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Antimicrobial studies of extended release amoxicillin trihydrate dental gels

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ABSTRACT

The aim of this study was to formulate and evaluate the novel amoxicillin trihydrate dental gel containing different hydrophilic polymers such as hydroxy propyl methyl cellulose (HPMC), methyl cellulose (MC), sodium carboxy methyl cellulose (Sod.CMC) and hydroxy propyl cellulose (HPC) in varied concentrations (5, 6, 6 and 9 %) for the treatment of periodontal diseases. Propylene glycol was used as a co-solvent in the concentration 15-25%. The formulated dental gels were found transparent, smooth and similar in color on physical observation. The pH of the dental gels was found to be in the range of 6.03 to 6.34. The spreadability of the gel was found to be in the range of 6.33 to 7.81 g-cm/sec. The viscosity values ranged from 4322.89 cps to 4665.78 cps. Drug content was found to be in the range of 95.93 to 99.31%. In-vitro drug release studies of formulations were carried out in the Kishery-Chein diffusion cell which exhibited an extended release of drug over a period of 7 hours. The pure drug and the best formulation were evaluated for antimicrobial studies by serial dilution method using nutrient broth medium. It was noticed that with increased drug concentration marked antimicrobial activity was achieved. Thus the present formulation of amoxicillin trihydrate dental gel could be suggested as a safe and beneficial dosage form for the treatment of periodontal infections.

Keywords: Dental gels, Periodontitis, hydrophilic polymers, antimicrobial activity.

INTRODUCTION

The word "periodontal" literally means "around the tooth". Periodontal disease (also known as gum disease, pyorrhea or periodontal infection) is an ongoing bacterial infection in the gums and bone around the teeth [1]. Periodontal diseases are gram-negative anaerobic infections leading to an inflammation under the gums. Periodontal diseases are generally divided into two groups:

Gingivitis, which causes lesions (wounds) that affect the gums and Periodontitis, which damages the bone and connective tissue that supports the teeth [2]. Periodontitis is caused by microorganisms that adhere to and grow on the tooth's surfaces, along with an overly aggressive immune response against these microorganisms. It can affect one tooth or many teeth [3].

Gels are transparent or translucent semisolid formulations containing a high ratio of solvent/gelling agent [4]. The U.S.P. defines gels as semisolids, either suspension of small inorganic particles or large organic molecules interpenetrated with liquid [5]. The scientific facts have clearly indicated that a formulation and development of a gel based topical dosage form for antimicrobial drug will be proved to be worthwhile like ability to deliver drug more selectively to a specific site, avoidance of gastro-intestinal incompatibility, providing utilization of drugs with short biological half-life, narrow therapeutic window, improving physiological and pharmacological response, improve patient compliance and provide suitability for self-medication [6]. Topical application of gels at pathological sites offer great advantage in a faster release of drug directly to site of action, independent of water solubility of the drug as compared to creams and ointments [7].

A dental gel is any gel that is designed to be applied to human teeth or gums. Gels are generally considered the best way to administer any sort of treatment to teeth, which is in large part owing to the porous composition of teeth. Most varieties of dental gel are chemically designed to effectively penetrate the teeth to strengthen or whiten them, or to dull pain [8].

Amoxicillin trihydrate was used as a model drug due to its bacteriostatic and bactericidal activity against gram negative bacteria. This drug acts by inhibiting the synthesis of bacterial cell walls. Amoxicillin trihydrate is not highly protein bound, with only 17% protein bound in serum. Its biological half–life have been reported to be 3 to 4 hrs, necessitates multiple daily dosing for maintaining therapeutic effect throughout the day. The oral use of amoxicillin trihydrate is associated with side effects like gastrointestinal disturbances, nausea, vomiting and diarrhea. Topical application of the drug prevents these side effects and offers potential advantage of delivering the drug at the site of action [9].

With these above considerations, amoxicillin trihydrate dental gels were formulated using different hydrophilic polymers and evaluated.

MATERIALS AND METHODS

Amoxicillin trihydrate(Yarrowchem Products, Mumbai). Methyl cellulose 400cp (MC); Hydroxy propyl methyl cellulose (HPMC); Sodium carboxy methyl cellulose (Sod.CMC); Hydroxy propyl cellulose (HPC) were purchased from Himedia Laboratories Private Limited, Mumbai; Aspartame, boric acid, propylene glycol, nutrient broth and all other solvents and reagents used were of analytical grade.

FORMULATION OF MEDICATED DENTAL GELS

Gels were formulated by dispersing the polymer at different concentrations in water by continuously stirring for a period of one hour. The drug was dissolved in a mixture of propylene glycol and little amount of water and this solution was added slowly to the above polymer

dispersion. The preservative boric acid and sweetening agent aspartame was also added to the polymer dispersion and stirred continuously till it forms a homogeneous product. The prepared gels were stored in a wide mouthed bottle at room temperature. The composition of the various gels is shown in Table 1.

