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FORMULATION AND EVALUATION OF ALOE VERA BASED LEVOFLOXACIN GEL FOR THE TREATMENT OF PERIODANTAL DISEASES

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ABSTRACT

Background: Levofloxacin is a novel fourth generation fluoroquinolone with a broad spectrum of antibacterial activity against Gram-positive and Gram- negative anaerobic and aerobic periodontal pathogens. Dental uses of Aloe vera are multiple. It is extremely useful in the treatment of gum diseases like gingivitis, periodontitis. So in the present study, Objective: An attempt was be made to investigate the effect synergistic Aloe vera and Levofloxacin for the treatment of periodontal disease. **Methods:** The Aloe vera gels for levofloxacin with HPMC and Carbapol polymers were formulated and evaluated, Aloe vera Based levofloxacin gel were prepared at concentration of Carbapol polymers 200mg, 300mg, 400mg with HPMC polymers 400mg, 500mg, 600mg. Methyl parabin and Propyleparabin (0.002%) as preservative, sodium meta bisulphate acts as antioxidant and disinfectant, Triethonalamine acts as pH adjusting agent. All the prepared aloe vera gel formulations were evaluated for percentage yield, Drug content, Measurement of viscosity, pH determination, Syringeability. **Results:** FT-IR and DSC studies revealed that drug was found to be compatible with formulation excipients. All the formulations showed satisfactory results

for pH, rheology and other physical properties. *In-vitro* release study revealed that release of drug from Aloe-vera gel was concentration dependent; as concentration of polymer increased the drug release rate was retarded. Based on maximum desirability and effectiveness formulation containing 200mg of carbapol polymer and 600mg HPMC polymer was consider as an optimized formulation. **Conclusion:** The Aloe-vera based levofloxacin gel is a promising approach for the treatment of periodontitis. This gel combines the advantage of an anti-inflammatory and antimicrobial activity.

KEYWORDS: Levofloxacin, Aloe-vera gel, Carbopol, HPMC, periodontitis.

INTRODUCTION

Periodontitis i.e. “peri” around, “odont” tooth, “itis” inflammation, refers to a number of inflammatory diseases affecting the periodontium, the supporting tissues around the teeth. Periodontitis involves progressive bone loss around the teeth, leads to the loosing and subsequent loss of teeth, and is characterized by periodontal pocket formation.[1] It is caused by microorganisms that adhere on the tooth’s surfaces, along with an overly aggressive immune response against these microorganisms. Periodontitis is a multifactorial infection with great complexity in the mechanisms of pathogenesis.[2] Periodontal disease is one of the world’s most prevalent chronic diseases, which has been considered as a possible risk factor in other systemic diseases such as cardiovascular disease, including coronary heart disease and stroke[3,4] and pre-term low birth weight infants, which can be characterized by a localized inflammatory response due to infection of periodontal pocket arising from the accumulation of periodontal plaque. Numerous epidemiological studies show that infectious dental diseases, tooth decay and diseases of periodontium are among the most common afflictions of mankind.[5] The appearance of periodontal pockets is the first clinical manifestation of periodontal disease which offers a favourable medium for bacterial colonization. The deeper periodontal pockets can be diagnosed by clinical examination using periodontal probe in combination with plaque X-ray imaging and microbiological techniques for a precise analysis of the infectious agents.[6] Strategies aiming at suppression or elimination of specific periopathogens from the periodontal pocket, drugs are chosen for their treatment. Earlier most frequently prescribed drugs by the periodontists include tetracycline, doxycycline, metronidazole, penicillin, amoxicillin, chlorhexidine etc. However providing pharmacological therapy to the periodontal pocket, the factors to be considered is effective therapy, predictable clinical results, low incidence of adverse side effects or interactions,

