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FORMULATION AND EVALUATION OF TOPICAL DOSAGE FORM CONTAINING MICROSPHERES FOR MODEL ANTI INFLAMMATORY DRUG

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ABSTRACT

The purpose of this study was to formulate a sustained release topical dosage form containing microspheres for Ketorolac Tromethamine (KT). Oral consumption of KT has significant gastric irritation, hence tried to prepare topical formulation to prevent possible side effects of oral consumption. The drug-excipients compatibility studies were carried out by FT-IR and DSC studies. Based on the results, excipients used were found to be compatible with KT. The formulations were prepared by using different concentration of polymer and plasticizer along with other excipients, and using 0.008% benzalkonium chloride as a preservative. The concentration of polymers was in the concentration range of 1.0%-2.5%, where plasticizer concentration range was 15-25%. The formulation F8 resulted in drug content 99%±0.36, spreadability 9±0.50gm-cm/sec, viscosity of 13420cps and maximum *in-vitro* diffusion of 47.37±0.39% over a period of 5 hrs which was selected as a best batch. The microspheres prepared by solvent evaporation method were evaluated and M3 batch was selected. M3 formulation of microspheres was incorporated into gel formulations F5 to F8. F11 selected as an optimized gel formulation containing KT microspheres which diffused 43.97±0.23% after 5 hrs. The selected optimized formulation F11 was subjected to stability studies as ICH guidelines and were found to be stable. Hence the formulation F11 will eliminate the GIT side effect, enhance the percutaneous absorption and exhibit sustained release anti-inflammatory activity.

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INTRODUCTION

Ketorolac tromethamine is a potent non narcotic analgesic with moderate anti-inflammatory activity. It has been investigated extensively for use in post-operative analgesia both as a sole agent and supplement opioid analgesics and excellent applicability in the emergency treatment of post operative cancer pain and in the treatment of migraine pain [2]. The biological half-life of KT is 4–6 h. Therefore frequent dosing is necessary to sustain the action of drug to alleviate pain in post operative patients with a possibility of patient non compliance. When administered as the conventional formulation, it causes gastro intestinal complications including irritation, ulcer, bleeding and perforation [3-5]. Therefore, recent focus of the researchers has been to deliver such potential NSAIDs in a controlled manner by using a dosage form that will minimize its release in stomach and thereby overcomes its chronic adverse effect [6].

Due to GIT upset which happens on oral consumption of KT, this research tried to provide a topical dosage form of KT to prevent GIT problems associated with the same drug. Recently several advancements have been made which have resulted in development of new techniques of drug delivery. These techniques are capable of controlling the rate or drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of the drug to the tissue [1]. The main aim of the research work is to develop a multiparticulate drug delivery system for topical dosage form, using model anti-inflammatory drug (Ketorolac tromethamine), in view to maintain sustained release action and reduce dosing frequency.

Several approaches have been tried to develop non-oral formulations in addition to injections. Among the non-invasive routes, topical administration has a promising potential as a viable alternative for systemic medication of drugs which shows merits such as non-invasive, easy, local effect, and high level of patient satisfaction [7].

Topical preparations circumvent GI irritation, prevent the metabolism of drug in the liver and increase the bioavailability of the drugs and provide its action directly at the site of action. Gel base formulations for dermatological use have several favorable properties such as greaseless, easily spreadable, easily removable, thixotropic, water soluble or miscible, non- staining and emollient [8,9].

Microspheres carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems [10]. They act as reservoir releasing an active ingredient over an extended period of time maintaining effective drug concentration in the skin and at the same time, reducing undesired side effects [11].

Hence an attempt was made to formulate Ketorolac tromethamine as gel incorporated in microspheres in order to eliminate the GIT side effect, to enhance the percutaneous absorption and to achieve sustained release anti-inflammatory activity.

MATERIALS AND METHODS

Materials

Ketorolac Tromethamine drug (molecular weight 376.403 gm/mol) received as a gift sample from Mylan Laboratories, Bengaluru (India). Carbopol 934P, ethyl cellulose, poly vinyl alcohol(PVA), dichloro methan, Benzalkonium chloride, sodium hydroxide flakes, were received from Central drug house (Pvt) Ltd., New Delhi (India). Propylene glycol, polyethylene glycol 6000, methanol, ethanol, glycerin, menthe oil, potassium Dihydrogen orthophosphate, were received from Industrial Estate, Mumbai (India) and Triethanolamine was received from Merck Limited, Mumbai (India). All other chemicals and reagent used in this study were of analytical grade.

