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FORMULATION DEVELOPMENT AND EVALUATION OF *INSITU* GELS FOR PERIODONTAL INFECTIONS

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Abstract:
Oral administration of drug dosage forms to treat dental problems yields slow action due to low onset of action and hepatic "first-pass." To overcome these problems in this work, it was planned to prepared and characterize the In-Situ Gels for periodontal applications for effective treatment at the site of the action. The technique (Sol-Gel transformed systems) includes the use of novel formulations allowing drugs to be delivered in a controlled manner over a prolonged period of time. Amoxicillin was used as a model drug which is acts on gram +ve and gram –ve organisms. Temperature and pH dependent systems for the treatment of periodontal infections were planned to prepare using poloxamer 407 (18% to 24%w/w) along with carbopol 934 (0.01 to 0.3 % w/w) and Amoxicillin (1%w/w). Poloxamer is a temperature sensitive hydrogel and carbopol 934 is the pH sensitive hydrogel by these combination in-situ gels were prepared and evaluated for physiochemical parameters like gelation Temperature, viscosity, content uniformity, FTIR. In-vitro drug release studies were carried out in Phosphate buffer (6.8 pH) for F_1, F_5, & F_6 formulations .The results of the present study revealed that Gelation temperature for the formulations were F_1-35°c , F_5-36°c and for F_6-38°c which were close to 37°c body temperature. The viscosity of prepared formulations showed that F1 was 29845 (Cps), F_5 was 42765 (Cps) and F_6 was 45732(cps). The drug uniformity for formulations were between 98.5% to 99.1%, pH of the formulations were F_1-6.8, F_5-6.9 and F_6-6.7, in FTIR showed no interactions between the drug and polymer. The in-vitro diffusion studies showed 79.6% of drug release for formulation F_6 when compared to F_1 was 95.8% and for F_5 was 89.6%. In-situ gels can be easily administered in to the Periodontal Pockets for efficient action of drug dosage forms.

Key words: *Periodontal infections; Amoxicillin, in-situ gel.*

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INTRODUCTION:

Dental diseases are recognized as the major public problems throughout the world. The region of the mouth that consists of the gum supporting structure is called the periodontium. It is made up of the following parts.

- Gum (gingival) is pale pink, firm, and immobile, with a smooth or stippled texture and the tissue between abutting teeth is shaped like a wedge.
- The crevice between the gums and tooth (the sulcus).
- Root surface (the cementum).
- Connective tissue attachments.
- The crest of supporting bone, which can be viewed on X-rays, is normally 2mm below the point where the crown of the tooth meets the root (the cementoenamel junction). Periodontal diseases refers to a group of problem that arise in the sulcus.

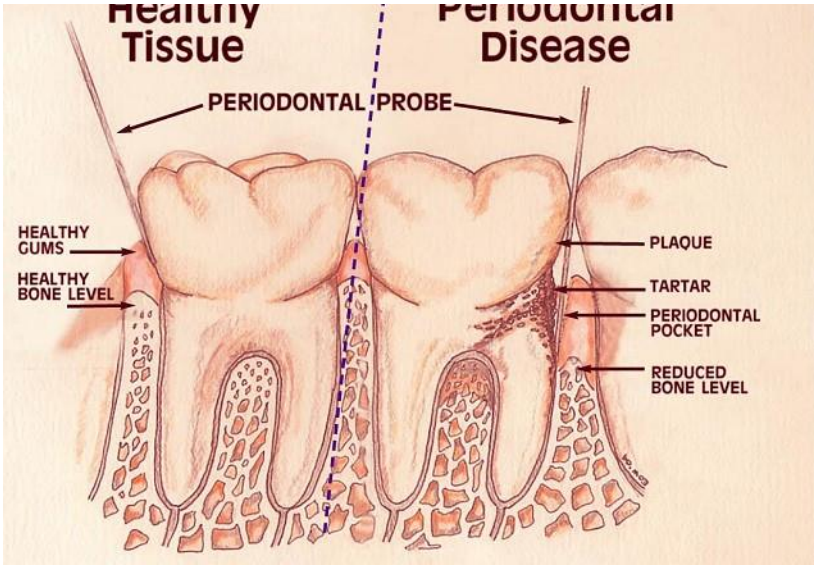
The crevice between the gum and the tooth. Periodontal diseases are generally divided into two groups.

- Gingivitis, which causes lesions (wounds) that affect the gums.
- Periodontitis, which damages the bone and connective tissues that supports the teeth.

Periodontal diseases has been consider as a possible risk factor in other systemic diseases such as cardiovascular diseases, including coronary heart diseases and stroke and pre-term low birth weight infants. Periodontal diseases is localized inflammatory response due to infection of a periodontal pockets arising from the accumulation of subgingival plaque. Untreated Periodontitis results in the loss of the supporting structure of the tooth through resorption of alveolar bone and loss of periodontal ligaments attachment. Clinically, as the diseases progresses, the periodontal pocket, which is somewhat deeper than the sulcus of healthy tooth, gets deeper with further destruction of the tooth's supporting structures, often resulting in tooth loss.

Even in healthy mouths, the suclus is teeming with bacteria, but they tend to be harmless varieties. Periodontal diseases develop usually becomes of two events in the oral cavity an increase in bacteria quantity and a change in balances of bacterial type from harmless to diseases causing bacteria. These harmful bacteria increase in mass and thickness until they form a film known as plaque. In healthy mouths, plaque itself actually provides some barrier against outside bacterial invasion. When it accumulates to excessive, however, plaque adheres to the surface of the teeth and adjacent gingival and causes cellular injury, with subsequent swelling, redness, and heat. When plaque is allowed to remain in the periodontal area, it transforms into calculus (commonly known as tartar). This material has a rock-like consistency and adheres tenaciously to the tooth surface. It is much more difficult to remove than plaque, which is a soft amorphous mass. The most important components in perpetuating the diseases process, however, is the body's own persistent immune response to the bacterial plaque. Scific immune factors are released that cause inflammation and damage that eventually destroy the support structure and bone and can lead to tooth loss.

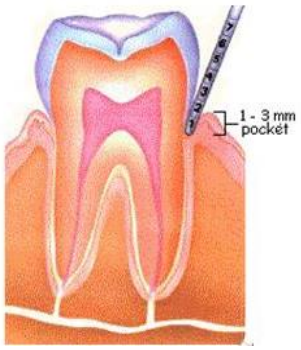
Figure no-1: Schematic representation of Normal tooth structure and Periodontal



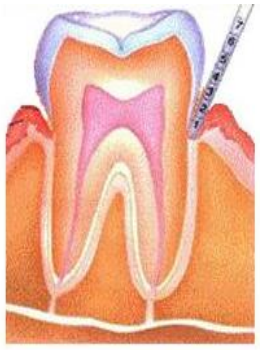
Gingivitis

The periodontal diseases are highly prevalent and can affect up to 90% of the worldwide population. The term periodontal diseases usually refers to common inflammatory disorders of gingivitis and Periodontitis that are causes by pathogenic microflora in the bioflim or dental plaque that forms adjacent to the teeth daily basis. Gingivitis, the mildest form of periodontal diseases, is highly prevalent and readily reversible by simple, effective oral hygiene. Gingivitis affects 50-90% of adults world wide, depending on its precise definition. Periodontitis results in the formation of soft tissues pockets or deepened crevices between the gingival and tooth root. Severe Periodontitis can result in loosening of teeth, occasional pain and discomfort, impaired manistication, and eventual tooth loss. One large survey estimated that about 22% of US adults had mild disease and 13% had moderate or severe disease. Gingivitis is a case when the gum are locally inflamed and become red, swollen and bleed easily. It is mild form of gums diseases that can be reversed with daily brushing and flossing and regular cleaning by dentist. This form of gum diseases usually is not associated with any type of bone or tissues loss. Gingivitis is an inflammation of the gingival, or gums, is nearly always chronic, but an acute from infrequently occurs. Chronic gingivitis affects over 90% of the population. It characterized by tender, red, swollen gums that bleed easily and may be responsible for bad breath (halitiosis) is some cases. Treatment is very effective if initiated early in the course of gingivitis. With good management, however, the problem can progress.

