Chapter 5
Biofortification of Food with Minerals and Vitamins Encapsulated in Silica

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Abstract Micronutrients such as minerals and vitamins are a class of food compounds that are essential for human health. Minerals and vitamins are indeed important for biological processes. The micronutrients actually occurring in dietary sources are not stable and get degraded during food processing and cooking. A major part of the population is suffering from vitamin deficiency due to change in the life style, food affordability, food habits, and food accessibility. As a consequence supplements have been made available in the market. Supplements are prepared by expensive formulations to increase vitamin bioavailability. Many additives are added to stabilize and enhance the delivery of active compounds. Last, people become reluctant to consume tablets because they do not trust what looks like pharmaceuticals. Hence, the alternative is the fortification of food, that is adding micronutrients to food. Examples include vitamin D fortified milk and iodized salt. For vitamins, biocompatible carrier molecules, such as silica, are needed because vitamins degrade easily in the presence of in light, pH, oxidation and reducing agents. Here we review the causes and consequences of micronutrient deficiencies, the bioavailability of nutrients, and vitamins, minerals and silica for food. We also studied of the release of nutrients entrapped in silica. Results show that the release of nutrients from silica in simulated intestinal fluid is better than in simulated gastric fluid.

Keywords Silica • Nutrition • Micronutrients • Vitamins • Minerals • Nutrient carrier • Nutrient release • Encapsulation • Gastro-intestinal tract

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5.1   Introduction

The understanding that micronutrients are essential for human growth and that they have to be part of the diet was a major stimulus for nutrition science. The importance of vitamins led to the development of processes for their large scale production, formulation and incorporation in food fortification so that the product could be ultimately consumed by humans. This inspired pharmaceutical and nutritional companies to develop different food formulations. Industrial production was not the complete solution yet, and new challenges arose with the incorporation of micronutrient and nutraceuticals in end-user applications such as tablets, vitamin water, beverages, yoghurts and other foodstuffs, as the nutrients in them are often chemically unstable. The nutrients could be easily degraded in natural form via oxidation, hydrolysis, light and heat (Gharsallaoui et al. 2007). New technologies had to be explored to maintain the stability of the micronutrients. Therefore, new approaches have been invented for the delivery of micronutrient by using carrier molecules to protect them from natural environmental condition. Thereby the stability and shelf-life of the compounds are prolonged and they can be released in a controlled and tailored manner. Further benefits of formulation and encapsulation processes include easier handling, improved sensory properties with respect to appearance and taste and uniform dispersion of low-concentrated actives (Gouin 2004). The food administrations already setup the dietary standards and nutrient requirements with recommended dietary allowance for the optimal intake of vitamins and minerals depending on age, gender and risk groups. In order to secure a sufficient intake of nutrients in diet, a number of countries implemented fortification programs of staple food. Examples are the fortification of flour with folic acid in the US, Canada and Latin American countries, the fortification of margarine with vitamin A and D or the fortification of milk and juices with vitamin D and fortification of flour or sugar with vitamin A especially in low-income countries.

5.2   Micronutrient Deficiencies in Developing Countries

Malnutrition in developing countries among women, children and adolescents is an emergency, needing immediate attention if these countries have to have inclusive growth and development. Without good nutrition, neither communicable nor non-communicable diseases can be controlled. Malnutrition is the worst form of non-communicable disease and is important risk factor for chronic diseases at a later stage. Nutrition is one of the bases of judging a national development. Besides deficiencies of calories and protein, deficiency of micronutrients like vitamins and minerals are also widespread (Indian national nutrition monitoring bureau 2003, 2006). Micronutrients deficiency is referred to as the hidden hunger since often at times it is a hidden killer or crippler, and extracts heavy human and economic cost. These contribute significantly in biological functions, because they are needed for

utilization of proteins and calories, they act as co-factors in functions of many enzymes (Bender 2003) and to fight infections from a young age. In addition to serious detrimental effects on bodily functions, the existence of malnutrition has profound implication on economic development and productivity in terms of huge public health costs. For example, Indian foods that are mostly cereal based are also deficient in micronutrient such as iron, folic acid, calcium, vitamin A, riboflavin. Among the micronutrient deficiencies, iron deficiency anaemia is the most serious public health problem (Indian national nutrition monitoring bureau, 2003, 2006). Estimates of iron deficiency anaemia in women and children have varied from 50 to 60 %; pregnant women being particularly susceptible. Iodine deficiency disease is another worrisome public health problem (National nutrition monitoring bureau 2006). Though, its magnitude has declined in recent years after the introduction of iodised salt, but the problem still persists, and not confined to the sub Himalayan regions as earlier thought. Fortunately, some of the severe vitamins- deficiency diseases such as beri-beri: thiamine-vitamin B1 deficiency, pellagra –niacin deficiency and scurvy –vitamin C deficiency has disappeared. Blindness due to vitamin A deficiency (Aykroyd 1930) and rickets due to vitamin D deficiency remain as clinical rather than public health problems. However, milder clinical manifestations and biochemical evidence of these deficiencies is rampant. Also osteoporosis in adults, particularly women after menopause due to calcium and vitamin D deficiency is common (Ross et al. 2011a, b).

5.3 Consequences of Micronutrients Deficiencies

The micronutrient deficiency results into severe depression related problem and mental illness. The affected person will develop poor appetite, anxiety. Nutrient such as amino acids, minerals, vitamin B, omega-3 fatty acids are known to be the precursors of neurotransmitters and various biological pathway and deficiency of these can lowers the secretion of the neurotransmitter with serious consequences of mental disorder (Sathyanarayana Rao et al. 2008). Micronutrient malnutrition is a major obstruction to socioeconomic development of the nation, which eventually has a deleterious effect over the underprivileged groups. Micronutrient malnutrition leads to high economic loss, reduction in the capability and potential of manpower, high rates of illness and mortality due to deficiency disease. It adversely affects human health, learning ability and productivity.

Apart from human suffering due to morbidity and mortality, malnutrition in general and micronutrient deficiencies in particular have high economic cost. Cost of treating malnutrition is 27 times more than investment required for its prevention (Leadership agenda 2010). According to a panel of Nobel laureates, the top 10 priorities selected for advancing global welfare using methodologies based on the theories of welfare economics, in Copenhagen Consensus 2008, five were in the area of nutrition – micronutrient supplements, micronutrients fortification, bio-fortifications, de-worming and other nutrient programs at school and community level (Copenhagen

Consensus 2008). These were also needed to achieve the millennium development goals.

5.4   Causes of Micronutrient Deficiency

The over exploitation of current agricultural resources has led to a development of nutrient deficient crops. The agricultural produce which traditionally had the necessary nutrients such as vitamins and minerals has progressively shown deficiency due to the inherently low nutrients in the soil or to the low application by farmer and low uptake by plants. The application of toxic chemical pesticides to the crops also affects the quality of soil and hinders the absorption of micronutrient by the plants and the crops remain nutrient deficient (Hornick 1992). Not only inherently low nutritional value of crops but also the lack of awareness of dietary rich food, malabsorption, & affordability are some of the major factors which responsible for the micronutrient deficiency.

5.5   Dietary Reference Intakes

Recommended dietary allowance is the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly 97–98 % healthy individuals in a particular life stage and gender group (Dietary Reference Intakes 2001). Adequate Intake is a recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group(s) of apparently healthy people, that are assumed to be adequate used when an recommended dietary allowance cannot be determined. Tolerable upper intake level is the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects for almost all individuals in the general population. As intake increases above the upper intake level, the potential risk of adverse effect increases. Estimated average requirement is the average daily nutrient intake level estimated to meet the requirement of half of the healthy individuals in a particular life stage and gender group.

The recommended dietary allowance is derived from (i) the individual variability, and (ii) the nutrient bio-availability from the habitual diet. Individual variability: Definition of recommended dietary allowance takes into account the variability that exists in the requirement of a given nutrient between individuals in a given population group. The distribution of nutrient requirement in a population group is considered normal and the recommended dietary allowance corresponds to a requirement, which covers most of the individuals (97.5 %) in a given population. This is termed as a safe level of intake of a nutrient, that is, the chances of individuals having requirements above the recommended dietary allowance is only 2.5 %. This principle is used in case of all nutrients except energy, since in the case of energy, intakes

either the excess or below the actual requirement of energy are not safe. In case of other nutrients the recommended dietary allowance is 25 % (+2SD) higher than the mean requirement, 12.5 % being considered as the extent of individual variability in the requirements of all those nutrients.

5.6 Bioavailability of Nutrients

The release of nutrient from food, its absorption in the intestine and bio response is the bioavailability of a given nutrient from a diet. This bioavailability factor is quite important in case of calcium and protein and trace elements like iron and zinc. In case of iron, the amount to be present in the diet is 20–30 times higher than the actual iron requirement to account for the low bio-availability of iron from a given diet, particularly a cereal-based diet. Recommended dietary allowance represents the level of the nutrient to be consumed daily to meet all the requirement of most of the individuals in a given population. However, it must be recognized that recommended dietary allowance is not meant to be used as standard to determine whether or not a given individual requirement has been met, since it is a level above the requirement of most individuals in a given population. Recommended dietary allowance value of a nutrient is valid only when all other dietary nutrient intakes are satisfactory.

5.7 Essential Nutrients and Importance of Vitamins and Minerals

Essential nutrients are nutrients that cannot be synthesized by the body, however it has an eminent role in normal body function and metabolism, therefore it must be obtained from dietary source. Categories of essential nutrients include vitamins, fats, essential amino acids and dietary minerals. Some essential nutrients may also be toxic when intake in high doses and can cause hyperactive diseases symptoms (Lichtenstein and Russell 2005).

Vitamins and minerals are the major constituent, which has the significant role in the metabolic and biological process of the body and also responsible for the reproductive function Hurley and Doane (1989). As vitamins are organic nutrient without caloric content and are essential for health, hence though minerals also perform diverse and critical roles in human health and disease. Vitamins can be obtained by dietary sources in adequate amount with the exception of vitamin D which are synthesized in the presence of sunlight. Dietary sources of vitamins, such as fruits and vegetables are preferable for vitamin and minerals supplements; perhaps this is because fruits and vegetables contain vitamins in useful proportions, as well as fiber and minerals, which together play a role in good nutrition and disease prevention

(Fragakis 2003). Vitamins come under two categories, fat soluble vitamins and water soluble vitamin. Both classes of vitamins are equally important for proper functioning of body.

What are vitamins? Vitamins are organic compounds which occur in very small quantities in food but are very important to life for specific regulatory functions and the maintenance of life and normal growth (Lieberman and Bruning 1990). All vitamins contain carbon, hydrogen, and oxygen but some of them contain nitrogen, sulfur and cobalt. Vitamins are measured also in terms of micrograms equivalent to one millionth (1/1,000,000) of a gram or in milligrams equivalent to one thousandth (1/1000) of a gram. Vitamins are divided into two groups: water-soluble (B-complex vitamins and C vitamins) and fat-soluble vitamins (A, D, E and K). Unlike water-soluble vitamins that need regular replacement in the body (Fukuwatari and Shibata 2008) fat-soluble vitamins are stored in the liver and fatty tissues, and are eliminated much more slowly than water-soluble vitamins (Maqbool and Stallings 2008). Complete classification and details of the vitamins are shown in Table 5.1.

Few vitamins in plants and animals are preformed, meaning that they are already in active form and ready for their biological function in the body. Some vitamins are present in plants as precursors, meaning that they have to be changed to active form to be ready for biological function. An example of this is carotene or pro-vitamin A. Carotenoid called a precursor of vitamin A (Wald 1935). There are also man-made vitamins which are synthesized in the laboratory. Hence, these are called synthetic vitamins. These synthetic vitamins are mostly used for therapeutic purposes. An individual who eats a well-balanced meal does not need synthetic vitamins because he is assured of the normal intake of vitamins from food sources. The condition that results from a deficient intake of vitamins is referred to as *avitaminosis*. On the other hand, too much intake results to excessive accumulation in the body resulting to *hypervitaminosis*. This is especially true with fat-soluble vitamins.

5.8 Fat-Soluble Vitamins

They constitute of vitamins that show solubility in fats like oil or organic solvents. They comprise mainly vitamin A, vitamin D, vitamin E and vitamin K. Vitamin A is an unsaturated hydrocarbon, which consist of retinol, retinal, retinoic acid and several provitamin A carotenoids and beta-carotene (Fennema 2008). Vitamin D is mainly a cholesterol derived vitamin. Vitamin E comprises of groups mainly: tocopherols and tocotrienols; each comprising of four forms (α) alpha, (β) beta, (γ) gamma and (δ) delta. Though the deficiency of fat-soluble vitamins were rare earlier, but now days due to the adaptation of a healthy life-style by many people their deficiencies are increasing. People avoid the intake of fat or fat containing compounds in any form resulting in the mal-absorption of the vitamins in the body.

Vitamin A All forms of vitamin A have a beta-ionone ring to which an isoprenoid chain is attached, called a retinyl group. Both structural features are essential for

Table 5.1 Classification of vitamins (Kutsky 1973)

Generic name	Chemical name	Solubility	Deficiency disease	Overdose disease	Food source
Vitamin A	Retinol, retinal	Fat	Night-blindness, Hyperkeratosis and Keratomalacia	Hypervitamin osis A	Orange, Ripe yellow fruits, carrot, liver, leafy vegetables, soya milk
Vitamin B_1	Thiamine	Water	Beriberi, Wernicke-Korsakoff syndrome	Drowsiness or muscle relaxation with large doses	Pork, oatmeal, brown rice, liver, egg
Vitamin B_2	Riboflavin	Water	Ariboflavinosis	–	Dairy products, beans
Vitamin B_3	Niacin, niacinamide	Water	Pellagra	Liver damage	Meat, Fish, eggs, mushrooms
Vitamin B_5	Pantothenic acid	Water	Paresthesia	Diarrhea, nausea, heartburn	Meat, broccoli, avocados
Vitamin B_6	Pyridoxine	Water	Anemia peripheral neuropathy	Impairment of proprioceptio n nerve damage	Meat, Nuts, banana
Vitamin B_7	Biotin	Water	Dermatitis, enteritis	–	Egg yolk, lover, peanuts
Vitamin B_9	Folic acid	Water	Megaloblast	–	Leafy vegetables, pasta, liver
Vitamin B_{12}	Cyanocobalami ne	Water	Megaloblastic anemia	Acne-like rash	Meat products
Vitamin C	Ascorbic acid	Water	Scurvy	Vitamin C megadose	Citrus fruits, liver
Vitamin D	Cholecalciferol	Fat	Rickets and Osteomalacia	Hypervitamin osis D	Fish, eggs, liver
Vitamin E	Tocopherols Tocotrienols	Fat	Hemolytic anemia	Heart failure	Fruits, vegetables, nuts, seeds
Vitamin K	Phylloquinone	Fat	Bleeding diathesis	Increased coagulation	Spinach, egg yolk, liver

vitamin activity. Many different geometric isomers of retinol, retinal and retinoic acid are possible as a result of either a trans or cis configuration of four of the five double bonds found in the polyene chain. The cis isomers are less stable and can readily convert to the all-trans configuration. Nevertheless, some cis isomers are found naturally and carry out essential functions (Nasab et al. 2013) (Fig. 5.1).

Fig. 5.1 Chemical
structure of vitamin A (US
National library of
medicine, NIH)
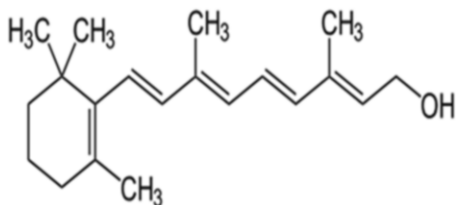
Significance of Vitamin A It is essential for normal vision, for maintaining the integrity of epithelial tissues and for a wide variety of metabolic functions (Tanumjhardjo 2011). Considerable amounts of vitamin A can be stored in the liver and made available for use as the need arises. Although the role of vitamin A in the visual process is well known, its mechanism of action in other metabolic processes is, as yet, not well understood. A series of studies (Sivakumar et al. 1997) on pregnant women were carried out at national institute of nutrition, correlating the duration of vitamin A supplementation, that was required to prevent the fall of plasma vitamin A, invariably occurring in the last few weeks of pregnancy, compared to those who did not receive any supplement. It was found that a daily dose of 780 RE was able to not only prevent the fall in plasma level but also increase neonatal status. Such amounts of intake were associated with improved fetus-placental function reversing the changes in protein catabolism and oxidative stress (Sivakumar et al. 1997).

