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Thermoreversible-pH sensitive cephalexin *insitu* gel for treating periodontal disease

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ABSTRACT

Controlled delivery of antimicrobials directly into periodontal pocket has received eminent interest and holds a level - headed promise in periodontal therapy. Cephalexin, a first generation cephalosporin antibiotic works by interfering with the bacteria's cell wall formation, which ruptures and kills the periodontal pathogen. Thermoreversible pH sensitive in situ *gel containing 0.1% w/v cephalexin was formulated by cold method using poloxamer and carbopol polymers. They were evaluated for clarity, pH, gelation temperature, spreadability, drug content, rheological studies,* in vitro *drug release, invitro anti bacterial activity, and stability studies. All formulations showed satisfactory physico-chemical characteristics. Further, all the formulations showed sustained drug release for a period of 24hours, which is beneficial to treat periodontal infection. The invitro antibacterial study shows that the optimized F3 formulation showed good zone of inhibition against microbial species of anaerobic Porphyromonas gingivalis, gram +ve Staphylococcus aureus and gram –ve Escherichia coli. The results of study indicate that, temperature sensitive poloxamer 407 and pH dependant mucoadhesive carbopol 934 are promising polymers to develop in-situ gel formulation containing cephalexin as a therapeutic agent for treating periodontal disease over conventional therapy. The formulation is stored at 4˚C before application, which is syringeable through 21 gauge needles, injected directly into periodontal pocket which immediately converts into gel form at the periodontal temperature and pH.*

Keywords: Gingival fluid, thermosensitive, syringeability, periodontal pocket, zone of inhibition

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INTRODUCTION

The periodontium also known as marginal periodontium which is the supporting structure of a tooth, or which help to attach the tooth to surrounding tissues and to allow sensations of touch and pressure. The word periodontium comes from the Greek terms *peri*, meaning “around” and *odons*, meaning “tooth” [1]. All together the word means that which is “around the tooth”. It is estimated that nearly 80% of adult Americans suffers some aspect of the disease's presence. Periodontitis is a chronic disease which is characterized by periods of exacerbation and remission[2]. Chronic periodontitis (formerly adult periodontitis) occurs in localized and generalized forms, and people with significant disease tend to be > 35 yr. About 85% of the population is affected to a mild degree, but the most advanced cases are seen in < 5% of the population.. Periodontal disease refers to a group of problems that arise in the sulcus, the gap between the gum and the tooth. The occurrence of periodontal disease is from a pre-existing gingivitis. Gingivitis is the inflammation of gingiva alone is and the severe inflammation of the periodontal structures with destruction of alveolar bone is called periodontal disease.[3-5] For an antimicrobial agent to be successful the pathogen must be known and it should be susceptible to the drug and not readily develop resistance for an adequate period of time.[6-9] The periodontal pocket provides a natural reservoir bathed by gingival crevicular fluid which is easily accessible for the insertion of a delivery device. [10-15] The gingival crevicular fluid provides a leaching medium for the release of a drug from the solid dosage form and for its distribution throughout the pocket.[16-19] More over the periodontal diseases are localized to the immediate environment of the pocket enabling the periodontal pocket a natural site for treatment with local sustained-release delivery systems. The sustained-

release dosage forms maximize the therapeutic effect of antimicrobials by maintaining a constant plasma drug concentration over MIC for a prolonged period of time in a controlled manner.[20] The aim of the present work is to develop a local controlled release drug delivery system containing cephalexin suitable antimicrobial agent directly into the periodontal pocket for the treatment of periodontitis.

EXPERIMENTAL SECTION

Albendazole which is obtained as gift sample from Cipla Ltd, Mumbai, polymers poloxamer 407 and carbapol 934 from Yarrow Chem. Products, Mumbai. And all other chemicals used in this study are of analytical reagent grade.

PREFORMULATION STUDIES

Preformulation studies are the first step in the rational development of dosage form of a drug substance. Preformulation investigations are designed to deliver all necessary data, especially physic-chemical, physico-mechanical and biopharmaceutical

Identification of drug

FTIR studies [21]

FTIR of the obtained pure drug of cephalexin, was compared with the FTIR of the standard spectrum.

Solubility [22]

Solubility of drug was determined in different solvents such as water, methanol, ethanol, chloroform, and buffers such as phosphate buffer of pH 7.2.

Λ max of the drug [23]

An absorption maximum of Cephalexin was determined using distilled water. Solution ranging from 2µg/ml - 10µg/ml were scanned from 200 – 400 nm using UV spectrophotometer.

Melting point [24]

Melting point of the obtained drug sample indicates the purity of the sample. The presence of relative small amount of impurity will lower the melting point. Melting Point was determined using Open Capillary Method.

Analytical methods [25]

Calibration curve was done using distilled water and Phosphate buffer of pH 7.2

Calibration curve of Cephalexin in distilled water

a) Preparation of standard stock solution

10mg of cephalexin was accurately weighed and taken in 100ml volumetric flask. The drug was dissolved and diluted to volume with distilled water to get the concentration of 100µg/ml.

b) Preparation of standard graph

From the standard stock solution, aliquots of 0.2, 0.4, 0.6, 0.8 and 1ml were withdrawn in to 10ml volumetric flask and diluted with distilled water to get the concentration 0f 2-10µg/ml. The absorbances of their solution were measured at 261.5nm by using UV Spectrophotometer, using distilled water as the blank. The absorbance was plotted against concentration (µg/ml) to obtain the standard graph.

