EVALUATION OF RELEASE PATTERN AND DEGRADATION PROFILE OF MODIFIED TRIPLE ANTIBIOTIC HYDROGEL AS AN INTRACANAL MEDICAMENT

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Abstract:

Objectives: To evaluate the drug release profile and degradation potential of carrageenan based modified triple antibiotic hydrogel at a concentration of 1 mg/ml.
Materials and Methodology: Using the ultraviolet (UV) spectrophotometric approach, the amount of antibiotic (ie, amoxicillin, metronidazole and ciprofloxacin) released from the hydrogel over a 7-day period was calculated.The assessment of drug release was continued for 7 days at a wavelengths specific to each antibiotic.At various times and with various pH levels, the hydrogel's ability to swell was investigated. 100 mL of distilled water were used to submerge the hydrogel.After removing the extra surface water with tissue paper, the weight of expanded hydrogels was measured at a specific time and temperature. Hydrogel's swelling ratio (Sr) and percent swelling (Ps) were computed.
Results: It was observed that antibiotics were successfully released from the hydrogel throughout the intended 1-week period. The burst effect was relatively minimal, as these profiles demonstrated. The modified triple antibiotic was released sustainably over a long period of time (7 days) in this system owing to biodegradable carrageenan hydrogel. It was also seen that there is minimal degradation associated with carrageenan hydrogel.
Conclusion: Our study's findings led us to the conclusion that the modified triple antibiotics' release pattern from hydrogel was acceptable . As a result, carrageenan hydrogel based intracanal medicament can be seen as a novel way to deliver antibiotics to the root canal area and offer localized and prolonged drug release.

Keywords: Antibiotics, degradation profile, drug release, Hydrogel, intracanal medicament

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1. Introduction

The major objectives of RCT are to eradicate all microorganisms from the inner surface of the root canal system, to prevent reinfection, and to establish or preserve healthy periapical tissues. There are still some restrictions to the endodontic disinfection process; viable biofilm cells can persist in undertreated and untreated locations of the root canal system due to the inherent difficulties associated with its complex anatomy.[1] Endodontic biofilms are a prevalent cause of persistent infections in the root canal due to their resistance to antimicrobial agents. Resistant microbial species such Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Streptococcus species, Actinomyces species, and Candida species, among others, may persist in root canals, which is of particular concern.[2][3] Modern techniques and equipment have greatly contributed to increased clinical success rates and significantly shortened the time needed to complete the RCT. It may be necessary to complete the RCT over the course of more than one appointment if the clinical situation is challenging and requires additional time, improved practitioner ability, and modern instruments for treatment. In order to achieve disinfection and prevent reinfection in between sessions, intracanal medicament is still a common clinical practise.[4]

An ideal intracanal medication would get rid of any residual microorganisms, reduce periapical tissue inflammation, render canal contents inert and neutralise debris, function as a barrier against leakage from temporary filling, and aid in drying persistently wet canals. This makes calcium hydroxide (CH) widespread use plausible[5]. Even so, E. faecalis and other tenacious intracanal microorganisms cannot be entirely eradicated by CH despite its good physical, biological, and pharmaceutical properties [6]. Chlorhexidine (CHX) in gel is an alternative to CH that has been proven to be highly efficient against E. faecalis. However, they also faced some drawbacks such as high cytotoxicity. An antibiotic combination known as "triple antibiotic paste" (TAP) was developed specifically for the treatment of open apex teeth with necrotic pulp as well as the regeneration and revascularization regimen. Additionally, this material has demonstrated other endodontic applications. TAP was initially developed by Hoshino and colleagues, who looked into the paste's efficacy in removing bacteria from root canals. Additionally, TAP has been utilized in vitro to clean dentine contaminated with Escherichia coli.[7] Later, the antibiotic paste's effectiveness in combating the bacteria found in carious dentine and diseased pulp received special attention. The results demonstrated that the bacteria had been completely eliminated from the radicular system.[8] However, a drug's effective antibacterial properties by themselves are insufficient to combat endodontic biofilms.

