CHAPTER 1

INTRODUCTION

1.1. INTRODUCTION TO MICRONUTRIENTS

Almost a third of the world’s populations suffer from micronutrient deficiency, also known as ‘hidden hunger’, which mostly affects those living in developing countries. The public health importance of vitamin and mineral deficiencies has been underscored through significant investment by national governments and donors, in combating micronutrient deficiency to achieve “Millennium Development Goals” relating to mother and child health (Jamil et al., 2008).

Micronutrient Deficiencies (MNDs) are of great public health and socio economic importance worldwide. They act as a significant factor in health problems in industrialized societies with impacts among vulnerable groups in the population, including women, children, the middle-aged and the elderly. They affect all populations in Europe and more severely in the transition Countries of Eastern Europe (CEE), the former Soviet Union, and Countries of Central Asia (CAR). They significantly contribute to chronic diseases as the major causes of morbidity and mortality in these countries.

The World Health Organization (WHO) considers that more than 2 billion people worldwide suffer from vitamin and mineral deficiencies, primarily iodine, iron, vitamin A and zinc, with important health consequences. In 2009, WHO published a landmark document entitled Guidelines for Food Fortification with micronutrients and introduced the publication as follows:

“Interest in micronutrient malnutrition has increased greatly over the last few years. One of the main reasons for the increased interest is the realization
that micronutrient malnutrition contributes substantially to the global burden of disease. In addition to the more obvious clinical manifestations, micronutrient malnutrition is responsible for a wide range of non-specific physiological impairments, leading to reduced resistance to infections, metabolic disorders, and delayed or impaired physical and psychomotor development. The public health implications of micronutrient malnutrition are potentially huge and are especially significant when it comes to designing strategies for the prevention and control of diseases such as HIV/AIDS, malaria, tuberculosis and diet related chronic diseases (Thulchinsky., 2006).

1.2. **IRON – THE MOST ESSENTIAL MICRONUTRIENT**

Iron is involved in many functions within the human body and is a vital constituent of hemoglobin and myoglobin, which are involved in oxygen transport and supply within the tissues. It is also involved in electron transfer, hydroxylation, and acts as catalyst for oxygenation, cell proliferation and disposal of oxygen radicals.

Its most important property is the reversible one electron oxidation reduction reaction between the two common oxidation states, Fe$^{2+}$ and Fe$^{3+}$, allowing it to coordinate electron donors and to participate in redox processes. Reactions with oxygen can lead to the formation of intermediates with unpaired electrons.

The human body has developed complicated metabolic processes to absorb, transport and store iron ensuring a ready supply for cellular growth and function, but limits its participation in reactions that produce free radicals and its availability to invading pathogens (Bashiri *et al.*, 2003).

Human beings normally have 40–50 mg Fe/kg body weight. Approximately 75% is present in metabolically active compounds. The remaining 25% constitutes a dynamic store that is turned over constantly. It ensures an adequate supply for normal physiological functions despite short term variations in absorption or loss.
from the body. The store also supplies the immediate needs when requirements are increased (e.g., by rapid growth or pregnancy). Iron reserves that have been utilized are then gradually replaced by increased absorption (Kraemer and Zimmermann., 2007).

1.2.1. Deleterious effects of iron deficiency

Many infants, childrens and women of childbearing age, particularly in the poorer countries of the developing world, are iron deficient. About half of these iron deficient individuals develop iron deficiency anaemia (IDA), the most advanced form of the disease, which has several major negative impacts on health and contributes substantially to the risk of early death and disability.

There are five major negative health consequences of IDA. Firstly in the pregnant woman, IDA leads to sub-optimal pregnancy outcome, including lower birth weight, increased morbidity in mothers and neonates, increased infant mortality and a greater risk of the infant developing iron deficiency after 4 months of age. Secondly, during infancy, IDA leads to delayed mental and motor development with effects on behaviour and cognitive performance when the child reaches school age. The effects of early IDA on brain development may not be reversible by subsequent treatments. In childrens, IDA can also lead to increased frequency and duration of upper respiratory infections and to increased risk for goiter due to diminished utilization of iodine for thyroid hormone production. Finally, physical work capacity is impaired for all individuals as IDA negatively affects aerobic capacity related to intense physical activity and reduces endurance capacity, voluntary activity and work productivity.

Iron deficiency is therefore a major health problem in the developing world and recently WHO ranked it as 7th out of the 10 major global preventable risks for disease, disability and death, that together account for 40% of the 56 million deaths that occur worldwide each year and for one third of the global loss of healthy life years.
In developing countries, underweight has been reported to be the greatest risk factor and accounts for 9.5% of the global DALY’s (disability adjusted life years, one DALY is equal to the loss of one year of healthy life). Iron deficiency is the next highest nutritional risk factor and accounts for 2.4% of global DALY’s, preceded only by sexually transmitted diseases, diseases related to unsafe water, poor sanitation and hygiene, alcohol abuse, and indoor smoke from solid fuels. It has been estimated that if iron deficiency were eliminated worldwide, more than 35 million people would have one additional year of healthy life (Hurrell et al., 2009).

1.2.2. Stages of iron deficiency

The first stage of iron deficiency is characterized by the absence of measurable iron store, the second (iron deficient erythropoiesis) by evidence of a restricted iron supply in the absence of anaemia and the third (iron deficiency anaemia) by a haemoglobin concentration that falls below the normal threshold for age and sex. The iron indicators that can be used to identify the three stages of iron deficiency are discussed.

In the first stage, a depletion of iron stores occurs with no apparent symptoms and hemoglobin levels in the normal range. Serum ferritin and bone marrow iron are decreased and there is a consequent increase in iron absorption. The second stage is when a slower erythropoiesis takes place due to the lack of iron availability, the hemoglobin levels start to decrease with the adjuvant decrease of serum ferritin, bone marrow iron, a low serum iron and an increase in total iron binding capacity. At this stage the hematocrit remains unchanged. The third and last stage is when iron deficiency anemia develops. Ferritin levels and transferrin saturation levels are very low, iron stores are depleted, serum iron and hemoglobin levels are low and the total iron binding capacity is elevated (Kraemer and Zimmermann., 2007).
Figure 1.1 Requirements for absorbed iron and energy consumption at different stages of the human life cycle

1.2.3. Iron deficiency anaemia

Anaemia is one of the most common and intractable nutritional problems in the world today. The World Health Organization (WHO) estimates that some two billion people are anaemic defined as haemoglobin concentrations that are below recommended thresholds. The main causes of anaemia are dietary iron deficiency, infectious diseases such as malaria, hookworm infections and schistosomiasis, deficiencies of other key micronutrients including folate, vitamin B_{12} and vitamin A or inherited conditions that affect red blood cells (RBCs). Iron deficiency affects energy metabolism, causing as a consequence symptoms like fatigue, lack of concentration and decreased mental, physical and cognitive performance.

Although anaemia has been recognized as a public health problem for many years, there has been little progress towards improvement and the global prevalence of anaemia, remains unacceptably high. It has been estimated that around two billion peoples in the world are anaemic, mostly in the countries of Africa and Asia (Jamil et al., 2008).
One of the reasons for the apparent failure to reduce the prevalence of anaemia is that many programmes and their interventions have been designed with the assumption that the only cause of anaemia is iron deficiency (Table 1.1). This has meant that, when trying to control anaemia, the role of other causes has been underestimated and that iron deficiency without anaemia has not been addressed as a major and common health problem (Worwood., 2007).

Table 1.1 Reasons behind the failure of iron bioavailability

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<th>Failure of effectiveness</th>
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<tr>
<td>• Use of iron compounds with low bioavailability or failure to enhance absorption from inhibitory diets</td>
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<tr>
<td>• Inadequate iron fortification</td>
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<td>• Consumption of fortified food too low to deliver adequate iron</td>
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<td>• High frequency of parasitic infections that cause blood loss (e.g. hookworm)</td>
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<td>• High frequency of infection, inflammation, or both, that impairs iron metabolism and erythropoiesis (e.g. malaria)</td>
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<th>Failure to detect effectiveness</th>
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<tr>
<td>• Failure to define iron status with specific indicators clearly</td>
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<tr>
<td>• Failure to recognise other causes of anaemia</td>
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<td>• Poor programme control and enforcement</td>
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To plan effective interventions to combat both iron deficiency and anaemia there is an urgent need to have better information on the iron status of populations.

**Etiology of Iron deficiency**

The prevalence of ID and IDA varies greatly from population to population according to a variety of host and environmental factors. The etiology of anemia is multifaceted and often several factors are at play in an anemic individual. Nutritional anemia as a result of iron deficiency is the most common cause of anemia worldwide, with approximately 50% of all cases attributed to a lack of iron
in the diet. A number of host and environmental factors are associated with iron deficiency, and in more severe forms contribute to IDA as well. These include:

1. **Inadequate dietary iron intake**: Diets low in iron or diets low in adequate amounts of bioavailable iron are a major cause of IDA, particularly in non-industrialized countries. Typically, high levels of IDA are also observed in old age when dietary quality and quantity deteriorates (Clark., 2008; Fiatarone *et al.*, 2000).

2. **Menstruation and pregnancy**: Blood losses associated with menstruation and pregnancy are common causes of ID and IDA. Typically non-menstruating women lose about 1 mg of iron per day, while menstruating women lose an additional 10 mg of iron per day during menses. Pregnancy is associated with an iron loss of approximately 1000 mg in a 55 kg woman (Bothwell., 1995).

3. **Infectious disease**: In the developing world common infections which may be both chronic and recurrent are associated with blood loss leading to iron deficiency, and ultimately to IDA. Hemolytic malaria and parasitic infections such as hookworm, trichuriasis, amoebiasis, and schistosomiasis are particularly common diseases that contribute to the depletion of iron stores and often result in IDA (Oppenheimer., 2001).

4. **Interactions with medication**: Several pharmacological agents can interfere with iron uptake and/or transport leading to iron loss or defective absorption. These include H₂ blockers, proton pump inhibitors, aspirin or non-steroidal anti-inflammatory drug use (Rockey and Cello., 1993).

5. **Gastrointestinal conditions**: Both acute and chronic gastrointestinal illness is associated with IDA and is an important consideration in clinical diagnosis of the condition (Figure 1.2). Duodenal or gastric ulcers, carcinoma, irritable bowel disease, erosive gastritis, celiac disease, altered hepatic function for any number of reasons, and/or compromised protein status may lead to IDA (Clark., 2008).
Figure 1.2  Fe circulation mechanism inside the human body

6. Periods of growth: Iron deficiency and IDA are particularly prevalent during peak periods of growth. Though full-term infants are normally born with adequate iron stores, if complementary foods containing iron are not introduced to the diet after six months of age then an infant is at risk of developing ID, and ultimately IDA. Iron requirements (Figure 1.1) on a body weight basis are proportional to growth velocity, thus iron deficiency and IDA are common in preschool years and during puberty (McLean et al., 2008).

7. Socioeconomic status: Iron deficiency and IDA are most common among groups of low socioeconomic status for a number of reasons, including but not limited to: malnutrition, poor education regarding health and hygiene, and greater presence of concomitant disease when compared to populations of higher socioeconomic status (Bhargava et al., 2001; Thankachan et al., 2007).
Hematological parameter associated with iron deficiency

The effects of iron depletion without anemia on adaptation to training have not been fully characterized but laboratory evaluation of iron status is necessary and helpful in defining anemic states. Iron containing compounds in the body are one of three types: a) functional compounds that serve in metabolic or enzymatic functions and b) compounds that serve as transport and c) storage forms for iron. There are a number of markers that describe these functional, transport and storage compartments for iron: serum iron, total iron binding capacity, red blood cell count, hemoglobin, hematocrit, red blood cell indices (MCV, MCH, MCHC, RDW), transferrin, transferrin saturation, and serum ferritin. These laboratory tests are essential to an accurate diagnosis of ID and the evaluation of therapy (Fallon. 2004). Three RBC measurements are routinely carried out: packed cell volume (PCV), the proportion of whole blood volume occupied by RBC; hemoglobin (Hb) concentration of whole lysed blood; and RBC count, the number of RBC per unit volume of whole blood.

**PCV**: PCV is the variable normally used to assess the basic status of the erythron increased in polycythemia, decreased in anemia although if a sample is too hemolyzed to allow measurement of PCV, a meaningful Hb measurement may still be obtained. RBC count as such should not be interpreted clinically. An abnormally high PCV (polycythemia) may be relative, due to a change in the proportion of circulating RBC to blood plasma without any alteration in the size of the erythron, or absolute, due to a real increase in erythron size. Absolute polycythemia may be primary (e.g. polycythemia vera or rarely, erythropoietin producing tumors) or secondary (a consequence of disease in another organ system).

