Nanofortification of vitamin B-complex in food matrix: Need, regulations, and prospects

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ABSTRACT

Micronutrient malnutrition (or hidden hunger) caused by vitamin B-complex deficiency is a significant concern in the growing population. Vitamin B-complex plays an essential role in many body functions. With the introduction of nanotechnology in the food industry, new and innovative techniques have started to develop, which holds a promising future to end malnutrition and help achieve United Nations Sustainable Developmental Goal-2 (UN SDG-2), named as zero hunger. This review highlights the need for nanofortification of vitamin B-complex in food matrix to address challenges faced by conventional fortification methods (bioavailability, controlled release, physicochemical stability, and shelf life). Further, different nanomaterials like organic, inorganic, carbon, and composites along with their applications, are discussed in detail. Among various nanomaterials, organic nanomaterials (lipid, polysaccharides, proteins, and biopolymers) were found best for fortifying vitamin B-complex in foods. Additionally, different regulatory aspects across the globe and prospects of this upcoming field are also highlighted in this review.

1. Introduction

Micronutrient malnutrition is widely known as hidden hunger caused due to the shortage of dietary micronutrients, including minerals and vitamins. It has a detrimental effect on human health as it has affected more than 2 billion people across the globe, and its high prevalence is majorly observed in Africa and South Asia. According to World Health Organization (WHO, 2019), more than 800 million people are undernourished, and more than 2 billion people are at risk of developing micronutrient malnutrition. Inadequate micronutrients mainly affect school-going children and women of reproductive age, resulting in stunted growth, generation of health problems, and severe birth-related defects. Vitamin B-complex is a micronutrient that comprises eight water-soluble vitamins, which form essential and closely interrelated roles. The complex involves vitamins B₁ (thiamine), B₂ (riboflavin), B₃ (niacin), B₅ (pantothenic acid), B₆ (pyridoxine), B₇ (biotin), B₈ (folate), and B₁₂ (cobalamin) (Bontu, Camacho, & Camacho, 2018; Xie et al., 2018). The main physiological processes regulated by these vitamins are the metabolism of carbohydrates, amino acids, fatty acids, and lipids and the synthesis of proteins, cholesterol, neurotransmitters, S-adenosyl methionine, and nucleotide bases (Romina Alina et al., 2019).

Moreover, it is known that vitamin B-complex deficiency leads to anemia, digestive issues, skin conditions, infections, peripheral neuropathy, and psychiatric disorders. The prevalence of deficiencies is differently present globally and discussed in Table 1.

Nanotechnological tools in fortification offer many benefits, including improved stability, shelf life extension, sustained release, organoleptic properties, and improved bioavailability due to better release profile kinetics. Various nanomaterials are used for fortification, including organic, inorganic, carbon, and composites, but among these, organic nanomaterials are used in large numbers because these are lipid-based nanomaterials (liposomes, solid lipid nanoparticles, nano-structured lipid complex, nanoemulsion, and cubosomes), biopolymeric nanomaterials, polysaccharides nanomaterials, and protein nanomaterials (Dima, Assadpour, Dima, & Jafari, 2020). Nanomaterials are becoming indispensable for fortifying food with vitamin B-complex to prevent and control micronutrient malnutrition in the growing population. Micronutrient deficiency poses a risk to health as well as to the global economy. Therefore, eliminating this disease has become of the utmost importance, and it also plays a vital role in fulfilling the United Nations (U.N.) designed sustainability goal number 2 (SDG-2), namely, Zero hunger.

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Many papers are on vitamin B-complex supplementation, fortification, biofortification, and post-harvest fortification (Tiozon, Fernie, & Sreenivasulu, 2021; Modupe & Diosady, 2021; Xie et al., 2021). But not much work on nanofortification of foods with vitamin B-complex is available; therefore, this review highlights the importance of nanofortification of vitamin B-complex, its journey, and the need for nanofortification methods, advanced nanotechnology tools, global regulations, and future trends. This work is one of its kind as it presents a comprehensive discussion on different nanomaterials used for vitamin B-complex fortification.

