



Study of the Antibacterial Activity of a New Prolonged-Release Dental Dosage form Containing Doxycycline and Lidocaine

Gourishankar Birtia¹ and Santosh Kumar Mahapatra^{2*}

¹*Siddhi Vinayaka Institute of Technology and Science (College of Pharmacy) 495001 Bilaspur, CG, India.*

²*Institute of Pharmacy and Technology Salipur 754202 Odisha, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Author SKM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GB managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i3630993

Editor(s):

(1) Dr. Syed A. A. Rizvi, Nova Southeastern University, USA.

Reviewers:

(1) Tsubanova Natalia, National University of Pharmacy, Ukraine.

(2) Sinar Yani, Universitas Mulawarman, Indonesia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63765>

Original Research Article

Received 01 October 2020

Accepted 06 December 2020

Published 22 December 2020

ABSTRACT

A dental infection, Gingivitis is a common and mild form of gum disease (periodontal disease) that causes irritation, redness and swelling (inflammation) of your gums. Gingivitis is able to escort to much more grave gum disease (periodontitis) and eventual tooth loss. To treat dental problems painkillers along with antibiotics and some dental paints are the commonly prescribed. But the common side effects of most of the painkillers are hyperacidity and gastric irritation most antibiotics due to slow onset of action and hepatic "first-pass" effect fail to produce prompt and prolong actions. Moreover, most of the dental formulations are washed out by saliva within a few hours of application. Hypothesis of the study is that if a soft moldable gummy material containing analgesic as well as antibiotic drugs is attached to an offending tooth and sustained drug release occurs from it, a extended local action of the drugs is achieved. Ethyl cellulose, gum tragacanth, hydroxyl propyl cellulose, PEG-400 and carbopol 934 were used to prepare denticap containing Doxycycline hydrate and lidocaine.

*Corresponding author: E-mail: drskm1966@gmail.com;

Keywords: Prolonged-release dental dosage form; antibacterial activity; in vitro study; Doxycycline; Lidocaine.

1. INTRODUCTION

Oral health is a fundamental module of general health. Oral health evils such as dental caries, periodontal diseases, and oral cancers are worldwide concerns restricting and confining the day-to-day errands and chores. Dental pain is caused by noxious pain stimuli such as bacterial infections, chemical or involuntary attrition of enamel, and depression of gingiva. Gingivitis is a widespread and placid form of gum disease (periodontal disease) that causes irritation, redness and swelling (inflammation) of your gums. Because gingivitis can be mild, it may not be aware of the condition occurred [1,2]. But it's significant to take gingivitis critically and delight it punctually. Gingivitis can lead to much more grave gum disease (periodontitis) and eventual tooth loss. Gingivitis always starts off with a buildup of plaque - an invisible, sticky film made up mostly of bacteria. When starches and sugars in foodstuff cooperate with bacteria usually found in our mouths, plaque can form on our teeth. Brushing our teeth twice a day and flossing once a day removes plaque. Plaque can re-form very rapidly - within 24 hours of combing your teeth. Plaque that remains in our mouths for longer than two or three days goes stiff under the gum line, and forms tartar (calculus). Tartar makes plaque greatly harder to brush away and acts as surroundings in which bacteria can flourish [3]. It is not generally probable to take away tartar by just brushing or flossing. It can only be effectively removed by a dentist or dental hygienist using a technique called scaling, scale, or polish - the tartar is scraped away using a special instrument. If there are any marks or stains the teeth are then polished [4]. If the plaque and tartar stay in the teeth, the gingiva (the gum) will turn out to be more irritated and inflamed. Ultimately, the gums will be engorged and more expected to bleed. If tartar build-up continues the circumstance can growth to Periodontitis.

To treat dental problems such as pain due to dental caries, periodonitis, gingivitis, and other gum infections, painkillers along with antibiotics and some dental paints are the commonly prescribed drugs by dentists as initial mode of treatment. But the common side effects of most of the painkillers are hyperacidity and gastric irritation upon oral administration [5]. On the other hand, the majority of antibiotics due to

dawdling onset of action and hepatic "first-pass" outcome fail to construct punctual and extend actions. Furthermore, the majority of the dental formulations are washed out by saliva within a few hours of appliance. Supposition of the study is that if a soft moldable gummy substance containing analgesic as well as antibiotic drugs is close to an aberrant tooth and sustained drug release occurs from it, a prolonged local action of the drugs is achieved [6]. The polymeric mold should have a suitable adhesiveness so that it may be effortlessly fixed on the exaggerated tooth and can be detached simply when required.

