Research Article

Preparation and Evaluation of Ketorolac Tromethamine Hydrogel

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ABSTRACT

Ketorolac tromethamine is a non-steroidal anti-inflammatory drug that has two major problems when administered orally; it has severe gastrointestinal side effects as bleeding, peptic ulcer, perforation. Second, it has short half-life (4hr) so require frequent administration. The aim of this study was to overcome these two problems through preparation of this drug as topical hydrogel. Hydroxypropyl, hydroxylpropylmethyl, and sodiumcarboxymethyl were the three cellulosic polymers used as gelling agents, the influence of type and concentration of them on the release of ketorolac was investigated. Oleic acid, tween 80, carveol, and isopropyl alcohol were used as penetration enhancers, and effect of these enhancers on diffusion of ketorolac across semi-permeable membrane was tested. The optimized formula was evaluated for its anti-inflammatory action on carragenan induced rat paw edema model. Results revealed that the hydrogel formulations exhibited high drug release especially at low polymer concentration and the release was follow diffusion controlled mechanism. All enhancers showed enhancement in ketorolac penetration specially the optimized formula contain Ketorolac 2%, HPMC 1%, and 0.5% Tween 80, which exhibited improved anti-inflammatory activity. So it can be concluded that topical hydrogel enhanced permeation of ketorolac and given an effective anti-inflammatory activity, with avoidance of GIT adverse effect.

Keywords: Ketorolac Tromethamine, Hydrogel, Penetration Enhancer, Rat paw, Tween 80.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) exert their effect due to inhibition of the cycloxygenase (COX) enzymes[1], several types of this class are given orally, but in last year's; several clinical data revealed that occurrences of several gastrointestinal side effects from oral administration of this class.

One approach try to eliminate or decrease this GIT adverse effects have been the development of transdermal preparation[2]. One of the best topical dosage forms is the gel.

Gels are semisolid systems consisting of dispersion of small or large molecules in an aqueous or non-aqueous liquid vehicle rendered jelly-like through the addition of a gelling agent[3]. Gels are classified according to vehicle into hydrogels or organogels. A hydrogel is defined as an aqueous phase with an interlaced polymeric component. An organogel is defined as an organic phase with an interlaced polymeric component[4].

According to colloidal phase, gelling agent may be inorganic as bentonite or organic as Carbomers, Hypromellose, Carboxymethylcellulose Sodium[5]. Another classification for gelling agents, regard any one of them belong to one of these four groups[6], 1) Materials which polymerize or alter viscosity as a function of temperature or shear or other physical forces. 2) Materials which polymerize in the presence of ions or removal of ions. 3) Materials that can be cross-linked chemically or with light, ultrasound or radiation. 4)

Materials that can be cross-linked by addition of covalent crosslinking agents such as glutaraldehyde[7].

In order to optimize the transdermal penetration, an approach to incorporate penetration enhancers inside the gel formula was developed, the ideal penetration enhancers should be: inert pharmacologically, non-toxic, non-allergenic, with immediate and suitable effect [8-9].

Ketorolac tromethamine is a (NSAID). It is a racemic mixture of with the S-form having analgesic activity. It exert its effect due to inhibition of both (COX-I and II)[10]. The drug is reported to cause severe GIT side effects such as GIT bleeding, peptic ulcer, perforation[11]. It has short half-life (4hr) so, frequent dosing is required which increase the incidence of the occurrence of adverse effect. To avoid these problems, dermal and transdermal delivery of ketorolac is an attractive alternative[12].

The main aim of this work is to formulate ketorolac tromethamine as hydrogel in order to eliminate the GIT side effect, and to enhance the percutaneous absorption and anti-inflammatory activity of this drug.

