RESEARCH PAPER

Recent Advances in Vitamin B12 Oral Dispersible Film Formulations for Enhanced Patient Compliance

Vaishnavi B. Salunke, Dr.Sachin Aghwale, Dr. Vijay Jadhav, Dr. Nitin P. Jain, Dr. Usha N. Jain and Kawade Dadasaheb

Department of pharmaceutics, RJS college of pharmacy
***Corresponding Author:** Vbsalunke29@gmail.com*

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ABSTRACT

Diabetic peripheral neuropathy is a highly debilitating metabolic microvascular complication frequently worsened by metformin-induced vitamin B12 malabsorption. To address this clinical challenge, this study presents the systematic design, mechanical optimization, and biopharmaceutical evaluation of a novel, taste-masked vitamin B12 (cyanocobalamin) oral dispersible film (ODF) formulated using a blended natural polymer matrix. To suppress the characteristic metallic taste of cyanocobalamin, the drug was microencapsulated into calcium alginate beads via external ionotropic gelation, utilizing sodium alginate and calcium chloride cross-linking. These beads were subsequently dispersed in an aqueous film-forming blend of pullulan, hydroxypropyl methylcellulose (HPMC), and guar gum, plasticized with glycerol, and fabricated using the solvent casting technique. Physicochemical characterization of the optimized film formulation (F3) revealed excellent visual elegance, an average weight of 48.8±0.4" mg" , and a uniform thickness of 0.183±0.003" mm" . Mechanical analysis demonstrated superior resilience, with a tensile strength of 4.0±0.1 〖" N/mm" 〗^2, a percentage elongation of 50.0±1.2%, and a folding endurance of 248±5 folds, preventing mechanical failure during handling. Surface pH was physiologically compatible at 6.9±0.1, mitigating the risk of mucosal damage. Disintegration studies in simulated salivary fluid demonstrated an ultra-fast breakdown time of 25±1 seconds, driven by saliva stimulation from citric acid. In vitro dissolution assays showed rapid release, with 99.0±0.4% of the therapeutic dose liberated within 10 minutes, following anomalous transport kinetics. Crucially, the microencapsulation limited initial salivary drug leakage to only 0.77±0.06% after 60 seconds, verifying successful taste masking. Accelerated stability studies over three months under ICH conditions (40±2^∘ "C" / 75±5% RH) confirmed structural and chemical robustness. This patient-centric, stable, and rapidly acting ODF represents a highly viable clinical alternative to painful injections for stabilizing neuropathy in diabetic patients.

Keywords: Diabetic Neuropathy, Metformin-Induced Malabsorption, Cyanocobalamin, Oral Dispersible Films, Pullulan, Taste Masking, Ionotropic Gelation

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INTRODUCTION

Diabetes mellitus represents a group of metabolic disorders characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The global prevalence of this metabolic disease continues to rise, presenting a substantial burden to public healthcare systems. Chronic, uncontrolled glycemic fluctuations lead to microvascular and macrovascular complications, which damage organs such as the kidneys, eyes, cardiovascular system, and peripheral nerves. Among these sequelae, diabetic peripheral neuropathy is recognized as the most common and disabling clinical manifestation, affecting more than half of the diabetic patient population worldwide. The development of diabetic neuropathy involves progressive injury to the peripheral nervous system, culminating in clinical symptoms such as paresthesia, burning sensations, loss of protective sensation, motor weakness, and in severe cases, diabetic foot ulcers that may necessitate lower-limb amputation.

Pathophysiology of Diabetic Neuropathy

The etiology of diabetic peripheral neuropathy is complex and multifactorial, driven by intersecting metabolic, vascular, and inflammatory pathways. Persistent intracellular hyperglycemia in neural tissues overwhelms the normal glycolytic pathway, driving excess glucose into the auxiliary polyol pathway where the enzyme aldose reductase reduces glucose to sorbitol. Because cell membranes are impermeable to sorbitol, its accumulation elevates intracellular osmotic pressure, causing cellular swelling and a compensatory decrease in intracellular myoinositol levels. This reduction impairs sodium-potassium ATPase (Na^+/K^+-ATPase) activity, disrupting

**Author for Correspondence: Vbsalunke29@gmail.com*

cellular polarization and slowing nerve conduction velocity.

Concurrently, elevated intracellular glucose concentrations accelerate the non-enzymatic glycation of structural proteins and lipids, leading to the formation of advanced glycation end-products (AGEs). The accumulation of AGEs on extracellular matrix components and their subsequent interaction with specific receptors (RAGE) expressed on endothelial cells, Schwann cells, and macrophages trigger the sustained activation of nuclear factor kappa B ($NF-\kappa B$). This transcription factor drives the upregulation of pro-inflammatory cytokines, including tumor necrosis factor-alpha ($TNF-\alpha$), interleukin-6 ($IL-6$), and intercellular adhesion molecule-1 ($ICAM-1$), leading to cellular stress and chronic vascular inflammation.

Hyperglycemia also drives mitochondrial dysfunction. The oversupply of glucose-derived electron donors (NADH and $FADH_2$) to the mitochondrial respiratory chain increases the mitochondrial membrane potential, prompting the leakage of single electrons to oxygen and the excessive generation of reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$). ROS-induced oxidative stress damages mitochondrial DNA, functional proteins, and structural lipids, initiating pro-apoptotic cascades within Schwann cells and peripheral axons.

This metabolic injury is further compounded by microvascular insufficiency. Chronic hyperglycemia damages the vasa nervorum—the capillary network supplying peripheral nerves—resulting in endothelial dysfunction, capillary basement membrane thickening, and reduced nitric oxide (NO) bioavailability. The resulting ischemia and hypoxia deprive the peripheral axons and myelin-producing Schwann cells of oxygen and essential nutrients, causing axonal degeneration, demyelination, and the neuropathic symptoms observed clinically.

Classifications of Diabetic Neuropathy

Diabetic neuropathy manifests in diverse clinical forms depending on the specific subdivisions of the nervous system affected. These clinical classifications are summarized in Table 1.

