Faculty of Pharmacy, Nursing and Health Professions

Master Program Industrial Pharmaceutical Technology

Preparation and Evaluation of Cyanocobalamin Mucoadhesive Sublingual dosage form

إعداد وتقييم شكل جرعات سيانوكوبالامين ملتصقة مخاطيا تحت اللسان

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Declaration

I hereby certify that the thesis I have submitted for my master's degree, titled " Preparation and Evaluation of Cyanocobalamin Mucoadhesive Sublingual dosage form" was carried out by me at Birzeit University's Pharmacy Department. Any information gleaned from the literature was cited in the references list and acknowledged throughout the text. There has never been a submission of this thesis, in whole or in part, for a diploma or other degree from any institution.

Signature:

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Date: 06/Jul/2023

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List of abbreviations

Abbreviation	Definition
Abs	Absorption
API	Active Pharmaceutical Ingredients
B_{12}	Cobalamin
B_{12}-IF	cobalamin-Intrinsic Factor Complex
B_{12}- R protein	cobalamin - transcobalamin I complex
Carb	Carbopol 940
Cbi/ CBI	Cobalamin
^{o}C	Celosias
cm^2	Centimetre square
CUB	Cubilin enterocyte receptor
Co A	
Con	Concentration
$CaCl_2$	Calcium chloride
CAS No	Chemical Abstracts Service Registry Number
Cl	Chloride
Da	Dalton
DC	Direct compression
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
DW	Distilled water
EC	Ethyl cellulose
EL 100	Eudragite L100
EL100-55	Eudragite L100-55
Eq	Equivalents
Es 100	Eudragite S100
GIF	Gene Intrinsic Factor
GIT	Gastrointestinal tract
Gut	Gastrointestinal tract
HPC	Hydroxypropyl cellulose
HPMC	Hydroxy propyl methyl cellulose
HCl	Hydrochloric acid
HCO3	Bicarbonate
h	Highest
IF	Intrinsic factor
IM	Intramuscular

ICH Q2B	International Council on Harmonisation Validation analytical procedure
J	Flux steady state
K+	Potassium
KD	Kilo Dalton
Kg	Kilogram
Kp	Kilopascal
LD	Loading Dose
L	Liters
LOQ	limit of quantification
LOD	limit of detection
MAN	Mannitol
MCC	microcrystalline cellulose
ml	Mlililiters
µg	Microgram
mg	Milligram
Mg.S	Magnesium sterate
MD	Maintenance dose
m^2	Meter sequaire
Min	Minutes
ml	Milliliters
µl	Microliters
mm	Milimeters
MTR	5-Methyltetrahydrofolate-homocystine methyltransferase reaction
Mosmol	Milliosmols
MUT	Methylmalonyl Coenzyme A mutase
MMA	methylmalonic acid
M.W	Molecular weight
Na+	Sodium
NHS	National Health Service
Ng	Nanogram
Nm	Nanometer
N	Normality
n	Number of values
NaCl	Sodium chloride
PA	Pernicious anemia
Pa	Pascal
P_{app}	Apparent permeability coefficient

PB	Phosphate buffer
PH	Potential of hydrogen
Pg	Picogram
Pka	Acid dissociation constant
Ppm	Part per million
Peg	Polyethylene glycol
PolyP	Polyplasidone
PVP	Polyvenyl pyrollidene
R^2	Coefficient of determination
r	Radius
Rpm	Round per minute
RSD	Relative standard deviation
SAM	s-Adenosylmethionin
Sec	Seconds
SEDDS	self-emulsifying agent
SD	Standard deviation
S	Slope
SSF	Simulates saliva fluid
TC	Holotranscobalamin
THF	Tetrahydrofolate
UV	Ultraviolet
USP	United State Pharmacopia
V	Volume
Vis	Visible
Vf	Volumetric flask
Wet	Wet granulation
XG	Xanthan gum
®	Trademark Symbols
#	Number
3D	Three dimensional
δ	Sigma

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Abstract

Vitamin B12 is an essential vitamin that plays a very important role in cell function and metabolism, such as DNA creation. There are many reasons for its deficiency, including intrinsic factor (IF) deficiency, which is often the main cause and is very critical for vitamin B_{12} absorption. Furthermore, intrinsic factor deficiency which usually occurred due to many causes, including disease, Gene Intrinsic Factor (GIF) deficiency, and side effects of certain drugs. Vitamin B_{12} deficiency has severe consequences such as an increased risk of cardiovascular disease, cognitive weakness, and loss of senses in the distal limbs. Oral administration is the most common route of use for vitamin B_{12} supplements available. However, it is sometimes not particularly useful for patients with intrinsic factor defects or deficiencies. Also, the injection route is invasive and requires a specialist.

This thesis aimed to enhance the absorption of vitamin B_{12} through the most preferred drug delivery route. The main idea is to increase the residence time of the drug at the administration site to improve absorption, whether an intrinsic factor is available or not, by passive diffusion absorption. It acts immediately after application of a dosage form containing a mucoadhesive polymer on the surface of the mucosa of the body, which immediately wets after contact with fluid on the surface, then penetrates deeply into the mucosa, forms chemical bonds, and remains adherent to the surface of the mucosa for a longer time. Sublingual mucoadhesive tablets are a good candidate alternative to conventional B_{12} supplementation because they will enhance B_{12} absorption through passive diffusion in the sublingual area.

A thesis involved the preparation and evaluation of different placebo formulas using molding, wet granulation, and direct compression methods. The disintegration time of each formula was assessed, and those meeting the accepted criterion of less than one minute proceeded to the next step, resulting in twenty selected formulas. The chosen formula was then used to incorporate cyanocobalamin through the direct compression method, a simple and cost-effective technique. Tablets obtained from this process were evaluated, and only those meeting the residence time criterion of greater than 15 min on bovine mucosa were passed to the next step, resulting in four formulas chosen. These four formulas were scaled up to 400 tablets and underwent various evaluation tests, including blend precompression for bulk and tapped density, Carr's index, and angle of repose to examine powder flowability. All formulas exhibited good and excellent flowability due to the presence of microcrystalline cellulose, which possesses excellent flowability characteristics.

A UV-Vis spectrophotometer method of analysis was developed and validated for the detection of cyanocobalamin. The validation process included an assessment of detection capability, calibration curve, accuracy, precision, robustness, and stability. Additionally, the post-compression characteristics of the formulated tablets were evaluated, including weight variation, thickness, diameter, friability, drug content, mucoadhesive strength, and dissolution. The results showed that all formulas were within an acceptable range and fitted to the Makoid-Banker and Peppas-Sahlin kinetic models, with R^2 values exceeding 0.99. This indicated that the drug release involved both Fickian kinetics and non-Fickian mechanisms, including diffusion, polymer matrix relaxation, swelling, and erosion.

The Permeapad® membrane, employed in the Franz diffusion cell, was utilized to evaluate the permeability of cyanocobalamin in the final formulas. The obtained results indicated that Eudragit L100-55 exhibited the highest permeability parameter. This finding suggests that the inclusion of Eudragit L100-55 in the formulation led to improved drug absorption and overall bioavailability.

ملخص

فيتامين ب 12 هو فيتامين أساسي يلعب دورًا مهمًا للغاية في وظيفة الخلية والتمثيل الغذائي، مثل إنشاء الحمض النووي. هناك العديد من الأسباب لنقصه، مثل نقص العامل الجوهري(IF) ، والذي غالبًا ما يكون السبب الرئيسي وهو بالغ الأهمية لامتصاص فيتامين ب 12. علاوة على ذلك، يرجع النقص في العامل الجوهري إلى العديد من الأسباب، بما في ذلك بعض الأمراض، نقص الجين المصنع للعامل الداخلي (GIF)والآثار الجانبية لبعض الأدوية. نقص فيتامين ب 12 له عواقب وخيمة مثل زيادة خطر الإصابة بأمراض القلب والأوعية الدموية، والضعف المعرفي، وفقدان الحواس في الأطراف البعيدة. الإعطاء الفموي للفيتامين هو أكثر الطرق شيوعًا لاستخدام مكملات فيتامين ب 12 المتاحة. ومع ذلك، فإنه في بعض الأحيان لا يكون مفيدًا بشكل خاص للمرضى الذين يعانون من عيوب أو خلل في العامل الجوهري. كما أن طريق الحقن مؤلم ويتطلب أخصائيًا.

تهدف هذه الأطروحة إلى تعزيز امتصاص فيتامين ب 12 من خلال طريق توصيل الدواء الأكثر تفضيلاً. الفكرة الرئيسية هي زيادة مدة بقاء الدواء في موقع الإعطاء لتحسين الامتصاص، سواء كان العامل الداخلي متاحًا أم لا، عن طريق الامتصاص بالانتشار السلبي. يعمل مباشرة بعد تطبيق شكل جرعة يحتوي على بوليمر لاصق مخاطي على سطح الغشاء المخاطي للجسم، والذي يبتل مباشرة بعد ملامسة السائل الموجود على السطح، ثم يخترق بعمق في الغشاء المخاطي، ويشكل روابط كيميائية، ويبقى ملتصقًا بسطح الغشاء المخاطي لوقت أطول. تعتبر الأقراص المخاطية اللاصقة تحت اللسان بديلاً مرشحًا جيدًا لمكملات ب12 التقليدية لأنها ستعزز امتصاص ب12 من خلال الانتشار السلبي في منطقة تحت اللسان.

تضمنت الأطروحة إعداد وتقييم صيغ وهمية مختلفة باستخدام طرق التشكيل والتحبيب الرطب والضغط المباشر. تم تقييم وقت تفكك كل صيغة، والصيغ التي استوفت المعيار المقبول وهو أقل من دقيقة واحدة للتفكك انتقلت إلى الخطوة التالية، مما أدى إلى عشرين صيغة مختارة. ثم تم دمج السيانوكوبالامين مع هذه الصيغ من خلال طريقة الضغط المباشر ، وهي تقنية بسيطة وفعالة من حيث التكلفة. تم تقييم الأقراص التي تم الحصول عليها من هذه العملية، وتم تمرير فقط تلك التي استوفت معيار وقت الإقامة لأكثر من 15 دقيقة على الغشاء المخاطي البقري إلى المرحلة التالية، مما أدى إلى نجاح أربع صيغ. تم تكبير هذه الصيغ

الأربعة حتى 400 قرص ومن ثم خضعت لاختبارات تقييم مختلفة، بما في ذلك تقييم الخليط قبل ضغطه كالكثافة الظاهرية، مؤشر كار، زاوية الراحة لفحص انسيابية المسحوق. أظهرت جميع الصيغ قابلية تدفق جيدة وممتازة بسبب وجود السليلوز دقيق التبلور، الذي يمتلك خصائص انسيابية ممتازة.

تم تطوير طريقة تحليل باستخدام مقياس الطيف الضوئي وتم التحقق من صحتها للكشف عن السيانوكوبالامين. تضمنت عملية التحقق من الصحة تقييمًا لقدرة الكشف عن السيانوكوبلامين ومنحنى المعايرة والدقة والمتانة والثبات. بالإضافة إلى ذلك، تم تقييم خصائص الاقراص المصنعة بعد الضغط، بما في ذلك اختلاف الوزن ، والسمك ، والقطر ، وفحص هشاشة الاقراص ، ومحتوى الدواء ، وقوة اللصق مع الغشاء المخاطي ، والذوبان. أوضحت النتائج أن جميع الصيغ كانت ضمن النطاق المقبول وملائمة للنماذج الحركية نموذج ماكويد-بانكر و نموذج بيباس-ساهلين، مع قيم معامل انحدار تتجاوز 0.99. يشير هذا إلى أن إطلاق الدواء شمل كلا بالانتشار الجزيء "Fickian " وآليات غير الانتشار الجزئي " non-Fickian" ، بما في ذلك استرخاء مصفوفة البوليمر، والتورم ، والتآكل

تم استخدام غشاء Permeapad في خلية انتشار فرانز، لتقييم نفاذية السيانوكوبالامين في الصيغ النهائية. أشارت النتائج التي تم الحصول عليها إلى أن Eudragit L100-55 أظهر أعلى معامل نفاذية. تشير هذه النتيجة إلى أن إدراج Eudragit L100-55 في المستحضر يؤدي إلى تحسين امتصاص الدواء والتوافر البيولوجي العام.

Chapter 1: Introduction

1. Introductions

Vitamins are mainly divided into two types: essential and non-essential vitamins. Essential vitamins are not produced in the human body and are obtained only from foods, while non-essential vitamins can be synthesized within the human body and can also be taken from nutrients.[1] Vitamin B_{12} (cobalamin) is one of the essential vitamins and plays a crucial role in human cell function and metabolism, such as DNA creation, especially for the rapidly regenerating organs, for example the nervous system and the digestive system.[2,3] The body requires 0.4 and 1.5 μg per day of vitamin B_{12} for infants and adults respectively. The main sources of vitamin B_{12} are animal sources such as eggs, meat, fish, and a few plants.[3,4] Vitamin B_{12} deficiency increases the risk of myocardial infarction and storke. There are many reasons for its deficiency, including a defect or deficiency in the intrinsic factor, which is often the main cause, as the intrinsic factor is very important to protect vitamin B_{12} from catabolism by intestinal bacteria, and, most importantly, it's critical in B_{12} absorption by binding enterocyte receptors in the terminal ileum. On the other hand, the uptake through passive diffusion is only about 1.2%, and that's not enough alone.[3,5]

1.1. Important of Vitamins B_{12}

As previously stated, vitamin B_{12} is required for DNA synthesis, myelin synthesis, and energy production in the human body. When vitamin B_{12} enters the bloodstream, it is converted to adenosylcobalamin coenzyme in the mitochondria

and methylcobalamin coenzyme in the cytoplasm, both of which are required for two enzymatic reactions (Figure 1). [2,6]

Firstly, the 5-methyltetrahydrofolate-homocystine methyltransferase reaction (MTR), which is also known as the methionine synthase reaction[2], converts homocysteine to methionine in the presence of the methylcobalamin cofactor and folate. The amino acid methionine is required for the production of s-adenosylmethionin (SAM). SAM is involved in methylation reactions in a wide range of biochemical reactions, including myelin sheath production, neurotransmitter synthesis, nervous system functions, and cysteine synthesis.[2,6] Additionally, methylcobalamin is necessary for the MTR reaction to produce tetrahydrofolate (THF), the folate active form that aids in DNA synthesis.[2]

When vitamin B_{12} deficiency develops, the MTR reaction is disrupted, resulting in an accumulation of homocysteine and 5-methyltetrahydrofolate precursors in the circulation. Methionine and THF can be obtained via diet or nutritional supplement sources, and at the right doses, SAM and DNA synthesis can be maintained in the human body. However, this cannot compensate for vitamin B_{12} insufficiency. Furthermore, these supplements will not be able to prevent the accumulation of 5-methyltetrahydrofolate and homocysteine, which are intermediate products of the metabolic processes, and therefore hyperhomocysteinemia will persist. [2]

Methylmalonyl Coenzyme A Mutase (MUT) is the second reaction:[2] The adenosylcobalamin cofactor is required for the MUT reaction to convert methylmalonyl coenzyme A to succinyl coenzyme A. In the mitochondrial citric acid cycle, this process is critical for energy extraction from fat and protein. In the

absence of vitamin B_{12}, this process does not take place, and methylmalonic acid (MMA) accumulates. The aggregation of MMA may interfere with the production of normal fatty acids and hinder myelin sheath synthesis, resulting in neurological impairment. The MUT reaction is mostly dependent on vitamin B_{12} coenzymes, and a lack of these coenzymes causes the reaction to stop. Vitamin B_{12} supplementation is the only one that can solve these issues (Figure 1). [2,6]

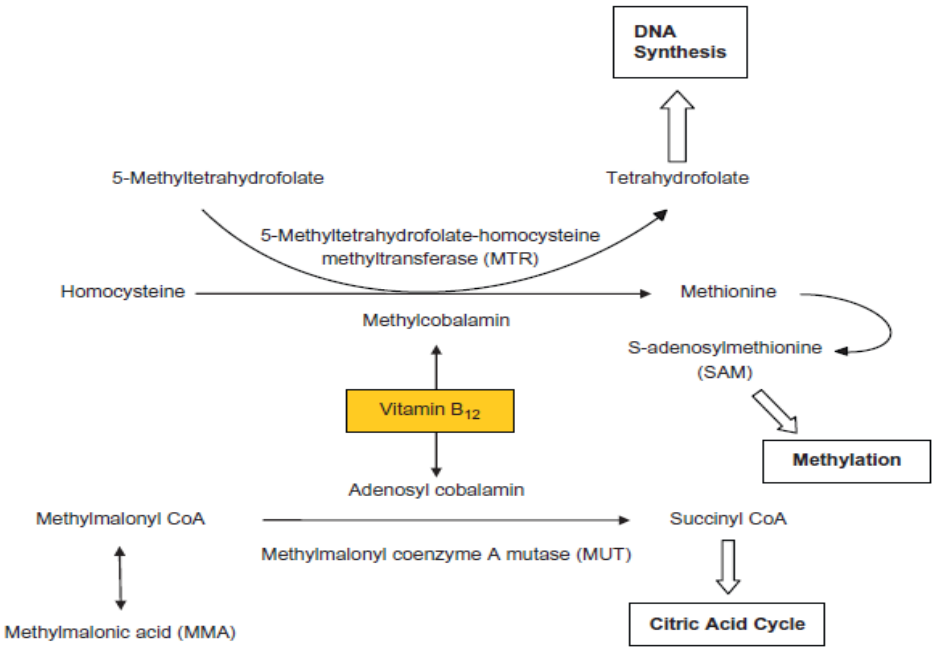


Figure 1: Vitamin B_{12} works as coenzyme in the human body [2]

1.2. Causes of vitamin B_{12} deficiency

The intrinsic factor deficiency is due to several factors. First of all, Gene Intrinsic Factor (GIF) deficiency in 10% of individuals since birth, which is responsible for the production of intrinsic factors.[5,7–9] GIF is located on chromosome 11 of the human genome. [5] Other congenital causes of deficiency include cobalamin mutation (C-G-1 gene), juvenile pernicious anemia, genetic transcobalamin insufficiency,

and Imerslund-Grasbeck syndrome, in which the passage of vitamin B_{12} through cells is limited. The intrinsic factor deficiency could be hereditary. [2,6,8,9]

In some cases, the intrinsic factor antibody is responsible for vitamin B_{12} deficiency. It can prevent vitamin B_{12} from attaching to the intrinsic factor and forming the B_{12}-IF complex.[10] Vitamin B_{12} deficiency, on the other hand, could be caused by an abnormal intrinsic factor. Although abnormal intrinsic factors bind to vitamin B_{12}, the resulting B_{12}-IF complex is unable to attach to the ileum's enterocyte receptor. [1,8] Antibodies against parietal cells also decrease the production of intrinsic factors.[2,3,6,11]

Second, vitamin B_{12} deficiency can be a result of insufficient intake, including malnutrition, reduce consumption of animal products, and adopt a strict vegetarian diet. [2,3,6,11] Malnutrition combined with chronic alcohol consumption and cystic fibrosis can cause pancreatic impairment. In this case, hydrochloric acid and pancreatic enzyme synthesis are reduced. As a result, the R protein- B_{12} complex break process fails, and thus the free vitamin B_{12} generation is inhibited, resulting in vitamin B_{12} malabsorption. [2,3,6,8,11]

Vitamin B_{12} can only be obtained from animal foods; vegetarians, on the other hand, have no vitamin B_{12} in their diet. Therefore, many vegetarians suffer from a vitamin B_{12} deficiency.[2,6] According to a systematic review of the literature, vegetarians have a vitamin B_{12} deficiency in roughly 45% of babies, 33.3% of children and adolescents, 86.5% of adult individuals, and 17–39% of pregnant women. [12] Vitamin B_{12} can be stored in the liver for up to 10-15 years. The efficient enterohepatic

circulation maintains a portion of the vitamin B_{12} level in these individuals (Figure 2).[8]

Third, vitamin B_{12} deficiency can be a result of stomach and intestinal malabsorption. [2,6] Despite the fact that humans consume animal diets, many patients, particularly the elderly, suffer from vitamin B_{12} deficiency related to the weakness of the extraction of vitamin B_{12} from cobalamin-bound protein compounds in food (Figure 2, Figure 10). As a result, intrinsic factor is unable to bind to B12 because it's not free, so it's not absorbed. On the other hand, free vitamin B_{12} stays absorbed. An oral dosage form of a vitamin B_{12} supplement can correct this deficit.[2] Additionally, food processing, such as cooking, pasteurization, and exposure to fluorescent light, can cause nearly half of the vitamin B_{12} found in the food to be lost.[12]

Pernicious anemia (PA) is the most common cause of malabsorption. [2,3,6] PA is an autoimmune illness that affects the stomach's mucosa and fundus. This disease results in a decrease in the number of parietal cells by destroying them. Those cells are responsible for the creating of intrinsic factor, which is required for B_{12} absorption.[2,3,6,8] Interruption of intrinsic factor production will prevents B_{12} binding to intrinsic factor, thereby preventing vitamin B_{12} absorption.[2,3,11] Then this condition may progress to megaloblastic anemia and neurological symptoms.[4] In addition, anti-parietal cell antibodies and anti-intrinsic factor antibodies were discovered in pernicious anemia patients. These antibodies lower and deplete the intrinsic factor level.[2,6,10] Pernicious anemia is associated with auto-antibodies that block the binding site of the intrinsic factor, which limits vitamin B_{12} absorption,

whether it's free or cobalamin-protein compound, resulting in a severe deficiency.[2,3,11] When comparing pernicious anemia with malabsorption, vitamin B_{12} dietary malabsorption develops more slowly than pernicious anemia.[2]

The cells of the gut are damaged in atrophic gastritis, which leads to a deficiency in the production of intrinsic factor.[2,3,6,7,11,13] Additionally, decrease the secretion of pepsin, pancreatic enzyme and hydrochloric acid. Alkaline environments prevent the release of vitamin B_{12} from the associated proteins in food (Figure 2).[2,11] Furthermore, it stimulates the overgrowth of gut bacteria which affects the transport of vitamin B_{12} in the gastrointestinal tract. Bacteria also consume the vitamin B_{12}-IF complex as nutrients.[1–3] These factors lower vitamin B_{12} levels in the blood, resulting in insufficiency.[2,3,11]

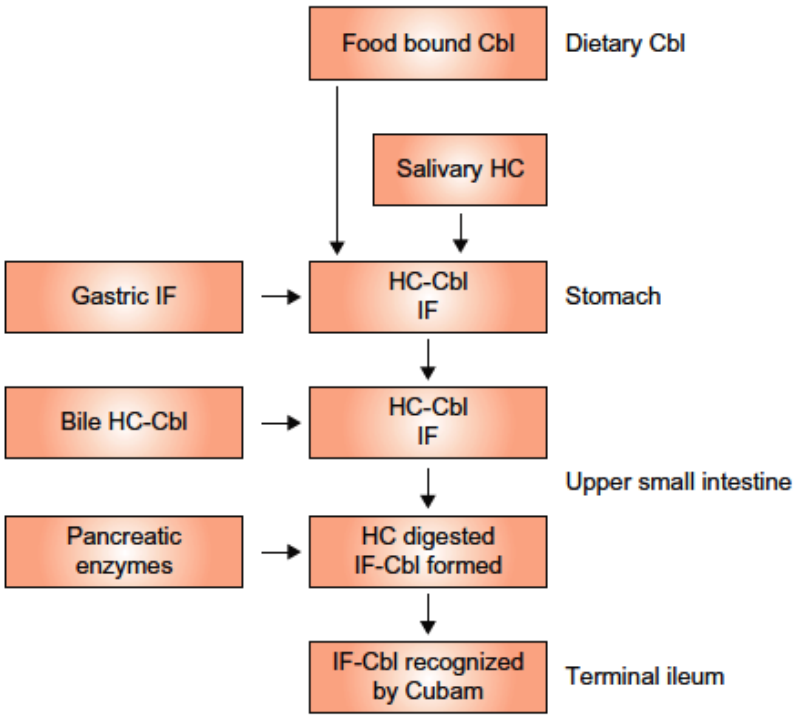


Figure 2. Vitamin B_{12} (Cobalamin/Cbi) absorption and transfer from the diet to the body's cells. Cbl is freed from food and bound to haptocorrin (HC). Furthermore, Cbl is recycled by the liver and supplied through bile coupled to HC. Cbl is linked to intrinsic factor (IF) when pancreatic enzymes enzymatically degrade HC in the upper intestine. The IF-Cbl complex is detected in the terminal section of the ileum by a unique receptor called cubam. [5]

Chronic condition of *Helicobacter pylori* [2,3,11] according to studies, 56% of patients have vitamin B_{12} deficiency, which has been linked to long-term *H. pylori* infection and atrophic gastritis.[2] After gastric bypass and partial or complete gastrectomy, the formation of intrinsic factor, stomach acid, and pancreatic enzymes will be reduced.[2,6,8] For example, in stomach antrum resection, the production of intrinsic factor will stop. This is because the antrum is the location of hydrochloric acid secretion and intrinsic factor generation.[4,8]

Malabsorption of the ileum, such as in tuberculous ileitis, Crohn's disease, inflammatory bowel disease, and ileal resection. In these conditions, the absorption receptor is removed.[2,3,6,11] There are also some congenital diseases that lead to reduction in vitamin B_{12}-IF complex ileal binding.[8] Other conditions that result in malabsorption of the ileum include, Blind loop syndrome, intestinal bacterial overgrowth, giardiasis, and fish tapeworm,[1–3,6,11] Luminal disturbances such as chronic pancreatic disease,[6] and Imerslund- Gräsbeck's syndrome. This later syndrome is caused by a genetic abnormality in cubilin and amnionless proteins in the ileum cubam receptor. These receptors are crucial for vitamin B_{12} absorption.[5,8,12]

Finally, vitamin B_{12} deficiency as a side effect of some drugs, such as cholestyramine, biguanides (metformin), aminoglycosides, nitrous oxide,[2–4,6,11] antacids (Histamine 2 antagonists and proton pump inhibitors), colchicines, [2–4,6,11] potassium chloride preparations and epileptic medications.[3,4,6] By interfering with vitamin B_{12} absorption from the gut, cholestyramine lowers vitamin B_{12} levels.[2] Metformin reduces B_{12} absorption when administered for longer than four months, by lowering the amount of free calcium ions in the gut. The uptake of vitamin B_{12}-IF complex in the terminal ileal receptor necessitates these ions. [2,14]

The generation of gastric juice is proportional to stomach acidity. Antacid medications inhibit the synthesis of hydrochloric acid, pepsin, and pancreatic enzymes. Low levels of hydrochloric acid cause the PH to rise to an alkaline level. The alkaline medium prevents the extraction of vitamin B_{12} from protein in the diet. [2,4,8,14] Furthermore, the alkaline medium inhibits the production of intrinsic factor

by parietal cells.[6] When an antiacid is used for more than 12 months, it reduces vitamin B_{12} absorption and contributes to insufficiency. [14] For these reasons, some patients who take vitamin B_{12} supplements orally do not benefit from them.[2,3,11]

1.3. Vitamin B_{12} deficiency manifestation

Vitamin B_{12} insufficiency causes a variety of symptoms in patients including.