decreased costs and patient acceptance.[7] The administration of systemic antibiotics may initially result in therapeutic drug levels at the gingival site, which decline to sub therapeutic levels over time. Hence to maintain the bactericidal drug concentrations, higher systemic drug concentrations may be required for which high serum drug concentrations due to poor bio-distribution of some systemic antibiotics to produce effective therapy, may lead to undesirable side effects. However, bacterial differences existing in etiological sub-forms of periodontitis renders the requirement of microbial testing before prescribing the adjunctive antibiotic and selecting the mode of delivery for the successful clinical management of periodontitis.[8] Data from prospective animal experiments and human studies support this concept, and indicate that NSAIDs can reduce gingival inflammation and reduce alveolar bone resorption. There is also evidence that systemic administration of antimicrobial and NSAIDs are effective in altering the progression of certain forms of periodontitis.[9] It is extremely helpful in the treatment of gum diseases like gingivitis, periodontitis. Bleeding, swelled gums are reduced and it also shows anti-inflammatory effect. It is a powerful antiseptic in pockets where normal cleaning is difficult. It is a powerful healing promoter and can be used following extractions. It has been used in root canal treatment as a sedative dressing and file lubrication during biomechanical preparation.[10] Levofloxacin is a fourth generation fluoroquinolone with a broad antibacterial activity against Gram-positive and Gramnegative bacteria. Moxifloxacin shows bactericidal, concentration dependent, antiinfective. It interferes with bacterial survival by binding to DNA gyrase (topoisomerase II) and topoisomerase IV, essential bacterial enzymes involved in the replication, translation, repair and recombination of deoxyribonucleic acid.[11] The main aim of the study was made to investigate the effect Aloe vera based levofloxacin gel for the treatment of periodontal diseases.

MATERIALS AND METHODS

The materials used in the formulation included Levofloxacin, procured from Yarrow Chem Products, Mumbai. The excipients used were Carbopol 940, HPMC, Triethanolamine, Methylparaben, Propylparaben, Sodium hydroxide (NaOH), and Potassium dihydrogen orthophosphate, all of which were obtained from S.D. Fine-Chem Ltd. These materials were selected based on their suitability for the formulation and their analytical grade quality. The instruments employed for the study included an Electronic Weighing Balance from Acculab and a Brookfield Digital Viscometer (Model D-220 LV) for viscosity determination. A UV–Visible Spectrophotometer (Shimadzu UV-Vis 1700) was used for spectrophotometric

analysis, while pH measurements were carried out using a Digital pH Meter from Hanna Instruments, Italy. The Digital Melting Point Apparatus was sourced from Analab Scientific Pvt. Ltd., and FTIR Spectrophotometric analysis was performed using the Shimadzu FTIR (Model 8400S), Japan. Additionally, a Magnetic Stirrer from Remi Instruments Ltd. was utilized for sample preparation and mixing.

Determination of melting point[12]

Melting point of Levofloxacin was determined by both capillary method and with digital melting point apparatus. In this method the capillary was sealed with gentle heating from one end. Then the small quantity of pure drug Levofloxacin was filled into the sealed capillary. Capillary was inserted into to the tube which was dipped in mineral oil phase. Gently the oil bath was heated, as soon as the powder had started melting the heating was stopped and the temperature was noted down.

Drug Excipients Compatibility Studies

A proper design and formulation of a dosage form requires consideration of the physical, chemical and biological characteristics of both drug and excipients used in fabrication of the product. Hence, before producing the actual formulation, compatibility of Levofloxacin with different polymers and other excipients was tested using FT-IR and DSC studies.

Fourier Transform Infra-Red Spectroscopy[13]

In the preparation of liquid formulation, drug and polymer may interact as they are in close contact with each other, which could lead to the instability of drug. Preformulation studies regarding the drug-polymer interaction are therefore very critical in selecting appropriate polymers. FT-IR spectroscopy was employed to ascertain the compatibility between Levofloxacin and the selected polymers. The pure drug and drug with excipient were scanned separately. Potassium bromide was mixed with drug and/or polymer in 9:1 ratio and the spectra were taken. FT-IR spectrum of Levofloxacin was compared with FT-IR spectra of Levofloxacin with polymer. Disappearance of Levofloxacin peaks or shifting of peak in any of the spectra was studied.