Table 1: Composition of amoxicillin trihydrate dental gels

INGREDIENTS (%w/w)	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
Amoxicillin trihydrate	1	1	1	1	1	1	1	1	1	1	1	1
HPMC	5	5	5	-	-	-	-	-	-	-	-	-
Sod.CMC	-	-	-	6	6	6	-	-	-	-	-	-
MC	-	-	-	-	-	-	6	6	6	-	-	-
HPC	-	-	-	-	-	-	-	-	-	9	9	9
Aspartame	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Boric acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylene glycol	0	15	25	0	15	25	0	15	25	0	15	25
Purified water(q.s)	100	100	100	100	100	100	100	100	100	100	100	100

HPMC: Hydroxy propyl methylcellulose; Sod.CMC: Sodium carboxy methyl cellulose; MC: Methyl cellulose; HPC: Hydroxy propyl cellulose.

EVALUATION OF AMOXICILLIN TRIHYDRATE DENTAL GELS

I. Drug-Excipient interaction studies: The drug and polymer interactions were studied by Fourier Transform Infrared Spectroscopy by KBr disc method. The spectra were recorded for pure drug and polymers in the ratio 1:1 at scanning range of 400-4000 cm^{-1} using FTIR-8400S, spectrophotometer (SHIMADZU, Japan).

II. Physico-Chemical properties:

The physical nature and **grittiness:** Physical appearances of all formulations were evaluated visually.

The pH: pH studies for all formulations were found out by using a digital pH meter (L1613, Elico) by dipping the electrode completely in to the gel and the readings were noted down.

Spreadability: For the determination of spreadability, 1g of dental gel was applied in between two glass slides and the weights were added to the pan until both slides detached. The time taken for the detachment of two slides was noted down and the weight was recorded and the spreadability was calculated using the formula

$$S = \frac{\text{Weight added} \times \text{Length of the glass slide}}{\text{Time taken for separation}}$$

Viscosity: Viscosity of the gel was evaluated using a Brookfield digital viscometer (DV-II) by applying increasing values of the shear rate. All the measurements were performed at a controlled room temperature.

Tube extrudability: It was determined by using a tube filled with the gel, having a tip of 5 mm opening and by measuring the amount of gel that extruded through the tip when a pressure was applied on the tube was noted down.

Drug content estimation: In this study, 1g of the dental gel was weighed in a 100ml volumetric flask, dissolved in 0.1N HCl and made upto the mark with 0.1N HCl. The solution was filtered through a Whatmann filter paper to get filtrate. To the filtrate further dilutions were made to get an appropriate concentration. The drug content was estimated by using UV spectrophotometer (Shimadzu) at λ_{max} 224 nm using 0.1 N HCl as blank.

***In-vitro* drug release studies:** *In-vitro* release studies were measured through a pretreated cellophane membrane using a modified Kishery-Chein cell. At pre-determined time intervals 1ml of the sample was withdrawn from the receptor compartment and replaced with the same volume of 0.1N HCl so as to maintain sink condition. The samples were analyzed by using UV spectrophotometer at λ_{max} 224 nm using 0.1 N HCl as blank for predicting percentage of drug release.

III. MICROBIOLOGICAL EVALUATION FOR ANTIMICROBIAL ACTIVITY

The Minimum inhibitory concentration (MIC) can be used as a quantitative parameter for comparison of antimicrobial activity amongst different formulations. The effects of release of drug from the medicated gels depend on the nature of the hydrophilic polymer and hence its effect on the sensitive organisms was screened, to establish the efficacy of the gels preparation in terms of antimicrobial activity.

Determination of Minimum Inhibitory Concentration using nutrient broth
Composition of nutrient broth

Beef extract: 10g/lit
Peptone : 10g/lit
NaCl : 5mg/lit
Water : 1000ml

- **Preparation of nutrient broth**

Dissolve all the ingredients in water. Adjust the pH to 8 to 8.4 with 5M NaOH and boil for ten minutes. Filter, sterilize at 115°C for 30 min's and adjust the pH to 7.3±0.1

- **Procedure for antimicrobial studies:** The evaluation was done by using serial dilution method for MIC (Minimum Inhibitory Concentration) as shown in Figure 1.

From the nutrient broth solution, 2ml of solution was transferred in to 9 sterilized test tubes. Antibiotic solution of 5µg/ml (2ml) was added to the 1st test tube to get a concentration of 2.5µg/ml. from this 1st test tube serial dilutions were made (transfer 2ml in a serial manner) to get concentrations of 1.25, 0.625, 0.3125, 0.15625, 0,078125, 0.0390625µg/ml. one tube containing the medium alone with the organism was kept as a positive control. Another uninoculated medium was kept as a negative control. Experiments were performed for amoxicillin formulations using sensitive organisms to establish the efficacy of the preparations, in terms of antimicrobial activity against *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis* microorganisms. All these test tubes were inoculated with the organisms and kept in an incubator at 37±0.5°C for overnight and checked for the growth of microbes.

