Chapter 5

Micro-structural Analysis of NiFe$_2$O$_4$ Nanoparticles
and its Suitability for BSA Adsorption

This chapter presents the experimental studies pertaining to the adsorption of Bovine Serum Albumin (BSA) on the nanoparticles of NiFe$_2$O$_4$ with a view of correlating the adsorption properties to their micro-structure and zeta potentials. Physical properties of two kinds of nickel ferrites, one synthesized by thermal plasma route and the other by chemical co-precipitation method, are compared in detail. The micro-structural analysis of NiFe$_2$O$_4$ nanoparticles is carried out with the help of TEM, HRTEM, XPS and Raman Spectroscopy. It is observed that the morphology and crystallinity of the nanoparticles play an important role in the adsorption of BSA. This chapter also reports the adsorption analysis of BSA onto NiFe$_2$O$_4$ nanoparticles by studying the Langmuir and Freundlich adsorption model.
5.1 Introduction

The nanoparticles of magnetic oxides have gained increasing interest because of their applications in biotechnology, especially in magnetic field assisted bio-separation, due to both their biocompatibility and stability in different environmental conditions. In addition, high magnetic susceptibility, low remanence, low coercivity and high saturation magnetization make them most suitable candidate for variety of applications. These include the biological separations, targeted drug delivery, magnetic resonance imaging (MRI), enzyme and protein immobilization, cell sorting process and biosensors. One of the prominent biological applications is the protein adsorption on solid surface which is observed in many natural processes. It has therefore promoted widespread interest in many research areas involving the separation of bio-molecules from the organic and inorganic matrix [1]. Here, the bio-molecules are attached onto the magnetic particles and then separated from the bulk solution by using magnetic field gradient [2,3]. Although pure metals (Fe, Co, Ni) possess the highest saturation magnetization, they are highly toxic and extremely sensitive to oxidation and hence such nanoparticles are least suitable for biotechnological applications [4]. Nanoparticles of ferrites are commonly used in biotechnology, since they possess low inherent toxicity, show ease of synthesis, exhibit physical and chemical stabilities and suitable magnetic properties [5]. Among different ferrite systems, NiFe$_2$O$_4$, having inverse spinel structure, is one of the most useful contenders, as a soft magnetic material. This is due to its low cost of synthesis, high electromagnetic performance, high electro-chemical stability, moderate saturation magnetization obtained at relatively small magnetic field and low coercivity [5,6]. Moreover, it is reported that the NiFe$_2$O$_4$ has high surface reactivity and high water adsorption capacity which makes it the potential candidate for biological applications [7]. The knowledge of protein adsorption on the surfaces of nanoparticles is relevant in many areas such as tissue regeneration, prosthetics, nanotoxicology, heterogeneous nucleation and therapeutic micro and nano devices [8]. Earlier reports suggest that the protein adsorption onto nanoparticles has inconsistent behavior i.e. few reported no influence of morphology on adsorption, while others reported an increase in the amount of protein adsorbed on the rough surfaces [9,10]. Therefore among various properties, the morphology and the surface chemistry of the nanoparticles are important due to difference in surface energies that arise from large fraction of edges, corners, vertices and particle
Moreover, there are many reports on the adsorption of protein on un-capped and capped nanocrystalline spinel ferrites. Koneraca et al. reported the effective covalent adsorption of BSA onto un-capped magnetic nanoparticles without losing the biological properties of the protein [12]. However, they have not quantified the amount of BSA adsorbed. Chun et al. reported the high adsorption capacity (110 µg/mg) of nano-NiFe₂O₄ synthesized by hydrothermal method for His-tagged proteins [13]. Similarly Gao et al. reported the fast and effective adsorption of protein onto highly crystalline aldehyde-functionalized magnetic nanoparticles synthesized by one pot solvo-thermal method [14]. However, there are not many attempts where the adsorption properties are assessed on the basis of the crystalline properties of the surface and its morphology. Here, we have made an effort to correlate the surface adsorption properties of nanocrystalline nickel ferrite with its surface morphology and crystallinity by studying the adsorption of BSA and magnetic field assisted bio-separation. The soft proteins such as Bovine Serum Albumin (BSA) is one of the most widely studied protein because it usually undergoes conformational re-orientations upon surface adsorption unlike hard proteins such as lysozyme. It has numerous biochemical applications including Enzyme Linked Immunosorbent Assay (ELISA), blot test etc. It is also used as a nutrient in cell and microbial culture because it does not affect other enzymes. Moreover, the high stability of BSA and structural homology with Human Serum Albumin (HSA) makes it the most suitable protein for study in the present case [15]. As discussed in the earlier chapter 4, the surface of the nanoparticle is either positive or negative depending upon the pH of the colloidal solution [16–18]. The aggregation of magnetic nanoparticles is observed at pH around the iso-electric point (IEP) due to very low charge density, whereas the colloidal solution is stable for pH far away from its IEP [19]. We have, therefore, compared the adsorption properties of two kinds of particles having different surface-morphologies, one highly crystalline and the other amorphous in nature. The crystalline structures of ferrite particles were synthesized by high temperature vapor phase condensation route using thermal plasma as a source for evaporation [20]. Their electro-kinetic properties are compared with those synthesized by chemical co-precipitation method. The quantitative evaluation of adsorbed BSA on the nanoparticles having approximate similar dimensions but varying in electro-kinetic properties forms the basis of the results reported in this chapter. Prior to investigating the adsorption properties, the micro-structural analysis of NiFe₂O₄ nanoparticles

