Journal of Drug Discovery and Therapeutics 1 (1) 2013, 30-36

RESEARCH ARTICLE

FORMULATION AND EVALUATION OF TRANSDERMAL GEL OF KETOROLAC TROMETHAMINE ALONG WITH NEEM OIL, TULSI OIL AND OLEIC ACID AS PENETRATION ENHANCERS.

[1]Dr. G. D. Gupta, [1]Prabhjot Singh Bajwa*, [2]Aman Sharma

[1]Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela, Ropar, Punjab, India

[2]Dept. of Pharmaceutical science, Guru Jambheshwar University of Science & Technology, Hisar

*Mr. Prabhjot Singh Bajwa, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela, Ropar, Punjab, India

ABSTRACT

The authors developed and evaluated a transdermal gel formulation of ketorolac tromethamine for the treatment of nociceptive somatic pain. The formulation was optimized for skin permeation enhancers, pH of the system, and dosage strength using *in vitro* techniques. Ketorolac tromethamine is a non-steroidal anti-inflammatory drug that belongs to class of heteroacetyl derivatives. In present study ketorolac tromethamine topical gel was formulated using Carbopol 940 and Carbopol Ultrez 10NF polymers and three penetration enhancers neem oil, tulsi oil and oleic acid were studied to see the permeation enhancement. Oleic acid was found to be the best penetration enhancer and the three different concentration of oleic acid were used 2%, 3% and 4% w/w to find the best concentration of oleic acid and the best concentration of oleic acid was found to be 3% w/w. Formulations were evaluated for drug content, pH, viscosity, spreadability and release. *In vitro* release studies were performed using Keshary-Chien diffusion cell. Release kinetic analysis was done to find the kinetics of drug release.

KEYWORDS: Ketorolac Tromethamine, Carbopol 940, Carbopol Ultrez 10NF, Topical gel

INTRODUCTION:

Non-steroidal anti-inflammatory drugs (NSAIDs) are established as first-line pharmacotherapy in the treatment of inflammation and pain associated with conditions such as rheumatoid arthritis, osteoarthritis, and musculoskeletal problems such as sprains and strains. Ketorolac tromethamine (± 5-Benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid) is a non-steroidal anti-inflammatory drug that belongs to class of heteroacetyl derivatives [1] . It is a non-selective cyclooxygenase (cox) inhibitor. The analgesic activity of ketorolac tromethamine is equivalent to morphine[2]. Oral administration of NSAID's is more prone to result into various complications (such as gastrointestinal injury, renal injury, hypertension, and platelet aggregation inhibition, the most common and severe are gastrointestinal complications, like mucosal erosion, ulcers, bleeding due to localization of high concentration of drugs in gastrointestinal lumen and cell membranes.[3] Parenteral administration too leads to distribution of these drugs throughout the body as well as into gastrointestinal tract by hepatobiliary secretion.[4] The Transdermal delivery system can be formulated to remove the above mentioned adverse effects: it avoids first-pass metabolism, it is easy to discontinue the administration whenever required[5]. Despite these advantages, only a limited number of drugs can be administered percutaneously, due to low skin permeability of most drugs through the skin. The stratum corneum was recognized as an excellent barrier against skin penetration to overcome these barrier properties various, vehicles, penetration enhancers, and electro-transport facilitated transdermal systems have been used[6]. Among various penetration enhancing methods used, use of chemical penetration enhancer is one of the most suited[7]. In present study penetration enhancers obtained from natural sources were employed to increase permeation of drug. Neem oil is a vegetable oil pressed from the fruits and seeds of neem (*Azadirachta indica)*. It comprises mainly triglycerides and large amounts of triterpenoid compounds [8]. Tulsi oil is oil extracted from tulsi plant (*Ocimum tenuiflorum*), it is an

*Corresponding author: Prabhjot Singh Bajwa,| Email: amansharma67@rediffmail.com,

aromatic plant in the family labiateae. It contains eugenol (volatile oil), ursolic acid (triterpenoid) and rosmarinic acid (phenylpropanoid). Other active compounds include caryophyllene, oleanolic acid, linoleic acid and linolenic acid[8-9]. Oleic acid is a monounsaturated omega-9 fatty acid found in various animal and vegetable fats[10]. In the present study various formulation were prepared and evaluated to optimize concentration of polymer and penetration enhancer for a successful novel transdermal drug delivery system.