1. Osteoporosis.[2,6]
2. Loss of appetites.[2]
3. Breathing problems.[2]
4. Weakness and fatigue.[2,6]
5. Suppression of the bone marrow.[6]
6. Hyperpigmentation of the skin. [2,6]
7. Infertility occurs in reproductive tissue.[6]
8. Low blood pressure and the risk of cardiomyopathy.[2,6]
9. Taste impairment, glossitis, and diarrhea.[2,6]
10. Blood disorders: Pancytopenia, macrocytosis, hypersegmented neutrophils, megaloblastic anemia, and other anemias.[2,6]
11. Neurological manifestations: Ataxia, confusion, myelopathy, irritability, psychosis, delusions, depression, memory loss, cognitive impairment, spasticity of hyporeflexia, loss of proprioception, autonomic dysfunction, motor disturbance, alteration in mental state, and spinal cord degeneration. [2,6,7]

1.4. Treatment options for vitamin B_{12} deficiency

Vitamin B_{12} is a nutrient that can be found in dietary supplements such as multivitamins and mineral supplements. It may be available as a B complex supplement with other B vitamins or as a vitamin B_{12} supplement separately. Multivitamin and mineral supplements have 5–25 µg, B–complex supplements contain 50–500 µg, and B_{12} pills contain 500–1000 µg. Cyanocobalamin is the most prevalent analog found in supplements. Adenosylcobalamin, hydroxocobalamin, and methylcobalamin are also available.[9]

Oral supplements are available in tablets, lozenges, and sublingual dosage forms. According to the literature, there appear to be no differences in efficacy between oral and sublingual dosages. Cyanocobalamin and hydroxocobalamin are utilized as intramuscular (IM) injections to treat vitamin B_{12} insufficiency in most countries. Hydroxocobalamin has totally replaced cyanocobalamin in some nations because it stays in the body for a longer time and can be given at regular intervals. [3,9,15]

In theory, oral vitamin B_{12} seems to have the same effect and is as safe as parenteral forms (IM). Passive diffusion without IF binding absorbs approximately 1.2 percent of total oral vitamin B_{12} intake in the GIT. A large dose of about 1 mg must be given to achieve adequate absorption, even in patients with ileal dysfunction or PA. As a result of that, the oral route is a feasible alternative to IM. [2,3,11,15]

In rare cases, intravenous cobalamin is used to treat vitamin B_{12} insufficiency.[11] Vitamin B_{12} nasal gel sprays are also available in 1000 µg nasal doses. In a brief

clinical investigation of 10 individuals, it was found that nasal dosage forms have similar bioavailability to oral dosage forms (2%).[9]

Approximately 1–5% of free cobalamin is absorbed by passive diffusion in the gastric system from the mouth to the intestinal mucosa. In addition, nasal mucosa. In recent years, the nasal route of administration has attracted attention.[11] The cyanocobalamin is produced as a gel or spray-dried powder via this route. After delivery through the nasal mucosa by passive diffusion, it reaches the serum in 1-2 hours.[11,16] Nascobal® (500 µg/0.1 ml) (Figure 3) is a commercial vitamin B_{12} intranasal spray that is administered once a week to treat cobalamin deficiency caused by a number of conditions, including pernicious anemia.[11]

CaloMist, intranasal pharmaceutical dosage of cyanocobalamin is commercially available. Each 0.1 ml of solution contains 25 µg, and each spray is about 0.1 ml (Figure 3). The recommended daily dose is 50 µg. If the patient does not respond, the dose can be increased to 100 µg a day.[17]

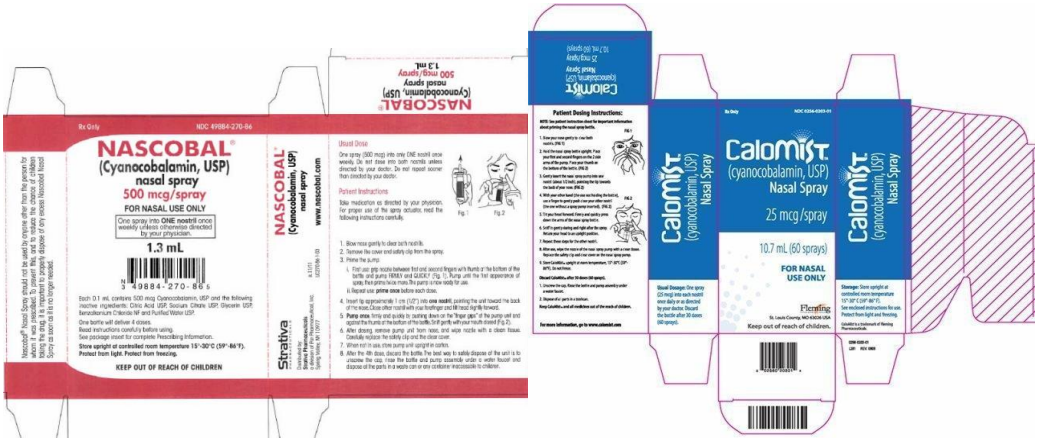


Figure 3: Intranasal cobalamin dosage forms (Nascobal and CaloMist respectively)[18,19]

There is currently no transdermal vitamin B_{12} product on the market. However, there is some research in the literature on this subject. The use of a transdermal product is non-invasive, overcomes the first pass effect, minimizes the number of doses, and improves patient adherence. The development of cobalamin microemulsions is one of these studies. Different solvents were used in these studies to improve drug penetration through a variety of mechanisms, including the fluidization of the stratum corneum, intercellular lipid extracting, and alteration of cellular proteins.[20,21]

The absorption of vitamin B_{12} supplements is affected by the dose and frequency of administration, which are dependent on the route's efficacy and the receptors saturable response. The following is the bioavailability of doses based on that: 56% for 1µg, 16% for 10µg, 3% for 50 µg, 2% for 500 µg, and 1.3% for 1000 µg.[9,12] About half of the dose is absorbed when the dose is less than 1-2 µg, because the quantity available does not surpass the intrinsic factor's cobalamin-binding capability. So when the dose is increased, the absorption decreases.[9]

Individuals' daily vitamin B_{12} requirement varies depending on their age and status (Table 1). For example, because of the expansion of tissue and the supply of B_{12} to the fetus and baby, pregnant and breastfeeding women require a higher amount.[12,22] The large oral doses, 500 µg sublingual or 500-1000 µg orally or two tablets of 250 µg B complex for eight weeks, with a passive diffusion absorption of 1%, meet this requirement.[22]

Table 1: Vitamin B_{12} recommended daily intakes[9]

Age	Male & Female	Pregnancy	Lactation
From birth to 6 months	0.4 μg	-	-
7-12 months	0.5 μg	-	-
1-3 years	0.9 μg	-	-
4-8 years	1.2 μg	-	-
9-13 years	1.8 μg	-	-
14-18 years	2.4 μg	2.6 μg	2.8 μg
19 and more	2.4 μg	2.6 μg	2.8 μg

When a deficiency occurs as a result of pancreatic insufficiency, vitamin B_{12} will be compensated by the combination of pancreatic enzyme and cobinamide. Cobinamide is a vitamin B_{12} analog that can replace vitamin B_{12}, which binds to the R protein. Vitamin B_{12} binds to the IF and is absorbed as a result.[8]

After diagnosis, if the patient exhibits neurological symptoms, treatment should begin immediately with IM vitamin B_{12}. If the patient has severe anemia, a packed red blood cell transfusion might be used as an emergency treatment.[6]

1.4.1. Recommended doses

Table 2 below summarizes the recommended vitamin B_{12} doses for various pharmaceutical dosage forms and illnesses when vitamin B_{12} deficiency is diagnosed.

Table 2: Vitamin B_{12} recommended doses

#	Vitamin B_{12} doses	Comments	Study duration	Ref
Hydroxocobalamin intramuscular injections				
1	Take 1mg daily for a week then 1mg every other day for 2 weeks as a loading dose (LD). Then 1mg weekly for one month as a maintenance dose (MD).	The patients have a neurological disorder.	Until the deficiency is resolved.	[2,6]
2	Take 1 mg every two or three months. Take 5 mg of folic acid with a B_{12} supplement. Also, if there is a deficiency of folate.	If a patient does not have any neurological problems, but there are some reasons for irreversible malabsorption, such as gastrectomy and PA.	For life	[6]
Cyanocobalamin intramuscular injections				
3	Take 1 mg daily for a week, then 1 mg weekly for a month as a LD.	In food malabsorption	Until the deficiency is resolved.	[11]

	Then 1 mg every 1 or 3 months as MD, depending on the cause of malabsorption			
4	Take 1 mg daily.	The patients have a neurological disorder.	At least for 1-3months	[11]
5	Take 1 mg daily for a week, then 1 mg weekly for a month as LD. Then 1 mg monthly as MD.	For the Biermer's disease (PA) patients.	For life	[11,23]
Oral cyanocobalamin products				
6	Take 50- 150 µg daily.	For malabsorption	Depends on the reason of deficiency	[6]
7	Take 1 mg daily for a month then 125 µg- 1 mg daily	For malabsorption	Until the deficiency is resolved.	[2,11]
8	Take 1 mg daily as MD	For irreversible malabsorption patients	For life	[2,6,11]
Sublingual vitamin B_{12} products				

9	Take 50- 350 µg daily	For malabsorption	Until the deficiency is resolved.	[11]

It was found that patients with PA responded to oral doses of 0.5 and 1 mg daily and achieved normal levels of vitamin B_{12} in the blood, whereas lower doses were ineffective.[15]

1.4.2. Advantages and limitations of B_{12} dosage forms

1.4.2.1. Intramuscular

Advantages of IM

1. Avoid exposure to gastrointestinal environments.[23,24]
2. Rapid absorption by diffusion.[23]
3. Bioavailability is predictable and nearly complete.[25]
4. Suitable for unconscious individuals who are unable to eat or drink.[25]

Limitation of IM:

1. Expensive and invasive.[26,27]
2. Need for qualified healthcare personnel such as nurses. [3,24,27]
3. Dangerous needle use and inappropriate disposal (such as needle).[24,25,27]
4. The size of the injection is restricted by the mass of the muscles that are available for injection.[25]

5. Bleeding can be a risk for individuals who have been taking anticoagulants for a long time[3,11,23,27]
6. Low patient compliance, discomfort and needle anxiety, as well as pain at the injection site, are all associated with injections. [3,11,24–27]
7. Nerve and bone injury, muscle atrophy, abscess, infection, and hemorrhage at the injection site are all possible consequences.[24,25,27]
8. Injections have a higher allergenic potential than tablets. Furthermore, hydroxocobalamin appears to be more allergic than cyanocobalamin. Despite the fact that all types of cobalamin can cause allergies.[23]
9. Some patients have side effects after administering IM vitamin B_{12}, including fever, chills, nausea, dizziness, itching, hot flashes, rash, breathing problems, polycythemia, pulmonary edema, hypokalemia, and joint tingling or numbness, as well as rapid weight gain. But in general, it's well tolerated.[6,23]

1.4.2.2. Oral dosage forms

The advantages of oral dosage forms

1. Painless and safe. [26,28–30]
2. The dosage's accuracy. [29–31]
3. Administration is simple. [24,30–34]
4. Fewer visits to doctors and nurses. [3]
5. There is very little patient discomfort. [30,33–36]
6. The most acceptable and compliant dosage form. [3,23,30,33–35,37]
7. Eliminates injection costs and is inexpensive. [11,23,28]

8. Utilized to deliver both traditional and innovative drugs. [25]
9. Reducing the risk of bleeding in anticoagulant-treated patients. [23]
10. Simple to manufacture, especially direct compression at a low cost [30–34,38]
11. There is about 300 m^2 of surface area for drug absorption, specifically passive diffusion. [24]

Limitations of oral dosage forms

1. The onset of action is slow. [29]
2. The acidic pH of the stomach may contribute to the degradation of the medication. [39]
3. Enzymatic degradation in the gut reduces the bioavailability of a variety of drugs, such as proteins. [29,30,40]
4. Almost 50% of people have difficulty swallowing, including the unconscious, elderly, and pediatric patients. [29,30]

Sublingual advantages

1. Facilitating self-management.[27]
2. Simple to reach the sublingual region. [31]
3. Avoid the first-pass hepatic metabolism. [25]
4. Patients with swallowing problems can use this route. [25]
5. If an unfavorable reaction occurs, the medication can be removed. [25]
6. Because of the extensive systemic veins in the mucosal network, there is a high rate of absorption. [25]

Sublinqual limitations

1. Expensive [23,41]
2. Not thoroughly researched. [23]
3. The bad-tasting pill has low acceptance.[25]
4. Ineffective for people with vomiting or diarrhea. [26]
5. Not suitable if a patient refuses to cooperate or is unconscious. [42]
6. When taking drugs, patients should not eat or smoke because it constricts the blood vessels. As a result, the drug's absorption will be reduced.[25,42]
7. Medication must not be chewed or swallowed, and the dosage form should be kept in place.[25]
8. Excessive saliva production results in quick tablet disintegration and absorption when swallowed.[25]

1.4.2.3. Intranasal

Advantages of intranasal

1. The first-pass metabolism is avoided. [24,25]
2. Avoid gastrointestinal complications. [24]
3. Absorption is quick, within half an hour. [25]
4. The subepithelial tissue is well vascularized. [25]
5. Patients who are used to intranasal products will find it simple to administer. [25]
6. The nasal mucosa has a higher permeability than the epidermis or gastrointestinal. [25]

Intranasal limitations

1. Expensive [23]
2. Not thoroughly researched. [23]
3. Not familiar to most individuals. [16]
4. As a result of nasal diseases, absorption is impaired.[25]
5. Mucus viscosity has an impact on bioavailability.[24]
6. Poor bioavailability is primarily due to mucociliary clearance. [24,25]
7. The metabolism of drugs is carried out by enzymes present in the nasal cavity. [24,25]
8. There is only a tiny space in the nose, so the dose and time for absorption are limited. [25]
9. The bioavailability of a drug decreases as the rate of mucus secretion increases. [25]
10. The drug molecule size causes a challenge. Only a drug with a molecular weight of less than 300 Daltons can be absorbed without being significantly affected by the drug's physicochemical characteristics.[24,25] However, the cobalamin molecular weight is 1355.4 Dalton. [17]

1.5. Studies to illustrate the efficacy of various pharmaceutical dosage forms of B_{12}:

1. In a prospective study, roughly 50 patients began the dosage protocol as follows:

1 mg of IM hydroxocobalamin as a loading dose (LD) until the serum vitamin B_{12} level reaches 418 pg/ml. If serum vitamin levels remain above 275 pg/ml, a maintenance dose (MD) of 1 mg of daily oral cyanocobalamin for 18 months is recommended. If the serum level falls below 275 pg/ml, the injection should be restarted. Outcomes of the study: all patients were able to continue taking cyanocobalamin orally without needing to restart their hydroxocobalamin injections.[11,43]

2. In prospective study, thirty individuals began the dosage protocol as follows:

   The participants were given 500 µg of cyanocobalamin in one of three dosage forms: 250 µg B complex in two tablets, 500 µg sublingual in one tablet or 500 µg in one oral tablet daily with breakfast for eight weeks. Outcomes of the study: after 4 weeks, all groups reached normal serum levels of vitamin B_{12}. [22]

3. Following gastric bypass surgery, patients were administered 1 mg of oral methylcobalamin or hydroxocobalamin in IM. After 6 months, all patients exhibited normal vitamin B_{12} levels with no significant differences. On the other hand, 46.6 percent of patients who took oral dosage forms had unacceptable low levels of vitamin B_{12} due to non-compliance. [11]
4. In a randomized control study, a comparison of two studies comparing oral and intramuscular delivery Cyanocobalamin was taken orally every day at

a dose of 1-2 mg, or 1 mg intramuscularly every day for ten days, then once weekly for four weeks, then once monthly for 90 days to 4 months.[3,44]

The oral group had considerably greater serum vitamin B_{12} levels than the intramuscular group. All neurological characteristics—memory, cognitive function, and sensory neuropathy were improved. Injections of 1 mg of cyanocobalamin resulted in 69 percent of doses being eliminated in the urine, while the elimination rate was 27 percent after administration of 1 mg of hydroxocobalamin. This could explain why, in this trial, oral efficacy was similar to or slightly better than that for parenteral.[3]

Despite the fact that some individuals were given 2 mg per day, while others were given 1 mg per day in this trial, there had been no reported negative effects in general. Although 1 or 2 mg were as effective as 1 mg IM, the effectiveness findings were not practical. Because the time given for patient follow-up is so limited.[3]

5. According to the literature, oral vitamin B_{12} was administered in five studies. In one study, 1000 µg was given sublingually, while in the other four studies, 1000 or 2000 µg was given daily as MD. All these studies suggest that taking vitamin B_{12} orally is sufficient to correct insufficiency as an alternative to IM in individuals with PA. For example, patients were administered 1000 µg of vitamin B_{12} sublingually for 7–12 days. All patients reached normal B_{12} levels. In most patients, B_{12} levels are four times higher than before treatment.[44]

6. In double-blind (participants and outcome judge) randomized, controlled, and parallel dietary intervention research. A total of 40 participants were enrolled in the research. The research was conducted between May 2015 and October 2016. One group was given a low dose (50 µg daily) of the supplement. The control group, on the other hand, was given a high dose (2000 µg weekly) of the supplement once a week. Outcomes of the study: Both types of supplementation significantly enhanced vitamin B_{12} levels in the blood.[26]

Even though the oral route is available and safe in most countries, it is not prescribed. Oral vitamin B_{12} in high-dose formulations was not available on NHS prescriptions in the United Kingdom in 2016. Because of the unpredictable absorption and lack of awareness of this alternative, oral vitamin B_{12} is prescribed and used less than other dosage forms. In Sweden, oral vitamin B_{12} accounts for about 73% of total B_{12} prescriptions. The oral route is commonly utilized in Canada.[3,44]

In a healthy patient, the daily requirement of vitamin B_{12} is about 2 µg/day, and the cobalamin dose intake of 100–250 µg is sufficient. In disorders when the intrinsic factor is missing, however, passive diffusion of 1% of the 1000 µg daily dose is sufficient.[44]

Despite its challenges, the oral drug delivery route has remained the most used route till now for many reasons, including the simplicity of administration, good patient compliance, safety, and non-invasiveness compared to the parenteral administration

technique and other benefits mentioned previously(Section1.4.2.2). Moreover, there are numerous enhancements that can be made to solve the obstacles that conventional oral dosage forms face (Section 1.4.2.2), such as controlled release formulations, licaps, liposomes, nanoparticles, and oral mucoadhesive formulations. [25,30,31,45]

1.6. Mucoadhesive sublingual dosage forms

1.6.1. Oromucosal drug delivery

Three categories exist for the delivery of drugs through oral cavity membranes: local, buccal, and sublingual deliveries (Figure 4). Drug administration is used for the localized treatment of bacterial and fungal infections and periodontal disease at the periodontal and gingival levels. To have a systemic effect, drugs must enter the capillary network below the mucosa. This is primarily accomplished by passing through the buccal or sublingual non-keratinized epithelium. [27,46] Buccal delivery refers to medication being delivered to the bloodstream via the mucosal membranes of the cheeks and the region of mucosa between the lips and the gums. Sublingual delivery is defined as drug delivery to the systemic circulation via the mucous membrane lining the mouth's floor. [27,31,39,40,42]

Oral mucosa with a 170 cm^2 total surface area has three primary layers that can be seen histologically. Squamous epithelium, commonly known as oral epithelium, was the top layer, with connective tissue beneath it separated by a basal membrane. Lamina propria is the deeper tissue layer, which is composed of dense, irregular

connective tissue that contains blood capillaries and muscles, commonly known as submucosa (Figure 4). [27,40,47]

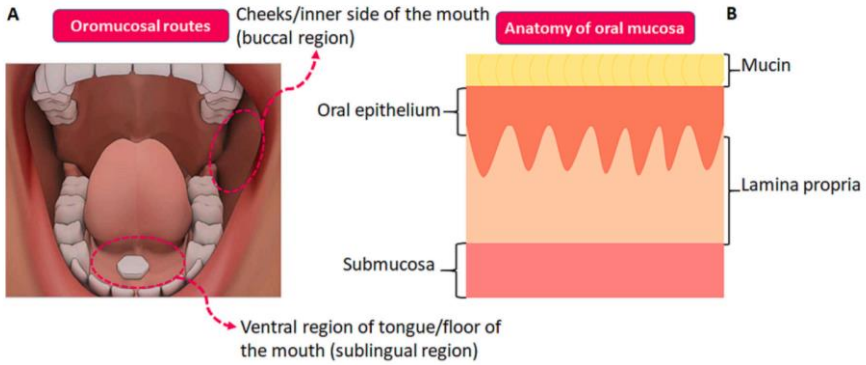


Figure 4 The oral mucosa regions for drug delivery and their anatomy [27]

Because the submucosa is lacking in some areas of the mouth cavity, the lamina propria is directly attached to the bones or muscles beneath. The thickness of the oral epithelium varies throughout the oral cavity, with the ventral side of the tongue, the ground of the mouth, the inside sides of the lips, and the cheeks being the thinnest. The oral epithelium secretes mucus and has sensory capabilities that enable the awareness of touch, taste, temperature, and pain. Mucin produced by the oral epithelium makes up the mucous layer and plays a critical role in maintaining the mouth cavity's interior environment, such as pH, lubrication, microbial flora, mastication, and enzymatic activities. [27]

1.6.2. Absorption of macromolecules

The selective permeability of biological membranes prevents larger molecules from passing through them. The large molecular weight (>1000 Da) of macromolecules, an important physicochemical property, is one of the main causes of this impaired

absorption. So, the oromucosal permeation of macromolecular compounds is irregular and partial, resulting in inadequate absorption and reduced bioavailability. Enzymatic instability in the oral cavity is another biopharmaceutical barrier to the administration of macromolecular compounds. [27]

Important methods for delivering macromolecules over the oral mucosa include receptor-mediated endocytosis, carrier-mediated diffusion, adsorptive endocytosis, and passive diffusion. [27] There are two main methods by which macromolecular compounds might pass the oral epithelium: paracellular (between the cells) or transcellular (via the cells), depending on a variety of macromolecular compounds' physicochemical characteristics, such as their size, molecular weight, and lipophilicity. [27,40,47] For instance, the paracellular pathway limits the penetration of hydrophilic or polar macromolecular compounds, while lipophilic (non-polar) compounds can diffuse passively across the mouth epithelium via transcellular pathways, either through facilitated or passive diffusion. [27,40,42] In contrast, drugs of an amphoteric nature display better penetration across the oromucosal epithelium. [27] Macromolecules can pass through connective tissue capillaries and enter the systemic circulation. [40]

Other parameters that could impact how macromolecules are delivered oromucosally across the oral mucosa include the thickness of the epithelium, the degree of stratified epithelium keratinization, and the lipid content. where oral epithelium thickness and keratinization level show an inverse connection with oromucosal permeability. [27]

Because of their thinnest epithelial layer, a squamous stratified epithelium that is non-keratinized, dense, and rich in vascularization, the sublingual and buccal mucosa locations are the most appealing for macromolecule delivery. [27,31,39,40,42,48] It provides quick access to blood vessels where drugs are directly passed into the reticulated vein and moved through the internal jugular vein, the brachiocephalic vein, and the facial veins to the bloodstream through that, causing the effect to start immediately, especially if necessary. [27,29–32,34,37,40,42,48–50] In addition, it features very little oral enzymatic inactivation[27] and bypasses the first-pass effect, drug breakdown in the stomach area, and intestinal hydrolysis caused by enzymes, thus improving drug bioavailability. [27,29–31,37,42,46,48–51] When necessary, simplified formulation removal, lesser adverse effects, and dosage reduction.[30,31,46,51] Additionally, it offers certain additional benefits. It is simple to reach the sublingual region. [31] facilitating self-management; patient adherence; higher tolerability and specificity to a location; increasing therapeutic effectiveness. [27]

Nevertheless, several variables may restrict drug absorption via the oral mucosa, including a small area for absorption and movement of the tongue, which produces shearing forces,[31] [46] short residence duration due to rapid turnover of saliva; involuntary swallowing of fluids greater than 200 µl; and/or involuntary swallowing of the dosage forms, so the drug leaves the mouth and moves to the gastrointestinal tract. [27,31,46,49] Moreover oromucosal drug delivery has many disadvantages.

