CHARACTERISATION OF MAGNETIC PARTICLES – DNA INTERACTION FOR IMOBILISATION OF VIRUSES AND THEIR SUBSEQUENT IDENTIFICATION BY ZETA POTENTIAL AND PARTICLE SIZE ANALYSES

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Abstract

Magnetic nanoparticles offer several advantages for isolation of biological materials. Since they are broadly used in diagnostic immunoassays and in various reactions involving enzymes, proteins, and DNA, this study considers the interaction of four surface modified magnetic microparticles with selected DNA fragments. The magnetic microparticle precursors are prepared by reduction of from iron(III)nitrate and sodium borohydride in ammonia solution. Different surface modifications, named as MAN 37, MAN 127, MAN 158 and MAN 164 were obtained by targeted chemical treatments. The zeta potential and particle size measurements were performed in a zetasizer MALVERN instrument. All considered magnetic microparticles are characterized by positive zeta-potentials, while the DNA fragments in solution show an approx. zeta-potential of -0.22 mV. All the particles were polycharged. MAN 37 and MAN 127 reveal the highest initial zeta potentials. Upon exposure to DNA, MAN 37 and MAN 127 particles lowered their zeta-potential near zero, meanwhile due to the DNA binding, in the case of MAN 164 the zeta potential decreases until -1.25 mV. All particles considered are classified as monodisperse systems. MAN 37 showed the smallest sizes of approx. 0.5 μm and almost no difference in size toward DNA exposure. MEN 158 distinguished for their initial high size of approx. 3.75 μm compared to MAN 127 and MAN 158, possessing sizes of approx. 3 μm each. MAN 164 particles exhibit the biggest change in contact to DNA, from approx. 3.0 μm up to 5 μm when exposed to DNA fragments. All results were confirmed by quantitative real-time PCR.

Keywords: Magnetic micro particles, DNA binding, zeta potential, particle size

1. INTRODUCTION

Biotechnology and nanotechnology are two of the 21st century's most promising scientific areas. Bionanotechnology is a new interdisciplinary area that integrates physics and chemistry for the development of multifunctional tools for medical, agricultural, and environmental applications. The study of interactions between the nanoparticles and nucleic acid molecules will provide new information on mechanisms that alter DNA molecular structure and highlight new insights for DNA detection and gene therapy [1, 2]. Research on nanoparticle interaction with DNA has started to explore the applications of gold nanoparticles [3], silver nanoparticles [4], carbon nanoparticles [5] and magnetic nanoparticles [6]. The interaction of DNA with unmodified Gold nanoparticles was shown to have more affinity to single stranded DNA when compared to double stranded DNA [3]. Functionalized gold nanoparticles with oligonucleotides can be used to specifically bind and elute DNA based on change in pH [7]. Railsback et al. investigated the mechanism of binding of

DNA to modified gold nanoparticles [8]. Their results showed that DNA bending and denaturation plays direct role in binding to weak cationic nanoparticles. Basu et al. showed that the binding of DNA bases to unmodified silver nanoparticles give different color for each base [4]. The authors reported change in nanoparticle color from red to purple to orange to blue in accordance to aggregation and orientation of individual nanoparticles in aggregate. In a different study, silver nanoparticles modified with oligonucleotides were shown to be ultrasensitive for detection of DNA [9]. An et al. showed that Lambda phage DNA binds randomly to carbon nanoparticles (~50 nm in size) while fine size (1 nm) nanoparticles bind on DNA like pearls on string [5]. However, carbon nanomaterials has been reported to inhibit DNA associated enzymes, e.g. blocking the polymerase chain reaction [1].
Magnetic nanoparticles offer more advantage for isolation of biological materials by a suitable immobilization process. Magnetic nanoparticles have been used in diagnostic immunoassays and in various reactions involving enzymes, proteins, and DNA for magnetically controlled target delivery of anticancerous drugs [10]. Pershina et al. provide comprehensive review of the literature regarding magnetic nanoparticle binding to DNA [11]. The structure and composition of nanoparticles, the pH at which DNA and nanoparticles interact, and the functionalization of the nanoparticle surface are among the major highlights of research in this field. Since the binding DNA-particles is of great interest for the separation of DNA from targeted systems, this study is intended to shed light on a reliable possibility of detection of the interaction between DNA fragments and magnetic microparticles based on zeta potential and particle size measurements.

