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RAPID COMMUNICATION

Synthesis of an antibiotic-preserving antimicrobial tetracycline-loaded gellan gum gel

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Abstract−Gels play a significant role in our daily lives as they provide stability and desired features to various items of regular use, such as cosmetics, pharmaceuticals, household items, and agricultural products. However, a microbial attack on gels commonly occurs during gel-based formulations. Antibiotic-loaded gels can reportedly be stored for a long time. In this study, we fabricated a tetracycline-loaded gellan gum (TC/GG) gel and assessed its long-term stability against microbial attack. Morphological analyses revealed a firm and smooth texture with multifold bumpy surfaces on the GG and TC/GG gels. Fourier transform infrared spectroscopic analysis showed that the gel was well hydrated and thus suitable for releasing antibiotics. The feasibility of inhibiting microorganisms was qualitatively examined using the disk diffusion method. Interestingly, the unloaded GG gel itself exhibited an antimicrobial effect, while the loaded TC/GG gel showed a better antibacterial effect on *S. aureus* than on *E. coli*. TC/GG can be used to store and protect gels from microbial attacks for a long duration. In addition, it is suitable for storing bioactive agents, which are used in the biomedical field.

Keywords: Gels, Gellan Gum, Tetracycline, Encapsulation, Antimicrobial Property, *E. coli*, and *S. aureus*

INTRODUCTION

Natural gums are widely used in our daily lives because of their unique properties, such as biocompatibility, easy availability, and affordable production costs. Polysaccharides are the major components of gels derived from plant tissues (e.g., gum acacia), seeds (e.g., guar gum) and microorganisms (e.g., gellan gum (GG) and xanthan gum) [1]. Typically, different types of gels and their derivates are investigated as fillers or extenders for pharmaceutical and biomedical applications [2-4]. Common examples of gellan-based excipients are matrices for tables, crosslinked or soft hydrogels, pellets, floating beads, and transdermal films [5]. Among these, GG has garnered considerable research attention owing to its ability to form hydrocolloids, with different morphologies and textures based on its concentration, in the presence of other ingredients and processing conditions [6]. At a low concentration, GG forms a viscous solution, which can be used as a thickening agent in food additives. As the concentration is increased to a high value, the morphology of GG gels varies from that of a soft gel to that of elastic gels. Therefore, gel formation depends on gellan gum's concentration, pH, and type of additive (such as salts and sugars) [7]. Low GG concentration, typically between 0.05% and 0.2%, results in the formation of soft gels with delicate textures, which are frequently used in confections and sweets [8]. In contrast, high GG concentrations, usually above 1.0%, lead to the formation of elastic gels, which are rigid and can be severely deformed without breaking. They are frequently utilized in food products, including gluten-free baked goods, vegan cheese, and vegan meats [9].

In addition to gel formation, GG can be used to prepare textures like foams, emulsions, and suspensions, which are frequently used in culinary techniques for providing better distinctive sensory experiences [10]. Moreover, a GG-based form of microbial polysaccharide is also employed as a thickening agent, gelling agent, and food ingredient [11]. Its linear chain-like structure is made up of repeated units of rhamnose, glucuronic acid, and glucose. Tetrasaccharides are the fundamental building blocks of GG and are composed of one glucose molecule, two glucuronic acid molecules, and one rhamnose molecule. To create the finished polymer, this tetrasaccharide unit is replicated numerous times [12]. Because of its distinctive structure, GG has special qualities, such as the ability to form solid gels at low concentrations and resilience to heat and acid [13]. GG has a highly intricate composition that can be altered depending on the type of microorganism and environmental conditions utilized in its production. Furthermore, the morphological features of GG, which is an anionic polysaccharide and forms rigid and brittle gels upon interaction cations (such as calcium and magnesium ions), are vastly different from those of xanthan gum and guar gum [14], which are typically viscous and colloidal dispersions [15]. Moreover,

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GG exhibits a firm and brittle texture that can withstand high temperature and acidic conditions. Therefore, GG is considered a suitable additive for a variety of products, including sauces, desserts and beverages [16].

