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FORMULATION OF KETOROLAC TROMETHAMINE IN SEMI-SOLID DOSAGE FORMS

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يعتبر الكيتورولاك تروميثامين من مضادات الالتهاب غير السيترويدية الذى له نفس الاثار الجانبية لهذة المجموعة عندما تعطى عن طريق الفم (قرحات المعدة - تغير فى وظائف الكلى والكبد). تهدف هذة الدراسة للتغلب على هذة الاثار الجانبية بصياغة هذا العقار فى صواغات موضعية (هلامات - هلامات مستحلبة- - مراهم مستحلبة). وقد تم دراسة كل من الخصائص الفيزيائية لهذة الصواغات (الشكل الخارجى - الاس الهيدروجين - اللزوجة) والتداخلات بين هذا العقار والبوليمرات المستخدمة وذلك باستخدام المسح الحرارى التفاضلى والاشعة تحت الحمراء ودراسة انطلاق العقار من الصواغات السطحية خلال غشاء سلوفان وباستخدام جلد فار كغشاء حيوى ودراسة سلوك انطلاق هذا العقار من هذة الصواغات.

وقد اوضحت النتائج ان لا يوجد اى تدخلات بين الكيتورولاك والبوليمرات المستخدمة ، وان هيدروكسى بروبيل ميثل سيللولوز وصوديوم كربوكسى ميثل سيللولوز قد أعطيا اعلى معدل لانطلاق العقار بينما بلورونيك ١٢٧ اعطى أبطأ معدل لانطلاق العقار. وفى حالة الهلامات المستحلبة وجد ان المستحلب الهلامى زيت فى ماء اعطي معدل لانطلاق العقار أعلى منه فى حالة المستحلب الدقيق. كما وجد فى حالة المراهم المستحلبة ان القاعدة زيت فى ماء اعطت معدل لانطلاق العقار اعلى منه فى حالة القاعدة ماء فى زيت.

باستخدام جلد فار كغشاء حيوى وجد ان هلام كاربوبول٩٣٤ والمستحلب الهلامى زيت فى ماء أعطيا اعلى نتائج لاختراق العقار مقارنة بهلام البلورينك ١٢٧ الذى اعطى اقل معدل لاختراق العقار.

Ketorolac tromethamine (KT) is one of NSAIDs that has GIT, renal and hepatic disorder if taken orally. The study aimed at avoiding the adverse effects of KT by formulating it in different topical dosage form such as gel (Sodium algnate, NaCMC, HPMC, Carbopol 934 and Pluronic F127), emulgel (O/W), microemulsion and cream (O/W and W/O). The interactions between KT, polymers and other ingredients used were studied using differential scanning calorimeter (DSC) and Infra red (IR) spectroscopy. The physical properties of these formulations appearance, homogeneity, pH and viscosity were studied. The in-vitro release of KT from these formulations through cellophane membrane was carried out. The kinetic study of KT release from these formulations was also studied. In-vitro study of KT permeation in diffusion cell using rat skin from the selected formulations was carried out.

Physical investigation of KT and polymers indicated that no interaction between KT and polymers. Among the polymers used in gel formulations, HPMC and NaCMC gave the highest release rate of KT in-vitro, while pluronic F127 gave promising sustained release. In case of emulgel formulations, O/W emulgel base gave higher release than microemulsion base. Also in case of emulsion ointment base formulations, the release of KT from O/W base was higher than W/O type which gave the lowest release.

In-vitro study of KT through the diffusion cell using rat skin as biological membrane, higher permeation was obtained in case of carbopol 934 gel and O/W emulgel comparison with pluronic F127 gel which gave the lowest permeation of KT.

INTRODUCTION

KT is often used for the treatment of acute and chronic rheumatoid arthritis or osteoarthritis. It is well absorbed following oral administration, although the usual oral dose of 10 mg of KT is well tolerated by the patients, yet several side effects have been reported

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including: gastrointestinal disturbances, edema, dizziness, headache, peptic ulcer, renal and liver side effects[1].

In the light of these side effects associated with the oral use of KT, it was proposed to formulate various topical dosage forms of the drug such as, gels, emulgels and cream as the topical dosage forms of NSAIDs have been explored as a potential methods to avoid the first pass effect and the gastric irritation that may occur when these drugs are administered orally The physical properties of these formulations (pH, viscosity, appearance and homogeneity), drug content, interaction between KT and other ingredients were studied. *In-vitro* release and *in-vitro* permeation of KT from these dosage forms and kinetic of KT release from these formulations was also studied.

EXPERIMENTAL

Materials

- Ketorolac Tromethamine Kindy supplied (Amriya Pharm. Ind. Co., Alex., Egypt).
- Standard cellophane membranes (molecular cut of range= 12000), pluronic F127, sodium hydroxide (Sigma Chem, Co., U.S.A).
- White petrolatum, polysorbate 80 (tween 80), stearyl alcohol, glycerol, liquid paraffin, sodium alginate and sodium carboxy methyl cellulose (NaCMC) disodium hydrogen orthophosphate and potasium dihydrogen orthophosphate (El-Nasr Pharm. Chem. Co., Cairo, Egypt).
- Carbopol 934, carbopol 940 (C. P. Evans Co., England).
- Hydroxypropylmethylcellulose (HPMC), (Aldirch Chem. Co., Inc., U.S.A.).
- Span 80 (Honil Ltd. Co., England).
- Hairless rat skin (Animal house of Assiut University).

Equipment

- Spectrophotometer (Shimadzu, UV-150-02, Seisakusho, Ltd., Kyoto, Japan).
- Magnetic stirrer with hot plate (Sybron /Thermolyne Co., Dubuque Iowa, U.S.A.).
- Thermostatically controlled shaking water bath (Gesellschaft labor technik M.B.H. & GFL, Germany).
- Digital pH meter (Tenway Ltd., Felsted, Dunmow, Essex, M63LB, U.K).
- Infrared spectrophotometer (IR-470, Shimadzu Co., Japan).
- Differential scanning calorimetry (DSC-50, Shimadzu Co., Japan).
- Brookfield DV-III ULTRA, Brookfield Engineering Laboratories, Inc., Stoughton, Manual No. M/98-211-B0104 (U.S.A).
- Micrometer.

Methods

Preparation of ketorolac tromethamine formulations

Preparation of ketorolac tromethamine gels (F1-F5)

The amount of polymer used, 8% w/w sodium alginate (F1) 4% w/w NaCMC (F2), 3% w/w HPMC (F3)[2] or 0.5% w/w carbopol 934 (F4)) were dispered in distilled water in which ketorolac (0.5% w/w) was previously dissolved. The required amount of NaOH was added to neutrised carbopol 934 (F4)[3]. The dispersion was mixed until a clear transparent gel was formed. In case of F127 (F5) the amount of pluronic F127 (20% w/w) is dispered in cold water (5-10°C) then left in a refrigerator over night. After that the preparation was left outside the refrigerator[4] with stirring, until clear transparent gel was obtained.

Preparation of ketorolac tromethamine emulgels (F6-F7)

O/W emulgel formulations were prepared by a three step method (i) polymer dispersion in water, (ii) neutralization of the polymeric aqueous dispersion, and (iii) emulsification of the oil phase. The polymer (0.5% w/w carbopol 934 (F6) or 0.5% w/w carbopol 940 (F7)) was suspended in distilled water in which KT (0.5% w/v) was previously dissolved. The resulting slurry was neutralized by the addition of a NaOH (0.8 gram of NaOH required to neutrised 2.0 gram carbopol 934). In order to obtain a complete polymer hydration, the forming stable gels were stored at 4°C for 24 h before the addition of the oil phase (liquid paraffin) was slowly added to the water phase (gel with tween 80). The addition was performed under stirring at 900 rpm at 80°C, then cooling to room temperature[5].

Preparation of ketorolac tromethamine microemulsion (F8)

A mixture of tween 80 and glycerin were heated at 90°C and stirred by using magnetic stirrer at 900 rpm for 20 min, liquid paraffin was added and the whole mixture was stirred at high speed. The aqueous phase (distilled water containing 0.5% w/w KT) was dissolved in the oily phase at 90°C until gel transparent microemulsion is formed (F8)[6].

