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DEVELOPMENT AND EVALUATION OF *IN-SITU* GELLING OTIC FORMULATIONS OF AZITHROMYCIN DIHYDRATE USING POLOXAMER

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ABSTRACT

Azithromycin dihydrate has been indicated in the treatment of chronic suppurative otitis media (CSOM). The aim of this study was to develop in situ gel forming ear drops of Azithromycin dihydrate. 1% w/v azithromycin dihydrate otic solution and *in situ* gel forming otic solutions were prepared. Poloxamer 407 forms thermoreversible gels so it was used as *in situ* gelling agent. Poloxamer concentration in the range of 15-20% w/v was tried. 18 and 19% w/v Poloxamer 407 gave satisfactory *in situ* gel forming formulations. Poloxamer 407 with viscosity increasing agents like Methocel K100M Premium and Carbopol 974 were also tried in range of 0.2-2% w/v. The formulations were evaluated for appearance/clarity, gelling time, pH, rheological characteristic and *in vitro* diffusion through synthetic membrane. The viscosity of formulations increased as concentration of Poloxamer 407 increased. When Poloxamer 407 concentration was kept constant, viscosity of formulations increased with increasing concentration of HPMC K4M and Cabopol 974. In vitro release from synthetic membrane showed that *in situ* gelling formulations were releasing the drug slowly as compared to drug solution. In case of ear drops, the drug released was more than 90% in 3h and from *in situ* gel formulation, drug released was less than 40% in 3h. From *in situ* gel formulations drug release was sustained upto 8h. Thus *in situ* gelling formulations are likely to give patient convenience and reduce frequency of ear drops administration.

INTRODUCTION

Antibiotics are also known as antibacterials and they are drugs used to treat infections caused by bacteria. Antibiotics are among the most frequently prescribed medications in modern medicine. Today, over 100 different antibiotics are available to cure minor, as well as life-threatening infections. Azithromycin dihydrate is a broad-spectrum antibiotic that is active against a wide variety of bacteria that cause a wide variety of infections. There are many diseases and disorders which can affect hearing. The otitis media is the commonest disease which is caused by viral and bacterial infection. Chronic suppurative otitis media (CSOM) is the result of an initial episode of acute otitis media (Jose, 2004) and caused by aerobic (e.g. *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes,* Klebsiella species) or anaerobic (e.g. Bacteroides, Peptostreptococcus, Proprionibacterium) (Jose, 2004, 2007). CSOM is characterized by a persistent discharge from the middle ear through a tympanic perforation (Jose, 2004). The CSOM can be treated with surgery. Local delivery of drugs to ear may be carried out with ear drops and sprays (British Pharmacopoeia, 2010). Microparticles gels made of biodegradable or nondegradable polymers have been proposed as effective methods to administer antimicrobial agents in otic therapy (Lichter *et al.,* 2009a and 2009b). Ear drop suffers from drawback such as shorter residence time in ear. Semisolid preparation has disadvantages such as difficulty in administration. Both of these problems can be overcome by formulating a solution which forms in situ gel after administration. Poloxamer 407 aqueous solutions (more than 18%w/v) can form in situ gel at body temperature (Bieberschulte *et al.,* 2003; Sawchuk *et al.,* 2004; Hunt, 2009; Meyer, 2009; Simons *et al.,* 2009). In past, in situ gelling formulations have been prepared for metronidazole (Mali *et al.*, 2011) and chloramphenicol (Kattekar, 2011). Azithromcyin dihydrate is used as antibacterial agent in the treatment of otitis externa and otitis media by topical and oral route. Azithromcyin dihydrate is having higher efficacy in treating aerobic and anaerobic bacterial infection. The greatest advantages of this drug are its unusual pharmacokinetics (high tissue distribution), metabolic stability and high tolerability. This drug is particularly noted for high and prolonged concentrations at the site of infection (Killion, 2003).

A need was felt to develop *in situ* otic gel of azithromycin dihydrate for sustained release of drug. So this study is devoted to formulation of Azithromycin dihydrate ear drops which are solution at room temperature and forms gel after administration in ear. For this purpose poloxamer 407 is used as a thermoreversible and sustained release polymer, Methocel K100M and Carbopol 974 are used as viscosity modifiers.

MATERIALS AND METHODS

Materials

Azithromycin dihydrate I. P. was obtained from Hindustan Antibiotics, Pune. Poloxamer 407 was obtained from BASF, Pvt. Ltd. Mumbai. Methocel K100M was obtained from Colorcon Asia Pvt. Ltd. Goa and Carbopol -974 was obtained from Vishal Chemicals, Mumbai.