Figure no-2: comparison between Healthy tissue and Gingivitis



Healthy tissues
No Bleeding or puffy gums,
Sulcus depth measures up to normal 3 mm or less

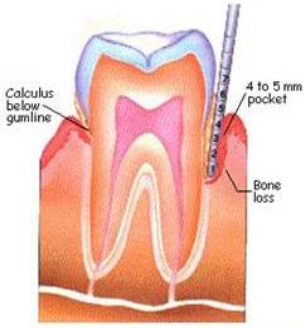


Gingivitis
Bleeding and puffy gums, sulcus depth measures up to 3 mm. No damage to supporting bone in this stage.

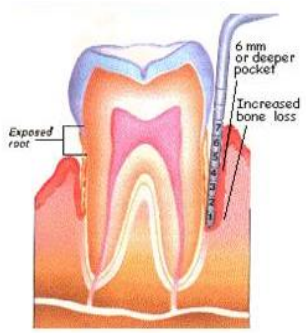
Periodontitis

Periodontitis describe a group of related inflammatory disease resulting in destruction of the tissues that support the tooth. It results form extension of the inflammatory process initiated in the gingival to supporting periodontal tissues. Clinical feature of Periodontitis include bleeding, pus discharge, halitosis, tooth mobility, functional impairment and ultimately tooth loss. The standard clinical measure for Periodontitis are bleeding on probing, clinical attachment level and depth pocket depth. Tooth loss especially in the anterior region can cause psychological trauma to the patient 5-20% of population suffers from severe generalized Periodontitis, through mild to moderate Periodontitis affects a majority of adults. The immediate goal is to prevent, arrest, control or eliminate Periodontitis and to restore the lost, form, function, esthetics and comfort. Periodontal diseases therapy has been directed at altering the periodontal environmental to one, which is less conductive to the retention of bacterial plaque in vicinity of gingival tissue. Active phase of the diseases can be reversed dramatically by reducing the plaque levels. Topical administration of antibacterial agents in the form of mouth washes, dentifrices or gels can be used effectively in controlling supragingival plaque. Periodontitis is a severe case of gum diseases where the gum start to pull away from the tooth and form a cavity or a “pocket”. The tooth eventually may become loose due to loss grip and may fall off or has to be removed. Another major difference between the two is that Gingivitis can affect only or two teeth gingival at a time, while Periodontitis can affect many teeth. Recently a new approach using local delivey system containing antimicrobial has been introduced. This produced more contant and prolonged concentration profile. Both topical delivery system and controlled release system have been termed as local delivery.

Figure no-3: Comparison between Healthy tissue and Periodontitis



Periodontitis
Bleeding and Puffy gums, Pocket depth



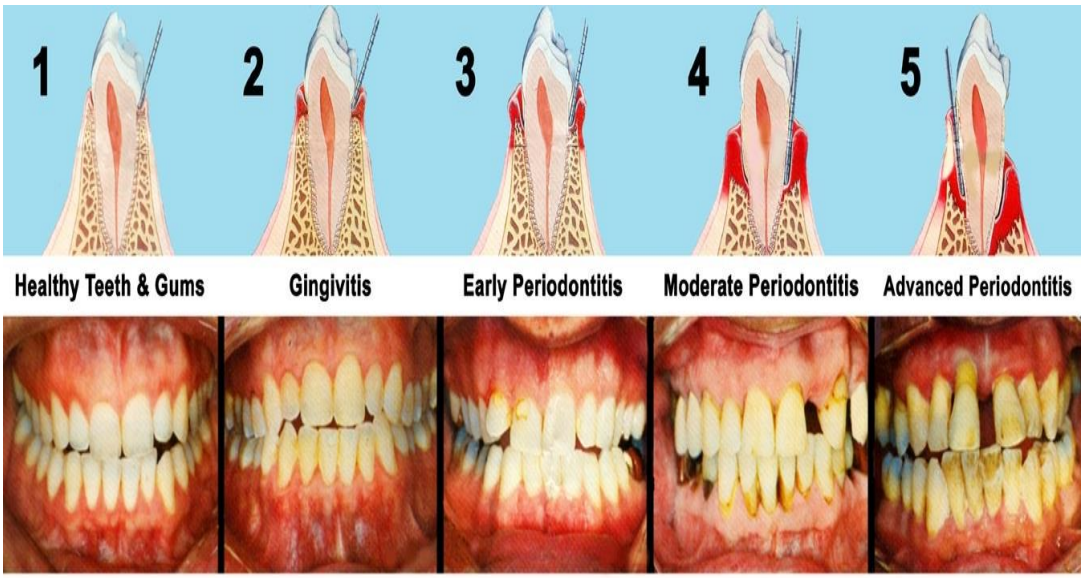
Periodontitis
Bleeding gums, Puffy gums, Pocket depth measure more than 5mm, Bone loss will be seen in this stage.

Periodontitis is characterized by following

Stages of Periodontal Disease

Below you will see five illustrations of a cross section of a tooth, the outer gingival or gum tissue, the inner connective tissue which supports the tooth and a depiction of a graduated probe being inserted into the crevice or periodontal 'pocket.' The probe measures the pocket depth, giving the hygienist an idea of the severity of the decayed area. The red color of the gum tissue at the neck of the tooth indicates inflammation, which increases with the severity of the condition. Notice how connective tissue is being destroyed during the progression. Periodontal disease is a separate issue from caries or 'tooth decay' and caused by the infection of different pathogens, although lack of proper and thorough oral hygiene will promote the disease process in both case – and one can *certainly* exist without the other. For this reason, many may feel that, because they are not or no longer developing cavities, their oral health is not at risk. However, studies show that between 75% and 95% of all adults are suffering some stage of periodontal disease.

Figure no-4: Stark comparison of the loss of binding tissue



Healthy Teeth & Gums	Gingivitis	Early Periodontitis	Moderate Periodontitis	Advanced Periodontitis
1. In healthy gums, the gingival or gum tissue is a pink or coral color. The tissue is firm and resilient and there will be minimal, if any, crevice or pocket depth.	**2.** At the stage referred to as 'gingivitis' the gingival or gum tissue will be inflamed at the neck of the tooth, as opposed to the pinkish color indicated in #1. There will be some pocket depth and gingival bleeding on probing (BOP). There will not be any deterioration of supporting tooth structure.	**3.** Inflammation of periodontal ligaments and minor loss of attachment or pocket development. No tooth mobility at this stage. No connective tissue loss.	**4.** Moderate loss of attachment and/or moderate to deep pocket formation. 30%-50% loss of bone support and slight tooth mobility.	**5.** Advanced breakdown of supporting periodontal tissues. Severe pocket depth or significant gingival recession. Severe loss of attachment. Greater than 50% loss of bone support and considerable tooth mobility.

GelsA gel is a solid or semisolid system of at least two constitutes, consisting of condense mass enclosing and interpenetrated by a liquid. When the coherent liquid is matrix and is rich in liquid, the product is often called a jelly. Examples are Ephedrine jelly and the common table jellies. When the liquid is removed leaving only the framework, the gel is known as xerogels. Examples are gelatin sheets, tragacanth ribbons and acacia tear.

In a typical polar gel, a natural or synthetic polymer builds a three-dimensional matrix throughout a hydrophilic liquid. Typical polymers used, include the natural gums Tragacanth, Pectin, Agar and Alginic acid, semi synthetic materials such as Methylcellulose, Hydroxy ethyl cellulose, Hydroxy propyl methyl cellulose and Carboxy methylcellulose and the synthetic polymers Carbopol.

In-Situ Hydrogels

In-Situ gelling systems improve the drug resistance time in periodontal pockets for effective treatment at the site of action. The technique (Sol-Gel transformed system) includes the use of novel formulations allowing drugs to be delivered in a controlled manner over a prolonged period of time.

