AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Preparation and Evaluation of *In Situ* Gelling Nimesulide Loaded Liquid Suppository using Poloxamer and Menthol

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ABSTRACT

The purpose of present work was to prepare a nimesulide-loaded liquid suppository using menthol and to study the effects of various penetration enhancers such as neem oil, tulsi oil and ethanol on the cumulative percentage release of nimesulide. The physicochemical properties such as gelation temperature and gel strength of various formulations composed of nimesulide, menthol and poloxamer 188 were investigated. The *in vitro* study of nimesulide delivered by the liquid suppositories composed of poloxamer 188, menthol and various concentrations of penetration enhancers were performed. The *in vitro* studies were performed using Keshary-Chein diffusion cell and Small wonder (Lyzer apparatus). The cumulative percentage release of nimesulide of each formulation were calculated and compared with the other formulations. The liquid suppository formulations containing neem oil 2% and tulsi oil 2% showed excellent release rate as compared to other formulations.

Keywords: Nimesulide, Neem oil, Tulsi oil, Ethanol, Liquid Suppository, *In vitro* release studies.

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Received 12 May 2014, Accepted 21 May 2014

Please cite this article in press as: Walia S *et al* Preparation and Evaluation of In Situ Gelling Nimesulide Loaded Liquid Suppository using Poloxamer and Menthol
. American Journal of PharmTech Research 2014.

INTRODUCTION

A conventional suppository is a semi solid dosage form which melts in the rectum at body temperature. The conventional suppository causes discomfort and sometimes refusal to the patient, possibly lowering patient compliance. Sometime semi solid suppository reach the end of the rectum and the drug delivered by them undergoes first pass metabolism[1].Therefore at the place of conventional suppository, an attempt was made to develop a liquid rectal dosage form which forms a gel at body temperature, has suitable gel strength does not leak out of the anus after administration and has suitable bio adhesive force so as not reach at the end of the colon[2].Liquid suppositories had been developed either to improve the local effect or to enhance the drug absorption. Poloxamer are the base of the liquid suppositories. Poloxamer solutions are known to exhibit phenomenon of reverse thermal gelation remaining as solution at low temperature (4^0C) and gelling upon increasing the temperature to 25-35^{0}C[3].

Nimesulide[4] is a non-steroidal anti-inflammatory analgesic agent given orally or rectally on a twice daily basis in a number of inflammatory and pain states, reducing the pain, fever and inflammatory symptoms of chronic rheumatoid arthritis or osteoarthritis. In a number of comparative studies, nimesulide has also been shown to be more effective than piroxicam, paracetamol, benzydamine or naproxen, serratiapeptidases, ketoprofen, mefenamic acid in following conditions respectively osteoarthritis, respiratory tract inflammation, otorhinolaryngological disease, postoperative or dental pain, trauma and phlebitis, postoperative dental pain dysmenorrhea[5]. Due to so much advantageous effects of nimesulide, nimesulide was incorporated in to the liquid suppository as an anti-inflammatory drug used against hemorrhoids and effect of three penetration enhancers[6,7](neem oil[8], tulsi oil[8] and ethanol) were studied.

MATERIALS AND METHODS

Materials

Poloxamers (P 407 and P 188) were purchased from the Sigma Aldrich,Menthol was purchased from Merck and Nimesulide was obtained as gift sample from Abhinanadan Rasayan Mumbai Ltd.

Method

Preparation of Liquid Suppository

Cold method[3] is the method of choice for formulation of liquid suppository and the formulation was done in the following phases.

A. **Achieving of required gelation temperature**- Precise gelation temperature between 34.4-26.8^{0}C was obtained by varying the concentration of Poloxamer 407 and Poloxamer 188.

B. **Solubility enhancement for nimesulide**- Nimesulide shows low solubility in water therefore varying concentration of menthol was used to enhance solubility of nimesulide.

C. **Final adjustment of polymer concentration**- The addition of menthol to the liquid suppository formulation caused the variation in the gelation temperature of the formulation, therefore again the ratio of P 188 was adjusted to set the gelation temperature within the range.