2. How does nanofortification come into play?

Codex Alimentarius, jointly led by Food and Agricultural Organization (FAO) and World Health Organization (WHO), defines the term ‘fortification’ as the process of purposely escalating the content of essential micronutrients, like, vitamins, minerals, amino acids, etc., to enhance the nutritional quality of food in a population (Cardoso, Fernandez, Gonzalez-Paramas, Barros, & Ferreira, 2019; Whiting, Kohrt, Warren, Kraenzlin, & Bonjour, 2016). It can be purely commercial, and sometimes it is a public health policy. This strategy can potentially benefit by reaching a large population (Dwyer, Wiemer, Dary, Keen, King, Miller, Philbert, Tarasuk, Taylor, Gaine, Jarvis, & Bailey, 2015). The fortification technique shifted to the nanofortification technique because the conventional techniques failed to provide satisfactory flavor profiles, good stability, and high bioavailability, resulting in elevated demand for nanofortification. Nanofortification is a technique that uses nanomaterials to encapsulate the nutrients because they have a small size that helps exhibit a high polydispersity index, high loading capacity,
and good encapsulation efficiency. The journey of nanofortification from fortification is shown in Fig. 1. Nanofortification programs are based on dietary recommendations, selecting food delivery vehicles, and choosing the target population to eradicate micronutrient malnutrition. The practical utilities of nanofortified food with various essential micronutrients, like vitamin B-complex, helps in improving human health by avoiding their deficiency in the body (Fig. 2). These deficiencies lead to the generation of diseases like the suppressed immune system, aging, cancer, anemia, depression, beriberi, diabetes, heart diseases, osteoporosis, osteomalacia, etc..

3. Need for nanofortification of food with vitamin-B complex

There are mainly four fortification methods of vitamin B-complex in foods: biofortification, microbial biofortification, commercial, and home fortification, but, among all these methods, commercial fortification is used in large quantities. However, these methods are insufficient to address the problem of vitamin B-complex fortification because vitamin B-complex comprises different vitamins, and the challenges faced by every vitamin are different. The challenges are categorized as the technological, sensory, safety, and cost of the type of fortificant to be used. One of the significant problems associated with adding thiamine is that it produces an unpleasant odor and easily breaks during heating. At the same time, riboflavin becomes unstable with increasing pH and is degraded by reduction. While riboflavin is heat stable during milk processing, it has been observed that when exposed to light, it loses approximately 20–80% of the riboflavin content within two hours. Moreover, many factors like light intensity, high temperature, and high surface area define the rate of loss of the contents. Furthermore, technical problems such as improving bioavailability, overcoming degradation, and improving organoleptic properties of certain fortificants are significant concerns that need to be resolved while directly adding vitamin B-complex in food products. However, these challenges could be addressed by using nanotechnology.

Nanofortification is an advanced method capable of handling the challenges of current fortification methods. It can mask the release of undesirable flavor, improve the stability of product, increase the bioavailability, and help improve the target population's micronutrient status. Before implementing any substance to the nanofortification program, its physical, chemical, and safety attributes should be examined and accepted by the respective agency. The general criteria for nanofortification are nutrient deficiency, regular consumption by the target population, food vehicle, non-toxicity by higher consumption, acceptable change in organoleptic properties, chemical stability, bioavailability, homogeneity, centrally controlled, and scalability. The cost of nanofortification should be affordable for the population, active participation among governmental organizations, academic institutions, research organizations, marketing specialists, and interested international organizations.

4. Advanced nanotechnology methods of fortification

The FAO report says that millions of people are undernourished globally (McGuire, 2015). Thus, it is essential to continue intensive work to overcome all food fortification challenges, and therefore, nanotechnology in food fortification is beneficial. One of the standard methods to provide vitamin B-complexes in the human body is nanoe encapsulation, as explained with the diagrammatic representation in Fig. 3. The nanoconjugated vitamin is added to the staple food in a defined amount. The human then consumes this nanofortified food, and the nanoparticle delivers the vitamin B-complex to the small intestine, where the blood can directly absorb it. However, designing and utilizing green-based nanosystems has been initiated rather than chemical approaches. Some nanomaterials used in the nanofortification process are discussed in this section.

