This chapter focuses on the structural, magnetic and anti-algal properties of cobalt substituted zinc ferrite nanoparticles. Cobalt substituted zinc ferrite was prepared by the sol-gel technique and the structural characterization was done using the XRD and WD-XRF. All the prepared samples showed an inhibitory effect on the green algae, Chlorella pyrenoidosa. The effect of cobalt substituted zinc ferrite nanoparticles on the algal cells was assessed by studying the algal cell density and the chlorophyll a content at a regular interval of 48 hours for a period of 14 days. Morphology of algal cells in response to cobalt substituted zinc ferrite was studied using the Phase Contrast Microscope and the Scanning Electron Microscope. Magnetic properties of the prepared samples were studied using the Vibrating Sample Magnetometer.
9.1 Introduction

Magnetic nanoparticles form an important platform for multidisciplinary research due to their diverse applications in different fields of science and technology. These diverse applications of nanoparticles are determined by the size, shape and morphology of the particles which in turn depend on the cationic distribution, chemical composition and method of preparation [1, 2]. The diamagnetic ion substitution in ferrites can make remarkable modifications to the properties of ferrites [3, 4]. The properties of ferrites depend on the ability of cations to distribute among the octahedral sites [5]. ZnFe$_2$O$_4$ possess a normal spinel structure with Zn$^{2+}$ ions in the tetrahedral site and Fe$^{3+}$ ions in the octahedral site. Whereas CoFe$_2$O$_4$ possess an inverse spinel structure, where Co$^{2+}$ ions and one half of the Fe$^{3+}$ ions occupy the octahedral site while the other half of Fe$^{3+}$ ions occupy the tetrahedral site [6]. But cobalt substituted zinc ferrite has a distorted spinel structure, thus making a remarkable difference in its properties. The structural and magnetic properties of Zn-Co ferrite have been studied in detail by many researchers [5 -7].

As mentioned earlier, study of the effect of nanoparticles on algae are of much importance. The toxicity of various nanoparticles on different algae has been reported by various researchers [8 - 13]. However, to the best of our knowledge, no studies have been reported on the effect of divalent ion substituted ferrites on algal cells.

9.2 Materials and Methods

9.2. Synthesis of Cobalt Substituted Zinc Ferrite Nanoparticles

Cobalt substituted zinc ferrite nanoparticles with general formula, Zn$_{1-x}$Co$_x$Fe$_2$O$_4$ (where x = 0.00, 0.25, 0.50, 0.75, 1.00), were synthesized using
the chemical precursors zinc nitrate, cobalt nitrate and ferric nitrate with ethylene glycol as the solvent. Details of the synthesis have been explained in section 2.2.1. The finely grounded powders were sintered at $400^\circ$C for two hours in a muffle furnace.

### 9.2.2 Experimental Setup

As mentioned in section 8.2.2., a culture of green algae *Chlorella pyrenoidosa H. Chick* [14] was used for the present experiment. A semi continuous test culture for the experiment was prepared by taking 15ml culture of *Chlorella pyrenoidosa H. Chick* [14] in a 250ml Erlenmeyer flask, containing 150ml of Walnes medium [15]. The prepared nanoparticles were then added to the cultures and the concentration of the nanoparticles in the culture was maintained at $1.0\mu\text{M}$. A culture without any nanoparticles was kept as the control. Each of the culture was then triplicated. All the cultures were incubated under two continuous cool fluorescent tubes of 1250 lux each (white light) for a light/dark period of 12:12 hours at a temperature of $(30\pm2)^\circ$C. The experimental setup is shown in figure 9.1.

![Figure 9.1: Experimental setup](image)
9.3 Structural Characterization

9.3.1 X-Ray Diffraction Studies

The X-Ray Diffraction method was used to characterize the crystal structure of the prepared samples. Figure 9.2 (a) shows the XRD pattern for the system Zn$_{1-x}$Co$_x$Fe$_2$O$_4$ (where $x = 0.00, 0.25, 0.50, 0.75, 1.00$) and figure 9.2 (b) presents the Rietveld plot for the sample Zn$_2$Fe$_2$O$_4$.

![Figure 9.2: (a) XRD Pattern of Zn$_{1-x}$Co$_x$Fe$_2$O$_4$ Series (b) Rietveld plot of Zn$_2$Fe$_2$O$_4$](image)

The absence of impurity peaks was confirmed, by comparing the experimental data with the standard data (JCPDS Card NO: 82 – 1042). The crystallite size with corresponding error factor was calculated using the Scherrer equation by fitting the most prominent peak (311) by the function PsdVoigt1.

