Chapter

Several Dosage Forms Containing Vitamin B and Their Use in Therapy

Özlem Çoban

Abstract

Vitamin B plays a critical role in the synthesis of DNA and maintaining the normal functioning of tissues. Therefore, its deficiency may lead to mental problems such as depression, schizophrenia, dementia, and systemic problems such as megaloblastic anemia and peripheral neuropathy. Vitamin B deficiency may be based on nutrition, as well as the use of some drugs such as metformin and omeprazole suppress the absorption of B vitamins, which may lead to deficiency. Since B vitamin is water soluble, it cannot be stored in the body. For this reason, it should be taken continuously with food. However, in cases where the vitamin B taken with food is not sufficient for the body, it should be reinforced with drugs or dietary supplements from outside. Studies have shown that the absorption of Vitamin B is 50% higher in food supplements than in foods. It can also be used as a targeting agent in tumor therapy, due to its overexpression in some tumor cells. Due to these properties of Vitamin B, various dosage forms are being developed. In this chapter, vitamin B-containing dosage forms, their production techniques, and their use in therapy will be mentioned.

Keywords: Liposomes, emulsions, microparticles, nanoparticles, encapsulation, vitamin B, 3D printing, targeting, tumor, electrospinning

1. Introduction

Vitamins cannot be synthesized by the human body and therefore must be obtained through the diet. Vitamin B complex is also a vitamin taken in this way, and there are various derivatives such as thiamine (B1), riboflavin (B2), niacin or nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folic acid (B9) and cobalamin (B12) [1, 2]. Foods containing Vitamin B can be: Lean pork, legumes and cereal grains (B1); milk, egg white, fish, roe, kidney and leafy vegetables (B2), especially yeast and liver; meat, fish or poultry, roasted coffee (B3); chicken, beef, potatoes, oat cereals, tomato products, liver, kidney, yeast, egg yolk, broccoli and whole grains (B5); fish and meat, seeds, non-citrus fruits such as bananas and watermelons (B6); liver, kidney, egg yolk, soybeans, nuts, spinach, mushrooms, lentils (B7); fruits and green-leafy vegetables, yeast, liver (B9); meat, fish, liver, dairy products (B12) [3].

B vitamins act as coenzymes for enzymes essential for cell function. Thanks to this their function, they take place in several physiological events including glucose,

fatty acid, amino acid and homocysteine metabolism, tryptophan metabolism in the kynurenine pathway and synthesis and metabolism of various neurotransmitters and neurohormones such as serotonin, dopamine, adrenaline, acetylcholine, gamma-Aminobutyric acid (GABA), glutamate, D-serine, glycine, histamine and melatonin. Some also play a role in the regulation of intestinal and blood–brain barrier permeability [4]. For example, thiamine and riboflavin are essential in the oxidative decarboxylation of multienzyme branched chain ketoacid dehydrogenase complexes in the citric acid cycle and flavoenzymes of the respiratory chain, respectively, while niacin provides protons in nicotinamide adenine dinucleotide (NADH) synthesis and consequent oxidative phosphorylation. Pantothenic acid is required for coenzyme A (CoA) formation, α-ketoglutarate and pyruvate dehydrogenase complexes, and fatty acid oxidation. Biotin is a coenzyme of decarboxylases required for gluconeogenesis and fatty acid oxidation [5]. Pyridoxine plays a role in the catabolism of cysteine and, finally, folic acid and cobalamin have an essential role in the remethylation of methionine [6].

Due to these critical functions of the vitamin B family in the metabolic activities of our body and the key roles it plays in the functions of the central nervous system and psychopathology, some disorders occur in their deficiency. It has been shown by clinical data to they have important roles in various psychiatric diseases such as major depression, bipolar disorder, schizophrenia, autism, Alzheimer's and Parkinson's [4]. For example; in a study conducted in 3 884 elderly people with depressive symptoms, it was observed that folate values, especially cobalamin values, were lower compared to the control group. As a result of this study, although it was found that especially cobalamin deficiency was associated with depression, a positive relationship was also found between folate deficiency and depression in 6 different clinical studies. In this case, it can be said that folic acid and cobalamin deficiency may contribute to mental illness and neurological disorders in older adults [7]. The reason for the emergence of this situation due to folate and cobalamin deficiency may be that these vitamins help the metabolism of monoamine neurotransmitters such as norepinephrine, and methylation and monoamine metabolism are impaired in their deficiency [7, 8]. In another study of 7 387 Iranian adults were found that a strong association had between high biotin intake and a lower probability of depression, anxiety, and stress for the entire population, but especially for women, and pyridoxine had a protective effect against stress. In addition, there were observed that an inverse association had between cobalamin and anxiety symptoms in men, women with high pantothenic acid intake had a lower rate of depression, but there was no statistically significant linear relationship for riboflavin in both genders [9].

High levels of intake of folic acid or vitamin B complex may be of use in Parkinson's disease due to decreased serum homocysteine levels or neuroprotective effects, respectively [6]. Similarly, since homocysteine is effective in the atherosclerosis process and accordingly hyperhomocysteinemia poses a cardiovascular risk, cobalamine and pyridoxine, especially folic acid may have efficacy in the prevention of cardiovascular diseases [10]. In addition to their effects on nutrition, axonal transport, the excitability of neurons or synthesis of neurotransmitters, studies are available showing that B vitamins (thiamine, pyridoxine and cobalamin) are clinically useful in the treatment of certain painful conditions such as lumbago, sciatica, trigeminal neuralgia, and chronic pain due to diabetic polyneuropathy and rheumatoid arthritis [11].

In conducted another study, it was shown that the risk of non-alcoholic fatty liver disease (NAFLD) was lower in subjects who followed a diet rich in vitamin D, thiamine, riboflavin, cobalamin, niacin and zinc [12]. Cobalamin deficiency is quite common in patients with type 1 and type 2 diabetes. Depending on this deficiency,

various clinical symptoms such as memory impairment, dementia, delirium, peripheral neuropathy, sub-acute combined degeneration of the spinal cord, megaloblastic anemia and pancytopenia are observed [13].

However, vitamin deficiency should not be attributed only to insufficient dietary intake of vitamins. For example, cobalamin deficiency can occur due to gastric atrophy, which causes absorption problems, and the use of gastric reflux medications, which lower acid levels in the stomach [7, 14].

As mentioned above, vitamin B has a protective effect in mental disorders such as schizophrenia, depression, neurological disorders such as Alzheimer's and Parkinson's, and metabolic disorders such as cardiovascular and diabetes. However, there is also the problem of absorption and stability of vitamin B. For this reason, these parameters should be taken into account when developing a formulation. In this section, the properties of various dosage forms containing vitamins belonging to the vitamin B family and the methods used during their preparation will be discussed.

2. Several dosage forms containing vitamin B and their use in therapy

Thiamine (vitamin B1) can improve immune system function and reduce the risk of type-2 diabetes, cardiovascular and kidney diseases, age-related, mental and neurodegenerative disorders and cancer. Therefore, its deficiency may affect the cardiovascular system, develop neuroinflammation, increase inflammation and cause atypical antibody responses [15]. Thiamine also acts as a carbonic anhydrase isoenzyme inhibitor [16, 17].

Riboflavin (vitamin B2) is the precursor of flavin adenine dinucleotide (FAD+) and makes folate coenzymes stabilize the C677T variant of 5,10-methylenetetrahydrofolate reductase (C677T MTHFR) by preventing the polymorphic enzyme from displacing the flavin cofactor [18]. In combination with UV light, riboflavin causes irreversible damage to nucleic acids such as DNA and RNA, rendering microbial pathogens unable to reproduce, and in this way may be effective against MERS-CoV virus as well as SARS-CoV-2 [19].