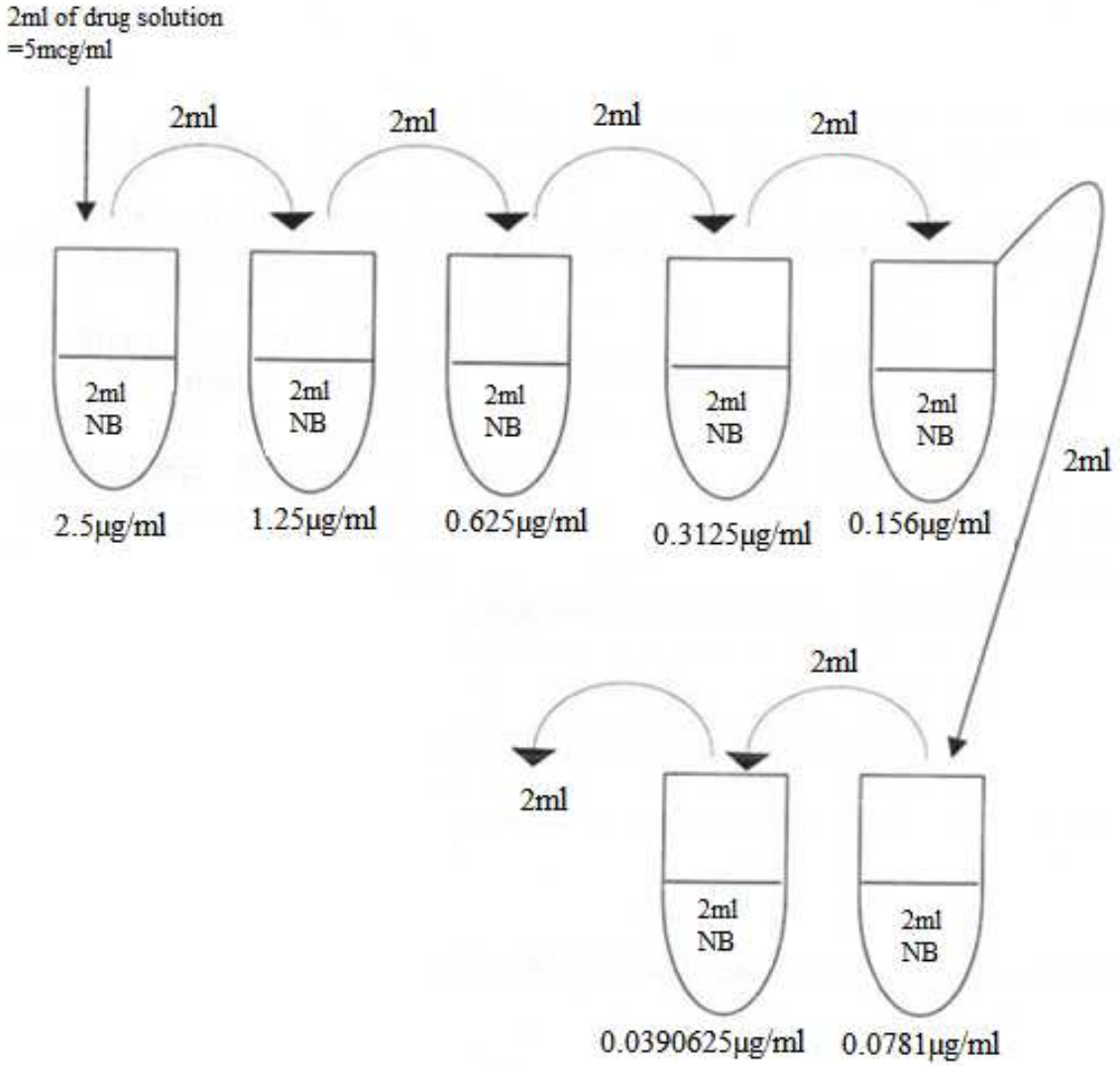

Figure 1: Serial dilution method

RESULTS AND DISCUSSION

The selected model drug passed various tests of identification and analysis.

The FTIR spectroscopy revealed the compatibility between the drug and various polymers as the wave number of principal peaks of the peak were almost intact as shown in Figure 2 to Figure 6.

