Journal of Population Therapeutics & Clinical Pharmacology

REVIEW ARTICLE
Doi : 10.53555/mjj3ey13

HYDROXOCOBALAMIN NASAL DELIVERY FOR VITAMIN B12 DEFICIENCY: OVERCOMING INSTABILITY WITH MICROENCAPSULATION

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ABSTARCT:
Vitamin B12 deficiency is a large scale global health issue which traditional delivery methods like intramuscular injections and oral supplements have little success in which we see poor patient compliance and very variable absorption. Hydroxocobalamin as a form of B12 has pharmacological benefits over other cobalamin forms in that it has a greater half life, more plasma protein binding, and better conversion into active coenzymes which makes it a very good option for alternate delivery systems. Also we see from clinical studies that the intranasal route of hydroxocobalamin is doable which reports in very quick and clinically relevant increases in serum B12 levels. But at present there is no marketed nasal formulation of this and we also have issues of hydroxocobalamin's instability to light, temperature, pH and oxidative and reducing agents. By using microencapsulation we may see to overcome these issues of stability, residence time, and absorption through the nasal mucosa. In order to potentially improve the treatment of vitamin B12 deficiency, future research should concentrate on creating reliable nasal delivery systems for hydroxocobalamin that combine pharmacological advantages with enhanced patient adherence.

1.0 INTRODUCTION:

Nutrients are chemical substances required by the body for the basic functions and are optimally obtained by eating a balanced & good diet. There are six major nutrients classes which essential for human health: carbohydrates, lipids, proteins, minerals, vitamins and water.
Carbohydrates, lipids, and proteins are macronutrients and identified as a source of energy. Water is required in large amounts but not provide energy. Vitamins and minerals are micronutrients and play roles in metabolism in human body.
Vitamins are organic micronutrients and they are classified in two class, water-soluble or fat-soluble. The essential water-soluble vitamins class have vitamins B1, B2, B3, B5, B6, B7, B9, B12, and C. The essential fat-soluble vitamins class have vitamins A, E, D, and K.
Minerals are inorganic micronutrients. Minerals can classify in macrominerals or microminerals. Macrominerals are required in large amount like, 100 mg per day in which it's include calcium, phosphorous, magnesium, sodium, potassium, and chloride.
Vitamins are organic compounds (or related set of molecules) which are must needed micronutrients that require in minute quantities, usually within micrograms (millionths of a gram) to milligrams (thousandths of a gram), for proper functioning of their metabolism in organism body.

Important nutrients cannot be made or synthesized into the human body (organism), so required all amount of sufficient amount, must be obtained by the diet, just as some beneficial phytochemicals are not synthesized in the body.

Not every vitamins are single molecules, rather they are groups of related molecules known as vitamers. For example, vitamin E be made up of four tocopherols and four tocotrienols. Thirteen essential vitamins required by human metabolic functions are:

- Vitamin A (as all-trans-retinyl-esters, all-trans-retinol, and all-trans-beta-carotene)
- (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitaminB6), biotin (vitamin B7), folic acid or folate (vitamin B9), cobalamins (vitamin B12)
- Ascorbic acid (vitamin C)
- Calciferols (vitamin D)
- Tocopherols and tocotrienols (vitamin E)
- Quinones (vitamin K).

Vitamins have many biochemical functions. Like, Regulation of cells and tissue growth and differentiation is function of Vitamin A. The B vitamins function as enzyme cofactors and coenzymes or their precursors. Vitamin D function as regulating mineral metabolism for the bones and other organs in the body. Vitamins C and E both act as antioxidants.[1][2][3]

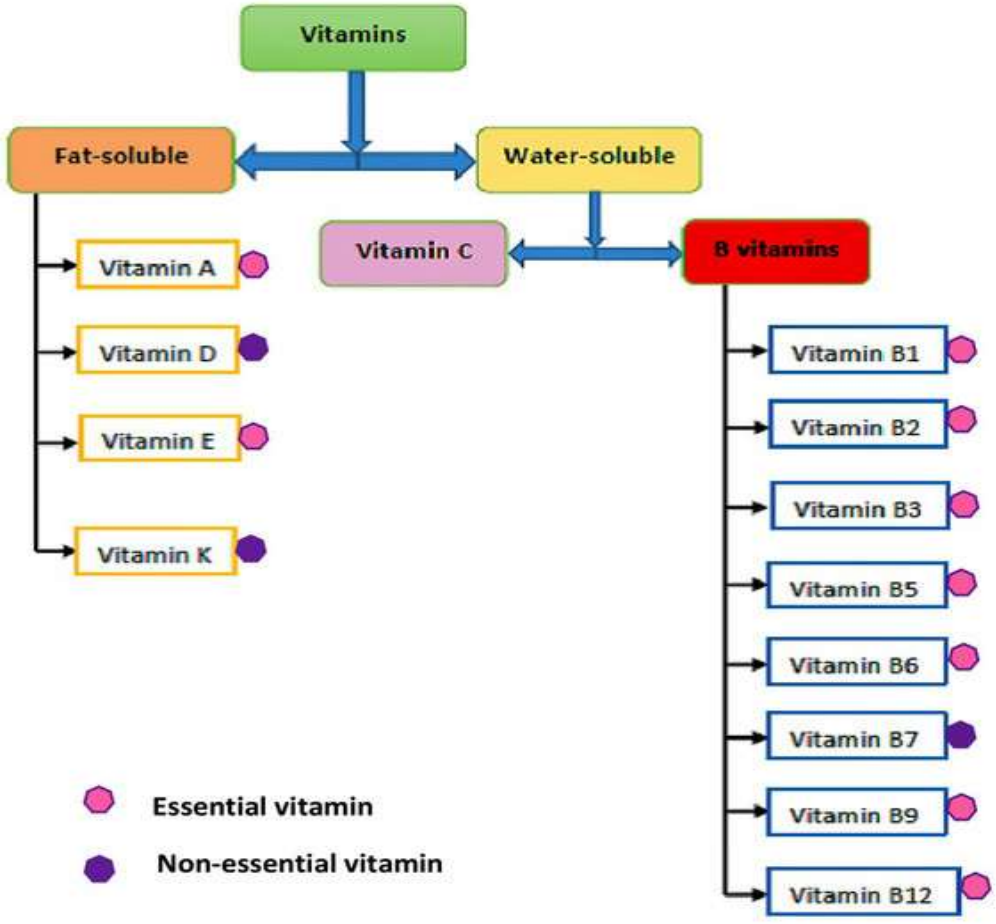

Fig1. Classifications of vitamins.[4]

1.1 VITAMINES FUNCTION IN BODY:[4]

VITAMINS	FUNCTIONS
A	Produces visual pigments in the retina of the eye, promotes good and participates in epithelial cell differentiation
D	Regulates bone metabolism, mineralization of bones and increases absorption of calcium and phosphate in the intestine