Disadvantages of oromucosal drug delivery

1. Weak control of the release rate. [27]
2. Masking issues with taste and smell [39,41]
3. Drug losses due to salivary turnover, especially if the drug takes a long time to absorb. [27,31,33,38,39,49]
4. The risk of drug loss by involuntary swallowing before absorption. [27,31,32,39,46,49]
5. Small absorption area [27,31,32,52]
6. Tongue motions' ability to produce shearing forces. [31]
7. Multilayered oral epithelium provides a physical barrier. [27]
8. Discomfort brought on by food intake and taste. [52]
9. For molecules of small to moderate weight [42]

A significant factor is how long the formulation stays at the absorption site (sublingual). Based on that, extending the duration of the drug's residence time with mucoadhesive compounds that are able to create molecular connections with mucosa constituents and thus immobilize dosage forms to extend the period of drug contact and create a longer time for drug release can overcome these difficulties. [31,40,46,49] Due to their low penetration resistance, sublingual and buccal administration are also the most preferred methods for delivering macromolecules. Additionally, due to its negative charge, mucin also contributes significantly to the promotion of macromolecular drug penetration into the oral mucosa via mucoadhesion, one of the main ways that macromolecules permeate the body. [27]

In addition, other techniques have been improved for macromolecule delivery through the mucosa, such as microneedles, modifying the structure, and using agents to increase penetration (for instance: surfactants, azones, and complexing agents) or stop proteolytic breakdown (by enzyme inhibitors). [27,46] The use of these techniques has enhanced the absorption rate and oral bioavailability of macromolecular compounds with optimized delivery.[27,32,33,40,50–52]

1.6.3. Mucoadhesive dosage forms

Mucoadhesive dosage forms for delivering macromolecules orally include wafers, tablets, patches, films, discs, sprays, sponges, gels, and ointments.[27,48] Mucoadhesive tablets swell, adhere to the mucosa surface that is humid and resistant to saliva's flushing effect, and are held there until the dissolution is finished[27,31,33,49] without impact the drug release significantly. [38,39] In contrast to traditional oral tablets, these allow you to speak and drink without feeling any discomfort. These tablets can be used on several oral cavity areas, including the palate, cheeks, and gums. The most researched and innovative dosage forms are mucoadhesive buccal tablets. It may be retained for up to 15 hours [27,53]

Compared to the buccal mucosa, the sublingual mucosa (100-190 μm) is thinner than the buccal mucosa (500–800 μm).[47] There are many smooth muscles and immobile mucosa present. Additionally, the sublingual region's abundant blood supply allows for efficient medication penetration. So the sublingual mucosa has a higher degree of permeability that allows for a quick onset of action and a high plasma drug concentration. [36,39,49,52,54] Additionally, the buccal formula has some

challenges: the continual salivation in the mouth dilutes the medication; swallowing; and mechanical stress. [36] In addition, buccal tablets have major limitations such as inadequate physical flexibility, which results in poor patient adherence for prolonged and recurrent use; mucoadhesive strength is poor, resulting in a short residential time; and a lack of macromolecule protection from enzymatic breakdown in the mouth. [27]

For sublingual drug delivery, several formulations such as tablets, films, gels, spray solutions, chewing gum, wafers, nanofibers, and patches are helpful. Compared to oral administration, sublingual administration increases drug absorption by 3–10 times.[27,35,40–42] It is only exceeded by a hypodermic shot.[42] Similar to regular tablets, sublingual fast-disintegrating tablets are a solid dosage form. In contrast, super-disintegrant agents are present. It aimed to spread out before swallowing as well as enhance oral absorption of the active component. So that it can dissolve and disintegrate quickly without the need for water within the low quantity of saliva in the mouth cavity between 3 seconds and 3 minutes, with direct rapid absorption into the bloodstream which accelerates the onset of the action. In addition, it has low degradation and enzymatic activity and bypasses the enterohepatic circulation which optimizes bioavailability. [32–35,37,38,54,55]

Sublingual rapid disintegrating drugs are easy, simple, self-administerable, and unremarkable to administer to people who have difficulty swallowing, such as children, elderly, dysphagic and/or unconscious patients, persistently nauseated people, and individuals who are afraid of choking. Moreover, they are advantageous for those with disabilities, paralyzed individuals, psychotics, Parkinson's disease,

multiple sclerosis, esophagitis, stroke, and bedridden individuals who do not have or have limited access to water. Given a greater level of compliance and acceptance, especially among children, because taste-masking excipients can produce a superior flavor. [29–31,33,35,37,38,42,46,50,51,54–56] This dosage form is localized and targeted to a specific area, either through local or systemic delivery.[35,52] Additionally, it has superior safety and efficacy compared to traditional oral dosage forms and allows for simple drug withdrawal if required. It is simply manufactured [54] and has a diversity of sizes and forms, [35,37] and a small package that is easy for patients to handle. [50,54]

Despite sublingual fast disintegrating tablets offering all these benefits, they also have some challenges related to the inherent physical characteristics of macromolecules, such as hydrophilic nature, high molecular weight, complexity of the structure, chemical instability, and low oral epithelial permeability. [27] These include being hygroscopic by nature as a result of the use of water-soluble excipients to speed up disintegration, [30,35,37] which makes them sensitive to environmental conditions and [41] require particular specifications, such as packaging, to ensure their stability and safety. [30,35,37] It can only load a reduced dose of a medication that is less than 20 mg. [30,35,37,42]. Taste-masking methods are required for drugs with a poor taste for the elderly and children in particular. [30,35,37,39,41] This location is not ideal for long-term drug delivery systems. [42] Get a short disintegration time. [41] On the other hand, the dosage form should not disintegrate and dissolve so quickly that an unwanted high percentage of the compounds is quickly removed into the digestive system by salivary washing. [33,38]

So, the suggested method of improving cyanocobalamin bioavailability is rapid disintegration and mucoadhesive sublingual formulations, which provide fast disintegration and dissolution with immediate blood circulation absorption and greater retention capacity, extending the time spent at the sublingual region, and thus the time of cyanocobalamin mucosa contact, decreasing the amount swallowed, and polymer encapsulation improves drug protection, provides the highest absorption rate, improves bioavailability to optimize medication administration, and enhances the therapeutic effectiveness of the medicine. [32,33,38–40,50–52,57]

1.6.4. Mucoadhesion

The word "adhesion" comes from the Latin word adhaerere (ad means to and haerere means to stick). is an assembly created by applying an adhesive substance between two surfaces of another material (the substrate), resulting in a joint that resists separation.[58] The term "bioadhesion" is used to explain how an adhesive particle interacts with any material that is biological or biologically generated.[32,53] The term "mucoadhesion" describes how a mucoadhesive substance adheres only to the biological mucous membrane through reciprocal penetration of mucoadhesive substance and glycoprotein chains (mucin). [27,51,53] According to several studies, mucin is the major agent inducing the mucoadhesion of compounds to mucous membranes. [27,53,58] And comprises a squamous epithelium stratified in humans that is made up of 8–12 cells that range in size from 0.1–0.2 mm. [40]

Usually, mucoadhesive dosage forms are applied to wet mucosal surfaces to facilitate wetting, followed by loss of solvent and water, which give it its gel-like structure to produce strong adhesive and cohesion based on the polymers' chemical nature, which is then strengthened by chemical and/or physical interactions.[27,39,53,58] The mucoadhesive substances and mucosal surface come into close contact as a result of this cohesion force, increasing the residence time of dosage forms at the site of administration.[31,52,58] As a result, the drug's residence time at the absorption site lengthens, enhancing absorption.[27,48,51] It facilitates the sustained and regular release of pharmaceutical ingredients depending on their particular swelling characteristics,[27,39,48,51] which can decrease the frequency of administration while also minimizing fluctuations in plasma concentration to achieve better therapeutic results.[27,48,51] Additionally, mucoadhesive can be utilized to target drug delivery to a specific area of the body. [48]

Goblet cells release mucous, a viscous, heterogeneous material that coats the epithelial surface, establishing the mucous membrane. [27,47] Goblet cells are present in a variety of physiological parts, including the sublingual, buccal, nasal, gingival, vaginal, rectal, ophthalmic, reproductive, and gastrointestinal systems.[27,48] The mucoadhesive drugs can be administered to all those systems.[36,39,51,52]

Where mucus is primarily composed of water (95%) and contains 2-3% hydrophobic glycosylated peptides (mucin), 0.3-0.5% lipids, inorganic salts, DNA and proteins.[27,40,53] The mucous layer has various functions, such as helping with mastication and food lubrication, defending against proteolytic deterioration, and functioning as a barrier against microbes. [27]

Due to their distinct features, polymers have distinguished themselves among numerous mucoadhesive compounds with their tensile strength, mechanical and great swelling characteristics, and flexible functionalization to increase mucin entanglement. [27]

1.6.4.1. Saliva

A biological fluid known as saliva is primarily secreted by the parotid, sublingual, and submandibular glands.[25,47] The majority of saliva is made up of water (99%), mucus proteins, ions such as K+, Na+, Cl, and HCO_3, hyaluronic acid and amylase.[40,59] With an average osmolality of 50–60 mosmol/kg, saliva is hypotonic as a result of Cl and Na+ reabsorption. Additionally, saliva has a role in the start of digestion. [59]

Humans can spread up to 6 ml of saliva over a 200 cm^2 mucosal surface area, salivary secretion into the mouth occurs continuously at a rate of 0.5 ml/min; when there is food present, this value rises quickly to greater than 7 ml/min. After swallowing, there is about 0.8–1.0 ml of residual saliva left in the oral cavity, which is lined with a thin layer that is around 100 micrometers thick. [59] Typically, saliva has a pH between 6.49 and 7.28, which is close to neutral. Its pH is stabilized by a variety of buffer systems, including proteins, hydrogen carbonate, and hydrogen phosphate. [25,59]

1.6.5. Mucoadhesion process

The mucoadhesion process involves three steps (Figure 5): the contact step (also called the wetting or swelling step), the diffusion step, and the consolidation step, which includes interactions between polymers and mucin. [27,52,53]

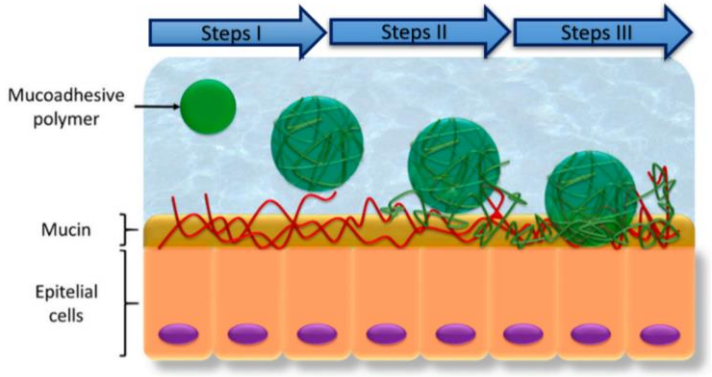


Figure 5 Steps in the mucoadhesion process [27]

Mucoadhesive materials come into contact with mucous membranes during the contact step and only establish weak adhesion (Figure 6). In the presence of saliva, rapid hydration, swelling, and spreading of the polymeric matrix are crucial during this step to create interpenetration between polymer chains and mucin (for example, electrostatic interactions, mechanical entanglement, or hydrogen bonds). [27,48,53,55,58]

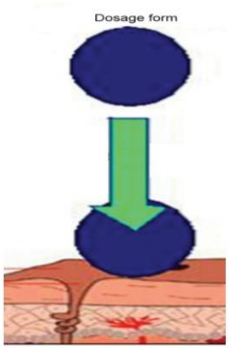


Figure 6 The contact step [52]

In the second step, polymeric chains extend deeply into the mucosal layer for greater entanglement, interpenetration or interdiffusion. [27,53,55,58]

Saliva plasticizes the system during the consolidation step, allowing the mucoadhesive polymers to dissociate, mucin and polymer chains to combine, where they solidly adhere (Figure 7). And the development of diverse binding types occurs during this step, for instance, through chemical bonding or weak attractive forces (for example, hydrophobic interactions, Van der Waals interactions, covalent bonds, hydrogen bonds, and ionic crosslinking), and then medication starts slowly releasing. [27,52,53,55,58]

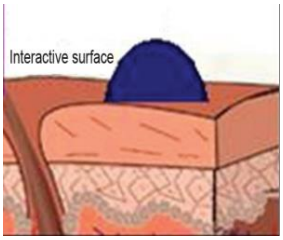


Figure 7 The consolidation step [52]

For oromucosal administration of macromolecules, mucoadhesive polymers have been widely employed in a variety of dosage formulations as excipients, either

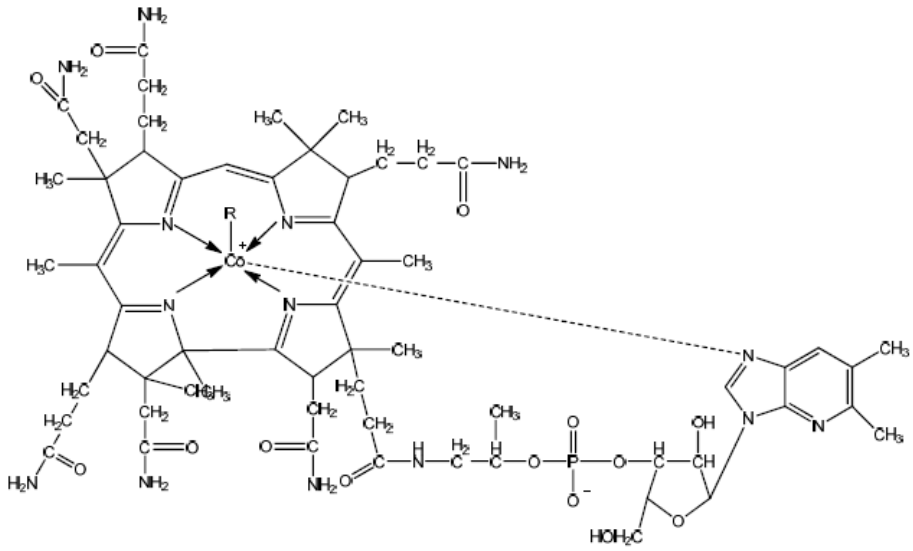
natural, synthetic, or semi-synthetic, including gellan, chitosan, carbomer, xanthan gum, guar gum, polyacrylic acid (PAA), hydroxylethyl cellulose (HEC), hydroxypropyl cellulose (HPC), hydroxypropylmethyl cellulose (HPMC), sodium carboxymethyl cellulose (SCMC), polyethylene oxide (PEO), and sodium alginate. [27,31,49,57]

Main criteria for sublingual fast disintegrating tablets

1. Good taste [29,42]
2. Drug must be water- and saliva-stable. [32,42]
3. Quickly disintegrate or dissolve in the saliva of the oral cavity. [29]
4. A drug must be soluble, and easily permeable through mucosal surfaces. [32]

1.7. Vitamin B_{12}

Vitamin B_{12} is a complex, water-soluble compound. It contains a corrin ring consisting of four reduced pyrroles with a cobalt atom in the center of the rings. Which is produced only by archaea and certain bacteria. It's obtained from the diet and the normal serum level should be higher than 200ng/L. Vitamin B_{12} has various analogs, including cyanocobalamin, methylcobalamin, hydroxocobalamin, and deoxyadenosylcobalamin (Figure 8), which play a crucial role as a cofactor in methyltransferase and isomerase. It is important to create precursors that enter the citric acid cycle (Kreb's cycle) and create DNA.[2,5]



-R	Name	Abbreviation
-CN	Cyanocobalamin	CN-Cbl
-OH	Hydroxycobalamin	OH-Cbl
-H2O	Aquocobalamin	HOH-Cbl
-5'-Deoxyadenosyl	5'-Deoxyadenosylcobalamin (Coenzyme B12)	AdoCbl
-CH3	Methylcobalamin	MeCBl
-SO3	Sulfitocobalamin	SO3Cbl

Figure 8: Analogues of vitamin B_{12} structures [7]

1.7.1. Description

Cyanocobalamin's chemical name is α-(5,6-dimethylbenzimidazolyl), molecular formula is $C_{63}H_{88}CoN_{14}O_{14}P$, molecular weight is 1355.4 Dalton, it appears like a dark red solid.[17] It's a potent drug with a daily commercial dose of 0.5-1mg.[5] It is a weak base vitamin with two Pka's 3.3 & 9.3, and a melting point of 300°C.[7]

1.7.2. Solubility

Vitamin B_{12} is soluble in organic solvents (e.g. ethanol and DMSO), moderately soluble in water, and insoluble in acetone and ether.[17] It's also soluble in different solvents that act as drug permeation enhancers through different mechanisms. The

solubility of vitamin B12 in hydrophilic solvents is higher than in lipophilic solvents. This is illustrated by the following values in Table 3. Cyanocobalamin is a biopharmaceutical classification system (BCS) class III molecule with high solubility and low permeability. [60]

Table 3: The solubility of vitamin B_{12} in various solvents

Agent	Solubility (mg/ml)	Temperature (^oC)	Ref
Water	79.780	37	[17,20]
Propylene glycol	52	37	[20]
Oleoyl macrogol-6-glycerides	0.4485	37	[20]
Propylene glycol monocaprylate	0.1507	37	[20]
Oleic acid	0.107	37	[20]
Oleic acid	1.07	25	[21]
Isopropyl myristate	0.42	25	[21]
Labrafil	0.048	25	[21]

1.7.3. Stability

Vitamin B_{12} is a highly sensitive substance, it breaks easily through oxidation and reduction via strong oxidizing and reducing agents such as vitamin C. It is also easily broken by light and heavy metals such as iron and sulfite. But it has a good UV light tolerance of about 15-20 minutes at 121^o C within a range of 4-7 PH.[7,17] In light, cyanocobalamin is converted into hydroxocobalamin, which is also

biologically active. The reaction of photolysis depends on the ionization of vitamin B_{12} molecules, this ionization relates to its structure, which consists of six weak base amide groups. Photostability is reached by increasing the viscosity of the medium by adding viscosity-increasing agents such as glycerol.[7,61] Cyanocobalamin is the most stable of these vitamin B12 analogs, so it is the most commonly used form in fortified and supplement preparations for animals and humans.[62]

1.7.4. Intrinsic factor

Intrinsic factor is a dimer glycoprotein molecule with a molecular weight of about 50 kD. It consists of a single chain of amino acids with the carbohydrate molecules necessary to protect it from enzymatic degradation.[4,8,13] The carbohydrate and amino acid content determine the molecular weight of a glycoprotein.[8] It is secreted by the salivary gland and parietal cells (oxyntic cells) in the stomach. Vitamin B_{12} binds with the intrinsic factor with a dissociation constant exceeding 10^{-12}mol/L to form a B_{12}-IF complex in the duodenum (Figure 9).[4,8,13] In addition, the composition of the complex depends on PH, and the highest correlation occurs at 6.5–10 PH in the presence of calcium. [63]

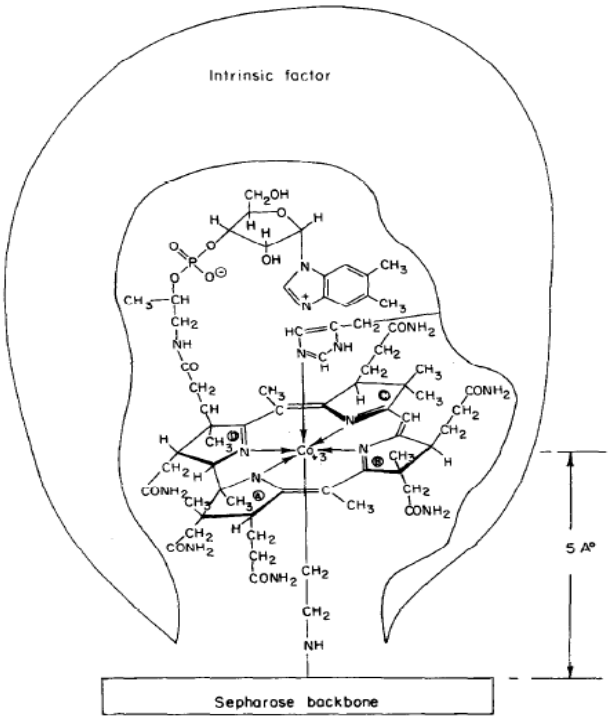


Figure 9: B_{12}-IF complex [64]

1.7.5. Vitamin B_{12} absorption

The absorption of vitamin B_{12} is a complicated process. [2,8] Cobalamin is usually introduced into the human body through food. It is found in food as a protein or peptide complex. Once a person consumes food in the mouth and stomach, the low pH caused by pepsin and hydrochloric acid breaks the cobalamin-bound protein, releasing free cobalamin.[2,5,8,13] Then it binds to transcobalamin I (also called haptocorrin or R protein) to form a complex where it is secreted by the salivary and stomach glands (Figure 10).[1,2,5,8,12,13] At acidic pH, the transcobalamin I affinity is three times that of the intrinsic factor to create a transcobalamin I-cobalamin complex.[1,8] Transcobalamin I in this complex acts as a protective agent, which prevents cobalamin from acid degradation in the stomach and eliminates useless or

harmful cobalamin analogues. It also has antimicrobial properties by restricting and prohibiting microorganisms in the gut from receiving vitamins and nutrients. [1,2,5,8,12,13]

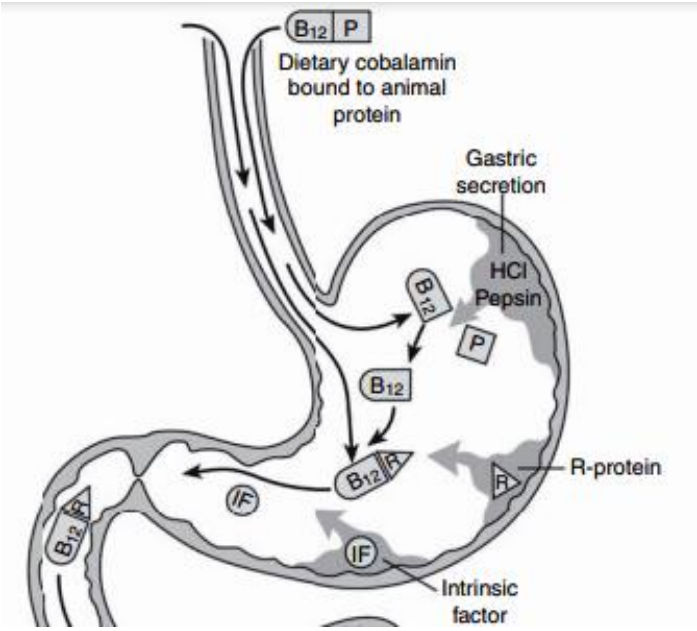


Figure 10: Cobalamin-bound protein breaks via pepsin and hydrochloric acid, then it forms a complex with the R protein. [65]

When the transcobalamin I-cobalamin complex arrives in the duodenum, cobalamin is separated from this compound by the pancreatic enzyme proteases to reintroduce free vitamin B_{12}. The free cobalamin is then associated with the intrinsic factor to form the B12-IF complex (Figure 11).[2,5,13] This intrinsic factor is generated by parietal cells in the stomach. These intrinsic factors protect vitamin B_{12} from catabolism by the gut microbes. It's also crucial for vitamin B_{12} absorption in the small intestine's terminal. Calcium ions and a neutral pH are necessary for cobalamin and intrinsic factor binding. [2,8,12]

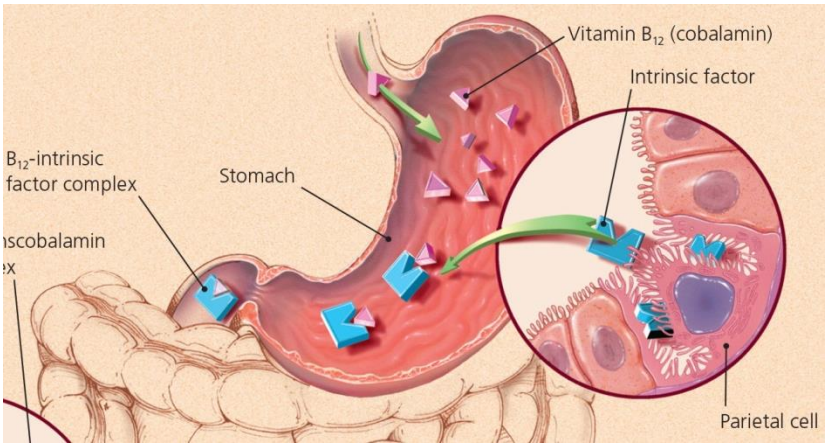


Figure 11: Cobalamin is linked to the intrinsic factor to form the B_{12}-IF complex. [14]