**Hemoglobin**: Hemoglobin constitutes the major fraction of body iron (functional iron) with a concentration of about 0.5 mg iron/mL blood. Iron is distributed within the body via transferrin in the plasma, a transport protein that mediates iron exchange between tissues. Ferritin is the primary storage compound for the body's
iron and serum ferritin concentration is a reliable index of iron stores (1 ng/ml of serum ferritin indicates about 8 mg of storage iron). Serum ferritin does not exhibit diurnal variations as are seen with serum iron levels (Fallon. 2004). The serum ferritin level is decreased in all stages of ID and may be the first indication of a developing ID. Serum ferritin is generally considered the single best test to detect iron deficiency. Although highly trained athletes usually have normal absolute levels of hemoglobin, they often have ID, generally latent, that implies no decrease in hemoglobin. Swimmers with low ferritin levels may not be anemic, but their performance at maximal intensities may be compromised. Anemia in swimmers, like in other athletes, has negative effects on physical exercise capacity and their ability to train from day to day.

1.2.4. Choice of the iron compounds

The choice of the iron compound is often a compromise between reasonable cost, bioavailability and the acceptance of any sensory changes. When selecting the most appropriate chemical form of a given micronutrient, the main considerations and concerns are thus (Kraemer and Zimmermann., 2007).

• Sensory problems. The iron compound fortified to the dietary substance must not cause unacceptable sensory problems (e.g. colour, flavour, odour or texture) at the level of intended fortification or segregate out from the food matrix and they must be stable within given limits.

• Interactions. The likelihood or potential for interactions between the added micronutrient and the food vehicle and with other nutrients (either added or naturally present), in particular any interactions that might interfere with the metabolic utilization of the iron compound supplement, needs to be assessed and checked prior to the implementation of a fortification programme.

• Cost. The cost of fortification must not affect the affordability of the food nor its competitiveness with the unfortified alternative.
• **Bioavailability.** Most importantly, the iron compound fortified must be sufficiently well absorbed from the food vehicle and be able to improve the micronutrient status of the target population.

Safety is also an important consideration. The level of consumption that is required for fortification to be effective must be compatible with a healthy diet. According to the conclusions of the Sharing United States Technology to Aid Improvement of Nutrition (SUSTAIN) Task Force reports (1997), only electrolytic iron powders (diameter <45 microns or 325 meshes) have been proven to be sufficiently bioavailable to humans. The data indicates that carbonyl iron and some hydrogen reduced (H-reduced) iron powders have comparable bioavailability to electrolytic iron.

**Table 1.2 Iron absorption rates of various conventional forms**

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<th>S.No.</th>
<th>Available form of Iron (Absorption rates)</th>
<th>Rate (%)</th>
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<tbody>
<tr>
<td>1.</td>
<td>Ferrous gluconate</td>
<td>17.0 – 25.0</td>
</tr>
<tr>
<td>2.</td>
<td>Ferrous Chloride</td>
<td>12.0 – 20.0</td>
</tr>
<tr>
<td>3.</td>
<td>Ferrous sulfate</td>
<td>12.0 – 16.0</td>
</tr>
<tr>
<td>4.</td>
<td>Ferrous carbonate</td>
<td>6.0 – 11.0</td>
</tr>
<tr>
<td>5.</td>
<td>Ferrous lactate</td>
<td>6.0 – 10.0</td>
</tr>
<tr>
<td>6.</td>
<td>Iron metallic</td>
<td>0.5 – 2.0</td>
</tr>
</tbody>
</table>

Atomized iron and carbon monoxide reduced (CO-reduced) iron are not recommended at the present time because of their lower bioavailability. Elemental iron with a large particle size (diameter >149 microns or 100 mesh) is probably too insoluble in the intestine and is therefore not generally recommended for use as a food fortificant (Hurrell et al., 2009).
There are several iron compounds subjected to the medication and food fortification in the later years using the ferrous salts with combinations. The absorption rates of the varied iron formulations available in the conventional form have been illustrated in the Table 1.2.

1.3. DIETARY DIVERSIFICATION OF IRON COMPOUNDS

Iron supplementation is a public health strategy designed for the prevention of iron deficiency and its consecutive anaemia. Standard practice has included screening for anaemia without requiring the determination of iron deficiency and oral supplementation as the treatment of choice due to its low cost and high effectiveness.

Technically, iron is the most challenging micronutrient to add to foods, because the iron compounds that have the best bioavailability tend to be those that interact most strongly with food constituents to produce undesirable organoleptic changes. When selecting a suitable iron compound as a food fortificant, the overall objective is to find the one that has the greatest absorbability i.e. the highest relative bioavailability (RBV), yet at the same time does not cause unacceptable changes to the sensory properties (i.e. taste, colour, texture) of the food vehicle. Cost is usually another important consideration (Hu., 2005).

The basic requirements on iron fortificants include:
1) Soluble to provide good bioavailability
2) Stable during storage and food processing to resist oxidation from moisture and heat and
3) Inexpensive and capacity available to be able for large-scale applications.

A wide variety of iron compounds are currently used as food fortificants at present. These can be broadly divided into three categories (Table 1.3).

— Water soluble
— Poorly water soluble but soluble in dilute acid
— Water insoluble and poorly soluble in dilute acid.
Water soluble iron compounds

Being highly soluble in gastric juices, the water soluble iron compounds have the highest relative bioavailabilities of all the iron fortificants and for this reason they are more often, the preferred choice. However, these compounds are also the most likely to have adverse effects on the organoleptic qualities of foods, in particular, on the colour and flavour. During prolonged storage, the presence of fortificant iron in certain foods can cause rancidity and subsequent off flavours. Moreover, in the case of multiple fortification, free iron, produced from the degradation of iron compounds present in the food, can oxidize some of the vitamins supplied in the same fortificant mixture.

The water soluble forms of iron are especially suited to fortifying cereal flours that have a relatively fast turnover, i.e. one month in warm, humid climates and up to 3 months in dry, cold climates. Water soluble iron compounds are also useful for dry foods, such as pasta and milk powder, as well as dried milk based infant formulas. Encapsulated forms, i.e. iron compounds that have been coated to physically separate the iron from the other food components, can be used for slowing down or preventing sensory changes.

Ferrous sulfate is by far the most frequently used water soluble iron fortificant, principally because it is the cheapest. It has been widely used to fortify flour. However, depending on its physical characteristics, the climate and the fat content of the flour to which it is added, ferrous sulphate can cause rancidity and therefore its suitability as a fortificant needs to be evaluated in trials before use. (Kraemer and Zimmermann., 2007).
Table 1.3 Iron compounds classified based on their solubility

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group Characteristics</th>
<th>Examples</th>
</tr>
</thead>
</table>
| 1.   | Iron compounds soluble in water | Ferrous sulphate  
Ferrous gluconate  
Ferrous lactate  
Ferrous ammonium citrate |
| 2.   | Iron compounds poorly soluble in water but soluble in dilute acids | Ferrous fumarate  
Ferrous succinate  
Ferrous saccarate  
Ferrous phosphate |
| 3.   | Iron compounds poorly soluble in water and poorly soluble in dilute acids | Elemetal iron powders  
Carbonyl iron  
Reduced iron  
Electrolytic iron |
| 4.   | Iron protected compounds | Hemoglobin  
EDTA  
Amini acid chelates  
Stabilized ferrous sulphate |

Iron compounds poorly soluble in water

Compounds that fall into the second category of iron fortificants are also reasonably well absorbed from food, as they are soluble in the gastric acids produced in the stomach of normal healthy adults and adolescents. Some concern has been raised about absorption in infants who may secrete less acid but further research is needed in this area.

In most people, however, with the possible exception of individuals who suffer from a lack of gastric acid due to medical problems, iron absorption from these compounds is likely to be similar to that from water soluble iron compounds. Poorly water soluble iron compounds, have the advantage of causing fewer sensory
problems in foods than the water soluble compounds and are generally next in line for consideration, especially if more water soluble forms cause unacceptable organoleptic changes in the chosen food vehicle.

Ferrous fumarate and ferric saccharate are the most commonly used iron compounds in this group. The former is frequently used to fortify infant cereals and the latter, chocolate drink powders. Ferrous fumarate is used to fortify maize flour in Venezuela and wheat flour in Central America, where it has also been proposed as a potential fortificant for maize masa. Ferrous fumarate can be used in an encapsulated form to limit sensory changes (Kraemer and Zimmermann., 2007).

**Iron compounds insoluble in water**

Relative to ferrous sulphate (water soluble), the absorption of iron from water insoluble compounds ranges from approximately 20% up to 75%. Despite their reduced absorbability, water insoluble iron compounds have been widely used by the food industry as fortificants because they have far less effect on the sensory properties of foods and because they are cheaper than the more soluble compounds. However, they are generally regarded as the last resort option, especially in settings where the diet of the target population is high in iron absorption inhibitors. If it is necessary to use a water insoluble iron fortificant, it should ideally have an absorption equivalent to at least 50% that of ferrous sulphate.

Elemental iron powders are used in a number of countries to fortify cereals, but the bioavailabilities of the different forms of elemental iron that are currently available are not well established. The solubility of elemental iron is very dependent on the size, shape and surface area of the iron particles (characteristics which are governed by the manufacturing process).

It is well known that food fortification with iron is the best long term approach in reducing iron deficiency and has been successfully practiced in developed countries for more than 60 years (Kraemer and Zimmermann., 2007).
1.4. INTRODUCTION TO NANOTECHNOLOGY

Nano iron materials have been advocated for use in biomedicine with grain sizes down to the nanoscale for longer than any other type of material due to the intrinsic behavior. Inorganic Nanoparticles are particularly attractive building blocks for bottom up approaches as they can readily be prepared in large quantities from various materials by simple methods. In addition, they have controllable sizes ranging from a few nanometers to several, which makes them smaller and comparable to the size of a cell (10-100 µm), a protein (5-50 nm) or a gene (2-10 nm). This means that particles, if successful in avoiding the immune system, have potential to get close to the biological entities of interest and if coated with biomolecules, interact with biological targets.

1.4.1. Nanoparticles in drug delivery

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, the nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer known as long circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time to target a particular organ, as carriers of DNA in gene therapy and their ability to deliver proteins, peptides and genes.

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation,
targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs / proteins and possess useful controlled release properties.

The advantages of using nanoparticles as a drug delivery system include the following:

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting.

2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.

3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction, this is an important factor for preserving the drug activity.

4. Site specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.

5. The system can be used for various routes of administration including oral, nasal, parenteral, intraocular etc.

The field of nanoparticle delivery systems for nutrients and nutraceuticals with poor water solubility has been expanding, almost exponentially, over the last five years and some of these technologies are now in the process of being incorporated in food products. The market projections for these technologies suggest a multifold increase in their commercial potential over the next five years.
The interest in the pharmaceutical and food related applications of these technologies have sparked tremendous developments in mechanical (top-down) and chemical (bottom-up) processes to obtain such nanoparticle systems. Mechanical approaches are capable of producing nanoparticles, typically in the 100–1000 nm range, whereas chemical methods tend to produce 10–100 nm particles. Despite these technological developments, there is a lack of information regarding the basis of design for such nanoparticle systems (Mohanraj and Chen., 2006).

1.4.2. Characteristics of nanoparticles

Particle size

Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the in vivo distribution, biological fate, toxicity and the targeting ability of nanoparticle systems. In addition, they can also influence the drug loading, drug release and stability of nanoparticles. Many studies have demonstrated that nanoparticles of sub micron size have a number of advantages over micro particles as a drug delivery system. Generally nanoparticles have relatively higher intracellular uptake compared to micro particles and available to a wider range of biological targets due to their small size and relative mobility.

Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out. Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability (Mohanraj and Chen., 2006).

Surface properties of nanoparticles

Nanoparticles have large specific surface area and hence their properties are dominated by the surfaces rather than bulk materials. The specific surface area
(SSA) is used as the basic unit for the particle properties of the nanomaterial. It is very important to access the influence of the particle shapes and particle size distribution for measuring the particle size from the specific surface area. Usually if the particles have the spherical or cubical shape with mono dispersed size distribution, the specific surface area can be related with the size of the particles. Lower the particle size of a material, then it will be larger the specific surface area.

Surface properties can have an enormous effect on the success or failure of a biomaterial. It is widely accepted that such factors as surface preparation and the subsequent characterization are central issues in biomaterials research. The properties that are of interest in the characterization of biomaterial surfaces include the chemical structure, the hydrophilicity or hydrophobicity, the presence of ionic groups, the morphology (i.e. the domain structure), and the topography (i.e. the surface roughness, planarity, and feature dimensions).

Choice of the surface characterization method used can be influenced by a excess of considerations including the type of measurement required, the extent of the analyzed surface region, the required precision and accuracy, the influence of the technique on the surface (i.e. does the probe, electron beam, ion beam, X-ray, required sample preparation, or the analysis environment induce undesirable effects on the surface of interest), the influence of the sample on the instrument, limitations imposed by the surface, as well as the ease of use and availability of equipment (Hosokawa et al., 2009).

1.4.3. Bioavailability enhancement with nanoparticles

The term bioavailability refers to the fraction of a dose that is available at the site of action in the body. For most oral doses this definition is interpreted as the fraction of the dose that enters the bloodstream. Uptake (or intestinal absorption), on the other hand, refers to fraction of the dose that is absorbed through the intestinal walls. Although both definitions are related, the entire dose that is absorbed through the intestine (uptake) may not be bioavailable due to the various processes involved
in the absorption of nutrients. To design effective nanoparticle delivery systems for nutrients, nutraceuticals and related active ingredients, it is necessary to understand the biological processes that regulate uptake and bioavailability (Acosta., 2009).