E	Possess membrane antioxidant capacity
K	Important for calcium metabolism for bone health and blood clotting
C	Promotes growth of tissues and also associated with repair of tissues, acts as an antioxidant in tissue and plasma, important for the synthesis of collagen and carnitine
B1	Important for energy metabolism, nerve and muscle activity and act as important coenzymes for decarboxylations of 2-keto-acids and transketolations
B2	An important coenzyme in oxidation-reduction reactions of fatty acids and tricarboxylic acid (TCA) cycle, important for synthesis of neurotransmitters
B3	Vital for energy metabolism and neurological processes, an essential coenzyme for several dehydrogenases
B5	Essential for wound healing and normalizing blood lipid profile, an important coenzyme in fatty acid metabolism
B6	Important for nerve activity, acts as coenzymes in amino acid metabolism, fundamental in the synthesis of neurotransmitters, DNA and haemoglobin
B7	Important coenzyme for carboxylation and in lipid metabolism, essential for hair, skin and nail regeneration
B9	Important coenzyme in single-carbon metabolism and synthesis of DNA, required for red blood cell production and also for neural tube formation
B12	Important for folate coenzyme function, red blood cell formation and also associated with absorption of iron, calcium and vitamin A, required in nerve activity and synthesis of neurotransmitters

1.2 VIT B12 INFORMATION:-

Vitamin B (recommended daily intake include B_1 (1.1–1.2 mg), B_2 (1.1–1.3 mg), B_3 (14–16 mg), B_5 (5.0 mg), B_6 (1.3–1.7 mg), B_7 (30 µg), B_9 (400 µg), and B_{12} (2.4 µg). Due to their unique physiological needs and critical roles in growth processes, Vitamin B requirements are higher for subjects, like children (for growth and development, energy metabolism, and nervous system development) and pregnant women (for fetal development, blood production, maternal health, energy and nutrient transfer, cell division and breastfeeding).

From many vitamin B forms, Vitamin B12 is a water-soluble vitamin and Vitamin B12 is naturally present in some foods (available as a dietary supplement) and a prescription medication. Cause of Vitamin B12 contains the mineral cobalt, compounds associated with Vitamin B12 activity are called as cobalamins.

From different forms of Vitamin B12 Methylcobalamin and adenosylcobalamin are the metabolically active forms. However, hydroxycobalamin and cyanocobalamin are other forms of the vitamin B12, which are biologically active after they are converted to methylcobalamin or adenosylcobalamin.

Vitamin B12 is essential for the development, myelinogenesis (process of forming myelin around axons of neurons), function of the central nervous system, healthy red blood cell formation and in DNA synthesis. Vitamin B12 serves as a cofactor for two enzymes: methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase is responsible for the conversion of homocysteine into the essential amino acid methionine.[5][6]

Breaking down the structure of Vitamin B12 unveils a three part arrangement of molecule. At the centre, there exists a modified tetrapyrrole engaging a central cobalt ion. This ring is unusual because it has gone through a ring-contraction process. As a result, one of the bridging carbon atoms that traditionally connects the four pyrrole rings has been removed.

This change creates a smaller, uneven ring called a corrin, which is different from the rings found in heme and chlorophyll. This ring structure is known as a corrin. The molecule also has a nucleotide loop with an unusual base which termed dimethylbenzimidazole (DMB) in the context of Vitamin B12. Attached to one of the corrin ring's side chains through an aminopropanol linker, the nucleotide loop extends below the plane of the corrin ring. This strategic arrangement allows the DMB base to function as a secondary ligand for the cobalt ion. Lastly, the third part is the upper ligand, which also attaches to the cobalt ion.

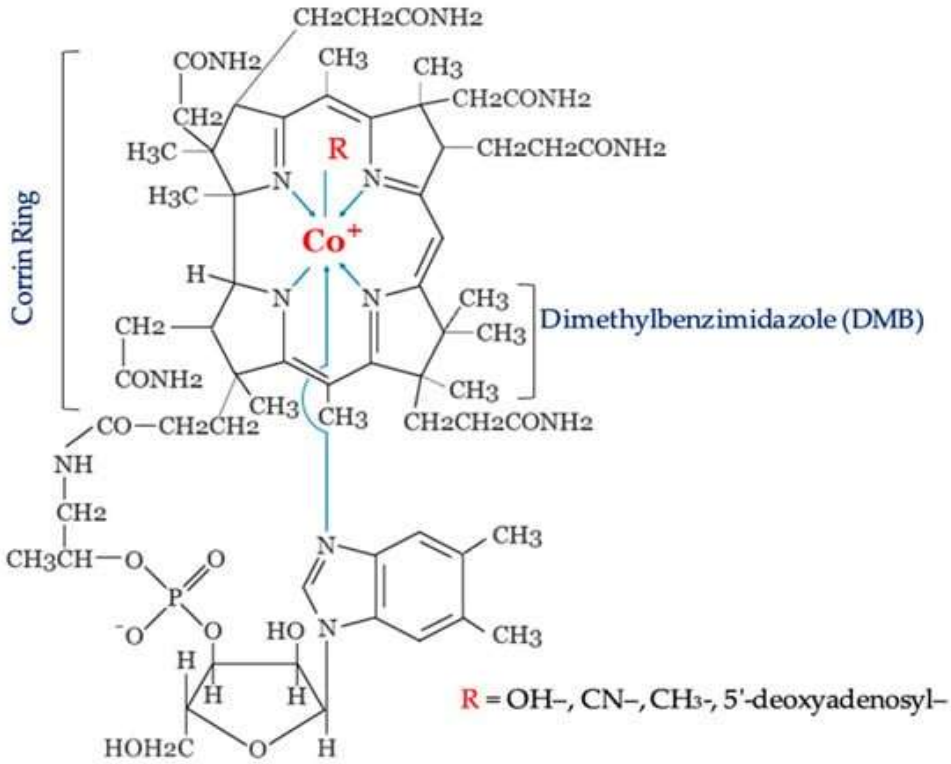

Fig 2. Chemical structure of VB_{12}. (Co^+ is central cobalt ion linked to the upper ligand (R)[52]

Within Vitamin B12, the upper ligand can take different biochemical forms like, cyanide, adenosyl, methyl and hydroxyl. From that adenosylcobalamin, cyanocobalamin, methylcobalamin, and hydroxycobalamin are the primary cobalamin compounds of Vitamin B12.
Addition of the "cyano" group in Vitamin B12 arises from the manner in which the molecule is isolated, involving the introduction of cyanide as an aid for its purification and extraction. This process finally yields cyanocobalamin as the dominant form of cobalamin.[7][8]

