EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 03
122-132
Year: 2017

VITAMIN B12 DEFICIENCY AND NOVEL DRUG DELIVERY STRATEGIES

[1]Bhagyesh Milan Pendse* and [2]Dr. Sujata Prahlad Sawarkar

[1]Research Scholar at SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Gate No.1, Mithibai College Campus, Vaikunthlal Mehta Road, Vile Parle (West), Mumbai, Maharashtra 400056.
[2]Associate Professor Department of Pharmaceutics, Dr. Bhanuben Nanavati College of Pharmacy. Address: Gate No.1, Mithibai College Campus, Vaikunthlal Mehta Road, Vile Parle (West), Mumbai, Maharashtra 400056.

***Corresponding Author: Bhagyesh Milan Pendse**
Affiliation: Research Scholar at SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Gate No.1, Mithibai College Campus, Vaikunthlal Mehta Road, Vile Parle (West), Mumbai, Maharashtra 400056. DOI: 10.20959/ejbps20173-2905

Article Received on 05/01/2016 **Article Revised on 25/01/2017** **Article Accepted on 06/02/2017**

ABSTRACT
Vitamin B12 (Cobalamin) is a vital micronutrient. Although, it is required in miniscule levels in the body, it performs many vital functions. It is found exclusively in animal sources with a very few plant sources available. Deficiency of Vitamin B12 is a very common cause for megaloblastic anaemia particularly affecting the elderly population and people on vegetarian diet. With very limited plant sources available, fortification of common food with Vitamin B12 supplements in form of Cyanocobalamin becomes the obvious choice for maintaining appropriate levels in vegetarian population to avoid deficiency. However, many challenges are posed when Vitamin B12 is given orally and intramuscularly. Thus, there is a continuous need for development of newer strategies to effectively deliver the appropriate levels of Vitamin B12. There are variety of causes for the deficiency which also includes certain drug induced causes. Many novel drug delivery strategies have been attempted to deliver cobalamin effectively viz. the sublingual route, intranasal route, transdermal route, and these drug delivery strategies have proven to give beneficial results in preclinical trials or in human subjects. This review article gives an overview of the sources, absorption, causes, chemistry, biochemistry, available treatments for deficiency and the recent methods that have evolved for mitigating the problem of Vitamin B12 deficiency.

KEYWORDS: Vitamin B12, Cyanocobalamin, transdermal, perineural, nanoparticles, microemulsions.

1. INTRODUCTION
Vitamin B12 is a vital micronutrient that is essential for reduction in size of erythrocytes which is required for transfer of Oxygen. It is also important for neuronal health[1] and it facilitates in DNA formation and energy generation.[2] The deficiency of Vitamin B12 causes megaloblastic anaemia as a primary disease along with disease of the nervous system like spina bifida, anencephaly.[3] The incidence of Vitamin B12 deficiency was reported for the first time in 1849 and up until 1926 it was a fatal deficiency. Post 1926, it was found that consumption of chicken or mutton liver can correct Vitamin B12 deficiency which reduced the frequency of mortality. Food cobalamin malabsorption is now considered as a primary cause for the deficiency.[4] With the establishment of biochemistry and metabolic studies available now, there is a change in the perspective for treating the deficiency.[2]

1.1 Sources of Vitamin B12
Vitamin B12 is naturally synthesized in the gastrointestinal tract of the animals by the action of some bacteria and it is then made available for absorption by the host.[5] The daily requirements of Vitamin B12 in normal healthy adults is about 1-2µg.[6] However, if normal healthy balanced diet is maintained the levels that are consumed are between 7-30µg.[7] The various sources of Vitamin B12 are summarized in Table 1.

Table 1: Source of Vitamin B12[5,8-10]

Sr. No.	Source	Vitamin B12 Level
1.	Mutton	5.11µg/300g
2.	Chicken liver pate	38µg per serve
3.	Chicken	1.3-1.9µg/300g
4.	Fish	13.3µg/300g
5.	Eggs	0.9-1.4µg/100g

1.2 Functions of Vitamin B12

The key functions of Vitamin B12 are highlighted in Table 2.

Table 2: Function of Vitamin B12[1,2,11-13]

Sr. No.	System/Organ/Cell	Function
1.	Cells	Acts as a cofactor for purine and pyrimidine synthesis
2.	Nervous System	Maintains the nerve tissue health and conduction velocity
3.	Cardiovascular System	Reduction in size of red blood cells to improve oxygen delivery
4.	Miscellaneous	Acts as an anti-oxidant due to its superoxide radical scavenging activity.

1.3 Chemistry and biochemistry of Vitamin B12

Vitamin B12 can be administered in 3 different forms viz. Cyanocobalamin, Hydroxycobalamin and Methylcobalamin. Cyanocobalamin is the most commonly used form. Cyanocobalamin compromises of a cobalt atom that is located centrally and is in direct conjugation with four nitrogen atoms which are equatorial and belonging to tetrapyrrolic corrin ring.[14] 5,6-dimethylbenzimidazole (DMB), a bulky base is co-ordinating with cobalt atom at the α-axial position and extends from one edge of the corrin ring. The most common form of Vitamin B12 used in vitamin supplements is the Cyanocobalamin form, which undergoes decyanation to release free cobalamin.[15] Several methyltransferases enzymes such as methionine synthase use methylcobalamin as a cofactor. Methionine is synthesised by the action of the enzyme methionine synthase in presence of methylcobalamin that serves as a methyl donor.[16] Methylcobalamin also acts as a cofactor for the ribonucleotide reductase which reduces the ribonucleotide di- or tri- phosphate into 2'-deoxyribonucleotide di- or tri- phosphate.[17] The biomarkers of Vitamin B12 which gives a direct indication about its levels are methylmalonic acid and homocysteine with the former being a metabolic indicator whereas latter acts as a deficiency indicator of cobalamin.[18,19]

1.4 Absorption of Vitamin B12

The non-vegetarian food is a rich source of Vitamin B12, however, it exists in bound form with the proteins. This bound form needs to be released to get absorbed and this process is done by the hydrochloric acid secreted by the gastric cells. This free form also called as cobalamin binds to R protein and then passes into the duodenum where it is released to its free form. This free form binds to the intrinsic factor (IF) which is a prerequisite for absorption. This cobalamin-IF complex is then absorbed in the presence of calcium in the distal part of ileum.[1,2] The receptor involved in this absorption is the IF-cobalamin ileal receptor.[5,20] The IF-cobalamin complex after reaching the ileal receptor is broken down and the free cobalamin binds to the holotranscobalamin or simply referred to as transcobalamin for absorption.[21] Transcobalamin is a carrier protein required for the transfer of Vitamin B12 to the various cells of the body. This transcobalamin-cobalamin complex is then transferred to all the tissues of the body.[22]

Kornerup LS et al[23] performed an experimental study using rat model to observe the absorption of different forms of cobalamin. In this study, they included Cyanocobalamin and hydroxycobalamin in free form, bound form and diluted with cow's milk. They concluded that liver and kidney accumulation as well as absorption profiles were identical irrespective of the form of Vitamin B12 delivered. The Figure 1 describes the absorption mechanism of Vitamin B12 from food.