Dietary Sources of Vitamin A Foods provide vitamin A either in the preformed state (directly as retinol or retinyl esters of fatty acids) from animal sources such as milk (28 µg 3 %), butter (684 µg 76 %), egg (140 µg 16 %) and carrot (835 µg 93 %) or its precursor carotenoids, especially carotene, derived from leafy vegetables and yellow- and orange-coloured fruits and vegetables. β-carotene and other carotenoids are variably converted into vitamin A in the body (Tang et al. 2005). Since β-carotene forms a major source of dietary vitamin A in many developing countries including India, the efficiency with which it is absorbed and utilized becomes important in translating β-carotene values into retinal equivalents. Although the enzymatic conversion of one molecule of β-carotene to retinol should theoretically result in two molecules of retinol, because of physiological inefficiency, the maximum conversion that has been shown in experimental animals is around 50 % (Olsan 1989). In addition, the efficiency of β-carotene absorption is variable, depending upon the food source. Red palm oil contains easily absorbable carotenes. Based upon available evidence, the food and agriculture organization –united nations/world health organization expert group (WHO Tech. Rep, 1967) assumed that about one third of dietary β-carotene is absorbed. As a result, a factor of 0.16 was used to convert β-carotene to retinol. In two studies carried out in India, one in adults (Rao and Rao 1970) and the other in children (Lala and Vinodini 1970) absorption of β-carotene from green leafy vegetables was found to be much higher, ranging from 50 to 99 %. Lower figures were obtained for the absorption of β-carotene from other sources

Table 5.2 Recommended intake of vitamin A (Retinol equivalents, RE, µg/d)

Group	Sub group	FAO/WHO[a] (2005) Retinol RE, µg/d	FAO/WHO (2011) Retinol RE, µg/d	B-carotene[b] RE, µg/d
Adult men		600	600	4800
Adult women		500		
Pregnant women		800	800	6400
Lactating women		850	950	7600
Infants	0–6 months	375	350	–
	6–12 months	400		2800
Children	1–6 years	400/450	400	3200
	7–9 years	500	600	4800
Adolescents	10–17 years	600	600	4800

[a]Food and agriculture organization –United Nations/World health organization, [b]A conversion ratio of 1:8 is used

such as carrots and papayas. There is epidemiological evidence to support this contention (Lala and Vinodini 1970; Pereira and Begum 1969) (Table 5.2).

Deficiency of Vitamin A One of the earliest manifestations of vitamin A deficiency is night blindness and more severe deficiencies include ocular changes leading to blindness, particularly in young children. Though there has been a significant reduction in severe clinical forms of Vitamin A deficiency over the last 3 decades, vitamin A deficiency is still a public health problem with 40–60 % child population in developing countries showing inadequate biochemical status of vitamin A (World Health Organization global database, 1995–2005). Very low intakes, poor bio-availability of pro-vitamin A from the predominantly vegetarian diet and recurrent infections are thought to be the main reasons for widespread prevalence of vitamin A deficiency.

Role of Vitamin A A generalized scheme for retinoid metabolism is that dietary retinyl esters, retinol, and provitamin A carotenoids (such as β-carotene) are taken into the body. Vitamin A (by definition all-trans-retinol) may be esterified into retinyl esters and stored. In times of dietary retinoid insufficiency, retinyl ester stores are hydrolyzed to retinol for delivery to peripheral tissues. Both all-trans-retinol and β-carotene may be converted enzymatically to all-trans-retinal. Retinal either can be enzymatically oxidized to retinoic acid, which regulates transcription of over 500 retinoid-responsive genes, or reduced enzymatically to retinol. When retinoic acid is no longer needed, it is catabolized and eliminated from the body (Li et al. 2014).

Vitamin D It refers to a group of fat-soluble seco-steroid compounds. Two nutritionally significant compounds are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D3 is metabolised to the active steroid hormone 1,25-dihydroxyvitamin D3 by successive hydroxylations in the liver and kidney. Vitamin D2

Fig. 5.2 Chemical
structure of vitamin D
(US National library of
medicine, NIH)
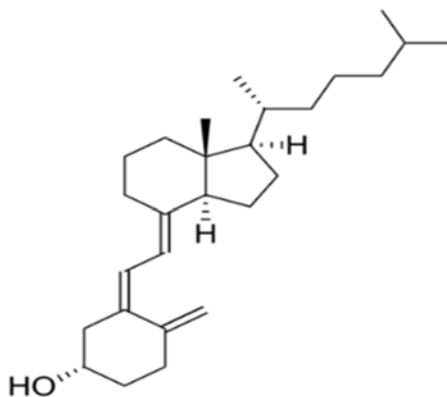
is metabolised to 1,25-dihydroxyvitamin D2 by the same enzyme systems. One
milligram of vitamin D is equivalent to 40,000 international units (Khandare et al.
2005) (Fig. 5.2).

Significance of Vitamin D It is a unique vitamin and its availability in the body
largely depends on its synthesis in the skin when exposed to sunlight and hence, its
dietary requirement is usually very small especially in the Indian context. In humans,
the most important related compounds of vitamin D are vitamin D2 and vitamin D3
(Holick 2006). Cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2) are
unique as they constitute the vitamin D and can be ingested from the diet and/or
supplements (Calvo et al. 2005; Norman 2008). Apart from its classical calcitropic
etiological role in the development of rickets (in children) and osteomalacia (in
adults) (Wolf 2004), leading to weak bones and physical deformation, vitamin D
has been found to have many non-clacitropic health promoting effect (Pittas et al.
2010). Diseases such as osteoporosis, diabetes, psoriasis, hypertension, arthritis,
multiple sclerosis and cardio vascular diseases are known to have the involvement
of vitamin D leading to the suggestion that the above spectrum of deficiency dis-
eases be called vitamin D deficiency diseases as in the case of other micronutrients
(Chung et al. 2009; McLaren 2006). The best evidence of benefit is for bone health
(Ross et al. 2011a, b) and a decrease in mortality in elderly women (Bjelakovic et al.
2011).

Dietary Sources of Vitamin D It can be photolytically formed from
7-dehydrocholesterol (as provitamin D3) in the skin when exposed to sunlight
(UV-B rays) (Houghton and Vieth 2006). It can be also obtained from the diet both
through plant sources as provitamin D2 (ergosterol) and get converted to D2 (ergo-
calciferol) in the skin or through animal foods as preformed vitamin D3 or its pro-
vitamin (Holick 2007). It is estimated that about one tenth of the body's requirement
is derived from dietary sources in the Indian context; mainly the exposure to sun-
light being the limiting factor (McLaren 2006). Vitamin D3 is also found in oily fish
and cod liver oil (Holick 2007) (Table 5.3).

Table 5.3 Recommended intake of vitamin D

Age	Male	Female	Pregnancy	Lactation
0–12 months	400 IU (10 mcg)	400 IU (10 mcg)	–	–
1–13 years	600 IU (15 mcg)	600 IU (15 mcg)	–	–
14–18 years	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)
19–50 years	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)
51–70 years	600 IU (15 mcg)	600 IU (15 mcg)	–	–
>70 years	800 IU (20 mcg)	800 IU (20 mcg)	–	–

IU international units, *mcg* micrograms

Deficiency of Vitamin D Its deficiency lead to abnormal calcium homeostasis resulting in defective mineralization of the growing long bones (rickets in children) or a decrease in the mineral content of the matrix of the bones (osteomalacia in adults) ending with weakened bones. A poor calcium deposition during growth phase and faulty economy in adults lead to osteoporosis and vitamin D and calcium supplementation is shown to be beneficial. While the rabid forms of bone disease are no longer prevalent in children now, growing urbanization, reduced physical activity and low exposure to sunlight are believed to contribute to a spurt of vitamin deficiency diseases as evidenced from low circulating vitamin D levels. The emergence of sub-clinical vitamin D deficiency in the country has been reviewed by Goswami (Goswami et al. 2008). In recent years, in endemic areas of fluorosis, excess of fluoride in drinking water was found to be associated with rachitic bone deformities and vitamin deficiency diseases in young children (Khandare et al. 2005)

Role of Vitamin D It is activated in two series of hydroxylation reactions in liver and kidney to active hormones endogenously which mediate increased intestinal absorption and renal tubular resorption of calcium and contribute to an increase in the availability of calcium (Cranney et al. 2007; Bischoff-Ferrari et al. 2012). The first hydroxylated compound, 25 hydroxy cholecalciferol and the next 1, 25 dihydroxy cholecalciferol are the well-known metabolites of vitamin D. The first one is the circulating and storage form and represents the status of vitamin D. 1, 25 dihydroxy cholecalciferol is the active hormone metabolite which induces the formation of calcium transport protein involved in calcium absorption (Cranney et al. 2008). Blood calcium is maintained in a narrow range by the concerted actions of parathyroid hormone, thyrocalcitonin and vitamin D. A lowering in calcium intake triggers an elevation in Parathyroid hormone levels which enhances the release of 1, 25 dihydroxy cholecalciferol so as to increase calcium absorption and thus normalize blood calcium. A lack of vitamin D will reflect in alterations in the calcitropic hormones and not always in serum calcium. Vitamin D is now considered more as a pro-hormone, than as a vitamin. It can be synthesized in the body in adequate amounts by simple exposure to bright sunlight even for 5 min per day (Crissey et al. 2003). Habitual Indian diets do not provide even 10 % of the requirement. Based on healing response in rickets, increased calcium absorption or circulating levels of 25
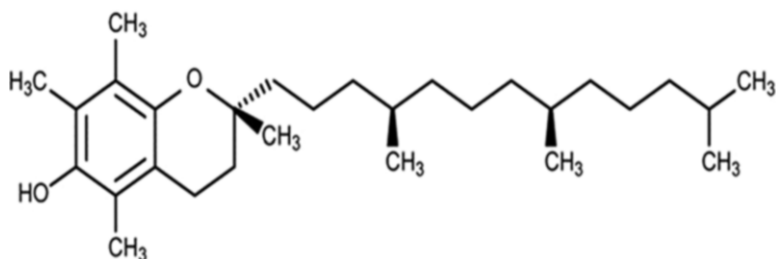
Fig. 5.3 Chemical structure of vitamin E (US National library of medicine, NIH)

hydroxy cholecalciferol and also turnover of isotopic vitamin D, the requirements of vitamin D have been determined over the years. The World Health Organization expert committees recommended 100 Units (2.5 µg)/d for adult males in 1988 (World Health Organization 1988) and increased them later in 2005 to 200 Units (5 µg)/d (Food and agriculture organization/World health organization 2004). This is obviously due to progressive decrease in the exposure to sunlight and the need to obtain the requirements from dietary and supplement sources. Hence, many foods such as milk and vegetable oils are subjected to mandatory fortification with vitamin D in the developed countries.

Vitamin E Vitamin E is not a single molecule, but a family of 8 related molecules called tocopherols and tocotrienols. Moreover, each of the different tocopherols exists in eight stereoisomers. However, having described this molecular complexity, it turns out that dietary vitamin E is predominantly alpha and gamma-tocopherol. The structure of alpha-tocopherol is depicted below; note the long hydrocarbon chain similar to the tail of a fatty acid (Fig. 5.3).

Significance of Vitamin E It is a form of fat-soluble compound that include both tocopherols and tocotrienols (Brigelius-Flohe and Traber 1999). It helps to stop the production of reactive oxygen species formed when fat undergoes oxidation (Herrera and Barbas 2001; Packer et al. 2001). Vitamin E has many biological functions, the antioxidant function being the most important one (Bell 1987). Other functions comprises of enzymatic activites, gene expression and neurobiological functions. It plays a very important role in cell signaling (Azzi 2007). Vitamin E mainly acts as a peroxyl radical scavenger, preventing the propagation of free radicals in cells. As it is fat-soluble it is incorporated into the cell membrane, which protects them from oxidative damage. Vitamin E plays an important role in gene expression too (Devaraj et al. 2001). It has an important role in neurological functions (Muller 2010) and inhibition of platelet aggregation (Atkinson et al. 2008). Vitamin E protects lipids and prevents the oxidation of polyunsaturated fatty acids too (Whitney et al. 2011).

Dietary Sources of Vitamin E Vegetable oils and invisible fat of cereals and other foods like nuts and vegetables contribute to adequate tocopherol content in Indian

Table 5.4 Recommended Intake of Vitamin E (National Institute of Health 2009)

Group	Sub-group	mg/day
Infants	0–6 months	4
	7–12 months	5
Children	1–3 years	6
	4–8 years	7
	9–13 years	11
Adolescents	14 and above	15

diets. About 60 % of vitamin E in the diet comes from vegetable oil (soybean, corn, cottonseed, and safflower). This also includes products made with vegetable oil (margarine and salad dressing). Vitamin E sources also include fruits and vegetables, grains, nuts (almonds and hazelnuts), seeds (sunflower) and fortified cereals (National Institute of Health 2009) (Table 5.4).

Deficiency of Vitamin E Its deficiency is rare. Cases of vitamin E deficiency usually only occur in premature infants and in those unable to absorb fats. Since vegetable oils are good sources of vitamin E, people who excessively reduce their total dietary fat may not get enough vitamins E. The deficiency is rare but if occurs leads to various problems like myopathies, peripheral neuropathy, ataxia impairment of the immune system, and red blood cell destruction (Kowdley et al. 1992). Vitamin E obtained from food usually does not pose a risk for toxicity. Supplemental vitamin E is not recommended due to lack of evidence supporting any added health benefits. Megadoses of supplemental vitamin E may pose a hazard to people taking blood-thinning medications such as Coumadin (also known as warfarin) and those on statin drugs.

Role of Vitamin E It is hydrophobic and is absorbed similarly to other dietary lipids. After getting soluble by bile acids, it is absorbed into small intestinal epithelial cells, incorporated into chylomicrons, and transported into blood via lymphatic. Once in the circulation, vitamin E is liberated from chylomicrons and much is taken up by the liver, where it is repackaged into very low density lipoproteins and secreted again into blood. Ultimately, vitamin E is transported in blood bound to a variety of lipoproteins, from which it is taken up by tissues throughout the body. Vitamin E is stored within the fat droplets of adipose tissue cells (Gagne et al. 2008).

5.9 Water-Soluble Vitamins

These are dissolved by the water and not stored by the body, henceforth required in adequate level in diet on daily basis. As excess of water-soluble vitamins gets eliminated in urine so therefore generally it's not toxic and harmful. The major dietary sources of water-soluble vitamins are fruit, vegetables and grains. Water-soluble vitamins are categorized into vitamin C, vitamin B and folic acid (Emerson and

folkers 1951). Although vitamins and dietary minerals are present in the foods however it is still found inadequate in major population and people become vulnerable to deficiency diseases. Preparation of food can also result in the loss of vitamins from vitamin enriched food because water soluble vitamins are heat labile and light sensitive so it can easily be degraded at high temperature or by exposure in light and air (Kreutler and Czajka-Narins 1987). In a survey low blood, folate level was found in most of the school going children (6–16), (Sivakumar et al. 2006) though their diet contained a mean intake of 155 % recommended dietary allowances for folic acid still they were found folate deficient. It was obvious that may be there is loss of dietary folate during cooking of food or in the processing of fortified food. Folate is essential in biological methylation reactions and nucleotide synthesis and impairment of these processes are thought to be involved in cancer development (Kim 2004)

Folic Acid Chemically folates are known as pteroyl-L-glutamic acid, its structure composed of three distinct moieties, heterocyclic pteridine ring, para-aminobenzoic acid and glutamic acid. Iwater-soluble vitamin and is member of vitamin B family (Blakley 1969) (Fig. 5.4)

It is an essential micronutrient in diet and is required for the healthy bodily function and metabolic process (Carmel 2005). Naturally, folate is present in very low concentration in food. Fortification can enrich the folic acid content by commercially adding the folic acid formulation in the dietary food (Cuskelly et al. 1996). Folic acid fortification is a process in which the stable formulation of folic acid is added to food so as to increase the blood folate levels. In USA, and other countries folic acid fortification is made mandatary so as to reduce the deficiency disease develops due to inadequate intake of folate (Bailey et al. 2010a, b). The Recommended dietary allowance for folic acid is age dependent like for infants it is 60 mcg/day, children 80–140 mcg/day, adults 200 mcg/day and for pregnant women 400 mcg/day (Subar et al. 1989).

Sources of Folic Acid Folate rich food includes leafy vegetables such as spinach, asparagus, lettuce, (Houlihan et al. 2011). Legumes such as dried fresh beans, Brussels sprouts peas and lentils, egg yolk, baker's yeast, fortified grain products, breakfast cereals fortified with folate, sunflower seeds (Table 5.5).
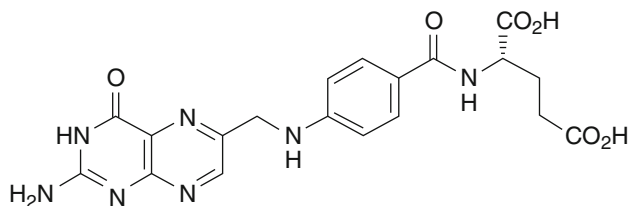
Fig. 5.4 Chemical structure of folic acid (US National library of medicine, NIH)

Table 5.5 Recommended dietary allowances for folic acid [Food and nutrition board, institute of medicine (1998)]

Age	Recommended dietary allowance (dietary folate equivalent-DFE)	Upper intake level
0–6 months	65 mcg DFE	–
7–12 months	80 mcg DFE	–
1–3 years	150 mcg DFE	300 mcg
4–8 years	200 mcg DFE	400 mcg
9–13 years	300 mcg DFE	600 mcg
14–18 years	400 mcg DFE	800 mcg
19+years	400 mcg DFE	1000 mcg
Pregnant women	600 mcg DFE	1000 mcg

*mcg microgram
1 mcg DFE=1 mcg food folate, 1 mcg DFE=0.6 micrograms folic acid from fortified foods or dietary supplements consumed with foods, 1 mcg DFE=0.5 micrograms folic acid from dietary supplements taken on an empty stomach

Functions of Folic Acid Folic acid is important in DNA synthesis and replication process. It has a biochemical role in mediating the transfer of single carbon moieties and act as cofactors for biosynthesis of metabolites (Wagner 1995). Human cannot synthesize folate, therefore folate has to be supplied through the diet to meet their daily requirement as folic acid is essential for its various biological functions. In aids to this folate is also important during the period of frequent cell division and growth, in infancy and pregnancy. Adequate folate intake during pregnancy would help to protect against neural tube defect (Shaw et al. 1995). Folic acid consumption also found in reducing the risk of colorectal cancer (Sanjoaquin et al. 2005). Human needs folate for the production of red blood cells and white blood cells as it helps in maintenance and production of new cells.

