Analysis of Electro-kinetic Properties of $NiFe_2O_4$ Nanoparticles Synthesized by Co-precipitation Method and its Effect on the Adsorption of BSA

S. V. BHOSALE[1, 2], V. C. HOLKUNDE[2], S. V. BHORASKAR[3], V. L. MATHE[3]

[1]Department of Physics, Sir Parashurambhau College, Pune, Maharashtra, India
[2]Department of Physics, Arts, Science and Commerce College, Indapur, Pune, Maharashtra, India
[3]Department of Physics, Savitribai Phule Pune University, Pune, Maharashtra, India
e-mail: vlmathe@physics.unipune.ernet.in

Abstract

Magnetic $NiFe_2O_4$ nanoparticles were synthesized by chemical co-precipitation method. The adsorption of Bovine Serum Albumin (BSA) protein onto nickel ferrite surfaces was systematically studied. The synthesized nickel ferrite nanoparticles were characterized by X-ray diffraction (XRD) and show the presence of single phase with spinel ferrite structure. The zeta potential measurements were carried out in order to study the variation of surface charge onto the nickel ferrite nanoparticles. It was observed that the maximum zeta potential of 40 mv and -42 mv was obtained at pH 3.64 and 9.66 respectively. The nickel ferrite nanoparticle has iso-electric point at pH 7.3. The measurements for adsorption of Bovine Serum Albumin on nanocrystalline nickel ferrites were carried out at body temperature (37 ^{o}C) for different pH values. The maximum adsorption of BSA was observed at pH 6.49. The high adsorption of BSA at pH 6.49 was observed due to electrostatic attractive interaction obtained due to opposite charges developed in the aqueous medium.

Keywords: $NiFe_2O_4$; Zeta potential; Iso-electric point; BSA.

Introduction

In recent years ferrite nanoparticles are increasingly being used for applications in biotechnology due to both their biocompatibility and attractive magnetic properties like high magnetic susceptibility, low remanence, low coercivity and high saturation magnetization. These properties are making them ideal candidates for applications in MRI, targeted drug delivery, biological separations, enzyme and protein immobilization, cell sorting, RNA and DNA purification and biosensors [1, 2]. The interaction of magnetic nanoparticles with protein is a fundamental phenomenon and many researchers have devoted their efforts in developing protein adsorption experiments and models. Basically, the protein adsorption is influenced by i) surface properties of nanoparticles (surface energy, polarity, charge and morphology), ii) protein properties (size, structural stability and composition and iii) external parameters (pH, ionic strength, temperature, and buffer composition) [3]. Model protein, BSA, has molecular weight of 67.00 kDa and isoelectric point (pI) at 4.7 [4]. Also BSA is the most abundant plasma protein and has an ellipsoidal shape with dimensions of 14 nm x 4 nm x 4 nm [5]. The BSA has amide and carboxylic functional groups, which develop net positive surface charge at pH values below its isoelectric point and net negative surface charge above its isoelectric point.

In the present study, we have analyzed the effect of zeta potential variation of $NiFe_2O_4$ nanoparticles on BSA adsorption. The understanding of this phenomenon will help us in improving the ability to control the conformation, orientation and stability, which allows us to design tailored surfaces for higher bioactivity. The $NiFe_2O_4$ nanoparticles were synthesized by chemical co-precipitation method. The particles develop surface charge in the aqueous solutions. The particles are positively charged for lower pH values due to the higher concentration of H^+ ions and negatively charged due to the higher concentration of OH^- ions [6 - 8].

Experimental Work

The $NiFe_2O_4$ nanoparticles were synthesized by chemical co-precipitation method. Here the nickel nitrate ($Ni(NO_3)_2.6H_2O$) and iron nitrate ($Fe(NO_3)_3.9H_2O$) salts were used as precursors. The precursors were dissolved in distilled water in the required mole proportion. The precipitation of reactant was carried out by adding the 2 M NaOH solution in the Ni and Fe nitrate solutions. The precipitate was then filtered and washed several times with distilled water until the pH of the filtrate become neutral (i.e. 7). The dried powder was then crushed into a fine powder using pestle mortar and then calcined at 800 ^oC for 10 hr. in the furnace.

