

Research article

Magnetite Beads Binding to Human Serum Protein

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Abstract.

Serum biomarker analysis of blood protein is often interfered with by the presence of dominant protein fractions such as albumin and globulin when analytical separation is performed. Magnetite solid phase extraction (MSPE) has been shown to be fast, effective and efficient to enable cleanup of proteins. Some studies have reported that magnetite adsorption to albumin is about 40-90% in a pure solution after several hours mixing and homogenizing. Few studies have examined the binding of magnetite to total protein in a human blood matrix. The magnetite coprecipitation method was performed in this study. Coprecipitation of magnetite was carried out by mixing 2:1 FeCl (III):(II) 0.01 M in an aqueous medium for 40 minutes at 90°C and pH 9. The serum protein used was Serodos Plus (lot005, Human.de). Protein determination was performed by the A280 spectrophotometric method. The FTIR pattern of magnetite showed OH stretch at wavenumber 3450-3200 cm^{-1} and Fe-O groups were detected at wave number 600-420 cm^{-1} . The XRD analysis displayed 7-8 peaks of standard magnetite according to the Crystallography Open Database. A particle size of 52 nm was calculated using the shearer equation. Simple SPE was carried out to adsorb about 10-20% of serum protein in a minute mixing vortex and 0.001786 L/mg adsorption capacity. Serum protein cleanup was successfully performed in minutes by magnetite nano particles in an aqueous medium.

Keywords: magnetite, SPE, protein cleanup, proteomics

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1. Introduction

Major problem in protein analysis or proteomics of low abundant protein was the need simple, efficient and faster sample preparation technique such as simplify depletion of unintended high abundant protein and minerals removal from human serum [1]

Magnetite (Fe_3O_3) is an inorganic molecule that has the peculiarities of magnetic force, surface functionalization and easy to synthesize by chemical method [2] or electrochemistry [3] even in facile aqueous medium without inert environment [4,5] These advantages is important to simplify the process of protein separation technology [6] Magnetite can bind directly to macromolecule such as albumin and globulin that high abundant protein fraction in human serum, through hydrophobic or van der Waals interactions as well as covalent bonds [7,8]. Pure albumin bonded to bare magnetite

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within an optimal 2 hours particles or without modification of the surface of the function group [3] or through modification of function groups on the surface [9,10]. Acidic pH of magnetite or surface coating with polymetacrylate is able to bind to fractions of globulin meaningfully [11]. Because the presence of these two fractions can interfere with the examination of low quantity protein in the blood, we need to remove fractions of protein by magnetic beads adsorbent, where it is possible to develop simple, faster and efficient extraction methods.

2. Methods

2.1. Study Design

2.1.1. Magnetite Preparation

Fe₃O₄ nanoparticles were synthesized using 1000 mL of precursor solution containing 0.004 mol iron (III) chloride hexahydrate (Merck, 103943 Supelco) and 0.008 mol iron (II) chloride tetrahydrate (Merck, 103861 Supelco), to which it was added 30 mL of 1 M sodium hydroxide. This mixture was left under constant agitation for 1 h at room temperature and adjusted to pH 9. The final precipitate was separated using a magnet and washed many times with distilled water and ethanol.

2.1.2. Nanoparticle Characterization

2.1.3. Adsorption Study of Serum Total Protein

Nanoparticles samples were characterized based on the X-ray diffractometer patterns obtained from a X-Ray Diffractometer (XRD) Bruker D8 Advance diffractometer with CuK α radiation. The FTIR spectrum was measured using a FTIR spectrometer (Simadzu instruments). The Zetasizer Horiba SZ-100 system (Horiba Instruments) was also used in the zeta potential measurements. Variations in pH were controlled using HCl/NaOH solution.

2.2. Data Analysis

Peaks spectra data analysis of FTIR was using Shimadzu Software. Zeta potential determination and particle size distribution in distilled water was performed in Horiba

software. Crystallinity of magnetite determination was observed in Bruker X-Ray Diffraction that compared to ICCD card phase identification of iron oxide predicted by origin lab software, while particle size estimation was calculated using Debye-Scherrer equation. Non-linear analysis of binding study between X concentration amount protein versus Y concentration of magnetite or iron oxide particle was performed in Ms. Excel with R^2 value > 0.9 as successful strong binding criteria with test of significant value < 0.05 .

