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QUANTITATIVE COMPARISON OF DIETARY FIBRES FOR VITAMIN B12 ENCAPSULATION

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ABSTRACT

Vitamin B12, also referred to as Cobalamin, is an essential nutrient for the human body. DNA synthesis, neurological functions, and haematopoiesis are a few of the vital roles vitamin B12 plays. However, it is concerning that a significant 47% of the Indian population is grappling with a deficiency in this vital nutrient. Vitamin B12 supplements in the form of capsules and tablets serve to solve this problem. We hereby explore a solution which is not only efficient but also sustainable, namely, Vitamin B12 encapsulation in environmentally friendly matrices via ionotropic gelation. Dietary fibres have been explored especially well in the food industry as encapsulating matrices and are known to have the potential for controlled release of bio actives in the pharmaceutical industry. This research work provides a comparison of the concentrations of different dietary fibres, namely alginate, pectin and cellulose required to encapsulate Vitamin B12. It serves as a starting point to discovering the full potential of these capsules in helping to solve the pressing issue of vitamin B12 deficiency.
Keywords: Vitamin B12, encapsulation, dietary fibre matrices

1. Introduction

1.1) Vitamin B12

Vitamin B12, scientifically termed cobalamin, is abundantly found in animal-derived foods like meat, fish, poultry, eggs, and dairy products. It is an essential water-soluble nutrient crucial for maintaining human health (Green et al., 2017d).

The importance of vitamin B12 spans various dimensions of human health. It plays key roles in red blood cell production, DNA synthesis, and maintaining optimal nervous system functionality. Additionally, it actively participates in the metabolism of carbohydrates, fats, and proteins, contributing to cognitive function and overall brain health. It is known to have potential in mitigating conditions like megaloblastic anaemia, pernicious anaemia, and cardiovascular diseases, emphasising its multifaceted role in human physiology (Weissbach & Taylor, 1969c) (Ling & Chow, 1954).

Vitamin B12 deficiency poses a prevalent health concern with multifaceted causes. Insufficient dietary intake is significant, particularly in individuals following vegetarian or vegan diets with limited natural sources. Compounding the issue is impaired absorption in the small intestine, often associated with gastrointestinal disorders like Crohn's disease and celiac disease, increasing the risk of deficiency. Extrinsic variables, such as excessive alcohol consumption, various malabsorption syndromes, specific medications, and the natural ageing process with associated physiological changes, contribute to the

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complexity of vitamin B12 deficiency. These diverse factors underscore the need for comprehensive assessment and management in clinical and public health contexts (Stabler, 2013) (Pawlak et al., 2013) (Ward et al., 2015).

Proactive intervention becomes imperative for individuals facing vitamin B12 deficiency challenges, leading healthcare practitioners to prescribe vitamin B12 injections or high-dose oral supplements as effective strategies. These therapeutic interventions are indispensable tools in mitigating the consequences of deficiency, ensuring the sustained provision of this essential nutrient, especially when conventional dietary sources or natural absorption mechanisms prove insufficient. The clinical deployment of these interventions emphasises their clinical significance and tailored use in managing vitamin B12 deficiency (Cyran, 2002).

1.2) Encapsulation

Encapsulation has emerged as a highly efficient tool for the targeted and precise delivery of sensitive biological compounds, proving to hold high potential in tackling vitamin B12 deficiency. This methodology offers a versatile means to govern the distribution, action, and metabolic fate of the encapsulated substances, thereby rendering it an asset across diverse domains, notably within the realms of pharmaceuticals and functional foods. The increasing popularity of encapsulation is associated with its intrinsic potential to optimise therapeutic or functional outcomes and to meet the contemporary demand for tailored and effective delivery systems (Klojdová et al., 2023).

The substances enveloped within protective matrices, whether they are individual pure compounds or intricate mixtures, are variably referred to by terms such as coated materials or core materials, among others. In contrast, the materials employed to envelop and safeguard these substances encompass a diverse nomenclature, encompassing designations such as shells, capsules, or membranes (Madene et al., 2005).

