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FORMULATION AND EVALUATION OF THERMOSENSITIVE *IN SITU* OFLOXACIN OTIC GEL FOR THE TREATMENT OF OTITIS MEDIA

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ABSTRACT

Conventional otic drug delivery has the drawbacks like short residence time, leakage of medicaments from ear, inconvenient administration and less patient compliances. Thermosensitive *in situ* otic gel overcome all these drawbacks as they exist as free flowing aqueous sol during administration and rapidly undergo phase transition when exposed to body temperature. Otits media is a group of inflammatory disease of middle ear accompanied by pain, dizziness and partial loss of hearing. Ofloxacin is recommended as most effective antibiotic against otitis media. *In situ* gel was prepared by blending drug with thermosensitive gelling agent in distilled water followed by addition of suitable viscosity enhancers and undergoes continuous stirring in a magnetic stirrer. The formulations were then kept aside overnight at 4°C to allow complete hydration of polymer. Out of the 18 preparations, formulation P_3K_1 containing 19% Poloxamer 407 and 0.05% hydroxypropyl cellulose was selected as best formulation as it exhibited suitable satisfactory physicochemical characteristics, with optimum drug content (92.166± 0.132%) and maximum in vitro drug release (75.608± 0.012 %) for 8hrs, showing first order kinetics and Higuchi model mechanism and was stable with no adverse effect of temperature and humidity during storage over three months.

KEYWORDS: Thermosensitive, *in situ* gel, otitis media, phase transition, Poloxamer, Sol-Gel transition.

INTRODUCTION

Middle ear drug delivery is the one of the most challenging problem facing pharmaceutical scientists. The main drawbacks of conventional dosage forms include spillage of medicament from ear, short residence time, inconvenient administration and less patient compliances. To overcome all these difficulties a new form of dosage form was introduced i.e., *in situ* gels. *In situ* gelation is a process of gel formation at the site of action when it is applied to particular site. Here the formulation will exist as free flowing aqueous sol before administration and undergo phase transition to gel when come in contact with site of action due to the presence of triggering factors. Triggering factors include pH, temperature, ions, photo polymerization, solvent exchange mechanism etc.

Thermosensitive *in situ* gel[1] are freely flowing liquid at the time of administration and when they come in contact with body temperature they undergo phase transition to gel. Presence of suitable thermosensitive gelling polymer is necessary to undergo phase transition in the formulation of thermosensitive *in situ* gel. Commonly used polymers include poloxamers, pluronics, gel rites etc. Addition of suitable viscosity modifiers is also important to attain better result.

Otitis media is a group of inflammatory diseases of the middle ear characterized by pain, dizziness and partial loss of hearing.[2] The two main types are acute otitis media (AOM) and otitis media with effusion (OME).[3] OME is defined as the presence of non-infectious fluid in the middle ear for more than three months. AOM include bulging or a lack of movement of the tympanic membrane from a puff of air.[4] Effusion within the middle ear can cause significant hearing impairment which may interfere with the development of normal speech in young children. Antibiotics are choice of treatment for otitis media. Ofloxacin is a second generation fluoroquinolone widely used in eye and ear drops. It has better efficacy and safety profile. In the current study, thermosensitive *in situ* otic gel of Ofloxacin was formulated. The thermosensitive gelling agent used was poloxamer 407. Different viscosity modifiers like hydroxyethylcellulose (natrosol 250M) and hydroxypropyl cellulose (klucel HF) were employed to prepare different formulations and formulation with better gelling ability and suitable viscosity was selected.

MATERIALS AND METHOD

Ofloxacin pure drug, poloxamer 407, hydroxyethyl cellulose and hydroxypropyl cellulose were obtained from Yarrow Chem Products, Mumbai and were proven that having analytical grade and also optimized. The

equipments and apparatus provided were with high standard and validated.

Preformulation studies

Pre-formulation testing was an investigation of physical and chemical properties of drug alone and also determination of the quality and purity of excipients used. It is the first step in the rational development of dosage form. Preformulation studies employed include determination of melting point, solubility and organoleptic properties. The melting point was determined by capillary fusion method. A capillary was sealed at one end filled with small amount of ofloxacin pure sample and the capillary was kept inverted that is sealed end towards into the melting point apparatus. Absorption maxima and calibration curve of Ofloxacin was prepared using 0.1N HCl as medium.