2. EXPERIMENTAL

2.1 Synthesis of magnetic microparticles

Nanomaghemite is prepared from iron(III) nitrate and sodium borohydride in ammonia solution. 400 ml of MilliQ water was poured in a 600 ml beaker and 7.48g of iron(III) nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$) was mixed on a magnetic rotor. 7 ml of 25% NH_3 was diluted with 43 ml of MilliQ water and poured in a separate beaker (of 150 ml). 1g of sodium borohydride ($NaBH_4$) was mixed with NH_3 solution in that small beaker. After 7-10 min of mixing, the solution was added to the large beaker containing iron nitrate. The color of the solution became black with an initial frothing. Then it was heated at 100°C for 2h. The mixture was stirred overnight. The magnetic particles were separated by external magnet and washed several times with MilliQ water and used for modifications.

2.2 Superparamagnetic iron oxide modifications

Man 37: was i-PrOH was added to the obtained product as obtained above followed by tetraethyl orthosilicate (TEOS) and later by 3-aminopropyl triethoxysilan (APS). The product was separated by magnet and washed several times with diluted EtOH.
Man 127: 1/5 part of prepared nanomaghemite was mixed with a PVP (10k, 0.2 g) solution and stirred overnight. The product was separated by magnet, washed several times with water and dispersed in PBS buffer (50 mL, p*H* = 7.4).
Man 158: 1/5 part of prepared nanomaghemite was stirred with $Na_2HPO_4 \cdot 2H_2O$ (1.2 M, 2 mL) and $Ca(NO_3)_2 \cdot 4H_2O$ (1 M, 4 mL). After 2 h NaOH (1 M, 5 mL) was added and stirred overnight. The product was separated by magnet, washed several times with water and left in water (50 mL). After that, half volumes of phosphate and nitrate were added to it.
Man 164: The surface of prepared nanomaghemite was modified by addition of sodium triphosphate followed by calcium nitrate. Product was separated by magnet and washed several times with water.

2.3 Preparation and binding of DNA to magnetic microparticles

Large amounts of DNA were prepared using PCR to amplify a 700 bp product from the Ebola glycoprotein GP gene (EBOV subtype Zaire, strain Mayinga 1976). The following primers were used for amplification of Ebola DNA from pCMV3-ZaireEBOV-U23187-GP-FLAG vector (Sino Biological Inc., North Wales, PA, USA): Forward: GACCCCCAAAAGCAGAGAAC. Reverse: ACGCCTGTAACTCCAATACCTG.

After purification of DNA fragments using MinElute kit (Qiagen, Venio, Netherlands), the concentration of DNA was first estimated using nanodrop-based method on Infinite 200 PRO NanoQuant instrument (Tecan, Maennedorf, Germany). Amounts of 100ng/µL is equivalent to $>10^{11}$ copies of DNA. Dilution of DNA to working solution of ~10^{10} copies/µL was enough for binding and quantitation using real-time PCR and also using nanodrop-based method. Binding mixture was prepared in 1.5 mL Eppendorf tubes: 40 µL of DNA (~10^{10} copies/µL) mixed with 40 µL Sodium acetate-HCl (0.75 M, p*H*=6), 32 µL magnetic particles, and 240 µL of EtOH 85%.