Capsules have established themselves as a ubiquitous and versatile encapsulation system, distinguished by their precisely tailored composition. This composition is meticulously designed to fulfil a dual role: safeguarding the integrity of the encapsulated sensitive compounds and enabling their deliberate release at precise locations within the intricate milieu of the gastrointestinal tract (Varankovich et al., 2015).

Ionotropic gelation is one of the methodologies used to encapsulate biologically active compounds. This technique relies on the cross-linking between polymer chains with the help of ions (Gadziński et al., 2022).
Hydration gelation is another process involved in the formation of gel matrices through the absorption of water. Initially the polymer is either exposed to a hydrated environment or water is introduced into it. This forms a protective matrix around the polymer substrate.

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1.3) Dietary fibres

Dietary fibres represent a significant component of plant-derived foods, characterised by their resilience to enzymatic digestion within the human gastrointestinal system. These fibrous materials are abundantly present in a spectrum of natural sources, notably encompassing whole-grain cereals, nuts, seeds, and an array of fruits and vegetables. Notable examples of dietary fibres include cellulose, pectin, alginate, lignin, among others. These compounds form a substantial component of the human diet, exerting diverse physiological effects and imparting essential health benefits.

The applications of dietary fibres span a multitude of dimensions, significantly impacting various aspects of human health. The several key applications of dietary fibres, elucidating their diverse and vital roles are digestive health and constipation prevention, facilitation of weight loss, maintenance of gut microflora and metabolites.

In our research, we are endeavouring to harness the potential of dietary fibres as encapsulating agents for vitamin B12 capsules. This approach involves the utilisation of distinct dietary fibres, namely Alginate, Cellulose, and Pectin as encapsulation matrices. These dietary fibres, renowned for their diverse physiological effects and health benefits, hold promise as suitable carriers for the encapsulation of vitamin B12, offering a range of possibilities for the controlled delivery and release of this essential micronutrient, combining the benefits of tackling vitamin B12 deficiency and the numerous advantages the fibres pose.

1.3.1) Alginate

Alginate, a naturally occurring polysaccharide derived from brown seaweeds, is a widely recognized dietary fibre with versatile applications in various industries, including food, pharmaceuticals, and biotechnology (Anderson et al., 2018). It is a linear polymer consisting of blocks of (1,4) - linked-β-D-mannuronate(M) and α-guluronate(G). The blocks are made up of consecutive G residues, consecutive M residues or alternating G and M residues. Its remarkable gelling and thickening properties make it a favoured choice for encapsulation purposes.

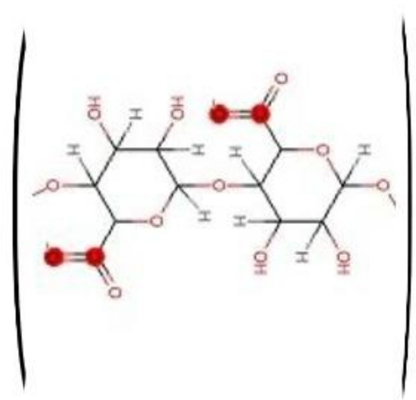

Figure1. Alginate structure

1.3.2) Cellulose

Cellulose, an abundant structural component in plant cell walls, is known for its insolubility and

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resistance to digestion by human enzymes. It has been employed in encapsulation due to its biocompatibility and the ability to provide a physical barrier for controlled release applications. It is a linear polysaccharide consisting of β-(1,4)-linked glucose units.

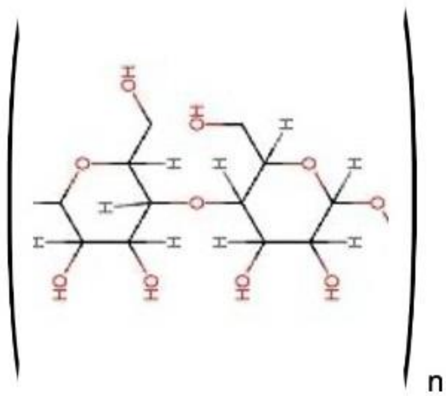

Figure2. Cellulose structure

1.3.3) Pectin

Pectin, another plant-derived polysaccharide, predominantly found in fruits, possesses excellent gelling properties attributed to its highly branched structure. It is an acidic polysaccharide that consists of 150 - 500 α-(1,4)-D-galacturonic acid (Lara-Espinoza et al., 2018). Pectin's unique gelling ability has made it a valuable candidate for encapsulation, enabling the development of controlled-release systems in the pharmaceutical and food industries.