Calibration curve of Cephalexin in Phosphate buffer pH 7.2

a) Preparation of Phosphate buffer 7.2

62.5ml of 0.2M Potassium dihydrogen phosphate was taken in 250ml volumetric flask, add specified volume containing 42.62ml of 0.2M NaOH (dissolve 8g NaOH in 1000ml water) followed by distilled water to the final volume.

b) Preparation of standard stock solution

10gm of Cephalexin was accurately weighed and taken in 100ml volumetric flask. The drug was dissolved and diluted to volume with phosphate buffer of pH 7.2 to get the concentration of 100µg/ml.

c) Preparation of standard graph

From the standard stock solution, aliquots of 0.2, 0.4, 0.6, 0.8 and 1ml were withdrawn in to 10ml volumetric flask and diluted to get the concentration of 2-10µg/ml. The absorbance of their solution was measured at 261.5nm by using UV Spectrophotometer, using phosphate buffer of pH7.2 as the blank. The absorbance was plotted against concentration (µg/ml) to obtain the standard graph.

Partition coefficient [26]

Partition coefficient of cephalexin in n-octanol-water was determined. Equal volume of water and n-octanol was taken in separately funnel. To this known amount of Cephalexin was added. The funnel was equiliberated for 2hrs at constant temperature with intermittent shaking at regular intervals. Then the aqueous layer was determined by UV Spectroscopy at 261.5 nm. The n-octanol –water partition coefficient of the drug was obtained using following equation:

Partition coefficient = Concentration of drug in organic layer / Concentration of drug in aqueous phase.

FORMULATION OF CEPHALEXIN LOADED *IN SITU* GEL

Optimization of carbopol 934

Different concentration of Carbopol 934 from 0.1 – 0.5% w/v was prepared using distilled water and allowed to hydrate till overnight. Then select the lowest carbopol concentration which forms sol – gel transition.

Optimization of poloxomer 407

Poloxomer 407 plain *in situ* gels were prepared by cold method. This method involves the slow addition of polymer poloxomer 407, in cold water with continuous agitation. The formed mixtures stored at 4°c and studied for effective *in situ* gel formulation. The concentration of poloxomer 407 was selected so as to obtain thermo reversible gel at minimum concentration. Poloxomer 407 vehicles with varying concentration 10%w/v to 20%w/v were screened preliminary to decide lowest possible concentration. Pluronic F 127, 15%w/v was selected to be lowest concentration that exhibited thermo reversible property at temperature 35.5°c (temperature of periodontal region).

Optimization of *in situ* gel by using cephalexin [27-28]

Thermoreversible gels were prepared using cold method. This method involved slow addition of polymer poloxomer 407, in cold water with continuous agitation. The formed mixtures were stored overnight at 4°C. The poloxomer 407 vehicles used throughout this study were composed of 18% wt/v of poloxomer 407 until a clear solution was obtained. Mucoadhesive anionic polymer carbopol 934 which was allowed to swell overnight was slowly added to the poloxamer solution with continuous agitation. Carbopol 934 was added in concentration range of 0.2% wt/v to 0.5% wt/v to poloxomer 407 solution. To the above solution add 10mg drug and dissolve it. The prepared gels were used for further evaluation.

Table 5: Composition of Cephalexin *in situ* gel

Formulation	Poloxamer 407 (w / v)	Carbopol 934 (%)	Drug (mg)	De-ionised water (ml)
F1	18	0.05	10	10
F2	18	0.1	10	10
F3	18	0.2	10	10
F4	18	0.4	10	10
F5	18	0.6	10	10
F6	18	0.8	10	10
F7	18	1	10	10

Evaluation of cephalexin *in situ* gel

Physicochemical properties [29-30]

Physicochemical properties such as surface pH, spreadabiliy, viscosity, gelation temperature, drug content of prepared *in situ* gel were determined.

Surface pH

An acidic or alkaline formulation is bound to cause irritation on mucosal membrane and hence this parameter assumes significance while developing a mucoadhesive formulation. A digital glass electrode pH meter was used for this purpose. pH was noted by bringing the electrode near the surface of the formulation and allowing it to equilibrate for 1 min.

Spreadability

The spreadability of the gel formulations was determined 48hours after preparation, by measuring the spreading diameter of 1g of the gel between two glass plates after 1 min. The mass of upper plate was standardized at 125g. The spreadability was calculated by using the formula S = m. l/t, where S is spreadability, m is the weight tied to the upper slide, l is the length of the glass slide, and t is the time taken. Homogeneity of gel formulation was tested by visual observations.

Gelation temperature
A 2ml aliquot of gel was transferred to a test tube, immersed in a water bath. The temperature of water bath was increased slowly and left to equilibrate for 5min at each new setting. The sample was then examined for gelatin, which was said to have occurred when the meniscus would no longer moves upon tilting through 90°c.

Syringeability
All prepared formulations were transferred into an identical 5 ml plastic syringe placed with 21 gauge needle to a constant volume (1 ml). The solutions which were easily passed from syringe was termed as pass and difficult to pass were termed as fail.

Drug content
1ml of each formulation was taken in 10ml volumetric flask, diluted with distilled water and volume adjusted to 10ml. 1ml quantity from these solutions was again diluted with 10ml of distilled water. Finally the absorbance of prepared solution was measured at 261.5 nm by using UV visible spectrophotometer.

Rheological studies
The rheological properties of all prepared formulations were measured using a Brookfield viscometer using spindle no.21. The specified volume of prepared *in situ* gel was transferred in sample cell which was placed carefully within the adaptor The viscosity of the sample solutions was measured at different speeds at a temperature of 25 ± 1°C. A typical run involved changing the speed from 10 to 100 rpm.