Doxycycline inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit; it has bacteriostatic activity against a board range of Gram positive and Gram-negative bacteria. The drug has been used to indulgence dental microbial plaques and periodontal diseases as bacterial infection. Lidocaine hydrochloride, an anesthetic agent, is available as dermal formulation, intravenous injection, intravenous infusion, nasal spray, oral gel, and topical gel. Lidocaine has been widely used as anesthetic agent for dental scaling, root planning, pain sensitivity, and early wound healing following nonsurgical periodontal therapy [7,8].

Sustained-release delivery systems apportion comprehensive drug action to indulgence dental and periodontal diseases compared to the conservative dosage forms. improved patient fulfillment in stipulations of submission regularity, healthier relief for longer period of time, and reduction in dose of drug leads to overcome the adverse reactions due to higher dose to achieve the same effectiveness when given orally [9,10]. As well quicker local action as compare to sluggish onset of action by oral route and evasion of hepatic "first-pass" consequence are significant compensation of this formulation. Investigate of sustained release devices is a relatively new area in dentistry [11-13]. Dental gels, mucoadhesive tablets, films, injectable semisolid systems, inserts and sponges are some of the sustain-release drug-delivery approaches for the treatment of periodontal diseases. Therefore, an effort was made here to develop and evaluate in vitro a Novel formulation for Periodontal disorder (since containing lidocaine hydrochloride and Doxycycline Hydrate as model drugs for sustained local action [14-16].

2. MATERIALS AND METHODS

2.1 Materials

Doxycycline hydrate and lidocaine were the gift sample. Ethyl cellulose, gum tragacanth, hydroxyl propyl cellulose, PEG-400 and carbopol 934 were obtained commercially.

2.2 Methods

Denticap was formulated with the combination of polymers such as ethyl cellulose, gum tragacanth, hydroxyl propyl cellulose, PEG-400 and carbopol 934. A paste was formed by homogenous mixing of drugs and polymers in ethanol (95%). This was then poured in cap-like ethanol-proof plastic moulds and thereafter solvent was evaporated at $37\pm 0.5^{\circ}\text{C}$, for 2h. Thus the formulations with the desired stickiness and consistency were achieved. The formulations had impermeable cover from all the sides other than the side intended to place on the affected tooth. Formulation was taken out from the moulds and at the bottom side adhesive tape was fixed and then formulations were coated by film coating method [17,18].

2.3 Drug Characterization

2.3.1 IR spectroscopy

The infrared spectroscopic analysis of doxycycline hydrate and lidocaine drug sample was performed on IR spectrophotometer (Shimadzu IR Affinity-1) and the spectrum obtained is shown in Fig. 1.

2.3.2 Swelling index

Denticaps were weighed (W_0) and allowable to puff up on a Petridis in replicated saliva, pH 6.8

at $37\pm 0.5^{\circ}\text{C}$. Samples were full out and surplus saliva was detached with filter paper. Then the weight (wet weight) of the formulation was taken and recorded. When the weight became constant (W_t), the weight taken was used for calculating the swelling index (S.I).

$$S.I. = \frac{(W_t - W_0) \times 100}{W_0}$$

2.3.3 Antimicrobial study

Antimicrobial activity of formulation was performed by using disc diffusion method. Firstly nutrient agar media was prepared for the growth of bacteria by taking different ingredients and then mix them in proper ratio. The formula was reported in Table 1.

Table 1. Formula of nutrient agar media

S. No.	Ingredient	Quantity
1.	Beef extract	10g
2.	Peptone	10g
3.	NaCl	5g
4.	Agar	1-2%
5.	Distilled water	1000ml

After preparation, autoclaved for suitable time at particular temperature to sterilize the medium. Then transfer the nutrient media into the petri plate in required quantity. After solidification of media inoculation of bacteria (*Staphylococcus aureus*) was done in an aseptic chamber under laminar air flow and incubate inside the incubator for specified period of time for the proper growth of bacteria. After the growth of the bacteria, antimicrobial activity of formulation was performed and check the Zone of inhibition after one day of incubation. The anti-microbial activity of the drug was determined by measuring the diameter of zone of inhibition [19].

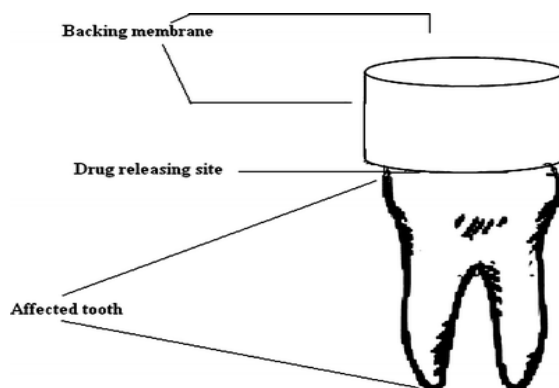


Fig. 1. Outline diagram of a denticap on an affected tooth

2.4 In-Vitro Drug Release Study

Drug release study is to know the release pattern of drug from a formulation, the rate and duration of drug release. Drug release study of novel formulation denticap containing doxycycline hyclate and lidocaine was carried out in a modified USP apparatus IV. This is a flow-through apparatus and the drug release occurs from only one side of the formulation, which remains open towards the reservoir containing simulated saliva, pH-6.8 as a dissolution media at 37 ± 0.50 C, with a flushing rate of 0.65 ml/min since this was reported to be the mean flow rate of saliva [15]. At predetermined time intervals samples were withdrawn, filtered and analyzed at 275 nm.