Methods

Pre-formulation studies

Preparation of calibration curve

A standard curve was prepared in the concentration range of 0-18 µ/ml with phosphate buffer pH 7.4. The absorbance of resulting solutions was measured at 324 nm and recorded. Concentrations versus absorbance values were plotted.

Drug-excipients Compatibility

The compatibility studies were carried out at room temperature by Fourier transform infrared (FTIR) spectroscopy to determine the interaction of drug with other excipients used in the formulation. The IR spectra of drug alone and physical mixtures of the drug with proposed excipients (ethyl cellulose) in the ratio of 1:1 were prepared. 1 part of this mixture was triturated with 100 parts of KBR (400–4000^{-1}) with a scanning speed of 2 mm/sec with normal slit and compressed to form pellet which was then analysed by FTIR (FTIR, 8400S, Shimadzu, Germany).

Differential Scanning Calorimetric (DSC)[12]

Samples were weighed and sealed in 40 ml aluminum crucibles with a pierced aluminum lid. The analyses were performed under nitrogen (nitrogen flow rate 50ml/min) in order to prevent oxidative effect at the standard heating rate of 10°C/min over a temperature range of 100°C-270°C, under static air, using a Mettler-Toledo STAR system.

Formulation of Ketorolac Tromethamine gel[13-16]

The formulations were prepared by using different concentration of polymer and plasticizer along with other excipients, and using 0.008% benzalkonium chloride as a preservative. The concentration of polymers was in the concentration range of 1.0%-2.5%, where plasticizer concentration range was 15-25%. The accurately weighed quantity of polymer was mixed in three by fourth quantity of distilled water. The resultant solution was viscous. The accurately weighed quantity of drug was dissolved in ethanol in separate beaker. Then the drug solution was mixed with polymer solution. Then the remaining excipients are mixed to the viscous solution. The above solution was thoroughly mixed by stirring for 30minutes. The prepared gel was evaluated for various parameters.

Table No. 1: Formulation of Ketorolac Tromethamine gels: F1-F8.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Ketorolac Tromethamine (gm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Carbopol 934P (gm)	1	1.5	2	2.5	2	2	1.5	1.5
Propylene Glycol (ml)	20	20	20	20	15	25	15	25
Polyethylene Glycol (ml)	5	5	5	5	5	5	5	5
Glycerine (ml)	10	10	10	10	10	10	10	10
Ethanol (ml)	10	10	10	10	10	10	10	10
Mentha Oil (ml)	1	1	1	1	1	1	1	1
Triethanolamine (ml)	1	1	1	1	1	1	1	1
Benzalkonium Chloride (ml)	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Distilled water (ml)	q.s to 100	q.s to 100	q.s to 100	q.s to 100	q.s to 100	q.s to 100	q.s to 100	q.s to 100

Preparation of sustained release Ketorolac Tromethamine microspheres [17-21]

Microspheres were prepared by solvent evaporation method. Drug and polymer (ethyl cellulose) were dissolved in 10 ml of Dichloromethane and methanol mixture which are present in the proportion of 8:2 as shown in Table No. 2. Then the slurry was slowly introduced into 50ml of 0.5% w/v of polyvinyl alcohol while being stirred at 900 rpm by a mechanical stirrer, equipped with a three bladed propeller at room temperature. The solution was stirred for 2 hrs to allow the solvent to evaporate completely and the microspheres were collected by filtration. The microspheres were filtered by using Whattman filter paper. The collected microspheres were dried for 1hr at room temperature and subsequently stored in desiccators over fused calcium chloride.

Table No. 2: Formulation of Ketorolac Tromethamine M1-M3.

