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Synthesis and Characterization of Iron Nanoparticles for Biological Applications

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Abstract

Iron (Fe) nanoparticles have been synthesized by simple co-precipitation method. The x-ray diffraction studies indicated the formation of Fe nanoparticles with cubic phase. Surface morphology of Fe and has been studied using scanning electron microscopy (SEM). Transmission electron microscopy (TEM) images reveal that Fe nanoparticle have size ranging from 25-41 nm. Also we have reported about the changes in the growth of piglets such as its body weight (kg), its haemoglobin (g %) level, its hematocrit (%) level and its erythrocyte count, when it was supplemented with different compositions of iron nanoparticles since iron is an integral component of hemoglobin which plays major role in supply of oxygen for normal functioning of body cells. Besides that iron is necessary for activity of several enzymes and catalytic pathways at cellular level.

Keywords: *Animal Study; Chemical method; Iron Nanoparticle; TEM.*

1. INTRODUCTION

In the rapidly emerging field of nano-biotechnology, metal nanoparticles are extensively used in drug delivery, biosensors, bio imaging, antimicrobial activities, food preservation etc. by exploiting their unique physical chemical and biological properties. There has been a great interest in using microorganisms as a tool for synthesis of new functional inorganic nanomaterials which are free from any kind of toxic chemicals and byproducts. Iron oxide (IO) has been widely used in biomedical research because of its biocompatibility (Mahdy *et al.* 2012). Nanoparticles of iron oxide, in its different phases, are being currently explored for their diverse range of applications such as magnetic storage media, environment protection, sensors, catalysis, clinical diagnosis and treatment (Cao and Zhang, 2006; Sarangi *et al.* 2009; Lee *et al.* 2008; Zelmanov and Samiat, 2008; Dengxin *et al.* 2008; Lin *et al.* 2009; Li *et al.* 2010). Iron oxide is a well-known semiconductor having negative temperature coefficient of resistance. The use of high resistance (~108 Ω) α-Fe_2O_3 thin films as humidity sensor has been demonstrated by Chauhan *et al.* (1999). However, in order to synthesize nano-sized iron oxide powders, the traditional high temperature solid state route is inappropriate, as it leads to coarsening of grains. In the pursuit to prepare homogenous nanoparticles of iron oxide, a variety of synthesis routes like precipitation, sol-gel, hydrothermal, combustion, solvent evaporation have been utilized. Raming et al have described a method to prepare nanocrystalline hematite powder in which ferric chloride was allowed to hydrolyze at 100 °C for a week in presence of hydrochloric acid (Raming *et al.* 2002). Ganguli *et al.* (2007) have reported a reverse micellar route to synthesize iron oxalate nanorods using cetyltrimethyl ammonium bromide as surfactant. Spherical α-Fe2O3 nanoparticles were obtained by decomposition of these nanorods at 500°C. Therefore, development of a simple synthetic methodology for preparation of pure and homogeneous Fe nano powders is a major challenge till now. In order to overcome the limitations of the existing methods, we have developed technically simple but cost effective chemical synthetic routes. Herein, we report synthesis methodology for the preparation of Fe nanoparticles.

2. EXPERIMENTAL

As reported from our previous work (Peula Kumari *et al.* 2013), Fe was synthesized by using aqueous solutions of ferric nitrate with sodium borohydride and ascorbic acid as a reducing agent. About 0.2M ferric nitrate is mixed with 0.5M ascorbic acid in deionisized water. The mixed solution was stirred at room temperature for 30 minutes. Then freshly prepared sodium borohydride was added drop-

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wise to the ferric nitrate and ascorbic acid mixer. Now reddish brown colored mixture turns into brick red, the stirring is continued until the reddish colour changes into black. The change in colour indicates the formation of metal particles in the solution and then the solution is stirred for 6 hrs at room temperature. After 6 hrs, the suspension was centrifuged and washed with water and ethanol several times. The samples were then suspended in ethanol and then allowed to age for 2 hrs without stirring. After centrifugation, the samples were dried at room temperature.