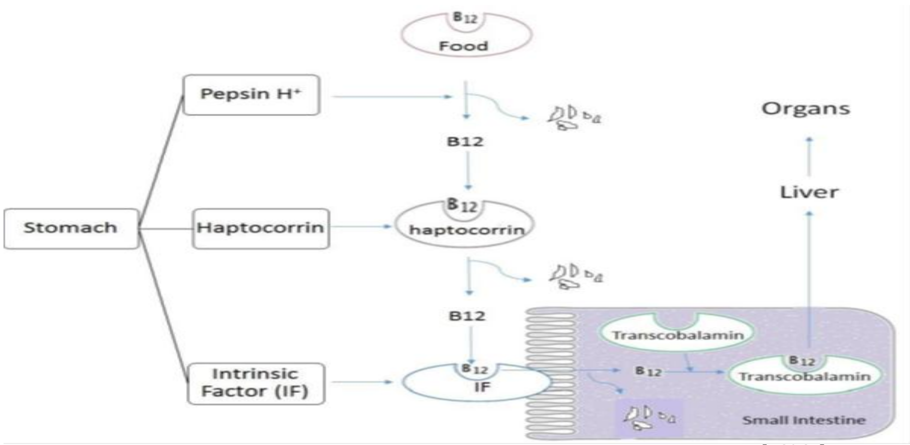

Figure 1: Absorption mechanism of Vitamin B12 from food[5,20,21]

1.5 Causes of Vitamin B12 deficiency

A patient is said to be Vitamin B12 deficient when the concentration of cobalamin in the serum is below 200 picogram/mL (pg/mL) as observed in two consecutive tests.[4,24] There is also a subclinical deficiency of Vitamin B12 that is highly prevalent in terms of the rate that it occurs in developing countries mainly concentrated in the geriatric and vegetarian population.[2]

Table 3 highlights the causes of Vitamin B12 deficiency

Sr. No.	Cause	Effect of cause
1	Food cobalamin malabsorption	Inability to absorb Vitamin B12 from food due to lower level or absence of intrinsic factor or gastric disturbances.
2	Drug induced	Long term use of Metformin may lead to Vitamin B12 deficiency
3	Dietary inadequacy	Inadequate intake of Vitamin B12 rich food specially in case of vegetarians.
4	Lower level or absence of Intrinsic factor	Intrinsic factor is an essential carrier for Vitamin B12. Its absence will lead to deficiency of Vitamin B12.
5	Gastrointestinal disorders	Gastritis or gastric ulcers can lead to improper absorption and hence deficiency.
6	Genetic defects	The genes responsible to produce transcobalamin or intrinsic factor may be mutated leading to deficiency of Vitamin B12.

1.6 Diseases and symptoms of Vitamin B12 deficiency

The main diseases and symptoms of Vitamin B12 deficiency are summarized in the table 4.

Table 4: Disease/ symptoms of Vitamin B12 deficiency[2,3]

Sr.no	Diseases	Symptoms
1	Megaloblastic Anaemia	Improper reduction of size of RBCs leading reduced Oxygen carrying capacity
2	Anencephaly	Absence of major portion of brain, skull and scalp during the embryonic stage.
3	Obesity	Abnormal increase in the weight of individual.
4	Spina Bifida	Improper closure of the backbone and membranes around the spinal cord.
5	Encephalocele	It is a neural tube defect characterized by sac-like protrusions of the brain and the membranes that cover it through openings in the skull.

2. Current treatments available for Vitamin B12 deficiency

Vegetarians are more prone to develop Vitamin B12 deficiency due to absence of animal food in their diet.[2] Lacto-ovo vegetarians can meet their requirements with the egg content rich in Vitamin B12 in their diet. The strict vegetarians must rely completely on the foods fortified with Vitamin B12.[28] Current research findings have indicated that white button mushrooms and Korean purple laver (nori) contain Vitamin B12 in trace amounts.[29,30]

The current treatment regime focusses on oral Vitamin B12 fortified foods or supplements. The oral fortified foods consist of soy milk, yeast spread, vegetarian meat and soy based burgers and sausages.[28] In case of severe deficiencies, intramuscular Vitamin B12 formulations are available at the dose of 1mg/day for a week.[4,31,32] The general intake of the fortified food includes fortified soy milk (2 servings each of 250mL/day) or Soy based meat analogues (One serving/day).[28] In a survey, it was proven that fortified food was more useful as supplement than the prescription tablets and intramuscular injections.[28] The commonly used form of Vitamin B12 in supplements is Cyanocobalamin.[20]

In one study, absorption of Vitamin B12 when administered by intramuscular route was compared with oral route. In this study, 33 patients were involved with 18 patients receiving 2000mcg Cyanocobalamin (2 tablets each containing 1000mcg Cyanocobalamin) with breakfast daily for 4 months and 15 patients receiving 1000mcg Cyanocobalamin intramuscularly for 3 months on days 1,3,7,10,14,21,30,60,90. The results showed that serum cobalamin levels and haematological and neurological response obtained by oral therapy were comparable to those of the standard intramuscular therapy. However, to achieve equivalent levels of response, the oral dose required was twice as that of the intramuscular route.[6]

Oral route is preferred over the conventional intramuscular route as the latter is painful and requires medical assistance.[33,34] Also, the oral route of administration is non-invasive, cost effective way to achieve the treatment in an efficient manner.[35] Oral supplements are preferred traditionally due to their advantages. The pregnant and breast feeding vegan are on these supplementations to maintain optimum Vitamin B12 levels.[28] However, higher doses of Vitamin B12 administered by oral route show variable blood levels and this level can vary from individual to individual. This could be due to the fact that absorption of Vitamin B12 is dependent on intrinsic factor and thus the process is saturable. Hence, lower dose supplements are always preferred.