EXPERIMENTAL:

MATERIAL AND METHOD:

Ketorolac tromethamine was obtained as gift sample from Oscar remedies Yamunanagar, Haryana, India. Carbopol Ultrez 10NF was obtained as gift sample from Lubrizol Advanced Material India Pvt. Ltd, Mumbai, India, Carbopol 940 was purchased from Loba chem. India. Other chemicals obtained were of analytical grade.

PREPARATION OF STANDARD CURVE:

Standard curve is prepared to study the release profile of formulations. Fifty mg of drug was dissolved in 100 mL of phosphate buffer pH 7.4. 20 mL of this solution was diluted up to 100 mL again with buffer. 20 mL of above solution was again diluted to 100 mL with buffer to get stock solution of 20µg/mL. Aliquots of 2, 4, 6, 8, 10, 12 and14 µg/mL were prepared **from** the stock solution. Absorbance was estimated by UV-Visible spectrophotometer (Shimadzu) at 323 nm[11].

FORMULATION OF KETOROLAC TROMETHAMINE GEL:

The formulations were prepared by using dispersion method. Weighed amount of polymer was dispersed in measured quantity of distilled water with stirring on mechanical stirrer. After complete addition of polymer, drug and Propyl paraben previously dissolved in water were added to the polymer dispersion, and then propylene glycol and penetration enhancer were added and mixed thoroughly. Dispersion obtained was neutralized with required quantity of triethanolamine to pH 7.4 to obtain gel[9].

Formulation batch G1 and G2 were prepared to find out the optimum concentration of Carbopol 940 and G3 was prepared to study the effect of drug concentration. Formulation G4, G5and G6 were formulated with three different penetration enhancer (neem oil, tulsi oil and oleic acid) and batch G7, G8 and G9 were prepared to find the optimum concentration of best enhancer chosen from previous batches. The varying formula of different ingredients has been listed in table 1. The same study was performed using Carbopol Ultrez 10NF by formulating batches F1-F9 listed in table 3.

Table1. Composition formula of Carbopol 940 NF formulations

Ingredients %w/w	G1	G2	G3	G4	G5	G6	G7	G8	G9
Ketorolac	1	1	1.5	1	1	1	1	1	1
Carbopol 940	1	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Propylene glycol	10	10	10	10	10	10	10	10	10
Propyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Neem oil	0	0	0	1	0	0	0	0	0
Tulsi oil	0	0	0	0	1	0	0	0	0
Oleic acid	0	0	0	0	0	1	2	3	4
Triethanolamine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Water QS	100	100	100	100	100	100	100	100	100

Table2. Composition formula of Carbopol Ultrez 10NF formulations

Ingredients %w/w	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ketorolac	1	1	1.5	1	1	1	1	1	1
Carbopol Ultrez 10NF	1	1.5	1	1	1	1	1	1	1
Propylene glycol	10	10	10	10	10	10	10	10	10
Propyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Neem oil	0	0	0	1	0	0	0	0	0

Tulsi oil	0	0	0	0	1	0	0	0	0
Oleic acid	0	0	0	0	0	1	2	3	4
Triethanolamine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Water QS	100	100	100	100	100	100	100	100	100

PHYSICO-CHEMICAL EVALUATION OF GEL:

pH:

The pH of formulations was determined by using digital pH meter. One gm of gel was dissolved in 100 mL of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and standard deviations were calculated[12].

DRUG CONTENT:

One gm of gel was taken and dissolved in 100 mL of phosphate buffer pH 7.4. The volumetric flasks were kept for 2 h and shaken well in a shaker to mix it properly. The solution was passed through the filter paper and filtered. One mL of above solution was taken and diluted to 10mL in 10mL volumetric cylinder; this solution was measured spectrophotometrically at 322 nm against corresponding gel concentration as blank[12, 9, 13].

VISCOSITY STUDY:

The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer. The gels were rotated at 2.5 rpm using spindle no. 64 and the corresponding dial readings were noted[9].

***IN VITRO* DIFFUSION STUDIES:**

The *in vitro* diffusion studies of prepared gel were carried out using Keshary-Chien diffusion cell.

