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# Galactomannan and Alginate Double Coat Encapsulation of Sulfamethoxazole-Trimethoprim for Treatment for Controlled Intestinal-Targeted Drug Delivery

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**Intracellular bacterial infections cause diseases that are hard to treat because of the anatomical barriers before the antibiotic reaches the target site and small intestine for absorption. Thus, the modulated release of the antibiotic with higher stability and drug retention needs to be achieved. Encapsulation using polymers retains the stability of drugs from the harsh environment of the stomach and delivers a drug to its intended site of release. This study aims to assess the drug release property and stability of galactomannan and alginate double coat encapsulation of SMX-TMP. The encapsulation of SMX-TMP using biopolymers was done through the extrusion method. The stability of the capsule was subjected to *in vitro* gastrointestinal simulation analysis and was analyzed using Fourier transform infrared spectrometer (FTIR) and scanning electron microscope (SEM) to confirm the successful encapsulation and its surface morphology. The delivery of the active drug, SMX-TMP, from the capsule was observed without significant degradation in the simulated gastric environment and disintegrates at the fasted state (pH 8) of the simulated intestinal environment, which is the target site of drug release. *In vitro* stability test validates the protection of SMX-TMP (encapsulated) from the gastrointestinal tract (GIT) conditions. The fingerprint of drug and encapsulating material was found to be present after drug encapsulation and intestinal disintegration. Morphological micrographs showed that the capsules were spherical that measures approximately 2.7 mm; ridges at the surface of the microcapsules were present for quick hydration and disintegration. This study reports the potential of galactomannan and alginate as candidates for encapsulating antibiotics for modulated and targeted drug delivery.**

Keywords: alginate, drug delivery, encapsulation, galactomannan, sulfamethoxazole-trimethoprim

## INTRODUCTION

Conventional antibiotics remain the first line of therapies against the intracellular bacterial pathogens (Salouti and Ahangari 2014). Intracellular bacteria are a broad group of Gram-positive and Gram-negative bacteria capable of invading the protective barrier and lining of the stomach and the small intestine, causing disruptions of the intestinal microflora (Todar 2009). Effective utilization of antibiotics against intracellular infections must address the inability of the drug to penetrate, accumulate in sufficient concentration, and be retained into the intended target site of release, which is commonly the small intestine. The

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majority of drug absorption processes occur in the small intestine because of its large surface area, and the drug spends longer in this organ (Masaoka 2006). Thus, the distribution of the active molecule or drug to the small intestine underscores the importance of assessing the physicochemical properties of the drug and its alternative delivery strategies (Atbiaw *et al.* 2018).

One of the most used antibiotics for intracellular infection is the fixed drug combination of SMX and TMP, also known as cotrimoxazole, which is classified as a folic acid inhibitor (NCBI 2020). The two chemically different drugs work synergistically against a broad spectrum of intracellular infections – including pneumonia, urinary tract infections, and intestinal infections (Hasan 2017). SMX-TMP is odorless with a bitter taste that typically appears yellowish to white crystalline powder. However, the drug is not soluble in water and organic solvents but is slightly soluble in binary solvents. SMX-TMP solubility in aqueous salt solutions depends on the pH of the solution (Aronson 2016). SMX or N1 -[5-methyl-3-isoxazolyl] sulfanilamide is an isoxazole (1,2-oxazole) sulfur-containing compound under the sulfonamides group. Sulfonamides are structural analogs of para-aminobenzoic acid, which functions as a cofactor for bacterial folic acid synthesis. TMP or 2,4-diamino-5- (3,4,5- trimethoxy benzyl) pyrimidine resembles pyrimidine structure with benzyl and methoxy substituents. The structure serves to block bacterial dihydrofolate reductase, halting the production of tetrahydrofolic acid required for DNA synthesis (Zinner and Meyer 2015).

Designing a drug delivery system formulation that can obstruct drug release in the stomach and subsequent degradation transiting into the small intestine is one of the most effective ways to control drug release into the target site with higher retention of the active drug molecule (Yoshida *et al.* 2013). The most feasible approach to this problem is through drug encapsulation. Encapsulation is the process of entrapping an antimicrobial agent with a submicroscopic carrier to protect it against the harsh biological conditions, while subsequently releasing it to the designated cells and tissues (Vyas and Khar 2003). Colloidal-based carriers such as biopolymers, liposomes, and nanoparticles are the main carriers developed to deliver antibiotics to improve their anti-microbial efficacy against intracellular infections. The ability of these drug formulations to deliver drugs on their target sites is highly dependent on the physicochemical properties of the carriers and the tissue characteristics (Abeylath and Turos 2008).

Biopolymer-based colloidal carriers are one of the most convenient and cheapest encapsulation drug formulations (Sharma 2017). Biopolymers are ubiquitous in nature, as they can be directly derived from biological sources or chemically synthesized in the laboratory using their building blocks as precursors (Singh 2011). They offer a lot of advantages such as biodegradability, biocompatibility, and non-toxicity (Cerquiera *et al.* 2019). Synthetic and semi-synthetic derivatives of polysaccharides such as starch, mucilage, gums, chitosan, *etc.* have been frequently used as biopolymers (Goudoulas 2012). Polysaccharide-based formulations have been found in many applications in drug delivery as films, micro-particles, implants, beads, solid monolithic matrix systems, and different multifaceted roles in drug dosage forms (Nitta and Numata 2013). Polysaccharide-based formulations in smart drug delivery is a growing technological advancement in the healthcare industry because, aside from being classified as food-grade and generally recognized as safe (GRAS), these polymers are employed to a wide variety of therapeutic applications such as protecting and delivering functional components of nutraceuticals (Matalanis *et al.* 2011).