Victor H. et al conducted a study to observe the effects of Vitamin B12 wherein dose ranges of 0.1-0.5µg (lower dose) and 1-5µg (higher dose) were selected. The lower dose group showed absorption ranges of 52-97% and higher dose showed lower absorption with the 5µg dose showing only 28% absorbance. Hence, the dose selection is critical while selecting an oral supplement for Vitamin B12.[36] Hence, to summarize the available treatment ranging from oral route comprises of oral supplements and fortified foods and intramuscular route consisting of Vitamin B12 injections.

The main drawback associated with the conventional intramuscular therapy is poor patient compliance. The conventional oral route is associated with a high dose intake of Vitamin B12 out of which only 2-3% is absorbed. This leads to loss of the remaining dose of Vitamin B12 and also adds up to the total cost of the therapy.

3. Novel strategies of treating Vitamin B12 deficiency

Since the conventional therapy is associated with a number of drawbacks, there is an increasing demand for the development of a non-invasive delivery system for delivering Vitamin B12 safely and effectively that can provide uniform levels of Vitamin B12 in systemic circulations. Some of the novel strategies reported in literature is summarized in Figure 2.

Figure 2: Novel strategies for Vitamin B12 delivery[37-43]

3.1 Sublingual Delivery

The advantages of administering Vitamin B12 by sublingual route include patient compliance, suitable for patients who are unable to take oral medication[38, 44] and patients with short bowel syndrome.[37]

Sharabi A. et al performed a study to compare the effects of oral and sublingual routes for delivering cobalamin. The study was conducted wherein equal dose of Cyanocobalamin (500µg) was administered by oral, sublingual route. This dose was selected to restore cobalamin concentrations in patients with mild cobalamin deficiency. The study was conducted on 30 patients with cobalamin levels <138pmol/L. The groups randomly received either sublingual or oral Cyanocobalamin at the dose of 500mcg. The study was performed for a period of 8 weeks. The patients randomly received one sublingual tablet or one oral tablet each of 500mcg Cyanocobalamin or two tablets of Vitamin B complex each containing 250mcg of Cyanocobalamin along with 100mg thiamine and 250mg pyridoxine. The bloodcobalamin levels were comparable in case of oral and sublingual route. This also lead to the conclusion that 500µg of cobalamin sublingually was enough to treat cobalamin deficiency rather than the previously used doses of 1000-5000µg given by the oral route.[44]

In another case study reported by Kotilea K. et al, a 9 year old child with short bowel syndrome was given sublingual nuggets of Cyanocobalamin with dose of 1000µg/day for a month. Care was taken that the nuggets were not swallowed and not administered in case of dry mouth. Therapy was continued for one month and a stable concentration of serum cobalamin (i.e. 790pg/ml) was achieved. A very good patient compliance was observed and maintenance of this level was ensured using sublingual nuggets equivalent to 1000mcg Cyanocobalamin per day for 10 months. The disadvantage associated with sublingual delivery is that the patient should adhere to the treatment. This type of formulation is not suitable for new born infants or 1-2 years old children.[37]

Some of the marketed products for sublingual delivery of cobalamin include drops, fast dissolve tablets as mentioned in Table 5.

Table 5: Marketed Sublingual products

Sr. No.	Product Name	Dosage form
1.	Vegan B12	Sublingual fast dissolve tablets
2.	Spring valley	Sublingual drops
3.	Sundown sublingual dots	Lozenges

3.2 Transdermal Delivery

Amongst the various novel routes for treating deficiency of Vitamin B12, the transdermal route has been accepted widely due to many advantages like high patient compliance, non-invasiveness, bypassing the gastrointestinal degradation and availability of large surface area.[39,45] However, the major barrier to deliver drugs through the skin is the stratum corneum. Vitamin B12 is a water-soluble vitamin making it difficult to deliver via transdermal route as lipid soluble drugs are more readily delivered through the skin. To circumvent this barrier efficiently, many strategies have been attempted that are broadly classified as physical permeation enhancement techniques and chemical permeation enhancement techniques. Thephysical permeation enhancement techniques include iontophoresis and use of microneedle drug delivery.[39] The chemical enhancement technique makes use of chemical penetration enhancers that makes transient passages into the stratum corneum for easy transport of drugs.[39,45]

The most widely used enhancement technique is use of chemical enhancers however recently microneedles have been found to be effective to deliver Vitamin B12 via the transdermal route.[39,46,47] Microneedles have shown to show increased permeation many folds for drugs irrespective of their molecular weight and molecular size and their solubility and permeability characteristics.[48]

Yang Y et al performed a study to evaluate the effects of permeation enhancers on transdermal delivery of cobalamin. Cyanocobalamin was selected as the model drug. The transdermal therapy using penetration enhancement technique, where iontophoresis and microneedles technique were compared for effective delivery of Vitamin B12. The passive control study was initially performed in which freshly excised abdominal skin was cleaned carefully to remove excess fat. The cleaned skin was then mounted between the receptor and donor chambers. The skin was mounted in such a way that the stratum corneum layer was in direct contact with the test formulation. The donor chamber was filled with 1.3mL of the test formulation at different concentrations of 0.5,1,5 and 10mg/mL of Cyanocobalamin without any chemical permeation enhancer in phosphate buffer. 7mL of phosphate buffer (pH 7.4) maintained at 37°C was filled in receptor chamber. Samples of 500µL were withdrawn at appropriate time intervals for a period of 24 hours. The analysis of these samples was done using HPLC. Amongst the various concentrations of Cyanocobalamin that were tested for permeation, the cumulative release at the end of 24 hours was obtained to increase with increase in concentration with the maximum permeation observed in case of 10mg/mL. This formulation was then taken ahead to evaluate the effect of chemical permeation enhancers and physical permeation enhancers. The various permeation enhancers evaluated were phosphate buffer + 20% ethanol, phosphate buffer + 50% ethanol, 10% oleic acid + propylene glycol, ethanol (20%) + Oleic acid (10%) + Propylene glycol (70%) and 50% ethanol + 10% oleic acid + 40% propylene glycol. The studies indicated that amongst all the combination of permeation enhancers attempted to deliver Cyanocobalamin, better results were observed using the combination of 50% ethanol + 10% oleic acid + 40% propylene glycol. The amount of Cyanocobalamin permeated using this chemical penetration enhancerwas about 162-164µg/cm2 at the end of 24 hours with permeation enhancer combination comprising of 50% ethanol + 10% oleic acid + 40% propylene glycol.[39]