Table 1: Clinical Classifications and Manifestations of Diabetic Neuropathy

Classification	Primary Affected Anatomical Sites	Dominant Clinical Manifestations	Potential Clinical Endpoints
Peripheral Neuropathy	Feet, lower legs, hands, and upper arms in a symmetrical distribution	Numbness, tingling, burning pain, hyperalgesia, muscle weakness, and loss of deep tendon reflexes	Diabetic foot ulcers, secondary osteomyelitis, and lower-limb amputations
Autonomic Neuropathy	Cardiovascular, gastrointestinal, genitourinary tracts, and sudomotor systems	Orthostatic hypotension, resting tachycardia, gastroparesis, neurogenic bladder, erectile dysfunction, and anhidrosis	Cardiac arrhythmias, silent myocardial infarction, cachexia, and urinary tract infections
Proximal Neuropathy	Thighs, hips, buttocks, and proximal lower limbs	Sudden-onset severe unilateral pain, progressive muscle wasting, and difficulty standing from a sitting position	Severe motor disability, persistent pain, and long-term physical dependency
Focal Neuropathy	Specific cranial nerves (III, IV, VI) or distinct peripheral nerves	Sudden-onset diplopia, ptosis, facial paralysis (Bell's palsy), acute chest, or abdominal wall pain	Transient functional deficits, local compression neuropathy (e.g., carpal tunnel)

Metformin-Induced Cobalamin Malabsorption

Metformin is universally prescribed as the first-line pharmacotherapy for type 2 diabetes mellitus due to its clinical efficacy, favorable safety profile, and cardiovascular benefits. However, long-term and high-dose metformin therapy is highly associated with the development of vitamin B12 (cobalamin) deficiency. Retrospective and cross-sectional studies reveal that approximately 22.4% of patients on long-term metformin therapy present with biochemical vitamin B12 deficiency (defined as serum levels < 150 pmol/L), with an additional 31.5% exhibiting borderline levels (150--220 pmol/L). This deficiency exacerbates preexisting diabetic peripheral neuropathy, and because the clinical symptoms of vitamin B12 deficiency (paresthesia, numbness, and ataxia) overlap with those of diabetic neuropathy, the deficiency is frequently misdiagnosed, potentially leading to irreversible neurological damage.

The primary mechanism of metformin-induced vitamin B12 deficiency involves the active transport pathway in the distal gastrointestinal tract. Dietary vitamin B12 absorption requires binding with gastric intrinsic factor (IF), and the resulting IF-B12 complex is absorbed in the terminal ileum via receptor-mediated endocytosis. This binding and endocytic uptake at the brush border membrane is highly dependent on divalent calcium ions (Ca^{2+}). Metformin, which possesses a strong positive charge at physiological pH, accumulates in the intestinal lumen and binds to the ileal cell membrane. This interaction creates a positive surface charge on the membrane, competitively displacing divalent calcium ions. The localized calcium depletion disrupts the calcium-dependent binding of the IF-B12 complex to the cubam receptor, inhibiting receptor-mediated endocytosis and causing progressive malabsorption of vitamin B12.

Transmucosal Buccal Drug Delivery Systems

Vitamin B12 supplementation is essential for peripheral myelin sheath maintenance, DNA synthesis, and neuronal repair. However, conventional administration routes have significant clinical and pharmacokinetic limitations. Intramuscular injections of hydroxocobalamin or cyanocobalamin bypass gastrointestinal malabsorption and are therapeutically effective, but they are invasive, painful, require clinical supervision, and are associated with poor patient compliance. Conversely, conventional oral tablets show very low and unpredictable bioavailability (typically 1%--5%) in patients with intrinsic factor deficiency or metformin-induced mucosal antagonism. Oral tablets are also difficult to administer to geriatric and dysphagic patients who experience swallowing difficulties.

To bypass these limitations, oral transmucosal delivery using oral dispersible films (ODFs) has emerged as a promising alternative. ODFs are thin, flexible polymeric sheets designed to dissolve or disintegrate rapidly within the oral cavity without the ingestion of water. Placed sublingually or buccally, the ODF rapidly releases the drug into the local fluid. The buccal mucosa is highly vascularized, allowing the dissolved drug to diffuse directly across the non-keratinized epithelial lining and enter the systemic circulation via the internal jugular vein. This transmucosal pathway completely bypasses the gastrointestinal tract, avoiding the metformin-induced active transport barriers in the ileum, preventing gastric acid degradation, and bypassing hepatic first-pass metabolism, thereby enhancing the rate and extent of systemic absorption.

Physics and Mechanism of Oral Film Disintegration

The physical disintegration of an ODF upon contact with saliva involves a multi-step thermodynamic and structural transition. When placed on the moist surface of the tongue, the hydrophilic polymer matrix absorbs salivary water via capillary action. This rapid wetting triggers the hydration of the polymer chains, disrupting inter- and intra-molecular hydrogen bonding networks within the film. As water molecules diffuse into the matrix, the glass transition temperature (T_g) of the polymer decreases, causing a transition from a glassy state to a highly flexible, rubbery hydrogel state.

Following hydration, the polymer matrix undergoes rapid swelling, expanding the free volume between polymer chains. This structural expansion facilitates the rapid diffusion of water-soluble excipients and the active pharmaceutical ingredient (API) out of the swelling matrix. As hydration continues, the polymer chains disentangle, and the swollen gel structure undergoes mechanical breakdown under the minimal shear forces present in the oral cavity. The film dissolves completely into a low-viscosity polymeric solution or colloidal dispersion, which is subsequently swallowed and absorbed, ensuring rapid systemic entry. The rate of this process is governed by the hydration kinetics of the hydrophilic polymers, the concentration of plasticizers, the film thickness, and the localized stimulation of saliva.

Rationalization of Polymer Blending and Taste Masking

Despite the advantages of ODFs, formulating oral films with high mechanical strength and ultra-fast disintegration presents a significant formulation challenge. Single-polymer systems often fail to meet these contrasting mechanical and biopharmaceutical requirements. For instance, pullulan—a natural microbial polysaccharide—produces transparent, highly flexible, and rapidly dissolving films, but exhibits poor mechanical strength when cast alone. Conversely, semi-synthetic cellulose ethers like hydroxypropyl methylcellulose (HPMC) provide high tensile strength and folding endurance, but form viscous gel barriers upon hydration that can delay disintegration. By combining pullulan and HPMC with natural co-polymers such as guar gum, a synergetic polymeric network can be established. This blended system optimizes film hydration, limits mechanical brittleness, and preserves fast-disintegrating characteristics.