GG is a water-soluble polysaccharide produced from the microorganism *Sphingomonas elodea*. Improper storage and handling of gellan-based gels are the major causes of gel degradation under microbial attacks, and mitigating this microbe-induced degradation of GG gels is challenging [17]. Microbial proliferation on GG gels spoil and degrade the final product by altering its texture, taste, and overall quality [18]. Thus, upholding appropriate hygiene and sanitation practices during the manufacturing process, storage, and handling of the finished product is crucial to prevent microbial contamination of GG gels [19]. These practices include keeping the equipment and facilities clean and dry, utilizing antimicrobial agents or preservatives, and regularly monitoring the temperature and pH of the product. Implementing and adhering to such guidelines ensures that the quality and safety of the GG gel are maintained and prevents deterioration or decomposition of the final product due to microbial proliferation [20]. In a recent study, GG and an evaporative casting approach were employed to prepare norfloxacin-loaded films with antibacterial activity. The evaluated morphology, water vapor transmission rates (WVTRs), water uptake, release kinetics, and antibacterial activity of the prepared films indicated the formation of a smooth film surface with a uniform distribution of the constituent materials. Evidently, high norfloxacin concentrations increased film swelling but decreased the WVTRs. Almost all the films showed a 5- to 20-min drug release rate [21]. Additionally, the antibacterial activity was proportional to the release rate, with higher norfloxacin concentration leading to stronger antibacterial properties. Coutinho et al. developed a GG hydrogel incorporated with ibuprofen and examined its mechanical and physical properties, drug release behavior, biocompatibility, and antibacterial activity. The GG hydrogel containing 5.0% ibuprofen displayed good mechanical properties, a low drug release rate, and a WVTR comparable to that of commercial wound dressing products. Furthermore, the hydrogel exhibited a low but noticeable antibacterial activity and was found to be biocompatible with human dermal fibroblast cells—a feature that makes it a potential wound dressing material [22]. In the current study, a tetracycline-loaded gellan gum (TC/GG) gel was synthesized, and its resistance to microbial attack was evaluated to assess the feasibility of using this proposed gel for transporting materials in various biomedical applications.

MATERIALS AND METHODS

GG, with specifications pH 5.0-7.5 (0.5% aq. sol.), a gelling temperature of 35-40 °C (2% sol.), a gel strength of 2%, maximum 15% loss on drying, and 95% TC HCl, was purchased from Sisco Research Laboratories Pvt. Ltd., India). All the commercial chemicals were used without further purification.

1. Preparation of Gel

GG was prepared by preheating 2 wt% of a 20 mL solution of GG to 60 °C with continuous stirring for 2 h, followed by cooling to 40 °C. The TC-loaded gel was prepared by adding 41.58 mM of TC to the prepared solution, followed by heating (60 °C) and then cooling (40 °C) the mixture to form an antibiotic-loaded gel.

2. Characterization of the Gel

The microstructure of the prepared gel was characterized by scanning electron microscopy (SEM). In SEM, a small amount of the GG gel is placed on a silicon wafer, and the gel is air dried overnight. The gel is fractured to reveal its internal structures, if required. The dried sample is mounted onto a stub using a conductive adhesive, such as a carbon tape (or silver paint). The stub is then placed into the SEM chamber. The SEM is operated at high vacuum and low temperature, and the sample is bombarded with a beam of electrons. The electrons interact with the sample surface and produce secondary electrons, which are detected by the SEM and used to create an image. The image can reveal the microstructure of the GG gel, such as the morphology and texture of the gel particles with their spatial arrangement. The SEM images might also show the presence of pores or voids within the gel matrix, which can affect the gel's texture and water-holding capacity. Overall, SEM analysis can provide valuable information about the microstructure of GG gels, which can be useful for understanding their physical and mechanical properties.