Figure 1: Keshary-Chien diffusion cell

In Keshary-Chien diffusion cell 500mg of gel containing 5mg of ketorolac tromethamine was spread uniformly on the cellophane membrane. In diffusion cell 110 mL of phosphate buffer 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 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[12].

The release study was done for 5 h. The cumulative amount of drug released expressed in % was plotted for each one of 18 studied formulation.

Drug release data were appropriately corrected for loss of drug and receptor medium volume during sampling by replacement using the following equation[14].

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

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.

RELEASE KINETIC STUDY[15, 16]:

Various *in vitro* release data various kinetic models were used to establish the order and mechanism of drug release. Release data of all the formulations were fitted to different kinetic models as zero order, first order and higuchi model. Regression Co-efficient ($\mathbf{r^2}$) values obtained from various Plots were studied to find the plot showing best linearity of data. The value which was closer to 1 was selected as the best fit model for the drug release.[17]

RESULT AND DISCUSSION:

The present investigation aims to develop transdermal gel of ketorolac tromethamine and to optimize the polymer and penetration enhancer. The mechanism of action of these penetration enhancers is not well established yet but it might be possible that they modify the barrier properties of stratum corneum temporarily to enhance percutaneous absorption. Formulations were evaluated for pH, viscosity and drug release studies.

Table 3. Concentration versus absorbance of ketorolac tromethamine in phosphate buffer pH 7.4

Sr. No	Conc. µg/mL	Absorbance
1	0	0
2	2	0.097
3	4	0.198
4	6	0.299
5	8	0.390
6	10	0.494
7	12	0.612
8	14	0.710

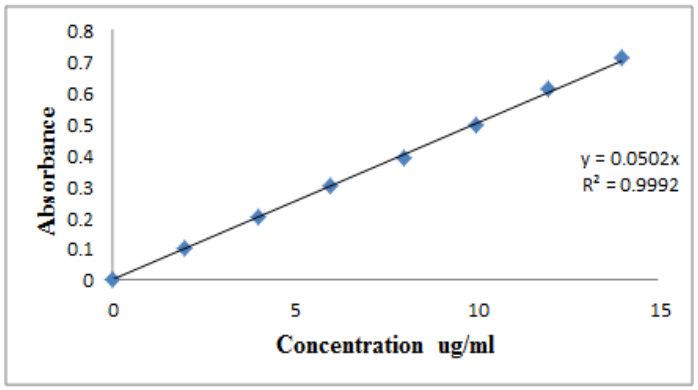

Figure 1: Standard calibration curve of ketorolac tromethamine

pH MEASUREMENT:

The pH values of all developed gels ranges 7.0-7.6 which lies in the normal pH range and would not produce any skin irritation as shown in Table 4.[16]

Table 4. pH values of the formulations measured after preparation

Code	pH ±SD	Code	pH ±SD
G1	7.4±0.17	**F1**	7.2±0.18
G2	7.3±0.05	**F2**	7.1±0.1
G3	7.5±0.20	**F3**	7.4±0.15
G4	7.1±0.2	**F4**	7.4±0.1
G5	7.2±0.11	**F5**	7.5±15
G6	7.4±15	**F6**	7.1±11
G7	7.6±0.17	**F7**	7.3±0.17
G8	7.5±0.05	**F8**	7.2±0.15
G9	7.4±0.11	**F9**	7.1±0.15

(SD: standard deviation, n=3).

VISCOSITY MEASURMENTS:

Table 5 exhibits the viscosity of each one of the prepared formulations. The formulations G1-G9 containing Carbopol 940 and formulation F1-F9 containing Carbopol Ultrez 10NF were found to give desirable viscosity with concentration 1.5 % w/v and 1% w/v respectively.

Table 5. Viscosity values of the formulations measured after preparation.

Code	Viscosity ±SD Cps	Code	Viscosity ±SD Cps
G1	38860±43.58	**F1**	40650±20.81
G2	45540±20.81	**F2**	55000±15.27
G3	45890±.20.00	**F3**	41500±.30.00
G4	46130±41.63	**F4**	42510±25.16
G5	45150±30.0	**F5**	40900±30.55
G6	45250±35.11	**F6**	42100±20.81
G7	45360±36.05	**F7**	42320±25.16
G8	45470±26.45	**F8**	42540±25.16
G9	45700±15.27	**F9**	43480±20.00

(SD: standard deviation, n=3).