***In vitro* drug release studies [31]**
In-vitro drug release study was performed by static dissolution method since the *in situ* gel should be immobile in the periodontal pocket. The pH of gingival fluid lies between 6.8 – 7.4, phosphate buffer pH 7.2 was used as simulated gingival fluid as a dissolution medium. Five ml of simulated gingival fluid placed in test tube and maintained at 37°± 1°C. Then one ml of the prepared formulation was placed in test tube maintained at 37° ± 1°C. At pre-determined time interval one ml of the sample was taken and analyzed spectrophotometrically at 261.5 nm.The dissolution medium was replaced with fresh medium after sampling.

***In vitro* Antibacterial studies [32-33]**
Test
Pure drug cephalexin in pH 7.2 buffer (+ve control), blank *in situ* gel formulation without drug (control) and optimized *in situ* gel (F3) formulation

Microorganisms used
- *Staphylococcus aureus*
- *Escherichia coli*
- *Porphyromonas gingivalis*

Determination of MIC and Zone of Inhibition.
Microbiological studies against *Staphylococcus aureus and Escherichia coli*
Nutrient agar medium was prepared and sterilized by autoclaving under aseptic condition and transfer the medium to sterile petri plates. After solidification of nutrient agar medium, lawn was made with 0.1 ml microorganism i.e. *S. aureus* and *E. coli* in separate petri plates. Cups were made on the solidified agar layer with the help of sterile borer of 6 mm diameter. Appropriate amount of drug solution was poured into the cups and incubated for 48 hours at 37°C. Finally zone of inhibition was measured.

Microbiological studies against *Porphyromonas gingivalis*
The procedure follows agar diffusion assay method. The samples were tested at different concentration. The medium was then poured in to sterile petridishes and allowed to set. Each sample with various concentrations (10 µl, 20 µl and 50µl) was added in to the well, prepared in the appropriate medium. The microbial culture was already spread plated onto blood agar. It was kept for incubation at anaerobic condition (48 hrs) at 37°C. After incubation, plates were examined for antibacterial Activity. The zone of inhibition of microbial growth around the well was measured in mm and also the MIC was calculated from the plates.

Stability studies [34]
The optimized *in situ* gel formulation (F3) were kept for stability studies for 45 days at room temperature (30 ± 2°c) and refrigerator temperature (4 ± 2°c) to determine physical and chemical stabilities. The formulation was evaluated visually and for drug content release after 7, 15, 30 and 45 days.

RESULTS AND DISCUSSION

PREFORMULATION STUDIES

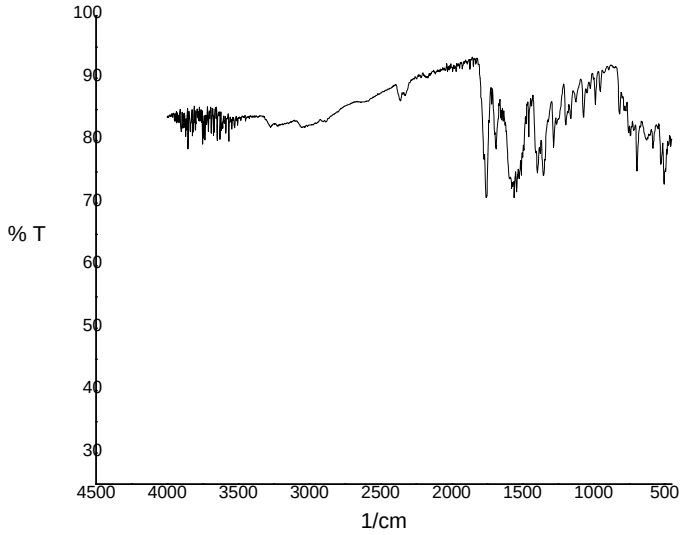
To confirm the identity, purity and suitability of drug for formulation and to establish a suitable drug profile, preformulation studies were undertaken.

Identification of drug

FTIR of drug

The FTIR of the drug was given in Figure 2, it was found in accordance with the reference standard.

Figure 2: FTIR of cephalexin



The FTIR spectra of cephalexin, showed all principle peaks of reference standard cephalexin at 3500 – 3000 cm^{-1} (series of broad bands, OH from H_2O and amide NH stretch), 2600 cm^{-1} (broad, NH_3), 1760 cm^{-1} (ß-lactam carbonyl stretch), 1690 cm^{-1} (Amide carbonyl stretch), 1600 cm^{-1} (very broad carboxylate stretch), 820-860 cm^{-1} (mainly skeleton vibrations including out-of-plane aromatic hydrogen bending).

Solubility of the drug

The solubility of the pure drug in various solvents were tabulated in Table 6**.** The result showed that the pure drug is completely soluble in distilled water, pH 7.2 and 0.1 N HCl.

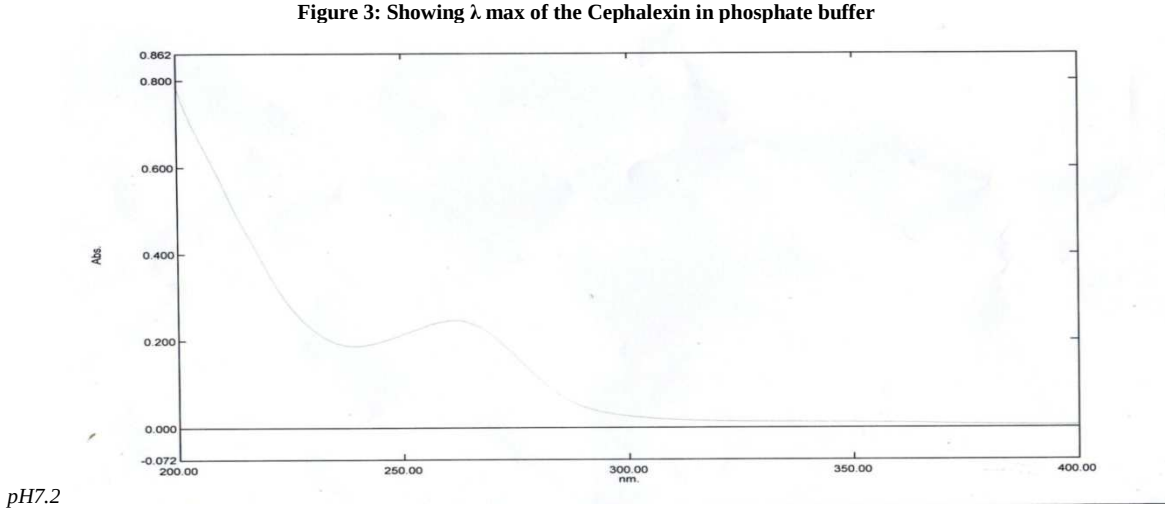
Table 6: Solubility comparison studies of pure drug in various solvents with reference

