# Vitamin B12 Deficiency in Older People a Practical Approach to Recognition and Management

Mrs.Sandhya S.Ahire[1], Mr.Sujeetkumar I.Ahire[1] ,Mr.Chetan S. Chaudhari[2], Mr.Vaibhav l.Chaudhari[2]
*[1] Assistant Prof. & [2] Students of KYDSCT COP, Sakegaon, Bhusawal.MH 425201.*

***Abstract: Vitamin B12deficiency is common in older people and is linked to anaemia, cognitive decline, and dementia. Clinical presentation is variable, ranging from no symptoms or subtle, non-specific symptoms, through to serious, irreversible neurological symptoms if left untreated.***

***Although common, it is often under-recognised, with diagnosis usually occurring on incidental blood test screening. Older people are at increased risk of vitamin B12deficiency due to insufficient dietary intake, malabsorption associated with aged-related changes in gastrointestinal function, higher incidence of pernicious anaemia, and chronic use of interfering medications such as metformin and proton-pump inhibitors. Early detection and treatment in symptomatic deficiency are crucial to prevent irreversible damage.***

***Vitamin B12intramuscular injection bypasses potential absorption issues and is traditionally the first-line treatment in older people. However, emerging evidence suggests that high-dose oral replacement may be as effective.***

***Keywords: Vitamin B12, deficiency, anaemia, malabsorption, neurological symptoms***

## INTRODUCTION

Vitamin B12, also known as cobalamin, is an essential water-soluble vitamin that plays a role in cell metabolism and function.1It is produced by microorganisms in the large intestines of animals.2,3For humans, vitaminB12is obtained solely from the diet, primarily through the consumption of animal products such as meat, sea-food, eggs, milk, and dairy products.

Adequate dietary intake and functioning mechanisms for absorbing vita-min B12are vital for maintaining adequate levels. Vitamin B12deficiency is common in people with vegetarian or vegan diets, people with gastrointestinal diseases, and older people.3

Older people are at risk of deficiency due to malabsorption associated with age-related patho-physiological changes in the gastrointestinal tract, inade-quate dietary intake, and higher incidence of perniciousanaemia, multiple comorbidities, and polypharmacy

Signs and symptoms of vitamin B12deficiency vary between individuals. Most older people with deficiency are either asymptomatic or have subtle, non-specific symptoms, which can make it difficult to recognise.1 If left untreated, vitamin B12deficiency can lead to significant haematological and neurological complications.

Inolder people,it can also contribute to frailty syndrome.6In symptomatic disease,early diagnosis and treatmentare important to prevent irreversible damage.1The aim of this review is to provide an overview of vita-min B 12 deficiency and its management in older people.

## PREVALENCE OF VITAMIN B12 DEFICIENCY IN OLDER PEOPLE

- The prevalence of vitamin B12deficiency is generally estimated at around 20% in people over 60 years, but may be as high as 40%.2,4,7-10. In Australian studies, the figure is around 20-25%in older people.11,12
- Institutionalised older people with multiple comorbidities may be at higher risk, with some studies reporting prevalence of 30-40%.1,2,13Higher incidence has been reported in men.

## ROLE OF VITAMIN B12

- Vitamin B12is essential for healthy red blood cell formation (erythropoiesis).It also has a key role in deoxyribo-nucleic acid (DNA) synthesis, nerve development, and maintenance of central nervous system function.7,14,15
- It is a necessary cofactor in three major enzyme reactions: the conversion of methylmalonic acid to succinyl coenzyme A; the conversion of homocysteine to methio-nine; and the conversion

of 5-methyl tetra hydro folate to tetrahydrofolate.7,14,15

- During vitamin B12deficiency,reductions in these reactions can lead to accumulation of the substrates methylmalonic acid and homocysteine in the plasma,and reduction in the release of active folate,leading to impaired DNA and mitochondrial cell synthesis.


Figure 01: ROLE OF VITAMIN B12

COBALAMIN (VITAMIN B12) VARIANTS

There are four main variants of vitamin B12,differing only slightly in their chemical structure.Active forms are ade-nosylcobalamin and mecobalamin (co-methylcobalamin).Supplemental forms are hydroxocobalamin and
cyanoco-balamin,which are precursors to the active forms.