1.3 PATHOPHYSIOLOGY OF VITAMIN B12:-

In humans, uptake of Vitamin B12 is a multiple stepped process, which is begin from the stomach with the release of Vitamin B12 from food cause by the gastric juices. This free Vitamin B12 binds to Haptocorrin (HC) (also named R-protein or Transcobalamin I), which is secreted by the oral mucosa. The Haptocorrin protein has a glycosylated structure which provide resistance to low pH, and helps to protecting Vitamin B12 from harsh gastric conditions.
When Vitamin B12-Haptocorrin complex is enters in the intestine this complex undergoes degradation in the duodenum. In duodenum Pancreatic proteases break down the Haptocorrin and the resulting free Vitamin B12, this free Vitamin B12 bind with Intrinsic Factor (IF) to form a complex. In the ileum, this new complex is recognized by the cubam receptor on enterocytes, which leads to its absorption into the cell. The complex is then sent to lysosomes for processing, while the cubam receptor returns to the cell surface for reuse.
Once the Vitamin B12- Intrinsic Factor complex reaches the lysosomes, Intrinsic Factor undergoes degradation and release the free Vitamin B12, which is then actively transported into the cytoplasm with the help of two transmembrane proteins LMBRD1 and ABCD4 genes.[9][10][11]
In the cytoplasm, free Vitamin B12 leaves the cell and enters the bloodstream, via active transport by a specific protein (mediated by multi-specific membrane transporter, multidrug-resistant protein 1, MRP1/ABCC1) or by passive movement.

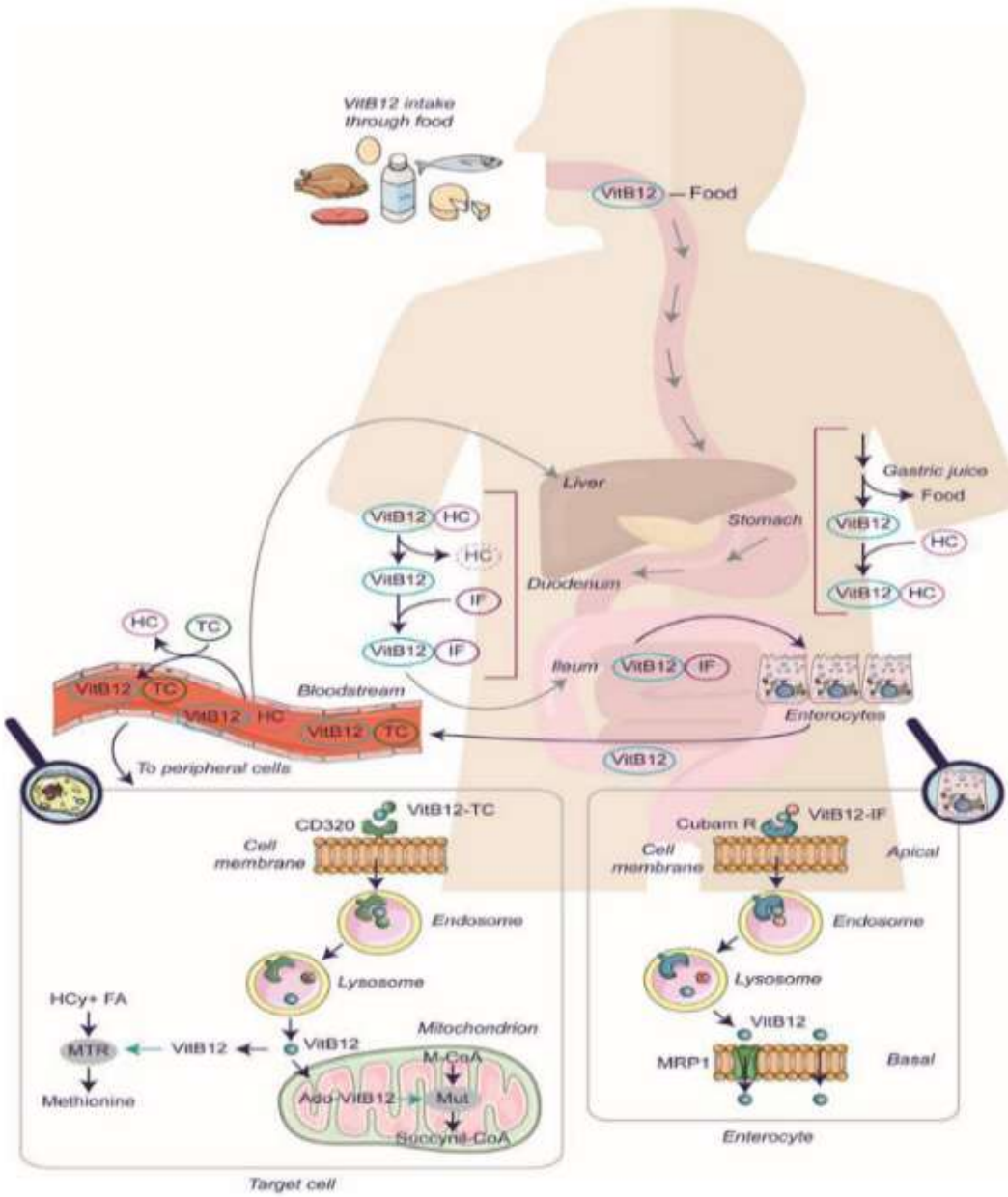

Fig 3. Diagram illustrates the key mechanisms (absorption, storage, and intracellular metabolism) of Vitamin B12 in human beings.[51]

In the blood, Vitamin B12 can bind with varying degrees of affinity to the known carriers, Transcobalamin (TC) (named Transcobalamin II and encoded by TCN2 gene) and Haptocorrin. Holotranscobalamin is form when Vitamin B12 bind with the Transcobalamin, this complex form (Holotranscobalamin) represents the circulating and bioavailable form of Vitamin B12. It is absorbed by the peripheral cells through endocytosis this process facilitated by the receptor CD320 (also known as Transcobalamin II Receptor).

