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Optimizing Vitamin B12 Delivery in Solid Pharmaceutical Formats: The Role of Gelatin in Stability and Formulation Performance

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ABSTRACT

Background: Cyanocobalamin delivery in solid oral formulations is limited by gastric acid instability and the dependence on impaired haptocorrin/intrinsic factor/pathways in groups at high-risk of vitamin B12 deficiency, such as metformin-treated diabetic patients (14% - 41% vitamin B12 deficiency) and older adults (10% - 40%). Gelatin encapsulation may enhance vitamin B12 delivery at therapeutic doses via acid protection and mucoadhesion. Methods: *In vitro* studies compared 1% w/w gelatinised cyanocobalamin with non-gelatinised vitamin B12 using simulated gastric fluid (SGF, pH 1.2; HPLC stability 0 - 3 h) and differentiated Caco-2 monolayers (24 h apical uptake ±SGF/SIF preexposure; LC17 MS/MS; TEER). Results: Gelatinised vitamin B12 retained 97.1% to 98.3%, whereas non-gelatinised vitamin B12 retained 90.0% to 93.7% of the total vitamin B12 content in SGF (3-fold less degradation, 1 - 3 h). Caco-2 monolayers presented 3-fold higher intracellular/basolateral vitamin B12 with gelatinised than with non-gelatinised vitamin B12, without TEER disruption. Conclusion: Gelatin encapsulation confers superior gastric stability and epithelial uptake, supporting progression to pharmacokinetic studies for improved therapeutic vitamin B12 delivery in absorption-compromised populations.

1. INTRODUCTION

Vitamin B12 (cobalamin) is an essential water-soluble vitamin required for red blood cell maturation,

DNA synthesis, methylation pathways, and normal neurological function [1, 2]. A deficiency disrupts erythropoiesis, impairs myelin integrity, and can manifest as megaloblastic anaemia, peripheral neuropathy, cognitive decline, or neuropsychiatric symptoms [1, 3]. Cobalamin exists in several chemical forms: cyanocobalamin and hydroxocobalamin are synthetic or modified forms widely used pharmaceutically, whereas methylcobalamin and adenosylcobalamin are the metabolically active cofactors in human tissue [2, 4, 5]. Among these different chemical forms, cyanocobalamin remains the most commonly used form in oral supplements, medicinal products and fortified foods because of its cost-effectiveness and greater chemical stability, with comparable absorption profiles when given at supplementation and therapeutic doses [4-7].

Clinically significant vitamin B12 deficiency is common in multiple risk groups: type 2 diabetes patients, especially those treated with metformin (14% - 41%), pernicious anaemia and post-gastrectomy patients (approximately 100%), and older adults (10% - 15% in community-dwelling; up to 40% in institutionalised), vegetarians or vegan, chronic alcoholism and others [8-13]. If left untreated, vitamin B12 deficiency can lead to serious haematological and neurological complications [3]. Standard management of vitamin B12 deficiency and peripheral neuropathy includes high-dose oral cyanocobalamin or intramuscular injections, in combination with other neurotropic B vitamins (B1, B6). Under normal physiological intake, vitamin B12 is absorbed through a combination of high-affinity, receptor-mediated routes with a low-efficiency passive diffusion at low microgram doses; at larger pharmacologic doses used in many oral neurotropic B-complex products, overall uptake is predominantly by passive diffusion, with approximately 1% - 2% of the ingested dose crossing the intestinal epithelium into the circulation [4, 14, 15]. However, conventional cyanocobalamin is chemically labile under strongly acidic conditions, such as those found in the gastric environment, and has a markedly shorter half-life at gastric pH than under near-neutral conditions [16-18]. In addition to gastric acidity, cobalamin is sensitive to light and humidity in solid pharmaceutical forms, and the relatively low amount used in solid pharmaceutical formats (microgram range per unit) creates additional challenges for ensuring content uniformity and maintaining potency throughout manufacturing and storage [6, 19].

