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CONTROLLED RELEASE OF RIBOFLAVIN FROM ALGINATE-CLAY COMPOSITES PREPARED BY AN INDIRECT ENCAPSULATION METHOD

Lamees Ali Omar ALSAHEELI

Master's Thesis

Supervisor

Assoc. Prof. Dr. Hakan KAYGUSUZ

İstanbul, 2025

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The thesis titled Controlled Release of Riboflavin from ALGINATE-CLAY COMPOSITES PREPARED BY AN INDIRECT ENCAPSULATION METHOD, prepared by LAMEES ALI OMAR ALSAHEELI and submitted on 02/07/2025, has been **accepted unanimously** for the degree of Master of Science in Biomedical Sciences.

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Assoc. Prof. Dr. Hakan KAYGUSUZ
Supervisor

Thesis Defense Committee Members:

Assoc. Prof. Dr. Hakan KAYGUSUZ	Department of Basic Sciences, Altınbaş University,	_____
Prof. Dr. Yasa Ekşioğlu ÖZOK,	Department of Electrical and Electronics Engineering, Altınbaş University,	_____
Asst. Prof. Dr. Bircan DINÇ	Department of Basic Sciences, Bahçeşehir University,	_____

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Lamees Ali Omar ALSAHEELI

Signature

DEDICATION

I dedicate this study to my thesis supervisor, Assoc. Prof. Dr. HAKAN KAYGUSUZ, and Arş. Gör. BURCU ORHAN for leading me to finish my thesis, and to my mother, father, husband, children, and everyone who supported me.

ABSTRACT

CONTROLLED RELEASE OF RIBOFLAVIN FROM ALGINATE-CLAY COMPOSITES PREPARED BY AN INDIRECT ENCAPSULATION METHOD

ALSAHEELI, Lamees Ali Omar

M.Sc. Biomedical Sciences Program, Altınbaş University,

Supervisor: Assoc. Prof. Dr. Hakan KAYGUSUZ

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Riboflavin is important for energy production and metabolic mechanisms and is considered an essential element. Alginate and montmorillonite are natural materials used as bio-based carriers due to their inherent properties, biocompatibility, and the ability to release active ingredients from the formulation in a controlled release. This study aimed to develop a controlled-release system for riboflavin using an encapsulation method using sodium alginate, which has high gelling properties, and montmorillonite (MMT), in the presence of carboxymethylcellulose (CMC), bound to calcium ions. The goal was to improve stability and bioavailability through a sustainable and biocompatible system. The release kinetics and encapsulation efficiency of beads were examined in simulated gastrointestinal media. The results show that hydrogel matrix encapsulation of riboflavin ensures a sustained release rate and good pH protection. The formula in this study has a significant potential for applications in the pharmaceutical and food industries, as well as high-efficiency packaging solutions.

Keywords: Controlled Release, Encapsulation, Hydrogels, Montmorillonite, Riboflavin, Sodium Alginate.

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ABBREVIATIONS

CMC	:	Carboxymethylcellulose
FAD	:	Flavin Adenine Dinucleotide
FMN	:	Flavin Mononucleotide
GO	:	Graphene Oxide
MMT	:	Montmorillonite
PHA	:	Polyhydroxyalkanoates
PLA	:	Polylacticacid
RF	:	Riboflavin

1. INTRODUCTION

Biodegradability, biocompatibility, and flexibility make biopolymers from plants, animals, and microbes popular in medicine. These polymers are crucial to drug delivery, tissue engineering, and regenerative medicine. Biopolymers' therapeutic and scientific uses have grown due to biotechnology and genetic engineering advances (Manivannan et al., 2024).

Biopolymers are widely used in medication delivery systems. Biopolymer-based drug delivery systems manage therapeutic agent release over time, improving treatment success and reducing adverse effects. Polyhydroxyalkanoates (PHA), biodegradable polymers generated by microorganisms, are beneficial for controlled medication release, surgical sutures, bandages, and bone plates (Çamlıbel, 2004). Polylactic acid (PLA) and its copolymer with polyglycolic acid (PGA) are often used in medical sectors since they decay naturally, removing the need for post-surgical removal (Çamlıbel, 2004).

Biopolymers scaffold tissue regeneration in tissue engineering. Collagen, a naturally occurring protein, is needed for tissue regeneration and wound healing. Cross-linked collagen hydrogels, delivering medicines or homeostatic agents, stimulate cell proliferation and are essential for tissue regeneration (Çamlıbel, 2004). Chitosan and then alginate are used to produce structures that stimulate tissue growth, particularly in wound care, and release active agents (Ahmad Raus et al., 2021; Feroz et al., 2020). Biopolymers also contribute to the development of regenerative medicine. Hydrogels and scaffolds made from natural gums such as xanthan gum and pectin promote tissue growth and healing (Piola et al., 2022; Eivazzadeh-Keihan, 2022). Cellulose, when combined with hyaluronic acid, can also help repair tissues that may be damaged (Yasin et al., 2022).

3D printing, when available, could open up many new possibilities, particularly for biopolymers within custom medical devices. Biocompatible materials are also being used to create implants and, subsequently, prosthetic limbs, as well as organs on chips (Shiva et al., 2023).

Polymers, because of their desired features such as biodegradability and biocompatibility, have been the focus of substantial study, especially for their uses in the medical and healthcare industries. This research has been conducted for polymers. Within the realm of medicine, it has been shown that the use of polymeric and synthetically generated materials

may bring about favorable effects (Shiva et al., 2023). Effective dry eye disease treatments include CMC-based enhanced-viscosity artificial tears. These products promote corneal epithelial repair and reduce pain, improving quality of life for those with this illness (Bruix et al., 2006; Noecker, 2006). Binding to corneal epithelial cells improved healing, making the chemical essential for ocular wound therapy (Garrett et al., 2007). CMC's versatility makes it useful in medicines, food, and cosmetics as well as ophthalmology. Pharmaceutical companies utilize CMC as a binder and excipient in tablet formulations to improve active ingredient distribution. It stabilises, emulsifies, and thickens food and maintains texture and moisture in cosmetics (Jones et al., 2017).

CMC has several uses outside of personal care and drugs. This biodegradable, non-toxic, and renewable substance is perfect for generating sustainable environmental remediation products. Recent research has combined CMC with graphene oxide (GO) to generate composite materials with improved characteristics. CMC/GO composite aerogel globules cross-linked with chitosan that adsorb methylene blue and chromium (VI), showing environmental cleaning potential (Huang et al., 2019).

The dairy, ice cream, water-based gule, and drilling sectors employ it (Pirsa & Hafezi, 2023). The compound's stability, emulsifier, and thickening properties improve consumer product quality and uniformity across various domains. CMC's potential to solve global healthcare, environmental, and material science problems is growing as research progresses. CMC-based composites, especially when combined with graphene oxide, may help provide creative, sustainable solutions across sectors (Abd-Elhamid et al., 2022).

Thus, the importance of Riboflavin (RF), generally known as Vitamin B2, was stressed. This water-soluble vitamin is required for many physiological activities. It is essential for energy metabolism as a cofactor for oxidation and reduction enzymes. Milk, organ meats (particularly calf liver), eggs, salmon, nuts, legumes, and green leafy vegetables contain it naturally. Riboflavin is found in foods, but the body cannot store it; thus, it must be eaten frequently to prevent deficiency (Cardoso et al., 2012).

Since Riboflavin is in ordinary foods, a balanced diet may include it. Riboflavin is available as a dietary supplement for those with inflammatory disorders or oxidative stress, or who need more. It is mostly harmless and eliminated in the urine when consumed in excess

(Buehler, 2011). Medical or nutritional demands may change the recommended daily dosage for Riboflavin (Powers, 2003).

Protecting Riboflavin from environmental conditions that diminish its potency improves bioavailability and efficacy. Light destroys light-sensitive Riboflavin. To avoid light exposure, it is usually stored in opaque or dark containers. Riboflavin also resists heat; therefore, cooking does not damage it (Cardoso et al., 2012). Riboflavin should be stored in a cold, dark area to stay effective (Buehler, 2011).

Due to its antioxidant and metabolic effects, Riboflavin helps health. It reduces oxidative stress, which is connected to diabetes, ischemia, and neurological illnesses, including ageing and cataracts (Zou et al., 2015; Cheung et al., 2014). SOD and catalase, activated by Riboflavin, decrease damaging free radicals in the body, protecting cells from oxidative damage (Pompella et al., 2003).

Riboflavin is an antioxidant and neuroprotective. Brain damage from ischemia or stroke may be reduced by regulating oxidative injury pathways (Tripathi et al., 2014). It activates neutrophils and macrophages, which fight infections and inflammation, to strengthen the immune system (Araki et al., 1995; Mazur-Bialy, 2013).

Riboflavin deficiency may cause riboflavinosis, which causes cheilitis, glossitis, and a scaly rash on the scrotum or vulva (Cardoso et al., 2012). Fatigue and weakness might result from low Riboflavin levels, which hinder energy metabolism. Since Riboflavin is needed for cell development and function, the deficit may impair the immune system and skin (Buehler, 2011).

Riboflavin, often known as vitamin B2, is a water-soluble vitamin that helps make the coenzymes Flavin mononucleotide (FMN) and Flavin adenine dinucleotide (FAD), which are essential for cellular metabolism and energy generation.(Suwannasom et al., 2020).Riboflavin cofactors enzymes involved in cellular respiration and repair, boosting metabolic health (Chaves et al., 2015; Thakur et al., 2017).

Riboflavin protects against cancer, cataracts, migraine, and anemia in clinical settings. It modulates folate metabolism and affects cell development and apoptosis enzymes, lowering cancer risk, notably colorectal and breast malignancies (Chaves Neto et al., 2015; Machado,

2013). High-dose Riboflavin inhibits tumour cell proliferation in animal experiments, suggesting cancer prevention and therapy. Riboflavin may improve chemotherapy effectiveness and minimise toxicity, making it a promising therapeutic agent (Thakur et al., 2017).