The zeta potential of as synthesized $NiFe_2O_4$ nanoparticles was systematically studied. Here nickel ferrite nanoparticles of 0.2 mg/ml concentration were added in already prepared buffer solutions of different pH values. Buffer solutions of pH 3.64, 4.59, 5.58, 6.49, 6.86, 7.86, 8.40, 8.96 and 9.66 were prepared with acetates, phosphates and tris - HCl. The solution was sonicated for 30 min. in order to remove the agglomeration of nanoparticles.

The adsorption of Bovine Serum Albumin (BSA) proteins on $NiFe_2O_4$ nanoparticles was carried out at body temperature (37 ^oC) for different pH values. In a small beaker, BSA was dissolved in distilled water to prepare the concentration of 1 mg/ml. Further, 10 mg of $NiFe_2O_4$ nanoparticles were added into it. The samples were placed and constantly stirred in an incubator for 3 hrs at 37 ^oC. Finally, the magnetic particles were removed from the solution using a permanent bar magnet. The experiment was repeated three times in order to reduce the error. The amount of BSA adsorbed on the nickel ferrite nanoparticles was calculated using mass balance equation [9].

$$q = \frac{(C_i - C_f)V}{m}$$

where, where 'q' is the amount of BSA adsorbed on the nanoparticles; 'C_i' is the initial BSA concentration; 'C_f' is the BSA concentration left in the supernatant after the nanoparticles were removed; 'V' is the total volume of the solution (10 ml); and m is the mass of the nanoparticles added into the solution. Here, C_i and C_f were determined from the absorbance intensity at 280 nm using calibration curve recorded for BSA. The X ray diffractometer (Bruker D8 advance), having CuKα radiations with wavelength of 1.54 Å, was used to record the powder diffraction pattern. The absorbance was observed at 280 nm using UV-Vis spectrophotometer (JASCO V-670). The zeta potential measurements were obtained using Brookhaven Zeta plus 90 analyzer.

Results and Discussions

Figure 1 shows the XRD pattern of the as-synthesized $NiFe_2O_4$ particles sintered at 800 °C for 10 hrs. The XRD data confirms the formation of phase, purity and crystalline properties of the sample. The diffraction lines were indexed by comparing the inter-planar distance 'd' with the JCPDS data for $NiFe_2O_4$ (File No. 74 - 2081). The XRD pattern shows the presence of (220), (311), (222), (400), (511), (440) and (533) planes, which correspond to the characteristic cubic spinel ferrite structure. The average particle size determined from the Debye Scherrer's formula is 28 nm. Figure 2 shows the variation of zeta potential of nickel ferrite nanoparticles at different pH values. From figure 1, the isoelectric point for $NiFe_2O_4$ nanoparticles is observed at 7.37.

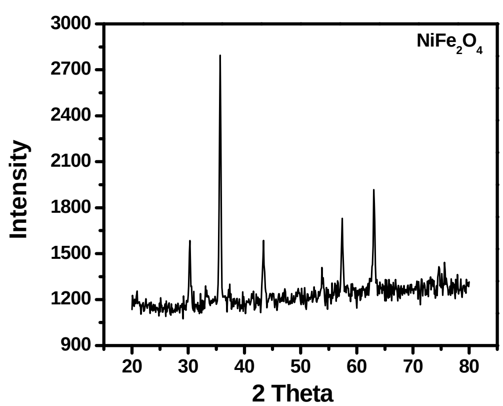

Figure 1. XRD pattern of $NiFe_2O_4$ nanoparticles sintered at 800 °C for 10 hrs

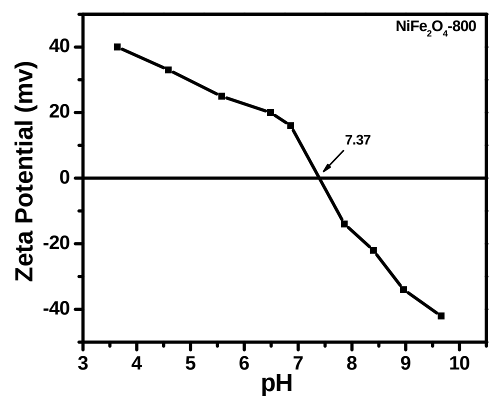

Figure 2. Variation of Zeta potential of $NiFe_2O_4$ nanoparticles for different pH values.