Gelatin is a collagen-derived, biodegradable excipient that is generally recognised as safe (GRAS) status and has a long history of use in pharmaceutical dosage forms, including capsules and matrix systems to improve ingredient stability and help with dosing uniformity, especially at small amounts [20-22]. Recent work has highlighted the ability of gelatin to form dense, pH-responsive matrices, interact with mucus, and act as a platform for controlled release and targeted delivery in oral preparations [23-25]. In commercial pharmaceutical products, gelatin-based capsules are used to maintain content uniformity and product stability [26]. Although gelatin is widely used in pharmaceutical formulations, the protective effects of gelatin encapsulation specifically on oral cyanocobalamin stability and bioavailability remain poorly characterised [25, 27, 28]. Recent research has highlighted the potential of gelatin in vitamin B12 delivery systems, particularly through nanofiber formulations and sustained-release mechanisms [27] and related lipid-based carriers have shown enhanced passive absorption of vitamin B12 via P-glycoprotein inhibition [29]. However, the dual protective effects of gelatin—acid stabilization combined with improved cellular uptake or absorption—have not been comprehensively characterised in complementary *in vitro* models.

Gelatinised vitamin B12 technology is designed to address several of these constraints simultaneously: by embedding cyanocobalamin (a common pharmaceutical form of vitamin B12) within a gelatin matrix, it can enhance stability against gastric acid and maintain higher free vitamin B12 concentrations in proximity to the intestinal epithelium, thereby maximising the contribution of passive diffusion at pharmacologic oral doses. This study therefore aims to characterise the role of gelatin in the protection of vitamin B12 during gastric transit using *in vitro* models by evaluating the impact of gelatin encapsulation on acid stability and epithelial uptake.

2. MATERIALS AND METHODS

2.1. Materials

Cyanocobalamin, active pharmaceutical ingredient (API, Hebei Huarong Pharmaceutical Company Ltd, China), was used as the reference vitamin B12 form. Gelatin (Rama Industries Ltd, bovine source) suitable for pharmaceutical use was employed to manufacture gelatinised vitamin B12 granules containing approximately

1% w/w vitamin B12 in a 99% gelatin matrix, which is consistent with established commercial formulations. Simulated gastric fluid (SGF; pH 1.2) was prepared according to the USP (US Pharmacopeial), and other reagents were of analytical grade.

2.2. Preparation of Gelatinised Vitamin B12

Gelatinised vitamin B12 can be manufactured via various methods such as spray drying or fluid-bed granulation. The gelatinised vitamin B12 granules (1%, Supreem Pharmaceuticals, India) used in this study were produced via fluid-bed granulation, in which small gelatin cores were sprayed with a solution of cyanocobalamin and gelatin under heated air (45˚C), followed by drying and application of a final gelatin coating (Figure 1). This process yielded granules with consistent particle sizes (80% - 100% passes through mesh 60#) and uniform vitamin B12 contents (1% loading, hereinafter referred to as 1% gelatinised vitamin B12) embedded within a continuous gelatin matrix and is analogous to the process used in marketed Neurobion® formulations containing 100mg of B1, 100 - 200 mg of B6, 200 - 5000 mcg of B12. For *in vitro* experiments, 1% gelatinised vitamin B12 granules were dispersed and diluted to the target vitamin B12 concentration in the relevant test media, and non-gelatinised vitamin B12 solutions were prepared at matched cyanocobalamin concentrations.

