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Corresponding Author: Dr. Zuzana Bilkova,

Corresponding Author's Institution: University of Pardubice

First Author: Lucie Holubova

Order of Authors: Lucie Holubova; Petr Knotek; Jiri Palarcik; Michaela Cadkova; Petr Belina; Milan Vlcek; Lucie Korecka; Zuzana Bilkova

Abstract: Iron oxide based particles functionalized by bioactive molecules have been utilized extensively in biotechnology and biomedicine. Despite their already proven advantages, instability under changing reaction conditions, non-specific sorption of biomolecules on the particles' surfaces, and iron oxide leakage from the naked particles can greatly limit their application. As confirmed many times, surface treatment with an appropriate stabilizer helps to minimize these disadvantages.
In this work, we describe enhanced post-synthetic surface modification of superparamagnetic microparticles varying in materials and size using hyaluronic acid (HA) in various chain lengths. Scanning electron microscopy, atomic force microscopy, phase analysis light scattering and laser diffraction are the methods used for characterization of HA-coated particles. The zeta potential and thickness of HA-layer of HA-coated Dynabeads M270 Amine were -50 mV and 85 nm, respectively, and of HA-coated p(GMA-MOEAA)-NH2 were -38 mV and 140 nm, respectively. The electrochemical analysis confirmed the zero leakage of magnetic material and no reactivity of particles with hydrogen peroxide. The rate of non-specific sorption of bovine serum albumin was reduced up to 50% of naked ones. The coating efficiency and suitability of biopolymer-based microparticles for magnetically active microfluidic devices was confirmed.

Highlights:

- Post-synthetic surface modification of magnetic microparticles by hyaluronic acid
- Hyaluronic acid – polymer of unique physicochemical and biological characteristics
- Panel of particles characterization methods was introduced
- HA-coated microparticles gain characteristics suited for microfluidic bioanalysis

Magnetic microparticles post-synthetically coated by hyaluronic acid as an enhanced carrier for microfluidic bioanalysis

Lucie Holubova[a,b], Petr Knotek†[c], Jiri Palarcik†[d], Michaela Cadkova[a,b], Petr Belina[e], Milan Vlcek[f1], Lucie Korecka[a], Zuzana Bilkova[a]*

[a]Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Studentska 573, 53210 Pardubice, Czech Republic
[b]Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentska 573, 53210 Pardubice, Czech Republic
[c]Joint Laboratory of Solid State Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentska 573, 53210 Pardubice, Czech Republic
[d]Institute of Environmental and Chemical Engineering, Faculty of Chemical Technology, University of Pardubice, Studentska 573, 53210 Pardubice, Czech Republic
[e]Department of Inorganic Technology, Faculty of Chemical Technology, University of Pardubice, Doubravice 41, 53210 Pardubice, Czech Republic
[f]Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovskeho sq. 2, 162 06 Prague, Czech Republic

*** Corresponding author:** Z. Bilkova
Department of Biological and Biochemical Sciences
Faculty of Chemical Technology, University of Pardubice
Studentska 573 (HB/C)
532 10 Pardubice
Czech Republic
Tel.: +420466037700
E-mail: Zuzana.Bilkova@upce.cz

† These authors contributed equally to this work

[1] Present address: Joint Laboratory of Solid State Chemistry, University of Pardubice, Studentska 573, 53210 Pardubice, Czech Republic

ABSTRACT

Iron oxide based particles functionalized by bioactive molecules have been utilized extensively in biotechnology and biomedicine. Despite their already proven advantages, instability under changing reaction conditions, non-specific sorption of biomolecules on the particles' surfaces, and iron oxide leakage from the naked particles can greatly limit their application. As confirmed many times, surface treatment with an appropriate stabilizer helps to minimize these disadvantages.

In this work, we describe enhanced post-synthetic surface modification of superparamagnetic microparticles varying in materials and size using hyaluronic acid (HA) in various chain lengths. Scanning electron microscopy, atomic force microscopy, phase analysis light scattering and laser diffraction are the methods used for characterization of HA-coated particles. The zeta potential and thickness of HA-layer of HA-coated Dynabeads M270 Amine were -50 mV and 85 nm, respectively, and of HA-coated p(GMA-MOEAA)-NH_2 were -38 mV and 140 nm, respectively. The electrochemical analysis confirmed the zero leakage of magnetic material and no reactivity of particles with hydrogen peroxide. The rate of non-specific sorption of bovine serum albumin was reduced up to 50% of naked ones. The coating efficiency and suitability of biopolymer-based microparticles for magnetically active microfluidic devices was confirmed.

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1. Introduction

Magnetic iron oxide particles in sizes ranging from nanometre to micrometre scales have been put to use in a number of areas: biotechnology, therapeutics, and *in vitro* and *in vivo* clinical diagnostics [1–3]. Many automatic immunoanalysers or multiplex systems in clinical laboratories utilize superparamagnetic micro- or nanoparticles with high specific surface area as a solid phase for creating efficient reaction conditions. Another rapidly developing field of application for magnetic particles is microfluidics [1–2]. In this field, such prerequisites as narrow particle size distribution, colloidal and reaction stability, and excellent superparamagnetic behaviour are necessary. To integrate the suspension of magnetic particles into microfluidic devices, it is necessary to eliminate any instability of magnetic particles during analytical steps caused by deviations from physiological reaction conditions (e.g. pH, ionic strength and density of particles). The tendency of particles to agglomerate and adhere to the inner surfaces of microfluidic devices and non-specific adsorption of biomolecules are significantly limiting factors in such routine applications [4–7]. It is well known that spontaneous aggregation of particles is accompanied by loss of their superparamagnetic behaviour [7]. Moreover, the hydrophilic or hydrophobic character of such particles affects the activity of biomolecules, resulting in a decrease or total loss of their activity due to their potential denaturation [6].

To minimize all the aforementioned disadvantages, post-synthetic coating with an appropriate stabilizer of natural or synthetic origin (e.g. using polymers, surfactants or other biomolecules with a hydrophilic nature) is one of the ways to overcome these obstacles. Such commonly used polymer stabilizers as poly(ethylenglycol), poly(vinyl)alcohol and poly(lactic acid) and such carbohydrates as dextran, chitosan and starch have been successfully applied [3,4,6,7]. Another potential low-fouling modifier is hyaluronic acid (HA). Among such surface stabilizers, HA has received much attention due to its unique characteristics [8].