Although a mechanism similar to what occurs in enterocytes takes place in peripheral target cells. Following receptor-mediated endocytosis, within the lysosome, Transcobalamin is degraded and the CD320 receptor is recycled to the plasma membrane. Cause of this Vitamin B12 is no longer complexed to protein and can be enter into the cytoplasm through the lysosomal transporters for utilization by some enzymes like, MTR and MUT and may even be exported. [12][13]

1.4 VIT B12 DEFICIENCY:-

Vitamin B12 deficiency has 4 primary etiologies:

Autoimmune: Pernicious anemia is an autoimmune disorder where antibodies to Intrinsic factors are produced. These Anti-intrinsic factor antibodies bind to and inhibit the action of intrinsic factors, which prevents the absorption of B12 in the terminal ileum.
Malabsorption: Intrinsic factors is generated by the parietal cells present in the stomach. So, some patients with a history of gastric bypass surgery may be at risk for occurring a B12 deficiency because their new nutrient pathway bypasses the site of intrinsic factor production. For that patients who produce normal intrinsic factor, any damage to the terminal ileum, like surgical removal cause of Crohn's disease, will affect the absorption of B12 and may cause Vitamin B12 deficiency. Likewise other damage to the small intestine, such as inflammation from celiac disease or infection cause by the tapeworm Diphyllobothrium latum, may also cause a Vitamin B12 deficiency.
Dietary insufficiency: Excess Vitamin B12 is stored in the liver. So, patients with the strict vegan diet for approximately 3 years may develop a Vitamin B12 deficiency due to lack of dietary intake of Vitamin B12.
Toxin exposure: Vitamin B12 deficiency also caused by exposure to the nitrous oxide and neurologic symptoms. Metformin treatment can cause B12 deficiency. [14]

2.0 TREATMENT FOR VITMINE B12 DEFICIENCY:-

2.1 FROM DIETARY ROUTE(VEG):-

Vegetarian diets are usually have large amount of carbohydrates, polyunsaturated fatty acids, dietary fibers, carotenoids, folic acid, Vitamin C, Vitamin E, and magnesium (Mg), but these diets have very low amount of proteins, saturated fatty acids, *n*-3 polyunsaturated fatty, Vitamin A, Vitamin B_{12}, Vitamin D_3, zinc, iron, and calcium.
In particular, Vitamins A, Vitamin B12, and Vitamin D_3 are present in large amount in animal-derived foods, while, Vitamin D_2 (ergocalciferol) and provitamin A (β-carotene) are present into mushrooms and vegetables.
All Vegetarians are at risk of Vitamin B12 depletion or deficiency regardless of their demographic groups, where they live, age or type of vegetarian diet they consume.
Various types of milk content very low (approximately 0.3–0.4 μg/100 g) amount of Vitamin B12 and noticeable losses of Vitamin B12 occur during the processing of milk. Approximately 20%–60% of the Vitamin B12 that is initially present in milk is recovered in cottage cheese, hard cheese, and blue cheese.
Many researchers tried to develop Vitamin B12 enriched vegetables by giving treatment with a solution that contains high levels of Vitamin B12. From this method significant increase in the plant Vitamin B12 contents are recorded, thereby that Vitamin B12 enriched vegetables may be beneficial to vegetarians. But, this artificial method of increase Vitamin B12 level in vegetables may not appropriate idea for vegetarians point of view.
The Vitamin B12 contents of soybeans are low or undetectable. But, fermented soybean product food called tempe have a good amount of Vitamin B12 (0.7 to 8.0 μg/100 gm). Bacterial contamination during it's production might be the reason for it's increased Vitamin B12 content of tempe. Other fermented soybean products contain small amounts of Vitamin B12.
Asparagus, broccoli, Japanese butterbur, mung bean sprouts, tassa jute, and water shield have only small amounts of Vitamin B12. Vitamin B_{12} is also found in various types of tea leaves like other source it's amount is very low (approximately 0.1 to 1.2 μg Vitamin B12 per 100 g dry weight).
In European countries wild edible mushroom species are very popular between vegetarians. In the dried fruiting bodies of porcini mushrooms (Boletus sp.), parasol mushrooms (Macrolepiota procera), oyster mushrooms (Pleurotus ostreatus), and black morels (Morchella conica) zero or less levels (approximately 0.09 μg/100 g dry weight) of Vitamin B12.
Various types of edible algae are consumed worldwide as food sources. Considerable amounts of Vitamin B12 (approximately 63.6 μg/100gm and 32.3 μg/100 gm) present into the dried green laver (Enteromorpha sp.) and purple laver and also they are the most widely consumed edible algae.[15]

2.2 FROM DIETARY ROUTE(NON-VEG):-

From the most nutrient packed foods organ meats are one of them. The liver and kidneys, especially from lamb are have high amount of vitamin B12. Higher amount of vitamin B12 is present in the beef or veal, but lamb liver have more amount of Vitamin B12 than beef or veal.

Sea food also have good amount of Vitamin B12, like Clams have good amount of Vitamin B12 which are chewy shellfish that are very small sized, but they are also packed with other essential nutrients. This mollusk is a good and healthy source of protein and also has very high concentrations of Vitamin B12. From 20 small clams (approximately 190 grams) you can get about 7000% of Daily Value.

Sardines have good amount of almost every single nutrients cause of this they are especially nutritious. 150 gram of drained sardines provides 554% of the Daily Value for vitamin B12.

From the excellent sources of Vitamin B12 beef is one of the most perfect source. About 190 grams of beef (grilled steak) provides 467% of the Daily Value for vitamin B12.

Another good source is Tuna. Tuna fish have a good amount of nutrients, like protein, minerals and vitamins. This nutrients are present in the muscles which are present under the skin, which are called as dark muscles. And Tuna have high level of vitamin B12. Tuna mostly consumed in canes and canned tuna also have good amount of vitamin B12. 165 grams of canned tuna contains 152% of the Daily Value of Vitamin B12.

Rainbow trout is a good source of Vitamins B, essential fats, and proteins. 100 gram of trout fillet has about 312% of the Daily Value for vitamin B12 and also has 1,171 mg of omega-3 fatty acids.

Salmon is another good source of nutrients. Salmon has higher amount of omega-3 fatty acids and Vitamins B. 178 grams of salmon (cooked) contains 208% of the Daily Value for vitamin B12 and 4,123 mg of omega-3 fatty acids.

Eggs are also one of the great source of protein and Vitamins B (like Vitamin B2 and Vitamin B12). 100 grams of eggs source nearby 46% of the Daily Value for vitamin B12 and provide Vitamin B2 around 39% of the Daily Value. Research has shown that higher level of Vitamin B12 present in the egg yolks than egg whites. Also Vitamin B12 present into the egg yolks is easier to absorb. So, eating whole eggs instead of only egg whites is recommended.[6][16]

2.3 FROM OTHER SUPPLIMENTS:-

The term vitamin B12 includes a number of chemical compounds with vitamin-B12 activity in humans and those compounds contain a common corrinoid group, centered on the mineral cobalt and various ligands, such as cyano, methyl, adenosyl, and hydroxyl ligands.

Cyanocobalamin	Hydroxocobalamin
Methylcobalamin	Adenosylcobalamin

Cyanocobalamin (CNCbl) is most commonly used synthetic form due to high stability and low cost; found in many supplements and fortified foods. Converted in the body to active forms (adenosyl- and methylcobalamin), but conversion may be less efficient in people with genetic differences. Higher urinary excretion and lower storage compared to natural forms.