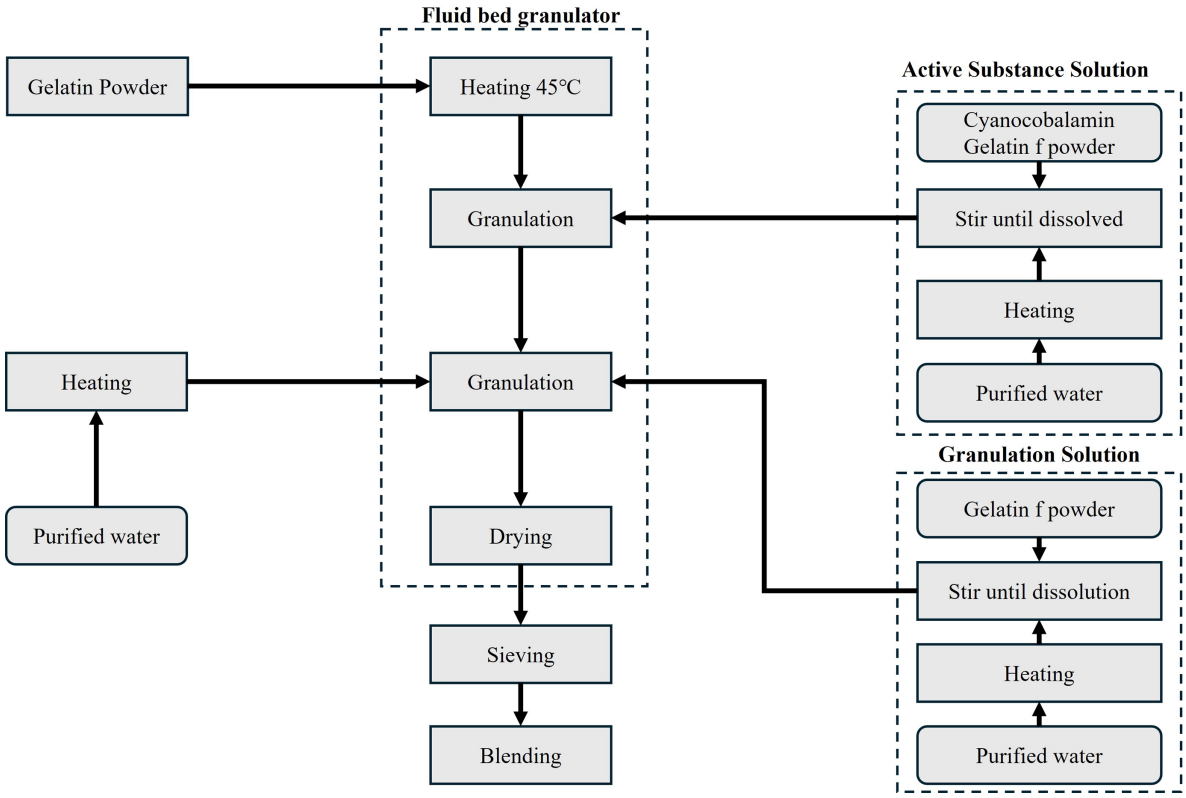

Figure 1. Manufacturing process flow chart for vitamin B12 embedding in gelatin.

2.3. Simulated Gastric Fluid (SGF) Stability Studies

The concentration of vitamin B12 (both non-gelatinised and 1% gelatinised) was adjusted based on the gastric volume, and label claim of vitamin B12 in the formulation (200 mcg). Stock solutions of 1% gelatinised vitamin B12 and non-gelatinised vitamin B12 were prepared in SGF pH 1.2 media to achieve concentration of 0.4 ppm (equivalent to 200 mcg dose, n = 3), similar to the concentration from Neurobion® and other preparations on the market containing 200 mcg vitamin B12. The vitamin B12 content was quantified

via reversed-phase high-performance liquid chromatography (HPLC) with 0.1% trifluoracetic acid (TFA) solution and acetonitrile as the mobile phase with a gradient pump mode via a phenyl column (150 mm × 4.6 mm) and UV detection at 361 nm. The amount of vitamin B12 was measured at various time points (initial, 1 hour, 2 hours and 3 hours) in the simulated setup until gastric emptying for both 1% gelatinised vitamin B12 and non-gelatinised vitamin B12.

2.4. Caco-2 Cell Culture and Uptake Assays

Human Caco-2 cells (American Tissue Culture Collection, Manassas, VA, USA, ATCC cat# HTB-37) were cultured on permeable inserts (growth area 3.14 cm^2, pore size 0.4 μm) at a density of approximately 1×10^5 cells per insert and maintained in standard growth medium (EMEM ATCC cat#30-2003 supplemented with 10% FBS-ATCC cat#30-2020). The medium was changed 24 h after seeding and every other day thereafter until the monolayers reached differentiation and confluence (18 to 21 days), as evidenced by stable transepithelial electrical resistance (TEER) readings of approximately 750. Before the experiments, the baseline TEER was recorded to confirm barrier integrity.

