

# Protein-functionalized magnetic iron oxide nanoparticles: time efficient potential-water treatment

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**Abstract** Recent advances in nanoscience suggest that the existing issues involving water quality could be resolved or greatly improved using nanomaterials, especially magnetic iron oxide nanoparticles. Magnetic nanoparticles have been synthesized for the development and use, in association with natural coagulant protein for water treatment. The nanoparticles size, morphology, structure, and magnetic properties were characterized by transmission electron microscope, X-ray diffraction, and superconducting quantum interference device magnetometry. Purified *Moringa oleifera* protein was attached onto microemulsions-prepared magnetic iron oxide nanoparticles (ME-MION) to form stable protein-functionalized magnetic nanoparticles

(PMO+ME-MION). The turbidity removal efficiency in both synthetic and surface water samples were investigated and compared with the commonly used synthetic coagulant (alum) as well as PMO. More than 90 % turbidity could be removed from the surface waters within 12 min by magnetic separation of PMO+ME-MION; whereas gravimetrically, 70 % removal in high and low turbid waters can be achieved within 60 min. In contrast, alum requires 180 min to reduce the turbidity of low turbid water sample. These data support the advantage of separation with external magnetic field (magnetophoresis) over gravitational force. Time kinetics studies show a significant enhancement in ME-MION efficiency after binding with PMO implying the availability of large surface of the ME-MION. The coagulated particles (impurities) can be removed from PMO+ME-MION by washing with mild detergent or cleaning solution. To our knowledge, this is the first report on surface water turbidity removal using protein-functionalized magnetic nanoparticle.

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## Introduction

Functionalized nanomaterials are known to display novel and significant physical, chemical, and biological properties due to their structure, size, and large specific

surface area (Liao and Chen 2002; Mikhaylova et al. 2004; Grecu et al. 2008). For this reason, device that encompasses nanomaterials/nanotechnology has the potential to improve water quality, viability, and accessibility of potable water (Li et al. 2008; Liu et al. 2008; Theron et al. 2008). The use of natural materials like coagulant protein from *Moringa oleifera* associated with eco-friendly compounds may represent another possible solution to the existing concerns in water treatment process. The availability of the natural material and competing food source is often a risk for long-term solution. The functionalization of magnetic iron oxide nanoparticle with protein is appealing; especially because of its several advantages; such as, low cost of materials, high efficiency, reduction of chemical usage including sludge volume, and fast separation due to its magnetophoretic properties (Okoli et al. 2011a). This method also has great potential for material recycling, which makes it very advantageous for large-scale treatment process. Moreover, natural protein incorporated on magnetic nanoparticles constitutes an excellent alternative for chemical usage in water treatment process.

Availability of clean and safe water is a major problem in both developed and developing countries. At present, the world is facing difficult challenges in meeting the increasing demands of potable water due to population growth, increasing pollution of water bodies from several industrial and agricultural activities, drought, and competing demands from a variety of users (Vorosmarty et al. 2000; Lee and Schwab 2005; Moe and Rheingans 2006; Coetser et al. 2007; Theron et al. 2008).

There are concerns regarding the quality and quantity of water globally. However, to provide potable drinking water, various technologies have been developed and used over a century (Cloete 2010).

The conventional method for water treatment involves the use of mechanical, physical, and some chemical approaches. The choice of chemicals in water treatment is decisive. The treatment process may add substances to the water, which can increase the risk of diseases to human health. Moreover, high impact on the production of large volume of sludge, increase in water pH as well as scarcity of foreign currency for importing these chemicals especially in third world countries are of great concern (Sanchez-Martin et al. 2010). Even though different treatment processes exist to improve the water quality, disease outbreaks is often reported worldwide.