In this study, galactomannans and alginates are the two polysaccharides employed in the double coating encapsulation of the antibiotic SMX-TMP. Galactomannans are commonly found in the endosperm cell wall storage in the seeds of several plant families, and they constitute the second-largest group of storage polysaccharides in terms of distribution (Dey 1978; Scherbukhin 1993; Daas *et al.* 2002; Srivastava and Kapoor 2005). Galactomannan contains a (1→4)-linked α-D-mannopyranosyl as its structural backbone, which is substituted by single units of β-D-galactopyranose at O-6. The mannose/galactose ratio and the number of α-D-galactopyranose residues in the side chains affect the water solubility of galactomannans. Galactose substituents contribution on conformational entropy in the solution, and blocking solid-state packing of mannan chains are presumably the reasons for increasing the water solubility of galactomannans (Bucke 1989).

Galactomannans have been widely used as excipients in solid pharmaceutical dosage forms, particularly tablets and capsules (Silveira and Bresolin 2011). The commercial galactomannan from guar gum was studied to provide a high level of hardness and compatibility in the formation of hydrophilic matrix tablets of theophylline with different dissolution rates. In this study, low concentrations (5–10%) of galactomannan was used as a disintegrating agent and high concentrations (25%) was used for binding purposes (Berta *et al.* 1994). Galactomannans use as a component of spherical cross-linked hydrogels of polyacrylamide-grafted guar gum was also explored, in which 10% of diltiazem hydrochloride was loaded by soaking the pH-sensitive hydrogels in an aqueous solution of the drug (Soppimath *et al.* 2001). In addition, galactomannan was also utilized in film coating, particularly for colon-specific drug delivery. One example is the cross-linking of galactomannan with butanediol

diglycidyl ether, which is microbiologically degradable and with a high degree of swelling (Hirsch *et al.* 1999).

Alginates are natural polysaccharides extracted mainly from brown seaweeds. It consists of a linear polymer chain of alginic acids arranged in blocks containing D-mannuronic acid and L-guluronic acid residues. These homogeneous blocks of either mannuronic or guluronic blocks alone are separated by blocks made of alternating units of mannuronic and guluronic acids (Smidsrod and Draget 1996). The ability of alginates to undergo proton catalyzed hydrolysis is dependent on time, pH, and temperature. Alginates can easily form high viscous acid gel due to intermolecular bonding upon hydration. Water molecules are trapped inside the gel matrix but are capable of migration due to capillary forces (Draget *et al.* 1994; Wang and Spencer 1998).

Alginates are one of the most versatile and established drug vehicles because of their biocompatibility, biodegradability, and abundance in nature. Alginates use in oral pharmaceutical dosage forms are some of the most documented applications. Traditionally, the salt or sodium alginate form is commonly used as a binding agent for tablets, while the free-form alginic acid is used as a disintegrating agent (Onsoyen 1996). Alginate has also been applied as part of the diffusion systems, primarily as a polymer membrane system. In this approach, the drug formulation – either in the form of a solid, a suspension, or an aqueous solution – is encapsulated within the drug reservoir compartment. The polymer membrane system controls the drug release with specific permeability (Tonnesen and Karlsen 2002). The drug formulation was designed either by spray-coating or microencapsulation. Alginate was also utilized in the preparation of gel capsules in one study, where the compound theophylline was encapsulated and the drug release rate was investigated. The drug release follows zero-order kinetics in which the release rate decreases as the thickness of the coat increases. The findings of the study showed a significant reduction of drug release compared to the matrix-type alginate gel beads (Tomida *et al.* 1993).

The concept of employing a dual biopolymer-based carrier has gained attention in the past year because it offers the advantage of enhancing the stability of the drug formulation and enhancing the significant reduction of drug disintegration as it reaches the small intestine or the target sites. In one study, lycopene was preserved with chitosan and trehalose beads and it was cross-linked with alginate-calcium beads. The results showed a proof-of-concept demonstration that the controlled release of lycopene and the stability toward isomerization were enhanced significantly (Aguirre Calvo and Santagapita 2017). The compatibility of mixing galactomannan and alginate as a new carrier system was investigated in one study where the viscoelastic properties of the drug formulation were assessed. Potential synergism as observed by the blending of viscoelastic properties of both polysaccharides and the apparent increase in viscosity of the resulting drug formulation (Valenga *et al.* 2011).

The combination of galactomannan and alginate to formulate a double coat encapsulation has attracted attention in the studies of alternative drug delivery systems. The employment of such alternative drug formulations combining two biopolymers was shown to improve the modulation of drug release and stability of the active molecule. Up to this, fewer studies have been conducted to explore the potential of galactomannan and alginate combination as the main biopolymers in double coat encapsulation of antibiotics, particularly for antibiotics targeting intracellular infections. Thus, the primary objective of the study is to characterize the feasibility of galactomannan and alginate double coat encapsulation of SMX-TMP for the treatment of intracellular infection by a preliminary assessment of its drug release and stability properties. Specifically, the encapsulation efficiency of the double coat of biopolymers on SMX-TMP was assessed by fingerprinting and *in vitro* drug release assay.