Gelatinised and non-gelatinised vitamin B12 formulations were prepared in cell culture medium with or without prior exposure to simulated gastric and intestinal fluids to mimic gastrointestinal transit [25]. Briefly, samples of gelatinised (0.05 w/v%-B12 content of gelatinised beads is 1% of total mass) or non-gelatinised B12 (0.005 w/v%) were incubated in the SGF for 1 hour at 37˚C, no agitation, then diluted 1:1 into simulated intestinal fluid followed by additional 1 hour incubation at 37˚C, no agitation followed by 1:1 dilution in Caco-2 medium and addition of protease inhibitor cocktail with 30 minute incubation at room temperature. The formulations were applied to the apical (mucosal) side (n = 4 for each treatment group), with fresh medium in the basolateral compartment, and the cells were incubated for 24 h at 37˚C [30]. After incubation, the cells were washed to remove extracellular vitamin B12 and lysed, and the basolateral vitamin B12 content was quantified via LC to MS/MS [25]. TEER was remeasured to assess whether barrier integrity was maintained during exposure [30]. SGF (VWR cat# RC7108-16) was prepared according to USP guidelines, with 3.2 g of pepsin (VWR cat#M142-250G)/Liter added to make complete SGF. For simulated intestinal fluid (VWR cat# RC7109-16), SIF, 10 g/Liter of pancreatin (VWR cat # 75875-592) was used. Prior to the application of SGF/IF-treated vitamin B12 solutions to Caco-2 cells, 30 μL of Protease Inhibitor Cocktail (ThermoFisher cat# 78429) was added to mitigate the deleterious impact of pepsin/pancreatin on Caco-2 cells.

2.5. Data Handling and Analysis

For each experiment, values were generated from replicate samples or inserts per condition [17, 25, 30]. The results are reported descriptively as approximate fold differences between gelatinised and non-gelatinised vitamin B12 based on mean values, reflecting the mechanistic focus and *in vitro* nature of the work [25]. Where relevant, literature was used to contextualise the magnitude of observed effects relative to physiological constraints and clinical dosing paradigms [4, 31].

3. RESULTS

3.1. Gelatin Encapsulation Enhances Vitamin B12 Stability in Simulated Gastric Fluid

The stability results of the sample solutions, with a concentration of 0.4 ppm (equivalent to a 200 mcg dose) of non-gelatinised vitamin B12 and 1% gelatinised vitamin B12 prepared in simulated gastric fluid (SGF) pH 1.2 media, were analysed by HPLC at different time intervals: initial, 1 hour, 2 hours and 3 hours. As shown in **Table 1**, for 1% gelatinised vitamin B12, 1.7%, 2.3% and 2.9% losses were observed at 1 hour, 2 hours and 3 hours respectively, whereas for non-gelatinised vitamin B12, 6.3%, 8.6% and 10.0% loss were observed from the initial assay at 1 hour, 2 hours and 3 hours respectively. The test data demonstrate that gelatin encapsulation (1% vitamin B12 and 99% gelatin matrix) protects vitamin B12 from degradation under acidic stomach conditions, with a mean result of 3.6 times less loss of vitamin B12 due to gelatin encapsulation.

Table 1. Gelatinised vitamin B12 loss vs non-gelatinised vitamin B12 loss at different time points.

Duration in SGF	1% gelatinised vitamin B12 loss % Solution concentration (200 mcg), n = 3 (A)	Non-gelatinised vitamin B12 loss % Solution concentration (200 mcg), n = 3 (B)	Total loss ratio [B/A]
1 hr.	Mean: 1.7% loss SD: 2.2	Mean: 6.3% loss SD: 4.4	3.7
2 hr.	Mean: 2.3% loss SD: 2.9	Mean: 8.6% loss SD: 5.9	3.7
3 hr.	Mean: 2.9% loss SD: 3.2	Mean: 10.0% loss SD: 6.7	3.4
			Mean: 3.6 SD: 0.2

The percentage loss (%) of vitamin B12 at 1 hour, 2 hours and 3 hours was compared against the initial assay results for both gelatinised and non-gelatinised vitamin B12. The HPLC method used for assessing vitamin B12 stability in simulated gastric fluid is operating at a low sample concentration of 0.4 ppm, which can lead to variability in the results, hence the probability of higher standard deviations cannot be eliminated.

3.2. Gelatinised Vitamin B12 Increases Cellular Passage in Caco-2 Monolayers without Compromising Barrier Integrity

Figure 2 shows that 1.2% of a dose of non-gelatinised vitamin B12 is absorbed, which is in line with clinical