3. Results

Magnetite nano particle was precipitated mixing chloride salt iron in aqueous medium to form black precipitate iron oxide at 500 rpm speed mixer. Zeta potential was stable at -37.7 mV that electronegatively higher compared to common standard bare magnetite in aqueous medium with zeta potential (> 35 mV). Table 1 showed the zeta potential and electrophoretic mobility.

TABLE 1: Zeta Potential Measurement of Magnetite nanoparticle.

Peak No.	Zeta potential	Electrophoretic Mobility
1	-37.7 mv	-0.000292 cm^2/Vs
2	— mv	— cm^2/Vs
3	— mv	— cm^2/Vs

To investigate the particle size distribution in aqueous medium, DLS analysis showed the total mean of the polydisperse magnetite was 378.2 nm, minimum 207.9 nm and maximum 500.4 nm. Z-average value 735.5 nm with polydisperse index (PI) 0.659. Interpreting standard of $\text{PI} > 0.5$ reflects high dispersity when particle size increased. However, the size by DLS (*Data Light Scattering*) was at submicron as shown in table 2.

TABLE 2: Particle Size Analysis of Magnetite nanoparticle (nm).

Peak No.	S.P. Ratio	Area	Mean	Standard Deviation	Mode
1	0.42		207.9	22.6	203.7
2	0.58		500.4	68.9	490.5
3	—		—	—	—
Total	1.00		378.2	154.2	490.5

Z-average: 735.5 nm, PI: 0.659

Groups functional assessment of hydroxyl -OH and iron oxide Fe-O from the spectra showed that hydroxyl stretch was at wave number 2962 cm^{-1} ($4400\text{-}4200 \text{ cm}^{-1}$),

while at 499.92 Fe-O was observed. We may conclude that magnetite particle was successfully synthesized and ready to use in experiments.

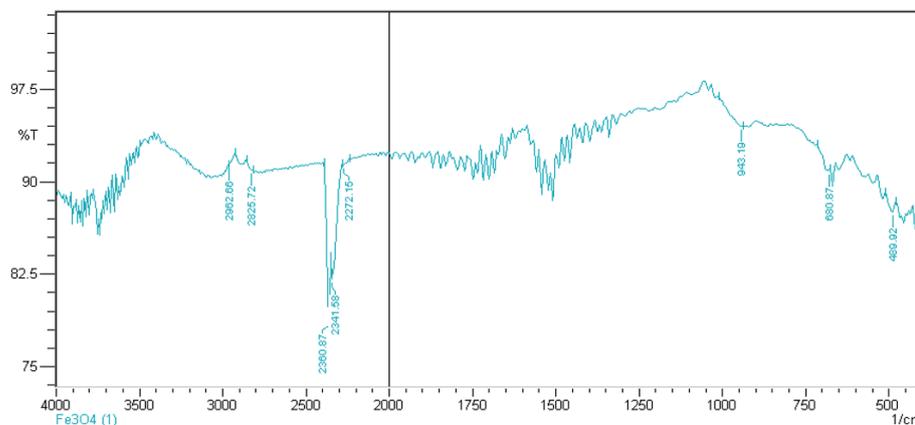
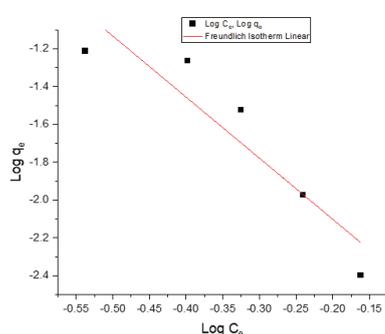


Figure 1: FTIR Spectra of Magnetite Nanoparticle.