Extracellular polysaccharides (EPS), which act as an affinity matrix, raise the medium's viscosity, and hence slow down the diffusion of antimicrobials, limit the penetration of a medicine into deeper layers of the biofilm. However, antimicrobial agents at higher concentrations in the biofilm's outer layer should result in faster overall diffusion rates into the deeper layers.[9][10] A drug can be incorporated into a locally-administrable type of formulation or device that controls its local release at the site of action in order to produce high local concentrations for a prolonged period of time. The application of local drug delivery systems based on hydrogel systems, microparticles, and nanoparticles constructed of biocompatible polymers is a novel approach. Such systems enable the direct placement of antimicrobial agents or other medications inside the root canal, as well as the sustained release of steady concentrations of these drugs for improved infection control.[11] In many areas of dentistry, numerous sustained release devices have previously been designed, examined, and made readily available for purchase. The root canal system can similarly use this principle of controlled release.[12]

Biopolymers are receiving a great deal of interest as a replacement for current fossil fuel supplies and as a cleaner source to comply with environmental requirements. They come from agricultural non-food crops and are sustainable, environmentally benign, renewable, and carbon neutral. Biopolymers also have the potential to cut carbon emissions and lessen atmospheric CO2 concentrations. Drugs can be encapsulated in biocompatible, environmentally friendly hydrogels using biopolymers, which can then be used in drug delivery systems.[13] Hydrogels are 3D structures which are hydrophilic in nature and have high molecular weight.They provide a number of benefits over traditional drug delivery methods, including sustained release and prolonged activity. They are less expensive, have less side effects, better drug utilization, target drugs to specific locations like the colon, etc.[14] Due to their numerous applications, hydrogels have drawn a lot of attention in recent years. Due to their high water content, they are extremely flexible and closely resemble natural tissues. Numerous studies on the use of hydrogels in various fields have been published.[15] Controlled drug delivery uses a multidisciplinary approach, which has several benefits over traditional dose forms, including increased efficacy, less toxicity, better patient compliance, and appropriateness. Depending on the hydrogel's composition and release kinetics, multiple mechanisms are involved in drug release

from hydrogel.[16] In this study, with the continuation of our previous work, we fabricated carrageenan/ xanthan gum loaded with modified triple antibiotic medicament, and evaluated the drug release and degradation potential of this drug delivery system.

2. Methodology:

Antibiotic Hydrogel Preparation:
In the present investigation, hydrogels containing carrageenan and xanthan gum were used to synthesize low concentrations of MTAP hydrogel(1, 5, and 10 mg/mL) as described in previous studies While MTAP contains metronidazole, ciprofloxacin, and amoxicillin, TAP contains metronidazole, ciprofloxacin, and minocycline. The powder was made into a stock solution with a concentration of 10 mg/mL using distilled water. The appropriate concentration of antibiotic solution (TAP or MTAP) was combined with 0.5% carrageenan and xanthan gum and allowed to form the hydrogel at 37 degrees Celsius. Equal amounts of amoxicillin, ciprofloxacin, and metronidazole were gradually dissolved in the corresponding amounts of sterile water while being constantly stirred in a sterilized environment to provide solutions with concentrations of 1, 5, and 10 mg/mL of MTAP. Then, 0.5% of Carrageenan and Xanthan gum powder was gradually added into each MTAP solution while stirring vigorously under control in order to reach a gel-like consistency of 1, 5, and 10 mg/mL, respectively.

Drug Release Assessment:
Using the ultraviolet (UV) spectrophotometric approach, the amount of antibiotic released from the hydrogel over a 7-day period was calculated.[17] Briefly, each capsule of hydrogel was transferred to a tube containing 3 mL PBS and placed in a room at 37°C. The overlaying solution on the hydrogel was extracted every 2 days and replaced with fresh solution (n = 3 in each group). At wavelengths specific to each antibiotic (231 nm for amoxicillin, 271 nm for ciprofloxacin, and 319 nm for metronidazole), the optical density (OD) of the centrifuged supernatant was evaluated using a spectrophotometer (UV/Vis; UNICO, Dayton, NJ, USA).