- The absorption of orally consumed vitamin B12is a com-plex,multistep process, involving a number of glycopro-teins including R-factor and intrinsic factor (summarisedin Figure1).
- Following absorption,vitamin B12is bound to carrier proteins (transcobalamin and haptocorrin)that transport it in the blood. Only transcobalamin-bound vitamin B12(holotranscobalamin)can enter cells,where it is converted to the active forms.14,15
- Excess vitamin B12that is not needed for immediate cell use is transported to the liver for storage (Figure1),where large reserves can be stored for many years.1Dis-ruptions in vitamin B12consumption or absorption can lead to deficiency,which typically takes years to develop as liver stores slowly deplete overtime.1,16
- Unabsorbed vitamin B12 is excreted mostly through the faeces and excess vitamin B12in the blood (e.g.fol-lowing injection supplementation) is excreted in the urine

CAUSES OF VITAMIN B12DEFICIENCY INOLDER PEOPLE

- The causes of vitamin B12deficiency can be divided into two main categories: inadequate dietary intake andi mpaired absorption
- Inadequate Dietary Intake Inadequate or restricted intake can be due to poor diet,difficulties in chewing or swallowing food,difficulties accessing food or deliberate exclusion of animal-sourced foods (e.g.due to vegetarian/vegan diet,high food pricing, or religious and cultural factors.

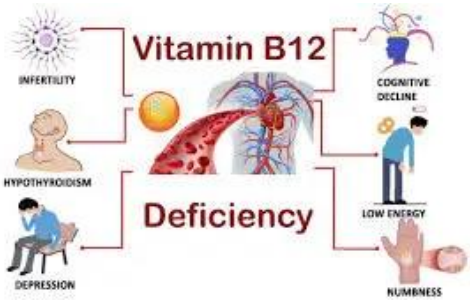


Figure 2: causes of vitamin b12deficiency

Impaired Absorption

- Even with good intake,impairment in theabsorption process may lead to vitamin B12deficiency.Malabsorption is thought to be the most common causeof vitamin B12 deficiency in older people,accounting for40-70% of cases.

Factors that contribute to malabsorp-tion are summarised below

Pathophysiological Changes

- Changes in the stomach,pancreas,and intestines ofolder people can lead to reduced production of acid andprotease enzymes.This can result in vitamin B12beingless effectively detached from food proteins,therebyreducing absorption.1,5
- Non-protein bound consumedvitamin B12(e.g.vitamin B12from oral supplementation)maybe less affected by these changes
- The absorption of orally consumed vitamin B12is a com-plex,multistep process,involving a number of glycoproteins including R-factor and intrinsic factor (summarisedin Figure1).
- Following absorption,vitamin B12is bound to carrier proteins (transcobalamin and

haptocorrin)thattransport it in the blood. Only transcobalamin-boundvitamin B12(holotranscobalamin)can enter cells,whereit is converted to the active forms.14,15

- Excess vitamin B12that is not needed for immediatecell use is transported to the liver for storage (Figure1),where large reserves can be stored for many years.1Dis-ruptions in vitamin B12consumption or absorption can lead to deficiency,which typically takes years todevelop as liver stores slowly deplete overtime.1,16
- Unabsorbed vitamin B12is excreted mostly through the faeces and excess vitamin B12in the blood (e.g.fol-lowing injection supplementation) is excreted in the urine

Factors that contribute to malabsorp-tion are summarised below

Pathophysiological Changes

- Changes in the stomach,pancreas,and intestines ofolder people can lead to reduced production of acid andprotease enzymes.This can result in vitamin B12beingless effectively detached from food proteins,therebyreducing absorption.1,5
- Non-protein bound consumedvitamin B12(e.g.vitamin B12from oral supplementation)maybe less affected by these changes

Pernicious Anaemia

- Pernicious anaemia is a common cause of vitamin B12malabsorption in older people and occurs due to intrin-sic factor deficiency.It is mainly driven by autoimmune destruction of parietal cells in the stomach that Produce intrinsic factor or by destruction of the protein itself.4,7,15
- Atrophic gastritis,occurring from chronic inflammation of the stomach lining, can cause parietal cell destructionleading to reduced intrinsic factor.1,5
- ChronicHelicobacterpylori(H.pylori)infection has been strongly associated with atrophic gastritis and vitamin B12deficiency,withone Study finding that 56%of people with vitamin B12deficiency had chronicH.pylori infection.