Hydroxocobalamin (OHCbl) Intramuscular injections are standard, especially in Europe, to treat severe deficiency and pernicious anemia. It has high plasma protein affinity and a longer half-life, allowing less frequent dosing than cyanocobalamin. Also used as first-line for cyanide poisoning, because it converts cyanide into non-toxic cyanocobalamin for excretion.

Methylcobalamin (MeCbl) is Bioactive coenzyme form, directly used by enzymes, notably methionine synthase in the cytosol. Some studies show greater tissue retention and potentially better utilization than cyanocobalamin. Generally considered equivalent in efficacy to other forms for deficiency correction, with no strong evidence favoring one over another.

Adenosylcobalamin (AdCbl) is a mitochondrial coenzyme form, essential for methylmalonyl-CoA mutase. Predominant form in muscle/meat (68%), whereas methylcobalamin is more abundant in milk and eggs. Supplementation may support energy metabolism, especially in conditions like chronic fatigue.[17]

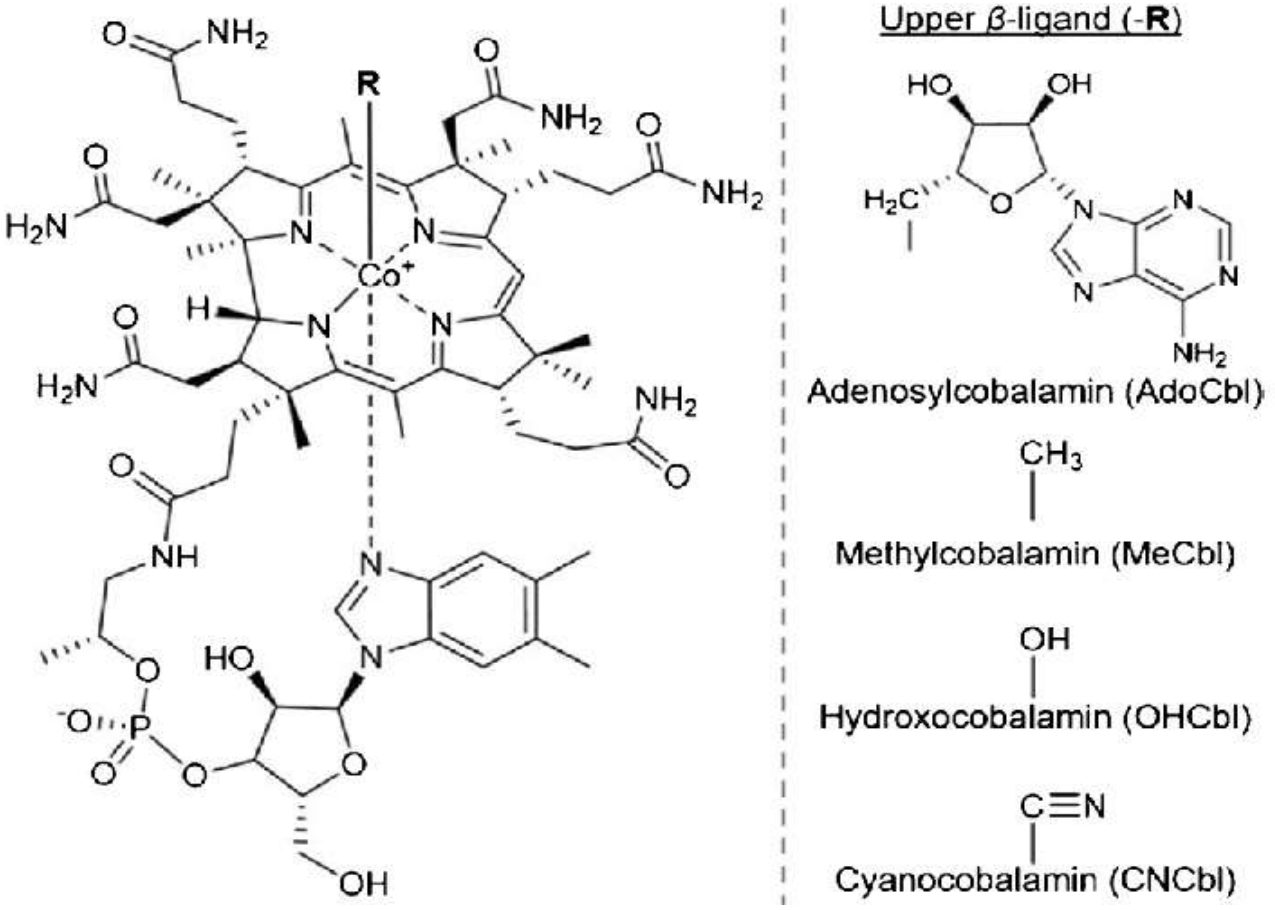

Fig 4 Chemical structure of cyanocobalamin, hydroxocobalamin, adenosylcobalamin and methylcobalamin[53]

3.0 ABOUT DRUGS & DRUGS DELIVERY:-

The intramuscular route of vitamin B_{12} administration has gained wide acceptance due to its quite constant bioavailability (10% of the injected dose) in comparison with the variable bioavailability of the oral route, which depends on various transporters. However, after the discovery of passive, transporter-independent vitamin B_{12} absorption, the effectiveness of its oral applications has been increasingly re-evaluated. Thus, high vitamin B_{12} doses (1000–2000 µg of CNCbl) are usually administered to ensure sufficient absorption to meet daily needs, even in the absence of transporter-mediated absorption.

The main conclusion of these studies, as well as of a Cochrane review on the efficacy of the oral versus the intramuscular route of vitamin B_{12} administration for the treatment of its deficiency, is that both application routes are similarly safe and effective in normalizing vitamin B_{12} serum concentrations. These studies also highlight the main benefits of oral over the intramuscular route of vitamin B_{12} application, such as reduced patient injection-related discomfort and increased patient convenience, reduced risk of bleeding in anticoagulated patients, and reduced number of hospital visits.