However, experimental data reveal that, in some cases, nanoparticles in the 100–1000 nm range are capable of producing substantial improvement in the bioavailability of the active ingredients. In most cases, this improvement in bioavailability seems to be linked to the direct uptake of the nanoparticle. Furthermore, direct nanoparticle uptake is controlled by the size and surface chemistry of the nanoparticle system. The use of this direct nanoparticle uptake, in particular for soluble but poorly absorbed ingredients, is one of the areas that needs to be explored in the future, as well as the potential side effects of these nanoparticle carriers (Mohanraj and Chen., 2006).

1.4.4. Iron nanoparticle supplement

Iron magnetic particles with appropriate surface coating are increasingly being used clinically for various biomedical applications, such as magnetic resonance imaging, hyperthermia, drug delivery, tissue repair, cell and tissue targeting & transfection. This is because of the nontoxicity and biocompatibility demand that mainly iron based materials are predominantly used.

Particle size is a determinant of iron (Fe) absorption from poorly soluble Fe compounds. Decreasing the particle size of metallic Fe added to food & medicines increases Fe absorption (Figure 1.3). The development and characterizing nanoparticles of Fe determines their bioavailability & potential toxicological effects and so these indications as well can serve as a good iron supplementation. A number of nutraceuticals & nutritional supplementation containing nano ingredients & additives (e.g.: vitamins, antimicrobials, inorganics, anti oxidants etc) are currently available. Virtually all these products claim enhanced absorption and bioavailability of nanosized ingredients in the body.
1.5. ATTEMPTS FOR PREVENTING IRON DEFICIENCY

The elimination of iron deficiency however has not proved easy, even after the improvements in supplementation. Dietary diversification (promoting the consumption of iron-rich foods) is hindered by the difficulty in achieving behavioural change as well as by the predominance in developing countries of plant based diets, deficient in the more bioavailable haeme form of iron. The choice of fortificant compound is often a compromise between reasonable cost, bioavailability from the diet and the acceptance of any sensory changes. Water soluble iron compounds were found to cause adverse organoleptic changes and poorly soluble Fe compounds although more stable tend to have low bioavailability. The development and characterizing the nanoparticles of Fe, determines their bioavailability & potential toxicological effects and so these indications as well can serve as a good iron supplementation.

This particular research mainly focuses on developing an iron compound that is highly bioavailable without producing high unacceptable sensory effects, while intracting with the food vehicle or matrix.
Ferrous phosphate is a poorly soluble iron compound, with high biological impact and unknown absorption values because of its bulk size. The scientific opinion of the European Food Safety Authority (EFSA) indicates the importance of ferrous phosphate compounds as a food additive and its unrevealed nature of absorption and acidic solubility (Aguilar et al., 2009). Most researchers have worked under the assumption that improvement in bioavailability comes from improvement in apparent solubility and there are no much investigation found on the direct nanoparticles uptake, which play a vital role in enhancing the bioavailability.

Thus, our research aims to synthesis the ferrous phosphate nanoparticles with low particle size and high surface area, thereby by it can produce high absorption with less sensory effects.

1.5.1. Encapsulation of active compounds

Encapsulation may be defined as a process to entrap one substance within another substance, thereby producing particles with diameters of a few nm to a few mm. The substance that is encapsulated may be called the core material, the active agent, fill, internal phase, or payload phase. The substance that is encapsulating may be called the coating, membrane, shell, carrier material, wall material, external phase, or matrix. The carrier material of encapsulates used in food products or processes should be food grade and able to form a barrier for the active agent and its surroundings (Figure 1.4).

Encapsulates might also be defined by their particle size, e.g., nanoparticles, microcapsules, micro reservoir, etc.

The possible benefits of microencapsulated ingredients in the pharmaceutical and food industry could be (Zuidam and Shimoni., 2010).
• Superior handling of the active agent (e.g., conversion of liquid active agent into a powder, which might be dust free, free flowing, and might have a more neutral smell).

• Immobility of active agent in food processing systems.

• Improved stability in final product and during processing (i.e., less evaporation of volatile active agent and/or no degradation or reaction with other components in the food product such as oxygen or water).

• Improved safety (e.g., reduced flammability of volatiles like aroma, no concentrated volatile oil handling).

• Creation of visible and textural effects.

• Adjustable properties of active components (particle size, structure, oil or water soluble, color).

• Off taste masking

• Controlled release (differentiation, release by the right stimulus)

1.5.2. Encapsulation methods

The production of the encapsulates can be achieved by a number of different methods. The most common encapsulation techniques used for the biodegradable polymers are described below.

1. Spray drying technique
2. Water-oil-water (w/o/w) triple emulsion technique
3. Phase separation technique

Spray drying technique

In principle, the biodegradable polymer is dissolved in a volatile organic solvent, such as dichloromethane or acetone, the drug in solid form is dispersed in the polymer solution by high speed homogenization and this dispersion is atomized in a stream of
heated air. From the droplets formed, the solvent evaporates instantaneously, yielding microspheres in typical size ranges from 1 to 100 μm, depending on the atomizing condition. The encapsulated products are collected from the airstream by a cyclone separator. Residual solvents are removed by vacuum drying. The process can be operated under aseptic condition and in a closed loop configurations, spray drying in a nitrogen atmosphere is technically feasible.

**Water in oil water (W/O/W) emulsion technique**

In principle, the drug in an aqueous solution is emulsified with the non miscible organic solution of the polymer to form water in oil (w/o) emulsion. The organic solvent dichloromethane is mainly used, and the homogenization step is carried out using high speed homogenizers, ultrasound or vortex mixing. The primary (w/o) emulsion is then rapidly transferred to a vast excess of an aqueous medium, containing a stabilizer, usually poly (vinyl alcohol). Again homogenization or intensive stirring is necessary to initially form a triple emulsion of w / o / w. The organic solvent is rapidly extracted from the O-phase, yielding solid microparticles that contain antigen in a polymeric matrix. In the hardening step, residual amounts of solvent are extracted and evaporated (solvent extraction or solvent evaporation).

**Phase separation technique**

In principle, the drug is dispersed in solid form into a solution containing dichloromethane, and the polymer. Silicon oil is added to this dispersion at a defined rate, reducing the solubility of polymer in its solvent. The polymer rich liquid phase (coacervate) encapsulates the dispersed drug particles and the “embryonic” microspheres are subjected to a hardening and washing step using organic solvents, such as heptane.
1.5.3. Preparation of microspheres

Microspheres are microbeads composed of a biopolymer gel network entrapping an active. Microspheres are commonly made via two different routes (Acosta., 2009)

(a) **The extrusion or dropping method:** This method consists of dropping droplets of an aqueous solution of 0.6 – 4.0 wt % sodium alginate and active compound into a gelling bath of 0.05 – 1.5 M calcium chloride solution. The dripping tool can be simply a pipette, syringe, vibrating nozzle, spraying nozzle, jet cutter, atomizing disk, coaxial air-flow, or electric field. In general, particles with a diameter between 0.2 and 5 mm can be made depending on the dripping tool and the visco elasticity of the alginate solution. Each of the technologies is suitable for the production of spherical microspheres (800 mm in diameter) from low viscous sodium alginate solutions (up to 2% w/w), whereas high viscous alginate solutions
cannot be processed with the vibration technology anymore. With the electrostatic, jet cutter, and coaxial air flow technologies microsphere production was possible and a narrow size distribution was always achieved.

(b) **The emulsion method:** This technique utilizes emulsions to make microspheres. Several variants exist. One may add calcium chloride to an emulsion of water droplets of an alginate solution and active in vegetable oil. This results in the “break-up” of the emulsion and microbeads are formed by the gelation of the alginate droplets. Alternatively, both alginate and calcium (in an insoluble form such as calcium carbonate) can already be present in the water phase of the emulsion.

The emulsion method has the advantage that it can produce smaller microspheres than the extrusion method (0.2 – 5.0 mm). It is also easier to scale up. However, the emulsion method might be more expensive if vegetable oil has to be removed and the microspheres have to be washed sufficiently to eliminate the residual vegetable oil on the surface.

### 1.6. POLYMERIC CARRIERS

Polymeric nanoparticles have been synthesized using various methods according to needs of its application and type of drugs to be encapsulated. These nanoparticles are extensively used for the nanoencapsulation of various useful bioactive molecules and medicinal drugs to develop nanomedicine. Biodegradable polymeric nanoparticles are highly preferred because they show promise in drug delivery system. Such nanoparticles provide controlled/sustained release property, subcellular size and biocompatibility with tissue and cells. Apart from this, these nanomedicines are stable in blood, non-toxic, nonthrombogenic, nonimmunogenic, noninflammatory, do not activate neutrophils, biodegradable, avoid reticuloendothelial system and applicable to various molecules such as drugs, proteins, peptides, or nucleic acids. The general synthesis and encapsulation of biodegradable nanomedicines
are represented in Figure 1.5. The drug molecules either bound to surface as nanosphere or encapsulated inside as nanocapsules.

For the past two decades, countless work has been conducted to develop most effective nanomedicines from biocompatible and biodegradable nanopolymers. The role of nanosystems for drug delivery through oral, nasal, ocular administration is reviewed with the various methods of synthesis and encapsulation of different bioactive molecules on nanoparticles. Most of the reported methods are frequently used for the synthesis of biodegradable nanomedicines. Some of the commonly used methods are concisely described in this review along with each section and their encapsulation. The administration, activity and therapeutic importance of some medicinal drugs on different nanosystems are different, for example taxol (anticancer drug) nanomedicines have 100% and 20% encapsulation efficiency on PLGA and PCL nanodevices respectively. However, the therapeutic activity and stability of PCL nanomedicines are reasonably high than PLGA nanomedicine. This part give a brief analysis and sound information about current research in nanobiotechnology and their impact on therapeutics, development of novel nanomedicines, preparation process and their surface modification for the improvement of therapeutics. The most commonly and extensively used polymeric nanoparticles (poly-d,l-lactide-co-glycolide, polylactic acid, poly-caprolactone, poly-alkyl-cyanoacrylates, chitosan and gelatin), their therapeutic advantages, general synthesis and encapsulation of various disease related drug have been described.

1.6.1. Biodegradable polymer – Chitosan

Chitosan is a linear polysaccharide, which can be considered as a copolymer consisting of randomly distributed β (1→4) linked D-glucosamine and N-acetyl-d-glucosamine. The composition is indicated by the degree of acetylation (DA), the fraction of acetyl-glucosamine units. The molar mass depends on the source and the isolation technology, but it can reach values of about $5 \times 10^5$ g/mol. Lower molar
masses and oligomers are obtained by the chain degradation under various conditions frequently yielding products, which differ from the composition and sequence arrangement of the parent molecules.

Chitosan can be classified as a non permanently charged cationic polyelectrolyte. Due to a pKa value of approximately 6.5, chitosan is positively charged and soluble in acidic to neutral medium. The charge density and solubility depend on the DA. Only chitosan with a DA not exceeding 40% is soluble in acidic aqueous medium. Exceptions are oligomers, which have a higher solubility across a broader pH range. Chitosan forms gels with tripolyphosphate and alginate. Moreover, it has a very good film forming ability (Zuidam and Shimoni., 2010).

Chitin, the main source of chitosan, has been evaluated to be as abundant as cellulose with an annual production of 1010–1012 tons in biomass. Chitosan itself is much less present in nature. It has only been observed in some microorganisms and certain fungi. Only very recently, the commercial isolation from fungi has started. The main process of the alkaline deacetylation of crustacean chitins remains.

1.6.2. Biodegradable polymer – PLGA

A considerable amount of research has been conducted on drug delivery by biodegradable polymers since their introduction as bioresorbable surgical devices about three decades ago. Amongst all the biomaterials, application of the biodegradable polymer poly lactic-co-glycolic acid (PLGA) has shown immense potential as a drug delivery carrier and as scaffolds for tissue engineering. PLGA is a family of Food and Drug Administration (FDA) approved biodegradable polymers that are physically strong and highly biocompatible and have been extensively studied as delivery vehicles for drugs, proteins and various other macromolecules such as DNA, RNA and peptides.

PLGA is most popular among the various available biodegradable polymers because of its long clinical experience, favourable degradation characteristics and possibilities for sustained drug delivery. Degradation of PLGA can be employed for
sustained drug release at desirable doses by implantation without surgical procedures. Additionally, it is possible to tune the overall physical properties of the polymer drug matrix by controlling the relevant parameters such as polymer molecular weight, ratio of lactide to glycolide and drug concentration to achieve a desired dosage and release interval depending upon the drug type.

Figure 1.5 Encapsulation of drug using polymeric carriers

PLGA can be processed into almost any shape and size and can encapsulate molecules of virtually any size. It is soluble in wide range of common solvents including chlorinated solvents, tetrahydofuran, acetone or ethyl acetate. In water, PLGA biodegrades by hydrolysis of its ester linkages.
The effect of these polymer properties on the rate of drug release from biodegradable polymeric matrices has been widely studied. The change in PLGA properties during polymer biodegradation, influences the release and degradation rates of incorporated drug molecules. PLGA physical properties themselves have been shown to depend upon multiple factors, including the initial molecular weight, the ratio of lactide to glycolide, the size of the device, exposure to water (surface shape) and storage temperature.