HA is a naturally occurring, linear, non-sulphated glycosaminoglycan consisting of a repeating disaccharide unit of β-1,3-N-acetylglucosamine linked to β-1,4-glucuronic acid. Among its other attributes, HA possesses highly hydrophilic and polyanionic characteristics under physiological conditions [8]. Its viscous solutions have unusual rheological properties and are exceedingly lubricious [9,11]. The naturally occurring locations and molecular mass of HA polymer chains (which can exceed even 10^6 Da) are the key factors affecting HA's broad spectrum of biological activities and features [12–14]. Namely high molecular mass extracellular HA is important structural element, acts as an signaling components [12,13] and it exhibits antiangiogenic properties whereas HA oligosaccharides support angiogenesis [13,14]. HA fragments also induce inflammatory, tumor growth and metastasis [13,14] and they are able to activate transcription factor like NF-κB, but high molecular mass HA has an inhibitory effect [12]. High molecular mass HA do not activate the inflammatory or proliferative genes [13]. HA is known for such excellent biological features as high biocompatibility, biodegradability, low immunogenicity, and high tolerance for the human body's immune system [9,15].

The aforementioned biological and physicochemical characteristics of this fascinating biomolecule have increased the interest in its safe utilization within the fields of modern biotechnology and biomedicine. The premise is that the HA-based and HA-coated materials gain the unique properties of HA. Its potential is certainly broad and versatile. HA is a beneficial structural component and basic building block of such HA-based carriers and biomaterials as HA-nanoparticles, hydrogels and scaffolds, and thus it offers great promise for diagnostics and even for therapy [10]. As already proven, HA also serves as an effective stabilizer for surface treatment and coating of a variety of medical devices and materials, including the likes of catheters [16], guidewires [17], superparamagnetic nanoparticles for *in*

vivo imaging [18,19], various types of artificial replacements such as dental, orthopaedic implants [20,21] or neuronal implants [22], and microfluidic channels [23].

The post-synthetic surface modification of superparamagnetic microparticles by HA was the aim of this study. Hyaluronic acid was chosen with respect to its unique biological and physicochemical characteristics contributing tissue integrity and cells communication. We assume HA imparts qualitatively new surface properties to the modified particles mainly for protein analysis performed in magnetically active microfluidic devices. No paper has yet been published describing such post-synthetic modification of magnetic microparticles for bioapplications. We have clearly demonstrated increased colloidal stability of HA-coated microparticles in aqueous solutions, their suppressed adhesion to the various materials used in microfluidic devices and reduced non-specific sorption of biomolecules.

2. Materials and methods

2.1. Chemicals

Hyaluronic acid (molecular weight = 10 kDa and 26 kDa) was a product of Contipro Group s.r.o (Dolní Dobrouč, Czech Republic). Oligo-HA4, fluorescein hyaluronic acid (≈800 kDa), hyaluronan biotin sodium salt (≥700 kDa), 2-(N-Morpholino)ethanesulfonic acid (MES), 1-ethyl-3-(3- dimethylaminopropyl)carbodiimide hydrochloride (EDC), bovine serum albumin (BSA), o-phenylenediamine (OPD), (+)-hydrazide biotin, streptavidin-peroxidase polymer (STR-HRP), cetyl trimethyl ammonium bromide (CTAB), and 30% hydrogen peroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Streptavidin-conjugated fluorescent dye Alexa Fluor 488 was obtained from Life Technologies (Carlsbad, CA, USA). *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS) was obtained from Fluka (Buchs, Switzerland), and a Micro BCA protein assay kit was purchased from Thermo Fisher

Scientific (Rockford, IL, USA). All other chemicals were of reagent grade and produced by PENTA (Chrudim, Czech Republic).

2.2. Magnetic particles

Dynabeads M-270 Amine magnetic particles (polystyrene, 2.8 μm) were purchased from Life Technologies (Carlsbad, CA, USA) and SiMAG-Amine magnetic microparticles (silica, 0.75 μm) from Chemicell GmbH (Berlin, Germany). Magnetic particles of poly[glycidylmethacrylate-(methacryloyloxy)ethoxy]acetic acid)amine (p(GMA-MOEAA)-NH_2) (4.5 μm) were obtained from the Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic (Prague, Czech Republic) [24].

2.3. Coating of magnetic particles by HA

One milligram of magnetic particles with $–NH_2$ functional groups was washed 5 times with MES buffer (0.1 M, pH 6.0). Subsequently, carboxylic groups located on HA chains were activated. Ten milligrams of EDC and 1.7 mg of sulfo-NHS dissolved in 400 μL of MES buffer (0.1M, pH 6.0) were successively added to HA (from 0.1 to 500 nM according to the molecular weight of the HA chain) dissolved in 200 μL of MES buffer (0.1 M, pH 6.0). The solution of activated HA was then added immediately to the pre-washed particles and the mixture was stirred for 3 h at room temperature. Particles modified by HA were washed 3 times with MES buffer (0.1 M, pH 6.0), then 3 times with 1M NaCl in the same buffer and 5 times with phosphate buffer (0.1 M, pH 7.0) to remove all unreacted or non-specifically adsorbed molecules. Phosphate buffer (0.1 M, pH 7.0) with sodium azide as a preservative was used as a storage solution.

2.4. Methods applied for characterizing HA-coated particles

2.4.1. Zeta potential measurement of magnetic microparticles

The electrophoretic mobilities and zeta potentials were determined by electrophoresis and phase analysis light scattering (PALS) using a ZetaPALS instrument (Brookhaven Instruments Corporation, Holtsville, NY, USA) equipped with Pd electrodes at 25 °C. The suspension of pre-washed naked/HA-coated magnetic particles in redistilled water at a concentration of 0.05 mg mL^{-1} was analysed. The zeta potential was calculated from the electrophoretic mobility using the Smoluchowski equation as the median from 42 experimental values.

2.4.2. Size measurement of magnetic microparticles

The size of the naked/HA-coated magnetic microparticles was measured by laser diffraction using the MasterSizer 2000 particle size analyser (Malvern Instruments Ltd., Malvern, UK). The magnetic carriers were thoroughly washed with redistilled water and ultrasonicated for 10 min. To analyse hydrodynamic diameter, 10 mL of magnetic carrier suspension was used at a concentration of 0.05 mg mL^{-1}. These samples were injected directly into the measuring cell. The laser obscuration was between 5% and 10%, and every sample was measured three times. The average particle size and its distribution were evaluated by red light (He-Ne laser with wavelength 633 nm) and blue diode (466 nm) on the basis of Fraunhofer bending.