and its suitability for BSA adsorption
nanoparticles synthesized by different routes was carried out using High Resolution Transmission Electron Microscopy (HRTEM). It has been shown that the adsorption efficiency of the (NiFe₂O₄) NFO-nanoparticles synthesized by high temperature plasma assisted route is superior to those synthesized by wet chemical method.

## 5.2 Experimental

The nanoparticles of NiFe₂O₄ were synthesized by dc thermal plasma arc and chemical co-precipitation route as discussed in the earlier chapter. The nickel ferrite nanoparticles prepared by thermal plasma route were termed as NFOTP whereas; those prepared by chemical co-precipitation route as NFOCP. The details of the operating conditions during the synthesis of NFOTP nanoparticles are summarized in Table 5.1. The yield obtained during the synthesis was 20g/hr. The NFOCP particles used in the experiment were calcined at 600°C for 10 hrs. The structural and micro-structural analysis of NiFe₂O₄ nanoparticles synthesized by different routes were carried out using XRD, TEM, HRTEM, AFM and Raman spectroscopy.

### Table 5.1: Summary of operating parameters used for the synthesis of NFOTP nanoparticles

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Operating Plasma Parameter</th>
<th>Operating Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Operating Current</td>
<td>150 A</td>
</tr>
<tr>
<td>2.</td>
<td>Operating Voltage</td>
<td>40 V</td>
</tr>
<tr>
<td>3.</td>
<td>Flow rate of Plasma</td>
<td>5 lpm</td>
</tr>
<tr>
<td></td>
<td>Forming Gas (Ar) through</td>
<td></td>
</tr>
<tr>
<td></td>
<td>plasma torch</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Base Pressure</td>
<td>0.01 Torr</td>
</tr>
<tr>
<td>5.</td>
<td>Arc length</td>
<td>5 cm</td>
</tr>
<tr>
<td>6.</td>
<td>Runtime</td>
<td>3-5 min</td>
</tr>
<tr>
<td>7.</td>
<td>Operating Pressure</td>
<td>500 Torr</td>
</tr>
<tr>
<td>8.</td>
<td>Flow rate of Ambient Gas</td>
<td>10 lpm</td>
</tr>
<tr>
<td></td>
<td>(O₂) Ambient Gas (O₂)</td>
<td></td>
</tr>
</tbody>
</table>

BSA is usually detected by measuring the photo-absorption spectrum between 200-500 nm since the intensity of absorption at around 280 nm indicates the characteristic peak of BSA. This measurement is therefore used to assess the presence of BSA in a solution and its quantitative evaluation is obtained by using a calibration curve (concentration of BSA versus
absorbance). The photo absorbance spectrum of native BSA and desorbed BSA for different pH values was obtained in the range from 200–500 nm by using UV-Vis Spectrophotometer.

The Zeta potential values were obtained for NFO particles dispersed in buffer solution; having different pH values ranging from 5.58 to 8.96. The ionic strength of the solution was maintained by adding 0.001M NaNO₃ because it yields higher values of zeta potential as discussed in chapter 4. Initially the zeta potential measurements were carried out for NFO concentration in the range of 0.1 – 0.6 mg/ml in the buffer solution at 25 °C. Further for fixed concentration of NFO particles (0.2 mg/ml), the zeta potential measurements were carried out for pH values in the range from 3.64 to 9.66. The buffer solutions of pH 3.64, 4.59 and 5.58 were prepared with 0.01 M sodium acetic acid, 0.01 M mono/di sodium phosphate buffer were used for pH 6.49, 6.86 and 7.86 and those corresponding to pH 8.40, 8.96 and 9.66 were prepared with 0.01 M tris-HCl. Because the particles have tendency to sediment under gravity due to their high density, the samples were sonicated for 30 minutes. The hydrodynamic particle size was determined immediately after the zeta potential measurements. Each experiment was repeated six times to insure the reproducibility of the measurements.

The infrared spectra of BSA and BSA adsorbed nickel ferrite nanoparticles were recorded in the range from 400 to 2500 cm⁻¹ by using Fourier Transform Infrared (FTIR) spectrometer. The surface area of the nanoparticles was determined by single point Brunauer-Emmett-Teller (BET) method using nitrogen adsorption/desorption method.