Niacin (vitamin B3, nicotinamide) serves as the building block of NAD and nicotinamide adenine dinucleotide phosphate (NADP), which are vital in chronic systemic inflammation [20]. In this respect, it serves as the sole substrate for poly (ADP-ribose) polymerase-1 (PARP), which is required for cell differentiation, DNA repair and expression, and apoptosis [18]. Since NAD+ acts as a coenzyme in various metabolic pathways, its high levels are required to treat a variety of pathophysiological conditions. For example, NAD+ has immunomodulatory properties, is released in the early stages of inflammation, and reduces serum levels of the proinflammatory cytokines IL-1β, IL-6, and TNF-α [7, 21, 22]. In addition, niacin reduces neutrophil infiltration and exerts anti-inflammatory effects in patients with ventilator-induced lung injury. It has been observed that niacin and nicotinamide prevent lung tissue damage in hamsters [23]. In addition, nicotinamide reduces viral replication and strengthens the body's defense mechanisms [24, 25].

Pantothenic acid (vitamin B5) has cholesterol and triglyceride lowering properties. It also speeds up wound healing, reduces inflammation and improves mental health [15]. However, there are limited studies showing its effects on the immune system [17].

Pyridoxine (vitamin B6, Pyridoxal 5′-phosphate) is required for the transsulfuration of vasculotoxic homocysteine and the activity of serine hydroxymethyl transferase (SHMT), which is the main entry point of one-carbon units into folate-dependent one-carbon metabolism [18]. Pyridoxal 5′-phosphate (PLP), the active form of pyridoxine, is an essential cofactor in various inflammatory pathways and

its deficiency leads to immune dysregulation. Also, thirty years ago, PLP levels were shown to reduce abnormalities in platelet aggregation and blood clot formation. During inflammation, the use of PLP increases and accordingly, its amount in the body decreases. PLP levels are low in patients with type-2 diabetes and cardiovascular disease, and the elderly [26–29]. Recently, researchers at the University of Victoria reported that pyridoxine (as well as riboflavin and folic acid) is a potent anti-inflammatory that can deactivate macrophages and monocytes, inhibit antigen-presenting cells and T cells, and upregulate the immunosuppressive cytokine IL-10 [30].

Biotin (vitamin B7) acts as a coenzyme for three important carboxylation reactions involving the conversion of pyruvate to oxalacetate, acetyl-CoA to malonyl-CoA, and propionyl-CoA to methylmalonyl-CoA in humans. These enzymes are vital in numerous metabolic processes. For example, these conversions break down food into glucose, the primary source of carbohydrates for the brain and body. Recently, the roles of biotin in cell signaling and epigenetic regulation have been recognized [31].

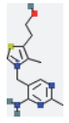
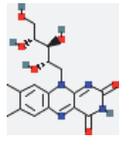
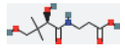
Folic acid (vitamin B9, folate) is an essential vitamin for DNA and protein synthesis and adaptive immune response [32]. It plays several essential and direct roles in the synthesis, repair, and expression of DNA [18]. Furin is an enzyme associated with bacterial and viral infections and is a promising target for the treatment of infections. Recently, it has been noted that folic acid can inhibit furin and thus preventing SARS-CoV-2 spike protein binding, cell entry and virus transformation [32].

Cobalamin (vitamin B12) is essential for red blood cell synthesis, nervous system health, myelin synthesis, cellular growth and rapid synthesis of DNA. Its active forms are hydroxo-, adenosyl- and methylcobalamin. Cobalamin acts as a modulator of the gut microbiota and its low levels elevate methylmalonic acid and homocysteine, resulting in increased inflammation, reactive oxygen species (ROS) and oxidative stress [22]. Hyperhomocysteinemia causes endothelial dysfunction, activation of platelet and coagulation cascades, megaloblastic anemia, disruption of myelin sheath integrity and decreased immune responses. In addition, cobalamin deficiency can cause disorders in the respiratory, gastrointestinal, and central nervous systems [17].

2.1 Preparation and characterization of the dosage forms containing vitamin B

In this title, particular attention has been given to drug delivery systems in nano/micro size, from formulations prepared to contain various vitamins from the vitamin B family alone or in combination. Various nano/microsystems containing vitamin B are summarized in **Table 1** in terms of preparation methods and characterization results. Apart from this, alternative dosage forms were also evaluated since nano/micro drug carrier systems were not encountered or rarely encountered in some vitamins.

Edible hydrogels containing thiamine were prepared by hot extrusion-based three-dimensional (3D) printing and casting method. In the 3D printing method, products are produced as three-dimensional sized on special printers by the layering of the models created in the digital environment. First of all, hydrogels containing thiamine based on agar or kappa-carrageenan were prepared at 70°C and 80°C, respectively. In the preparation of these gels, 1 M NaOH was added to the medium to protect agar, kappa-carrageenan and thiamine from degradation, and the pH was maintained at 5.5. The prepared hydrogels were filled into the syringe of the printer while they were hot, the temperature of the environment where the plate was located was adjusted to 20°C, and various formulations were prepared by changing

Vitamin B Type	Molecular Stracture	Formülation Type	Preparation Method	Characterization				References
				Particle Size	Polydispersity Index	Zeta Potential	Encapsulation Efficiency	
Thiamine		• Microparticle • Liposome	• Self-emulsion polymerization • High speed homogenization	• 681–1604 nm • 108–151 nm	• nd • 0.264–0.308	• (−)5.69 - (−) 16.90 mV • (−)21 - (−)34 mV	• 120–140 mg/g • 97%	• [33] • [34]
Riboflavin		• Microparticle • Y/S emulsion • Liposome	• Emulsion-enzymatic gelation • High shear mixer/ Microfludization • Thin film	• 31.7–151.6 μm • 45–216 nm • 113–121 nm	• nd • nd • nd	• nd • (−)5 - (−) 7 mV • nd	• 56.5%–84.1% • nd • 25.86%–42.34%	• [35] • [36] • [37]
Niacin		• Dendrimer • Microsphere • Microsphere • Microemultion	• Simple esterification reaction • W/O/O double emulsion-solvent diffusion • Chemical denaturation • Dilution	• nd • 202–560 μm • 54–94 μm • 13.6–78.9 nm	• nd • nd • nd • nd	• nd • nd • nd • nd	• nd • 37–86% • 18.22%–70.42% • nd	• [38] • [39] • [40] • [41]
Pantothenic acid		• Liposome/ Microparticle • Nanofiber	• Proliposome/Encapsulator • Electrospinning	• 100–240 nm/240–300 μm • 625 nm	• nd/nd • nd	• nd/nd • nd	• 0.75 ± 0.02/0.60 ± 0.02 • nd	• [42] • [43]
Pyridoxine		• Liposome • Microsphere • Nanoparticle	• Ethanol injection/Thin film / Modified thin film /Reverse phase evaporation • Spray drying • Emultion-solvent evaporation	• 176.70–260.30/164.80–256.40/177.80–223.20/153.60–235.00 nm • 4.5–4.8 μm • 585.7 nm	• 0.22–0.49/ 0.23–0.39/ 0.19–0.57/ 0.18–0.59 • nd • nd	• (+)4.7 – (+) 5.31 mV • (+)37 – (+)44 mV • (+) 8.98 mV	• 33.83%–34.94%/ 30.42%–34.31%/39.17%–43.69%/29.17%–40.12% • 83 ± 3.17% • nd	• [44] • [45] • [46]
Biyotin		No biotin encapsulated nanoformulation was found.						