D. **Optimization of penetration enhancer**- Three penetration enhancers (neem oil, tulsi oil and ethanol) were used in different concentrations and the best of these were selected as final formulation.

Details of the above phases are given in result and discussion section.

Evaluation of the Liquid suppository

Gelation temperature

For assessing gelation temperature the tube tilting method was used[9]. In this 2ml of aliquot was transferred to test tubes, immersed in a water bath 4 ^{0}C and sealed with the aluminum foil. The temperature of the water bath was increased in increments of 1^{0}C and left to equilibrate for 5 minutes at each new setting. The samples were than examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting through 90^{0}C.

Gel strength

Liquid suppository weighing 50 g was put into 100 ml graduated cylinder and gelation was accomplished using thermostat at 36.5^{0}C. The apparatus used for measuring the gel strength weighed 35 g and was placed on to the top of liquid suppository. The gel strength which is considered as the viscosity of the liquid suppository at physiological temperature was determined by the time (sec) the apparatus took to sink 5 cm down through the liquid suppository[10].

***In vitro* release studies**

The *in vitro* studies were carried out using two apparatus i.e. Keshary-Chein diffusion cell and Small wonder. 7 formulations which were evaluated for *in vitro* release using Keshary-Chein diffusion cell. The 1 ml of the suppository was put on the cellophane membrane (0.45 µm, Millipore)and then the membrane was affixed in the diffusion cell. After the interval of 30 minutes the 3 ml of the sample was withdrawn from the diffusion cell and the cells were again replenished with fresh phosphate buffer (pH 7.4) 3 ml. the sample withdrawn was diluted 10 times and was analyzed on UV spectrophotometer (Shimadzu, UVIDEC-1600, Japan). The sampling procedure was same for the both diffusion cells.

Keshary-Chein diffusion cell

For the *in vitro* study the Keshary-Chein diffusion cell(Figure1) was used **.**In Keshary-Chein diffusion cell 1 ml of liquid suppository containing 50 mg of Nimesulide was spread uniformly on the cellophane membrane. In Keshary-Chein diffusion cell 55 ml of phosphate buffer 7.4 was used as receptor compartment. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.5^{0}C. The solution on the receptor side was stirred by externally driven Teflon coated magnetic stirrer using a small bead. Sample of 3ml were withdrawn at different time interval and replacement was done with 3ml of fresh buffer. The drug concentration on the receptor fluid was determined spectrophotometrically against blank.

Small wonder apparatus (Lyzer apparatus)

In Lyzer apparatus (Figure 2) 1 ml of liquid suppository containing 50 mg of Nimesulide was spread uniformly on the membrane. Lyzer apparatus 55 ml of phosphate buffer 7.4 was used as receptor compartment. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.5°C. The solution on the receptor side was stirred by externally driven Teflon coated magnetic stirrer using a small bead. Sample of 3 ml were withdrawn at different time interval and replacement was done with 3 ml of fresh buffer. The drug concentration on the receptor fluid was determined spectrophotometrically against blank.

Drug release data were appropriately corrected for loss of drug and receptor medium volume during sampling by replacement using the following eq. (1).

$$Ci = Ai + \left(\frac{Vs}{Vt}\right).\sum_{t=1}^{n-1} Ai\left(\frac{Vt}{Vt-Vs}\right) \qquad (1)$$

Where, Ci is the corrected absorbance of ith observation, Ai is the observed specific absorbance, Vs is the sample volume, and Vt is the total volume of dissolution medium[11].

Figure1:Keshary-Chein diffusion CellFigure2: Small wonder apparatus

RESULTS AND DISCUSSION

Preparation of liquid suppository

The first phase of the formulation comprised of addition of a definite concentration poloxamer 407 which showed gelation property at lower temperatures (below 25^{0}C) in 5 ml of distilled water. The

formulation was prepared by measuring the required quantity of poloxamer 407 in varying concentration and then adding it into the test tubes containing 5 ml of water and then it was stored in room temperature where temperature existed below 10^0C. The table1 consisting varying concentrations are as under.