Ingredients	M1	M2	M3
KT (gm)	0.1	0.1	0.1
Ethyl cellulose (gm)	0.20	0.30	0.40
Dicholoromethan (ml)	8	8	8
Methanol (ml)	2	2	2
0.5% W/V Polyvinyl Alcohol (ml)	50ml	50ml	50ml

Preparation of sustained release Ketorolac Tromethamine gels containing microspheres

Best formulation of prepared gels and microspheres were selected to prepare Ketorolac Tromethamine gel containing microspheres in different ratios. The formulated microspheres were uniformly dispersed in the gel base by mechanical stirring for 30 minutes to get microspheres loaded gel. The prepared gels were packed in wide mouth glass jar covered with screw capped plastic lid, covering the mouth with an aluminum foil and were kept in dark and cool place.

Table No. 3: Formulation of Ketorolac Tromethamine gel containing microspheres (F9-F12).

Ingredients (gm or ml)	F9	F10	F11	F12
Prepared Microspheres of Ketorolac Tromethamine (1:4)	0.1	0.1	0.1	0.1
Carbopol 934P	1.5	2	2	1.5
Propylene glycol	20	20	15	15
Polyethylene glycol	5	5	5	5
Glycerin	10	10	10	10
Ethanol	10	10	10	10
Mint oil	1	1	1	1
Triethanolamine	0.5	0.5	0.5	0.5
Benzalkonium chloride	0.008	0.008	0.008	0.008
Distilled water	q.s to 100	q.s to 100	q.s to 100	q.s to 100

Evaluation of gel:

Appearance of gel

The prepared gels were inspected visually for clarity, color and presence of any particle. The test is important regarding patient compliance.

pH

Measurement of pH for all the formulations were done by dissolving one gram of formulation in 100ml distilled water for 2hrs and pH was measured by digital pH meter.

Drug content

One gram of gel was taken and dissolved completely in phosphate buffer of 7.4 pH. Made up the volume to 50ml by phosphate buffer and withdrawn 1, 2 and 3ml of the stock solution and made up the volume to 10ml with the phosphate buffer of same pH. Then it was analyzed by validated UV spectrophotometric method at λ_{max} of 324nm.

Spreadability[22]

Spreadability of the formulations was determined by an apparatus suggested by Multimer, which was fabricated itself in laboratory and used for slide fixed on wooded block and upper slide with one end tied to glass slide and other end tide to weight pan. Excess of gel (2gm) was placed between two glass slides and then 100gm weight was placed on slides for 5mins to compress the sample to a uniform thickness. Weight in the increasing order i.e. 20gm, 40gm, 80gm was added to pan. The time (sec) required to separate the two slides was taken as a measure of spreadability. It was calculated using the below formula:

$$S = L \times M / T$$

Where,

M = Weight tied to the upper slide.

L = Length of glass slide moved.

T = Time taken (sec).

Shorter time interval to cover the distance of 6.5cm indicates better spreadability.

Extrudability

For a good gel formulation, it should extrude easily from the selected container. This is also type of measurement of viscosity of formulation. In this test, sample is extruded from the tube by the visual procedure. A closed collapsible tube containing gel was passed firmly at crimped end. When the cap was removed, the gel extrudes until pressure was dissipates. The result for each formulation were recorded as extrusion pressure.

Viscosity

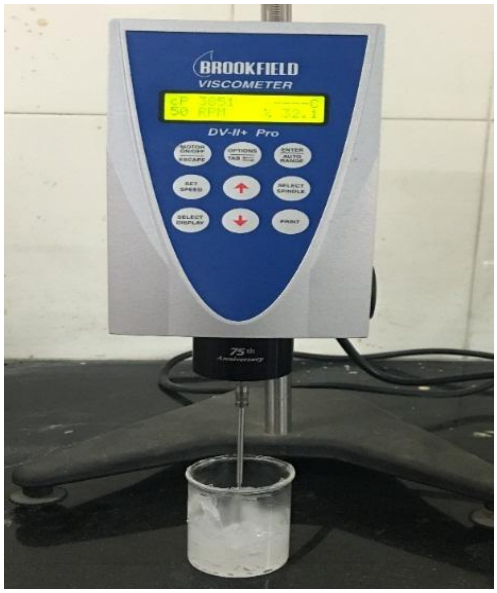

Figure No. 1: Measurement of viscosity by Brookfield Viscometer.

The viscosity of the gels prepared was determined using Brookfield Viscometer model. The gel sample is filled in the sample holder and a particular spindle (No.64) inversed into the sample. The spindle is attached to the viscometer and it is allowed to rotate at particular speed (50rpm). The viscosity of the formulation is measured after 2mins.