2.4 Zeta potential and particle size analyses

The zeta potential measurements were performed in a Zetasizer MALVERN, (Malvern Instruments Ltd. Worcestershire, United Kingdom), considering the same refraction index and absorption coefficient as described in particle size measurements. Furthermore, the measuring parameters such as, temperature and viscosity were the same as in particle size measurements. Calculations considered the diminishing of particles concentration based Smoluchowsky model, with a F(κa) of 1.50 and an equilibrating time of 120 s. For the measurements, a disposable cell DTS1070 was employed. In each case, the measurement duration depended on the number of runs, which varied between 20 and 40. The measurements were triplicate and were performed under the automatic setting of attenuation and voltage selection.
The particle size measurements were performed considering a refraction index of the dispersive phase of 3.00 and 1.333 for the dispersive environment. The absorption coefficient in both cases was 10^{-3} respectively. The measuring temperature was set at a constant value of 25°C, while the viscosity was 0,8872 cP. For each measurement, disposable cuvettes type ZEN 0040, were used, containing 40 µL of sample. The equilibration time was 120 s, at a measurement angle of 173° backscatter. The measurements were triplicate.

3. DISCUSSION OF THE RESULTS

All the magnetic microparticles in this study are characterized by positive zeta-potentials (Fig. 1 left), which enable them to bind to DNA fragments in solution with an approx. zeta-potential of -0.22 V. The magnetic particles entitled as MAN 37 and MAN 127 show the highest initial zeta potentials, followed by MAN 158 and MAN 164. The attachment of DNA fragments to all of them lowers their zeta potential, but at the same time ensures particle protection due to the particle surface coverage. The exposure of DNA to MAN 37 and MAN 127 particles lowers their zeta-potential near zero, meanwhile due to the DNA exposure, in the case of MAN 164 the zeta potential is decreased until -1.25 mV (Fig. 1 left). All the particles were polycharged and their charge configuration, depends on the electrical double layer around them and changes upon the DNA fragment exposure. An illustrative example is depicted in Fig. 2, showing three consequent zeta potential measurements of Man 128 particles without and with DNA. Upon the DNA binding, the particles stabilize and turn from poly-charged to mono-charged (Fig. 2).

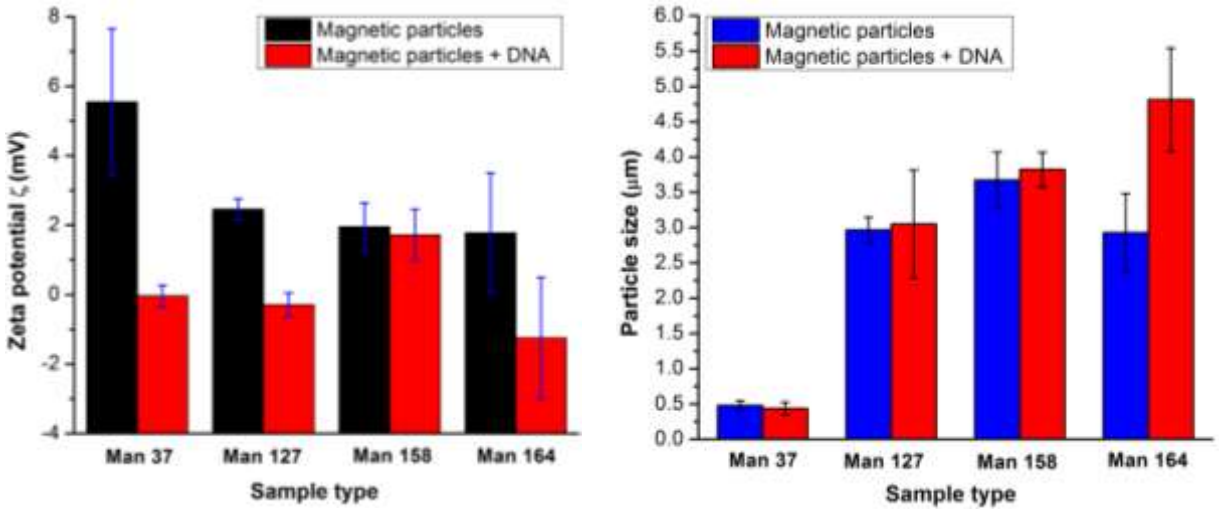
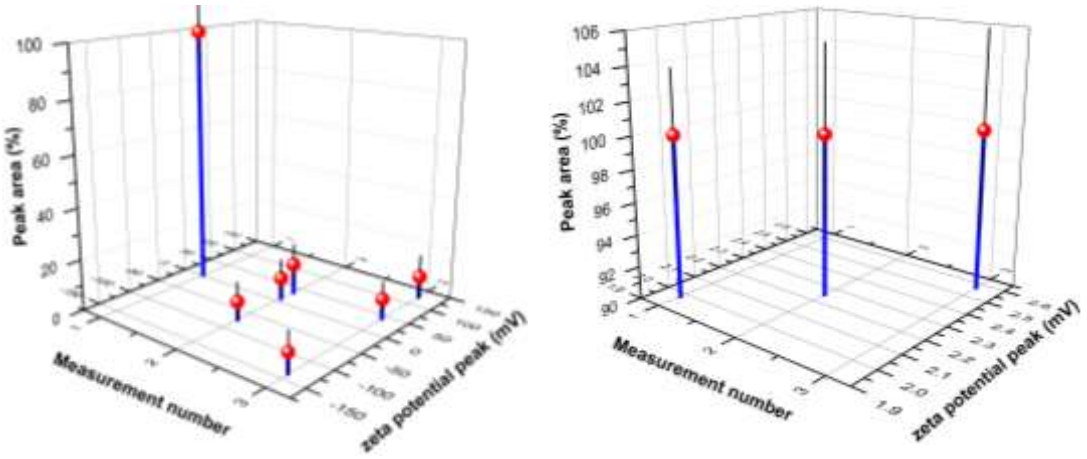