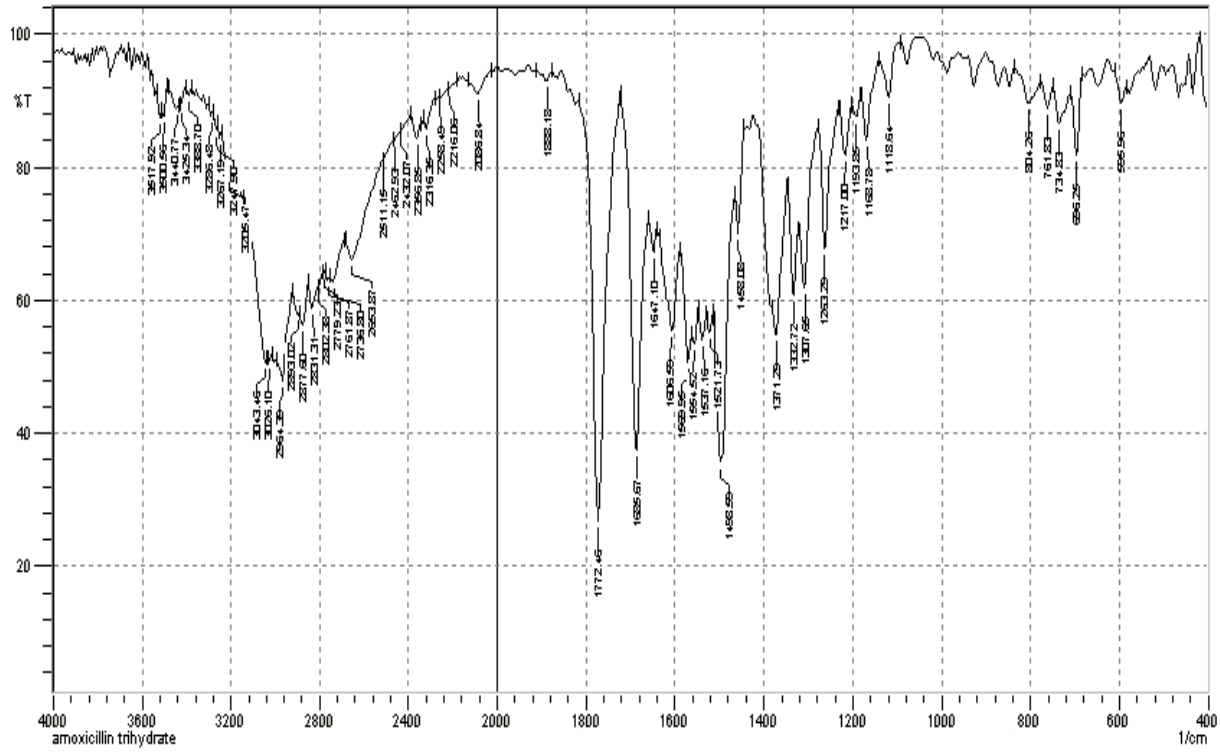

Figure 2: Infrared spectrum of pure drug amoxicillin trihydrate

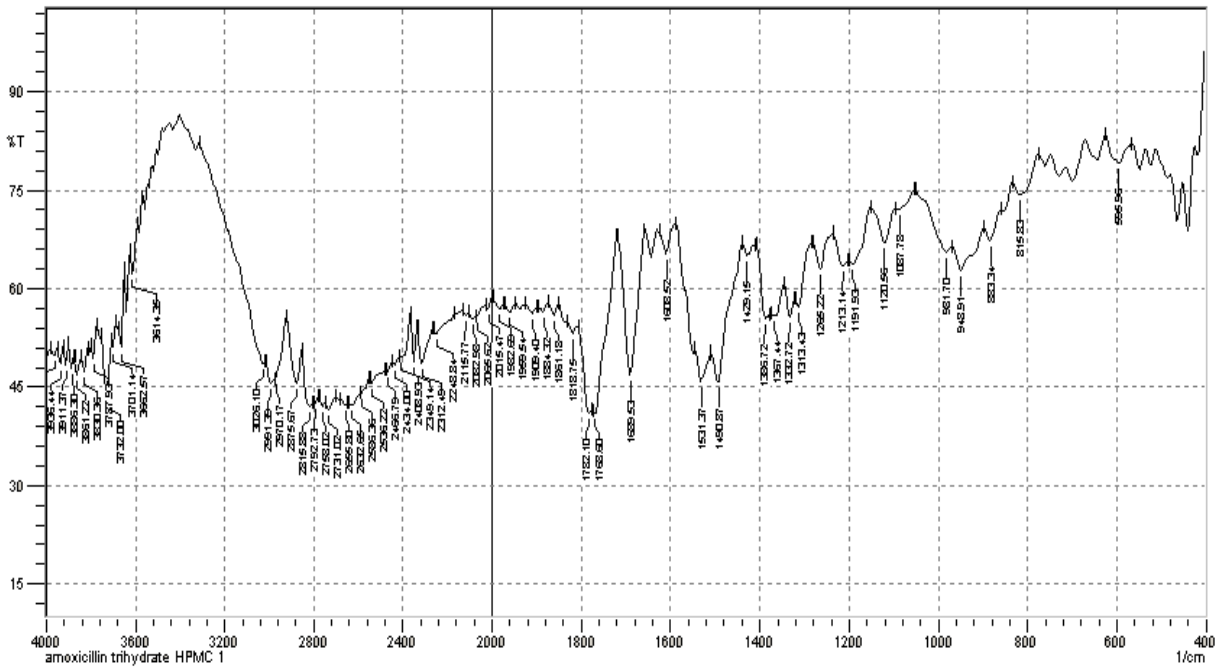

Figure 3: Infrared spectrum of physical mixture of amoxicillin trihydrate with HPMC (1:1)