In the same experiment Cyanocobalamin permeation was evaluated using iontophoresis. Iontophoresis is an active permeation enhancement technique. A donor chamber containing 0.5 ml of Cyanocobalamin formulation (10 mg/mL) was mounted over the pre-treated stratum corneum. Silver electrode was used in the donor chamber and silver chloride electrode was placed in the receptor chamber. Anodal iontophoresis was performed wherein a current of 0.3mA/cm2 was applied for 4 hours. 300µL of sample was withdrawn at different time intervals over a period of 24 hours. The permeation level of Cyanocobalamin was observed to be 27.4 µg/cm2/hr, which was significantly higher than the passive control group. A 17-fold increase in the steady state flux of cobalamin was observed as compared to the passive control. When ethanol (20%) was used before applying iontophoresis, it led to further increase in penetration as compared to passive control but comparable to iontophoresis alone. When the formulation was administered in combination with the best permeation enhancer earlier (50% ethanol + 10% oleic acid + 40% propylene glycol) observed, increase in the permeation was observed as compared to the passive control but the results were comparable with iontophoresis alone. This suggested that there was no synergism in permeation of Cyanocobalamin between iontophoresis and use of chemical permeation enhancers.[39]

In the same experiment, Cyanocobalamin was administered as maltose microneedles to evaluate improvement in permeation of Cyanocobalamin. Maltose microneedles were attempted because they are soluble and have been used widely for transdermal delivery of many drugs.[48,49,50] In this case, skin samples were stretched on a flat platform. Microneedles were incorporated manually for microporation and held for about one minute. Calcein imaging studies were done to confirm that microporation has occurred. Microneedles were incorporated in the abdominal skin to give micropores and this skin was then sandwiched between donor and receptor compartments. Permeation studies were carried out as earlier for a period of 24 hours. Upon use of microneedles, the steady state flux of cobalamin was increased by 13-fold as compared to passive control and the cumulative release was 459.69µg/cm2. The best combination of penetration enhancer as mentioned above when tried before using microneedles showed no further improvement in the permeation of cobalamin.[39] Out of the three techniques attempted to improve permeation, maltose microneedles were most effective.

The disadvantages of transdermal delivery are difficulty to deliver ionic drugs (except for iontophoresis), a high dose cannot be delivered and high molecular weight drugs cannot be administered effectively.[51,52,53] Although, these are the disadvantages but cobalamin can be delivered successfully as it is excluded from most of the above listed disadvantages. The marketed product for transdermal delivery of Vitamin B12 is B12 patch which is manufactured by Vita Sciences.

3.3 Perineural Injections

Cobalamin therapy has proven in the past to be effective in treating peripheral neuropathy in animals.[54] Since oral delivery gives moderate levels of cobalamin, there is a necessity to find a novel delivery for treatment of peripheral neuropathy using cobalamin.

Development in the field of ultrasound has led to an increase of its use in peripheral nerve assessment. Using a similar technique, ultrasound guided peripheral nerve block can be used as a tool to deliver Vitamin B12 precisely around the nerve.[55] Chen CH et al discussed a case report where in a 37 year old patient was diagnosed with dropped foot. Using ultrasound, a focal peroneal nerve swelling was diagnosed at a level of popliteal fossa. On diagnosis of peroneal nerve swelling, 500µg of methylcobalamin was administered around the peroneal nerve under the guidance of ultrasound. The administration was done twice in an interval of two weeks. An improvement in the muscle power was observed within two weeks. The entire muscle strength was regained after 3 months of therapy. Ultrasound imaging for more than 3 months showed significant reduction in peroneal nerve swelling.[40]

The case report concluded that ultrasound guided perineural injections can provide basis for a novel way of treating peripheral neuropathy using cobalamin. Cobalamin was found to promote myelination and hence nerve transmission in the peripheral nerves.[55-59] It was then reported that high amounts of cobalamin can be delivered precisely and efficiently using ultrasound guided perineural injections. However, further clinical studies are needed to fix the exact dose of cobalamin that can be administered safely using perineural injection.[40]

3.4 Microemulsion

Microemulsions represent a class of dispersions which are thermodynamically stable and contain mixture of oil and water. The stability of microemulsions is attributed to the use of combination of surfactants and cosurfactants.[41,60,61] The various advantages provided bymicroemulsions are ease of preparation, high stability, increased drug solubility, improved bioavailability of both hydrophilic and lipophilic drugs and flexibility of administration through various routes efficiently.[60,61] Several studies have also proven efficacy of microemulsions for delivery of therapeutically active moiety via the transdermal route. This is attributed to the fact that microemulsion show better solubility and penetration enhancement.

Salimi A et al have reported a microemulsion formulation of Vitamin B12 which was intended to be administered by the transdermal route. The oils, surfactant and cosurfactant were selected on the basis of their Vitamin B12 solubilizing capacity, hydrophilic lipophilic balance (HLB) value and microemulsion forming ability. The various oils screened for optimum solubility of Cyanocobalamin were Mineral oil, Isopropyl myristate, Oleic acid and Labrafil M 1944 and their combination with Transcutol P (10:1). The saturation solubility was assessed in individual oils as well as combination with Transcutol P using UV method at 362nm wavelength. Oleic acid showed the highest solubilizing capacity (~1.07mg/mL). However, it was observed that when used in combination with Transcutol

P, the solubility of Cyanocobalamin in Oleic acid was improved (~7.53mg/mL). Hence, Oleic acid and Transcutol P (10:1) was selected as the oil phase.