Mechanical strength of the PLGA is affected by physical properties such as molecular weight and polydispersity index. These properties also affect the ability to be formulated as a drug delivery device and may control the degradation rate and hydrolysis (Makadia and Siegel., 2011). Mechanical strength, swelling behavior, capacity to undergo hydrolysis and subsequently biodegradation rate of the polymer are directly influenced by the degree of crystallinity of the PLGA, which is further dependent on the type and molar ratio of the individual monomer components in the copolymer chain.

The main advantage of Fe encapsulation is that it may allow addition of Fe compounds of high bioavailability to difficult-to-fortify food vehicles, such as cereal flours, milk products and low grade salt (Zimmermann., 2004). Fe encapsulation may decrease Fe catalyzed oxidation of fatty acids, amino acids, and other micronutrients that can cause adverse sensory changes and decrease the nutritional value of these foods (Schrooyen et al., 2001). Also, it may reduce interactions of Fe with food components that cause color changes and lower Fe bioavailability, such as tannins, polyphenols and phytates (Hurrell., 2002). A new concept goes beyond regarding microcapsules as a container from which functional components can be released in a controlled manner within the gastro intestinal tract. With respect to micronutrient encapsulation, typical synergistic reactions between the micronutrient component and the reaction partner component are the suppression of oxidation and complexation, but it could as well be a synthesis reaction generated by the functional molecules. The major objective of this approach is the improvement of
bioavailability and the reduction of functional component losses during storage, preparation and perception.

1.7. INTRODUCTION TO RESPONSE SURFACE METHODOLOGY (RSM)

Response surface methodology (RSM) is a widely practiced approach in the development and optimization of drug delivery devices. Based on the principle of design of experiments (DoEs), the methodology encompasses the use of various types of experimental designs, generation of polynomial equations and mapping of the response over the experimental domain to determine the optimum formulation. The technique requires minimum experimentation and time, thus proving to be far more effective and cost effective than the conventional methods of formulating dosage forms.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques for empirical model building. By careful design of experiments, the objective is to optimize a response (output variable) which is influenced by several independent variables (input variables). An experiment is a series of tests, called runs, in which changes are made in the input variables in order to identify the reasons for changes in the output response.

Originally, RSM was developed to model experimental responses, and then migrated into the modelling of numerical experiments. The difference is in the type of error generated by the response.

Design of Experiments

An important aspect of RSM is the design of experiments, usually abbreviated as DoE. These strategies were originally developed for the model fitting of physical experiments, but can also be applied to numerical experiments.
The objective of DoE is the selection of the points where the response should be evaluated.

Most of the criteria for optimal design of experiments are associated with the mathematical model of the process. Generally, these mathematical models are polynomials with an unknown structure, so the corresponding experiments are designed only for every particular problem. The choice of the design of experiments can have a large influence on the accuracy of the approximation and the cost of constructing the response surface.

Experimentation is needed for development of pharmaceutical manufacturing processes. The requirement of pharmaceutical development is to design a quality product and its manufacturing process to consistently deliver the intended performance of the product. One of the major challenges in developing a highly effective experimental plan to optimize the design space of a manufacturing process is the highly complex nature of the pharmaceutical manufacturing processes (Nair et al., 2011).

Thus, Response surface methodology (RSM) technique is widely used in pharmaceutical research and is the method of choice for demonstrating robust process which is as per regulatory requirements. RSM is useful for the modelling and critical analysis of problems in which a response of interest may be altered by several variables and the objective is to optimize this response. The methodology includes the use of various experimental designs, generation of polynomial equations, and mapping of the response over the experimental domain to determine the optimum formulation. As the experimentation procedure and time requirement is very less, it is more effective and cost effective than the other conventional methods of dosage form formulating methods.

Therefore, concluding the introduction section it is learned that, the world population consequently suffer from the micronutrient deficiencies. Also, a major health crisis arises due to the iron deficiency problems all over the world, even after the development of several supplementation technologies. It is known that, the only
way to eradicate these iron deficiency is by the food fortification with proper iron compound. It is noticed that EFSA have acknowledged the importance of ferrous phosphate and their unnotified nutritional values. Keeping in view of these points and the advantages of nanotechnology in the food and medicinal applications, the literature survey has been done, to know the existing research developments and status of the iron compounds used for the fortification process.

NATIONAL STATUS OF IRON DEFICIENCY

National “Anaemic free India” campaign launched at 2011 reports that the dietetic and nutrition surveys in India reveal that 87 per cent of pregnant women suffer from anaemia. According to the Nutrition Foundation of India, 90 per cent of adolescent girls, women and children suffer from iron deficiency. Every age group is vulnerable to iron-deficiency anaemia. Almost 20 per cent of maternal deaths are because of iron deficiency anaemia and it is a contributory factor in 20 per cent more deaths.

Iron deficiency anaemia (IDA) is a significant public health problem in India. National and regional surveys indicate that the prevalence of anaemia could be as high as 74 percent in children below three years of age, 85 percent in expectant mothers and 90 percent among adolescent girls in some population groups. It has been estimated that iron deficiency costs India about 5 percent of its gross national product annually from loss of lives, resources and productivity. The main reasons for IDA have been determined to be inadequate intake of iron, low bioavailability (1–6 percent) of dietary iron from plant foods due to inhibitory factors, low levels of absorption enhancers in the diet, repeated pregnancies, increased needs during growth and development among children and adolescents, parasitic infestations and chronic blood loss. Poverty compounds these factors through inadequate access to dietary diversity, safe water, knowledge about safe food handling and proper feeding practices (Sanghvi., 2010).
The major approaches to controlling IDA, which are not mutually exclusive, are medicinal supplementation with iron and folic acid and food-based approaches, i.e. dietary diversification and fortification of foods, both complemented by programmes to counter parasitic infestations. While supplementation with iron is considered necessary for groups at high risk as a short-term emergency measure, it fails to address the root causes and cannot provide the overall long-term benefits of economy and sustainability. Evaluation studies of India’s nationwide and long-standing supplementation programme showed irregular supplies, non-compliance by the beneficiaries, poor counselling, etc. As such, the supplementation strategy has proved to be inadequate (Vijayaraghvan., 2002). Food-based approaches to addressing IDA in India are being promoted, but information on which and to what extent food combinations would improve the bioavailability of dietary iron is fragmentary. Long-term controlled consumption and feeding studies are lacking owing to the difficulty and costs of dealing with several variables in large populations.

Repeated surveys have shown that the magnitude of nutritional anaemia is of public health concern in India. Though reduced intake of iron is a major etiological factor, low intake or an imbalance in the consumption of other haematopoietic nutrients, their utilization; increased nutrient loss and/or demand also contribute to nutritional anaemia. In India, cereals and millets form the bulk of the dietaries and are major sources of non haeme iron. According to the current estimates, the intake of iron is less than 50 per cent of the recommended dietary allowance (RDA) and iron density is about 8.5 mg/1000 Kcal. It is now well established that iron bioavailability from habitual Indian diets is low due to high phytate and low ascorbic acid/iron ratios. These factors determine iron bioavailability and the RDA. There are striking differences in the iron RDAs among the physiological groups, which need to be validated. The other dietary factors affecting iron status are inadequate intake of folic acid and vitamins B₁₂, A, C and other vitamins of the B-complex group. Chronic low grade inflammation and infections, and malaria also contribute significantly to iron malnutrition. Recent evidence of the interaction of
hepcidin (iron hormone) and inflammatory stimuli on iron metabolism has opened new avenues to target iron deficiency anaemia. Food-based approaches to increase the intake of iron and other haematopoietic nutrients through dietary diversification and provision of hygienic environment are important sustainable strategies for correction of iron deficiency anaemia.

GLOBAL STATUS OF IRON DEFICIENCY

Globally, nearly two billion people are affected by anemia (McLean et al., 2008). The majority of those affected live in developing countries where the problem is exacerbated by limited access to inadequate resources and appropriate treatment (Baltussen et al., 2004). IDA is unique in that it is the only nutrient deficiency which is significantly prevalent in virtually all industrialized nations as well. Currently there are no figures specifically for IDA, but it is widely accepted that approximately 50% of all cases of anemia are caused by iron deficiency.

The global prevalence of anaemia was calculated by combining the estimates for all population groups, which covered the entire population except for one segment (women 50.0 – 59.9 yrs). The estimate of anaemia prevalence in the elderly was applied to this segment of the population.

However, countries without survey data should be encouraged to collect data, since regression based estimates are good at the regional and global level, but may not be the most accurate reflection of the situation for an individual country. The generation of these estimates and the maintenance of the anaemia database provide a reliable tool to track the global progress towards the elimination of anaemia and the effectiveness of the current strategies for anaemia control. However, since information on causal factors is not routinely collected, the database does not provide information on the ability of the strategies to address these factors. Hopefully, these estimates will encourage countries to plan surveys which assess the prevalence of factors that contribute to anaemia not only iron deficiency, but also infectious diseases and other
micronutrient deficiencies. The understanding of how these factors vary by geography, level of development and other social and economic factors will make it easier to design interventions that are more effective and integrative in addressing multiple contributing factors at the same time.

The widespread prevalence of anemia, both in the developed and developing worlds, is great cause for concern. Although we have made strides, there is still much that we do not understand about iron deficiency and anemia, especially in relation to treatment and prevention. A renewed effort to find effective ways to combat this problem is needed, as anemia is unique and complex public health crisis that is of global proportions.

1.8. REVIEW OF LITERATURE

1.8.1. Status on iron deficiency – An overview

Zimmermann, (2003) in his review illustrated that the Vitamin, mineral and/or trace element supplements are beneficial, if they supply a nutrient that is deficient in the diet. That is, when dietary intake is lower than the amount needed to provide maximum benefit as judged from all biological perspectives. It is difficult to accurately define nutrient in competitive athletes, for several reasons. Dietary iron (Fe) intake is marginal or inadequate in many females who engage in regular physical exercise. Basal obligatory losses in adults are ~1 mg Fe d⁻¹ and must be replaced by absorbed Fe to maintain balance. In many athletes, poor food choice and/or energy restriction to reduce body mass contributes to negative Fe balance.

Iron deficiency is one of the leading risk factors for disability and death worldwide, affecting an estimated 2 billion people. Nutritional iron deficiency arises when physiological requirements cannot be met by iron absorption from diet. Dietary iron bioavailability is low in populations consuming monotonous plant based diets. The high prevalence of iron deficiency in the developing world has substantial health and economic costs, including poor pregnancy outcome, impaired
school performance and decreased productivity. Targeted iron supplementation, iron fortification of foods, or both, can control iron deficiency in populations. Although technical challenges limit the amount of bioavailable iron compounds that can be used in food fortification, studies show that iron fortification can be an effective strategy against nutritional iron deficiency. Specific laboratory measures of iron status should be used to assess the need for fortification and to monitor these interventions (Zimmermann and Hurrell, 2007).

Hurrell et al. (2009) summarized that the major approaches for controlling IDA, which are not mutually exclusive, are medicinal supplementation with iron and folic acid and food-based approaches, i.e. dietary diversification and fortification of foods, both complemented by programmes to counter parasitic infestations. While supplementation with iron is considered necessary for groups at high risk as a short term emergency measure, it fails to address the root causes and cannot provide the overall long-term benefits of economy and sustainability. Food based approaches to addressing IDA in India are being promoted, but information on which and to what extent food combinations would improve the bioavailability of dietary iron is fragmentary. Several experimental studies on the availability of food iron and related aspects have been reported, which showed the possibility of assessing how to improve the bioavailability of iron in plant foods, which should reduce the prevalence of IDA in the long run.

Brenda et al. (2009) suggested that deficiencies of iron and folic acid during pregnancy can lead to adverse outcomes for the foetus, thus supplements are recommended. Adherence to current tablet based supplements is documented to be poor. The objective was to measure the relative bioavailability of iron and folic acid from a powdered supplement that can be sprinkled on semi solid foods or beverages versus a traditional tablet supplement in pregnant women. The unexpected lower bioavailability of iron from the powdered supplement is contrary to previously published reports. However, since pills and capsules are known to be poorly
accepted by some women during pregnancy, it is reasonable to continue to explore alternative micronutrient delivery systems and forms of iron for this purpose.

Walter, (2007) categorised the causes underlying the pathology and the subsequent contribution of absolute or functional iron deficiency anemia which include:

- Inadequate intake of dietary iron
- Blood loss during the extra corporeal procedure in hemodialysis patients
- Blood loss from the gastrointestinal tract (bleeding)
- (Too) frequent diagnostic blood tests
- Inadequate intestinal iron absorption and inhibition of iron release from macrophages (anemia of chronic disease)
- Increased iron requirements during therapy with erythropoiesis stimulating agents (ESAs).

