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Research Article

PREPARATION AND EVALUATION OF IN-SITU ORAL TOPICAL GEL OF LEVOFLOXACIN BY USING COMBINATION OF POLYMERS

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ABSTRACT

Gel dosage forms are successfully used as drug delivery systems to control drug release and protect the medicaments from a hostile environment. The main objective is to formulate and evaluate in situ oral topical gels of levofloxacin. The system utilizes polymers that exhibit sol-to-gel phase transition due to change in specific physico-chemical parameters. Conventional oral formulations like solution, suspension, and ointments have many disadvantages which result into poor bioavailability of drug. The poor bioavailability and therapeutic response may be overcome by the use of mucoadhesive *in situ* gel forming systems that are applied as liquid and undergo a sol-gel transition which have good mucoadhesion. In-situ gel were prepared by using carbopol 934P and using sodium carboxy methyl cellulose along with hydroxylpropylmethylcelluose was used to prolong the release of levofloxacin. Formulations were evaluated for gelling capacity, viscosity, gel strength, bioadhesive force, spreadability, microbiological studies and *in vitro* release. Levofloxacin from the mucoadhesive system in simulated salivary fluid was influenced significantly by the properties and concentration of carbapol 934 and sodium CMC showed to enhance bioavailability through its longer oral residence time and ability to sustain the release of the drug.

KEY WORDS: Mucoadhesive, Levofloxacin, sodium CMC, Carbapol, hydroxypropylmethylcellulose, insitu gel, ion sensitive, Oral drug delivery, prolonged release.

INTRODUCTION

Periodontal diseases are groups of infections and inflammatory conditions, including gingivitis and periodontitis that affect teeth supporting structures (Luana et al.2004). Gingivitis can and does occur in all groups, ethnicities, races, genders and socioeconomic levels (Michael et al. 1988). These diseases occur when bacteria from dental plaque invade surrounding tissues and from the accumulation of plaque at the gingival margin, which, in turn, induces an inflammatory response. The result is the formation of pockets between gingiva and tooth that causes gingival margin retraction and the development of an ideal environment for anaerobic bacteria growth responsible for the disease. The progression of this destructive process can cause tooth loss (Sreeja et al. 2012). According to estimates by Government of India-World Health Organization collaborative programme, about 50% of school children are suffering from Dental caries and

more than 90% of adults are having periodontal diseases (Morrison et al. 1999). Levofloxacin is L-isomer of fluoroquinolon antibiotic ofloxacin and it is found to be two fold more potent than ofloxacin in the treatment of periodontal diseases was chosen for present study. In the form of conventional dosage form such as tablets, parentrals and capsules Levofloxacin is available for the treatment of bacterial infection, but not available for treatment of infection locally. Hence it was a challenge to formulate in-situ periodontal gel containing Levofloxacin with rate controlling polymers which provides a longer duration of action and local antibacterial effect without loss of dosage (Sapra et al. 2013). In-situ is a Latin word which means 'In its original place or in position'. The gelation can be triggered by temperature, pH change, ionic change & also UV induced gelation, Solvent exchange induced gelation. In situ drug delivery system offers advantages such as reduced frequency of administration, improved patient compliance, and comfort. An in situ gel formulation provides an interesting alternative for achieving effective plasma drug concentration, an advantage over conventional delivery systems (Divya et al. 2013). The stimuli that induces various responses to form hydrogels includes: Physical stimuli such as change in temperature, electric fields, light, pressure, sound, and magnetic fields; chemical stimuli such as change in pH and ion activation from biological fluids; and biological or biochemical stimuli such as change in glucose level. Out of these different environmental conditions only pH, ion activated, and temperature stimuli are used for dental drug delivery system (Upendra et al. 2013).

MATERIALS AND METHODS

Material:

Levofloxacin was obtained as the gift sample from Psychotropic ltd.Haridwar, and carbopol 934 P, sodium CMC, HPMC, ethanol, sodium citrate, and calcium chloraide was obtained from Central Drug House Pvt. Ltd., New Delhi (IND).