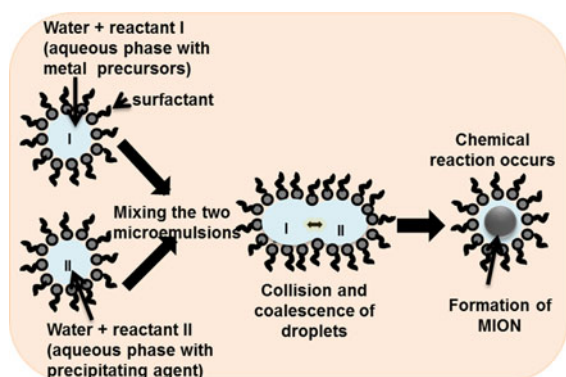
Considering the importance of potable drinking water globally, and keeping in mind concerns regarding the viability of recent practices of meeting the rising water demands, there is a pressing need to develop novel technologies and materials that will tackle the challenges associated with safe drinking water. While new technologies are being developed today for example nanofiltration, reverse osmosis, etc., (Cloete 2010) the need to have cost-effective, user-friendly, robust, and more efficient systems than the existing techniques for the removal of contaminants from drinking water either in water treatment process or in situ are considered paramount. The emergence of nanoscience has been identified as a promising technology that could play a major role in solving several complications involving water treatment and quality (Theron et al. 2008).

In this study, the development and application of magnetic iron oxide nanoparticles prepared from microemulsion that incorporates natural protein for coagulation/flocculation in water treatment is reported. The magnetic nanoparticles were characterized by transmission electron microscope (TEM), X-ray diffraction (XRD), and superconducting quantum interference device magnetometry (SQUID). Purified *Moringa oleifera* (PMO) coagulant protein was incorporated onto the magnetic nanoparticles to form PMO+ME-MION. The comparison of coagulation activity in terms of turbidity reduction for synthetic and surface waters (high and low turbidity) using PMO+ME-MION, PMO, bare ME-MION, and aluminum sulfate (alum) was also investigated. Effect of separation with magnetic field and force of gravity was compared and reported. Regeneration potential of the PMO+ME-MION was evaluated and discussed.

## Materials and methods

### Microemulsion-prepared magnetic iron oxide nanoparticles

The synthesis of ME-MION was carried out by *water-in-oil* microemulsion method. Two microemulsions were mixed (Fig. 1). Each microemulsion system consists of a surfactant, cetyl trimethyl ammonium bromide (CTAB), a co-surfactant (1-butanol), oil phase (hexanol), iron precursors (FeCl<sub>3</sub> and FeCl<sub>2</sub>), precipitating agent (32 % NH<sub>3</sub>), and water (Milli-Q



**Fig. 1** Scheme for the preparation of ME-MION by *w/o* microemulsion reaction approach mixing two microemulsions of identical composition except for reactant I (metal precursors) and reactant II (precipitating agent)

water). The first microemulsion consists of aqueous solution of iron precursor while the second microemulsion contains a precipitating agent (aq.  $\text{NH}_3$ ). Both microemulsion mixtures were homogenized. Precipitation of ME-MION was achieved by adding the first microemulsion into the latter at room temperature upon vigorous stirring for 1 h. The obtained ME-MION was washed and either dried at 70 °C or suspended in Milli-Q water until further use. More detailed synthetic procedures are given (Okoli et al. 2011a).

#### Characterization techniques

The size and morphology of the ME-MION were examined by high-resolution transmission electron microscopy (HRTEM), which was carried out using a field emission transmission electron microscope, JEM-2200FS, 200 kV, with 0.19 nm resolution in TEM mode. For this purpose, dry powder sample was dispersed in isopropanol and deposited onto a formvar carbon copper grid. Selected area electron diffraction patterns (SAED) of the nanoparticles were also investigated from the electron micrographs (data not shown). X-ray diffraction data of the microemulsion-prepared nanoparticles were performed between 20 and 70° on Siemens D5000 diffractometer using  $\text{Cu K}\alpha$  radiation. Room temperature magnetic hysteresis loops of the nanoparticles were measured using a superconducting quantum interference device (SQUID) magnetometer, quantum design MPMS XL. The magnetic moments of the particles were determined in field over a range  $\pm 60\text{kOe}$ .

#### Analytical water source

The water samples for this study were collected from different lakes in Sweden. Raw surface waters with initial turbidity ranging from 7 to 17 NTU were collected in autumn from Lake Mälaren, west of Stockholm. Arboga (Västmanland) and Vättern-Lake (Göteborg) water were collected during spring. The choice of seasonal variation in the collection of raw water samples is essential to evaluate the performance of coagulants with different turbidity/contaminants. The samples once collected were stored at 4 °C to prevent any bacteria growth. The model water sample was prepared from kaolin clay [14] with initial turbidity range of 150–170 NTU for comparison purposes. Aluminum sulfate (alum) was prepared (5 %) and used as a control. All experiments were carried out in a batch system.