SL NO:	Solvents	Reference	Observation
1	Distilled water	+	+
2	Methanol	-	-
3	Chloroform	-	-
4	Phosphate buffer pH 7.2	+	+
5	0.1N HCl	+	+

Determination of λ max

The λ max of the drug was found to be 261.5 nm Figure 3 at an absorbance of 0.248, it was in accordance with the official standard.

Figure 3: Showing λ max of the Cephalexin in phosphate buffer



pH7.2

Table 7: Table showing the absorption maxima of cephalexin in Phosphate buffer 7.2

SL. NO:	Wavelength (nm)	Absorbance
1.	403.50	0.033
2.	**261.5**	**0.248**
3.	398.50	0.006
4.	239.50	0.189

Melting point

The melting point of the obtained drug sample was found to be 323-327°c by using open capillary method, it was found in accordance with the reference standard.

Analytical methods

Calibration curve of Cephalexin in distilled water

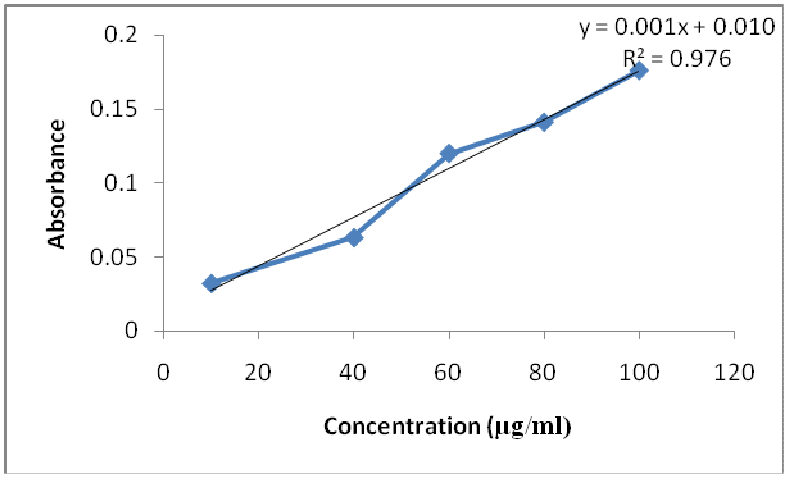
Table 8 shows the absorption reading of standard cephalexin in distilled water and Figure 4 shows the standard calibration curve of cephalexin in distilled water.

Table 8: Table showing calibration curve of cephalexin in distilled water

SL NO:	Dilutions (ml)	Absorbance
1	0.2	0.032
2	0.4	0.063
3	0.6	0.120
4	0.8	0.141
5	1	0.176

(Presented values; mean ±SD, n = 3)

Figure 4: Calibration curve of cephalexin in distilled water



Calibration curve of cephalexin in phosphate buffer pH 7.2

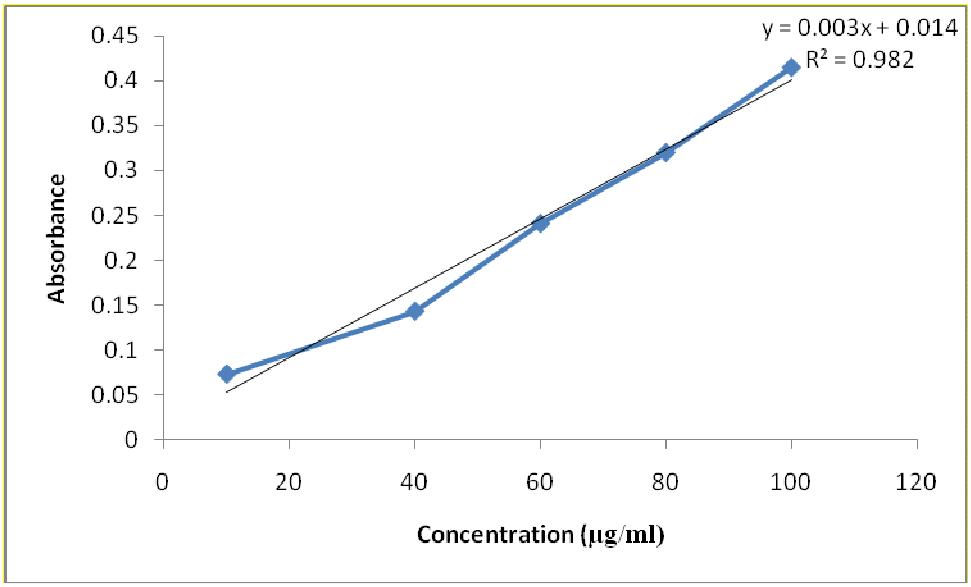
Table 9 shows the absorption reading of standard cephalexin in phosphate buffer pH 7.2 and Figure 5 shows the standard calibration curve of cephalexin in phosphate buffer pH 7.2.