Method:

Polymerization in situ method: This method is used for preparation of sheets of cross-linked polymer sheets in which drug can be incorporated. A liquid polymer or pre polymerized inside a suitable mould. The release from monolithic devices depends on diffusion of drug through matrix. By manipulating the system, selecting the ideal polymer, adjusting the cross-linking, fillers, and plasticizers and by using co-polymers, release of some low molecular drug can be achieved. For an antimicrobial agent to be successful the pathogen must be known, it must be susceptible to the drug. It should not readily develop resistance for an adequate period of time. Also the drug should have little or no side effects (Varun et al. 2011). Preparation of in-situ gel: Aqueous solutions of varying concentration containing Carbopol 934P and HPMC were prepared and evaluated for gelling capacity and viscosity in order to identify the composition suitable for as in situ gelling systems. (Formulation codes CF1, CF2, CF3, CF4 and CF5). Many experiments were conducted by varying the concentration of these polymers in order to identify the optimum concentration required for the gel forming solution. Dispersion containing carbopol 934P were initially prepared in pH 4.5 phosphate buffer solutions. Sodium carboxy methyl cellulose solutions of various concentrations were prepared by adding the sodium CMC to deionised water containing 0.17% w/v sodium citrate and heated to 90° while stirring. After cooling to below 40° appropriate amounts of calcium chloride (0.05% w/v) was added into the sol (formulation codes NF1, NF2, NF3, NF4, NF5). Levofloxacin was dissolved in ethanol (2% w/w) and was added to the solution. The

mixture was stirred by using a magnetic stirrer to ensure thorough mixing. (Table. 1).

Table 1: Formulation chat of in situ gel.

S.No	Formulation	Drug:Polymer
1.	CF1	1:1
2.	CF2	1:1.5
3.	CF3	1:2
4.	CF4	1:2.5
5.	CF5	1:3
6.	NF1	1:1
7.	NF2	1:1.5
8.	NF3	1:2
9.	NF4	1:2.5
10.	NF5	1:3

EVALUATION PARAMETER:

1. Determination of visual appearance and clarity: Gel formulations were visually inspected for clarity, color, homogeneity, presence of particles and fibers by under black and white background (Mahakalkar et al. 2013).

2. Determination of PH: Weighed 50 gm of each gel formulation were transferred beaker and measured it by using the digital pH meter.

3. Spreadability: The spreadability of the gel formuation was determined 48h after preparation, by measuring the spreading diameter of 1gm of the gel between two glass plates after 1min. The mass of the upper plate was standardized at 125 g. The spreadability was calculated by using the formula S = m.l/t

Where, S is spreadibility, m is weight tied to the upper slide, length of the glass slide, and t is the time taken (Singla et al. 2002 and Keller et al. 1982).

4. Drug content uniformity: The container containing formulations were properly shaken for 2–3 min. One milliliter of the formulation was transferred into a 50 ml volumetric flask with a 1 ml calibrated graduated pipette. Twenty five milliliters of simulated saliva with pH 6.8 was added. The formed gel was completely crushed with the help of a glass rod followed by vigorous shaking until the formed gel was completely dispersed to give a clear solution. Final volume was adjusted to 50 ml with simulated saliva. Obtained solution was filtered through Whatman filter paper. One milliliters of this solution was transferred to a 10 ml volumetric flask and volume was adjusted with simulated saliva and the drug concentration was determined at 290 nm by using UV-Visible Spectrophotometer (Agilent) (Dabhi et al. 2010).

5. Gelling capacity:The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of simulated salivary fluid (pH 6.8) freshly prepared and equilibrated at 37º and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. Different grades were allotted as per the gel integrity, weight and rate of formation of gel with respect to time (Harish et al. 2009).

6. Viscosity Estimation: The viscosity of gel was determined by using advance version of bookfield viscometer (Fungilab) (Chu et al. 1991 and Younggon et al. 2007).

7. Evaluation of antimicrobial activity: The ability to inhibit the growth of microbes of in situ gel containing Levofloxacine was determined by using an agar-cup diffusion method. Culture medium was prepared by mixing beef extract, peptone, D-mannitol, agar and sodium chloride. Petri dishes were filled with melted agar medium. After the agar settled, the microorganisms were streaked on the whole agar surface by using cotton swabs. In Agar plate gel was kept inside using disc. The plates were incubated at 37°C for bacteria. The inhibition zone was measured. The organisms studied were S. Aureus (Patel et al. 2013).