Other Condition

- Other diseases or surgeries impacting gastric, intestinal,or pancreatic function can also cause vitamin B12malab-sorption

Medications

- A significant contributor to vitamin B12deficiency inolder people is long-term use of certain medications. Reduced vitamin B12absorption is a well- established riskwith metformin use,with studiesfinding it can affect upto 30%of users.17,18
- Risk is higher with larger doses and longer durations of use.18-20 Symptomatic deficiency is more likely to present after 5-10 years of use,but onestudy reported depletion in serum vitamin B12levels afteras little as 3-4 months.7
- The mechanism is unknown butis thought to be related to altered calcium homeostasis inthe ileum,leading to mal absorption Proton-pump inhibitors and histamine H2-receptorblockers suppress gastric acid secretion and can impairvitamin B12release from food protein.Long-term use,greater than 12 months,has been associated with increased risk of vitamin B12deficiency.
- Other medications that are less strongly associated with vitamin B12deficiency include colchicine and chole-styramine(thought to interfere with intestinal vitaminB12absorption),nitrous oxide,and antibiotics such as aminoglycosides.

➢ Clinical signs and symptoms of vitamin B12deficiency

- Most older people havemild,subclinical deficiency identifiedviaincidental labo- ratory findings,which is usually asymptomatic.1,7,19
- Non-specific symptoms such as nausea,vomiting,diar-rhoea,and stomach pain can occur,making initial diagnosis difficult.1Classical features are anaemia andneuropathy,but these are uncommon.

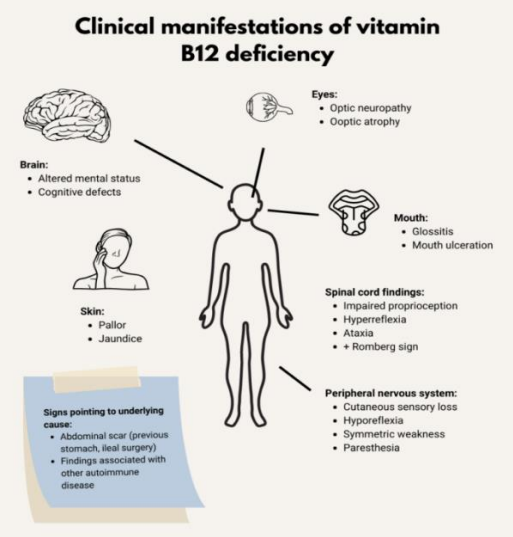


Figure 3: clinic al manifestation of vit B12

➢ Haematological FeaturesIn

- Anaemia can occur in severe vitamin B12deficiency.This usually presents as
- macrocytic or megaloblastic anaemia(associated with abnormally large red blood cells
- ,low haemoglobin, and high mean cell volume),often withcoexisting folate deficiency.1,7,24
- Patients may present with fatigue,lethargy, headaches, light-headedness, and unexplained weight loss.1,7
- If anaemia is severe,symp-toms attributable to tissue hypoxia and organ is chemiasuch as chest pain,shortness of breath,tachycardia,and skin pallor can occur

➢ Neurological Features

- The most common neurological symptoms associated with vitamin B12deficiency are paraesthesia or Numbnessin the hands and feet and gait problems.other symptoms included peripheral neuropathy,vision disturbance and memory loss 1,7.
- Cognitive decline,dementia, psycho-sis, mania,and agitation can occur,with one studyfind-ing that low serum vitamin B12levels was associated witha 2-to 4-fold increased risk of cognitive impairment.2,27In the most severe form of vitamin B12deficiency,degen-eration of the spinal cord and paralysis can develop andcan be permanent if left untreated

DIAGNOSIS

Serum Vitamin B12Testing

Diagnosis is based mainly on serum measurements of total vitamin B12concentration.
This is the most com-mon assay and is widely available at low cost.14TheWorld Health Organization defines deficiency as a serum level below 150 pmol/L and this is generally accepted in practice for diagnosis.28Some have argued this cut-off is too low,as neurological symptoms can occur at levels above this range.