DRUG RELEASE STUDIES:

EFFECT OF POLYMER AND DRUG CONCENTRATION ON DRUG RELEASE:

Maximum cumulative % release was obtained at 3.5 hours which was 74.78 for G1 and 62.71 % for G2 and formulation G3 had different drug concentration of 1.5 % w/w and maximum cumulative % release was obtained at 3.5 hours which was 60.27 % as shown in Fig 3. Study showed that increase in polymer concentration decreases drug release while drug concentration didn't have any effect on drug release. Fig. 4 shows the result of diffusion studies from Carbopol Ultrez 10NF formulations F1, F2 and F3. Maximum cumulative % release was obtained at 3.5 hours which was 76.38 and 62.03 % respectively for F1, F2. F3 having different drug concentration of 1.5 % w/w showed maximum cumulative release of 61.18 %. It was interesting to note that formulations with Carbopol 940 yielded better release at 1.5% w/w concentration of polymer whereas Carbopol Ultrez 10NF formulation yielded better release at 1% w/w concentration with a good viscosity.

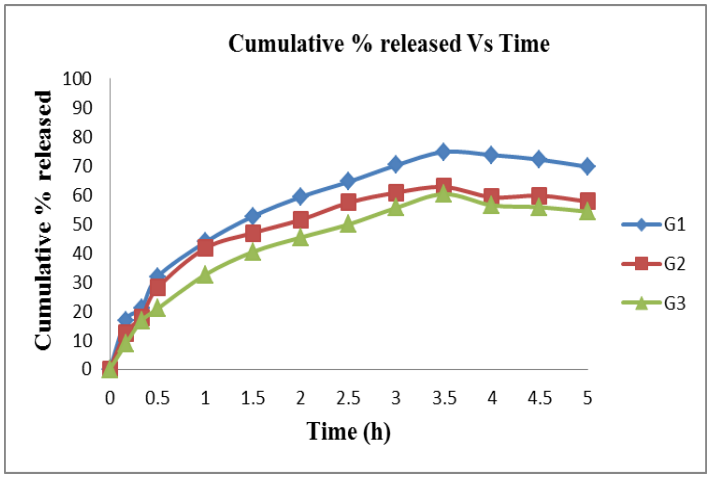

Figure 3: Results of *in vitro* release of formulation G1, G2, and G3.

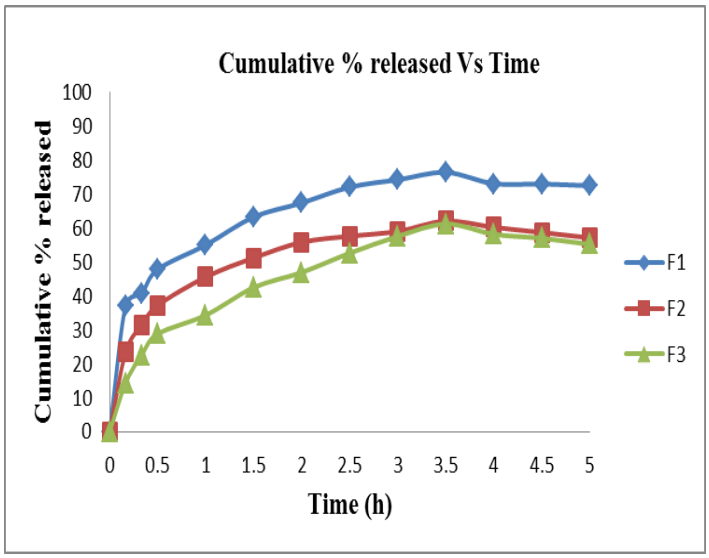

Figure 4: Results of *in vitro* release of formulation F1, F2, and F3.