X-ray diffraction studies have been carried out using PANalytical x-ray diffractometer, surface morphology of the samples has been studied using scanning electron microscope (JEOL JSMS 800-V). Transmission electron microscope (TEM) images of the prepared Fe have been recorded using a Philips TECNAI F20 microscope.

3. RESULTS & DISCUSSION

Fig. 1a shows the X-ray diffraction patterns of Fe. The diffraction peaks at 2θ (degrees) value of 43.63° corresponds to (400) plane of Fe. The sharpness of the diffraction peak suggest that the product is well crystallized.

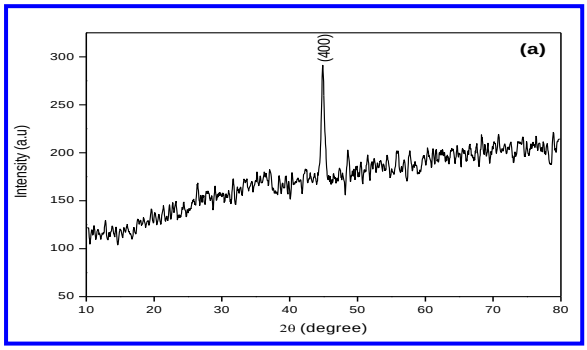

Fig. 1: X-ray diffraction pattern of Fe nanoparticles.

The average size of Fe have been calculated using Debye Scherrer's equation,

$$D = \frac{K\lambda}{\beta \cos\theta}$$

where, D is the grain size, K is a constant taken to be 0.94, λ is the wavelength of the x-ray radiation, β is the full width at half maximum and θ is the angle of diffraction. The crystallite size of Fe is found to lie in the range of 30-45 nm (±0.1nm).

Fig. 2(a) shows the scanning electron microscope (SEM) image of Fe nanoparticles. SEM image (fig. 2a) shows clearly the formation of Fe nanocluster. It clearly indicates that the particles are very small in size.

Fig. 2(b) shows the transmission electron microscope (HRTEM) image of Fe nanoparticles. It can be clearly seen that all the Fe particles have agglomerated. Using the particle number and the average particle diameter of the particles in the TEM image the particle size has been calculated. The particle size of Fe is found to lie in the range of 25-41 nm (±0.1nm).

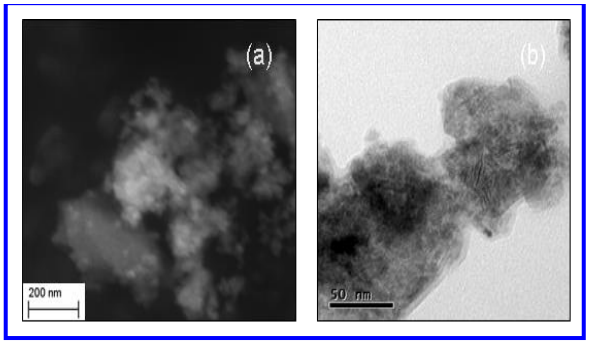

Fig. 2: (a) SEM image of Fe (b) TEM image of Fe

4. ANIMAL STUDY

Further the as prepared iron oxide nanoparticles were used to evaluate the effect on five groups of piglets was observed entirely for four weeks which showed that the nanoparticles have moderate the piglet's body weight, hematological parameters and total erythrocytes count which are compared, tabulated and discussed below.

4.1 Experimental Details

The study was conducted on piglets born to Large White Yorkshire sows. Individual piglets born in each litter were assigned to one of the five groups as detailed below.

Group I - No supplementation of iron
Group II -Iron injection
Group III -Oral supplementation of ferric ammonium citrate
Group IV -Oral supplementation of nano iron (3mg/day)
Group V -Oral supplementation of nano iron (6mg/day)

Piglets of group II were injected with single injection (i/m) of iron-dextran on day-3 after birth. Piglets of group III were fed orally ferric ammonium citrate equivalent to elemental iron (10 mg) /day given on alternate days from 3rd day to 28 days of age.