By protecting lens proteins and reducing oxidative damage, Riboflavin consumption reduces cataract risk, especially in the elderly. Its use in migraine prevention, particularly in children and adolescents, suggests it may also treat neurological problems (Sherwood, 2014; Condó et al., 2009).

Riboflavin is found in dairy, eggs, green leafy vegetables, and fortified cereals. Supplementation may be needed for people. Poor diets, malabsorption diseases, and rice dependence may need extra Riboflavin (Ashoori & Saedisomeolia, 2014). Riboflavin-enriched meals like fermented dairy and sourdough bread may boost consumption in these circumstances. According to studies, Lactobacillus plantarum and Propionibacterium freudenreichii may create Riboflavin during fermentation, making these foods a natural source of the vitamin. Riboflavin supplementation might also help prevent iron-deficient anaemia by improving iron absorption (Ashoori & Saedisomeolia, 2014). Studies demonstrate that Riboflavin improves osteoblast differentiation and bone production (Machado et al., 2013).

Riboflavin is susceptible to light, heat, and alkalinity, which destroy the vitamin and diminish its efficacy. Therefore, Riboflavin stability throughout storage, packing, and consumption is crucial. Nano/micro-encapsulation has been investigated to shield Riboflavin from environmental influences that might degrade it. Riboflavin is protected from light and temperature extremes in protective coatings, retaining its bioactivity and enhancing bioavailability (Thakur et al., 2017)

Functional meals and dietary supplements often include Riboflavin to maximize consumption. Functional foods like riboflavin-enriched yoghurts, fermented milks, and fortified cereals make it easy to get this vitamin and improve absorption and retention (Ashoori & Saedisomeolia, 2014). Biotechnological methods like encapsulation may better transport Riboflavin to target tissues, improving its therapeutic effects in cancer, neurological disease, and cardiovascular health (Machadeo et al., 2013)

Riboflavinosis, or Riboflavin deficiency, may be dangerous. Sore throat, oral and mucous membrane swelling and redness, cheilitis, glossitis, and cataracts are deficient symptoms (Toyasaki, 1992; Skalka & Prchal, 1981). Lack of Riboflavin may also impede iron absorption, alter tryptophan metabolism, enhance oxidative damage, and impair cellular and mitochondrial function (Ashoori & Saedisomeolia, 2014). Where staple diets lack riboflavin-rich items, including milk, beef, and fortified cereals, deficiency is frequent. Supplementation and nutrition are crucial for riboflavin-deficient treatments. Fortified foods and supplements are necessary to provide nutritional demands and prevent deficiencies in malnourished or poor-diet settings (Jacques et al., 2001).

Riboflavin (Vitamin B2), a water-soluble vitamin, helps the body produce red blood cells, grow tissues, and release energy from proteins. Riboflavin cannot be synthesized or stored; hence, it must be eaten. Milk and dairy products are the major sources of Riboflavin. However, plant-based eaters, pregnant people, youngsters, and athletes may be more susceptible to Riboflavin insufficiency (Peechakara et al., 2024).

Although uncommon, Riboflavin deficiency may cause anaemia, a painful throat, skin fissures, and a swollen tongue. This is why high-risk persons are advised to take Riboflavin supplements. The U.S. also approves Riboflavin. FDA-approved for treating corneal ectasia post-refractive surgery and keratoconus, a degenerative eye disease. Riboflavin is used off-label for migraine prophylaxis and phototherapy in newborns (Peechakara et al., 2024).

Although found in many foods, RF's bioactivity is vulnerable to light, heat, and oxidation. This susceptibility may reduce its efficacy when eaten unprotected, particularly under stressful gastrointestinal settings. Thus, improved micro/Nano encapsulation of Riboflavin is essential for bioavailability. These approaches regulate vitamin release, prevent breakdown, and distribute it to intestinal absorption locations (Suwannasom et al., 2020).

Cold-set gelation with whey protein isolate may improve Riboflavin stability and bioavailability. Microbeads protect riboflavin against gastric breakdown. In vivo studies showed that these microbeads were partly disintegrated after ingestion, allowing riboflavin to reach the intestines. Riboflavin release from microbeads followed a pseudo-first-order kinetic model, allowing prolonged and controlled release (Vicente et al., 2014; Hu et al., 2015).

Using an intensification technique in ultrasonication, transglutaminase is cross-linking soy protein isolate to encapsulate riboflavin, which improves the efficiency of encapsulation and gelation yield because it retards riboflavin release in simulated stomach and intestinal fluids. This longer release profile enhances bioavailability of riboflavin through stabilization and facilitates absorption (Hu et al., 2015). Added the ionotropic gelation with alginate and chitosan nanoparticles, which are ideal for riboflavin encapsulation. These are 119.5nm-sized nanoparticles, possessing a negative zeta potential, that are stable and can release the vitamin slowly for preventing early degradation and increasingly improving the intestinal absorption (Azevedo et al., 2014).

Riboflavin has been encapsulated in nanogels derived from ultrasonicated soy protein () and dextran. These 143.3-nm nano gels encapsulate riboflavin and continuously release it in simulated stomach fluid-in effects. They surround the vitamin to protect it from stomach degradation while delivering riboflavin using an intestinal absorption mechanism (Jin et al., 2016). In addition, using phenylalanine ethyl ester-alginate bio conjugate from self-assembly nanoparticles are self-assembled under neutral pH-adjusted environment levels of the intestine (Zhng et al., 2016).

Hydrogenated canola oil solid lipid nanoparticles (SLNs) encapsulate Riboflavin inside them. Lipid-based nanoparticles tend to be very stable in the environment. The prolonged release characteristics carry Riboflavin to the mid-intestinal area for absorption; the lipid matrix also elevates the solubility and bioavailability (Couto et al., 2017).

They have also been developed using the Co-precipitation crosslinking process of dissolution (CCD). These submicron particles involve human serum albumin as a wall material. These biocompatible and controlled-release riboflavin particles increase absorption in the gastrointestinal tract and bioavailability (Suwannasom et al., 2019).

Montmorillonite (MMT), being biocompatible and cost-effective, is one of various phyllosilicate clay minerals widely used in industrial and medical applications, primarily for its nanolayer characterization, having high cation exchange capacity and adsorption abilities. Such properties make MMT useful in the areas of drug delivery and dental materials (Prasad et al., 2024). Besides montmorillonite, also improves collision efficiencies, although very high projection still decreases the release rates significantly (Kaygusuz et al., 2015).

MMT has so far been extensively investigated for the regulated absorption and sustained release of medicine. This can, however, increase the bioavailability and solubility of a hydrophobic drug by forming complexes with other polymers. The polymeric matrices are entrapped through electrostatic interactions, especially with cationic medicine, and liberated (Park et al., 2016).

Property of detoxification, apart from medicines, can even adsorb some dangerous heavy metals such as arsenic, cadmium, lead, undesirable anions in water such as fluoride (Thakre et al., 2010). The detoxifying characteristics make it suitable for environmental and medicinal applications, such as managing water-borne pollutants. It is also said that it may detoxify by adsorbing aflatoxins and hazardous chemicals like (Desheng et al., 2005; Shi et al., 2005)..

MMT may be used in food formulations as well as medicine. GI troubles are treated with this natural clay. Sodium MMT has been shown to help beneficial probiotics such as Lactobacillus casei survive in the gut (Li et al., 2014). When MMT is incorporated into an alginate hydrogel, it traps protein in the composite matrix and provides controlled intestinal release of protein from that matrix. BSA exhibits a strong interaction with Alg-MMT by penetrating the clay layers. In this way, the composite beads exhibit high BSA loading efficiency and low protein release in both acidic gastric and intestinal media, compared to when using pure alginate, where protein release is very low (Kaygusuz & Erim, 2013). MMT has also been shown to treat irritable bowel syndrome (IBS) and diarrhea (Khediri et al., 2011; Ducrotté et al., 2005).

MMT is gaining popularity in dentistry as a restorative material reinforcer. Its high aspect ratio and polymer interfacial bonding make it a good dental composite filler, improving mechanical qualities and lifespan. Adding calcium, fluoride, silver, and zinc to MMT-based materials improves their remineralization and antibacterial capabilities, which improves dental treatments (Prasad Kumara et al., 2024). Due to its potential properties, MMT's inclusion into tooth restorative materials is still under study. The unique adsorption capacity of MMT and its versatility in diverse applications clearly identify MMT as the material of choice for future revolutions in various fields. In the field of pharmaceuticals, the applications of MMT address bioavailability, drug release, and improvement issues. In

environment-related applications and in dentistry, such applications of MMT are becoming popular (Park et al., 2016).

1.1 STUDY OBJECTIVE

The present study is aimed at assessing the controlled release of riboflavin from alginate-clay composites that were prepared by the indirect encapsulation technique.

1.2 SIGNIFICANCE OF THE STUDY

Really important for study is CMC applications, which along with riboflavin in terms of preparations, is important medically and cosmetically as well as agriculturally (Pourjavadi et al., 2006; El-Naggar, 2016). On top of all these are its uses in engineering, specifically in medicine.It becomes more relevant for the study because of the importance of applications of CMC and riboflavin medically, cosmetically, and agriculturally according to the preparations used (Pourjavadi et al., 2006; El-Naggar, 2016). Plus all these are incorporated into engineering, mainly in medicine (Kalaycıoğlu et al., 2020; Lin & Chiu, 2021).

2. GENERAL INFORMATION

2.1 RIBOFLAVIN

Riboflavin, otherwise known as vitamin B2, is categorized as a water-soluble compound and an essential member of the B-vitamins group. It is crucial in maintaining health and has proven to have protective effects from many medical conditions, such as ischemia and sepsis, when taken in sufficient amounts either through diet or supplementation. Also, studies illustrate riboflavin intake correlates with a lower risk for particular cancers in both men and women (Suwannasom et al., 2020).