Swelling Assessment:
At various times and with various pH levels, the hydrogel's ability to swell was investigated. 100 mL of distilled water were used to submerge the hydrogel.After removing the extra surface water with tissue paper, the weight of expanded hydrogels was measured at a specific time and temperature. Hydrogel's swelling ratio (Sr) and percent swelling (Ps) were computed.

3. Results:

Drug Release Assessment:
Figure 1 shows the drug release profile over a period of 7 days. It is evident that the hydrogel exhibits a pronounced initial burst release on the first day of the one week release period from the hydrogel. On day 1, the highest releases of ciprofloxacin, metronidazole, and amoxicillin were 28.48 %, 17 %, and 21.1 %, respectively. Ciprofloxacin and metronidazole and amoxicillin release patterns reached a plateau after the first day. As observed, antibiotics were successfully released from the hydrogel throughout the intended 1-week period. The burst effect was relatively minimal, as these profiles demonstrated. The modified triple antibiotic was released sustainably over a long period of time (7 days) in this system owing to biodegradable carrageenan hydrogel.

	AMOX
1 HR	21.10%
3 HRS	12.80%
6 HRS	10.00%
24 HRS	10.00%
72 HRS	6.52%
120 HRS	5.08%
168 HRS	4.36%

Table 1- Denotes the release profile of amoxicillin over a period of 7 days

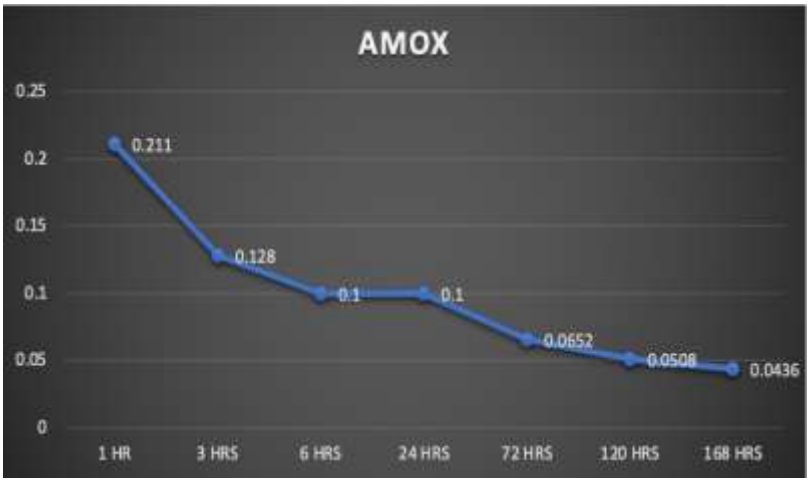


Figure 1- Demonstrates graphic representation of release profile of amoxicillin

	CIPRO
1 HR	28.48%
3 HRS	15.60%
6 HRS	15.60%
24 HRS	11.40%
72 HRS	5.68%
120 HRS	5.68%
168 HRS	5.68%

Table 2- Denotes the release profile of ciprofloxacin over a period of 7 days

Figure 2- Demonstrates graphic representation of release profile of ciprofloxacin