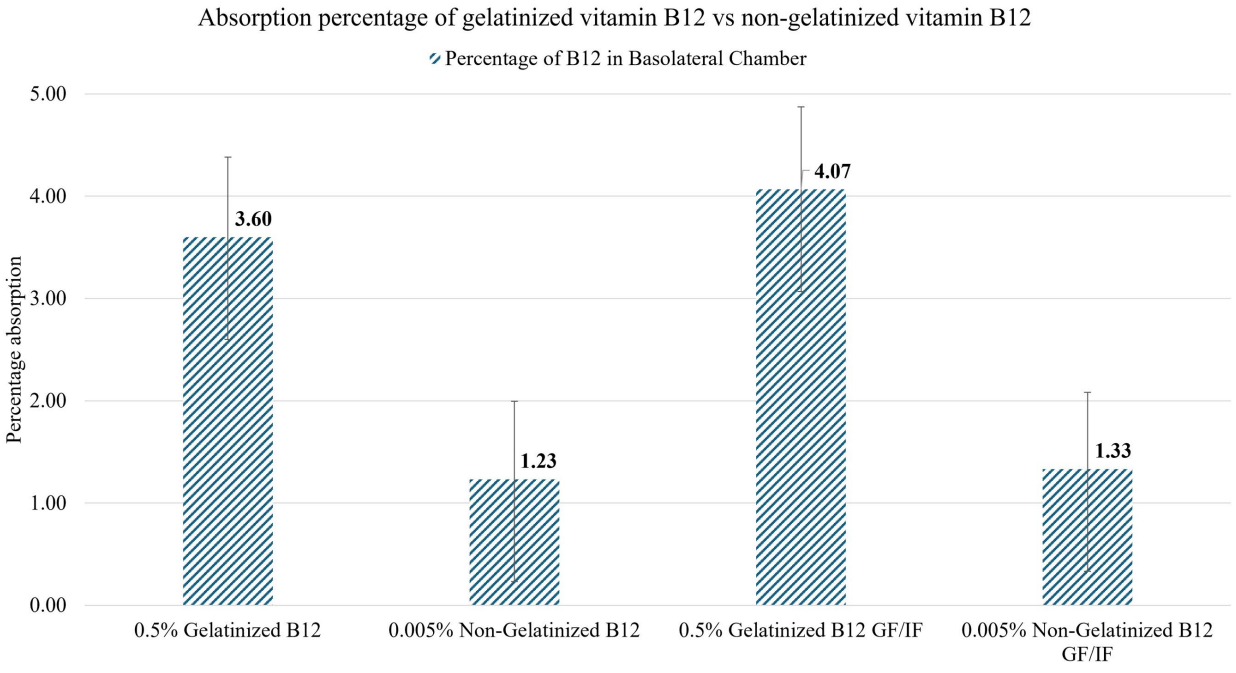

Figure 2. Intracellular vitamin B12 levels in Caco-2 monolayers after 24 h of incubation with gelatinised vitamin B12 vs pure vitamin B12, with and without simulated gastric fluid (GF) and intestinal fluid (IF) Note that 0.05% gelatinised vitamin B12 has 0.005% actual vitamin B12, so the levels are matched.

observations showing that 1% - 2% of a dose of vitamin B12 is absorbed via the passive absorption pathway. When the same amount of vitamin B12 embedded in gelatin (1% vitamin B12 and 99% gelatin matrix) was added to the cells, a 3-fold increase in basolateral recovery was noted (3.6%). This result was achieved both when gelatinised vitamin B12 was added directly to cells or when it was pre-incubated with simulated gastric and simulated intestinal fluids for 1 hour each (to approximate the conditions of the stomach and first parts of the small intestine).

4. DISCUSSION

Exposure to simulated gastric fluid (SGF; pH 1.2, 37˚C) for 3 hours led to substantial degradation of non-gelatinised vitamin B12, with only approximately 90.0% to 93.7% of the initial vitamin B12 content remaining at matched starting concentrations. In contrast, gelatinised vitamin B12 granules (1% vitamin B12 in a 99% gelatin matrix) retained approximately 97.1% to 98.3% of their initial vitamin B12 content under identical conditions, corresponding to an approximate threefold reduction in vitamin B12 loss compared with non-gelatinised vitamin B12 (cyanocobalamin). This observation aligns with the hypothesised dual protection mechanism, which comprises first a dense, minimally swollen gelatin matrix that physically limits hydrogen ion penetration into the core for approximately 60 min at pH 1.2 [32] and, second, proton-accepting amino acid residues (approximately 13% of the polypeptide chain of gelatin are positively charged) within the gelatin that buffer the local microenvironment around the encapsulated vitamin B12 [25, 33]. Gelatin-based matrices have been reported to provide sustained protection over several hours; with crosslinked gelatin hydrogels remaining mechanically stable for 4 to 6 hr under physiological conditions, which is consistent with the gastric residence time of 1 to 3 h [33, 34].