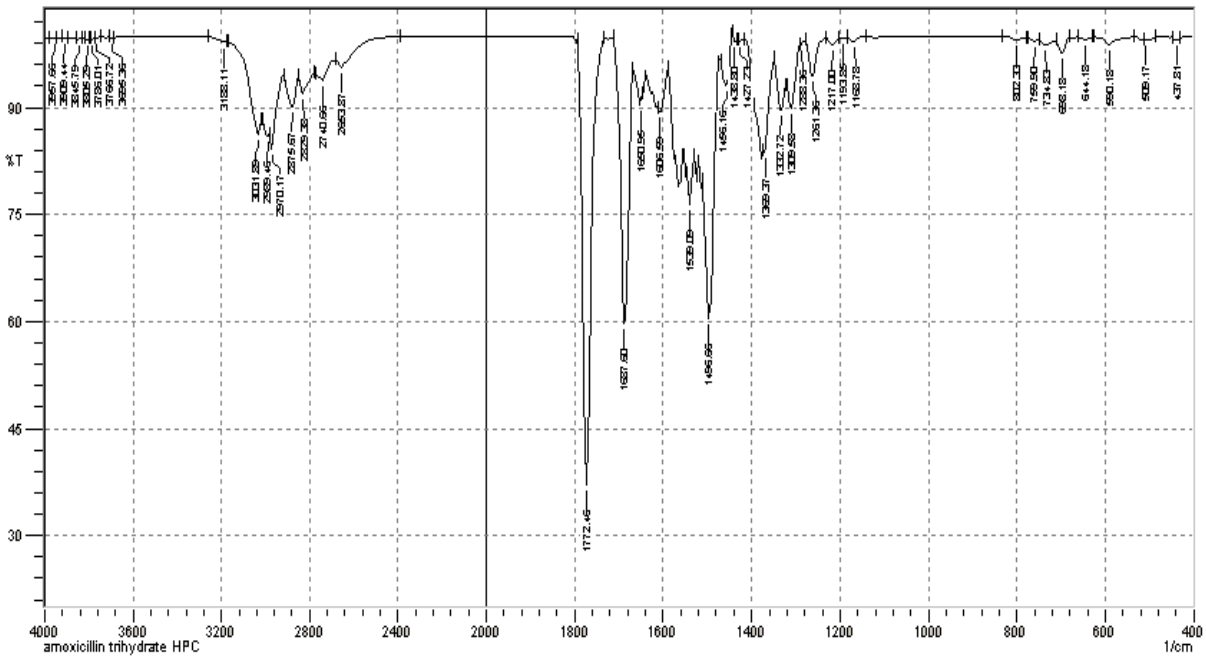

Figure 4: Infrared spectrum of physical mixture of amoxicillin trihydrate with HPC (1:1)

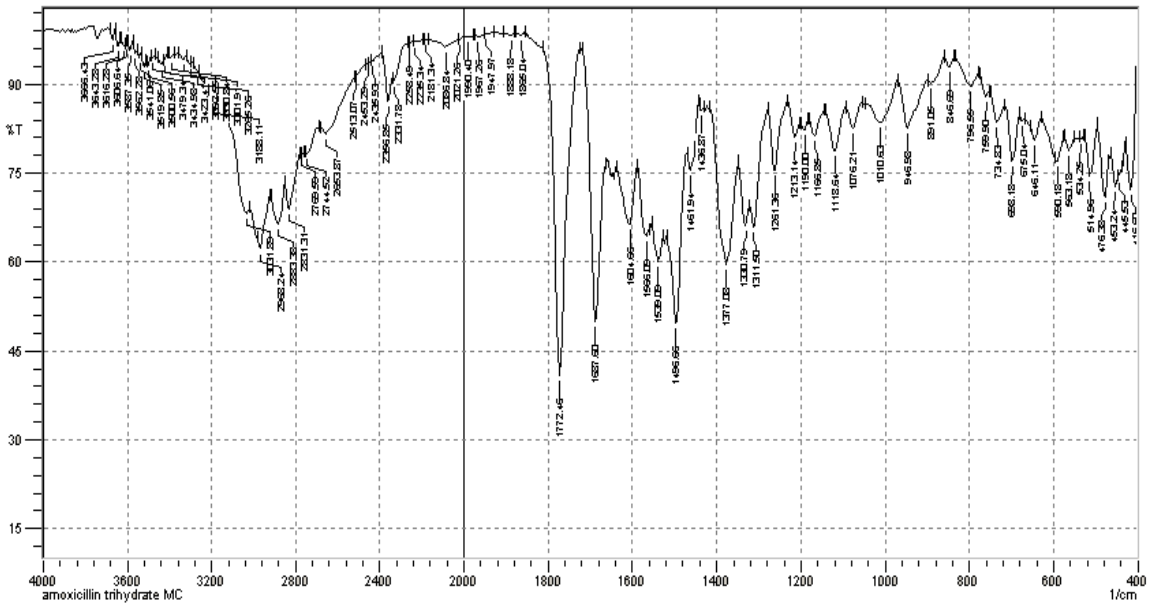

Figure 5: Infrared spectrum of physical mixture of amoxicillin trihydrate with MC (1:1)