#### Binding of PMO onto ME-MION

The purification of *Moringa oleifera* coagulant protein was achieved according to the method already described (Okoli et al. 2011a; Okoli et al. 2011b). Purified *Moringa oleifera* protein was used for the binding assay. Briefly, ME-MION was equilibrated with 10 mM ammonium acetate buffer, pH 6.7. The binding of PMO protein onto the ME-MION was performed in ammonium acetate buffer followed by 1–2 h incubation period in a rotary mixer (20 rpm) at room temperature. The PMO was adsorbed to the ME-MION. The supernatant was removed by magnetic separation and the resultant PMO+ME-MION was washed with ammonium acetate three times to remove the non-adsorbed protein and other impurities. The protein-functionalized ME-MION was suspended in ammonium acetate buffer and this was termed PMO+ME-MION system.

#### Turbidity removal study with PMO+ME-MION

The turbidity removal efficiency of the PMO+ME-MION has been examined in synthetic turbid water (clay solution). In a typical experimental setup, PMO+ME-MION was added to clay suspension and then mixed instantly. Absorbance at 500 nm was measured with Thermo Spectronic UV–visible spectrophotometer at time 0 and after 60 min settling time. In order to estimate the percentage turbidity removal

for different samples after sedimentation by force of gravity, the following equation was used:

$$\text{Turbidity removal (\%)} = \frac{I_T - F_T}{I_T} \times 100 \quad (1)$$

where  $I_T$  is the initial turbidity and  $F_T$  is the final turbidity.

#### Time kinetics investigation

A simple and quick method to verify the performance of PMO+ME-MION in turbid waters has been developed. This technique was utilized for easy screening of active coagulant from different coagulant samples over a range of time. The following coagulant agents *i.e.*, alum, PMO, PMO+ME-MION, and bare ME-MION were investigated.

For the kinetic study, each coagulant agent was mixed with the turbid water source in a vial. The sample solution in each vial was mixed rapidly at 50 rpm for 5 min to allow the optimal contact between the sample waters and the coagulants. Initial turbidity of the solution was measured. The measurements were carried out periodically (0–240 min) during their settling time to monitor the optimal time needed to achieve good turbidity reduction. The synthetic clay solution, different surface water samples, and bare ME-MION was used as a negative control.

#### Separation by magnetic field or force of gravity

The magnetization effect of PMO+ME-MION was compared with the force of gravity (alum) in coagulation/flocculation of particles in turbid water samples. For this reason, a quick method was developed to achieve efficient turbidity reduction and separation with the aforementioned system over a short period of time using applied external magnetic field. Synthetic clay solution and natural surface water samples (SW1; 7 NTU and SW2; 17 NTU) from Lake Mälaren were used. PMO+ME-MION and alum were added to the turbid water sample and mixed instantly; the initial turbidity of the mixture was measured. The resultant mixture was allowed to interact for 10 min at room temperature. After the interaction time, an external magnetic field was applied for 1–2 min to separate the clean water solution from the PMO+ME-MION system while the water samples containing alum were decanted due to settling by gravity. Final turbidity of the treated water was measured.