The B_{12} -IF complex is absorbed by binding to the intestinal cubam enterocyte receptors in the ileum (Figure 12). The cubam receptors contain cubilin and amnionless proteins. After binding to these receptors, the B_{12}-IF complex is internalized via receptor-mediated endocytosis. [1,5,6,12,13,28,66] The intrinsic factor is then degraded by a peroxisome lysosomal enzyme. The receptor is then recycled to the side of the apical intestinal membrane (Figure 13) and the vitamin cobalamin is transported into the bloodstream. [2,8,12] After the B_{12}-IF complex binds to the cubam receptor, vitamin B_{12} takes roughly 3-4 hours to enter the bloodstream and bind to transcobalamin II.[4,6] The absorption capacity of vitamin B_{12} needs 4-6 hours to recover before it is ready to absorb the next dose.[2,4,5,13] Because the number of receptors is limited and in tiny quantities, it easily reaches saturation. Only a small amount of vitamin B_{12} is absorbed.[1] More than half of the vitamins in diets are absorbed through these routes, and 1% of free B_{12} is absorbed through passive diffusion in the intestines. [2]

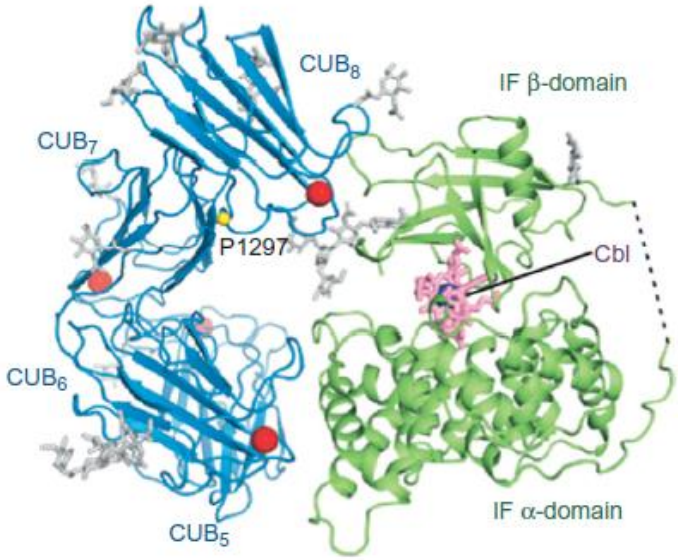


Figure 12: Vitamin B12-IF complex binds to the intestinal cubilin enterocytes receptor as 3D structure, where the IF: intrinsic factor , CBI: cobalamin , CUB : cubilin enterocyte receptor.[5]

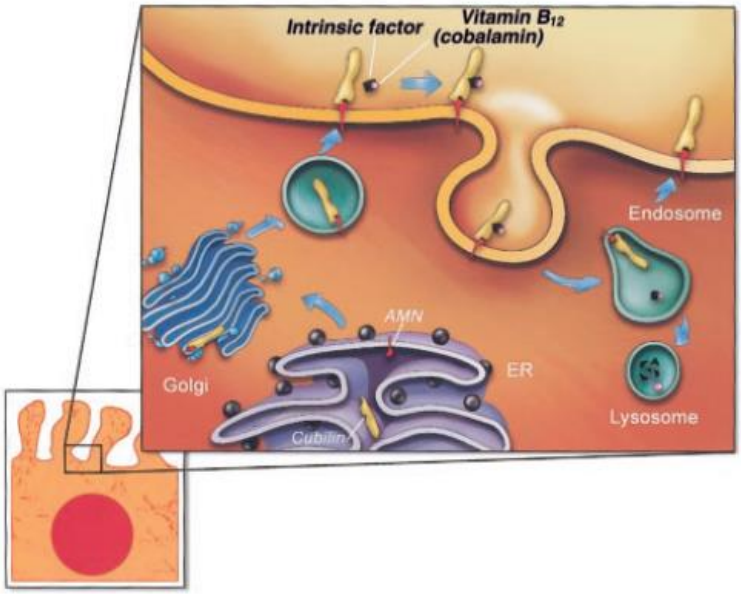


Figure 13: Internalization of the B_{12}-IF complex occurs via receptor-mediated endocytosis. [66]

In plasma, cobalamin binds to the transcobalamin II transporter, which is a glycoprotein. Then it's transported as methylcobalamin and adenosylcobalamin

coenzymes to particular receptors on every cell membrane in the body via the portal system (Figure 14). [2,8]

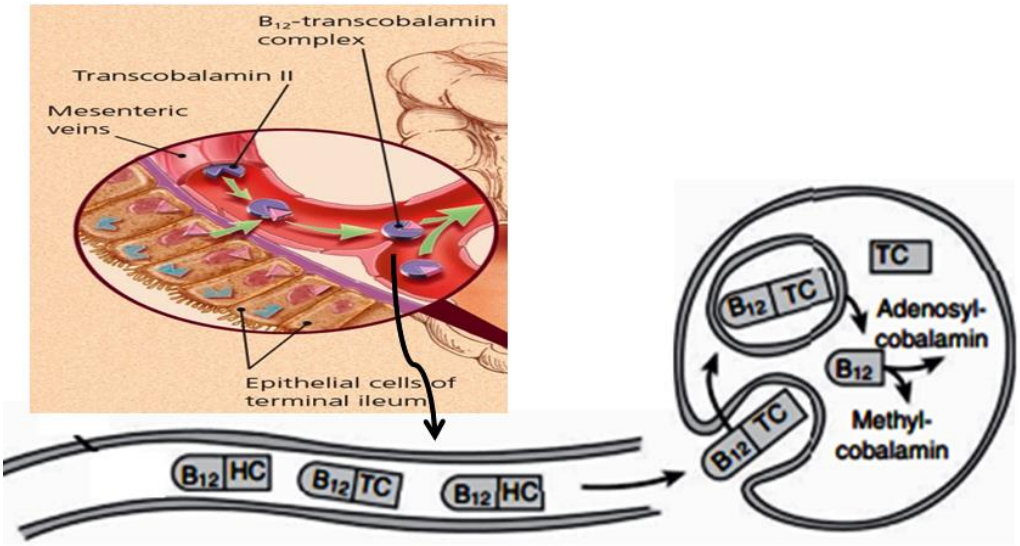


Figure 14: B_{12} binds to the transcobalamin II transporter in the bloodstream, where TC: holotranscobalamin and HC: transcobalamin II. [14]

Cobalamin can be stored in the liver for up to 2–5 mg, and the liver excretes approximately 0.1–0.2% of this amount per day via renal and biliary excretion, and biliary excretion of vitamin B_{12} is greater. The majority of B_{12} excreted in the bile is reabsorbed by the enterohepatic circulation, [1,7–9,12] where it is excreted as a B_{12}-R protein complex in the duodenum and separated by the pancreatic enzyme. The free B_{12} then binds to intrinsic factor and is reabsorbed in the terminal ileum. (Figure 2). This enterohepatic circulation makes B_{12} vitamin insufficiency take many years to develop, even if all diets containing vitamin B_{12} are eliminated.[1,2,8,12] Eventually, the bioavailability of vitamin B_{12} from absorption is varied and based on a person's gastrointestinal capability. [4]

Chapter II: Problem Justification

2. Problem justification

After absorption, when vitamin B_{12} reaches the circulatory system, intracellular cobalamin converts to the coenzyme methylcobalamin in the cytoplasm and adenosylcobalamin in the mitochondria; they are essential cofactors for homocysteine and methylmalonic acid, respectively. It's required to create methionine by acting as a cofactor by transferring the methyl group from methyl tetrahydrofolate to homocysteine, which is important for the creation of pyrimidine and purine, which is very critical as a precursor to DNA creation. Also, it is necessary as a cofactor for the creation of succinyl CoA, which acts as a precursor to the Krebs cycle of cell metabolism for energy generation (Figure 1).[4,6]

As previously indicated, the main source of vitamin B_{12} is the animal diet, such as meat, liver, eggs, and a few plant foods.[4] Vitamin B_{12} takes 12 years to develop deficiency due to malabsorption, lack of absorption occurs mainly as a result of a lack of intrinsic factor because of its important role in absorption. The shortage of this vitamin and its critical function may have serious consequences associated with the basic functions it performs.[3,4] Vitamin B_{12} uptake was previously tested by the Schilling test using radioactive Co^{57} cyanocobalamin. Currently, this method is not being used, and there's no alternative method. Several different individual tests are therefore used to diagnose the main causes of vitamin B_{12} non-absorption and deficiency.[4,6,67]

Vitamin B_{12} deficiency is diagnosed when the serum level is less than 200 ng/L (148 ppm/mL), vitamin B_{12} deficiency has many complications such as

megaloblastic anemia, high risk of cardiovascular disease and osteoporosis, cognitive weakness, and nervous system weakness that characterized by sensory loss in the distal limbs and neural tube defect where the vitamin is needed as a cofactor to methionine formation for the folate cycle as previously mentioned.[3,4,6]

To compensate for the cobalamin deficiency, the patient takes vitamin B_{12} as a dietary supplement. Based on the specialist's diagnosis and patient history, hydroxocobalamin is given as an injection treatment or cyanocobalamin as an oral and nasal treatment. In addition, other anlage are available.

Generally, vitamin B12 is available only in oral, intranasal, and parenteral dosage forms. Injection dosage forms, which are invasive and uncomfortable and need a specialist to administer them, and many other limitations previously discussed (Section 1.4.2.1). [3,6] While oral (tablets and sublingual) and intranasal also have many limitations discussed previously (Section 1.4.2.1 and 1.4.2.3), and the most important of these limitation is the poor bioavailability; where about 1–5% of free cobalamin is absorbed by passive diffusion in the gastric system from the mouth to the intestinal mucosa. [11] In addition, endocytosis absorption has a better bioavailability by utilizing intrinsic factor but there are many reasons for intrinsic factor deficiency discussed in detail previously [66] (Section 1.2).

Because of that, the main objective was to enhance the absorption of vitamin B_{12} by prolonging the residence time of the drug available at the absorption site to increase the percent of the drug absorbed by passive diffusion. One of these suggested methods is the cyanocobalamin mucoadhesive sublingual dosage form, which we will study in this thesis.

Chapter III: Objectives

3. Objectives

3.1. Objective

1. To the development of sublingual mucoadhesive tablets using different methods and different polymers.
2. To examine the residence time of a fast-disintegrating mucoadhesive formula on ex-vivo bovine sublingual tissue.
3. To develop an analytical method for determination of vitamin B12 using a UV-Vis spectrophotometer
4. To study the release profile of the different preparation formulas and drug permeation through a Permeapad® membrane.
5. To evaluate the stability of the drug in simulating salivary fluid.

Chapter IV: Research Methodology

4. Research Methodology

4.1. Formulation material, equipment, and tools

4.1.1. Material

All materials used in this thesis are displayed in the Table 4.

4.1.1. Equipment and tools

Table 5 shows the equipment and devices used in this research for preparation and evaluation.

Table 4 Materials required for formulation experiments with their function.

#	Materials	CAS #	Lot numbers	Company	Country	Functions
1	Cyanocobalamin	68-19-9	2009002	Planet Pharma	China	Active ingredients
2	Poly vinyl pyrrolidine MW 40,000	25232-41-1	2011k300603	Chem-Impex Int'l. Inc.	United state	Binder
3	Xanthan Gum	11138-66-2	35191196	SUN PHARM drug store LTD.	Palestine	Polymer
4	Carbopol 940	9003-01-4	C15092701	IndiaMART	India	Polymer
5	Eudragite S100	25086-15-1		Evonik Industries	Germany	Polymer
6	Eudragite L100	25806-15-1		Evonik Industries	Germany	Polymer
7	Eudragite L100-55	25212-88-8		Evonik Industries	Germany	Polymer
8	Hydroxy propyl cellulose (M.W.100,000)	9004-64-2	Z27D005	Alfa Aesar	United state	Polymer
9	Ethyl cellulose	9004-57-3		Colorcon®	Italy	Polymer
10	Hydroxy propyl methyl cellulose Viscosity (2% aq. Soln., 20 ^{o}C) 7500-14000 mPa.s (Methocel)	9004-65-3	Q10D058	Alfa Aesar	United state	Polymer
11	Polyplasdone	9003-39-8		Colorcon®	Italy	Disintegrant
12	Magnesium stearate	557-04-0		Colorcon®	Italy	Lubricant
13	Ethanol absolute anhydrous 100%	64-17-5	V3E472258E	Madi for laboratories	Palestine	Wet granulation solvent

#	Materials	CAS #	Lot numbers	Company	Country	Functions
14	Mannitol	69-65-8	S00190MS	Donated from pharmacare Co., Ltd.	Palestine	Filler
15	Microcrystalline cellulose PH 101	9004-34-6		Colorcon®	Italy	Filler
16	Acetonitrile	75-05-08	2049762	Sigma-Aldrich ,	Untied state	Molding solvent
17	Hydrochloric acid 37%	7647-01-0	V4N503104N	CARLO ERBA Reagents S.A.S	France	PH adjustment
18	Sodium hydroxide Pellets	1310-73-2	V5I978136D	CARLO ERBA Reagents S.A.S	France	PH adjustment
19	Sodium chloride	7647-14-5	S0125PI1	KOSDAQ listed company	Korea	SSF
20	Disodium hydrogen phosphate	7558-79-4	V6F631077B	CARLO ERBA Reagents S.A.S	France	SSF, PB
21	Potassium dihydrogen phosphate	7778-77-0	V6H654206N	CARLO ERBA Reagents S.A.S	France	SSF, PB
22	Sublingual and cheek bovine mucosa	-		From a local butcher	Palestine	For residence time test
23	Potassium chloride	7447-40-7	SLCC4713	Sigma-Aldrich	United state	Evaluation test

Table 5 Equipment and tools used for evaluation experiments with function

#	**Equipment and tool**	**Uses**	**Model / Manufacturer**	**Company & country**
1.	Analytical balance	Weighting	METTLER TOLEDO balance (5 digits),	OHAUS®, Switzerland
2.	Mortar and postal	Mixing	From Samih Darwazah institute	Palestine
3.	Single punch manual compression machine	Tablet compression	From Samih Darwazah institute	Palestine
4.	Sieve #16 Sieve # 40	Wet granule sieving Formulation	From Samih Darwazah institute	Palestine
5.	Amber glass volumetric flask	Analysis test	From Samih Darwazah institute	Palestine
6.	Beaker different volumes	Formulation	From Samih Darwazah institute	Palestine
7.	Plastic dropper	Formulation	From Samih Darwazah institute	Palestine
8.	Cylinder different volumes	Formulation and evaluation test	From Samih Darwazah institute	Palestine
9.	Plastic dishes (different size)	Formulation	From Samih Darwazah institute	Palestine
10.	Thermos scientific Hot plates	Evaluation test	From Samih Darwazah institute	Palestine
11.	Thermometer	Temperature measurement	From Samih Darwazah institute	Palestine

#	Equipment and tool	Uses	Model / Manufacturer	Company & country
12.	Syringes	Formulation	From Samih Darwazah institute	Palestine
13.	Spatula	Formulation	From Samih Darwazah institute	Palestine
14.	USP II dissolution apparatus	Residence time evaluation test	DT70 Pharma test	Pharma Test Apparatebau AG Germany
15.	UV-Visible double beam spectrophotometer	Analysis test	UVS035 PerkinElmer	PerkinElmer, Canada
16.	Micropipette	Analysis and evaluation tests	KIRGEN®	China
17.	Tablet Hardness tester	Hardness evaluation test	PTB 111 Pharma test	Pharma Test Apparatebau AG Germany
18.	Friability tester	Friability evaluation test	FRT012 Pharma Test	Pharma Test Apparatebau AG Germany
19.	Bath sonicator	Solubilization	ELMA S300H, BAS008	Elmasonic, Germany
20.	Manual compression machine	Tablet compression	From Samih Darwazah institute	Palestine
21.	Modified Franz Diffusion Cell	Permeation evaluation test	ORCHID ScientificTM	India
22.	PH/ORP meter	PH measurement and adjustment	HANNA instruments	Indonesia
23.	Parafilm	Formulation test	Bemis	United States
24.	Scissors	Evaluation test	Local market	Palestine

#	Equipment and tool	Uses	Model / Manufacturer	Company & country
25.	Empty strip	Mold for casting suspension preparation until dry	Jerusalem Pharmaceuticals Co. Ltd.	Palestine
26.	Plastic rubber	Residence time & dissolution evaluation tests	Local market	Palestine
27.	Plastic slide	Residence time evaluation test	Local market	Palestine
28.	Scalpel	Evaluation test	Birzeit University labs	Palestine
29.	Tray	Formulation	From Samih Darwazah institute	Palestine
30.	Super glue	Residence time evaluation test	Local market	Palestine
31.	Plastic thread	Mucoadhesive strength evaluation test	Local market	Palestine
32.	Wooden balance model scale	Mucoadhesive strength evaluation test	Designed by a carpenter	Palestine
33.	Fixed weights scale	Mucoadhesive strength evaluation test	From Samih Darwazah institute	Palestine
34.	Tapped density tester	Formulation and blend evaluation	Copley® aHas AT2000	England

#	Equipment and tool	Uses	Model / Manufacturer	Company & country
35.	Plastic funnel	Formulation and blend evaluation	From Samih Darwazah institute	Palestine
36.	Plastic ruler	Formulation and blend evaluation	From local market	Palestine
37.	Funnel stand	Formulation and blend evaluation	From Samih Darwazah institute	Palestine
38.	Friability tester	Friability test	IS0 9001 Pharma PTF test	Pharma Test Apparatebau AG Germany
39.	Nylon Syringe-driven filters 0.45 µm	Evaluation test	FNY-422-025 Lot no: 160406-150	Jet Bio-Filtration Co., Ltd China
40.	Water bath shaker	Evaluation test	Mrc laboratory instruments	England
41.	Caliper	Evaluation test	From Samih Darwazah institute	Palestine
42.	Plastic cup	Mucoadhesive strength evaluation test	Local market	Palestine
43.	Dialysis tubing Cellulose membrane	Drug release evaluation test	D9402-100FT Lot#: 3110	Sigma-Aldrich , United state
44.	Pump	Drug release evaluating test	Local market	JAJALE®, China
45.	Multi stirrer and stir bar	Drug release evaluation test	VELP Scientifica MST019	Italy

#	**Equipment and tool**	**Uses**	**Model / Manufacturer**	**Company & country**
46.	Cap of 100ml glass bottle	Drug release evaluation test S	Madi for laboratories	Palestine
47.	Glass dish	Drug release evaluation test	Birzeit University laboratories	Palestine
48.	CellStar tubes 50ml	Drug release evaluation test	E19043GC	Greiner bio-one North America
49.	Test tube	Evaluation test	From Samih Darwazah institute	Palestine
50.	PermeaPad® membrane	Drug permeation test	2023-0002	innoME GmbH Germany

4.2. Operational methodology

4.2.1. Formulation development

Initially, a variety of formulations were developed using different methods without the addition of active ingredients (Table 6). The strengths of used materials such as diluent, binder, disintegrant, and polymer were modified. Three methods were used to prepare the suggested sublingual tablet formulations: direct compression, wet granulation and molding.

4.2.1.1. Direct compression

The excipients were mixed with a mortar and pestle for 5 minutes before being compressed by a manual single-punch tablet compression machine, which was fed by a manual feeder. (Figure 15)

Figure 15 Manual single-punch tablet compression machine

4.2.1.2. Wet granulation

The excipients (including 50% diluents, disintegrant, and the entire amount of binder) were mixed with a mortar and pestle followed by gradually addition 100% ethanol until granules were formed. The granules then sieved through mesh #16 and allowed to dry before being compressed. A manual compression machine was used after being fed by a manual feeder for tablet compression.

4.2.1.3. Molding

Microcrystalline cellulose, polyplasidone, and polyvinylpyrrolidone were dispersed in an acetonitrile solvent until a homogenous suspension was formed.[27] A portion of the produced suspension was placed into empty medication strips. The volume of the suspension used was modified to form a 50 mg tablet after drying. After 24 hours, whereas the tablets were completely dried, they removed from the mold.[27,30] (Figure 16)

Figure 16 An example of a molding method: a strip into which the first formula was poured.

Table 6 below presents the quantity of each material per tablet as a percentage, where the total weight of each tablet is 50 mg. Approximately 50 tablets of each

formula were prepared. Finally, many formulations were chosen, each with an optimal disintegration time of less than one minute.

Table 6 The composition of mucoadhesive sublingual tablet formulations without cyanocobalamin

Materials / Formula	PVP	XG	Carb	Es100	EL 100	EL 100-55	HPC	EC	HPMC	MAN	MCC	PolyP	Mg.S	Method
1	1%	-	-	-	-	-	-	-	-	-	70	29	-	Molding
2	1%	-	-	-	-	-	-	-	-	-	70%	29%	-	Wet
3	-	-	-	-	-	-	-	-	1%	-	70%	29%	-	Wet
4	3%	5%	-	-	-	-	-	-	-	-	86.5%	5%	0.5%	DC
5	3%	5%	-	-	-	-	-	-	-	-	83.5%	8%	0.5%	DC
6	3%	5%	-	-	-	-	-	-	-	-	76.5%	15%	0.5%	DC
7	0%	5%	-	-	-	-	-	-	-	-	79.5%	15%	0.5%	DC
8	0%	5%	-	-	-	-	-	-	-	-	74.5%	20%	0.5%	DC
9	0%	3%	-	-	-	-	-	-	-	-	76.5%	20%	0.5%	DC
10	0%	3%	-	-	-	-	-	-	-	-	66.5%	30%	0.5%	DC
11	3%	5%	-	-	-	-	-	-	-	86.5%	-	5%	0.5%	DC
12	0%	5%	-	-	-	-	-	-	-	79.5%	-	15%	0.5%	DC
13	1%	5%	-	-	-	-	-	-	-	-	67%	27%	-	Wet
14	1%	3%	-	-	-	-	-	-	-	-	69%	28%	-	Wet
15	1%	4%	-	-	-	-	-	-	-	10%	79.5%	5%	0.5%	DC
16	1%	4%	-	-	-	-	-	-	-	25%	64.5%	5%	0.5%	DC
17	1%	2%	-	-	-	-	-	-	-	-	70%	27%	-	DC
18	-	1%	-	-	-	-	-	-	-	-	70%	29%	-	DC
19	1%	-	-	-	-	-	-	-	1%	-	70%	28%	-	DC
20	1%	-	-	-	-	-	-	-	5%	-	67%	27%	-	Wet
21	1%	-	-	-	-	-	-	-	5%	-	67%	27%	-	DC
22	1%	-	-	-	-	-	-	-	10%	-	61%	28%	-	DC

23	1%	-	-	-	-	-	-	-	15%	-	56%	28%	-	DC
24	-	0.5%	-	-	-	-	-	-	-	-	70%	29.5%	-	Wet
25	-	0.5%	-	-	-	-	-	-	-	-	70%	28.5%	-	DC
26	1%	-	5%	-	-	-	-	-	-	-	67%	27%	-	DC
27	-	-	0.5%	-	-	-	-	-		-	70%	29.5%	-	DC
28	-	-	1%	-	-	-	-	-	-	-	70%	29%	-	DC
29	1%	-	-	5%	-	-	-	-	-	-	66%	28%	-	DC
30	1%	-	-	10%	-	-	-	-	-	-	61%	28%	-	DC
31	1%	-	-	15%	-	-	-	-	-	-	56%	28%	-	DC
32	1%	-	-	-	5%	-	-	-	-	-	66%	28%	-	DC
33	1%	-	-	-	10%	-	-	-	-	-	61%	28%	-	DC
34	1%	-	-	-	15%	-	-	-	-	-	56%	28%	-	DC
35	1%	-	-	-	-	5%	-	-	-	-	66%	28%	-	DC
36	1%	-	-	-	-	10%	-	-	-	-	61%	28%	-	DC
37	1%	-	-	-	-	15%	-	-	-	-	56%	28%	-	DC
38	1%	-	-	-	-	-	5%	-	-	-	66%	28%	-	DC
39	1%	-	-	-	-	-	10%	-	-	-	61%	28%	-	DC
40	1%	-	-	-	-	-	15%	-	-	-	56%	28%	-	DC
41	1%	-	-	-	-	-	-	5%	-	-	66%	28%	-	DC
42	1%	-	-	-	-	-	-	10%	-	-	61%	28%	-	DC
43	1%	-	-	-	-	-	-	15%	-	-	56%	28%	-	DC

Where PVP: polyvenyl pyrollidene; XG: xanthan gum; Carb: Carbopol 940, Es 100: Eudragite S100, EL 100: Eudragite L100, EL100-55: Eudragite L100-55, HPC: hydroxypropyl cellulose, EC: ethyl cellulose, HPMC: hydroxypropyl methyl cellulose, MAN: mannitol; MCC: microcrystalline cellulose; PolyP: polyplasidone, Mg.S: magnesium sterate

Based on the assessment of the prepared tablets without active ingredient (Table 6), the formulation development process was followed by incorporating the API into the successfully prepared formulations, and several formulations were prepared (Table 7). Because of the very low amount of active pharmaceutical ingredients (API) (2%) provided to the formula, the addition of powder materials during the mixing process was done by geometric mixing in all formulas produced using the direct compression method (Figure 17). [68] Initially, cyanocobalamin was mixed with an equal quantity of polymer, then a binder was added, then a filler and disintegrating agent, and finally a lubricant. All processes were done in dim light.