3. RESULTS AND DISCUSSION

In this study the novel formulation was prepared as denticap containing doxycycline and lidocaine. The formulation was sustained release so the drug was release from the formulation for prolong period and show action at application site. Optimization of different polymer combination and evaluation of physicochemical parameters and in vitro release was done. The selected formulation reported here consisted of doxycycline hyclate, lidocaine, ethyl cellulose, gum tragacanth, hydroxyl propyl cellulose, PEG-400 and carbopol 934.

Drug and excipient was characterized by IR Spectroscopy. Fig. 2-8 shows the IR Spectra of doxycycline hyclate, lidocaine, ethyl cellulose, gum tragacanth, hydroxyl propyl cellulose, PEG-400 and carbopol 934 respectively. The IR Spectra of pure doxycycline hyclate (Fig. 2) and lidocaine (Fig. 3) shows that all the characteristic peaks of doxycycline hyclate and lidocaine are present. Fig. 4 to Fig. 8 shows the spectra of ethyl cellulose, gum tragacanth, hydroxyl propyl cellulose, PEG-400 and carbopol 934 respectively. Fig. 9 shows IR Spectra of drug, anesthesia and polymer.

3.1 Swelling Index

The swelling index was determined in terms of percentage water uptake at 37°C . Fig. 10 shows swelling index of formulation containing doxycycline+ lidocaine+ polymer. The results show that the percentage swelling of the formulation was 70% in 6 hour.

3.2 Antimicrobial Activity

Antimicrobial activity of formulation was performed by using disc diffusion method. Different concentration of formulation was put on the cultured petri plates to check the Zone of inhibition after one day of incubation. The antibacterial activity of the drug was determined by measuring the diameter of zone of inhibition. 100 $\mu\text{g/ml}$ shown highest zone of inhibition that is 21.33 mm. Fig. 11-15 shows zone of inhibition.