In addition to these advantages, the use of orally administered vitamin B_{12} also results in a significant reduction in healthcare costs. Therefore, some countries, such as Sweden and Canada, have implemented the routine use of oral vitamin B_{12} for deficiency treatment, and others, such as the UK, consider the use of oral vitamin B_{12} acceptable for the treatment of asymptomatic deficiencies, as a maintenance treatment, or for the prevention of vitamin B_{12} deficiency. In addition to intramuscular and oral vitamin B_{12} administration, new routes, such as sublingual and nasal administration, have recently become available. In addition to the obvious advantages over the oral and intramuscular routes (patient convenience and good adherence, safety, cost-effectiveness), sublingual and nasal treatments also provide a promising alternative for specific populations (infants, children, and the

elderly) and patients with some specific conditions (swallowing disorders or malabsorption due to intestinal surgery, inflammatory intestinal diseases, or short bowel syndrome), for which oral and intramuscular routes are less appropriate.
Their efficacy has been extensively evaluated in recent years and has been demonstrated to be comparable to those of oral or intramuscular vitamin B_{12} administration. Namely, the administration of sublingual vitamin B_{12} was shown to be comparable or even superior to the intramuscular route in infants, children, adults, and some specific population groups (patients with type 2 diabetes treated with metformin, and vegans and vegetarians). Similarly, nasal vitamin B_{12} has a demonstrated comparable efficacy to intramuscular vitamin B_{12} in children, adults and the elderly.[18]

3.1 ADVANTAGE OF NASAL OVER OTHER ROUTE

The administration of intramuscular injections of Vitamin B12 is inconvenient for patients, primarily due to the pain at the injection site and the necessity for frequent visits to healthcare professionals. Oral Vitamin B12 formulations do not consistently raise the amount of Vitamin B12 in the body, even after extended treatment. To overcome the limitations of current Vitamin B12 supplementation options, alternative formulations like nasal spray and sublingual tablets (novel Methylcobalamin nasal spray and Methylcobalamin sublingual tablet) have been made available.
It's proven by some study that nasal delivery for vitamin B12 drug is quickly and significantly absorbed after intranasal administration, while the absorption from the sublingual tablet is very low. Vitamin B12 is stored in the liver, and adequate levels therein decrease the likelihood of Vitamin B12 deficiency. However, in Vitamin B12-deficient patients, Liver stores get depleted, and to replenish the stores, high levels of Vitamin B12 are required.

High Vitamin B12 levels achieved by nasal delivery of B12 are adequate to replenish the liver stores and correct the deficiency, as well as provide sustained level. So nasal B12 demonstrated rapid and predictable absorption of Vitamin B12 required to replenish liver stores and hence effectively treats Vitamin B12 deficiency.[19]

The FDA label for Nascobal® states that after a month of weekly dosing, intranasal Vitamin B12 achieved significantly higher serum levels compared to intramuscular administration, indicating that nasal delivery may be more effective in certain maintenance scenarios even though intramuscular administration offers higher single-dose bioavailability.[20]

3.2 STABILITY CHALLENGES AND DEGRADATION OF VITAMIN B12 FORMS

The stability of vitamin B12 is influenced by various environmental and chemical factors, and other forms of this vitamin display different levels of sensitivity. Even though OHCbl, AdoCbl, and MeCbl are gaining popularity due to their progress in food, supplement, and pharmaceutical applications, CNCbl, the most stable form, remains widely used in supplementation compared to the other forms of vitamin B12.
Vitamin B12 is an essential nutrient, but it is prone to photolytic, hydrolytic, oxidative, and thermal degradation. Some of the byproducts of these degradation reactions still retain biological activity and influence health. Several factors can aggravate the instability. They are as follows: [20]

- Temperature
- pH of the Solution
- Light Exposure
- Oxidising and Reducing Agents
- Presence of Reducing Sugars
- Drug and Vitamin Interactions

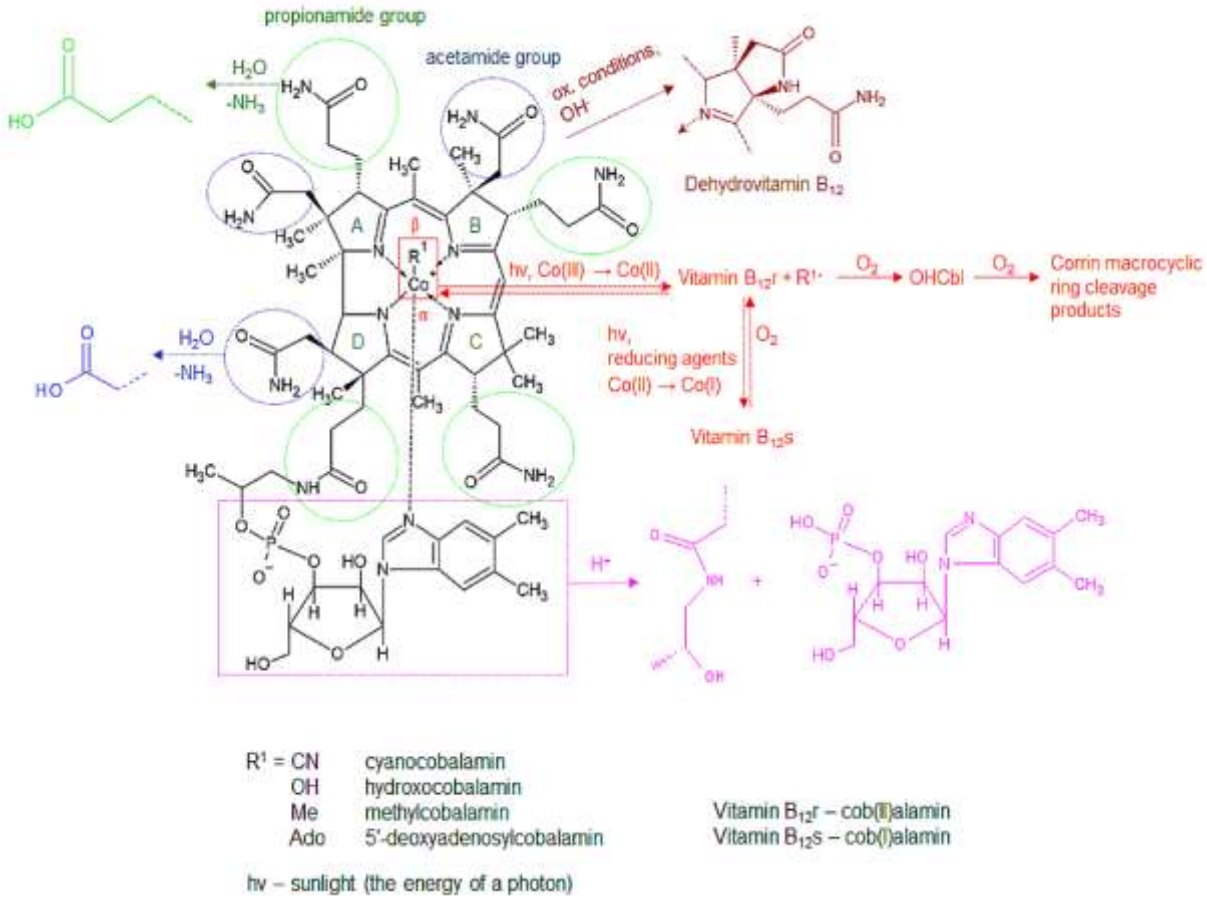

Fig 5 Typical degradation reactions of vitamin B_{12}[18]

4.0 STABILITY PROFILE AND DEGRADATION OF HYDROXOCOBALAMIN

Hydroxocobalamin is a precursor of the active coenzymes of vitamin B12, methylcobalamin, and adenosylcobalamin. These derivatives serve as essential cofactors in key biochemical pathways, including DNA and amino acid synthesis, metabolism of fatty acids, and neurological health maintenance. During the early stages of life, hematopoiesis and nervous system development are supported by methylcobalamin, whereas adenosylcobalamin contributes to processes that are fundamental for myelin synthesis, such as carbohydrate, fatty acid, and amino acid metabolism. Methylcobalamin acts as a cofactor for methionine synthase, an enzyme that is responsible for the conversion of homocysteine to methionine. This reaction is crucial in the synthesis of purines and pyrimidines, which are essential for DNA synthesis and the formation of red blood cells.