Differential Scanning Calorimetry (DSC) Analysis[14,15]

DSC (Perkin-Elmer thermal analysis) studies were carried out in order to characterize the physical state of drugs. Sample of pure drug and physical mixture were placed in the aluminium pans and thermatically sealed. The heating rate was 10^{o}C per min using nitrogen

as pure gas. The DSC instrument was calibrated for temperature using indium. In addition, the enthalpy calibration indium was sealed in aluminium pan with sealed empty pans as reference.

Determination of analytical wavelength of levofloxacin

From the stock solution 2ml was pipetted out into 100 ml volumetric flask. The volume was then made up to 100 ml with phosphate buffer pH 6.6. The resulting solution containing 20µg/ml was scanned between 200-400 nm. The wavelength of drug as λ max was selected.

Calibration curve of levofloxacin in phosphate buffer pH 6.6

From the standard stock solution, a series of dilutions were prepared using phosphate buffer pH 6.6 to get concentration of 2-10µg/ml as given in the table no.7.The absorbance of these solutions was measured using UV-spectrophotometer. Standard curve was obtained by plotting absorbance vs. drug concentration.

Preparation of gel from aloe vera juice

The central parenchymatous pulp was removed from Aloe leaves with the help of spatula and the pulp was washed repeatedly with water and finally treated with 0.1 N sodium hydroxide to avoid acidity in preparation. Juice was obtained by putting treated pulp in a blender. The juice obtained was filtered under vacuum. Different concentrations of HPMC/Carbopol 940 were added to the clear liquid obtained and dispersed uniformly without any lumps respectively. Levofloxacin, methyl paraben and propyl praben were dissolved in small volume of ethanol and added to the above mixture. A solution of triethanolamine was added dropwise until a gel was formed. The prepared Levofloxacin Aloe vera gel was weighed and stored in air tight containers in dark room to prevent photo-oxidation.

Table No. 1: Composition of Aloe vera Based Levofloxacin Gel.

Ingredients (mg)	Formulations					
	F1	F2	F3	F4	F5	F6
Levofloxacin	100	100	100	100	100	100
HPMC K4M	400	500	600	-	-	-
Carbopol-940	-	-	-	200	300	400
Sodium meta bisulphate	0.02	0.02	0.02	0.02	0.02	0.02
Methyl paraben	0.002	0.002	0.002	0.002	0.002	0.002
Propyl paraben	0.002	0.002	0.002	0.002	0.002	0.002
Aloe Vera extract (ml)	7.5	7.5	7.5	7.5	7.5	7.5
Triethanolamine	Qs	Qs	Qs	Qs	Qs	Qs

Physical appearance and pH[16]

Prepared *Aloe vera* based Levofloxacin gels were checked for their clarity and the pH was measured using a calibrated digital pH meter at 37°C. All measurements were made in triplicate and the results are given in table no. 8.

Viscosity measurement of Aloe vera based Levofloxacin gels[17]

The viscosity of Aloe vera based Levofloxacin gel were determined by using Brookfield digital viscometer (Model no LVDV 2P230) using 10g of gel. Measurements were performed using suitable spindle and the temperature was maintained at 37±1°C. The viscosity was read directly from the viscometer display. All measurements were made in triplicate and the results are given in table no. 8.

Determination of drug content[18]

1gm of *Aloe vera* based Levofloxacin gels was dissolved in 5ml of 6.6 ph buffer solution and volume was made up to 100ml phosphate buffer pH 6.6. After suitable dilution the absorbance was measured by UV spectrophotometer (Shimadzu) at 285 nm.

Syringeability[19]

All prepared formulations were transferred into a 5 ml syringe placed with 20 gauge needle to a constant volume (2 ml). The solutions, which easily passed from syringe were termed as pass and difficult to passed were termed as failed.