Absorption Mechanism of Folic Acid In the diet, folates are present in the conjugated form of polyglutamates with variable number of glutamate residue (Bernstein et al. 1970) Polyglutamate is converted into monoglutamate by folate reductase in the intestine before absorption. Folic acid is more stable than the natural food folates; however it loses its activity during the processing of food. Natural food folate/folic acid is not biologically active, but is converted into dihydrofolate by dihydrofolate synthase in the liver and tetrahydrofolate by tetrahydrofolate reductase and become active for biological function (Crider et al. 2011).

Deficiency of Folic Acid Folic acid deficiency is rare and uncommon. Chronic and severe forms of folic acid deficiency lead to hemopoiesis and megaloblastic anemia characterized by large, abnormally, nucleated erythrocytes. Both vitamin B12 and folic acid were found to be associated with elevated blood homocysteine level, which was reported as a risk factor for cardiovascular diseases (Brattstrom 1996). Since folate deficiency limits cell division, erythropoiesis, production of red blood cells is hindered and leads to megaloblastic anemia. In addition, inadequate intake

of folic acid in pregnant women causes fetal neural tube defect and other birth defects (Crider et al. 2011) neural tube defect occurs when the neural tube fails to close early in embryonic stage. Folate deficiency may also lead to diarrhea, soreness and ulceration of tongue, depression. (Bailey and Gregory 2006). The deficiency in dietary folate have also been reported to increase the risk of Colorectal cancer (Kim 2004).

5.10   Minerals

Minerals are nutrient ions present in the soil and cannot be synthesized by living being, such as plants and animals, however it is absorb by plants from soil and aided to the food web Minerals are one of the most important constituent require for metabolism and all biochemical functioning of body (Underwood and Suttle 1999). Main dietary source of minerals includes fruits and vegetables. Minerals are also present in drinking water depending upon the geographical location.

Significance of Minerals   They are minute amounts of inorganic nutrient that are essential for bone and tooth formation. They also help in biological activity as enzyme action, muscle contraction, nerve reaction, and blood clotting. Minerals play an important role in regulation of intracellular and extracellular body fluid and Generation of energy from the food (Soetan et al. 2010). Minerals enriched food includes meat, cereals, fish, milk and dairy foods, vegetables, fruit (especially dried fruit) and nuts. Essential minerals include calcium and iron, although there are also many other types of minerals that are an important part of a healthy diet.

Minerals are broadly classified into macro-minerals and trace minerals. Body requires macro-minerals in large quantity than trace mineral. The macro-mineral group includes calcium, phosphorus, magnesium, sodium, potassium, chloride, and sulfur (Eruvbetine 2003). A trace mineral are those, which required by the body in small quantity. So even though your body needs trace minerals, it needs just a tiny bit of each one. Trace minerals includes iron, manganese, copper, iodine, zinc, cobalt, fluoride, and selenium.

Calcium is a mineral nutrient that plays an important role in bone health, muscle contraction, blood clotting, nerve conduction, enzyme regulation, (Wosje and Specker 2000) and required in substantial amounts, but many diets are deficient in calcium making supplementation necessary or desirable. Similarly, iron deficiency is one of the leading risk factors for death world- wide, affecting an estimated 2 billion people (Zimmermann and Hurrell 2007). 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. Knowing the benefits of mineral nutrients and the fact that there is a low intake via food, novel strategies have been aimed to supplement these nutrients to meet the dietary recommendations.

Calcium It is an essential nutrient for human metabolism. It is a major element in the human body. The key role of calcium is to maintain the health and strength of bones. It also has other important functions like muscle contraction, nerve induction, blood coagulation, membrane permeability (Berivan and Nuray 2005). The recommended dietary allowance of calcium can be met by the consumption of healthy calcium rich diet. In some individual particularly in, elderly people calcium supplements required to achieve their recommended dietary calcium intake (McCabe et al. 2004). Calcium fortified food and beverages are the other option for the people who are lactose intolerant and those who does not consume dairy products to meet their calcium levels by these means. People of different age groups require different level of calcium, it is especially important for children of growing age, older women, pregnant women (Heyman 2006). The recommended dietary allowance for calcium varies, for children it is 600–800 mg/day, adults 1000 mg/day, pregnant women 1200 mg/day (Bailey et al. 2010a, b). The deficiency leads to severe disorder like osteoporosis. Calcium plays a key role in wide range of biological function either in the free form or bound complexes. Approximately 99 % of the calcium is present in the skeleton system as calcium phosphate composition and the remaining one percent is present in soft tissue and blood.

Calcium Sources Calcium enriched food includes dairy based products such as milk, yoghurt, cottage cheese. These are the major contributor of calcium. Other natural calcium rich food sources are vegetables such as broccoli, cabbage, spinach but the bioavailability is poor. Fortified foods with good calcium content are Tofu, cereals, fruit juices. The two main forms of calcium precursors are calcium citrate and calcium carbonates, which are used as calcium supplements (Straub 2007). The bioavailability of calcium carbonate is high when consumed with food in comparison to calcium citrate. Other calcium supplements include gluconate, lactate, and phosphate. In fortified juices calcium malate is well absorbed (Andon et al. 1996) (Table 5.6).

Functions of Calcium Calcium is required for the development and maintenance of skeleton (Heaney 2002). Calcium helps in construction, formation of bone and teeth. Apart from these, calcium plays a crucial role in regulation of cardiovascular

Table 5.6 Recommended dietary allowance for calcium (Bailey et al. 2010a, b)

Age	Recommended dietary allowance (RDA)	Upper intake level (UL)
0–6 months	200 mg	1000 mg
7–12 months	260 mg	1500 mg
Children (1–3 years)	700 mg	2500 mg
Children (4–6 years)	1000 mg	2500 mg
Children (9–13 years)	1300 mg	3000 mg
Adolescents (10–18 years)	1300 mg	3000 mg
Adults (19–50 years)	1000 mg	2500 mg
Pregnant women	1300 mg	3000 mg

and neuromuscular function. All muscles use Ca^{2+} as their main regulatory and signaling molecule (Berchtold et al. 2000). It acts as an important co-factor in the blood clotting mechanism and it also help in wound healing. Calcium is an important component in the production of enzymes and hormones, which are essential for metabolic process. Calcium regulates normal functioning of kidney. Extracellular calcium is essential for maintaining the potential difference across the cell membrane. Calcium is also important in the functioning of parathyroid, thyroid gland and regulation of hormones (Brown 1991). Vitamin D3 (calcitriol) plays a pivotal role in calcium absorption.

Absorption Mechanism of Calcium   Parathyroid hormone and calcitonin are the two hormones, which regulates calcium metabolism cascade. The Parathormone is secreted by parathyroid gland; it increases the calcium concentration in blood whereas calcitonin secreted by thyroid gland decreases the calcium concentration in the blood. The fall in the calcium level increases the secretion of PTH from parathyroid gland. PTH acts directly on bone to stimulate resorption and release of Ca^{2+} into the extracellular space and acts on kidney to increase calcium reabsorption and phosphate excretion (Potts and Gardella 2011). It enhances the absorption of calcium in intestine by increasing the production of vitamin D by up regulating the enzymatic process of conversion of vitamin D2 to Vitamin D3 (Peacock 2010).

Calcium Deficiency   Calcium is very vital in strengthening of bones and proper functioning of body. Its deficiency diseases may lead to skeleton abnormalities, osteoporosis in adults and rickets in children. Apart from loss in bone density, muscle cramp, hypertension (McCarron, 1989) and impairment of nervous system are the symptoms of calcium deficiency. Major part of the calcium is deposited as bone mineral; its inadequate intake may lead to accelerated bone loss and osteoporosis (Parfitt et al. 1995). In mechanism of muscle contraction, calcium is essential and lack of which may results into painful muscle cramp. Deficiency of calcium also causes neuromuscular irritability as it maintains the ionic channels within the nervous system.

Iron   It is an essential micronutrient, which is responsible for various metabolic processes in human body. Iron is essential for many complex biological processes that occur at molecular level like transportation of oxygen to red blood cells and muscles (Anderson and Fitzgerald 2010). Iron is a major part of hemoglobin structure, which carries oxygen to the red blood cells and myoglobin, which carries oxygen to the muscles. Iron also act as important co-factor for various enzymes in the food iron is present in two form heme iron and non-heme iron. Almost two third of the body iron is present as hemoglobin as red blood cells. In addition, iron is involved in energy producing reaction in the body. Excess iron is stored in the body in the form of ferretin protein in the liver (Ponka et al. 1998). The rich source of iron includes meat, fish, dried beans, spinach, dried fruits, and spinach. Adequate iron intake is important, particularly in childhood and pregnancy, where the developing baby is dependent upon its mother's iron supplies. To meet the recommended iron

level adults need to absorb 1–1.5 mg of iron per day. The recommended dietary allowance for iron varies considerably based on the age, for children its 7–8 mg, adults 15–18 mg, pregnant women 27 mg per day (Institute of Medicine, Food and Nutrition Board 2001). Iron deficiency anemia occurs when there is inadequate amount of iron in erythrocytes due to blood loss, pregnancy, poor absorption. Iron absorption is inhibited by many factors and food sources. Phosvitin protein present in eggs binds to iron and prevents the body from absorbing from food. Daily consumption of calcium in large quantity reduces the iron absorption (Halberg et al. 1992) Oxalate present in tea, coffee, and cocoa impairs the absorption of iron.

Sources of Iron The food sources rich in iron occur in two form heme form and non-heme form. Heme iron is rich in food that contains natural hemoglobin such as in red meat, fish (Hurrell 1997). Vegetables such as lentils, beans contain iron in non-heme form. Iron in heme form has high bioavailability than those in non-heme form. Fortified iron based food contain non-heme form of iron (Miret et al. 2003) (Table 5.7).

Functions of Iron The main role of iron in the body is in the production of red blood cells, a process known as haematopoiesis. It combines with globin protein to form hemoglobin, five membered ring structures which is the pigment of erythrocytes (Webb 1992). Iron regulates the proper functioning of immune system and helps in maintenance of the physical growth of cells (Beard 2001). Iron act as a catalyst and regulates the oxidation and reduction reaction in the body.

Absorption Mechanism of Iron Iron metabolism is a set of chemical reactions in body, which helps in the maintaining of human homeostasis. The rate of dietary iron absorption in human body is a set of many interdependent factors and is a dynamic process (Herndon et al. 1957). The efficiency of dietary iron is dependent on food source, as Iron absorption is decreased with the food rich in oxalate, phytate, polyphenol as these binds with it and reduces its absorption. Absorption of iron takes place in duodenum of intestine (Anderson et al. 2009). The iron in ferrous state is in the form of heme iron and absorbed more efficiently than non-heme iron, which is in ferric state. The enzyme Duodenal cytochrome B present in the enterocytes cells

Table 5.7 Recommended dietary allowance for iron (Food and Nutrition Board, Institute of Medicine 2001a)

Age	Recommended dietary allowance (RDA)	Upper intake level (UL)
7–12 months	11 mg	40 mg
Children (1–3 years)	7 mg	40 mg
Children (4–8 years)	10 mg	40 mg
Children (9–13 years)	8 mg	40 mg
Adolescents (14–18 years)	11 mg	45 mg
Adults (19–50 years)	8 mg	45 mg
Pregnant women	27 mg	45 mg

of intestinal lining plays an essential role in the reduction of iron from its ferric to ferrous state. The non-heme iron binds to apoferritin and stored as ferretin protein (Dennis et al. 1999).

Iron Deficiency Iron deficiency anemia is one of the most severe and important nutritional deficiency, affecting all the age groups, especially pregnant women, infants, adolescent boys and girls Pollitt et al. (1976). Anemia is a blood disorder occurs when there is insufficient hemoglobin in erythrocytes. Inadequate intake of dietary iron will eventually results in the depletion of stored iron in the body and the person develops anemia. Apart from anemia iron deficiency also impairs the cognitive development in children (Seshadri and Gopaldas 1989). It affects the immune system mechanism (Beard 2001). Iron deficiency in pregnant women causes problems of hemorrhage, birth defects and both maternal and prenatal are affected. Iron deficiency occurs due to abnormal blood loss, bleeding can be caused due to ulcers, hemorrhoids, cancer. In a survey conducted by the National Institute of Nutrition it was reported that more than 70 % of the children in India are suffering from anemia. (Gomber et al. 1998). In infants, iron deficiency also results in poor brain development (Grantham McGregor and Ani 1999).

5.11 Silica as a Nutrient

In 1939, the Nobel Prize winner chemistry, Professor Adolf Butenant, proved that life cannot exist without Silica. Silica has been long suspected to be important in maintaining health in humans (Becker et al. 1983). Silica plays an important role in many body functions and has direct effect to mineral absorption. The average human body holds approximately seven grams of Silica, quantity more than important mineral such as iron. As we age, scientific measurements have shown that the human body retains less and less silica. Silica does not occur in sufficient amounts in a wide variety of foodstuffs due to food processing in modern food. Hence silica supplementation is essential. Silica is needed for carrying out ongoing metabolic work that is vital to life. Many studies that prove the favorable influence of vegetal silica on the development of animals have been under taken. It is essential to the development of the skeleton and mineralization. Silica's absence results in skeletal deformities. Average daily intakes of silicon have been suggested to range from about 20–50 mg/day (Pennington 1991). Ample evidence exists to indicate that silicon can be accepted as an essential nutrient for higher animals, including humans. Findings from animals indicate that silicon nurtures apparently affects macromolecules, such as glycosaminoglycan, collagen and elastin, and thus is needed for healthy bones, brains and blood vessels. Although more should be known about the physiologic functions and requirement for silica before doing so, it is seductive to speculate about specific disorders that can be augmented or caused by inadequate silicon nutrition; those that have been proposed are atherosclerosis, osteoarthritis and hypertension. Even if these speculations are not found to be true, because the silicon

content in human diets can be easily be lower than that inducing changes in animals especially those containing refined and animal product foods, and because the response of animals to silicon deprivation can be enhanced by stressors commonly found with humans, such as low dietary calcium, high dietary aluminum and low estrogen status, finding pathologic conditions caused by silicon deprivation would not be surprising. Thus, silicon probably should be considered a nutrient of concern for humans

5.12 Silica as a Carrier Molecule

Till date a lot of search has been done in encapsulating the vitamins or minerals on materials like chitosan (Britto et al. 2012) gelatin or polylactidecoglycolide (Ignjatovic et al. 2013). Silica has attracted considerable attention due to their ability to enhance solubility of poorly water-soluble compounds, as well as to achieve sustained release, leading to improved bioavailability (Kapoor et al. 2010; Zhang et al. 2010). The advantages of silica particles as a carrier molecule is that it provides large surface area, high pore volume, tunable pore size, and well defined pore structure, as well as mechanical and chemical stability and protection of the integrity of the molecular structure of the compound (Kilpelainen et al. 2009; Slowing et al. 2008; Salonen et al. 2005). Recently porous silica particles were developed for intracellular controlled drug/gene delivery (Vivero-Escoto et al. 2010; Hom et al. 2011) and targeted drug delivery to cancer cells (Mamaeva et al. 2011; Rosenholm et al. 2010). Moreover the safety of silica as delivery systems has been highlighted in many reports, which makes it desirable as perfect delivery system (Chervenkov et al. 2007). Studies have even found that oral administration of silicon dioxide did not result in significant changes in the biochemical parameters characterizing liver functions and gastric mucosal damage (Chervenkov et al. 2007; Tanaka et al. 2010).

Silica surface chemistry is very well studied, thus suitable surface modifications enables the loading of diverse nutrients and minerals, through the non-covalent electrostatic, hydrogen bonding and van der walls force of interaction. Characteristic property of silica having high porosity enables the nutrients to be loaded within the pores, canals or adsorbed on the surface. The advantage of silica particle is that it itself is an inherent nutrient which is essentially required for our body. Probably one of the most challenging tasks in the formulation and loading of micronutrients is to control the release rate while assuring protection of the active compound during processing, transport and storage. Generally, the release of an entrapped compound from a dense particle is slower than from a highly porous structure, which also inversely affects the stability of the active compound (Matalanis et al. 2011). In this report, silica is used as a carrier molecule for the loading of nutrients, increase the efficiency and stability of nutrients and study this formulation through sustained release profile in in-vitro gastro-intestinal conditions.

5.13   Silica as a Carrier for Delivery of Nutrients

Though nutrients are available in natural food sources but still there is a huge percentage of population suffering from their deficiency. A number of supplements are available in the market but these are mainly pharmaceutical supplements (Yuan et al. 2010). To increase the bioavailability of the vitamins in these supplements they are prepared by expensive formulations like micro encapsulations. There are many additives added to them to stabilize the molecules and enhance their delivery. These additives need not have any biological importance and the body needs to spend extra energy in their excretion. And people are usually reluctant to consume these tablets. Hence a need of a carrier molecule which serves the purpose of both carrier and nutrient was needed. The carrier would not only deliver the vitamins in a stable form but also show biological functions in the body, and its preparation should be cost effective.

The main concern though becomes providing the right nutrition at the affordable cost. We propose herein the use of nutrient loaded silica particles to provide the much needed nutrition for the society. The choice of the material comes from the facts that (i) Silicon in silica is inherently a nutrient that is essential for our bodies for bone and cartilage development (Jugdaohsingh 2007; Vasanthi et al. 2012) (ii) Nutrient loaded silica nanoparticles serve dual purpose: inherent nutrient as well as carrier –a novel way of providing quick nutrition in the form of food additives or health drinks, (iii) Highly porous food grade silica provides high surface area suitable to load large quantities of nutrients both water soluble or insoluble (Ciriminna et al. 2011). The concept of our work is centered on using these silica particles which by themselves act as inherent nutrient, as carriers of other nutrients such as vitamin A, D3 and E and calcium, iron, vitamin B9 (folic acid) –a novel way of providing quick nutrition in the form of food additives or health drinks.