2.4.3. SEM and AFM measurement

The naked/HA-coated magnetic microparticles were prepared for atomic force analysis (AFM) and scanning electron microscopy (SEM) imaging by spin-coating on the glass substrate according to a procedure described previously [25,26]. Gold-coated samples were visualized using the SEM instrument Jeol JSM 5500 LV (JEOL Ltd., Tokyo, Japan). The

topography and force spectroscopy modes of the AFM were executed using the Solver Pro M instrument (NT-MDT, St. Peterburg, Russia). Data for diameter, topography and force spectroscopy were determined according to procedures described previously [27–29].

2.5. Electrochemical validation of HA-layer compactness

All electrochemical measurements were performed using Pt-Pt-Ag/AgCl screen-printed electrodes (BST, Berlin, Germany) and a PalmSens potentiostat (PalmSens, Utrecht, Netherlands).

One hundred micrograms of pre-washed naked/HA-coated magnetic particles were resuspended in 800 µL of 1 mg L^{-1} of hydrogen peroxide in phosphate buffer (0.1 M, pH 7.3) with 0.15 M NaCl. Forty microlitres of hydrogen peroxide substrate solution were dropped onto the electrode surface. The electrochemical response of hydrogen peroxide was measured by linear sweep voltammetry (LSV) in 5-minute intervals for 15 min in total.

2.6. Behaviour of HA-coated particles in the channel of a microfluidic device

The naked/HA-coated magnetic particles were integrated into the hydrophilized rhombic channels chip eP1 made from such various materials as polymethylmethacrylate (PMMA), cyclo-olefin polymer (COP, zeonor) and cyclo-olefin copolymer (COC, topas) (Microfluidic ChipShop GmbH, Jena, Germany). All such experiments were performed using ChipGenie edition P on-chip sample-preparation systems (Microfluidic ChipShop GmbH, Jena, Germany). The channels of commercial microfluidic devices were filled with 0.5 mL of a suspension containing magnetic particles in phosphate buffered saline at a concentration of 4 mg L^{-1}. The particles were trapped inside the channel for 10 min by an external magnetic field and then rinsed out. The behaviour of the tested particles in the microfluidic channel was monitored during the experiment using an AM-7013MZT microscope (Dino-Lite Digital

Microscope, Naarden, the Netherlands). Representative images were evaluated using ImageJ analysis software (version 1.42) from the National Institutes of Health (Bethesda, Md., USA) [30].

3. Results and discussion

The main goal of this work was to modify the surface of magnetic microparticles to attain suitable characteristics as required for bioanalysis to be performed in microfluidic devices. It should be emphasized that the adhesion of magnetic beads to various materials, their agglomeration and formation of large clusters, the high rate of non-specific adsorption of proteins and other biomolecules on the surface of particles and possible toxic impact of magnetic materials are unfavourable properties limiting their routine use in bioapplications.

Despite the availability of a wide range of simple, non-covalent binding methods, covalent binding was applied to prevent the accidental release of HA molecules and layer disruption as a result of environmental changes. Given the structure of HA and the easily accessible reactive carboxylic groups located within its structure that are extremely convenient for coupling with carriers, magnetic particles with $–NH_2$ functional groups differing in size and material were chosen for testing. We selected for our experiments either the commercially available Dynabeads M-270 Amine (2.8 µm) and SiMAG-Amine (0.75 µm) magnetic particles or poly[glycidylmethacrylate-(methacryloyloxy)ethoxy]acetic acid)amine p(GMA-MOEAA)-NH_2 magnetic particles (4.5 µm) specially developed for microfluidic applications [24].

For covalent binding of HA chains via carboxylic functional groups, we used the slightly modified carbodiimide technique with EDC and sulfo-NHS as enhancing agents adapted from

Staros et al. [31] (see 2.3). The selected technique was optimized with the aim of fulfilling the maximal binding capacity and achieving the greatest stability and homogeneity of the surface HA-layer. The main experimental parameters (i.e. composition of reaction mixture, ratio of reaction constituents, type of HA, incubation time and temperature during all experimental steps) were optimized. In order to evaluate the efficiency of HA-coating, zeta potential measurement (see 2.4.1) was performed. The zeta potential closely relates to surface charge and functional groups. The tested naked particles are amine-functionalized (Dynabeads M-270 Amine) or functionalized by both amine and carboxylic groups (p(GMA-MOEAA)-NH_2). The molecules of HA contain large amount of carboxylic functional groups, at neutral pH they are polyanions. The HA-layer created on the surface of particles installs carboxylic functional groups instead of amine and changes totally surface charge [32]. The results thus obtained demonstrated a positive correlation between the quantities of HA in the original sample and the absolute zeta potential values. The maximum absolute value of zeta potential was obtained using 200 µg HA per mg of particles. Figure 1 displays the increased colloidal stability of HA-coated p(GMA)-MOEAA-NH_2 microparticles compared with naked ones. The significantly changed zeta potential values of HA-coated Dynabeads M-270 Amine magnetic particles (from positive to negative values) demonstrate the occurrence of a compact HA-layer on their surface. The similar zeta potential values of three various type of HA-coated Dynabeads M-270 Amine particles differing in molecular weight of used HA signify the similar total amount of negatively charged carboxylic groups. These results indicate the complete coverage of particles by HA. We also observed that the zeta potential of HA-coated particles was not influenced by the chain lengths of HA used.

The efficiency of covalent binding and characterization of HA-coated particles were evaluated also by microscopic techniques and particle size measurement using laser diffraction. To evaluate coating procedures, techniques based on bioaffinity interaction (the

commonly used biotin–streptavidin interaction) with spectrophotometric (streptavidin-Allexa Fluor 488) and fluorescent detection (streptavidin-HRP + OPD) were also performed. The results thus obtained unequivocally confirmed the presence of an HA-layer on the surface of particles, although these techniques cannot be used for quantitative evaluation. Also tested was the turbidimetric analysis described by Ferrante et al. [33] for determining the HA concentration in a reaction solution before and after immobilization using CTAB as a cationic agent, but this method proved to be inappropriate for quantification.