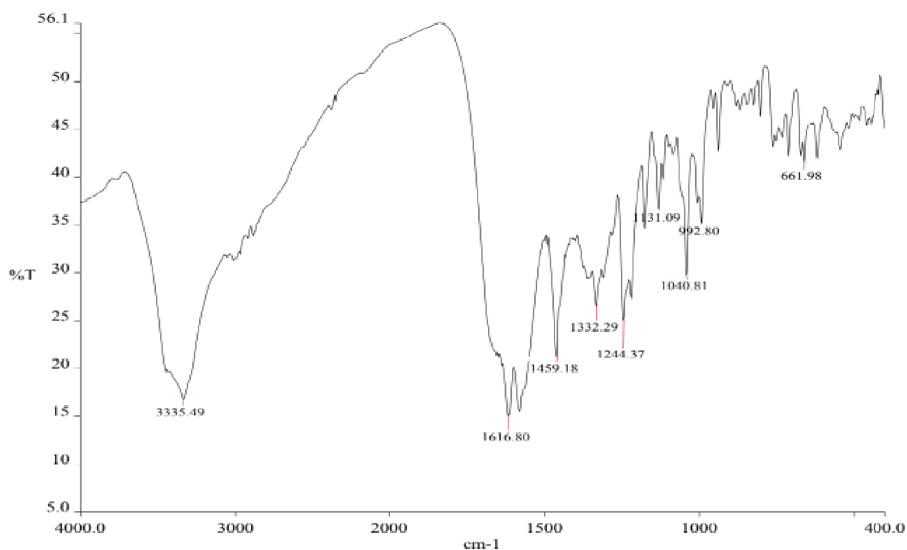


Fig. 2. IR Spectra of Doxycycline hyclate

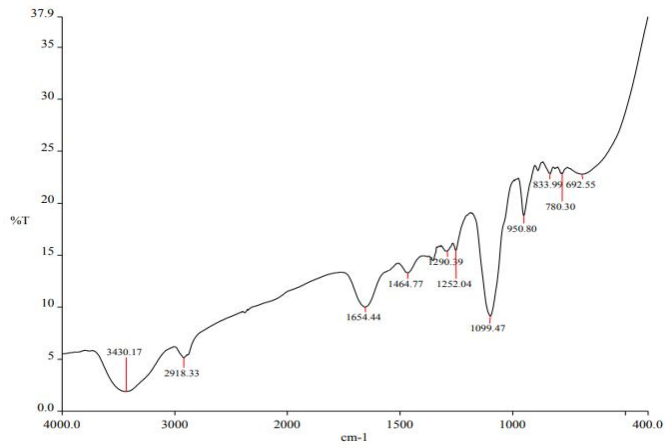


Fig. 3. IR Spectra of Lidocaine

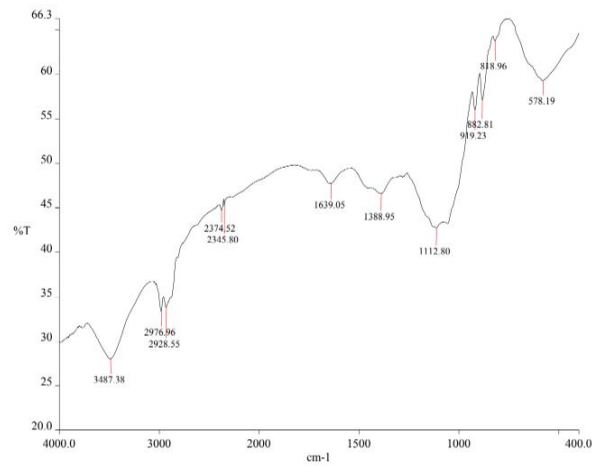


Fig. 4. IR Spectra of Ethyl cellulose

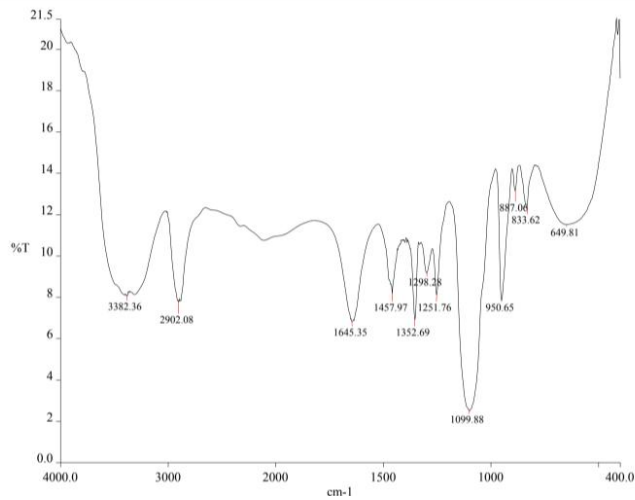


Fig. 5. IR Spectra of Gum tragacanth

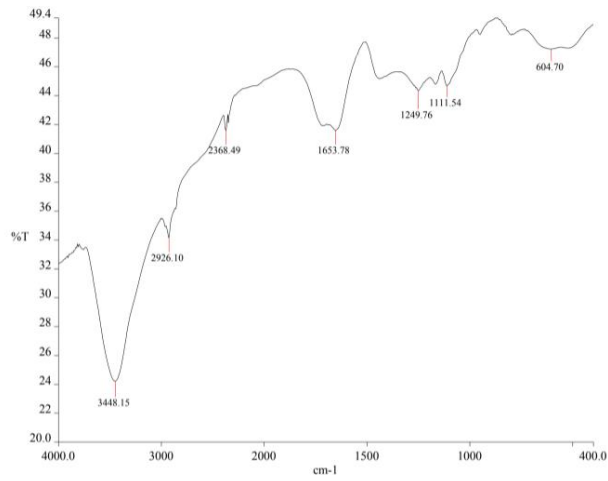


Fig. 6. IR Spectra of PEG 400

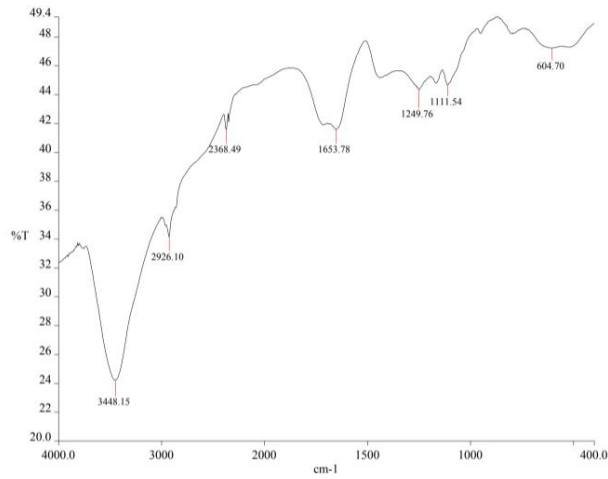