Table 9: Table showing calibration curve of cephalexin in phosphate buffer pH 7.2

SL NO:	Dilutions (ml)	Absorbance
1	0.2	0.073
2	0.4	0.143
3	0.6	0.241
4	0.8	0.320
5	1	0.415

(Presented values; mean ±SD, n = 3)

Figure 5: Calibration curve of cephalexin in phosphate buffer of pH 7.2



(Each values; mean ±SD, n = 3)

Partition Coefficient

The partition coefficient of the drug cephalexin was found to be 5.65 indicating that the drug is highly soluble in water.

FORMULATION

Formulation of Cephalexin loaded *in situ* gel.

In the present investigation, an attempt was made to develop and evaluate *in situ* gel formulation of cephalexin having controlled release characteristics for direct placement into the periodontal pocket. In this study, the thermo gelling polymer, poloxamer 407 and a mucoadhesive polymer carbopol 934 has been used for formulation of *in situ* gel of cephalexin by cold method (Figure 6). This formulation can be directly injected in to periodontal pocket where it will immediately convert in to gel form at periodontal temperature and pH. In general, the gelation temperatures have been considered to be suitable for preparing *insitu* periodontal gel if they are in the range of 35°c to 37°c. If the gelation temperature of a thermo gelling formulation is lower than 25°c, a gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administering. If the gelation temperature is higher than 37°c, a liquid dosage form still exists at the body temperature, resulting in the loss of the administered drugs at an early stage. As the temperature of the periodontal cavity is 36- 37°c, this study aimed at preparing the liquid formulations of poloxamer 407 and carbopol 934 that may gel below 36°c.

Figure 6: Figure showing the sol to gel transition of *in situ* gel formulation

Evaluation of cephalexin *in situ* gel

Physicochemical properties

The physicochemical properties such as surface pH, spreadability, gelation temperature, drug content of prepared *in situ* gel were determined and depicted in Table10.

Surface pH

The value represent the mean of three replicates were well within the range of neutral pH. This indicates that formulation can be used and will not cause any irritation in the periodontal cavity.

Spreadability

The formulated gel shows good spreadability indicates uniform spreading of drug when applied.

Gelation temperature

At gelation temperature, liquid phase makes transition into gel. Thermo gelling polymer-based liquid formulations that provide *in situ* gelling property in periodontal cavity were designed to delay clearance of the formulations from the oral cavity. In these studies, a minimum concentration of polymers is required to become gel having temperature below 36°c. In general, the gelation temperatures have been considered to be suitable if they are in the range of 35°c to 37°c.

Syringeability

The syringeability of each formulation is represented. As the concentration of polymer increases, the viscosity of formulations were increased and increase the force required to expel each formulation from the syringe equipped with 21 gauge needle. Formulation F6, F7 and F8 fail the syringeability test because they contain higher amount of polymer

Drug content

Drug content uniformity in the drug delivery system is an important aspect that determines the performance of the system in vivo conditions. If the drug is not disturbed uniformly throughout the formulation, it could either lead to availability of sub therapeutic dose or toxic dose. Drug content uniformity was also performed to ensure minimum batch to batch variations. The formulations exhibit fairly uniform drug content. This is because of easy and single step preparation i.e. addition of drug to the polymer solution accounted for minimal or no drug loss.

5.4. Rheological studies

This is an important parameter for the *in situ* gels, to be evaluated. The rheological properties of the polymeric formulations were determined with Brookfield Viscometer. The viscosity of these formulations should be such that no difficulties are envisaged during their administration by the patient, especially during injectable preparation. The flow curve (viscosity against speed) of all prepared formulations indicates that, at the examined polymer concentration pseudoplastic systems were obtained. The prepared formulations tend to thin when being exposed to

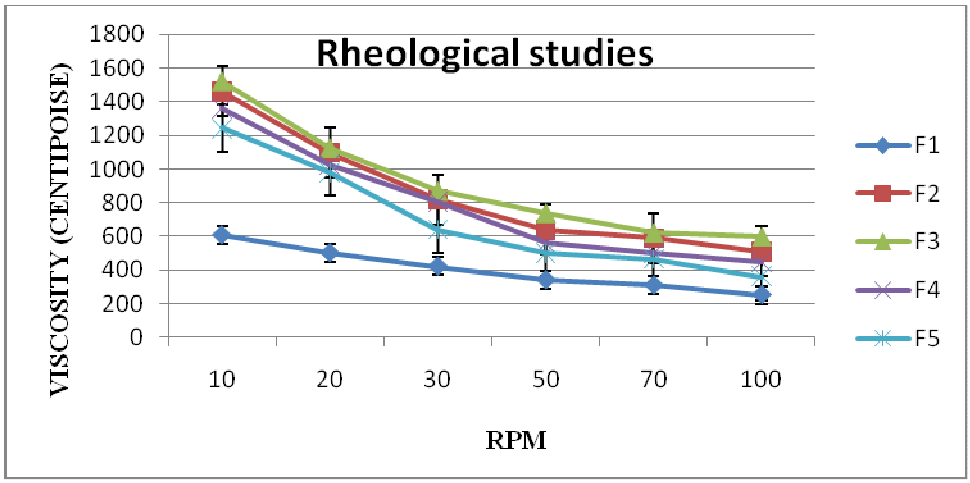
shearing force. Figure 7 compares the shear dependent viscosity of prepared formulations containing poloxamer 407 and carbapol 934.