5.14   Experimental Section

5.14.1   Loading of Fat Soluble Nutrients onto Silica

Fat soluble vitamins such as retinyl acetate (vitamin A), cholecalciferol (vitamin D_3) and α-tocopherol acetate (vitamin E) were obtained from sigma Aldrich. The commercial silica used was of food grade quality. It was amorphous in nature and did not contain any heavy metal. Other reagents used for various experiments & analysis were of analytical grade.

Ex-Situ **Loading of the Vitamin E**   6.39 g of vitamin E was weighed and dissolved in 100 ml of absolute Ethanol. To this homogenous solution 10 g of commercial food grade Silica was weighed and added slowly with continuous stirring. The mixture was kept in rotatory shaker for overnight stirring at 120 rpm. The samples were

then centrifuged at 4000 rpm for 5 min and supernatant was collected for analysis. The solids which settled down were washed again with ethanol to remove any unbound vitamin E by centrifuging at 4000 rpm for 5 min. Again the supernatant was collected for analysis and the powder was dried in a desiccator for 24 h. Once the samples dried it was grinded and used for further analysis by particle size analyzer, Fourier transform infrared spectroscopy, UV-vis spectroscopy, thermo gravimetric analysis, BET surface area and high performance liquid chromatography. 0.1 mg of samples was taken and dissolved in absolute ethanol. This dispersed sample was used to measure the particle size and the zeta potential of the silica before and after loading of vitamin. IR spectra of the samples were recorded in the range of 400–4000 cm^{-1}. The UV-vis absorbance of the supernatant collected was measured using a UV-vis spectrometer and the amount of vitamin present was calculated by plotting against a suitable standard. High performance liquid chromatography in a mobile phase used for quantification of vitamin loaded onto silica particles. Quantification studies resulted in 20 % loading of vitamin E onto silica particles.

Ex-Situ **Loading of Vitamin A and D onto Silica Matrix**   2 g of vitamin A and 5 g of vitamin D were weighed separately and dissolved separately in 2 conical flasks containing 100 ml of absolute ethanol (100 %) each. To each of these homogenous solution 10 g of silica powder was added slowly with continuous stirring. The mixtures were kept in rotatory shaker for overnight stirring at 120 rpm. The samples were then centrifuged at 4000 rpm for 5 min and supernatant was collected separately for analysis. The powder which settled down was washed again with ethanol to remove any unbound vitamin A and D by centrifuging at 4000 rpm for 5 min. Again the supernatants were collected for analysis and the powders were dried at 40 °C under vacuum. Once the samples were dried they were grinded using a mortar and pestle, and were further characterized. Quantification studies resulted in 13–15 % loading of vitamin A and 7–8 % loading on vitamin D3 onto silica particles.2

5.14.2 In-Vitro *Release Studies of Fat Soluble Vitamins*

The *in-vitro* release profile of the vitamins A, D and E loaded onto silica were determined by gastric and intestinal release processes. Samples were subjected to simulated environments of the gastro-intestinal tract by preparation of simulated fluids, maintenance of pH and temperature and time estimation.

Release at Gastric Conditions   Accurately weighed 0.1 mg nutrient loaded silica was transferred to a beaker containing 15 ml of simulated gastric juice fluid. The simulated gastric fluid was prepared by dissolving 2 g of sodium chloride in 100 ml of distilled water. 7 ml of concentrated hydrochloric acid was added drop wise. 3.2 g of pepsin was added to the solution and the volume is made up to 1000 ml and

checked for the pH. The gastric fluid is acidic so the pH was maintained between 1 and 1.3. The test solution had a pH of about 1.3. Initially the mixture was mixed slightly and placed in a shaker incubator rotating at 50 rpm and maintained at 37 °C. The supernatant was removed at a time interval of 30 min for next 2 h. The supernatant was filtered through 0.45 μm Millipore membrane filter and then was analyzed for the amount of vitamin present by high performance liquid chromatography method.

Release at Intestinal Conditions For intestinal release study the pH of the solution obtained after gastric digestion was adjusted to neutral pH by addition of simulated intestinal fluids and the above gastric juice treated sample was added to it after filtration through 0.45 μm Millipore membrane. The intestinal simulated fluid was prepared by dissolving 6.8 g of monobasic potassium phosphate in 250 ml of water, mix properly and 77 ml of 0.2 N sodium hydroxide and 500 ml of water was added. 10 g of pancreatin was added. Mixture was mixed thoroughly and pH was adjusted to 6.8 with either 0.2 N sodium hydroxide or 0.2 N hydrochloric acid. The volume was made up to 1000 ml with distilled water. The bottles were again placed in shaker incubator rotating at 50 rpm and maintained at 37 °C. The supernatant was removed at a time interval of 30 min for next 2 h. The supernatant was filtered through 0.45 μm Millipore membrane filter and then was analyzed for the amount of vitamin present by high performance liquid chromatography method.

5.14.3   Loading of Water Soluble Nutrients onto Silica

Folic acid, calcium chloride, ferrous sulfate was obtained from Sigma Aldrich. The commercial silica used was of food grade quality. It was amorphous in nature and did not contain any heavy metal.

Ex-Situ **Loading of Folic Acid** 2 g of folic acid is dissolved in aqueous solution of 6 % sodium hydrogen carbonate and to this homogenous solution 10 g of food grade silica was added while stirring. The mixture was kept in rotatory shaker for overnight stirring at 120 rpm. The samples were then centrifuged at 4000 rpm for 5 min and supernatant was collected for analysis. The solids which settled down were dried under vacuum at 30 °C. Once the samples dried it was grinded and used for further analysis by Fourier Transfer Infrared Spectroscopy, UV-Spectroscopy, Thermo gravimetric analysis, BET surface area and high performance liquid chromatography 0.1 mg of samples was taken and dissolved in water. This dispersed sample was used to measure the zeta potential of the Silica before and after loading of the folic acid. IR spectra of the samples were recorded in the range of 400–4000 cm^{-1}. The UV-vis absorbance of the supernatant collected was measured using UV-vis spectrometer and the amount of folate present was calculated by plotting against a suitable standard. High performance liquid chromatography in a mobile

phase used for quantification of folates loaded onto silica particles. Quantification studies resulted in 19 % loading of folates onto silica particles.

In-Situ **Loading of Folic Acid** 2 g of folic acid was added to 100 ml of 14 % commercial food grade sodium silicate and stirred for 5 min. In 100 ml of 2.5 M of sulfuric acid, 2 % of gelatin was dissolved and stirred for 10 min. Thereafter, 54 ml of folic acid dissolved in sodium silicate solution is added drop wise to sulfuric acid-gelatin solution until neutralization (pH ~ 6.5–7). The samples obtained were centrifuged at 4000 rpm for 5 min and was dried under vacuum at 30 °C. Once the samples dried it was grinded and used for further analysis. Quantification studies resulted in ~18 % loading of folates onto silica particles. The samples were analyzed by Fourier transfer infrared spectroscopy, UV-vis spectroscopy, Thermo gravimetric analysis, BET surface area and high performance liquid chromatography.

Both in-situ and ex-situ loading methods were employed for the loading of nutrients. Water soluble folic acid was loaded on to the silica carrier molecule by both *in-situ* and *ex-situ* techniques. Iron and calcium were loaded using *in-situ* method. As loading efficiency was very poor using *ex-situ* method for minerals.

5.15 Loading of Calcium onto Silica

Calcium Chloride was used as precursor for the loading of calcium. Calcium chloride (20 g) was dissolved in 100 ml of 2.5 M Sulfuric acid and 2 % gelatin solution. 10 g of silica synthesized from sodium silicate was added to the solution and left for overnight stirring. The samples were centrifuged at 4000 rpm for 5 min and were dried under hot air oven. The dried samples were grinded into fine powder. The powder generated was analyzed by different characterization technique to ensure the loading efficiency.

5.16 Loading of Iron onto Silica

Method 1 Ferrous sulfate was used as precursor for the loading of iron. To 100 ml of 1.5 M of sulfuric acid 2 % gelatin was dissolved and stirred for 10 min. 20 g of ferrous sulfate was added to the above solution. Ascorbic acid was used as capping agent for iron to maintain it in its ferrous state. It was taken in a ratio of ferrous sulfate (1): ascorbic acid (0.5). The reaction mixture was left for stirring. Sodium Silicate solution was added drop wise and checked for neutralization (pH = 6.5–7). The samples were centrifuged at 4000 rpm for 5 min and were dried under hot air oven. The samples were dried and grinded into fine powder. The powder generated was analyzed by different characterization technique. 68 ml sodium silicate was consumed in reaction.

Method 2 100 ml of 0.5 M ortho-phosphoric acid (H_3PO_4) was taken in a 1 liter beaker and 20 g of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) was added slowly while stirring using magnetic stirrer. Added sodium silicate (7 % SiO_2 with pH value of ~13) to the above solution until pH 7 was achieved. The above solution was stirred for 1 h to facilitate precipitation. The precipitated solution was centrifuged at 4000 rpm for 5 min. The supernatant was separated and solid cake collected after centrifugation. The cake was washed twice with distilled water while centrifuging at 4000 rpm for 5 min each time, to remove any free uncoordinated iron. The pellet from the centrifuge was dried in an oven at 60 C for 12 h. Ground the dry powder obtained using a kitchen grinder. The silica-iron complex powder obtained was characterized by inductively coupled plasma (ICP-MS) method.

5.16.1   In-Vitro *Release Studies of Water Soluble Vitamins*

The *in-vitro* study was conducted to quantify the amount of folic acid, Iron and calcium leached out from the matrix in the fluid during the gastric and intestinal digestion. The recommended amount of folic acid, iron and calcium was added to the fluid. The release amount of folic acid was measured by high performance liquid chromatography (high performance liquid chromatography) and iron, calcium by inductively coupled plasma spectroscopy.

Gastric Digestion Gastric fluid was prepared to conduct the digestion process. 2 g of sodium chloride was added to 100 ml of water. 7 ml of concentrated hydrochloric acid was added drop wise. 3.2 g of Pepsin was added to the solution and the volume is made up to 1000 ml and checked for pH. The gastric fluid is acidic so the pH was maintained around 1–1.3. Pepsin was added prior to the acidification of the samples to pH2 to pH4. Acidification of the samples to pH2 or 4 is important, because pepsin begins to denature itself and thus will 1.lose its activity at pH ≥5.

Digestion Process The procedure used for in-vitro gastric digestion. Weighed amount of samples was added in 50 ml of gastric juices. The mixture was stirred for 2 min. The beaker was placed in a shaker incubator at 50 rpm. The temperature of shaker incubator was maintained at room temperature 37 °C. The reaction mixture was stirred continuously for 2 h and the supernatant was collected in the interval of half an hour and kept for analysis. The nutrient components present in the supernatant represent the soluble components were quantified by inductively coupled plasma spectroscopy and High performance liquid chromatography.

Intestinal Digestion Intestinal fluid was prepared to setup an experimental digestion process. 6.8 g of monobasic potassium phosphate was dissolved in 250 ml of water. To this 77 ml of 0.2 N sodium Hydroxide dissolved in 500 ml of water was mixed and stirred. 10 g of pancreatin was added and the volume was made up to 1000 ml with distil water. The pH was adjusted to 6.8–7 with 0.1 N sodium

hydroxide solutions. Before the start of the intestinal digestion, the samples were neutralized to pH5.5–6 prior to the addition of pancreatin which includes pancreatic enzymes such as pancreatic amylase, lipase, ribonuclease, and proteases such as trypsin and bile salts. The final pH is adjusted between 6.5 and 7.

Digestion Process Weighed amount of mineral were added to 50 ml of intestinal juices in a beaker. The mixture was stirred for 2 min. The beaker was placed in a shaker incubator which was set at 50 rpm. The temperature of shaker incubator was maintained at room temperature 37 °C. The reaction mixture was stirred continuously for 2 h and the supernatant was collected in the interval of half an hour and kept for analysis. The nutrient components present in the supernatant represent the soluble components and were analysed by inductively coupled plasma spectroscopy (ICP), high performance liquid chromatography (high performance liquid chromatography).

5.17 Results and Discussion

5.17.1 Characterization and Analysis of Fat Soluble Nutrients on Silica Matrix

Thermo gravimetric analysis of silica loaded with vitamin E is studied. As shown in Table 5.8 in control sample which consisted of only bare food grade silica sample 1 (CS-1) the weight loss was observed in one step from 30 °C–192 °C which is due to loss of water. For bare food grade silica sample 2 (CS-2) the weight losses were seen between 30 °C–172 °C which is also due to loss of water; as shown below in Fig. 5.5a, b. After loading of the vitamins onto the silica the weight loss was seen in three steps, addition to water loss two more steps of weight loss was noticed. For CS-1 loaded with vitamin E loss was seen in between 201.82 °C–362.73 °C and 362.73 °C–515.45 °C as shown in Fig. 5.6a. For CS-2 loaded with Vitamin E the weight loss was seen in between 202.67 °C–346.93 °C and 346.93 °C–451.42 °C as shown in Fig. 5.6b.

From the thermo gravimetric measurement data one can clearly see that after loading of vitamin E onto silica matrix there is a significant weight loss which was not seen in bare silica, and the weight loss is around the temperature corresponding to the boiling point of vitamin E i.e. 201 °C. Hence the percentage loss in weight can be due to the loss of vitamin E from the silica matrix, as silica itself is very stable at high temperature. Hence from the TGA analysis it was found that loading percentage of vitamin E on CS-1 was 19.937 % and on CS-2 was 18.994 %. TGA could not be conducted for vitamin A and vitamin D due to its low melting point.

UV-Visible (UV–vis) Spectroscopy Once vitamins are loaded onto silica matrix, it is washed as per procedure explained in the experimental section. The absorbance of the supernatant obtained after centrifugation and after washing was taken.

Table 5.8 Thermo gravimetric analysis

Sample code	Temperature (°C)	Percentage loss (%)	Percentage remaining (%)
Bare food grade commercial silica (CS-1)	30.00	–	99.791
	30–192.66	6.506	93.285
	217.63–573.68	1.607	91.582
Bare food grade commercial silica (CS-2)	30.00	–	99.637
	30–172.76	6.373	93.264
	316.21–713.26	2.259	90.124
	30.7–179.64	7.592	91.628
	274.22–725.78	3.753	86.644
Vitamin E loaded on CS-1 Silica (ex-situ)	30.2	–	98.611
	30.2–149.09	3.007	95.604
	201.82–362.73	15.296	79.876
	362.73–515.45	4.641	75.235
Vitamin E loaded on CS-2 Silica (ex-situ)	30.00	–	100.026
	30–135.17	2.892	97.133
	202.67–346.93	13.604	82.818
	346.93–451.42	4.188	78.63
	561.47–742.71	1.202	75.162
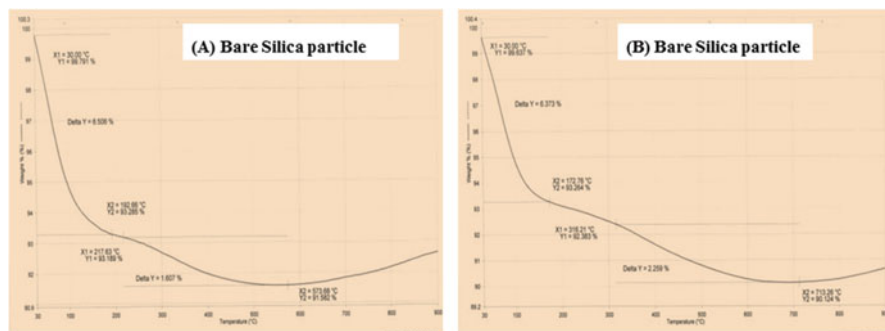
Fig. 5.5 (**a**) Thermo gravimetric measurement of commercial food grade silica particles (CS-1); (**b**) Thermo gravimetric measurement of commercial food grade silica particles (CS-2)

Vitamin A showed absorbance at 325 nm, vitamin D at 264 nm and vitamin E at 285 nm. The absorbance was quantified against a standard. The absorbance and standards of the vitamins are shown below in Figs. 5.7, 5.8, and 5.9. From the UV-vis analysis the percent of vitamins in the solution was calculated, and from that the percent remaining in the powder was estimated. Table 5.9 lists the percent of vitamins loaded onto silica matrix.