Preparation of ketorolac tromethamine creams (F9-F10)

(O/W and W/O) Cream were prepared by placing all the aqueous-phase (containing 0.5% w/w KT) and oil-phase ingredients into separate beakers and heated to 70°C, O/W emulsion base (F9) was prepared by the addition of the oil phase to water-surfactant (tween 80) mixture[7] while W/O emulsion base (F10) was prepared by addition of the water phase to oil-surfactant (span 80) mixture[8] and stirred until cold[9].

Evalution of ketorolac tromethamine formulations

1- Interaction between KT and polymers

Differential scanning calorimetry (DSC)

DSC studies were carried out for each of KT, the polymer separately and the corresponding physical mixture (the drug and each polymer) in order to determine the extent of crystallinity of the drug in presence of the studied polymers.

Samples of about 5 mg were accurately weighed and encapsulated into flat-bottomed aluminum pans with crimped-on lids. The scanning speed of 10 C/min from 30°C to 300°C was used in presence of nitrogen at flow rate of 40 ml/min.

IR-spectroscopy

IR spectra for each of KT, the polymer and the corresponding physical Mixture of the drug and the polymer were done at a range 4000-400 cm^{-1} using disk method. The samples were ground, mixed thoroughly with KBr and compressed at a pressure of 6 ton/cm^2 using Shimadzu SSp^{-10} A IR compression machine.

2- Physical properties of KT formulations

Visual inspection

The prepared formulae were examined for their physical characteristics, color and homogeneity.

pH measurements

pH was measured for each formulation directly after preparation using a pH meter which was calibrated before use with standard buffered solution at pH 7, comparison of pH of formulation containing KT with that of placebo (formula contain no drug)[2].

Viscosity measurement

The viscosity of KT formulations was determined using Brookfield DV-III at temperature 25°C. Fifty grams of the sample was tested using a 50 ml capacity vessel using spindle 94 at speed 20 rpm.

Drug content studies

Drug content was determined by dissolving an accurately weighed quantity of the formulations (one gram) in 100 ml distalled water. 10 ml was quantitatively transferred to 50 ml volumetric flask and appropriate dilutions were made with distilled water. The resulting solution was then filtered using 0.45 cm membranefilters before subjecting the solution to UV spectrophotometric analysis for KT at λ_{max} 322 nm[2].

3- *In-vitro* release of KT from the prepared formulations

One gram sample of the formulation was placed on acircular area (6 cm^2 diameter) of cellophane membrane previously moistened with the receptor phase. The loaded membrane was firmly stretched over one end of a glass tube. The tube was then immersed in a 250 ml beaker containing 100 ml of the release media (phosphate buffer pH 6.8) and placed in thermostatically water bath at 37°C at 30 strock / min. An aliquot of 5 ml sample was withdrawn at different time intervals, and replaced by equal volume of the release medium maintained at the same temperature. The amount of KT released at each time interval was determined spectrophotometrically at λ_{max} 322 nm against blank similarly treated[3].

4- *In-vitro* skin permeation

Hairless rats were sacrificed by an overdose of halothane anesthesia. The skin from the dorsal surface excised and the adherent fat and subcutaneous tissue were removed. The skin was mounted on diffusion cell (6 cm^2 tube as *in-vitro* release) with the epidermis facing the donor compartment. The skin permeation studies were performed as mentioned under (the *in-vitro* release studies) but using rat skin (6 cm^2 section area, 0.2 cm thickness) instead of cellophane membrane[10].

5- Kinetic studies

Kinetic analysis was carried out by the data to determine the release model which describes the release pattern of the drug, formulations were analyzed to zero order, first order[11], Higuchi diffusion model[12].

RESULTS AND DISCUSSION

1- Differential scanning calorimetry (DSC) and IR spectroscopy

The DSC studies showed the weakness of the drug melting point peak in case of KT - HPMC mixture (Fig. 3) and in case of KT–Carbopol934 mixture (Fig. 4) but still in the same region of KT melting point (165-167°C)[13] while in case of KT-sod alg (Fig. 1), KT-NaCMC (Fig. 2) and KT-Pluronic (Fig. 5) mixtures, the melting point of KT is still identified. These results indicates that no interaction between KT and these polymers.

The IR studies (Figs. 6-10) show that no chemical interaction has occurred between KT and the polymers as there is no remarkable shifting in characteristic peaks of KT (carbonyl group stretching band peak at 1600 cm^{-1}, hydroxyl group and amine group stretching bands at 3400-3500 cm^{-1}) in both KT and physical mixture spectra. From DSC & IR studies, the results confirmed the compatibility between KT and polymers.

2- Physical properties of KT formulations

Visual Inspection

Visual inspection showed that all formulae were homogeneous and appearance was acceptable and its colour different from formula to other, brown (F1), transparent (F2, F3, F4, F5 and (F8), white (F6, F7, F9 and F10).

Drug content, pH and viscosity measurements

Table (1) shows that the drug content of KT in different formulations was between 4.84 mg/gram (F10) and 4.98 mg/gram (F1) (96.8-99.6%). pH measurements of formulations were 6.30 (F9) and 7.1 (F4) and this less than that of placebo which referred to the acidic NSAIDs. The viscosity of KT formulations were between 6800 cp (F1) and 42000 cp (F10), rheological properties of KT formulations showed psuedoplastic flow.

3- Release of KT from the prepared formulations

Figure (11) shows that within the time of release (4 hrs) at first two hrs there is similar in release of KT from sodium alginate, NaCMC, HPMC and Carbopol 934 while the release from pluronic 127 is less than those polymers, but after 4 hrs there is different in release of KT from polymers as follow order. HPMC > NaCMC > Carbopol 934 > Sodium alginate > Pluronic 127.

The difference in drug release from gels formulations may be due to a decrease in the availability of free drug as a result of micellar entrapment, i.e. the tendency of the drug to leave the vehicle is strongly dependent on its microstructure[14].

The release of KT from emulgels (Fig. 12) shows that within the time of release, first 0.5 hr no significant different in release of KT from emulgels, after 0.5 hr significant different in release between F6 and (F7, F8), but no significant different in release between F7 and F8. The different in release of KT from emulgel bases may be due to different in viscosity of bases and different in oil content (↑ oil content decrease release) as KT is Fairly soluble in water[13].

The release of KT from emulsion ointment base (Fig. 13), from first 0.25 hr to end of time release O/W cream base (F9) gave higher release than W/O cream base (F10) which can be explained as mentioned under emulgel release.

Kinetic studies

Mathematical treatment of the release patterns of KT formulations through cellophane membrane indicated that, the release patterns follow First order for F1, F2,

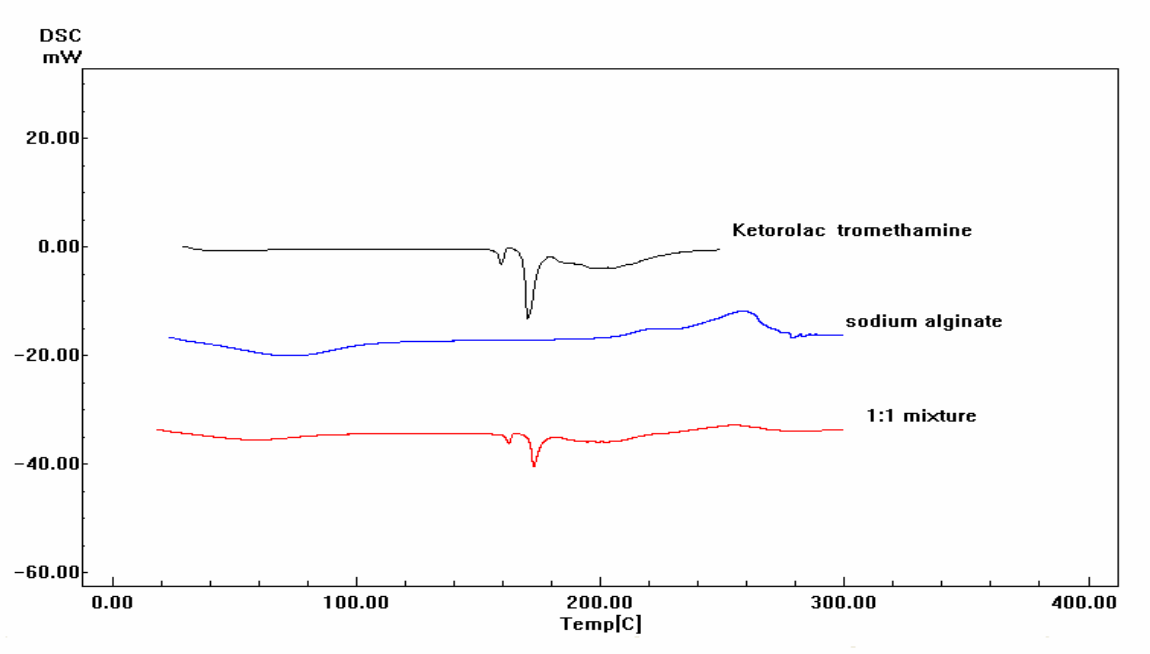

Fig. 1: DSC thermogram of KT, sodium alginate and mixture 1:1.