MATERIALS AND METHODS

Materials

Ketorolac tromethamine was purchased from Sigma (St. Louis, MO, USA). Hydroxypropylmethylcellulose (HPMC), Hydroxypropylcellulose (HPC), Na-carboxymethylcellulose (Na-CMC)Aqualon, U.K. Tween 80, C.B.H. Lab Chemicals, Nottingham, U.K. Sodium chloride, disodium hydrogen

phosphate and potassium dihydrogen phosphate, Prolabo, France. Cellulose nitrate membrane filter, diameter pore: 10 µm pore size, Albet Company, Spain. Isopropyl alcohol, Merck, Germany. Dimethylformamide, Merck, Germany. Oleic acid, Merck, Germany.

Methodology

Preparation of ketorolac tromethamine Hydrogel

1) Preparation of Hydroxypropyl methylcellulose (HPMC) gel

The weighed quantity of HPMC powder was sprinkled gently, into the vortex of 50 ml keterolac tromethamine solution at concentration 20 mg/ml in 200ml beaker, using homogenizer for stirring, until thin dispersion, without lumps was formed. The solution was kept overnight in the refrigerator to form the gel. HPMC gel bases were Prepared at concentrations of 1% (F1), 3% (F2) and 6 % (F3),

2) Preparation of Hydroxypropyl cellulose (HPC) gel

The weighed amount of HPC powder was sprinkled gradually into the vortex of 50 ml ketorolac tromethamine solution at concentration 20 mg /ml, placed in a 200 ml beaker, and stirred with a mechanical stirrer at a high speed until no lumps was observed . HPC gel bases were prepared at concentrations of 1% (F4), 3% (F5) and 6% (F6),

3) Preparation of Sodium carboxymethylcellulose (Na CMC) gels

The weighed amount of NaCMC was sprinkled gradually into the vortex of 50 ml ketorolac tromethamine solution at concentration 20 mg/ml, placed in a 200 ml beaker, and stirred with a mechanical stirrer at a high speed until no lumps was observed. NaCMC gel bases were prepared at concentrations 1% (F7), 3 % (F8) and 6 % (F9).

In-Vitro Release of Ketorolac from Different Hydrogel

This test was done by using modified USP Dissolution test (Apparatus I). A glass cylindrical tube (6 cm in length and 2.25 cm in diameter) containing the hydrogel was hunged in place of the basket and was tightly covered with a semipermeable membrane (100 µm pore size).

1 gm of hydrogel contain 2% ketorolac tromethamine was placed in the cylindrical tube covered with the semipermeable membrane. The glass tube dipped in a 500 ml 0.2M phosphate buffered saline (pH 7.4). The test was carried out at $37^{o}C \pm 0.5^{o}C$, and rotation speed of 50 r.p.m.

A liquots of 2 ml were withdrawn from the dissolution medium at 5, 15, 30, 60, 90, 120,150, and 180 minutes time intervals, replaced at each time with 2ml of buffer to maintain a constant volume.

The sample diluted with equal volume with buffered and The absorbance of the collected samples were measured spectrophotometrically at λ max 323 nm using phosphate buffered saline as a blank.

Kinetic Analysis of the *In-Vitro* Release Data of Ketorolac from different gel formulae

The data were analyzed to establish the order of drug release. The linear regression was used for analysis of the released data according to:

A) $Ct = Co – Kt$ -- For Zero order kinetics

B) $Log\ Ct = -Kt/2.303\ + Log\ Co$ -- For First order kinetics

C) $Q = [Dt\ (\ 2A – Cs\ )\ Cs]1/2$ -- For Higuchi diffusion model

Where

A =total amount of drug in base per unit volume

D =diffusion coefficient

Cs=drug solubility in the base.

Q =amount released per unit area at time t

This equation gives a linear relationship between Q and square root of time and (r) was determined in each case

Effect of Addition of Different Penetration Enhancers to A selected Formula on Permeation of Ketorolac

Permeation enhancers such as 0.5% carveol, 0.5% terpene, 5% oleic acid, 5% isopropyl alcohol, were incorporated separately into a selected gel prepared according to Formula 1 (2% Ketorolac Tromethamine + 1% HPMC) because it gave the most optimum results in all previous tests.