Vitamin B Type	Molecular Stracture	Formülation Type	Preparation Method	Characterization				References
				Particle Size	Polydispersity Index	Zeta Potential	Encapsulation Efficiency	
Folic acid		• Nanoemultion • Nanoparticle • Microparticle	• Double emultion-spray drying • Ionic gelation • Spray drying	• 32.5–90 nm • 209.5–479.4 nm • 5.4–267.5 μm	• nd • 39.9–120.4 • nd	• nd • nd • (−)6.7 - (−) 29.7 mV	• 86.60% • 43.7%–92.1% • ~100%	• [47] • [48] • [49]
Cobalamin		• Microstructure • Protein-lipid nanoparticle • Nanoemultion	• Electrospinning/spray drying • Emultion-solvent evaporation • solvent evaporation	• 0.25–1.25 μm, 0.63–20 μm/2.23–6.42 μm • 243–255 nm • 210.8–1947 nm	• nd • 0.17–0.19 • 0.21–0.85	• nd • (−)7.5 - (−)20 mV (for pH 6) • (−)9.67 - (−) 34.9 mV	• 71.0%–94.6% /61.3%–100.0% • 69–71% • 105.89%–110.65%	• [50] • [51] • [52]

Table 1.
Various nano/micro systems containing vitamin B, preparation methods and characterization results (The molecular stractures of the vitamins are taken from the PubChem database. nm; nanometer, μm; micrometer, nd; no data, mV; millivolt).

the parameters in terms of % filling, printing speed, flow percentage and layer height. Cast samples, on the other hand, were obtained by pouring the hydrogel solutions into the mold and leaving them to gel for 2 minutes (close to the printing time) at ambient temperature. When these two preparation methods were compared in terms of hardness, Young's modulus and release rate, it was observed that cast samples were harder and had higher Young's modulus than 3D ones, while their release rate was lower. Since it is based on layering, the microstructure of 3D products differs from cast samples. Printed gels are less resistant to external damage as they are a discontinuous network with several semi-fused small meshes, while cast gels can resist greater amounts of force since they are a single continuous mesh [53]. Thiamine-containing microparticles were prepared by the self-emulsion polymerization method. According to this method, first of all, empty polycitric acid (CA), polymaleic acid (MA) or polycitric acid-co-maleic acid (CA-MA) microparticles were synthesized at 25°C using a mechanical stirrer. For this, CA or MA, or CA/MA and 20 mL of deionized water were added to the reaction vessel, and then 10% mol of N,N′-methylenebisacrylamide (MBA), 0.08 mL of N,N,N′,N′-tetramethylethylenediamine (TEMED) and finally 3 mol% of ammonium persulfate (APS) aqueous solution were added to the reaction vessel, respectively, and finally mixed. The prepared poly(MA) or poly(CA) microparticles were washed with water and acetone and centrifuged for 20 minutes to remove the remaining chemicals without reacting. Thiamine was adsorbed to these prepared microparticles and loaded [33]. Finally, liposomes containing thiamine were prepared using lecithin, L-α-phosphatidylcholine and high-speed homogenization. Phosphatidylcholine was dissolved in chloroform and chloroform was removed with a fast evaporator to form a phosphatidylcholine layer. Thiamine solution was added to the layer and hydrated, and this mixture was homogenized at room temperature using a highspeed homogenizer. The nanoliposome solution was passed through a 0.2 μm syringe filter to collect small particles [34].

Studies as systems containing riboflavin include microparticles prepared using pea protein as carrier and transglutaminase as cross-linker. Emulsion-enzymatic gelation was preferred as the method. In this method, riboflavin was dissolved in the solution of pea proteins in phosphate buffer saline (PBS). Transglutaminase was added to this solution. The water phase thus formed was added to the preheated oil phase consisting of Mgliol under stirring. This mixture was left to incubate for a few more hours under stirring to allow gelation to occur. Microparticles formed over time were separated from the oil phase by centrifugation, washing with 2% Tween 80 solution, and filtration, respectively, and were lyophilized [35]. In addition, liposome formulations of riboflavin were prepared by the thin-film method using phosphatidylcholine and cholesterol. During the preparation, phosphatidylcholine and cholesterol were dissolved in methanol: chloroform mixture and the organic phase was removed in a rotary evaporator. The solution of riboflavin in PBS was added to the thin film and hydrated by rotation at ~40°C. The centrifugally formed liposomes were removed from the dispersion medium and extruded to reduce their size [37].

Microspheres were prepared by Water/Oil/Oil (W/O/O) double emulsion solvent diffusion method using ethyl cellulose for niacin. For this, niacin and ethyl cellulose were dissolved in an acetonitrile-dichloromethane mixture and deionized water was added to this mixture under a stirrer to prepare a Water/Oil (W/O) primer emulsion. Afterwards, this emulsion was added into liquid paraffin containing Span 80 under stirring. Pet ether was then added to the double emulsion to harden the microspheres. To obtain the resulting microspheres, filtration, washing with pet ether (to remove liquid paraffin) and then drying were performed, respectively. Size, encapsulation efficiency and release studies were performed on

the obtained microspheres. As the polymer-drug ratio increased up to a certain value, the encapsulation efficiency increased but decreased after a certain value. The reason for this may be that the viscosity of the solution increases due to the increase in the polymer ratio, and accordingly the formation of large polymer/solvent droplets, in this case, the curing of the microspheres is delayed and drug diffusion occurs during [39]. Niacin microemulsions were prepared by dilution method using the triangular phase diagram. Labrasol was used as a surfactant, Peceol as cosurfactant, isopropyl myristate as oil phase and water as water phase. Dimethylformamide (DMF), propylene glycol and lauric acid were preferred as penetration enhancers. In this method, the oil phase and the surfactant: cosurfactant mixture were mixed first and then titrated with water to form microemulsions. Particle size, rheological properties, conductivity and pH, refractive index, physical stability and in vitro skin penetration studies were performed on the formulations. In these studies, the highest permeation values were obtained with DMF in pH values ranging between 4.32 and 5.09 [41].

To protect pantothenic acid from environmental factors and improve its stability, liposomes, and alginate and alginate-pectin microparticles loaded liposomes have been developed. Liposomal formulations were prepared by the proliposome method. After dissolving Phospholipon 90 G (phosphatidylcholine) in ethanol, the vitamin was added to this solution. This mixture was raised to 60°C for a few minutes and then cooled to room temperature. Liposomes were obtained by adding citric acid solution dropwise to this mixture under stirring. Microparticles were prepared using an encapsulator with a 120 mm nozzle. For liposome-loaded microparticles, the pantothenic acid-loaded liposome suspension was mixed with 3% alginate or alginate-pectin mixture at a ratio of 1:1 (v/v), then these mixtures were passed through the encapsulator and sent to a solution containing 1.5% $CaCl_2$ (w/v) for hardening. The formulations prepared were evaluated in terms of morphology, encapsulation efficiency and release rate. Liposomes containing encapsulated pantothenic acid showed higher stability at moderate-acidic pH (4.0). However, microencapsulation of pantothenic acid-loaded liposomes using alginate or alginate pectin mixtures did not increase the encapsulation efficiency and retention of pantothenic acid [42]. Since pantothenic acid can be used in wound healing due to its fibroblast migration and proliferation effect, silk nanofibers containing pantothenic acid have been produced. Pantothenic acid and silk fibroin were dissolved in ultrapure water and nanofibers were produced by spraying this solution in an electrospinning device at a distance of 20 cm from the collector and a rate of 0.3 mL/h. The produced nanofibers were then exposed to 75% ethanol vapor. Morphological analyzes, cell viability and antioxidant activity were examined as characterization studies [43].