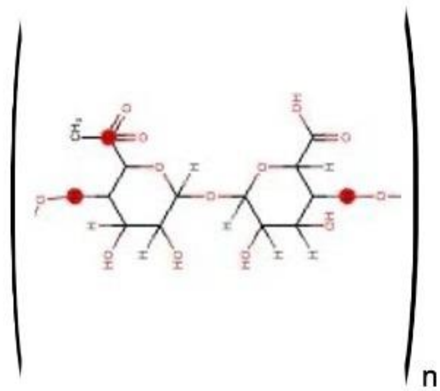

Figure3. Pectin structure

These dietary fibres, Alginate, Cellulose, and Pectin, collectively represent a rich resource for encapsulation technologies, offering diverse functionalities and potential applications in tackling vitamin B12 deficiency.

2. Methodology

*(All concentrations are expressed as weight/volume %)

2.1) Preparation of Cross-linking solution:

A 100 mM solution of calcium chloride was prepared in distilled water to serve as the cross-linking agent for the fibre encapsulation of Vitamin B12 via ionotropic gelation.

2.2) Encapsulations of Vitamin B12 at 2% concentration of Dietary fibres:

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Sodium alginate, pectin, and hydroxypropyl methylcellulose, each of which was weighed to 2 grams and dissolved in 100 ml distilled water to obtain the 2% solutions.

Vitamin B12 powder corresponding to the recommended concentration of 2.4 micrograms was added to each and mixed well (Office of Dietary Supplements - Vitamin B12, n.d.).

The mixture was pipetted out dropwise into the prepared calcium chloride solution.

2.3) Encapsulations of Vitamin B12 at 3%, 4%, 5%, 6%, 7% concentrations of Dietary fibre:

The previous steps were repeated for higher concentrations of the fibres in increments of 1 % (3%, 4%, 5%, 6%, 7% and 8%) with corresponding weights of alginate, pectin, and cellulose in distilled water as long as the capsules were obtained.

The obtained bead capsules were washed with distilled water to remove excess calcium chloride.