Microscopic techniques with sufficient resolution, namely AFM and SEM, were used as the first-choice methods for characterizing the HA-layer on the particles [34] (see 2.4.2). The results of this analysis are therefore described in full detail.

The topography of naked Dynabeads M-270 Amine particles was detected to be that of the smooth single or dimer particles as illustrated in Fig. 2, panels A (AFM topographical scan) and B (created topographical profile). The separated particles kept their original spherical shape, whereas the shape of agglomerates was deformed by the magnetic forces occurring between particles. The shape of the naked particles was spherical with a height of 2.85 µm and standard error 0.02 µm. As typical for AFM topographical profile the spherical particles are located on the flat surface and the profile is imaged as only upward facing surface [35]. The diameter of HA-coated particles (by oligo-HA4) increased in comparison to that of the naked particles by 40–200 nm and the surface was rough (compare the dotted curve and the solid curve in panel B of Fig. 2), as HA does not cover particles with a layer of homogenous thickness. The typical maximum height of HA-coated particles was determined to be 3.02 µm. The maximum thickness of HA-coating determined as $(d_{HA\text{-}coated} - d_{naked})/2$ was 85 nm. Similar results for diameter increase were also obtained using a laser diffraction technique to determine their hydrodynamic diameter (data not shown).

The topography of the naked SiMAG particles was very rough, as it was formed by the aggregate with inner structure (Fig. 2, panel C, and the black profile in panel D). The typical topographical profiles of naked and HA-coated particles are illustrated in panel D of Fig. 2. The values of height and diameter of SiMAG particles coated by HA (using oligo-HA4) typically increased in order of tens of nanometers with respect to the naked ones (see. Fig. 2, panel D). Unfortunately, due to the inner structure as well as broad distribution of the shapes and diameters of naked particles with respect to the assumed HA-coating thickness (85 nm for Dynabeads and 140 nm for p(GMA-MOEAA)-NH_2 particles), statistical evaluation provided non-relevant results.

Due to limited prolongation of AFM piezoceramics (see the limits for crystals with heights >4 μm [36]), the p(GMA-MOEAA)-NH_2 particles were analysed using SEM in a similar procedure as described previously [27]. The naked particles were smooth, and after coating by oligo-HA4 we detected no perturbations (Fig. 2, panel E), similarly as with the Dynabeads M-270 Amine particles. The microspheres' diameters were statistically analysed similarly as in [27] and the diameters of the HA-coated spheres increased compared with those of the naked spheres. The microparticle diameter values fit a normal statistical distribution with the average of the diameters of HA-coated particles (d = 4.82 μm) > naked (d = 4.54 μm) microspheres with a standard error of 0.03 μm (Fig. 2, panels E and F). This result shows the thickness of the HA-coating to be approximately 140 nm. All of these findings together clearly confirmed that the surfaces of all tested magnetic particles were modified by an HA-layer with stable linkage.

The mechanical behaviour and thickness of the water layer on the surface of the naked/HA-coated Dynabeads M-270 Amine particles were analysed in force-spectroscopy mode [28,29]. The stiffness expressed by the slope of curves (Fig. 3, panel A) was not

changed within the detection limits for this experimental set-up (the values of the slope are similar). A statistically significant increase was detected in the thickness of the adsorbed water layer determined as the length between the jump-to-contact point and the point of equality between the attractive and repulsive forces (see arrows in Fig. 3, panel B) determined for >30 nm particles. The mean values of water layer thickness increased from 14 nm to 36 nm with standard error of 0.5 nm for the naked and HA-coated particles, respectively, which corresponds to the high hydrophilic character of HA in comparison to other biopolymers [9]. The narrow distribution of water layer thickness on the HA-coated particles is a consequence of the fully HA-covered surface of the microparticles.

Similar to the aforementioned results, an increase in water layer thickness was detected on the SiMAG particles. The thickness was significantly increased for the HA-coated particles (24 nm) in comparison to the naked particles (13 nm with standard error of 0.5 nm), as can be seen in Fig. 3, panel B. Thus, the presence of a homogeneous HA-coating was proven also for SiMAG particles.

In addition, the level of non-specific sorption, the tendency of particles to agglomerate, the degree of adhesion to various types of solid planar materials, and also the compactness of the HA-layer were evaluated as characteristics important for verifying the suitability of utilizing HA-coated particles in bioaffinity assays, primarily for protein analysis performed even in a microfluidic layout. Knowing most of proteins is slightly negatively charged under the physiological conditions (approx. pH 6.8 – 7.5) and assuming that the carrier is also negatively charged, the non-specific interaction between the surface of HA-coated microparticles and biomolecules from sample has to be reduced significantly. Under such reaction conditions the priority is given to the specific interaction between ligands and target analytes. Our experiments with bovine serum albumin (BSA) as an inert protein usually

applied for monitoring of non-specific sorption have confirmed our premise. The amount of BSA non-specifically adsorbed onto the surface of naked and HA-coated magnetic carriers was quantified by Micro BCA protein assay. The results (Fig. 4) indicate a significant reduction of non-specific sorption on the surface with HA-layer, as values were approximately half those of the naked microparticles.

Particles for microfluidic applications must have excellent superparamagnetic characteristics [37]. Increased iron content with no leaking of magnetic materials is a prerequisite for microparticles intended for microfluidic integration. Only such particles can be fixed in the separation channel of a microfluidic device through all steps of procedures. One method for preparing such highly magnetized particles is that of multistep swelling and polymerization followed by repeated precipitation of iron oxides inside the pores [24]. In this case, however, the iron ions are easily accessible on the surface of particles and they may be responsible for undesired interference reactions, such as decrease or even absolute loss of bioactivity as a result of redox reaction. It is therefore necessary to ensure that labile biomolecules will not be in direct contact with such metal ions. We had expected that HA-coating could efficiently suppress this side effect. Newly synthesized p(GMA-MOEAA)-NH_2 microparticles having such characteristics were used for the next set of experiments to evaluate the electrochemical response stability of hydrogen peroxide over time after contact with such magnetic particles (see 2.5). The results obtained (Fig. 5) illustrate the protective effect of HA-coating. The LSV voltammogram of hydrogen peroxide (panel A) demonstrates a stabilized response through time due to the suppression of redox reactions. The interaction of naked particles with hydrogen peroxide (1 mg L^{-1}) caused instability of electrochemical response as a result of redox reaction with accessible iron ions (panel B).