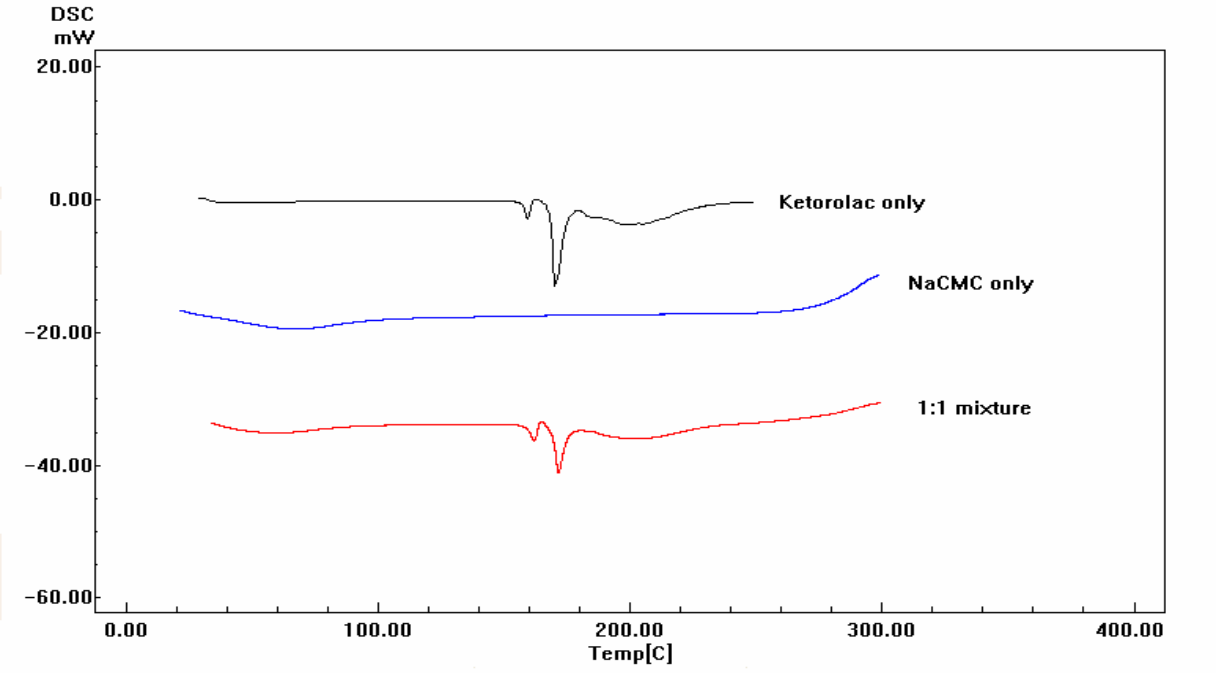

Fig. 2: DSC thermogram of KT, NaCMC and mixture 1:1.

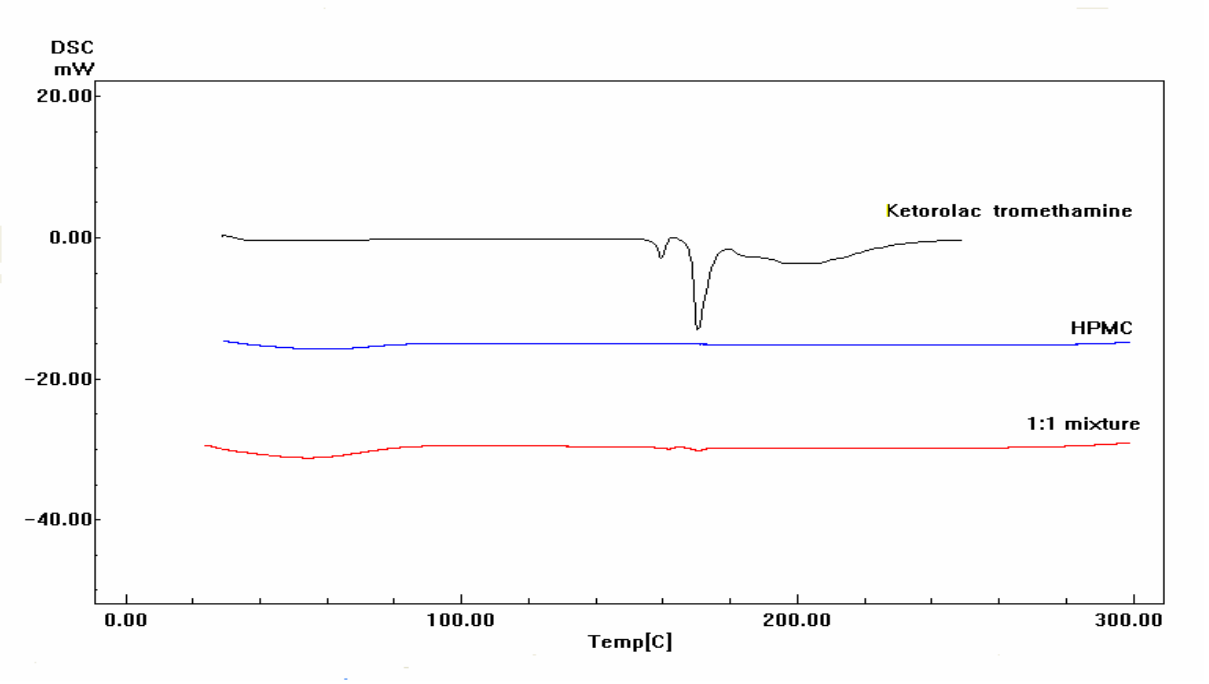

Fig. 3: DSC thermogram of KT, HPMC and mixture 1:1.

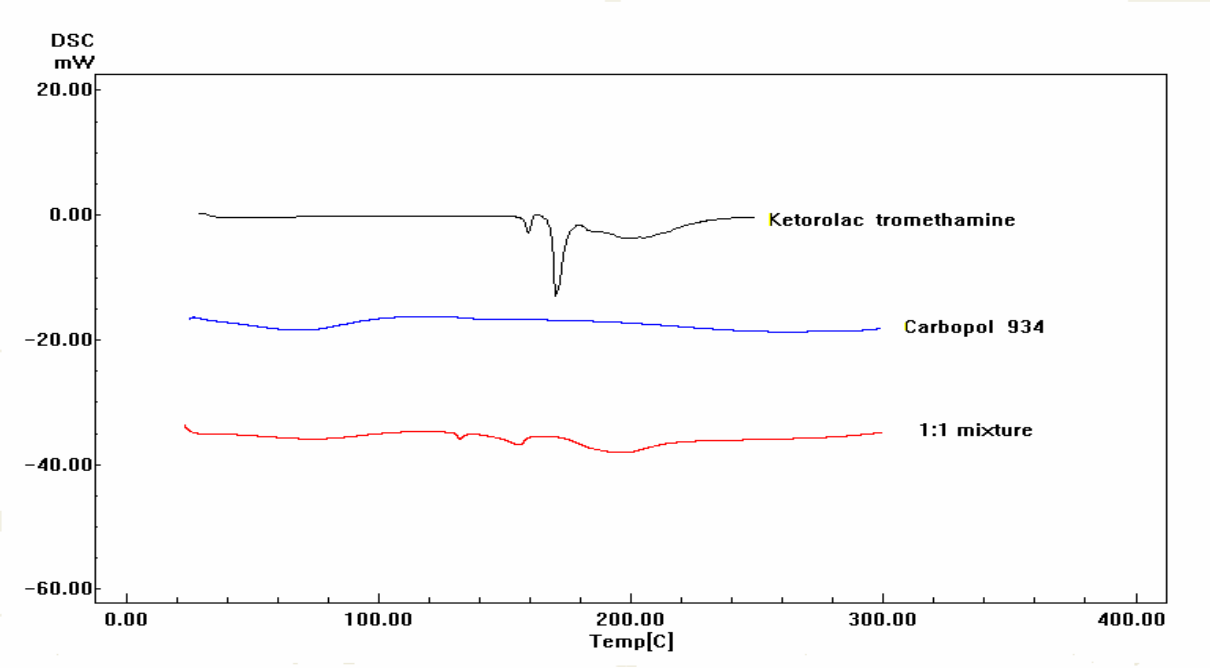

Fig. 4: DSC thermogram of KT, Carbopol 934 and mixture 1:1.

