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# DEVELOPMENT AND EVALUATION OF *IN-SITU* GELLING OTIC FORMULATIONS OF METRONIDAZOLE USING POLOXAMER

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**ABSTRACT**
The purpose of this study was to develop a sustained release in situ gel formulations for the treatment of chronic suppurative otitis media with the aim to increase drug concentration in the middle ear fluid. Poloxamer 407 was used as in situ gelling agent and Metronidazole was used as an active ingredient. In an attempt to reduce the concentration of Poloxamer 407 without compromising the *in situ* gelling capabilities, various viscosity increasing agents were added to Poloxamer 407 solution containing 0.75% Metronidazole. The formulations were evaluated for appearance / clarity, sol-gel transition temperature, gelling time, pH, spreadability, rheological characteristic and *in vitro* diffusion through synthetic as well as biological membrane. The viscosity of formulation increased as the concentration of Poloxamer 407 increased. The viscosity of formulation increased as the concentration of viscosity increasing agent was increased in the formulation containing constant amount of Poloxamer 407.

## INTRODUCTION

There are many diseases and disorders which can affect hearing of patient. Out of which the otitis media is the commonest disease which is caused by viral and bacterial infection. There are two types of otitis media (i) acute and (ii) chronic otitis media. Chronic suppurative otitis media (CSOM) is the result of an initial episode of acute otitis media (Jose, 2004) and caused by aerobic (e.g. *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Klebsiella* species) or anaerobic (e.g. Bacteroides, Peptostreptococcus, Proprionibacterium) (Jose, 2004, 2007; Yeo *et al.,* 2007). CSOM is characterized by a persistent discharge from the middle ear through a tympanic perforation. It is an important cause of preventable hearing loss, particularly in the developing world (Jose, 2004).

Prevalence surveys show that the global burden of illness from CSOM involves 65–330 million individuals with draining ears, 60% of whom (39–200 million) suffer from significant hearing impairment. CSOM accounts for 28,000 deaths and a disease burden of over 2 million DALYs. Over 90% of the burden is borne by countries in the South-east Asia (India) and Western Pacific regions, Africa, and several ethnic minorities in the Pacific rim. CSOM is uncommon in the Americas, Europe, the Middle East, and Australia. Among the South-East Asian countries, prevalence rates in Thailand ranged from 0.9 to 4.7% while the Indian prevalence of 7.8% is high. The CSOM can be treated with surgery. Mastoidectomy with or without tympanoplasty eradicates mastoid infection in about 80% of patients and may be combined with surgical drainage of otogenic abscesses elsewhere. However, such treatment is costly and does not always lead to satisfactory hearing improvement, and is inaccessible in many developing countries (Jose, 2004).

The systemic use of antibiotics results in certain disadvantages such as antibiotic resistance and adverse effects like nausea, diarrhoea and pseudomembranous colitis (Sato *et al*., 2008). Hence, the development of localized drug delivery systems for the release of antimicrobials in the ear is attempted. Local delivery of antimicrobials in CSOM may be carried out with ear drop and sprays (solution, suspension, emulsion), ear powders, ear washes, ear tampons, semisolid ear preparation (British Pharmacopoeia, 2010) and 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, 2009b and 2009c and 2010). 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; Simons *et al*., 2009; Hunt, 2009; Meyer and Thomas, 2009). Use of Metronidazole by oral and injectable application has been indicated in treatment of CSOM (Jokipii and Jokipii, 1981; Thore *et al*., 1985). CSOM was treated with systemic combinations of one of Clindamycin, Metronidazole, Lincomycin with Gentamycin out of these therapy the