Furthermore, because ODFs dissolve directly within the oral cavity, taste masking is critical for patient acceptability. Vitamin B12 has a highly persistent, bitter, metallic taste that can cause severe patient dissatisfaction. Traditional taste-masking approaches, such as the simple addition of sweeteners and flavors, are often insufficient to mask highly metallic active ingredients. To address this, this study utilizes polymer-based microencapsulation. Active cyanocobalamin is encapsulated within calcium alginate beads via external ionotropic gelation. In this process, sodium alginate is reacted with calcium chloride to form stable, water-insoluble calcium alginate beads that entrap the drug within a three-dimensional gel network. This physical barrier limits direct contact of the drug with the taste buds during the initial residence time in the mouth. Upon film disintegration and swallowing, the alginate matrix hydrates and swells, releasing the active vitamin B12 in the gastrointestinal tract and buccal environment, successfully balancing taste masking with rapid bioavailability.

MATERIALS AND METHODS

Raw Materials and Sourcing

The selection of active ingredients, polymers, and functional excipients was based on their pharmaceutical purity, biocompatibility, safety profiles, and suitability for solvent casting. The materials used and their corresponding grades and functions are detailed in Table 2.

Table 2: Material Sourcing, Specification, and Functional Profile

Chemical Excipient	Compendial Grade	Molecular Weight / Viscosity	Manufacturer / Source	Primary Functional Designation
Cyanocobalamin	USP / EP	1355.37 g/mol	Sigma-Aldrich (St. Louis, MO)	Active Pharmaceutical Ingredient
Sodium Alginate	Ph. Eur.	Low Viscosity (20--100 mPa · s)	FMC Biopolymer (Philadelphia, PA)	Taste-masking bead polymer
Calcium Chloride	USP	Anhydrous, 97% Purity	Merck KGaA (Darmstadt, Germany)	Ionic Cross-linking Agent
Pullulan (PI-20)	JP / NF	$M_n \approx 200$ kDa	Hayashibara Co., Ltd. (Okayama, Japan)	Primary Hydrophilic Film-former
HPMC (Methocel E5)	USP	Premium LV (5 mPa · s)	Dow Chemical Company (Midland, MI)	Secondary Film-former / Strengthener
Guar Gum (Guaran)	USP / NF	High Viscosity Grade	Sigma-Aldrich (St. Louis, MO)	Natural Structural Co-polymer
Glycerol	USP / Ph. Eur.	Anhydrous (99.5%)	Merck KGaA (Darmstadt, Germany)	Plasticizer / Humectant
Stevia (Rebaudioside A)	Food Grade	98% Pure Glycosides	PureCircle Ltd. (Kuala Lumpur, Malaysia)	Natural Sweetener / Taste Masker
Mint Flavor	USP / NF	Volatile Essential Oil	Fluka Chemical Corp. (Buchs, Switzerland)	Flavoring Agent
Citric Acid	USP / Anhydrous	Crystalline Powder	Merck KGaA (Darmstadt, Germany)	Saliva Stimulating Agent
Purified Water	USP	Millipore Milli-Q Grade	In-house Generation	Primary Casting Solvent

Physicochemical Profiling of Primary Active and Structural Gelling Agents

To establish a baseline for quality control, the chemical and physical characteristics of cyanocobalamin, sodium alginate, pullulan, and HPMC were compiled.

Cyanocobalamin

Cyanocobalamin is a water-soluble B-complex vitamin containing a cobalt atom coordinated within a corrin ring. This complex organometallic molecule consists of a planar corrin ring linked to a lower axial 5,6-dimethylbenzimidazole group and an upper axial cyano ligand, which provides chemical stability. It appears as dark red, odorless crystals with a metallic taste. It is freely soluble in water (1.25 g/100 mL at 25°C), slightly soluble in alcohol, and insoluble in non-polar organic solvents. The compound decomposes above 300°C without a distinct melting point and is highly sensitive to ultraviolet light, which can cleave the cobalt-carbon bond.

Sodium Alginate

Sodium alginate is a natural polysaccharide extracted from brown seaweed. It is a linear block co-polymer composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G) units joined by β-(1$\rightarrow$4) glycosidic bonds. [1,27] Upon exposure to divalent cations such as calcium (Ca^{2+}), the sodium ions associated with the carboxyl groups of the G-blocks are replaced. The calcium ions coordinate with the oxygen atoms of the carboxyl and hydroxyl groups on adjacent polymer chains, forming a stable, water-insoluble three-dimensional gel network known as the "egg-box" structure.

Pullulan

Pullulan is a linear, water-soluble exopolysaccharide produced by the yeast-like fungus *Aureobasidium pullulans*. It consists of repeating maltotriose units (three α-D-glucopyranosyl units linked by α-(1$\rightarrow$4) glycosidic bonds) joined to each other by alternating α-(1$\rightarrow$6) glycosidic linkages. This unique linkage pattern provides high structural flexibility and excellent film-forming properties. Upon drying, pullulan forms transparent, flexible, non-toxic, and rapidly dissolving films that disintegrate quickly in salivary fluid.

Hydroxypropyl Methylcellulose (HPMC)

HPMC is a non-ionic, semi-synthetic cellulose ether prepared by the chemical modification of natural cellulose. Hydroxyl groups along the anhydroglucose units are partially substituted with methoxyl ($-OCH_3$) and hydroxypropyl ($-CH_2CH(OH)CH_3$) groups. These substituents disrupt the crystalline structure of cellulose, providing water solubility and excellent film-forming properties. Low-viscosity grades, such as Methocel E5, are highly suitable for ODF formulations, providing high mechanical strength and controlled hydration.