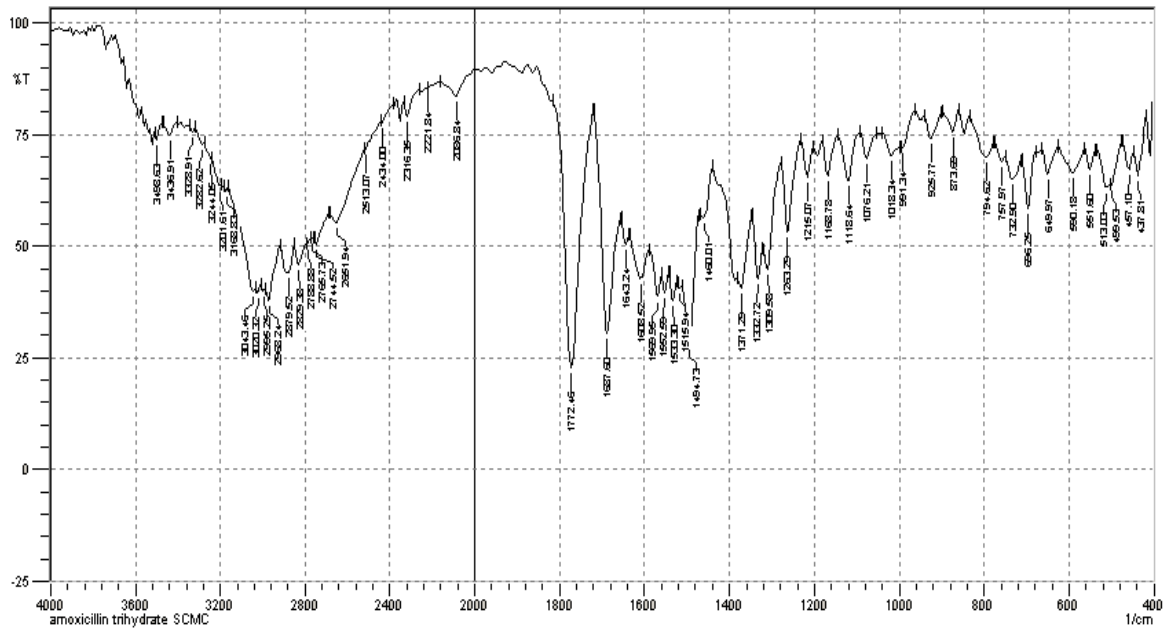

Figure 6: Infrared spectrum of physical mixture of amoxicillin trihydrate with S.CMC (1:1)

The physico-chemical characteristics of the prepared dental gels is as shown in the Table 2, revealed that the dental gels were found to be transparent, smooth and similar in color.

Table 2: Physical properties of amoxicillin trihydrate dental gels

Formulation Code	pH	Spreadability (g-cm/sec)	Viscosity (cps)	Tube extrudability (%)	Drug content (%)	Percentage drug release at 7 hrs
A1	6.32	7.81	4413.64	96.11	96.32	75.90
A2	6.28	7.14	4540.47	94.78	99.01	81.05
A3	6.34	6.53	4569.43	92.34	96.43	76.00
A4	6.25	7.64	4479.34	94.47	97.42	71.10
A5	6.28	7.0	4587.59	92.35	98.34	71.22
A6	6.27	6.66	4598.94	90.65	99.31	81.27
A7	6.03	7.35	4322.89	94.26	99.04	66.07
A8	6.04	6.87	4356.38	93.59	99.31	71.22
A9	6.16	6.33	4394.64	92.63	97.84	69.30
A10	6.05	7.81	4645.43	95.57	96.91	66.37
A11	6.07	7.36	4654.64	92.67	95.93	81.65
A12	6.11	6.42	4665.78	90.87	98.56	76.6

The pH of the dental gels was found to be between 6.03 to 6.34 which was well within the normal pH range of buccal cavity 6 to 7, which substantiates that the prepared dental gels will be irritation free. The spreadability of the gels was found to be in the range of 6.33 to 7.81 g-cm/sec, confirming that the gels may spread smoothly and uniformly. Spreadability values decreased as the concentration of propylene glycol increased in the formulations. The viscosity values ranged from 4322.89 cps to 4665.78 cps, and it got increased with the increase in propylene glycol concentration. Tube extrudability of the dental gels was not influenced by the type of hydrophilic polymer used. The gel was passable through the tube and its value decreased with an increase in

concentration of propylene glycol, which was probably due to the change in viscosity. Drug content estimation of the formulations indicated the uniform distribution of drug and it was found to be in the range of 95.93 to 99.31%.