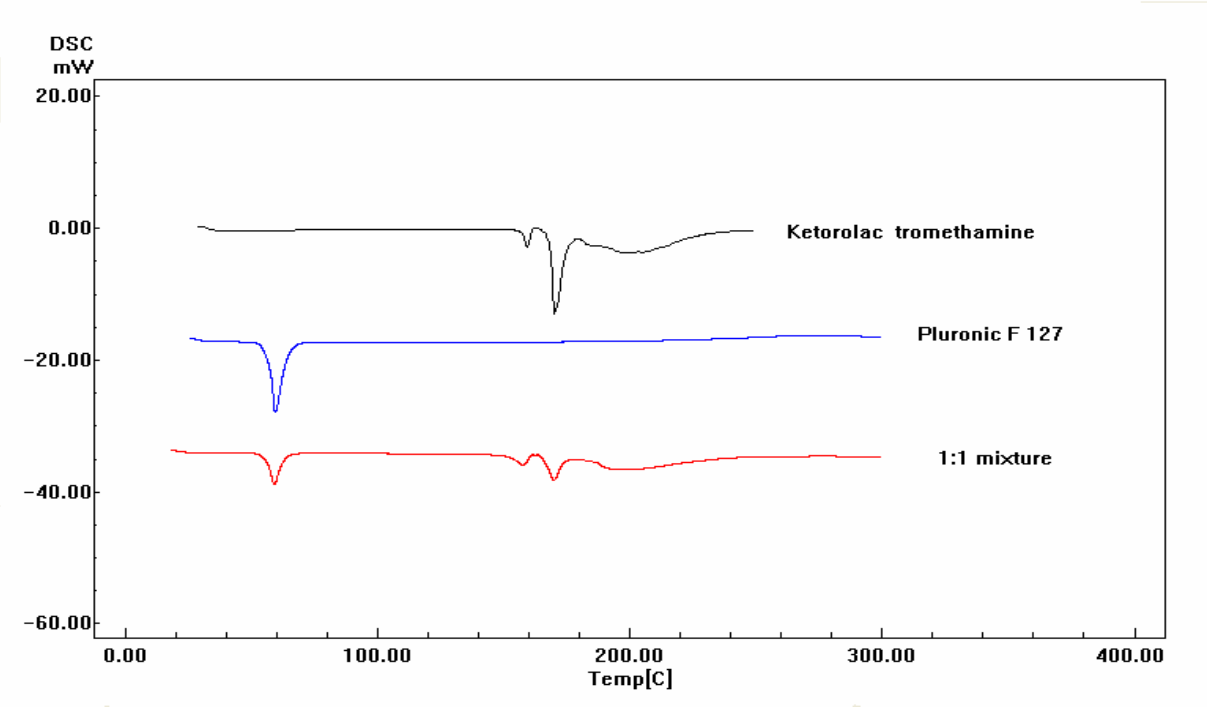

Fig. 5: DSC thermogram of KT, Pluronic 127 and mixture 1:1.

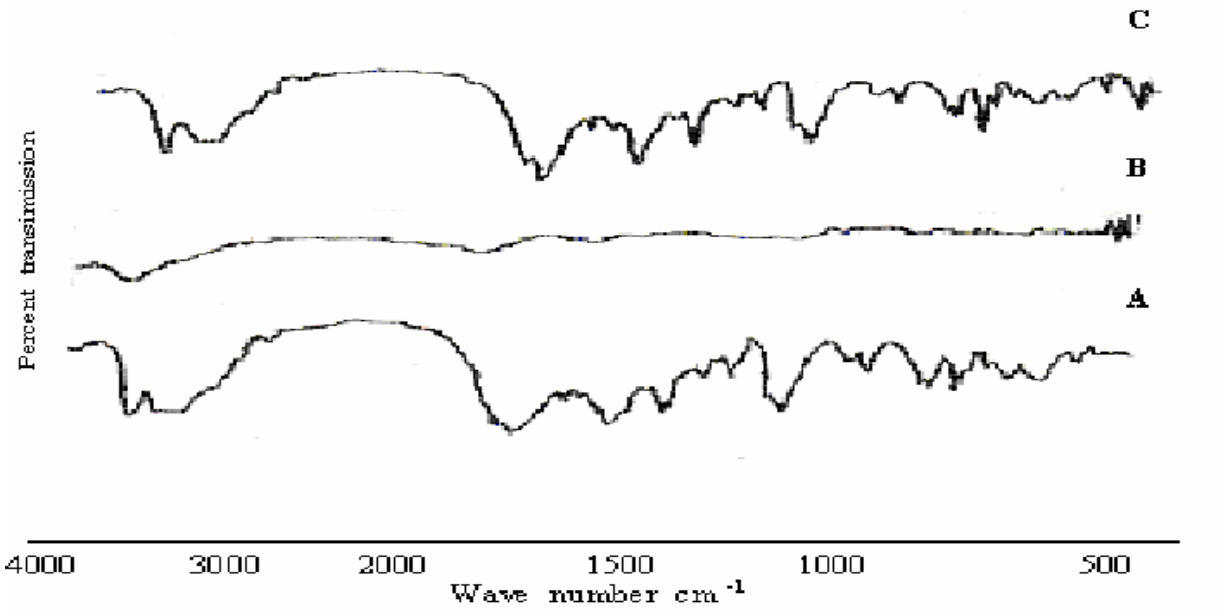

Fig. 6: The IR absorption spectra of KT /sodium alginate 1:1 ratio.
where A= KT, B= Sodium alginate, C= Physical mixture.