4.1. Organic nanomaterials

Organic nanomaterials are most commonly used to fortify vitamin B-complex due to their simple design, range of biochemical properties, payload flexibility, and high bioavailability. They are classified as lipids, polysaccharides, proteins, and biopolymeric nanomaterials, as explained in Fig. 4. The section below discusses its detailed applications.

4.1.1. Lipid-based nanomaterials

Lipid-based nanomaterials are typically spherical platforms that consist of more than one lipid bilayer surrounding an internal aqueous compartment. They are generated using top-down (energy-intensive) techniques such as spray drying, extrusion, or bottom-up (low-energy) techniques such as self-assembly. They have shown improved stability, sustained delivery, bioavailability by reducing side-effects, shielding the entrapped vitamin from free radicals, metal ions, pH variations, and enzymes (Mendes et al., 2016).

4.1.1.1. Nanoliposomes. Nanoliposomes are biocompatible, easy to reduce, and stable, making them a suitable carrier agent for the fortification process. One of the remarkable works noted till now is the development of thiamine-loaded nanoliposomes prepared by high-speed homogenization utilizing phosphatidylcholine (Juveriya Fathima, Fathima, Abhishek, & Khanum, 2016). Further, another experiment was
performed in which nanoliposomes were synthesized ultrasonically with different hydrophobicity and were used as carriers to deliver cobalamin, tocopherol, and ergocalciferol. Characterization based on size, encapsulation efficiency, and stability was also performed. Moreover, small unilamellar vesicles (SUVs) and multilamellar vesicle (MLVs) are the types of liposome-based nanomaterials whose diameter ranges from 40 to 51 nm and 2.9–5.7 μm, respectively. It was observed that MLVs exhibited a higher encapsulation efficiency for all kinds of vitamins, making them stable carriers. On the other hand, SUVs showed an encapsulation efficiency of 56%, 76%, and 57% for cobalamin, α-tocopherol, and ergocalciferol, respectively. Additionally, it was also noted that high encapsulation efficiency could increase the hydrophobicity of the encapsulated vitamin (Bochicchio, Barba, Grassi, & Lamberti, 2016).

4.1.1.2. Lipid nanoparticles. Lipid nanoparticles are gaining interest as a novel and promising carrier system for micronutrients as SLNs and NLCs are the primary lipid nanoparticles (Katouzian, Faridi Esfanjani, Jafari, & Akhavan, 2017). For instance, interesting work was done for riboflavin encapsulation using fully hydrogenated canola oil, sodium lauryl sulfate, and polyethylene glycol (PEG) (stabilizer). Owing to the hydrophilic nature of lipid nanoparticles, they can readily absorb the vitamin, making them a good encapsulating agent, as encapsulation controls its release along with protection from light (Couto, Alvarez, & Temelli, 2017).

4.1.1.3. Nanoemulsion nanomaterials. Nanoemulsions are small droplets (from 10-few hundred nanometers), which are thermodynamic unstable but are kinetically stable in colloidal systems. Both high energy and low energy methods can be used to prepare them. They can be single, double, and pickering nanoemulsions. Whey protein concentrate and pectin have been explored for nanofortification of folic acid using the spray drying method, and researchers showed that these nanomaterials have immense potential for controlled delivery of micronutrients (Assadpour & Jafari, 2017). Similarly, another successful study was done with a whey protein complex with maltodextrin for nanofortification of folic acid (Assadpour, Maghsoudlou, Jafari, Ghorbani, & Aalami, 2016).