Hydroxocobalamin clinically serves as an antidote for cyanide poisoning. The toxic effects of cyanide are exerted because of its binding to cytochrome c oxidase, an essential enzyme in the electron transport chain, which thereby leads to the inhibition of ATP production and ceases the utilization of cellular oxygen. The cobalt compounds present in hydroxocobalamin bind with cyanide and form cyanocobalamin, which is then eliminated by renal excretion, preventing the lethal inhibition of cellular respiration.

Hydroxocobalamin in circulation binds to plasma proteins, primarily transcobalamin-II, which mediates its transport to tissues. After absorption, vitamin B12 is mainly stored in the liver, where it undergoes recycling through the enterohepatic circulation. It is excreted predominantly through feces; however, administering it parenterally may lead to elevated serum levels, with excess vitamin being cleared by the kidneys.

In comparison to cyanocobalamin, hydroxocobalamin exhibits stronger plasma protein binding, which prolongs its half-life and sustains serum concentrations. Hydroxocobalamin readily transforms into methylcobalamin and adenosylcobalamin, unlike cyanocobalamin, which must undergo cyanide group removal before its conversion to active enzymes. Although cyanocobalamin is more cost-

effective and pharmaceutically stable, hydroxocobalamin is considered more efficient physiologically due to its easy metabolic activation and superior systemic retention.

From a stability perspective, cyanocobalamin remains the most stable form of vitamin B12, particularly under conditions of heat and light exposure. This enhanced stability explains the widespread use of cyanocobalamin in supplements where a longer shelf life is crucial. On the other hand, hydroxocobalamin is less stable as an aqueous formulation under light exposure, although its stability can be improved by incorporating appropriate buffer systems.[21][22][23]

4.1 ADVANTAGES OF THE INTRANASAL ROUTE OF ADMINISTRATION OF HYDROXOCOBALAMIN

The intranasal route offers an alternative approach that is clinically valuable for patients who are unable to absorb vitamin B12 through the gastrointestinal tract. The nasal pathway bypasses the gastrointestinal barriers entirely, unlike other oral administrations that rely mainly on factor-mediated active transport. Thus, hydroxocobalamin is directly absorbed in rapid systemic uptake via highly vascularized nasal mucosa. Clinical studies stipulate that hydroxocobalamin administration through the intranasal route achieves a therapeutic serum concentration comparable to, and in some cases exceeding, those attained via intramuscular injection.

Hydroxocobalamin, due to its strong protein-binding capacity, prolonged tissue retention, and longer plasma half-life compared to cyanocobalamin, is more suited for intranasal delivery. These pharmacokinetic benefits enable less frequent dosing while maintaining stable serum concentrations, which makes it more efficient for chronic management of vitamin B12 deficiency. The intranasal hydroxocobalamin efficacy is supported by evidence showing that doses of up to 1500 µg produce a significant rise in serum B12 levels in both elderly and deficient populations.

Additionally, intranasal formulations offer advantages that enhance long-term patient adherence. Being non-invasive, painless, and eliminating the inconvenience associated with repeated intramuscular injections makes intranasal hydroxocobalamin advantageous, especially for elderly patients and those requiring sustained therapy, as it combines efficacy with patient compliance and comfort.[24][25][26]

4.2 COMPARISON OF B12 FORMS FOR NASAL DELIVERY

Parameter	Hydroxocobalamin (OH-Cbl)	Cyanocobalamin (CN-Cbl)	Methylcobalamin (Me-Cbl)
Plasma half-life	6-9 days	24-48 hours	~24–48 hours
Protein binding	High (90%)	Moderate(~50–60%)	Moderate (~60%)
Conversio to active form	Direct precursor of active forms	Requires conversion	Already active
Stability	Less stable (light/pH sensitive)	Most stable form	Moderate stability
Suitability for nasal route	High(better retention and longer systemic effect)	Moderate (shorter-effect)	Moderate

Comparison of the vitb12 forms[5][25][27][28]

4.3 PROBLEMS RELATED TO HYDROXOCOBALAMIN

4.3.1 CAUSE BY TEMPERATURE

CNCbl deterioration in aqueous solution after 24 h and 48 h of storage at 60 °C (5.3% and 8.6% deterioration, respectively), which did not take place at room temperature (<1% deterioration after 48 h) where OHCbl is documented to be stable in a reconstituted solution for injection for up to 6 h at 40 °C. Nevertheless, the literature findings regarding the comparative thermal stability of OHCbl are somewhat inconsistent. Accordingly, Mander et al. indicate that the effect of temperature on the stability of OHCbl mirrors that on CNCbl and MeCbl, producing a similar rate of degradation under equivalent conditions. [29][30][31]

4.3.2 CAUSE BY PH OF THE SOLUTION

Considering the liability of vitamin B_{12} to both acidic and alkaline hydrolysis, the effect of pH is important for its stability. Different vitamin B_{12} forms are generally unstable in both strongly acidic or alkaline media. Degradation of CNCbl takes place in highly alkaline environments. In particular, after 48 hours of room temperature storage, CNCbl was completely destroyed in 0.1 M NaOH (85% loss after 1 hour at 80°C), whereas under the same conditions of concentration, storage time, and temperature, 11.2% degradation occurred in 0.1 M HCl (61% degradation after 1 hour at 80°C). However, after being stored at ambient temperature for 24 hours (20% at pH 3.0 and 24% at pH 9.0), OHCbl was evaluated at pH 3.0, 4.5, 8.0, and 9.0 in comparison to CNCbl. [29][32][33]

4.3.3 CAUSE BY EXPOSURE TO LIGHT

From the all forms of Vitamin B12, particularly MeCbl and AdoCbl, are vulnerable to photodegradation. Findings indicate that each hour of sunlight or UV exposure leads to roughly 20% CNCbl decomposition. CNCbl shows markedly lower susceptibility to photolysis than OHCbl and MeCbl (4% CNCbl degradation after 60 min of UV exposure compared with 28% for OHCbl and 39% for MeCbl in aqueous solutions under the same conditions). [33][34]