Figure 17 Cyanocobalamin with an equivalent amount of polymer to achieve geometric mixing.

Table 7 below presents the quantity of each material per tablet as a percentage, where the total weight of each tablet is 50 mg. Approximately 50 tablets of each formula were prepared.

Table 7 The composition of suggested cyanocobalamin mucoadhesive sublingual tablet formulas

Formula #	B_{12}	PVP	EC	HPMC	HPC	EL100-55	EL100	ES100	XAN	CR940	MCC	POLY	MG.S
S1	1	0.5	2.5	-	-	-	-	-	-	-	31.75	14	0.25
S2	1	0.5	5	-	-	-	-	-	-	-	29.25	14	0.25
S3	1	0.5	7.5	-	-	-	-	-	-	-	26.75	14	0.25
S4	1	0.5	-	0.5	-	-	-	-	-	-	33.75	14	0.25
S5	1	0.5	-	2.5	-	-	-	-	-	-	31.75	14	0.25
S6	1	0.5	-	-	2.5	-	-	-	-	-	31.75	14	0.25
S7	1	0.5	-	-	5	-	-	-	-	-	29.25	14	0.25
S8	1	0.5	-	-	7.5	-	-	-	-	-	26.75	14	0.25
S9	1	0.5	-	-	-	2.5	-	-	-	-	31.75	14	0.25
S10	1	0.5	-	-	-	5	-	-	-	-	29.25	14	0.25
S11	1	0.5	-	-	-	7.5	-	-	-	-	26.75	14	0.25
S12	1	0.5	-	-	-	-	2.5	-	-	-	31.75	14	0.25
S13	1	0.5	-	-	-	-	5	-	-	-	29.25	14	0.25
S14	1	0.5	-	-	-	-	7.5	-	-	-	26.75	14	0.25
S15	1	0.5	-	-	-	-	-	2.5	-	-	31.75	14	0.25
S16	1	0.5	-	-	-	-	-	5	-	-	29.25	14	0.25
S17	1	0.5	-	-	-	-	-	7.5	-	-	26.75	14	0.25
S18	1	-	-	-	-	-	-	-	-	0.25	33.75	14.75	0.25
S19	1	-	-	-	-	-	-	-	-	0.5	33.75	14.5	0.25
S20	1	-	-	-	-	-	-	-	0.25	-	33.75	14.75	0.25

4.2.2. Tablets evaluation:

4.2.2.1. Disintegration:

The disintegration test was done by adding the compressed tablets to a beaker containing 100 ml of distilled water (DW) at 37 °C [57], stirring somewhat to simulate the disintegration tester, then turning on the timer and recording the disintegration time for each formula.

4.2.2.2. Adhesive properties

All prepared tablets were tested for hardness, disintegration by the method prescribed previously, and mucoadhesive residence time. First, a formula containing 5% polymers (EC, HPMC, and HPC) was put in 10 ml of simulated saliva fluid at PH 6.8 to monitor adhesive properties initially (Figure 18), and then a mucoadhesive residence time test was done.

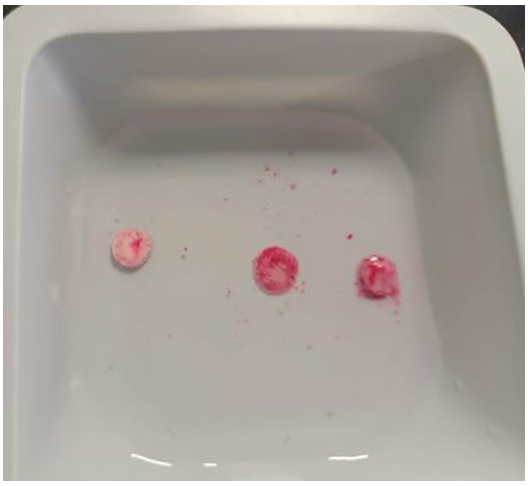


Figure 18 A dish contains S1, S4, and S6 (EC, HPC, and HPMC) was put in SSF at PH 6.8.

4.2.2.3. Ex vivo Mucoadhesive Residence time

The mucoadhesive residence time was performed on excised sublingual mucosa from bovines, [34] where the sublingual mucosa was cut by a scalpel into appropriate small pieces (1*3 cm), then washed. [48,69] After that, the sublingual mucosa was fixed to a plastic slide with superglue adhesive. [38,69] Then it was fixed to the paddle of the dissolution test by plastic rubber (Figure 19). [48] Afterward, one face of the tablet was wetted with approximately 200 µL of DW and was then pressed gently for 30 seconds on the excised tissue to start the adhesion process for the mucoadhesive tablet for residence time evaluation.[39,69] After that, the plastic slide containing the attached tablet was immersed into dissolution vessels containing 900

ml of DW. [38] The temperature of the apparatus was maintained at 37 °C during the experiment at a speed of 50 rpm. The test was performed for 120 minutes.[69] whereas the time at which a tablet either detached or disintegrated from the mucosal surface was considered mucoadhesive residence time. Each tablet formula was assessed for three measurements. [38]

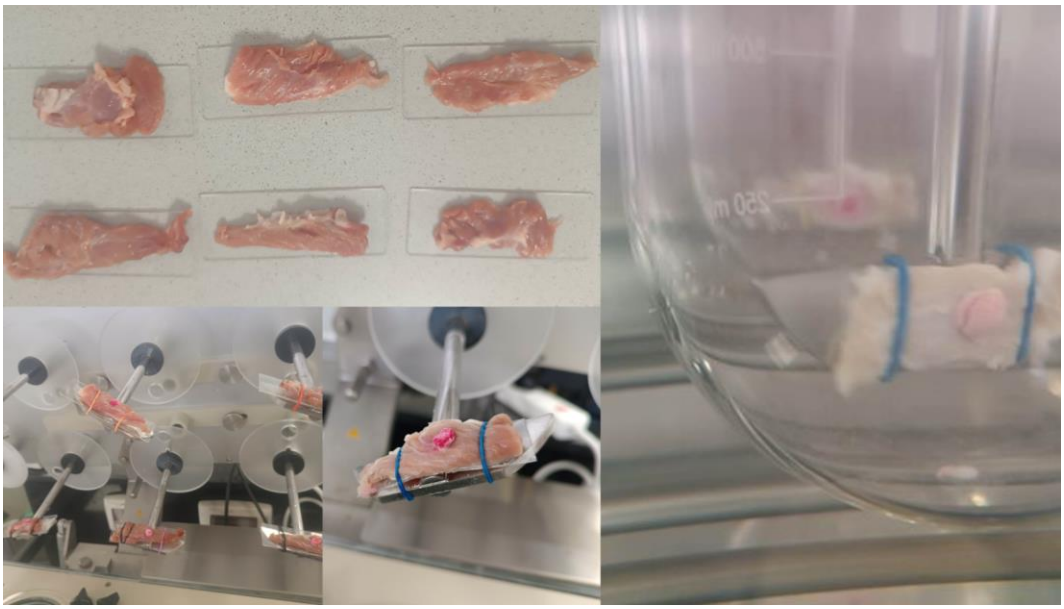


Figure 19 Sublingual mucosa fixed to the slide.

The formulations that passed were chosen based on their hardness, disintegration, and residence time results. Where the disintegration time is less than 1 minute, which was adopted previously, and the residence time for the formula is more than 10 minutes. After that, all formulas that succeeded were scaled up from 50 to 400 tablets.

4.2.3. Development of UV-Vis spectrophotometer analysis methods and validation

4.2.3.1. UV – Vis spectrophotometer analysis

The analysis of cyanocobalamin was performed using a UV-Vis spectrophotometer. The analytical method was validated according to ICH Q2B, including limit of detection (LOD), limit of quantification (LOQ), accuracy, linearity, and precision. [63]

Development of an UV-Vis spectrophotometer analysis method to detect vitamin B_{12} analogues (cyanocobalamin). A calibration curve for the B_{12} analogues was created where the range covered the detection and assay of vitamin B_{12}, dissolution, stability study, and compatibility study. Distilled water (DW), phosphate buffer saline and simulated saliva fluid (SSF) were used as solvents for all preparations.

All processes were done in dim light and using the amber volumetric flasks.

Distilled water

Distilled water was taken directly from a special tap at the Samih Darwazah Institute of Pharmaceutical Industries at Birzeit University.

Simulated saliva fluid. (SSF)

Simulated saliva fluid was prepared by adding, for each liter, 1.79 g of disodium hydrogen phosphate, 1.36 g of potassium dihydrogen phosphate, and 7.02 g of sodium chloride to distilled water, then sonicating until all amounts were dissolved. After that, the pH was adjusted to 6.8 with sodium hydroxide and hydrochloric acid. [31,55]

Phosphate buffer saline (PBS)

Phosphate buffer saline was prepared by adding, for each litter, 8 g of sodium chloride, 0.2 g of potassium chloride, 1.44 g of disodium hydrogen phosphate, and 0.24 g of potassium dihydrogen phosphate in distilled water, then sonicating until all amounts were dissolved. After that, the pH was adjusted to 7.4 with sodium hydroxide and hydrochloric acid.[70]

4.2.3.2. Stock solution preparation

Stock solutions were prepared using different solvents, including distilled water (DW) and simulated saliva fluids in the same method.

A stock solution was made to obtain several standards through solvents. A quantity of 500mg of vitamin B_{12} (cyanocobalamin) was dissolved in an appropriate amount of solvent 500-ml volumetric flask (VF), then the volume was adjusted up to 500 ml with solvent, then sonicated for 10 minutes to ensure total dissolution. The strength of the stock solution prepared was 1000 µg/ml.

4.2.3.3. Standard solution preparation

Standard solutions were prepared by transferring various volumes from the stock solutions into 100 ml volumetric flasks and subsequently diluting them with SSF solution to reach a final volume of 100 ml. The solutions were then sonicated for 15 minutes to ensure complete dissolution of the components. Equation 1 was utilized to calculate the required volume for each standard concentration (5, 10, 20, 25, 30, and 40 µg/ml).

$$M_1 V_1 = M_2 V_2 \qquad Equation\ 1$$

Where M refers to molarity and V to volume, 1 refers to the solution before dilution, and 2 refers to the solution after dilution.

4.2.3.4. UV analysis methods and validation

Scanning

From the stock solution prepared by distilled water (DW), 1000 µl of the solution was transferred to a 25-ml volumetric flask (VF), and the volume was adjusted to 25 ml with DW. The solution was then sonicated for 10 minutes. For the analysis of cyanocobalamin at a concentration of 40 µg/ml, the UV-vis spectrophotometer was used to scan the solution from 200 to 700 nm. DW was used as a blank in the spectrophotometer.

Calibration curve and linearity

From a stock solution, various standard concentrations 5, 10, 20, 25, 30 and 40 µg/ml were produced in triplicate by using DW and SSF solvent. The wavelength detected from scanning that has the highest peak was used for analysis.

A calibration curve was prepared in SSF solvent by measuring the absorbance of various concentrations using a UV-vis spectrophotometer at 361 nm within the range of 5 to 40 µg/ml. Subsequently, the calibration curve was generated by plotting the average absorbance against the corresponding concentrations of the standards.

Accuracy

Accuracy refers to the degree of closeness between the measured value and the true value in analytical methods.[71] Standard deviation (SD), percent recovery, and

relative standard deviation (RSD) were used to examine the method's accuracy at various concentration levels (80%, 100%, and 120%). A known quantity of stock solutions (1.6, 2, 2.4 ml in 100 ml VF) was applied, and the specified method was used to analyze the solutions.

Precision study

Precision is the degree to which an analytical process may be repeatable under existing operational conditions. The precision of the study was done at interday (intermediate precision or reproducibility). Three replicates of samples at various levels (80%, 100%, and 120%) were analyzed. For the intraday variation (repeatability) six samples at a level of 100% are prepared at the same concentration, and then the specified measurement techniques are used to analyze them.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD indicates the lowest concentration of an analyte in a sample that can be detected by an analysis method. The LOQ is known as the lowest level of an analyte in a sample that can be quantitatively quantified with acceptable accuracy, precision, and variability. The absorbance standard deviation (SD) and the slope of the related calibration curve were used to calculate the LOD and LOQ, which were determined by the following equations [71,72]:

$$\boldsymbol{LOD = 3.3 * \frac{\delta}{s}} \qquad Equation\ 2$$

$$\boldsymbol{LOQ = 10 * \frac{\delta}{s}} \qquad Equation\ 3$$

$$\boldsymbol{\delta} = \sqrt{\frac{\sum(\boldsymbol{X} - \boldsymbol{mean})^{\boldsymbol{2}}}{\boldsymbol{n}}} \qquad Equation\ 4$$

where δ is Standard Deviation SD of the y intercept for 3-time linearity, s is the slope of the corresponding calibration curve, X is each value of the example, the mean is the average of the values, and n is the number of values.

Robustness test

Robustness refers to the ability of an analytical method to remain unaffected by small, deliberate changes in parameters. This characteristic ensures the reliability of the method under normal usage conditions. [72]

To evaluate the robustness of the methods, a concentration of 20 µg/ml was prepared in triplicate, as mentioned earlier. Subsequently, the solution was measured using a UV-vis spectrophotometer at both 361 nm in SSF, PBS, and distilled water, as well as at 364 nm in SSF. Finally, the relative standard deviation (RSD) was calculated to assess the variability between the measurements.

Stability

To evaluate the stability of cyanocobalamin in the solution, a stock solution was prepared (1000 µg/ml) in the same method mentioned previously by SSF solution, and then absorption was measured at a concentration of 20 µg/ml. Stock solution was stored at room temperature for a week and for 90 days in SSF solution in a closed brown volumetric flask; after that, the absorption was measured at a concentration of 20 µg/ml.

4.2.4. Evaluation of mucoadhesive sublingual tablets

4.2.4.1. Evaluation of cyanocobalamin sublingual mucoadhesive blend.

The scale-up process of the formulas involves utilizing the same geometric mixing technique (Section 4.2.1) with the addition sieving steps using a #40 sieve after incorporating magnesium stearate. The final mixture, was underwent evaluation for various parameters, including the angle of repose, tap density, and bulk density.

Carr's index and Hauser ratio were calculated to assess the flowability characteristics of the powder. Ultimately, the powder underwent compression.

1. Angle of repose

The angle of repose test was conducted by placing a funnel on a stand positioned 5 cm above the floor. The average diameter and height of the resulting cone-shaped pile were measured. Subsequently, the average values from three measurements were used in Equation 5 to calculate the angle of repose. [29,37]

Angle of repose = $\tan^{-1}$ h/r $\quad\quad$ $Equation\ 5$

where h is the high and r is the radius in centimetres.

2. Tapped and bulk density

Tapped and bulk density: Initially, the bulk volume of the materials was recorded, followed by the calculation of bulk density using Equation 6 and 7 respectively. Subsequently, a tap density tester was used to continuously tap the powder until the volume remained constant. [37]

Bulk density = $\frac{weight\ of\ powder}{bulk\ volume}$ $\quad\quad$ $Equation\ 6$

Tapped density = $\frac{weight\ of\ powder}{tapped\ volume}$ $Equation\ 7$

3. Carr's index

Carr's index = $\frac{100\ (Vb-Vf\ )}{Vb}$ *Equation 8*

where Vb is the bulk powder volume and Vf is the final tapped volume. [29]

4. *Hauser ratio*

Hauser ratio = $\frac{Vb}{Vf}$ *Equation 9*

Where Vb is the bulk powder volume and Vf is the final tapped volume. [29]

The flow characteristics of the powder were assessed by analysing the angle of repose, as referenced in Table 8. Additionally, Carr's index and Hauser ratio, as presented in Table 9. [29]

Table 8 The impact of repose angle on flow characteristics

Angle of repose	**Flow character**
<25º	Excellent
25-30º	Good
30-40º	Passable
>40º	Very poor

Table 9 The impact of carr's index and hauser ration on flow characteristics

Carr's index	**Flow character**	**Hauser ratio**
<10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
>37	Very very poor	>1.6

4.2.4.2. Evaluation of cyanocobalamin sublingual mucoadhesive tablets.

The prepared cyanocobalamin sublingual mucoadhesive tablets were evaluated. Several quality control test were conducted such as weight variation, thickness, diameter, hardness, friability, mucoadhesive strength, content uniformity, and surface pH analysis of the tablets.

1. Weight variation

Weight variation was performed on a random selection of 20 tablets, from which the mean and standard deviation (SD) were calculated. The tablet weight was found to be within the acceptable limit of less than 130 mg, with a standard deviation of 10% (45-55 mg). Additionally, the thickness and diameter of the tablets were measured using a caliper, and the mean and SD were determined. [34,38,49]

2. Hardness

A hardness tester was utilized to measure the hardness of 10 randomly selected tablets, with the results recorded in kilopascals (KP). The mean and standard deviation (SD) of the hardness measurements were subsequently calculated. [30,34,38,59]

3. Friability

For the friability test, a total weight of 6.5 g (each tablet weighing 50 mg) was used. The tablets were placed in the pharma-test friability drum and subjected to rotation at 25 rpm for 4 minutes. Once the test was completed, the tablets were meticulously inspected for any signs of cracks, capping, or breakage. Subsequently, tablets were reweighed, and the weight loss percentage was calculated using Equation 10. It is

important to note that the weight loss should not exceed 1% of the tablet's initial weight.

$$\% \text{ Friability} = \frac{W0-W1}{W0} \qquad Equation\ 10$$

Where W0 weight before test , W1 weight after test

4. Content uniformity

The content uniformity test was performed by crushing 10 tablets from each formula. Subsequently, a weight equivalent to 1 mg of cyanocobalamin was added to 50 ml of simulated saliva fluid (SSF), followed by sonication for 10 minutes. The mixture was then filtered, and the concentration was determined by measuring the absorption using a UV spectrophotometer at 361 nm. The obtained value was then compensated using a calibration curve equation to calculate the assay percentage. [50,68]

5. Surface PH

A surface pH test was conducted to assess the potential in vivo side effects associated with alkaline and acidic pH values, which may cause mucosal irritation. The target pH range was determined to be 6.2–7.6 in normal saliva, indicating a nearly neutral pH. Six tablets were immersed in 20 ml of distilled water adjusted to a pH of 6.8 and maintained at 37°C in a water bath for a duration of 2 hours. Subsequently, the pH was measured using a digital pH meter once the reading reached a constant value.[36,51,68]

6. Mucoadhesive strength

For the mucoadhesive strength testing, a specially designed model resembling a balance was utilized. The test involved the incorporation of bovine sublingual mucosa into the balance model. One mucosal surface was fixed to a wooden square attached to the floor of the balance model, while the other mucosa was secured to a plastic cup-tied by a thread. A wet tablet of simulated saliva fluid (SSF) was placed between the two mucosal surfaces and gently compressed for 30 seconds (Figure 20). On the opposite side of the thread, additional plastic-cup were placed. A weight scale was used to gradually increase the force until the tablet detached from the mucosa. [48,68] The test was conducted in triplicate (n = 3) for each formula.

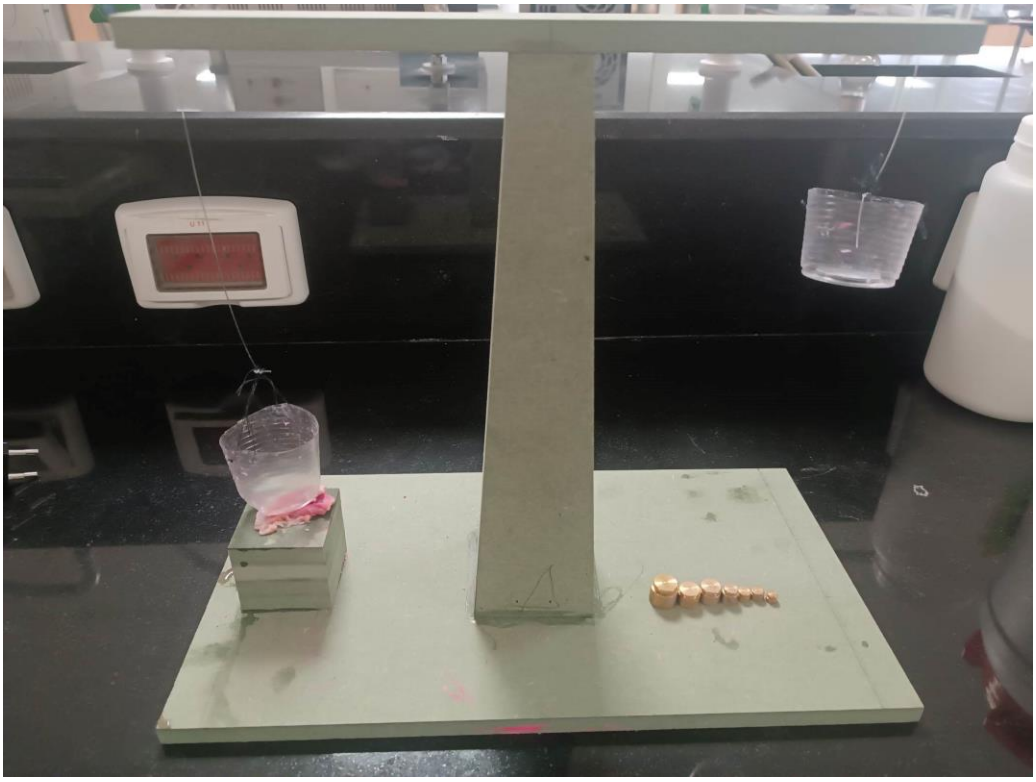


Figure 20 The balance model that was used to check the tablet's mucoadhesive strength

7. Drug release test

The drug release test was conducted using a modulated system, comprising a fixed plastic tube with a 2.5 cm diameter immersed in a 100-ml glass beaker. The lower open end of the tube was covered with a cellulose dialysis membrane secured by a plastic rubber, which was pre-wetted in SSF buffer at pH 6.8 for 30 minutes. Subsequently, one tablet was added to the interior of the tube along with 5 ml of SSF, while 50 ml of SSF at pH 6.8 was added to the beaker. The SSF in the beaker was agitated using a magnetic stirrer. [73]The beaker was placed in a water bath at 37 °C, which was maintained by using two pumps that circulated water from the bath into the glass dish and vice versa (Figure 21). The drug release test was conducted for both the standard and the final four formulas. The time intervals for the standard samples were 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, and 23 hours. For the samples of the final formulas (n=3), the time intervals were 1, 1.5, 2.5, 3.5, 4.5, 5.5, and 22.5 hours. At each time interval, a 10-ml sample was collected, and the volume was replenished with fresh SSF solution. The drug content released in the collected samples was analyzed using a UV-vis spectrophotometer at a wavelength of 361nm.

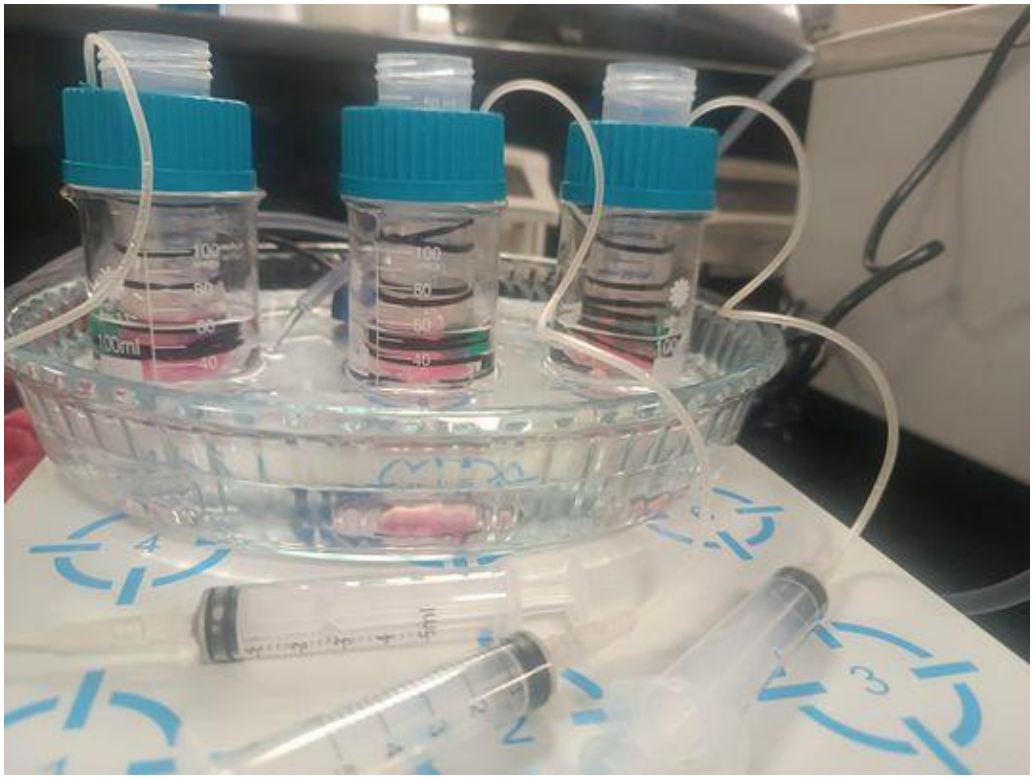


Figure 21 A model system for drug release test

The concentrations of the released drug were determined by using a calibration curve equation. Subsequently, the cumulative amounts of the drug released at each time point were calculated. These values were then used to plot a graph of the amount of drug released versus time. This graph was analyzed to study the kinetic model of drug release.