Fig. 1: Graphical visualization of zeta potential (left) and particle size (right) of unbound and DNA-bound magnetic particles

In parallel to zeta potential determination, the particle size measurements revealed considerable size changes of the samples without and with contact to DNA fragments (Fig. 1 right). In spite the significant content of polycharged particles, with the mathematical model used for data calculations they were classified as monodisperse systems. The magnetic particles of MAN 37 showed the smallest sizes among the other particles of approx. 0.5 μm (Fig. 1 left, blue bar), which were comparable in size to the DNA-bound particles (red bar). Anyway, these particles change considerably when compared to MAN 127, MAN 158 and MAN 164 (Fig. 1 right). The particles of Man 158 distinguished for their initial high size of approx. 3.75 μm compared to MAN 127 and MAN 158 which possessed sizes of approx. 3 μm each.



(a) **(b)**

Fig. 2: Zeta potential variation for three consequent measurements of Man 158 samples: a) without and b) with contact to DNA

Interestingly, MAN 164 particles revealed the biggest change in contact to DNA. Their size increased considerably, from approx. 3.0 μm up to 5 μm when exposed to DNA fragments. The DNA binding efficiency regarding the particle size variation is illustrated for all particle types by the plots of Fig. 3. The particle size distribution showed in Fig. 3, represented as Number (%) *vs*. Particle size for the triplicate experiments in each case, exhibited no visible change between MAN 37; Man 37-DNA, MAN 127; MAN 127-DNA and MAN 158; MAN 158-DNA within the measurement accuracy. Meanwhile, particles of Man 164 exhibited a

considerable increase, where the triplicate measurement MAN 164-DNA showed a clear shifting toward higher numbers compared to the triplicate of MAN 164, leading to the conclusion of a significant binding efficiency of DNA fragments by them, which leads to further particle agglomeration.
Since MAN 37 particle agglomerates are smaller in size compared to the other particles considered agglomerates, they exhibit as a consequence a higher surface area. Therefore, certain amounts of DNA fragments attached to MAN 37 particles decrease their zeta potential more than in the case of bigger particles. Due to this fact, the decrease of zeta-potential for MAN 37 particles is more significant than for all the others (Fig. 1 left). Anyway, since MAN 37 particles surface can accommodate much less DNA fragments than other particles, their increase in size is not significant when compared to the other particles. Since MAN 127 and MAN 164 have similar sizes but different zeta potentials and behave differently (Fig. 1), it seems that the particle size alone does not play the greatest role on the DNA fragments binding. A crucial factor which influences the zeta potential and the binding in this process is the particle surface modification, though, the respective double layer.