Finally, we tested the behaviour of such magnetic particles in the separation channel of various microfluidic devices (see 2.6). Frequently occurring phenomena in such procedures include plaque formation and blocking of the inlet and outlet by large clusters of magnetic particles due to agglomeration and surface adhesion, especially in cases of microfluidic devices having intricate geometry. These problems can arise particularly with newly developed, home-made microfluidic devices, but their occurrence is not rare even in using commercially available chips. Naked and HA-coated (by oligo-HA4) p(GMA)-MOEAA-NH_2 magnetic particles were optically detected in the channels in order to test their behaviour. We used commercially available microfluidic devices with a hydrophilic-treated channel, where optimal behaviour is assumed. We monitored the behaviour and the rate of adhesion of HA-coated as well as naked particles. In comparison with naked particles, those which were HA-coated evinced easier filling further into the channel, formation of more homogenous suspensions, and prompt response to an external magnetic field. The degree of adhesion of the tested microparticles was assessed according to the size and optical density of plaque formation on the inner surface of the channel after rinsing. The rate of this parameter was densitometrically evaluated using ImageJ analysis software.

Figure 6 shows an image of a microfluidic channel after the filling and removing of naked magnetic particles versus those coated using oligo-HA4 (panel A) and densitometric evaluation as to the presence of adhered particles within the channel represented as the length profiles of relative optical density through the channel (panel B). The relative optical density corresponds with the rate of particle adhesion. The plot shows substantially decreased optical density in the defined part of the channel after the experiment with the HA-coated particles compared with the naked particles. This indicated significantly suppressed adhesion of HA-coated particles on the inner surface of microfluidic channels. The behaviour of the tested particles in the other microfluidic devices utilized, which were made of various materials,

namely topas and zeonor, showed a similar positive effect of HA-coating, and the greater suitability of HA-coated particles for integration into the microfluidic devices was thereby proven. Additionally, considering the reduced non-specific sorption, such particles can be applied in any lab-on-a-chip even for analysing complex biological samples.

4. Conclusion

In summary, we have demonstrated a universal procedure for enhanced post-synthetic coating of various commercial as well as lab-prepared superparamagnetic microparticles. We chose HA, which is a beneficial polymer for surface modification owing to its remarkable physicochemical and biological properties. Due to the compact HA-layer, the HA-coated particles gained qualitatively new characteristics convenient for biomolecules analysis even performed in microfluidic devices. In addition, the hydrophilic HA-layer protects bioactive molecules from toxic metal ions. In this case the functional activity of ligands is protected against steric hindrance and denaturation which can be induced by the contact of ligand molecules with hydrophobic clusters on the surface of naked particles.

The post-synthetic surface treatment and the HA-layer on the surface of microparticles were qualitatively and quantitatively evaluated using various techniques and a panel of characterization methods was introduced. Microscopic techniques, namely SEM, AFM and zeta potential and hydrodynamic diameter measurement were used for characterizing the HA-coated particles. The panel of these techniques proved the presence of a compact HA-layer on the entire surface of the microparticles and the enhanced behaviour of HA-coated microparticles specifically within the channels of magnetic-based microfluidic devices.

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Abbreviations:

AFM – atomic force microscopy

BCA – bicinchoninic acid

BSA – bovine serum albumin

COC – cyclo-olefin copolymer

COP – cyclo-olefin polymer

CTAB – cetyl trimethyl-ammonium bromide

EDC – 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride

HA – hyaluronic acid

LSV– linear sweep voltammetry

MES – 2-(N-Morpholino)ethanesulfonic acid

OPD – o-phenylendiamine

p(GMA-MOEAA)-NH_2 – poly[glycidylmethacrylate-(methacryloyloxy)ethoxy]acetic acid)amine