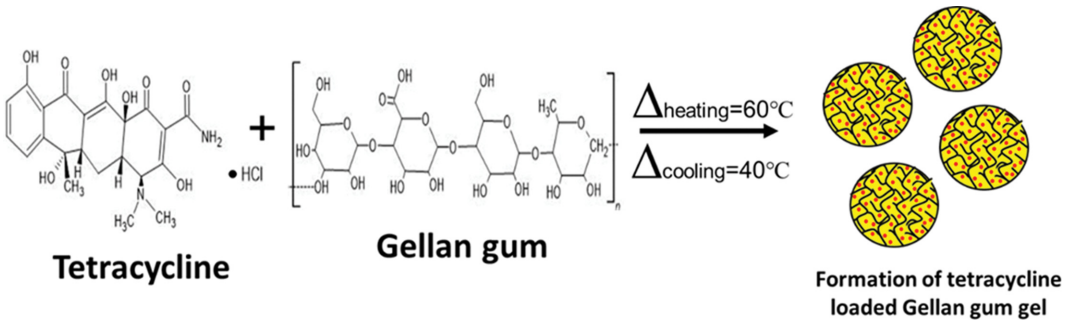
3. Antibacterial Activity

The disk diffusion test is commonly used to determine the antibacterial activity of compounds. The effectiveness of the antibacterial substance was evaluated by conducting tests on both gram-positive bacteria, specifically *Staphylococcus aureus* (*S. aureus*), and gram-negative bacteria, particularly *Escherichia coli* (*E. coli*). Muller-Hinton (MH) agar, which is a standard growth medium, was sterilized using an autoclave at a temperature of 120 °C for 15 min. The bacteria were cultivated in the MH agar and placed in an aerobic incubator at a temperature of 37 °C for 24 h. After the 24-h aerobic incubation period, the petri dishes were inspected to observe whether a clear zone had formed around the disk containing the specimens. A camera was then used to take photographs of the petri dishes for further analysis and evaluation. The size of the zone of bacterial growth inhibition (ZOI) depends on various factors, including the potency of the antibacterial compound, concentration of the compound on the disk, and susceptibility of the bacterial strain being tested. Therefore, the disk diffusion test provides a qualitative measure of the antibacterial activity of the compound and not a quantitative measure.

RESULTS AND DISCUSSION

1. Fabrication of Antibiotic-loaded Gel

The antibiotic-loaded gel was fabricated by dropwise mixing of aqueous TC with preheated GG; the final gel was prepared by heating GG and then cooling it as shown in Scheme 1 [23]. GG offers several advantages, including the ability to form gels at low concentration, resulting in a finer texture of the final product, while minimizing the overall amount of thickener required. Moreover, because of thermal reversibility, GG can be melted and re-gelled multiple times without compromising its texture or stability. This property is particularly useful in food processing or cooking, which requires frequent heating and cooling. Additionally, GG gels have a consistent and smooth texture, which can enhance the sensory experience of the product user. They can also be tailored to achieve a range of



Scheme 1. Scheme for fabricating a TC-loaded GG gel.

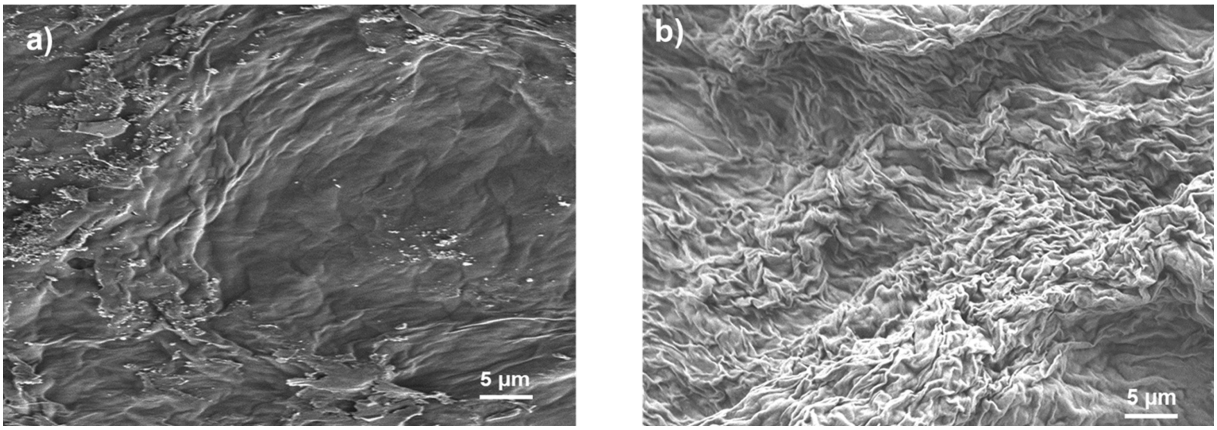


Fig. 1. SEM image of the fabricated gel. a) Unloaded GG gel, b) TC-loaded GG gel.

textures, from soft and creamy to firm and chewy, making them versatile for use in various food and beverage products.