In some situations,total serum vitamin B12can be misleading,as it measures both holo trans cobalamin(the biologically active form of vitamin B12, bound to trans- cobalamin)and the inactive forms (vitamin B12boundto other carrier proteins or unbound).A low total serum vitamin B12 level may be related to disruptions in vitamin B12metabolism and may not necessarily represent tissue deficiency.
1A false normal or false high total vita-min B12level can occur in patients with conditions such as alcoholism,liver disease or myelo proliferative disorder due to reduced hepatic clearance of transcobalamin.1,7
Therefore, patients who return a borderline result (e.g.serum level between 150 and220 pmol/L)or a result which does not appear compati-ble with clinical findings,should have confirmatory additional testing completed
Holotranscobalamin Testing.
Holotranscobalamin,being the biologically active formof vitamin B12in the serum,represents a more sensitive marker for vitamin B12deficiency.2,30,31Measuringserum holotranscobalamin levels can confirm deficiency when serum vitamin B12levels are indeterminate or borderline.24 If the holotranscobalamin level is low,the patient should be considered vitamin B12deficient.

Holotranscobalamin measurements are more expen-sive and not as readily available as serum vitamin B12levels,but some laboratories are beginning to routinely check holo transcobalamin when serum vitamin B12levelis borderline or there is concern about a false low.1Emerging evidence indicates that a low level of holo-transcobalamin has higher diagnostic accuracy for vita-min B12deficiency compared to serum levels of total vitamin B12and other metabolites.

Homocysteine and Methylmalonic Acid Testing

- Other second-line tests sometimes used to assist diagno-sis are homocysteine and methylmalonic acid,as levelsof these are elevated in vitamin B12deficiency.4,7,19,24
- The role of these tests in practice is not clearly definedand availability is limited.19
- Total plasma homocysteine may be a more sensitivemarker than serum vitamin B12for diagnosing defi-ciency, but several limitations reduce its utility 14
- The test is non-specific and measurements can be con-founded by factors such as folate deficiency,vitamin B6deficiency,and renal impairment, which are especially relevant in older people.14
- Another drawback is that the sample must be processed within 2 h of collection Measurement of methylmalonic acid may also be a more sensitive measure of vitamin B12status,but levels can be falsely elevated in older people and those with renal impairment,and it is more expensive

Schilling Test

- The Schilling test is an older measurement traditionally used to assess vitamin B12absorption and diagnose intrinsic factor-related malabsorption.The test had poor sensitivity and is no longer recommended in Australia

WHEN TO TEST FOR VITAMIN B12 DEFICIENCY

- Testing should be completed in all patients with unex-plained anaemia or neurological symptoms and patientswith risk factors for deficiency such as malabsorption, insufficient dietary intake,or long-term use of metfor-min,proton- pump inhibitors,or histamine H2-receptorantagonists (see Table1for other risk factors).7,19
  - Inolder patients with cognitive impairment, delirium, orrecurrent falls, vitamin B12testing is usually included inthe geriatric assessment. Screening in older people with-out other risk factors has been debated and may be war-ranted, although there is no consensus.6Annual testing should be considered for at-risk patients

CONCOMITANT DEFICIENCIES

- Vitamin B12is closely linked with folate. The biochemical pathways for bothvitamins are intertwined and deficiencies often coexist ,particularly in situations of insufficient dietary intake or malabsorption.19,24If vita-min B12deficiency is diagnosed, folate levels should be checked to exclude coexisting deficiency.
- If co existing folate deficiency is confirmed, vitamin B12treatmentmust be commenced first to minimise the risk of sub-acute combined degeneration of the spinal cord.7,14
- Many causes of vitamin B12and folate mal absorption can also impair iron absorption. If vitamin B12 deficiency is confirmed, iron studies should also be checked to exclude coexisting iron deficiency.

TREATMENT OF VITAMIN B12 DEFICIENCY

Vitamin B12deficiency should always be treated with pharmacological replacement unless there is strong rationale not to do so In patients with symptomatic anaemia or neurological symptoms,treatment should commence promptly to reduce progression of symptoms and irreversible damage.

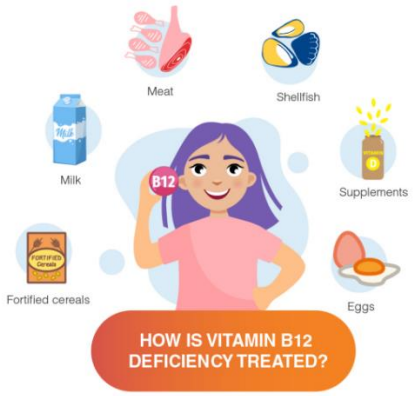


Figure 04: treatment of vit B12

PHARMACOLOGICAL REPLACEMENT

  - There are four formulations of vitamin B12currentlyavailable in Australia. Hydroxocobalamin and cyanocobalamin are used most commonly.
  - Mecobalamin(co-methylcobalamin)and adenosylcobalamin are the physiological forms of vitamin B12and have recently emerged as alternative options.