#### Regeneration of PMO+ME-MION

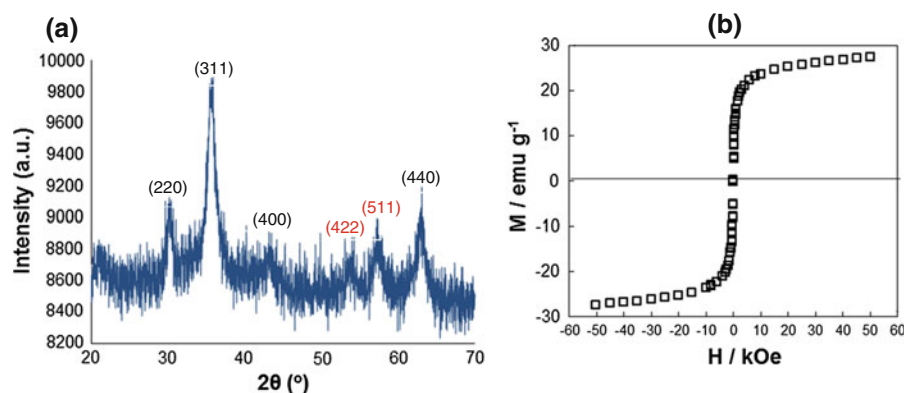
The possibility of regenerating the PMO+ME-MION system was investigated in this work. Reusability potential of PMO+ME-MION (*i.e.*, after removal of flocculated impurities) was studied in three successive reuse. PMO+ME-MION were cleaned from the impurities adsorbed in turbid water sample (synthetic clay solution) by washing with mild detergent or cleaning solution. Two different cleaning agents were compared (20 % ethanol and 0.1 wt% Tween 20 in water). The mixture was left for 10 min at room temperature. The supernatant was removed by magnetic separation and the washing was repeated once again followed by 2× wash with 10 mM ammonium acetate buffer. The turbidity removal assay was performed as described before (Eq. 1). The regeneration step was repeated twice; the control sample was calculated initial percent turbidity removed using PMO+ME-MION in water sample before cleaning.

## Results and discussion

#### Synthesis and characterization techniques

The formation of ME-MION in *w/o* microemulsions can be achieved by mixing two *w/o* microemulsions of identical composition, one comprising the inorganic salt precursor and another one comprising the precipitating agent in the aqueous phase nano-droplets. The size, morphology, and agglomeration of prepared ME-MION were examined by HRTEM (Okoli et al. 2011a). The obtained data depicts that ME-MION consisted mainly of elongated, rod-like morphologies with widths ranging from 5 to 10 nm (Okoli et al. 2011a). Diffraction pattern of the dry powder indicates the presence of a magnetic phase of either  $\text{Fe}_3\text{O}_4$  or  $\gamma\text{-Fe}_2\text{O}_3$ ; which is quite typical at  $35^\circ$  for (311) and  $63^\circ$  for (440). The XRD pattern of ME-MION (Fig. 2a) shows that the nanoparticles correspond to crystalline magnetite which reflects to the well matching of the diffraction peaks with magnetite pattern (Deng et al. 2005; Okoli et al. 2011a).

Magnetic moment of the ME-MION was determined at room temperature (300 K) in field over a range  $\pm 60\text{kOe}$ . It can be clearly seen that the magnetization curve for ME-MION displayed no magnetic remanence and coercivity, confirming a



**Fig. 2** X-ray diffraction pattern (a); and SQUID showing magnetization as a function of magnetic field (b); of ME-MION

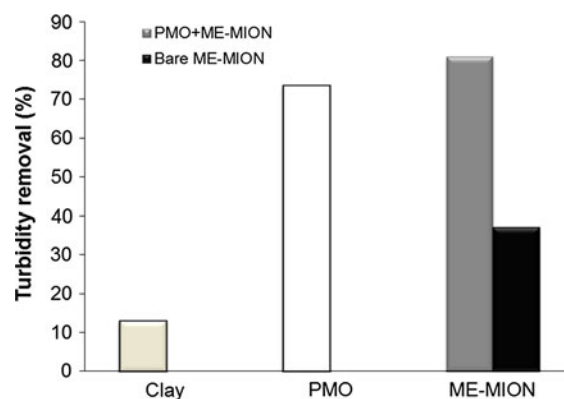
superparamagnetic behavior and nanoscale dimensions of the particles (Fig. 2b). The recorded saturation magnetization ( $M_s$ ) value for ME-MION is 30 emu/g.

#### Turbidity removal study with PMO+ME-MION

The PMO+ME-MION and PMO demonstrated significant coagulation capacity when assayed on the synthetic water sample. The efficiency of PMO+ME-MION improved after binding with PMO. Formation of flocs and reduction in turbidity of the clay suspension indicate better performance of the coagulants. The weight effect of PMO+ME-MION improved the separation speed of the flocs as the magnetized flocs formed around them are much heavier than suspended particles. Results from Fig. 3 also suggest that bare ME-MION can aggregate and flocculate the suspended particles in turbid water samples. Larger flocs can be formed by the collisions of bare nanoparticles with the turbid water sample, resulting in high density mass of ME-MION.