Group IV and group V piglets were administered orally nano iron from 3rd day to 28 days of age on alternate days @ 3 mg/day and 6 mg/day respectively. While piglets of group I were not received any iron supplementation and served as control.

4.2 Body weight

It was observed from the results that at the end of 2nd week of age, body weight of piglets group V of supplemented with nano iron (6 mg/day) differed significantly ($P<0.01$) with all other groups. At 4th week of age, supplementation of nanoiron at both

levels (3 mg and 6 mg/day) improved weight gain (P<0.01) over control, iron injection and ferric ammonium citrate fed group. However, the body weight did not differ significantly at 1st as well as 3rd week of age. Iron dextran injection and oral ferric ammonium citrate did not influence the body weight gain in piglets compared to unsupplemented group. Some of the reported iron dextran injection lead to higher weight gain over the untreated piglets are similar to the results we observed (Pollmann *et al.* 1983; Svoboda *et al.* 2007).

Table 1. The mean body weight (kg) of piglets supplemented with different iron preparations.

Age (Weeks)	Body weight (Kg)				
	Group I	Group II	Group III	Group IV	Group V
First	2.29	2.39	2.31	2.33	2.31
Second	3.59	3.72	3.64	3.79	4.22
Third	4.79	5.25	5.16	5.84	5.98
Fourth	6.73	7.09	7.11	7.44	7.69

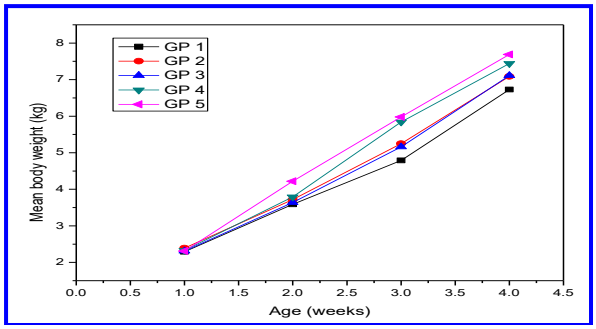

Fig. 3: Graphical representation of the mean body weight (kg) with respect to ages (weeks)

4.3 Hematological parameters

4.3.1 Hemoglobin

In Iron supplementation increased the hemoglobin content from first week of age onwards in all the groups except group I which shows reduced in hemoglobin rate when weeks increases. At the 4th week the hemoglobin content of iron nanoparticle supplemented group was higher (P<0.01) than oral ferric ammonium citrate fed group. At 3rd and 4th week of age, the iron supplementation effect was superior in group IV and V followed by group II and III. However, hemoglobin level was similar at both the levels of iron nanoparticle supplementation

Table 2. The mean hemoglobin (g%) of piglets supplemented with different iron preparations.

Age (Weeks)	Hemoglobin (g%)				
	Group I	Group II	Group III	Group IV	Group V
First	9.62	11.09	10.43	11.72	11.83
Second	7.51	11.74	11.21	12.65	12.72
Third	6.27	11.83	11.21	13.19	13.33
Fourth	5.98	12.59	11.57	13.86	14.26

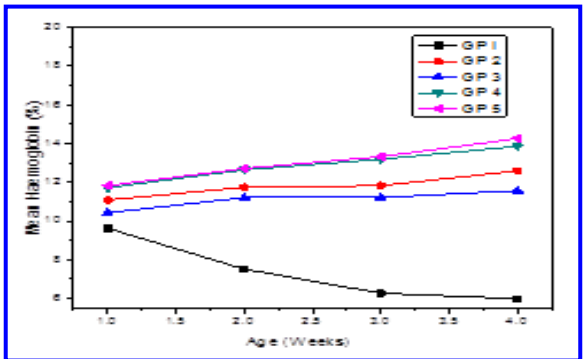

Fig. 4: Graphical representation of the mean hemoglobin (g%) with respect to ages (weeks)