Scintigraphic and transit studies indicate that, depending on the prandial state and formulation, the upper gastrointestinal residence time (stomach plus small intestine) for solid and drinkable dosage forms typically lies within a 1 - 3 hr window in adults, extending toward 3 - 4 hr in the fed state [35-38]. The observed threefold reduction in cyanocobalamin degradation within 1 - 3 hours in SGF could warrant further clinical evaluation, as this period corresponds to the typical duration during which most of an orally administered dose is subjected to gastric conditions prior to reaching the small intestine, where predominantly passive absorption occurs at high oral doses [14, 39]. As the product is taken with or shortly before a meal, the gastric pH may be transiently elevated, but tablet disintegration and early dissolution still occur during this 1 - 3 hr residence window, thus reducing acid-mediated cyanocobalamin degradation over this period is expected to translate into a higher intact dose reaching the small intestine for absorption [35, 40]. The observed threefold reduction in degradation is in line with literature showing greater cyanocobalamin stability at moderately acidic pH and with reports of pH-responsive behaviour in gelatin hydrogels [16-18, 23]. Taken together, these data indicate that improving cyanocobalamin stability over the physiologically relevant 1 - 3 hr gastric residence period may increase the amount of intact vitamin B12 reaching the small intestine, where absorption at pharmacologic doses is driven predominantly by passive diffusion.

The Caco-2 experiments evaluate the next step of the physiological journey of vitamin B12 toward absorption by demonstrating approximately threefold greater basolateral recovery of vitamin B12 from gelatinised formulations compared with non-gelatinised vitamin B12 formulations, without evidence of reduced transepithelial electrical resistance (TEER) or barrier breakdown [25, 30]. This experimental model approximates the gastrointestinal tract through three key features: the epithelial monolayer reflects the absorptive surface; pretreatment with simulated gastric and intestinal fluids mimics physiological pH conditions and the enzymatic milieu; and the measurement of TEER confirms preservation of tight junction integrity throughout the incubation period. The observed threefold increase in transcellular vitamin B12 uptake with gelatin encapsulation may reflect several hypothesized mechanisms: first, prolonged contact of the gelatinised formulation with the apical epithelial surface, potentially mediated by mucoadhesive interactions [41]. Second, sustained local vitamin B12 availability from the protective gelatin matrix, maintains a favourable concentration gradient for passive diffusion [25]. Third, the chemical integrity of vitamin B12 is protected during transit through the acidic gastric phase, preventing premature degradation [17].

This pattern suggests that gelatin promotes epithelial transport under preserved tight junction function, likely via prolonged apical surface contact, maintenance of local vitamin B12 gradients, and protection of vitamin B12 integrity during simulated gastric transit [25, 41]. Gelatin peptides contain collagen-derived motifs (Pro-Gly sequences and RGD-like epitopes, which are arginine-glycine-aspartic acid sequences) that may interact with epithelial integrins and subtly modulate claudin-2 tight junction proteins, potentially enhancing paracellular transport; however, this mechanism remains speculative and requires targeted experimental validation [25, 42-45].

This work provides convergent *in-vitro* evidence that encapsulating cyanocobalamin (a common pharmaceutical form of vitamin B12) in a gelatin matrix confers meaningful advantages for gastric stability, improved availability for intestinal absorption and enhanced epithelial uptake, mechanisms that are directly relevant to oral vitamin B12 replacement at therapeutic doses [17, 25, 30].

Clinical relevance

The clinical relevance of these findings lies in the different absorption pathways of vitamin B12. In the stomach, vitamin B12 binds to a glycoprotein called haptocorrin (also referred to as R-factor or R-protein), forming the haptocorrin-vitamin B12 complex, which protects vitamin B12 from acid degradation. Once the haptocorrin-vitamin B12 complex reaches the duodenum, pH changes favour the degradation of haptocorrin and cleavage of the haptocorrin-vitamin B12 complex, releasing the free form of vitamin B12. In the active absorption pathway, the free vitamin B12 can then bind to intrinsic factor (IF), a glycoprotein secreted by gastric parietal cells, to form an IF-vitamin B12 complex and that travels to the ileum for receptor-mediated absorption into the blood [15, 46].