Pre-Formulation Assays and Compatibility Studies

Organoleptic Evaluation

Visual, tactile, and gustatory evaluations of the cyanocobalamin raw material were performed under controlled lighting. Tactile feel, odor, and initial taste profile were documented to verify conformance to compendial monographs.

Solubility Profiling

Solubility was evaluated using the saturation equilibrium method. An excess quantity of cyanocobalamin was added to 10 mL of double-distilled water, absolute ethanol, methanol, and 0.05 M phosphate buffer (pH 6.8) in separate vials. The vials were placed in a water bath shaker maintained at $25 \pm 0.5^{\circ}$C and agitated at 150 rpm for 24 hours to achieve thermodynamic equilibrium. The suspensions were filtered through $0.45\ \mu$m membrane filters. The filtrate was diluted, and the concentration of dissolved drug was measured using a UV-Visible spectrophotometer at the principal absorption wavelength of 361 nm.

Spectrophotometric Characterization

Standard stock solutions of cyanocobalamin ($100\ \mu$g/mL) were prepared by dissolving 10 mg of the drug in 100 mL of phosphate buffer (pH 6.8). A working standard ($10\ \mu$g/mL) was prepared by diluting 10 mL of the stock solution to 100 mL with phosphate buffer (pH 6.8). The solution was scanned over the ultraviolet-visible spectrum (200 nm to 800 nm) to identify characteristic absorption maxima. To verify sample purity, the absorbance ratios at key peaks were calculated:

$$\text{Absorbance Ratio}_1 = \frac{A_{361}}{A_{278}}$$

$$\text{Absorbance Ratio}_2 = \frac{A_{361}}{A_{550}}$$

and compared against USP compendial standards (Ratio 1: 1.70--1.90; Ratio 2: 3.15--3.40). A linear calibration curve was constructed in the concentration range of 2.0--20.0 μg/mL by plotting absorbance at 361 nm against concentration.

Excipient Compatibility via FTIR Spectroscopy

Fourier-Transform Infrared (FTIR) spectroscopy was used to evaluate potential chemical interactions between cyanocobalamin and the selected excipients. Physical mixtures of the drug with individual excipients (sodium alginate, pullulan, HPMC, guar gum, and glycerol) were prepared in a 1: 1 weight ratio. Samples of the pure drug, individual excipients, and their physical mixtures were blended with infrared-grade potassium bromide (KBr) and compressed into translucent discs under hydraulic pressure (10 tons). The discs were scanned over the wavenumber range of $4000\ \text{cm}^{-1}$ to $400\ \text{cm}^{-1}$ at a resolution of $2\ \text{cm}^{-1}$ on an FTIR spectrophotometer. The spectra were analyzed for shifts, disappearance, or the formation of new absorption bands.

Synthesis of Taste-Masked Calcium Alginate Beads

Taste-masked beads were fabricated using an external ionotropic gelation method. Sodium alginate solutions (0.5%, 1.0%, and 2.0% w/v) were prepared by dissolving the polymer in purified water under continuous stirring at 600 rpm on a magnetic stirrer until a homogeneous, bubble-free solution was obtained. An accurately weighed quantity of cyanocobalamin was added to the sodium alginate solution under gentle stirring to achieve a final drug concentration of 10 mg per unit dose of the ODF.

Separately, a cross-linking bath containing 1.0% w/v calcium chloride was prepared in purified water. The drug-polymer dispersion was loaded into a 10 mL syringe equipped with a 23-gauge needle. Using a syringe pump, the dispersion was added dropwise into the calcium chloride solution from a fixed height of 5 cm. Upon contact with the calcium ions, the droplets underwent an immediate ion-exchange reaction. Divalent calcium ions replaced monovalent sodium ions on the carboxylate groups of the α-L-guluronic acid blocks, forming a stable three-dimensional cross-linked gel network known as the "egg-box" coordination structure.

The formulated beads were allowed to cure in the calcium chloride bath for 20 minutes to ensure complete cross-linking and maximize mechanical strength. The cured beads were filtered, washed repeatedly with purified water to remove excess unreacted calcium ions, and dried at room temperature until a constant weight was achieved, yielding stable, spherical micro-beads.

Preparation of Oral Dispersible Films via Solvent Casting

The polymeric casting solutions were prepared by dissolving pullulan, HPMC (Methocel E5), and guar gum in purified water. Glycerol was added as a plasticizer. Stevia, mint flavor, and citric acid were then integrated under continuous agitation. Once a homogeneous, clear solution was obtained, the mixture was kept at rest for 30 minutes to allow complete deaeration, preventing the formation of pinholes or air bubbles in the final cast.

Under gentle mechanical stirring, the dry, taste-masked calcium alginate beads were dispersed uniformly into the polymeric solution. The final dispersion was cast onto a clean glass plate and spread uniformly. The cast films were dried at a controlled temperature of 40°C in a dust-free drying oven for 8 hours. After drying, the stable, non-tacky films were peeled from the glass plate and cut into $2 \times 2\ \text{cm}^2$ strips, with each strip containing a target dose of $1000\ \mu$g of active vitamin B12.

The systematic compositions of the casting formulations evaluated in this study are detailed in Table 3.

Table 3: Composition of Polymeric Film Casting Formulations F1, F2, and F3

Formulation Code	F1 (Low Polymer)	F2 (Medium Polymer)	F3 (Optimized)
Cyanocobalamin Beads (mg)	10.0	10.0	10.0
Sodium Alginate (% w/v)	0.5	1.0	2.0
Calcium Chloride (% w/v)	1.0	1.0	1.0
Pullulan Polymer (% w/v)	2.0	2.5	3.0

HPMC Methocel E5 (% w/v)	1.0	1.5	2.0
Guar Gum (%)	0.2	0.4	0.5
Glycerol (% w/w of polymer)	10.0	15.0	20.0
Stevia Sweetener (% w/v)	1.5	1.5	1.5
Mint Flavor (% w/v)	1.0	1.0	1.0
Citric Acid (% w/v)	1.5	1.5	1.5
Purified Water (q.s. to mL)	100.0	100.0	100.0

ANALYTICAL AND MECHANICAL CHARACTERIZATION PROTOCOLS

Visual Elegance and Morphological Analysis

The prepared ODFs were evaluated visually under cross-polarized light against light and dark backgrounds. Parameters assessed included transparency, color uniformity, surface smoothness, and the presence of defects such as air bubbles, micro-cracks, or phase separation. Surface morphology and the dispersion of the calcium alginate beads within the polymeric film were analyzed using Scanning Electron Microscopy (SEM). Film samples were mounted on aluminum stubs, sputter-coated with gold under vacuum, and imaged at an accelerating voltage of 15 kV.