Fig. 7. IR Spectra of Carbopol 934

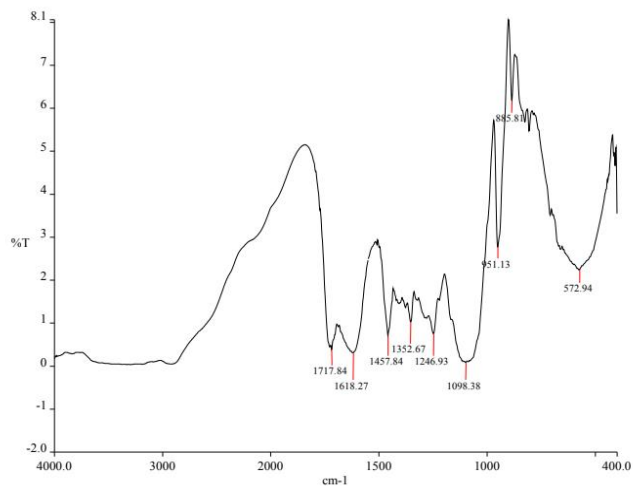


Fig. 8. IR Spectra of drug+anesthesia+polymer

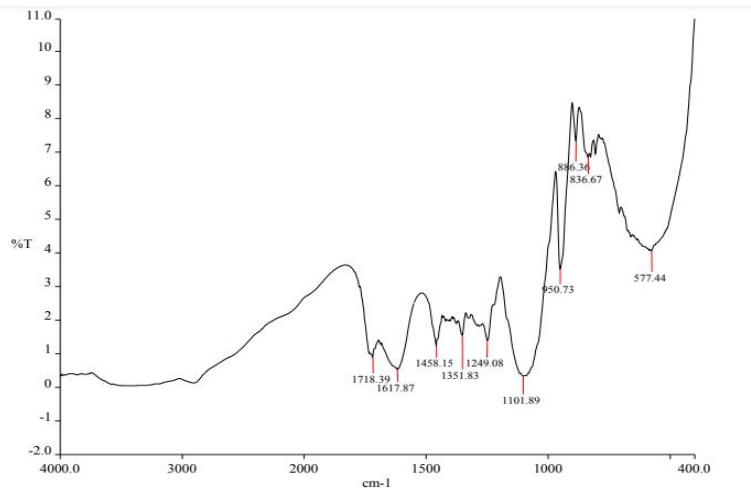


Fig. 9. IR Spectra of Drug + Polymer

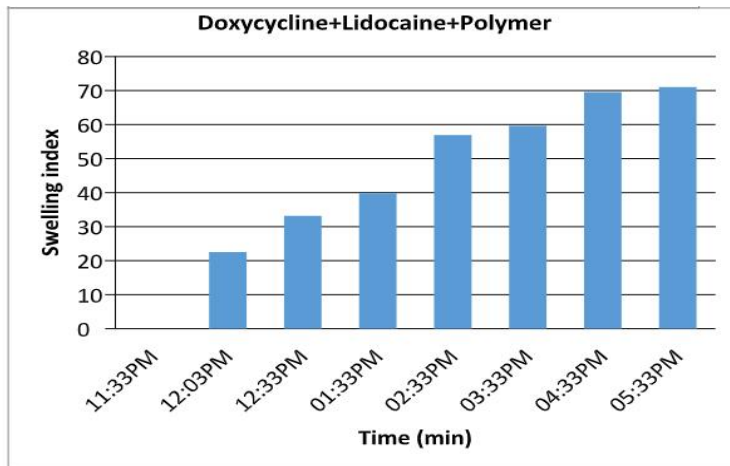


Fig. 10. Swelling Index of the formulation

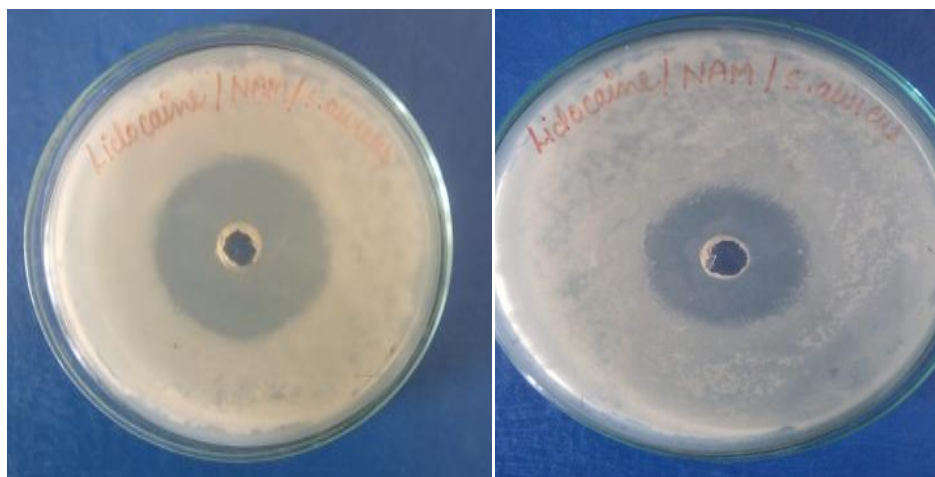


Fig. 11. Zone of inhibition of different concentration of the formulation (Lidocaine)