While the active mechanism saturates at low physiological levels and therefore contributes only a small fraction of the total absorbed vitamin B12, passive diffusion plays an important role in uptake at pharmacological doses. At pharmacological oral doses of vitamin B12, such as those used in neurotropic B-complex preparations (200 - 5000 mcg), total absorption is achieved primarily through passive diffusion across the intestinal epithelium, which is generally inefficient, accounting for approximately 1% - 2% of the pharmacologic dose and has considerable inter-individual variability [15]. In this dose range, the amount of vitamin B12 exceeds the haptocorrin binding capacity, leaving significant unbound vitamin B12 vulnerable to acid degradation [39]. Clinical effectiveness therefore depends on the amount of intact vitamin that reaches the ileum [2].

By stabilizing cyanocobalamin throughout production and storage, ensuring consistent microgram-level dosing of vitamin B12, protecting it from stomach acid during digestion, and maintaining higher concentrations near the absorption sites, gelatinised vitamin B12 formulations are specifically designed to support its absorption [25]. Formulations that reliably deliver vitamin B12 and support intestinal absorption are important to restoring deficient vitamin B12 levels and contribute to improving deficiency-related neuropathic symptoms.

These benefits are particularly pertinent for common patient groups in whom therapeutic doses of vitamin B12 are frequently indicated. These include patients with a clinical or subclinical deficiency in vitamin B12 and patients suffering from peripheral neuropathy. Common target groups requiring a therapeutic dose of vitamin B12 are patients with type 2 diabetes treated with metformin, who have a higher prevalence of vitamin B12 deficiency and peripheral neuropathy; older adults, in whom multiple age-related factors reduce vitamin B12 status; individuals following vegetarian or vegan diets, who have low dietary intake of cobalamin; people with chronic alcoholism, where malnutrition and malabsorption coexist; and patients receiving chronic haemodialysis, who often exhibit low or borderline serum vitamin B12 levels and others [8-13, 47]. In these populations, high-dose oral vitamin B12 is routinely used to correct deficiency and support nerve function, and a gelatinised formulation that stabilises vitamin B12 in the stomach and enhances passive uptake in the small intestine can provide a practical advantage over non-gelatinised preparations [48, 49].

Metformin-treated type 2 diabetic patients: Approximately 14% to 41% of patients on long-term metformin therapy develop biochemical vitamin B12 deficiency, with the risk increasing with increasing metformin dose and cumulative exposure [8, 9, 50-52]. The underlying mechanism involves calcium antagonism:

the positive charge of metformin competes with vitamin B12 for the calcium binding sites required for intrinsic factor-B12-Ca^{2+}-cubilin complex formation in the terminal ileum [51]. Consequent impairment of active absorption forces greater reliance on passive diffusion through the gastrointestinal tract. Additionally, metformin use decreased plasma levels of total cobalamin [53, 54], total haptocorrin and haptocorrin-bound cobalamin in diabetic patients taking metformin, revealing the effect of metformin on haptocorrin glycoprotein leading to vitamin B12 degradation in the stomach.

Gelatinised vitamin B12 may help mitigate some of these absorption barriers through mechanisms independent of haptocorrin status, including acid protection of unbound vitamin B12, mucoadhesion-mediated epithelial contact, and sustained release that maintains concentration gradients for passive absorption [25]. The passive pathway becomes functionally relevant when gelatin-encapsulated cyanocobalamin is provided at pharmacologic concentrations [4]. This mechanism is particularly valuable in populations where active absorption is compromised, such as metformin-treated diabetic patients (calcium antagonism), pernicious anaemia and post-gastrectomy patients (impaired haptocorrin and absent intrinsic factor), and older adults (age-related receptor density reduction) [11, 51].

Older adults with age-related absorption decline: Approximately 10% to 15% of community-dwelling individuals and up to 40% of institutionalised older persons exhibit vitamin B12 deficiency [10, 11]. While the haptocorrin level might be lower in healthy elderly people, it is generally not considered the primary cause of vitamin B12 deficiency. Age-related absorption impairment is multifactorial, with increased gastric hypochlorhydria impairing food-bound vitamin B12 release, reduced ileal cubilin receptor density, and altered intestinal microbiota composition [10, 11, 31]. Through the three complementary mechanisms identified in the Caco-2 model (prolonged apical contact via mucoadhesion, sustained local vitamin B12 availability, and acid protection), gelatin-mediated enhancements in passive uptake, mucoadhesion, and acid protection collectively address these multifactorial absorption deficits [4, 25].