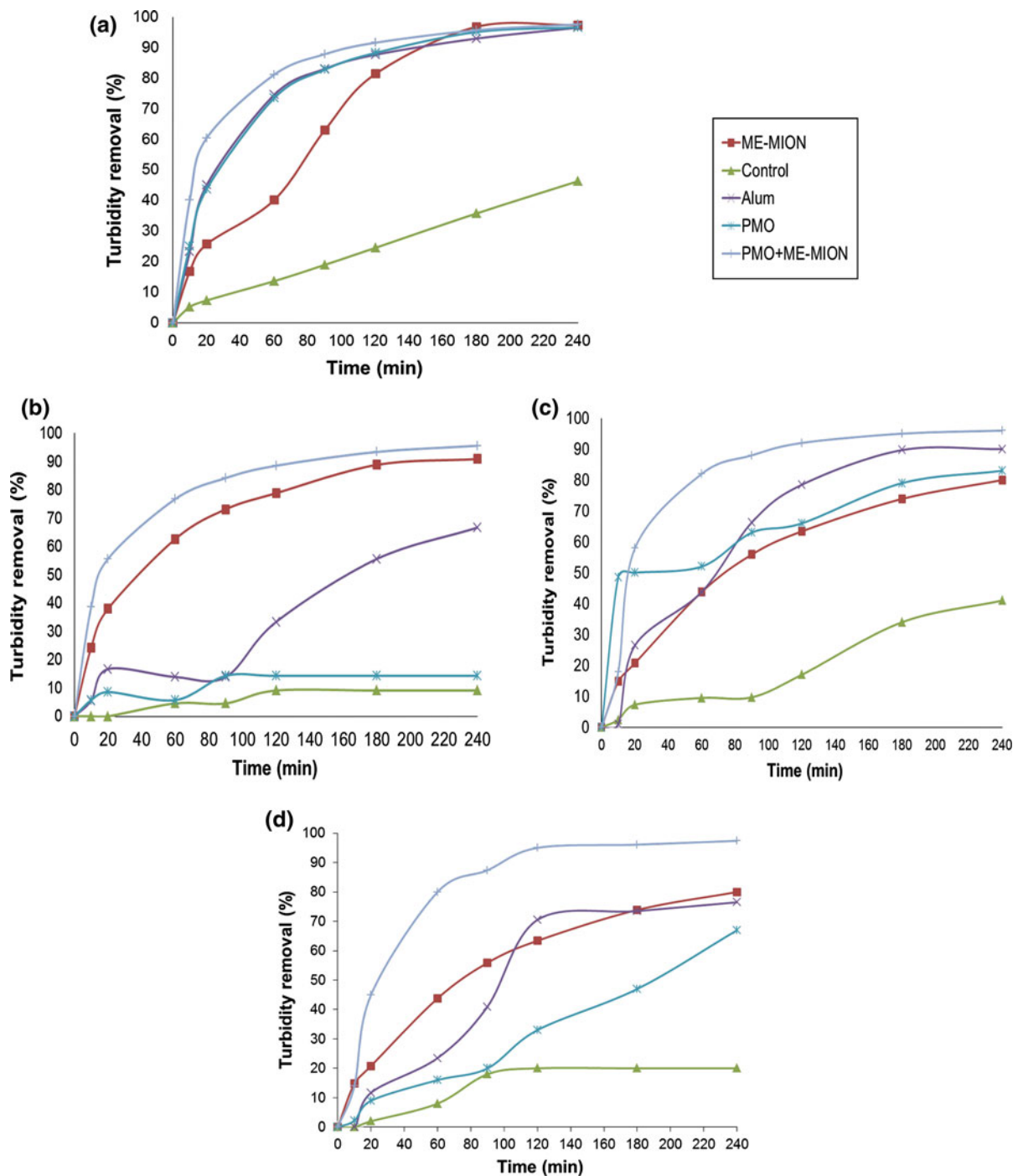
#### Time kinetics investigation

The present study was carried out to elucidate the performance of some coagulant agents in raw surface waters during different seasons. Results showing the optimal time required to achieve an efficient turbidity reduction with different coagulant agents have been shown in Fig. 4. The PMO+ME-MION showed a significant decrease in turbidity when tested on both synthetic clay solution (Fig. 4a) and natural water