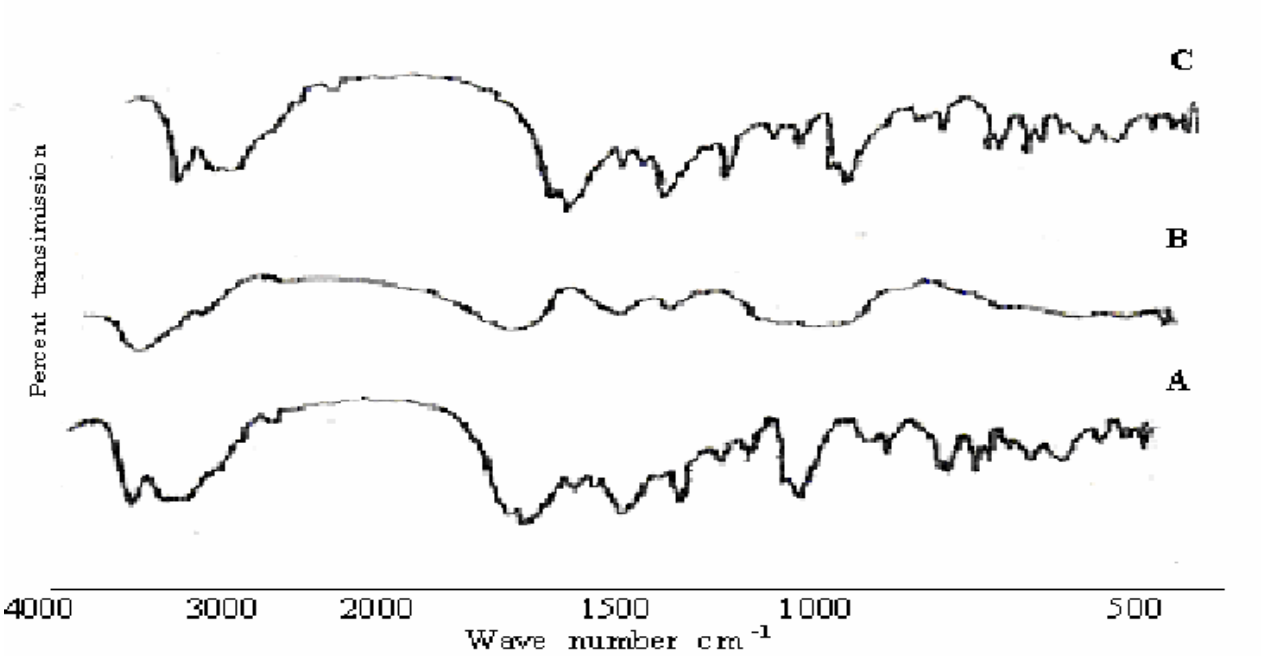

Fig. 7: The IR absorption spectra of KT /NaCMC 1:1 ratio.
where A= KT, B= NaCMC, C= Physical mixture.

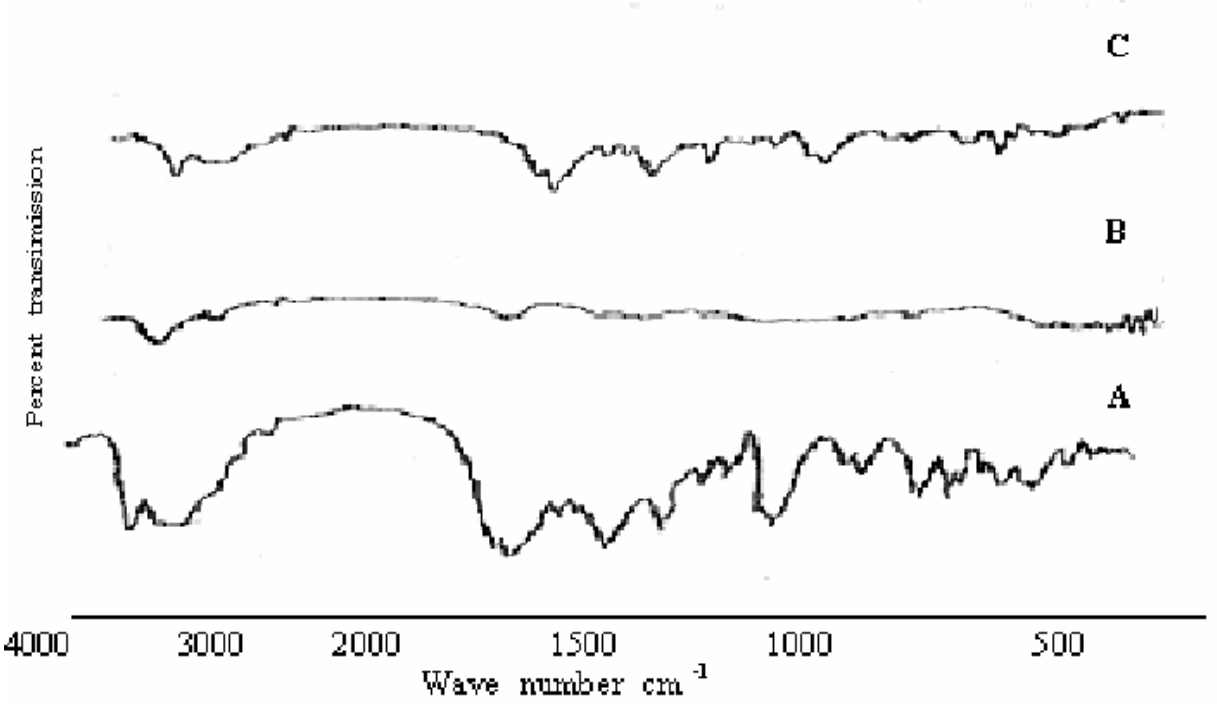

Fig. 8: The IR absorption spectra of KT /HPMC 1:1 ratio.
where A= KT, B= HPMC, C= Physical mixture.

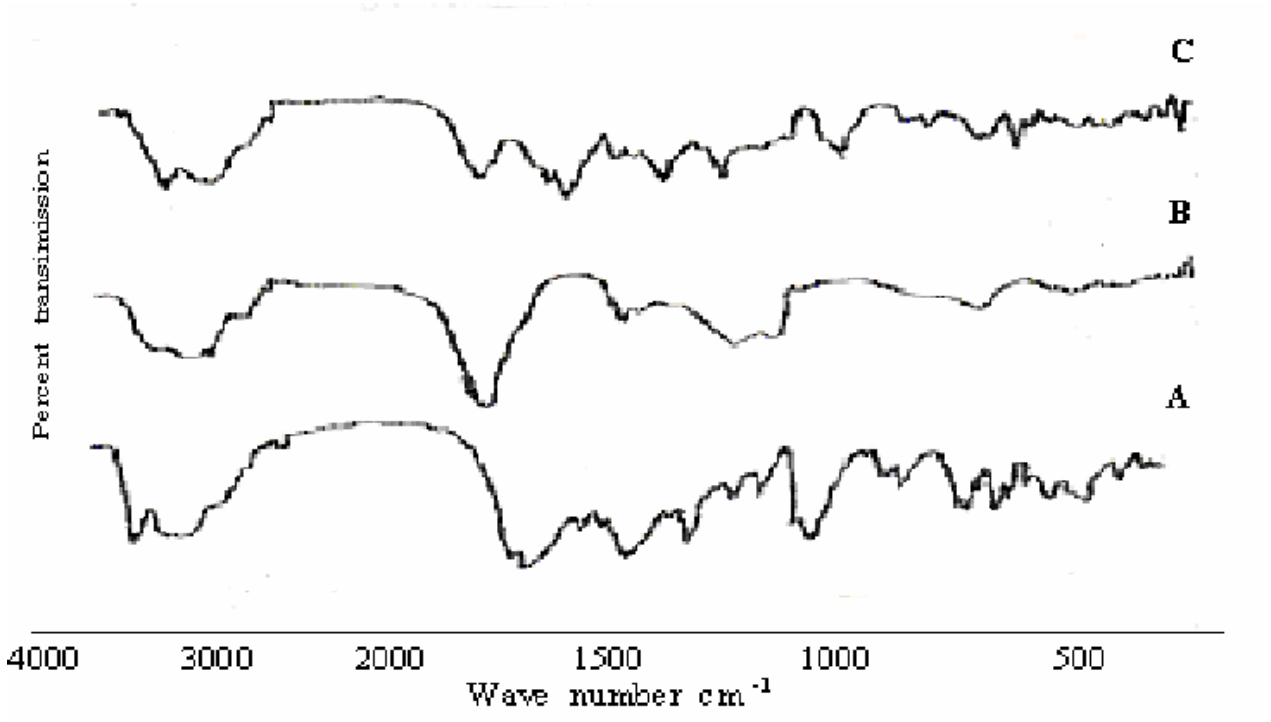

Fig. 9: The IR absorption spectra of KT /Carbopol 934 1:1 ratio.
where A= KT, B= Carbopol 934, C= Physical mixture.