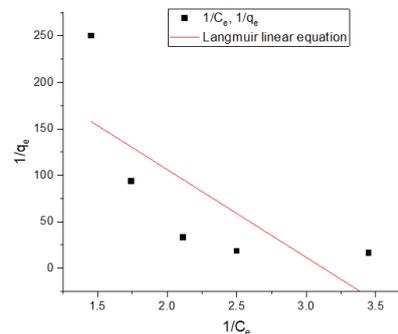
Stretch of hydroxyl groups of magnetite particle has a role in hydrogen bonding to molecules such as protein capture in the sample via hydrogen binding mechanism. Study adsorption isotherm of magnetite and protein will use Freundlich and Langmuir linear equation model.

$$\text{Log } q_e = \text{log } K f + \frac{1}{n} \text{log } C_e \text{ Freundlich linear equation (1)}$$

$$\frac{1}{q_e} = \frac{1}{K_L \cdot q_{max}} \times \frac{1}{C_e} + \frac{1}{q_{max}} \text{ Langmuir linear equation (2)}$$



2a. Freundlich model isotherm



2.b Langmuir model of isotherm

Figure 2: Linear model of Magnetite-protein adsorption.

Figure 2 showed two linear model of adsorption isotherm Freundlich and Langmuir model. Summary of the equation parameters detailed in the table 3.

From table 3, Freundlich equation showed linear model, where K_F is adsorption capacity (L/mg) and $1/n$ is adsorption intensity, The Freundlich adsorption model is used if it is assumed that there is more than one surface layer (multilayer) and the site is heterogeneous, i.e. there is a difference in binding energy on each site. Exponents of

TABLE 3: Summary of linear model in adsorption study of magnetite and serum protein.

Magnetite Protein	Intercept	Slope	1/n	K_f		R^2
Freundlich Linear	-2.74811	-3.23142	-3.23142	0.001786		0.81565
			q_{max}	K_L	R_L	
Langmuir Linear	294.95698	-94.4734	0.0033903	-3.12212	-0.00645	0.40178

1/n are usually less than 1.0 because the site with the largest binding energy is used first, followed by a weaker site and so on. It also indicates the relative distribution of the energy and the heterogeneity of the adsorbate sites. In Langmuir equation model, RL values indicate the adsorption to be unfavourable when $RL > 1$, linear when $RL = 1$, favorable when $0 < 1$, and irreversible when $RL = 0$.

The results table 3, showed that adsorption of serum protein on magnetite surface followed linear model with R square 0.81565 with Freundlich constant value 0.001786 better compared to Langmuir model. However, Freundlich linear equation R square close to 0.9 as strong adsorption criteria. In synthesis, the room temperature environment consistent to other findings that the geometric of particle was cubic [12]. pH controlling and shorter time of homogenizing when Coprecipitation method used is fundamental for faster synthesis in high yield of magnetite. Furthermore, the results reflect high homogeneity in nanoparticle shape and size in the samples studied.

4. Discussion

Several independent factors were fixed variable such as temperature was set to 20-25°C at pH 9 and 500 rpm speed agitation for an hour. The only dependent variable is before and after magnetite-protein binding and interaction after a minute incubation since there was no significant different on qualitative and quantitative incubation between 0.5 to 240 minutes [13]. Zeta potential seems to be charge negatively consistent with degree of pH more basic 9 or 10 [14,15,13]. Particle size showed sub-micron scale to meet term “beads” for the micro-adsorbent [16].

Absorption study was consistent with previously studied by [17] for albumin [18,3] and globulin plasma [13]. A280 nm determination of protein before and after incubation was showed logarithmic regression. Mean of absorbance before magnetite mixing was 0.555 and 0.481 after removing the magnetite beads. This report a fact the facile coprecipitation method was easily to follow [12,5]. Pearson correlation showed 0.973 with significant p-value 0.01. Yield of precipitate of black magnetite about 10-20% adsorption

reported that protein plasma contains several coagulation protein such as fibrinogen, Factor VII that bind to magnetite nanoparticle [13].

The limitation of this study is phase of magnetite particle was not showing high purity <99% because the present of hematite phase. Size of particle was narrow in particle size. Matrix serum was not from native human origin, however it was coming from animal blood and modified by several additives that was causing interferences. R square value was < 0.9, in the future research, this should be longer than a minute to have maximum absorption for multilayer and heterogeneity sample.

5. Conclusions

There was a significant adsorption of magnetite beads to serum protein in minutes and could be used as removal agents for protein contaminants depletion.

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