### 5.2.1 Adsorption of BSA onto NiFe₂O₄ nanoparticles

The procedure followed for studying the adsorption of protein (BSA) onto the magnetic nanoparticles is shown schematically in Fig. 5.1. The concentration of NFO particles in the buffer solution was fixed at 0.2 mg/ml. The external parameters which influence the adsorption of protein like temperature, ionic strength and buffer composition were kept constant while performing the adsorption experiment. The effect of BSA concentration on the adsorption was studied by changing the concentration of BSA in the range from 0.2 – 1.4 mg/ml for different pH values of 5.58, 6.86 and 8.96. Further in order to study the effect of pH on adsorption of BSA onto the NFO nanoparticles, concentration of BSA was kept constant (1mg/ml) and adsorption studies were carried out by varying pH in the range of
3.64-9.66 at body temperature (37°C). The amount of BSA adsorbed onto the NFO nanoparticles ‘q’ (µg/mg) was indirectly calculated by using the mass balance equation [21] given by,

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

where ‘C_i’ (mg/ml) is the initial BSA concentration; ‘C_f’ (mg/ml) 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 NFO 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. Further in order to investigate the relationship between the adsorbed species and their equilibrium concentration the Langmuir and Freundlich adsorption isotherms were plotted.

Fig. 5.1: Schematic showing the procedure of adsorption of BSA onto the NiFe_2O_4 nanoparticles at constant temperature

5.3 Results and Discussion

5.3.1 Structural and Morphological Analysis

The structural characterization of the as synthesized nanoparticles was carried out by X-Ray powder diffraction analysis. The XRD pattern for NFOTP is shown in Fig. 5.2(a) and exhibits typical reflections of (220), (311), (222), (400), (511), (440) and (533) indicate the
presence of cubic spinel structure. These diffraction lines provide clear evidence of the formation of NiFe$_2$O$_4$. All the diffraction lines match well with the reported values (JCPDS: File No. 74-2081). No secondary phase was detected in XRD, ensuring the phase purity of the final product. Fig. 5.2(b) shows the XRD pattern for NFOCP which is seen to consist of similar Bragg reflection lines and thus again confirm the formation of NiFe$_2$O$_4$ phase. However, in case of NFOCP few additional diffraction lines belonging to hematite ($\alpha$-Fe$_2$O$_3$) is observed which is attributed to loss of Ni$^{+2}$ ions during washing the precipitate [22]. The average crystallite size was calculated by measuring the XRD line widths and by using the Debye Scherrer’s formula:

$$d = \frac{k \lambda}{\beta \cos \theta},$$  

5.2

The average crystallite size of NFOTP and NFOCP nanoparticles determined is summarized in Table 5.2. These results confirm the formation of single phase and crystalline particles for NFOTP.

![XRD pattern of NFOTP and NFOCP](image)

**Fig. 5.2:** The XRD pattern of (a) NFOTP and (b) NFOCP. The asterisks indicate the peaks corresponding to the $\alpha$-Fe$_2$O$_3$ phase.
Fig. 5.3: TEM image and histogram of particle size distribution (a) and (b) for NFOTP, (c) and (d) for NFOCP particles respectively.

The morphologies of two kinds of nanoparticles were studied by TEM analysis by recording series of images at different sites. Most of the micrographs were similar in nature thus confirming that the one presented here are true representative of the synthesized particles. Fig. 5.3 refers to the TEM image of thermal plasma synthesized and chemically synthesized nanoparticles of NiFe$_2$O$_4$. The low magnification TEM image (Fig. 5.3(a)) indicates that NFOTP are clearly non-agglomerated in nature and possess sharp boundaries. The size histogram derived from this image shows wide particle size distribution in the range from 10 to 200 nm and is reflected in Fig. 5.3(b). Similar TEM analysis has been carried out for the chemically synthesized particles. Fig. 5.3(c) shows the low magnification image of NFOCP having narrow particle size distribution. The particles are seen to be agglomerated and therefore clear boundary of the particles cannot be seen. It is therefore difficult to see any crystalline facets in these particles. The size histogram is plotted in Fig. 5.3(d) in the range from 5 to 50 nm.
5.3.2 Magnetic Properties Analysis

Fig. 5.4 show the magnetic hysteresis curve of the nanoparticles and their magnetic properties are summarized in Table 2. The factors which decide the magnetic properties of ferrite nanoparticles are the surface defects, size, stoichiometry and their cationic occupancy in the specific sites [12]. Also the nanocrystalline spinel ferrite magnetic properties are adjudicated mainly by i) crystallinity of nanostructures and ii) cation redistribution in a given sample. Out of these two factors high crystallinity in nanostructures is one of the prominent factors. In case of NFOTP, nanostructures are highly crystalline which leads to superior magnetic properties whereas poor crystallinity in NFOCP leads to poor magnetic properties. Thus the higher value of saturation magnetization for NFOTP is attributed to the wider particle size distribution and high crystallinity which is expected in high temperature thermal plasma synthesis process [18,23]. The low value of saturation magnetization observed in case of NFOCP as compared to NFOTP is also attributed to the presence of non-magnetic $\alpha$-Fe$_2$O$_3$ phase [24]. The values of coercivity observed for both NFOTP and NFOCP nanoparticles are small as should be observed for soft magnetic materials and is given in Table 2. The higher value of coercivity obtained for NFOTP than NFOCP is attributed to the variation in the particle size distribution and the presence of lattice defects which are expected to result from the steep temperature gradient and high collision rate at the time of synthesis [20].