Unilamellar liposomes containing pyridoxine were prepared using ethanol injection, thin film, modified thin film and reverse phase evaporation methods. In the ethanol injection method, Lipoid S 100 was dissolved in ethanol and injected dropwise into an aqueous medium containing pyridoxine under stirring. In the thin film method, Lipoid S 100 was dissolved in chloroform and the organic solvent was evaporated in a rotary evaporator at low pressure at a temperature above the phase transition temperature of the lipid. The resulting dry lipid film was hydrated with an aqueous solution of pyridoxine. In the modified thin film method, a small amount of ethanol was used to dissolve the pyridoxine. Lipoid S 100 was dissolved in this ethanolic solution and the ethanol was evaporated at reduced pressure. The resulting lipid film was hydrated with deionized water. And finally, in the reverse phase evaporation method, Lipoid S 100 was dissolved in chloroform and pyridoxine was dissolved in 10% distilled water. Then, these solutions were mixed in a bath sonicator for 5 minutes to obtain an emulsion. The organic solvent was evaporated

at reduced pressure and the remainder of the aqueous phase was added. Deionized water was used instead of buffer in all methods as the dispersion medium because when the buffer is used, the electrostatic interaction between pyridoxine and liposome decreases and accordingly the encapsulation efficiency decreases [44]. Within the scope of personalized treatment, the tablet formulation containing 4 different substances (thiamine, niacin, pyridoxine and caffeine) together and in which vitamins are released rapidly and caffeine is released in a sustained was prepared using a semi-solid pneumatic activated paste extrusion 3D printer at room temperature. Weight variation, moisture content, hardness, in vitro dissolution and active ingredient content analyzes were performed on 3D tablets [54].

The tablet dosage form in the form of a combination of biotin with various vitamins and minerals, although not alone, was prepared by the researchers. Prepared as a bilayer, this tablet contains biotin (with A, D, E, C and various B vitamins) in one layer and minerals in another layer. Since the active ingredients can be included in the form of premixes, the direct tableting method was used in the preparation of bilayer tablets. For this formulation, powder blends were evaluated in terms of angle of repose, bulk density, tapped density, Hausner ratio and Carr Index, while tablets were evaluated in terms of weight uniformity, diameter-thickness, friability, hardness, disintegration time and dissolution [55]. Unfortunately, no studies have been found on biotin loaded nano/microsystem formulations. However, there are studies on nano/microsystems where biotin is used as a ligand for active targeting. As an example of these; is the design of biotin-conjugated sunitinib-loaded nanostructures for use in lung cancer therapy. The reason why biotin is conjugated to the surface of the nanocarrier is that it is overexpressed by various cancer cells such as lung cancer [56]. Thus, it is envisaged that the nanosystem can only be delivered to tumor cells. In this study, after the preparation of biotin-sterylamine conjugate, nanostructured lipid carriers were prepared by emulsification-solvent evaporation method. In the conjugation process, the amine group of stearylamine and the activated carboxyl group of biotin were reacted in the medium of dicyclohexylcarbodiimide (DCC) and 4-N-hydroxysuccinimide (NHS) [56]. To increase oral penetration of insulin by targeting it to enterocytes, biotin-conjugated muco-inert nanocomplexes were prepared by Cui and coworkers. Although there are many ligands such as biotin, transferrin, and folic acid in enterocytes, the reason for choosing biotin in this study is that biotin receptors are widely located on the surface of enterocytes and that it has specific intracellular traffic and basal exocytosis through the transport of sodium-dependent multivitamins. In this study, after the chitosan-biotin copolymer was synthesized, insulin-loaded and hyaluronic acid (HA) coated chitosan-biotin nanocomplexes were prepared by self-assembly method (HA was used to increase mucus penetration) [57]. Apart from these studies, there are several studies in which biotin is used in surface modification; photoactive gold nanoparticles for improving the photothermal therapy of brain cancer, liposomes for use in targeted breast cancer treatment, and polymeric micelles to release paclitaxel [58–60].

Folic acid-containing nanoemulsion was prepared by Assadpour and coworkers by double emulsion method to protect folic acid from environmental and production conditions. According to this method, first of all, the W/O primary microemulsion was obtained. For this, the aqueous phase consisting of folic acid solution and Span 80 was added dropwise to the oil phase consisting of canola oil at 1 000 rpm under magnetic stirring. This primer emulsion was then added to the aqueous solution containing maltodextrin-whey protein, and the Water/Oil/Water (W/O/W) double emulsion was obtained mixing with a homogenizer at 12 000 rpm-10°C for 5 min, and then in the same homogenizer at 15 000 rpm-10°C for 8 min. In this way, nanoemulsions in which folic acid is encapsulated have been

obtained [47]. Pamunuwa et al., on the other hand, encapsulated folic acid into alginate-pectin nanoparticles using the ionic gelation method to provide controlled release of folic acid. Accordingly, a small amount of Span 80 and then folic acid was added to the aqueous solution of the polymer adjusted to pH 5, and the mixture was stirred at 1 500 rpm for 30 min. $CaCl_2$ solution was added dropwise to this solution to ensure nanoparticle formation by gelation [48]. Apart from these, various nanosystems have been developed in which folic acid is conjugated to the surface, as in biotin. The general reason for using folic acid in these studies is the widespread presence of folic acid receptors in cancer cells. Examples of these studies are doxorubicin-loaded chitosan nanospheres for use in nuclear targeted cancer therapy, spiropyran and imidazole-loaded photoactive gold nanoparticles to increase the radiosensitivity of prostate cancer cells, and chitosan-coated trans-resveratrol-ferulic acid-loaded solid lipid nanoparticles to act on colon cancer cells [61–63].

Cobalamin loaded zein microstructures were prepared by Coelho et al. using electrospinning and spray drying methods. In the electrospinning method, 3 different microstructures were obtained as film, bead and fiber, while only microparticles were obtained in spray drying. A solution of zein in ethanol was used for both methods. However, while absolute ethanol is used in spray drying, aqueous alcohol is preferred in electrospinning. The formulation solutions were prepared by adding cobalamin to the solutions of zein in alcohol [50]. As a non-invasive supporting approach in cobalamin deficiency, cobalamin-loaded buccoadhesive films were prepared using solvent casting as a method, and poly(vinyl alcohol) (PVA) and chitosan as polymers. For this, glycerin or poly(ethylene glycol) 400 (PEG 400) as a plastisizer, propylene glycol as a penetration enhancer and maleic acid for chemical polymerization were added to the PVA: chitosan solution in various proportions in addition to cobalamin. After the prepared mixture was poured into the mold, it was kept at ambient temperature for 24–48 hours to form a film. Thickness, weight variation, drug content, moisture uptake and moisture content percentage, surface pH, mechanical properties, in vitro release and mucoadhesion tests were performed on the prepared formulations. In addition, in vivo pharmacokinetic studies on rabbits showed that the AUC value of bucoadhesive films was approximately 1.5 times higher than the market preparation administered intramuscularly [64]. As with other B vitamins, cobalamin has been used in the functionalization of various nanosystems. For example, calcium phosphate nanoparticles coated with cobalamin-chitosan conjugate and sodium alginate were prepared to increase receptor-mediated endocytosis and thus absorption of insulin from epithelial cells. For this, first of all, insulin-loaded calcium phosphate nanoparticles were prepared by microemulsion method, and then layer-by-layer coating process was performed [65]. In addition, solid lipid nanoparticles loaded with amphotericin B and coated with cobalamin-stearic acid conjugate were prepared to evaluate the anti-leishmanial effect of amphotericin B in vitro [66].