The values of crystallite size, lattice constant, X-ray density and volume of the unit cell for all samples are given in table 9.1.
Table 9.1: Effect of cobalt substitution on the crystallite size, lattice constant and volume of unit cell of zinc-cobalt system.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Crystallite Size (nm)</th>
<th>Lattice Constant (Å)</th>
<th>Volume of Unit Cell (Å³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnFe₂O₄</td>
<td>18.7 ± 0.4</td>
<td>8.427</td>
<td>598.44</td>
</tr>
<tr>
<td>Zn₀.₇₅Co₀.₂₅Fe₂O₄</td>
<td>15.4 ± 0.3</td>
<td>8.380</td>
<td>588.48</td>
</tr>
<tr>
<td>Zn₀.₅₀Co₀.₅₀Fe₂O₄</td>
<td>13.2 ± 0.4</td>
<td>8.343</td>
<td>580.72</td>
</tr>
<tr>
<td>Zn₀.₂₅Co₀.₇₅Fe₂O₄</td>
<td>14.9 ± 0.2</td>
<td>8.310</td>
<td>573.86</td>
</tr>
<tr>
<td>CoFe₂O₄</td>
<td>12.2 ± 0.3</td>
<td>8.298</td>
<td>571.37</td>
</tr>
</tbody>
</table>

The crystallite size showed a decreasing tendency with increase in the cobalt concentration. The lattice constant and unit cell volume were found to decrease with increase in the Co²⁺ ion concentration in ZnFe₂O₄. This could be attributed to the fact that the substitution of larger Zn²⁺ ions (ionic radius, 0.074nm) by smaller Co²⁺ ions (ionic radius, 0.072nm) leads to a shrinkage of the unit cell which in turn decreases the lattice constant and unit cell volume.

### 9.3.2 WD-XRF Analysis

The stoichiometry of the ZnFe₂O₄ nanoparticles and CoFe₂O₄ nanoparticles were checked using the Wavelength Dispersive X-Ray Fluorescence (WD-XRF) Spectrometer. The composition of the elements present in ZnFe₂O₄ nanoparticles and CoFe₂O₄ nanoparticles are presented in table 9.2.

Table 9.2: Values of concentration of each elements present in samples ZnFe₂O₄ and CoFe₂O₄ compared with expected concentration.

<table>
<thead>
<tr>
<th>Elements present</th>
<th>ZnFe₂O₄ Atomic mass percentage from XRF (%)</th>
<th>Expected Concentration (%)</th>
<th>CoFe₂O₄ Atomic mass percentage from XRF (%)</th>
<th>Expected Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>45.370</td>
<td>46.331</td>
<td>47.323</td>
<td>47.608</td>
</tr>
<tr>
<td>O</td>
<td>26.500</td>
<td>26.548</td>
<td>27.351</td>
<td>27.261</td>
</tr>
<tr>
<td>Zn</td>
<td>26.170</td>
<td>27.121</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Co</td>
<td>Nil</td>
<td>Nil</td>
<td>25.230</td>
<td>25.110</td>
</tr>
</tbody>
</table>
The percentage of the constituent elements present in the samples (ZnFe$_2$O$_4$ and CoFe$_2$O$_4$) as observed by the WD-XRF spectrometer was found to be consistent with the theoretically expected values, which confirms the purity of the prepared samples.

9.4 Effect of Nanoparticles on Algae

9.4.1 Chlorophyll $a$ content in algal cultures

The variation of chlorophyll $a$ content with cobalt ion substitution in zinc ferrite observed at a regular interval of 48 hours is depicted in figure 9.3.

**Figure 9.3:** Chlorophyll $a$ content in the cells of *Chlorella pyrenoidosa*, H. Chick [14] in cultures containing Zn$_{1-x}$Co$_x$Fe$_2$O$_4$ nanoparticles. Values are reported as mean of three replicates ± standard deviation (SD).

The chlorophyll $a$ content of the algal cells were measured using the modified Strickland and Parson method [16] applying the equation [17]

\[
\text{Chlorophyll } a = 11.85 (O.D \ 665) - 1.54 (O.D \ 645) - 0.08 (O.D \ 630) \quad \text{----(9.1)}
\]

\[
\text{Chlorophyll } a \ (\mu g/mL) = \frac{\text{Chl } a \ \times \text{Extract Volume (ml)}}{\text{Volume of sample (L)}} \quad \text{-------------------------(9.2)}
\]
Details of the method used for the measurement of chlorophyll \( a \) content in the algal cells are explained in section (2.7.3).

In the present study, chlorophyll \( a \) content of algal cells was found to be lower in cultures containing nanoparticles (with different concentrations of zinc and cobalt) when compared to the chlorophyll \( a \) values of the control. The reduction of chlorophyll \( a \) content of the test culture with respect to the control, was found to reduce as \( x \) increased from \( x=0.00 \) to \( x=0.75 \). But a prominent decrease in chlorophyll \( a \) content was shown in all the cultures grown with pure cobalt ferrite nanoparticles.