**Fig. 3** Results showing turbidity removal at 60 min of separation time with purified moringa protein (PMO) and PMO+ME-MION in synthetic clay solution. Clay solution and bare ME-MION was used as controls

samples (Fig. 4b–d). The efficacy of the coagulant agents depend on the initial turbidity of the water sample (Ghebremichael et al. 2005; Sanchez-Martin et al. 2010). In this study, it is observed that the efficiency of PMO+ME-MION is not influenced by the initial turbidity of the water sample; for instance in 60 min, PMO+ME-MION showed approximately 80 % removal efficiency in both waters collected during autumn and spring seasons. However, it was evident that the high density mass of the magnetized flocs induced by electrostatic interaction resulted in fast flocculation of impurities in the water sample. On the contrary, alum and PMO were found to be less effective, especially in water sample collected during autumn season (Fig. 4b). However, alum and PMO were effective on surface waters collected during spring season (Fig. 4c, d). In addition, it can be

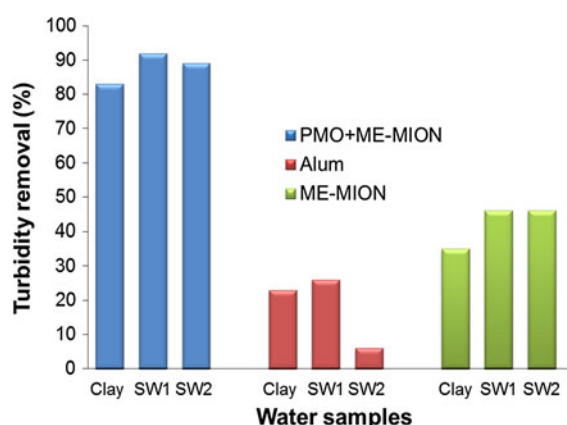


**Fig. 4** Kinetic studies showing percent turbidity removal with: **a** synthetic clay solution and surface waters from **b** Lake Mälaren (autumn season), **c** Arboga water (spring season), and

**d** Vättern-Lake (spring season). Control represents water sample without coagulant agents

observed that the efficiency of alum and PMO during spring season also depends on the composition of the source water. For instance, both alum and PMO

showed 40–50 % turbidity removal with Arboga water (Fig. 4c) in 60 min; whereas, 10–20 % removal was observed with Vättern-Lake (Fig. 4d) in 60 min.

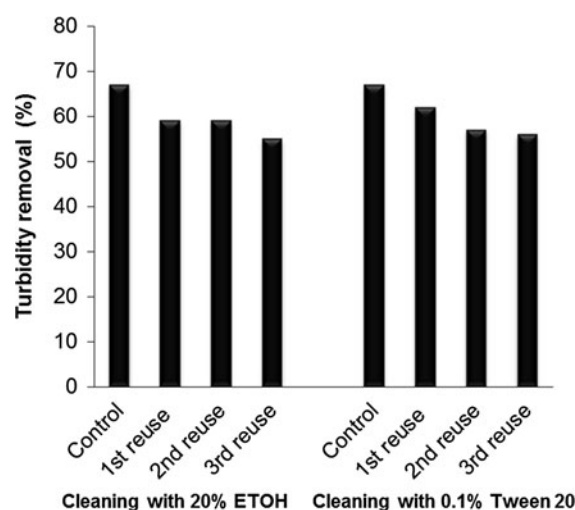


**Fig. 5** Turbidity removal result showing the effect of magnetic field separation of suspended particles in natural and synthetic turbid water samples, with PMO+ME-MION and alum after 10 min interaction time and 2 min separation. SW1 and SW2 represent surface water (7 and 17 NTU, respectively) from Lake Mälaren. ME-MION was used as control

Experimental results indicate that the performance of PMO+ME-MION, alum, PMO, and bare ME-MION follow a similar trend in natural surface waters while the bare ME-MION showed lower removal performance in synthetic clay solution. Nonetheless, bare ME-MION showed good performance in water collected during autumn season than that collected during the spring season. From this study, it can be established that the PMO+ME-MION is very efficient irrespective of the season or composition of the raw water. The experimental data clearly depicts that the developed PMO+ME-MION may serve as a viable complementary coagulant agent in water treatment process.