***In-vitro* drug release[20, 21]**

The dialysis technique using cellophane membrane was used to study *in-vitro* release. Prior to diffusion studies; the dialysis membrane was soaked overnight in pH 6.6phosphate buffer solution. 1gm of gel was placed on dialysis membrane which was sealed on one side and one side opened. The dialysis tube was placed in a glass beaker containing 50 ml of pH 6.6phosphate buffer solution. The release studies were performed at 37±0.5°C and stirred at 50 rpm using magnetic stirrer. The 5 ml of sample was pipetted out at different time interval and was replaced with same volume of pH 6.6phosphate buffer to maintain the sink condition. The samples were filtered through whatman filter paper (No.41) and solutions were analyzed using UV Spectrophotometer after suitable dilution. Cumulative percentage drug release was determined by using standard graph.

RESULT AND DISCUSSION

Melting point

The melting point of Levofloxacin was determined by capillary method using digital melting point apparatus (in triplicate) and found to be in the range of **225-227°C.** Thus obtained melting point is in agreement with literature melting point which confirms the purity of drug.

Compatibility study

FT-IR studies

The pre formulation study was carried out to study the compatibility of the pure drug Levofloxacin with the polymers Carbopol, and HPMC. The individual spectra of the pure drug and in combination with excipients are shown in Fig. 1 and 2. In IR spectra of Levofloxacin major peaks of functional groups were found at various wavelengths. In the physical mixture, the principle peaks were approximately matched with the pure drug principle peaks. Hence, it can be concluded that there were no possible interactions between the drug and excipients.

Table No. 2: Major peaks of Levofloxacin and physical mixture of IR spectra.

INGREDIENTS	C-H Stretching	COOH Stretching	C=C Stretching	C-X stretching	C-H_2 Bending
Levofloxacin	2937.83	2359.75	1604.51	1030.81	1467.82
Physical mixtures of Levofloxacin + HPMC K4M	**1701.56**	**3531.49**	**1621.69**	**2691.41**	**1062.19**
Physical mixtures of Levofloxacin + Carbopol	**1710.12**	**-**	**1621.69**	**2759.84**	**1009.89**

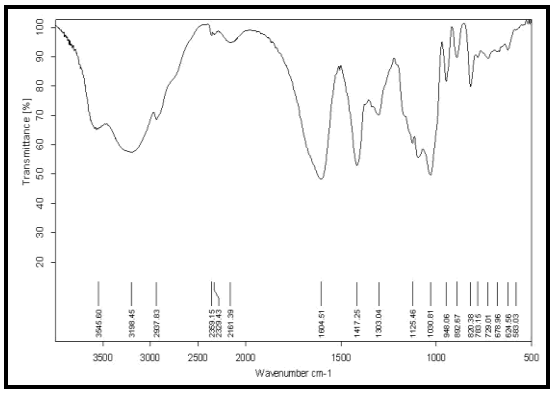

Fig. 1: FTIR Spectra of Pure Drug Levofloxacin.

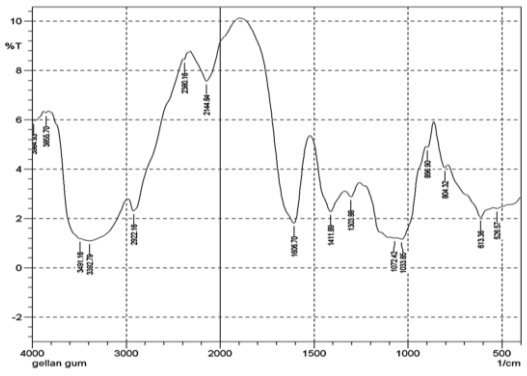

Fig. 2: FTIR Spectra of Polymer Carbopol.

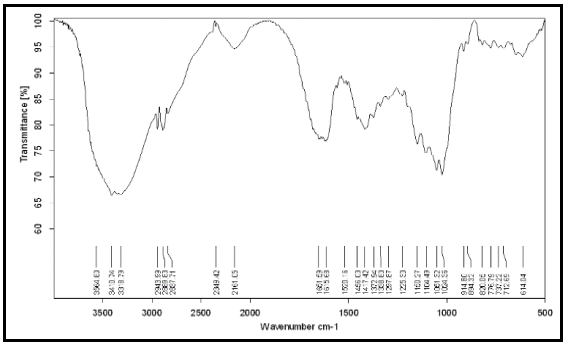

Fig. 3: FTIR Spectra of Polymer HPMC.