The tolerance level of algal cells to heavy metals depend on several factors like composition of culture and test medium, temperature, incident light etc. [18]. There are reports regarding reduction in the chlorophyll \( a \) content due to heavy metal toxicity caused by the presence of nano particles containing heavy metals in the culture medium [19]. The above observed difference in the reduction of chlorophyll \( a \) content in the test samples (with respect to control) may be because of the antagonistic effect of the different heavy metals (Co, and Zn) present in the used nanoparticles. Antagonistic effect of two metals can reduce the deleterious effect of individual heavy metals [20]. However the prominent decrease in the chlorophyll \( a \) content shown in all the cultures grown with pure cobalt ferrite nanoparticles could be because of the absence of the antagonistic effect mentioned above (because of the absence of Zn). In such a situation, there is the possibility of expression of cobalt toxicity, and this could be the reason for the observed large reduction in the chlorophyll \( a \) content in cultures grown with pure cobalt ferrite nanoparticles.
9.4.2 Cell Density in Algal Cultures

A hemocytometer was used here to measure the cell density. Figure 9.4 represents the cell density in algal cultures containing nanoparticles with different concentrations of zinc and cobalt for a period of 14 days.

![Graph showing cell count of Chlorella pyrenoidosa](image)

**Figure 9.4**: Cell count of *Chlorella pyrenoidosa*, H. Chick [14] in cultures containing Zn$_{1-x}$Co$_x$Fe$_2$O$_4$ nanoparticles. Values are reported as mean of three replicates ± standard deviation (SD).

The percentage of inhibition of algal growth in cultures containing nanoparticles was calculated using the equation [21].

\[
\text{Inhibitory Rate (IR\%)} = \left(1 - \frac{N}{N_0}\right) \times 100 \%
\]

where, N is the algal cell density of the cultural solution with nanoparticles (whose inhibitory rate is calculated), $N_0$ is the algal cell density of the control.

The percentage of inhibition of algal growth in cultures containing nanoparticles is presented in table 9.3.
Table 9.3: Percentage of inhibition of algal growth in cultures with Zn–Co mixed ferrite.

<table>
<thead>
<tr>
<th>Nano Particles</th>
<th>Percentage of inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnFe$_2$O$_4$</td>
<td>25%</td>
</tr>
<tr>
<td>Zn$<em>{0.75}$Co$</em>{0.25}$Fe$_2$O$_4$</td>
<td>23%</td>
</tr>
<tr>
<td>Zn$<em>{0.50}$Co$</em>{0.50}$Fe$_2$O$_4$</td>
<td>14%</td>
</tr>
<tr>
<td>Zn$<em>{0.25}$Co$</em>{0.75}$Fe$_2$O$_4$</td>
<td>7%</td>
</tr>
<tr>
<td>CoFe$_2$O$_4$</td>
<td>43%</td>
</tr>
</tbody>
</table>

In this study, the algal cell density was found to be lower in the cultures containing nanoparticles (with different concentrations of zinc and cobalt) when compared to the cell density values of the controls. The reduction of cell density of the test cultures with respect to the control, was getting lesser as the value of $x$ increased from 0.00 to $x=0.75$. However a prominent decrease in cell density was seen in all cultures grown with pure cobalt ferrite nanoparticles. This observed pattern of variation in the cell density is similar to the observed variation in chlorophyll $a$ content mentioned earlier suggesting very strongly that the variation in chlorophyll $a$ content could be due to the variation in the cell density rather than a reduction of the chlorophyll $a$ content in the algal cells.

9.4.3 Morphological Analysis of the algal cells.

A Scanning Electron Microscope was used to study the morphology of the algal cells both in the control and in the algal cultures, containing nanoparticles. Figure 9.5(a) shows the SEM images of ZnFe$_2$O$_4$ nanoparticles. Figure 9.5 (b) depicts the algal cells in the presence of ZnFe$_2$O$_4$ nanoparticles. The arrow mark indicates the zinc ferrite nanoparticles. Figure 9.5 (c) presents the algal cells in the control (in the absence of nanoparticles) and the inset shows an intact algal cell. Figure 9.5 (d) clearly shows the disruption, shrinkage, cell wall rupture and degradation of algal cells when in contact with cobalt ferrite nanoparticles.
A significant decrease in the growth of algal cells was observed in the algal culture with pure cobalt ferrite. The cell damage was also very prominent in the algal culture containing cobalt ferrite. There are reports stating that there are pores on the algal cell walls and the size of these pores range from 5nm to 20nm [21]. Nanoparticles in these size ranges can plug the pores on the algal cell walls thus interfering in the normal metabolism of algal cells which in turn may result in a reduction in their reproduction and hence lowering cell count in the cultures containing nano particles. However, further studies are required to find the exact reason for the inhibition of algal growth in algal cultures containing nanoparticles.
9.5 Magnetic Characterization

Magnetic characterizations at room temperature for all samples were carried out using a VSM, with maximum applied field of 15kOe. Figure 9.6 shows the typical magnetic hysteresis loops of Zn$_{1-x}$Co$_x$Fe$_2$O$_4$ system.