EXPERIMENTAL (MATERIALS AND METHODS)

Materials And Equipment's Used:

Table No. 1: Materials used

Sl. No.	Materials	Source
1	Amoxicillin Trihydrate	EURO DRUGS LAB, HYD
2	Poloxamer 407	Asha Analytical, Hyderabad
3	Carbopol 934P	S,D.Fine Chem.Ltd Mumbai
3	Triethanolamine	Loba Chemie, Mumbai
4	Sodium Hydroxide	Ranbaxy fine chemicals, New Delhi
5	Potassium Dihydrogen Phosphate	Ranbaxy fine chemicals, New Delhi
6	DM001 Dialysis Membrane 50	Hi-Media Labs, Mumbai

Table No. 2: Equipment's used

Sl. No.	Equipment / Instrument	Source
1	Digital Balance	Contech, CA 223
2	Digital pH meter	Secor India
3	Magnetic Stirrer	Remi-Bombay
4	FTIR Spectrophotometer	Shimadzu, Model-8400s
5	Refrigerator	Godrej Ultra, India
6	UV-Visible Spectrophotometer	ELICO-INDIA, SL-164
7	Water Bath	Dolphin- Bombay
8	Incubator	Dolphin- Bombay

Phase-I: Preparation of In-Situ Gels[45]
Formulation of In-Situ Gels
Total nine formulation was prepared

Table No. 3: formulation of in-situ gels

Ingredients	FORMULATIONS								
	F1	***F2***	***F3***	***F4***	***F5***	***F6***	***F7***	***F8***	***F9***
Amoxicillin Trihydrate	5g	5g	5g	5g	5g	5g	5g	5g	5g
Poloxamer-407	9g	10g	12g	9g	10g	12g	9g	10g	12g
Carbopol 934-P	-	-	-	0.050g	0.100g	0.150g	0.500g	1g	1.5g
Methyl paraben	0.005 g	0.005g	0.005g	0.005g	0.005g	0.005g	0.005g	0.005g	0.005g
Water(Q.s)	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml
0.1N HCl	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml
Triethanolamine	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml
Flavor (orange flavor)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Method of preparation of in-situ gels
Preparation of In-situ gels are prepared by the "cold technique".
Different formulations were prepared with various ratios of poloxamer 407 and carbopol 934.
Step I: A solution of poloxamer 407 and methyl paraben were solubilized in 17.5ml of cold distilled water.
Step II: A solution of carbopol 934 was made in 17.5ml of water kept overnight for hydration
Step III: Mix both the solutions on continuous stirring for one hour and kept a side at room temperature for 24hr
Step IV: the drug Amoxicillin trihydrate was dissolved in 10ml of 0.1N HCl and added to the above mixture on continuous stirring.

Step IV: The preparation was adjusted to the pH 7 with the addition of 5ml of Triehanolamine on stirring.

PHASE: II

Calibration of Standard Graph of Amoxicillin Trihydrate

In the present study, the UV-Visible spectrophotometric method of analysis of Amoxicillin Trihydrate was employed.

Determination of λ_{max} of Amoxicillin Trihydrate:

Stock Solution: Accurately weighed 100 mg of Amoxicillin Trihydrate was dissolved and the volume was made to 100 ml with 6.8 pH (1000 µg/ml).

Scanning: : Firstly a standard solution of 10ppm was prepared by taking 1ml of stock solution and diluted up to 10ml then the solution is kept in the test solution chamber and its absorbance was measured at various wave lengths ranging from 200-400nm at an intervals of 10nm.

Standard plot of Amoxicillin Trihydrate in 6.8P^{H} Phosphate Buffer:

Preparation of standard solution:

- 100 mg of Amoxicillin Trihydrate was accurately weighed in to 100ml volumetric flask and dissolved in small quantity of methanol. The volume was made up with the methanol to get a concentration of 1000µg/ml (SS-I). From this 10ml was withdrawn and diluted to 100ml to get a concentration of 100µg/ml (SS-II).

Preparation of working standard solutions:

- From (SS-II) aliquots pipetted out 10ml to 100ml volumetric flasks. The volume was made up with Phosphate Buffer to get a concentration of 10µg/ml (SS-III).
- From this pipette out 2, 4, 6, 8, 10, 12, 14, 16 ml respectively. And up to 10ml with Phosphate Buffer to optain 2, 4, 6, 8, 10, 12, 14, 16 µg/ml.
- Absorbance of solution is measured at 229nm using Phosphate Buffer as blank. Plot a graph of concentrations vs. absorbance to get calibration curve.

Characterization of prepared In-Situ gels:

Gelation temperature:

Preliminary evaluation of gel (gelation temperature): The different formulations of poloxamer 407 and Carbopol 934 combinations were evaluated for gelation temperature. The gelation temperature was determined by heating the solution (1-2ºc) min in a test tube with gentle stirring until gel was formed. The gel was said to have formed when there was no flow after container was overturned.

Determination of Drug Content

The prepared formulations were analyzed for the drug content by taking 1 ml of the smart gel in 100 ml volumetric flasks, 5 ml of 6.8pH was added and shaken to dissolve the drug, the volume was made up to the mark by 6.8 pH and the solution was left overnight. The drug content was determined by measuring the absorbance at 229 nm using UV-Visible spectrophotometer.

Determination of pH

Take prepared gel of in-situ gel in a beaker and determination of pH was by using Digital pH meter.

Determination of Viscosity

The viscosity studies of all the formulations were measured by using Brookefield DV-III+ programmable rheometer using spindle no: 21 at 250 rpm. The viscosity measurements were done at 37ºC.

Evaluation of Prepared In-Situ Gels

Microbiological Evaluation

Testing of formulations

Culture Media for aerobic bacteria

Two media were used for the test, one for aerobic and other for anaerobic bacteria.

I. Nutrient broth and Nutrient Agar media are used for aerobic bacteria.

II. Fluid Thioglycollate media is used for anaerobic bacteria**.**

Quantity of sample used: 1ml

Apparatus used: Conical Flasks, Petri dish, Test tubes, Beakers, Fluid, Disposable Syringes (2 ml), Measuring Cylinder.

Note: All the glasswares were sterilized (Dry-heat) in an oven at 170ºC for 2 hrs.

Formula for Nutrient Broth media

Sl No.	Ingredients	Quantity
1.	Peptone	5.0 gm
2.	Sodium Chloride	5.0 gm
3.	Yeast Extract	2.0 gm
4.	Beef Extract	1.0 gm
5.	Distilled Water Q.S.	1000 ml
pH adjusted to 7.4 ± 0.2		

Formula for Nutrient Agar media

Sl No.	Ingredients	Quantity
1.	Agar	15.0 gm
2.	Peptone	5.0 gm
3.	Sodium Chloride	5.0 gm
4.	Yeast Extract	2.0 gm
5.	Beef Extract	1.0 gm
6.	Distilled Water Q.S	1000 ml
pH adjusted to 7.4 ± 0.2		

Procedure

- Dissolve nutrient broth (13 gm/lt) and nutrient agar media (28 gm/lt) in distilled water and sterilize the media in autoclave for 30 min at 121ºC.
- The medium was poured into sterilized petri dishes and agar tubes.
- Inoculate the culture on the petri dish and into the broth media.
- Incubate the media at 37ºC for 14 days.

Formula for Fluid Thioglycollate media

Sl No.	Ingredients	Quantity
1.	Pancreatic digest of Caesin	15.0 gm
2.	Glucose	5.5 gm
3.	Yeast Extract	5.0 gm
4.	Sodium Chloride	2.5 gm
5.	Agar	0.75 gm
6.	L-Cystine	0.5 gm
7.	Sodium Thioglycollate	0.5 gm
8.	Resazurin	0.001 gm
9.	Distilled Water q.s	1000 ml
pH adjusted to 7.1 ± 0.2		

Procedure

- Melted Fluid Thioglycollate media (29.75 gm/lt) in distilled water and sterilized the media in autoclave for 30 min at 121ºC.
- The medium was poured into sterilized Thioglycollate tubes.
- Inoculate the culture into the media.
- Incubate the tubes at 37ºC for 14 days.