Table 10: Summary of evaluated parameters for F1 to F7 *in situ* gel

Formulation code	Surface pH	Spreadability (g cm/sec)	Gelation temperature (°c)	Syringeability	Drug content (%)
F1	6.81± 0.37	15.24 ± 0.72	34.33 ± 1.52	Pass	89.24 ± 2.25
F2	7.13 ± 0.14	21.38 ± 1.19	35.67 ± 1.53	Pass	95.32 ± 1.16
F3	7.28 ± 0.157	25.15 ± 1.24	36.24 ± 1.73	Pass	98.416± 0.29
F4	6.84 ± 0.091	29.76 ± 1.36	35.6 ± 2.08	Pass	92.44 ± 1.18
F5	6.24 ± 0.12	34.33 ±1.42	35.00 ± 1.08	Fail	90.65 ± 0.84
F6	5.89 ± 0.24	42.71 ± 1.57	34.5 ± 0.92	Fail	87.21 ± 1.12
F7	5.15 ± 0.38	56.19 ± 1.66	34.12 ± 0.72	Fail	86.87 ± 1.11

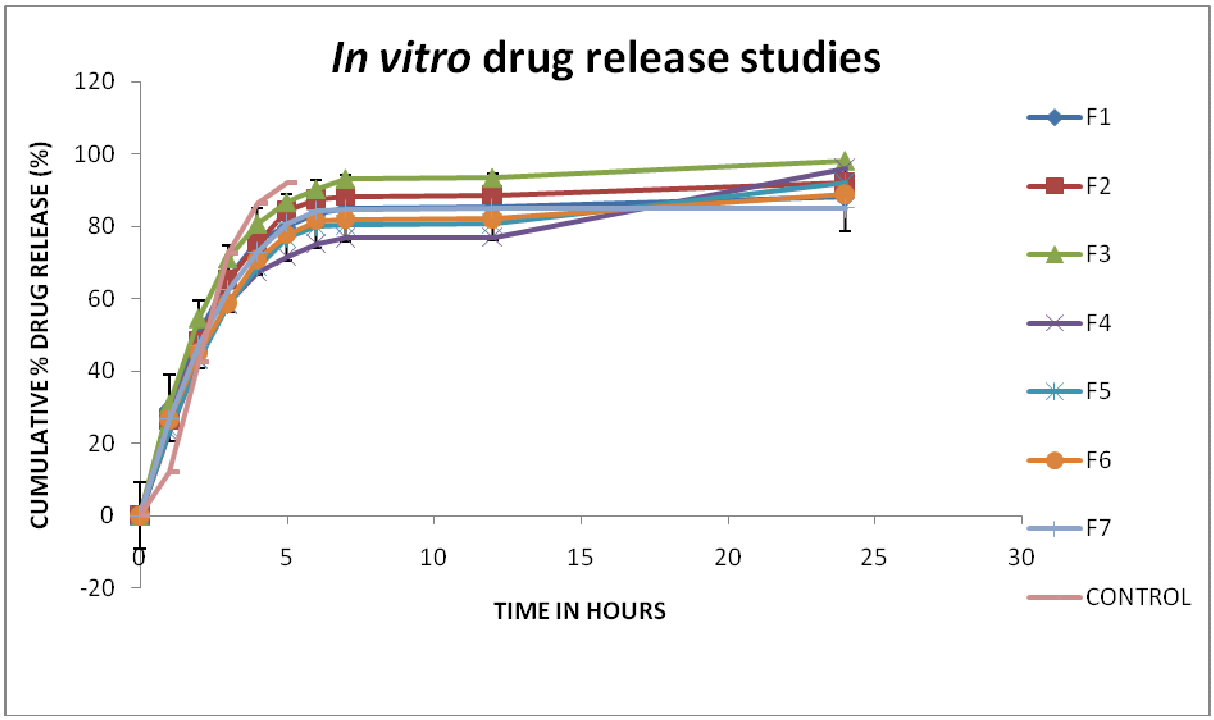
(Presented values; mean ±SD, n = 3)

Figure 7: Rheological properties of prepared formulations



(Each values; mean ±SD, n = 3)

Figure 8: *In vitro* drug release profile of F1 to F7 formulations



(Each values; mean ±SD, n = 3)

5.5. *In vitro* drug release studies

The release profile of a drug predicts how a delivery system might function and gives valuable insight into its *in vivo* behaviour. The release time profile of cephalexin from different concentration of poloxamer 407 and carbopol 934 are shown in Figure 8.The release rate was found to be increased with increasing polymer concentration upto a certain concentration of carbapol, then graduallydecreases . The pH of the gingival fluid lies between 6.8-7.4, phosphate buffer pH 7.2 was used as simulated gingival fluid for the dissolution studies. The *in vitro* drug release studies showed slow and controlled drug release for 24 hours. Since the formulation remains immobile in the periodontal pocket, a static dissolution model was adopted in this work. The release profile exhibited rapid initial release of the drug at the beginning (due to initial burst effect) because of elution of the drugs from the outer surface and cut edges of the matrix, which is beneficial to kill majority of periodontal pathogens. Once the burst effect was completed, slow and sustained release was seen up to 24 hours. The percentage cumulative drug release was greater in F3 formulations than other formulation and was comparable with positive control.