First isolated in 1879 as a yellow pigment occurring naturally in milk, riboflavin was first used as a dye (Cardoso et al., 2012). This chemical structures defined as 7,8-dimethyl-10-ribityl-isoalloxazine consists of a flavin-isoalloxazine ring with ribitol-side chains (Dym & Eisenberg, 2001). Heat-stable riboflavin endures severe conditions of degradation during cooking; it is sensitive to light, which can lead to its breakdown upon exposure. Natural sources rich in riboflavin include dairy products such as milk, as well as eggs, organ meats, and green leafy vegetables. Due to its vital role in human metabolism and its prevalence in various foods, riboflavin is widely recognized as a key nutrient for maintaining overall health.(Buehler, 2011).

2.2 BENEFICIAL HEALTH EFFECTS OF RF

2.2.1 Antioxidant Properties

Oxidative stress is a critical factor implicated in the development and progression of numerous human diseases, including ischemia-reperfusion injury, type 1 and type 2 diabetes, and angina pectoris (Toyasaki, 1992). It also contributes significantly to the aging process and the onset of various degenerative conditions. Scientific evidence suggests that supplementation with riboflavin can enhance longevity and reproductive capacity in fruit flies, primarily by boosting the activity of key antioxidant enzymes (Zou et al., 2015). These effects are largely attributed to its role in extending lifespan through improved oxidative balance. In addition, riboflavin therapy has been proposed to reduce oxidative stress in cases of keratoconus by promoting the formation of a healthy extracellular matrix and decreasing levels of intracellular reactive oxygen species (ROS)(Cheung et al., 2014).

2.2.2 Reperfusion Oxidative Injury

Oxidative reperfusion means that this kind of injury arises from damage, either directly or indirectly, to tissues that leads to a lack of perfusion or lessened perfusion. More so at the time when free radicals increase, as do the inflammatory cytokines (Suwanasom et al., 2020). This can be greatly reduced by riboflavin, which has an overall high efficacy in reducing oxidant injury. This is by virtue of its ability to scavenge the resulting free radicals, mainly those free radicals responsible for injury due to reoxygenation. Scientists have also discovered that the presence of reactive oxygen species has a powerful physiological impact on diseases, particularly injuries resulting from ischemia or possibly resulting from reperfusion. Furthermore, animal research has demonstrated that" RF" has a profound impact on the entire body, including the protective effects it has on nerves when ischemia occurs (Tripathi et al., 2014).

2.2.3 Immune System

Riboflavin is critically involved in regulating immune function by promoting the proliferation of key immune cells such as neutrophils and monocytes, while also enhancing the phagocytic activity of both macrophages and neutrophils (Qureshi et al., 2011). Experimental evidence indicates that the viability and replication of RAW 264.7 macrophage cells are highly dependent on sufficient levels of riboflavin. In environments with limited riboflavin availability, these cells exhibit impaired proliferation (Verdrengh & Tarkowski, 2005). Moreover, in a poultry model, a nutrient formulation comprising quercetin, riboflavin, and delta-tocotrienol effectively decreased circulating levels of key inflammatory mediators, including tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO) (Qureshi et al., 2011).

Beyond systemic immune modulation, riboflavin influences localized inflammatory processes. It has been shown to limit early neutrophil migration, thereby restricting the accumulation of activated granulocytes in peripheral tissues—a mechanism that likely contributes to its inflammation-reducing properties (Verdrengh & Tarkowski, 2005). Riboflavin possesses photoactive and cytotoxic properties and is being studied for potential application as a virus-inactivating agent and as adjunct therapy in neoplastic treatments like chemotherapy and radiotherapy. Moreover, riboflavin has demonstrated the potential to

decrease the infiltration of T cells and suppress the generation of donor-specific antibodies during the early post-transplant period (Iwanaga et al., 2007).

At the molecular level, riboflavin can inhibit inflammatory signals by impeding the nuclear factor kappa B (NF-κB) route. Under normal circumstances, NF-κB activation relies on its inhibitor, IκB's degradation, which allows it to migrate into the nucleus for the transcriptional start of pro-inflammatory protein-encoding genes. Acting as a proteasome inhibitor, riboflavin blocks this pathway by restricting the oxidant-mediated activation of NF-κB, which would further reduce the expression of inflammatory-responsive genes (Qureshi et al., 2011).

Reactive oxygen species (ROS) are synthesized in a broad variety of chemically reactive forms of oxygen, such as superoxide anion (O_2-), singlet oxygen (O_2•), hydrogen peroxide (H_2O_2), or ozone (O_3), with all of them having unpaired valence electrons and thus being highly nucleophilic (Sies & Jones, 2020). These species play vital roles in innate immunological defense, such as activation of their inflammasome and destroying pathogen invasion using phagocytes (Bassoy et al., 2021), yet they are also vital as signaling molecules for the essential biological functions of life, such as embryogenesis (Elhiti & Stasolla, 2014) and tissue regeneration (Vullien et al., 2024).

As modern biomedical research continues to change, more people are becoming interested in exploring using micronutrients such as riboflavin for therapeutic effects. Riboflavin may be the potential remedy for inflammatory and immune-related disorders either given in combination with conventional antibiotics or as a singular therapeutic measure (França et al., 2001; Bertollo et al., 2006).

2.2.4 Migraine

Most patients diagnosed to suffer from mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome indicate disturbances in mitochondrial energy metabolism accompanied by migraine-like headache. Clinical observations reveal that riboflavin supplementation eases migraine symptoms attributed to the enhancement of mitochondrial function. Riboflavin in particular, among other vitamins, has been shown to lead to the prevention of migraines as a result. Effectively attributed to support of cellular

energy production and lessening of mitochondrial dysfunction, which is a core pathophysiological factor in migraines, is the major thrust of this action (Shaik et al., 2015).

2.2.5 Cataract

Studies indicate that cataracts can develop as a result of large amounts of proteins clustering together to cause cloudiness in the lens. This is where the importance of RAF comes in; intake of it from natural, unprocessed food or supplements can lower a person's risk of cataract formation in the lens (Jacques et al., 2001). A high quantity of RAF yields protection against cataracts and helps patients prevent civilizations' progress in the advancement of their disease, normally related to aging.The cloudiness of the lens can sometimes appear developed when a considerable amount of protein accumulates. This is what RAF concerns. Consumption of it through fresh foods or supplements is said to reduce the risk of someone's forming cataracts in the lens (Jacques et al., 2001). A very high dose of RAF also has its protective measures against cataracts, and if the patient has already caught the disease, this has curbed its advancement, which usually is age-induced (Seetharam Bhat, 1987).

2.2.6 Premenstrual Syndrome

Premenstrual syndrome (PMS) comprises a plethora of symptoms, both physical and emotional, that usually occur in the luteal phase of the menstrual cycle and subside with the onset of menstruation. The effects of PMS can be quite strong on one's quality of life and daily activities. It is found that increased dietary consumption of riboflavin is protective against PMS. For instance, it was noted that people who consumed about 2.52 mg of riboflavin daily were 35% less likely to develop PMS than people who had lower intakes around 1.38 mg a day. The above illustrates adequate riboflavin consumption's contribution towards reducing the risk and severity of PMS symptoms (Chocano-Bedoya et al., 2011).

2.2.7 Bone

Osteoporosis is a bone disease. Therefore, the intake of vitamins is an important factor and one of the most decisive ones that may affect fracture risk. According to the study by Chaves Neto et al. (2010), Riboflavin effectively promoted osteoblast differentiation via ascorbate and beta-glycerophosphate in MC3T3-E1 cells. The action includes a G0/G1 arrest enhancement (Chaves Neto et al., 2010).

2.2.8 Neuropathy

There is sufficient evidence that RF plays an important role in early brain development immediately after birth (Foley et al., 2014). The understanding of the condition was seen wherein mutations in the SLC52A2 gene can lead to a process that reduces Riboflavin uptake and production of the Riboflavin transporter protein (RFVT2). Following treatment with very high dosages of oral Riboflavin, patients with such mutations were shown to improve significantly over a long duration of time concerning both their clinical and biochemical states (Foley et al., 2014).In an experiment of treating the C57/B mice with riboflavin, it was demonstrated that riboflavin has a protective action against the glutamate/NMDA-induced excitotoxicity of cerebellar granule neurons (Powers et al., 2011).

2.2.9 Diabetes Mellitus

Bestowing unto the state of the art in scientific investigation in the contemporary world is a plethora of factors in relation to how oxidative stress leads to the pathogenesis of Type 2 Diabetes Mellitus. Reactive oxygen species contributions, when chronicized, occur together with inflammation, impairing the insulin signaling pathway and glucose metabolism, worsening the spectrum of complications. Known riboflavin, a dietary antioxidant, can offer some possible benefits against oxidative stress and inflammatory symptomology. In fact, it assists in blood glucose regulation, in part by enhancing glucose absorption in intestines and uptake by peripheral tissues such as skeletal muscle and white adipose tissue. The data from studies in diabetic mouse models show that riboflavin treatment reduces oxidative stress significantly, lowers levels of tissue and DNA damage, and exerts effect in keeping the blood sugar levels as required. Besides, there are indications that its action through antioxidant properties may exhibit some cardioprotective advantages, as some evidence has been found in animal models of type 1 diabetic cardiomyopathy showing improved cardiac function after riboflavin treatment. Collectively, these findings indicate that riboflavin may serve as a supplementary agent in the management of diabetes and its cardiovascular complications (França & Vianna, 2010).

2.2.10 Cardiac Abnormalities

Riboflavin significantly reduces acute rejection and early oxidative stress of the graft after organ transplantation. Additionally, Riboflavin significantly reduces graft-induced coronary vasculopathy, which results from cardiac ischemia and reperfusion. In a mouse heart transplant model, Riboflavin pre-treatments may protect tissues from oxidative damage by reducing myocardial lipid peroxidation, leukocyte infiltration, cytokine production, and vasculopathy in the heart graft during transplantation (França & Vianna, 2010).