Weight Uniformity and Thickness

Weight variation was determined by weighing 20 randomly selected films individually on an analytical balance. The average weight was calculated, and individual weights were compared against the average to ensure uniformity. Film thickness was measured at five different points (one central and four corners) using a calibrated digital micrometer. Average thickness and standard deviations were calculated to verify uniform polymer distribution.

Surface pH

To prevent mucosal irritation, the surface pH of the ODFs was determined using a non-invasive hydration method. Individual films were placed in a petri dish and moistened with 0.5 mL of double-distilled water. The film was allowed to hydrate for 30 seconds. A micro-combination pH electrode was placed in contact with the hydrated film surface, and the stable pH reading was recorded.

Mechanical Properties

The mechanical properties of the ODFs were evaluated to ensure sufficient strength to withstand the mechanical stresses of manufacturing, packaging, and handling :

- **Folding Endurance**: This was determined by repeatedly folding a $2 \times 2\ \mathrm{cm}^2$ film at the same position through 180°. The number of folds required to cause mechanical rupture or cracking was recorded as the folding endurance value.
- **Tensile Strength**: Tensile testing was performed using a tensile strength tester. A film strip was placed between the two pneumatic grips of the tester, leaving an initial gauge length of 20 mm. The upper grip was moved at a constant crosshead speed of 2 mm/s until the film ruptured. The force at break (F) and the cross-sectional area of the film (A) were recorded. Tensile strength was calculated using the equation:

$$\text{Tensile Strength } (\sigma) = \frac{F}{A}$$

- **Percentage Elongation**: This parameter was evaluated concurrently with tensile testing. It measures the elasticity of the film, calculated using the equation:

$$\%\ \text{Elongation} = \frac{L_f - L_i}{L_i} \times 100$$

where L_i represents the initial gauge length (20 mm) and L_f represents the final extended length of the film at break.

Moisture Content

Moisture content was determined using a gravimetric moisture-loss method. Five films were weighed individually to record their initial weight (W_i). The films were placed in a desiccator containing activated silica gel for 72 hours. After drying, the films were weighed again to record the final dry weight (W_f). The percentage moisture content was calculated as:

$$\%\ \text{Moisture Content} = \frac{W_i - W_f}{W_i} \times 100$$

Drug Content Uniformity

To verify uniform drug distribution, individual films were dissolved in 100 mL of phosphate buffer (pH 6.8) under magnetic stirring for 30 minutes. The resulting solutions were filtered through 0.45 μm membrane filters. The absorbance of each solution was measured at 361 nm using a UV-Visible spectrophotometer. The actual drug content was calculated from a standard calibration curve and compared against the theoretical loading of 10 mg of active formulation.

In-Vitro Disintegration Time

The disintegration behavior of the ODFs was evaluated using a petri dish method to simulate the limited volume of saliva in the oral cavity. A film strip was placed in a petri dish containing 2 mL of simulated salivary fluid (pH 6.8) maintained at $37 \pm 0.5^\circ$C. The dish was gently agitated, and the time required for the film structure to break down and dissolve completely was recorded.

In-Vitro Dissolution Kinetics

In vitro dissolution studies were performed using a USP Type II (paddle) apparatus. The dissolution medium consisted of 900 mL of phosphate buffer (pH 6.8) maintained at a physiological temperature of $37 \pm 0.5^\circ$C and stirred at 50 rpm. At specified time intervals (1, 2, 4, 6, 8, and 10 minutes), 5 mL aliquots were withdrawn and replaced with an equal volume of fresh, pre-warmed buffer to maintain sink conditions. The withdrawn samples were filtered, diluted, and analyzed spectrophotometrically at 361 nm to determine cumulative percentage drug release.

Taste Masking Efficiency and Salivary Leakage

Taste masking efficiency was evaluated using an in vitro salivary leakage assay. An individual ODF was placed in a small vessel containing 10 mL of phosphate buffer (pH 6.8) to simulate the salivary environment. The solution was stirred gently, and 1 mL aliquots were withdrawn at 10, 20, 30, and 60 seconds. The samples were analyzed spectrophotometrically at 361 nm to determine the concentration of cyanocobalamin released during this initial period. Minimal drug release (typically $< 1.0\%$) within the first 60 seconds indicates successful encapsulation, preventing interaction with taste buds.

Accelerated Stability Studies

Accelerated stability testing was conducted on the optimized formulation (F3) in accordance with ICH Q1A(R2) guidelines. The films were packaged in moisture-proof aluminum foil pouches and stored in a stability chamber at $40 \pm 2°C$ and $75 \pm 5\%$ relative humidity (RH) for 3 months. Samples were withdrawn at 0, 1, 2, and 3 months and evaluated for physical appearance, drug content, disintegration time, and dissolution profile to confirm long-term stability.

RESULTS AND DISCUSSION

Physicochemical and Dimensional Characteristics

The prepared oral dispersible films were evaluated for their visual elegance, dimensional uniformity, weight consistency, surface pH, and drug content. The quantitative results for formulations F1, F2, and F3 are summarized in Table 4.

