_016433 Sesamum indicum chloroplast, complete genome. Figure from Geneious 5.5.6

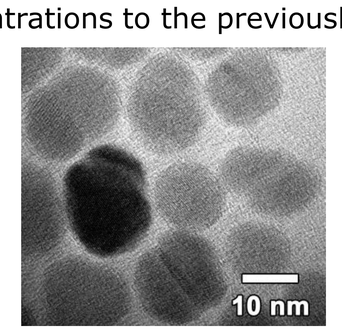
qPCR method

The qPCR method used was designed for the amplification of a fragment of 68bp from maturase K gene of sesame

≈20-nm Fe_3O_4 NPs were added in different concentrations to the previously optimized qPCR protocol

Fe_3O_4@OL@TMAOH





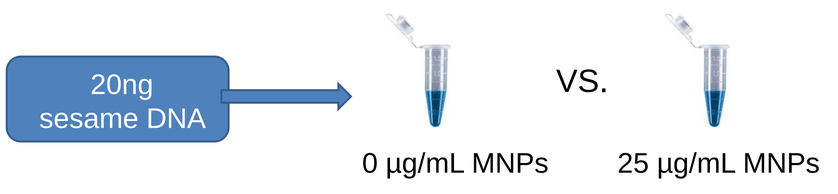
SYBR® Green I dye based protocol

Taqman® MGB probe based protocol

Concentrations of MNPs on qPCR solutions:
0 µg/mL
0.25 µg/mL
2.5 µg/mL
25 µg/mL

Standard curves were prepared with 10 fold dilutions of sesame DNA extract ranging from 0.5e-3 to 50 ng of total DNA in order to evaluate the effect of different concentrations of MNPs on qPCR efficiency and sensitivity

High Resolution Melting (HRM) was used to evaluate the effect of NPs on the stability of dsDNA and possible alteration of thermal dissociation behavior of dsDNA



Results

Taqman® MGB probe based protocol

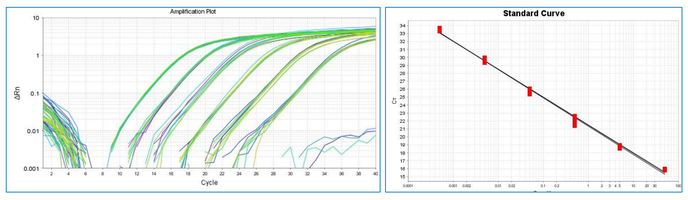


Fig 1: amplification plot and standard curves corresponding to 0, 0.25 and 2.5 µg/mL of MNPs. 0.5e-3 to 50 ng of DNA

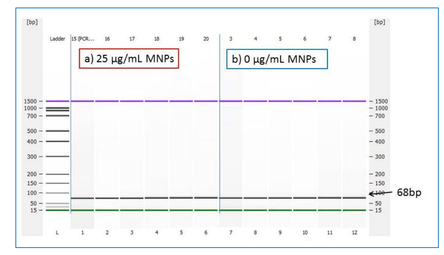


Fig 2: Gel view (2100 Bioanalyzer): L DNA ladder; 1-12 PCR products from 0.5e-3 to 50 ng of sesame DNA a) with 25 µg/mL; b) 0 µg/mL of MNPs

	0 µg/mL	0.25 µg/mL	2.5 µg/mL	25 µg/mL
Intercept	21.51	21.36	21.46	21.09
Slope	-3.5	-3.56	-3.52	-3.51
Efficiency	92.84%	90.76%	92.23%	92.43%
R2	0.9971	0.9945	0.997	0.9942

Table 1: Evaluation of the effect of different concentrations of MNPs on the amplification profile of the Taqman MGB probe based protocol

SYBR® Green I dye based protocol

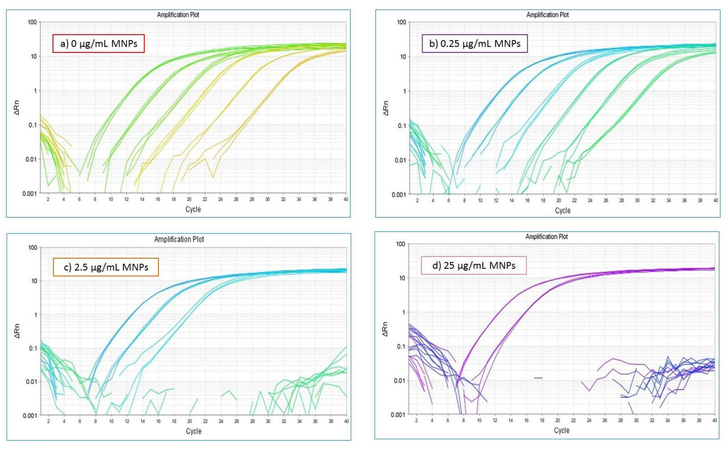


Fig 3: Comparison of amplification plots of 0.5e-3 to 50 ng of sesame DNA with different concentrations of MNPs: a) 0 µg/mL; b) 0.25 µg/mL; c) 2.5 µg/mL; d) 25 µg/mL

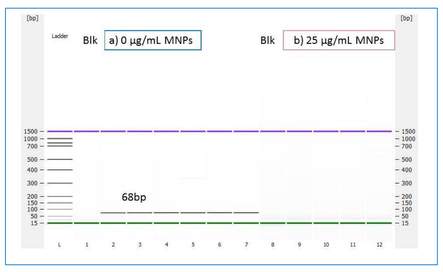


Fig 4: Gel view: L DNA ladder; 1 and 8 Blk (PCR blank); a) PCR products from 0.5e-3 to 50 ng of sesame DNA with 0 µg/mL of MNPs; b) PCR products from 0.5e-3 to 0.5 ng of sesame DNA with 25 µg/mL of MNPs

	0 µg/mL	0.25 µg/mL	2.5 µg/mL
Intercept	19.316	16.035	15.42
Slope	-3.56	-3.71	-3.42
Efficiency	90.70%	85.89%	96.08%
R2	0.9948	0.9972	0.9774

Table 2: Evaluation of the effect of different concentrations of MNPs on the amplification profile of the the SYBR Green I dye based protocol

HRM

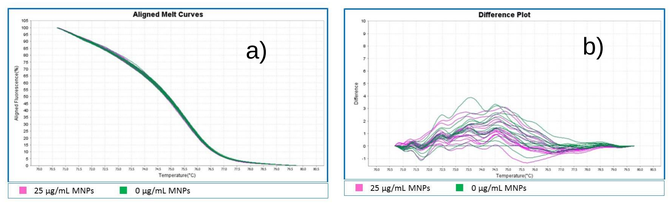


Fig 4: HRM results from 0 µg/mL MNPs versus 25 µg/mL MNPs. a) Aligned Melt Curve; b) Difference Plot

Conclusions

- Amplification profile of Taqman® MGB probe based protocol is not affected by MNPs at the tested concentrations
- Amplification profile of SYBR® Green I based protocol is affected by the addition of increasing concentrations of MNPs, specially sensitivity of the method which is reduced with increasing concentration of MNPs
- Preliminary HRM results indicate that MNPs do not alter the thermal dissociation behaviour of dsDNA
- These resuts confirm the need for careful evalution of the effect of nanomaterials on qPCR

References

[1] Rivas, J., Bañobre-López, M., Piñeiro-Redondo, Y., Rivas, B., & López-Quintela, M. A. (2012). Magnetic nanoparticles for application in cancer therapy. Journal of Magnetism and Magnetic Materials, 324(21), 3499–3502.

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