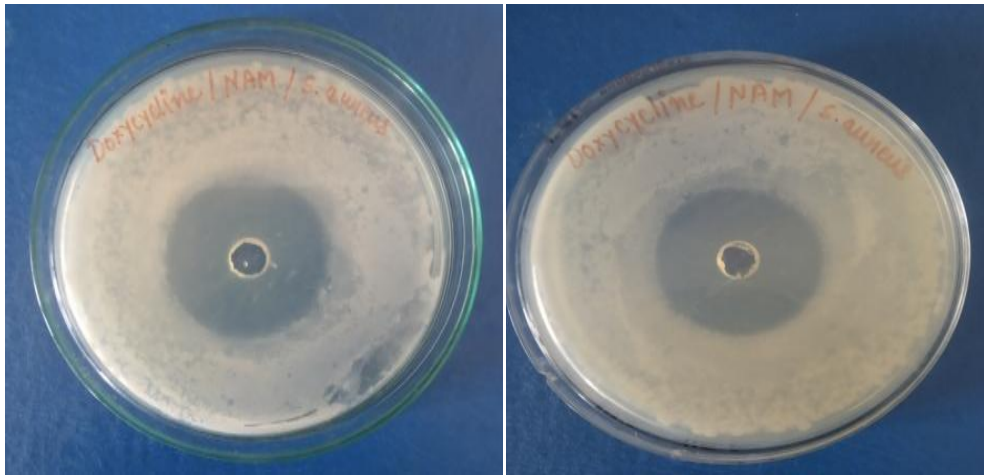


Fig. 12. Zone of inhibition of different concentration of the formulation (Doxycycline+ Polymer)

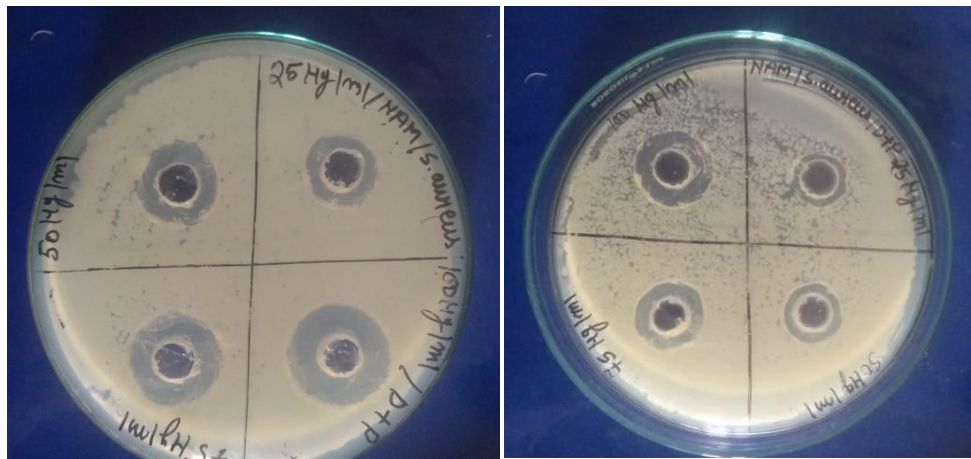


Fig. 13. Zone of inhibition of different concentration of the formulation (Doxycycline+ Polymer)

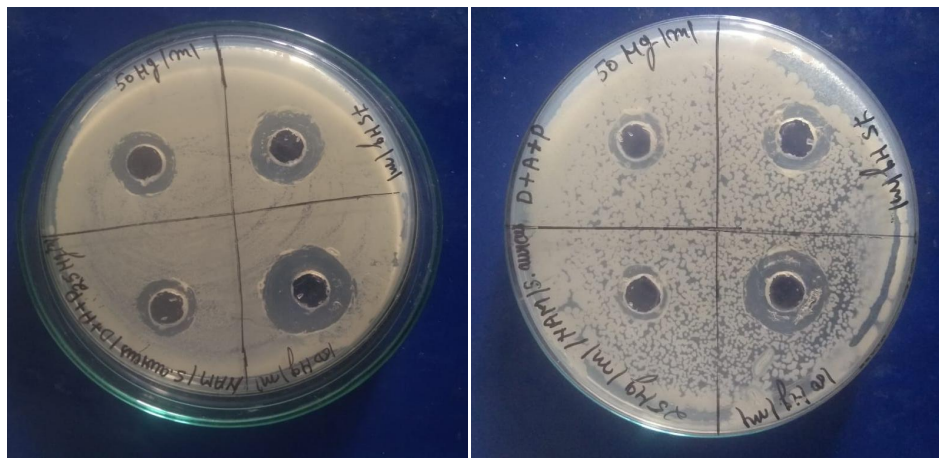


Fig. 14. Zone of inhibition of different concentration of the formulation (Doxycycline + Anesthesia + Polymer)

