Evaluation of the effect of magnetic nanoparticles as additives on qPCR

Marta Prado, Yury V. Kolen’ko, José Rivas
International Iberian Nanotechnology Laboratory, Avenida Mestre José Veiga s/n, 4715-330 Braga, Portugal

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 makes 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, Fe3O4, 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 2002/89/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.

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

20-nm Fe3O4 NPs were added in different concentrations to the previously optimized qPCR protocol

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 results confirm the need for careful evaluation of the effect of nanomaterials on qPCR

References