- Supplementation is most commonly administered by the parenteral or oral routes.

PARENTERAL REPALCEMENT

- Intramuscular injection is the most commonly used treatment for vitaminB12deficiency.It provides rapid and effective correction of vitamin B12deficiency as It bypasses absorption barriers.
- In Australia, hydroxocobalamin and cyanocobalaminare available as parenteral therapy.
- Hydroxocobalaminis the preferred first choice as it is retained in the bodyfor a longer period and maintenance doses can be administered every 2-3 months, compared to cyanoco-balamin, which delivers a smaller and shorter effect and requires administration every month For patients with severe anaemia or neurologicalsymptoms, aggressive initial intramuscular dosing is recommended.
- Various dosage regimens have been suggested but generally a high total dose of either hydroxycobalamin or cyanocobalamin such as 3-10 mg over 2-4 weeks is recommended 19,35.
- In patients with neurological features, alternate day dosing may need to continue until symptom improvement occurs.19,24
- For patients without severe anaemia or neurological symp-toms,a less intensive initial vitamin B12 loading approach may be adopted (shorter duration, longer interval between doses,or a lower dose)
- Most patients with vitamin B12deficiency require ongoing maintenance therapy after initial treatment.19,34,35
- Inpatients without neurological symptoms,hydroxocobalamin1 mg intramuscularly every 3 months is recommended.19,3
- Intramuscular injections usually require administration by healthcare providers.In community settings, alternate day injections may be challenging to achieve and a pragmatic approach should be adopted that may include less frequent dosing Or oral replacement therapy.
- On the other hand,compli-ance may be better with parenteral therapy due to fre-quent contact with the healthcare provider.

ORAL SUPPLEMENTS.

- Concerns regarding unpredictable absorption of oral vita-min B12supplements have traditionally led clinicians to avoid this treatment route.4
- However,it is now thought that adequate absorption of oral supplements can be achieved through passive absorption mechanisms (with-out binding to intrinsic factor).
- In a Cochrane review comparing high-dose oral vitamin B12and intramuscular vitamin B12,it was found that both forms had similar effects in correcting serum vitamin B12levels,even in patients with pernicious anaemia or ileal resection.4
- High-dose oral vitamin B12may be considered a suit-able alternative in cases where intramuscular injections are not tolerated or compliance with regular injections is problematic.
- The British Society for Haematology suggests that oral replacement may be considered inpatients with a symptomatic ,mild disease.19
- Administration via the oral route is easier and less invasive,but it increases the pill burden,which may be problematic for some people.2,38Reduced physician visits associated with injections may also be more cost-effective.39In Australia,cyanocobalamin is available in oral preparations over the counter. Suggested dosing regimens vary.
- In patients with suspected mal absorption,high initial doses of 1 mg daily for a month are recommended, followed by maintenance dosing of 50-200lg orallydaily.1,35Higher maintenance doses of 1 mg daily have been recommended in patients with pernicious anaemia

OTHER REPLACEMENT

- FormulationsVarious other dose forms of vitamin B12are availableover the counter,including liquid,nasal,transdermal,and sublingual forms,but most lack sufficientevidence.2,7,15,29 Of these options,sublingual formulations have the most evidence from small studies absorption,evidence has not found them to be superior to oral vitamin B12.41
- The active variants of vitamin B12,mecobalamin and adenosylcobalamin often Formulated in sublingual options,have also not been found to be superior to cyanocobalamin or hydroxocobalamin.Intranasal formulations are not widely used because high cost,risk of

rhinorrhoea, and lack of evidence limit their use.2,38,40

- Transdermal forms are available but rarely used. Low dose vitamin B12is also available in multivitamin and vitamin B complex supplements, but these are not recommended for the treatment of vitamin B12 deficiency.