Table 1: Gelation property varying with different concentrations of poloxamer 407

Formulation	Poloxamer 407 (mg)	Observation
F 1	400	No gelation property
F 2	600	No gelation property
F 3	800	No gelation property
F 4	1000	No gelation property
F 5	**1200**	**Gelation property**
F 6	1400	Gelation property

From the above table formulation no. 5 was selected and was treated with the varying concentrations of poloxamer 188. The poloxamer 188[12] is commonly used as a solubility enhancer and in the liquid suppository formulations it is used to modify the gelation temperature. Keeping the concentration of poloxamer 407 unchanged, varying concentrations of poloxamer 188 were added and new formulations were developed which showed variable gelation temperatures. The varying gelation temperature was due to the varying concentration of poloxamer 188. The gelation temperatures are as shown in table 2.

Table 2 Varying gelation temperatures with different concentrations.

Formulation	P 407 (mg)	P188 (mg)	Gelation Temp.
F 7	1200	0	Below 25^0C
F 8	1200	100	At 25^0C -26^0C
F 9	**1200**	**200**	**At 35^0C -36^0C**
F 10	1200	400	At 37^0C -38^0C
F 11	1200	600	At 39^0C -40^0C

The F9 was selected. Then nimesulide was added to the poloxamer 188 solution, agitated and stored in the refrigerator for half an hour. Then after some time the weighed quantity of the poloxamer 407 was added to the test tube and test tube was agitated for 15 minutes and then stored in refrigerator for 12 to 18 hours. After the time period the test tube containing the drug was observed, it showed sedimentation of the drug at the bottom of the test tube which was not acceptable.

Therefore it was proposed to develop a dispersion of the drug using menthol or to enhance the solubility of the drug. Keeping the concentration of poloxamer 407, poloxamer 188 and drug constant, the concentration of the menthol was varied. The different ratios of menthol and drug were taken in the mortar and pestle and the concentration of poloxamer 188 was kept constant,

were triturated for half an hour and then simultaneously 5 ml of the distilled water was added to the mixture in the mortar. After the time period of half an hour the solution was transferred in to the test tubes and stored in the refrigerator till its temperature drops below 10 ^{0}C, then after some time the weighed quantity of the poloxamer 407 was added to all the formulations and were agitated for 15 minutes. Finally all were stored in refrigerator for 12 to 18 hours. The results obtained were as in table 3

Table 3: Represents varying ratios of Nimesulide and Menthol.

F. No.	P 188	Menthol	Drug	Water	Ratios	Observation
F 12	200	50	50	5	1:1	Disper. not formed
F 13	200	100	50	5	1:2	Disper. not formed
F 14	200	150	50	5	1:3	Disper. not formed
F 15	200	200	50	5	1:4	Disper. not formed
F 16	**200**	**250**	**50**	**5**	**1:5**	**Disper. Formed**

The F 16 formulation showed the satisfactory dispersion of the nimesulide in the liquid suppository formulation but it was yet not acceptable because due to the addition of the menthol in the formulation it affected the gelation temperatures of the F 16 formulation and the gelation temperature showed by the F 16 was not with in the required range. Therefore again the ratio between the poloxamers was adjusted. Keeping the concentration of poloxamer 407 constant, the concentration of poloxamer 188 was varied as shown in table 4

Table 4: Represents varying poloxamer 188 concentrations.

F. No.	P 407	P 188	Menthol	Drug	Water	Observation
F 17	1200	200	250	50	5	Gelation at 25^{0}C
F 18	1200	400	250	50	5	Gelation at 25^{0}C
F 19	1200	600	250	50	5	Gelation at 33^{0}C -34^{0}C
F 20	**1200**	**800**	**250**	**50**	**5**	**Gelation at 35^{0}C -36^{0}C**
F 21	1200	1000	250	50	5	Gelation at 38^{0}C -39^{0}C

Table 5: Represents the Percentage Formula of various formulations

Name (%w/w)	F 22	F 23	F 24	F 25	F 26	F 27	F 28
P 407	24	24	24	24	24	24	24
P 188	16	16	16	16	16	16	16
Nimesulide	1	1	1	1	1	1	1
Menthol	5	5	5	5	5	5	5
Neem oil	0	2	0	0	4	0	0
Tulsi oil	0	0	2	0	0	4	0
Ethanol	0	0	0	2	0	0	4
Water (QS)	100	100	100	100	100	100	100

The formulation F 20 was selected as the main formulation to which various penetration enhancers were added in concentrations of 2% and 4%. On the addition of neem oil, tulsi oil and ethanol in concentration of 2% and 4% showed minor fluctuation in gelation temperature of these formulations which was not considerable. The table 5 represents the percentage formula of the formulation containing 2% and 4% neem oil, tulsi oil and ethanol and the formulation containing no penetration enhancer.