This was followed by construction of a pseudoternary phase diagram. The water titration method was used for determining the volume of water that would give the desired microemulsifying range. Initially stock solution of Tween 80 and Span 20 as non-ionic surfactants at ratios of 1:1 and 3:1 were prepared. Propylene glycol was used as a cosurfactant. The formulations that were attempted by using these ratios were labelled as traditional formulation. Additionally, another stock solution of Stearylamine (cationic surfactant) and Labrafil (cosurfactant) in the ratio of 1:4 and 1:8 were prepared. The formulations that were attempted by using these ratios were labelled as novel formulation. Various ratios of oil and surfactant-cosurfactant were prepared in both the cases (novel and traditional) and titrated with water. Same procedure was repeated with 0.07% Vitamin B12. The percent compositions of all the samples attempted were calculated and pseudoternary phase diagram were constructed. Out of the four pseudoternary phase diagram constructed (two each of novel and traditional), the traditional formulation containing non-ionic surfactants with propylene glycol as cosurfactant showed the maximum water solubilizing capacity. However, higher microemulsion region was observed at higher levels of surfactant. The difference in traditional and novel formulations was the surfactants + cosurfactants (Smix) whereas the oil phase in both cases was the same. The Smix concentrations in the range of 20-90% and 5-70% showed the microemulsion region for traditional and novel formulations. The microemulsions were found to be stable at various storage conditions like 4°C, 25°C and 40°C. Droplet size analysis of the microemulsions showed that the novel formulation had a lower mean droplet size range (17nm-210nm) as compared to traditional formulation (49nm-209nm). Both formulations showed a polydispersity index of 0.5. The interfacial tension in both the formulations was in the range of 3.9-5mN/m.[41]

Although microemulsions offer a wide range of advantages there are certain disadvantages like a high concentration of surfactant and cosurfactant is generally needed for droplet stabilization and this may lead to toxicity, substances with high melting point cannot be solubilized efficiently, temperature and pH during delivery of microemulsions to patients is very critical as it may affect the eventual stability.[62,63]

3.5 Nanoparticles

Nanonization of any drug increases the efficiency of the drug to get absorbed and hence it shows better pharmacological potential.[64] The field of nanomedicine is rapidly evolving and hence it has also been tried with Vitamin B12 recently by Yarif I. et al, 2015. The technique used for nanonization of Cyanocobalamin included the sonication technique.[42]

Sonochemistry deals with the science and technology of using ultrasound waves for a variety of applications, one of which being preparation of nanoparticles and their fabrication.[42,65-67] The ultrasound works using the principle of acoustic cavitation.[42] The ultrasound wave acts on to a small bubble formed in the liquid medium that eventually grows and then collapses. The volume of bubble initially increases due to the entrapment of solid and solvent vapours which then collapses once reaching the maximum size. This collapse brings about the breakdown of many chemical bonds that gives rise to a great increment in the temperature temporarily. The increase in the temperature is thought to be of about the levels of 5000°C.[65] This increase in temperature causes size reduction and hence nanoparticles are formed.[42] Sonochemistry has already been used for the formation of nanoparticles of inorganic salts like sodium chloride, potassium iodide, potassium bromide and copper sulphate and the medium used for them were aqueous solutions of the respected salts.[68] Apart from this, nanoparticles of organic materials like amylase enzyme, tannic acid and RNA are also prepared.[69-72]

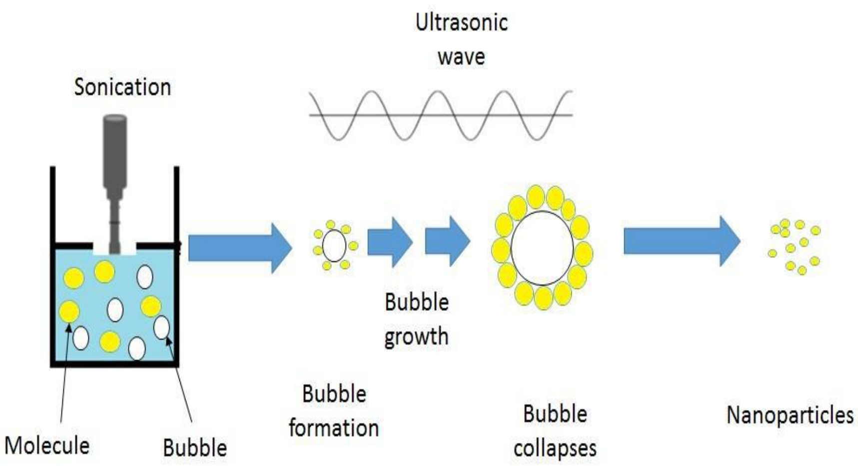

Figure 3: Acoustic cavitation[42]

Figure 3 represents acoustic cavitation. The dispersion is bombarded with ultrasonic waves which leads to bubble formation in the solution and eventual collapse to form the resultant nanoparticles.

The distinct advantages offered by use of nanoparticles are reduction in size increasing the effective surface area and hence also increases the activity of the molecules[42,73] and it also offers good penetration enhancing properties through the skin which acts as a semisolid matrix.[73]

Yariv I et al reported use of Cyanocobalamin in nanoparticulate form for enhancing its antioxidant property. As mentioned above sonication was the technique used for preparing nanoparticles. Aqueous solution of Cyanocobalamin was prepared in bulk at a concentration of 36.9μM. Vitamin B12 was obtained as a solution and diluted with double distilled water to get the appropriate concentration. This solution was then subjected to ultrasonic sound waves using Ti-horn, 20KHz, 100 W/cm2 at 25% efficiency for a time span of 10 minutes.

Size characterization was performed using a Malvern zetasizer. Sonication time directly affected the size reduction process as an average particle size of 179 nm ±27 nm and 124 nm ±19 nm was obtained at 3 and 5 minutes of sonication time respectively. Also antioxidant activity of nanoparticles of Cyanocobalamin was evaluated. Electron paramagnetic resonance (EPR) technique was used to measure the antioxidant property of Cyanocobalamin. In this test, the level of reactive oxygen species (ROS) was determined. The presence of hydroxyl free radical produced by Fenton reaction was determined in the presence of bulk Vitamin B12 and nanoparticles of Vitamin B12. The results showed that the hydroxyl free radicals produced with the use of Vitamin B12 nanoparticles were twice as low as produced with the use of bulk Vitamin B12. This proved the increase in antioxidant activity when in nanoparticles and was attributed to the increased surface area of the nanoparticles of Cyanocobalamin. Thus nanoparticles of Vitamin B12 were found to possess a higher biological activity as compared to the bulk form. The authors concluded that nanoparticles of Cyanocobalamin and other drugs can change the perspective of treatments for many diseases.[42]