Five different formulae containing enhancers were prepared:

Formula 1-A: containing Ketorolac Tromethamine 2% + 1% HPMC (Control hydrogel)

Formula 1-B: containing Ketorolac Tromethamine 2% + 1% HPMC+ 0.5% carveol

Formula 1-C: containing Ketorolac Tromethamine 2% + 1% HPMC+ 0.5% tween 80

Formula 1-D: containing Ketorolac Tromethamine 2% + 1% HPMC+ 5% Oleic acid

Formula 1-E: containing Ketorolac Tromethamine 2% + 1% HPMC+ 5% isopropyl alcohol

Different formulations then subjected to test effect of those permeation enhancers on permeability of ketorolac across semi-permeable membrane (0.01 cm thickness). The membrane samples were mounted on diffusion cells (2.25 cm^2 surface area). Weighted amount of gel contain 10 mg of ketorolac putted on a side that facing upward. The other side of the membrane directing toward the buffer compartment filled with 5 ml buffer, which kept at $37^{o}C$ and shaked at 50 rotations per minute. Then samples were withdrawn at time intervals 15, 30, 60, 120, 150, 180 and 240 minutes and replaced with equal volume of fresh phosphate buffered saline to maintain a constant volume.

The absorbance of the collected samples was measured spectrophotometrically at λmax 323 nm using phosphate buffered saline as a blank. Experiments were run in triplicates.

Calculation of the Permeation Parameters of ketorolac Across Semi- permeable Membrane

The permeation parameters of ketorolac Tromethamine (Permeability coefficient [P], partition coefficient [K], diffusion coefficient [D], Apparent steady state flux [Jss], enhancing factor [Fen], lag time [tL], relative permeation rate [RPR]) were calculated from the penetration data.

Plotting the cumulative amount permeated versus time, and the slop represent [Jss]

$P = Jss / Co$

Plotting amount permeated versus square root of time [D] was calculated from the slope obtained according to the following equation

$D = (slope / 2\ Co\ )2\ .\ \pi$

[K] was calculated from P and D using the penetration barrier L with known thickness of semipermeable membrane (0.01 cm) from equation :

$K = P.\ L / D$

[tL] was calculated from equation : $tL = L^2 / 6\ D$

[Fen] = Cumulative amount permeated from formula / Amount permeated from control gel

[RPR]= P of the formula / P of the Commercial product

Pharmacodynamic of Ketorolac from Different Hydrogel Formulae

Adult male albino rats weighing 150-200gm will use. They will divide into 2 groups each of 3 animals. Inflammation was induced by a subcutaneous injection of 0.05 ml of 1% carageenan solution into the planter tissue of the two hind paws before the application of the topical drug to the animals.

The topical products were applied to the left hind paw only of the rats after a period of 15 minutes and leave for 30 minutes insure contact of the formulae to the inflammed left hind paw of the rats. The mean increase in the volume of carageenan injected paws of rats treated with the drug (Vt) as well as that of the control right paw (Vc) were calculated by subtracting the volume of the left hind paw from the Volume of the right hind paw. After 0.5 hr, 1 hr, 1, 5 hr, 2 hr and 2.5hr.

The percentage inhibition of the inflammation for each group was calculated from the mean effect in control and treated paws according to the following equation:

% Inhibition in Edema volume = [(Vc-Vt) / Vc] x 100

RESULTS AND DISCUSION

In-Vitro Release of Ketorolac from Different Hydrogels in saline phosphate buffer pH 7.4

The release of ketorolac from the prepared hydrogels was performed in order to study the effect of different polymers types and concentration on the release of ketorolac aiming to select the best formula. Figures (1-3) show the results of *in vitro* release. It appear that formulae F1,2,4,5,7, and 8 enhanced release of Ketorolac when compared with other formulae and the percentage of ketorolac release after 180 minutes from these formulations was 89.2% for formula F1 which showed higher value and 63.1% for F5 which showed the lower value, while formulae F3, F6, F9 decreased extent of ketorolac released, the percentage of ketorolac released after 180 minutes from these formulations was 42.6%, 45.9%, and 53.6%, respectively.