4.1.1.4. Cubosomes. Cubosomes are developed from the lipid cubic phase and are highly stable because of the polymer-based outer corona. An experiment consisted of two alternative carriers of cobalamin: phy-tantrol (PHYT) cubosomes and nano-engineered polymeric capsules. Both were incorporated with magnetic nanoparticles in the bilayer of cubic lipid nanoparticles and the shell of polymeric nanocapsules. These magnetic nanoparticles helped achieve the targeted drug delivery when an external magnetic field was applied. Moreover, the structure of the cubosome provides a high membrane surface area that can be used for loading small drug molecules and membrane proteins. This experiment showed that the cubosome-based drug delivery was biocompatible, feasible, and stable and can be used for the delivery of vitamins (Maiorova et al., 2019).

4.1.2. Protein nanomaterials

Food-grade proteins are adequate materials for preparing nanoparticles with good digestibility, low price, biocompatibility, and interaction with other nutrients. They are explored for the nanofortification of micronutrients, especially vitamin B-complex. For instance, zein nanoparticles of size 200 nm were developed efficiently to deliver the folic acid at the targeted site without getting damaged in the stomach’s atmosphere. The experiment reported that the release depended on the pH conditions, concluding that the conjugate was not affected under simulated gastric conditions (Kasai, 2018). Moreover, these zein nanoparticles can fortify various food products, but more studies are still needed to explore the potentialities of zein nanoparticles in this domain. A schematic representation of the overall experiment is illustrated in Fig. 5 (Penalva et al., 2015a). Another work utilizing soy protein nanoparticles for fortification improved the intestinal absorption of cobalamin (Zhang, Field, Vine, & Chen, 2014). Recent work showed that bovine serum albumin-nanoparticle (BSA-NPs) were used for folic acid and iron fortification in stirred functional yogurt. Coating BSA-NPs with amino acids (lysine) allows the positive/negative charge
of molecules to absorb electrostatically without the intervention of any other compounds (Darwish, Soliman, Elhendy, & El-Kholy, 2021). Similarly, casein nanoparticles of size 150 nm were also studied for nanofortification of folic acid. The coacervation process was used to synthesize them, and amino acids (lysine, arginine) helped stabilize them. Consequently, the oral bioavailability of folic acid was 50% higher than the traditional aqueous solution (Penalva et al., 2015b). In a study reported by (Madalena et al., 2016), β-lactoglobulin (β-Lgb) were suitable carriers for riboflavin delivery.

4.1.3. Polysaccharide nanomaterials

Polysaccharide nanomaterials are used in nanofortification, pickering emulsion stabilization, and material enforcing agent due to their environmentally friendly properties. They are alginates, chitin, chitosan, cellulose, dextran, starch, hyaluronic acid, pectin, and pullulan (Plucinski, Lyu, & Schmidt, 2021). A recent study (Jhan, Gani, Noor, & Ashraf, 2021) was done by fabricating starch nanoparticles from underutilized cereal grains for nanofortification of folic acid. They reported controlled release and were more resistant to in-vitro digestion. Recently, studies have started to focus on conjugation complexes of polymers with polysaccharide nanomaterials with improved properties.

4.1.4. Biopolymer-based nanomaterials

Biopolymeric nanomaterials are present in nanocapsules, nanosphere, nanomicelle, and nanogels. They can be made up of single or complex biopolymers and precisely control particle characteristics, flexible payload, and accessible surface modifications. Bottom-up approaches generally form their composites. One study conducted using thymol-loaded chitosan-based nano scaffolds for coating fish fillets showed the effectiveness of improving unstable vitamin B-complex stability (Ceylan, Yaman, Sądıç, Karabulut, & Yılmaz, 2018). Another study reported that alginate-pectin nanoparticles enhanced the regulated release of folic acid at gastrointestinal pH (Pamunuwu et al., 2020). In another exciting work, polyacetic co-glycolic acid (PLGA) was used for co-administrating folic acid and cobalamin, which showed improved bioaccessibility. This approach showed promising results as it can release the vitamin at a low pH, enhance the bioavailability, and stabilize it by protecting against specific conditions (Ramalho, Loureiro, & Pereira, 2021). Additionally, water-soluble β-cyclodextrin, the novel polymer, was effectively used to encapsulate riboflavin to enhance physicochemical characteristics. An experiment was performed in which complexes between water-soluble cationic poly(β-CD-co-guanidine) and riboflavin were synthesized through a co precipitation method. The experimental results exhibited that the solubility of riboflavin drastically increased in the complex form. Therefore, this finding provides ample room for the researchers to explore its utilities in various domains like food industries, pharmacology companies, drug development, etc. (Heydari, Doostan, Khoshoood, & Sheibani, 2016). Food-grade alginate/chitosan complex nanolaminates obtained using the layer-by-layer technique showed controlled release at pH 7 than pH 3, making them a good choice for nanofortification and novel functional food development (Acevedo-Fani, Soliva-Fortuny, & Martín-Belloso, 2018).