Preparation of Poloxamer 407 Azithromcyin dihydrate formulations

Azithromycin dihydrate 1% w/v aqueous solution was prepared by using citric acid monohydrate for adjusting pH. To this, sodium chloride (0.5% w/v) and methyl paraben (0.01%) were dissolved as tonicity adjusting agent and preservative respectively. The gels were made on w/v basis using the modified cold method. Poloxamer 407 was mixed with aqueous solution of Azithromycin dihydrate and refrigerated at 4ºC and was sonicated until a homogenous solution was obtained. Poloxamer 407 concentrations of 18, 19, 20% w/v were prepared. Formulations containing Poloxamer 407 and viscosity increasing agent Methocel K100M and Carbopol 974 were also prepared. Table 1 gives formulation details.

Evaluation of formulations

Appearance/ clarity

The otic formulations were observed carefully for color, odour and presence of suspended particulate matter if any. The clarity of solutions was further assessed by observing them against a dark and white background.

Sol-Gel transition temperature

The test for gelling ability (Sol-Gel transition temperature) was conducted in test tubes which were put in low temperature bath and temperature was increased at rate of 1ºC every 5 minutes from 33ºC to 40ºC. Test tubes were observed for formation of viscous gel.

Gelling time

The gelling time was measured by using glass plate with slope same as ear slope and maintained at 37ºC ± 0.5ºC temperature. The individual otic formulations (100-200μL) were dropped on the glass plate (37ºC ± 0.5ºC) and gelling time was measured. The transition of solution to viscous gel was observed visually. The negative score (-) was assigned to those solutions which did not form gel. The lowest score of (+) was assigned to those solutions which exhibited phase transition only after 90 sec. and the formed gels which collapsed within 1-5 min. The moderate score of (++) was assigned to those solutions, which formed the gels in between 30-90 sec. The highest score of (+++) was assigned to those solutions which exhibited phase transition within 30 sec. and the gels so formed remained stable for more than 30 min.

Determination of pH values of otic formulations

The pH of each of prepared otic formulation was recorded using previously calibrated digital pH meter. The pH values were recorded immediately after preparation as well as after storage for 24h at room temperature.

Rheological study

The viscosity was estimated by using Brookfield viscometer (RVDV-II + Pro) for otic solutions of Azithromycin dihydrate at temperature below 10ºC by using small sample adaptor spindle no. S21. Viscosity of preformed gels of these solutions at temperature 35-37ºC was measured by using spindle no. S93 at 1-100 RPM. Evaluations were conducted in triplicate.

***In-vitro* drug release studies from gel formulations**

Preparation of standard curve

For analysis of Azithromycin dihydrate released, a method reported by Abdul-Wahab El-Rjoob *et al*. (2008) was used. Accurately weighed quantity (10mg) of Azithromycin dihydrate was transferred to 100mL volumetric flask. It was dissolved in 10mL methanol. Then volume was made up with Phosphate buffer pH 7.4. This gave concentration of 100μg/mL. From this 1, 2, 3, 4, 5 and 6mL were taken into 10mL volumetric flask. In each flask 1mL of 0.001 M $FeSO_4.7H_2O$ in methanol was added and volume was made with phosphate buffer pH 7.4 and mixed. Solutions were incubated at 25ºC for 1h. The dilutions prepared were in concentration range from 10-60μg/mL. Spectrum was recorded from 500 to 200nm (UV- visible spectrophotometer). Absorbances were recorded at 281nm against solvent blank.

***In-vitro* diffusion study through synthetic membrane**

For *in vitro* diffusion, procedure reported by Mali *et al*. (2011) was used. The micro test tube (1mL) having 6.5mm internal diameter was used as donor compartment for in vitro diffusion study. 0.1mL of formulation was placed in micro test tube in solution form. The pretreated synthetic membrane (high media dialysis membrane, 20μm pore size) was mounted carefully on the rim of the micro test tube. The formulation was allowed to form gel then donor compartment placed in receptor compartment which contained 3mL Phosphate Buffer (7.4 pH). The whole diffusion assembly was then placed in the thermostatically controlled orbital shaking incubator set at 37ºC and 70 rpm. 0.5mL aliquots were withdrawn carefully from the receptor compartment at different time intervals and were replaced immediately with the same volume of fresh PBS (maintained at 37ºC). This was transferred to 10mL volumetric flask. To it, 1mL methanol, 1mL 0.001M $FeSO_4.7H_2O$ in methanol were added and diluted upto the mark with Phosphate buffer pH 7.4 and incubated at 25ºC for 1h in orbital shaking incubator. The absorbances of samples were measured at 281.0 nm. Azithromycin dihydrate concentration was determined by using standard curve.