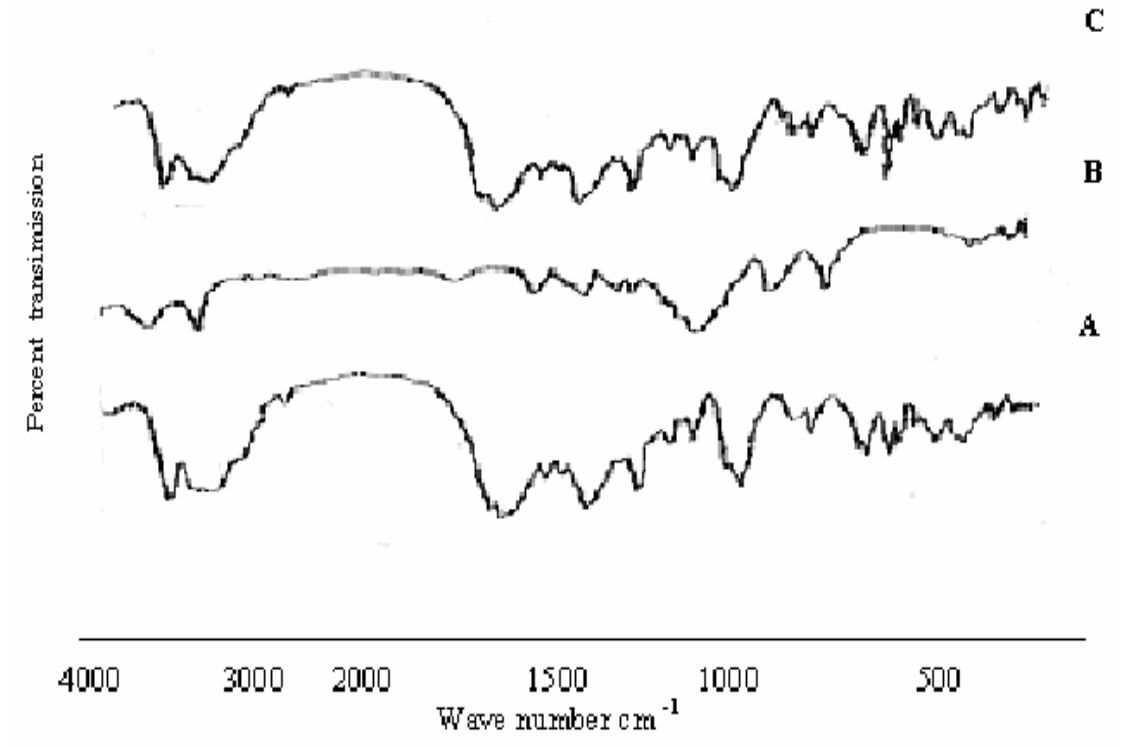

Fig. 10: The IR absorption spectra of KT /Pluronic 127 1:1 ratio.
where A= KT, B= Pluronic 127, C= Physical mixture.

Table 1: drug content, pH and viscosity of 0.5% w/w KT formulations.

Formula	Drug content (mg/gram) ±SD	pH		Viscosity (cp) at 25°C±SD
		Placebo formula	KT formula	
F1	4.98±0.08	6.72	6.50±0.08	6800±150
F2	4.92±0.08	6.74	6.55±0.06	13000±200
F3	4.97±0.06	6.70	6.45±0.06	15500±240
F4	4.95±0.06	7.18	7.10±0.08	12000±220
F5	4.90±0.06	6.98	6.65±0.08	19000±280
F6	4.93±0.08	6.89	6.65±0.06	10200±230
F7	4.89±0.06	6.86	6.58±0.08	14500±270
F8	4.95±0.08	6.76	6.45±0.04	17000±210
F9	4.89±0.05	6.78	6.30±0.06	28000±230
F10	4.84±0.08	6.75	6.40±0.06	32000±300

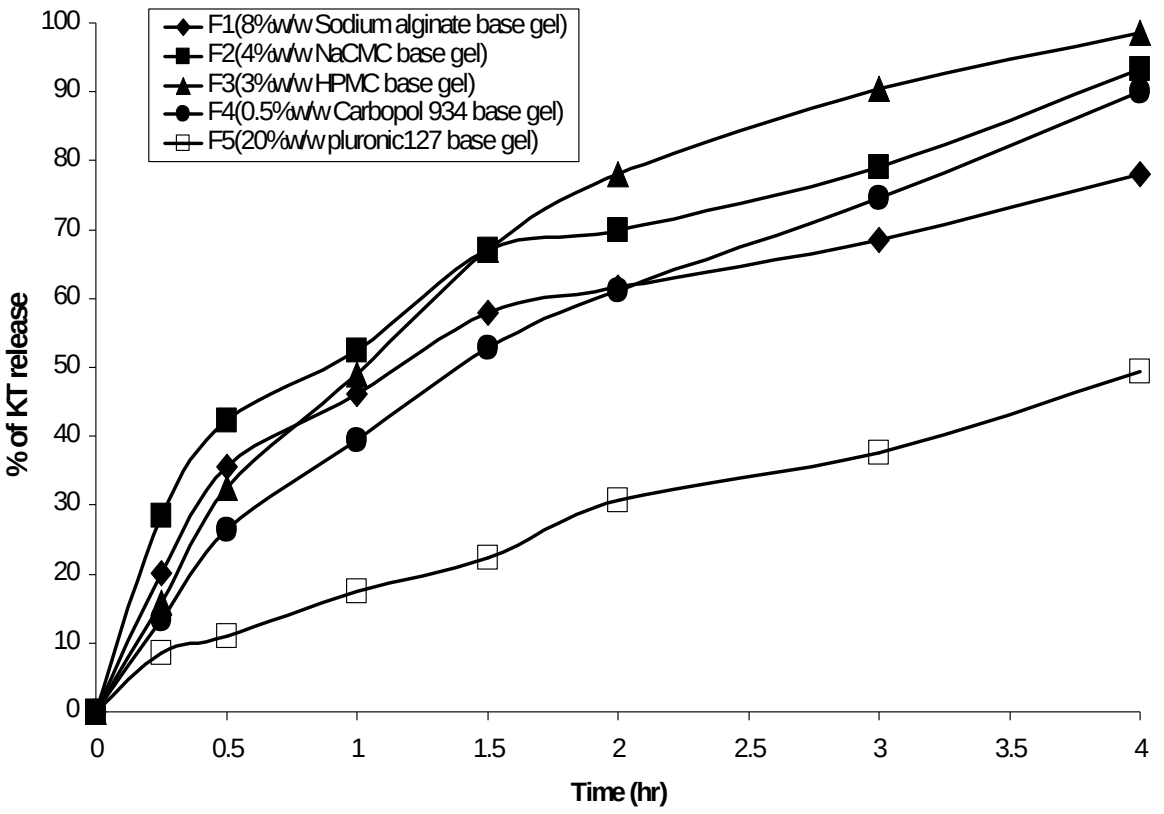

Fig. 11: Release profile of KT from gel formulations.

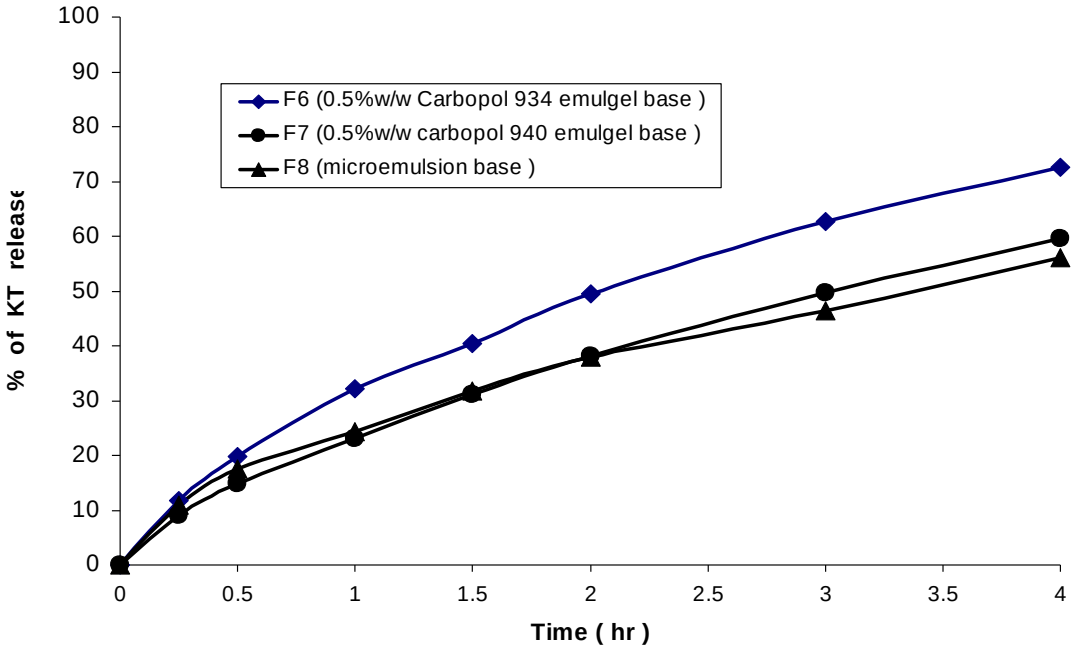

Fig. 12: Release profile of KT from emulgel and microemulsion formulations.