4.4 Hematocrit

Table 3. The mean hematocrit (%) level of piglets supplemented with different iron preparations

Age (Weeks)	Hematocrit (%)				
	Group I	Group II	Group III	Group IV	Group V
First	29.39	34.94	31.97	32.66	34.18
Second	22.43	35.18	34.27	38.24	38.92
Third	19.27	37.88	36.15	41.26	40.98
Fourth	16.47	40.70	39.16	42.83	43.71

Iron supplementation increased hematocrit level (P<0.01) over unsupplemented group from second week of age onwards. At 1st week of age, there was no difference in hematocrit level among all the groups. In subsequent weeks, group IV and V had higher hematocrit level than group II and III.

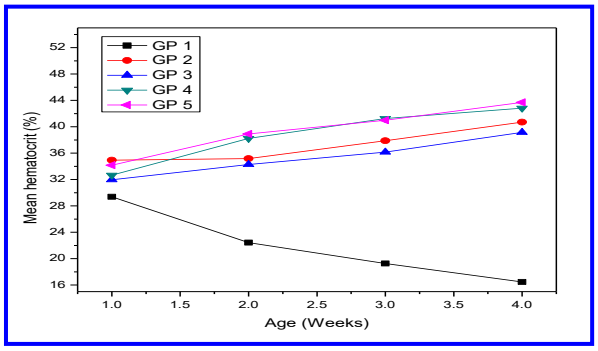

Fig. 5: Graphical representation of the hematocrit (%) level with respect to ages (weeks)

4.5 Total erythrocytic count

From 2nd week of age onwards, iron supplementation to the piglets increased (P<0.01) the total erythrocyte count compared to control group. At 1st week of age, there was gradual decrease in the group I piglets for the weeks passed and there is no difference in total erythrocyte count among all the other groups. From second to fourth week of age, iron nanoparticle fed groups had higher (P<0.01) total erythrocyte count than ferric ammonium citrate fed groups At second and fourth week of age, significant difference was noticed between group IV and V. It was observed that iron nanoparticle administration produced effect similar to parenteral administration of iron.

Table 4. The mean total erythrocytic count (106/µl) level of piglets supplemented with different iron preparations

Age (Weeks)	Total erythrocytic count(106/µl)				
	Group I	Group II	Group III	Group IV	Group V
First	6.38	6.41	6.35	6.39	6.50
Second	5.19	6.59	6.32	6.49	6.57
Third	4.51	6.77	6.69	7.01	6.97
Fourth	4.04	6.72	6.63	7.27	7.29

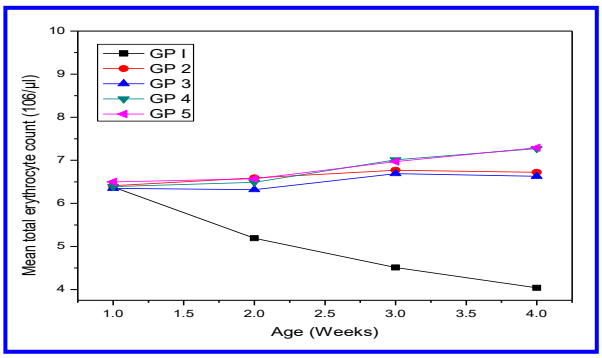

Fig. 6: Graphical representation of the erythrocytic count with respect to ages (weeks)

5. CONCLUSION

Fe nanoparticles have been prepared by a simple chemical method. X-ray diffraction pattern reveals that Fe nanoparticles exhibit cubic structure and the average particle size of the nanoparticles is in the range of 30-45 nm. The TEM studies show that the average particle size of Fe nanoparticles is in the range of 25-41 nm. The prepared Fe nanoparticles have been used to study the improvement in the pig model's body weight, hematological parameters, hematocrit and total erythrocytic count in biological system (pig model) and the variation table shows the effect of iron nanoparticles.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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