High Performance Liquid Chromatography The high performance liquid chromatography analysis of the vitamins loaded onto silica powder was carried out by extracting the vitamins from the powder by standard extraction technique.
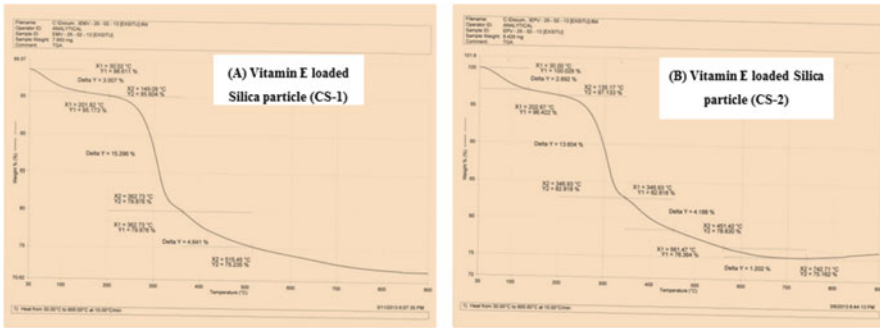
Fig. 5.6 (**a**) Thermo gravimetric measurement of silica particles (CS-1) loaded with vitamin E; (**b**) Thermo gravimetric measurement of silica particles (CS-2) loaded with vitamin E
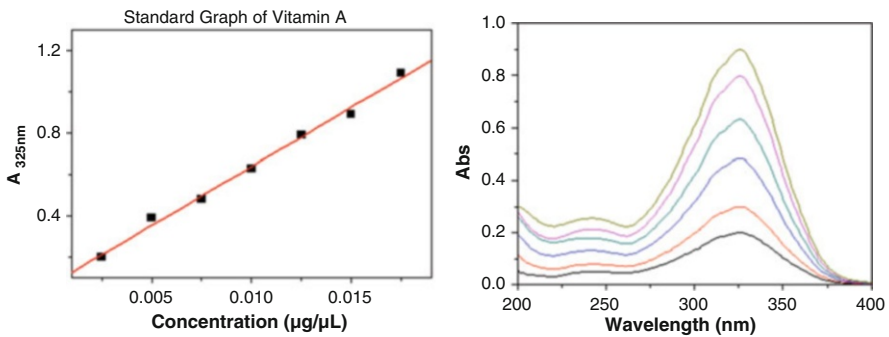
Fig. 5.7 The absorbance of the supernatant and washed solution of vitamin A was quantified using a standard plotted absorbance against concentration as shown above
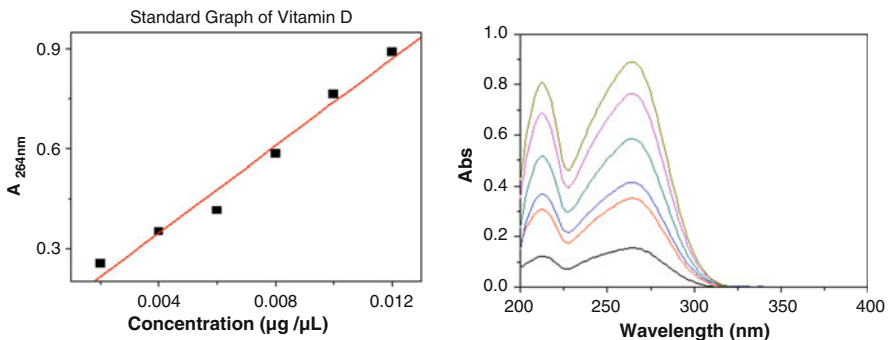
Fig. 5.8 The absorbance of the supernatant and washed solution of vitamin D was quantified using a standard plotted absorbance against concentration as shown above
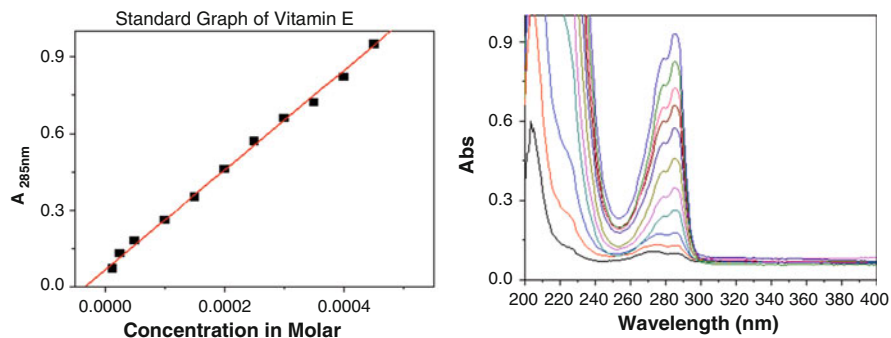
Fig. 5.9 The absorbance of the supernatant and washed solution of Vitamin E was quantified using a standard plotted absorbance against concentration as shown above

Table 5.9 Estimation of vitamins loaded onto silica matrix through UV–vis spectroscopy analysis

Sample	Silica	Loaded percent (%)
Vitamin A	CS-1	13.7
	CS-2	15.2
Vitamin D	CS-1	8.3
	CS-2	7.2
Vitamin E	CS-1	21.38
	CS-2	20.49

Table 5.10 Loading percentage estimated by high performance liquid chromatography (HPLC)

Samples		Percentage estimated by HPLC method (%)
Vitamin A	CS-1	9.87
	CS-2	8.78
Vitamin D	CS-1	6.33
	CS-2	5.21
Vitamin E	CS-1	17.79
	CS-2	15.27

Calibration curve was obtained analyzing five times each, five different solutions of known concentrations of analytes which included 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm of concentration. The curve equation $y = bx + m$ calculated with linear regression method was used to determine samples concentration. Results are reported in Table 5.10.

Fourier Transform Infrared Spectroscopy (FTIR) In order to understand the characteristic of vitamins loaded onto silica, FTIR spectroscopy was used. FTIR spectroscopy will help to understand whether there is any interaction between the

loaded vitamin molecules with the silica matrix. The FTIR spectra of different vitamins loaded onto silica are shown below in the figures (Figs. 5.10, 5.11, 5.12, and 5.13).

5.17.2 Stability Studies of Fat Soluble Vitamins–Silica Complex

FTIR spectrum of the same sample was taken after an interval of time to check the stability of the samples. It was noticed that there was no change in peaks in the IR spectrum for any of the samples of silica loaded with vitamins. As shown in Figs. 5.14, 5.15, and 5.16, all the significant peaks of the vitamins observed in the loaded silica are retained by the silica even after ~2 months under storage at the room temperature.

5.18 Zeta Potential Measurements

The results of zeta potential for silica particles without and with different vitamins are shown in Table 5.11. Zeta potential was reported as the average and standard deviation of measurements, with five reading taken per sample. From the table below we can see that zeta potential of silica particles after vitamin loading clearly
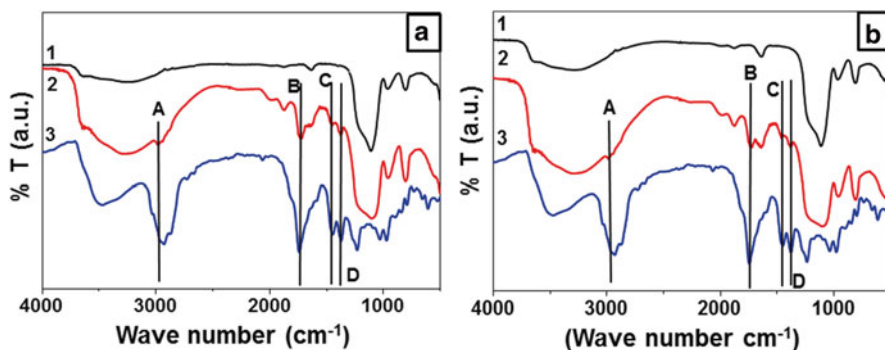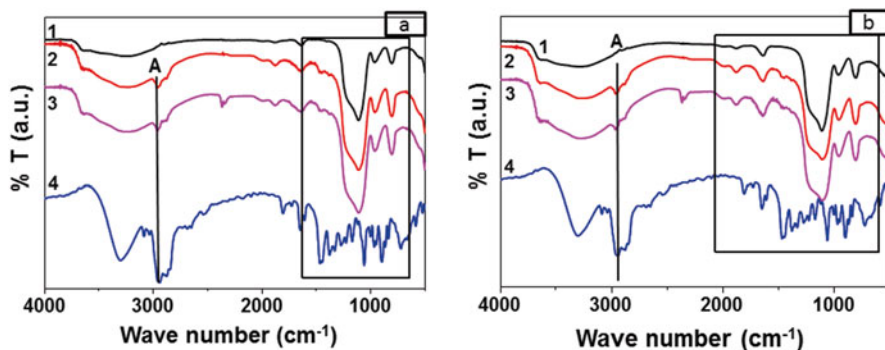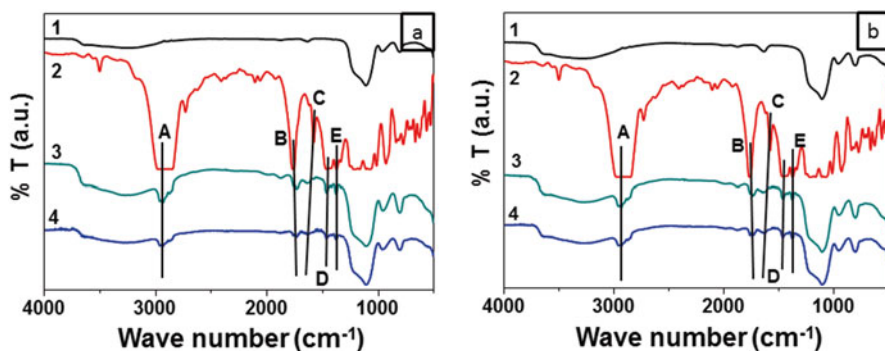

Fig. 5.10 (**a**): CS-1 loaded with vitamin A-acetate via *ex-situ* method. Here *curve 1* belongs to bare silica, *Curve 2* belongs to vitamin A loaded onto CS-1 and *curve 3* is for standard vitamin A. *A* CH_2/CH_3 asymmetrical stretching was seen at 2978 cm^{-1} which slows a slight shift which may be due to interaction between the CH/CH and the silica molecules. *B* C=O stretching was seen at 1735 cm^{-1}. *C* C-CH bending at 1439 cm^{-1}. D) OH bending at 1372 cm^{-1}
(**b**): CS-2 loaded with vitamin A-Acetate via ex-situ method. Here also *curve 1* belongs to bare silica, *curve 2* belongs to vitamin A loaded onto CS-2 and *curve 3* is for standard vitamin A. *A* CH2/CH3 asymmetrical stretching was seen at 2978 cm^{-1} which slows a slight shift which may be due to interaction between the CH2/CH3 and the silica molecules. *B* C=O stretching was seen at 1735 cm^{-1}. *C* C-CH bending at 1439 cm^{-1}. *D* OH bending at 1372 cm^{-1}
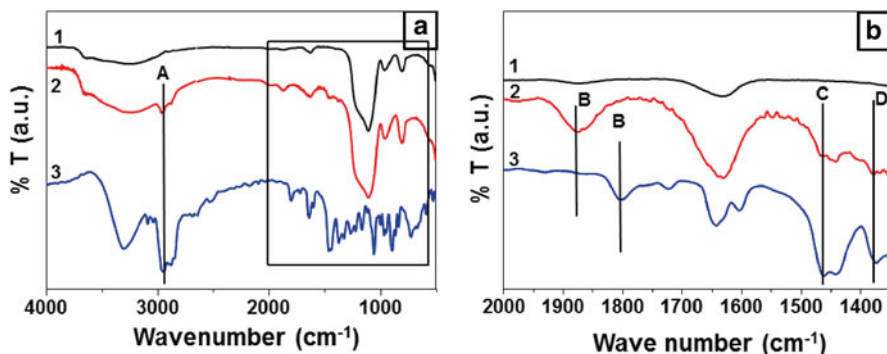
Fig. 5.11 (**a**): CS-1 loaded with vitamin D via *ex-situ* method. Here *curve 1* belongs to bare silica, *Curve 2* belongs to vitamin D loaded onto CS-1 and *curve 3* is for standard vitamin D. *A* CH₂/CH₃ asymmetrical stretching at 2948 cm⁻¹. Squared region expanded in Fig. 5.8b
(**b**): *B* C=C stretching shifts from 1800 to 1870 cm⁻¹; *C* CH bending at 1450 cm⁻¹. *D* OH bending at 1370 cm⁻¹
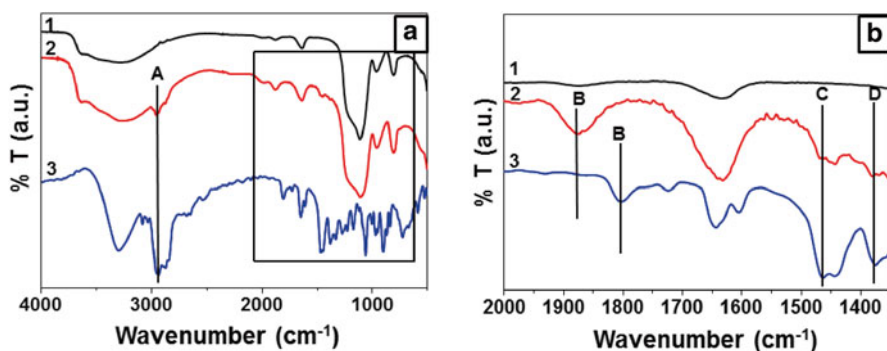
Fig. 5.12 (**a**): CS-2 loaded with vitamin D via *ex-situ* method. Here *curve 1* belongs to bare silica, *curve 2* belongs to Vitamin D loaded onto CS-1 and the *curve 3* is for standard vitamin D. *A* CH₂/CH₃ asymmetrical stretching at 2948 cm⁻¹. Squared region expanded in Fig. 5.9b
(**b**): C=C stretching shifts from 1800 cm⁻¹ to 1870 cm⁻¹. *C* CH bending at 1450 cm⁻¹. *D* OH bending at 1370 cm⁻¹

shows significance change. This change is clearly due to the attachment of the vitamins onto silica surface as all the vitamins itself bear a negative zeta potential.

5.18.1 Release Profile Studies of Fat Soluble Vitamins from Silica Matrix

Bioavailability is defined as the amount of an ingested nutrient that is absorbed and available for physiological functions, is dependent on digestion, release from the food matrix, absorption by intestinal cells, and transport to body cells. *In-vitro*
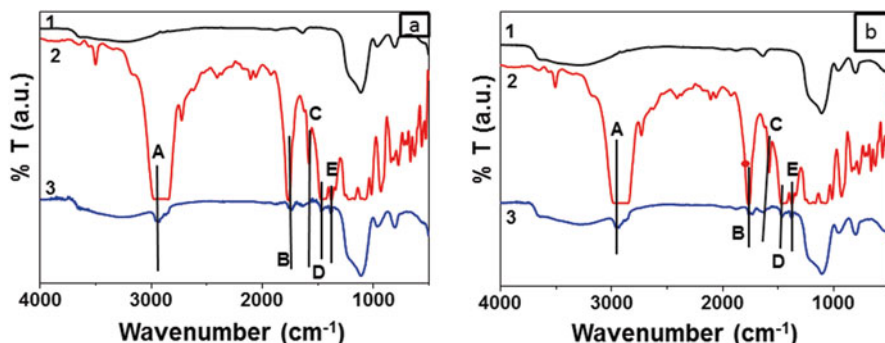
Fig. 5.13 (**a**) CS-1 loaded with vitamin E-Acetate via *ex-situ* method. Here *curve 1* belongs to bare silica, *curve 2* belongs to vitamin E loaded onto CS-1 and *curve 3* is for standard vitamin E. *A* CH$_2$/CH$_3$ asymmetrical stretching at 2946 cm^{-1}. *B* C=O stretching with a slight shift from 1763 cm^{-1} to 1735 cm^{-1}. *C* C-C=C stretching shifts from 1577 cm^{-1} to 1633 cm^{-1}. *D* Phenyl skeletal bending at 1465 cm^{-1}. *E* Methyl symmetrical bending at 1372 cm^{-1}
(**b**) CS-2 loaded with vitamin E-Acetate via *ex-situ* method. Here *curve 1* belongs to bare silica, *curve 2* belongs to vitamin E loaded onto CS-1 and *curve 3* is for standard vitamin E. *A* CH$_2$/CH$_3$ asymmetrical stretching at 2946 cm^{-1}. *B* C=O stretching with a slight shift from 1763 cm^{-1} to 1735 cm^{-1}. *C* C-C=C stretching shifts from 1577 cm^{-1} to 1633 cm^{-1}. *D* Phenyl skeletal bending at 1465 cm^{-1}. *E* Methyl symmetrical bending at 1372 cm^{-1}

Fig. 5.14 (**a**): CS-1 loaded with vitamin A-Acetate via *ex-situ* method. Here *curve 1* belongs to bare silica, *curve 2* belongs to vitamin A loaded onto CS-1 on Day 1, *curve 3* is vitamin A loaded onto CS-1 on 60th day and *curve 4* is for standard vitamin A. As compare to previous results no loss of peaks are observed
(**b**): CS-2 loaded with vitamin A-Acetate via ex-situ method. Here *curve 1* belongs to bare silica, *curve 2* belongs to vitamin A loaded onto CS-2 on Day 1, *curve 3* belongs to vitamin A loaded onto CS-2 on 60th day and *curve 4* is for standard vitamin A. As compare to previous results no loss of peaks are observed

Fig. 5.15 (**a**): CS-1 loaded with vitamin D via ex-situ method. Here *curve 1* belongs to bare silica, *curve 2* belongs to vitamin D loaded onto CS-1 on Day 1, *curve 3* belongs to vitamin D loaded onto CS-1 on 60th day and *curve 4* is for standard vitamin D. As compare to previous results no loss of peaks are observed

(**b**): CS-2 loaded with vitamin D via ex-situ method. Here *curve 1* belongs to bare silica, *curve 2* belongs to vitamin D loaded onto CS-2 on Day 1, *curve 3* belongs to vitamin D loaded onto CS-2 on 60th day and *curve 4* is for standard vitamin D. As compare to previous results no loss of peaks are observed

Fig. 5.16 (**a**): CS-1 loaded with vitamin E via ex-situ method. Here *curve 1* belongs to bare silica, *curve 2* belongs to vitamin E loaded onto CS-1 on Day 1, *curve 3* belongs to vitamin E loaded onto CS-1 on 60th day and *curve 4* is for standard vitamin E. As compare to previous results no loss of peaks are observed

(**b**): CS-2 loaded with vitamin E via ex-situ method. Here *curve 1* belongs to bare silica, *curve 2* belongs to vitamin E loaded onto CS-2 on Day 1, *curve 3* belongs to vitamin E loaded onto CS-2 on 60th day and *curve 4* is for standard vitamin E. As compare to previous results no loss of peaks are observed

bioaccessibility/bioavailability methods are useful to understand interactions between nutrients and food components. The effects of luminal factors such as pH and enzymes, nature of the matrix, processing protocols on micronutrient absorbability can be well understand by in-vitro bioavailability methods. *In vitro* methods are less expensive, faster, and offer better controls of experimental variables than

Table 5.11 Zeta potential of silica with and without vitamins

Sample	Zeta potential
CS-1	−40.2 ± 0.6
CS-2	−30.6 ± 0.7
Vitamin A	−7.36 ± 0.1
Vitamin A + CS-1	−23.2 ± 0.3
Vitamin A + CS-2	−25.1 ± 0.2
Vitamin D3	−12.5 ± 0.28
Vitamin D3+ CS-1	−4.32 ± 0.96
Vitamin D3 + CS-2	−8.44 ± 0.56
Vitamin E	–
Vitamin E + CS-1	−2.51 ± .036
Vitamin E + CS-2	−8.44 ± 0.98

human or animal studies (Sandberg 2005). However, *in-vitro* studies cannot be substituted for *in vivo* studies, and should be therefore regarded as a screening, or categorizing method. These are methods that can provide knowledge, when vast number of factors is considered that can affect nutrient absorption. The release of nutrient from matrix of the carrier molecule silica is conducted through in in-vitro studies. An *in-vitro* digestion includes the experimental setup of human digestive system through two-step digestion process that includes a gastric and intestinal digestion. The nutrients were first dissolved in gastric fluid for 2 h. The mixture was centrifuged and the pellet was added to the intestinal fluid.