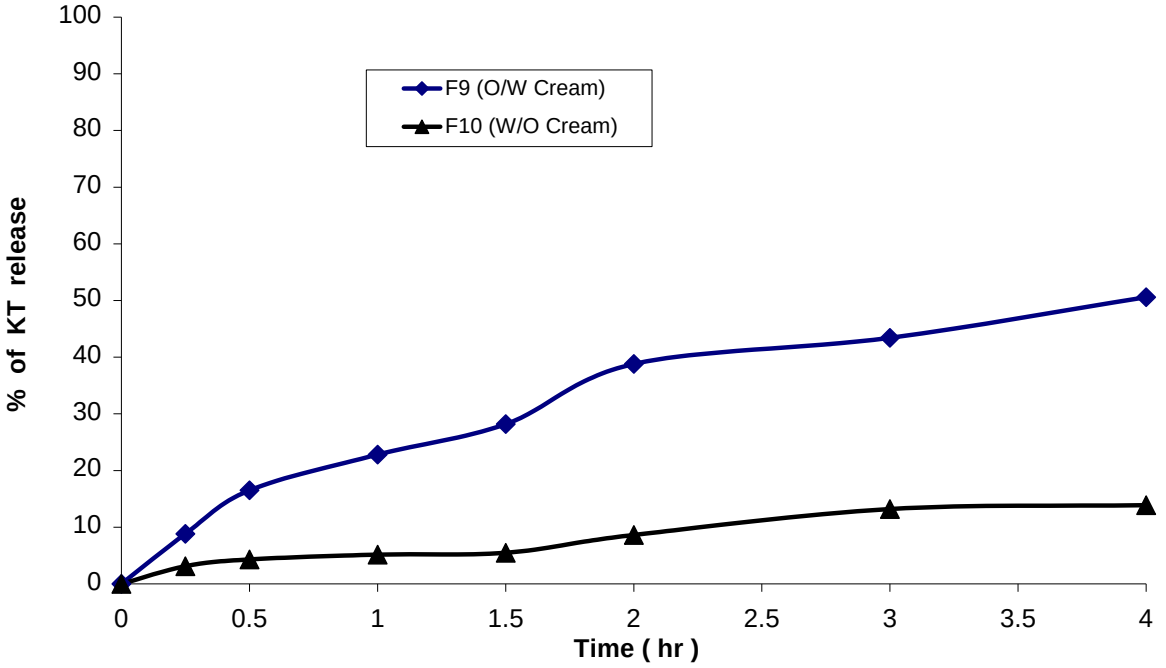

Fig. 13: Release profile of KT from emulsion ointment base formulations.

Zero order for F5, F7, F10, Higuchi diffusion for F3, F4, F6, F8, F9. These results are shown in Table (2).

4- *In-vitro* skin permeation

Three gel formulations (F3, F4, F5), one emulgel formula (F6), micromulsion base (F8) and one cream base (F9) are used in studying skin permeation of KT. The permeation rate of the drug from the donor through the skin into the acceptor compartment is determined by measuring the amount permeated as a function of time. The cumulative drug permeated ($\mu g.cm^{-2}$) was plotted against time (Fig. 15) and the steady state permeation rate J ($\mu g.cm^{-2}.h^{-1}$) was calculated from the slope of the linear portion of the curve[15]. The diffusion coefficient was calculated from the slope obtained by plotting the cumulative amount of permeated drug ($\mu g/cm^2$) versus square root of time (Fig. 16) according to Higuchi diffusion equation.

$$D = (\text{slope} / 2\, C_v)^2\, \pi$$

The partition coefficient K was calculated from P and D using the penetration barrier L with known thickness hairless rat skin (0.2 cm).

Figure (14) shows, within the time of the permeation of KT, at first two hrs no significant different on permeation of KT from all formulations while at end of time of permeation (4 hrs), permeation of KT from carbopol 934 gel and HPMC gel was higher than that of other formulations.

The difference in KT permeation could be mainly attributed to the difference in the diffusion through the skin barrier (D) and to some extent to the partitioning between the vehicle and the stratum corneum (K), as the high value of K indicates that the vehicle has poor affinity for the drug. A low K value which indicates a high degree of mutual interaction, reflects the tendency of the drug to remain in the vehicle. Hence, the release of a substance will be favored by selecting with low affinity for the drug.

KT gels formulations; F3, F4 gave higher permeation rate across the hairless rat skin; 33 $\mu g.cm^{-2}.h^{-1}$, 34 $\mu g.cm^{-2}.h^{-1}$ respectively than F5; 6.5 $\mu g.cm^{-2}.h^{-1}$. This observation was attributed mainly to the difference in K; F3 (0.7×10^2), F4 (0.6×10^2) which low value comparsion with that in case of F5 (2.9×10^2). Also the difference of K values explain the difference in KT permeation in other formulations (Table 3).

Diffusion coefficient (D) reflects the facility of drug move through the various membrane strata. The KT flux through rat skin from F6 was higher than F8. The higher diffusion coefficient (18.8 cm^2/h) of KT from F6 compared to that of from F8 (5.45 cm^2/h) can explain this observation.

Table 2: Kinetic parameters of the release data of KT from formulations.

Formula	R (Correlation coefficient)			Order of release	Intercept	Rate constant (K)	$t_{1/2}$
	Zero	First	Diffusion				
F1	0.978	**0.992**	0.984	First	1.89	0.399 hr^{-1}	1.740 hr
F2	0.957	**0.997**	0.958	First	1.93	0.685 hr^{-1}	1.012 hr
F3	0.954	0.945	**0.993**	Diffusion	4.36	54.98%$hr^{-1/2}$	0.827 hr
F4	0.933	0.982	**0.997**	Diffusion	1.52	40.714%$hr^{-1/2}$	1.510 hr
F5	**0.992**	0.979	0.986	Zero	4.63	10.74%hr^{-1}	4.655 hr
F6	0.987	0.993	**0.997**	Diffusion	8.54	48.47%$hr^{-1/2}$	1.064 hr
F7	**0.997**	0.991	0.985	Zero	4.20	11.44%hr^{-1}	4.370 hr
F8	0.982	0.975	**0.990**	Diffusion	9.04	36.8%$hr^{-1/2}$	1.846 hr
F9	0.986	0.989	**0.996**	Diffusion	4.39	34.48%$hr^{-1/2}$	2.100 hr
F10	**0.994**	0.985	0.988	Zero	2.24	3.111%hr^{-1}	16.07 hr

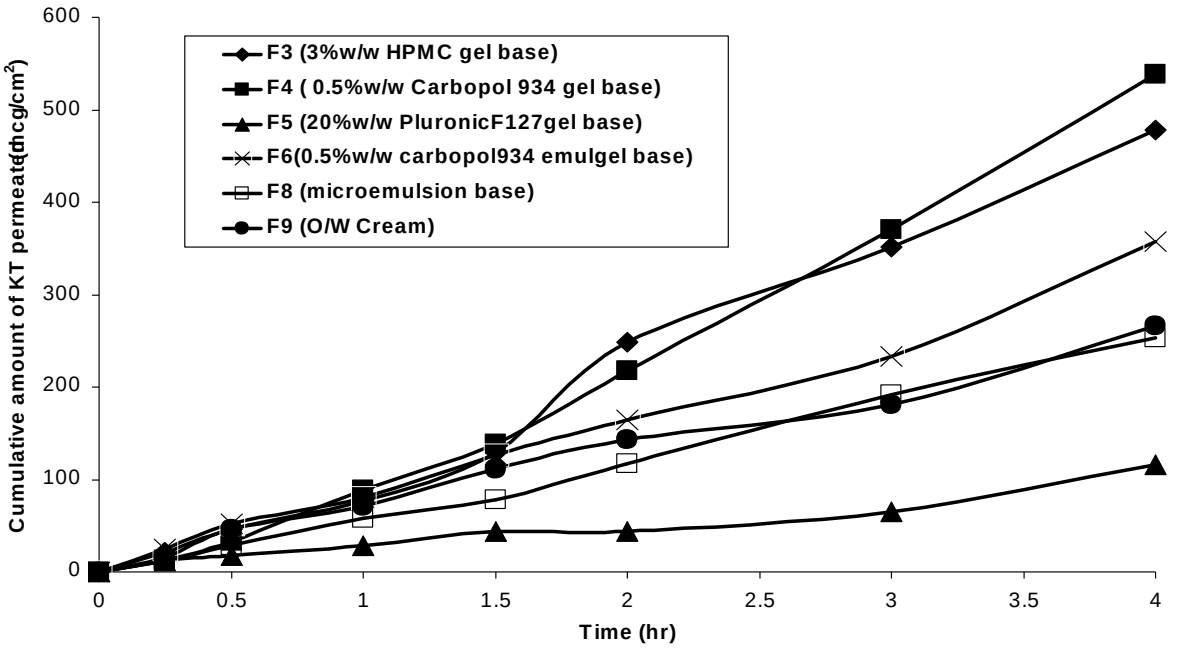

Fig. 14: Permeation profile of KT from selected formulations.