Gelation temperature

Gelation temperature is the temperature at which the liquid phasemakes a transition to gel. For liquid suppository, gelation temperature ranged between 34^0C -36^0C. If it is lower than the 33^0C gelation occurs at room temperature leading to difficulty in manufacturing, handling and administering, while higher than 36^0C, the liquid suppository would remain as a liquid within the body resulting in leakage from the anus. When the poloxamer 188 and poloxamer 407 were mixed in ratio of 2:3 they showed the gelation at temperature 36^0C. This ratio of poloxamers was set only after studying the effects of the individual ingredient on the gelation temperature of the formulation. The gelation temperature found was between 34^0C and 36^0C of all the formulations as shown in Table 6.

Table 6: Gelation temperature and Gel strength of various formulations.

Formulation	Gelation temperature (^{0}C)	Gel strength (sec)
F 22	35.1±0.15	25
F 23	35.2±0.11	31
F 24	36.9±0.15	22
F 25	34.3±0.21	21
F 26	33.6±0.45	29
F 27	34.6±0.26	20
F 28	33.3±0.39	18

It was noticed that the formulation containing the 2% of the penetration enhancers showed the minimum deviation but as the concentration of the penetration enhancers was increased from the 2% to 4% then there was change in the gelation temperature, the gelation temperature decreased slightly. The gelation temperature lowering could be explained by their ability to bind to the polyoxyethylene chains of the Poloxamer molecules which promotes dehydration and causes an increase in entanglement of adjacent molecules with more extensive intermolecular hydrogen bonding thus producing gelation at lower temperature[13]. The formulation containing 6% of penetration enhancers were also formulated but they showed gelation temperature below 30^0C therefore these formulations were not considered for study.

Measurement of gel strength

When the liquid suppository is developed, the gel strength is the most important characteristic property of the dosage form because it allows the easy insertion of the suppository and no leak from the anus. At high gel strength, it is difficult to insert the suppository and if the suppository has lower gel strength then suppository would get leaked from the anus. It is reported that the optimal liquid suppository must have suitable gel strength (10-50 s) [3].

The gel strength of the various formulations is as shown in Table 6. From the data obtained it was concluded that the addition of the penetration enhancers to the formulation did not significantly affect the gel strength.

***In vitro* drug release studies**

The seven formulations which were evaluated for the *in vitro* studies are as shown in table All the formulations were studied using the Keshary-Chein diffusion cell. The graphical representation of cumulative %releases of the drug obtained are displayed in figure 3