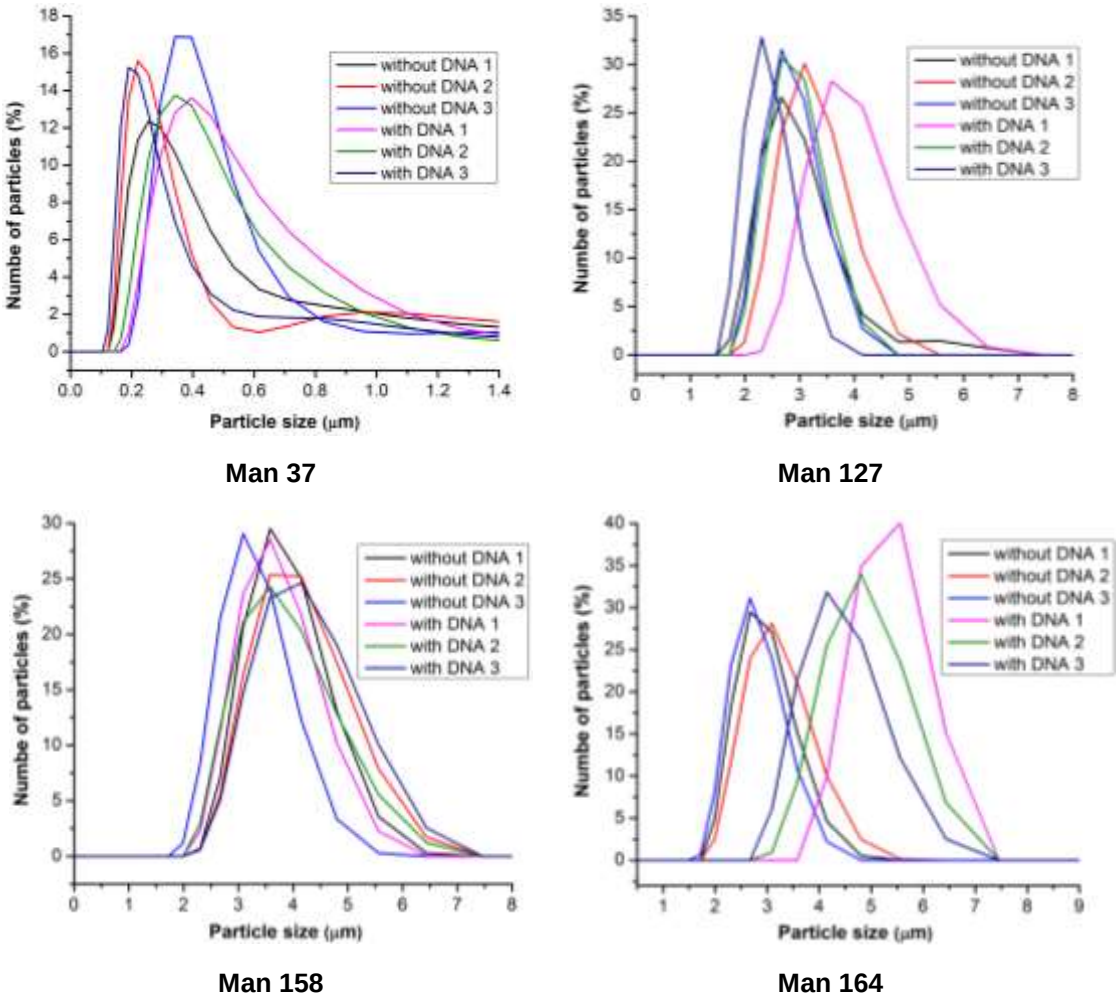


Fig. 3: Number of particles in % versus particle size for unbound and DNA-bound magnetic particles

All of these data evidencing the binding of DNA fragments to the magnetic micro particles are confirmed by quantitative real-time PCR measurements too, and are in full agreement to the showed results.