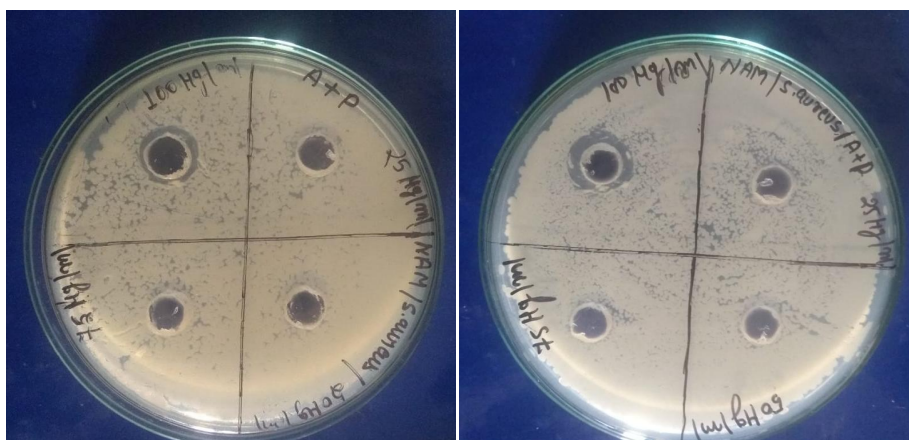


Fig. 15. Zone of inhibition of different concentration of the formulation (Anesthesia + Polymer)

3.3 In-Vitro Drug Release

Fig. 16 shows release of doxycycline hyclate from the prepared denticap was studied in simulated saliva (pH 6.8). The cumulative percentage release of doxycycline hyclate was about 99.583 % over a time period of 12 h. There are conventional dosage forms present in the market with some limitations like washout with saliva, hypersensitivity etc. To overcome these limitations denticap was prepared containing doxycycline hyclate and lidocaine. The IR Spectra of drug and excipients similar to the standard spectra. The percentage swelling of the formulation was 70% in 6 hour. The anti-bacterial

activity of the drug was determined by measuring the diameter of zone of inhibition. Different concentration of formulation (shown in the table 2) was put on the cultured petri plates to check the Zone of inhibition after one day of incubation. 100µg/ml showed highest zone of inhibition that is 21.33 mm. The In-vitro was carried out in a modified USP apparatus IV. The cumulative percentage release of doxycycline hyclate was about 99.583 % over a time period of 12 h. The stability study was accepted out subsequent ICH guideline for stability testing of new drug substances and products. The result shows the formulation was stable at 40°C± 2°C and 25°C± 2°C.

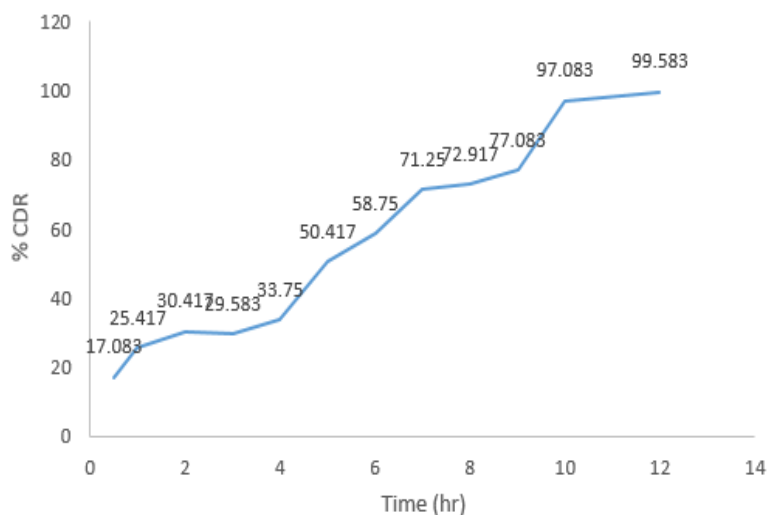


Fig. 16. In vitro release of doxycycline hyclate and lidocaine from the denticap

Table 2. Zone of inhibition

D+A+P	25 ug/ml	50 ug/ml	75 ug/ml	100 ug/ml
1	09.00	13.00	14.30	23.00
2	07.00	10.00	12.00	22.00
3	12.00	12.03	14.00	19.00
Mean	09.33±2.516	1.761	13.43±1.250	21.33±2.081