Thus the tested formulations can be arranged according to their efficiency in increasing release of ketorolac as following Formula 1 (89.2%) > formula 7 (86.9%) > formula 2 (78.2%)
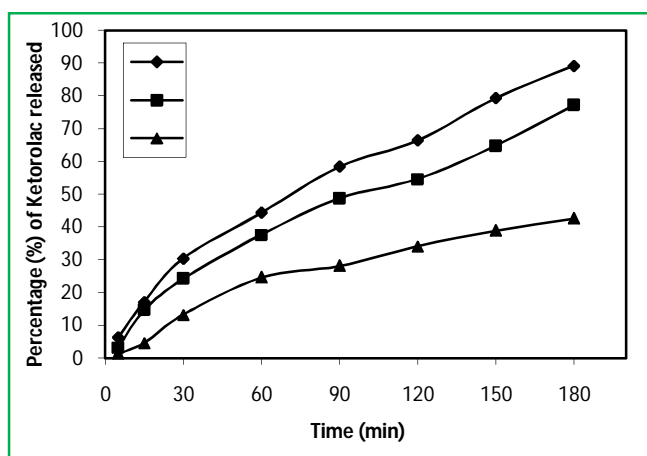

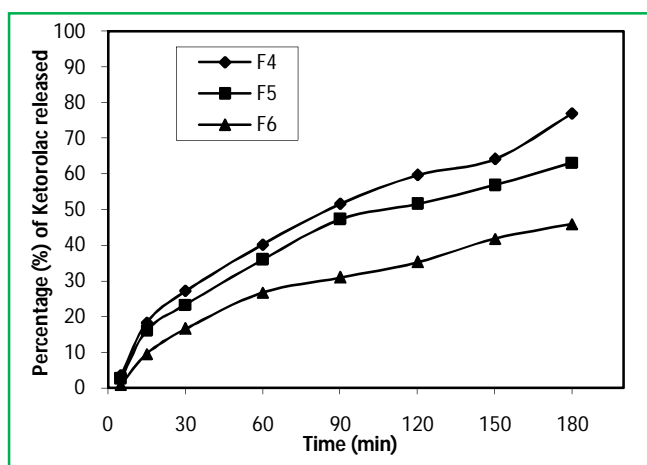**Figure 1:** *In Vitro* Release of Ketorolac from HPMC Gels in Saline Phosphate Buffer pH 7.4
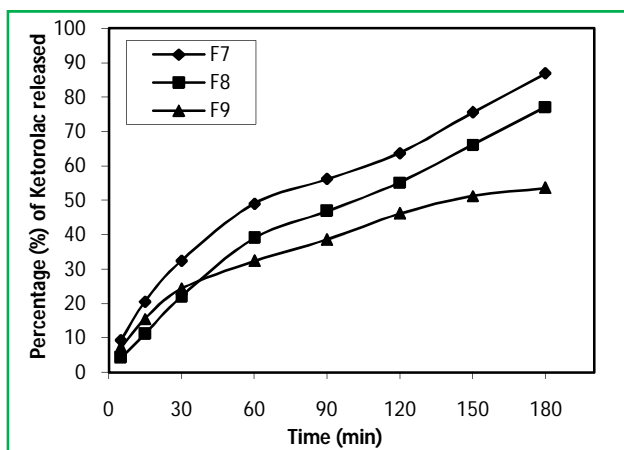

Figure 2: *In Vitro* Release of Ketorolac from HPC Gels in Saline Phosphate Buffer pH7.4

Figure 3: *In Vitro* Release of Ketorolac from NaCMC Gels in Saline Phosphate Buffer pH 7.4.