Metronidazole plus Gentamycin therapy was more effective than other combinations (Rotimi *et al*., 1990). Anaerobic bacteria are most common causes of CSOM and their treatment with Metronidazole is recommended (Beeden and Willis, 1980; Browning *et al*., 1983). Complicated otitis media caused by Fusobacterium nacrophorum was succisively treated with intravenous Metronidazole and surgery (Giridharan *et al*., 2004). In paediatric CSOM, *Staphylococcus aureus* was the commonest aerobic while in adult CSOM, *Pseudomonas aeruginosa* was commonest one. Among anaerobes *peptostreptococcus* spp. was commonest. Anaerobes have higher sensitivity to Metronidazole (98.6%) than other antibiotics like Clindamycin and Chloramphenicol (Saini *et al*., 2005) and good uptake of Metronidazole was seen in the cholesteatomatous membranes of patients with chronic otitis media (Kristensen *et al*., 1986). Metronidazole was recommended for the treatment of periodontitis and rosecea by topical route and was found safe drug for topical administration (Sato *et al*., 2008; Yilmaz *et al*., 1996; McClellan and Noble, 2000; Schmadel and McEvoy, 1990).

So this study is devoted to formulation of Metronidazole ear drops which are solution at room temperature and form gel after administration in ear. For this purpose poloxamer 407 is used as a thermoreversible and sustained release polymer, Methocel E4M, Methocel K100M and Natrosol 250M are used as viscosity modifiers.

## MATERIALS AND METHODS

### Materials

Metronidazole I.P. was obtained from Aarti Drugs Limited Mumbai. Poloxamer 407, Natrosol 250M, Klucel HF were obtained from Signet Chemical Corporation Pvt. Ltd. Mumbai. Methocel E4M, Methocel K100M were obtained from Colorcon Asia Pvt. Ltd. Goa.

### Preparation of Poloxamer 407 Metronidazole formulations

The gels were made on w/v basis using the modified cold method (E1-Kamel, 2002). Poloxamer 407 was mixed with aqueous solution of Metronidazole and refrigerated at 4ºC and stirred periodically until a homogenous solution was obtained. Poloxamer 407 concentrations of 18, 19, 20%w/v were prepared.

Formulations containing Poloxamer 407 and viscosity increasing agents, Methocel E4M, Methocel K100M, Methocel K4M, Natrosol 250M and Klucel HF were also prepared. In this case, the required amount of selected viscosity increasing agent was dissolved in cold water except Methocel which was dissolved in hot water. Benzalkonium chloride was added as preservative in all formulations. The pH of formulation was in the range of 5.0-7.0. All formulations contained Metronidazole (0.75% w/v) and BKC (0.01 % w/v) Table 1 gives formulation details.

### Evaluation of formulations

### Test for 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 and gelling time

Sol-Gel transition temperature was measured for a solution in test tube which was put in low temperature cryostat bath and temperature was increased at rate of 1ºC every 5 minutes from 33ºC to 40ºC. Test tube was observed for formation of viscous gel.

The gelling time was measured by using glass plate with slope same as ear slope and maintained at 37ºC ± 0.5ºC temperature (Simons *et al*., 2009). The individual otic formulations (200$\mu$L) 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 in 90-300 sec. 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 gel formation in less than 30 sec.

### Determination of pH of formulations

The pH 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 24 hr at room temperature.

### Measurement of spreadability

The spreadability of otic formulations was measured by using modified glass assembly in which warm water (37ºC ± 3ºC) was filled. The slope of assembly was 45° and the ear membrane was pasted on the assembly and then 4 drops (200$\mu$L) of formulation were placed on the membrane. The distance travelled by the individual formulation before gelling was measured.

### Rheological study

The viscosity was estimated by using Brookfield viscometer (RVDV-II +Pro) for otic solutions of Metronidazole at temperature below 10ºC by using small sample adaptor spindle no. S 21 as well as the preformed gels of these solutions at temperature 35-37ºC by using spindle no. S 93 at 1-100 RPM. Evaluations were conducted in triplicate.

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

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

Fig. 1 depicts the *in vitro* diffusion assembly. The micro test tube (1mL) having 6.5mm internal diameter was used as donor compartment for *in vitro* diffusion study. 0.2 mL of formulation was placed in micro test tube in solution form. The pretreated synthetic membrane (high media dialysis membrane, 20$\mu$m 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 3 mL Phosphate Buffer (7.4 pH). The whole diffusion assembly was then placed in the thermostatically controlled orbital shaking incubator set at 37ºC ± 0.5ºC and 70 rpm. 0.1 mL 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 37 ± 0.5ºC). The samples were diluted suitably and the release

of Metronidazole was analyzed by UV spectrophotometer at 320.0 nm.