Evaluation of In-Situ gels:

Phase: III: In-Vitro Drug Diffusion Studies:

Release of Amoxicillin Trihydrate from various gel formulations were studied employing slightly modified permeation apparatus as described by Fites. A glass cylinder with both ends open, 10 cm height and 3.7 cm outer diameter was used as a permeation cell. A Dialysis membrane (soaked in phosphate buffer of pH 6.8 over a night) was fixed to one end of the cylinder by adhesive tape 1ml. of the prepared gel was taken in the cell (donor compartment) and the cell was placed in glass beaker containing 100ml of pH 6.8 phosphate buffer solution as receptor compartment. The cell was immersed to the depth of 1cm below the surface of receptor fluid. The medium in the receptor compartment was agitated using magnetic stirrer and temperature of 37±0.5ºc was maintained. Sample 5 ml of recipient sample was withdrawn for 8 hrs at 1-hour intervals and replaced with same volume of buffer solution. Samples were analyzed for Amoxicillin Trihydrate content by at 229nm U.V-visible spectrophotometer.

The data obtained was graphed as followed

1. Percentage cumulative drug release versus time
2. Log percentage cumulative drug remaining versus time (first order)

Drug excipients compatibility studies

Compatibility between the drug and polymer was studied by FT-IR spectra. The position of peaks in FT-IR spectra of pure Amoxicillin Trihydrate is compared with those peaks of Amoxicillin Trihydrate plus excipients (in 1:1:1 ratio). It was observed that, there was no disappearance or shift in peak position of Amoxicillin Trihydrate in spectra of drug and excipients, which proved that drug and excipients were compatible. Hence, it can be concluded that the drug is in free State and can release easily from the polymeric network in the free form.

Stability studies

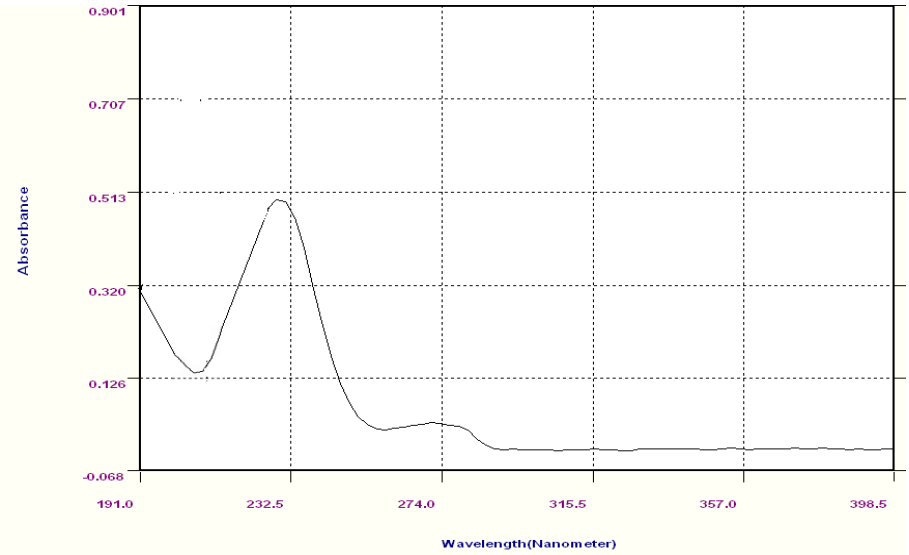
The stability studies were carried out according to ICH to assess the drug formulation stability.

Optimized F_1, F_5 and F_6 formulation was sealed in aluminum packaging laminated with polyethylene. Sample were kept at 40 ^{0}C and 75% RH for 3 months. At the end of the study period, the formulation was observed for change in physical appearance, color, drug content and drug release characteristics.

5.1 Calibration of Standard Graph

The construction of standard calibration curve of Amoxicillin Trihydrate was done by using 6.8 pH phosphate buffer as the medium. Amoxicillin Trihydrate concentration of 50ug/ml in 6.8 pH phosphate buffer was scanned under UV- Visible spectrophotometer over a range from 200 nm to 400 nm. And found to have the maximum absorbance at 229 nm.The standard graph of Amoxicillin Trihydrate in 6.8 pH phosphate buffer was developed in the concentration range of 2 – 16 ug/ml with suitable dilutions of same medium. And aliquots are observed for their absorbance under UV- spectrophotometer at an absorption maximum of 229 nm. The standard graph of Amoxicillin Trihydrate in 6.8 pH phosphate buffer showed a good linearity with R^2 of ***0.993***, and the equation of graph is ***y = 0.025x + 0.0228***

Figure-5: Absorption spectrum of Amoxicillin Trihydrate in pH 6.8 phosphate Buffer



Characterization of prepared In-Situ gels

Gelation Temperature:

Gelation temperature of In-situ gels are tabulated. The data indicates that F1, F5 and F6 formulations are very close to 37ºc. Hence these formualtions were selected for further studies.

Table No. 4: Gelation Temperature of prepared in-situ gel formulation

FORMULATION	GELATION TEMPRATURE
F1	35ºC
F2	25ºC
F3	23ºC
F4	28ºC
F5	36ºC
F6	38ºC
F7	42ºC
F8	50ºC
F9	60ºC

*Each sample of 1ml of gel contain 100mg of drug

Drug content uniformity:

The drug content uniformity was performed for the optimized formulations results are tabulated. Three trails are done for each batch formulation and analyzed by spectrophotometrically. The average value of all formulations are calculated. The drug content in In-situ gels were found to be between 99.5% to 99.1% of Amoxicillin Trihydrate. The results are with in the range and that indicated uniformity of mixing

Table no. 5: Drug content uniformity data of prepared in-situ gel formuation

Sl. no.	Formulation code	Drug content(%)			
		Trail I	Trail II	Trail III	Average
1	F1	99%	97.5%	98.5%	98.5%
2	F5	99.5%	98%	98.5%	98.5%
3	F6	99%	99%	99.5%	99.1%

*Each sample of 1ml of gel contain 100mg of drug

Determination of pH

pH of the formulation are tabulated. Tha data indicates that the prepared in-situ gel are nearly neutral pH range.

Table No.6 : pH of the prepared in-situ Gel formulation

Sl. no.	Formulation code	Ph
1	F1	6.8
2	F5	6.9
3	F6	6.7

*Each sample of 1ml of gel contain 100mg of drug

Determination of viscosity:

Viscosity of formulation are tablulated. As the tempeature was increase the viscosity was also increases.

Table No. 7: Viscosity data of prepared In-situ gel formulation

Sl. No.	Formulation code	Viscosity (cps)
		37°c
1	**F1**	**29845**
2	F5	42765
3	F6	45732

*Each sample of 1ml of gel contain 100mg of drug

Microbial Test

The final formulations were tested for any contimation and it was found that there was no growth in the nutrient broth and nutrient agar media after 14 days. Hence the formulations were confirmed to be free from aerobic bacteria as shown in the Figure 9 & 10. Similarly, it was observed that no growth was found in the fluid thioglycollate media after 14 days. Hence the formulations were confirmed to be free from anaerobic bacteria as shown in the Figure 11.

Evaluation

Invitro drug release:

The invitro drug release studies was carried out in P^H 6.8 phosphate buffer. The invitro drug release profile of Amoxicillin Trihydrate (1%) from In-situ gels were shown in tables no 9 to 12 and to figure 2 to 9.

1. Percent cumulative drug release Vs time
2. Log percent cumulative drug release Vs square root of time.

At the end of 8hr the percentage amount of drug release from F1, F5 and F6 were found to be 95.8%, 89.6%, 79.6% respectively. The Formulation F6 shown prolong release of action.