## METHODOLOGY

### Standards, Reagents, and Chemicals

The chemicals and reagents used were 1.0 M $CoCl_2$, 1.0 M $CaCl_2$, and 1.0 M $MgCl_2$; 5% w/v NaOH; buffer 1 (KCl-HCl buffer at pH 2.4 with 3.0 g/L pepsin, 125 mM NaCl, 75 mM KCl, and 75 mM $NaHCO_3$), buffer 2 (KCl-HCl buffer at pH 1.4 with 3.0 g/L pepsin, 125 mM NaCl, 75 mM KCl, and 75 mM $NaHCO_3$), buffer 3 (phosphate buffer at pH 5.4 with 1% w/v pancreatin enzymes, 6.5 g/L NaCl, 0.835 g/L KCl, 0.22 g/L $CaCl_2$, and 0.15% bile salts), buffer 4 (phosphate buffer at pH 8.0 with 1% w/v pancreatin enzymes, 6.5 g/L NaCl, 0.835 g/L KCl, 0.22 g/L $CaCl_2$, and 0.15% bile salts) and buffer 5 (phosphate buffer at pH 7.4 with 0.20 g/L KCl and 0.8 g/L NaCl); sodium alginate; and guar gum (galactomannan). The aforementioned were all analytical grade and were purchased from Sigma-Aldrich, USA.

### Encapsulation of SMX-TMP

***Matching of metal-ion crosslinker.*** The preparation of metal-ion crosslinker was adapted from the procedure of Reddy and Tammishetti (2002). Solutions of 1.0 M $CoCl_2$, 1.0 M $CaCl_2$, and 1.0 M $MgCl_2$ were prepared to serve as potential metal-ion crosslinker. A 1% w/v of galactomannan (guar gum) solution was prepared by mixing galactomannan

with 5% w/v NaOH and was added dropwise into each crosslinker solution. The beads or microcapsules formed in mixing the galactomannan and crosslinkers were assessed through its shape and rigidity to determine the best metal-ion crosslinker to be used for encapsulation.

***Encapsulation using guar gum and alginate.*** Encapsulation of SMX-TMP was done by means of a manual extrusion method. Different concentrations (1.9%, 2.1%, 2.3% w/v) of galactomannan solution was prepared by mixing the polymer with 5% w/v NaOH solution. On each galactomannan solution, the antibiotic was added until a final concentration of 0.3% w/v was achieved. The co-extrusion method was done by adding the galactomannan-antibiotic solution dropwise in the crosslinker solution and stand at room temperature for 4 h for complete matrix formation. The produced capsules were double-coated by submerging the microcapsules of galactomannan-antibiotic in 2% w/v sodium alginate solution and dropped in 1.0 M $CaCl_2$ solution and placed at the refrigerator for 24 h for complete matrix formation. The resulting capsules were freeze-dried at –51.5 ºC, 58 mTorr using the FD5 Series Freeze Dryer for further analysis.

***In vitro gastrointestinal simulation for drug release.*** This method was based on the study of Santiago and Devanadera (2016). In order to imitate the human gastrointestinal environment, buffers containing salts with enzymes were utilized for the dissolution tests. Simulated fed and fasted gastric fluids were made of KCl-HCl buffer at pH 2.4 and 1.4, respectively, and both contained 3.0 g/L pepsin, 125 mM NaCl, 75 mM KCl, and 75 mM $NaHCO_3$. On the other hand, both simulated intestinal fluids in fed (pH 5.4) and fasted (pH 8.0) states were composed of phosphate buffer containing 1% w/v pancreatin enzymes, 6.5 g/L NaCl, 0.835 g/L KCl, 0.22 g/L $CaCl_2$, and 0.15% bile salts. Moreover, colonic fluids – both fed and fasted – contained phosphate buffer at pH 7.4 with 0.20 g/L KCl and 0.8 g/L NaCl.

The temperature of the medium was kept at 37 ºC for 3 h, which was similar to the physiological temperature, and was shaken using Thermo Scientific MaxQ 4000 (Thermo Scientific, USA) incubated orbital shaker at a constant rotation speed of 100 rpm that resulted in a high-intensity shear force that was comparable to the peristaltic movement in the GIT. The double-coated microcapsules of the antibiotic were placed in both fasted and fed states of simulated gastric conditions for 3 h of incubation. After incubation, microcapsules were collected and transferred to simulated intestinal conditions (fed and fasted state) and incubated for 3 h. All undissolved capsules were transferred to the colonic environment as the final part of the gastrointestinal simulation. Solutions from simulated gastric, intestinal, and colon conditions were used for the detection of drug release.

***In Vitro* Drug Release**

Aqueous solutions of the encapsulated drug traveling from the simulated *in vitro* gastric, intestinal, and colonic conditions were collected. All the solutions gathered were read at 300 nm using ultraviolet spectrophotometry (Thermo Scientific Multiskan Go) to determine drug release concentration upon exposure to the different gastrointestinal conditions. Drug concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL were used in the standard curve. Distilled water served as the blank.

**FTIR Analysis**

FTIR studies were carried out to determine the peak of functional groups present in the samples (unencapsulated drug, encapsulating material, and encapsulated drug) and to evaluate the encapsulation of the drug with the polymers using Bruker Alpha II FTIR (Bruker, Singapore). The solid samples were ground until fine powder form whereas liquid samples were retained as-is. One at a time, a small amount of sample was placed into the diamond plate and the percentage transmittance was read. The IR spectrum that was used was mid-IR with a wavenumber ranging from 4000–600 $cm^{-1}$ and a resolution of 4 $cm^{-1}$. The sample scan time was 24 scans with a measurement time of > 30 s. Running of samples were done in triplicates. The locations of important functional groups of the drug found in the FTIR spectra were identified and cross-checked with the FTIR spectra of drugs with carrier materials.