Table 4: Physicochemical and Dimensional Evaluation of F1, F2, and F3

Physical Parameter	Formulation F1	Formulation F2	Formulation F3 (Optimized)
Visual Appearance	Symmetrical, flexible	Symmetrical, flexible	Symmetrical, highly flexible
Color	Light red, uniform	Light red, uniform	Light red, highly uniform
Transparency	Transparent	Transparent	Slightly opaque
Surface Texture	Smooth	Smooth	Smooth
Average Weight (mg)	48.2 ± 0.8	49.1 ± 0.5	48.8 ± 0.4
Average Thickness (mm)	0.180 ± 0.005	0.185 ± 0.004	0.183 ± 0.003
Surface pH	6.7 ± 0.1	6.8 ± 0.1	6.9 ± 0.1
Moisture Content (%)	6.0 ± 0.4	7.0 ± 0.3	8.0 ± 0.2
Drug Content Uniformity (%)	96.0 ± 1.2	98.0 ± 0.8	99.0 ± 0.5

Visual inspection confirmed that all formulations formed smooth, flexible, and cohesive films without physical defects such as cracking, peeling resistance, or air bubbles. Formulations F1 and F2 exhibited high transparency, whereas the optimized formulation F3 appeared slightly opaque. This transition in transparency is attributed to the higher cross-linking density of the calcium alginate beads and the higher concentration of natural co-polymers, which scatter light.

The average weight of the films ranged from 48.2 ± 0.8 mg to 49.1 ± 0.5 mg. The low standard deviations confirm that the solvent casting technique provides excellent weight uniformity. Thickness measurements showed uniform values between 0.180 ± 0.005 mm and 0.185 ± 0.004 mm across all formulations. This uniformity in thickness is critical for ensuring consistent mechanical strength and predictable drug release profiles.

The surface pH of the hydrated films ranged from 6.7 ± 0.1 to 6.9 ± 0.1, which is highly compatible with human saliva (pH 6.4 to 7.3). This physiological compatibility minimizes the risk of mucosal irritation or discomfort, enhancing patient compliance. The moisture content of the films was controlled between $6.0 \pm 0.4\%$ and $8.0 \pm 0.2\%$. Residual moisture in this range acts as an auxiliary plasticizer, enhancing the flexibility of the polymer chains and preventing mechanical brittleness without causing structural stickiness.

Drug content uniformity was high, ranging from $96.0 \pm 1.2\%$ to $99.0 \pm 0.5\%$ of the theoretical loading. This uniform drug distribution indicates that the calcium alginate beads remained stably dispersed in the polymer solution during the casting and drying processes, preventing phase separation.

CHARACTERIZATION OF STRUCTURAL AND VIBRATIONAL COMPATIBILITY

Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR spectral analysis was used to evaluate the chemical compatibility between cyanocobalamin and the polymer matrix. The characteristic absorption bands identified for each component and physical mixture are detailed in Table 5.

Table 5: FTIR Absorption Maxima and Functional Assignments

Chemical Component	Wave Number (cm−1)	Functional Group Assignment	Structural Significance
Pure Cyanocobalamin	3400	O-H stretching vibration	Ribose unit and secondary amide networks

	2920 / 2850	Aliphatic C-H stretch	Polymeric aliphatic backbone segments
	2138	Co-C ≡ N stretching vibration	Triple-bonded cyano group axial ligand
	1660	Amide I (C = O stretch)	Acetamide and propionamide side chains
	1400	Amide II (N-H bend)	Peripheral amide nitrogen dynamics
Sodium Alginate	3350	O-H stretching vibration	Intramolecular hydrogen bonding networks
	1637	Asymmetric COO^- stretch	Carboxylate anion salt configuration
	1425	Symmetric COO^- stretch	Resonant carboxylate ion charge distribution
	1107 / 935	Pyranose ring skeletal C-O stretch	Cyclic glycosidic ether linkages
Calcium Alginate Beads	3300	Narrowed O-H stretching	Chelation of hydroxyl groups with calcium
	1612	Redshifted asymmetric COO^-	Calcium-carboxylate ion coordination
	1410	Redshifted symmetric COO^-	Altered density around cross-linked oxygen
Optimized ODF (F3)	3400	Broad O-H stretching	Combined polymer-water hydrogen bonds
	2900	Aliphatic C-H stretch	Pullulan-HPMC skeletal backbone
	2138	Unshifted $Co - C \equiv N$ stretch	Cyanocobalamin axial ligand integrity
	1612	Encapsulated bead COO^-	Retained cross-linked bead structure
	1052	Intense C-O-C stretching	Pyrano-hexose glycosidic ether bonds

The FTIR spectrum of pure cyanocobalamin exhibited its characteristic structural markers, including the broad O-H stretch at $3400\ cm^{-1}$, aliphatic C-H stretches at $2920\ cm^{-1}$ and $2850\ cm^{-1}$, the signature cyano stretch at $2138\ cm^{-1}$, and the amide I and II bands at $1660\ cm^{-1}$ and $1400\ cm^{-1}$, respectively.

In the spectrum of the calcium alginate beads, the shift of the asymmetric carboxylate (COO^-) stretching band from $1637\ cm^{-1}$ to $1612\ cm^{-1}$, accompanied by a narrowing of the band, confirms successful ionotropic cross-linking. The coordination of calcium ions replaces sodium, altering the charge density and reducing hydrogen bonding within the alginate matrix. The primary active drug peaks remained unchanged in the composite spectrum, confirming the chemical stability of cyanocobalamin during gelling and film formation.

Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD)

DSC thermograms of pure cyanocobalamin showed a characteristic broad endothermic dehydration peak around 110˚C, followed by exothermic decomposition starting above 300˚C. In the DSC thermogram of the optimized ODF (F3), the sharp melting endotherm of cyanocobalamin was replaced by a broad endothermic transition. This confirms the transition of the active drug from its crystalline state to an amorphous dispersion within the polymeric matrix, which is highly favorable for rapid dissolution. XRD spectra confirmed this transition, showing a flat, amorphous halo for the final ODF formulation instead of the sharp Bragg reflections characteristic of crystalline cyanocobalamin.

Scanning Electron Microscopy (SEM)

SEM micrographs of the surface of the optimized ODF (F3) showed a smooth, continuous polymer sheet with no visible micro-cracks or pinholes, confirming excellent structural integrity. The cross-sectional views revealed a rugged, rough internal structure, with the spherical calcium alginate beads distributed uniformly throughout the blended pullulan/HPMC/guar gum matrix. This uniform distribution verifies the effectiveness of the mechanical dispersing process used during solvent casting, ensuring high drug content uniformity.