Table No. 8: In-vitro drug release profile of Formulation-1 Amoxicillin Trihydrate (1%) In-Situ gel

Sl. No.	Time (hr)	Cumulative percentage drug release	Log Cumulative percentage drug remaining
1	0	0.00	2
2	1	38.3	1.788
3	2	50.1	1.698
4	3	61	1.591
5	4	69.5	1.484
6	5	80.2	1.296
7	6	87.7	1.089
8	7	91.2	0.944
9	8	95.8	0.623

*Each sample of 1ml of gel contain 100mg of drug

Table No. 9: In-vitro drug release profile of Formulation-5 Amoxicillin Trihydrate (1%) In-Situ gel

Sl. No.	Time (hr)	Cumulative percentage drug	Log Cumulative percentage drug
1	0	0.00	2
2	1	26.9	1.863
3	2	37.7	1.793
4	3	48.4	1.712
5	4	59.3	1.609
6	5	68	1.505
7	6	72.6	1.33
8	7	80	1.255
9	8	89.6	0.973

*Each sample of 1ml of gel contain 100mg of drug

Figure. No-6: In-vitro drug release profile of Amoxicillin Trihydrate (1%) for Formulation-5

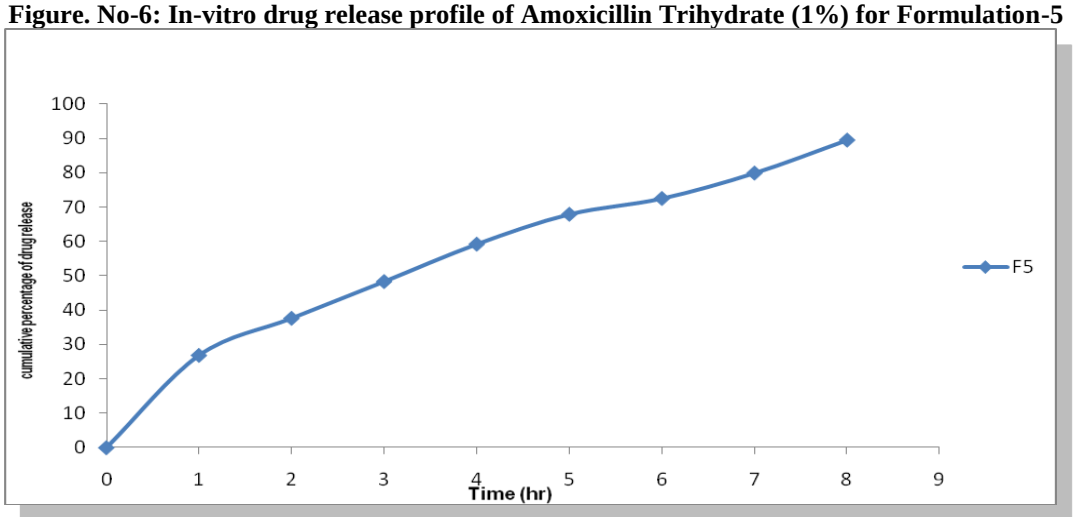


Figure. No-7: In-vitro drug release profile of Amoxicillin Trihydrate (1%) for Formulation-6

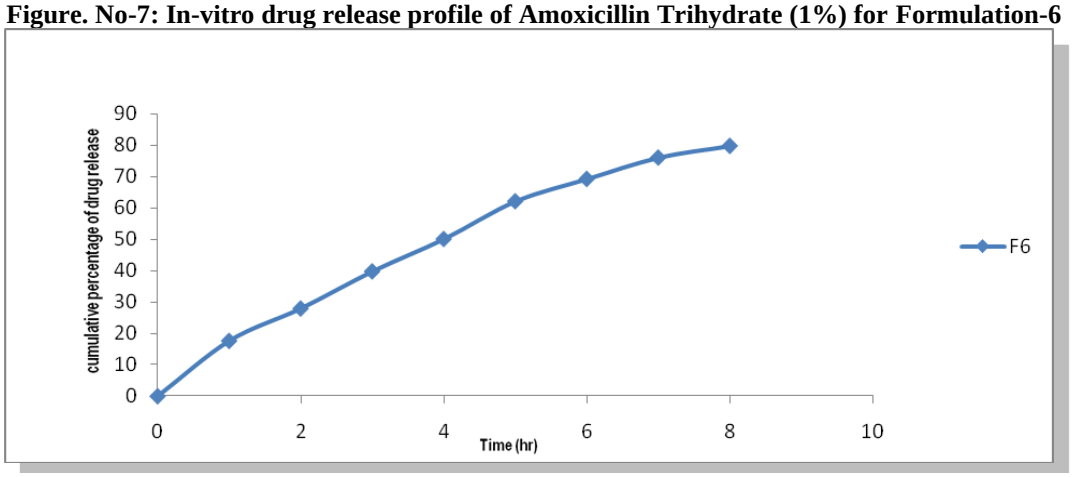


Figure. No-8: In-vitro drug release profile of Amoxicillin Trihydrate (1%) for Formulation-6

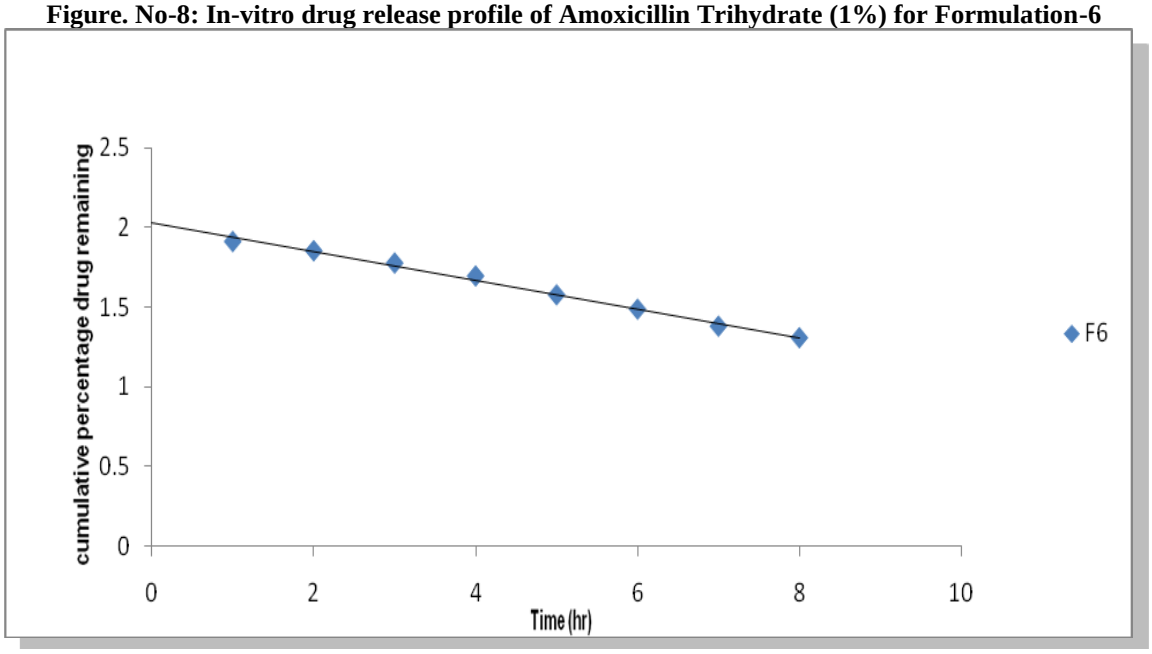
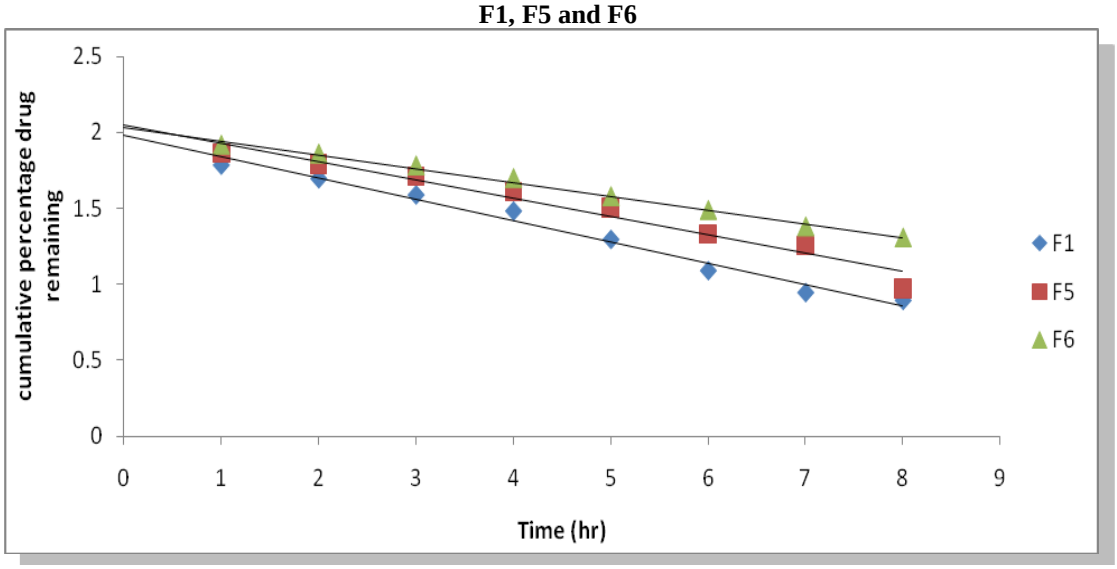