![Magnetization curves of the NFOTP and NFOCP magnetic nanoparticles.](image)

**Fig. 5.4:** Magnetization curves of the NFOTP and NFOCP magnetic nanoparticles.
Table 5.2 gives the summary of the particle size obtained from different techniques. It also gives the average hydrodynamic particle size (µm) along with specific surface area (m²/g) for both the nanoparticles.

**Table 5.2:** Summary of structural and magnetic properties of NFOTP and NFOCP nanoparticles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NFOTP</th>
<th>NFOCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRD- average particle size (nm)</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>TEM- mean particle size (nm)</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Average Hydrodynamic Particle size (µm)</td>
<td>1.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Specific Surface Area (m²/g)</td>
<td>54</td>
<td>81</td>
</tr>
<tr>
<td>Saturation Magnetization Mₛ (emu/gm)</td>
<td>46.53</td>
<td>28.48</td>
</tr>
<tr>
<td>Retentivity Mᵣ (emu/gm)</td>
<td>5.15</td>
<td>3.36</td>
</tr>
<tr>
<td>Coercivity Hc (Gauss)</td>
<td>91.20</td>
<td>79.89</td>
</tr>
<tr>
<td>Squareness Ratio Mᵣ/Mₛ</td>
<td>0.11</td>
<td>0.12</td>
</tr>
</tbody>
</table>

### 5.3.3 Raman Spectroscopic Analysis

The Raman spectra of NFOTP and NFOCP nanoparticles are shown in Fig. 5.5. Both the spectra consist of five peaks, the details of the peak positions and their possible assignments as inferred from the reported literature [25–31] are listed in Table 5.3. It is known that the Raman spectroscopy is a sensitive tool to understand the short range site ordering of the cations in the spinel ferrites. Since NiFe₂O₄ has a cubic –inverse spinel structure belonging to the point group Oₖ; the group theory predicts five Raman-active phonons (A₁g + E₉ + 3F₂g) in the cubic spinel structure [26]. In accordance to the assignments proposed by Lazarevic et al. the A₁g mode corresponds to the symmetric stretching of Fe-O and Ni-O bonds at the tetrahedral group site. E₉ mode corresponds to symmetric bending of Fe-O and Ni-O bonds at the tetrahedral site. The F₂g(2) mode is assigned to the asymmetric stretching of Fe–O and Ni-O bonds at the octahedral site. F₂g(3) mode is caused due to asymmetric bending of Fe-O and Ni-O bonds at octahedral site and F₂g(1) mode is due to translational motion of the whole tetrahedral group.
Further from Fig. 5.5 and Table 5.3 it is observed that (a) some of the Raman signals are very broad, suggesting that they may be composed of more than one raman bands (b) there is shift of raman peaks towards higher wave number values in NFOTP as compared to those in NFOCP. (c) broad shoulders below $A_{1g}$ and $E_g$ peaks at 648, 283 cm$^{-1}$ and 644, 269 cm$^{-1}$ for NFOTP and NFOCP nanoparticles respectively (d) weakening of $F_{2g}(3)$ mode in NFOCP at 570 cm$^{-1}$ (e) sharper Raman peaks in NFOTP as compared to NFOCP (f) suppressed $F_{2g}(1)$ mode in both the cases (g) NFOCP has an additional peak at 614 cm$^{-1}$.

![Raman Spectra of NFOTP and NFOCP nanoparticles](image)

**Fig. 5.5**: The Raman Spectra of NFOTP and NFOCP nanoparticles

The broad shoulders observed below $A_{1g}$ and $E_g$ peaks in both the cases is attributed to the second overtone of the corresponding modes and indicates the presence of inverse spinel structure. The weakening of $F_{2g}(3)$ mode in NFOCP indicates dilution of the long range order of cations in B sites. The sharp peaks observed in NFOTP particles on the other hand
is attributed to the effect of densification of long range order of cations as a result of better crystallization and ordering of cations in the material which is also supported by the XRD data. Suppression of $F_{2g}$ mode in both the cases suggests that there is less translational motion of the whole tetrahedral group in both the cases [28–31].

Table 5.3: The assignment of Raman modes to the observed peaks

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Peak position NFOTP (cm$^{-1}$)</th>
<th>Peak position NFOCP (cm$^{-1}$)</th>
<th>Raman Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>196</td>
<td>183</td>
<td>$F_{2g}(1)$</td>
</tr>
<tr>
<td>2.</td>
<td>326</td>
<td>308</td>
<td>$E_g$</td>
</tr>
<tr>
<td>3.</td>
<td>482</td>
<td>467</td>
<td>$F_{2g}(2)$</td>
</tr>
<tr>
<td>4.</td>
<td>570</td>
<td>Suppressed</td>
<td>$F_{2g}(3)$</td>
</tr>
<tr>
<td>5.</td>
<td>690</td>
<td>688</td>
<td>$A_{1g}$</td>
</tr>
</tbody>
</table>