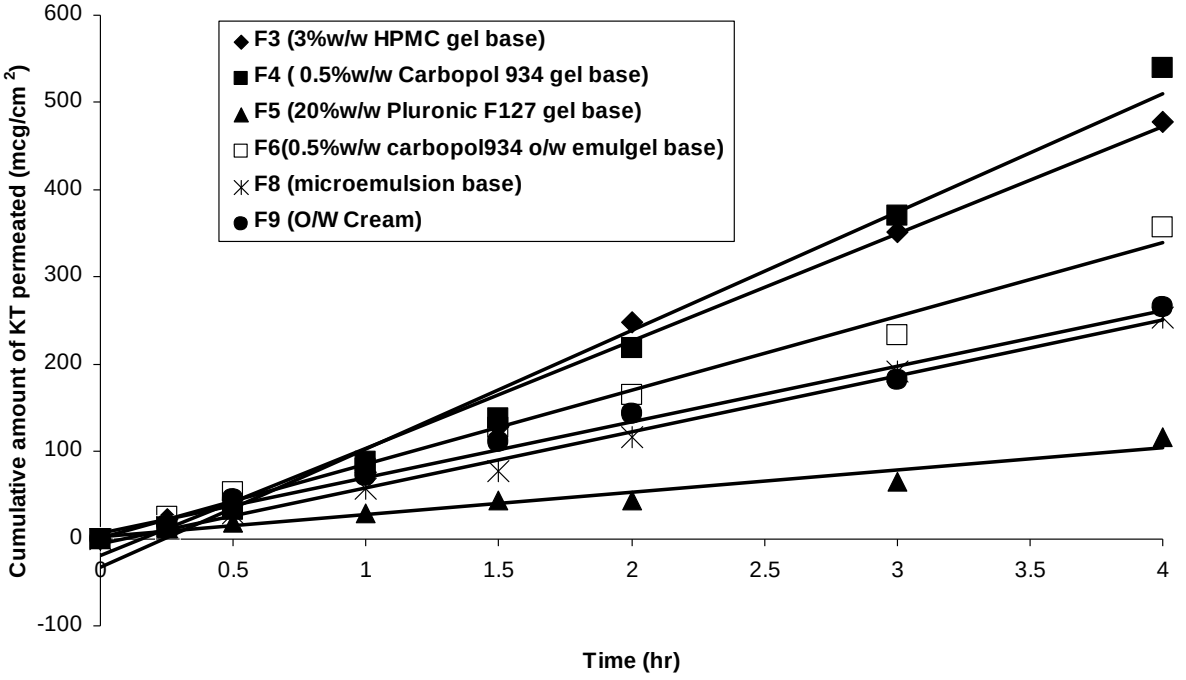

Fig. 15: Amount of KT permeated across hairless rat skin from selected formulations.

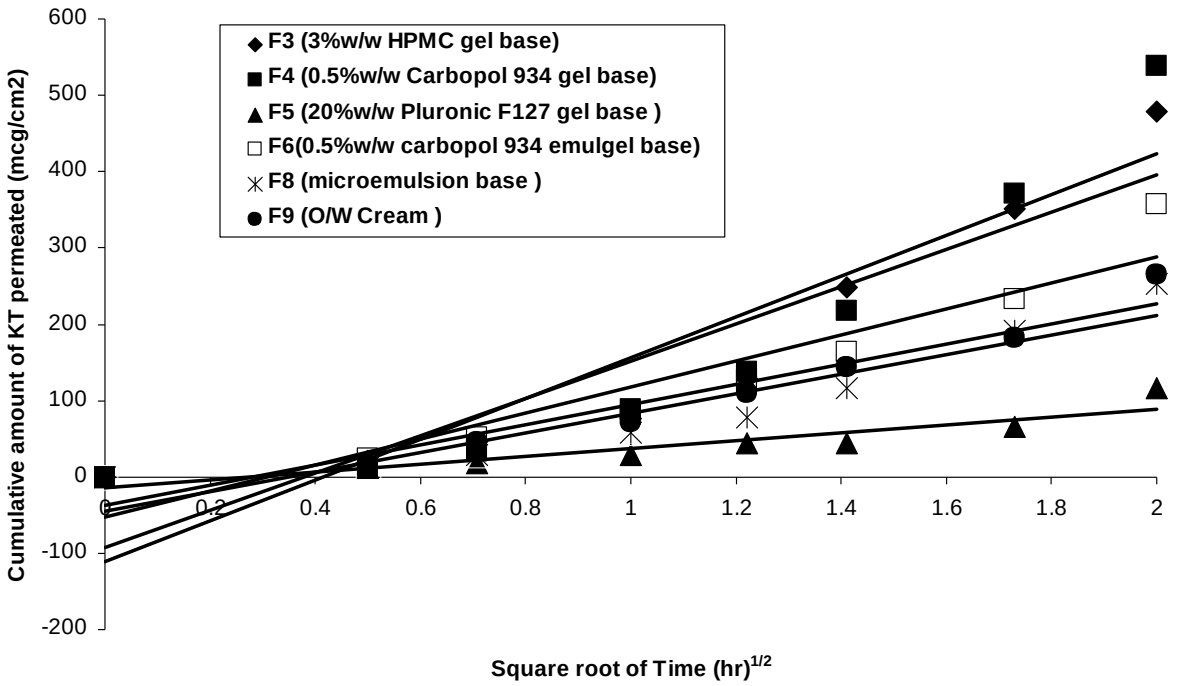

Fig. 16: Amount of KT permeated per unit surface area vs. Square root of time profile of KT from selected formulations.

Table 3: Percutanous penetration parameters of KT ketorolac formulations through hairless rat skin.

Formula	Flux (J) ($\mu g/cm^2 h$) ±SD	Permeability Coefficient(P) (cm/h) × 10^4 ±SD	Diffusion Coefficient(D) (cm^2/h) ±SD	Partition Coefficient (K) ×(10^2) ±SD
F3	33.0 ± (0.57)	0.66 ± (0.01)	18.8 ± (0.44)	0.70±(0.001)
F4	21.0 ± (0.81)	0.68 ± (0.012)	22.4 ± (0.42)	0.60±(0.001)
F5	34.0 ± (0.91)	0.13 ± (0.001)	0.83 ± (0.001)	2.90±(0.005)
F6	6.50 ± (0.32)	0.32 ± (0.005)	5.20 ± (0.08)	1.23±(0.003)
F8	16.0 ± (0.63)	0.32 ± (0.004)	5.45 ± (0.05)	1.17±(0.001)
F9	16.0 ± (0.49)	0.42 ± (0.005)	9.20 ± (0.15)	0.91±(0.001)

Conclusion

KT has been successfully prepared in various topical Formulations. The prepared formulations showed acceptable consistency and usage criteria. Gel formulations gave high release of KT *in-vitro* and more KT permeation through hairless rat skin than other formulations except pluronic F127 which gave promising sustained release in both *in-vitro* and *in-vitro* permeation study. From the results obtained, Carbopol 934 gel gave also good results concering high release and good appearance.

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