DURATION OF TREATMENT

- The duration of vitamin B12replacement depends on the underlying cause of deficiency.
- Lifelong treatment is usually required for conditions that are irreversible,such as gastric surgery,age-related mal absorption or pernicious anaemia.
- In cases where the cause of deficiency can be reversed (e.g.by avoiding restrictive diet or implicated medications),treatment can be stopped once the deficiency is corrected .

RESPONSE AND MONITORING

- Haematological response begins several days after commencing treatment, but it can take up to 2 months for complete response.7,14-16
- Neurological symptoms resolve more slowly and can take 6 weeks to 3 months.1,7,14Adverse effects from vitamin B12supplements are reven at high doses.1,4,34
- Hypokalaemia can occur(production of new blood cells results in marked intra-cellular uptake of potassium)and may require monitoring, particularly in patients receiving high-dose parenteral treatment.1,14,16,34
- Excessive replacement is not likely to be a major concern as the body can adjust to high intakes by decreasing absorption and increasing excretion

Retesting Serum Vitamin B12 Level

- Retesting during or following treatment is not usually required. If repeat vitamin B12testing is indicated,a suggested interval is 3-6 months.1,35
- Earlier testing at4 weeks can be considered in some cases (e.g.patients with ongoing symptoms of deficiency,including anae-mia,and patients with suspected mal absorption or non-compliance with treatment).concern as high levels are not considered toxic. Very high levels have been reported in some cases and are thought to be related to the formation of antibodies to the carrier protein,transcobalamin.44,45
- Formation of these immunoglobulin transcobalamin B12 complex more commonly occur in the initial weeks following vit b12 injection.
- The complexes cannot enter cells and are not thought to have any clinicalconsequences.16,45
- High serum levels in patients not receiving supple-mentation is more concerning and may be an early marker for underlying pathologies such as liver disease,kidney failure, haematological malignancies and solid organ cancers.45
- Elevated levels are also frequently reported in critically ill patients and are associated with higher mortality.

PREVENTION OF VITAMIN B12DEFICIENCY

Increasing Dietary Intake.

- Patients with risk factors for vitamin B12deficiencyshould be encouraged to increase intake of vitamin B12-containing foods.2In older people,this may not be achievable due to the factors relating to reduced intake mentioned earlier.Increased intake of foods fortified with vitamin B12(i.e.vitamin B12added by food manufacturers) is another approach.
- In Australia,only a limited numberof foods are permitted to be fortified with vitamin B12,such as selected soy milks,yeast spreads,and meat ana-logues such as soy based burgers and sausages.3Regu-lar consumption of fortified foods has been shown tolead to higher serum vitamin B12levels.47,48 .Some evidence suggests vitamin B12from fortified foods may be absorbed more effectively than from meat,fish, andpoultry sources.47
- Effectiveness of increased intake relies on effective absorptive mechanisms. In patients with confirmed vita-min B12deficiency,increased dietary intake may not reverse deficiency and supplementation with pharmaco-logical doses of vitamin B12is essential


Figure 5: prevention of vit b12 deficiency

## CONCLUSION

With the recent developments,bioadhesive drug delivery system looks a promising approach to achieve a targeted and sustained release of drug while maintaining patient compliance.However,it needs to address the regulatory hurdles in order to be widely accepted as a major drug delivery system.

## REFERENCES

[1] Andrews,Gavin P.,Thomas P.Laverty,and David S.Jones."Mucoadhesive Polymeric Platforms for Controlled Drug Delivery."European Journal of Pharmaceutics and Biopharmaceutics 71.3 (2009):505-18.

[2] Asane,GS,et al.,"Polymers for Mucoadhesive Drug Delivery System:A current status drug development and insustrial pharmacy,34.11.2008,1246-66.

[3] Chanburee,Sanipon,and Waree Tiyaboonchai."Mucoadhesive Nanostructured Lipid Carriers (NLCs)as Potential Carriers for Improving Oral delivery of curcumin drug development and industrial pharmacy 43.3 2017,432-40.

[4] Dodou,Dimitra,Paul Breedveld,and Peter A.Wieringa."Mucoadhesives in the Gastrointestinal Tract:Revisiting the Literature for Novel Applications."European journal of pharmaceutics and biopharmaceutics 60.1 (2005):1-16.

[5] Jain,Shashank,et al.,"Formulation and Rheological Evaluation of Ethosome- Loaded Carbopol Hydrogel for Transdermal Application."Drug development and industrial pharmacy 42.8(2016):1315-24.