5.3.4 Atomic Force Microscopy (AFM) Analysis

The surface roughness of NFO particles were characterized by using atomic force microscopy. The representative AFM micrograph and its 3D view for NFOTP particles are shown in Fig. 5.6(a) and 5.6(b) and that corresponding to NFOCP is shown in Fig. 5.6(c) and 5.6(d). The RMS surface roughness obtained for NFOTP and NFOCP nanoparticles was 8.94 nm and 4.10 nm respectively. It is observed that the NFOTP particle has higher surface roughness than NFOCP.
5.3.5 Micro-structural analysis using HRTEM

Micro-structural analysis of two kinds of nanoparticles was studied by using HRTEM, by recording the series of images at different sites. Most of the micrographs were similar in nature thus confirming that the one presented here are true representative of the synthesized particles. Fig. 5.7 and Fig. 5.8 show the HRTEM images of NFOTP and NFOCP nanoparticles respectively. The highly resolved image of NFOTP (Fig. 5.7 (a)) show clear faceted surfaces with sharp boundaries. Moreover, it can also be seen that most of the smaller particles exhibit hexagonal faceting whereas the larger ones exhibit square or rounded facets. It is well known that the highly crystalline particles show such facets when grown into much smaller sizes. Fig. 5.7(b) is a typical HRTEM microgram of NFOTP, exhibiting lattice planes having ‘d’ values of 0.2916 nm corresponding to (220) planes. Fig. 5.7(c) shows the Selected Area Electron Diffraction (SAED) pattern with labeled indices for
the prominent spots corresponding to (311) and (440) planes. Appearance of clear spots confirms the single crystal nature of these particles.

**Fig. 5.7:** NFOTP: (a) TEM image exhibiting facets (b) HRTEM image showing the crystal planes (c) SAED pattern along with planes

**Fig. 5.8:** NFOCP: (a) HRTEM image (b) SAED pattern indicating crystalline planes

Similarly, the HRTEM image (having the same resolution as that in Fig. 5.7(b)) of NFOCP is shown in Fig. 5.8(a) which does not provide any information about its crystallinity and no lattice planes are seen. The surface is seen to be very hazy, probably indicating an amorphous layer. The SAED pattern shown in Fig. 5.8(b) indicates ring pattern which is consistent with the poly-crystalline nature of the particles. The miller indices of the planes have been labeled as (311), (400) and (440). In conclusion it is clear from HRTEM analysis that each particle of NFOTP is single crystal in nature and the boundaries are very well...
defined whereas the particles in NFOCP possess poor crystallinity and are not defined by clear crystalline surfaces. Moreover, Fig. 5.9 shows the highly resolved image exhibiting stepped surface for NFOTP nanoparticles where step like structure is seen at the surface. Presence of inter-planar distance of 0.208 nm corresponds to (400) plane of spinel ferrite. Observed feature is useful in understanding the surface chemistry, electro-kinetic properties and adsorption properties which is discussed in the next section.

Fig. 5.9: HRTEM image exhibiting stepped surface for NFOTP nanoparticles

5.3.6 Analysis of Electro-kinetic Properties

The value of zeta potential obtained by varying the pH value of the buffer solution for different NFO concentrations is shown in Fig. 5.10. It is seen that i) the magnitude of zeta potential remains unchanged for the measured range of concentrations of nanoparticles in the solution for different pH values. ii) The value of zeta potential for NFOTP is higher than that of NFOCP for all the pH values used in the present study. iii) The value of zeta potential is positive for low pH values and negative for higher pH values.
Fig. 5.10: Effect of NFO concentration on the zeta potential for different pH values

The first feature is seen to be as expected and is aimed at finding the suitable concentration of NFO particles which can produce consistent results and further can be used for adsorption analysis. Tantra et al. [32] suggests that there is a region in which the zeta potential value is independent of the nanoparticle concentration, whereas it was observed that for extreme dilution there was change in the zeta potential value which is attributed to the contribution of signal from extraneous particles in the liquid, rather than the nanoparticles themselves and the higher concentration causes multiple scattering which makes the interpretation more difficult [33].

The explanation of higher zeta potential values for NFOTP particles is also discussed in Chapter 4. However, this chapter discusses the role of morphology, crystallinity and thermodynamics of the particles due to which the higher values of zeta potential are obtained. The second feature is associated with the higher surface reactivity of NFOTP particles as compared to NFOCP. This is naturally a specific property associated with reduced dimensions. However, out of the two kinds of nanoparticles studied in the present experiments, those synthesized by chemical co-precipitation method have been characterized to exhibit larger surface area and smaller average particle size. In spite of these characteristics, NFOCP particles are seen to exhibit smaller values of zeta potential than that
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of NFOTP. The observed fact definitely indicates that the particles synthesized by thermal plasma route should have higher specific surface energy. It is therefore necessary to review the thermodynamic properties of these adsorbents since the surface energy is an important thermodynamic characteristic of a crystalline particle and is expressed in terms of $\gamma$ (the surface energy per unit area). It is included in the total free energy of a system having a surface area ‘A’ and is given by