To determine the kinetic behaviour of drug release from four different formulas, various mathematical kinetic models were fitted to the data. The models used included the first-order, zero-order, Higuchi model, Quadratic model, Makoid-Banker model, and Peppas-Sahlin model. The selection of the most suitable model was based on the R^2 value, as a higher R^2 value indicates a better fit and provides

insight into the release mechanism of cyanocobalamin from sublingual mucoadhesive tablets.

8. PermeaPad® permeation test

The drug permeation test was performed using a Franz diffusion cell, with a Permeapad® membrane having a diameter of 25 mm, out of which 20 mm was exposed. The experiment involved two compartments - the donor compartment and the acceptor compartment. In the donor compartment, one tablet of the samples was added with 2 ml of simulated saliva fluid at PH 6.8, mimicking the conditions of the oral mucosa. The acceptor compartment was filled with 20 ml of phosphate buffer saline at pH 7.4. These buffer solutions represented the physiological conditions under which the drug would be released and permeate across the membrane.[74 68 46]

The system was maintained at a temperature of 37 ± 0.5 °C with continuous stirring at 250 rpm using a magnetic stirrer. To ensure a closed system, the sample port and donor compartment were covered with Parafilm. At each hour interval, 1 ml of samples was collected from the acceptor compartment and replaced with fresh phosphate buffer saline. The collected 1 ml sample was then diluted with 2 ml of phosphate buffer and measured at 361 nm using a UV-Vis spectrophotometer (Figure 22).

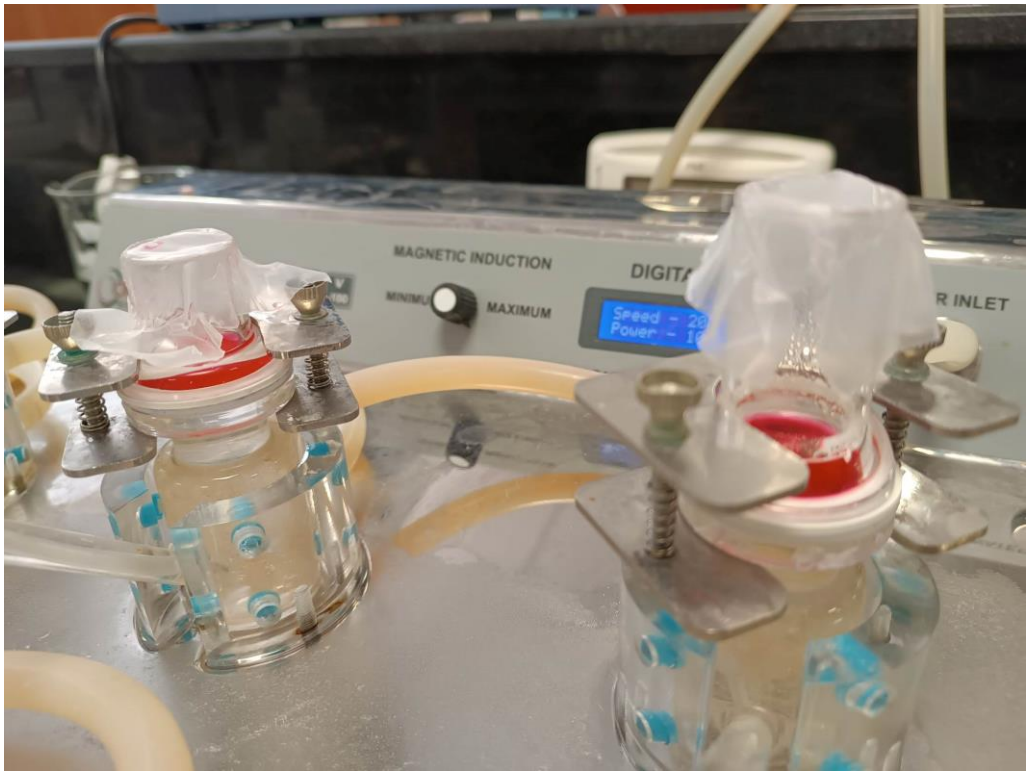


Figure 22 The Franz diffusion cell used in the cyanocobalamin permeation test.

The drug concentration was determined by using the calibration curve equation, and the amount of drug that crossed the Permeapad® membrane was calculated for each sample. The experiment was performed in triplicate.

The steady-state flux (J) of the Permeapad® membrane was determined by calculating the slope of the linear regression of the cumulative cyanocobalamin amount versus the time of sample collection. [74]

$$J = \frac{dn}{(A * dt)} \qquad Equation\ 11$$

Where J: steady state flux , dn : cumulative amount of drug permeated , dt: time, A: area of permeability [75]

Subsequently, the flux value (J) was used to calculate the apparent permeability coefficient (P_{app}) using Equation 12. By calculating the apparent permeability coefficient, you can quantitatively assess the permeability of cyanocobalamin through the sublingual mucosa. This information is valuable in understanding the drug's ability to cross the sublingual mucosa and potentially reach systemic circulation.[75 76] The equation is given by:

$$P_{app} = \frac{J}{C_0} \qquad Equation\ 12$$

Where:

P_{app} is the apparent permeability coefficient, J is the flux of the cyanocobalamin through the membrane, and C_0 is the initial concentration of cyanocobalamin in the donor compartment.

9. Drug Stability test in simulated saliva fluid

For stability assessment, a solution of cyanocobalamin with a concentration of 20 µg/ml was prepared in SSF buffer. To prepare the solution, 10 mg of cyanocobalamin was added to 50 ml of SSF in a 500 ml volumetric flask, and the volume was adjusted to the mark. The solution was then incubated in a water bath at 37 °C for 24 hours.

Samples were collected at specific time intervals, including 0, 1, 2, 3, 4, 5, 6 and 24 hours. The absorbance of each sample was measured at 361 nm using a visible spectrophotometer. By applying the calibration curve equation, the drug concentration, % recovery, and drug amount in each sample were determined.

Chapter V: Result and discussion

5. Chapter V: Result and discussion

5.1. Formulation developments

5.1.1. Formulation developments without API

The formulation development procedure started with preparation and evaluation of severer formulations without of active ingredients. As shown in Table 6, multiple formulations have been developed in order to investigate the effect of different components and technologies on tablet properties. Table 10 presents the results of tablet weight, hardness, and disintegration time. Accordingly, successful formulas have been chosen, where the ideal disintegration time is less than three minutes for the tablets that are designated to be placed under the tongue.[38,49] All formulas that had a disintegration time less than a minute were considered successful formulas. [30] Then cyanocobalamin was added to the successful direct compression formulas to conduct additional tests to determine the best formula for our purpose.

During tablets preparation using the molding method, part of the acetonitrile was volatilized, and as a result, the concentration continuously changed (increased) in a specific volume. Accordingly, it was difficult to estimate, control, or calculate the concentration precisely and to prepare a homogeneous solution or suspension using acetonitrile. Due to acetonitrile volatilization, it is continuously separated from the mixture so that it goes down under and the powder stays up in the syringe during dropping.

This method is used where quick disintegration is needed due to the dispersion matrix.[30] We note that these tablets are very fragile; they crumble when handled, and the surface is irregular from above due to the evaporation process (Figure 23, Figure 24). This problem is considered a challenge in the molding method. As stated in a study by Mutasem Rawas-Qalaji and others, they mentioned in their study that the mechanical properties were a challenge faced in this method, and this agrees with our results. [27] The mold needed to be deeper to accommodate weight correctly with an appropriate thickness.

Figure 23 Molding formula after 40-second disintegration test

Figure 24 Mucoadhesive sublingual tablets after 24 hours by molding method

Formulas which contain 5% of xanthan gum (4, 5, 6, 7, and 8) needed a long time for disintegration (Figure 25). Additionally, when the tablets from these formulas was transferred and immersed in 10 ml of DW, an hour later the center of the tablet stayed powdery with no wetting.

Xanthan gum is well-known for its high water solubility in both cold and hot water. [77] When the concentration of xanthan gum increases, it is suggested that the mucoadhesive characteristics improve until reaching a critical concentration. This behavior is attributed to the polymer's unique properties. Beyond this critical concentration, the polymer enters an "unperturbed" state with a significant solid structure. This structure restricts the penetration of polymer chains due to reduced solvent accessibility. [52]

The wetting behavior of tablets is influenced by multiple factors, including the concentration of the polymer, the pore structure within the tablet matrix, and the crosslinking density. Increasing the crosslinking density results in reduced water penetration into the tablet. This relationship has been observed in previous studies. Additionally, the retardation of wetting can be attributed to the unique behavior of xanthan gum. Xanthan gum has the ability to rapidly form a gel-like structure upon contact with water. This gel undergoes a transformation into a more porous and rubbery state. At higher concentrations of xanthan gum, the viscosity increases and crosslinking density, creating barriers that impede further water penetration into the system. These barriers contribute to the delay in wetting observed in tablets with

higher concentrations of xanthan gum. Therefore, the concentration of the polymer is an important factor in determining the wetting properties of the tablets. [52] [78]

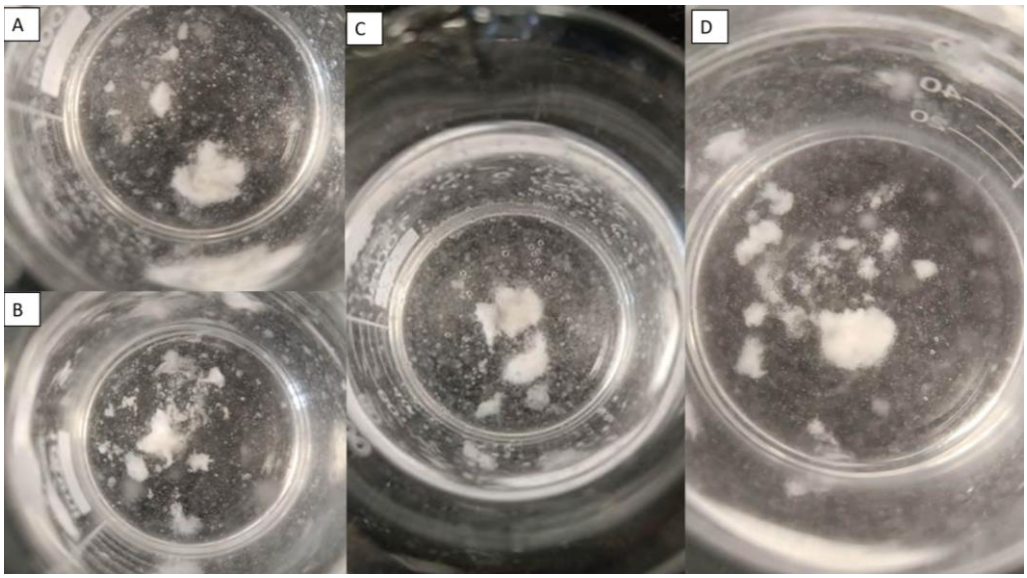


Figure 25 Formula 5, 6, 7, and 8 after a 1-hour disintegration test, respectively

It was observed that an increase in the concentration of mucoadhesive polymers resulted in a prolonged disintegration time. For instance, when the concentration of HPMC polymer exceeds 10% (Table 6, formula 23), the disintegration time extends to approximately 13 minutes (Table 10). This phenomenon is explained in the literature, which discusses how the polymer behaves when it absorbs water and forms gel layers on the tablet's surface at this concentration, thus delaying the disintegration time. [47] Additionally, the concentration of xanthan gum positively influences the disintegration time (Table 6, formulas 4 to 18). At concentrations exceeding 3%, it takes more than 30 minutes for disintegration. The longer disintegration time is attributed to the increased viscosity resulting from the formation of a gel matrix on the tablet, which gradually erodes. [50] [78]

Furthermore, all formulations that achieved disintegration times of less than one minute underwent preparation using either the direct compression or wet granulation methods. Among these methods, the direct compression method was chosen as the preferred approach to complete the formulation process. This selection was based on its simplicity, cost-effectiveness, and reduced number of steps involved compared to the wet granulation method. Importantly, the direct compression method eliminates the need for solvents and drying steps. [32,35] As a conclusion formulas 19, 21, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 were selected for the next stage.

Table 10 The result of the weight, hardness, and disintegration tests for the initial formulas of sublingual mucoadhesive tablets

Formula	Weight (mg) ± SD	Hardness ± SD	Disintegration time ± SD	Note	Result
1	39.711± 3.666941914	Very friable	97.426667 ± 15.97018 sec		Fail
2	49.38667± 1.806248	4.93333 ± 0.4041	47.7733 ± 7.410 sec		Pass
3	50.96667± 0.602771	6.367 ± 0.5132	29.735 ± 1.534 sec	HPMC is added as a solution in a 100% ethanol.	Pass
4	50.47667± 1.581339	3.86667 ± 0.3512	1.0867 ± 0.0808 hr		Fail
5	50.04333± 0.568888	4.267 ± 0.3215	1.0667 ±0.0611 hr	Take a very long time before completely wetting	Fail
6	50.53333± 1.234234	4.7 ± 0.4	1.00667 ± 0.005 hr	Take a very long time before completely wetting	Fail
7	52.36667 ± 1.193035	4.8333 ± 0.5686	1.0923 ± 0.0849 hr	Take a very long time before completely wetting	Fail
8	50.23333 ± 0.85049	5.0667 ± 0.2517	46.667 ± 2.88 min	Take a very long time before completely wetting	Fail
9	51.63333 ± 0.83865	5.1333 ± 0.3512	50 ± 2min		Fail
10	50.56667 ± 1.530795	5.8667 ± 0.3055	15 ± 1 min		Fail
11	50.66667 ± 1.167619		15 ± 2 min	The rods of the hardness tester almost hit each other because they were extremely friable.	Fail
12	50.33333 ± 1.289703		14.667 ± 2.5167 min	The rods of the hardness tester almost hit each other because they were extremely friable.	Fail
13	49.46667 ± 0.378594	5.7 ±0.436	55 ± 2 min	When added to 10 ml of distilled water, after 10 minutes, the surface remained a powder with no wetting or swelling.	Fail

Formula	Weight (mg) ± SD	Hardness ± SD	Disintegration time ± SD	Note	Result
14	50.43333 ± 1.001665	3.833 ± 0.2082	32.33 ± 2.517 min	Thirty minutes later, it was wet—even the center of the tablet was wet, but the tablet did not disintegrate. It was like a dissolving	Fail
15	50.1 ± 0.984886	4.2 ± 0.3	40.167 ± 0.763 min	Thirty minutes later, it was wet—even the center of the tablet was wet, but the tablet did not disintegrate. It was like a dissolving	Fail
16	50.1 ± 1.03923	5.267 ± 0.3512	37 ± 2 min	Thirty minutes later, it was wet—even the center of the tablet was wet, but the tablet did not disintegrate. It was like a dissolving	Fail
17	50.2 ±0.69282	5.1 ± 0.2646	10.0067 ±1.55 min		Fail
18	51.09667 ± 0.700024	4.167 ± 0.4509	5 ± 1.5 min		Fail
19	50.83333 ± 0.503322	4.0333 ± 0.1528	14.053 ± 1.0533 sec		Pass
20	50.43333 ± 2.200757	5.233 ± 0.2517	40 ± 0.540 sec		Pass
21	49.83333 ± 1.93477	3.8 ± 0.2	30.04 ±1.100 sec		Pass
22	50.56667 ± 1.504438	5.333 ± 0.2517	99.67 ± 10.066 sec		Pass
23	50.5 ± 1.473092	4.067 ± 0.5033	13.71 ± 1.391 min		Fail
24	50.43667 ± 0.753016	5.1 ± 0.3	61.28 ± 11.289 sec		Pass
25	49.7 ± 1.1	5.2 ± 0.2646	50.743 ± 1.19 sec		Pass
26	52.3 ± 0.754983	6.533 ±0.3055	46.0933 ± 2.22 min		Fail
27	50.53333 ± 0.907377	5.067 ± 0.2517	40 ± 2 sec	At first, it turned into two separate layers, then disintegrated completely.	Pass
28	49.46667 ± 0.85049	6.033 ± 0.5508	155.22 ± 9.662 sec	At first, it turned into two separate layers, then disintegrated completely.	Pass
29	51.2 ± 0.52915	4.833 ± 0.2082	11.52 ± 0.984 sec		Pass

Formula	Weight (mg) ± SD	Hardness ± SD	Disintegration time ± SD	Note	Result
30	51.3 ± 0.818535	3.67 ±0.2517	14.0933 ±0.5401 sec		Pass
31	50.56667 ± 0.907377	4 ± 0.2	15.57 ± 2.014sec		Pass
32	50.3 ± 1.769181	5.933 ± 0.404	19.667 ± 0.956 sec		Pass
33	50.36667 ± 0.68252	4.3 ± 0.4	16.58 ± 1.402 sec		Pass
34	50.7 ± 1.4	3.733 ± 0.3055	9.377 ±0.725 sec		Pass
35	50.40667 ± 1.484632	5.2 ±0.265	11.633 ± 0.839 sec		Pass
36	49.96667 ± 2.759227	5.167 ± 0.416	13.483 ± 2.0189 sec		Pass
37	51.26667 ± 1.167619	4.433 ± 0.503	15.94 ± 1.614 sec		Pass
38	50.06667 ± 1.040833	4.8 ± 0.2	12.38± 1.619 sec		Pass
39	51.6 ± 1.081665	5.4 ± 0.46	26.463 ± 3.609 sec		Pass
40	51.46667 ± 1.069268	5.267 ± 0.252	21.657 ± 2.129 sec		Pass
41	52.06667 ± 1.101514	3.966 ± 0.3511	14.677 ± 1.115 sec		Pass
42	50.6 ± 0.4	5.1 ± 0.265	18.577 ± 1.981 sec		Pass
43	51.53333 ± 0.550757	5.6 ± 0.4	15.84 ± 2.049 sec		Pass

* Average of triplicate

5.1.2. Formulation developments with API

Based on the prior evaluations of prepared tablets without cyanocobalamin, the formulation development process was proceeded by incorporating the API into the formulations. Accordingly, numerous formulations were developed in order to formulate a cyanocobalamin mucoadhesive sublingual tablet (Table 7). Table 11 presents the results of weight, hardness, and disintegration time prepared formulas of cyanocobalamin mucoadhesive sublingual tablets.

Tablet prepared using eudragite S100, L100, and L100-55 (S9 to S17) at first exploded into two layers and then disintegrated. The carbopol crumbled in the form of blocks or grains (Figure 26), and the xanthan gum was closer to the dissolving state (Figure 27). Additionally, it was observed that when the concentration of EC, HPMC, and HPC polymers increased, the disintegration time increased (S1 to S8).

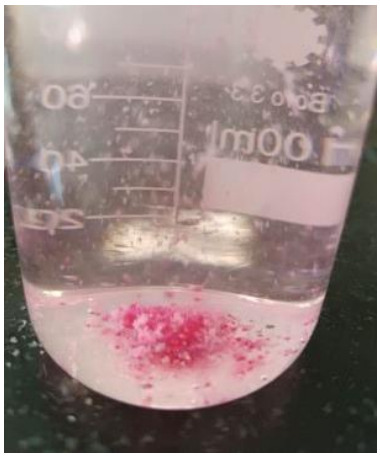


Figure 26 Carbopol after disintegration

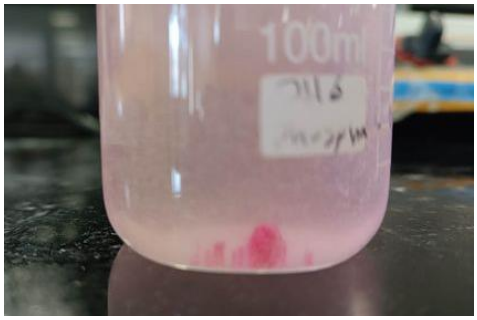


Figure 27 xanthan after disintegration

In 10 ml of simulated saliva fluid containing 5% polymer tablets, the EC tablet (S1) detached after 2 hours, the HPC tablet (S6) after 2.5 hours, and the HPMC tablet (S5) after more than 3 hours (Figure 28).

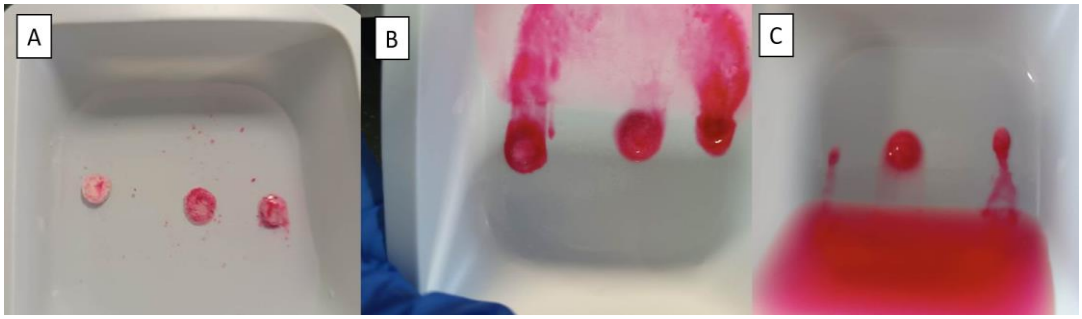


Figure 28 Tablets in SSF for the initial adhesion test: A at zero-time, B at 54 minutes, and c after 2 hours, where tablets rank S1, S2, and S3 (EC, HPMC, and HPC), respectively.

During the residence time test, it was observed that ethyl cellulose and Carbopol 490 polymers (formula S1, S2, S3, S18, and S19) exhibited rapid swelling followed by an explosion, resulting in a residence time of fewer than 6 minutes. On the other hand, hydroxypropyl methylcellulose, hydroxypropyl cellulose, eudragit, and xanthan (S4-S17 and S20) exhibited less swelling compared to the previous polymers, leading to a longer residence time. This is a normal outcome since

excessive swelling can create a slippery mucilage, causing easy detachment from the mucosal surface. [39,79,80]

The residence time of the preparation was found to increase with an increase in polymer concentration, as observed in Table 11. This relationship is attributed to the characteristic behavior of the polymer. At low concentrations of polymers (S1 to S4, S6, S7, S9, S10, S12-S17), there are relatively fewer polymer chains available to penetrate the mucosal surface, resulting in weaker interactions and lower mucoadhesion strength. However, as the concentration of the polymer increases, more chains can penetrate the mucosal surface, leading to stronger mucoadhesive properties (S5, S8, S11). This increase in mucoadhesion strength follows a certain trend until a critical concentration is reached. [52]

Importantly, among all these formulas, S5, S8, S11, and S20 demonstrated a significantly longer residence time, exceeding 15 minutes. These formulas can be considered successful formulations due to their extended residence time.