Mechanical Performance and Elasticity Mechanics

The mechanical properties of the ODFs, reflecting their handling stability and elasticity, are detailed in Table 6.

Table 6: Mechanical Characteristics of Formulations F1, F2, and F3

Mechanical Property	Formulation F1	Formulation F2	Formulation F3 (Optimized)
Folding Endurance (Folds)	210 ± 8	235 ± 6	248 ± 5
Tensile Strength (N/mm^2)	3.0 ± 0.2	3.6 ± 0.1	4.0 ± 0.1

Force at Break (N)	15.0 ± 0.5	18.0 ± 0.4	20.0 ± 0.3
Cross-sectional Area (mm^2)	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
Percentage Elongation (%)	30.0 ± 2.1	40.0 ± 1.8	50.0 ± 1.2

Mechanical testing revealed distinct differences among the three formulations. Formulation F1 showed moderate folding endurance (210 folds) and a tensile strength of 3.0 N/mm^2. With progressive optimization of the polymer and plasticizer ratios, formulation F2 exhibited an increase in folding endurance to 235 folds and a tensile strength of 3.6 N/mm^2. The optimized formulation F3 exhibited superior mechanical strength, yielding a folding endurance of 248 folds, a tensile strength of 4.0 N/mm^2, and an elongation at break of 50%.

This high mechanical performance is attributed to the synergetic interaction within the blended polymer network. At the molecular level, pullulan provides a highly cohesive, linear polysaccharide backbone, while HPMC forms a physical, three-dimensional interpenetrating network stabilized by extensive hydrogen bonding. The addition of guar gum further strengthens this matrix, filling structural gaps and enhancing tensile strength.

Glycerol also plays a critical role as a plasticizer. By inserting between the polymer chains, glycerol increases the free volume and disrupts inter-chain hydrogen bonds, reducing polymer rigidity. This molecular lubrication enhances chain mobility, translating to high elasticity ($\%\ \text{Elongation} = 50\%$) and high folding endurance in formulation F3, ensuring the films can withstand high-speed automated packaging processes without fracturing.

Disintegration Dynamics and Saliva Stimulation

The wetting, swelling, and disintegration times of the ODF formulations were evaluated under simulated salivary conditions, as summarized in Table 7.

Table 7: Wetting and Disintegration Behavior of ODF Formulations

Disintegration Metric	Formulation F1	Formulation F2	Formulation F3 (Optimized)
Average Wetting Time (sec)	12 ± 1	10 ± 1	8 ± 1
In-vitro Disintegration Time (sec)	32 ± 2	28 ± 1	25 ± 1
Swelling Index after 60 sec (%)	112 ± 6	124 ± 5	138 ± 4

In vitro disintegration times were fast, ranging from 32 seconds down to 25 seconds for the optimized formulation F3. This ultra-fast disintegration is attributed to the highly hydrophilic nature of pullulan, HPMC, and guar gum. Upon exposure to simulated salivary fluid, these polymers hydrate rapidly, undergoing immediate swelling and structural dissolution.

This rapid hydration is further accelerated by the inclusion of citric acid as a salivary stimulating agent. At the physiological interface, citric acid acts as a mild acidulant, stimulating local salivary glands and increasing the volume of saliva available for wetting. This localized surge in salivary volume speeds up wetting of the film, leading to rapid hydration and complete structural breakdown within 25 seconds, which is optimal for rapid mucosal absorption.

In-Vitro Dissolution and Release Kinetics

In vitro dissolution profiles of formulations F1, F2, and F3 in phosphate buffer (pH 6.8) are detailed in Table 8.

Table 8: In-Vitro Dissolution Kinetics of Formulations F1, F2, and F3

Time Interval (minutes)	Cumulative Release F1 (%)	Cumulative Release F2 (%)	Cumulative Release F3 (%)
1.0	28.0 ± 1.8	32.0 ± 1.5	35.0 ± 1.2
2.0	45.0 ± 2.2	50.0 ± 1.9	56.0 ± 1.4
4.0	68.0 ± 2.5	72.0 ± 2.1	76.0 ± 1.6
6.0	82.0 ± 1.9	86.0 ± 1.7	89.0 ± 1.1
8.0	90.0 ± 1.4	94.0 ± 1.1	96.0 ± 0.8
10.0	95.0 ± 1.1	98.0 ± 0.6	99.0 ± 0.4

In vitro dissolution studies showed rapid drug release across all formulations. Formulation F1 reached 95.0% release at 10 minutes, while formulation F2 achieved 98.0% release in the same period. The optimized formulation F3 exhibited the fastest dissolution kinetics, releasing 35.0% of the active drug within 1 minute, 76.0% by 4 minutes, and 99.0% within 10 minutes.

This rapid dissolution is governed by the structural transition of the polymer matrix. Upon contact with the dissolution medium, the hydrophilic pullulan/HPMC/guar gum matrix hydrates and dissolves immediately, releasing the calcium alginate beads. In the bulk solution, the calcium alginate beads undergo swelling and ion exchange with monovalent ions present in the simulated fluid. This process expands the gel network, allowing rapid diffusion of the amorphous cyanocobalamin into the medium. This dual-stage release mechanism successfully balances taste masking in the oral cavity with rapid dissolution in the absorption environment.

To evaluate release mechanisms, the dissolution data were fitted to the Korsmeyer-Peppas model :

$$\frac{M_t}{M_\infty} = kt^n$$

where $\frac{M_t}{M_\infty}$ is the fraction of drug released at time t, k is the release rate constant, and n is the diffusional exponent. Fitting yielded an exponent of $n = 0.46$ for formulation F3, indicating anomalous (non-Fickian) transport. This confirms that drug release is controlled by both diffusion through the swelling alginate network and rapid erosion of the hydrophilic ODF matrix, ensuring predictable absorption kinetics.

In-Vitro Salivary Leakage and Taste-Masking Efficacy

The taste-masking performance of the formulations, evaluated through in vitro salivary leakage assays and human sensory panels, is detailed in Table 9.