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

Goat ear with tympanic membrane was procured from a nearby slaughter house and carefully transported to laboratory in an airtight container containing cold (2-8ºC) normal saline solution. Intact tympanic membrane with adjacent external auditory metus (EAM) was separated and washed with cold saline. Tympanic membrane with adjacent EAM was preserved in freshly prepared PBS (7.4 pH) maintained at 2-8ºC. 0.2 mL of formulation was placed in EAM with intact tympanic membrane in solution form and allow to form gel, then it was placed in receptor compartment which contained 3 mL PBS (7.4 pH). The whole diffusion assembly (Fig. 2) was then placed in the thermostatically controlled orbital shaking incubator set at 37ºC± 0.5ºC and 70 rpm. 0.1 mL 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 37 ± 0.5ºC). The samples were diluted suitably and the release of Metronidazole was analyzed by using UV spectrophotometer at 320.0 nm

## RESULTS AND DISCUSSION

**Test for appearance/ clarity**

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

**Sol-Gel transition temperature and gelling time**

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 33°C to 37°C. 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 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. Hence the formulations P2, P3, PH3, PH4, PH5, PH K2, PH K3, PH K4, PH K5, PN3, PN4, PN5 and PK6 (having gelling time between 30-90 sec.) were selected for further evaluation.

**Determination of pH of formulations**

The pH of formulations was in the range 6.7-7.0. This indicated that the pH of formulations were suitable for administration in the ear.

**Measurement of spreadability**

The spreadability of selected formulations is shown in Table 2. The formulations showed sufficient spreadability (more than 1.5cm) to reach tympanic membrane (the length of external ear is 1.5 cm).

**Rheological studies**

**Effect of Poloxamer 407 concentration**

As the concentration of Poloxamer 407 was increased from 19%w/v to 20%w/v, viscosity of formulation increased (Table 2). All gel formulations exhibited pseudoplastic (shear thining) flow behavior. Fig. 3 shows typical rheogram of Poloxamer 407 gels.

**Effect of additives**

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 0.75% Metronidazole and their effect on the rheological behavior of prepared gels was investigated. The additives used in this study were Methocel E4M (0.5-1.5%w/v), Methocel K100M (0.5-1.5%w/v), Natrosol 250M (0.5-1.5%w/v) and Klucel HF (0.5-1.5%w/v). The concentration of the additives used in this study maintained the thermoreversible sol-gel transition of the Poloxamer 407 formulations.

The viscosity of solution as well as gel was increased as the concentration of viscosity enhancing agents increased with constant concentration of Poloxamer 407 (PF-127) (Table 1and 2).

***In-vitro* release studies of Metronidazole from different formulations**

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

Figure 4 shows release of Metronidazole through synthetic membrane.

**Effect of Poloxamer 407 concentration**

Average cumulative percent Metronidazole release (after 4 and 8 hr.) from gel formulations is given in Table 2. The rate of drug release was retarded with addition of Poloxamer 407 when compared with Metronidazole solution. As Poloxamer concentration was increased from 19% to 20% average