In these high-risk groups, gelatinised vitamin B12 could potentially improve delivery efficiency at existing therapeutic doses while maintaining therapeutic vitamin B12 status, with implications for improved adherence, reduced pill burden, improved treatment compliance, and enhanced clinical outcomes [25, 55].

Limitations

This work has recognised limitations typical of mechanistic *in vitro* studies. Simulated gastric fluid assays use simplified media lacking dynamic peristalsis, enzymatic processes and variable pH microenvironments characteristic of living gastric tissue [30]. Caco-2 monolayers, although widely predictive of intestinal permeability *in vivo*, lack full mucus layers, peristaltic stimulation, and complete proteolytic milieu of the living intestinal tract; consequently, the observed threefold increase in cellular uptake should be interpreted as a qualitative indicator of potential bioavailability enhancement rather than a direct *in vivo* prediction [30]. Although passive diffusion efficacy remains low, a modest increase in amount of intact vitamin B12 could potentially influence total absorbed dose at pharmacologic oral concentrations. The clinical significance requires further research to understand whether the observed *in-vitro* improvements translate to clinically meaningful improvement to vitamin B12 status. Despite these constraints, the data provide internally consistent mechanistic support that warrants progression to *in vivo* studies [25]. Another limitation of the HPLC method used for assessing vitamin B12 stability in simulated gastric fluid is that operating at a low sample concentration of 0.4 ppm can lead to variability in results. With a small sample size (n = 3), the standard deviation of the sample result tends to be wider with more variability and can lead to less precise outputs in terms of mean vitamin B12 loss.

5. CONCLUSION

This study evaluated the impact of gelatin encapsulation on cyanocobalamin stability and cellular uptake via two complementary *in vitro* systems: simulated gastric fluid stability assays and Caco-2 intestinal epithelial monolayers. Compared with non-gelatinised vitamin B12, gelatinised vitamin B12 resulted in approximately threefold less vitamin B12 loss via acid-mediated degradation, and approximately threefold greater

intracellular uptake through Caco-2 cells without compromising barrier integrity. These converging findings support gelatin as a multifunctional excipient that simultaneously protects vitamin B12 from gastric acid, sustains availability via controlled release and mucoadhesion, and enhances passive epithelial transport-all mechanisms particularly relevant to populations dependent on passive absorption pathways, including metformin-treated diabetic patients, older adults and peripheral neuropathy patients. The *in vitro* evidence suggests that progression to human pharmacokinetic studies in healthy volunteers and clinical efficacy trials in target populations are needed to establish the magnitude of benefit and to evaluate the role of gelatinised vitamin B12 in improving formula performance *in vivo*.

AVAILABILITY OF DATA AND MATERIALS

All data supporting the findings of this study are available within the manuscript.

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AUTHOR CONTRIBUTIONS

Study conceptualization: GC, YL, JTS. Design of study: GC, TG, PA, JZW, TL, LJL, JTS. Acquisition and analysis: HR, JZW. Interpretation of data: GC, YL, TG, PA, HR, JZW, TL, LJL, JTS. Drafted work: GC, YL, TG, PA, JZW, TL, LJL. Revision and review: GC, YL, TG, PA, TL, LJL, JTS.

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DECLARATION

GC, TG, PA, HR, JZW, TL, LJL and YL are employed by and own stocks in Procter and Gamble. JTS is a former employee of Procter and Gamble and owns stocks in Procter and Gamble.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredient
B1	Thiamine (Vitamin B1)
B6	Pyridoxine (Vitamin B6)
B12	Cobalamin (Vitamin B12)
ATCC	American Type Culture Collection
Caco-2	Human Colorectal Adenocarcinoma Cells
DNA	Deoxyribonucleic Acid
EMEM	Eagle's Minimum Essential Medium
FBS	Fetal Bovine Serum
GRAS	Generally Recognized as Safe
HC-Cbl	Haptocorrin-Bound Cobalamin
HPLC	High-Performance Liquid Chromatography
IF	Intrinsic Factor
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
pH	Potential of Hydrogen
ppm	Parts per Million
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
TEER	Transepithelial Electrical Resistance
TFA	Trifluoroacetic Acid
USP	United States Pharmacopeia
UV	Ultraviolet
w/w	Weight per Weight