$$ G = G_0 + A\gamma, \quad 5.3 $$

where $G_0$ is the free energy of the system; assuming that all the material in the system has the properties of the bulk. $\gamma$ is therefore the excess free energy arising from the material lying at the interface. From the basic definition of the Gibb’s free energy the surface free energy is related to the temperature ‘T’ and entropy ‘S’ of the system by

$$ \gamma = H – TS, \quad 5.4 $$

where, $H$ is the enthalpy, and, for a system with volume $V$ and pressure $P$ it can be written as

$$ \gamma = E + PV – TS, \quad 5.5 $$

where, ‘$E$’ is the internal energy of the system arising from the total kinetic and potential energy. Thus, even if the PV term is ignored for a given volume of the particle at pressure ‘P’; the effect due to the surface entropy must be taken into consideration. This is so since the surface atoms have more freedom of movement and therefore possess higher thermal entropy compared to atoms in the bulk. Moreover, extra configurational entropy can also be introduced into the surface by the formation of surface vacancies. The surface of the crystal should therefore be associated with positive excess entropy which will partly compensate for the high internal energy and thus stabilize the system. This implies that a single crystalline particle will tend to have larger surface defects.

On the other hand if a particle has poor crystallinity in the bulk, the surface defects get self-passivated. It is clear from the TEM images that NFOTP is single-crystalline in nature and exhibits a sharp crystalline boundary whereas NFOCP do not provide any lattice features and appears amorphous in nature as evidenced from Fig. 5.7 and Fig. 5.8. However, the fact that both provide clear X-Ray Diffraction patterns would definitely pose a question regarding the lack of crystallinity in the latter. Reason behind this might be the presence of amorphous layers on the NFOCP particles. The amorphous layers also help in passivating the surface and thus are responsible in reducing the surface energy. Further, in NFOTP, different crystal
surfaces should have different values of surface free energy, depending on the number of broken bonds, since there is a clear indication of faceted surfaces in these particles. A crystal plane at an angle ‘θ’ with respect to the close packed plane will contain additional broken bonds due to the atoms at steps as shown in Fig. 5.9 which consequently increase the configurational entropy. This will prompt to create greater number of defects on the inclined surface. The step like feature is clearly seen in Fig. 5.9 and the schematic of surface is also shown in the same figure. Therefore, NFOTP should have larger surface defects as compared to NFOCP making them more reactive. Such properties of NFOTP must have emerged from the high temperature present during the nucleation inside the plasma plume. Close to the periphery of the plasma, the metallic species evaporated from the anode are allowed to react with oxygen ambience which results in the formation of thermodynamically stable ferrite phase. Due to the steep temperature gradient and high velocity of the metallic species evaporated from the anode the NFO particles experience rapid quenching, which contributes towards the modified surface properties. In case of NFOCP nanoparticles the process involves the reaction of salts of Ni and Fe with base, NaOH in the present case, which results in formation of corresponding amorphous hydroxides in the initial stage. Further, the heat treatment (i.e. calcination at 600°C for 10 hr.) causes dissociation of hydroxide and subsequent formation of spinel oxide phase. Here, the heat treatment is provided for a longer duration of few hours (in contrast to that of thermal plasma route) and therefore less surface modification is expected. This low surface modification is one of the reasons for comparatively low value of zeta potential in case of NFOCP [34].

The third feature is already discussed in chapter 4, and is due to the adsorption of counterions of H⁺ and OH⁻ ions for low and high pH values respectively.

5.3.7 Analysis of Protein Adsorption

Fig. 5.11(a) shows the calibration curve recorded for BSA protein. It is seen to follow a linear variation of absorbance as a function of concentration. The absorption coefficient ‘α’ is calculated to be 0.78 mg ml⁻¹cm⁻¹. This value of ‘α’ was used to determine the concentration of supernatant BSA. Fig. 5.11(b) shows the adsorption equilibrium of BSA on to the fixed concentration of NFO (0.2 mg/ml) at different pH values in the range of 5.58 – 8.96. It is also seen that the adsorbed quantity of BSA increases significantly with concentration up to 1
mg/ml and afterwards it almost remains constant. Therefore for further studies the concentration of BSA was fixed as 1 mg/ml and that of NFO at 0.2 mg/ml.

**Fig. 5.11:** (a) calibration curve for BSA (b) adsorption equilibrium at different pH values

Further, the effect of pH on the adsorption of BSA onto NFO nanoparticles was studied. It is seen from Fig. 5.12(a) that the adsorbed quantity of BSA onto NFO nanoparticles increases with increasing pH from 3.64 attains a maximum at pH value of 5.58 and decreases with further increase in pH values. In this way it was found that the maximum adsorption of 231.57 µg/mg was observed for NFOTP at pH value of 5.58 whereas for the same pH value the adsorption of BSA was found to be 178.71 µg/mg for NFOCP. Higher adsorption of BSA onto NFOTP than that of NFOCP is due to the dependence of adsorption on protein properties as well as surface properties of the material (surface charge, surface energy, polarity and morphology) [35–37]. In order to understand it in more detail, the effect of pH on the zeta potential of both types of nickel ferrite particles and BSA was obtained and is shown in Fig. 5.12(b). The iso-electric point (IEP) of NFOTP and that of NFOCP particles obtained from the zeta potential curve is at pH value of 7.25 and 7.41 respectively. These values are in close agreement with the literature value of pH 7.7 for ferrites [38–40]. Similarly, the IEP of BSA is obtained at pH 4.78 and is very close to the values reported in the literature [35].
Fig. 5.12: (a) Effect of pH on the adsorption of BSA (1 mg/ml) onto NFO particles (0.2mg/ml) (b) variation of zeta potential as a function of pH