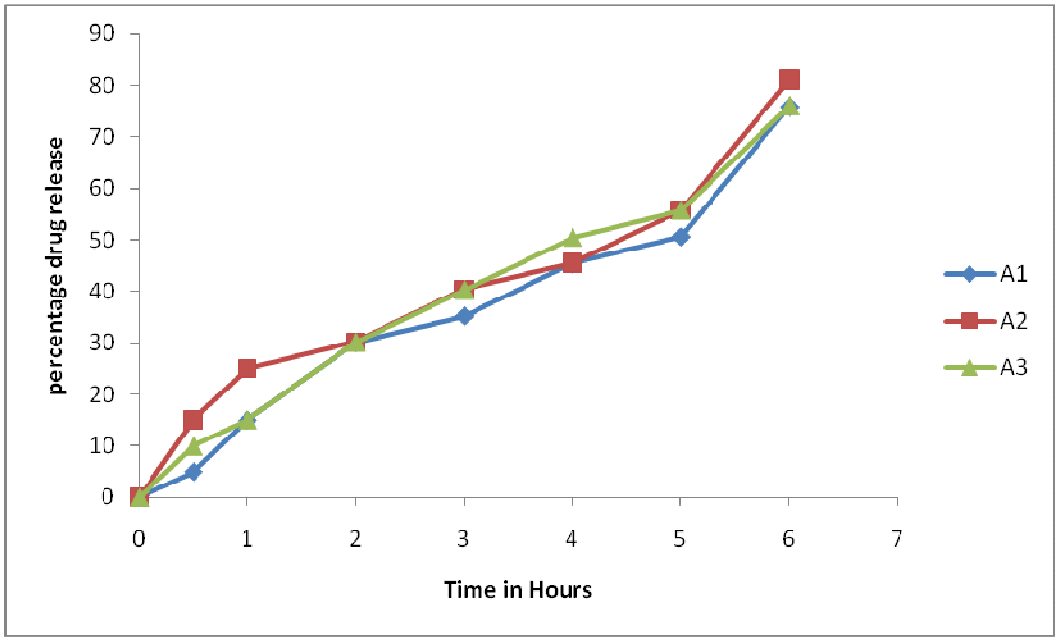

Figure 7: Cumulative percentage drug release of formulations containing HPMC

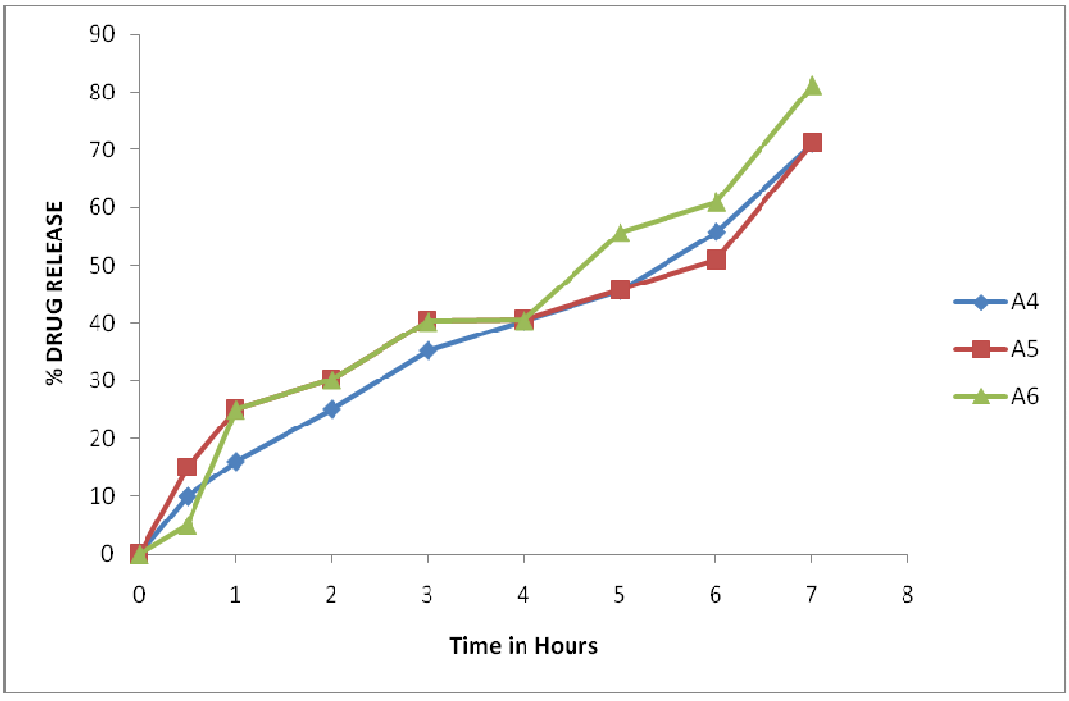

Figure 8: Cumulative percentage drug release of formulations containing S.CMC

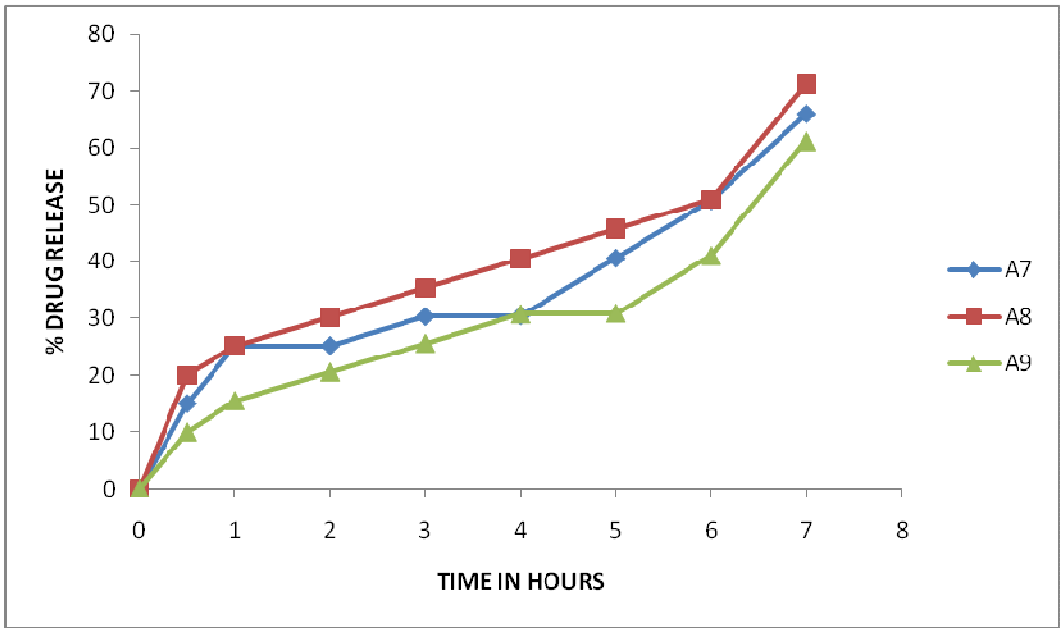

Figure 9: Cumulative percentage drug release of formulations containing MC

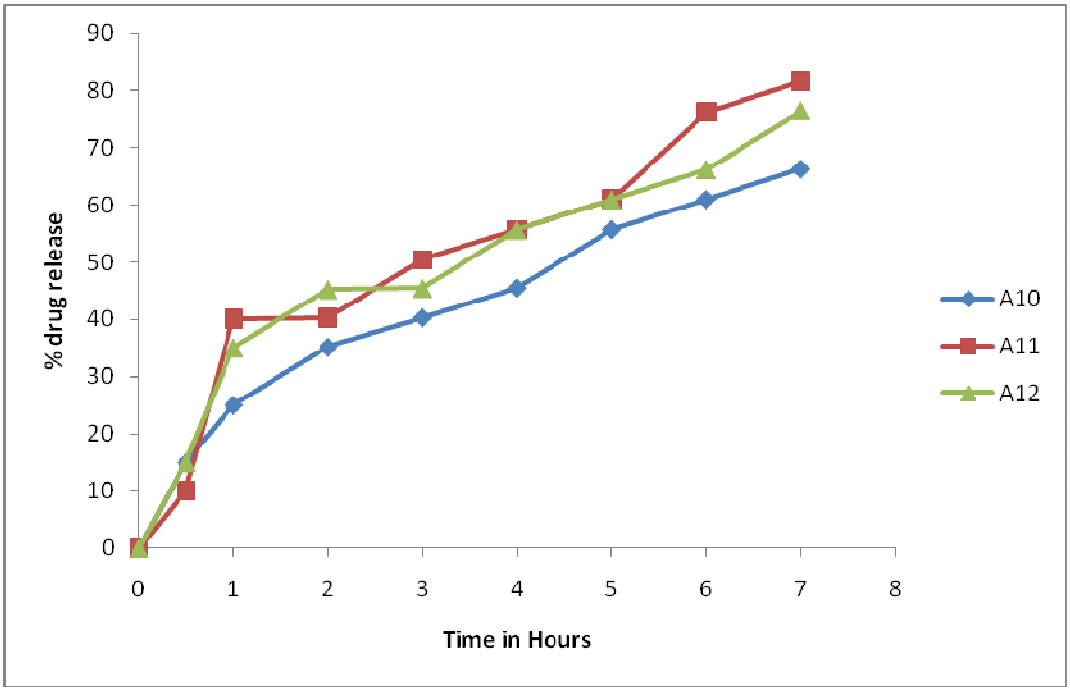

Figure 10: Cumulative percentage drug release of formulations containing HPC

The influence of different hydrophilic polymers on the release of amoxicillin trihydrate through the cellophane membrane was examined. The release of amoxicillin trihydrate containing dental gels was found to be slower. Co-solvents propylene glycol (15-25%) was used as a permeation enhancer enhanced the drug release considerably. Propylene glycol concentration showed an inverse relationship on the drug release from formulations except S.CMC which showed direct

relationship on the drug release with the increase in propylene glycol concentration as depicted in the Figure 7– Figure 10. The reports suggested that the drug release from A1, A4, A7 and A10 was comparatively slower than the other 8 formulations; this might be due to the absence of propylene glycol in these 4 formulations.