Davidsson et al. (2005) stated that a major problem related to the potential effect of iron-fortified complementary foods such as infant cereals is that unacceptable organoleptic changes may occur during storage or during food preparation of fortified products containing water-soluble iron compounds with high relative bioavailability. Consequently, non-water-soluble iron compounds are often used to fortify infant cereal products, although some of the most commonly used iron compounds have been shown to have low relative bioavailability and can therefore be expected to have only a limited effect on the iron status of the consumers. Clearly, the use of an iron compound with high relative bio-availability whose absorption is not susceptible to the negative effects of inhibitory ligands would be a useful way of providing iron via fortified foods. Also, the researchers in their earlier studies evaluated the enhancing effect of ascorbic acid and Na$_2$EDTA (Sodium feredetate) on iron bioavailability from a cereal-based Peruvian school breakfast meal fortified with ferrous sulphate. It was concluded clearly that, the development of a food fortification strategy and, in particular, the selection of an
approach to optimize iron bioavailability from the fortified food need careful consideration for the specific conditions relevant to the food fortification vehicle and the target population group.

1.8.2. Reports on the studies of iron compounds

Davidsson et al. (2000) indicated that infant cereals are commonly fortified with insoluble iron compounds with low relative bioavailability, such as ferric pyrophosphate, because of organoleptic changes that occur after addition of water soluble iron sources. Iron bioavailability was measured as the incorporation of stable iron isotopes into erythrocytes 14 days after administration of labeled test meals (25 g dry wheat and soy infant cereal, 100 g water, and 2.5 mg Fe as $^{57}$Fe ferric pyrophosphate or $^{57}$Fe ferrous fumarate). Iron bioavailability from iron fortified infant cereals can be improved by using an iron compound with high relative bioavailability and by ensuring adequate ascorbic acid content of the product.

Moretti et al. (2006) compared the Relative Bioavailability Value (RBV) of iron compounds fortified with different food vehicles. One of the important findings from this study is that the Relative Bioavailability Value (RBV) of ferric pyrophosphate (particle size: 0.77 µm) varied according to the food vehicle, it was 62% in a wheat-milk infant cereal and only 15–25% in a rice meal. This finding compares with previously reported RBV values (particle size: 0.3 µm) of 95% from a yogurt drink and 83% in a wheat-milk cereal. Therefore, at last the finding says, for poorly water soluble iron compounds, the use of a single RBV value to set a fortification level and predict potential efficacy in all food vehicles, may be of limited value.

Fox et al. (1998) studied the bioavailability of iron glycine added to a vegetable infant weaning food compared with ferrous sulfate. Stable, isotopically labeled compounds ($^{57}$Fe or $^{58}$Fe) were mixed into the midday meal (1.4 mg added Fe/serving) and fed to 9 months old infants on alternate days for 8 days.
Bioavailability, expressed as a percentage of the dose consumed, was measured from isotopic enrichment of hemoglobin 14 days after the last test meal. There was no difference between iron glycine and ferrous sulfate: 9.0 ± 0.7% and 9.9 ± 0.8%, respectively. The results showed no significant difference in bioavailability between the two forms of iron when added to infant weaning foods, suggesting that the glycine complex was fully or partially dissociated in the gastrointestinal tract. It was concluded that chelation does not improve the bioavailability of iron in the presence of dietary inhibitors. It has been suggested that the higher bioavailability of iron glycine is due to the fact that it is absorbed intact by the epithelial cells of the intestine, probably via an active transport mechanism.

Forbes et al. (1989) determined the solubility of the Fe sources in duplicate using 20-mg Fe samples in 250 ml 0.02M HCl/L at 37°C. The electrolytic Fe was much more soluble than the FePO₄. Relative biological values (RBV) of electrolytic Fe were determined and compared with the reference FeSO₄. They compared the bioavailability values of radiolabeled and nonradiolabeled electrolytic Fe and FePO₄ and reported that the elemental Fe source was not as well absorbed as FeSO₄. It was concluded that the most widely accepted method for predicting Fe bioavailability in human subjects is Hb repletion. This technique measures the Hb response to graded amounts of Fe in rats with induced Fe-deficiency anemia.

Mudge et al. (2009) reported that post-transplant anaemia remains a common problem after kidney transplantation, with an incidence ranging from nearly 80% at day 0 to about 25% at one year. This study is a single centre, prospective, open label, randomised, controlled trial of oral versus intravenous iron supplements in renal transplant recipients and recruits approximately 100 patients over a 12 month period. Patients were randomised to receive a single dose of 500 mg iron polymaltose (intravenous iron group) or two ferrous sulphate slow release tablets daily (oral iron group). The primary outcome is time to the normalisation of haemoglobin post transplant. The trial showed a reduction in the time to correction
of anaemia with intravenous iron or less side effects than oral iron, indicating intravenous iron may become the standard of treatment in this patient group.

Nguyen et al. (2008) compared the rate of adherence and reported adverse events among pregnant women who were randomized to commence supplementation with a small tablet prenatal multivitamin, containing either low or high iron content. Women were randomized to take a small size (16 mm × 9 mm × 4 mm), low elemental iron content (35 mg as ferrous fumarate) multivitamin (35 mg group) or a small size (5 mm radius, 5 mm thickness), high elemental iron content (60 mg as ferrous sulphate), multivitamin (60 mg group). Despite ideal conditions and regular follow ups, mean adherence based on pill intake recall, in both groups were approximately 50%. No statistically significant difference was detected in proportions of women who actually started taking either multivitamins. The present result suggests that iron content is not a major determinant of adherence to prenatal multivitamins.

Benjamin et al. (2000) compared the iron absorption capacity from ferrous sulfate, ferrous bisglycinate and ferric trisglycinate in whole maize meal, to determine whether iron from ferrous bisglycinate and ferrous sulfate exchanges in the intestinal pool and to assess iron absorption from ferrous bisglycinate and ferric trisglycinate over a range of iron status. Excessive iron absorption can cause a variety of diseases in humans, including hepatic cirrhosis and diabetes mellitus. Although the mechanism has not been completely elucidated, it is known that iron absorption is regulated by iron status and is considerably lower in persons with normal or high iron stores. The researchers are unable to predict the behavior of bisglycinate in the presence of other inhibitors of iron absorption but this seems to vary (e.g, from milk compared with whole maize meal porridge). It appears that ferric trisglycinate is not a useful iron fortificant for maize in humans.

Swain et al. (2006) proposed that the elemental iron powders are relatively economical, without adverse organoleptic effects on fortified foods, but their
usefulness for fortification of agricultural products is uncertain because few bioavailability or efficacy studies have been conducted in humans. Elemental iron powders are generally characterized by production method as carbonyl, electrolytic, or reduced iron and are composed of relatively pure (>98% iron; zero oxidation state) metallic iron. Compared with iron salts, the elemental iron powders have lower bioavailability for absorption, which is directly associated with their lower solubility and surface area. Iron absorption by 56 volunteers were measured from a farina cereal breakfast radiolabeled with $^{59}$FeSO$_4$ or an electrolytic $^{55}$Fe powder irradiated by neutron activation. Despite a much higher bioavailability (50% relative to FeSO$_4$) of this same electrolytic iron when tested previously in a pig model, the bioavailability of the irradiated electrolytic iron was poor in humans.

Baltussen et al. (2004) estimated the costs, effects and cost effectiveness of iron supplementation and iron fortification interventions in four regions of the world. The population model took into consideration were effectiveness, patient adherence and geographic coverage. At 95% geographic coverage, iron supplementation had a larger impact on population health than iron fortification. Iron supplementation would avert 12,500 disability adjusted life years (DALY) annually in the European subregion, with very low rates of adult and child mortality, to almost 2.5 million DALYs in the African and Southeast Asian subregions, with high rates of adult and child mortality. On the other hand, fortification is less costly than supplementation and appears to be more cost effective than iron supplementation, regardless of the geographic coverage of fortification. They concluded that iron fortification is economically more attractive than iron supplementation.

Ward et al. (2003) evaluated the effects of a single intramuscular iron dose, 10mg, to pregnant rats on Day of pregnancy, on the outcome of pregnancy, with respect to foetal weight and mother’s immune function has been investigated. Despite significantly elevated hepatic iron stores after iron supplementation in pregnant rats this had no significant effect upon blood haemoglobin or transferrin
saturation levels. However, the mean weight of the foetus at Day 20-21 was significantly lower than that of the non-supplemented pregnant rats.

Hurrell *et al.* (1991) identified the usefulness of ferrous fumarate as an iron fortificant for an experimental chocolate drink powder targeted to children and adolescents. Organoleptically ferrous fumarate was acceptable when the chocolate drink powder was reconstituted in milk or water that was heated to < 80°C. Unacceptable colour changes occurred, however, when boiling milk or water was used. In human Fe absorption studies, when the Fe compounds were added to the chocolate drink immediately before consumption, ferrous fumarate was 3.31% absorbed compared with 2.82% for ferrous sulphate and 2.11% for ferric pyrophosphate. When the Fe compounds were processed during the manufacture of the chocolate drink powder, the absorption of ferrous fumarate was 5.27%, ferrous sulphate 2.62% and ferric pyrophosphate 0.55%. Ascorbic acid had little or no effect on the absorption of ferrous fumarate. It is concluded that food processing can influence the relative absorption of fortification Fe and that, if not reconstituted with boiling milk or water, ferrous fumarate could be a useful compound for the fortification of chocolate drink powders.

Hicks *et al.* (2004) pointed that, studies in humans suggest that ferritin iron in soybeans has high bioavailability. However, direct evidence for this is lacking because the soybeans were intrinsically labelled. They evaluated the absorption of iron from extrinsically labeled, purified ferritin (horse spleen) reconstituted with either high phosphate iron mineral (plant-type) or low phosphate iron mineral (animal type) and compared it with iron absorption from ferrous sulfate. Nonanemic, healthy young women were fed with a standard breakfast meal supplemented with $^{59}$Fe-labeled ferritin or ferrous sulfate, in randomized order. Fifteen subjects received ferritin with the low-phosphate iron mineral and 15 subjects received ferritin with the high-phosphate iron mineral. Iron absorption was measured in a whole-body counter after 14 and 28 days and by red blood cell incorporation after 28 days. There was no significant difference in iron absorption
between ferritin and ferrous sulfate: low-phosphate iron mineral ferritin (21.4 ± 14.7%) compared with ferrous sulphate (21.9 ± 14.6%), or high-phosphate iron mineral ferritin (22.2 ±19.2%) compared with ferrous sulfate (16.7 ± 7.1%). Results obtained by using whole body retention of iron and red blood cell incorporation differed with the type of iron, which suggests that pathways for iron uptake and utilization differed for the 2 forms. Iron is equally well absorbed from ferritin and ferrous sulfate independent of the phosphate content of the ferritin iron mineral.

Park et al. (1983) evaluated the bioavailability of iron from ferrous sulfate sources that had been stored for unknown periods. The efficiency of converting dietary iron from these sources into hemoglobin varied from 60 to 84%. This variability was not explained on the basis of percent of iron in the ferrous state nor on the percent of iron in the source. The one fresh source used was reevaluated 3 months later and its bioavailability had been reduced from 84 to 65%. The bioavailability of six fresh ferrous sulfate samples varied from 50 to 59%. It was concluded that, whenever ferrous sulfate is to be used as a reference source, fresh salt should be obtained to reduce variability among experiments.

Jacobsa et al. (2000) suggested that the utilisation of iron from polymaltose might be enhanced by glycerophosphate. The data of three polymaltose groups were combined and compared to ferrous sulphate. The rate at which haemoglobin level improved, red cell indices returned to normal and the number of hypochromic and microcytic red cells fell was not significantly different between the groups. Similarly the serum iron, percentage saturation of transferrin and red cell ferritin were comparable. In contrast the serum ferritin levels were higher for those receiving ferrous sulphate. These data demonstrate that the addition of glycerophosphate, observed to be beneficial in rats, which did not occur in humans. Secondly, in the blood donors, equivalent amounts of iron provided as the polymaltose, with or without glycerophosphate or ferrous sulphate, corrected haemoglobin concentration and morphologically abnormal erythropoiesis at comparable rates. Interestingly, there is a discrepancy in the serum ferritin which is
higher with the salt and this may reflect oxidative stress. Finally it is postulated that
the iron polymaltose complex formulation more closely approximates the way in
which enterocytes handle dietary iron and thus physiologic regulatory mechanisms
would be expected to reciprocally slow down absorption as stores expand.
Logically, therefore, the latter finding suggests that this formulation may have a
potential role in longer term supplementation programmes.

It was found that ascorbic acid and Na$_2$EDTA enhance Fe absorption from
the water soluble Fe compound FeSO$_4$ but their effect on poorly water soluble Fe
compounds such as ferrous fumarate is less well established. In the study, the effects
of ascorbic acid and Na$_2$EDTA on Fe absorption from ferrous fumarate were
evaluated in adult women (ten women / study) from the erythrocyte incorporation of
Fe stable isotopes (57 Fe or 58 Fe) 14 days after administration. Two separate
studies were made with test meals of Fe-fortified infant cereal (5 mg Fe/meal).
The results of the present studies showed that Fe absorption from ferrous fumarate is
enhanced by ascorbic acid but not by Na$_2$EDTA, thus emphasising that not all
findings from Fe absorption studies made with FeSO$_4$ can be extrapolated to Fe
compounds with different solubility properties (Fidler et al. 2003).