2.2.11 Hypertension

High blood pressure is considered the most important risk factor for death worldwide (Lopez et al., 2006). It is estimated to be responsible for 8 million premature deaths annually (Lawes et al., 2008). One study suggests a link between Riboflavin use and blood pressure regulation. Riboflavin supplements did not cause any side effects in animal experiments, but they did cause a significant reduction in systolic blood pressure, which was independent of age in both young and adult rats (França & Vianna, 2010).

2.3 MECHANISM OF ANTIOXIDANT PROTECTION

Riboflavin's potent antioxidant properties primarily stem from its function as a precursor to the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes play a crucial role in the activity of various enzymes involved in oxidative metabolism, notably glutathione reductase (GR). GR, which acts as a free radical scavenger, depends on FAD as a necessary cofactor. The protective effects of Riboflavin against numerous diseases have been well documented (Ashoori & Saedisomeolia, 2014). Research has demonstrated Riboflavin's capacity to reduce reactive oxygen species (ROS) across multiple pathological conditions, including its anti-aging effects and its ability to mitigate drug-induced toxicity during treatments.

Furthermore, further study has been done about various vitamins including vitamins E and C, carotenoids, and Riboflavin, with a special focus on their antioxidant properties. The mechanisms of the antioxidant effect of Riboflavin are still in a partially understood realm. As a coenzyme in the forms of FMN and FAD, Riboflavin lowers oxidative stress by combating lipid peroxidation and oxidative damage inflicted during reperfusion. Riboflavin,

in conjunction with GR, catalyzes a reaction where oxidized glutathione (GSSG) is converted back to reduced glutathione (GSH). The role of FAD is to transfer the hydrogen from NADPH to GSSG and in doing so is involved in the restoration of GSH. This reduced glutathione is an important, endogenous antioxidant that acts mainly inside the cell, neutralizing ROS. By using glutathione peroxidase (GPx), GSH provides detoxification of peroxides, including lipids, hydroperoxides, and conversion into lipid alcohols while generating GSSG. It follows that any deficiency of Riboflavin will increase lipid peroxidation and oxidative damage (Suwannasom et al., 2020).

2.4 ENCAPSULATION TECHNIQUES

An interesting number of encapsulation practices have made alginate a primary polymer for hydrogel formation of micro to macro size. In recent years, alginate is regarded very much as a good encapsulating medium on account of its biodegradability, biocompatibility, and non-toxic properties. Applications of alginate encapsulations are tremendous and are likely to rise tremendously in the coming years due to the increased global demand for sustainable materials in varied technological fields (Colin et al., 2024). Alginates held distinct advanta for encapsulation and have been thus recognized (Benacief et al., 2021).

The substrate alginate was isolated and patented by Edward C. C. Stanford in 1881, a British chemist and pharmacologist (Donati & Paoletti, 2009). When utilized for encapsulation, the active ingredient becomes entrapped within the alginate gel matrix, safeguarding it from environmental factors while allowing controlled and gradual release. Alginate encapsulation holds particular promise, as it provides means to control the release rate of the encapsulated substance, which is conducive to its biotechnological application that includes controlled drug delivery (Grøndahl, Lawrie, Anitha, & Shejwalkar, 2020; El-Aassar, Hafez, El-Deeb, & Fouda, 2014; Ulag, Celik, Sengor, & Gunduz, 2022; Bankole et al., 2022).

The alginate-based encapsulation methods have gained avenues across different fields of medical and healthcare industries (Lee & Mooney, 2012; Baruch & Machluf, 2006; Smidsrød & Skjåk-Braek, 1990; De Vos, Hoogmoed, & Busscher, 2002; Kang, Lee, Huh, & Takayama, 2021; Dodero et al., 2021), food industry (De Vos, Faas, Spasojevic, & Sikkema, 2010; Ramos, Cerqueira, Teixeira, & Vicente, 2018), and agricultural function in crop protection and biocontrol as well (Pour, Saberi-Riseh, Mohammadinejad, & Hosseini,

2019; De Oliveira, Fraceto, Bravo, & Polanczyk, 2021; Schoebitz, López Belchí, & Roldán, 2013; Meftah Kadmiri et al., 2021; Szopa et al., 2022; Barrera-Cortés et al., 2017; Zhang et al., 2023). Materials such as SA and montmorillonite clay (MMT) are used in packaging:

2.4.1 Sodium Alginate (SA)

Sodium alginate (SA) is generally found in nature in its anionic form and is found in large quantities on the seafloor. It has been isolated from the production of brown algae like Macrocystis aeruginosa and Sargassum, in addition to bacterial species such as Pseudomonas aeruginosa and nickel-fixing bacteria. SA was extracted from other seaweeds for industrial purposes some time back. There has been increasing interest by scientists in sodium alginate, which is incorporated in some processes such as condensation, followed by synthesis and crystallization, as well as in its application as an alginate-based edible film and coating from millet (Sellimi et al., 2015).

It is also called SA alginate known for its properties like expected intracellular toxicity and huge potential for membrane formation, which may possibly qualify it as a natural polysaccharide prophylactically desired in food and pharmaceutical industries. However, the utilization of SA alginate in conditions involving temperature does not happen because the material becomes unstable and soluble at low temperatures (Nezamdoost-Sani et al., 2023; Riseh et al., 2021). For example, modified SA alginates and their complex packaging enable protection, and customization of special materials and pharmaceuticals (Andrade et al., 2004; Szekalska et al., 2016).

Moreover, as it has high hydrophilicity and long-lasting hydroxyl groups, a single SA can be more suitable in increasing the hydrophobicity (Hecht & Srebnik, 2016).

Bacteria are formed when molecules with a biocompatible structure of citric acid are easily decomposed into carbon dioxide at high temperatures (Yan et al., 2024). Furthermore, in addition to their poor biocompatibility, they cannot be prepared from common cations. The main plan is to rapidly explore the positive effects of comprehensive research and cross-linking between them, exhibiting different properties depending on the different ionic cross-linking. Its most important function is the coagulation property of citric acid (Nezamdoost-Sani et al., 2023),

Ca2+ specifically. The cavity that serves as a Ca2+ binding site is created by the conjugation of the adjacent guluronic acid. This cavity binds to the carboxyl group on the SA core, resulting in the formation of an eggshell-like spatial structure. The physical, chemical, or both properties of SA are influenced to varying degrees by the M/G ratio and the block length of the different molecular chains. This is a general statement. As the concentration of Ca2+ ions increases, SA with a higher G-block ratio (M/G < 1.0) produces a stiff gel. On the other hand, SA with a higher M-block ratio (M/G > 1.0) forms a soft, elastic gel. This results in a higher M-block ratio in the gel, making it softer and more elastic. Because citric acid is sensitive to changes in pH, when the pH is lower than 3.6 (the pKa value of the uronic acid residue), the citric acid molecular chain contracts due to the protonation of the carboxyl group. Furthermore, the degree of ionization decreases, resulting in the formation of an acidic gel through intermolecular hydrogen bonding. When the pH is higher than 11, the opposite is true. The resulting carboxylate anion causes the molecular chain to stretch, making the system more susceptible to collapse and resulting in a decrease in viscosity (Chen et al., 2017).

2.4.2 Carboxymethyl Cellulose (CMC)

Carboxymethyl cellulose (CMC), sometimes called cellulose gum (FAO, 2024), is a derivative of cellulose, with carboxymethyl groups (-CH2-COOH) directly linked to hydroxyl groups within the glucopyranose monomers that form the backbone of cellulose. It is most commonly used in its solid form, the sodium salt, and in the form of sodium carboxymethyl cellulose. It was formerly marketed under the name Tylose, which is a registered trademark of SE Tylose (ShinEtsu, 2022)

2.4.3 Montmorillonite

Clay minerals are important components that can be found naturally. With a significant increase in understanding of clay structure, montmorillonite has the potential to be used to enhance the performance of a variety of materials and products within specific catalytic applications. It is also used in food additives, has antibacterial properties, is used in polymers, and is also used in other materials (Uddin, 2018).

Additionally, MMT functions very well as an additive in the field of smart coating technology for metal alloys. One of its benefits is that it is known to have qualities that guard against corrosion and resist corrosion. According to Park and Jana (2004), the most common kind of silicate is layered silicate. The layered structure of its nanoparticles may have the potential to provide enhanced corrosion protection properties (Kumar et al., 2023; Chen et al., 2022; Xing et al., 2022), according to Kumar et al. (2023, Chen et al. (2022, and Xing et al. (2022. In addition, this location is distinguished by the presence of extensive interaction locations, a broad variety of resources, and a low cost (Sun et al., 2022; Nematollahi et al., 2010; Sun et al., 2021).

2.4.4 MMT Structures

There is a 2:1 ratio between tetrahedral and octahedral sheets in MMT. A tetrahedral sheet is composed of silicon tetrahedra and oxygen, connected to the surrounding tetrahedral sheets by sharing three corners. This configuration produces a hexagonal lattice (Massaro et al., 2020). During the part formation process, the remaining tetrahedra join the adjacent octahedral sheet. Oxygen from the tetrahedral sheet and hydroxyl are often present in the octahedral sheet, which may typically consist of magnesium or aluminium in a hexagonal arrangement (Massaro et al., 2020).

2.5 IMPORTANCE AND APPLICATIONS OF RIBOFLAVIN

Encapsulation Riboflavin (7, 8-dimethyl-10-ribityl isoalloxazine, Vitamin B2) is a water-soluble organic compound with ribitylaminoantaretine heterocyclic structure and a molecular weight of 376.37 g/mol. Riboflavin is an essential nutrient for human growth, development, and maintenance. It is also required for vision, lipid metabolism, and skin health. Water-dispersed Riboflavin is applied for the irradiation of formulated foods, agricultural products, and food packaging materials. Moreover, Riboflavin is added to dairy, bakery, and baby foods. Riboflavin is also used in medicinal supplements. The synthesis of Riboflavin is not found in flora or fauna and needs to be obtained exogenously, where the daily need of Riboflavin is around 2â€- 3 mg. In the synthesis of a number of important enzymatic functions in vital processes, such as cellular respiration and lipid metabolism, play a role. Riboflavin is actively involved in enzymes, and has a low turnover time compared to other vitamins; therefore, it needs to be taken in sufficient amounts to provide.