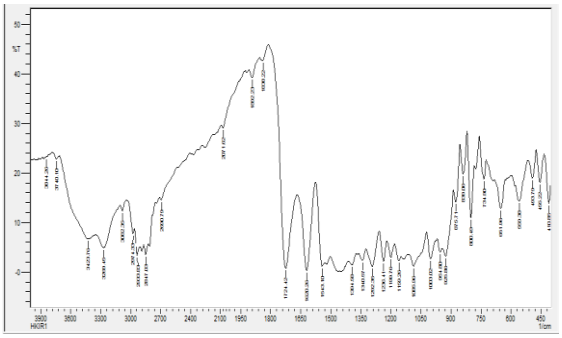

Fig. 4: FTIR spectra of Physical Mixer of Drug & HPMC Polymer.

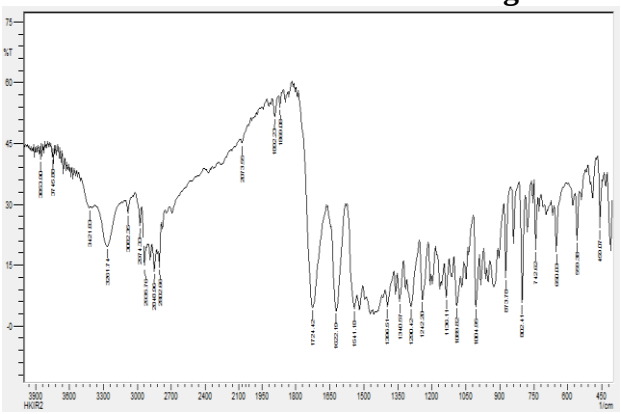

Fig. 5: FTIR spectra of Physical Mixer of Drug & Carbopol Polymer.

Differential scanning calorimetry (DSC)

Any possible drug polymer interaction can be studied by thermal analysis. The DSC patterns of pure Levofloxacin and its physical mixture are shown in Fig. 11,12 and 13 Pure Levofloxacin showed a sharp endothermic peak at **230.79°C** corresponding to its melting point. There was negligible change in the melting endotherms of the physical mixture of drug with HPMC **(242.87°C)** and Carbopol **(241.04^{0}C),** compared to pure drug. This may be due to engulfing of drug in the polymer. This observation further supports the IR spectroscopy results, which indicated the absence of any interactions between the drug and additives used in the preparation.

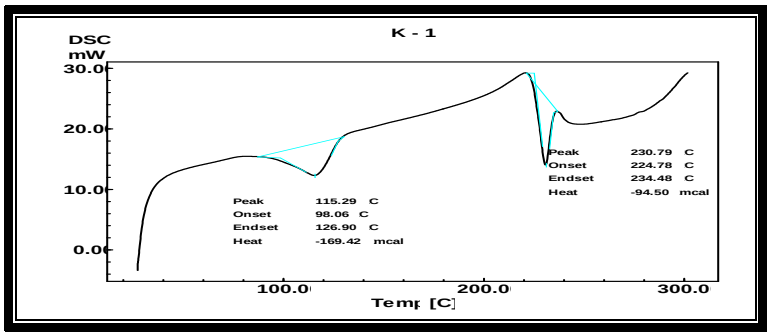

Fig. 6: DSC of Pure drug Levofloxacin.

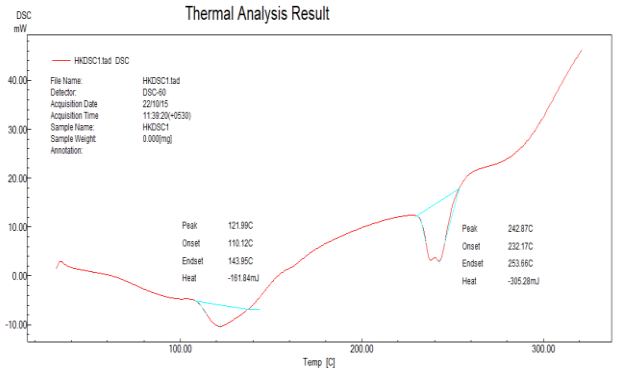

Fig. 7: DSC of Physical Mixer of Drug and HPMC polymer.