3.6 Intranasal Delivery:[43]

Pharmacological agents that can be used to relieve from neuropathic pain are evaluated considerably but very little success has been obtained in this field.[74] Cobalamin therapy is known for its neuropathic treatment from a variety of conditions like diabetes neuropathy, carpal tunnel syndrome[74-76], it is proved by the study performed that Cyanocobalamin may also have a role in restoring the nerve function post therapy even in people who are not deficient. Ceib P et al performed an exploratory randomized clinical trial for evaluating the putative effects of intranasal cobalamin therapy in reducing the impairment caused from orthognathic surgery on large and small diameter nerve fibre function. Prior to the main clinical trials the authors demonstrated the tolerability of Vitamin B12 intranasal spray in healthy individuals that begun 2-3 weeks prior to the orthognathic surgery and continued for six months post-surgery. The spray was well tolerated with occurrence of only a rash without fever as an adverse event. The subjects were randomized based on the number of jaws operated (both jaws vs mandibular alone) as well as the addition of genioplasty (yes vs no).

In the study Nascobal®, a Cyanocobalamin intranasal spray which is prescribed for Vitamin B12 deficiency was evaluated. The nasal spray contained 2.3mL of a 500mcg/0.1mL solution of Cyanocobalamin with sodium citrate, citric acid, glycerine and benzalkonium chloride in purified water. The pH of the solution is adjusted between 4.5-5.5. The usual dose of 500mcg/spray (containing Cyanocobalamin) was administered. Twice weekly dosing was started 2-3 weeks before the orthognathic surgery and continued for 6 months post-surgery. Subjects receiving the spray were instructed about the activation and the use of intranasal spray. Subjects were observed and monitored for 30-minute post the first administration. Dosing was repeated twice weekly for about six month post the surgery. One group received the therapy while the other group did not receive any therapy. Serum B12 assays were performed for a period of six months post-surgery. The Vitamin B12 assays showed that there was a significant difference in the average Vitamin B12 levels between the two groups. The Vitamin B12 level was about 40% higher in the group receiving the spray during the surgery as compared to the group not receiving the spray (control group). The Vitamin B12 levels were about 35% higher in the group receiving the spray for six months post-surgery as compared to the control group. The result showed reduction in neuropathic pain for the group that received the treatment and desired therapeutic levels of Vitamin B12 were achieved successfully.[43]

CONCLUSION

Vitamin B12 deficiency continues to be a consistently rising problem even of the modern era. The deficiency continues to be a problem in vegetarians as they have negligible access to the vitamin and hence should rely on fortified foods as an alternate. The conventional techniques of fortification of food, oral supplements and intramuscular injections continue to dominate the therapy regime for correcting the deficiency. However, with advances in the techniques to deliver therapeutically active agents, delivery of Vitamin B12 is continuing to improve. There is still further scope for improvement in the field of delivering Vitamin B12 and alternate routes are continuously being reviewed to improve the therapeutic condition. The various approaches attempted for the improved delivery of Vitamin B12 are having their respective pros and cons. The transdermal, sublingual and intranasal delivery seems to be very promising for patients with a gastrointestinal problem for erratic absorption of Vitamin B12. With the rapid evolution of science and technology in the field of drug delivery, many new ways are being researched across the globe. Out of the various methods that have been attempted, the transdermal route is relatively better as compared to the others. The microemulsion approach if combined with the transdermal delivery approach can lead to an even better drug delivery strategy. The newer research should be more focussed towards the use of various combination drug delivery strategies in order to improve the absorption of Vitamin B12. The newer combination methods to deliver Vitamin B12 may lead to better management and treatment of the deficiency.

REFERENCES

1. Gibson RS. Principles of Nutritional Assessment. 2nd edition, Oxford University Press: New York, NY, USA, 2005.
2. O' Leary F, Samman S. Vitamin B12 in Health and Disease. Nutrients, 2010; 2: 299-316.
3. Thompson MD, Cole DE, Ray JG. Vitamin B-12 and neural tube defects: the Canadian experience. Am J Clin Nutr, 2009; 89: 697S-701S.
4. Dali YN, Andres E. An update on cobalamin deficiency in adults. Q J Med., 2009; 102.
5. Heyssel RM, Bozian RC, Darby WJ, Bell MC. Vitamin B12 turnover in man. The assimilation of Vitamin B12 from natural foodstuff by man and estimates of minimum daily requirements. Am J Clin Nutr, 1966; 18: 176-184.
6. Kuzminski AM, Del Giacco EJ, Allen RH, Stabler SP, Lindenbaum J. Effective treatment of cobalamin deficiency with oral cobalamin. Blood, 1998; 92(4): 1191-1198.
7. Vidal-Alaball J, Butler CC, Cannings JR, Goringe A, Hood K, McCaddon A, et al. Oral Vitamin B12 versus intramuscular Vitamin B12 for Vitamin B12 deficiency. Cochrane Database Syst Rev., 2005; 3(3).
8. Doscherholmen A, McMahon J, Ripley D. Vitamin B12 absorption from chicken meat. Am J Clin Nutr, 1978; 31: 825-830.
9. Doscherholmen A, McMahon J, Ripley D. Vitamin B12 absorption from fish. Proc Soc Exp Biol Med., 1981; 167: 480-484.
10. Doscherholmen A, McMahon J, Ripley D. Vitamin B12 absorption from eggs. Proc Soc Exp Biol Med., 1975; 149: 987-990.
11. Rostand SG. Vitamin B12 levels and nerve conduction velocities in patients undergoing maintenance hemodialysis. Am J Clin Nutr., 1976; 29: 691-7.
12. Moreira ES, Brasch NE, Yun J. Vitamin B12 protects against superoxide-induced cell injury in human aortic endothelial cells. Free Radic. Biol. Med., 2011; 51(4): 876-883.
13. Manzanares W, Hardy G. Vitamin B12: the forgotten micronutrient for critical care. Curr Opin Clin Nutr Metab Care., 2010; 13(6): 662-668.
14. Carmen, Michael L, Ruma B. Navigating the B12 Road: Assimilation, Delivery, and Disorders of Cobalamin. J Biol Chem., 2013; 288(19): 13186–13193.
15. Kim J., Gherasim C., Banerjee R. Decyanation of Vitamin B12 by a trafficking chaperone. Proc Natl Acad Sci U S A., 2008; 105: 14551–14554.
16. Ragsdale, S.W. The acetogenic corrinoid proteins. In Chemistry and Biochemistry of B12; Banerjee, R., Ed. John Wiley & Sons; New York, NY, USA, 1999; 633-653.
17. Fontecave, M.; Mulliez, E. Ribonucleotide reductases. In Chemistry and Biochemistry of B12; Banerjee, R., Ed.; John Wiley & Sons; New York, NY, USA, 1999; 731-756.
18. Carmel R. Mild transcobalamin I (haptocorrin) deficiency and low serum cobalamin concentrations. Clin Chem., 2003; 49: 1367-1374.
19. Carmel R., Green R, Rosenblatt DS, Watkins D. Update on cobalamin, folate and homocysteine. Hematology, the American Society of Hematology. Education Program, 2003; 62-81.
20. Scott JM. Bioavailabilty of Vitamin B12. Eur J Clin Nutr., 1997; 51: S49-53.
21. Hin H, Clarke R, Sherliker P, Atoyebi W, Emmens K, Birks J, Schneede J, Ueland PM, Nexo E, Scott J, et al. Clinical relevance of low serum Vitamin B12 concentrations in older people: the Banbury B12 study. Age Ageing, 2006; 35: 416-22.
22. Hvas AM, Nexo E. Diagnosis and treatment of Vitamin B12 deficiency: an update. Haematologica, 2006; 91(11): 1506-12.
23. Kornerup LS, Juul CB, Fedosov SN, Heegaard CW, Greibe E, Nexo E. Absorption and retention of free and milk protein-bound cyano- and hydroxocobalamins. An experimental study in rats. Biochimie, 2015.
24. Andres E, Vogel T, Lang PO. Food-Cobalamin Malabsorption: A Controversial Etiology of Symptomatic Vitamin B12 Deficiency. Journal of Blood Disorders and Transfusions, 2015; 6: 301.
25. Mazokopakis EE, Starakis IK. Recommendations for diagnosis and management of metformin induced Vitamin B12 (Cbl) deficiency. Diabetes Res Clin Pr., 2012; 97: 359-367.
26. DeFronzo RA, Goodman AM. The Multicenter Metformin Study Group. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. New Engl J Med., 1995; 333: 541–549.
27. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Metabolic abnormalities in cobalamin (Vitamin B12) and folate deficiency. Federation of American Societies for Experimental Biology., 1993; 7: 1344-1353.
28. Carol LZ, Bevan DH, Kate AM, Angela VS, Michelle AR, Melinda RR. Vitamin B12 and vegetarian diets. Med J Australia, 2012; 2: 27-32.
29. Koyyalamudi SR, Jeong SC, Cho KY, Pang G. Vitamin B12 is the active corrinoid produced in