The most important characteristic of Riboflavin is that it is effective in the emission region of the UV spectrum, increasing this wavelength and promoting photochemical reactions; it absorbs and emits colored light. SA is a water-soluble biocompatible polymer containing a copolymer of Î±-L-guluronic and Î²-D-mannuronic acid residues. In recent years, SA has been used in the encapsulation of a variety of compounds, including enzymes, proteins, and drugs. SA can be extruded in an appropriate pH (â‰ˆ4.0â€- 5.0) to achieve a desired viscosity and spread dropwise onto a cation-exchanged solution to form beads by ionic cross-linking with calcium. Beads consisting of SA are mechanically strong and are useful for the controlled release of encapsulated compounds. Optionally, for further tailoring the release profile more finely towards particular applications, second-order (commonly used), one of the models, may be used to describe the release kinetics of Riboflavin more closely. Here, riboflavin-release kinetics are examined through beads made of SA or a blend of SA and carboxymethylcellulose (CMC) cross-linked with calcium ions (Simões et al., 2019).

2.6 APPLICATIONS OF MMT IN SMART COATINGS FOR CORROSION PROTECTION

Recently, MMT-based nanoclays have seen widespread use in smart coating technology, particularly for modifying the functionality and performance of polymers (Uddin, 2018). Several significant experiments have been conducted on integrating MMT with urushiol via in situ polymerization to improve the lifespan, efficiency, and self-healing ability of smart corrosion-resistant coatings (Chen et al., 2022). Tannic acid, modified Cerium-MMT (Li et al., 2023), and a mixture of benzimidazole, sodium, and zinc (Shchukina et al., 2019) are examples of compounds used by scientists over the past several years, providing an overview of the uses of MMT in multifunctional smart coatings for a variety of institutions. This material provides a polymerization reaction for 2-fluoroaniline (PFA) (Sun et al., 2023).

To develop high-performance multifunctional coatings against metal corrosion, the PFAM-Ce nanohybrid in the coating acts as an effective additive for protection against corrosive media and ultraviolet radiation. The incorporated additives, which include MMT in the epoxy resin, provide strong water-repellent properties, excellent dispersion, and UV-protective capabilities. Furthermore, the coating heals gradually, avoiding any future damage that could lead to substrate corrosion. Since ultraviolet radiation had a greater impact on the corrosion resistance of the pure epoxy resin coating compared to the coating containing

MMT as an additive, the corrosion protection effectiveness of this coating after UV exposure was evaluated using EIS (Sun et al., 2023).

Not only that, but MMT technology has also been used in many different ways for corrosion protection and, subsequently, for the development of anticorrosive coatings. Environmentally friendly and ideal for corrosion (Sun et al., 2021).In another method, also known as the ion exchange method, cerium was introduced into the gaps of MMT layers (Sun et al., 2021; Nematollahi et al., 2010).

It was also decided to use modified MMT to study the possibility of improving corrosion protection and water resistance on steel substrates. MMT, as a nanoclay, has been shown to increase structural cohesion within the coating. Additionally, it has been found to help create a labyrinth effect within the coating, which helps extend the diffusion channel for corrosive chemicals. This, in turn, enhances the barrier properties of the coating (Mo et al., 2019).

2.7 RELEASE DYNAMICS

2.7.1 Kinetics of Riboflavin

Humans and other animals require vitamin B2, also known as Riboflavin, to survive. Healthy, well-nourished adults require between 0.9 and 1.3 milligrams of this vitamin per day. This vitamin must be obtained through meals (meat, eggs, milk, dairy products, beef liver, broccoli, kale, soybeans, peanuts, etc.) or dietary supplements, and any excess is excreted in the urine (Schwechheimer et al., 2016). Vitamin B2 plays a key role as a precursor to the coenzymes Flavin adenine dinucleotide (FAD) and Flavin mononucleotide (FMN) (Fedorovych et al., 2021).

Riboflavin is widely used in the cosmetics, pharmaceutical, food, and feed sectors (Lim et al., 2001). Annual production of Riboflavin reached 10,000 tons, and global sales exceeded US$250 million in 2020 (Zhao et al., 2021). Only about 10% of this production is used in the production of pharmaceuticals and multivitamins, while between 10% and 20% is used as colorants, and the remainder is used as animal feed (Zhang et al., 2022).

In biotechnology, Riboflavin requirements have been met, as solely fermenting microorganisms perform manufacturing. In recent decades, Bacillus subtilis, A. gooseberry, and Candida albicans have been the main models for industrialization (Schwechheimer et

al., 2016; Fedorovych et al., 2021; Lim et al., 2001; Zhao et al., 2021; Zhang et al., 2022; Liu et al., 2020; Revuelta et al., 2017). However, a wide range of other microorganisms, such as yeasts, filamentous fungi, and bacteria, are also interesting candidates for Riboflavin overproduction (Schwechheimer et al., 2016; Fedorovych et al., 2021; Lim et al., 2001; Zhao et al., 2021; Zhang et al., 2022; Liu et al., 2020; Revuelta et al., 2017).

Yeast is known to be capable of producing sufficient Riboflavin for its growth through biosynthetic processes. Some species even produce excess Riboflavin and secrete it into the fermentation broth (Russo et al., 2014). Thanks to their high specific growth rate, rapid multiplication rate, ease of cultivation and processing, metabolic diversity, low maintenance requirements, safety, and ease of genetic modification, they offer several advantages that are beneficial for large-scale production (Revuelta et al., 2017; Russo et al., 2014).

2.7.2 Nano Encapsulation of Vitamin B2

Vitamin B2, also known as riboflavin, is essential for maintaining healthy cellular metabolism and functions. However, it has the disadvantage of being highly photosensitized and poorly soluble in water. It also has the disadvantage of having a gastric barrier in the intestine, which limits nutrient storage, transport, and absorption. Selecting nanomaterials that may be suitable for encapsulating vitamin B2 is even more important to overcome these challenges. Encapsulation may improve the photo stability of vitamin B2 under ultraviolet light and prolong its release into body fluids, which may be compared to encapsulated vitamin B2. CS-PLGA nanoparticles have also shown significantly higher uptake in intestinal epithelial cells (Caco-2), suggesting improved transport and potential use in fortified food systems. In addition, CS-PLGA nanoparticles have great potential for delivering vitamin B2 into food and pharmaceutical applications. Therefore, they are used for patients with gastrointestinal diseases or malabsorption, as this vitamin B2 works to deliver it through the gastrointestinal tract (Sathiensathaporn et al., 2025). Moreover, it has been established that the CS modification of PLGA nanoparticles may boost cellular absorption in a range of cell types, such as hepatocytes C3A and MDCK, 3T6 fibroblasts (Chronopoulou et al., 2013), and intestinal epithelial cells (Caco-2) (Guo et al., 2013).

2.8 CONTROLLED-RELEASE SYSTEMS PROLONG RIBOFLAVIN AVAILABILITY

The chemical formula used for Riboflavin is the sequence 7,8-dimethyl-10-ribityl-isoaloxazine, which may indicate that it consists of a flavin-isoalloxazine ring connected via a sugar side chain called ribitol(Dym & Eisenberg, 2001). Riboflavin, a water-soluble and heat-resistant vitamin, is also known as an important form of vitamin B2. Cooking does not reduce Riboflavin levels; however, exposure to light may damage this vitamin. A wide range of foods and natural sources contain Riboflavin, such as yeast, cheese, and many dietary supplements (Cardoso et al., 2012; Buehler, 2011).

The reduced absorption of Riboflavin in humans, specifically, leads to its poor storage in vertebrates. Therefore, to prevent riboflavinosis, which is characterized by inflammation of the lips, tongue pain, and a scaly rash on the scrotum or vulva, it is necessary to take Riboflavin orally or sometimes through a nutritious diet (Buehler, 2011). This is because large amounts of Riboflavin are excreted directly in the urine rather than being stored within the body (Buehler, 2011).

Riboflavin can be detected in a variety of fluids and within organs of the human body, at varying levels. Several studies have discussed recent analytical methods developed to detect Riboflavin in biological samples (Zhang et al., 2018; Gul, Anwar, & Qadeer, 2014; Antal, Bazel, & Kormosh, 2013).

2.9 RIBOFLAVIN ENCAPSULATION TECHNIQUE

2.9.1 Nano Encapsulation and Nanoparticles

Nanoencapsulation is the technique of packaging drug molecules within particles with sizes ranging from 1 to 1000 nanometers. Nanoparticles are solid carriers for the drug, which may be either biodegradable or non-biodegradable. Generally, they are divided into two categories, namely nanospheres and nanocapsules. Nanospheres are solid particles composed of drug molecules either adsorbed onto the surface or encapsulated within the particle's matrix. On the other hand, nanocapsules consist of a vesicular system where the drug is entrapped in a central liquid core surrounded by a polymeric membrane. Thereby allowing drugs either dissolved in the core or adsorbed on the surface of the capsule. The

use of nanoparticles for the delivery of drugs has been increasingly researched and found to be effective in their delivery to the therapeutic sites. One advantage of their smaller nano-size is that this gives them a far better intracellular uptake as compared to larger microparticles, thus enhancing the bioavailability of these drugs. In addition, the absorption of drugs through the intestines from these nanoparticles varied and depended on their size, charge, and nature, with hydrophobic nanoparticles often exhibiting greater absorption than hydrophilic ones. All these properties make hydrophobic polymer-based nanoparticles like poly(styrene) ideal candidates for absorption by intestinal cells as compared to hydrophilic or negatively charged particles (Reis et al., 2006).