8. In vitro diffusion study: In vitro release study was carried out using the egg membrane. An egg membrane (of suitable size) stored in phosphate buffer pH 6.8 for 24 hours before

use. Release of drug from various gel formulations was studied using Franz's diffusion cell. A egg membrane was tied to one end of donor compartment. 1 gm of gel was accurately weighed containing 10mg of gel taken in donor compartment. Receptor compartment was filled with phosphate buffer pH 6.8 previously heated to 37±1°C. Phosphate buffer was agitated using magnetic stirrer and temperature maintained at 37±1°C. 1ml of sample was withdrawn from reservoir compartment at 60 min interval and absorbance was measured spectrophotometrically at 290 nm. Each time the reservoir compartment was replenished with the 1ml volume of phosphate buffer pH 6.8 solutions to maintain constant volume.

9. In vitro drug release kinetic studies:The release data obtained were treated according to zero order (cumulative amount of drug release versus time), first order (log cumulative percentage of drug remaining versus time), Higuchi (cumulative percentage of release versus square root of time) and korsmeyer-Peppas (log cumulative percentage of drug release versus log time) equation models (Baksh et al. 2012).

RESULT AND DISCUSSION

Appearance and Clarity

All the formulations of in-situ gel shows clear appearance.

Determination of pH

The pH was measured with pH meter and all the pH range of all the formulations were within range i.e 4 to 7.5 which is the required range for the dental formulation. The pH of all the formulations was adjusted to 6.5 to 7 with triethanolamine. The prepared in-situ gel formulations were evaluated for variable parameters such as pH and the evaluations parameters of all the formulations were shown in Table. 2.

Spreadability

Table .2 shows the spredability for formulaton CF1 to CF5 and NF1 to NF5. Formulation NF3, NF4, NF5 showed good spreadability as compared with the any other formulation. Comparing NF1 to NF5 showed good spread ability by comparing with CF1 and CF5.

Drug content

Table 2. Shows the percent drug content for formulations CF1to CF5 and NF1 to NF5. The drug content was found to be in acceptable range for all the formulations. Percent drug content of formulations of all (CF1-CF5) and (NF1-NF5) was found to be 95.59% - 98.67% and 92.88% - 98.54%. This indicate that process employed to prepare gels in this study was capable of producing gels with uniform drug content and minimal gel variability.

Gelling capacity

The two main prerequisites of an in situ gelling system are viscosity and gelling capacity. To instill easily at the affected site the formulation must possess optimum viscosity. Further, the formulation should undergo rapid sol to gel transition upon contact at the affected site. All the optimized formulation was found to be good gelling capacity (Table 2).

(-) no gellation;

(+), gels after few minutes, dispersed rapidly;

(++), gelation immediate, remains for few hours; and

(+++), gelation immediate, remains for an extended period

Table 2: Characteristics of various Levofloxacin gel formulation

S.No.	Formulation	pH	SPREADABILITY(g/cm^2)	Drug content	Gelling capacity
1	CF1	6.8	20.21	95.83	–
2	CF2	6.5	20.01	96.81	+

3	CF3	6.2	20.63	95.59	+
4	CF4	6.2	21.89	97.82	++
5	CF5	6.1	22.72	98.67	+++
6	NF1	6.9	21.16	98.25	–
7	NF2	7.5	22.49	97.49	–
8	NF3	6.5	24.11	94.72	+
9	NF4	7.1	24.73	92.88	+
10	NF5	7.2	25.09	98.54	++

Antimicrobial activity

Antimicrobial activity of all batches reveals that *in situ* gel containing 1.0% Levofloxacin shows good zone of inhibition. Furthermore, we could say that Levofloxacin was effective against Staphylococcus aureus (bacteria). So the formulation can be used for the treatment of gingivitis. (Table 3)

Table 3: Antimicrobial activity of in situ gel formulation.