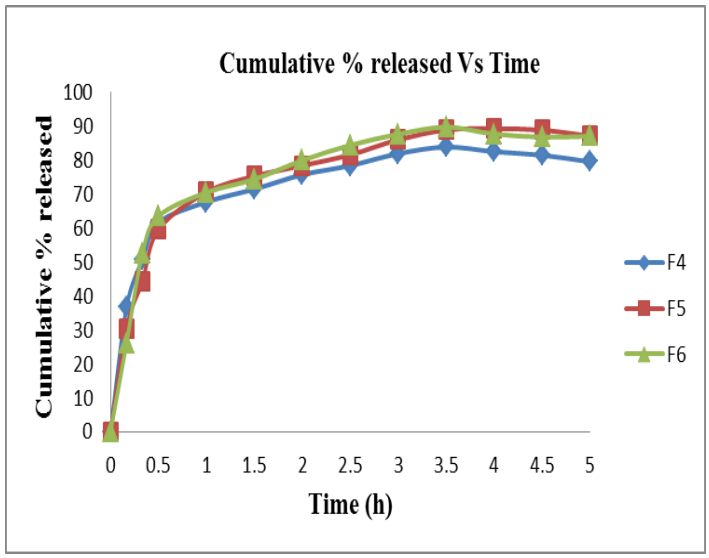

Figure 6: Results of *in vitro* release of formulation F4, F5 and F6

EFFECT OF PENETRATION ENHANCER:

Three penetration enhancer's neem oil, tulsi oil, and oleic acid were used with optimized polymer concentration to enhance the release. Release studies were performed to find the best penetration enhancer form the formulations G1-G9 and F1-F9. Fig 5 and Fig 6 shows the result of different penetration enhancers on formulation G4, G5, G6 and formulation F4, F5, F6 respectively. The maximum release was obtained 75.98% (G4), 78.39% (G5), 81.31 % (G6) and 83.82% (F4), 88.84% (F5), 89.71 % (F6) respectively. The study showed that for the both studies Oleic acid was found to be best penetration enhancer.

EFFECT OF CONCENTRATION OF OLEIC ACID PENETRATION ENHANCER:

Four different concentration of oleic acid as penetration enhancer were evaluated 1, 2, 3, and 4 % w/w. Fig 7 and Fig 8 shows the result of release study of formulation G6-G9 and F6-F9 respectively. It was interesting to note that the release first increased with increase in concentration of oleic acid from 1 % to 3% w/w and then decreased on further increase to 4% and the respective maximum release obtained was 81.31 % (G6), 83.45% (G7), 87.52 % (G8), 85.22 % (G9) and 89.71 % (F6), 90.33 %(F7), 95.67 % (F8) and 91.59 % (F9). The study showed that the maximum release was obtained 3%w/w concentration of oleic acid.