4.2. Inorganic nanomaterials

There are various types of inorganic nanomaterials like mesoporous silica/ amino-silicate composites, clays, calcium carbonates, calcium phosphate, and layered double hydroxides (LDH) that are promising candidates for fortification (Jampilek, Kos, & Kralova, 2019). Among these, mesoporous silica nanoparticles are explored for fortification purposes. A recent study based on gated mesoporous silica particles (TEM particle size of 862 nm) was used to fortify folic acid in apple and orange juices. Simulated digestion studies showed the release of encapsulated folic acid at the intestinal stage, longer shelf life over the free form, and better stability on exposure to UV-visible light (Ruiz-Rico et al., 2017). Simple powders and tablets of nanostructured hybrids exhibited enhanced release compared to crystalline folic acid (Pagan, Tiralti, & Perioli, 2016). Nanoclays (montmorillonite) are also explored for nanofortification purposes of cobalamin that exhibited better absorption (Akbari Alavijeh, Sarvi, & Ramazani Afarani, 2017).

4.3. Carbon-based nanomaterials

Carbon-based nanomaterials include carbon nanotubes, carbon nanoparticles, and carbon nano-dots that can also be used in the nanofortification process. However, very little work on these nanomaterials for fortification purposes is available; therefore, it gives a bigger room for researchers to explore their potentialities in vitamin fortification (Zawari, Aghaei, & Monajjemi, 2015).

4.4. Composite nanomaterials

Composites of proteins and lipids enhance delivery systems with desirable characteristics. For example, lipid-protein composite systems showed increased targeted delivery, sustained release, and reduced cytotoxicity (Dissanayake, Sun, Abbey, & Bandara, 2022). Food protein and lipid-based nanoparticles have allured a lot of interest for efficient delivery of micronutrients. A novel protein-lipid composite nanoparticle consisting of a three-layered structure (barley protein layer, α-tocopherol layer, and phospholipid layer) and an inner aqueous compartment was designed to load hydrophilic micronutrients. Its unique design helped to absorb hydrophilic micronutrients with high encapsulation efficiency. Therefore, this delivery system exhibited excellent efficiency in encapsulating cyanocobalamin (vitamin B12) and regulated the release profile in a simulated gastrointestinal environment. Overall, this novel oral protein-lipid composite nanoparticle showed remarkable capability, which can be used as the primary platform for delivering many different hydrophilic micronutrients (Liu et al., 2018). Another extended study based on succinylation of protein chain improved the nanoparticle stability, cellular uptake (increased 20 fold), mucosalhesive ability, and prevented leaking in the gastric environment due to increased surface charge. (Liu et al., 2019). A few examples of the recent

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**Table 2**

Examples of nanomaterials used for vitamin B complex fortification.