Formulation	Zone of inhibition (Diameter in mm) (S.aureus)
CF1	25.0
CF2	24.9
CF3	22.0
CF4	20.1
CF5	20.0
NF1	25.9
NF2	25.2
NF3	23.5
NF4	21.7
NF5	20.3

Viscosity Estimation

The viscosity of gel was determined by using a advance version of Brookfield viscometer at different RPM and it was concluded that the rate of shearing is directly proportional to shearing stress, which shows that the formulation is non-Newtonian in nature. When the rate of shear is increase viscosity decreases that prove that the formulation is shear thinning pseudo-plastic in nature. The rheological studies of the prepared levofloxacin in-situ gel was carried out and as shown in table 4 the rate of shearing is directly proportional to shearing stress, which shows that the formulation is non-Newtonian in nature. When the rate of shear is increased as shown in graph a (1a, 1b and 1c, 1d) viscocity decrease that proves that the formulation is shear thinning pseudoplastic in nature. (Table 4)

Table 4: Measurement of viscosity of gel

S. No.	RPM	TORQUE (for sodium CMC)	VISCOSITY(cps) (for sodium CMC)	TORQUE(for carbopol 934P)	VISCOSITY (cps)(for caobopol 934P)
1.	12	68.6	26773	23.7	9260.0
2.	10	67.2	31499	21.9	10265
3.	8	60.8	35626	18.8	11032
4.	6	49.2	38394	–	–
5.	4	43.9	51376	–	–
6.	2	29.1	68212	–	–