Table 11: Showing the antibacterial activity of optimized formulation of *Stapylococcus aureus, Escherichia coli and Porphyromonas gingivalis*

Plate no: with microbial strain	Formulation	Concentration (mg)	Zone of inhibition (mm)
Plate 1 *Staphylococcus aureus*	a) Optimized *in situ* (F3) formulation	0.1	20
		0.15	30
		0.2	45
	b) Pure drug in pH 7.2 (+ve control)	0.15	20
	c) Control	0.2	0
Plate 2 *Escherichia coli*	a) Optimized *in situ* (F3) formulation	0.2	23
		0.4	35
		0.6	40
	b) Pure drug in pH 7.2 (+ve control	0.4	31
	c) Control	0.6	0
Plate 3 *Porphyromonas gingivalis*	a) Optimized *in situ* (F3) formulation	0.25	15
		0.5	18
		0.75	21
	b) Pure drug in pH 7.2 (+ve control)	0.5	17
	c) Control	0.75	0

(Each values; mean ±SD, n = 3)

Figure 9: Photograph of *in vitro* antibacterial studies using *Staphylococcus aureus*

5.6. *In vitro* Antibacterial studies

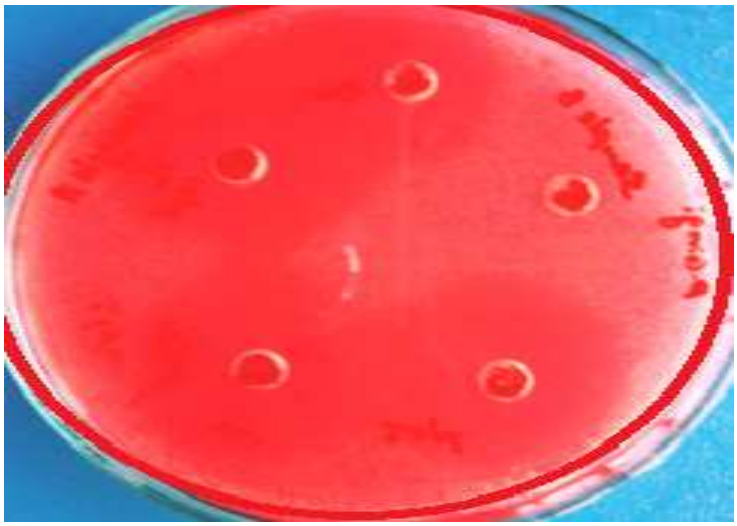
5.6.1. Determination of microbial growth inhibitory properties by Zone of inhibition.

The *in vitro* antibacterial activity of optimized *in situ* gel (F3) formulation against gram positive *Staphylococcus aureus*, gram negative *Escherichia coli* and anaerobic *Porphyromonas gingivalis* was compared with pure drug cephalexin in pH 7.2 buffer (positive control) and blank *in situ* gel formulation without drug (control) The antibacterial activity was carried out at four different concentrations (10 µl , 20 µl and 50 µl). The antibacterial activity of optimized formulation was tabulated in the Table 11. The photographs were shown in Figure 9 to 11. For Staphylococcus aureus, the minimum inhibitory concentration was found to be 0.1mg/ml. For E.coli the minimum inhibitory concentration was found to be 0.2 mg/ml and for Porphyromonas gingivalis the MIC value obtained at

0.25mg/ml. The optimized *in situ* gel (F3) confirmed good anti bacterial activity against *Stapylococcus aureus, Escherichia coli* and *Porphyromonas gingivalis.*

Figure 10: Photograph of *in vitro* antibacterial studies using *Escherichia coli*

Figure 11: Photograph of *in vitro* antibacterial studies using *Porphyromonas gingivalis*



5.7. Stability studies

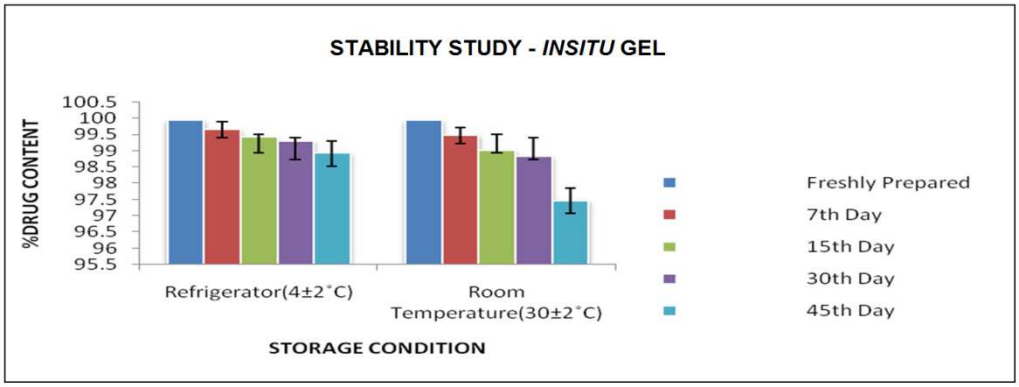
The stability studies of the optimized *in situ* gel formulation (F3) at refrigerator temperature (4 ± 2°c), room temperatures (30 ± 2°c) were carried out for 45 days and drug content was estimated at an interval of one week. The physical appearance showed that it does not show any changes when compared to the freshly prepared formulations at refrigerator and room temperature. The drug content were evaluated on 7^{th} , 15^{th} , 30^{th} , 45^{th} day is represented in the Table 12 and showed that there are no significant changes in the drug content during the storage for 45 days in refrigerator conditions. From the stability studies (Figure 12) it was confirmed that the optimized *in situ* gel formulations of cephalexin remained stable at room temperature (30 ± 2°c) and at refrigerator temperature (4 ± 2°c).