Here it is also observed that the zeta potential values of NFOTP are larger than NFOCP particles for all the selected pH values. It is reported earlier that if the values of the zeta potential are higher than ± 30 mV the particles are stable and have less tendency to agglomerate which clearly indicates that in the colloidal solutions NFOTP particles are highly stable and has less tendency to agglomerate as compared to NFOCP particles. Thus the large value of zeta potential and high stability of NFOTP nanoparticles is one of the reasons which are responsible for more adsorption of protein on them as compared to NFOCP particles. In addition, understanding of electrostatic interaction between magnetic particles and BSA at different pH values is also an important issue. For pH values less than IEP of BSA, both NFO and BSA have positive charge and therefore, the electrostatic repulsion does not favor the adsorption. Similarly for pH values greater than IEP of NFO, both NFO and BSA have negative charge, resulting in the decrease of adsorption. However the region where the BSA has negative charge and the NFO has positive charge, the electrostatic attraction can promote the adsorption.

Further to understand the higher adsorption of BSA onto NFOTP as compared to NFOCP at pH value of 5.58, the average hydrodynamic particle size was determined by DLS. It is observed that the average hydrodynamic particle size of NFOCP (2.2 μm) is larger than that of NFOTP (1.4 μm) which confirms that the latter particles agglomerate to a lesser extent and are thus more stable (reported in Table 5.2). Also high value of zeta potential in case of NFOTP than that of NFOCP indicates larger repulsive interaction between the particles,
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which inhibits the agglomeration and subsequently reduce the hydrodynamic size resulting in stable suspension. These stable nanoparticles thus provide more effective surface sites for the adsorption of BSA as compared to NFOCP. The difference in the surface morphology of the two kinds of particles has already been confirmed from the HRTEM images. Particles of NFOTP have free dangling bonds and possess higher surface adsorption capacity. Comparison of Fig. 5.7 and 5.8 reveals that in case of NFOCP the surface is passivated by an amorphous structure whereas NFOTP surface does not have such an amorphous passivation. Such a surface feature also leads to different surface chemistry. In case of NFOTP, the metal ions such as Ni\(^{+2}\) or Fe\(^{+3}/Fe^{+2}\) are expected to be present at the surface which will come in contact with BSA directly whereas in case of NFOCP the metal ions are already passivated by the amorphous structure and therefore, will not come in contact with BSA directly.

The metal ions present at the surface leads to enhancement in the positive surface charge whereas BSA has got negative surface charge at pH 5.58 which is one of the reasons for enhanced adsorption in case of NFOTP that of NFOCP. Hence the adsorption for NFOTP is higher than the NFOCP nanoparticles. These results are in agreement with earlier reported values where the maximum adsorption of 418.9\(\mu\)g/mg at pH value of 4.64 for Fe\(_3\)O\(_4\) nanoparticles with 10 nm particle size was reported in the presence of carbodiimide [2]. Thus the maximum adsorption of BSA is obtained at pH value of 5.58 for both the NFO particles.

In order to confirm the adsorption of BSA onto nickel ferrite nanoparticles FTIR analysis has been carried out. Fig. 5.13 shows the FTIR spectra of NFOTP, NFOCP, BSA, BSA-NFOTP and BSA-NFOCP. The absorption peak observed at 463 or 536 cm\(^{-1}\) is associated with the vibrations of Ni-O bonds at the tetrahedral site and that observed at 707 and 713 cm\(^{-1}\) are associated with the vibrations of Fe-O bonds at octahedral sites.

The observed shift of 73 cm\(^{-1}\) is attributed to the change in force constant in case of NFOCP due to poor crystallinity and different chemical environment as compared with that of NFOTP. According to the FTIR spectrum the characteristic transmission peaks of BSA are observed at 1695 and 1557 cm\(^{-1}\) [18]. The adsorption of BSA on magnetic particles shows both the characteristic peaks of BSA and nickel ferrite (NFOCP and NFOTP) nanoparticles. This indicates that the BSA was successfully adsorbed on the surfaces of NiFe\(_2\)O\(_4\) (NFOCP and NFOTP) magnetic nanoparticles.
5.3.8 Adsorption Isotherm

Initially the BSA adsorption equilibrium isotherm was studied for the particles at different pH values (5.58, 6.86 and 8.96) and is shown in Fig. 5.11(b). All the curves in Fig. 5.11(b) are identical in nature however the quantity of saturated adsorbed BSA varies significantly with the history of the samples. A maximum quantity of adsorbed BSA in each curve was fitted according to the different theoretical model and the best fit was determined.