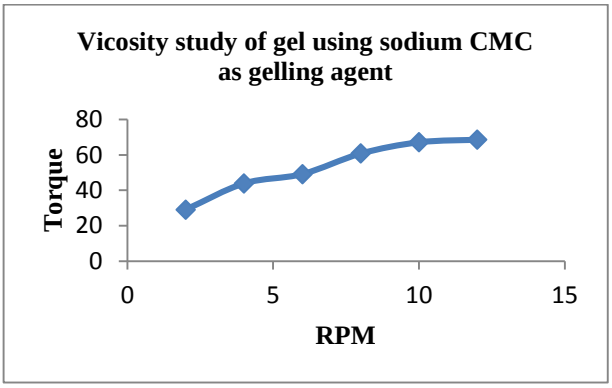

Fig 1(a): Graph b/w Torque v/s RPM

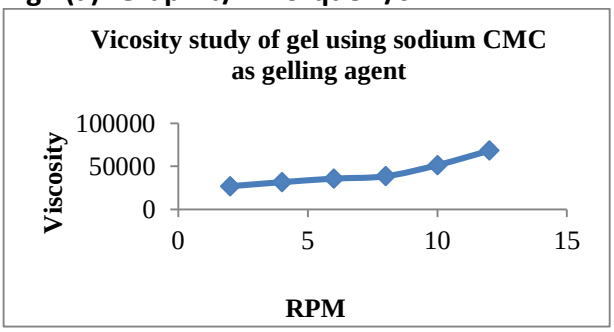

Fig 1(b): Graph b/w average viscosity v/s RPM

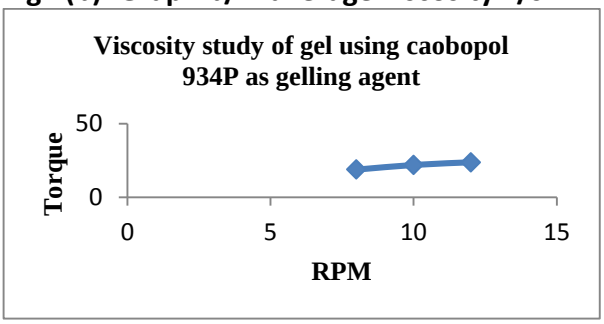

Fig 1(c): Graph b/w Torque v/s RPM

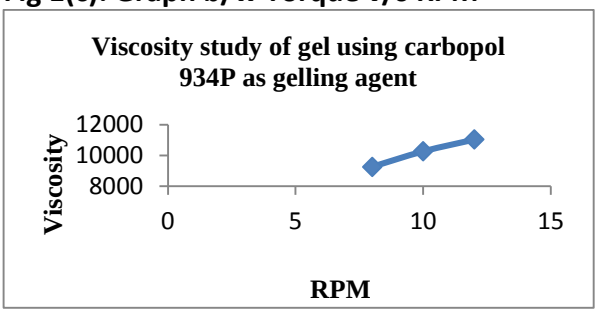

Fig 1(d): Graph b/w average viscosity v/s RPM

In vitro diffusion studies

The *in vitro* dissolution profile of Levofloxacin from the gels containing different concentration of sodium carboxy methyl cellulose and carbopol based gels. The release of drug from these gels was characterized by an initial phase of high release (burst effect) and as the gelation proceeded, the remaining drug was released at a slower rate (second phase). This bi phasic pattern of release is a characteristic feature of matrix diffusion kinetics. The initial burst effect was considerably reduced with increase in polymer concentration. The formulation NF1and NF2 containing the lower polymer ratio (0.1: 0.5 and 0.2: 0.5) showed the release profile only up to 4 h, whereas formulation having higher polymer ratio i.e., NF6, showed only 50% release at the end of 6 h. Since we were inclined to formulate *in situ* gel which show 90% release profile within 6 h, NF1, NF2 and NF5 formulations were not found to be ideal formulations for *in situ* gels. In comparison, the gels containing carbopol having the maximum concentration CF5 could show release only up to 5 h, though, the other formulations of carbopol disintegrated rapidly and released the drug within 4 h. However, these findings clearly showed that the gels have the ability to retain Levofloxacin at higher concentration of carbopol (CF5) and premature release of drug can be avoided. Hence NF3, NF4 and CF5 formulations were chosen to meet the above said criterion (Table 5). It is known that the peppas model is widely used to confirm whether the release mechanism is Fickian diffusion and non-Fickian diffusion. The 'n' (release exponent of korsmeyer-peppas model) value could be used to characterize different release mechanisms. The interpretation of n value was done in the following manner.

- n<0.5 (0.45) - quasi-Fickian Diffusion
- n=0.5 (0.45) - Diffusion mechanism
- 0.5<n<1 - Anomalous (non Fickian) Diffusion – both diffusion and relaxation (erosion)
- n=1 (0.89) - Case 2 transport (Zero order release)
- n>1 (0.89) – Super case 2 transport (relaxation)

Table 5: Drug release studies of different formulations

S.No.	Time (hrs)	CF1	CF2	CF3	CF4	CF5	NF1	NF2	NF3	NF4	NF5
1	0	0	0	0	0	0	0	0	0	0	0
2	1	4.62	6.43	12.92	13.61	14.77	15.55	11.05	8.32	6.97	12.62
3	2	14.93	15.93	25.78	27.89	29.83	23.26	20.57	12.30	11.51	22.10
4	3	19.13	21.69	34.76	35.81	37.41	34.73	33.15	28.41	25.69	35.44
5	4	29.78	32.74	47.92	49.95	50.29	43.21	42.62	34.73	31.2	44.20
6	5	40.60	44.92	56.46	57.15	59.10	48.51	50.42	42.89	39.11	52.11
7	6	51.39	54.17	67.82	68.29	69.75	55.82	58.52	50.21	48.20	58.99
8	7	59.99	63.71	72.91	74.80	76.13	74.78	69.74	61.20	59.18	72.24
9	8	67.14	70.11	80.61	83.36	84.19	86.83	81.24	72.11	69.11	85.57

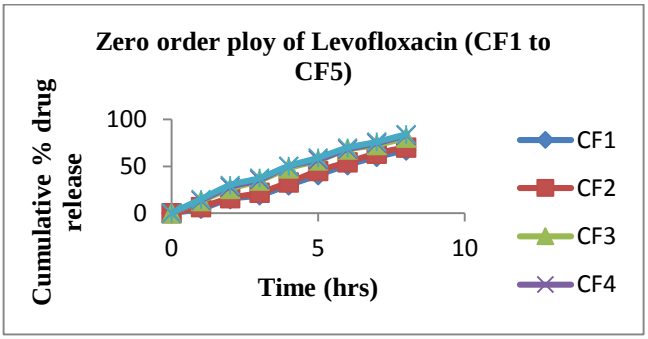

Fig 2(a) Plot for cumulative % drug release Vs time (zero order kinetic) of Levofloxacin for CF1 to CF2 formulation.