2.9.2 Liposome Encapsulation

These liposomes are tiny, imitated phospholipid vesicles forming bilayered membranes. They have been designed to deliver the drugs to the specific targeted sites in the body while providing solutions to stability and toxicity issues. Liposomal formulations have found special use in stabilizing photodegradable drugs such as riboflavin, where they protect the drug from light degradation. Various methods to enhance riboflavin's photo protection have been studied, such as using stabilizers like citrate buffers or complexing agents. Furthermore, the presence of antioxidants such as α-tocopherol in liposomes has been reported to enhance the stability of lipid bilayers against oxidation. Nevertheless, accurate measurement of riboflavin in liposomal formulations can be quite complicated due to the interference of riboflavin's degradation products. Therefore, multicomponent spectrometric methods are used to monitor the degradation and stabilization of riboflavin in these formulations to ensure reliable results (Ahmad et al., 2015).

2.9.3 Nanoparticle Encapsulation

Even Nanoencapsulation enables the proper loading of pharmaceuticals into nanocarriers such as liposomes, micelles, carbon nanotubes, dendrimers, and magnetic nanoparticles, thus yielding enormous therapeutic benefits. It also minimises the systemic toxicity of the drug by specific targeting of the drug release, and improves the therapeutic activity of the therapeutics. Another advantage of this class of nanocarriers is the possibility of modifying their capabilities to release drugs, thus improving the bioavailability of the drug and reducing side effects. There is a considerable number of nanocarriers studied for drug loading, each

having its unique advantages for targeting, sustained delivery, and improved stability of drugs (Kumari et al., 2014).

2.9.4 Microencapsulation

Microencapsulation is the technique that gives the active site within a protective membrane, which may include drugs and nutrients. It is mainly used to prevent factors from affecting the encapsulated substances, such as light, heat, moisture, or oxygen, and sometimes to mask taste or odour. For instance, the microencapsulation process in food industries includes modification of sensitive properties for solubility, dispersibility, or stability, enabling them to be incorporated into food products. It is essential, especially for controlled release of bioactive compounds, to enhance the exact site, and thereby, improve their efficiency (Comunian & Favaro-Trindade, 2016).

2.9.5 Spray Drying

Rapid drying of liquids or slurries with hot air leads to the formation of powders: this transformation is known as spray drying and is a common approach. Thermally sensitive materials, for example, certain pharmaceuticals and food ingredients, benefit greatly from spray drying. The fine powders can then be employed for different applications, such as drug formulations, all while ensuring the stability and bioactivity of sensitive compounds during processing (Campbell et al., 2020).

2.9.6 Coacervation Complex Coacervation

One method of microencapsulation through complex coacervation is that of two oppositely charged biopolymers through electrostatic attraction that results in a coacervate or precipitate. Bioactive compounds are mostly encapsulated using this technique. Encapsulation improves the stability and increases the efficiency of the delivery and release of the encapsulated material. Factors such as mixing ratio, ionic strength, and pH can be optimized to achieve effective encapsulation of sensitive ingredients in food and drugs through complex coacervation. With time, this technique has been used frequently in the food industry, where it stabilizes emulsions and protects other active ingredients such as phytosterols and omega-3 fatty acids (Hua et al., 2022).

It is known that complex coacervation involves the application of the electrostatic attraction created by two oppositely charged biopolymers to make a coacervate or a precipitate. Such fabrication of coacervate primarily serves to encapsulate bioactive compounds. Generally, the release of the encapsulated material is made easier, while stability is achieved through encapsulation. Sensitive ingredients from food products and drugs are successfully encapsulated using this complex coacervation technique by optimizing factors that determine the quality of coacervate formation, such as mixing ratio, ionic strength, and pH. Increasingly, this technique has been utilized in the food industry, where it is applied to protect active ingredients such as phytosterols and omega-3 fatty acids and stabilize emulsions (Hua et al., 2022).

3. MATERIAL AND METHODS

3.1 MATERIALS

Main ingredients of the composite formulation, which are alginic acid sodium salt (from brown algae), carboxy methyl cellulose sodium salt (low viscosity), calcium chloride dihydrate (CaCl2 · 2H2O), and montmorillonite (MMT), are obtained from Sigma Aldrich.

If pH adjustments were necessary, these are done by using sodium hydroxide and hydrochloric acid solutions. All solutions were prepared using deionized water.

3.2 SGF PREPARATION

3.2.1 Preparation of SGF

Material needed: hydrochloric acid (HCl) 1M, distilled water, sodium chloride (NaCl) 0.2 g, PH check. By using the dilution formula, calculate the required volume. For 100 mL, add 10 mL of HCl to 90 mL of distilled water, stirring with caution, and add the acid to the water, not the opposite. Add 0.2 g of sodium chloride and stir until dissolved. Using the PH meter, check the PH to ensure it is the same as natural gastric fluid (PH 1-2)

$$C_1V_1 = C_2V_2 \rightarrow 1M \times V_1 = 0.1M \times 0.1L \rightarrow V_1 = 0.01 = 10mL \quad (3.1)$$

3.2.2 SIF Preparation

For the preparation of 1 Liter of SIF, we need potassium dihydrogen phosphate, sodium Hydroxide Weight 6.8 g of KH_2PO_4 equivalent to (0.05 mol) put in 1 Liter a volumetric flask prepper NaOH solution by dissolving 0.8 g of NaOH in 100 ml of distilled water then add slowly add the NaOH solution to the flask contains 6.8 g KH_2PO_4 stir until opting homogenous solution add the distilled water to the mixture until reach 1 liter.

During the sample preparation step, a precision balance (Ohaus), a magnetic stirrer (Haidolf), and a vibrating water bath (Nüve ST-30) were used. Preparation of the samples was conducted at the Chemistry Laboratory of Altınbaş University, Faculty of Engineering and Architecture.

3.3 PREPARATION OF ALGINATE-MMT BEADS

3.3.1 Alginate Solution Preparation

A 2% (w/v) SA solution was prepared by dissolving 1 g of SA in ionized water under constant stirring. CMC was then added and stirred until a homogeneous mixture was achieved. Subsequently, Riboflavin was introduced into the solution, followed by continuous stirring to ensure uniform dispersion. The mixture was then divided into equal volumes, and MMT at a concentration of 1% (w/v) was incorporated to formulate the clay solution.

3.3.2 Bead Formation and Cross-Linking

To obtain cross-linked beads, 20 mL of the prepared alginate-MMT mixture was carefully introduced dropwise into 40 mL of a $CaCl_2$ gelling solution using a syringe with an inner diameter of 0.85 mm. The solution was stirred at 200 rpm for 1 hour to facilitate the cross-linking process, as shown in Figure 3.1.

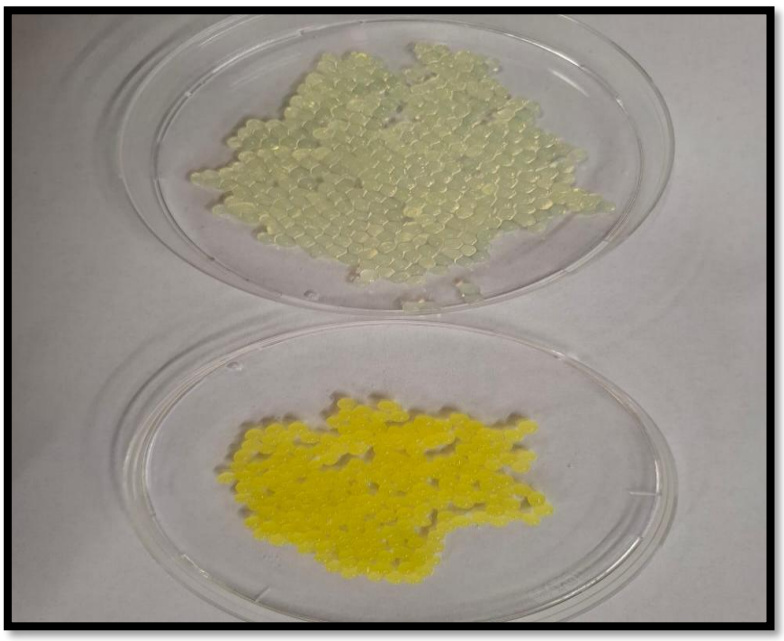

Figure 3.1: Freshly Prepared Samples Containing Riboflavin.

3.3.3 Bead Washing and Drying

After the gelling step, the formed beads were filtered from the gelling medium and thoroughly washed with pure water to remove any excess Riboflavin from the surface. The lost Ca^{2+} content in both the gelling solution and washing water was quantified using UV-Vis spectroscopy at a wavelength of 445 nm. Prepared samples are shown in Figure 3.2.

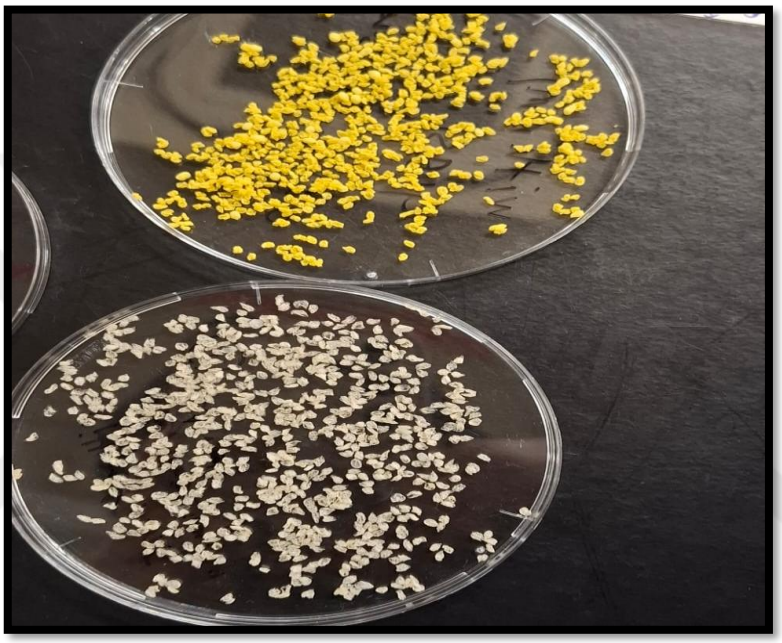

Figure 3.2: Dried Samples Containing Riboflavin.