Figure. No-9: Comparative In-vitro drug remaining profile of Amoxicillin Trihydrate (1%) formulation F1, F5 and F6



FT-IR studies:

FTIR sepectra in figure showed no degradation of drug during formulation. Beta lactum peak(co stretch) at 1774 cm^1. A amide peak at 1685 cm^1. The nature of peak did not vary for drug or polymer and In-situ gels indicated that therir was no interaction between the drug and polymer in the formulation were shown in figures 10 to 13.

Figure. No-10: FTIR Spectra of carbopol- 934

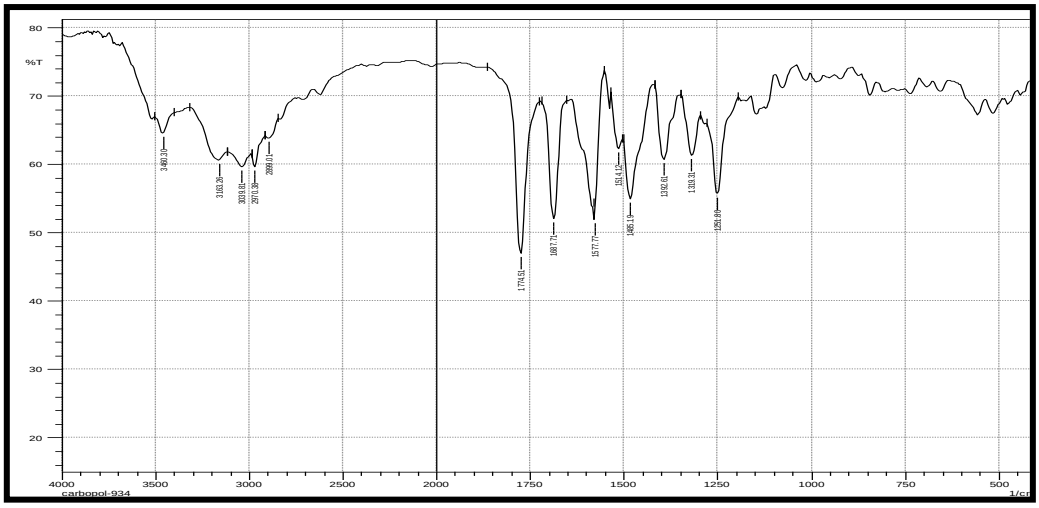


Figure No-11: FT-IR Spectra of Amoxicillin Trihydrate and Polymers

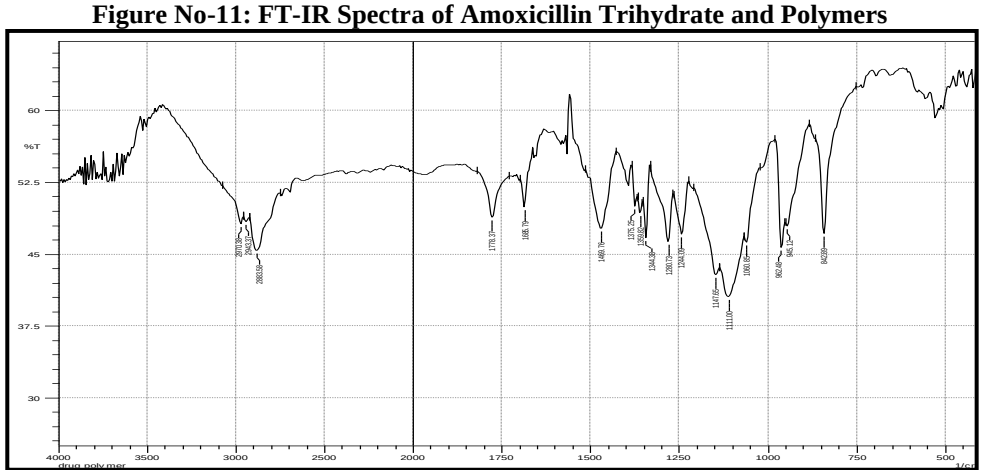
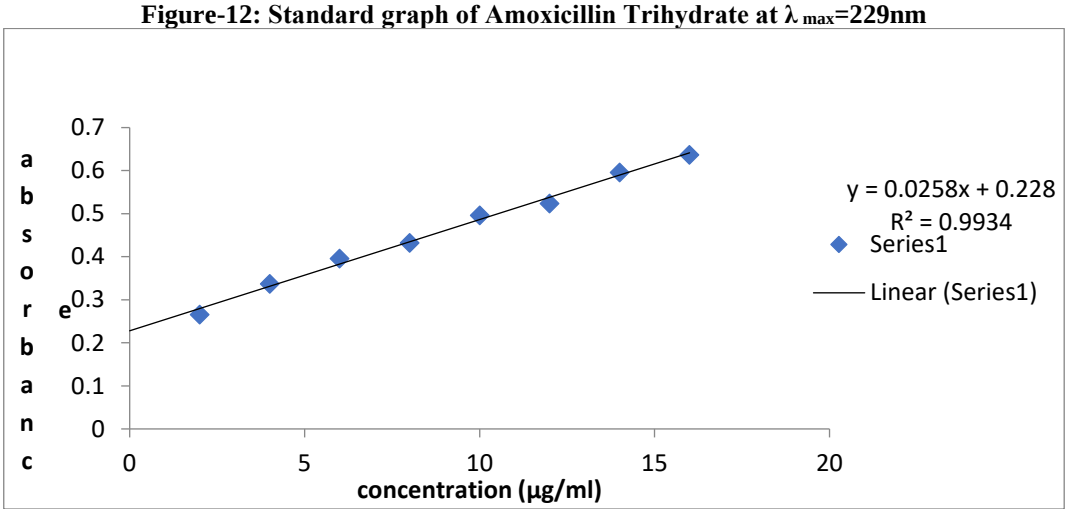


Table No.10: Standard Graph of Amoxicillin Trihydrate in pH 6.8 Phosphate Buffer

Concentration	Absorbance(nm)
2µg/ml	0.266
4 µg/ml	0.337
6 µg/ml	0.396
8 µg/ml	0.432
10 µg/ml	0.496
12 µg/ml	0.524
14 µg/ml	0.596
16 µg/ml	0.637

Figure-12: Standard graph of Amoxicillin Trihydrate at λ_{max}=229nm



5.2 Characterization of prepared In-Situ gels

Gelation Temperature:

Gelation temperature of In-situ gels are tabulated. The data indicates that F1, F5 and F6 formulations are very close to 37ºc. Hence these formualtions were selected for further studies.