cultivated white button mushrooms (Agaricus bisporus). J Agr Food Chem, 2009; 57(14): 6327-6333.
30. Miyamoto E, Yabuta Y, Kwak CS, Enomoto T, Watanabe F. Characterization of Vitamin B12 compounds from Korean purple laver (Porphyra sp.) products. J Agr Food Chem, 2009; 57(7): 2793-2796.
31. Andres E, Federici L, Affenberger S, Vidal-Alaball J, Loukili NH, Zimmer J et al. B12 deficiency: a look beyond pernicious anemia. J Fam Practice. 2007; 56(7): 537–542.
32. Andres E, Dali-Youcef N, Vogel T, Serraj K, Zimmer J.Oral cobalamin (vitamin B(12) treatment An update. Int J Lab Hematol., 2009; 31(1): 1–8.
33. Middleton J, Wells W. Vitamin B12 injections: considerable source of work for the district nurse. Brit Med J., 1985; 290: 1254-1255.
34. Lederle FA. Oral cobalamin for pernicious anemia. Medicine's best kept secret?. JAMA., 1991; 265(1): 94–95.
35. Lederle FA. Oral cobalamin for pernicious anemia: Back from the verge of extinction. J Am Geriatr Soc., 1998; 46: 1125–7.
36. Herbert V. Staging vitamin B-12 (cobalamin) status in vegetarians. Am J Clin Nutr., 1994; 59: 1213S–1222.
37. Kallirroi K, Stefanie Q, Valérie D and Dominique AH. Successful Sublingual Cobalamin Treatment in a Child with Short-Bowel Syndrome. J Pediatr Pharmacol Ther., 2014; 19(1): 60–63.
38. Sharabi A, Cohen E, Sulkes J et al. Replacement therapy for Vitamin B12 deficiency: comparison between sublingual and oral route. Bri J Clin Pharmaco., 2003; 56: 635-638.
39. Ye Yang, Haripriya K and Ajay KB. Effects of Chemical and Physical Enhancement Techniques on Transdermal Delivery of Cyanocobalamin (Vitamin B12) In Vitro. Pharmaceutics., 2011; 3: 474-484.
40. Chien-Hua Chen, Yin-Kai Huang, Fu-Shan Jaw. Ultrasound-guided Perineural Vitamin B12 Injection for Peripheral Neuropathy. J Med Ultrasound., 2015; 23: 104-106.
41. Anayatollah S, Behzad S, Makhmal Z, Eskandar M. Preparation and Characterization of Cyanocobalamin (Vit B12) Microemulsion Properties and Structure for Topical and Transdermal Application. Iran J Basic Med Sci., 2013; 16: 865-872.
42. Inbar Y, Anat L, Aharon G, Rachel L, Dror F. Enhanced pharmacological activity of Vitamin B12 and Penicillin as nanoparticles. Int J Nanomedicine, 2015; 10: 3593-3601.
43. Phillips C, Essick GK, Chung Y, Blakey G. Non-invasive therapy for altered facial sensation following orthognathic surgery: an exploratory randomized clinical trial of intranasal Vitamin B12 spray. J Maxillofac Trauma, 2012; 1(1): 20-29.
44. Delpre G, Stark P, Niv Y. Sublingual therapy for cobalamin deficiency as an alternative to oral and parenteral cobalamin supplementation. Lancet, 1999; 354: 740.
45. Thong HY, Zhai H, Maibach HI. Percutaneous penetration enhancers: an overview. Skin Pharmacol Physiol, 2007; 20: 272-282.
46. Donnelly RF, Raj Singh TR, Woolfson AD. Microneedle-based drug delivery systems: microfabrication, drug delivery and safety. Drug Deliv, 2010; 17: 187-207.
47. Prausnitz MR. Microneedles for transdermal drug delivery. Adv Drug Deliver Rev., 2004; 56: 581-587.
48. Kalluri H, Banga AK. Microneedles and transdermal drug delivery. J Drug Deliv Sci Technol, 2009; 19: 303-310.
49. Kolli CS, Banga AK. Characterization of solid maltose microneedles and their use for transdermal delivery. Pharmaceut Res., 2008; 25: 104-113.
50. Li G, Badkar A, Kalluri H, Banga AK. Microchannels created by sugar and metal microneedles: characterization by microscopy, macromolecular flux and other techniques. J Pharm Sci., 2010; 99: 1931-1941.
51. Vikas CJ, Vipin S, Sunil K, Nancy M. Transdermal Drug Delivery Systems: Approaches and Advancements in Drug Absorption through Skin. Int J Pharm Sci Rev Res., 2013; 20(1): 47-56.
52. Sandhu P, Bilandi A, Kataria S, Middha A. Transdermal Drug Delivery System (patches), Applications in Present Scenario. Int J Res Pharm Chem., 2011; 1(4): 1139-1151.
53. Patel D, Chaudhary SA, Parmar B, Bhura N. Transdermal Drug Delivery System: A review. Pharma Innovation., 2012; 1(4): 66-75.
54. Sun H, Yang T, Li Q. et al. Dexamethasone and Vitamin B(12) synergistically promote peripheral nerve regeneration in rats by upregulating the expression of brain-derived neurotrophic factor. Arch Med Sci., 2012; 8: 924-930.
55. Williams SR, Chouinard P, Arcand G. et al. Ultrasound guidance speeds execution and improves the quality of supra-clavicular block. Anesth Analg, 2003; 97: 1518-1523.
56. Dror DK, Allen LH. Vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutr Rev., 2008; 66: 250-255.
57. Okada K, Tanaka H, Temporin K, et al. Methylcobalamin increases Erk1/2 and akt activities through the methylation cycle and promotes nerve regeneration in a rat sciatic nerve injury model. Exp Neurol, 2010; 222: 191-203.
58. Xu G, Lv ZW, Feng Y, et al. A single-centre randomized controlled trial of local methylcobalamin injection for sub-acute herpetic neuralgia. Pain Med, 2013; 14: 884-894.
59. Liao WC, Wang YJ, Huang MC et al. Methylcobalamin facilitates collateral sprouting of donor axons and innervation of recipient muscle in end-to-side neurorrhaphy in rats. PLoS One. 2013; 8: e76302.
60. Bagwe RP, Kanicky JR, Palla BJ, Patanjaili PK,