While designing a formulation of thermosensitive *in situ* otic gel it is necessary to give importance on drug-polymer interaction within the system. So it is important to find out whether there is any interaction between Ofloxacin and polymers used in the formulation. The study was performed using Fourier Transform Infra-red Spectroscopy.

Formulation of thermosensitive *in situ* gel[5][6][7]

A typical thermosensitive *in situ* gel formulation consisted of ofloxacin 0.3% w/v, thermosensitive gelling polymer Poloxamer 407 (17 – 19 % w/v), viscosity modifiers Natrosol 250M and Klucel HF (0.25 – 1 % w/v) and distilled water as solvent. (table.no:1 &2).

Preparation include specified amount of poloxamer 407 was weighed and dispersed in sterile water with continuous stirring in a magnetic stirrer. When a white thick mass is obtained, weighed amount of different viscosity modifiers are added to each preparation and stirring continued. It was then followed by addition of specified amount of Ofloxacin to the mixture and stirred well. The mixtures were stored overnight at 4˚C to allow complete hydration of polymer. The obtained solution were stored in airtight container covered with aluminium foil and was kept in refrigerator at 4˚C to obtain its liquid consistency. Eighteen formulations with three different concentrations of thermosensitive *in situ* gelling polymer poloxamer 407 and two different viscosity modifiers Natrosol 250M (hydroxyethyl cellulose) and Klucel HF (hydroxypropyl cellulose) with three different concentrations keeping the level of all other ingredients constant with final volume made up to 10ml were prepared.

Table 1: Formulation of thermosensitive *in situ* gel containing natrosol.

SI.No	Formulations	Poloxamer 407(g)	Natrosol250M(g)
1	P_1N_0	1.7	0.025
2	P_1N_1	1.7	0.05
3	P_1N_2	1.7	0.1
4	P_2N_0	1.8	0.025
5	P_2N_1	1.8	0.5
6	P_2N_2	1.8	0.1
7	P_3N_0	1.9	0.025
8	P_3N_1	1.9	0.05
9	P_3N_2	1.9	0.1

Table 2: Formulation of thermosensitive *in situ* gel containing klucel HF.

SI.No	Formulations	Poloxamer 407 (g)	Klucel HF (g)
10	P_1K_0	1.7	0.025
11	P_1K_1	1.7	0.05
12	P_1K_2	1.7	0.1
13	P_2K_0	1.8	0.025
14	P_2K_1	1.8	0.05
15	P_2K_2	1.8	0.1
16	P_3K_0	1.9	0.025
17	P_3K_1	1.9	0.05
18	P_3K_2	1.9	0.1

Evaluation of thermosensitive *insitu* gels

Visual inspection of formulation[8]

The prepared formulations were evaluated visually for its clarity, transparency and stickiness. If it was satisfactory, then it was taken for further evaluations. If the formed gel were not satisfactory they were discarded.

Sol – gel transition temperature[9]

For this, about 2-3 ml of each of the sol formulation was taken in glass test tubes. The test tubes was exposed to gradually increasing temperature in the range of 22˚ - 45˚ (increment of 1˚ every 5 min) and the temperature at which sol was transformed to semisolid gel was noted.

Gelling time[5]

The gelling time was measured by using glass chamber tilted at an angle of 45˚ containing water, which was maintained at 37 ± 0.5˚. The individual otic formulations (100 -200 µl) were dropped on the outer surface of the glass chamber and time required for phase transition was noted. The transition of solutions to viscous gel was observed visually and grades were assigned depending upon, time taken for gel formation and collapsing of gel structure.

Mucoadhesive strength[10][11]

For determination of mucoadhesive strength of otic gel an apparatus was fabricated in the laboratory. The apparatus was modified dispensing balance consisting of central liver, on one arm with pan and other arm with glass vial. Another glass vial in inverted position was fixed to the wooden base of the balance using double sided adhesive tape. The membrane was fitted in the gap between lower surface of suspended vial and upper surface of fixed vial. Weighed quantities (0.5g) of individual samples of otic gel formulations were applied to the base of inverted glass vial using double sided adhesive tape to secure the gel in position. The distances between two vials were adjusted in such a way that the gel samples remain adhered to mucosal membrane. Sufficient pressure was applied on both of the vials for 10 sec to allow proper adhesion of gel to mucosa. A constant weight was added to the pan to pull the vial away from the vial. The weight required for detaching the two vials was noted down. The mucoadhesive force, expresses as the detachment stress in dynes/cm^2.