Table No. 11: Gelation Temperature of prepared in-situ gel formulation

FORMULATION	GELATION TEMPRATURE
F1	35ºC
F2	25ºC
F3	23ºC
F4	28ºC
F5	36ºC
F6	38ºC
F7	42ºC
F8	50ºC
F9	60ºC

*Each sample of 1ml of gel contain 100mg of drug

Drug content uniformity:

The drug content uniformity was performed for the optimized formulations results are tabulated. Three trails are done for each batch formulation and analyzed by spectrophotometrically. The average value of all formulations

are calculated. The drug content in In-situ gels were found to be between 99.5% to 99.1% of Amoxicillin Trihydrate. The results are with in the range and that indicated uniformity of mixing

Table no. 12: Drug content uniformity data of prepared in-situ gel formulation

Sl. no.	Formulation code	Drug content (%)			
		Trail I	Trail II	Trail III	Average
1	F1	99%	97.5%	98.5%	98.5%
2	F5	99.5%	98%	98.5%	98.5%
3	F6	99%	99%	99.5%	99.1%

*Each sample of 1ml of gel contain 100mg of drug

Determination of pH

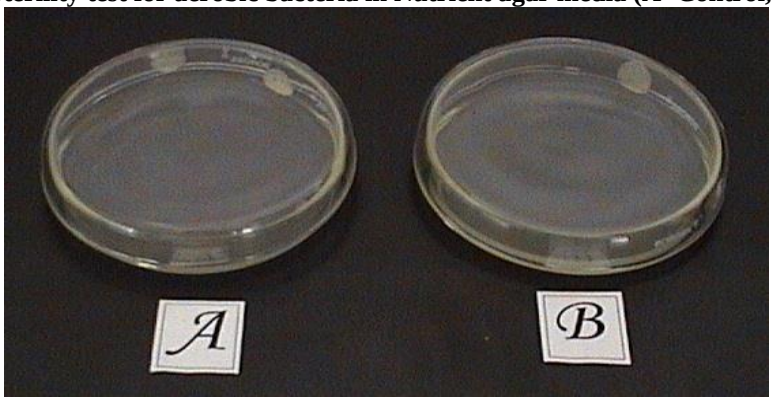
pH of the formulation are tabulated. Tha data indicates that the prepared in-situ gel are nearly neutral pH range.

Table No.13: pH of the prepared in-situ Gel formulation

Sl. no.	Formulation code	Ph
1	F1	6.8
2	F5	6.9
3	F6	6.7

*Each sample of 1ml of gel contain 100mg of drug

Fig-13: Sterility test for aerobic bacteria in Nutrient agar media (A=Control, B=Test)



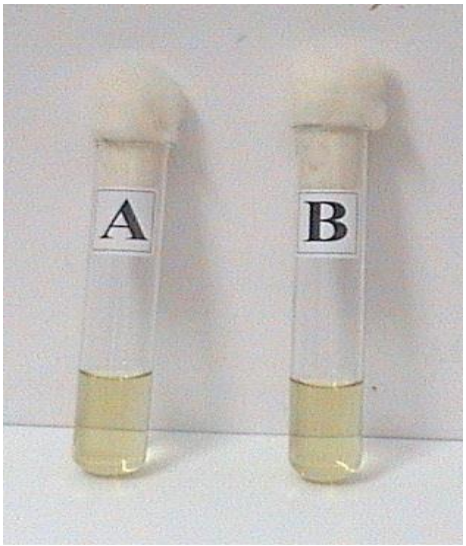


Fig no-14: **Sterility test for aerobic bacteria in Nutrient broth media A = Control, B = Test.**

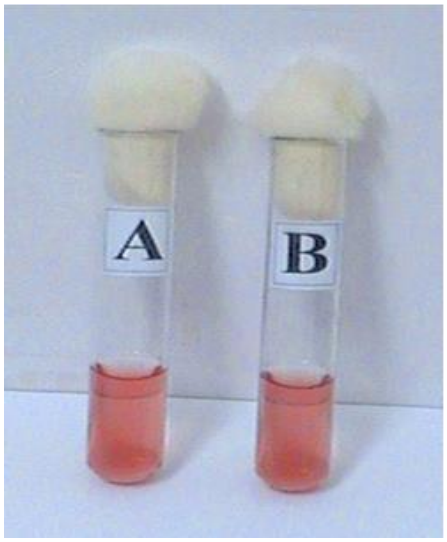


Fig no-11: **Sterility test for anaerobic bacteria in Fluid Thioglycollate media A = Control, B = Test.**

Figure No- 15: Invitro drug release profile of Amoxicillin Trihydrate (1%) for formulation-1

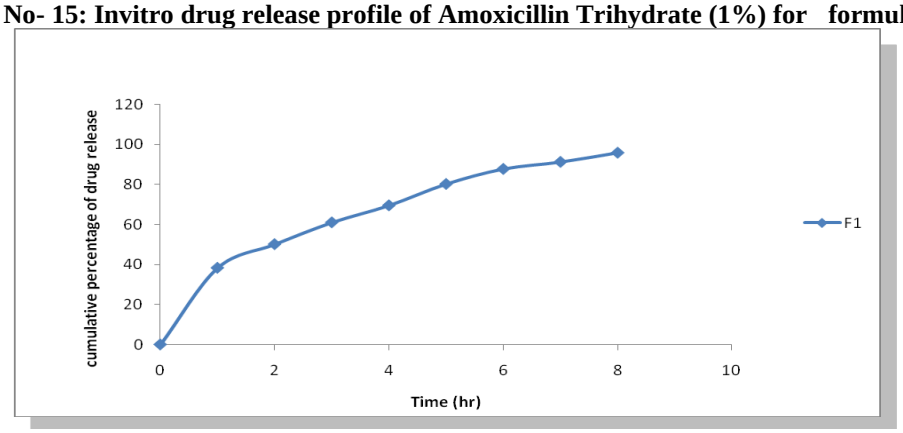


Figure.No-16: In-vitro drug release profile (first order) of Amoxicillin Trihydrate (1%) for formulation-1

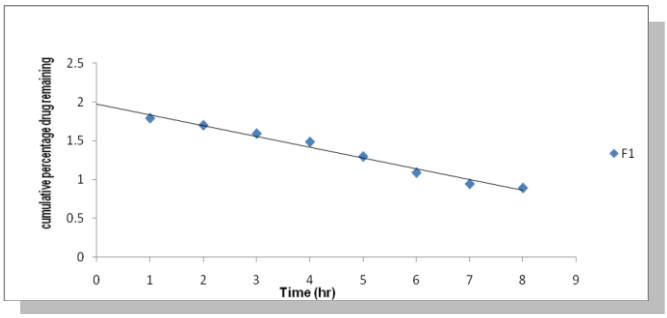


Figure. No-17: In-vitro drug release profile of Amoxicillin Trihydrate (1%) for Formulation-5

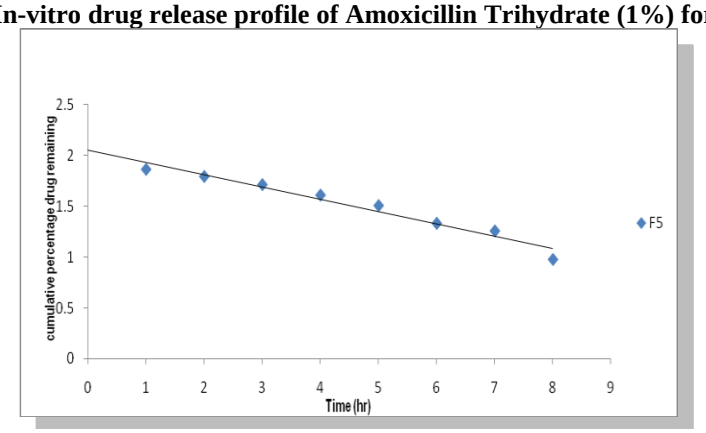


Table No. 14: In-vitro drug release profile of Formulation-6 Amoxicillin Trihydrate (1%) In-Situ gel

Sl. No.	Time (hr)	Cumulative percentage drug release	Log Cumulative percentage drug remaining
1	0	0.00	2
2	1	17.6	1.915
3	2	27.9	1.857
4	3	39.7	1.78
5	4	50	1.698
6	5	62	1.579
7	6	69.1	1.489
8	7	75.9	1.382
9	8	79.6	1.309