Casal et al. (2004) revealed that Ethylenediaminetetraacetic acid (NaFe-
EDTA) is a chelator capable of binding a wide variety of metals, with a high affinity
constant for Fe$^{3+}$. NaFe-EDTA has been extensively studied and validated as an
excellent choice for iron fortification programs and extensive research has
demonstrated its high bioavailability specially for cereal based foods. To further
evaluate the usefulness of this compound, iron uptake experiments with EDTA
using the Caco-2 cell system was performed. Cells were incubated in PBS at pH 5.5
or 7.0, containing or not ascorbic acid. Different sources of EDTA, different
concentrations of NaFe-EDTA and the inclusion of another iron compound as
electrolytic iron, were tested. Iron uptake from electrolytic iron was inhibited when
Na$_2$ or K$_2$-EDTA were included.
Uicich et al. (1999) observed that, it is possible to fortify food with 15 mg of iron per liter by a new technological procedure in which ferrous sulphate is microencapsulated with phospholipids. Therefore, the absorption of this novel iron compound called SFE-171 by means of the classical double isotopic method. This study was made in fifteen healthy adult men, none of them were anemic and all of them had normal iron stores. Iron absorption from SFE-171 in milk was 9.2% when it was standardized to 40% absorption of the reference dose of ferrous ascorbate. In conclusion the iron from ferrous sulfate microencapsulated with phospholipids has a good bioavailability and it is an effective alternative for the fortification of fluid cow’s milk without affecting its shelf life and its sensorial properties.

Jadayil et al. (1999) evaluated the bioavailability of iron from local plants (black cumin seeds, milk thistle seeds, sesame seeds and thyme leaves). Apparent absorption of iron was calculated by subtracting fecal iron (using total collection of feces) from iron intake in Sprague-Dawley rats. Two trials of animal feeding were performed. Liver and serum concentrations of iron and serum hemoglobin concentration were taken as response parameters for the bioavailability. Iron intake and total iron absorption were highest for the rats fed the dry thyme egg white diet. Liver weights for the groups fed black cumin seeds and dry thyme were significantly higher (p < 0.05) than those for the groups fed milk thistle and sesame seeds. It is concluded from this study that iron was better utilized from black cumin seeds as indicated by liver storage of iron. On the other hand, thyme had the highest iron absorption but lowest utilization.

Lee et al. (2003) carried out an experiment to examine the stability of microencapsulated ascorbic acid in simulated gastric and intestinal situation in vitro and the effect of microencapsulated ascorbic acid on iron bioavailability. When ascorbic acid was microencapsulated by triacylglycerol, the release of ascorbic acid was 6.3% at pH 5 and 1.32% at pH 2 in simulated-gastric fluids during 60 min. When ascorbic acid was microencapsulated by polyacylglycerol, the more ascorbic
acid was released in the range of 9.5 to 16.0%. Comparatively, ascorbic acid release increased significantly as 94.7% Therefore, the present data indicated that microencapsulated ascorbic acid with both forms were effective means for fortifying ascorbic acid into milk and for enhancing the iron bioavailability and 83.8% coated by MCT and PGMS, respectively.

Chiploankar et al. (2003) examined the influence of micronutrient status in diagnosed anemic patients, a cross-sectional survey on adults from rural and urban parts of Western India was undertaken. Iron deficiency anemia (IDA) patients (81 men, 102 women) and age-sex matched healthy controls (80 men, 100 women) were studied for their blood status of iron and seven micronutrients and nutrient intakes. Median levels of serum iron (647 g/L), serum ceruloplasmin (192 mg/L), ascorbic acid (2.3 mg/L) and B12 (368 mg/L) were significantly lower in anemic subjects than the control group (750 g/L, 251 mg/L, 3.2mg/L, 416 mg/L respectively, p<0.01).

Stein et al. (2008) proposed a methodology for ex ante impact assessment of iron biofortification, building on a disability-adjusted life years (DALYs) framework. This methodology is applied in an Indian context. Using a large and representative data set of household food consumption, the likely effects of iron rich rice and wheat varieties are simulated for different target groups and regions. The results indicate size able potential health benefits. Depending on the underlying assumptions, the disease burden associated with iron deficiency could be reduced by 19-58%. Due to the relatively low institutional cost to reach the target population, the expected cost-effectiveness of iron biofortification compares favourably with other micronutrient interventions. Nonetheless, biofortification should not be seen as a substitute for other interventions. Each approach has its particular strengths, so they complement one another.
1.8.3. Studies on nanomaterial synthesis

Nanotechnology is beginning to allow scientists, engineers, and physicians to work at the cellular and molecular levels to produce major advances in the life sciences and healthcare. Real applications of nanostructured materials in life sciences are uncommon at the present time. However, the excellent properties of these materials when compared with their bulk counterparts provide a very promising future for their use in this field. In almost all applications the preparation method of the nanomaterials represents one of the most important challenges that will determine the particle size and shape, the size distribution, the surface chemistry of the particles and consequently their magnetic properties. Also, depending on the mechanism of formation, spherical particles obtained in solution can be amorphous or crystalline if they result from a disordered or ordered aggregation of crystallites, respectively. In addition, the preparation method determines to a great extent the degree of structural defects or impurities in the particle, as well as the distribution of such defects within the particle and therefore its magnetic behaviour. (Tartaj et al., 2003)

The field of nanoparticle delivery systems for nutrients and nutraceuticals with poor water solubility has been expanding, almost exponentially, over the last five years, and some of these technologies are now in the process of being incorporated in food products. The market projections for these technologies suggest a multifold increase in their commercial potential over the next five years. The interest in the pharmaceutical and food-related applications of these technologies has sparked tremendous developments in mechanical (top-down) and chemical (bottom-up) processes to obtain such nanoparticle systems. Mechanical approaches are capable of producing nanoparticles, typically in the 100 – 1000 nm range, whereas chemical methods tend to produce 10 – 100 nm particles. Despite these technological developments, there is a lack of information regarding the basis of design for such nanoparticle systems. Fundamental thermodynamic and mass transfer equations reveal that, in order to generate a broad spectrum delivery system,
nanoparticles with 100 nm diameter (or less) should be produced. However, experimental data reveal that, in some cases, even nanoparticles in the 100 – 1000 nm range are capable of producing substantial improvement in the bioavailability of the active ingredients (Acosta., 2009).

Chiriac et al. (2008) synthesised and investigated the magnetic properties of nanostructured magnetic materials present interest from both fundamental and technological point of view. Magnetic nanoparticles are used and are explored for use in fields as diverse as biology and data storage. In these applications, the ability to control particle size, shape, composition and surface chemistry is critical in obtaining the desired magnetic properties. For biomedical applications the use of particles that present superparamagnetic behaviour at room temperature (no remanence along with a rapidly changing magnetic state) is preferred. In biomedicine, superparamagnetic particles are used for cell sorting and are explored for radiation treatment. Such particles are also being explored for use in drug delivery and gene therapy.

Yang et al. (2005) studied the direct formation of iron phosphate nanoparticles on hydroxyl terminated SiO$_2$/Si substrates with a narrow size distribution (average diameter of 2.2 nm), achieved by a simple room temperature spontaneous reaction of ferric chloride and phosphoric acid. Single-walled carbon nanotubes (SWNTs) are grown in high yield from the synthesized iron phosphate nanoparticles by the thermal chemical vapour deposition (CVD) method, as confirmed by atomic force microscopy (AFM) and Raman spectroscopy. The reduced solubility of Fe (III) ions when they form iron phosphate salts in aqueous media is the main driving force for the nanoparticle formation. Systematic control experiments revealed that the surface property, concentration, and pH of the reaction solution play equally important roles in the formation of nanoparticles.

Mizutani et al. (2008) prepared the magnetic nanoparticles by hydrothermal synthesis under various initial ferrous/ferric molar ratios without adding any oxidizing and reducing agents in order to clarify effects of the molar ratio on the
reaction mechanism for the formation of magnetite nanoparticles. On the other hand, at higher molar ratios, the particle size and crystallinity increased with increasing molar ratio because using surplus ferrous hydroxide the crystallites of magnetite nanoparticles grew up slowly under hydrothermal conditions according to the Schikorr reaction. The magnetite nanoparticles prepared under various molar ratios had good magnetic properties regardless of the molar ratio.

Iida et al. (2007) synthesized nanoparticles of Fe₃O₄ by hydrolysis in an aqueous solution containing ferrous and ferric salts at various ratios with 1,6-hexanediame as a base. It was found that the ferrous to ferric ratio influence the reaction mechanism for the formation of Fe₃O₄. When the ratio of ferrous to ferric ions was increased, the formation of large hydroxide particles as a precursor of Fe₃O₄ was promoted, which resulted in an increase in the size of Fe₃O₄ nanoparticles. As a result, the mean diameter of Fe₃O₄ nanoparticles increased from ∼9 to ∼37 nm as the molar percentage of ferrous ions with respect to the total iron ions was increased from 33 to 100%. Furthermore, it was demonstrated that magnetic properties of Fe₃O₄ nanoparticles can be controlled by adjusting the molar ratio of ferrous to ferric ions as well as the particle diameter.

Wang and Jiang (2009) stated that much interest has been attracted to the magnetic materials with porous structure because of their unique properties and potential applications. In this report, Fe₃O₄ nanoporous particles assembled from small Fe₃O₄ nanoparticles have been prepared by thermal decomposition of iron acetylacetonate in the presence of polyethylene glycol 4000. The size of the spherical nanoporous particles is 100–200 nm. Surface area measurement shows that these Fe₃O₄ nanoporous particles have a high surface area of 87.5 m²/g. It is found that the morphology of the products is greatly influenced by polyethyleneglycol concentration and the polymerization degree of polyethylene glycol.

Mohapatra and anand (2010) synthesised nano iron oxides by almost all the known wet chemical methods which include precipitation at ambient/elevated
temperatures, surfactant mediation, emulsion/micro-emulsion, electro-deposition etc. Iron oxides in nanoscale have exhibited great potential for their applications as catalytic materials, wastewater treatment adsorbents, pigments, flocculants, coatings, gas sensors, ion exchangers, magnetic recording devices, magnetic data storage devices, toners and inks for xerography, magnetic resonance imaging, bioseparation and medicine. Nano sized magnetite Fe$_3$O$_4$, and maghemite $\gamma$-Fe$_2$O$_3$ exhibiting excellent magnetic properties find applications for biomedical purposes and as soft ferrites.

Li et al. (2007) developed an ecofriendly technology in material synthesis is of considerable importance to expand their biological applications. Nowadays, a variety of inorganic nanoparticles with well defined chemical composition, size and morphology have been synthesized by using different microorganisms and their applications in many cutting edge technological areas have been explored. This review highlights the recent developments of the biosynthesis of inorganic nanoparticles including metallic nanoparticles, oxide nanoparticles, sulfide nanoparticles, and other typical nanoparticles. The conditions to control the size/shape and stability of particles are summarized. The applications of these biosynthesized nanoparticles in a wide spectrum of potential areas are presented including targeted drug delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, enhancing reaction rates, separation science and magnetic resonance imaging (MRI).

Lee et al. (2008) investigated the synthesis of nano-size iron particle using a borohydride reduction of ferric ion in the presence of solvent containing carbonyl groups. When the varied contents of solvent were used, there is a corresponding variation of synthesis and iron form. Each sample of synthesized iron particles was characterized by transmission electron microscope (TEM) and powder X-ray diffractometer (XRD). The contents of zero valent iron (ZVI) in the synthesized particles were measured to investigate the effect of solvent proportions in aqueous organic solvent system reducing iron particles.
Bisht et al. (2007) developed a nanoparticle based drug delivery approach that have the potential for rendering hydrophobic agents like curcumin dispersible in aqueous media, thus circumventing the drawback of poor solubility. They synthesized polymeric nanoparticle encapsulated formulation of curcumin - nanocurcumin utilizing the micellar aggregates of crosslinked and random copolymers of N-isopropylacrylamide (NIPAAM), with N-vinyl-2-pyrroldione (VP) and poly (ethyleneglycol) monoacrylate (PEG-A). Physicochemical characterization of the polymeric nanoparticles by dynamic laser light scattering and transmission electron microscopy confirms a narrow size distribution in the 50 nm range. The authors concluded that the nanocurcumin provides an opportunity to expand the clinical repertoire of this efficacious agent by enabling ready aqueous dispersion. Future studies utilizing nanocurcumin are warranted in pre-clinical in vivo models of cancer and other diseases that might benefit from the effects of curcumin.