PALS – phase analysis light scattering

PMMA – polymethylmethacrylate

SEM – scanning electron microscopy

sulfo-NHS – N-hydroxysulfosuccinimide sodium salt

Figure 1

Click here to download high resolution image

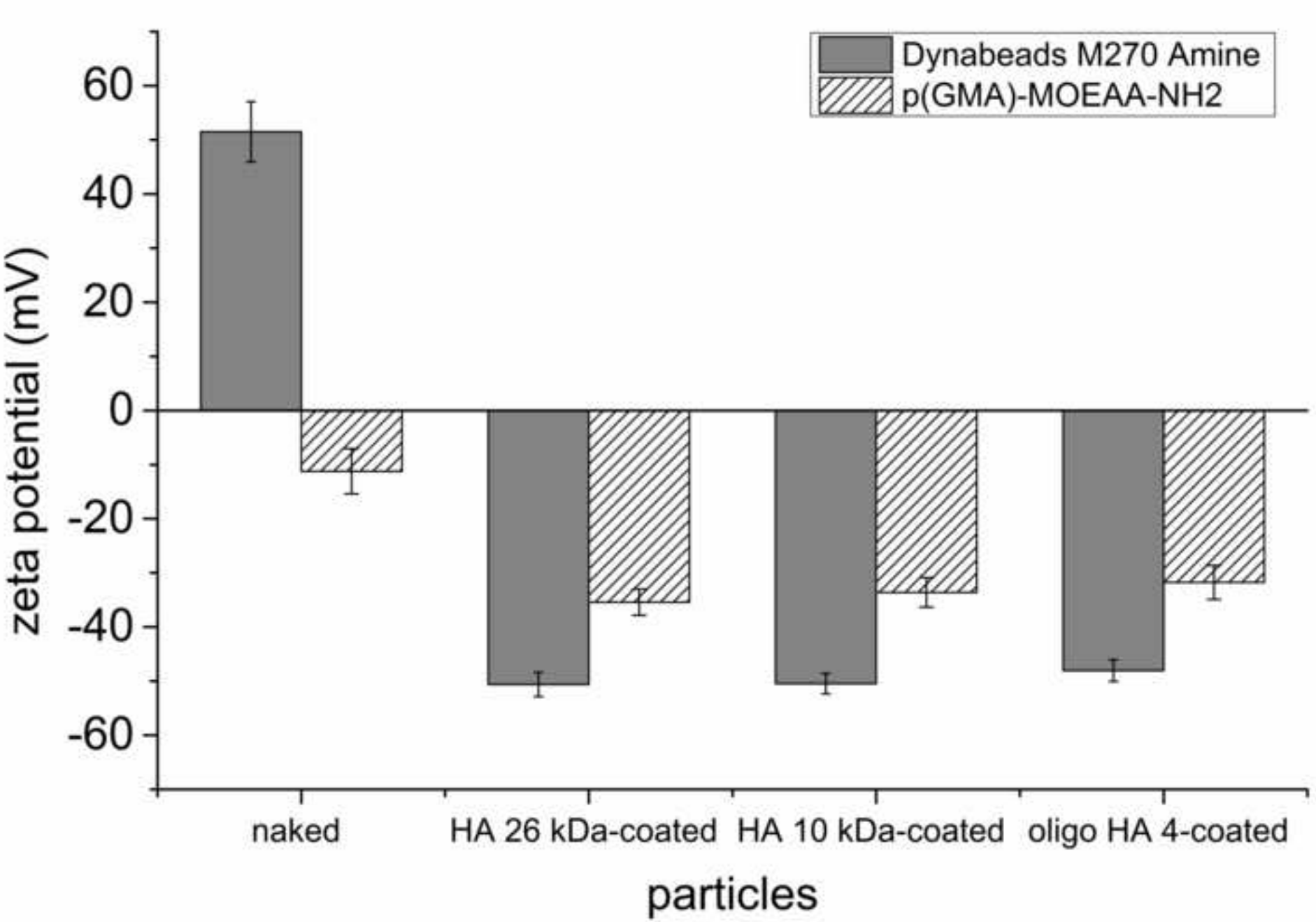
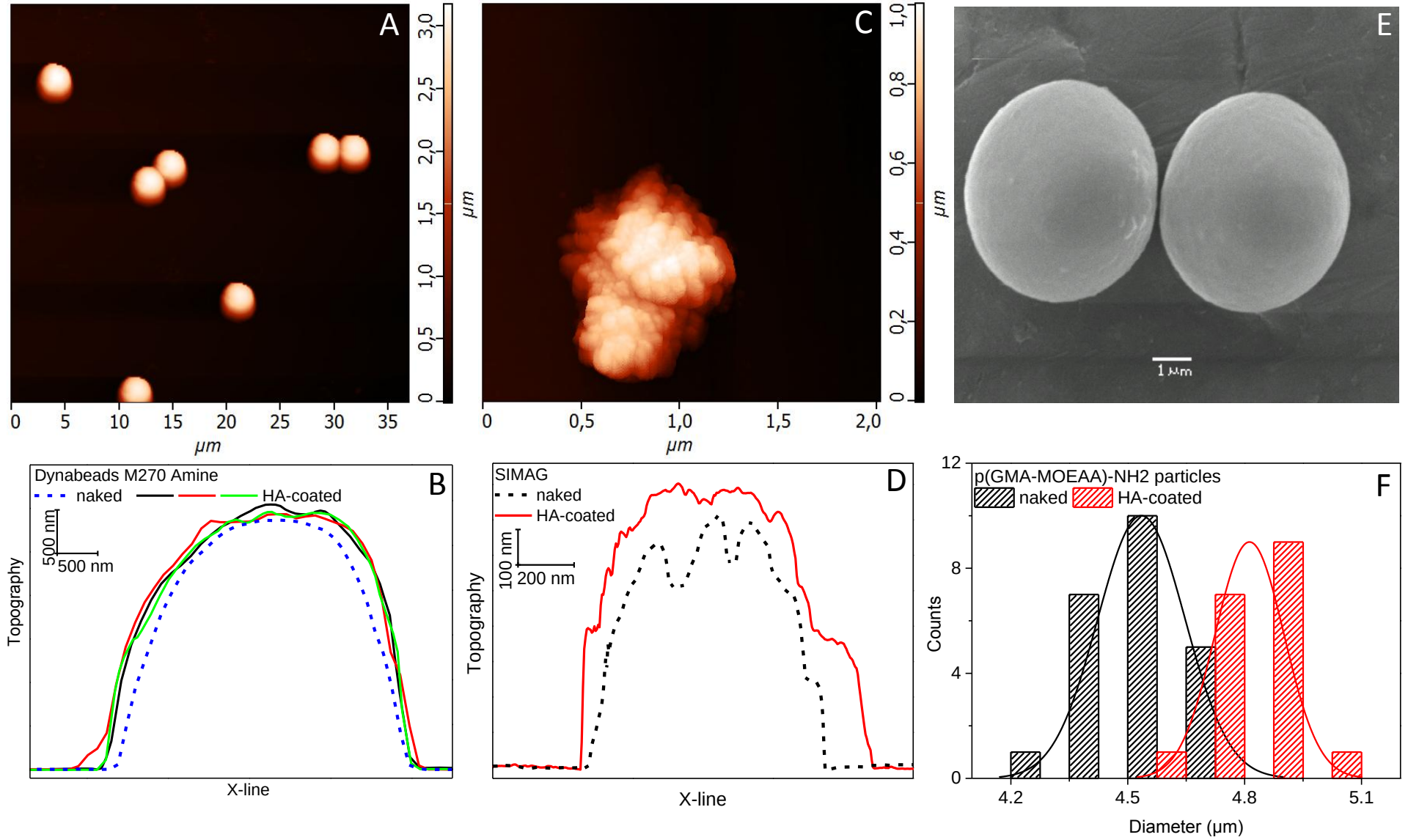