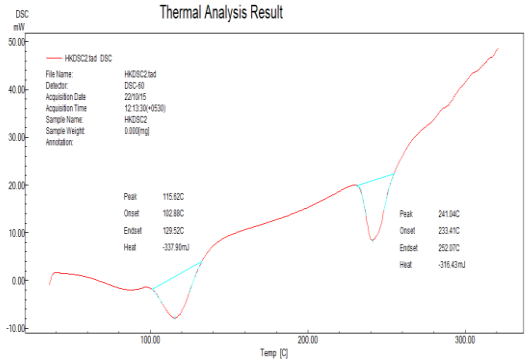

Fig. 8: DSC of Physical Mixer of Drug and Carbopol polymer.

Determination of analytical wavelength (λ max)

The absorption maximum of the standard solution of Levofloxacin was scanned between 200-400 nm regions on UV-visible spectrophotometer (Shimadzu UV-Vis 1800). The wavelength of maximum absorbance (λ max) was found to be 285 nm in pH 6.6phosphate buffer (Fig no. 14).

Fig. 9: UV absorption spectrum of Levofloxacin.

Standard Calibration of Levofloxacin in pH 6.6

The calibration curve of drug was obtained in pH 6.6phosphate buffer. First, Beer-Lambert's range was determined by preparing series of solutions of various concentrations and it was found that Beer's law was obeyed within the concentration range of 2-10 µg/ml. The data obtained (shown in table no. 11) was statically evaluated to calculate the standard deviation of the said values and correlation coefficient (R^2), it was found to be 0.999 respectively.

Table No. 3: Standard Calibration data of Levofloxacin in pH 6.6phosphate buffer.

Sr. No.	Concentration (µg/ml)	Absorbance AM±SD
1.	0	0
2.	2	0.155± 0.016
3.	4	0.294 ± 0.017
4.	6	0.438 ± 0.025
5.	8	0.576 ± 0.029
6.	10	0.726 ± 0.027

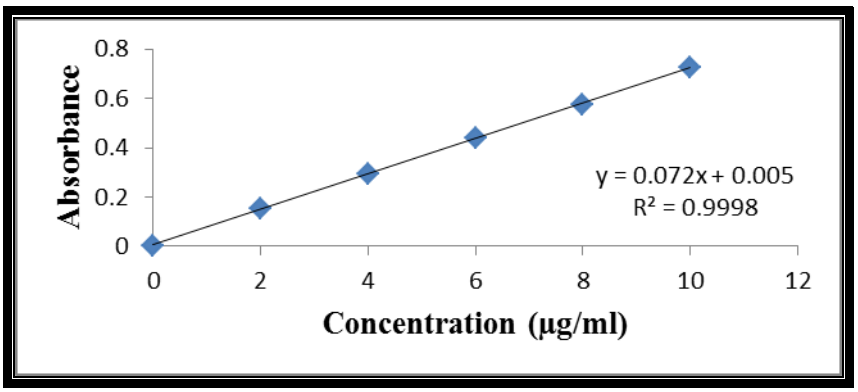

Fig. 10: Standard Calibration curve of Levofloxacin in pH 6.6 Phosphate buffer.

EVALUATION OF ALOE VERA BASED LEVOFLOXACIN GEL

In the present investigation, an attempt was made to develop and evaluate *Aloe vera* based Levofloxacin gel for controlled release for direct placement into the periodontal pocket.

Appearance

All the formulation developed has a light yellow colour with clear appearance without any lumps in the gel when stored at room temperature.

Table No. 4: Evaluation Parameters of Levofloxacin Gel.

Formulation code	Surface pH	Viscosity (Cp)	Drug content (%)	Syringeability (Cm)
F1	6.23	89	72.67 ± 1.55	Pass
F2	6.44	98	89.15 ± 1.11	Pass
F3	6.57	106	93.75 ± 1.11	Fail
F4	6.66	92	86.48 ± 0.94	Pass
F5	6.21	102	94.95± 1.05	Pass
F6	6.43	112	95.62± 1.05	Fail

Diffusion Study

Table No. 5: *In-vitro* drug release profile of Aloe vera based Levofloxacin gel (F1 to F6).