| Nanofortification technique | Wall material | Vitamin | Purpose | Ref. |
|-----------------------------|---------------|---------|---------|-----|
| Coacervation                 | Lactoferrin   | Folic acid | Fabricating a naturally occurring carrier. | (Chapeau et al., 2016) |
|                             | β-lactoglobulin | Folic acid (vitamin B9) | Potential carrier for development of novel functional foods | (Bandara et al., 2018) |
| Ultrasonication             | Chitosan      | Folic acid (vitamin B9) | Analyzing the encapsulation yield and stability. | (Pérez-Masiá et al., 2015) |
| Electrospraying and         | Zinc          | Folic acid (vitamin B9) | Using this process as functional healthier-fat food ingredients. | (Bou, Cofrades, & Jiménez-Colmenero, 2014) |
| Nano emulsification         | Whey protein concentrate (WPC). | Folic acid (vitamin B9) | Encapsulation. | (Azevedo, Bourbon, Vicente, & Cerqueira, 2014) |
| Ionotropic-gelation         | Alginate      | Vitamin B12 | | |
| Cold gelation               | Chitosan      | Vitamin B12 | | |
|                             | Soybean protein | Cobalamin | Improved intestinal transport. | (Zhang, Field, Vine, & Chen, 2015) |
nanofortification of vitamin B-complex are given in Table 2.

5. Regulatory guidelines across the globe

Whenever we talk about nanomaterials, their regulations are always a concern and should be a priority. Globally, no specific legislation is applied regarding the regulation of nanomaterials in the food fortification and medical sectors. Many countries still have no particular regulations for the risk assessment of nanomaterials (Nel & Malloy, 2017). Recently, the European Union (EU) has taken the lead and made improvements in the annexes for nanomaterials to provide more clarity to manufacturers, importers, and users (Clausen & Hansen, 2018). It has established regulations that any food ingredient derived from nanotechnology applications must undergo a safety assessment before its use (Bazana, Codevilla, & de Menezes, 2019). European Chemical Agency (ECHA), the competent authority for the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH), and the Classification, Labelling, and Packaging (CLP) Regulation legal acts contain several product-specific regulations, including food.

Furthermore, the REACH Regulation’s recent revision on chemical substances introduced nano-specific provisions (Miernicki, Hofmann, Eisenberger, von der Kammer, & Praetorius, 2019). Different organizations like Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the European Food Safety Agency (EFSA), and Organisation for Economic Cooperation and Development (OECD) are working in this direction in the EU (Scott-Fordsmand et al., 2021). Some guidelines have also been released by the U.S. Food and Drug Administration regarding nanotechnology in food. Other organizations such as International Standard Organization (ISO), WHO, and Scientific Committees are working in this direction in the USA (Amenta et al., 2015). Globally there is a need for proper food nanofortification with Vitamin B-complex. The overall process for implementing the food nanofortification initiative and the role of external factors are schematically presented in Fig. 6.

6. Conclusion and future directions

Vitamin B-complex benefits human health as they are present in many foods. However, they present a challenge in the food industry because of manufacturing and storage conditions. However, nanotechnology has opened several possibilities to improve their stability and benefits according to their structure storage conditions. Although nanofortification is an alternative to the current fortification process, however, a gap persists in long-term health and environmental impact. But it has been believed that the nanofortification will help achieve the UN sustainable development goal of zero hunger. Although, many countries like Switzerland, Nigeria, America, etc., have already started nanofortifying their food items with different micronutrients, vitamins, etc. Further, with the onset of the current global pandemic caused by SARS-CoV-2, it has generated health and medical concerns among people leading to rising global food concerns. Moreover, much studies are still required to know the excretion routes of nanoparticle from the body, and toxicity, which demands rigorous and in-depth study to lay down basic biosafety and bioregulatory protocols on their applications.

However, much research is still needed in the nanofortification of vitamin B-complex. The future of this field is very bright and could be used to develop more improved fortification techniques and help eradicate malnourishment from society. Thus, this review summarizes the role of nanotechnology in the fortification of the vitamin B-complex in food. It also discusses some associated regulations of nanotechnology. Organic nanomaterials are mainly used for the fortification process among the various discussed nanomaterials. Hence, this review aims to deliver a detailed discussion on the various methods of nanofortification of vitamin B-complex along with its recent trends and advancements.

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Declaration of Competing Interest

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