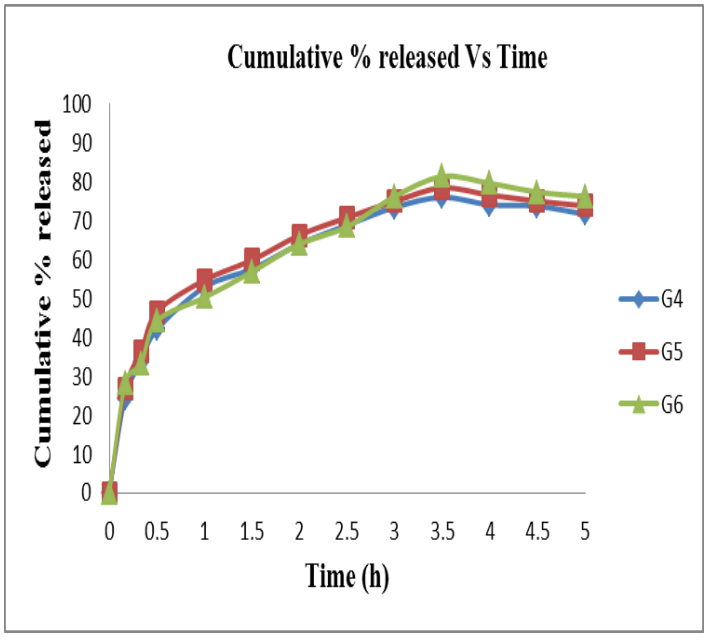

Figure 5: Results of *in vitro* release of formulation G4, G5, and G6

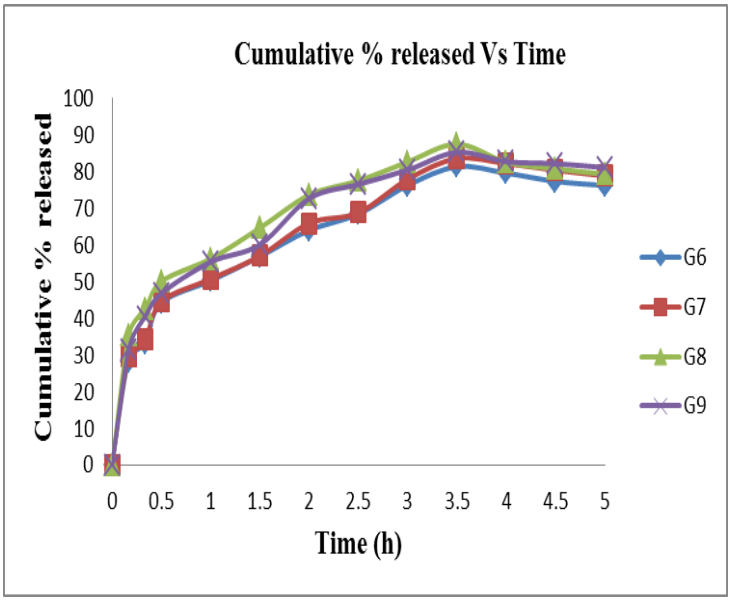

Figure 7: Results of *in vitro* release of formulation G6, G7, G8 and G9

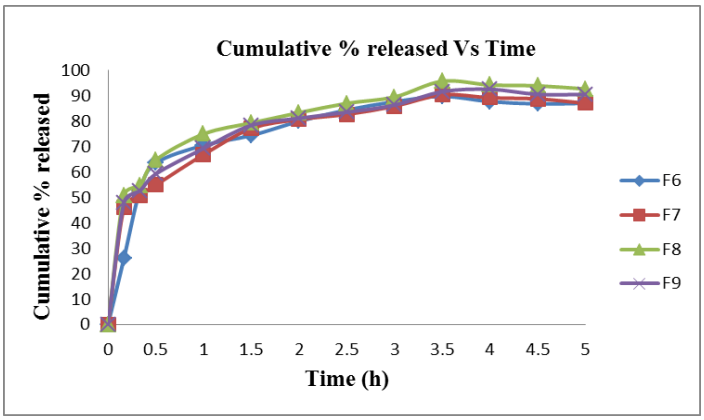

Fig. 8: Results of *in vitro* release of formulation F6, F7, F8 and F9

RELEASE KINETICS OF VARIOUS FORMULATIONS [17-18]

The various kinetics models like zero order, first order and higuchi release were applied on release profile of various formulations. The regression co-efficient (r^2) values were studied and results concluded that higuchi release kinetic model was obeyed by the both (G1-G9 and F1-F9 formulations). Regression coefficient values of formulations G1-G9 and F1-f9 are displayed in table 6 and 7 respectively.

Table 6. Regression Co-efficient (r^2) values of Carbopol 940 formulations

Formulations	Zero order release r^2	First order release r^2	Higuchi release r^2
G1	0.905	0.9817	0.9943
G2	0.8736	0.9432	0.9814
G3	0.8963	0.9433	0.9954
G4	0.7892	0.915	0.9657
G5	0.7903	0.9371	0.9521
G6	0.8439	0.9623	0.9708
G7	0.8477	0.9614	0.9701
G8	0.7992	0.9655	0.9514
G9	0.8177	0.9653	0.9616

Table 7. Regression Co-efficient (r^2) values of Carbopol Ultrez 10NF formulations.

Formulations	Zero order release r^2	First order release r^2	Higuchi release r^2
F1	0.7247	0.8967	0.9108
F2	0.7486	0.8561	0.9322
F3	0.8952	0.9614	0.9924
F4	0.6398	0.8617	0.855
F5	0.7077	0.9202	0.9032
F6	0.6808	0.9175	0.8782
F7	0.6947	0.9343	0.8905
F8	0.6464	0.9275	0.8544
F9	0.6654	0.9254	0.8698

CONCLUSIONS

From the above study we have concluded that the transdermal gel prepared along with Carbopol 940 and Carbopol Ultrez 10NF by using natural penetration enhancers can be used to prepare an ideal transdermal gel preparation. All developed gels showed good homogeneity with absence of lumps. *In vitro* drug release study showed that the formulations containing oleic acid releases the drug faster as compared to other penetration enhancers. It may be concluded from the results that as the concentration of oleic acid increases from 1-3% w/w in the formulations the rate of drug release also increases and decreases on further increase of concentration to 4%. Carbopol Ultrez 10 NF is having better formulating properties over Carbopol 940 because of easy dispersion, less aggregation property and good viscosity with less concentration. These composition also had propylene glycol which acts as humectant and propyl paraben which act as preservative. Formulations viscosity shows inverse relationship with the amount of drug released and this observation is according with a vast previous literature. The required viscosities for formulating the topical gel