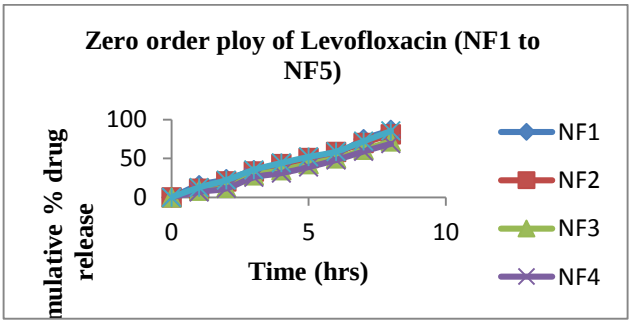

Fig 2(b) Plot for cumulative % drug release Vs time (zero order kinetic) of Levofloxacin for NF1 to NF2 formulation.

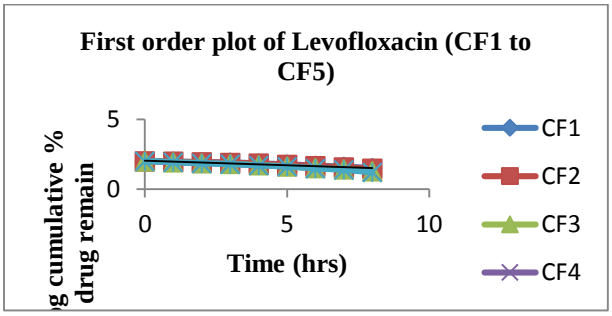

Fig 2(c) Plot for log cumulative % drug remain Vs time (first order kinetic) of Levofloxacin for CF1 to CF2 formulation.

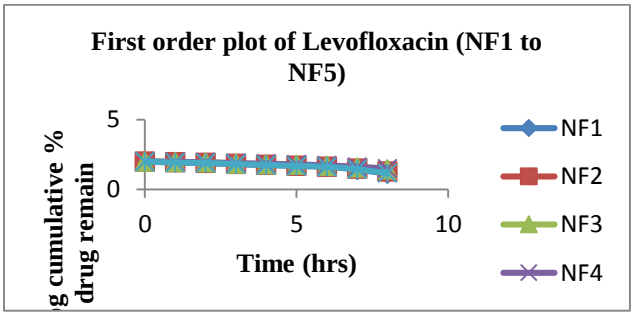

Fig 2(d) Plot for log cumulative % drug remain Vs time (first order kinetic) of Levofloxacin for NF1 to NF2 formulation.

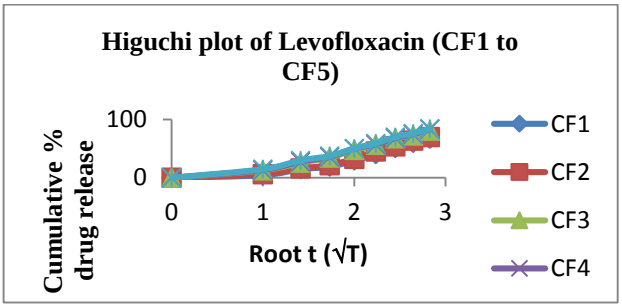

Fig 2(e) Plot for cumulative % drug release Vs square root time (Higuchi matrix) of Levofloxacin for CF1 to CF2 formulation.

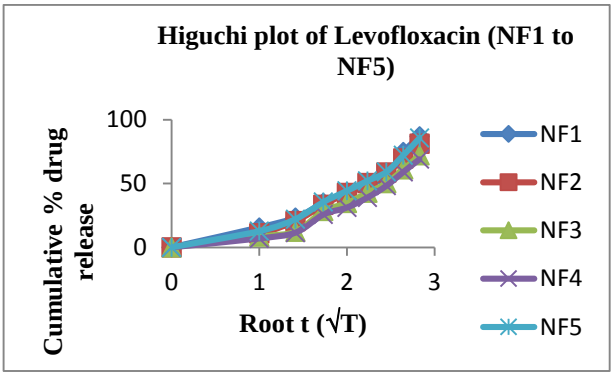

Fig 2(f) Plot for cumulative % drug release Vs square root time (Higuchi matrix) of Levofloxacin for NF1 to NF2 formuation.