Detachment stress = mg/A , where, m is the weight required for detaching the two vials,g is the acceleration due to gravity, taken as 980 cm/s^2 ,A is the area of membrane exposed and is equal to $A= \pi r^2$ (r is the radius of the exposed membrane).

Gel strength[11][5]

For this, 8ml of each of the gel bases (sol phase) was transferred into 10ml glass measuring cylinder. The cylinder was fixed to burette stand using a three way clamp. A scale of 15cm was placed at the back drop of the cylinder. A stainless steel ball bearing (1cm diameter and 3.48g weight) was dropped from height of 5cm into the bulk of the gel mass contained in the cylinder with proper care of not allowing the ball to touch the sides of the cylinder. The distance travelled by the ball within 30 sec was noted down to the nearest value in cm.

Viscosity measurements and rheological behaviour[12][13]

The viscosity of each formulation at room temperature (32±5˚C) was noted using Brookfield viscometer DV-I-LV. The guard leg was kept in place and the volume of the sample was stirred slowly using a motor driven stirring spindle LV4 with spindle marking 64. The viscosity values are noted at different rpm. A typical run involved changing the angular velocity of spindle from 1 – 100 rpm. The viscosity values at each rpm were noted.

Estimation of drug content[7][5]

The content of Ofloxacin in various formulations was estimated by UV spectrophotometric method. For this, 1ml of each of the otic formulations was taken and diluted upto 100ml with distilled water to form solution A. From the solution A 1ml was withdrawn and diluted to 50ml using distilled water. Then the samples were analyzed spectrophotometrically at λmax of Ofloxacin (293nm). The concentration of drugs in samples was determined from a previously developed calibration curve of Ofloxacin and percentage drug content in each formulation were recorded.

***In vitro* release of drugs**[14][7][5]

The recipient compartment was filled with phosphate buffer saline (PBS) PH 7.4 (30ml). The pretreated egg membrane of appropriate diameter (2.5cm) was mounted carefully on the rim of the diffusion tube , 2ml of sample was placed in the diffusion tube and left to form gel then donor compartment placed in receptor compartment , which contain 30ml phosphate buffer. The whole diffusion assembly was then placed in the thermostatically controlled magnetic stirrer at 37±0.5˚C and 70 rpm. About 2ml quantities of aliquots were withdrawn carefully from the receptor compartment and were replaced immediately with the same volume of fresh phosphate buffer (maintained at 37±0.5˚C). About 1ml of samples were diluted suitably and the content of drugs in each samples was estimated from the absorbance at 293nm using the previously developed calibration curve. The percentage of drug release was calculated by using the standard graph of Ofloxacin.

Stability studies[14][6]

The stability studies of optimised thermosensitive *in situ* gel were carried out at refrigerated temperature 4˚C for 3 months. The formulation was kept in airtight container covered with aluminium foil and placed away from sunlight in refrigerator. The formulations were evaluated for appearance, drug content, viscosity, gelation behaviour, gel strength and *in vitro* drug release.

Release studies[15]

To analyze the mechanism of drug release kinetics of dosage form, the obtained data were fitted to various kinetic equation of zero order, first order, Higuchi model and Korsmeyer-Peppas model and plotted as: Cumulative percentage of drug release Vs time (zero order plots), Log cumulative percentage drug remaining Vs time (first order plots), Cumulative percentage drug release Vs square root of time (Higuchi plots), Log cumulative percentage drug release Vs log time (Korsmeyer-Peppas plots).

RESULTS

From the Preformulation studies performed, the melting point of Ofloxacin obtained was 254 ± 0.04512˚C and absorption maxima of the drug were 293nm. FTIR spectra of the drug and polymer show that no major difference was seen in the characteristics absorption peaks of the pure drug.

Table 3: Sol-gel transition temperature and gelling time.