Table 11 The result of the weight, hardness, and disintegration tests for the formulas of sublingual mucoadhesive cyanocobalamin tablets

Formula	Polymers	Hardness, ±SD *	Weight, ±SD (mg)*	Disintegration time, ±SD (sec) *	Residence time, ±SD *	Result
S1	**EC 5%**	5.2 ±0.6	49.6 ± 3.5	16.29 ± 4.05	1.24 min ± 0.085	Fail
S2	**EC 10%**	4.2 ± 0.3	51.56 ± 1.3	24.59 ± 6.19	1.35 min ± 0.28	Fail
S3	**EC 15%**	5.1 ± 0.9	51.23 ± 2.52	30.53 ± 8.84	2.19 min ± 0.612	Fail
S4	**HPMC 1%**	4.5 ± 0.1	49.47 ± 0.45	24.37 ± 7.02	5.11 min ± 2.15	Fail
S5	**HPMC 5%**	5.77 ± 0.378	49.17 ± 3.54	42.45 ± 16.43	86.40 min ±48.17	Pass
S6	**HPC 5%**	4.733 ± 0.321	53.2 ± 1.72	25.36 ± 2.25	4.2 min ± 4.79	Fail
S7	**HPC 10%**	5.8 ± 0.608	51.83 ± 1.3	31.98 ± 3.96	3.55 min± 1.34	Fail
S8	**HPC 15%**	5.6 ± 0.4	49.63 ± 1.77	42.78 ± 9.34	22.45 min ± 6.47	Pass
S9	**EL100-55 5%**	4.3 ± 0.608	50.1 ± 1.81	18.87 ± 2.31	5.26 min ± 10.56	Fail
S10	**EL100-55 10%**	6 ± 0.6	50.63 ± 2.52	14.41 ± 3.07	4.16 min ± 3.81	Fail
S11	**EL100-55 15%**	3.5 ± 0.458	50.13 ± 2.69	16.68 ± 1.75	118.20 min ± 2.89	Pass
S12	**EL100 5%**	4.7 ± 0.7	50.97 ± 1.46	17.6 ± 3.25	44 sec ± 6.38	Fail
S13	**EL100 10%**	5.7 ± 0.435	51.33 ± 1.62	22.31 ± 1.50	1.47 min ± 20.82	Fail
S14	**EL100 15%**	3.7 ± 0.435	52.5 ± 2.15	30.72333 ± 9.83	5.66 min ± 4.13	Fail
S15	**ES100 5%**	4.4 ± 0.624	53.2 ± 3.16	20.00333 ± 1.37	2.18 min ± 1.14	Fail
S16	**ES100 10%**	3.1 ± 0.1	51.2667 ± 1.54	16.25667 ± 1.47	1.41 min ± 0.43	Fail
S17	**ES100 15%**	3.7 ± 0.556	52.1 ± 2.44	17.72 ± 0.54	4.45 min ± 0.98	Fail
S18	**CR490 0.5%**	3.8 ± 0.608	52.733 ± 0.75	20.24667 ± 0.69	5.33 min ± 3.47	Fail
S19	**CR490 1%**	4.6 ± 0.458	50.733 ± 1.5	58.34 ± 1.48	4.65 min ± 2.11	Fail
S20	**XAN 0.5%**	5.2 ± 0.1	51.3 ± 1.63	53.93 ± 2.37	57.40 min ± 19.66	Pass

* Average of triplicate

5.2. UV-Vis Spectrophotometer analysis result

The methods were validated in accordance with ICH guidelines. The sample solutions were made in accordance with the previously used method described in the study.[71]

1. Scanning

According to Figure 29 below, the curve displays the greatest absorbance of cyanocobalamin at 361 nm.

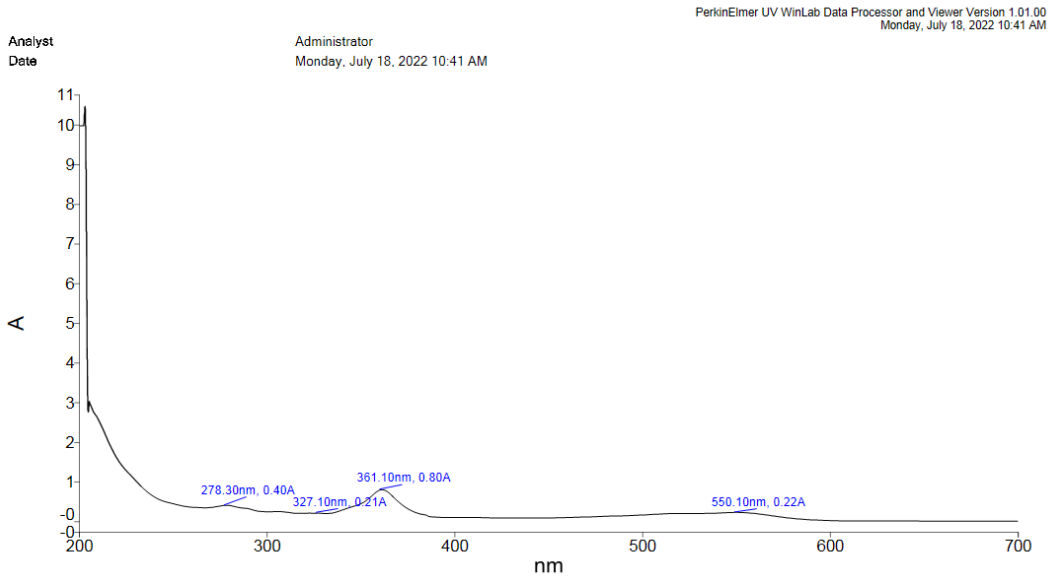


Figure 29: UV spectrum of cyanocobalamin

2. Calibration curve and linearity

As a result of the scanning process, all absorption of the concentrations was measured by UV-Vis spectrophotometry at 361 nm. The findings of a study on the linearity of cyanocobalamin concentration are displayed in Table 12. The average of three samples' absorbance for each concentration was calculated.

Using the R^2 of the calibration curve's regression line, it was confirmed that the linearity matches the investigated concentration range.

The cyanocobalamin calibration curves' linear regression results revealed a strong linear relationship over a range of 5-40 µg/ml. The results of a linear regression equation were y = 0.0181x + 0.0155, R^2 = 0.9997 (Figure 30).

Table 12 Absorption result of cyanocobalamin calibration curve in SSF at PH 6.8

Con (µg/ml)	ABS			Abs mean*	SD	RSD
	Abs1	Abs2	Abs3			
5	0.102	0.099	0.1	0.100333	0.001528	1.523
10	0.204	0.199	0.198	0.200333	0.003215	1.605
20	0.38	0.378	0.377	0.378333	0.001528	0.4039
25	0.463	0.475	0.465	0.467667	0.006429	1.37
30	0.565	0.559	0.56	0.561333	0.003215	0.573
40	0.737	0.738	0.729	0.734667	0.005033	0.6854

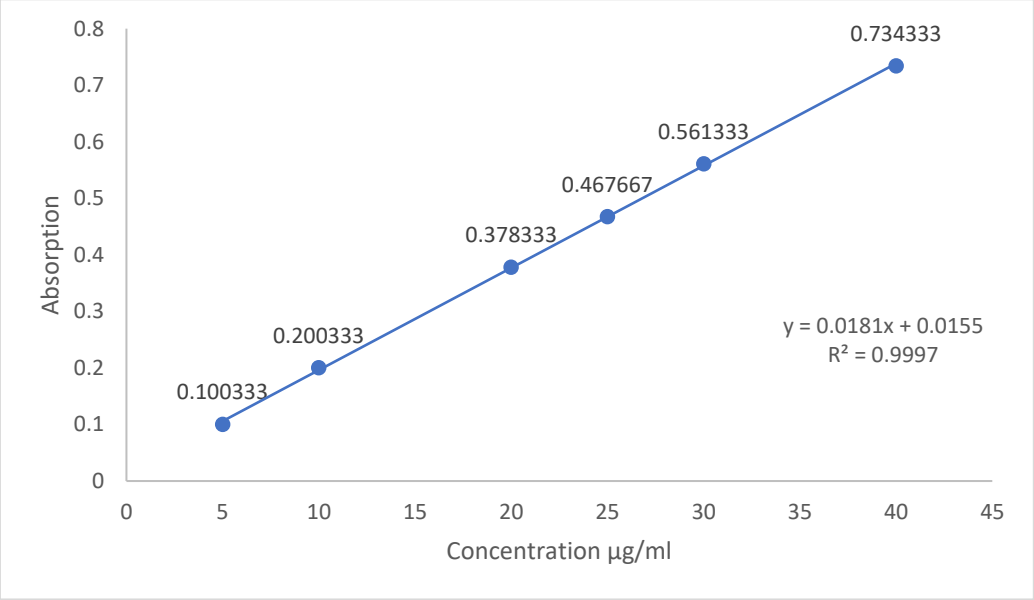


Figure 30 Calibration curve of cyanocobalamin in SSF at PH 6.8

3. Accuracy

The suggested method was used to analyze the solutions. The recovery percentage values were calculated. The recovery results in Table 13 indicated that the suggested method has a satisfactory level of cyanocobalamin accuracy in the range of 98-99.68% with a RSD of less than 2.

Table 13 Accuracy of cyanocobalamin in SSF

Con (µg/ml)	**Abs SSF1**	**Abs SSF 2**	**Abs SSF 3**	**Mean ± SD**	**RSD**	**Con recovered**	**%Recovered**
16	0.303	0.298	0.297	0.299333± 0.003215	1.074	15.68	98
20	0.38	0.376	0.373	0.376333± 0.003512	0.9332	19.936	99.68
24	0.446	0.444	0.447	0.445667± 0.001528	0.34285	23.77	99.02

4. Precision

The obtained results are expressed as the RSD% of triplicate measurements that are statistically significant. These, along with the 95% confidence interval and standard error, were tabulated (Table 14, Table 15).

Interday

The interday precisions were calculated by observing absorbance on three separate days at three different concentrations. Table 14 shows the mean, concentration found, SD, and percent RSD of the determined absorbance result. The RSD values that were shown to be less than 2 demonstrate that this method is reproducible and precise for the evaluation of drugs.

Table 14 Cyanocobalamin interday precisions studies

Con (µg/ml)	Abs SSF day 1	Abs SSF day 2	Abs SSF day 3	Mean ± SD	RSD	Con recovered	%Recovered	Confidence interval 95%	Standard error
16	0.2993	0.30967	0.3093	0.30611± 0.00587	1.91	16.056	100.35	0.306111 ± 0.00664 (0.299 to 0.313)	0.00339
20	0.3763	0.3773	0.38	0.377899± 0.001896	0.502	20.021	100.105	0.377889 ± 0.00215 (0.376 to 0.38)	0.00109
24	0.4457	0.44867	0.4513	0.448556± 0.002835	0.632	23.926	99.688	0.448556 ± 0.00321 (0.445 to 0.452)	0.00164

*Average of triplicate

Repeatability (Intraday)

By measuring the concentration of the cyanocobalamin solution six times, repeatability was verified. The percentage amount recovered was 100.275% with RSD less than 2. (Table 15)

Table 15 Cyanocobalamin repeatability study.

Concentration (20µg/ml)						
Abs	0.378	0.377	0.38	0.376	0.373	0.387
Mean ± SD	0.3785± 0.004764					
RSD	1.2586					
Con found	20.055					
%Recovered	100.275					
Confidence interval 95%	0.3785 ± 0.00381 (0.375 to 0.382)					
Standard error	0.001945					

5. LOD and LOQ

After application to Equation 2 and Equation 3, it was found that LOD = 0.19216 µg/ml and LOQ = 0.58232 µg/ml for cyanocobalamin.

6. Robustness test

After calculating the percent recovery for the robustness methods at 361 nm in both DW and SSF solvents and at 364 and SSF solvent, it was determined that the recovery percentage ranged from 97.099% to 100.69%, with a relative standard deviation (RSD) of less than 2% (Table 16). These results confirm the reliability of the method.

Table 16 The result of robustness

Con (20 µg/ml)	Abs at 361 nm DW solvent	Abs at 361 nm SSF solvent	Abs at 364 nm SSF solvent	Abs at 361nm PBS
Mean abs of triplicate ± SD	0.3677 ± 0.00698	0.38 ± 0.005888	0.37067± 0.003859	0.381125 ±0.0021
RSD	1.871	1.549	1.041	0.551
%Recovery	97.099	100.69	98.112	101
Mean	0.37487375			
SD	0.00669554			
RSD	1.7862			

7. Stability

As shown in the results of Table 17 below, the drug is stable after three months of storage in DW with a RSD of less than 2.

Table 17 The result of cyanocobalamin Stability after stored in distilled water

At zero time		After 1 week		After 3 months	
Con (20 µg/ml)		**Con (20 µg/ml)**		**Con (20 µg/ml)**	
Mean abs of triplicate	0.381	**Mean abs of triplicate**	0.380	**Mean abs of triplicate**	0.36467
SD	0.005568	**SD**	0.007211	**SD**	0.0012472
RSD	1.461	**RSD**	1.898	**RSD**	0.342
SE	0.003215	**SE**	0.004163	**SE**	0.00072
CI	0.381 ± 0.0063 0.3747-0.3873	**CI**	0.380± 0.00816 0.3718 to 0.3882	**CI**	0.36467± 0.00141 0.363 to 0.366
Con found. % Recovery	20.193 100.96	**Con found. % Recovery**	20.138 100.69	**Con found. % Recovery**	19.29 96.456

5.3. Evaluation of mucoadhesive sublingual tablets

Based on the values presented in Table 18, the blend exhibited excellent flow characteristics, as indicated by the angle of repose. Additionally, S5, S8, and S20

demonstrated good flow properties, while S11 showed a fair flowability characteristic, as determined by Carr's index and Hauser ratio. This is related to the presence of a high percent of microcrystalline cellulose, which has excellent flow properties.

Table 18 The evaluation of the final formula blend

Formula	Polymer	Angle of repose	Carr's index	Bulk density	Tapped density	Hauser test
S5	**HPMC**	21.047	15.789	0.3496	0.4152	1.187
S8	**HPC**	15.836	12.766	0.3625	0.4159	1.146
S11	**Eudragite L 100-55**	23.400	17.857	0.3554	0.4332	1.27
S20	**Xanthan gum**	23.505	12.727	0.3619	0.4147	1.146

*Average of triplicate

The physical evaluation of cyanocobalamin mucoadhesive tablets is presented in Table 19. The weight variation of all formulations falls within the range of 49.935 to 50.53 mg. Tablets diameter and thickness are similar for all formulations, except for S5, which contains HPMC polymers and has a lower thickness. The average hardness of the tablets ranges from 4.28 to 4.94 KP, with all tablets exhibiting a hardness between 3 and 7. The friability percentage of all formulations is less than 1%, ranging from 0.2619 to 0.6802. These results indicate that all formulations exhibit good mechanical properties, making them suitable for mechanical shipping and storage. [30,69]

The assay of all formulas confirmed the uniformity of content, with values ranging from 93.508 to 103.91. The surface pH of the tablets falls between 5.35 and 6.63, with most tablets maintaining a relatively stable surface pH, except for S11, which showed a slight decrease of approximately 1 pH unit. This decrease is a normal result attributed to the acidic properties of the eudragit L100-55 polymer. [81,82] None

of the formulas would cause irritation, as observed in vitamin B_{12} buccal mucoadhesive films with a pH of 5.1 that did not exhibit any irritation, [73].

All formulas exhibited mucoadhesive strength within the range of 11–18.67 g, representing the force required to detach the tablets from the mucosal layers. The mucoadhesive strength can be arranged in ascending order as follows: S8 < S5 < S20 < S11. Among these, S8, which contains HPC polymer, displayed the lowest mucoadhesive strength, while S11, containing eudragit L100-55, exhibited the highest mucoadhesive strength. Additionally, S11 demonstrated the longest mucoadhesive time, lasting for 118.2 minutes in a previous result (Table 11).

The mucoadhesive strength of S5 (HPMC) is indeed higher compared to S8 (HPC). This difference in mucoadhesive strength can be attributed to several factors, including the swelling properties and viscosity characteristics of the cellulose polymers. Cellulose derivatives polymers are known for their excellent swelling properties, which promote the entanglement of polymer chains and enable strong interactions with the mucin present in the mucosal layer. [83] [52]

As observed in Table 11 and Table 19, the S5 formula, which contains HPMC, exhibits superior mucoadhesive characteristics in terms of strength and time compared to the S8 formula, which contains HPC polymer. Both polymers are hydrophilic, non-ionic, cellulose derivative, and water-soluble polymers. However, the differences in mucoadhesive performance between the two polymers can be attributed to factors such as the rate of tablet uptake by the polymers and the viscosity characteristics. [83]

HPMC has a more complex structure compared to HPC. This structural difference may contribute to HPMC's superior mucoadhesive properties, as it allows for stronger interactions with the mucosal surface. [84] The presence of hydroxypropyl and methoxyl groups along increases the polymer's hydrophilicity with a hydrophobic group. This structure allows for the formation of hydrophobic interactions in addition to fast wetting and spreading of the polymer upon contact with the mucin surface. [84] Additionally, enhances its ability to form hydrogen bonds with mucin, the major component of mucus. These interactions promote adhesion and prolong the residence time of the formulation on the mucosal surface. HPMC also possesses the ability to hold fluid within its structure through pores, [85] forming a hydrogel structure that maintains its three-dimensional integrity through cross-linking by creating hydrogen bonds, ultimately contributing to increased mucoadhesive strength. [56,69]

In contrast, HPC, with its simplified structure, may have lower mucoadhesive strength and shorter residence time compared to HPMC. Its lower viscosity characteristics may result in weaker interactions with the mucosal surface, leading to reduced mucoadhesive performance. [83]

The S20 formula (Xanthan gum) demonstrates superior mucoadhesive characteristics compared to the S8 formula (HPC). This can be attributed to the inclusion of xanthan, an anionic hydrophilic natural mucoadhesive polymer known for its pseudoplastic behaviour. [78,86] The mucoadhesive properties of xanthan are primarily attributed to its charge and ionization properties. The ionic nature of xanthan allows for stronger electrostatic interactions with mucin, making it a more

effective mucoadhesive characteristic when compared to the S8 formula, which contains natural non-ionic polymer. [27,53]

Additionally, the superior mucoadhesive strength of xanthan gum can be attributed to its high wetting properties. Xanthan gum is a water-soluble hydrophilic polymer that rapidly dissolves in hot and cold water, allowing the polymer chains to quickly diffuse into the mucosal surface. This fast diffusion and wetting process facilitate the creation of a strong interaction between the matrix and the mucosa, resulting in enhanced mucoadhesive strength. [68,87] A key factor that enhances the mucoadhesive properties of xanthan gum is its high molecular weight, which typically ranges from 2,000,000 to 20,000,000. Compared to other cellulose derivative polymers, xanthan gum exhibits a significantly higher molecular weight. [87] The high molecular weight of xanthan gum plays a crucial role in its mucoadhesive behavior. Research studies have indicated that polymers with molecular weights exceeding 100,000 generally demonstrate improved mucoadhesive properties. With molecular weights in the range mentioned above, xanthan gum exhibits an exceptional capacity for mucoadhesion.[87] [52] The S11 formula, containing Eudragit L100-55, exhibits the best mucoadhesive characteristics. Eudragit L100-55 is a polymer derived from acrylic and methacrylic acid, with different types classified based on alkaline and acidic groups. Specifically, Eudragit L100-55 is an anionic, hydrophobic, soluble polymer that demonstrates solubility at pH levels above 5.5, such as in saliva. [59,80,82] Due to its anionic nature and charged properties, Eudragit L100-55 generates a stronger electrostatic interaction in comparison to natural non-ionic HPMC polymers. [53]

Its mucoadhesive strength can be attributed to the presence of carboxylic acid groups in the polymer structure allows it to form strong hydrogen bonds with the mucin in mucosa layer. These hydrogen bonds contribute to the adhesion of the polymer to the mucosa, resulting in mucoadhesion. Furthermore, the high molecular weight of Eudragit L100-55 also contributes to its mucoadhesive strength. Research has shown that polymers with molecular weights higher than 100,000 exhibit greater mucoadhesive strength. The long polymer chains of Eudragit L100-55 can entangle with the mucus layer, increasing the overall adhesive forces between the polymer and the mucosal surface. [79] [52]

Table 19 Evaluation of mucoadhesive cyanocobalamin sublingual tablets

Formula	Weight variation mg ± SD	Diameter mm ± SD	Thickness mm ± SD	Hardness Kp ± SD	Friability %	Assay % ± SD	Surface PH	Mucoadhesive strength (g) ± SD
S5	50.32±0.591	5± 0	1.5 ± 0	4.94±0.742	0.4802	93.508 ± 0.001247	6.63	14 ±1.73205081
S8	49.935±0.668	5± 0	2± 0	4.28±0.933	0.3667	103.91 ± 0.003559	6.41	11± 1
S11	50.155± 0.638	5± 0	2± 0	4.91± 0.935	0.3043	99.124 ± 0.001699	5.35	26 ± 1
S20	50.085 ±0.774	5± 0	2± 0	4.609± 0.943	0.2619	96.362 ±0.0008165	6.49	18.67 ± 1.527525

Drug release test

To control drug release from polymer matrices, polysaccharide polymers such as HPMC (hydroxypropyl methylcellulose), Eudragite L100-55, xanthan gum, and HPC (hydroxypropyl cellulose) are commonly employed. These polymers facilitate drug release through a dissolution process involving solvent diffusion and/or disentanglement of polymer chains. [88]

A comparison of the drug release profiles between standard cyanocobalamin and the four samples (S5, S8, S11, and S20) revealed that the standard cyanocobalamin exhibited detectable absorption after 15 minutes, whereas in samples S5, S8, and S11, the absorption was delayed until 1 hour. In the case of sample S20, the burst effect of xanthan gum polymer resulted in drug release being detected after 30 minutes (Figure 31, Figure 32). Therefore, the presence of the polymer in the tablets effectively retards the release of the drug from the tablet matrix.[78] [87]

Throughout the duration of the study (up to 23 hours), the release of standard cyanocobalamin remained higher than the release of all samples (S5, S8, S11, and S20). However, both the samples and the standard exhibited release percentages lower than 82.75% within this timeframe. This may be attributed to the loss of force that was responsible for transferring the drug from the donor to the acceptor compartment. Additionally, during the stability test conducted for standard cyanocobalamin in SSF at 37 °C, which mimicked the conditions of the drug release test, a loss of approximately 5.34% was observed (Table 21).

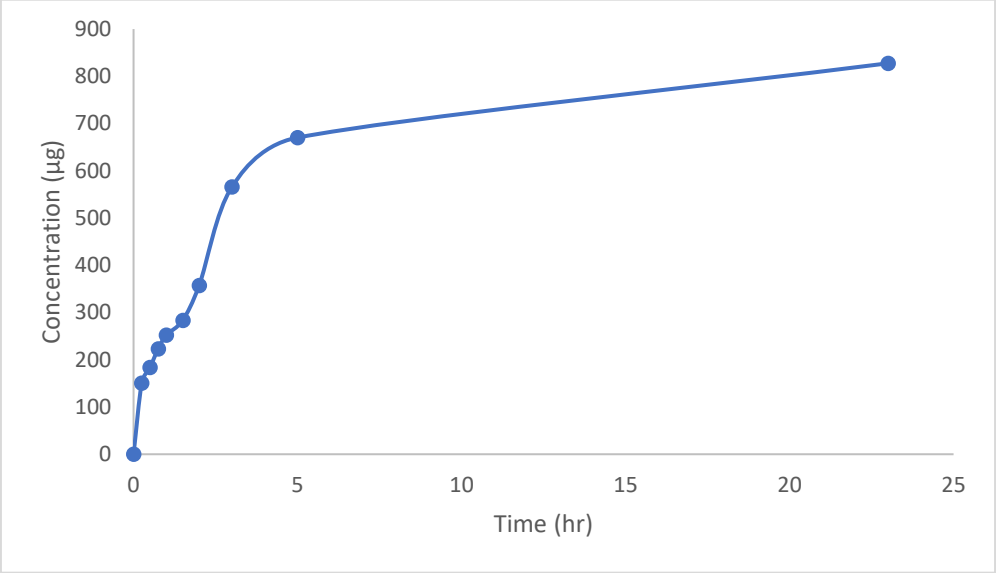


Figure 31 The result of standard cyanocobalamin drug release

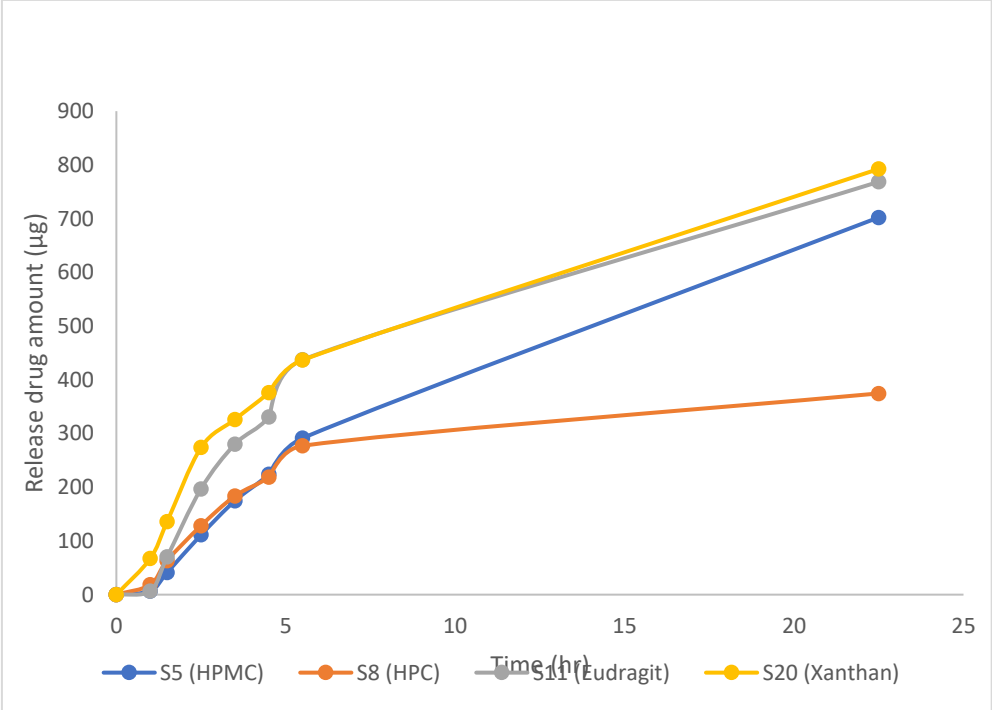


Figure 32 The result of the drug release test for final formulas S5, S8, S11, and S20 (HPMC, HPC, Eudragite L100-55, and xanthan), respectively.