**Figure 1: *In vitro* diffusion assembly**

**Figure 2: (A) goat external ear with tympanic membrane. (B) *In vitro* diffusion through biological membrane**

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

| Formulation code | Concentration (% w/v) Poloxamer 407 | Methocel E4M | Methocel K100M | Natrosol 250M | Klucel HF | Sol-gel transition temperature | Gelling time at 37ºC |
|---|---|---|---|---|---|---|---|
| P1 | 18 | - | - | - | - | 37ºC | + |
| P2 | 19 | - | - | - | - | 36ºC | + + |
| P3 | 20 | - | - | - | - | 34ºC | + + |
| PH1 | 17 | 0.5 | - | - | - | 37ºC | + |
| PH2 | 17 | 1.0 | - | - | - | 37ºC | + |
| PH3 | 17 | 1.5 | - | - | - | 36ºC | + + |
| PH4 | 18 | 0.5 | - | - | - | 36ºC | + + |
| PH5 | 18 | 1.0 | - | - | - | 35ºC | + + |
| PH6 | 18 | 1.5 | - | - | - | 34ºC | + + + |
| PH K1 | 18 | - | 0.5 | - | - | 37ºC | + |
| PH K2 | 18 | - | 1.0 | - | - | 36ºC | + + |
| PH K3 | 18 | - | 1.5 | - | - | 34ºC | + + |
| PH K4 | 19 | - | 0.5 | - | - | 35ºC | + + |
| PH K5 | 19 | - | 1.0 | - | - | 34ºC | + + |
| PH K6 | 19 | - | 1.5 | - | - | 33ºC | + + + |
| PN1 | 18 | - | - | 0.5 | - | 37ºC | + |
| PN2 | 18 | - | - | 1.0 | - | 37ºC | + |
| PN3 | 18 | - | - | 1.5 | - | 36ºC | + + |
| PN4 | 19 | - | - | 0.5 | - | 36ºC | + + |
| PN5 | 19 | - | - | 1.0 | - | 34ºC | + + |
| PN6 | 19 | - | - | 1.5 | - | 33ºC | + + + |
| PK1 | 18 | - | - | - | 0.5 | 37ºC | + |
| PK2 | 18 | - | - | - | 1.0 | 37ºC | + |
| PK3 | 18 | - | - | - | 1.5 | 37ºC | + |
| PK4 | 19 | - | - | - | 0.5 | 37ºC | + |
| PK5 | 19 | - | - | - | 1.0 | 37ºC | + |
| PK6 | 19 | - | - | - | 1.5 | 36ºC | + + |

*all formulations; + gelling time 90-300 sec.; + + gelling time 30-90 sec.; + + + gelling time less than 30 sec.

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 concentration of Poloxamer 407 with varying concentration of viscosity increasing agents shown in Fig. 4 and Table 2. The release of drug decreased as the concentration of viscosity increasing agent increased due to the hydrophilic nature of viscosity increasing agent. The polymer swelling increases the viscosity which may retard the rate of drug release. The order of drug release retardation was PH5 (PF 18% with Methocel E4M 1%) > PH K5 (PF 19% with Methocel K100M 1%) > PH K4 (PF 19% with Methocel K100M 0.5%) > PN5 (PF 19% with Natrosol 250M 1%) > PK6 (PF 19% with Klucel HF 1.5%). The slowest rate of drug release was obtained from the formulation containing Poloxamer 407 18% and Methocel E4M 1%

***In-vitro* diffusion through biological membrane**

Fig. 5 shows release profiles of Metronidazole through biological membrane. The diffusion of Metronidazole through goat tympanic membrane (ear drum) was slower than its diffusion through synthetic membrane (Fig. 4 and 5). The rate

**Table 2: Spreadability, viscosity and average cummulative % drug release of selected formulations**

| Formulation code | Spreadability [Distance travelled before gelling (cm)] | Viscosity of solution*(cps) | Viscosity of gel** (cps) | Avg. ±SD cummulative % drug Release after 4 hr*** | Avg. ±SD cummulative % drug Release after 8 hr*** |
|---|---|---|---|---|---|
| M | - | - | - | 79.06±0.66 | - |
| P2 | 3.0 | 40 | 38500 | 42.41±0.57 | 75.32±0.29 |
| P3 | 1.5 | 45 | 44700 | 36.54±0.53 | 70.80±0.41 |
| PH3 | 2.6 | 90 | 42200 | 53.98±0.53 | 73.58±0.59 |
| PH4 | 2.4 | 65 | 24700 | 37.81±0.41 | 65.41±0.38 |
| PH5 | 2.3 | 75 | 42800 | 38.02±0.46 | 61.78±0.69 |
| PH K2 | 4.8 | 50 | 36300 | 53.72±0.49 | 72.94±0.52 |
| PH K3 | 4.5 | 95 | 44500 | 47.64±1.03 | 71.39±0.46 |
| PH K4 | 2.2 | 45 | 40100 | 44.69±0.48 | 63.66±0.63 |
| PH K5 | 2.0 | 65 | 55600 | 38.48±0.54 | 62.25±0.67 |
| PN3 | 2.5 | 90 | 32100 | 73.10±0.29 | 93.39±0.81 |
| PN4 | 2.7 | 75 | 32600 | 48.16±0.46 | 74.15±0.44 |
| PN5 | 2.4 | 80 | 47200 | 39.75±0.72 | 63.74±1.05 |
| PK6 | 2.6 | 150 | 47200 | 38.34±0.36 | 71.25±0.71 |