**Physical Property of Encapsulated Drug**

***Surface morphology using SEM.*** The size and morphological surface of the encapsulated SMX-TMP were determined using a Hitachi TM3000 SEM (Hitachi, Japan). The bead was taped on the sample holder and was observed at specified magnifications.

***Stability analysis of material using differential scanning calorimetry.*** Thermal analysis was utilized to analyze the thermal behavior of the drug-loaded capsules. DSC studies were carried out using the DSC 4000 thermal analyzer (Perkin Elmer, USA). Samples were run in a nitrogen atmosphere at a heating range of 25–400 °C with a heating rate of 10 ºC/min.

## RESULTS AND DISCUSSION

**Microencapsulation of SMX-TMP**

Encapsulation is a convenient tool to improve the delivery of active agents as it slows down the degradation process until the product is completely delivered at the desired sites, thus controlling their release at recommended rates (Bakry *et al.* 2015). In pharmaceutical industries,

this could help increase the retention of the delivered active drug molecule to the intended site of release. Ionic cross-linking aids in the formation of the functional membrane or coating that will protect the inner substance. It involves the interconnection between polymer chain and suitable metal ion, which is often utilized as a method of encapsulation of non-stable drugs. Interactions between the metal ion and functional group of a certain polymer are used as a crosslinking junction to produce a solid bead. However, each metal ion behaves differently and does not have the same capacity in forming a solid bead. Thus, the success of encapsulation depends on the structural integrity of the crosslinking junction formed.

Among all the divalent metal ion solutions tested for galactomannan, only calcium chloride and cobalt chloride help to form a rigid spherical bead (Figures 1B and 1C). This means that the metal ions were able to bond and cross-linked to the galactomannan. However, cobalt chloride is not an ideal metal ion crosslinker due to the harmful effects associated with it when it is exposed to the bloodstream (Czarnek *et al.* 2015). In the case of magnesium chloride, the galactomannan was not able to form a firm structure as it refused to harden (Figure 1A). It remained open and can easily disintegrate. It proved that this metal ion lacks the structural integrity to form a crosslinking junction. Finally, zinc chloride did not even produce a distinguishable formation of beads (Figure 1D). The calcium chloride solution was chosen as a crosslinker for the encapsulation of SMX-TMP since the crosslinking effect of calcium ion exhibited the most stable, compact beads compared to the other metal ions. Crosslinking of functional groups of alginates (guluronate and mannuronate) and galactomannan with the calcium ion was presented in Figure 2.

Galactomannan and sodium alginate served as the encapsulating material as they enclosed the drug with a membrane. The drug was composed of alginate as the outer membrane (Figure 3A) and galactomannan as the inner layer (Figure 3B). Calcium ions liberated from calcium chloride solution were able to bond to the free hydroxyl groups of the galactomannan, thus forming ionic cross-links to its polymer chain. It is reported that the presence of sodium chloride salt alters the structure and charge density of the galactomannan, hence aiding the intermolecular interactions in the formation of junctions in the polymer membrane (Mudgil *et al.* 2011).

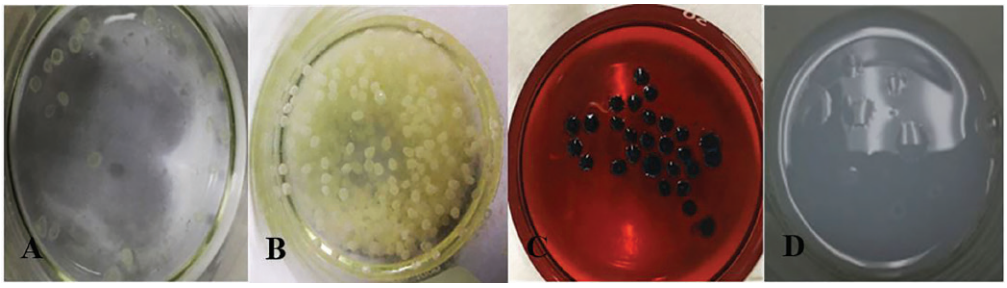


**Figure 1.** Evaluation of microcapsules formed in mixing galactomannan with divalent metal ions cross linker such as (A) 1M $MgCl_2$, (B) 1M $CaCl_2$, (C) 1M $CoCl_2$, and (D) 1M $ZnCl_2$.

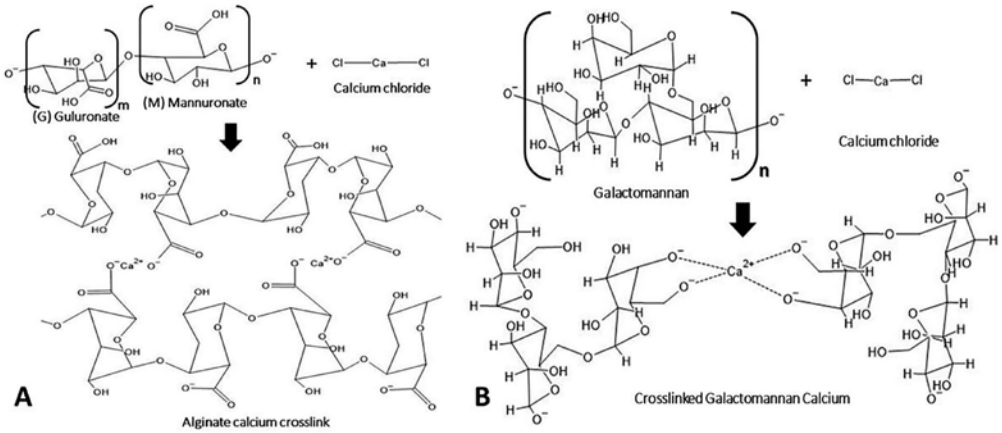


**Figure 2.** Crosslinking illustration of (A) alginate with calcium ion and (B) galactomannan with calcium ion in the formation of microcapsules.