Tripathi et al. (2010) produced Poly lactic-co-glycolic-acid (PLGA) nanoparticles using the single emulsion solvent evaporation method. In most cases poly vinyl alcohol (PVA) is used as stabilizer of the emulsion. The rationale of this study was to develop PLGA nanoparticles loaded with rifampicin, intended to be intravenously administered and able to improve the therapeutic index of the drug. The influence of the concentration of PVA and the polymers was tested on particle size of prepared particles. PLGA-based rifampicin nanoparticles were prepared by single and double evaporation method, solvent diffusion and ionic interaction method. The incorporation efficiency of rifampicin was higher with the single emulsion evaporation method in the nanosize range particles. The processing parameters involved in the method were optimized, including drug / polymer ratio, concentration of surfactant, phase ratio (organic phase/ aqueous phase) and sonication time to obtain small nanoparticles with maximum drug entrapment.
1.8.4. Studies on polymers and RSM

Zhenga et al. (2004) studied and developed alginate–chitosan–poly (lactic-co-glycolic acid) (PLGA) composite microspheres to elevate protein entrapment efficiency and decrease its burst release. Bovine serum albumin (BSA), which used as the model protein, was entrapped into the alginate microcapsules by a modified emulsification method in an isopropyl alcohol-washed way. The rapid drug releases were sustained by chitosan coating. The average diameter of the composite microcapsules was 31 ± 9 µm and the encapsulation efficiency was 81–87%, while that of conventional PLGA microspheres was just 61–65%. Furthermore, the burst releases at one hour of BSA entrapped in composite microspheres which containing PLGA (50:50) and PLGA (70:30) decreased to 24% and 8% in PBS and further decreased to 5% and 2% in saline.

Kim et al. (2004) developed and optimized oral controlled release formulations for tamsulosin hydrochloride using a combination of two cellulose ester derivatives, hydroxypropyl methylcellulose (HPMC) and hydroxypropyl methylcellulose phthalate (HPMCP), with Surelease as a coating material. A three factor, three-level Box-Behnken design was used to prepare systematic model formulations, which were composed of three formulation variables, the content of HPMC (X1) and HPMCP (X2) and the coating level (X3), as independent variables. The response surface methodology (RSM) and multiple response optimizations utilizing the polynomial equation were used to search for the optimal coating formulation with a specific release rate at different time intervals. The drug release percentages at 2, 3 and 5 hours were the target responses and were restricted to 15–30% (Y1), 50–65% (Y2) and 80–95% (Y3), respectively. The optimal coating formulation was achieved with 10% HPMC and 20% HPMCP at a coating level of 25% and the observed responses coincided well with the predicted values from the RSM optimization technique. The drug release from pellets coated with the optimized formulation showed a controlled release pattern (zero-order), in comparison with a commercial product (Harunal capsule). In conclusion, a novel,
oral, controlled release delivery system for tamsulosin hydrochloride was successfully developed by incorporating HPMC and HPMCP as coating additives into aqueous ethylcellulose dispersion.

Mengatto et al. (2012) reported the study of estradiol permeation in chitosan membranes. A fractional factorial design was built for the determination of the main factors affecting estradiol permeation. The independent factors analysed were: concentration of chitosan, concentration of cross-linking agent, cross linking time and thermal treatment. It was found that concentration of chitosan and cross linking time significantly affected the response. The effects of thermal treatment and concentration of cross linking agent were not significant. An optimization process based on response surface methodology was carried out in order to develop a statistical model which describes the relationship between active independent variables and estradiol flux. This model can be used to find out a combination of factor levels during response optimization. Possible options for response optimization are to maximize, minimize or move towards a target value.

Mothilal et al. (2012) optimized and characterized the controlled release microspheres of Aceclofenac using “Box-Behnken experimental design”. The microspheres were prepared by using the natural polymer chitosan in different ratios with glutaraldehyde as the cross linking agent. The microspheres were prepared by ionic cross linking technique. The microspheres were evaluated for particle size, drug content, drug loading efficiency, in vitro drug release surface morphology. The particle size of the prepared microspheres was between 3 to 800µm in diameter. In conclusion NSAID controlled release delivery system utilizing natural polymer i.e. chitosan was successfully developed. Further parameters for dosage form designing can be identified for optimum formulation in terms of desirable long term stability and to study the therapeutic effects of these particles in vivo.
Nair et al. (2011) developed a central composite design based on response surface method used to prepare experimental trials using different ratio of etodolac and chitosan to optimize the microsphere formulations. Different Formulations were prepared by Gluteraldehyde cross linking method. In this investigation $3^2$ full factorial design was used to investigate the joint influence of two variables: concentration of chitosan ($X_1$), and stirring speed ($X_2$), on the time for % of drug release after 12 hours and entrapment efficiency. Potential variables such as stirring time, volume of volatile solvent were kept constant in the experimental design. A statistical model with significant interaction terms was derived to predict the % drug release. The prepared microspheres were discrete and free flowing and indicated that the concentration of polymer, stirring rate significantly influenced the formation of microspheres and drug entrapment. The results demonstrated a good relationship between the predicted and experimental values, confirming the validity of the model. Drug release mechanism indicated a best fit model of zero order release. The model F values were found to be significant in nature. Response surface plots are presented to show the effect of $X_1$, $X_2$ on the % of drug release and entrapment efficiency. Acceptable batches were identified with the help of experimental design.

Nair et al. (2011) developed a highly effective experimental plan to optimize the design space of a manufacturing process is the highly complex nature of the pharmaceutical manufacturing processes. Response surface methodology (RSM) techniques are widely used in pharmaceutical research and are the method of choice for demonstrating robust process which is as per regulatory requirements. Microspheres were prepared by using ethyl cellulose as a polymer by solvent evaporation method. In this investigation $3^2$ full factorial design was used to investigate the joint influence of two variables: the stirring speed ($X_1$), concentration of ethylcellulose ($X_2$), on the time for % of drug release after 12 hours and entrapment efficiency. Potential variables such as stirring time, volume of volatile solvent were kept constant in the experimental design. A statistical model with significant interaction terms is derived to predict the % drug release.
results of F statistics revealed that for obtaining controlled drug release, the microspheres should be prepared using relatively high stirring speed and high concentration of ethylcellulose.

Wang et al. (2009) reported that the control of size and size distribution of microspheres is necessary for obtaining repeatable controlled release behavior. The microspheres with different size were prepared by using the membranes with different pore size, and there was a linear relationship between the diameter of microspheres and pore size of the membranes when the microspheres were in the range of micron size. The smallest chitosan microspheres obtained was 0.4 Am in diameter. This is the first report for preparing the uniform-sized chitosan microspheres by membrane emulsification technique. Uniform chitosan microspheres were further used as a carrier of protein drug. Bovine serum albumin (BSA) as a model drug was loaded in the microspheres and released in vitro. The effects of pH value, diameter and crosslinking degree of microspheres, and BSA concentration on loading efficiency and release behavior were discussed.

Chang et al. (2006) studied the influences of combination of different mechanisms of penetration enhancers (such as azone, sodium lauryl sulfate and menthol) on the percutaneous absorption of meloxicam formulations through rat skin were investigated using uniform design and response surface methodology. The uniform design was applied to prepare systematic model formulations which were composed of three formulation factors: azone (x1), sodium lauryl sulfate (x2), and menthol (x3). The result showed that azone had the highest potential influence on the penetration absorption of meloxicam, followed by sodium lauryl sulfate and menthol. With zero-order delivery, the required flux of meloxicam gel to maintain a therapeutic concentration was about 400 μg/hr/cm². The result showed that the optimal addition concentration of azone at 4% to 6% could be obtained at high penetration rate and short lag time of meloxicam gel. It was shown that as the concentration of sodium lauryl
sulfate increased from 0% to 12% the flux and cumulative amount at 48 hr increased and lag time decreased.

Deveswaran et al. (2010) developed a central composite design based on response surface method employed to prepare experimental trials using different ratio of drug and polymer at varying r/min. to optimize the microsphere formulations. Formulations were prepared by emulsion solvent evaporation method using a mixture of dichloromethane: chloroform (2:3) as solvent system for the drug and polymer. The solution was added to 0.5%w/v sodium carboxy methyl cellulose solution and stirred at varying r/min. until all the organic solvent was evaporated completely resulting in the formation of microspheres. The prepared ketoprofen microspheres were discrete and free flowing and indicated that the concentration of polymer, stirring rate significantly influenced the formation of microspheres and ketoprofen entrapment while concentration of the polymer have a significant positive impact on ketoprofen release over a period of 12 hours and the stirring rate have minimal effect on the drug release. The results demonstrated a good relationship between the predicted and experimental values, confirming the validity of the model. Drug release mechanism indicated a best fit model of zero order release. The optimized final formulation KEC1 showed better analgesic and anti-inflammatory activity as compared with standard drug ketoprofen. The optimized formulation was found to be stable when subjected to accelerated and long term stability studies as per ICH guidelines. The results obtained indicated that response surface methodology can be successfully used to analyze the effect of formulation variables and develop an optimized formulation thereby reducing the number of trials, time and cost of formulation development.

1.8.5. Reports on in vitro and in vivo studies

Rao and Prabhavathi (1978) developed an in vitro method for the determination of availability of nonheme iron from foods and diets. Food was extracted with pepsin-HCI at pH 1.35 and subsequently the pH was adjusted to
pH 7.5 and filtered. Ionizable iron was determined in the pH 7.5 filtrate by the 
α, α′-dipyridyl method. The percent ionizable iron at pH 7.5 in a number of diets 
was shown to correlate highly with percent iron absorption from the same diets 
observed in the adult males. Ionizable iron at pH 7.5 was shown to increase in 
presence of ascorbic acid and meat extract while it decreased in presence of phytate 
and tannins, similar to the effects of these factors on iron absorption in human 
subjects. Based on these observations it is proposed that ionizable iron at pH 7.5 
determined as described in this study can be used as a reliable measure of 
bioavailability of nonheme iron in foods.

A new method for direct determination of dissolved Fe (III) in acid mine 
water has been developed by To et al. (1999). In most present methods, Fe (III) is 
determined by computing the difference between total dissolved Fe and dissolved 
Fe (II). For acid mine waters, frequently Fe (II). Fe (III); thus, accuracy and 
precision are considerably improved by determining Fe (III) concentration directly. 
The new method utilizes two selective ligands to stabilize Fe (III) and Fe (II), 
thereby preventing changes in Fe reduction-oxidation distribution. Complexed 
Fe (II) is cleanly removed using a silicabased, reversed-phase adsorbent, yielding 
excellent isolation of the Fe (III) complex. Iron (III) concentration is measured 
colorimetrically or by graphite furnace atomic absorption spectrometry (GFAAS). 
The method requires inexpensive commercial reagents and simple procedures that 
can be used in the field. Calcium (II), Ni (II), Pb (II), Al- (III), Zn (II), and Cd (II) 
cause insignificant colorimetric interferences for most acid mine waters. Waters 
containing >20 mg of Cu/L could cause a colorimetric interference and should be 
measured by GFAAS. Cobalt (II) and Cr (III) interfere if their molar ratios to Fe 
(III) exceed 24 and 5, respectively. Iron (II) interferes when its concentration 
exceeds the capacity of the complexing ligand (14 mg/L). Because of the GFAAS 
elemental specificity, only Fe (II) is a potential interferent in the GFAAS technique. 
The method detection limit is 2 g/L (40 nM) using GFAAS and 20 g/L (0.4 M) by 
colorimetry.
Bagherian et al. (2009) developed a simple, selective and sensitive kinetic spectrophotometric method with no need for removing iron (III) interference is proposed for determination of iron(II) in pharmaceutical and water samples. This method is based upon the catalytic effect of iron (II) on the sodium bromated crystal violet system in acidic media. Decolourization of crystal violet was used to monitor the reaction spectrophotometrically at 630 nm. The influence of various foreign species was studied and it was found that without addition of a masking agent, Fe (III) did not interfere with the Fe (II) determination up to 50-fold concentration of this ion. This method could be used successfully for determination of the iron (II) content of spiked water and pharmaceutical samples.

Mophan et al. (2010) reported that the Iron (III) is sparsely soluble in aqueous solutions even in an acidic condition. The solubility of iron (III) can be enhanced by complexing it with saccharides. Iron-dextran and iron-sucrose have been used to treat iron-deficiency anaemia. His work investigates the enhancement of the solubility of iron (III) in aqueous solution by complexing it with polysaccharides from locally available starch. Iron (III) was complexed with cassava and arrowroot starch and the state of iron (III) in the solutions was examined by UV-Vis spectrophotometry. The results showed that the solubility of iron (III) could be enhanced by cassava and arrowroot starch. It was asserted that these polysaccharides prevent the hydrolysis and precipitation of the iron (III) as iron-oxide. These finding might lead to an alternative treatment for iron-deficiency anaemia.

Raval and Patel (2011) aimed to enhance the dissolution of poorly water soluble meloxicam by preparing stable nanoparticles. Meloxicam nanoparticles were produced by combining antisolvent precipitation and high pressure homogenization approaches in presence of selected stabilizers and converting into dry powders by spray-drying. An increase in the stability of the nanoparticles was also assured by the sufficient adsorption of the stabilizers onto the drug surface. Meloxicam nanoparticles increased the saturation solubility of drug almost
fourfold. The *in vitro* studies at Q5min showed a marked increase in the drug release from just 7% (raw drug) to 82% (Meloxicam nanoparticles). The combining of both the methods was a promising method to produce uniform and stable nanoparticles of meloxicam with remarkable improvement in dissolution rate due to an increased solubility by the effect of increased surface area and change to amorphous form of the drug.