The zeta potential values are positive below this iso-electric point and negative for pH values above this. The maximum positive zeta potential of +40 mV and maximum negative zeta potential of - 42 mV was obtained at pH 3.64 and 9.66 respectively. The magnitude of zeta potential is also important in the adsorption process. Higher adsorption of BSA is observed for higher values of zeta potential. It is observed that the maximum adsorption of 178.3 μg/mg is obtained at pH 6.49 as shown in Table 1. The higher adsorption of BSA at 6.49 can be explained on the basis of interaction mechanism between the particle surface and the active functional groups of BSA.

Table 1. The quantity of adsorbed BSA onto nickel ferrite nanoparticles at different pH values

Sr. No.	pH of the solution	Quantity of adsorbed BSA q (µg/mg)
1.	3.64	70.23
2.	4.59	98.30
3.	5.58	126.4
4.	6.49	178.6
5.	6.86	168.3
6.	7.86	156.8
7.	8.40	121.5
8.	8.96	87.70
9.	9.66	58.30

The overall schematic representation of protein adsorption on the $NiFe_2O_4$ nanoparticles is shown in Figure 3. Figure 3 is divided into three regions. In region I, both the particle and BSA are positively charged, whereas in region II, the particles are positively charged and BSA is negatively charged. In region III, the particle and BSA both have negative charges. The pH 6.49 lies in the region II, where both the particles are oppositely charged.

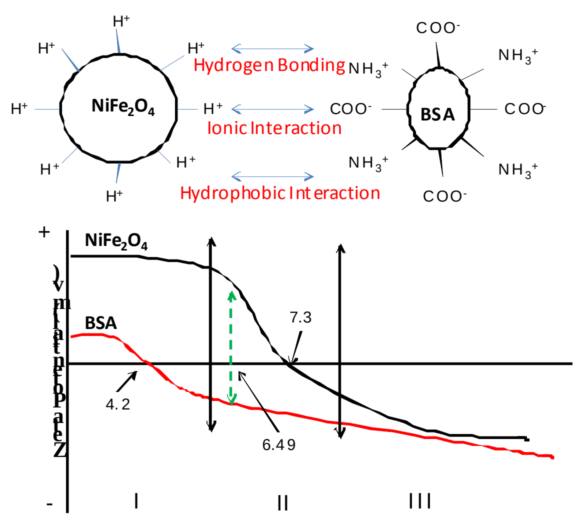

Figure 3. Overall schematic representation of the protein adsorption on the $NiFe_2O_4$ nanoparticles.

The maximum adsorption of BSA is observed at 6.49. The BSA has positive NH^{3+} and negative COO- functional groups. However, at pH values above its isoelectric point, the density of negative charges is more than the positive charges and hence overall the BSA is negatively charged. Although different interaction mechanisms (hydrogen bonding, ionic interaction and hydrophobic interaction) are involved during the adsorption process maximum adsorption at pH 6.49 takes place due to electrostatic ionic interaction.

Conclusions

The nickel ferrite nanoparticles synthesized by chemical co-precipitation method shows high adsorption capacity for BSA. The zeta potential analysis shows the isoelectric point at 7.37 for $NiFe_2O_4$ nanoparticles. The high electrostatic attractive forces involved at pH 6.49 results in high adsorption capacity of BSA onto nickel ferrite nanoparticles as compared to other pH values. Thus, nickel ferrite nanoparticles could be effectively used for magnetic separation of BSA from the aqueous solutions.

Acknowledgement

The author S. V. Bhosale acknowledges University Grants Commission (UGC), New Delhi for providing financial assistance under minor research scheme and sanctioning teachers leave under faculty improvement programme (FIP). Author V. C. Holkunde acknowledges University Grants Commission (U.G.C.), New Delhi for providing financial assistance under minor research scheme.

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