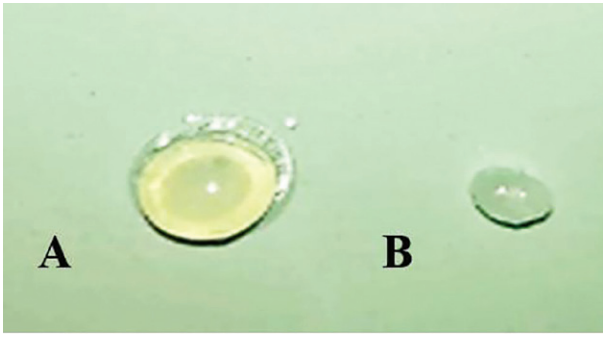


**Figure 3.** Encapsulated SMX-TMP: (A) spherical beads of galactomannan and alginate, and (B) spherical beads of galactomannan alone.

This binding interaction formed between the hydroxyl groups of the galactomannan and calcium chloride was responsible for the entrapment of SMX-TMP. There is no direct interaction between the drug and galactomannan as it produced insoluble, gel-like beads. For further stability of the drug, a secondary membrane was added. Based on the structure of alginate, it is mainly made up of guluronic acid and mannuronic acids. With the presence of divalent calcium ions, it was able to cross-link to the carboxylate groups of guluronate groups on the polymer backbone. This interaction entrapped the SMX-TMP encapsulated in galactomannan.

**Drug Release by Gastrointestinal Simulation**

Encapsulated drugs are highly affected by environmental changes when exposed to certain conditions. These are sensitive to chemical stimuli such as pH (Rizwan *et al.* 2017) and are very prone to degradation when exposed to the harsh environment of the GIT. The pH varies along the length of the GIT, as well as between fed and fasted states. To ensure that the encapsulated SMX-TMP was able to withstand the acidic condition of the gastric environment, the capacity of the polymers to disintegrate was analyzed. The beads remained intact and stable upon exposure to both fed (pH 2.4) and fasted states (pH 1.4) in the gastric environment (Figures 4A and 4B). However, when it comes to the higher pH of the intestinal environment, there was a partial disintegration of the beads at the fed state (pH 5.4) and completely dissolved beads at the fasted state (pH 8.0) (Figures 4C and 4D). The residues of the partially disintegrated beads were only completely dissolved upon reaching the higher pH of the colonic fluids (Figure 4E).

Further quantification of the drug release in the gastrointestinal simulation was used to determine if the encapsulated SMX-TMP was able to maintain its integrity at the gastric environment's acidic condition and control its drug release to the intestine (Figure 5). As observed, the concentration of the encapsulated drug at both fasted (pH 1.4) and fed (pH 2.4) states at the gastric environment was nearly zero, indicating that the galactomannan-alginate coating has been able to withstand the acidic condition without releasing its drug content. However, upon exposure to the higher pH condition of the intestinal environment, there was an increased concentration of drug in the solution of both fed (pH 5.4) and fasted (pH 8.0), showing that the galactomannan-alginate coating started to release its drug content. In the first 30 min, there was an immediate increase in the drug release since the encapsulated drug was exposed to the intestine's new high pH condition after it passed the gastric environment. After 3 h, the sudden increase in drug concentration in the solution indicated the total disintegration and release of the drug content of the encapsulated drug at the intestinal environment. Thus, the encapsulation using galactomannan and alginate was able to modulate the drug's delivery to the intestine. The delivery of the encapsulated drug without being absorbed in the gastric environment permits a higher concentration of drug to its target site of release.

The pH varies along the length of the GIT. In the gastric environment, the pH is very acidic and ranges between 1.5–3.5. Meanwhile, the intestinal pH ranges from slightly acidic at the duodenum to basic at the ileum (pH 5.5–8.0). Galactomannan and alginate have ionizable groups attached to their backbone that can accept or donate $H^+$ ions. The change in the pH of the GIT environment causes either protonation or deprotonation of the ionizable groups. This shift in ionic charge of the polymer leads

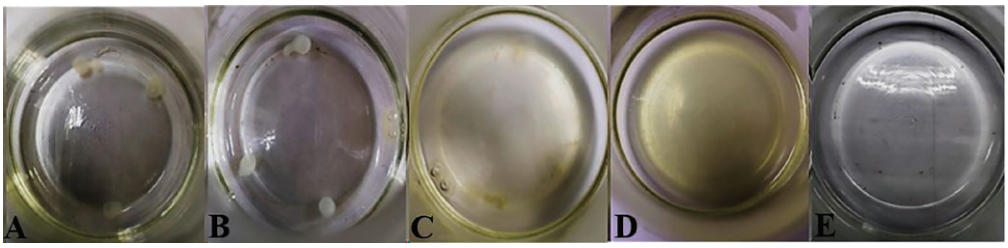


**Figure 4.** *In vitro* gastrointestinal simulation of galactomannan-alginate encapsulated SMX-TMP at (A) gastric fed state, (B) gastric fasted state, (C) intestinal fed state, (D) intestinal fasted state, and (E) colon fed state.