5.3.8.1 Langmuir Adsorption Isotherm

The linear Langmuir isotherm equation is expressed as

\[
\frac{C_e}{q_e} = \frac{1}{q_m K} + \frac{C_e}{q_m}
\]
where ‘$C_e$’ is equilibrium concentrations of the BSA solution (mg/ml), ‘$q_e$’ is the amount of BSA adsorbed at equilibrium (µg/mg), ‘$K$’ is a measure for the intensity of the adsorption process (adsorption capacity) and ‘$q_m$’ is the constant related to the area occupied by a monolayer of adsorbate, reflecting the adsorption capacity. The Langmuir plot of the experimental data is given in the Fig. 5.14. The Langmuir Constants $q_m$ and $K$ are summarized in Table 5.4.

![Fig. 5.14: Langmuir plot for the adsorption of BSA onto NFOTP and NFOCP nanoparticles](image)

### 5.3.8.2 Freundlich Adsorption Isotherm

The linear Freundlich isotherm equation is expressed as:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e$$  \hspace{1cm} (5.7)

where, $q_e$ is the amount BSA adsorbed (µg/mg), $C_e$ is the equilibrium concentration (mg/ml), $K_f$ is adsorption coefficient and represents quantity of BSA adsorbed onto adsorbent for a unit equilibrium concentration and ‘$n$’ is the measure of adsorption intensity or surface heterogeneity [1]. The Freundlich plot of the experimental data is given in the Fig. 5.15 and the constants are summarized in Table 5.4.
Fig. 5.15: Freundlich plot for the adsorption of BSA onto NFOTP and NFOCP nanoparticles

Table 5.4: Langmuir and Freundlich adsorption constants

<table>
<thead>
<tr>
<th>Model</th>
<th>pH→</th>
<th>NFOTP</th>
<th>NFOCP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.58</td>
<td>6.86</td>
<td>8.96</td>
</tr>
<tr>
<td>Langmuir</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$q_m$ (µg/mg)</td>
<td></td>
<td>0.251</td>
<td>0.222</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.220</td>
<td>0.194</td>
</tr>
<tr>
<td>$K$ (ml/mg)</td>
<td></td>
<td>10.22</td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.00</td>
<td>2.94</td>
</tr>
<tr>
<td>$R^2$</td>
<td></td>
<td>0.9989</td>
<td>0.9845</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9989</td>
<td>0.9670</td>
</tr>
<tr>
<td>Freundlich</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_f$ (µg/mg)</td>
<td>227.88</td>
<td>164.47</td>
<td>75.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>175.34</td>
<td>154.81</td>
</tr>
<tr>
<td>$n$ (ml/mg)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>5.24</td>
<td>2.71</td>
</tr>
<tr>
<td>$R^2$</td>
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<td>0.9688</td>
<td>0.9549</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9026</td>
<td>0.9337</td>
</tr>
</tbody>
</table>

From fitting of both the models it is observed that the $R^2$ values are higher for Langmuir model and thus it fits better than the Freundlich model for both the particles at wide range of pH values.

### 5.4 Summary and Conclusion

This chapter systematically reports the analysis of the characteristic adsorption properties and its correlation with structural, morphological and electro-kinetic properties of nickel ferrite nanoparticles synthesized by different routes. The difference in the surface structure as evidenced by several microscopic techniques such as AFM, TEM, HRTEM and SAED is
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Responsible for differences in the electro-kinetic properties under identical measurement conditions. It is observed that the faceted nanocrystals, synthesized by high temperature vapor phase condensation method, are more suitable for adsorbing protein than those which have poor crystallinity. The surface chemistry of nanoparticles having highly crystalline surface and those having poor crystalline surface is different. Highly crystalline surface provide active surface sites, exhibit higher zeta potential and hence higher adsorption than their counterparts and hence it is more suitable for BSA adsorption. The important conclusions from this chapter are listed below:

1. Micro-structural analysis suggests that the method of synthesis affects the physico-chemical properties of NiFe$_2$O$_4$ nanoparticles.
2. It is observed that the NFOTP particles are non-agglomerated in nature and possess sharp boundaries with high crystallinity as compared to NFOCP particles.
3. Although the NFOCP have smaller particle size and higher specific surface area as compared to NFOTP, however it has higher value of average hydrodynamic particle size which is attributed to its nature of agglomeration.
4. The NFOTP particles are found to have higher surface roughness than NFOCP.
5. The NFOTP has higher value of saturation magnetization as compared to NFOCP and is attributed to the high degree of crystallinity and wide particle size distribution.
6. The NFOTP particles have larger surface defects making them more reactive as compared to NFOCP.
7. The NFOTP has higher adsorption capacity than NFOCP for all the pH values under study. The maximum adsorption (231.57µg/mg) of BSA at 37°C was observed at pH value of 5.58 for thermal plasma synthesized particles than those synthesized by wet chemical means (178.71µg/mg).
8. The adsorption analysis confirms the fitting of experimental data to Langmuir model than to the Freundlich model. This suggests that there is higher probability of formation of monolayer onto the surface of NiFe$_2$O$_4$ nanoparticles.
References

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