2.2 Vitamin B use in therapy

When cobalamin is metabolically significantly deficient, the methylation process, which is also essential in the conversion of dietary folate (methyl-tetrahydrofolate) to its active metabolic form (tetrahydrofolate), will be disrupted and the amount of intracellular and serum homocysteine will increase. The state of hyperhomocysteinemia has a potentially toxic effect on neuron and vascular endothelium. Also, as a cofactor, cobalamin helps convert methylmalonyl CoA to succinyl-CoA. Therefore, in its deficiency, this conversion decreases and an increase in serum methylmalonic acid (MMA) occurs. As a result, a defect develops in fatty acid synthesis in neuronal membranes [13]. Because cobalamin plays a vital

role in the metabolism of fatty acids, which are necessary for the protection of nerve myelin [67]. Cobalamin is essential in the synthesis of monoamines or neurotransmitters such as serotonin and dopamine, and its deficiency disrupts this synthesis [13]. Similarly, pyridoxine plays a role in the production of serotonin and norepinephrine, chemicals that transmit signals in the brain, and in the formation of myelin [67].

Diabetes mellitus is one of the most common and serious metabolic disorders characterized by hyperglycemia, altered lipid, carbohydrate and protein metabolism, leading to oxidative stress and cell death in the brain, resulting in cognitive and behavioral disorders. The disease is associated with peripheral neuropathy and dysfunctions in the central nervous system. Patients have moderate changes in memory and cognitive functions, poor motor coordination and reduced motor activity. It is also associated with progressive end-organ damage in "diabetic encephalopathy" in the central nervous system. When the role of vitamin B complex in the prevention of neuronal death is examined, it has been observed that thiamine supplementation can improve and usually completely resolve the symptoms associated with Wernicke's encephalopathy (ophthalmoplegia, ataxia and confusion triad), folic acid improves memory in the elderly, and vitamin B complex protects Purkinje neurons, which is the main relay neurons of the cerebellum and play a very important role in motor coordination and learning, from degeneration and loss in diabetes [67–69].

There is substantial evidence to suggest that one-carbon metabolism is associated with cardiovascular disease, and particularly stroke. A one-carbon metabolism requires adequate folate along with cobalamin, pyridoxine, and riboflavin. The suboptimal state of any of these B vitamins and/or genetic polymorphisms on B-vitamin-dependent enzymes in one-carbon metabolism may cause disruption of one-carbon metabolic pathways that can lead to adverse phenotypes even if dietary folate and vitamin B intake are adequate. However, riboflavin supplementation may offer an effective personalized approach to managing hypertension in genetically at risk individuals. In fact, according to the findings from the 5-year Chinese Stroke Primary Prevention Study (CSPPT), which included 20 702 hypertensive patients, it was observed that intervention with folic acid did not reduce blood pressure but reduced the incidence of first stroke by 21% [70].

Because neuropathic pain that develops after nerve injury is severe and persistent, current drugs and non-drug treatments cannot provide significant pain relief in most patients. In a study investigating the analgesic effects of B vitamins (thiamine, pyridoxine and cobalamin) in rats with neuropathic pain due to spinal ganglion compression (CCD) or loose ligation of the sciatic nerve (CCI), the results showed that intraperitoneal injection of thiamine/pyridoxine or cobalamin significantly reduced thermal hyperalgesia; when used in combination synergistically inhibited thermal hyperalgesia; when administered repeatedly, it provided prolonged inhibition of thermal hyperalgesia and did not affect mechanical hyperalgesia or normal pain sensation, but showed similar effects on CCD and CCI-induced hyperalgesia. In conclusion, the effects of B vitamins on pain and hyperalgesia following primary sensory neuron injury have been demonstrated, and the possible clinical use of B vitamins has been suggested in the treatment of neuropathic pain conditions following injury, inflammation, degeneration or other disorders in the nervous system for humans [11].

Because vitamin B plays a crucial role in cell function, energy metabolism and immune function, it helps activate both the hereditary and adaptive immune response, reduces proinflammatory cytokine levels, improves respiratory function, maintains endothelial integrity and prevents hypercoagulability. Because of these effects, it can be used in the prevention or reduction of COVID 19 symptoms, or in

the treatment of SARS-CoV-2 infection. For this reason, the vitamin B status of COVID-19 patients can be evaluated and their existing treatments can be supplemented [17].

It is known that behavioral symptoms mimicking schizophrenia and long-term changes in brain function are observed in animals exposed to maternal deprivation (MD) during early development, and the positive effect of vitamin B complex on schizophrenia. In an experiment in Wistar rats, a decrease in long and short-term memory was observed in addition to anxiety-like behaviors in subjects exposed to maternal deprivation. However, it was determined that rats receiving vitamin B complex showed a decrease in behavioral decline, histomorphological deterioration and oxidative stress caused by MD due to the enhancement of endogenous antioxidant defense, and thus nootropic behavior and reduced anxiety. In conclusion, it has been confirmed that the vitamin B complex is neuroprotective against neuropathological changes caused by maternal deprivation [71].

Vitamin B has effects not only on humans but also on animal and plant health. For example, B vitamins mainly have an indirect effect on the immune function of the gut and their interaction with the bacterial cells and network in the gut. However, impaired redox balance and uncontrolled inflammation can often occur in pigs, especially due to rapid genetic improvement and intensive production methods. Therefore, antioxidant vitamins, such as vitamin B, can be used for the control of reactive oxygen species and peroxides and, accordingly, improved enteric immunity and inflammation control [72]. As in humans, pigs have a decrease in plasma homocysteine due to folic acid and/or cobalamin supplements (although not as much as in humans) [73, 74]. To evaluate the effect of high homocysteinemia on piglets (birth to 8 weeks of age), two piglet populations with high or low homocysteinemia were used in one study. The low homocysteine group was formed by giving sows folate and cobalamin during pregnancy and lactation, and by intramuscular injection of cobalamin into piglets during lactation. As an indicator of cell-mediated immunity, the proliferative response of lymphocyte to mitogen activation was investigated. However, as a positive correlation between growth rate and feed conversion (gain:feed) and plasma homocysteine was observed in piglets, young "high performance" piglets producing high levels of plasma homocysteine may appear immunologically suppressed [75] and are may be less prone to develop an optimal adaptive immune response to antigenic challenges. However, the disease resistance of high-performing piglets is also high in the clinic. This may be due to homocysteine being an important metabolite that plays a central role in the interface between transsulfuration and trans- and re-methylation, linking dietary intake of at least three B vitamins and regulation of endogenous antioxidation activity, innate and adaptive immune responses [72]. Although ultraviolet-B radiation is essential for plant growth at low doses, it can have serious adverse effects on plants at high doses. Reactive oxygen species (ROS) can form in some plants in response to UV-B, and pyridoxine is known to be a quencher of ROS [76].

3. Conclusions

Vitamin B is a family of vitamins that are water-soluble and cannot be accumulated and synthesized in the body, is essential for vital functions in the body, therefore it must be taken with food. It mostly functions as a coenzyme and is also responsible for DNA repair. It has antioxidant activity. It has various activities in metabolic disease, inflammatory conditions, and mental disorders such as schizophrenia, dementia, Alzheimer's. In this respect, its deficiency may trigger these conditions in people with a genetic predisposition. Also, although it can be taken

with food, some gastrointestinal conditions and medications can reduce its absorption. Therefore, external reinforcement is recommended. However, effective formulations are needed due to their low in vitro stability and limited in vivo bioavailability. In this respect, it is aimed to develop nano/microsystems that are known to be more effective rather than conventional dosage forms such as tablets and capsules. However, when we look at the literature, there are not enough studies on these drug delivery systems containing vitamin B. Therefore, researchers can be advised to develop dosage forms containing vitamin B with high bioavailability and in vitro stability.

Acknowledgements

The work was not funded by any institute or person.