![Figure 9.6: Room temperature hysteresis curves of Zn$_{1-x}$Co$_x$Fe$_2$O$_4$](image)

The variation of saturation magnetization ($M_s$), coercivity ($H_c$) and remanence ($M_r$) of all the samples are presented in table 9.4.

### Table 9.4: Effect of Co$^{2+}$ doping on the magnetic parameters of ZnFe$_2$O$_4$

<table>
<thead>
<tr>
<th>$x$</th>
<th>$M_s$ (emu/gm)</th>
<th>$M_r$ (emu/gm)</th>
<th>$R = M_r/M_s$</th>
<th>$H_c$ (Oe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>4.70</td>
<td>0.05</td>
<td>0.01</td>
<td>5.68</td>
</tr>
<tr>
<td>0.25</td>
<td>22.06</td>
<td>0.33</td>
<td>0.02</td>
<td>23.91</td>
</tr>
<tr>
<td>0.50</td>
<td>48.87</td>
<td>2.48</td>
<td>0.05</td>
<td>71.39</td>
</tr>
<tr>
<td>0.75</td>
<td>77.62</td>
<td>13.83</td>
<td>0.18</td>
<td>306.96</td>
</tr>
<tr>
<td>1.00</td>
<td>42.30</td>
<td>11.20</td>
<td>0.27</td>
<td>1182.50</td>
</tr>
</tbody>
</table>

In a cubic system, the magnetic properties mainly depend on the super exchange interactions between the metal ions in the A and B sublattices[22]. The saturation magnetisation was found to increase with increase in the cobalt...
content and reached a maximum value of 77.62 emu/gm, when \( x = 0.75 \). The increase in saturation magnetisation with increase in the Co\(^{2+}\) ions can be explained to be the result of an enhancement in the magnetic properties, caused by the replacement of non-magnetic Zn\(^{2+}\) ions by magnetic Co\(^{2+}\) ions. However a low value of saturation magnetisation was observed for pure cobalt ferrite. The coercivity value increased from 5.68Oe to 1182.50Oe with increase in the Co\(^{2+}\) ion content. Coercivity depends on various factors like micro strain, size distribution, magneto–crystalinity, magnetic domain size etc [23, 24]. It has been reported by various researchers that the coercivity is inversely proportional to the grain size [24]. Larger the grain size, easier will be the motion of domain walls and smaller will be the coercivity. This could be the reason for the increase in coercivity with increase in the Co\(^{2+}\) ion content, as the crystallite size decreases with increase in the cobalt content. The remanent ratio was found to be low indicating isotropic nature of the prepared samples [25].

9.6 Conclusions

Sol-gel technique was used to prepare Zn\(_{1-x}\)Co\(_x\)Fe\(_2\)O\(_4\) (with \( x = 0.00, 0.25, 0.50, 0.75, 1.00 \)) nanoparticles. X-Ray Diffraction technique was used to study the crystal structure of the prepared samples. The lattice constant was found to decrease continuously with increase in the cobalt ion substitution. XRF analysis confirmed the absence of any impurity elements and confirmed the purity of the samples. All the ferrite nanoparticles showed an inhibitory effect on *Chlorella pyrenoidosa*, *H. Chick*. The inhibitory effect was found to decrease from 25% to 7% with cobalt substitution in zinc ferrite (for \( x = 0.00 \) to \( x = 0.75 \)). However pure cobalt ferrite showed 43% inhibition on the growth of the green algae. The SEM images of algal cells in cultures with cobalt ferrite nanoparticles showed disruption, shrinkage and damage of cell walls of...
The effect of cobalt substitution on the structural, magnetic, and anti-algal properties of the algal cells. An increase was observed in the saturation magnetisation with increase in Co\(^{2+}\) ion concentration, which can be explained as the enhancement of the magnetic properties by the replacement of non-magnetic Zn\(^{2+}\) ions by magnetic Co\(^{2+}\) ions. The change in coercivity with cobalt content is attributable to the variation of coercivity of nanoparticles in the multi domain regime. The magnetic properties of Zn-Co mixed ferrite, can be explored further as a tool for a quick and cheap method for separating the algal cells from water bodies. For a deeper understanding on the nature of toxicity of zinc-co cobalt ferrite nanoparticles on algal cells, further studies are needed.

References