Yamini *et al*. 2011 designed a study with an objective to chemically evaluate the popular Siddha formulation “Tapyadi Lauha” by determining the total iron content using spectrophotometric method. The standard stock solution of iron was prepared using ferric ammonium sulphate. It was then diluted to give 0.1, 0.2, 0.3, 0.4 & 0.5mg/ml respectively. After 15-20 minutes, the absorbance was noted at 515nm. The standard curve of concentration Vs absorbance was plotted. The *Tapyadi Lauha* was incinerated to ash. The ashes obtained were acidified with 50ml of 6N HCl and dissolved in distilled water to prepare sample solution. The absorbance was noted at 515 nm. From the absorbance, the corresponding concentration was determined by extrapolation of calibration curve. Thus, the total iron content of *Tapyadi Lauha* was found to be as 0.410mg/ml. Hence, the data from this study suggested that the spectrophotometric method was rapid, reliable and accurate for the estimation of iron content. Hence, this method can be used for the determination of total iron content of related Siddha formulation useful in the treatment of iron deficiency anemia.

Iwalewa *et al*. (2009) determined the concentrations of 15 elements and heavy metals in the stem bark of *Harungana madagascariensis* using an energy-dispersive X-ray fluorescence (EDXRF) spectrometer. The anti-anaemic activity was done using the changes in hematological parameters (PCV, RBC and Hb) influenced by phenylhydrazine HCl (80 mg/kg) and malaria parasites-induced anemia. Results showed Cd, Ni, Mo, Cr and Br were in the range of 0.021–0.94 mg/g, while Pb, Zn, Fe, Cu and Hg were in the range of 1.50–7.24 mg/g. The elements with very high concentration were Ca, K, Sr, Mn and Cl and were in the range of
10.5–774.3 mg/g. Remarkable anti-anaemic activity was obtained with PCV of 40–48%, RBC count of $81-155 \times 10^4$ and Hb value of 57-66 g/dl after treatment; compared with 30% PCV, $67 \times 10^4$ RBC count and 36.5 g/dl Hb value obtained for the untreated control animals. The results suggested that *H. madagascariensis* stem bark extract constituents exhibit anti-anaemic activity.

Berger (2007) elucidated that, Phenylhydrazine (PHz) and its derivatives were first given a medical application at the end of the 19th century but with very little benefit. However, this compound seems to be very useful in models studying mechanisms of haemolytic anaemia. Phenylhydrazine induces a reactive oxygen species formation, peroxidation of lipids and oxidative degradation of spectrin in the membrane skeleton. PHz-induced haemolytic injury seems to be derived from oxidative alternations to red blood cell proteins. This compound can modulate immune reactions. Phenylhydrazine induces the destruction of red blood cells by oxidation stress and many joint changes at cellular levels resulting in haemolytic anaemia. PHz-induced toxic anaemia offers a model for research into the pathogenesis of haemolytic anaemia and the influence of anaemia on other physiological processes or the course of associated diseases. PHz-induced oxidative stress may serve as the model of the increased likelihood of cancer. Although changes in red blood cells after PHz treatment are showed in many published papers, little seems to be known of PHz effects on different types of cells.

Ogbe *et al.* (2010) investigated the antianaemic potential of three plant extracts on phenyl hydrazine-induced anaemia in rabbits. Anaemia was induced in rabbits with phenyl hydrazine hydrochloride at a dose of 30 mg kg$^{-1}$ b.wt by subcutaneous administration. Treatment of anaemia was done with ethanolic extract of *Mangifera indica* stem bark, aqueous leaves extract of *Telfairia occidentalis* and *Amaranthus hybridus*. *In vivo* investigation showed that oral daily dose of 20 mg kg$^{-1}$ b.wt of the ethanolic extract of *M. indica* stem bark and aqueous leaves extract of *T. occidentalis* produced a significant ($P < 0.05$) antianaemic effect. The aqueous leaves extract of *A. hybridus* only produced a minimal antianaemic effect,
reflected by a significant increase (P < 0.05) in haemoglobin concentration. Phytochemical analysis of the plant extracts detected saponins, tannins, cardiac glycosides, flavonoids and alkaloids in the 3 extracts. This study therefore, shows that *M. indica* and *T. occidentalis* extracts have antianemic potential.

Biapa *et al.* (2011) evaluated the hematological parameters of *Amphimas pterocaroides*, *Harungana madagascariensis*, *Myrianthus arboreus* and Cussonia barteri which are medicinal plants commonly used in Cameroon for the management or reversal of anaemia, were screened for their scavenging radical kinetic and the antianemic properties. The scavenging radical kinetic was defined as the inhibition rate of DPPH (2, 2 diphenyl 2 picrylhydrazyl hydrate) using methanolic extract of each plant. The antianemic property of fractions was also evaluated by measuring the haemoglobin (Hb), red blood cell (RBC) and haematocrit (HCT) levels. *A. pterocarpoides* and *M. arboreus* inhibits the DPPH rapidly with time and dose. The total inhibition of *A. pterocarpoides* was successful at 90 min with the concentration of 0.5 mg / ml and at 30 min when 2 mg / ml was used. *M. arboreus* and *H. madagascariensis* and *C. barteri* have presented their total inhibition at 60 min, 90 min and 180 min respectively. Though all plant fractions tested gave an increase in the content of Hb, RBC and HCT, the increase was most rapid for the hydroethanolic extract of *H. madagascariensis*. The presence of active phytochemical substances with antioxidant activities may provide a substantial basis for the use of these plants in ethnomedicine.

Yakubu *et al.* (2012) evaluated the haematological indices (Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Platelets, Neutrophil and Eosinophil) following oral administration of aqueous extract of *Fadogia agrestis* stem at the doses of 18, 50 and 100mg/kg body weight in male albino rats were evaluated progressively on daily basis at 24hrs after 1, 7, 14 and 21days. Extract administration significantly altered (P<0.05) WBC count and those
relating to it while it produced no significant change on RBC count and its related indices (P>0.05). The result suggest that aqueous extract of *Fadogia agrestis* stem has exhibited localized systemic toxicity which will impair the normal functioning of the WBC and its related indices.

Adeneye (2008) evaluated the blood-forming effects of (100% methanol seed extract) of *Citrus paradisi* Macfad in adult Wistar rats for 30 days as a way of evaluat-ing its traditional use in the treatment of blood deficiencies. Acute oral toxicity study was also conducted using limit dose test of the Up and Down Procedure statistical program (AOT425 PgmStat, Version 1.0) at a dose of 2000 mg/kg body weight/oral route. Results showed significant (p<0.05) progressive and dose dependent elevations in total leucocyte count (TLC), lymphocyte differentials (Lymph.), red blood count (RBC), haemoglobin con-centration (Hb), packed cell count (PCV), mean corpuscular volume (MCV), mean corpus-cular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count (PL). Reversed effect was recorded for the neutrophil (Neutro.) and monocyte (Mono.) differentials which were significantly (p<0.05) decreased in the treated rats. Acute oral toxicity showed the extract to be relatively safe at 2000 mg/kg on acute exposure.

Fermented seeds of *P. biglobosa* and leaves of *G. latifolium* were collected, sun dried, ground into powder and extracted using 98% ethanol. They were phytochemically screened and administered orally to male rats at doses of 0, 200, 300 and 400mg/kg b.wt, respectively for 60 days. Phytochemistry of the plants showed that alkaloids, glycosides, reducing compounds and polyphenol were present in the different extracts though in varying proportions. Tannins and flavonoids were only present in the leaf extract of *G. latifolium* but completely absent in seed extract of *P. biglobosa*. Additionally, saponins, phlobatanins, anthraquinones and hydroxymethyl anthraquinones were not identified. Result on haematological parameters revealed that the administration of the extracts caused significant effects (P < 0.05) on all the blood parameters except the packed
cell volume (PCV) that showed no significant difference (P >0.05). Generally, the results indicated that the two spice plants can enhance the production of blood cells, especially at the doses of 200mg/kg and 300mg/kg BW, respectively though seed extract of *P. biglobosa* was seemingly more toxic than the leaf extract of *G. latifolium* (Ikpeme *et al*., 2012).

From the above literature screening, it was revealed that the iron deficiency is a global health problem, which should be addressed immediately before leading to severe complications. It shows that, the existing problem can be well rectified only through the fortification process rather than the supplementation. Also, choosing the iron compound for the fortification is a critical parameter which determines the bioavailability and iron absorption. The existing ferrous sulphate which is a water soluble compound, serves to be a good supplementation but it fails to satisfy the long term storage process. Though ferrous fumarate assures the long term storage process, it does not result with a good bioavailability values.

Therefore, it is noticed that nanotechnology means of product development aims to provide solutions to several types of prevailing scientific problems. The synthesis of iron compounds through the nanotechnological process may bring a proper response to the existing iron deficiency problem. Also the literature survey shows the importance of the encapsulation in the drug delivery. The drugs encapsulated with the polymers and liposomes, were found to have a better targeted approach and provide good bioavailability to the drugs through slow release. Relating the above said technology with the iron compounds, it can be concluded that the encapsulation of the iron compounds may protect the iron from the sensory effects and leads to better absorption.

1.9. **OBJECTIVES OF THE STUDY**

Keeping in view for the need to improve the bioavailability of the iron compound, the formulations involving biodegradable polymers like Chitosan and PLGA were tried in this study.
➢ To synthesis the ferrous phosphate nanoparticles through controlled hydrolysis method.

➢ To characterize the ferrous phosphate nanoparticles for Spectroscopic analysis:
  • XRF spectroscopy for elemental analysis.
  • FTIR spectroscopy to know the functional elements involved.

➢ To characterize the ferrous phosphate nanoparticles for morphological analysis:
  • Scanning electron microscopy to study the bulk nature of the particles.
  • Atomic force microscopy to know the individual nature of the particles.

➢ To characterize the ferrous phosphate nanoparticles for surface characteristic analysis:
  • BET study to know the specific surface area of the particles.
  • Zeta potential study to know the surface charge of the particles.

➢ To analyse the *in vitro* solubility of the particles in varying pH.

➢ Preformulation studies
  • To develop an optimized formulation of Chitosan encapsulated drug using Response Surface Methodology.
    ➢ Response effect on particle size.
    ➢ Response effect on drug entrapment efficiency.
    ➢ Response effect on drug release.
• To develop an optimized formulation of PLGA encapsulated drug using Response Surface Methodology.
  ➢ Response effect on particle size.
  ➢ Response effect on drug entrapment efficiency.
  ➢ Response effect on drug release.

➢ To identify the ideal batch of Chitosan encapsulated drug using desirability values of RSM by numerical and graphical methods.

➢ To identify the ideal batch of PLGA encapsulated drug using desirability values of RSM by numerical and graphical methods.

➢ To evaluate the \textit{in vivo} effect (Haematological parameters) of the ferrous phosphate nanoparticle drug, Chitosan encapsulated drug, PLGA encapsulated drug and standard drug using a rat model.
  • Determination of packed cell volume (PCV levels).
  • Determination of RBC levels.
  • Determination of Haemoglobin levels.
  • Determination of ferritin levels.

**SCOPE OF THE STUDY**

The development of highly bioavailable iron preparations and their use as food supplements and fortiﬁcants for food, presents a challenge for the capacity of iron homoeostasis. At the same time, iron deﬁciency is still widely distributed in developing countries. In the attempt to balance the risk of iron deﬁciency and iron overload in the face of highly bioavailable iron sources, a number of national and regional bodies developed recommendations for iron intake that are highlighted in relation to their physiological, epidemiological or toxicological background.

This study aims to bring out a proper iron drug formulation that could serve as a better iron supplement in a simple form, than the available means. The aim of synthesising a simple form of iron signiﬁes for the better digestion and absorption
in the gastrointestinal tract. This task is to be achieved by reducing the particle size of the iron compound and increasing its surface area. Thus, the significant high surface area can lead to high solubility and thereby can bring high relative bioavailability.

The encapsulation of the particles with polymer based materials which are highly biocompatible and biodegradable, can serve to be a protective barrier from the iron absorption inhibitors. Such type of formulation can be attributed to avoid the unnecessary organoleptic changes, inside the gastrointestinal tract. This signifying task will avoid hindering the iron absorption. Such an advantageous and favourable characteristics iron compounds will provide a better iron supplementation in both pharmaceuticals and also for the food fortification. Since these formulations are obtained through the simple controlled hydrolysis reactions, the drug components are less cost effective and will be easily available for all population sectors to combat iron deficiency.

Our study will consolidate the status of iron supplementations and iron deficiency in India and worldwide, with all possible effects which are unpreventable. The formulation development with this study can fetch a new iron compound with beneficial characteristics to eradicate iron deficiency. The knowledge of sensory outcomes in relation to the drug formulations was taken into considerations with effect to Indian dietary substances. The significant involvement of nanoscale science in the present study, can pave way to identify much more drug ingredients for most prevailing diseases.