Conflict of interest

The authors declare no conflict of interest.

Author details

Özlem Çoban[1,2]

1 Department of Pharmaceutical Technology, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Turkey

2 Drug and Pharmaceutical Technology Application and Research Center, Karadeniz Technical University, Trabzon, Turkey

*Address all correspondence to: ozlemcoban@ktu.edu.tr

References

[1] Chantereau G, Sharma M, Abednejad A, Vilela C, Costa EM, Veiga M, Antunes F, Pintado MM, Sèbe G, Coma V, Freire MG, Freire CSR, Silvestre AJD. Bacterial nanocellulose membranes loaded with vitamin B-based ionic liquids for dermal care applications. Journal of Molecular Liquids. 2020;302:112547. DOI: 10.1016/j.molliq.2020.112547

[2] Kennedy DO. B vitamins and the brain: Mechanisms, dose and efficacy—A review. Nutrients. 2016;8(2):68. DOI: 10.3390/nu8020068

[3] Bonetti F, Brombo G, Zuliani G. The role of B group vitamins and choline in cognition and brain aging. Nutrition and Functional Foods for Healthy Aging. 2017;139-158. DOI: 10.1016/b978-0-12-805376-8.00015-0

[4] Rudzki L, Stone T, Maes M, Misiak B, Samochowiec J, Szulc A. Gut microbiota-derived vitamins–underrated powers of a multipotent ally in psychiatric health and disease. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2021;107:110240. DOI: 10.1016/j.pnpbp.2020.110240

[5] Depeint F, Bruce WR, Shangari N, Mehta R, O'Brien PJ. Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism. Chemico-Biological Interactions. 2006;163(1-2):94-112. DOI: 10.1016/j.cbi.2006.04.014

[6] Haghdoost-Yazdi H, Fraidouni N, Faraji A, Jahanihashemi H, Sarookhani M. High intake of folic acid or complex of B vitamins provides anti-Parkinsonism effect: no role for serum level of homocysteine. Behavioural Brain Research. 2012;233(2): 375-381. DOI: 10.1016/j.bbr.2012.05.011

[7] Mikkelsen K, Stojanovska L, Apostolopoulos V. The effects of vitamin B in depression. Curr Med Chem. 2016;23(38):4317-4337. DOI: 10.2174/0929867323666160920110810

[8] Bottiglieri T, Laundy M, Crellin R, Toone BK, Carney MW, Reynolds EH. Homocysteine, folate, methylation, and monoamine metabolism in depression. Journal of Neurology, Neurosurgery & Psychiatry. 2000;69(2):228-232. DOI: 10.1136/jnnp.69.2.228

[9] Mahdavifar B, Hosseinzadeh M, Salehi-Abargouei A, Mirzaei M, Vafa M. Dietary intake of B vitamins and their association with depression, anxiety, and stress symptoms: A cross-sectional, population-based survey. Journal of Affective Disorders. 2021;288:92-98. DOI: 10.1016/j.jad.2021.03.055

[10] Ntaios G, Savopoulos C, Grekas D, Hatzitolios A. The controversial role of B-vitamins in cardiovascular risk: an update. Archives of Cardiovascular Diseases. 2009;102(12):847-854. DOI: 10.1016/j.acvd.2009.07.002

[11] Wang ZB, Gan Q, Rupert RL, Zeng YM, Song XJ. Thiamine, pyridoxine, cyanocobalamin and their combination inhibit thermal, but not mechanical hyperalgesia in rats with primary sensory neuron injury. Pain. 2005;114(1-2):266-277. DOI: 10.1016/j.pain.2004.12.027

[12] Vahid F, Hekmatdoost A, Mirmajidi S, Doaei S, Rahmani D, Faghfoori Z. Association between index of nutritional quality and nonalcoholic fatty liver disease: the role of vitamin D and B group. Am J Med Sci. 2019;358(3):212-218. DOI: 10.1016/j.amjms.2019.06.008

[13] Kibirige D, Mwebaze R. Vitamin B12 deficiency among patients with diabetes mellitus: is routine screening and

supplementation justified?. Journal of Diabetes & Metabolic Disorders. 2013; 12(1):1-6. DOI: 10.1186/2251-6581-12-17

[14] Marcuard SP, Albernaz L, Khazanie PG. Omeprazole therapy causes malabsorption of cyanocobalamin (vitamin B12). Annals of Internal Medicine. 1994;120(3): 211-215. DOI: 10.7326/0003-4819-120-3-199402010-00006

[15] Mikkelsen K., Apostolopoulos V. Vitamin B1, B2, B3, B5, and B6 and the immune system. In: Mahmoudi M, Rezaei N, editors. Nutrition and Immunity. Cham: Springer; 2019. p. 115-125. DOI: 10.1007/978-3-030-16073-9_7

[16] Özdemir ZÖ, Şentürk M, Ekinci D. Inhibition of mammalian carbonic anhydrase isoforms I, II and VI with thiamine and thiamine-like molecules. Journal of Enzyme Inhibition and Medicinal Chemistry. 2013;28(2):316-319. DOI: 10.3109/14756366.2011.637200

[17] Editorial. Be well: A potential role for vitamin B in COVID-19. Maturitas. 2021;144:108-111. DOI: 10.1016/j.maturitas.2020.08.007

[18] Lucock M, Yates Z, Glanville T, Leeming R, Simpson N, Daskalakis I. A critical role for B-vitamin nutrition in human developmental and evolutionary biology. Nutrition Research. 2003;23 (11):1463-1475. DOI: 10.1016/S0271-5317(03)00156-8

[19] Ragan I, Hartson L, Pidcoke H, Bowen R,Goodrich R. Pathogen reduction of SARS-CoV-2 virus in plasma and whole blood using riboflavin and UV light. PLoSONE, 2020;15(5): e0233947. DOI: 10.1371/journal.pone.0233947

[20] Boergeling Y, Ludwig S. Targeting a metabolic pathway to fight the flu. The FEBS Journal. 2017:284(2):218-221. DOI: 10.1111/febs.13997

[21] Mikkelsen K, Apostolopoulos V. B Vitamins and ageing. In: Harris J, Korolchuk V, editors. Biochemistry and Cell Biology of Ageing: Part I Biomedical Science. Subcellular Biochemistry. Vol 90. Singapore:Springer; 2018. p. 451-470. DOI: 10.1007/978-981-13-2835-0_15

[22] Mikkelsen K, Stojanovska L, Prakash M, Apostolopoulos V. The effects of vitamin B on the immune/cytokine network and their involvement in depression. Maturitas. 2017;96:58-71. DOI: 10.1016/j.maturitas.2016.11.012

[23] Nagai A, Matsumiya H, Hayashi M, Yasui S, Okamoto H, Konno K. Effects of nicotinamide and niacin on bleomycin-induced acute injury and subsequent fibrosis in hamster lungs. Experimental Lung Research. 1994;20 (4):263-281. DOI: 10.3109/01902149409064387

[24] Zhang L, Liu Y. Potential interventions for novel coronavirus in China: A systematic review. Journal of Medical Virology. 2020;92(5):479-490. DOI: 10.1002/jmv.25707

[25] Mehmel M, Jovanović N, Spitz U. Nicotinamide riboside—the current state of research and therapeutic uses. Nutrients. 2020;12(6):1616. DOI: 10.3390/nu12061616

[26] Merigliano C, Mascolo E, Burla R, Saggio I, Vernì F. The relationship between vitamin B6, diabetes and cancer. Frontiers in Genetics. 2018;9: 388. DOI: 10.3389/fgene.2018.00388