A release profile of the vitamins D was studied under gastric and intestinal simulated conditions. The values obtained by high performance liquid chromatography analysis are plotted as time versus concentrations as shown in Fig. 5.17a, b.

From the graph one can clearly understand that the release of the vitamin occurs mostly in the intestinal fluids and not in the gastric fluids. A continuous release profile of the vitamins E was studied under gastric and intestinal simulated conditions. The values obtained by high performance liquid chromatography analysis are plotted as time versus concentrations as shown in Fig. 5.18a, b.

5.18.2   Discussion

Simple homogenization process of vitamins solutions stirred with silica was used for loading of the silica particles. The characterization of vitamin samples by various techniques showed that the vitamins are loaded onto silica particles. Though
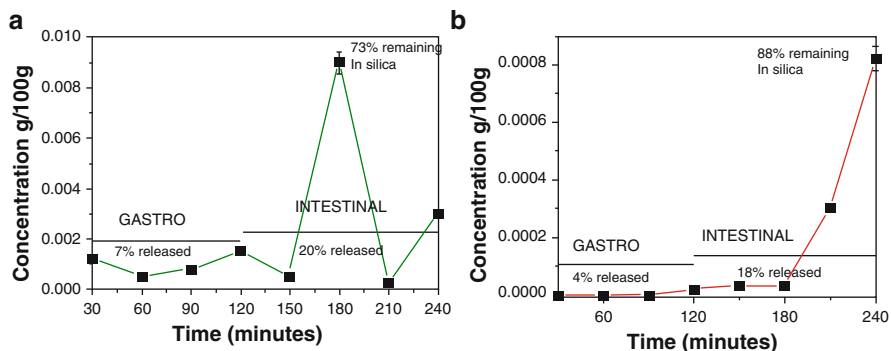
Fig. 5.17 (**a**) shows the release profile of vitamin D from CS-1; (**b**) shows the release profile of vitamin D from CS-2
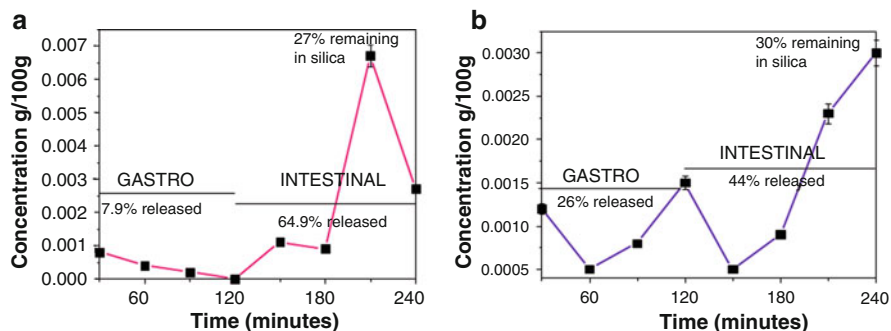
Fig. 5.18 (**a**) Shows the release profile of vitamin E from CS-1; (**b**) Shows the release profile of vitamin E from CS-2

structural features of the loaded silica does not show any visible change before and after loading, however FTIR and Zeta potential results show distinct changes. This clearly indicates the silica particles retain their structural functionality even after loading and there is surface interaction between the silica and vitamins. The stability studies of vitamins loaded silica matrix show that natural vitamins which are easily destroyed in natural environment due to light or oxidation, are found to be stable up to 60 days even after storage at room temperature. These samples were then subjected to various simulated environments to test for their release from silica matrix. The release profile studies were carried out in simulated gastric and intestinal environments. All the vitamins showed negligible release in the simulated gastric juice whereas considerable amount was released in the intestinal simulated fluids. This characteristic is an important feature as all the fat-soluble vitamins are absorbed in the intestinal region of the human gastro-intestinal tract. Bile forms a micelle and helps in the transport of these vitamins into the blood vessels via the villi of small intestinal region.

5.18.3 Analysis of Water Soluble Nutrients on Silica Matrix

Loading of nutrient on to silica was performed using both *in-situ* as well as *ex-situ* process. Calcium, folic acid loading was done by *ex-situ* method while iron loading was done using *in-situ* method. The loading percentage of calcium and iron was estimated by inductively coupled plasma spectroscopy (ICP). Folic acid loading percentage was determined by high performance liquid chromatography (Tables 5.12 and 5.13).

UV- Visible Spectrophotometry The supernatant obtained from silica/folic acid synthesis was analyzed by UV–vis spectrophotometry to determine the amount of folic acid loaded within the silica matrix. Folic acid showed absorbance at 280 nm. The absorbance was quantified against a standard. The absorbance and standard of the folic are shown below in Fig. 5.19. From the UV–vis analysis the percent of folic acid in the solution was calculated, and from that the percent remaining in the powder was estimated. The percentage loading result is shown in Table 5.14.

The graph of the linear relationship between folic acid concentration (mg/10 mL) and absorbance at 280 nm as obtained from the samples with known folic acid concentration is shown in the Fig. 5.19a and UV spectra of the solution with a known concentration of folic acid is in Fig. 5.19b.

Fourier Transformation Infrared Spectroscopy The structural characteristics of silica particles with folic acid loaded onto it and the qualitative composition of the sample is confirmed by IR spectroscopy. The IR spectra of folic acid loaded on CS-1 silica particles are presented in Fig. 5.20a. The IR spectra of iron and calcium loaded on silica are presented in Fig. 5.20b, c.

Besides the characteristic groups for silica, the spectra also shows characteristic groups for folic acid loaded on Silica (CS-1). The significant peaks of folic acid are found at 3545 cm^{-1} belongs to the hydroxyl (O-H) stretching, while the peaks at 3328 cm^{-1} are N-H stretching vibration. C-O bond stretching vibration of carboxyl group appears at 1695 cm^{-1}, while the band at 1644 cm^{-1} belongs to C-O bond

Table 5.12 Loading of iron and calcium percentage onto silica matrix as estimated by inductively coupled plasma spectroscopy

Sample	Loading percentage (%)
Iron loading on to food grade silica	21
Calcium loading on to food grade silica	10

Table 5.13 Loading folic acid percentage onto silica matrix as estimated by high performance liquid chromatography

Sample	Loading percentage (%)
Folic acid loading on to commercial food grade silica (CS 1)	10
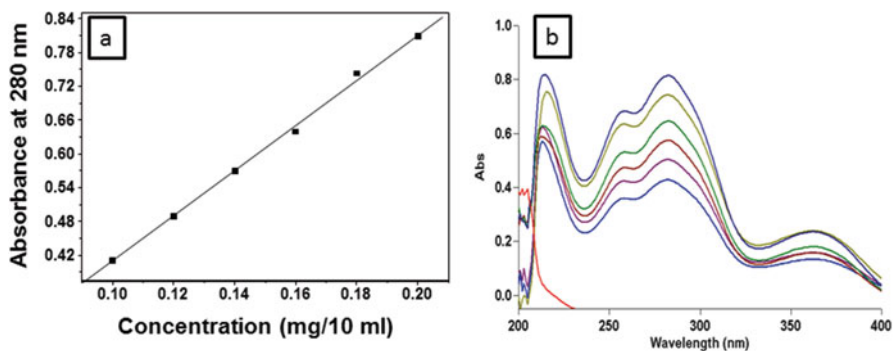
Fig. 5.19 (**a**) Graph of the linear relationship between absorbance and known folic acid concentration; (**b**) UV spectra of the solution with a known concentration of folic acid

Table 5.14 Folic acid loading percentage estimated by UV–vis spectrophotometer

Sample	Percentage loading (%)
Folic acid loading on to commercial food grade silica (CS 1)	10.6

stretching vibration of -CONH$_2$ group. The band at 1602 cm^{-1} relates to the bending mode of N H vibration and at 1482 cm^{-1} demonstrates the characteristic absorption band of phenyl ring. All these peaks are visible and shown in Fig. 5.20a.

In Fig. 5.20b, the band at 3545 cm^{-1} which belongs to the hydroxyl (O-H) stretching, 3328 cm^{-1} belongs to N-H stretching vibration peak, 1695 cm^{-1} belongs to C-O bond stretching vibration of carboxyl group, 1644 cm^{-1} belongs to C-O bond stretching vibration of -CONH$_2$ group and 1602 cm^{-1} band relates to the bending mode of N-H vibration. There is band shift of Si-O-Si asymmetrical bond at 1100 cm^{-1} in the (calcium loaded silica sample) and there is a loss of peak at 800 cm^{-1} belongs to Si-O-Si asymmetrical bond.

Iron does not have its characteristic peaks until it is not present in its complex form. The Fig. 5.20c shows that there is no major change in the significant peaks of silica and hence its stability is not affected by the minerals loaded onto it.

Zeta Potential Measurement The determinations of zeta potential for silica particles without and with loaded nutrient are shown in Table 5.15. Zeta potential was reported as the average and standard deviation of measurements, with five readings taken per sample. Zeta potential is an important property of the particle in dispersion as it has exerts a significant influence on their stability based on surface charge.

From Table 5.15 we can observe that there is fluctuation in the charge distribution of nutrient loaded silica when compared with their respective bare silica. Folic acid is negatively charged particles so it develops a net charge on the surface of silica. It affects the ion distribution and increases the charge of silica loaded with folic acid. Both iron and calcium are divalent cations and so it affects the charge

Fig. 5.20a (**a**) Spectra 1- FTIR spectra of commercial silica (CS-1); Spectra 2- FTIR spectra of folic acid loaded onto commercial Silica; Spectra 3- FTIR spectra of pure folic acid
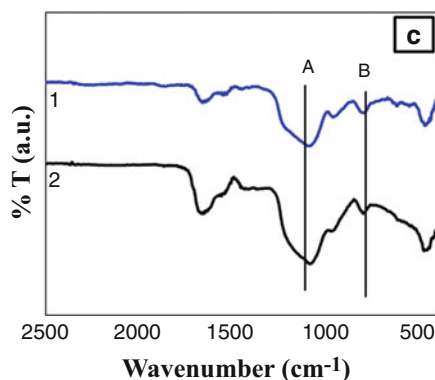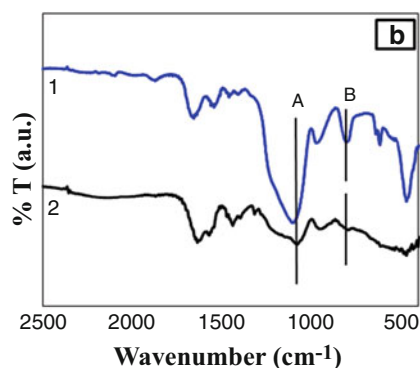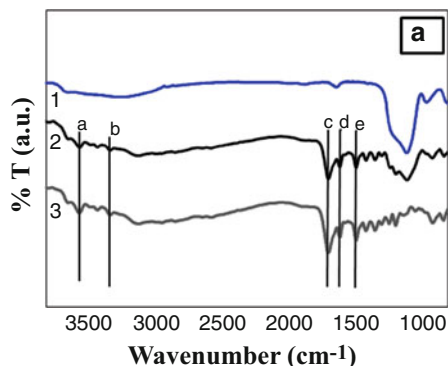
Fig. 5.20b–c (**b**) FTIR spectra of bare silica (*Curve 1*) and FTIR spectra of calcium loaded onto silica matrix (*Curve 2*). (**c**) FTIR spectra of bare silica (*Curve 1*) and FTIR spectra of iron loaded onto silica matrix (*Curve 2*)

Table 5.15 Zeta potential measurement of silica and silica loaded with folic acid, iron and calcium

Sample	Zeta potential
Silica (CS1)	(−) 38.8 ± 0.1
Folic acid + Silica	(−) 49.5 ± 0.1
Iron + Silica	(−) 24 ± 0.1
Calcium + Silica	(−) 14.9 ± 0.1

distribution of silica. The sample calcium loaded silica showing reduction in the charge as calcium itself is positively charge and stabilizes with negatively charged silica.

Energy-Dispersion Analysis of x-ray (EDAX) EDAX is a qualitative analysis technique for chemical composition of a sample. A chemical mapping of the nutrient loaded onto silica matrix was done using EDAX and the presence of element in each sample was analyzed (Figs. 5.21a–c, 5.21d–e, and 5.21f–g.
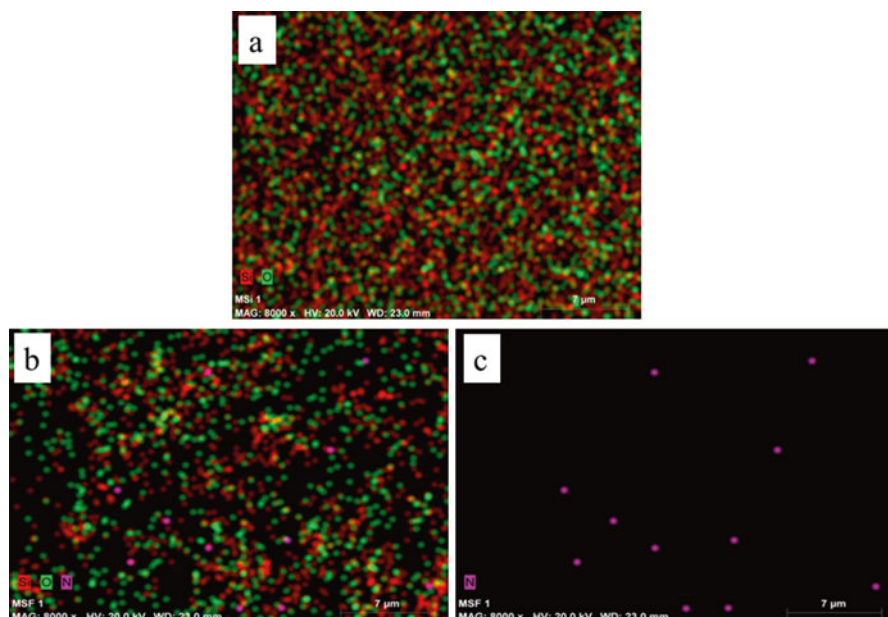
Fig. 5.21a–c (**a**) Chemical mapping (EDAX) of silica powder depicts presence of Si and O elements; (**b**) Chemical mapping of silica loaded with folic acid depicting presence of 3 elements such as Si, Oand N; (**c**) Chemical mapping of only nitrogen in the silica loaded folic acid sample (by masking Si and O elements)
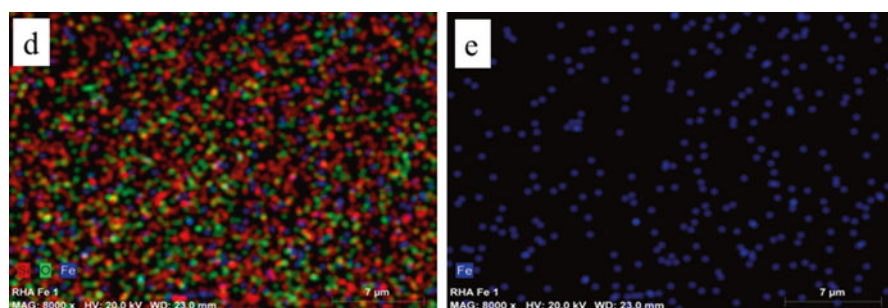
Fig. 5.21d–e (**d**) Chemical mapping (EDAX) of silica loaded with iron depicting presence of all 3 elements in the sample such as Si, O and Fe; (**e**) Chemical mapping of iron present in the iron loaded silica sample by masking Si and O in the image

From the micrographs (Fig. 5.21), it is clearly seen that the nutrient loaded samples shows the presence of individual signature elements such as N (nitrogen signal coming from amide group of folic acid), iron, and calcium when compared with the standard silica. The EDAX spectra recorded ~7 % percentage of nitrogen present in the folic acid loaded silica sample, ~21 % iron recorded in the iron loaded silica sample, and ~12 % calcium recorded calcium loaded silica sample.
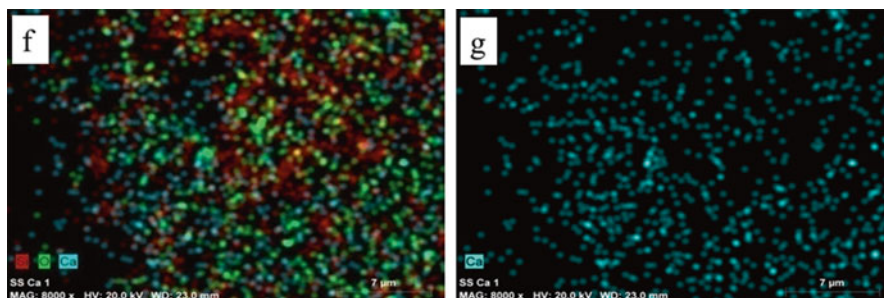
Fig. 5.21f–g (**f**) Chemical mapping (EDAX) of silica loaded with calcium depicting presence of Si, O and Ca elements. (**g**) Chemical mapping of calcium present in the calcium loaded silica sample by masking Si and O in the image

5.18.4 Release Profile Studies of Water Soluble Vitamins from Silica Matrix

The experimental study was conducted to quantify the release of loaded nutrients from the silica matrix. The protocol includes two steps process, gastric digestion and intestinal digestion. The sample is first dissolved in gastric fluid and then in intestinal fluid. After gastric digestion the sample were centrifuged and supernatant is removed and sent for analysis. The pellet obtained is then dissolved in intestinal fluid. The nutrients or compounds present in the supernatant represent the soluble components and are measured by inductively coupled plasma mass spectroscopy, high performance liquid chromatography. It is a kinetic study done over 4 h and is represented as graph in Fig. 5.22.