It is clear from the above results that increasing polymer concentrations in formulae F3, F6, and F9 which contain polymers in their highest concentrations resulted in decreased percentage of drug release for all types of polymers. This is due to high viscosity, which will retard release of drug from hydrogel base, and by increasing concentration of polymer this leads to an increase viscosity of gel and thus retards release of drug. While formulae F1, F7 which contain polymers in low concentrations, resulted in low viscosity and higher release rate.

Kinetic Analysis of the Release Data

The data is illustrated in tables (1-2). It appear that the release of ketorolac follows diffusion controlled mechanism. The cumulative amount of ketorolac released from different hydrogel bases was plotted against square-root of time. Linear plots (r > 0.9) for formulations were produced, indicating that the release mechanism is in compliance with the Higuchi equation.

Table 1: Linear Regression of Release Data of Ketorolac from Different Hydrogel Bases.

Formulae	Linear Regression analysis Using correlation Coefficient (r^2) According to		
	Zero Order	First Order	Diffusion model
F1	0.9651	0.9125	0.9973
F2	0.9542	0.9601	0.9936
F3	0.9751	0.9798	0.9913
F4	0.9198	0.9801	0.9932
F5	0.9694	0.9796	0.9906
F6	0.9701	0.9812	0.9922
F7	0.9598	0.9612	0.9920
F8	0.9612	0.9642	0.9932
F9	0.9501	0.9713	0.9942

The results are in agreement with Hassan et al.,[13] who reported that that the mechanism of release of ciprofloxacin and norfloxacin from gel bases obeyed Higuchi diffusion model mechanisms. Our findings are also in agreement with Fergany A.[14], who reported

that the mechanism of release of diclofenac sodium from NaCMC gel base follows Higuchi diffusion model mechanisms.

**Table 2**: Kinetic Analysis of Release Data of Ketorolac from Different Hydrogel Formulations.

Formula Number	Order of Release	r^2	K (mg%. min –1/2)
F1	Diffusion	0.9973	7.301
F2	Diffusion	0.9936	6.266
F3	Diffusion	0.9913	3.824
F4	Diffusion	0.9932	6.146
F5	Diffusion	0.9906	5.255
F6	Diffusion	0.9922	3.844
F7	Diffusion	0.9920	6.618
F8	Diffusion	0.9932	6.427
F9	Diffusion	0.9942	4.163

Effect of Penetration Enhancers on Permeation of Ketorolac A cross Semi-Permeable membrane

Figures (4) show the effect of incorporation of different permeation enhancers such as 0.5% carveol, 0.5% tween 80, 5% oleic acid, 5% isopropyl alcohol on release of ketorolac from hydrogels prepared according to formula 1 (2% Ketorolac+1%HPMC)

The cumulative amount permeated after 240 minutes were 1118, 1227, 1450, 1332, and 1273 µg/cm2 for F1- A to E, It appear from the obtained results that all prepared formulation with enhancers gave higher results than control hydrogel.

The maximal enhancement of ketorolac permeation was obtained with formula F1-C (Contain Ketorolac 2% + HPMC 1% + 0.5% Tween 80).
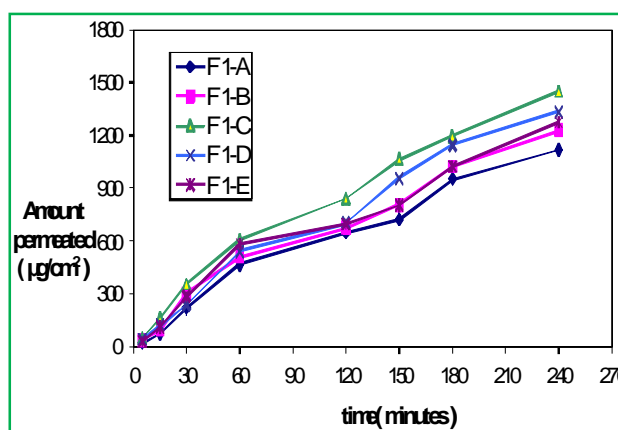
Figure 4: Permeation of ketorolac across semipermeable membrane from the selected gels

One attributes the penetration enhancing effect of tween 80 to its ability to decrease interfacial tension between drugs in topical products and membrane, thereby greatly increasing their permeability.