Figure 2


A
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Topography
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naked
HA-coated
100 nm
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D
Topography
X-line
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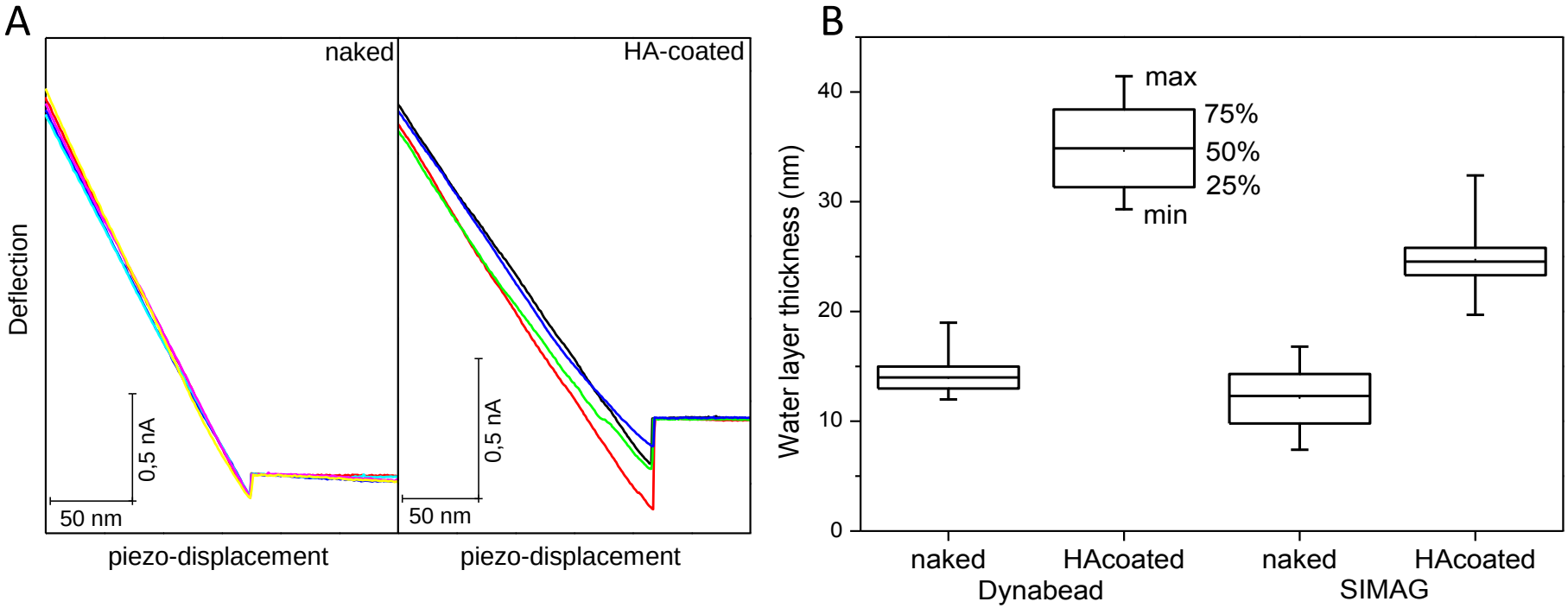
naked
HA-coated
F
Counts
Diameter (μm)

Figure 3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30
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naked
HAcoated
Dynabead
SIMAG

Figure 4

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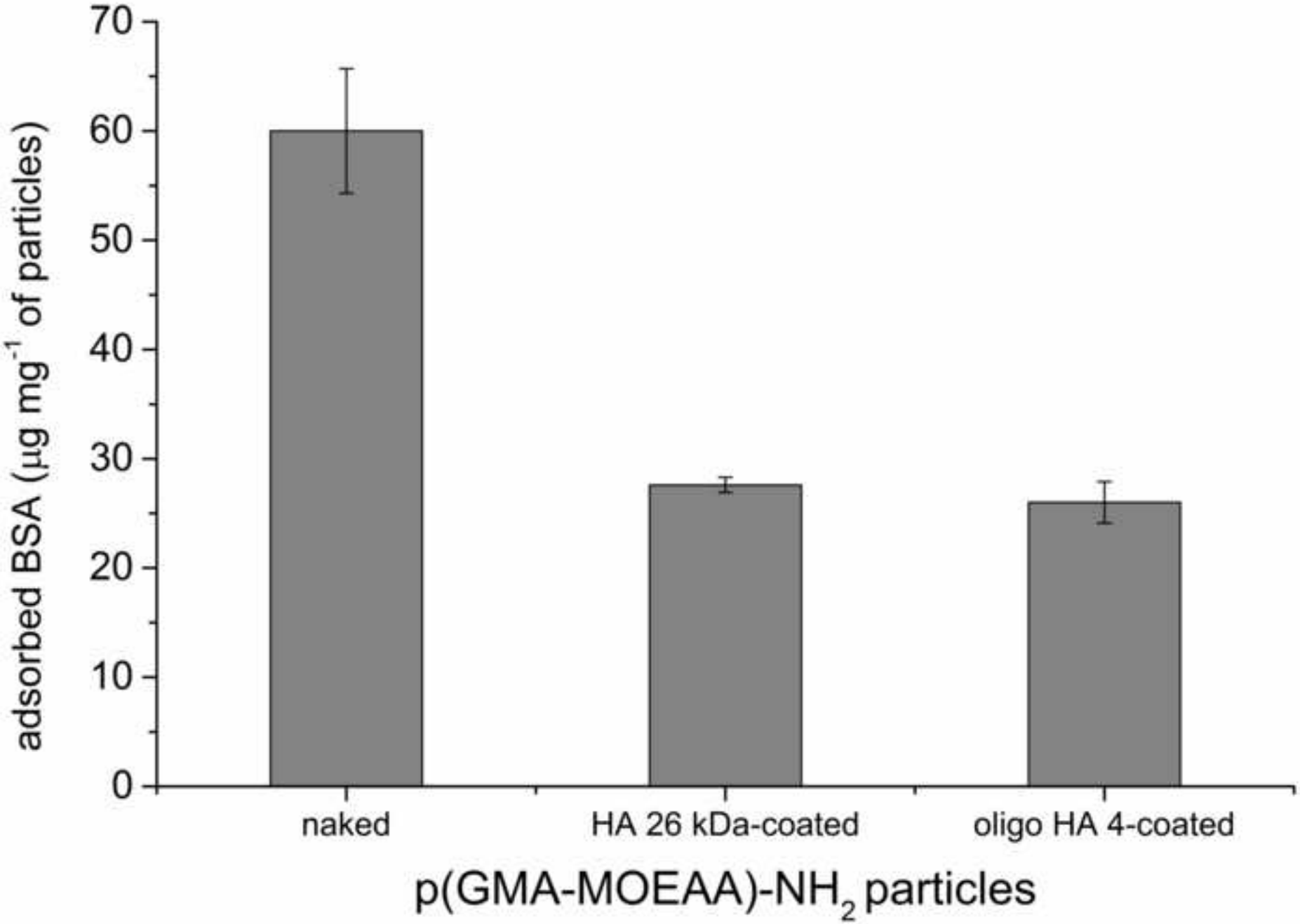
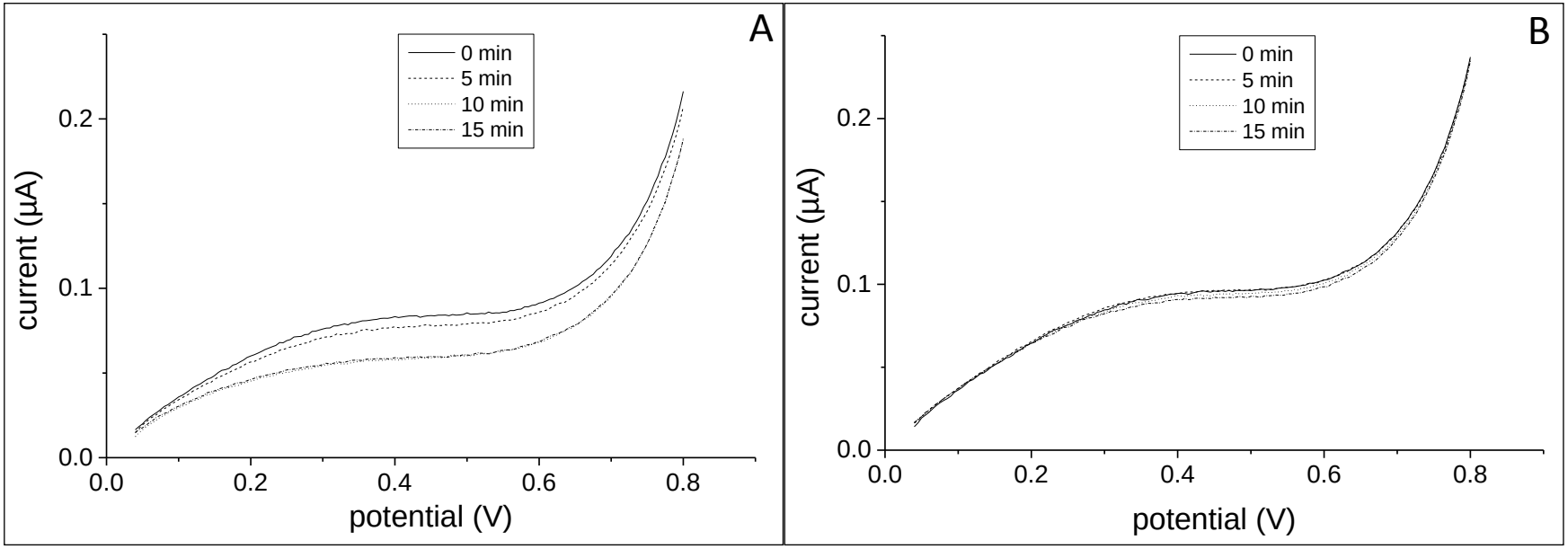