Following the washing step, the beads were immersed in deionized water and frozen at 21°C for 24 hours. They were then melted, filtered, and dried at room temperature for 24 hours. This process is called the Quasi-cryogelation method. Riboflavin Calibration was calculated as in Figure 3.2.

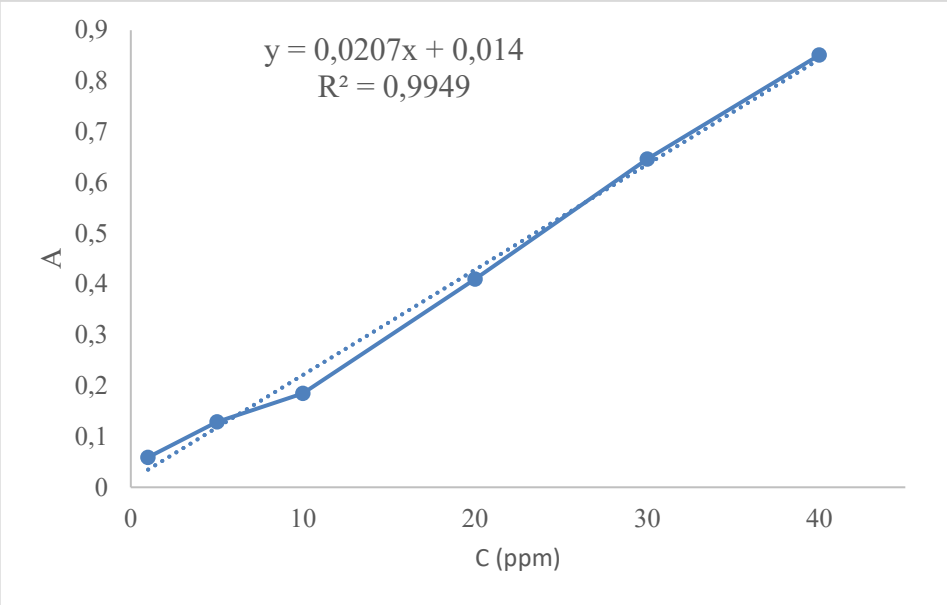

Figure 3.3: Riboflavin Calibration

3.4 ENCAPSULATION EFFICIENCY CALCULATION

The encapsulation efficiency (EE) of the riboflavin-loaded beads was calculated using the equation:

$$EE(\%) = \frac{(Mi - Mf)}{Mix100} \tag{3.2}$$

Where (Mi) is the initial amount of Riboflavin added to the formulation, and (M_f) represents the total amount of Riboflavin lost in the gelling solution and washing water.

3.5 CONTROLLED RELEASE STUDY

To evaluate the controlled release of Riboflavin, an in vitro release study was conducted in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) at 37 °C. A predetermined quantity of beads was immersed in 20 mL of SGF and SIF solutions. The dissolution vessels were subjected to shaking at 37 °C, and at specific time intervals, samples were withdrawn to measure absorbance at 445 nm using UV-Vis spectroscopy.

3.6 CHARACTERIZATION OF BEADS

Fourier Transform Infrared Spectroscopy (FTIR) was performed to analyze the effect of MMT incorporation on the structural properties of the alginate beads, using a device Shimadzu IRAffinity-1S Fourier Infrared Spectrometer

4. RESULTS AND DISCUSSION

4.1 ENCAPSULATION EFFICIENCY

The encapsulation efficiency is shown in Table 4.1 below.

Table 4.1: Encapsulation Efficiency of the Samples.

Sample	Encapsulation Efficiency (%)
Alg-MMT	96
Alg	< 1

Table 4.1 shows that the presence of MMT significantly increased the encapsulation efficiency of riboflavin-loaded granules. This result provides further evidence that MMT plays a role in improving Riboflavin retention. On the other hand, due to the absence of MMT, encapsulation did not occur, resulting in the complete loss of Riboflavin during preparation.

4.2 CONTROLLED RELEASE RESULTS

Controlled release was performed in simulated gastric fluid, and the results are shown in Table 4.2.

Table 4.2: The Result of Serum Gastric Fluid (SGF) Control Release.

Time (min)	Absorption (Alg-MMT)	Absorption (Alg)
15	0	0.003
30	0.011	0.009
60	0.011	0
120	0	0
180	0.003	0.000
210	0.003	0

In Table 4.2, the absorption data from SGF indicate minimal release of Riboflavin from the MMT-loaded granules, indicating strong protection from acid degradation. The MMT-free sample also showed irregular absorption values, indicating instability and potential degradation.

Table 4.3: The Result of Serum Intestinal Fluid (SIF) Control Release.

Time (min)	Absorption Alg-MMT	Absorption Alg
15	0.273	0.155
30	0.374	0.222
60	0.440	0.267
120	0.516	0.291
180	0.544	0.317

In Table 4.3, the SIF-controlled release study showed a gradual increase in Riboflavin release in the MMT-loaded beads, confirming their sustained release pattern. The unloaded sample showed a lower release rate, likely due to the absence of a protective coating.

Table 4.4: Cumulative Release Efficiency.

Time (min)	Cumulative Release (Alg-MMT)%	Cumulative Release (Alg)%
15	86%	N/A
30	100%	N/A

In Table 4.4, the MMT-loaded beads demonstrated an efficient controlled release mechanism, reaching 100% cumulative release within 30 minutes in SIF. The unloaded sample showed no release, indicating immediate loss at the first stage.

4.3 ENCAPSULATION AND RELEASE CALCULATIONS

4.3.1 Encapsulation Mass Balance Equation

$$M_{encapsulated} = M_{initial} - \left(C_{washing} \times V_{washing}\right) - \left(C_{crosslinking} \times V_{crosslinking}\right) \quad (4.1)$$

Where:

a. $M_{initial}$: Initial Mass

b. $C_{washing}$: Lost concentration in washing step.

c. $V_{washing}$: Total volume of washing solution.

d. $C_{crosslinking}$: Lost concentration in crosslinking step.

e. $V_{crosslinking}$: Total volume of crosslinking solution.

The initial mass of the substance ($M_{initial}$) is 0.0025g during the washing step, the concentration of the substance in the washing solution ($C_{washing}$) is 0, indicating that no detectable amount of the substance was released from the MMT loaded beads. The volume

concentration of the substance in the crosslinking solution ($C_{crosslinking}$) was 0.024, with the volume of 30mL ($V_{crosslinking}$)

4.3.2 Percentage Release Calculation

$$\% \, Released = \frac{C_t \times V_t}{M_{encapsulated}} \tag{4.2}$$

Encapsulation and release calculations demonstrate the protective role of MMT in controlled-release formulations. The optimized formulation enhances bioavailability by preventing premature degradation in the gastric phase, while allowing for gradual release in the intestinal phase.

The study results showed that the MMT incorporation process significantly improved encapsulation efficiency and controlled Riboflavin release. Montmorillonite-loaded granules demonstrated effective protection against gastric degradation, while allowing sustained release in the intestinal environment. These results highlight the potential of montmorillonite-based encapsulation systems to improve the bioavailability of sensitive bioactive compounds.

Carboxymethyl cellulose is a hydrophilic biopolymer that, when added to alginate, increases the surface area of the beads. This is what was observed when crosslinking the solution with $CaCl_2$ by following the quasi-cryogelation method. CMC is considered a superabsorbent, increasing the swelling degree of the beads in release media and decreasing the degradation of the beads over time

Figure 4.1. Indicates the FTIR results of the samples. Typical riboflavin peaks for -N-H and -O-H groups are identified, although they are not clearly visible in the samples, probably due to hydrogen bonding (Chakraborty et al., 2014). The presence of MMT did not result in a significant change in functional groups, indicating the physical effect of bead-barrier protection.

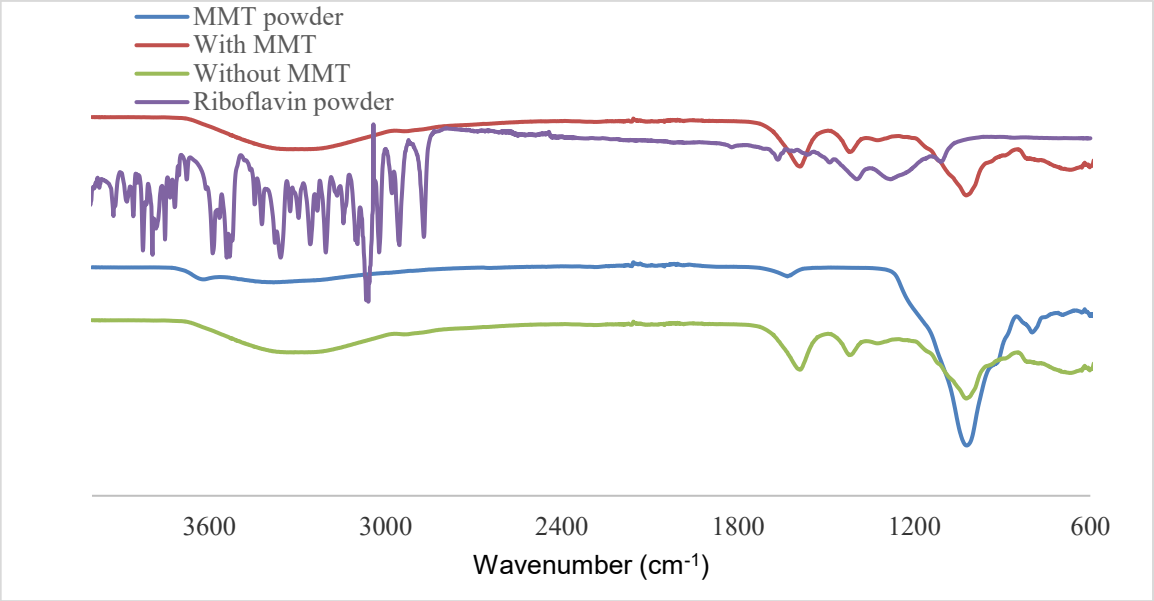

Figure 4.1: FTIR Spectrum of the Samples.