M = Metronidazole (0.75%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

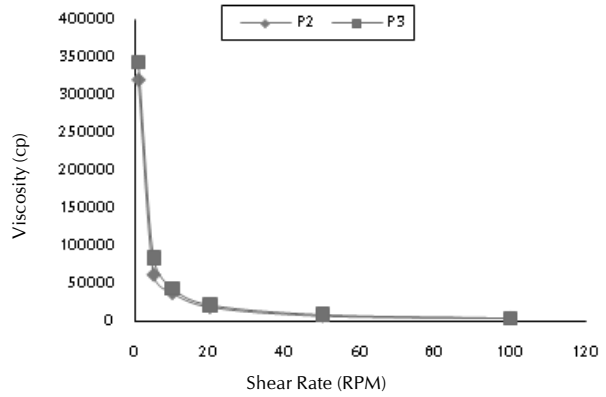


**Figure 3: Viscosity–shear rate graph of medicated gel containing 19%w/v (P2) and 20%w/v (P3) of Poloxamer 407 (n = 3) by Brookfield viscometer (spindle no. S93)**

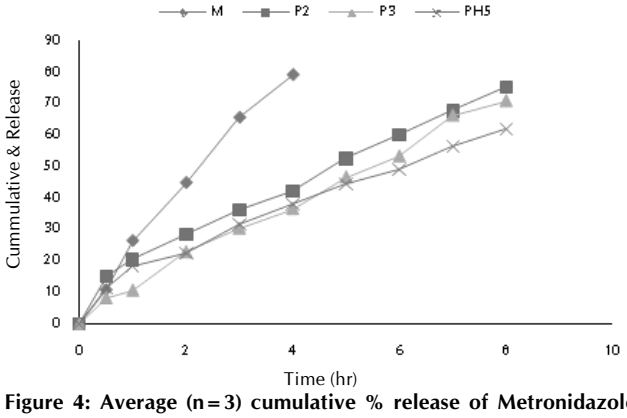


**Figure 4: Average (n = 3) cumulative % release of Metronidazole through synthetic membrane from formulations** (i) M = solution without any polymer (ii) P2 = formulation containing Poloxamer 407 19% w/v (iii) P3 = formulation containing Poloxamer 407 20% w/v (iv) PH5 = formulation containing Poloxamer 407 18% w/v and Methocel E4M 1% w/v

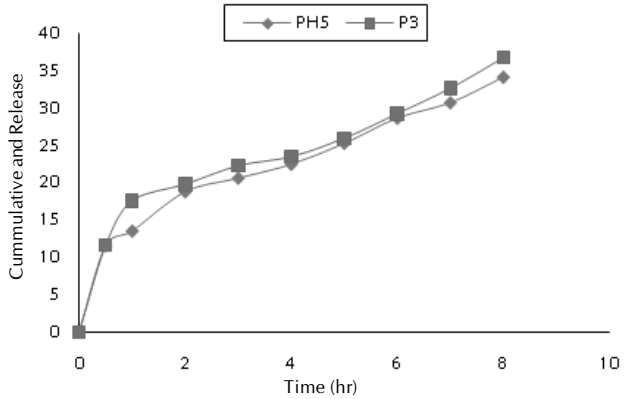


**Figure 5: Average (n = 3) cumulative % release of Metronidazole through biological membrane from gel formulations** (i) P3 = containing Poloxamer 407 20% (ii) PH 5 containing Poloxamer 407 18% & with Methocel E4M 1%

of release of Metronidazole from gel formulations through biological membrane was in the order PH5 < P3. Hence, the formulation containing Methocel E4M (PH5) is considered to be most effective in sustaining the release of Metronidazole.

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