Figure 5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B
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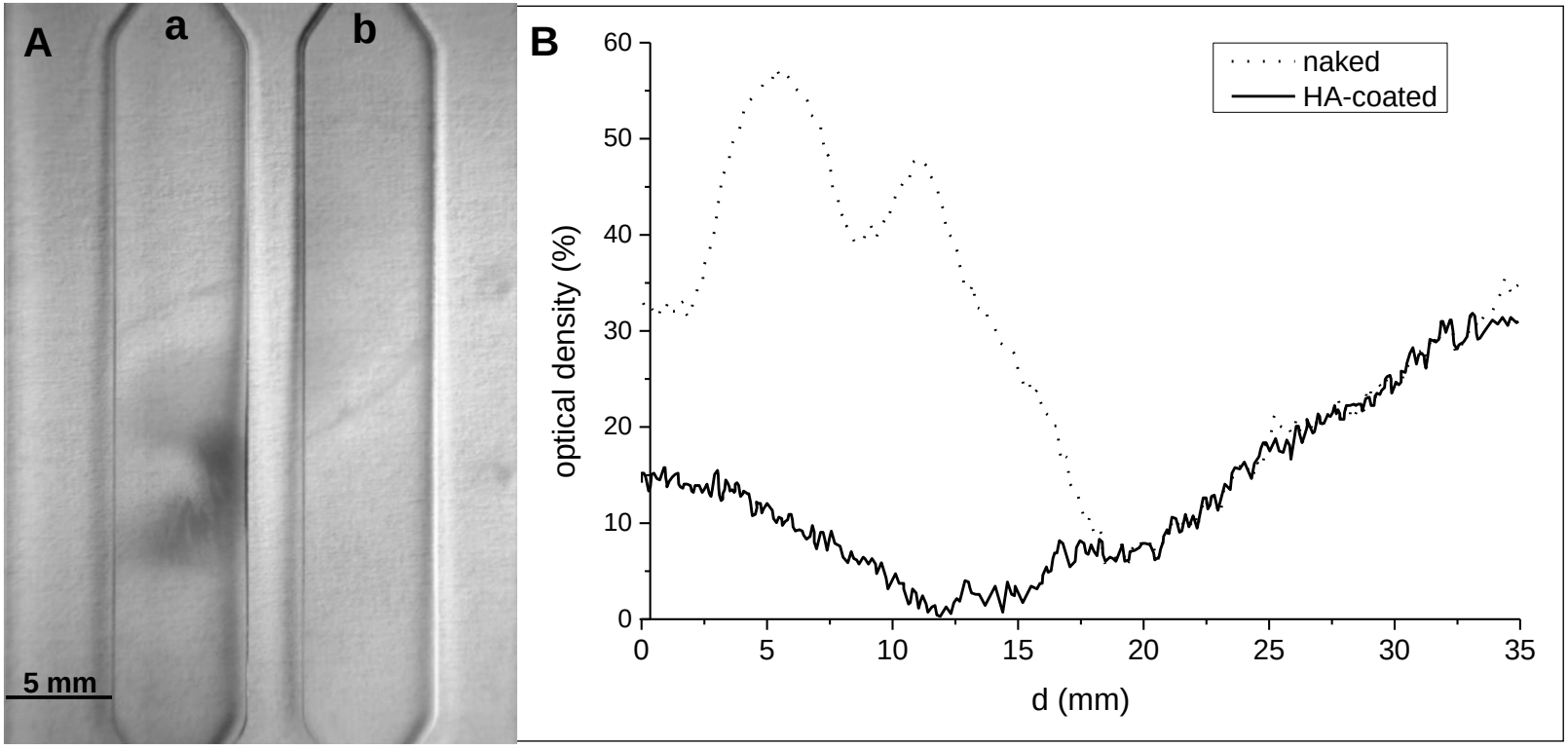
Figure 6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