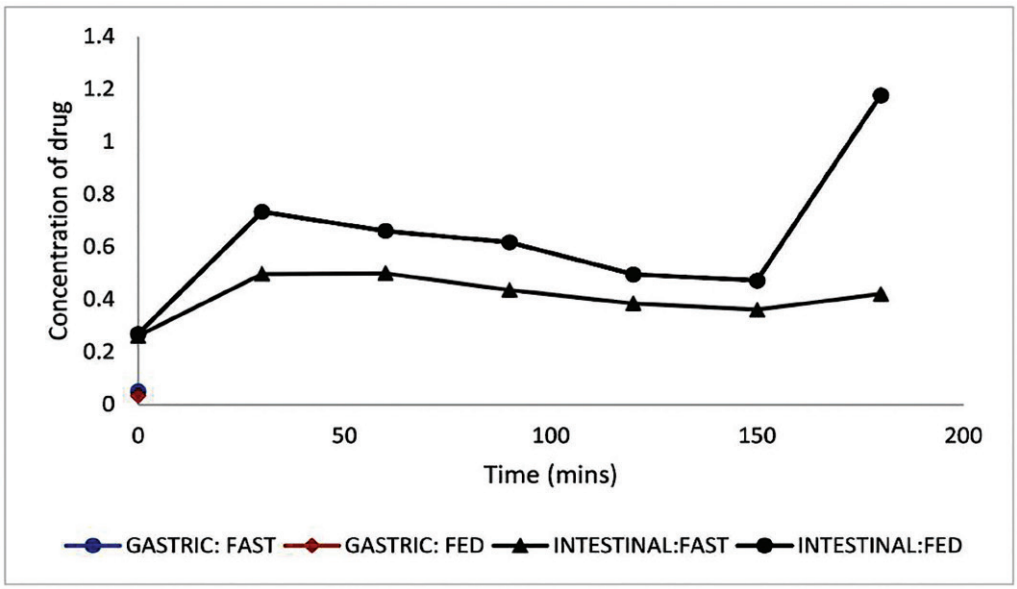


**Figure 5.** *In vitro* drug release of galactomannan-alginate encapsulated SMX-TMP.

to a change in conformation and can result in either a collapsed state or an expanded state (Liu *et al.* 2017; Tayo 2017). Alginate – having pKa values of 3.38 and 3.65 for mannuronic and guluronic residues, respectively (Chuang *et al.* 2017) – swells at the basic condition of the intestinal environment. This is due to the ionization of its acidic groups, causing it to disintegrate and release its components. There was an electrostatic repulsion between the carboxyl groups of galactomannan-alginate membrane that leads to the change of carboxyl groups into $-COO^-$ at higher pH. The higher the repulsion among $-COO^-$ groups, the higher the tendency of the alginate membrane to dissociate (Seeli and Prabaharan 2016). On the contrary, the disintegration of the inner coating was mainly due to the swelling and retarding characteristics of the galactomannan (pKa range = 6.0–7.0) (Kaur and Kaur 2018). As this polysaccharide gets hydrated, it swells and forms a viscous layer that helps in controlling the release of the drug at the intended site of release. At low pH, the alginate was compacted and stable while the galactomannan could easily disintegrate because of the dissociation of the crosslinking junction in the polymer. The use of alginate as a secondary membrane for the drug helps in the stabilization of the galactomannan at acidic pH (Rizwan *et al.* 2017).

**FTIR Analysis**

FTIR spectroscopy analysis was used to characterize the complex structure of the encapsulating agents, the drug, and the encapsulated drug itself. It identifies the functional groups of each compound and confirms if the SMX-TMP is really incorporated inside the polymers. The FTIR spectra of galactomannan and alginate are almost the same since they are both polysaccharides in nature with a distinguishable -OH at 3400 $cm^{-1}$, -CH at 2900 $cm^{-1}$, and -C-O-C- at 1000 $cm^{-1}$ (Figures 6A and 6B). The only difference is that alginate has a distinct presence of the carboxylate group (O=C-O) in its polymer backbone, which is visible at 1400 $cm^{-1}$ and 1600 $cm^{-1}$. Furthermore, the FT-IR spectrum of the drug produced several peaks of functional groups since this drug is a combination of SMX and TMP that produce a synergistic effect. The functional groups -NH at 3400 $cm^{-1}$ comes from SMX alone and $-NH_2$ at 3200 $cm^{-1}$, -CH (aromatic) at 3100 $cm^{-1}$, and -C=C- at 1700 $cm^{-1}$ come from both compounds (Figure 6C). Upon encapsulation, the FT-IR spectrum of the final product validated that the drug was successfully entrapped by the polymers (Figure 6D). The broad peak at 3400 $cm^{-1}$ was attributed to the -OH group of both polymers while the -O=C-O- at 1400 $cm^{-1}$ came from alginate alone. Finally, -C=C- represented the aromatic group of the drug. The overlapping peaks of these components to the final product just proved that each component was embodied in the encapsulated drug.