Mechanism of drug release

Drug release from matrix systems is influenced by various processes, including diffusion, swelling, erosion and degradation of the drug within the polymer matrix. Fickian diffusion is the primary mechanism of drug release, which follows Fick's law. This diffusion-based drug release occurs when the time required for solvent diffusion is much smaller than the polymer relaxation time. However, as the relaxation time of the polymer becomes comparable to the diffusion time of the drug, non-Fickian (anomalous) drug release mechanisms may become more dominant. [88]

To determine the specific mechanism of drug release from a matrix system, mathematical models are employed. These models simplify the drug release process and focus on specific formulas that describe the release kinetics. By fitting experimental drug release data to these mathematical models, insights into the underlying mechanisms at play can be obtained. These mathematical models can be applied to assess whether drug release follows Fickian diffusion, non-Fickian diffusion, a combination of both, or other mechanisms.[88]

As shown in Table 20, the Makoid-Banker model and the Peppas-Sahlin model exhibit the best fit to the kinetic release data, with R^2 values exceeding 0.99. Notably, both models demonstrate R^2 values that are very close to each other, indicating their comparable performance in describing the release kinetics.

The Makoid-Banker Model is a kinetic mathematical model used for drug release testing, particularly when multiple release mechanisms are involved.[89] its equation is

$$\frac{Mt}{M\infty} = K_{MB}\, t^{n}\, e^{(-ct)} \qquad \textit{Equation 13}$$

Where Mt/M∞ represents the fraction of drug released at time t. $K_{MB,}$ n and c are empirical parameter used in Makoid-Banakar model. [90 91]

The R^2 value exceeds 0.99, indicating a strong fit of the model and suggesting a complex combination of two release mechanisms: Fickian kinetics and non-Fickian kinetics. In all formulas, the n value is greater than 1, while the K constant value for all formulas is close to zero. These observations suggest a close resemblance of the model to the Korsmeyer-Peppas model and indicate a distinct release mechanism involving Fickian diffusion and super Case II transport, which is associated with polymer swelling, relaxation and erosion. [89 90]

Peppas-Sahlin Model

The Peppas-Sahlin Model is a model that combines the effects of Fickian diffusion and case II transport in drug release. It utilizes the exponent coefficient (n) from the Krosmeyer-Peppas drug release model to calculate the constants (K1, K2). In this model, K1 represents the contribution of Fickian diffusion to drug release, while K2 represents the contribution of non-Fickian release mechanisms such as polymer chain relaxation. The Peppas-Sahlin Model offers a comprehensive approach to understanding the complex drug release behavior by incorporating multiple release mechanisms.[92]

Its equation is

$$\frac{Mt}{M\infty} = K1\, t^{m} + k2\, t^{2m} \qquad Equation\ 14$$

Where Mt/M∞ represents the fraction of drug released at time t. The model includes two constants, K1 and K2. K1 is associated with Fickian diffusion, which involves drug release through diffusion processes. On the other hand, K2 represents case II transport, which is related to mechanisms such as polymer relaxation that influence drug release. [92]

The R^2 value exceeds 0.99, indicating a good fit of the model and suggesting a complex combination of two release mechanisms: Fickian kinetic and non-Fickian kinetic. In the case of the S5 formula, it is observed that the K2 constant has a higher value compared to the K1 constant (which is negative), indicating that the dominance of polymer relaxation over drug diffusion release (non-Fickian) is more pronounced. Conversely, for S8, S11, and S20, the K1 constant has a higher value compared to the negative K2 constant, indicating that diffusion drug release is dominant with a lesser effect of polymer relaxation drug release. [92]

From both the Makoid-Banker Model and the Peppas-Sahlin Model, it is evident that the drug release mechanisms in formulas S5, S8, S11, and S20 involve both Fickian diffusion and polymer relaxation. The presence of mucoadhesive polymers plays a significant role in these release mechanisms.

In the case of S5, which contains HPMC polymers, the drug release is primarily influenced by the swelling effect of the polymers. It be When the HPMC polymer matrix comes into contact with the dissolution medium, the solvent diffuses into the matrix, leading to swelling of the polymer and hydration, resulting in the formation of a viscous gel. [93]

Initially, there is a burst effect observed, which could be attributed to the rapid release of the drug from the surface of the swollen polymer matrix. As the release continues, the drug release is controlled by diffusion, following the erosion process of the HPMC polymer matrix. The presence of solid bridges formed between the polymers and drugs supports sustained drug release over time. [94] [93]

This dissolution process plays a vital role in drug release kinetics and is characterized by both Fickian and non-Fickian release kinetics. Fickian diffusion refers to the release mechanism where the drug diffuses through the swollen polymer matrix following Fick's law. Non-Fickian release kinetics, on the other hand, are associated with more complex release patterns, including relaxation of polymers. The behavior observed in S5 is related to the swelling and relaxation characteristics of the HPMC polymer matrix within the dosage form. [94] [93]

For S8, which contains HPC polymer, the hydrophilic nature of the polymer results in rapid hydration and swelling. The drug release from the polymer depends on pore formation and the erosion rate of the polymer, which is influenced by the concentration of the polymer and the resulting viscosity. The more viscous hydrophilic polymer leads to slower swelling and resistance to erosion processes, thereby retarding drug release. This behaviour is observed in the drug release profile (Figure 32) . [94] [95]

In the case of S11, the formula incorporates eudragite (Polymethacrylates) hydrophobic polymers, which exhibit an initial burst effect, along with the drug release properties of the adhesive polymers based on diffusion. The polymer forms

hydrogels that entrap the drug within them. When the hydrogels swell, the drug is released through diffusion within the polymer chains, following a Fickian mechanism. Subsequently, the entrapped drug is slowly released through polymer erosion and degradation, which follows a non-Fickian drug release pattern. [94 96 97]

S20, which contains xanthan gum, demonstrates Fickian diffusion with non-Fickian Case II transport. This is attributed to the swelling and relaxation of xanthan gum, along with the drug diffusion through the hydrophilic polymeric matrix. As the concentration increases, the viscosity also increases, leading to retarded drug release. This behaviour is supported by Figure 32. [98]

Table 20 The kinetic result of the drug release test for final formulas (S5, S8, S11, and S20)

Model	**Zero order**		**First order**		**Higuchi Model**		**Quadratic Model**			**Makoid-Banakar Model**				**Peppas-Sahlin Model**			
Formula	**K0**	**R2**	**K1**	**R2**	**KH**	**R2**	**K1**	**K2**	**R2**	**KMB**	**n**	**K**	**R2**	**K1**	**K2**	**m**	**R2**
STD	4.5127	-0.4548	0.252	0.8908	21.492	0.3708	-0.007	0.188	0.8989	27.254	0.704	0.048	0.9767	27.980	-2.066	0.707	0.9767
S5	3.3483	0.9345	0.055	0.9868	12.3169	0.8631	-0.010	0.0533	0.988	2.7208	1.681	0.088	0.9984	-69.2624	69.0538	0.157	0.9985
S8	2.0768	0.4148	0.030	0.5956	8.6188	0.8744	-0.018	0.0574	0.9829	4.0039	1.470	0.104	0.9952	4.0627	-0.0721	1.221	0.9935
S11	3.9362	0.7488	0.083	0.9579	15.3694	0.9034	-0.023	0.0984	0.9785	5.0007	1.593	0.099	0.9930	5.1365	-0.0617	1.334	0.9915
S20	4.1526	0.6197	0.100	0.9651	16.7279	0.9602	-0.020	0.0736	0.9917	10.4454	1.022	0.051	0.9929	10.5911	-0.3362	0.951	0.9922

PermeaPad® permeation result

Permeapad® membrane is an artificial biomimetic membrane commonly employed to investigate drug permeation from dosage forms. It is particularly relevant for studying the permeability of drugs through mucosal surfaces such as the buccal and gastrointestinal mucosa. Permeapad® membrane is designed with two supported hydrophilic sheets, within it a phospholipid layer " sandwich structure" which is formed using Soy phosphatidylcholine (PC) S-100.[74] [75]

By incorporating a phospholipid layer, the Permeapad® membrane mimics the lipid composition and structure of biological membranes. This unique characteristic makes it highly suitable for reliably assessing passive drug permeation behavior and evaluating drug delivery systems. [76]

As represented in Figure 33, the permeation of cyanocobalamin from S5 (HPMC) and S8 (HPC) polymers is lower compared to that from S11 (Eudragit L100-55) and S20 (xanthan gum) polymers. This is consistent with the drug release profile shown in Figure 32. The observed difference in permeation can be attributed to the varying amounts of drug available in the donor compartment for permeation through the Permeapad® membrane.

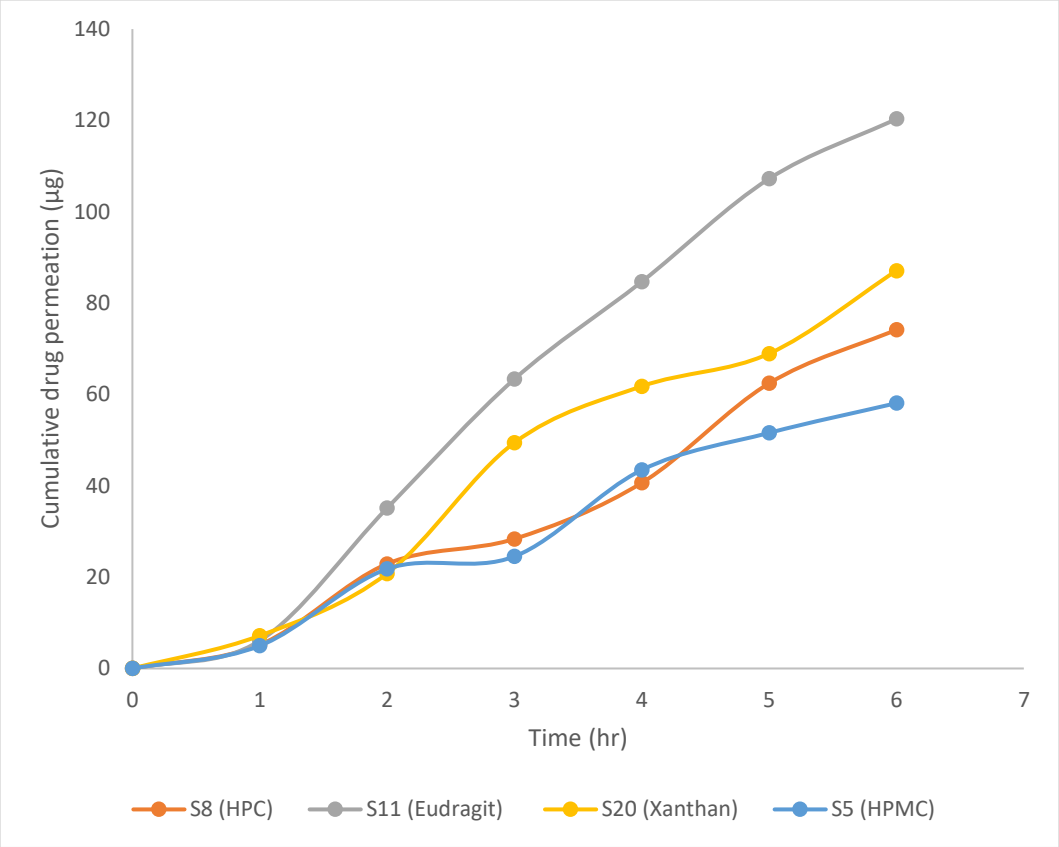


Figure 33 The result of Permeapad® cyanocobalamin permeation test

In addition, Table 21 presents the R^2 values for cyanocobalamin permeability, steady-state flux, and apparent permeability coefficient (P_{app}) through the Permeapad® membrane. Notably, the highest values for all parameters are observed in the case of the S11 formula, which contains Eudragit L100-55. This indicates that S11 exhibits the highest cyanocobalamin permeability among the tested formulations. Specifically, the P_{app} value of cyanocobalamin for S11 is approximately two-fold higher compared to S5, 1.6-fold higher to S8, and 1.38-fold higher for S20.

Table 21The result of the cyanocobalamin permeability test, including R^2 for the Makoid-Banakar model, flux steady state, and apparent permeability coefficients (P_{app})

Formula	R^2	Flux (µg/hr/cm²)	P_{app} (cm/hr) ± SD	P_{app}(cm/sec)
S5	0.9825	3.083542	0.006167 ± 0.0007527	1.71306 *10^{-6}
S8	0.9875	3.93749	0.007875 ± 0.00048066	2.1875*10^{-6}
S11	0.9987	6.387022	0.012774 ± 0.00372004	3.5483*10^{-6}
S20	0.9863	4.6231	0.009246 ± 0.00264401	2.5683*10^{-6}

The apparent permeability coefficient (P_{app}) values for all formulas are higher than 1.5 *10^{-6}, with a standard deviation (SD) of less than 27%. These values indicate good permeability as they exceed the cut-off value for classifying permeability.[99] However, it is important to note that the results of all drug permeation parameters consistently demonstrate that the permeation of S11 (Eudragit L100–55) is higher compared to S20 (Xanthan gum) in the tested formulas. This conclusion is supported by the findings presented in Figure 33 and Table 21, which show higher permeation levels for S11 compared to S20.

By referring to the drug release profile in Figure 32, it can be observed that S20 (Xanthan gum) initially exhibits slightly higher drug release behavior during the first 5 hours, after which the drug release profile becomes similar to that of S11 (Eudragit L100-55). This difference in early drug release behavior could be attributed to a burst effect associated with the higher swelling of Xanthan gum compared to Eudragit L100-55. [98]

While the permeation test shows that S11 (Eudragit L100-55) has higher permeability compared to S20 (xanthan gum), this difference in drug permeability behaviors can be attributed to several factors.

Firstly, the higher viscosity of xanthan gum compared to Eudragit L100-55 may play a role. Xanthan gum is a hydrophilic polysaccharide compound, and its viscosity tends to increase with higher pH values and in the presence of salts such as NaCl or KCl at elevated temperatures. On the other hand, Eudragit L100-55 is an acrylic and methacrylic acid derivative, which is an acidic polymer that contains carboxylic acid groups. It exhibits solubility at pH values higher than 5.5. Changes in pH can affect the solubility and ionization of polymers. Furthermore, Eudragit L100-55 generally has a lower viscosity compared to xanthan gum. [75,87]

Additionally, the higher affinity of Eudragit L100-55 for cyanocobalamin, which is a weak base, could contribute to its higher permeability. Eudragit L100-55 is an amphiphilic methacrylic acid polymer, meaning it contains both hydrophilic and hydrophobic groups. This characteristic promotes the migration of the soluble polymer from the simulated saliva fluid (pH 6.8) through the Permeapad® membrane to the phosphate buffer saline (pH 7.4) along with cyanocobalamin. In contrast, xanthan gum has more hydrophilic groups, and the Permeapad® membrane consists of hydrophilic supported sheets and phospholipid layers. For the drug to cross this membrane, it should possess a balance of lipophilic and hydrophilic properties. Eudragit L100-55 exhibits a better balance of these properties compared to xanthan gum, which may enhance the interaction and increase the permeability of cyanocobalamin from the Eudragit L100-55 matrix. [75,82,87,100–102]

The bioavailability of S11 (Eudragit L100-55), as estimated by the cumulative amount of drug passing through Permeapad® membrane into the donor compartment, is approximately 12.03%. When comparing this bioavailability with that of conventional oral dosage forms (1 mg) by passive diffusion, which typically has a bioavailability of 1.3%, it is evident that the cyanocobalamin mucoadhesive sublingual tablets formula in S11 is a promising approach to significantly increasing the bioavailability of cyanocobalamin and enhancing its therapeutic efficacy. [9,12]

Drug Stability test in simulated saliva fluid

As per the British Pharmacopoeia, the acceptable range for the drug content of cyanocobalamin in tablets is 90-115%.[103] Based on the observations presented in Table 22, the drug amount loss after 24 hours under conditions mimicking the site of administration was found to be a maximum of 672.195 µg (5.34%). This indicates that the drug loss remains within acceptable limits, with 94.66% of the initial drug amount remaining.

Considering these findings, it can be recommended to initially add 110% of the desired cyanocobalamin amount in the tablet formulation. This ensures that even after the expected drug loss, the remaining amount will not fall below 100% (1 mg/tablet), which is the desired target. By accounting for the anticipated drug loss, the formulation can be optimized to maintain the desired drug content throughout the shelf life of the tablet.

Table 22 Result of the drug stability in the simulated salivary fluid solution at 37 C

Time (hr)	Concentration (µg/ml) ± SD	% Recovery ±SD	Drug amount (µg) ± SD	RSD of % recovered
Zero	19.659±0.084393659	100 ± 0	9829.65 ± 42.1968	0
1	19.567±0.084393659	99.537 ± 0.4293439	9783.61± 42.1968	0.431361713
2	19.457±0.287080244	98.957 ± 1.5965141	9820.442 ± 143.540	1.597979036
3	19.37±0.031897805	98.53 ± 0.423159419	9673.112 ± 15.9489	0.430002414
4	19.51±0.084393659	99.24 ± 0.85421803	9755.985 ± 42.1968	0.860646803
5	19.309±0.084393659	99.92 ± 0.835617011	9663.904 ± 42.1968	0.849928413
6	19.198±0	99.35 ± 0.418675269	9599.448 ± 0	0.428710183
24	18.297±0.055248619	94.66 ± 0.619888167	9157.459 ± 27.6243	0.665378354

*Average of triplicates

Chapter IV: Conclusion

6. Chapter six: conclusion

6.1. Conclusion

In conclusion, this study provides valuable insights into preparing and evaluating sublingual cyanocobalamin mucoadhesive tablets using different polymers. By utilizing appropriate techniques and analytical methods, the formulated tablets achieved an appropriate residence time and optimized the drug release mechanism. These results will have important implications for the development of optimized formulations for sublingual drug delivery, which could make cyanocobalamin more bioavailable and help it work better in therapy.

Among the tested formulas, S11 (Eudragit L100-55) has the best mucoadhesive properties in terms of both time and strength. It also has a good drug release profile, and most cyanocobalamin molecules can pass through it. These results show that the choice of polymer greatly affects mucoadhesive properties, drug permeation, and drug release. This suggests that it could be used to make dosage forms that are more bioavailable.

7. Recommendation

To further improve the bioavailability of sublingual cyanocobalamin mucoadhesive tablets, it is recommended to explore the use of different permeation enhancers in the formulation. Future research should focus on incorporating various permeation enhancers into the successful formulas to assess their efficacy in enhancing drug absorption.

Moreover, conducting in vivo studies using animal models such as sheep or rabbits is suggested. These studies can evaluate the absorption of the formulated mucoadhesive tablets by measuring plasma concentrations at various time points. The results can then be compared with those obtained from commercially available conventional sublingual tablets.

By conducting such investigations, valuable insights into the bioavailability of the formulated tablets can be obtained and compared. This will provide a better understanding of the effectiveness and potential advantages of the developed formulation in enhancing cyanocobalamin absorption, thus facilitating its potential therapeutic applications.

These studies will contribute significantly to the scientific understanding of sublingual drug delivery and may serve as a guide for future advancements in this field.

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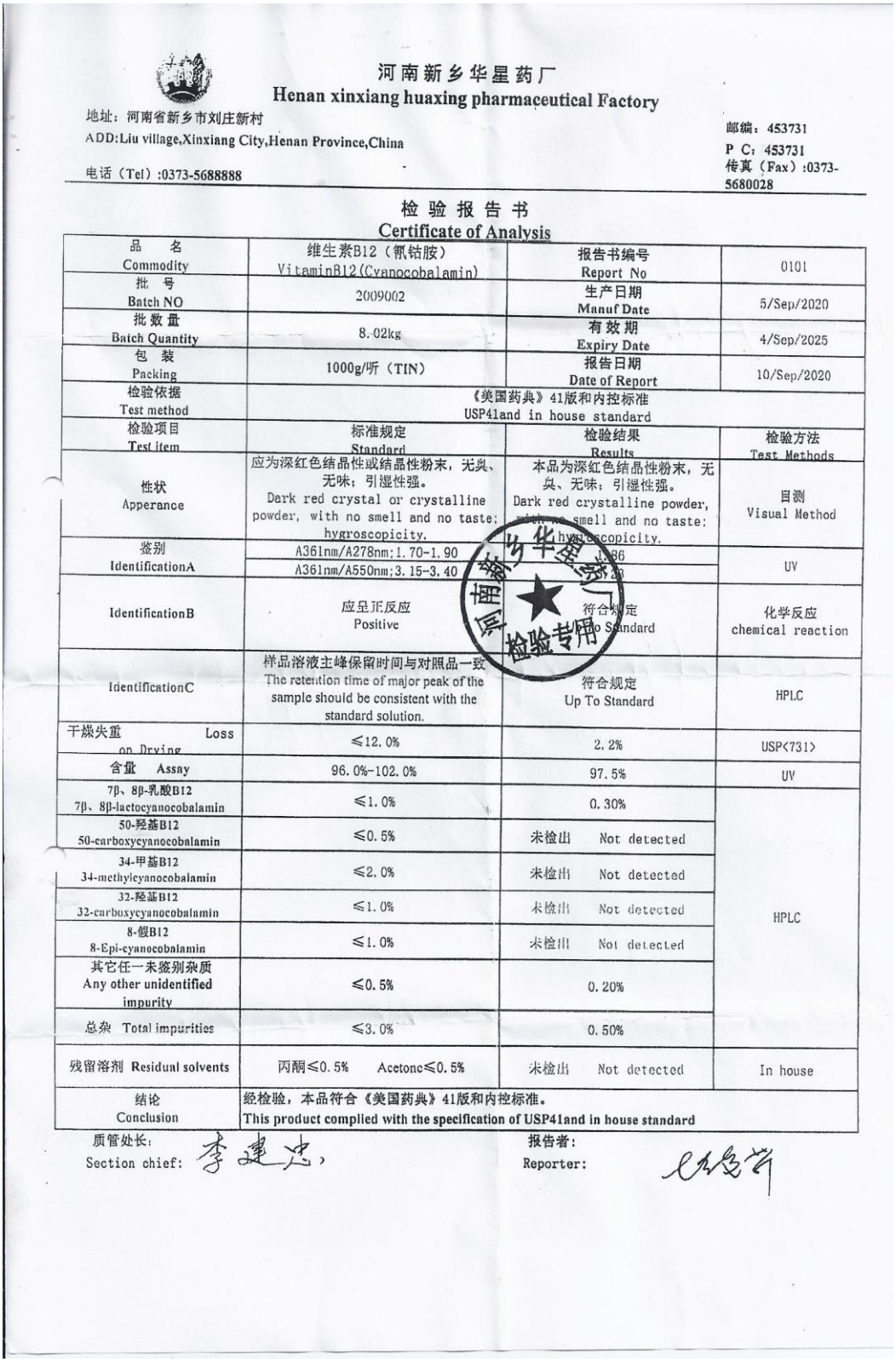
9. Appendix



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检验报告书
Certificate of Analysis

品　名 Commodity	维生素B12（氰钴胺） VitaminB12(Cyanocobalamin)	报告书编号 Report No	0101
批　号 Batch NO	2009002	生产日期 Manuf Date	5/Sep/2020
批数量 Batch Quantity	8.02kg	有效期 Expiry Date	4/Sep/2025
包　装 Packing	1000g/听（TIN）	报告日期 Date of Report	10/Sep/2020
检验依据 Test method	《美国药典》41版和内控标准 USP41and in house standard		
检验项目 Test item	标准规定 Standard	检验结果 Results	检验方法 Test Methods
性状 Apperance	应为深红色结晶性或结晶性粉末，无臭、无味；引湿性强。 Dark red crystal or crystalline powder, with no smell and no taste; hygroscopicity.	本品为深红色结晶性粉末，无臭、无味；引湿性强。 Dark red crystalline powder, with no smell and no taste; hygroscopicity.	目测 Visual Method
鉴别 IdentificationA	A361nm/A278nm:1.70-1.90	1.86	UV
	A361nm/A550nm:3.15-3.40	3.20	
IdentificationB	应呈正反应 Positive	符合规定 Up To Standard	化学反应 chemical reaction
IdentificationC	样品溶液主峰保留时间与对照品一致 The retention time of major peak of the sample should be consistent with the standard solution.	符合规定 Up To Standard	HPLC
干燥失重 Loss on Drying	≤12.0%	2.2%	USP<731>
含量 Assay	96.0%-102.0%	97.5%	UV
7β、8β-乳酸B12 7β、8β-lactocyanocobalamin	≤1.0%	0.30%	HPLC
50-羟基B12 50-carboxycyanocobalamin	≤0.5%	未检出 Not detected	
34-甲基B12 34-methylcyanocobalamin	≤2.0%	未检出 Not detected	
32-羟基B12 32-carboxycyanocobalamin	≤1.0%	未检出 Not detected	
8-假B12 8-Epi-cyanocobalamin	≤1.0%	未检出 Not detected	
其它任一未鉴别杂质 Any other unidentified impurity	≤0.5%	0.20%	
总杂 Total impurities	≤3.0%	0.50%	
残留溶剂 Residual solvents	丙酮≤0.5% Acetone≤0.5%	未检出 Not detected	In house
结论 Conclusion	经检验，本品符合《美国药典》41版和内控标准。 This product complied with the specification of USP41and in house standard		

质管处长：
Section chief: 李建忠

报告者：
Reporter:

Figure 34 Cyanocobalamin Certificate of analysis