[27] Lengyel CO, Whiting SJ, Zello GA. Nutrient inadequacies among elderly residents of long-term care facilities. Can J Diet Pract Res. 2008;69(2):82-88. DOI: 10.3148/69.2.2008.82

[28] Nix WA, Zirwes R, Bangert V, Kaiser RP, Schilling M, Hostalek U, Obeid R. Vitamin B status in patients with type 2 diabetes mellitus with and

without incipient nephropathy. Diabetes Research and Clinical Practice. 2015;107(1):157-165. DOI: 10.1016/j.diabres.2014.09.058

[29] Van Wyk V, Luus HG, du P. Heyns A. The in vivo effect in humans of pyridoxal-5′-phosphate on platelet function and blood coagulation. Thrombosis Research. 1992;66(6): 657-668. DOI: 10.1016/0049-3848(92)90042-9

[30] Mikkelsen K, Prakash MD, Kuol N, Nurgali K, Stojanovska L, Apostolopoulos V. Anti-tumor effects of vitamin B2, B6 and B9 in promonocytic lymphoma cells. International Journal of Molecular Sciences. 2019;20(15):3763. DOI: 10.3390/ijms20153763

[31] Bistas KG, Tadi P. Biotin [Internet]. 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554493/

[32] Sheybani Z, Dokoohaki MH, Negahdaripour M, Dehdashti M, Zolghadr H, Moghadami M, Masoompour SM, Zolghadr AR. The role of folic acid in the management of respiratory disease caused by COVID-19. ChemRxiv. Cambridge: Cambridge Open Engage. DOI: 10.26434/chemrxiv.12034980.v1

[33] Alpaslan D, Dudu TE, Kubilay Ş, Aktaş N. Synthesis and characterization of biocompatible poly (maleic acid-co-citric acid) microparticles as a smart carrier for thiamine. Polymer Bulletin. 2020;1-16. DOI: 10.1007/s00289-020-03405-y

[34] Fathima SJ, Fathima I, Abhishek V, Khanum F. Phosphatidylcholine, an edible carrier for nanoencapsulation of unstable thiamine. Food Chemistry. 2016;197, 562-570. DOI: 10.1016/j.foodchem.2015.11.005

[35] Djoullah A, Saurel R. Controlled release of riboflavin encapsulated in pea protein microparticles prepared by emulsion-enzymatic gelation process. Journal of Food Engineering. 2021;292: 110276. DOI: 10.1016/j.jfoodeng.2020.110276

[36] Uluata S, McClements DJ, Decker EA. Riboflavin-induced oxidation in fish oil-in-water emulsions: Impact of particle size and optical transparency. Food Chemistry. 2016; 213:457-461. DOI: 10.1016/j.foodchem.2016.06.103

[37] Ahmad I, Arsalan A, Ali SA, Sheraz MA, Ahmed S, Anwar Z, Munir I, Shah MR. Formulation and stabilization of riboflavin in liposomal preparations. Journal of Photochemistry and Photobiology B: Biology. 2015;153: 358-366. DOI: 10.1016/j.jphotobiol.2015.10.017

[38] Sharma A, Khatchadourian A, Khanna K, Sharma R, Kakkar A, Maysinger D. Multivalent niacin nanoconjugates for delivery to cytoplasmic lipid droplets. Biomaterials. 2011;32(5):1419-1429. DOI: 10.1016/j.biomaterials.2010.10.025

[39] Maravajhala V, Dasari N, Sepur A, Joginapalli S. Design and evaluation of niacin microspheres. Indian Journal of Pharmaceutical Sciences. 2009;71(6): 663-669. DOI: 10.4103/0250-474X.59549

[40] Vidyavathi M, Papishetty S. Design and evaluation of niacin microspheres with a biopolymer. Trends in Biomaterials & Artificial Organs. 2013; 27(2):81-87.

[41] Tashtoush BM, Bennamani AN, AL-Taani, BM. Preparation and characterization of microemulsion formulations of nicotinic acid and its prodrugs for transdermal delivery, Pharmaceutical Development and Technology. 2013;18(4):834-843. DOI: 10.3109/10837450.2012.727003

[42] Ota A, Istenič K, Skrt M, Šegatin N, Žnidaršič N, Kogej K, Ulrih NP.

Encapsulation of pantothenic acid into liposomes and into alginate–pectin microparticles loaded with liposomes. Journal of Food Engineering. 2018;229:21-31. DOI: 10.1016/j.jfoodeng.2017.06.036

[43] Fan L, Cai Z, Zhang K, Han F, Li J, He C, Mo X, Wang X, Wang, H. Green electrospun pantothenic acid/silk fibroin composite nanofibers: Fabrication, characterization and biological activity. Colloids and Surfaces B: Biointerfaces. 2014;117:14-20. DOI: 10.1016/j.colsurfb.2013.12.030

[44] Abd-El-Azim H, Ramadan A, Nafee N, Khalafallah N. Entrapment efficiency of pyridoxine hydrochloride in unilamellar liposomes: experimental versus model-generated data, Journal of Liposome Research. 2018;28(2):112-116. DOI: 10.1080/08982104.2016.1275679

[45] Chatterjee NS, Anandan R, Navitha M, Asha KK, Kumar KA, Mathew S, Ravishankar CN. Development of thiamine and pyridoxine loaded ferulic acid-grafted chitosan microspheres for dietary supplementation. Journal of Food Science and Technology. 2016;53(1):551-560. DOI: 10.1007/s13197-015-2044-4

[46] Vigneshwaran R, Sankar M, Vigneshkumar S. Synthesis and characterization of gellan gum-pyridoxine nanoparticle for treatment of localized peripheral neuropathy. International Journal of Engineering and Advanced Technology (IJEAT). 2019;9 (1S4):576-580. DOI:10.35940/ijeat.A1127.1291S4194

[47] Assadpour E, Maghsoudlou Y, Jafari SM, Ghorbani M, Aalami M. Optimization of folic acid nano-emulsification and encapsulation by maltodextrin-whey protein double emulsions. International Journal of Biological Macromolecules. 2016;86: 197-207. DOI: 10.1016/j.ijbiomac.2016.01.064

[48] Pamunuwa G, Anjalee N, Kukulewa D, Edirisinghe C, Shakoor F, Karunaratne DN. Tailoring of release properties of folic acid encapsulated nanoparticles via changing alginate and pectin composition in the matrix. Carbohydrate Polymer Technologies and Applications. 2020;1:100008. DOI: 10.1016/j.carpta.2020.100008

[49] Estevinho BN, Lazar R, Blaga A, Rocha F. Preliminary evaluation and studies on the preparation, characterization and in vitro release studies of different biopolymer microparticles for controlled release of folic acid. Powder Technology. 2020; 369:279-288. DOI: 10.1016/j.powtec.2020.05.048

[50] Coelho SC, Laget S, Benaut P, Rocha F, Estevinho BN. A new approach to the production of zein microstructures with vitamin B12, by electrospinning and spray drying techniques. Powder Technology. 2021; 392:47-57. DOI: 10.1016/j.powtec.2021.06.056

[51] Liu G, Yang J, Wang Y, Liu X, Chen L. Protein-lipid composite nanoparticles for the oral delivery of vitamin B12: Impact of protein succinylation on nanoparticle physicochemical and biological properties. Food Hydrocolloids. 2019;92: 189-197. DOI: 10.1016/j.foodhyd.2018.12.020

[52] Çoban Ö, Yıldırım S, Bakır T. Alpha-lipoic acid and cyanocobalamin co-loaded nanoemulsions: Development, characterization, and evaluation of stability. Journal of Pharmaceutical Innovation. 2021;1-11. DOI: 10.1007/s12247-020-09531-4