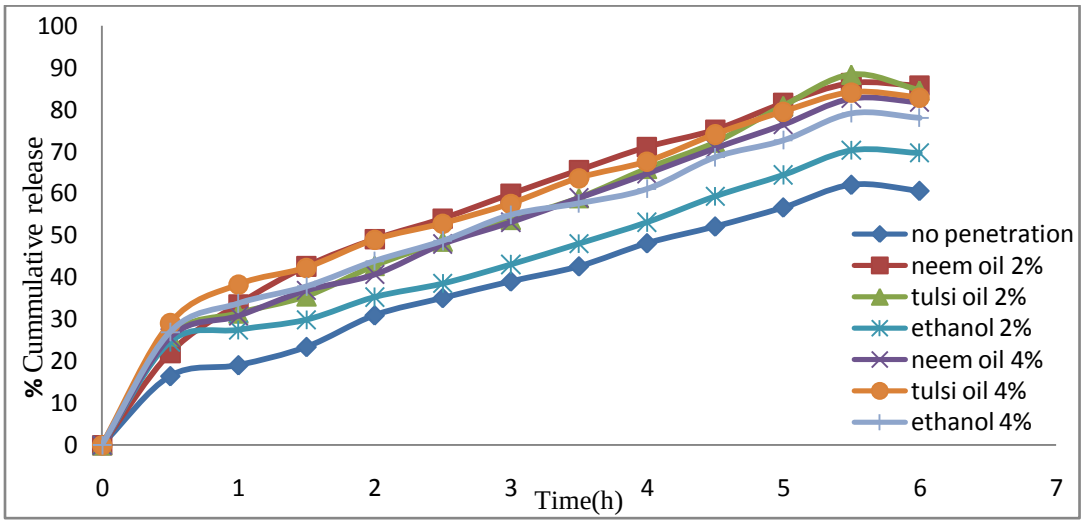

Figure 3: Cumulative % drug release of the formulations using Keshary-Chein diffusion cell

From the above results the best releases were found to be of F 23(86.3%) and F 24(88.3%). On the basis of these results, these formulations were selected for further drug release studies using Small wonder apparatus. The cumulative % release of formulations consisting of neem oil and tulsi oil are shown as under in table 7 & Figure 4

Table 7: Showing Cumulative % release of F 23 and F 24

S. No.	Time (hr)	Cumulative %release of Neem oil 2% (F 23)	Cumulative %release of Tulsi oil 2% (F 24)
1	0	0	0
2	0.5	25.86977648	27.58017
3	1	28.09329446	31.14869
4	1.5	35.92225462	36.94849
5	2	42.07580175	43.34694

6	2.5	47.12536443	47.79397
7	3	54.65889213	52.13605
8	3.5	61.98250729	60.6414
9	4	70.79105928	69.60933
10	4.5	81.77259475	82.89602
11	5	87.51409135	89.31778
12	5.5	93.37220603	94.8105

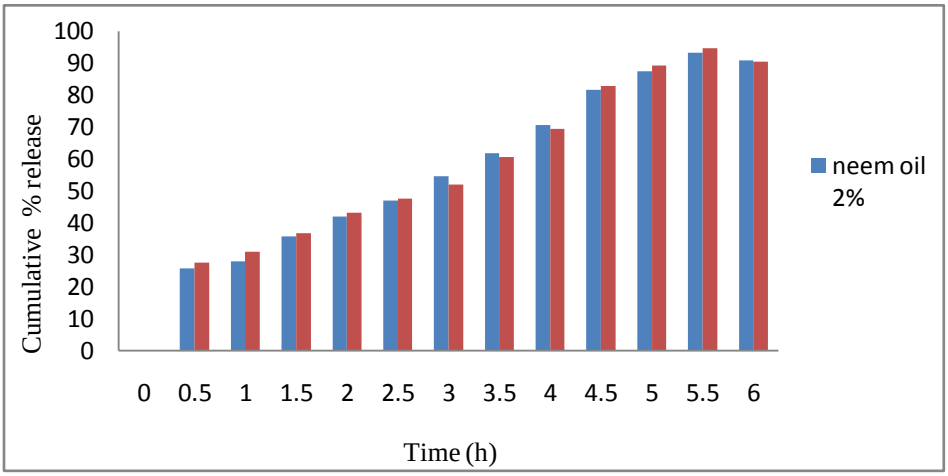

Figure 4: Represents the cumulative % release of F 23 and F 24

From the results obtained the formulation containing neem oil showed the 93 % release and tulsi oil showed 94% release on the Small wonder apparatus.

CONCLUSION

It is concluded that menthol plays a significant role in the formulation of liquid suppository as it enables the formation of dispersion of Nimesulide and increases the solubility of the drug in the formulation. The ratio of P 188 and P 407 plays a significant role in the maintenance of the gelation temperature. The F 23 and F 24 formulation showed the satisfactory results, when studied using the Keshary-Chein diffusion cell and Small wonder apparatus. Thus, the liquid suppository system with poloxamer 188 and menthol was found to be convenient and effective rectal dosage form for nimesulide along with neem oil and tulsi oil.

ACKNOWLEDGMENT

The authors are thankful to Abhinanadan Rasayan Mumbai Ltd. for providing nimesulide for completion of research work.

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