Moreover, when GG is heated to approximately 60 °C, the polymer molecules separate from the chain and interconnect to form a three-dimensional structure, which helps in the formation of a gel. When the temperature rises above 60 °C, the mobility of the molecules increases, thereby inhibiting the formation of a gel network. However, as the temperature drops, the mobility of the polymer chains decreases, allowing the molecules to intertwine and create a gel. The exact temperature at which GG forms a gel depends on several factors, including the concentration and pH of the gum as well as the presence of other ingredients or additives. Nonetheless, in general, GG starts to form a gel at approximately 60 °C or less.

TC is a broad-spectrum antibiotic that can be used to treat a variety of bacterial infections, including respiratory, urinary tract, and sexually transmitted infections (like chlamydia and gonorrhea). TC inhibits bacterial protein synthesis and thus prevents bacterial multiplication and subsequent spread. Notably, TC is an attractive choice for patients with limited financial resources because of its cost-effectiveness. Additionally, TC is available in both oral and topical forms and can thus be used to treat different types of infections.

2. SEM-based Morphological Analysis of the TC/GG Gel

SEM-based imaging is widely employed to analyze the physical characteristics and internal structure of gels. Gels are soft materials containing a three-dimensional network of polymer chains or other molecules linked together by weak bonds. In SEM, an electron beam is irradiated on the gel sample to produce high-resolution images that reveal the surface features, porosity, and pore size distribution of the gel. Fig. 1a shows an SEM image of the unloaded GG gel, revealing a firm and smooth surface without any interconnected channel and porous structure. The surface of the gel appears bumpy and uneven due to the gel network formed by the individual polymer chains (or molecules). In Fig. 1b, the TC/GG gel appears to be soft and undulating as well as microbe-free.

3. FTIR Spectroscopy of the Synthesized Gel

Fourier transform infrared (FTIR) spectroscopy is commonly employed to investigate the chemical structure of materials, such as gels. GG is a polysaccharide that forms gels when are exposed to divalent cations, and FTIR spectroscopy can be performed to evaluate the constituent functional groups and gel structure of GG. For the FTIR analysis, a small dried gel sample was placed in the FTIR spectrometer to measure the absorbance/transmittance of the sample at different wavelengths. The resulting FTIR spectrum, shown in Fig. 2, displays peaks at 1,600, 3,400-3,600, and 1,000-1,100 cm^{-1}, which can be ascribed to the functional groups present in the polysaccharide chain of GG, i.e., the carboxyl group (C=O), hydroxyl group (-OH), and glycosidic bonds (C-O-C), respectively. Additional peaks corresponding to divalent cations, like calcium (Ca^{2+}) or magnesium (Mg^{2+}) ions, which are typically utilized to crosslink the GG chains and form a gel network, are also visible. Further, the FTIR spectrum of TC HCl shows peaks at approximately 1,660-1,680, 1,500-1,600, and 3,000-3,100 cm^{-1}, which