SI.No	Formulation	Sol-gel transition temperature (°C)	Gelling time (sec)
1	P_1N_0	42±1	88.287±0.015
2	P_1N_1	33.33±1.404	53.667±0.577
3	P_1N_2	40±1	55±1
4	P_2N_0	30±1	40.667±0.577
5	P_2N_1	33.33±0.0234	16.33±0.577
6	P_2N_2	31±1	17.33±1.155
7	P_3N_0	30.667±0.577	19.33±0.577
8	P_3N_1	28.667±1.157	16.667±0.577
9	P_3N_2	25±1	15.33±0.577
10	P_1K_0	-	-
11	P_1K_1	-	-
12	P_1K_2	-	-
13	P_2K_0	28.667±0.577	18.667±0.577
14	P_2K_1	32±1.153	16±0.672
15	P_2K_2	30.33±0.577	33.667±0.577
16	P_3K_0	28.667±0.577	13.33±0.577
17	P_3K_1	32.667±1.528	22.667±0.577
18	P_3K_2	29.33±0.557	17.33±0.577

Values are mean±SD, n=3.

From the formulation P_1N_0 to P_3K_2**,** three formulations (P_1K_0, P_1K_1 and P_1K_2) were rejected as they doesn't form gel and formulations P_2N_1 (containing 18% poloxamer 407, 0.05% natrosol), P_2N_2 (containing 18% poloxamer 407, 0.1% natrosol), P_2K_1 (18% poloxamer 407,0.05% klucel) and P_3K_1 (19% poloxamer 407, 0.05% klucel) were selected (table 3) for further evaluation studies based on their sol – gel transition temperature and gelling behaviour.

Table 4: Evaluation tests of selected formulation.

SI.No	Formulation	Mucoadhesive strength	Gel strength	Viscosity measurement	Drug content	*In vitro* drug release
1	P_2N_1	5.64±0.041	1.4±0.023	46646±300.88	91.488±0.945	68.8±0.062
2	P_2N_2	2.82±0.080	4.76±0.0577	38800±700	89.318±0.598	50.7±0.034
3	P_2K_1	2.79±0.039	3.63±0.0577	44347±225.22	88.149±0.234	51.4±0.092
4	P_3K_1	5.52±0.040	1.23±0.0577	52183±175.59	92.166±0.132	69.9±0.057

Values are mean±SD, n=3

From the evaluation (table 4) conducted on the optimized formulations of Ofloxacin, the formulation P_3K_1 containing 19% poloxamer 407 and hydroxypropyl cellulose (klucel HF) as viscosity modifier with concentration 0.5% was found to be the best formulation and it was chosen for kinetic release study and stability studies. The linear regression coefficient of each kinetic model was calculated and pattern of drug release from the dose was predicted (table 5). It was found that the optimized formulation P_3K_1 follows first order kinetics and Higuchi model mechanism (figure 1). From the stability studies, it was found that there is no significant change in appearance, drug content, viscosity, gelation behaviour, gel strength and *in vitro* drug release.

Table 5: Regression coefficient of different kinetic models of P_3K_1

Formulation	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Korsmeyer – Peppas (R^2)
P_3K_1	0.9326	0.9769	0.9026	0.8926

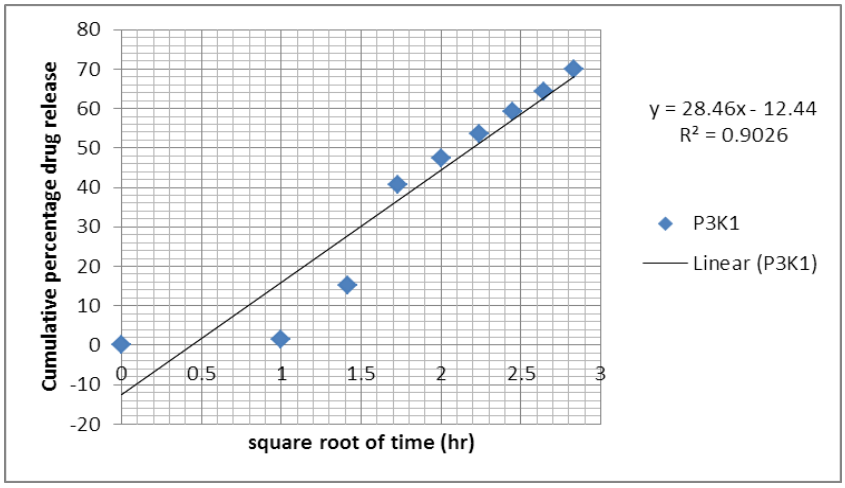

Figure 1: Higuchi model of P_3K_1.