4 CONCLUSIONS

In this study magnetic microparticle precursors are prepared by reduction of from iron(III) nitrate and sodium borohydride in ammonia solution. By targeted chemical treatments, four different particle types with respective surface modifications were obtained. The interaction between the particles and DNA fragments was investigated by zeta potential and particle size analyses. All considered magnetic microparticles revealed positive zeta-potentials, while the DNA fragments in solution were slightly negatively charged, causing electrical interaction between them. Zeta potential characterization of all samples showed polycharged particles with MAN 37 and MAN 127 revealing the highest initial zeta potentials. Due to the interaction with DNA, the zeta potential decreased near zero for MAN 37 and MAN 127 particles, meanwhile in the case of MAN 164 the zeta potential decreased even below zero. With the selected mathematical calculation model, all the considered particles are classified as monodisperse systems. Despite of their initial size, the particle behavior was strongly influenced by their surface modification and consequently by the electrical double layer. Man 37 showed the smallest sizes and almost no size difference in contact to DNA. Unlike that, MAN 158 was distinguished for their initial high size particles compared to MAN 127 and MAN 158, but nevertheless they did not reveal significant particle size variations in contact to DNA. The most significant size increase was observed for Man 164 particles in contact to DNA. All these results were confirmed by parallel quantitative real-time PCR measurements, making therefore zetasizer analyses as a reliable tool for the detection of magnetic microparticles – DNA interaction.

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5 REFERENCES

1. An, H.J. and B. Jin, *Prospects of nanoparticle-DNA binding and its implications in medical biotechnology.* Biotechnology Advances, 2012. **30**(6): p. 1721-1732.
2. Fakruddin, M., Z. Hossain, and H. Afroz, *Prospects and applications of nanobiotechnology: a medical perspective.* Journal of Nanobiotechnology, 2012. **10**.
3. Gaylord, B.S., A.J. Heeger, and G.C. Bazan, *DNA detection using water-soluble conjugated polymers and peptide nucleic acid probes.* Proceedings of the National Academy of Sciences of the United States of America, 2002. **99**(17): p. 10954-10957.
4. Basu, S., et al., *Interaction of DNA bases with silver nanoparticles: Assembly quantified through SPRS and SERS.* Journal of Colloid and Interface Science, 2008. **321**(2): p. 288-293.
5. An, H.J., et al., *DNA binding and aggregation by carbon nanoparticles.* Biochemical and Biophysical Research Communications, 2010. **393**(4): p. 571-576.
6. Heger, Z., et al., *Paramagnetic Nanoparticles as a Platform for FRET-Based Sarcosine Picomolar Detection.* Scientific Reports, 2015. **5**.
7. Sun, L.P., et al., *Effect of pH on the Interaction of Gold Nanoparticles with DNA and Application in the Detection of Human p53 Gene Mutation.* Nanoscale Research Letters, 2009. **4**(3): p. 216-220.
8. Railsback, J.G., et al., *Weakly Charged Cationic Nanoparticles Induce DNA Bending and Strand Separation.* Advanced Materials, 2012. **24**(31): p. 4261-+.
9. Thompson, D.G., et al., *Ultrasensitive DNA detection using oligonucleotide-silver nanoparticle conjugates.* Analytical Chemistry, 2008. **80**(8): p. 2805-2810.
10. Heger, Z., et al., *Fluorescence resonance energy transfer between green fluorescent protein and doxorubicin enabled by DNA nanotechnology.* Electrophoresis, 2014. **35**(23): p. 3290-3301.
11. Pershina, A.G., A.E. Sazonov, and V.D. Filimonov, *Magnetic nanoparticles - DNA interactions: design and applications of nanobiohybrid systems.* Russian Chemical Reviews, 2014. **83**(4): p. 299-322.