In Figs. 5.22, 5.23 and 5.24, it is observed that the release of nutrients from the silica CS-1 is constant till 2 h, after which a sudden increase in the release was observed with time interval. The first 2 h study was under in-vitro gastric condition between pH 1 and 2. The nutrient released in the gastric condition is negligible amount. However, in the next 2 h of study under *in-vitro* intestinal condition, the release of the micronutrient was increased. The release of nutrients in the intestinal region is more important that in the gastric region. The uptake of iron, folic acid and calcium, was of particular interest due to the way it is absorbed in the human body and processed through the intestines.

In Fig. 5.22, folic acid loaded on silica (CS-1) found to be constant in first half study then there is increase in release with time interval. The total input of the sample was 0.05 g which contains 4.8 mg of folic acid according to 9.6% loading. The total amount released 1.07 mg. The percentage released in the leaching study was found 22.29% which meets typical recommended dietary allowance values for folic acid.

Figure 5.23 shows the release profile of calcium from silica matrix. The release is seen to be constant in the first half study then there is increase in release with time. The total input of the sample was 1 g which contains 0.1 g of calcium accord-

**Fig. 5.22** Shows release profile of folic acid from silica matrix
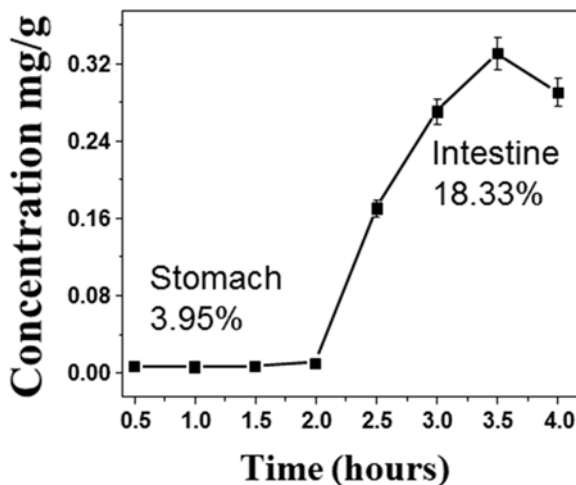
Fig. 5.23 Shows release profile of calcium from the silica matrix
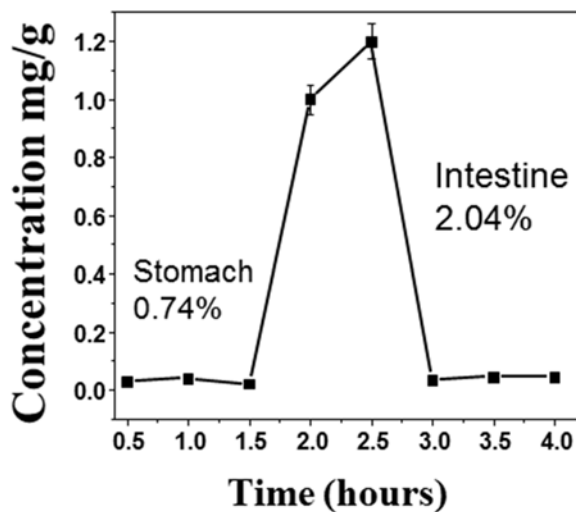
ing to 10 % loading. The total amount released 2.78 mg. The percentage released in the leaching study was found 2.78 % which is found to be lower than specified recommended dietary allowance limits for calcium. Theoretically, if 10 g of sample is added to meet the recommended dietary allowance for calcium i.e. 1 g, the release will be 27.8 mg.

Figure 5.24 shows release profile of iron from silica matrix. The release is seen to be constant in the first half study and we observed variation in the release with time. The total input of the sample was 0.1 g which contains 0.01 g of iron according to 10 % loading. The total amount released 5.1 mg. The percentage released in the leaching study was found 5.1 %.

Fig. 5.24 Shows release profile of iron from the silica matrix
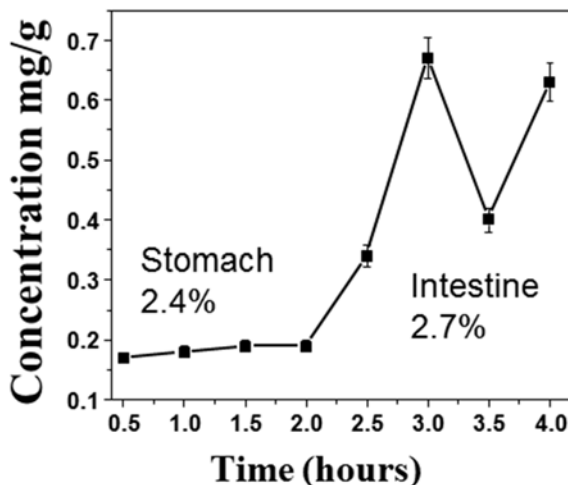
5.18.5   *Discussion –Water Soluble Vitamin Silica Complex*

The loading efficiency and percentage of folic acid was found to be increased when attempted with ex-situ process. Folic acid is very sensitive to light, so the samples were dried in room temperature in the dark. In this work the folic acid and silica ratio was taken as 2:10 and the percentage loading was analyzed to be 9.6 %. Calcium loading was carried out using ex-situ process. In most of the published data focuses on the use of calcium carbonate as a precursor or source for loading or encapsulation Han et al. (2007) because it is the most bioavailable form. In the present work three salts calcium carbonate, calcium chloride and calcium gluconate were attempted for the loading of calcium onto the silica matrix out of which calcium chloride shows the highest percentage loading i.e. 10 %. Though calcium chloride has low bioavailability, in the release profile study it was observed that the 2.78 % of calcium was released from the matrix. Iron loading was carried out by using both in-situ as well as ex-situ methods with and without surface modifications of silica matrix. In the ex-situ process the percentage loading was found to be very low, therefore in-situ process of loading iron onto silica was developed. Ascorbic acid was added as surface modifying agent so as to prevent the oxidation of iron from its ferrous form to ferric form. Ferrous sulfate was used as the precursors for iron, a 10 % loading of iron onto silica matrix was observed.

Silica due to its characteristic features like high porosity, negative surface charge, stability and biocompatibility forms a good substrate that can be used for carrier applications. Silica itself is a nutrient and believed to be required by body for maintenance of bone health. Two commercial food grade silica samples were used are amorphous in nature with surface area of 185 m²/g (CS-1) and 133 m²/g (CS-2). Based on the results presented above we have made an attempt to load both fat soluble and water soluble nutrients onto silica matrix and also demonstrated their

in-vitro release profile under simulated gastro-intestinal tract fluids. The future aspect of the work would include the bioavailability study of these loaded silica particles and its application as food fortifications.

5.19   Conclusion

An attempt was made for the development of a nutrition loaded silica additive where the silica matrix provides chemical and physical stabilization of the entrapped/coated nutrients. Simple methods were employed for loading of nutritional molecules onto silica matrix. Detailed analysis of the nutrient loaded silica particles show stability of nutrient even after storage under ambient conditions for many weeks. The release of nutrients from silica particles in simulated gut-intestine fluids was studied. The idea of combining the inherent nutrition properties of silica with its ability to load essential nutrients could wide open the possibilities of potential use in food as an additive or in the form of a nutritional drink.

References

Anderson GJ, Fitzgerald C (2010) Iron: an essential nutrient. Colorado State University Extension. A division of the office of Engagement No. 9:356

Anderson GJ, Frazera DM, McLarenb GD (2009) Iron absorption and metabolism. Curr Opin Gastroenterol 25(2):129–135. doi:10.1097/MOG.0b013e32831ef1f7

Andon MB, Peacock M, Kanerva RL, De Castro JAS (1996) Calcium absorption from apple and orange juice fortified with calcium citrate malate. J Am Coll Nutr 15:313–316

Anonymous (2008) The Copenhagen consensus 2008-results. The Copenhagen Consensus cent, Frederiksberg, pp 1–6. Available at http://www.copenhagenconsensus.com/Home.aspx, Accessed 24 Apr 2015

Atkinson J, Epand RF, Epand RM (2008) Tocopherols and tocotrienols in membranes: a critical review. Free Radic Biol Med 44(5):739–764. doi:10.1016/j.freeradbiomed.2007.11.010

Aykroyd WR (1930) Functional night-blindness due to vitamin deficiency. Lancet 1:824

Azzi A (2007) Molecular mechanism of alpha-tocopherol action. Free Radic Biol Med 43(1):16–21. doi:10.1016/j.freeradbiomed.2007.03.013

Bailey LB, Gregory JFR (2006) Folate. In: Bowman B, Russell R (eds) Present knowledge in nutrition, vol 1. International Life Sciences Institute, Washington, pp 278–301

Bailey RL, Dodd KW, Gahche JJ et al (2010a) Total folate and folic acid intake from foods and dietary supplements in the United States. Am J Clin Nutr 91(1):231–237. doi:10.3945/ajcn.2009.28427

Bailey RL, Dodd KW, Goldman JA, Gahche JJ, Dwyer JT, Moshfegh AJ, Sempos CT, Picciano MF (2010b) Estimation of total usual calcium and vitamin D intakes in the United States. J Nutr 140(4):817–822. doi:10.3945/jn.109.118539

Beard JL (2001) Iron biology in immune function: muscle metabolism and neuronal functioning. J Nutr 131(2S-2):568S–580S

Becker CH, Matthias D, Wossmann H, Schwartz A, Engler E (1983) Investigations on a possible medical importance of silicon. In: Anke M, Baumann W, Briunlich H, Bruckner C (eds) Spurenelement-symposium, 4th edn. Friedrich-Schiller-Universitat, Jena, pp 142–148

Bell EF (1987) History of vitamin E in infant nutrition. Am J Clin Nutr 46:183–186

Bender DA (2003) Nutritional biochemistry of the vitamins. Cambridge University Press, Cambridge. ISBN 978-0-521-80388-5

Berchtold MW, Brinkmeier H, Muntener M (2000) Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Am Physiol Soc 80(3):1215–1265. PMID: 10893434

Berivan T, Nuray N (2005) Importance of calcium. Turk J Med Sci 35:197–201

Bernstein LH, Gutsein S, Weiner S, Efron G (1970) The absorption and malabsorption of folic acid and its polyglutamates. Am J Med 48(5):570–579. doi:10.1016/0002-9343(70)90006-9

Bischoff-Ferrari HA, Willett WC, Orav EJ, Oray EJ, Lips P, Meunier PJ, Lyons RA, Flicker L, Wark J, Jackson RD, Cauley JA, Meyer HE, Pfeifer M, Sanders KM, Stahelin HB, Theiler R, Dawson-Hughes B (2012) A pooled analysis of vitamin D dose requirements for fracture prevention. N Engl J Med 367(1):40–49. doi:10.1056/NEJMoa1109617

Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, Bjelakovic M, Gluud C (2011) Vitamin D supplementation for prevention of mortality in adults cochrane database of systematic reviews July. Cochrane Database Syst Rev. doi:10.1002/14651858.CD007470.pub2

Blakley RL (1969) The biochemistry of folic acid and related pteridines. North Holland research monographs. In: Newbergen H, Taton EL (eds) Frontiers of biology, vol 13. North Holland Publishing Company, Amsterdam, p 569

Brattstrom L (1996) Vitamins as homocysteine-lowering agents. J Nutr 126(4):1276S–1280S

Brigelius-Flohe B, Traber MG (1999) Vitamin E: function and metabolism. FASEB 13(10):1145–1155

Britto D, Moura MR, Aouada AF, Mattoso HCL, Assis OBG (2012) N, N, N-trimethyl chitson nanoparticles as a vitamin carrier system. Food Hydrocoll 27:487–493. doi:10.1016/j.foodhyd.2011.09.002

Brown EM (1991) Extracellular Ca2+ sensing, regulation of parathyroid cell function, and role of Ca2+ and other ions as extracellular (first) messengers. Am Physiol Soc 71(2):371–411. PMID: 2006218

Calvo MS, Whiting SJ, Barton CN (2005) Vitamin D intake: a global perspective of current status. J Nutr 135(2):310–316. PMID: 15671233

Carmel R (2005) Folic acid. In: Shils M, Shike M, Ross A, Caballero B, Cousins R (eds) Modern nutrition in health and disease. Lippincott Williams and Wilkins, Baltimore, pp 470–481

Chasteen ND, Harrison PM (1999) Mineralization in ferritin: an efficient means of iron storage. J Struct Biol 126(3):182–194. doi:10.1006/jsbi.1999.4118

Chervenkov T, Ivanova D, Galunska B, Gerova D, Yankova T (2007) Toxicity of polymeric nanoparticles with respect to their application as drug carriers. In: Simeonova P, Opopol N, Luster M (eds) Nanotechnology- toxicological issue and environmental safety. Springer, Dordrecht, pp 111–118

Chung M, Balk EM, Brendel M, Ip S, Lau J, Lee J, Lichtenstein A, Patel K, Raman G, Tatsioni A, Terasawa T, Trikalinos TA (2009) Vitamin D and calcium: a systematic review of health outcomes. Evid Rep/Technol Assess 183:1–420

Ciriminna R, Sciortino M, Alonzo G, Schrijver AD, Pagliaro M (2011) From molecules to systems: sol-gel microencapsulation in silica-based materials. Chem Rev 111:765–789

Cranney A, Horsley T, O'Donnell S, Weiler H, Puil L, Ooi D, Atkinson S, Ward L, Moher D, Hanley D, Fang M, Yazdi F, Garritty C, Sampson M, Barrowman N, Tsertsvadze A, Mamaladze V (2007) Effectiveness and safety of vitamin D in relation to bone health. Evidence report/technology assessment August (158):1–235

Cranney A, Weiler HA, O'Donnell S, Puil L (2008) Summary of evidence-based review on vitamin D efficacy and safety in relation to bone health. Am J Clin Nutr August 88(2):513S–519S

Crider KS, Bailey LB, Robert J, Berry L (2011) Folic acid food fortification—its history, effect, concerns, and future directions. Nutrients 3(3):370–384. doi:10.3390/nu3030370

Crissey SD, Ange KD, Jacobsen KL, Slifka KA, Bowen PE, Stacewicz-Sapuntzakis M, Langman CB, Sadler W, Kahn S (2003) Serum concentrations of lipids, vitamin D metabolites, retinol, retinyl esters, tocopherols and selected carotenoids in twelve captive wild felid species at four zoos. J Nutr 133(1):160–166

Cuskelly GJ, McNulty H, Scott JM (1996) Effect of increasing dietary folate on red-cell folate: implications for prevention of neural tube defects. Lancet 347(9002):657–659. doi:10.1016/S0140-6736(96)91205-2

Devaraj S, Hugou I, Jialal I (2001) Tocopherol decreases CD36 expression in human monocyte-derived macrophages. J Lipid Res 42:521–527

Emerson G, Folkers K (1951) Water soluble vitamins. Annu Rev Biochem 20:559–598. doi:10.1146/annurev.bi.20.070151.003015

Eruvbetine D (2003) Canine nutrition and health. A paper presented at the seminar organized by Kensington Pharmaceuticals Nig. Ltd., Lagos on August 21, 2003

Fennema O (2008) Fennema's food chemistry. CRC Press Taylor and Francis, Boca Raton, pp 454–455

Food and Agriculture Organization/World Health Organization (1967) Report of the Joint FAO/WHO Expert Group. Requirements of vitamin A, thiamine, riboflavin and niacin, WHO technical report series no. 362. WHO, Geneva

Food and Agriculture Organization/World Health Organization (2004) Vitamin and mineral requirements in human nutrition, 2nd edn, WHO, Geneva. Annex 2

Food and Nutrition Board, Institute of Medicine (1998) Dietary reference intakes: thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline. National Academy Press, Washington. doi:10.17226/6015