	METRO
1 HR	17%
3 HRS	12.30%
6 HRS	8.60%
24 HRS	6.12%
72 HRS	2.44%
120 HRS	2.44%
168 HRS	2.44%

Table 3- Denotes the release profile of metronidazole over a period of 7 days

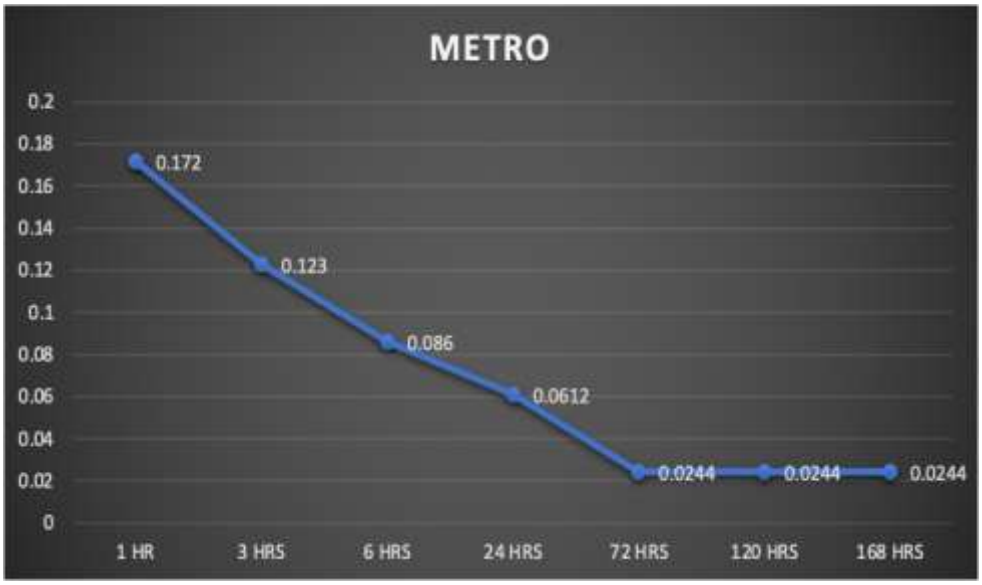


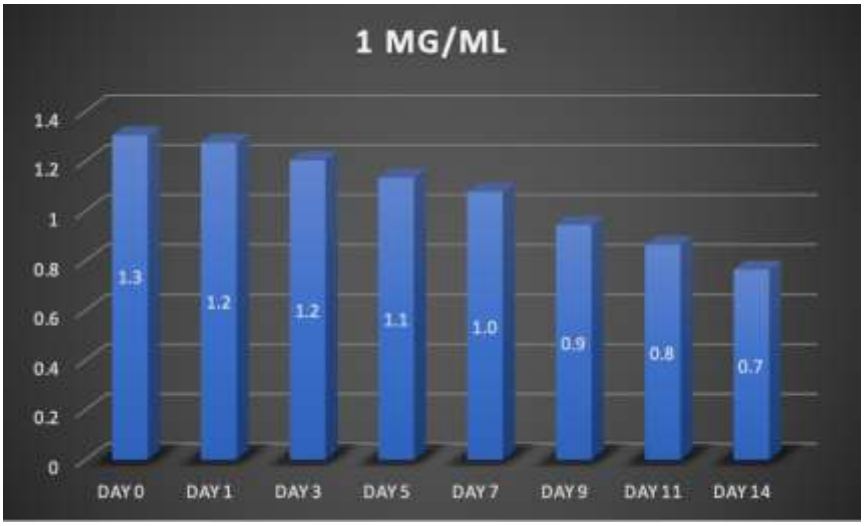
Figure 3- Demonstrates graphic representation of release profile of metronidazole

Swelling Assessment:

From the studies it is seen that there is minimal degradation associated with carrageenan hydrogel. These materials could be used to make biomedical devices like drug delivery capsules or dermatological patches due to their low degradation potential.These hydrogels are great candidates for usage in certain applications like drug release because of the low material degradability, the presence of pores, and the antibiotics' controlled release qualities (see above).

	1 MG/ML
DAY 0	**1.30333333**
DAY 1	**1.27333333**
DAY 3	**1.20333333**
DAY 5	**1.13333333**
DAY 7	**1.07666667**
DAY 9	**0.94333333**
DAY 11	**0.86333333**
DAY 14	**0.76333333**

Table 4- Denotes degradation profile of the hydrogel at 1 mg/ml



Graph 4- Demonstrates graphic representation of degradation profile at 1 mg/ml

	5MG/ML
DAY 0	1.36666667
DAY 1	1.34
DAY 3	1.3
DAY 5	1.2
DAY 7	1.17333333
DAY 9	1.09666667
DAY 11	1.02
DAY 14	0.93

Table 5- Denotes degradation profile of the hydrogel at 5 mg/ml

Graph 5- Demonstrates graphic representation of degradation profile at 5 mg/ml

4. Discussion:

Modified Triple Antibiotic Hydrogel based on carrageenan and xanthan gum were developed in the current study as a novel drug delivery system; it proved to be bioactive, biocompatible, and capable of delivering antibiotics when loaded. The Modified Triple Antibiotic Hydrogel contained Amoxicillin, Metronidazole and Ciprofloxacin at a concentration of 1 mg/ml. In our study, Carrageenan was the polymer of choice for formulating the hydrogel.