The antimicrobial activity of different dental gel formulations is as shown in the given Table 3. In case of all the microbial strains, the antimicrobial activity was achieved at a slightly higher concentration but not above the pure drug concentration. Thus, the formulations were found to be possessing prominent antimicrobial activity against some of the important bacterial strains (*E.coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis*).

Table 3: Microbiological studies of amoxicillin dental gels

CONCENTRATION(µg/ml)	**2.5**	**1.25**	**0.625**	**0.3125**	**0.1562**	**0.0781**	**0.0390**
Organism: *E.coli*							
Pure drug	-	-	-	-	+	+	+
Amoxicillin trihydrate gel	-	-	-	+	+	+	+
Organism: *St. aureus*							
Pure drug	-	-	-	-	+	+	+
Amoxicillin trihydrate gel	-	-	-	+	+	+	+
Organism: *Ba. Subtilis*							
Pure drug	-	-	-	-	+	+	+
Amoxicillin trihydrate gel	-	-	-	+	+	+	+
Organism: *Ps. aeruginosa*							
Pure drug	-	-	-	-	+	+	+
Amoxicillin trihydrate gel	-	-	-	+	+	+	+

CONCLUSION

The amoxicillin trihydrate dental gels were formulated using different hydrophilic polymers. The *in-vitro* drug release was found extended up to 7 hrs and dependent upon the formulation variables like concentration of propylene glycol and the type of hydrophilic polymers used. The dental gel possessed a marked anti-microbial activity. Hence, it can be concluded that an extended release dental gels can be formulated using different hydrophilic polymers for the treatment of periodontal disease.

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