[6] Luo,Yangchao,et al.,"Solid Lipid Nanoparticles for Oral Drug Delivery: Chitosan Coating Improves Stability,Controlled Delivery,Mucoadhesion and Cellular Uptake."Carbohydrate Polymers 122 (2015):221-9.

[7] Mazzarino,Leticia,et al.,"Xyloglucan-block-Poly (e-Caprolactone)Copolymer Nanoparticles Coated with Chitosan as Biocompatible Mucoadhesive Drug Delivery System."Macromolecular bioscience 14.5(2014):709-19.

[8] Mikos,AG,and NA Peppas."Scaling Concepts and Molecular Theories of Adhesion of Synthetic Polymers to Glycoproteinic Networks."Bioadhesive Drug Delivery Systems.CRC Press Boca Raton,FL,1990.25-42.

[9] Montenegro-Nicolini,Miguel,and Javier 0.Morales."Overview and Future Potential of Buccal Mucoadhesive Films as Drug Delivery Systems for Biologics."AAPS PharmSci Tech 18.1(2017):3-14.

[10] Park,Kinam,and Joseph R.Robinson."Bioadhesive Polymers as Platforms for Oral-Controlled Drug Delivery:Method to Study Bioadhesion."International journal of pharmaceutics 19.2 (1984):107-27.

[11] Patel,Niketkumar,et al.,"Influence of Electronic and Formulation Variables on Transdermal lontophoresis of Tacrine Hydrochloride."Pharmaceutical development and technology 20.4 (2015):442-57.

[12] Patel,Niketkumar,et al.,"Application of Design of Experiments for Formulation Development and Mechanistic Evaluation of lontophoretic Tacrine Hydrochloride Delivery."Drug development and industrial pharmacy 42.11 (2016):1894-902.

[13] Ponchel,Gilles,and Juan-Manuel Irache."Specific and Non-Specific bioadhesive particulate systems for oral delivery to the gastrointestinal tract advanced drug delivery reviews 34.2 1998:191-219.

[14] Rieger,Christiane,et al.,"Characterization of Different Carbon Nanotubes for the Development of a Mucoadhesive Drug Delivery System for Intravesical Treatment of

Bladder Cancer."International journal of pharmaceutics 479.2(2015):357-63.

[15] Serra,Laura,Josep Domenech,and Nicholas A.Peppas."Engineering Design and Molecular Dynamics of Mucoadhesive Drug Delivery Systems as Targeting Agents."European journal of pharmaceutics and biopharmaceutics 71.3 (2009):519-28.

[16] Shah,Mansi K.,Parshotam Madan,and Senshang Lin."Preparation,in Vitro Evaluation and Statistical Optimization of Carvedilol-Loaded Solid Lipid Nanoparticles for Lymphatic Absorption Via Oral Administration."Pharmaceutical development and technology 19.4 (2014):475-85.

[17] Shelke,Santosh,et al.,"Thermo reversible Nanoethosomal Gel for the Intranasal Delivery of Eletriptan Hydrobromide."Journal of Materials Science:Materials in Medicine 27.6 (2016):1-13.

[18] Vasir,Jaspreet Kaur,Kaustubh Tambwekar,and Sanjay Garg."Bioadhesive Microspheres as a Controlled Drug Delivery System."International journal of pharmaceutics 255.1 (2003):13-32.

[19] Vora,Namrata,S.Lin,and PL Madan."Development and in-Vitro Evaluation of an Optimized Carvedilol Transdermal Therapeutic System using Experimental Design Approach."Asian Journal of Pharmaceutical Sciences 8.1 (2013):28-38.

[20] Xu,Jinke,et al.,"Genipin-Crosslinked Catechol-Chitosan Mucoadhesive Hydrogels for Buccal Drug Delivery."Biomaterials 37 (2015):395-404.

[21] Jain,Shashank,et al.,"Quality by Design Approach for Formulation,Evaluation and Statistical Optimization of Diclofenac-Loaded Ethosomes via transdermal route ,pharmaceutical development and technology 20.4,2015,473-89.

[22] Woodley john bioadhesion clinical pharmacokinetics 40.2,2001,77-84.

[23] Jain,Shashank,et al.,"Recent Advances in Lipid-Based Vesicles and Particulate Carriers for Topical and Transdermal Application."Journal of pharmaceutical sciences (2016)