were achieved at concentration of 1.5 % and 1% for Carbopol 940 and Carbopol Ultrez 10NF respectively. Study showed that the topical gel formulation F8 formulated using Carbopol Ultrez 10 NF with polymer concentration of 1%, and oleic acid 3% is having the maximum release of 95.67 % and observed as optimized batch. The best kinetic model which described the release of drug is Higuchi model which states the release from insoluble matrix as a square root of time dependent process based on Fickian diffusion model.

REFERENCES:

1. Chandra A and Kumar SP (2009). Transdermal Delivery of ketorolac. Yakugaku zasshi, 129(3): 373-379.
2. Dubey R, Bommagani M, Venkateswarlu V, Mullangi R, Karnati, Thammera RK and Menon VCA (2009). Ketorolac Tromethamine Transdermal Gel, Development, In Vitro and In Vivo Evaluation. Journal of Pain & Palliative Care Pharmacotherapy, 23(1): 26-34.
3. Whittle BJR. Cyclooxygenase and nitric oxide systems in the gut as therapeutic targets for safer anti-inflammatory drugs. CurrOpin Pharmacol. 2004; 4:538–545.
4. Whittle BJR. Mechanisms underlying intestinal injury induced by anti-inflammatory COX inhibitors. Eur J Pharmacol. 2004; 500:427–439.
5. Puglia C, Filosa R, Peduto A, Carariis P, Rizza L, Bonina F and Blasi P. Evaluation of Alternative Strategies to Optimize Ketorolac Transdermal Delivery. AAPS Pharm SciTech., 7(3)64: 1-9.
6. Choi JS and Cho AY (2007). Formulation and evaluation of ketorolac transdermal systems. Drug Delivery, 14: 69–74.
7. Sinha VR and Kaur MP (2000). Permeation enhancers for transdermal drug delivery. Drug Dev. Ind. Pharm., 26(11): 1131–1140.
8. Kokate CK, Purohit AP and Gokhale SB (2003). Pharmacognosy, 23rd ed., Pune: Nirali prakashan, pp. 250-303.
9. Gupta V, Dwivedi A, Trivedi N, Jain NK, Graud N and Jain Dk (2009) Formulation and Evaluation of Naproxen Gel Containing Tulsi Oil as Penetration Enhancer. Int. J. Pharm. and Clinical Research, 1(3): 153-155.
10. Rowe RC and Sheskey PJ (2006). Hand book of Pharmaceutical Excipients. 5th ed. Pharmaceutical Press. London, pp. 111-114.
11. El-setouhy DA and El-Ashmony SMA. Ketorolac Trometamol topical formulations: release behavior, physical characterization, skin permeation, efficacy and gastric safety (2010). Jour. Pharm. and Pharmacology, 62: 25–34.
12. Shivhare UD, Jain KB, Mathur VB, Bhusari KP and Roy AA (2009). Formulation Development And Evaluation Of Diclofenac Sodium Gel Using Water Soluble Polyacrylamide Polymer. Digest Journal of Nanomaterials and Biostructures, 285-290.
13. Joshi M and Patravale V (2006). Formulation and Evaluation of Nanostructured Lipid Carrier (NLC)–based Gel of Valdecoxib. Drug Development and Industrial Pharmacy, 32: 911–918.
14. Singh B, Kaur T and Singh S. 1997.Correction of raw dissolution data for loss of drug and volume during sampling. Indian J Pharm Sci., 59:196–9.
15. Kumar L and Verma R (2010). In vitro evaluation of topical gel prepared using natural polymer. International Journal of Drug Delivery, 58-63.
16. Shoaib MH, Tazeen J, Merchant HA, Yousuf RI. Evaluation of drug release kinetics from Ibuprofen matrix tablet using HPMC. Pak J Pharm Sci 2006; 19(2):119-124.
17. S. Verma, V. Valecha, S.K. Singh, N. Syan, P. Mathur (2011). Development and evaluation of montelukast sodium colon targeted matrix tablets based on pulsatile approach for nocturnal asthma. IJPSRR, 8,1: 129-137