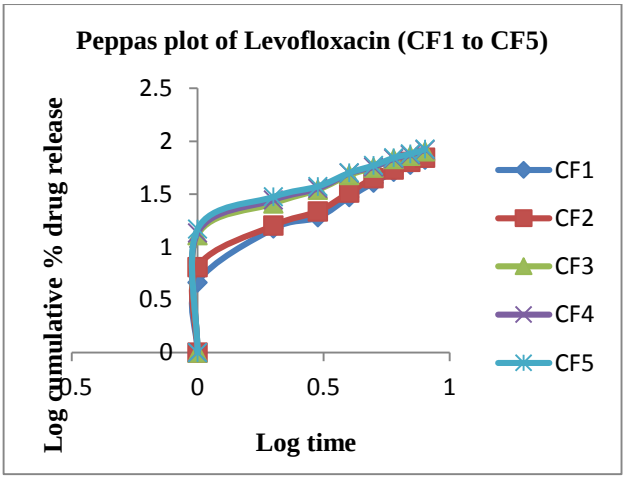

Fig 2(g) Plot for log cumulative % drug release Vs log time (Korsmeyer peppas) of Levofloxacin for CF1 to CF2 formulation.

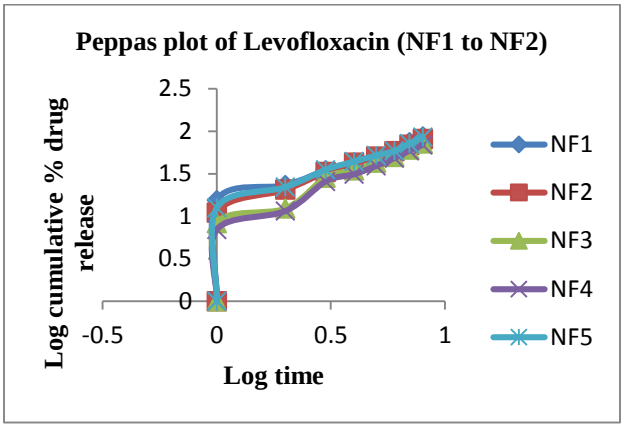

Fig 2(h) Plot for log cumulative % drug release Vs log time (Korsmeyer peppas) of Levofloxacin for NF1 to NF2 formuation.

Table: Model fitting release profile of formulation.

Formulation	Zero order	First order	Higuchi matrix	Korsmeyer peppas	'n' value
CF1	0.9918	0.9614	0.8713	0.89	1.6518
CF2	0.9944	0.966	0.8856	0.8543	1.6025
CF3	0.9892	0.9831	0.9494	0.7301	1.4933
CF4	0.9894	0.9742	0.9529	0.7186	1.4868
CF5	0.987	0.9761	0.9591	0.7017	1.4707
NF1	0.9829	0.8663	0.907	0.7031	1.4361
NF2	0.9973	0.9419	0.9247	0.7648	1.5146
NF3	0.9923	0.9507	0.8926	0.8228	1.5508
NF4	0.9924	0.9491	0.8793	0.8495	1.5706
NF5	0.994	0.8999	0.9239	0.7423	1.4953

CONCLUSION

The present work was carried out to develop a Novel in situ gel based dental drug delivery system by using thermo reverse gelation technique. The methodology Adopted for preparation of in situ gel solution was very simple and cost effective. It is newer approach to improve easy dental instillation residence time and prolong drug release. In research work in situ dental gel containing Levofloxacin was developed with combination of carbopol 934P and sodium carboxy methyl cellulose. Effect of calcium carbonate and other process parameters optimized and found that increase in calcium ions produced stronger gels. By doing compatibility study, drug was found to be compatible with formulation excipients, it is concluded that the selected polymers are likely to be suitable for preparation of in situ dental gel formulation. The developed formulations shows satisfactory results for gelling capacity, pH, spreadability, and other physical properties. From the study conducted, the following conclusion were drawn the gels which was prepared by using the technique thermo reverse gelation with Levofloxacin shown good antimicrobial activity. The In situ systems showed increased residence time and prolonged drug release for over 8 hrs.

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