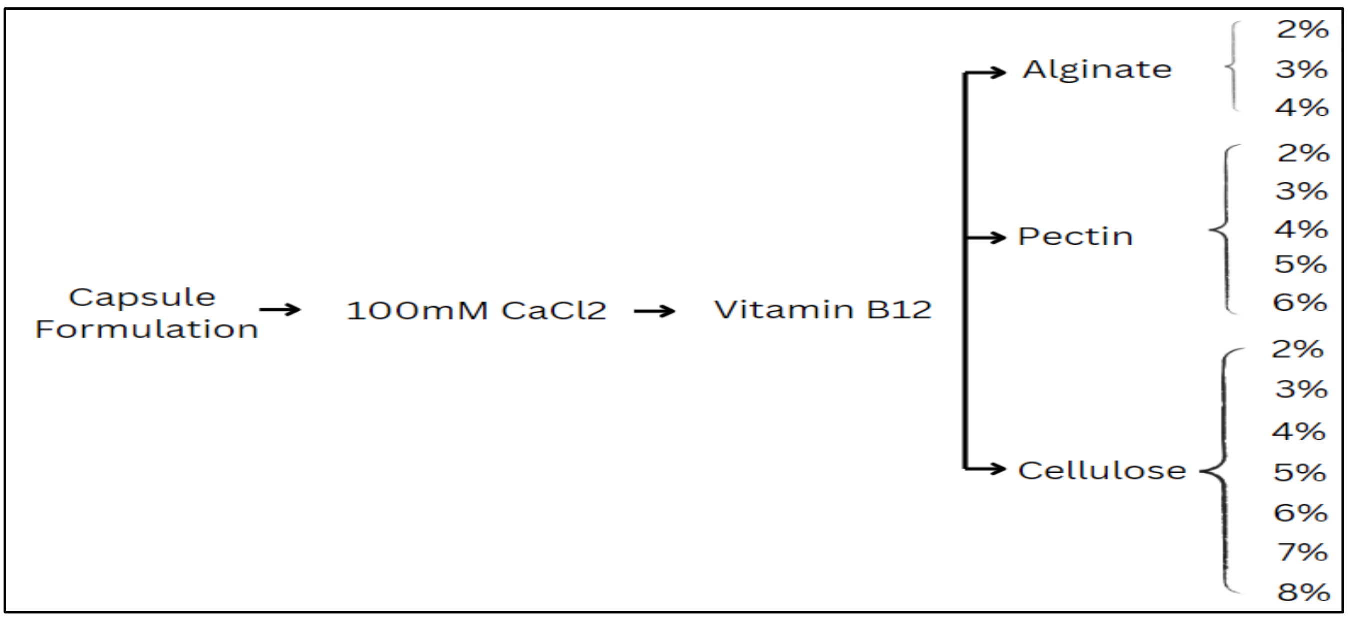

Figure 4. Experimental design for vitamin B12- dietary fibre encapsulate formulation.

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3. Results and Discussion

Figure 5. Encapsulation of Vitamin B12 with 4% Alginate

Figure 6. Encapsulation of Vitamin B12 with 6% Pectin

Bead capsules of Vitamin B12 were obtained in alginate at 4% concentration.

Bead capsules of Vitamin B12 were obtained in pectin at 6% concentration.

Bead capsules of Vitamin B12 were not obtained in cellulose up to a concentration of 8%, following which the matrix turned very thick making it unsuitable for obtaining capsules.

The wet laboratory observations can be potentially justified as follows.

Alginate and Pectin follow the gelation mechanism of ionotropic gelation whereas Cellulose follows the simple mechanism of hydration for encapsulation rather than ionotropic gelation. Ionotropic gelation involves cross linking of the fibre material with divalent calcium ions which results in it being a stronger gelation approach than hydration, wherein the material absorbs water due to its hydrophilicity and swells up forming jelly capsules. This probably explains the reason as to why alginate and pectin could form capsules of vitamin B12 at lower concentrations unlike cellulose (Smrdel et al., 2008) (Aguilar et al., 2015) (Smyth et al., 2018).

Next, within alginate and pectin, the higher threshold concentration of pectin can be explained as follows.

Pectin is a complex polymer of galacturonic acid with a higher degree of methyl esterification of the carboxyl groups compared to that in alginate, a linear polysaccharide of guluronic acid and mannuronic acid. This higher extent of esterification of the carboxyl groups in pectin compared to alginate leads to lower availability of free carboxyl groups for interaction with the calcium ions, thus resulting in a much higher concentration of pectin being required for

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encapsulating vitamin B12 compared to alginate via ionotropic gelation (Shen et al., 2022).

Table 1: Comparison of concentrations % of different dietary fibres required to obtain capsules of Vitamin B12

Concentration (w/v)	*Alginate*	*Pectin*	*Cellulose*
2%	-	-	-
3%	-	-	-
4%	+	-	-
5%		-	-
6%		+	-
7%			-
8%			-

LEGEND:

+	**Obtained Capsules**
-	**Capsules Not Obtained**

4. Conclusion

The concentration of Alginate that is required to encapsulate vitamin B12 is 4%. On the other hand, the concentration of Pectin required to encapsulate vitamin B12 is 6% and no capsules were obtained with cellulose. Correspondingly, the order of quantity of each of the three dietary fibres required for encapsulating vitamin B12 using a fixed volume of distilled water is Alginate followed by Pectin, with no vitamin B12 capsulation observed with cellulose as a matrix.

Thus, the conclusion drawn is that alginate would be the best suited matrix material among the three for vitamin B12 encapsulation based on the quantity of the dietary fibres required alone, as a sole parameter of comparison.

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