**Morphological Characterization of Drug Encapsulated**

Further analysis of the encapsulated drug showed a tightly packed surface morphology, indicating that the outer membrane alginate was able to entrap properly the galactomannan containing the drug (Figure 7A). The figure also proved that the gelation of the alginate with the divalent $Ca^{2+}$ was compatible enough to produce solidified spherical beads. The size of the encapsulated drug was

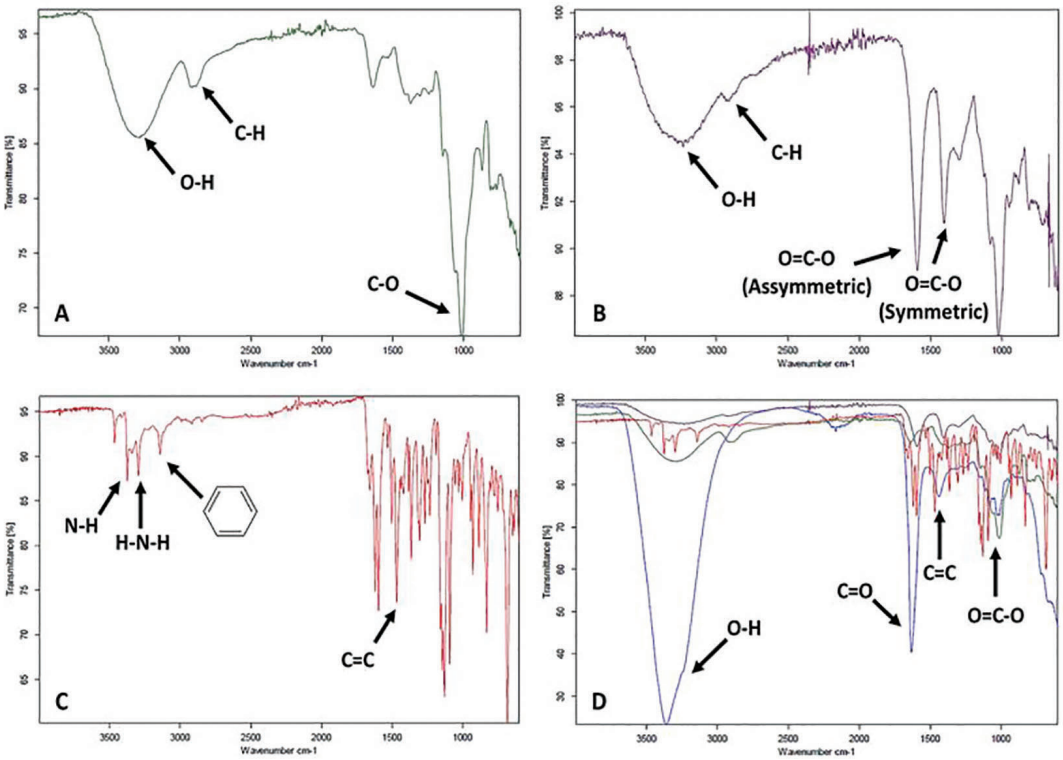


**Figure 6.** FT-IR spectrum of each component of the encapsulating materials, drugs, and encapsulated drugs such as (A) galactomannan, (B) sodium alginate, (C) SMX-TMP, and (D) encapsulated SMX-TMP (blue).

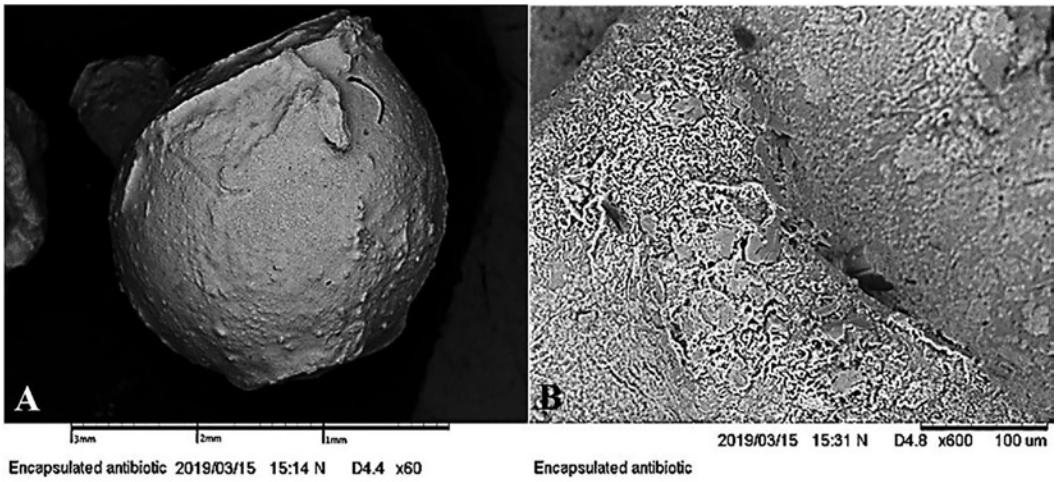


**Figure 7.** Microscopic analysis of encapsulated SMX-TMP showing the surface morphological characteristics of (A) encapsulated SMX-TMP at 60X magnification and (B) surface of the capsule at 100X magnification.

approximately 2.7 mm. The average size is critical in the encapsulation efficiency and integrity because it affects the thickness of the encapsulated drug and its dispersion. If the size of the beads gets larger or smaller than the average size, it would increase or decrease the thickness of the membrane of the encapsulated drug, which could lead to poor dispersion of the content. Moreover, the surface of the final product was a little rough due to the presence of pores (Figure 7B). These pores allow the drug to diffuse in or out of the gel beads, depending on their size. Despite the property of alginate to become biocompatible material that could hold the inner components, it is still unable to form complex and only acts as an extracellular matrix (Sanchez *et al*. 2013).