#### Separation by magnetic field or force of gravity

The influence of external magnetic field in the performance of protein-functionalized nanoparticles has been compared with gravitational force, in the clarification of turbid waters (Fig. 5). Experimental results show that PMO+ME-MION tested on natural water samples exhibit good turbidity removal. With PMO+ME-MION, turbidity removal of 75–90 % can be achieved within 12 min, due to the effect of magnetic field. According to magnetization curve theories, when nanoparticles are placed within a steady external magnetic field, the internal magnetic moment will spin in the same direction as the external magnetic



**Fig. 6** Regeneration of PMO+ME-MION after turbidity removal assay with clay solution. Cleaning capacity of 20 % ETOH and 0.1 % Tween 20 were compared after 90 min of coagulation activity test. Control sample represents the initial coagulation activity of PMO+ME-MION before cleaning

field. This magnetophoretic occurrence will boost the magnetic properties of the nanoparticles thereby causing the aggregation of the water impurities (Lu et al. 2007; Faraji et al. 2010). The existence of this dynamics will induce the aggregation of the water contaminants by nanoparticles. The combination of PMO+ME-MION and applied magnetic field enhanced the effectiveness of the system in coagulating/flocculating impurities in different turbid water samples. Bare ME-MION showed lower turbidity removal as compared to PMO+ME-MION. This suggests that the binding of the protein onto the ME-MION was critical in enhancing their performance. Comparing the time kinetics study and separation by magnetic field (Figs. 4, 5), the time efficient removal of turbidity with PMO+ME-MION was evident. Moreover, in contrast to aluminum sulfate (alum), the turbidity removal of PMO+ME-MION presented high level of consistency in all the assayed water samples.

#### Regeneration of PMO+ME-MION

In order to examine the reusability of the PMO+ME-MION, the developed system was washed with mild detergent or cleaning solution after separation. Turbidity removal assay reveals that it is possible to reuse the PMO+ME-MION three times (Fig. 6). In comparison with the control sample (initial percentage

removal of PMO+ME-MION before washing), cleaning with 20 % EtOH and 0.1 % Tween 20 showed almost similar turbidity removal in the clay solution. PMO+ME-MION, on cleaning with both ethanol and Tween 20, showed a small reduction in performance (removal efficiency) after the first wash; however, a high level of consistency is seen in the successive washing. The most probable reason for the slight reduction in removal efficiency after first cleaning step could be that the clay particles were not completely removed from PMO+ME-MION. However, it is unlikely that the detergent or cleaning solution will desorb the electrostatic interaction between the negatively charged nanoparticles and the positively charged protein. Results from this investigation indicate that 20 % EtOH and 0.1 % Tween 20 have the capacity to regenerate the PMO+ME-MION after several reuses. More investigation is being conducted to fully understand the mechanism of cleaning to improve the efficiency of the system. One advantage of this technique is the reduction in cost of materials and treatment process.

## Conclusions

The prepared magnetic nanoparticles size, morphology, structure, and magnetic properties were characterized by TEM, XRD, and SQUID. The development of protein-functionalized magnetic nanoparticles from microemulsion-prepared nanoparticles (ME-MION) and PMO protein have demonstrated creditable potential in the treatment of high and low turbid surface water samples irrespective of the season or source of water. The present investigation underscores the advantages of water treatment approach that encompasses magnetic nanoparticles with natural coagulant, which offers a novel water treatment approach; since the usage of chemical will be reduced by more than 60 % during the water treatment process. The treatment of surface waters with PMO+ME-MION showed better turbidity removal efficiency when compared to either alum or PMO. In contrast to alum and PMO, reduction in water turbidity is not affected by the initial turbidity of the water sample. The PMO+ME-MION could effectively remove more than 90 % of turbidity in surface waters under the influence of an external magnetic field within 12 min. The combination of PMO+ME-MION and magnetic

field enhanced the effectiveness of the system in coagulating/flocculating impurities in different turbid water samples. Present investigation also suggests the possibility of regenerating PMO+ME-MION in more than three consecutive steps after turbidity removal assay.

The PMO+ME-MION developed in the work will be the first report, to our knowledge, that could potentially offer a cost-effective, robust, and environmentally friendly water treatment process.

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