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Effect of surface functionalisation on the interaction of iron oxide nanoparticles with polymerase chain reaction

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ABSTRACT

The combination of nanoparticles with the polymerase chain reaction (PCR) can have benefits such as easier sample handling or higher sensitivity, but also drawbacks such as loss of colloidal stability or inhibition of the PCR. The present work systematically investigates the interaction of magnetic iron oxide nanoparticles (MIONs) with the PCR in terms of colloidal stability and potential PCR inhibition due to interaction between the PCR components and the nanoparticle surface. Several types of MIONs with and without surface functionalisation by sodium citrate, dextran and 3-aminopropyl-triethoxysilane (APTES) were prepared and characterised by Transmission Electron Microscopy (TEM), dynamic light scattering (DLS) and Fourier Transform Infrared (FT-IR) spectroscopy. Colloidal stability in the presence of the PCR components was investigated both at room temperature and under PCR thermo-cycling. Dextran-stabilized MIONs show the best colloidal stability in the PCR mix at both room and elevated temperatures. Citrate- and APTES-stabilised as well as uncoated MIONs show a comparable PCR inhibition near the concentration 0.1 mg ml^{-1} while the inhibition of dextran stabilized MIONs became apparent near 0.5 mg ml^{-1}. It was demonstrated that the PCR could be effectively carried out even in the presence of elevated concentration of MIONs up to 2 mg ml^{-1} by choosing the right coating approach and supplementing the reaction mix by critical components, *Taq* DNA polymerase and Mg^{2+} ions.

1. Introduction

The use of nanoparticles in molecular biology can bring significant benefits such as improved efficiency, miniaturisation or parallelisation [1]. One of the most important techniques in molecular biology is polymerase chain reaction (PCR), which allows in vitro amplification of a defined DNA region by means of a thermostable DNA polymerase and a pair of short specific oligonucleotides exposed to a specific temperature program [2]. Due to the importance of PCR in areas such as forensic research or personalized medicine where only very small sample quantities are often available, ways of improving the efficiency of the reaction became the subject of increased interest from both chemical engineering [3] and application point of view [4]. In particular, various low- and high-molecular weight additives have been explored to enhance the PCR specificity and efficiency [5–17].

Recently, it was shown that colloidal nanomaterials such as gold and silver nanoparticles, graphene oxide, quantum dots, carbon nanotubes, or magnetic iron oxide nanoparticles could also have PCR enhancement abilities [18]. For example, 13 nm gold nanoparticles at concentration 0.1–1 nmol l^{-1} could improve PCR sensitivity by a factor of 10^4–10^6 in addition to decreasing the non-specific PCR product formation [19,20]. Single-walled carbon nanotubes at less than 3 μmol l^{-1} were shown to improve the efficiency of the PCR; however, larger concentrations were found to suppress the PCR [21].

Magnetic iron oxide nanoparticles (MIONs) represent a special category of interest for PCR due to the possibilities they offer in areas such as magnetic separation, actuation, magnetic resonance imaging, or the remote control of local reaction-diffusion processes [22–24], which is also the motivation of the present work. At the same time, the combination of MIONs with the PCR environment represents a challenge due to a large diversity of surface functionalisation approaches that are available in order to achieve colloidal stability of the nanoparticles. A typical PCR mixture contains multiple components that can potentially interact with the nanoparticle

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surface, thereby affecting both colloidal stability and the PCR efficiency.

However, only a few studies on the interaction of MIONs with the PCR can be found in the literature. It was shown that uncoated or cationic/anionic detergent-coated MIONs tend to inhibit PCR depending on their size and structure [25]. It was postulated that MIONs could decrease the activity of the *Taq* DNA polymerase by capturing it in the spaces of nanoparticle clusters. The suggested solution was to apply an external magnetic field [26]. The inhibitory effect of MIONs was also reduced by aggregating the nanoparticles with Bovine Serum Albumin (BSA) in a PCR heated by alternating magnetic field [27]. Various types of amino-modified MIONs have shown different PCR inhibitory effect. While amino-modified silica coated magnetic nanoparticles inhibited the PCR completely, silica coated magnetic nanoparticles were less prone to causing PCR inhibition. A similar trend was also observed in the case of amino-modified and unmodified non-magnetic silica nanoparticles [28]. Besides, it was reported that sub-nanomolar concentration of oleate coated MIONs showed a PCR enhancing effect [29].

The above studies hint at interesting but complex interaction patterns. However, the lack of basic physico-chemical characteristics such as particle size distribution, surface charge, or even concentration renders a direct comparison or generalisation of the published results difficult. Therefore, the purpose of the present work is to fill the gap in the understanding of MION behaviour in the PCR system by performing a systematic study where several common types of MIONs were synthesised and characterised. Their behaviour in the PCR reaction system such as colloidal stability as function of concentration and temperature, inhibitory effect as function of concentration and surface functionalisation, and interaction with the *Taq* DNA polymerase or Mg^{2+} ions was then systematically investigated.