Food and Nutrition Board, Institute of Medicine (2001a) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. National Academy Press, Washington. doi:10.17226/10026

Food and Nutrition Board, Institute of Medicine (2001b) Dietary reference intakes. National Academy Press, Washington. doi:10.17226/10026

Fragakis AS (2003) The health professional's guide to popular dietary supplements. The American Dietetic Association, Chicago

Fukuwatari T, Shibata K (2008) Urinary water-soluble vitamins and their metabolic contecnts as nutritional markers for evaluating vitamin intakes in young Japanese women. J Nutr Sci Vitaminol 54(3):223–229. doi:10.3177/jnsv.54.223

Gagne A, Wei SQ, Fraser WD, Julien P (2008) Absorption, transport, and bioavailability of vitamin E and its role in pregnant women. J Obstet Gynaecol Can 31(3):210–217

Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R (2007) Applications of spray-drying in microencapsulation of food ingredients. Food Res Int 40:1107–1121. doi:10.1016/j.foodres.2007.07.004

Gomber S, Kumar S, Rusia U (1998) Prevalence and etiology of nutritional anemias in early childhood in an urban slum. Ind J Med Res 107:269–273. PMID: 9701895

Goswami R, Mishra SK, Kochupillai N (2008) Prevalence and potential significance of vitamin D deficiency in Asian Indians. Indian J Med Res 127:229–238. PMID: 18497436

Gouin S (2004) Microencapsulation: industrial appraisal of existing technologies and trends. Trends Food Sci Technol 15:330–347

Grantham McGregor SM, Ani CC (1999) The role of micronutrients in psychomotor and cognitive development. Br Med Bull 55(3):511–527. doi:10.1258/0007142991902583

Halberg L, Rossander- Hultein L, Brune M, Gleerup A (1992) Calcium and iron absorption: mechanism of action and nutritional importance. Eur J Clin Nutr 46(5):317–327. PMID: 1600930

Han MR, Kwon MC, Lee HY, Kim JC, Kim JD, Yoo SK, Sin IS, Kim SM (2007) pH-dependent release property of alginate beads containing calcium carbonate particles. J Microencapsul 24(8):787–796. doi:10.1080/02652040701657820

Heaney RP (2002) The importance of calcium intake for lifelong skeleton health. Calcif Tissue Int 70(2):70–73. doi:10.1007/s00223-001-0032-3

Herndon JF, Rice EG, Tucker RG, Van Loon EJ, Greenberg SM (1957) Iron absorption and metabolism. J Nutr 64(4):615–623. PMID: 13549994

Herrera E, Barbas C (2001) Vitamin E: action, metabolism and perspectives. J Physiol Biochem 57(2):43–56. PMID: 11579997

Heyman MB (2006) Lactose intolerance in infants, children, and adolescents. Am Acad Pediatr 118:1279–1286. doi:10.1542/peds.2006-1721

Holick MF (2006) High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc March 81(3):353–373. doi:10.4065/81.3.353

Holick MF (2007) Vitamin D deficiency. N Engl J Med 357(3):266–281. doi:10.1056/NEJMra070553

Holick MF, Biancuzzo RM, Chen TC, Klein EK, Young A, Bibuld D, Reitz R, Salameh W (2007) Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J Clin Endocrinol Metab 93(3):677–681. doi:10.1210/jc.2007-2308

Hom C, Lu J, Liong M et al (2011) Mesoporous silica nanoparticles facilitate delivery of siRNA to shutdown signaling pathway in mammalian cells. Small 6(11):1185–1190. doi:10.1002/smll.200901966

Hornick SB (1992) Factors affecting the nutritional quality of crops. Am J Altern Agric 7(1–2):63–68

Houghton LA, Vieth R (2006) The case against ergocalciferol (vitamin D2) as a vitamin supplement. Am J Clin Nutr 84(4):694–697

Houlihan A, Pyke M, Scheelings P, Graham G, Eaglesham G, Wong L, Puwastien P, Jongjitsin W (2011) Folate content of Asian vegetables, Rural Industries Research and Development Corporation, 10/16

Hurley WL, Doane RM (1989) Recent developments in the roles of vitamins and minerals in reproduction. J Dairy Sci 72(3):784–804. doi:10.3168/jds.S0022-0302(89)79170-0

Hurrell RF (1997) Preventing iron deficiency through food fortification. Nutr Rev 55(6):210–222. PMID: 9279057

Ignjatovic N, Uskokovic V, Ajdukovic Z, Uskokovic D (2013) Multifunctional hydroxyapatite and poly (D, L–lactide–co–glycolide) nanoparticles for the local delivery of cholecalciferol. Mater Sci Eng C 33(2):943–950. doi:10.1016/j.msec.2012.11.026

Institute of Medicine, Food and Nutrition Board (2001) Dietary reference intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc : a report of the panel on micronutrients. National Academy Press, Washington, DC

Jugdaohsingh R (2007) Silicon and bone health. J Nutr Health Aging 11(2):99–110. PMCID: PMC2658806

Kapoor MP, Vinu A, Fujii W et al (2010) Self-assembly of mesoporous silicas hollow microspheres via food grade emulsifiers for delivery system. Microporous Mesoporous Mater 128:187–193. doi:10.1016/j.micromeso.2009.08.019

Khandare AL, Harikumar R, Sivakumar B (2005) Severe bone deformities in young children from vitamin D deficiency in fluorosis in Bihar-India. Calcif Tissue Int 76:412–418. doi:10.1007/s00223-005-0233-2

Kilpelainen M, Riikonen J, Vlasova MA et al (2009) In vivo delivery of a peptide, ghrelin antagonist, with mesoporous silica nanoparticles. J Control Release 137(2):166–170. doi:10.1016/j.jconrel.2009.03.017

Kim YI (2004) Folate, colorectal carcinogenesis and DNA methylation: lessons from animal study. Environ Mol Mutagen 44(1):10–25. doi:10.1002/em.2002

Kowdley KV, Mason JB, Meydani SN, Cornwall S, Grand RJ (1992) Vitamin E deficiency and impaired cellular immunity related to intestinal fat malabsorption. Gastroenterology 102(6):2139–2142

Kreutler PA, Czajka-Narins DM (1987) Nutrition in perspective, 2nd edn. Prentice Hall, Englewood Cliffs

Kutsky RJ (1973) Vitamin B6.7n handbook of vitamins and hormones. Van Nostrand Reinhold Co, New York, pp 60–61

Lala VR, Vinodini R (1970) Absorption of β-carotene from green leafy vegetables in undernourished children. Am J Clin Nutr 23(1):110–113. PMID: 5412643

Leadership agenda (2010) Coalition for sustainable nutrition security for India. http://www.nutritioncoalition.in/publication.asp

Li Y, Wongsiriroj N, Blaner WS (2014) Evolving insight into vitamin a transport. Sight and Life 28(2):36–43

Lichtenstein AH, Russell RM (2005) Essential nutrients: food or supplements? Where should the emphasis be? JAMA 294(3):351–358. doi:10.1001/jama.294.3.351

Lieberman S, Bruning N (1990) The real vitamin and mineral book. New york, Avery Group, 3, ISBN 0-89529-769-8.

Mamaeva V, Rosenholm JM, Bate-Eya LT et al (2011) Mesoporous silica nanoparticles as drug delivery system for targeted inhibition of notch signaling in cancer. Mol Ther 19(8):1538–1546. doi:10.1038/mt.2011.105

Maqbool A, Stallings VA (2008) Update on fat-soluble vitamins in cystic fibrosis. Curr Opin Pulm Med 14(6):574–581. doi:10.1097/MCP.0b013e3283136787

Matalanis A, Jones OG, McClements DJ (2011) Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. Food Hydrocoll 25(8):1865–1880. doi:10.1016/j.foodhyd.2011.04.014

McCabe LD, Martin BR, McCabe GP, Johnston CC, Weaver CM, Peacock M (2004) Dairy intakes affect bone density in the elderly. Am J Clin Nutr 80(4):1066–1074. PMID: 15447921

McCarron DA (1995) Calcium metabolism in hypertension. Kieo J Med 44(4):105–114. doi:10.2302/kjm.44.105

McLaren DS (2006) Vitamin D deficiency disorders (VDDD): a global threat to health. Sight Life Mag 3:6–15

Miret S, Simpson RJ, McKie AT (2003) Physiology and molecular biology of dietary iron absorption. Annu Rev Nutr 23:283–301. doi:10.1146/annurev.nutr.23.011702.073139

Muller DP (2010) Vitamin E and neurological function: review. Mol Nutr Food Res 54(5):710–718. doi:10.1002/mnfr.200900460

Nasab MK, Davoudi SM, Hamedani MA, Eshagian M, EdalatiNasa M (2013) Metabolism and deficiency of retinol in ruminant. J Am Sci 9(7s):57–65. doi:10.7537/j.issn.1545-1003

National Nutrition Monitoring Bureau (NNMB) (2003) Prevalence of micronutrient deficiencies. Technical Report No 22, National Institute of Nutrition, ICMR, Hyderabad

National Nutrition Monitoring Bureau (NNMB) (2006) Diet and nutrition status of populations and prevalence of hypertension among adults in rural areas. Technical Report No 24, National Institute of Nutrition, ICMR, Hyderabad

Norman AW (2008) From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr August 88(2):491S–499S. PMID: 18689389

Olsan JA (1989) Provitamin A function of carotenoids: the conversion of beta-carotene into vitamin A. J Nutr 119(1):105–108. PMID:2643691

Otten JJ, Hellwig JP, Meyers LD (eds) (2001) Dietary reference intakes: the essential guide to nutrient requirements. Institute of the medicine of the national academies, The National Academies Press, Washington, DC

Packer L, Weber SU, Rimbach G (2001) Molecular aspects of α-tocotrienol antioxidant action and cell signaling. J Nutr 131(2):369S–373S. PMID: 11160563

Parfitt AM, Villanueva AR, Foldes J, Rao DS (1995) Relations between histologic indices of bone formation: implications for the pathogenesis of spinal osteoporosis. J Bone Miner Res 10(3):466–473. doi:10.1002/jbmr.5650100319

Peacock M (2010) Calcium metabolism in health and disease. Clin J Am Soc Nephrol 5:S23–S30. doi:10.2215/CJN.05910809

Pennington AT (1991) Silicon in foods and diets. Food Addit Contam 89(1):97–118. PMID: 2015936

Pereira SM, Begum A (1969) Studies in the prevention of vitamin A deficiency. Indian J Med Res 22(7):858–862, PMID: 5797053

Pittas AG, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, Lichtenstein AH, Lau J, Balk EM (2010) Vitamin D and cardiometabolic outcomes: a systematic review. Ann intern med, March 152(5):307–314. doi:10.1059/0003-4819-152-5-201003020-00009

Pollitt E, Rudolph MD, Leibel L (1976) Iron deficiency and behavior. J Pediatr 88(3):372–381. doi:10.1016/S0022-3476(76)80250-8

Ponka P, Beaumont C, Richardson DR (1998) Function and regulation of transferrin and ferritin. Euro Pubmed Cen 35(1):35–54. PMID:9460808

Potts JT, Gardella TJ (2011) Parathyroid hormone and calcium homeostasis. Pediatric bone, 2nd edn, Biology and diseases, science direct, pp 109–140. doi:10.1016/B978-0-12-382040-2.10006-1

Rao CN, Rao BS (1970) Absorption of dietary carotenes in human subjects. Am J Clin Nutr 23(1):105–109. PMID: 5412642

Rosenholm J, Sahlgren C, Linden M (2010) Cancer-cell targeting and cell-specific delivery by mesoporous silica nanoparticles. J Mater Chem 20:2707–2713. doi:10.1039/B920076B

Ross AC, Manson JE, Abrams SA (2011a) Report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know. J Clin Endocrinol Metab 96:53–58. doi:10.1210/jc.2010-2704

Ross AC, Taylor CL, Yaktine AL, Del VHB (2011b) Dietary reference intakes for calcium and vitamin D. National Academies Press, Washington

Salonen J, Laitinen L, Kaukonen AM et al (2005) Mesoporous silicon microparticles for oral drug delivery: loading and release of five model drugs. J Control Release 108(2–3):362–374. doi:10.1016/j.jconrel.2005.08.017

Sandberg AS (2005) Methods and options in vitro dialyzability; benefits and limitations. Int J Vitam Nutr Res 75(6):395–404. doi:10.1024/0300-9831.75.6.395

Sanjoaquin MA, Allen N, Couto E, Roddam A, Key TJ (2005) Folate intake and colorectal cancer risk: a meta-analytical approach. Int J Cancer 113(5):825–828. doi:10.1002/ijc.20648

Sathyanarayana Rao TS, Asha MR, Ramesh BN, Jagannatha Rao KS (2008) Understanding nutrition, depression and mental illnesses. Indian J Psychiatry 50(2):77–82

Seshadri S, Gopaldas T (1989) Impact of iron supplementation on cognitive functions in preschool and school-aged children. Am J Clin Nutr 50(3):675–686. PMID: 2773845

Shaw GM, Schaffer D, Velie EM, Morland K, Harris JA (1995) Periconceptional vitamin use, dietary folate, and the occurrence of neural tube defects. Epidemiology 6(3):219–226. PMID: 7619926

Sivakumar B, Panth M, Shatrugna V, Raman L (1997) Vitamin A requirements assessed by plasma response to supplementation during pregnancy. Int J Vitam Nutr Res 67(4):232–236. PMID:9285251

Sivakumar B, Nair KM, Sreeramulu D (2006) Effect of micronutrient supplement on health and nutrition status of school children: biochemical status. Nutrition 22(1):S15–S25. doi:10.1016/j.nut.2005.07.012

Slowing II, Vivero–Escoto JL, Wu CW, Lin VSY (2008) Mesoporous silica nanoparticles as controlled release drug delivery and gene tranfection carriers. Adv Drug Deliv Rev 60(11):1278–1288. doi:10.1016/j.addr.2008.03.012

Soetan KO, Olaiya CO, Oyewole OE (2010) The importance of mineral elements for humans, domestic animals and plants. Afr J Food Sci 4(5):200–222

Straub DA (2007) Calcium supplementation in clinical practice: a review of forms, doses, and indications. Nutr Clin Pract 22:286–296

Subar AF, Block G, James LD (1989) Folate intake and food sources in the US population. Am J Clin Nutr 50:508–516

Tanaka T, Godin B, Bhavane R et al (2010) In vivo evaluation of safety of nanoporous silicon carriers following single and multiple dose intravenous administration in mice. Int J Pharm 402(1–2):190–197. doi:10.1016/j.ijpharm.2010.09.015

Tang G, Qin J, Dolnikowski GG, Russell RM, Grusak MA (2005) Spinach or carrots can supply significant amounts of vitamin A as assessed by feeding with intrinsically deuterated vegetables. Am J Clin Nutr 82(4):821–828. PMID: 16210712

Tanumjhardjo SA (2011) Vitamin A: biomarkers of nutrition for development. Am J Clinl Nutr 94(2):658S–665S. doi:10.3945/ajcn.110.005777

U.S National Library of Medicine. National Institute of Health, The Brooklyn Hospital Center, Brooklyn, NY

Underwood EJ, Suttle NF (1999) Mineral nutrition of livestock, 4th edn. Moredun Research Institute, Cabi Publishing, Midlothian. doi:10.1079/9780851991283.0000

Vasanthi N, Saleena LM, Raj SA (2012) Silicon in day today life. World Appl Sci J 17(11):1425–1440

Vitamin E: fact sheet for health professionals (2009) National Institute of Health, office of dietary supplements, US department of health and services

Vivero-Escoto JL, Slowing II, Trewyn BG, Lin VSY (2010) Mesoporous slica nanoparticles for intracellular controlled drug delivery. Small 6(18):1952–1967. doi:10.1002/smll.200901789

Wagner C (1995) Biochemical role of folate in cellular metabolism. In: Bailey LB (ed) Folate in health and disease. Marcel Dekker, New York, pp 23–42

Wald G (1935) Caroteniod and the visual cycle. J Gen Physiol 19(2):351. doi:10.1085/jgp.19.2.351

Webb EC (1992) Anemia and physical work capacity. Enzyme Nomenclature Academic Press, San Diego

Whitney E, Sharon RR (2011) In: Peggy W (ed) Understanding normal and clinical Nutrition, 9th edn. Wadsworth, Cengage Learning, Belmont

Wolf G (2004) The discovery of vitamin D: the contribution of Adolf Windaus. J Nutr 134(6):1299–1302. PMID: 15173387

World Health Organization (1988) Calcium requirements, WHO technical report series no 230. WHO, Geneva

World Health Organization (1995–2005) Global prevalence of vitamin A deficiency in populations at risk, WHO global database on vitamin A deficiency, Geneva, WHO, 2009

Wosje K, Specker BL (2000) Role of calcium in bone health during childhood. Nutr Rev 58(9):253–268. doi:10.1111/j.1753-4887.2000.tb01879

Yuan H, Luo K, Lai Y, Pu Y, He B, Wang G, Wu Y, Gu Z (2010) A novel Poly(l-glutamic acid) dendrimer based drug delivery system with both pH-sensitive and targeting functions. Mol pharm 7(4):953–962. doi:10.1021/mp1000923

Zhang YN, Zhi ZZ, Jiang TY, Zhang JH, Wang ZY, Wang SL (2010) Spherical mesoporous silica nanoparticals for loading and release of the poorly water-soluble drug telmisartan. J Control Release 145(3):257–263. doi:10.1016/j.jconrel.2010.04.029

Zimmermann MB, Hurrell RF (2007) Nutritional iron deficiency. Lancet 370(9586):511–520. doi:10.1016/S0140-6736(07)61235-5