**Thermal Stability of the Drug Encapsulated**

Differential scanning calorimetry measured the stability of the spherical beads in response to heat exposure. It shows the thermal transition temperature (Tm) as the peak of the data plot that signifies the complete melting point of the polymers, whereas the area under the peak corresponds to the total energy change needed to the unfolding of the polymers. Measuring the thermal stability of the drug's encapsulating polymers is a factor that determines how stable they are against temperature. The higher the Tm, the more stable the polymers are at elevated temperatures. Likewise, DSC is also used to determine the physicochemical interaction between the drug and the encapsulating polymers (Mello and Ricci-Júnior 2011). The thermal behavior of the galactomannan and alginate as encapsulating materials was analyzed wherein the difference in the amount of heat needed to elevate the temperature of the standard and the sample was evaluated as a temperature's function (Karoui 2012). For the galactomannan-alginate alone, two separate endothermic peaks were exhibited because of the presence of two different polymers (Figure 8A). As observed, the two peaks have shown high melting temperatures indicating that both galactomannan and alginate have high thermal stability. These polymers are made up of long molecules of carbon attached together by strong bonds. This is an indication that they have resilient intermolecular forces, which give them a higher melting point. Likewise, in Figure 8B, two separate endothermic peaks are observed. The absence of the melting peak of the drug on the DSC thermogram suggested that the polymers inhibited the degradation and alteration of the drug's structure upon exposure to high temperature. The abrupt increase in the thermal behavior of the first peak from Figure 8A (122.82 °C) to Figure 8B (138 °C) indicated the interaction of the SMX-TMP to the polymers. The sudden shift within the polymers' thermal behavior and the drug indicated that the encapsulating polymers were able to entrap and interact with the drug, respectively (El-Houssiny *et al.* 2016). The endothermic peaks and melted enthalpy are essential in ascertaining the compatibility of the polymers with the drug.

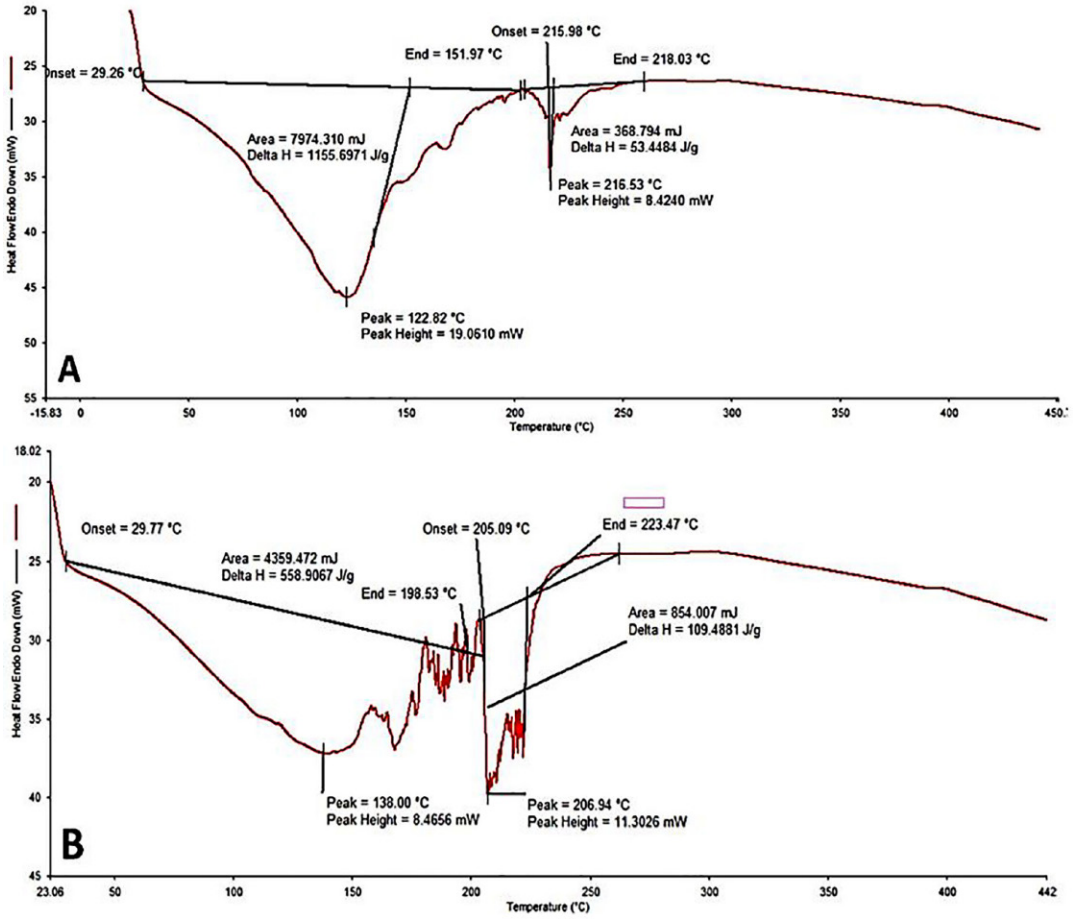


**Figure 8.** Differential scanning calorimetry thermogram of (A) galactomannan and alginate alone, and (B) galactomannan and alginate with SMX-TMP.

## CONCLUSION

Natural polymers like galactomannan and alginate have shown an extensive application in pharmaceutical industries as they were used as a material to help control the drug delivery system. These natural polymers were able to encapsulate SMX-TMP to withstand the acidic condition of the gastric environment. The encapsulated drug exhibited integrity and stability after being subjected to *in vitro* gastrointestinal simulation. Its coating only disintegrated in the intestine, enabling the drug to deliver its maximal therapeutic efficacy at the intended site of release. The FTIR spectra confirmed that the drug was successfully incorporated within the encapsulating materials. It was also observed that the final product was spherical in shape with some ridges at the surface and has a size of approximately 2.7 mm, as shown on the SEM micrographs.

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