[53] Kamlow MA, Vadodaria S, Gholamipour-Shirazi A, Spyropoulos F, Mills T. 3D printing of edible hydrogels containing thiamine and their comparison to cast gels. Food Hydrocolloids. 2021;116:106550. DOI: 10.1016/j.foodhyd.2020.106550

[54] Goh WJ, Tan SX, Pastorin G, Ho PCL, Hu J, Lim SH. 3D printing of four-in-one oral polypill with multiple release profiles for personalized delivery of caffeine and vitamin B analogues. International Journal of Pharmaceutics. 2021;598:120360. DOI: 10.1016/j.ijpharm.2021.120360

[55] Rajakumari R, Oluwafemi OS, Thomas S, Kalarikkal N. Dietary supplements containing vitamins and minerals: formulation, optimization and evaluation. Powder Technology. 2018; 336:481-492. DOI: 10.1016/j.powtec.2018.06.026

[56] Taymouri S, Alem M, Varshosaz J, Rostami M, Akbari V, Firoozpour L. Biotin decorated sunitinib loaded nanostructured lipid carriers for tumor targeted chemotherapy of lung cancer. Journal of Drug Delivery Science and Technology. 2019;50:237-247. DOI: 10.1016/j.jddst.2019.01.024

[57] Cui Z, Qin L, Guo S, Cheng H, Zhang X, Guan J, Mao S. Design of biotin decorated enterocyte targeting muco-inert nanocomplexes for enhanced oral insulin delivery. Carbohydrate Polymers. 2021;261:117873. DOI: 10.1016/j.carbpol.2021.117873

[58] He Y, Gao Q, Lv C, Liu L. Improved photothermal therapy of brain cancer cells and photogeneration of reactive oxygen species by biotin conjugated gold photoactive nanoparticles. Journal of Photochemistry and Photobiology B: Biology. 2021;215:112102. DOI: 10.1016/j.jphotobiol.2020.112102

[59] Tang B, Peng Y, Yue Q, Pu Y, Li R, Zhao Y, Hai L, Guo L, Wu Y. Design, preparation and evaluation of different branched biotin modified liposomes for targeting breast cancer. European Journal of Medicinal Chemistry. 2020;193:112204. DOI: 10.1016/j.ejmech.2020.112204

[60] Wang Y, van Steenbergen MJ, Beztsinna N, Shi Y, Lammers T, van Nostrum CF, Hennink WE. Biotin-decorated all-HPMA polymeric micelles for paclitaxel delivery. Journal of Controlled Release. 2020;328:970-984. DOI: 10.1016/j.jconrel.2020.09.013

[61] Ren X, He L, Tian X, Zhang P, Chen Z, Mei X. pH and folic acid dual responsive polysaccharide nanospheres used for nuclear targeted cancer chemotherapy. Colloids and Surfaces B: Biointerfaces. 2019;178:445-451. DOI: 10.1016/j.colsurfb.2019.03.028

[62] Changizi O, Khoei S, Mahdavian A, Shirvalilou S, Mahdavi SR, Rad JK. Enhanced radiosensitivity of LNCaP prostate cancer cell line by goldphotoactive nanoparticles modified with folic acid. Photodiagnosis and Photodynamic Therapy. 2020;29: 101602. DOI: 10.1016/j.pdpdt.2019.101602

[63] Kumar CS, Thangam R, Mary SA, Kannan PR, Arun G, Madhan B. Targeted delivery and apoptosis induction of trans-resveratrol-ferulic acid loaded chitosan coated folic acid conjugate solid lipid nanoparticles in colon cancer cells. Carbohydrate Polymers. 2020;231:115682. DOI: 10.1016/j.carbpol.2019.115682

[64] Mohamad SA, Sarhan HA, Abdelkader H, Mansour HF. Vitamin B12-loaded buccoadhesive films as a non-invasive supplement in vitamin B12 deficiency: In vitro evaluation and in vivo comparative study with intramuscular injection. Journal of Pharmaceutical Sciences. 2017;106(7): 1849-1858. DOI: 10.1016/j.xphs.2017.03.040.

[65] Verma A, Sharma S, Gupta PK, Singh A, Teja BV, Dwivedi P, Gupta GK, Trivedi R, Mishra PR. Vitamin B12 functionalized layer by layer calcium phosphate nanoparticles: A mucoadhesive and pH responsive carrier for improved oral delivery of insulin. Acta Biomaterialia. 2016;31:

288-300. DOI: 10.1016/j.actbio.2015.12.017

[66] Singh A, Yadagiri G, Parvez S, Singh OP, Verma A, Sundar S, Mudavath SL. Formulation, characterization and in vitro anti-leishmanial evaluation of amphotericin B loaded solid lipid nanoparticles coated with vitamin B12-stearic acid conjugate. Materials Science and Engineering: C. 2020;117:111279. DOI: 10.1016/j.msec.2020.111279

[67] Eltony SA. Histological study on the protective role of vitamin B complex on the cerebellum of diabetic rat. Tissue and Cell. 2016;48(4):283-296. DOI: 10.1016/j.tice.2016.06.009

[68] Galvin R, Bråthen G, Ivashynka A, Hillbom M, Tanasescu R, Leone MA. EFNS guidelines for diagnosis, therapy and prevention of Wernicke encephalopathy. European Journal of Neurology. 2010;17(12):1408-1418. DOI: 10.1111/j.1468-1331.2010.03153.x

[69] Reynolds EH. Folic acid, ageing, depression, and dementia. BMJ. 2002; 324(7352):1512-1515. DOI: 10.1136/bmj.324.7352.1512

[70] Psara E, Pentieva K, Ward M, McNulty H. Critical review of nutrition, blood pressure and risk of hypertension through the lifecycle: do B vitamins play a role?. Biochimie. 2020;173:76-90. DOI: 10.1016/j.biochi.2020.03.016

[71] Omotoso GO, Abdulsalam FA, Mutholib NY, Bature AI, Gbadamosi IT. Cortico-hippocampal morphology and behavioural indices improved in maternal deprivation model of schizophrenia following vitamin B complex supplementation. Neurology, Psychiatry and Brain Research. 2020; 38: 74-82. DOI: 10.1016/j.npbr.2020.10.007

[72] Lauridsen C, Matte JJ, Lessard M, Celi P, Litta G. Role of vitamins for gastro-intestinal functionality and health of pigs. Animal Feed Science and Technology. 2021;273:114823. DOI: 10.1016/j.anifeedsci.2021.114823

[73] Simard F, Guay F, Girard CL, Giguère A, Laforest JP, Matte JJ. Effects of concentrations of cyanocobalamin in the gestation diet on some criteria of vitamin B-12 metabolism in first-parity sows. J. Anim. Sci. 2007;85(12): 3294-3302. DOI: 10.2527/jas.2006-523.

[74] Giguère A, Girard CL, Matte JJ. Methionine, folic acid and vitamin B12 in growing-finishing pigs: Impact on growth performance and meat quality. Archives of Animal Nutrition. 2008;62 (3):193-206. DOI: 10.1080/17450390802027494

[75] Audet I, Girard CL, Lessard M, Lo Verso L, Beaudoin F, Matte JJ. Homocysteine metabolism, growth performance, and immune responses in suckling and weanling piglets. Journal of Animal Science. 2015;93(1):147-157. DOI:10.2527/jas.2014-7872

[76] Ristilä M, Strid H, Eriksson LA, Strid Å, Sävenstrand H. The role of the pyridoxine (vitamin B6) biosynthesis enzyme PDX1 in ultraviolet-B radiation responses in plants. Plant Physiology and Biochemistry. 2011;49(3):284-292. DOI: 10.1016/j.plaphy.2011.01.003