RESULTS AND DISCUSSION

Test for appearance/clarity

Formulations were observed against dark and white background visually. All the formulations were clear and transparent solutions.

Sol-Gel transition temperature

The gelling properties of formulations containing Poloxamer 407 alone or in combination with different viscosity increasing agents are shown in Table 1. Formulations formed gels between 33ºC to 37ºC.

Gelling time

The formulations were selected on the basis of gelling time between 30 to 90 sec. (++) for further study. The reason behind this is that the formulation applied as solution should

Table 1: Formulations details and gelling parameters of different formulations*

Formulation code	Concentration (%w/v)Poloxamer 407	Methocel K100M	Carbo pol 974	Gelling time at 37^{0}C**
P1	15	-	-	-
P2	16	-	-	-
P3	17	-	-	+ +
P4	18	-	-	+ +
P5	19	-	-	+ +
P6	20	-	-	+ + +
PH K100M1	18	0.1	-	+ +
PH K100M2	18	0.2	-	+ +
PH K100M3	18	0.5	-	+ +
PH K100M4	18	1.0	-	+ +
PH K100M5	18	1.5	-	+ + +
PH K100M6	18	2.0	-	+ + +
PH K100M7	19	0.1	-	+ +
PH K100M8	19	0.2	-	+ +
PH K100M9	19	0.5	-	+ +
PH K100M10	19	1.0	-	+ + +
PH K100M11	19	1.5	-	+ + +
PH K100M12	19	2.0	-	+ + +
PCA1	18	-	0.1	+ +
PCA2	18	-	0.2	+ +
PCA3	18	-	0.3	+ +
PCA4	18	-	0.4	+ +
PCA5	18	-	0.5	+ +
PCA6	19	-	0.1	+ +
PCA7	19	-	0.2	+ + +
PCA8	19	-	0.3	+ +
PCA9	19	-	0.4	+ + +
PCA10	19	-	0.5	+ + +

*all formulations contained 1% Azithromycin dihydrate and 0.1 % citric acid, 0.1% methyl paraben and 0.5% w/v sodium chloride ; **- no gel formation; + gelling time 90-300 sec.; + + gelling time 30-90 sec.; + + + gelling time less than 30 sec.

form gel between 30 to 90 sec. in ear. If formulation gels in less than 30 sec.; it may form gel before reaching tympanic membrane. If formulation takes more than 90 sec. to form gel then the patient needs to keep head inclined for longer time. Formulations P4, P5, PHK100M8 and PCA6 (having gelling time between 30-90 sec.) were selected for *in-vitro* diffusion study.

Determination of pH of formulations

The pH of formulations was in the range 6.5-7.4. This indicated that the pH of formulations were suitable for administration in the ear.

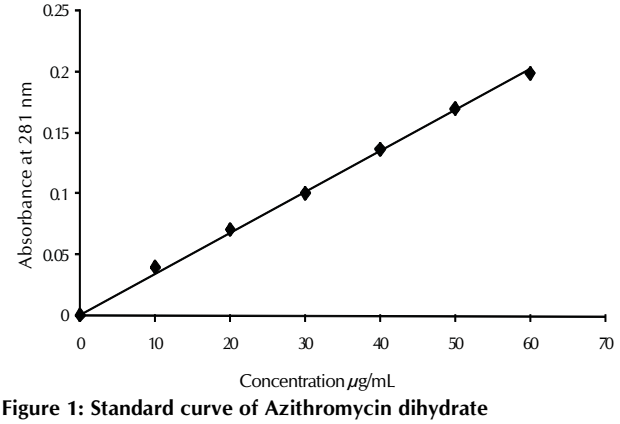

Figure 1: Standard curve of Azithromycin dihydrate

Rheological studies

Effect of poloxamer 407 concentration

As the concentration of Poloxamer 407 was increased in 18%w/v and 19% w/v, viscosity of formulation increased (Table 2). All gel formulations exhibited pseudoplastic (shear thining) flow behavior. Fig. 2 shows typical rheogram of Poloxamer 407 gels. Similar results were reported by Mali *et al*. (2011).