2. Materials and methods

2.1. Overall experimental strategy

The experiments consisted of the following sequence of steps: *(i)* synthesis and physico-chemical characterisation of four types of magnetic iron oxide nanoparticles (MIONs) with different functional groups attached to the surface; *(ii)* investigation of colloidal stability of the MIONs in the PCR buffer at both room and elevated temperature; *(iii)* determining the inhibitory concentration of each type of MION by performing PCR in the presence of MIONs at increasing concentrations; *(iv)* investigation of the specific interaction of the individual PCR components with the MIONs and variation of the PCR mixture composition in order to mitigate the inhibitory effect of the MIONs. The overall aim was to identify such conditions (concentrations of PCR components, type of MION surface functionalisation) that enable the PCR to proceed even in the presence of high concentrations of MIONs.

2.2. Materials

Iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) and iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), Dextran 70 (from Leuconostoc spp., approx. M_W 70 kDa) and 3-aminopropyl-triethoxysilane (APTES) were purchased from Sigma-Aldrich (USA); sodium citrate dihydrate, ammonium hydroxide (NH_4OH) and ethanol (99.8%) were purchased from Penta (Czech Republic). Demineralised water was produced by a water purification system Aqual 25. The DNA primers and deoxynucleotide triphosphates mixture (dNTPs) were purchased from Sigma-Aldrich (USA).

2.3. Synthesis of magnetic iron oxide nanoparticles (MIONs)

Uncoated MIONs were formed by the co-precipitation method: 0.75 g of $FeCl_3{\cdot}6H_2O$ and 0.375 g of $FeCl_2{\cdot}4H_2O$ was dissolved in 105 ml of demineralised water and heated to 80 °C for 10 min in a 250 ml three-necked flask equipped with a reverse cooler and nitrogen atmosphere. Under vigorous stirring, 15 ml of NH_4OH (25% aq.) was dropped to the solution. The reaction mixture was stirred for 2 h and then left to cool to ambient temperature. The nanoparticle suspension was dialyzed for 24 h against demineralised water, the dialysate was sonicated for 10 min and centrifuged at 1000 rpm for 5 min to remove any larger agglomerates. After centrifugation, the supernatant was filtered by a 0.2 μm PVDF filter to obtain a nanoparticle suspension. These purification steps were applied for all types of nanoparticles.

Citrate coated MIONs were synthesised by a similar procedure: 0.75 g of $FeCl_3{\cdot}6H_2O$ and 0.375 g of $FeCl_2{\cdot}4H_2O$ was dissolved in 50 ml of distilled water and mixed for 10 min at 80 °C in a 100 ml three-neck flask equipped with a reverse cooler and nitrogen atmosphere. After 10 min, 5 ml of NH_4OH (25% aq.) was added dropwise and another 2.5 ml of NH_4OH was added to the reaction mixture after one hour. After 2.5 h from the beginning of the reaction, a previously prepared solution of 2.0 g of $Na_3C_6H_5O_7{\cdot}2H_2O$ in 10 ml of distilled water was poured into the reaction mixture and stirred for a further 1 h. The nanoparticle suspension was then cooled down and purified as indicated above.

Dextran coated MIONs [30] were synthesised as follows: 0.75 g $FeCl_3{\cdot}6H_2O$ and 0.375 g of $FeCl_2{\cdot}4H_2O$ were dissolved in 15 ml distilled water and kept in a 100 ml three-neck flask equipped with a reverse cooler and nitrogen atmosphere under vigorous stirring. 500 mg of 70 kDa dextran dissolved in 25 ml of distilled water was added, the mixture was heated to 85 °C and 2.5 ml of 25% NH_4OH was added dropwise into the reaction vessel. The reaction mixture was kept at 85 °C for 1 h and then cooled to room temperature. The nanoparticles were separated by magnetic decantation, washed three times with distilled water and purified as indicated above.

Finally, MIONs functionalised by 3-aminopropyltriethoxysilane (APTES) were synthesized by the following procedure: 0.75 g of $FeCl_3{\cdot}6H_2O$ and 0.375 g of $FeCl_2{\cdot}4H_2O$ was dissolved in 55 ml of distilled water and mixed for 10 min at 80 °C in a 100 ml three-neck flask equipped with a reverse cooler and nitrogen atmosphere. Under vigorous stirring, a previously prepared mixture of 2 ml of APTES, 15 ml of 25% NH_4OH and 50 ml of ethanol was added to the solution. The reaction was completed after 2 h. The solution was left to cool to ambient temperature then purified as indicated above.

2.4. Characterization of MIONs

The morphology and size of the nanoparticles were determined by Transmission Electron Microscopy (TEM). A droplet of the nanoparticle solution was deposited on a carbon-coated copper grid, left to adhere for 2–3 min and the excess solution was removed by a filtration paper. After drying, the grid was inserted into a JEOL JEM-1010 TEM and observed at 80 kV acceleration voltage at various magnifications. The images were taken by a CCD camera MegaView III and analysed by AnalySIS v2.0 software (Olympus, Japan).

The surface functionalisation of MIONs by citrate, APTES and dextran was studied by Fourier Transform Infrared (FTIR) spectroscopy as specified in Supplementary Information 1. The size distribution and the zeta potential of the MIONs were evaluated by Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering (ELS), respectively, using the Zetasizer Nano-ZS (Malvern Instruments, UK). Before the measurement, 10 μl of the sample was diluted by water to 2 ml and placed into a disposable cuvette.