In this study, an enhancement of encapsulation efficiency and controlled release behavior of riboflavin by montmorillonite (MMT) in alginate matrices was observed. Encapsulation efficiency was found to be 96% in MMT-loaded beads, while the control plain alginate sample retained virtually nothing (<1%). Controlled release testing in simulated gastric fluid (SGF) established that Alg-MMT beads protect riboflavin from acid degradation, showing almost no or little release in 180 min. On the other hand, the observations made in the MMT-free formulation revealed erratic and unstable absorption profiles, raising suspicions of degradation. The slow, sustained release of riboflavin from the Alg-MMT system was witnessed in simulated intestinal fluid (SIF) with 100% cumulative release within 30 minutes, while the control sample released less and very rapidly lost the active compound. These results therefore support the conclusion that MMT is protecting riboflavin, both structurally and chemically, for a site-specific delivery to further its bioavailability.

Likewise, Orhan et al. (2023) prepared a biopolymer-based superabsorbent that uses alginate and carboxymethyl cellulose (CMC), crosslinked with calcium ions, processed by an innovative quasi-cryogelation method. Results confirmed that swelling capacity in conditions like pure water, isotonic solution, as well as SGF and SIF, was significantly enhanced in the quasi-cryogels. The quasi-cryogels retained their integrity in SGF for some time but swelled more than the standard beads. The swelling in SIF and citrate-PBS was as high as 2598%, but degradation started after 30-90 minutes due to phosphate interactions.

Importantly, the beads showed good reusability and significant swelling capacity after five cycles. The FTIR studies confirmed that these modifications were physical and did not involve the formation of any new covalent bonds between alginate and CMC (Orhan et al., 2023)

Our research was successful in developing a biopolymer-based superabsorbing material in calcium-ion crosslinked alginate and carboxymethyl cellulose (AlgCMC) by quasi-cryogelation, wherein gelation was permitted to go to completion before freezing. The resultant QuasiAlgCMC beads were able to absorb water at capacities that were up to 1652% swelling in distilled water and 2343% in isotonic solution, showing a retention of performance above 1200% even after five reusability cycles. This swelling behavior was described by a pseudo-second-order kinetic model ($R^2 > 0.99$), indicating some interactions of water uptake by strong types of chemisorption-like adsorptions. FTIR and SEM analyses confirmed that hydrogen bonding exists between alginate and CMC, with a significant structural transformation after freezing, giving a roughened surface with snowflake-like characteristics. These alterations within the structure must have contributed to their improvement in swelling capacity.

These findings are pretty much in line with what Orhan et al. (2022) found in developing superabsorbents by a quasi-cryogelation method that yielded QuasiAlg CMC. The same study also demonstrated the swelling potential by the alteration in morphology resulting from the freezing, with swelling approximating 1652% in water and 2343% in isotonic solutions, almost equal to our findings. Both studies supported a 1:1 Alg CMC ratio and 3% $CaCl_2$ crosslinker concentration for best gel integrity and absorbency. Orhan et al. also demonstrated the reusability of the quasi-cryogels, and three cycles of swelling performance were cited: that finding approximated what we observed in sustainably absorbent up to the fifth cycle. The observation about degradation of phosphates induced in SIF and PBS adds power to the strand placed under the ion-exchange mechanism of crosslinker destabilization. Overall, both studies strengthen the assurance that quasi-cryogelation is easy to accomplish and green in creating superabsorbents out of purely biopolymers without synthetic grafting or chemical cross-linking (Orhan et al., 2022)

In this study, the presence of Alg-MMT resulted in a considerable improvement in the encapsulation effectiveness of particles loaded with radiofrequency. The discovery of this

result offers more proof that Alg-MMT plays a part in enhancing the retention of Riboflavin information." On the other hand, since Alg-MMT was not present, encapsulation did not take place, which led to the total loss of Riboflavin. This finding is in agreement with the findings of Malektaj (2024), who discovered that the incorporation of Alg-MMT clay into alginate gel matrices resulted in an increase in the effectiveness of encapsulation when compared to alginate gels that did not include Alg-MMT (Malektaj, 2024). According to Lueckgen et al. (2019) and Seddiqi et al. (2021), who discovered that the incorporation of Alg-MMT clay into gel matrices greatly increased the encapsulation effectiveness of diclofenac sodium in comparison to gels that did not include Alg-MMT, this finding is also compatible with the findings of the aforementioned researchers (Lueckgen et al., 2019; Seddiqi et al., 2021).

Because of this investigation, significant insights into the protective capacities of Alg-MMT against acid-induced degradation were revealed by the controlled release of riboflavin from Alg-MMT-loaded granules into SGF. According to the findings, the "RF" release from Alg-MMT-loaded granules was significantly decreased, which suggests that the granules provide efficient protection against acidic environments. Samples that did not include MMT, on the other hand, had erratic uptake levels, which indicated instability and the possibility of deterioration. In line with the findings of Kevadiya et al. (2010), who discovered that the presence of Alg-MMT enhanced the controlled release of the medication in SIF, it may be concluded that MMT can adjust the kinetics of drug release efficiently. Clay minerals have been shown to have a favorable impact as a drug carrier for "TM," which is a non-selective beta-adrenergic receptor blocking agent. This finding is in line with Josh et al. (2009), who discovered that clay minerals play a vital role in modifying medication delivery. It was established that timolol maleate could be intercalated into the Alg-MMT interlayer at a variety of pH levels and starting concentrations (Josh et al., 2009).

In this study, results showed a significant discrepancy in drug release performance between Alg-MMT-loaded and non-loaded Riboflavin formulations in SIF. The Alg-MMT-loaded granules achieved 86% cumulative release after 15 minutes, followed by 100% release after 30 minutes, highlighting the effectiveness of Alg-MMT as a controlled-release carrier.

The non-loaded formulation, on the other hand, exhibited a release rate of zero percent, which indicates that the formulation underwent either rapid degradation or loss during the

initial phase, most likely in the acidic environment of the stomach, prior to reaching the appropriate absorption site. When it comes to oral medication administration, Alg-MMT has a twofold benefit: it provides protective encapsulation under gastric circumstances and it provides efficient and targeted release throughout the gastrointestinal system. These features are especially useful for medications like Riboflavin, which are prone to acid degradation and need precise distribution in order to preserve their effectiveness.

5. CONCLUSION

In this study, indirect encapsulation of Riboflavin was studied. The results show that the addition of the coating showed significantly higher effectiveness than alginate alone. In gastric media (simulated gastric fluid), the acidity of the medium prohibits the release of riboflavin; therefore, as a drug carrier, it can be protected in the stomach and reach the intestines effectively. In simulated intestinal fluid, riboflavin is released from the sample. The study suggests further research on riboflavin (RF), particularly ALG-MMT, to explore its additional benefits for the human body and to compare the effects of encapsulation for broader pharmaceutical applications.

These results provide strong evidence of the critical role of montmorillonite (MMT) in improving both encapsulation efficiency and the controlled release of riboflavin-loaded granules based on the alginate system. It is evident that 96% encapsulation efficiency in the Alg-MMT samples is much higher than that of less than 1% in the alginate-only group, confirming MMT's stabilizing and protective role. During the preparation of this system, the MMT retained the active compound, while in the free formulation, there was an immediate degradation and loss.

Controlled release studies in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) further demonstrated the obvious superiority of the MMT-loaded system in functionality. The release in SGF is minimal evidence of strong acid resistance, necessitating adaptation for the protection of sensitive bioactives to acid, such as riboflavin. Sustained and complete release in SIF, on the other hand, indicates the formulation's potential in the target environment. These results, corroborated with FTIR analysis, point to more physical interaction owing to no major chemical modification, making this green, cost-effective approach to encapsulation.

Moreover, the study briefly captured the functionality of carboxymethyl cellulose (CMC) as a polymeric additive. Include higher surface area and superabsorbent properties, especially under quasi-cryogelation processing in beads. It is expected to improve the swelling behavior of beads and their structural integrity for release, which could still experience optimization for prolonged delivery.

5.1 RECOMMENDATIONS

a. Riboflavin Further Structural Optimization Riboflavin: Incorporation of CMC along with other biopolymers such as xanthan gum or pectin in the formulation with MMT may improve bead integrity, along with control in release and mechanical strength.

b. Riboflavin up model and stability test Riboflavin: Promising lab-scale data should encourage pilot-scale trials and an extended period in stability studies to see how formulations hold up to conditions of real-life storage and handling.

c. Riboflavin Studies on Bioavailability. In Riboflavin: Proof of the controlled release represented in vitro will be followed next by pharmacokinetic testing in vivo.

d. Riboflavin Application in Other Bioactives Riboflavin: This encapsulation strategy should also be considered for other acid-sensitive compounds such as probiotics, peptides, and vitamins, since the formulation has effects of protection and release.

e. Riboflavin: Because this system doesn't use harsh chemicals or complicated methods, a lifecycle assessment (LCA) and cost analysis would help show that it can be used for sustainable delivery of drugs or nutrients.

f. This work highlights the promise of MMT-based encapsulation systems as a potential controlled oral delivery system for unstable bioactives and is consistent with current trends in green chemistry and designing such functional materials.

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