TIME	FORMULATION CODE (Cumulative release %)					
	F1	F2	F3	F4	F5	F6
0	0.000±0.00	0.000±0.00	0.000±0.00	0.000±0.00	0.000±0.00	0.000±0.00
10	13.2±2.34	11.08±0.57	10.87±0.36	7.36±2.37	4.94±0.90	4.14±1.40
20	20.8±3.199	16.65±0.22	14.23±0.42	9.66±3.78	7.46±1.55	6.32±2.16
30	29.2±4.08	21.94±0.68	19.29±0.82	11.98±4.13	9.34±3.84	8.84±2.11
45	35.6±4.67	33.77±1.11	27.21±0.64	13.9±5.31	11.54±3.553	11.92±0.73
60	40.4±5.39	38.17±1.04	35.78±1.90	18.54±4.48	17.86±6.55	16.06±1.71
90	55.6±7.56	46.97±1.63	43.46±2.45	23.52±9.55	21.2±5.89	20.92±6.50
120	62.16±8.67	58.70±1.27	51.52±3.86	37.02±7.41	32.4±4.91	30.86±3.30
150	72.8±9.23	68.87±2.44	62.08±3.31	44.8±7.81	40.4±5.85	35.28±2.43
180	80±6.33	76.86±5.63	73.08±2.87	55.8±6.86	49.14±3.84	42.68±3.93
210	86±2.19	81.49±5.28	76.36±1.59	67.00±2.50	60.2±3.95	56.9±5.45
240	89.2±1.43	85.68±4.13	80.74±2.16	72.2±1.67	68.06±2.88	62.36±1.02
270	93.12±1.75	90.88±1.31	88.45±1.48	83.6±2.62	78.04±2.71	73.38±1.52
300	95.78±1.95	94.32±1.513	92.44±1.68	89.2±2.12	84.32±2.91	80.32±1.82
330	97.42±1.22	95.02±1.44	94.78±0.88	94.2±0.88	90.12±1.81	86.38±1.54
360	98.78±1.66	96.32±1.564	94.44±1.48	96.23±1.72	93.4±2.14	92.6±1.22

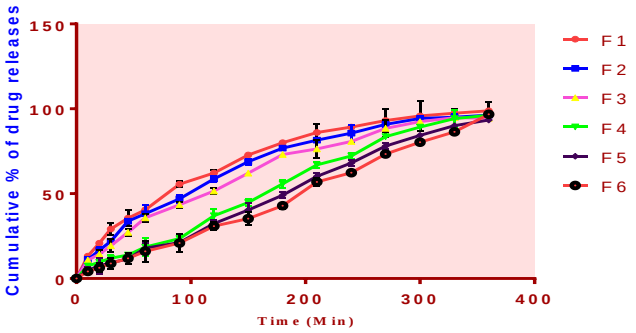

Fig. 11: *In-Vitro* diffusion profile of Formulation F1 to F6.

CONCLUSION

The aim of the present research work was to develop and Aloe vera based levofloxacin gel for the treatment of periodontal diseases. The method employed for the preparation of gel was simple and reproducible. The concentration of HPMC and Carbopol chosen for the formulations found to have good gelling capacity. IR and DSC spectra revealed that there were no possible interactions between the drug and polymer used in the formulation. All the formulations were found to have desired amount of drug content, indicating that the method adopted for making of the formulation was suitable. The pH of the formulations varied from 6.2 –7.2 which is considered safe for oral delivery. Increase in the polymer concentration increases the viscosity and thereby increases strength of the formed gel. The *in-vitro* release

followed a controlled release pattern. The results showed that the amount of drug released was decreased with increasing polymer concentration, and the trend continued for the entire duration of the study. Based on visual observation and drug content the optimized *Aloe vera* based Levofloxacin gel (F5) was stable for a period of 3 months.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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