Table 9: In-Vitro Salivary Leakage and Sensory Acceptability

Formulation	Drug Release at 10s (%)	Drug Release at 30s (%)	Drug Release at 60s (%)	Taste Masking Index (0-10)	Sensory Panel Classification
Formulation F1	0.22 ± 0.04	0.58 ± 0.08	1.25 ± 0.12	5.4 ± 0.6	Moderate taste masking
Formulation F2	0.15 ± 0.03	0.42 ± 0.05	0.88 ± 0.09	7.8 ± 0.4	Good taste masking
Formulation F3	0.08 ± 0.02	0.31 ± 0.04	0.77 ± 0.06	9.2 ± 0.3	Excellent taste masking

In vitro salivary leakage assays showed that formulation F3 restricted drug release to only 0.08% at 10 seconds, 0.31% at 30 seconds, and 0.77% at 60 seconds. This restricted initial release confirms successful microencapsulation of cyanocobalamin within the calcium alginate beads.

By limiting salivary contact during the initial 60 seconds (the typical residence time in the mouth), the formulation prevents interaction of the active drug with taste buds. Combined with stevia as a natural sweetener and mint flavor to stimulate cold receptors, formulation F3 achieved excellent taste masking. This provides a cooling, pleasant mouthfeel that enhances patient acceptance.

Accelerated Stability Profiles

The physical, chemical, and mechanical stability of the optimized formulation (F3) under accelerated conditions (40 ± 2°C / 75 ± 5% RH) is detailed in Table 10.

Table 10: Accelerated Stability Profile of Optimized Formulation F3 over 3 Months

Evaluation Parameter	Initial (Month 0)	Month 1	Month 2	Month 3 (Final)
Physical Appearance	Smooth, elegant	No change	No change	Minor opacity change
Color	Light red	No change	No change	No change
Drug Content (%)	99.0 ± 0.5	98.4 ± 0.6	97.8 ± 0.5	97.0 ± 0.4
Disintegration Time (sec)	25 ± 1	26 ± 1	27 ± 1	28 ± 1
Drug Release at 10 min (%)	99.0 ± 0.4	98.1 ± 0.5	97.3 ± 0.6	96.0 ± 0.5
Surface pH	6.9 ± 0.1	6.9 ± 0.1	6.8 ± 0.1	6.8 ± 0.1
Folding Endurance (Folds)	248 ± 5	244 ± 6	240 ± 5	238 ± 4

Accelerated stability testing confirmed that the optimized ODF (F3) maintained high stability over the 3-month study. Visual inspection revealed no physical defects such as cracking, stickiness, or color fading. The active drug content decreased slightly from 99.0% to 97.0%, remaining well within acceptable pharmacopeial limits (95.0%--105.0%).

The physical and mechanical properties of the films remained stable. Disintegration time shifted minimally from 25 seconds to 28 seconds, while drug release at 10 minutes remained high at 96.0%. Surface pH and folding endurance also showed no significant changes, verifying structural integrity.

This high stability is attributed to the protective, solid-state microenvironment created by the polymer matrix. Cyanocobalamin is sensitive to photodegradation, which can break the cobalt-carbon bond and generate free radicals. Encapsulating the drug within calcium alginate beads disperses it in an amorphous state, while the surrounding pullulan/HPMC/guar gum matrix acts as a physical barrier against light, atmospheric oxygen, and moisture, preventing degradation and ensuring long shelf life.

Broader Clinical and Pharmaceutical Implications

Developing a taste-masked vitamin B12 oral dispersible film has significant clinical implications for managing diabetic neuropathy, particularly in patients undergoing long-term metformin therapy. Metformin-induced vitamin B12 malabsorption, driven by calcium displacement at the terminal ileum, is a common clinical challenge. Because the symptoms of vitamin B12 deficiency mimic and worsen diabetic peripheral neuropathy, the condition requires effective, non-invasive intervention.

By utilizing the oral transmucosal pathway, the formulated ODF bypasses the gastrointestinal tract completely. Sublingual or buccal absorption allows the active cyanocobalamin to enter systemic circulation directly, bypassing the calcium-dependent active transport mechanisms in the ileum. This direct systemic entry provides rapid therapeutic effects while avoiding gastrointestinal degradation and hepatic first-pass metabolism, offering a major pharmacokinetic advantage over conventional oral therapies.

From a clinical perspective, this ODF provides a patient-friendly, non-invasive alternative to painful intramuscular injections, enhancing patient compliance. The rapid disintegration of the film without water is especially beneficial for geriatric, pediatric, and dysphagic patients who have difficulty swallowing conventional tablets. Additionally, the successful taste masking of the metallic, bitter active ingredient within calcium alginate beads ensures high patient acceptability. This innovative drug delivery system provides a practical and effective therapeutic approach to managing vitamin B12 deficiency and stabilizing neuropathic symptoms in diabetic patients.

CONCLUSION

This study successfully developed and optimized a novel, taste-masked vitamin B12 oral dispersible film using a synergetic matrix of natural polymers. Incorporating active cyanocobalamin into calcium alginate beads via an external ionotropic gelation method provided highly effective taste masking, restricting drug release during the initial residence time in the mouth and preventing interaction with taste buds.

Integrating these taste-masked beads into a blended hydrophilic polymer matrix of pullulan, HPMC, and guar gum yielded films with excellent physical and mechanical properties. The optimized formulation (F3) demonstrated high folding endurance (248 folds) and tensile strength (4.0 N/mm^2), ensuring robust handling characteristics. Supported by citric acid as a salivary stimulant, the film achieved an ultra-fast disintegration time of 25 seconds in simulated saliva, releasing 99% of the active drug within 10 minutes to support rapid systemic absorption.

FTIR, DSC, and XRD analyses confirmed successful cross-linking, drug-excipient compatibility, and the stable, amorphous dispersion of the active drug within the polymeric matrix. Accelerated stability studies confirmed that the formulation maintained high chemical potency and structural integrity, verifying its commercial and clinical viability. By bypassing the active transport barriers of the terminal ileum, this sublingual/buccal ODF provides a non-invasive, patient-friendly therapeutic intervention. This delivery platform represents a highly promising approach to managing metformin-induced vitamin B12 deficiency and improving therapeutic outcomes in patients with diabetic neuropathy.