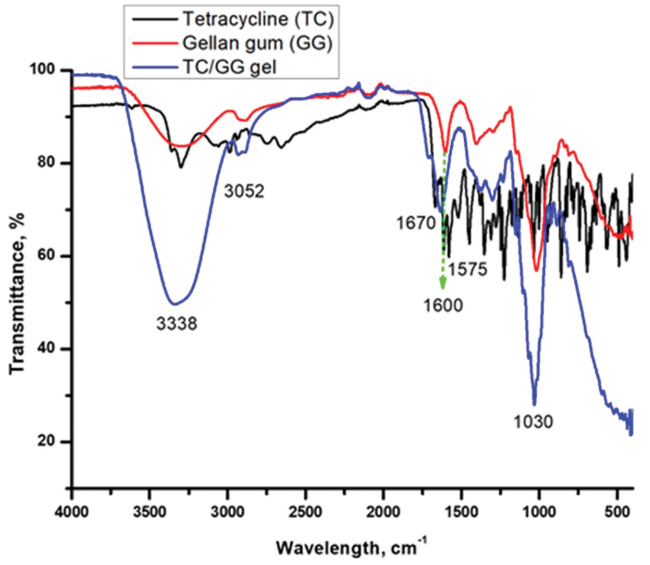


Fig. 2. FTIR spectra of the loaded and unloaded TC/GG for the freeze-dried gel.

originate from the amide group (C=O-NH), phenyl group (C=C), aromatic ring (C-H) present in TC, respectively. In addition, a peak corresponding to Cl^- is observed. The spectrum of the hydrated gel (Fig. 2) exhibits peaks at 3,335, 1,635, and 428 cm^{-1}, which correspond to -OH, C-C, and C-H, respectively.

4. Antibacterial Activity of the Fabricated TC/GG Gel

The antibacterial activity of a gel is typically assessed by various methods, among which the disk diffusion assay is the most widely used. In the disk diffusion assay, the target gel is placed into a bacterial culture on an agar medium, the ZOI around the gel is measured. In our study, we qualitatively estimated the antibacterial activity of TC, unloaded GG, and the TC/GG gel using the disk diffusion method, and the corresponding results are displayed in Fig. 3. The TC-induced ZOI was measured against *E. coli* and *S. aureus* after 24 h. For a 2 g TC/GG gel sample loaded with 1 mg of TC, the ZOI are 20±1 and 34±1.7 mm for *E. coli* and *S. aureus*, respectively (Table 1). The control sample, i.e., a sample loaded with 100 μg of

Table 1. ZOI of TC, the GG gel, and the TC/GG gel

S. No.	Material	ZOI (mm)	
		E. coli	*S. aureus*
1	TC (100 μg)	30±1.5	35±1.75
2	Unloaded GG gel	12±0.6	10±0.5
3	Loaded TC/GG gel (1 mg)	20±1	34±1.7

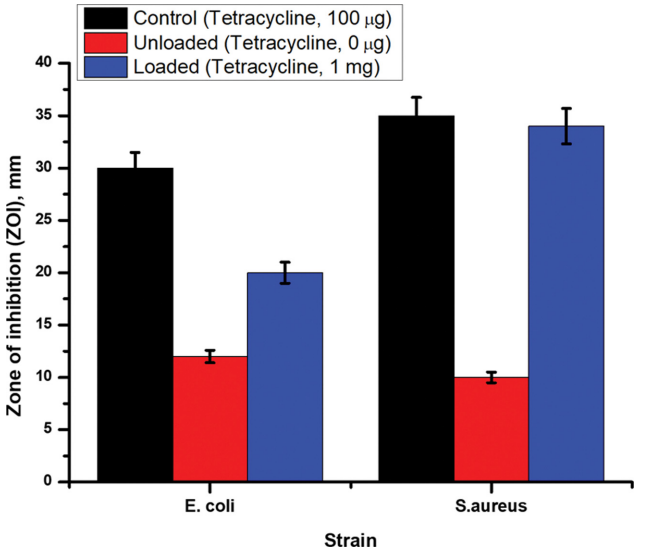


Fig. 4. ZOI of TC, GG, and the TC/GG gel for *E. coli* and *S. aureus* strains.

TC, shows the maximum antimicrobial activity against *E. coli* and *S. aureus*. The antimicrobial activity of TC against *S. aureus* is 17% higher than that against *E. coli*. The measured ZOI of the TC/GG gel reveals a 67% jump in antimicrobial activity compared to that of the control. Notably, the antimicrobial activity of the unloaded GG gel is 40% higher than that of the control and is suitable for developing GG-based formulations for biomedical applications. Moreover, the antimicrobial activity of the loaded TC/GG gel against *S. aureus* is 70% higher than that against *E. coli* (Fig. 4).