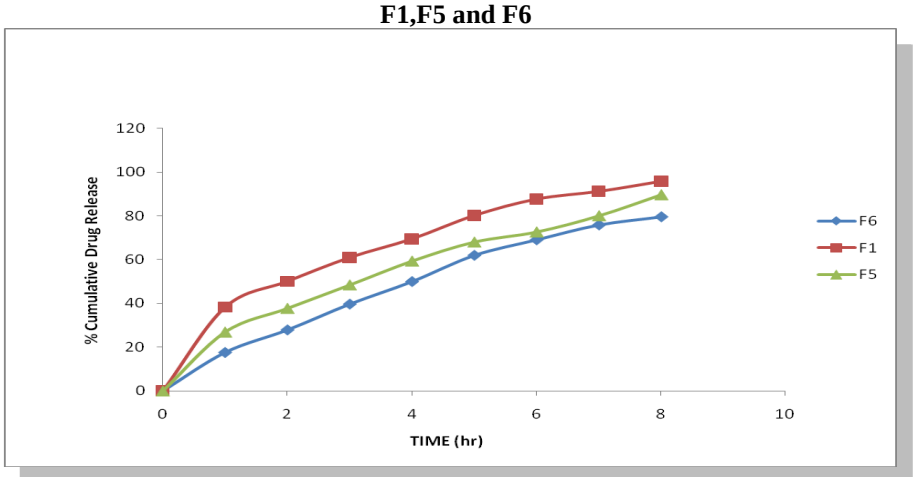
*Each sample of 1ml of gel contain 100mg of drug

Table No.15: Comparative In-vitro drug release and drug remaining profile of Formulations of F1,F5&F6 containing Amoxicillin Trihydrate (1%) in-situ gel

Sl. No.	Time (hr)	Cumulative percentage drug release			Log Cumulative percentage drug remaining		
		F1	F5	F6	F1	F5	F6
1	0	0.00	0.00	0.00	2	2	2
2	1	38.3	26.9	17.6	1.788	1.863	1.915
3	2	50.1	37.7	27.9	1.698	1.793	1.857
4	3	61	48.4	39.7	1.591	1.712	1.78
5	4	69.5	59.3	50	1.484	1.609	1.698
6	5	80.2	68	62	1.296	1.505	1.579
7	6	87.7	72.6	69.1	1.089	1.33	1.489
8	7	91.2	80	75.9	0.944	1.255	1.382
9	8	95.8	89.6	79.6	0.623	0.973	1.309

*Each sample of 1ml of gel contain 100mg of drug

Figure. No-18: Comparative In-vitro drug release profile of Amoxicillin Trihydrate (1%) Formulation F1,F5 and F6



Drug excipients compatibility studies

Figure No-19: FT-IR Spectra of Amoxicillin Trihydrate.

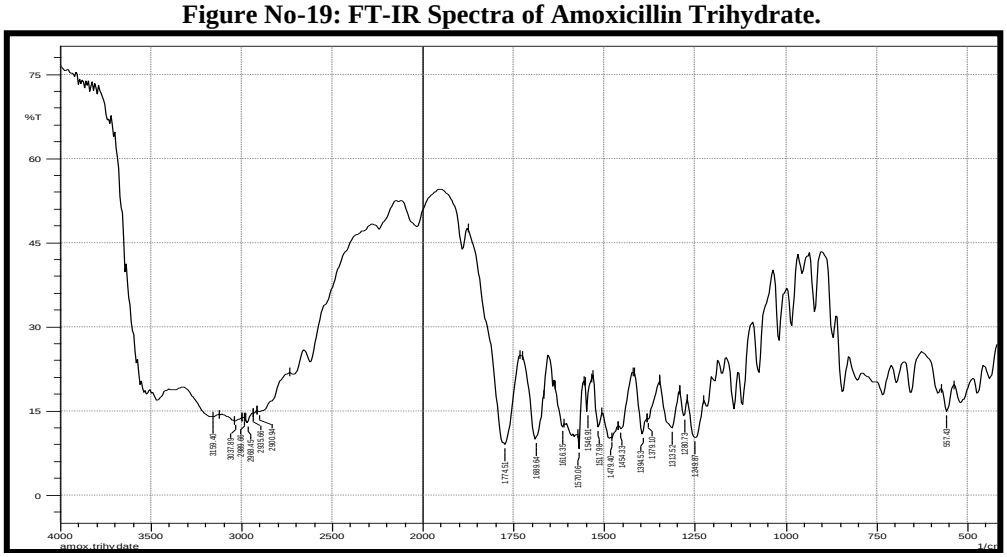
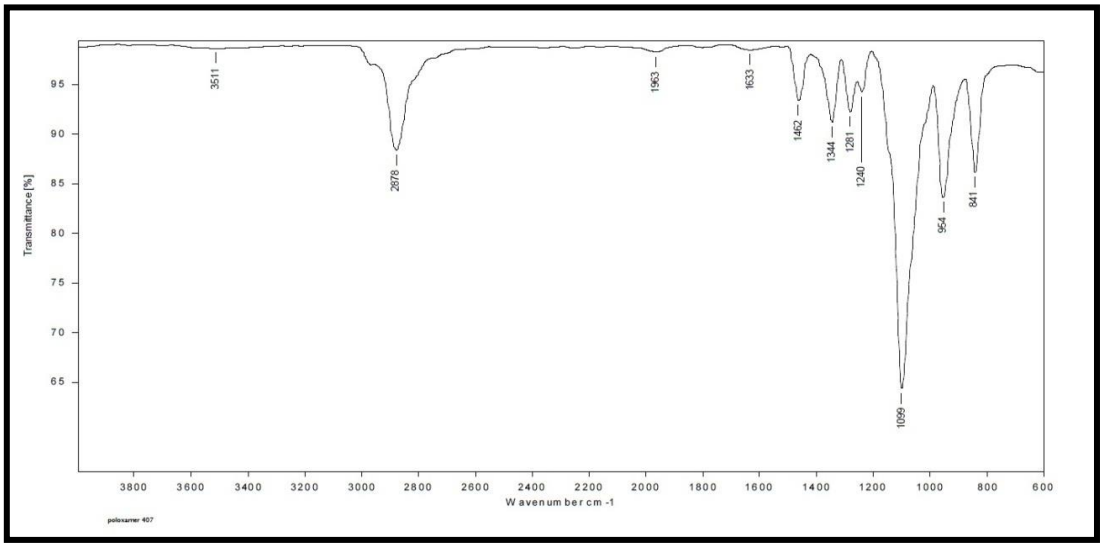


Figure No-20: FT-IR Spectra of poloxamer 407.



Stability studies

The optimized in situ from batch F_1, F_5 and F_6 were charged for stability studies. There was no change in physical appearance, color, Viscosity. Formulations were analysed at the end of 3 months for the assay and dissolution studies. In vitro dissolution profile showed that there was no significant change in the release rate of the drug at the end of 3 months.

CONCLUSION:

The main objective of the experimental study was to formulate and evaluate smart gels of Amoxicillin Trihydrate using different concentrations of Poloxemer, carbopol. The prepared smart gels were evaluated for various properties such as sterility test, viscosity, content uniformity, Gelation temperature and drug release characteristics. The results of the investigation undertaken are summarized as follows

1. The preformulation studies of Amoxicillin Trihydrate showed that it possess required properties to be formulated as smart gels.
2. The drug excipient compatibility studies showed that there was no interaction between the Amoxicillin Trihydrate and Pluronic F-127, carbopol.
3. The microbial studies showed that the product was free from bacteria.
4. Amoxicillin Trihydrate is potential candidate for the formulation of smart gel delivery system exhibiting uniform distribution in the smart gels.
5. Viscosity of the smart gels increased with increase in polymers concentration and temperature. Drug concentration in the smart gels had little / no effect on the viscosity of formulations.
6. The *in vitro* release studies showed that Amoxicillin Trihydrate was released from smart gels in prolong manner. I*n vitro* studies Formulation 6 shows controlled release.

This study revealed that smart gels formulation was simple, easy to administer, comfortable, with less side effects, has increased compliance and also enhance the antimicrobial activity by releasing the drug in prolong manner. It may be concluded that in situ gel delivery system is a novel approach that can be developed for the treatment of Periodontitis.

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