4.3.4 CAUSE BY PRESENCE OF OXIDIZING AND REDUCING AGENTS

Vitamin B12 exhibits instability when exposed to both oxidizing and reducing agents. Consequently, its incompatibility with ascorbic acid is the most widely recognized. More broadly, reducing agents such as ascorbic acid, reducing sugars, thiols, formaldehyde, mercaptide ion, NaHSO3, or FeSO4 usually induce reduction of the cobalt ion from Co(III) to Co(II), producing vitamin B12r by cleavage of the β-ligand. In air, the vitamin B12r generated in this way is oxidized to OHCbl. As with the oxidation of other vitamin B12 forms, in some conditions (e.g, low oxygen levels and presence of hydrogen peroxide),Vitamin B12r oxidation may also lead to irreversible cleavage of the corrin ring.. To maintain the oxidative stability of vitamin B12 active ingredients (CNCbl, OHCbl, and MeCbl) and their preparations, regulatory authorities advise storage in airtight containers.[23][35][36][37]

5.0 METHOD TO OVERCOME PROBLEMS RELATED HYDROXOCOBALAMIN

The encapsulation of VB_{12} holds significant scientific merit, primarily stemming from its susceptibility to environmental degradation and the intricate physiological dynamics underlying its absorption. VB_{12} is notably labile, subject to degradation in the presence of factors like light, heat, oxygen, and moisture. Encapsulation provides a protective microenvironment that shields the vitamin from these detrimental influences, thereby preserving its structural integrity and bioactivity.

The controlled release properties conferred by encapsulation are of paramount importance. By governing the release kinetics, encapsulation can synchronize the availability of VB_{12} with the physiological processes responsible for its absorption, facilitating optimized uptake and utilization. Where precise dosing and prolonged bioavailability are critical for ensuring therapeutic or nutritive efficacy.

Therefore, encapsulation strategies not only safeguard VB12 from degradation but also harness its delivery dynamics to align with its intricate metabolic pathways, substantiating its significance in diverse applications. Encapsulation techniques for VB12 encompass a diverse array of approaches, spanning both micro- and nanoencapsulation strategies. These methodologies capitalize on distinct mechanisms to confer protection, controlled release, and enhanced bioavailability of VB12.[38][39]

5.1 MIROENCAPSULATION

5.1.1 SINGLE-CORE MICROCAPSULES

Through controlled-release testing of samples both freshly prepared and after four months of storage, Carlan et al. done some wall materials performance evaluation which including sodium alginate, carrageenan, gum arabic, maltodextrin, modified starch, xanthan, and pectin for microencapsulation of VB12 (particle size 3.17–6.67 μm) using spray-drying. The spherical microparticles formed showed either smooth surfaces (in the case of sodium alginate, carrageenan, maltodextrin, and pectin) or rough surfaces (with the other polymers). These systems exhibited satisfactory storage stability (up

to 120 days) and enabled controlled release of VB12 over time, although the powder recovery was moderate (27–50%).

Coelho and co-workers employed electrospinning and spray-drying to design zein-based microstructures incorporating VB12. Electrospinning produced films, microbeads (around 3 μm), and fibrous structures, with encapsulation efficiencies (EEs) of 90, 91, and 100%, respectively. In contrast, spray-drying generated wrinkled-surface microparticles that provided higher product yields (67–83%) along with EEs ranging between 71 and 95%.

Sugandhi et al. developed spray-dried spherical VB12 microparticles (0.59 μm) for pulmonary administration using a mixture of pullulan (a mucoadhesive polymer) and bovine serum albumin (BSA) as encapsulating agents. When administered intratracheally to male Wistar rats, pharmacokinetic data showed enhanced permeability and a 4.5-fold improvement in in vivo bioavailability, while in vitro studies indicated a bioavailability of 64.1%.

In another approach, Vitamin B12 was combined into solid lipid microparticles (SLMs) via spray-chilling using formulations containing 0.1–1% Vitamin B12 and 0–5% soy lecithin. This encapsulation method, which avoids organic solvents and high temperatures, resulted in spherical microparticles with smooth morphology, good yields, stable encapsulation efficiency, and prolonged storage stability. Combining soy lecithin with spray-chilling markedly improved both the stability and controlled release of VB12.

Additionally, VB12 was encapsulated into W/O/W emulsions using a mixture of soybean lipophilic protein (SLP) and methyl cellulose (MC). The formation of a strong gel-like barrier on droplet surfaces increased the viscoelastic strength of the emulsions and maintained high encapsulation efficiency during storage. [40][41][42][43][44]

5.1.2 DOUBLECORE AND MULTIPLECORE MICROCAPSULES

Using spray-drying to co-microencapsulate vitamins B_{12} and D_3 into an optimal biopolymeric matrix comprising gum acacia, Hi-Cap® 100, and maltodextrin with a ratio of 38:60:2. The spherical micro-particles produced with a smooth surface could well maintain vitamins' stability with a slow release rate under the in vitro digestion conditions. A remarkably enhanced bioavailability of VB_{12} (151%) and vitamin D_3 (109%) compared to the control was reported. Interestingly, the in vivo studies in rats revealed a slower release rate and more superior absorption potential of encapsulated VB_{12} than vitamin D_3 in serum.

A stable double emulsion (~96% in a 30 d storage) containing highly nutritious ingredients (i.e., vitamins B_6, B_{12}, and C) was prepared using a two-step mechanical emulsification. An EE (75.0–99.3%) and encapsulation stability (74.0–95.9%) of all the bioactive compounds with controlled release during digestion were recorded.[45][46]

6.0 FUTURE PROSPECTS RELATED HYDROXOCOBALAMIN

Future in the research of hydroxocobalamin nasal forms we should see the integration of microencapsulation technologies with mucoadhesive polymers like chitosan to improve stability and extend residence time in the nose. Hydroxocobalamin's light, pH and oxidative sensitivity puts forward the need for protective encapsulation which in turn preserves potency through storage and application. We do see that intranasal hydroxocobalamin is a doable option which achieves therapeutic serum levels, what we do not have is a marketed nasal product. That said, use of encapsulation strategies with nasal delivery may present a controlled release feature, better patient compliance, and in turn better therapeutic results which is a very promising direction for future study.[25][47][48][49][50]

7.0 CONCLUSION

Using hydroxocobalamin via the intranasal route has potential as an intranasal vitamin B12 delivery system with better patient acceptance and absorption than the existing routes. It is further supported for nasal dosage forms due to hydroxocobalamin's better pharmacokinetics, including longer half-life and greater protein binding. But, critical issues around stability as a light-sensitive oxidizable drug,

flux pH environments, and drug pH remain. While nasal hydroxocobalamin is feasible as a dosage form, there are no products available on the market. Further research with an emphasis on formulation technologies, such as microencapsulation improved drug stability, is needed to fill this gap. Hydroxocobalamin nasal sprays could become practical, safe, and effective with a combination of clinical testing and innovative delivery systems for managing vitamin B12 deficiency.

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