DISCUSSIONS

The FTIR spectra of the drug and physical mixture of drug and polymer showed that no interaction had taken place between the drug and polymer. Hence there is no reaction between drug and polymer exists. From the visual inspection of prepared formulations it was found that the formulation with lower concentration of gelling polymer forms an easily breakable soft gel with very low consistency. As the concentration of poloxamer increases the gelling ability of the formulations were also improved. The addition of the hydroxyethyl cellulose and hydroxypropyl cellulose were aimed to impart mechanical strength to form in situ gel by increasing their viscosity. And it was also found that improved gelling ability was found with poloxamer 407 concentrations above 17% in the total preparation volume.

The transition temperatures of the formulations were found within the range between 25±1 - 42±1˚C. With increase in temperature the gelling ability of the formulation increases to some extend but with further increase in temperature cause collapse of gel structure. Gelling time is another important factor in the evaluation studies of thermosensitive in situ gel formulations. Improved gelling ability depends on reduced gelling time of the formulations. The gelling time of the formulations ranges between 88.87±0.015 – 16.667±0.577 sec. From the sol-gel transition temperature and gelling time determination, three formulations doesn't form gel at any suggested temperature (P_1K_0, P_1K_1 and P_1K_2). It indicates that below a particular concentration of poloxamer 407, klucel HF together fails to form gel even if the concentration of viscosity modifier's concentration is increased.

The mucoadhesive strength of the formulations were in the range between 2.79 -5.64(dynes/cm^2 $\times 10^3$). There was a corresponding increase in mucoadhesive strength with increase in concentration of individual polymers. Mucoadhesive involves association of reactive functional groups of polymers with those of mucin layer lining the organ. However the extent of association depends on the degree of freedom available for the polymer chain. Gel strength of the optimized formulation were in the range between 1.4±0.023 – 4.76±0.057 cm. Sufficient gel strength is required to prevent leakage of formulation from ear. The increase in polymer concentration leads to increase in gel strength of the formulation. The *in situ* gel bases clearly indicated the shear thinning behaviour. As the concentration of viscosity increasing agent was increased, the viscosity was also increased. The viscosities of the formulations were lies in the range between 38800±700 – 52183.33± 175.594 cp. The content of Ofloxacin in the selected *in situ* gelling system lies within the range between 88.318± 0.234 % -92.166± 0.132%.

The *in vitro* drug release study was carried out for all selected formulations. All batches showed a sustained drug release profile for 8hr. Formulation P_3K_1 showed 69.9± 0.012, P_2K_1 showed 51.46±0.034, P_2N_1 showed 68.868±0.034 and P_2N_2 showed 50.784±0.029 in vitro drug release respectively for a period of 8hrs. From the data obtained it was clear that formulation P_3K_1 has good *in vitro* drug release. It was noted that, the drug release of drug is not only affected by poloxamer concentration but also the concentration of viscosity modifiers. The linear regression coefficient of each kinetic model was calculated and pattern of drug release from the dose was predicted. It was found that the optimized formulation P_3K_1 follows first order kinetics and Higuchi model mechanism. The optimized formulation P_3K_1 was selected for the stability studies which were stored at refrigerated temperature 4˚C for three months. From the evaluation, it was found that there is no significant change in parameters during the storage period.

CONCLUSION

Formulation P_3K_1 containing 19% Poloxamer 407 and 0.05% Klucel HF was selected as best formulation as it exhibited suitable satisfactory physicochemical characteristics, with optimum drug content (92.166± 0.132%) and maximum in vitro drug release (75.608± 0.012 %) for 8hrs, showing first order kinetics and Higuchi model mechanism and was stable with no

adverse effect of temperature and humidity during storage over three months. The formulation suggests the management of otitis media could be possible with *in situ* otic gelling system. However, *in vivo* studies are needed to ascertain efficacy of the experimental formulations.

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