*D+A+P (Doxycycline hyclate +Anesthesia+ Polymer)

4. CONCLUSION

In the present study the aim of developing sustained release device drug was fulfilled. The delivery device provides initial high release and moderate release on the later time in-vitro study. The developed dosage form was satisfactory in terms of drug release. The local delivery of antimicrobials into the periodontal pocket has opened up a new arena for the management of periodontal diseases. This type of drug delivery devices is likely to be well received by dental profession in future because it can produce much higher levels of drugs at the site of interest with no side effects in comparison to conventional oral therapy for prolonged periods. "Denticap" is a novel sustained release formulation give action for extended period. This formulation possibly will supply patient fulfillment and marketable use.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Ahuja A, Ali J, Rahman S. Biodegradable periodontal intrapocket device containing metronidazole and amoxicillin: formulation and characterisation. *Pharmazie*. 2006;61:25–29.
- Ang CY, Samsudin AR, Karima AM, Nizam A. Locally produced bovine bone sponge as a haemostatic agent. *Med J Malaysia*. 2004;59:149–150.
- Bahrami F, Morris DL, Pourgholami MH. Tetracyclines: drugs with huge therapeutic potential, *Mini-Reviews in Medicinal Chemistry*. 2012;12(1):44–52.
- Barat R, Srinatha A, Pandit JK, Ridhurkar D, Balasubramaniam J, Mittal N, Mishra DN. Niridazole biodegradable inserts for local long-term treatment of periodontitis: possible new life for an orphan drug. *Drug Deliv*. 2006;13:365–173.
- Brännström M, Garberoglio R. The dentinal tubules and the odontoblast processes. A scanning electron microscopic study. *Acta Odontol Scand*. 1972;30:291–311.
- Caprio RD, Lembo S, Costanzo LD, Balato A, Monfrecola G. Anti-inflammatory properties of low and high doxycycline doses: An *In Vitro* study, *Mediators of Inflammation*; 2015;2015. Article ID 329418
- Colin D. Salivary flow patterns and the health of hard and soft oral tissues. *J Am Dent Asso*. 2008;139:18–24.
- Dandi KK, Rao EV, Margabandhu S. Dental pain as a determinant of expressed need for dental care among 12-year-old school children in India. *Indian J Dent Res*. 2011;22:611.
- Griffin MO, Fricovsky E, Ceballos G, Villarreal F. Tetracyclines: a pleiotropic family of compounds with promising therapeutic properties. Review of the literature, *The American Journal of Physiology—Cell Physiology*. 2010;299(3):C539–C548.
- Kasaj A, Heib A, Willershausen B. Effectiveness of a topical salve (Dynexan) on pain sensitivity and early wound healing following nonsurgical periodontal therapy. *Eur J Med Res*. 2007;12:196–199.
- Letendre L, Scott M, Dobson G, Hidalgo I, Aungst B. Evaluating barriers to bioavailability *in vivo*: validation of a technique for separately assessing gastrointestinal absorption and hepatic extraction. *Pharm. Res*. 2004;21:1457–1462.
- Murakami S, Mealey BL, Mariotti A, Chapple ILC. Dental plaque-induced gingival conditions *J Clin Periodontol*. 2018;45(20):S17-S27.
- Perioli L, Ambrogi V, Rubini D. Novel mucoadhesive buccal formulation

- containing metronidazole for the treatment of periodontal disease. *J Control Release.* 2004;95:521–533.
14. Roy G, Ghosh S, Sinha B, Mukherjee B. In vivo evaluation and the stability study of the formulation “Denticap” intended for local application to relieve dental pain. *Int J Pharm Sci. Tech.* 2009;2:18-24.
 15. Scannapieco F. An Inflammatory Periodontal Disease: A Supplement to compendium of continuing education in dentistry. *L* 2004;25(7 Suppl 1):60-69.
 16. Schwach-Abdellaoui K, Vivien-Castioni N, Gurny R. Local delivery of antimicrobial agents for the treatment of periodontal diseases. *Eur J Pharm Biopharm.* 2000;50:83–99.
 17. Semalty A, Bhojwani M, Bhatt GK, Gupta GD, Shrivastav AK. Design and evaluation of mucoadhesive buccal films of diltiazem hydrochloride. *Indian J Pharm Sci.* 2005;67:548–552.
 18. Stoltze K, Stellfeld M. Systemic absorption of metronidazole after application of a metronidazole 25% dental gel. *J. Clin. Periodontol.* 1992;19:693–697.
 19. Vishnu MP, Bhupendra GP, Harsha VP, Karshanbhi MP. Mucoadhesive bilayer tablets of propranolol hydrochloride. *AAPS Pharm Sci Tech.* 2007;8(3):77.

© 2020 *Birtia and Mahapatra*; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/63765>