Table 12: Stability study of Optimized *In situ* gel (F3)

Temperature	Drug content (%)				
	Freshly prepared	7^{th} Day	15^{th} Day	30^{th} Day	45^{th} Day
Refrigerator (4 ± 2°c)	99.62	99.50	99.28	99.00	98.50
Room temperature (30 ± 2°c)	99.48	99.34	99.00	98.56	97.34

(Presented values; mean ±SD, n = 3)

Figure 12 : Stability study of optimized *in situ* gel (F3)



(Each values; mean ± SD, n = 3)

CONCLUSION

Periodontitis is a chronic inflammatory condition of the periodontal tissues, which leads to apical migration of the junctional epithelium along the root surface and progressive destruction of the periodontal ligament and the alveolar bone. Eradicating microbes from the periodontal pocket is a crucial task in treating periodontitis. Hence local delivery of an anti-infective agent to infection sites with effective levels for a sufficient time while concurrently evoking minimal or no side effects is highly essential. Novel therapeutic agents are being explored in the arena of local drug delivery system to ensure maximum benefit. In this study, in *situ* periodontal gel of cephalexin was successfully formulated using poloxamer 407 (thermoreversible) and Carbopol 934 (pH sensitive) approaches. The developed formulations showed satisfactory results for pH, viscosity, drug content, in-vitro gelling capacity, rheology and other studies. From the present study it can be concluded that the developed formulation is having enough bioadhesive property, will remain in to the periodontal cavity for sufficient time and can release the drug at controlled rate for prolonged duration. Its syringeability allows easy insertion of gel formulation in to periodontal pocket. The results also indicated that these local drug delivery devices for the treatment of periodontal diseases showed significant advantages and effective/ prolonged local levels of an anti-microbial could be achieved without much systemic load with comparatively less frequency of administration. In conclusion, cephalexin loaded local site-specific *insitu* gel formulations directly into periodontal pocket proves to be a viable and effective alternative to conventional periodontal therapy. Obviously, its clinical use in the fast-growing field is promising.

REFERENCES

[1] Reddy RJ, Anjum M, Hussain MA. *Amer J Adv Deliv* **2013**; 1(3):300-312.
[2] Rams T, Slots J. *Periodont* **2000** 1996; 10:139-159.
[3] Greenstein G. *J Periodontol* **1993**; 64(1):1-15.
[4] Goodson JM, Haffajee A, Socransky SS. *J Clin Periodontol* **1979**; 6(2):83-92.
[5] Madhusudan RY, Vani G, Rameshachary BR. *Ind Drug* **1998**; 35(9):134-141.
[6] Armitage GC. *Ann Periodontol* **1999**; 4:1–6.
[7] Neha B, Laxmi G, Preeti K. *J Appl Pharm Res* **2014**; 9(2):1-17.
[8] Prakash S, Dhavel P, Moinuddin S, Jayant C. *J Sci Innov Res* **2013**; 2(3):607-626.
[9] Kevin G, Parth J, Malay S, Ramkishan A, Jaydeep P. *Int J Pharm Investig*. **2013**; 3(1):29-41.
[10] Tomas I, Rubido S, Donos N. *Curr Res Tech Adv* **2011**; 1(1):530-541.
[11] Swati R, Sandeep W, Swaroop L. *Curr Pharm Res* **2010**; 1(1):60-69.
[12] Vikesh S, Vasudha M, Vineet B, Masareddy RS, Manvi FV. *Der Pharm Lettre* **2010**; 2 (1):61-69
[13] Sudipta G, Alekha KD.. *Inter J Pharma* **2004**; 276(2):83-92.
[14] Kamal AH, Ashri LY, Alsarra IA. *AAPS Pharm Sci Tech* **2007**; 8(3):1-11.
[15] Saima A, Saeid R, Kanchan K. *Sci Res* **2009**; 3(11):1175-1183.
[16] Fariba G, Samira VF, Ebrahim VF. *Rew Iran Polym* J **2010**; 19(5):375-398.
[17] Griffith RS. *Post grand Med J* **1993**; 59(5):16-27.
[18] Ceulemans J, Ludwig A. *Eu J Pharm Biopharm* **2002**; 54:41-50.
[19] Gourav R, Arushi G. *Am J Pharm Tech Res* **2012**; 2:25-53.
[20] Obaidat AA, Hammad MM. *J Appl Polym Sci* **2010**; 116:333-336.
[21] Kumar P, Awasthi R, Kumar PR, Kumar M, Kumar MP. *Der Pharm Lettre*. **2010**; 2:28–39.
[22] Warve JR, Bakliwal SR, Rane BR, Pawar SP. *Int J App Bio Pharm Tech*. **2011**; 2(1):204-206.
[23] Deepak VB, Avinash SP, Vineetha VK, Vilasrao JK. *Int J Pharm Pharma Sci* **2010**; 2(3):64-72.

[24] Jones DS, Woolfson AD, Brown AF, O'Neill MJ. *J Control Rel* **1997**; 49:71–79.
[25] Shailesh TP, Charmi GP. *Asian J Biochem Pharm Res* **2011**; 2(1):507-524.
[26] Golomb G, Friedman M, Soskolne A, Stabholz A, Sela MN. *J Dent Res* **1984**; 63:1149–115.
[27] Varshosaz J, Tavakoli N, Saidan S. *Drug Deliv* **2002**; 9:127–33.
[28] Sang CS, Joon O, Seong JC. *Arch Pharmacol Res* **1996**; 19:1-5.
[29] Badgujar SD, Sontakke MA, Narute DR, Karmarkar RR, Tupkar SV, Barhate SD. *Int J Pharm Res Dev* **2010**; 2:1–8.
[30] Pisal S, Shelke V, Mahadik K, Kadam S. *AAPS Pharm Sci Tech* **2004**; 2: 51-9
[31] Majithiya RJ, Ghosh PK, Umrethia ML, Murthy SR, *AAPS Pharm Sci Tech* **2006**; 7(3):E1-E7.
[32] Islam MA, Alam MM, Choudhury ME, Kobayashi N, Ahmed MU. *Bangel J Vet Med* **2008**; 6: 121-126.
[33] Kamal RA, Rhadhika J. *Jundishapur J Micro* **2009**; 2(3):105-111.
[34] Mohammed GA, Harish NM, Narayana C, Prabhakar P. *Trop J Pharm Res* **2009**; 8:33-41.