Table 3 shows the permeation parameters of ketorolac from hydrogels prepared according to formulae F1-A to E. It appear that for all prepared formulae; the amount permeated at 240 minutes was higher compared to control gel. The tested formulae could be ranked as following: F1-C >1-D >1-E >1-B >1-A. The steady state flux [Jss] was highest (5.8451 $\mu g/cm^2.min$) for formula F1-C and was lowest with F1-A (4.6444 $\mu g/cm^2min$). The permeabilty coefficient [P] was highest (7.793 cm/min x 10^{-4}) for formula F1-C and was lowest with F1-A (6.192cm/minx10^{-4}) The Lag time [t lag]

was 12.89 min., for formula F1-C compared to 20.69,18.36, 14.54, and 17.59 min., for Formula 1-A,B,D,E, respectively. The decrease in [Lt] of the tested formulae as in F1-C, lead to rapid onset of effect. Enhancing factor [Fen] was >1 for all tested hydrogels, which prove the usefulness and superiority of HPMC as a base for ketorolac specially for formula F1-C (Ketorolac 2% + HPMC 1% + 0.5% Tween 80) which gave best results in all tests. Therefore, this formula was chosen for in-vivo investigation.

Table 3: Permeation Parameters of Ketorolac Across semi-permeable From Different Hydrogel Formulations

Formula	Cumulative amount at 240 min ($\mu g/cm^2$)	Jss $\mu g/cm^2min$	t lag (min) x10^{-4}	P Cm/minx10^{-4}	K	D cm^2/min x 0^{-5}	Fen
F1-A	1118	4.6444	20.69	6.192	0.76	8.053	——
F1-B	1227	4.9263	18.36	6.568	0.72	9.076	1.097
F1-C	1450	5.8451	12.89	7.793	0.60	12.92	1.296
F1-D	1332	5.5712	14.54	7.428	0.64	11.46	1.191
F1-E	1273	5.0128	17.59	6.683	0.70	9.473	1.138

Pharmacodynamic of Ketorolac From Different Hydrogel Formulae

It is clear from table (4) that different formulae inhibited the carageenan- induced paw edema compared to the control to a varying degree depending on the type of formula compared. In this respect the control formula [F1-A] inhibited the carageenan- induced paw edema by 23.324% compared to the control value (table 8 and figure 10). Formula (F1-C) showed a remarkable reduction in the paw edema by 54.382 % compared to the control value., which indicate that this optimize formula has an anti-inflammatory activity and enhanced percutaneous absorption.

Table 4: Antinflammatory activity of different formulation of Ketorolac using carrageenan-induced paw oedema in rats

Formulation	Mean edema volume in ml	% Inhibition of control value
Control	0.892 ± 0.02	0.000
Formula 1-C	0.406 ± 0.01	54.382
F 1-A [Control Gel]	0.683 ± 0.02	23.324

CONCLUSION

On the basis of the previous finding it can be concluded that the nature of the polymers used in preparation of gels and their concentrations showed an effect on release of ketorolac from hydrogel base. The maximal enhancement of ketorolac permeation was obtained with formula F1-C (Contain Ketorolac 2% + HPMC 1% + 0.5% Tween) which showed maximum cumulative amount permeated at 240 minutes, highest steady state flux [Jss], highest permeabilty coefficient [P], lowest Lag time [t lag], and highest Enhancing factor [Fen], and

significantly inhibited the carrageenan-induced paw edema and gave comparatively superior results in all in-vitro tests and gave comparatively superior antinflammatory activity.

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