Effect of additives

Table 2: Viscosity and average cumulative % drug release of selected formulations

Formulation code	Viscosity of solution *(cps)	Viscosity of gel* *(cps)	Diffusion through synthetic membrane	
			Avg.± SD cumulative% drug Release after 3 h***	Avg.± SD drug cumulative % Release after 8 hr***
A	-	-	98.41± 0.45	-
P4	50	65000	32.17±0.2	87.12±0.7
P5	70	75200	33.90±2.1	81.19±0.2
PHK100M8	130	72500	23.48±2.5	69.50±0.2
PCA6	105	71550	23.15±0.7	71.31±0.4

A = Azithromycin dihydrate (1%w/v) Solution without any polymer; *Viscosity of formulation in solution form using small sample adaptor spindle (S21) at 10 rpm.; **Viscosity of formulation in gel form using T-bar spindle (S93) at 10 rpm.; *** n = 3

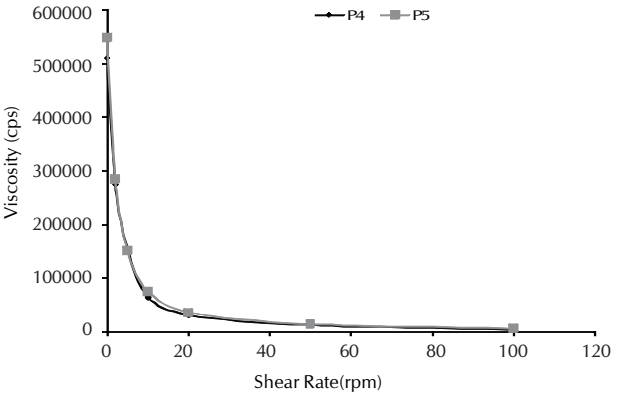

Figure 2: Viscosity–shear rate graph of medicated gel containing 18%w/v (P4) and 19%w/v (P5) of Poloxamer 407 (n = 3) by Brookfield viscometer (spindle no. S93)

To reduce the concentration of Poloxamer 407 and to obtain reasonable viscosity for the prepared formulations, some viscosity increasing agents were added to Poloxamer 407 gel containing 1% Azithromycin dihydrate and their effect on the rheological behaviour of prepared gels was investigated. The additives used in this study were Methocel K100M (0.1-2.0%w/v) and Carbopol 974 (0.1-1.0%w/v). The concentration of the additives used in this study maintained the thermoreversible sol-gel transition of the Poloxamer 407 formulations.

In-vitro release studies of Azithromycin dihydrate from different formulations

Effect of Poloxamer 407 concentration

Average cumulative percent Azithromycin dihydrate release (after 3 and 8 h.) from gel formulations is given in Table 2. The

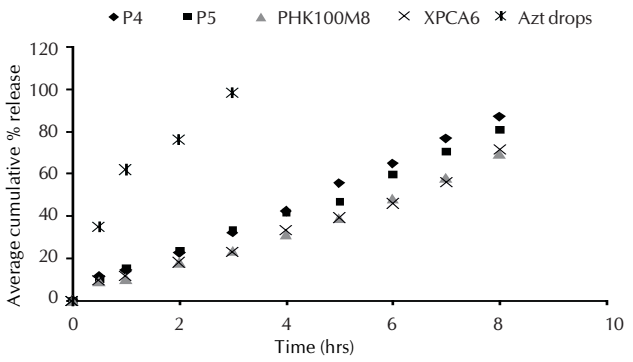

Figure 3: Average (n = 3) cumulative % release of Azithromycin dihydrate through synthetic membrane from formulations (i) A = solution without any polymer (ii) P4 = formulation containing Poloxamer 407 18% w/v (iii) P5 = formulation containing Poloxamer 407 19% w/v (iv) PHK100M8 = formulation containing Poloxamer 407 19% and 0.2%w/v Methocel K100M (v) PCA6 = formulation containing Poloxamer 19 % + 0.1% w/v Carbopol 974

rate of drug release was retarded with addition of Poloxamer 407 when compared with Azithromycin dihydrate solution. As Poloxamer concentration was increased from 18% w/v to 19% w/v average cumulative percent drug release decreased. This may be because of increased viscosity of formed gel.

Effect of additives

The drug release from formulations containing varying concentrations of Poloxamer 407 with varying concentration of viscosity increasing agents is shown in Fig. 2 and Table 2. The release of drug decreased with addition of viscosity increasing agent due to the hydrophilic nature of viscosity increasing agent. The polymer swelling increases the viscosity which may retard the rate of drug release.

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