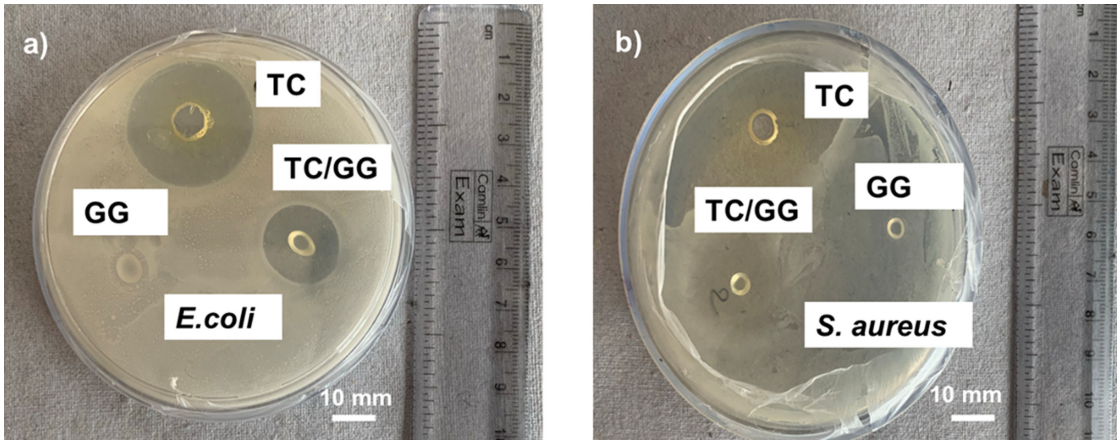


Fig. 3. Disk diffusion assay of TC (control), GG, and the TC/GG gel against *E. coli* and *S. aureus*.

CONCLUSIONS

We developed TC/GG and GG gels and analyzed their morphology and antibacterial activity. GG exhibited a firm and smooth surface, whereas the TC/GG gels exhibited bumpy textures with undulating surfaces. The FTIR analysis showed that the gels were hydrated and loaded with TC. The antibacterial activity of the TC/GG gel against *S. aureus* was better than that against *E. coli*. After loading, no microbe was detected on the TC/GG gel. The unloaded GG showed an antimicrobial activity of approximately 40% compared to the control. However, the poor solubility and dispersion of GG in water can hinder its implementation in certain applications. Additionally, changes in pH can affect gel formation ability of GG and thus limit its effectiveness in certain formulations. Current antibiotic formulation has certain drawback such as systemic side effects by disrupting natural microbial balance in various organs that leads to allergy and organ toxicity. In addition, the major issue on antibiotic formulation stimulates the development of antibiotic resistance at various microorganisms. Other limitations, such as poor targeting, destroys pathogenic as well as beneficial microbes and it is limited for local applications.

The biocompatibility of gellan gum-based formulations is suitable to use in biological systems. It is non-toxic, non-cytotoxic and non-immunogenic, which makes it suitable for biomedical applications. The degradation rate can be controlled over time by modulating various factors, such as concentration, presence of enzyme, pH and temperature. After degradation, the end product must be a simple sugar that will not harm the human body. The mechanical strength and elasticity can be altered by changing concentration and cross-linking agent which is used to make soft and flexible gel to apply in tissue engineering applications. While TC can be effective in treating a wide range of bacterial infections, it is not universally effective against all types of bacteria, and certain strains of bacteria have developed resistance to this antibiotic over time. Further, TC can cause side effects, such as gastrointestinal problems, photosensitivity, and tooth discoloration, which may limit its effectiveness in some patients. The rise of antibiotic resistance among pathogens poses a significant challenge in healthcare, highlighting the need for alternative strategies to combat multi-drug resistant microorganisms. Here are a few examples of advanced materials that could offer protection against multi-drug resistant microorganisms such as antimicrobial peptides (AMPs) (e.g., LL-37, cathelicidin) and antibacterial nanomaterials (e.g., silver nanomaterials). Despite these limitations, the proposed TC/GG gel can be used in various applications with long-term storage without degradation due to microbial attack.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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