Biopolymers, or natural polymers, can be chemically or biologically manufactured from biological material or biosynthesized from live organisms like algae.The primary focus in the research of biopolymers is on marine polysaccharides because of their significant importance in perspective of their sources and their convenience of acquisition as a renewable resource. The most widely used sulfated polysaccharide derived from red marine algae is carrageenan. Carrageenans' unique qualities, such as biocompatibility, consolidation behavior, ability to interact with other polymers, and high water absorption capacity, are currently being used by the pharmaceutical industry to build drug delivery systems with pH and temperature sensitivity, prolonged drug release profile, and enhanced solubility and bioavailability of poorly-water soluble medicines.As a result of its use to enhance drug formulation and controlled release, Carrageenan has emerged as one of the most important biomaterials in the pharmaceutical industry for a variety of applications.[18][19] Carrageenan has also shown a variety of bioactive applications that have been researched, including their function as antioxidants, antiviral, antibacterial, antitumor, antihyperlipidemic, anticoagulant, and immunomodulatory agents that allow them to be used in the biomedical field as potential pharmaceutical formulations.[20][21][22]

The goal of the current study was to formulate a modified triple antibiotic hydrogel that has sustained release properties for prolonged action. A goal of endodontic treatment is to eliminate as many microorganisms as possible using various techniques, including aseptic treatment protocols, chemomechanical preparation, and so on, because infected root canal systems contain a variety of pathogens. Antimicrobial medications taken locally have been thought of as a supplement to conventional and systemic antibiotics. On the other hand, it is extremely unlikely to eliminate the root canal microbiota with a single antibiotic because of the complexity and type of the microorganisms found in the radicular system. A combination of antibiotics should be explored for root canal treatment because facultative and obligate aerobes and anaerobes can be detected there.[23]

Due to the antibiotics' solubility in water and increasing hydrophilicity, there was a burst release, which was followed by a gradual and then continuous release. This could have been caused by the carrageenan biopolymer's slow breakdown. It appears that the release of antibiotics from hydrogel is influenced by the interplay of the polymer's diffusion and degradation processes, and is principally reliant on the mass degradation of carrageenan. The three antibiotics had different rates of release, with metronidazole having the slowest rate. This might be associated with the different biochemical properties of antibiotics and the type of bond each antibiotic had with the biopolymer.For the extracts used in the study, PBS was chosen as the release medium. Studies by Torshabi and Tiwari had also employed PBS for the same technique since it may simulate bodily circumstances in humans.[17] In another investigation, the drug-release medium was distilled water. [24]

For almost thirty years, researchers have been looking for sustained release drugs that can replace popular intracanal medications. Calcium hydroxide, which has a long history of usage, may be appropriate in the majority of clinical instances. However, when faced with resistant periapical lesions brought on by the persistence of E. faecalis, a different intracanal medication should be used. The modified triple antibiotic hydrogel described in this study has potential pharmacological and physical characteristics with sustained release capabilities that are essential against endodontic E. faecalis biofilms.

5. Conclusion:

The purpose of the current investigation is to transfer medicaments locally to the root canal system in accordance with modern concepts. Antimicrobials can be delivered to the root canal system via the novel hydrogel. Our study's findings led us to the conclusion that the modified triple antibiotics' release pattern from hydrogel was acceptable . As a result, carrageenan hydrogel based intracanal medicament can be seen as a novel way to deliver antibiotics to the root canal area and offer localized and prolonged drug release. With regard to the present methods of drug administration to the radicular area and for regenerative endodontics, this novel drug delivery system provides a promising new future.

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