Shah DO. Improved drug delivery using microemulsion: rationale, recent progress and new horizons. Crit Rev Ther Drug, 2001; 18: 77-140.
61. Tosmic M, Podlogar F, Gasperlin M, Bester-Rogac M, Jamink A. Water- tween 40/imwitor 308-isopropyl myristate microemulsions as delivery systems for ketoprofen: Small-angle X-ray scattering study. Int J Pharm., 2006; 327: 170-177.
62. Shaji J, Reddy MS. Microemulsion as drug delivery system. Pharma Times, 2004; 36(7): L 17-24.
63. Om Prakash Agrawal, Satish Agrawal. An overview of New Drug Delivery System: Microemulsion. Asian J Pharm Sci Tech, 2012; 2(1): 5-12.
64. Ansari AA, Khan MN, Alhoshan M, Aldwayyan AS, Alsalhi MS. Nanostructured materials: classification, properties, fabrication, characterization and their applications in biomedical sciences. In: Kestell AE, DeLorey GT, editors. Nanoparticles: Properties, Classification, Characterization, and Fabrication. Hauppauge, NY: Nova Science Publishers, Inc, 2010.
65. Gedanken A. Using sonochemistry for the fabrication of nanomaterials. Ultrason Sonochem. 2004; 11(2): 47–55.
66. Zheng YY, Zhu TJ, Zhao XB, Tu JP, Cao GS. Sonochemical synthesis of nanocrystalline Bi2Te3 thermoelectric compounds. Mater Lett, 2005; 59(23): 2886–2888.
67. Bang JH, Suslick KS. Sonochemical synthesis of nanosized hollow hematite. J Am Chem Soc. 2007; 129(8): 2242–2243.
68. Kiel S, Grinberg O, Perkas N, Charmet J, Kepner H, Gedanken A. Forming nanoparticles of water-soluble ionic molecules and embedding them into polymer and glass substrates. Beilstein Journal of Nanotechnology, 2012; 3(1): 267–276.
69. Meridor D, Gedanken A. Forming nanoparticles of α-amylase and embedding them into solid surfaces. J Mol Catal B: Enzymatic, 2013; 90: 43–48.
70. Meridor D, Gedanken A. Preparation of enzyme nanoparticles and studying the catalytic activity of the immobilized nanoparticles on polyethylene films. Ultrason Sonochem, 2013; 20(1): 425–431.
71. Perelshtein I, Ruderman E, Francesko A, Fernandes MM, Tzanov T, Gedanken A. Tannic acid NPs – synthesis and immobilization onto a solid surface in a one-step process and their antibacterial and anti-inflammatory properties. Ultrason Sonochem., 2014; 21(6): 1916–1920.
72. Benisvy-Aharonovich E, Shimanovich U, Kronfeld N, et al. Pre-miRNA expressing plasmid delivery for anti-cancer therapy. Med Chem Commun., 2014; 5(4): 459–462.
73. Gelperina S, Kisich K, Iseman MD, Heifets L. The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. Am J Resp Crit Care., 2005; 172(12): 1487–1490.
74. Finnerup NB, Sindrup SH, Jensen TS. The evidence for pharmacological treatment of neuropathic pain. Pain, 2010; 150: 573–581.
75. Sun Y, Lai M-S, Lu C-J. Effectiveness of Vitamin B12 on diabetic neuropathy: systematic review of clinical controlled trials. Acta Neurologica Taiwanica., 2005; 14: 48–54.
76. Sato Y, Honda Y, Iwamoto J, Kanoko T, Satoh K. Amelioration by mecobalamin of subclinical carpal tunnel syndrome involving unaffected limbs in stroke patients. J Neurol Sci., 2005; 231: 13–8.