Preservation Efficacy Test

Weighed 3.7gm of nutrient agar was dissolved in 100ml distilled water, sterilized the media and pour 20ml into each petri-dish which were previously sterilized. The petri-dishes containing nutrient agar media were refrigerated for 2hours for solidification. Using a sterile swab, a suspension of the pure culture (*S. aureus*) is spread evenly over the face of sterile solid agar medium. A hole is bored in the center of the solid agar medium and 1gm of gel was added. Incubated at 30℃-35℃ and the product testing was done after 24hrs, 48hrs and 72 hrs. The zone of growth of microorganism is measured and noted.

***In-Vitro* diffusion study**

The apparatus used is Franz diffusion cell that is consists of donor and receive compartment. One gm gel equivalent to 100mg of KT, spread uniformly on the surface of cellophane membrane (previously soaked in phosphate buffer pH of 7.4 for overnight) and was fixed to the end of donor compartment such that the preparation occupied inner circumference of the tube. The whole assembly was fixed in such a way that the low end of the tube containing gel was just touched the surface of diffusion media i.e.50ml phosphate buffer of pH of 7.4, and maintained at 37± 2ºC. A quantity of 2ml of samples was withdrawn from receptor fluid at the time intervals of 30, 60, 90, 120, 150, 180, 210, 240, 270, 300 min. The released drug was estimated by using Shimadzu UV – Visible spectrophotometer at 324 nm.

Evaluation of microspheres

Particle size analysis of microspheres[23]

The particle size of the microspheres was determined by using optical microscopy method. Approximately 100 microspheres were counted for particle size analysis by using calibrated optical microscopy.

Scanning Electron Microscopy[24]

The shape and surface morphology of the dried microsphere was examined by scanning electron microscopy. Prior to examination, samples were gold coated under vacuum using 10.0-KV accelerating voltage, a 30mm working distance, 50μm objective aperture and a probe current of 6×10^{-11} amps. The object was exposed to two different magnifications to show the nature of the surface of the microspheres. The samples after 10hrs dissolution was also magnified at two different magnifications to show the formation of pores and the nature of release of drug from the matrix.

***In-vitro* drug release of Microspheres[25]**

Microspheres equivalent to 100mg of KT were subjected for dissolution. Dissolution tests were carried out using USP Type-I rotating basket (40 mesh). The stirring rate was 50 rpm. Dissolution medium was 900ml Phosphate buffer 7.4 pH and the temperature was maintained at 37±0.5℃. Aliquots of 1ml were withdrawn at predetermined intervals for 6hrs with pipette, filtered and volume was made to 10ml using Phosphate buffer 7.4 pH. The collected samples were analysed spectrophotometrically at 324nm.

Evaluation of gel loaded microspheres

***In-vitro* diffusion studies[26]**

The apparatus used is Franz diffusion cell that is consists of donor and receive compartment. One gm gel equivalent to 100mg of KT, spread uniformly on the surface of cellophane membrane (previously soaked in phosphate buffer pH of 7.4 for overnight) and was fixed to the end of donor compartment such that the preparation occupied inner circumference of the tube. The whole assembly was fixed in such a way that the low end of the tube containing gel was just touched the surface of diffusion media i.e.50ml phosphate buffer of pH of 7.4, and maintained at 37± 2ºC. A quantity of 2ml of samples were withdrawn from receptor fluid at the time intervals of 30, 60, 90, 120, 150, 180, 210, 240, 270, 300 min. The released drug was estimated by using Shimadzu UV – Visible spectrophotometer at 324 nm.

Stability studies[27]

Stability studies were carried out at 0℃, 25℃/60% RH and 40℃/75% RH for the optimized formulation for one month. The selected formulation was kept in suitable container and tightly closed. They were then stored at 0℃, 25℃/60% RH and 40℃/75% RH for 1 month and evaluated for drug content.

RESULTS AND DISCUSSION

Pre-formulation studies

A standard curve ranging from 0-18 µ/ml with phosphate buffer pH 7.4 at 324 nm was found to be linear with y=0.0549x and R^2=0.9994 as shown in Figure No. 2.

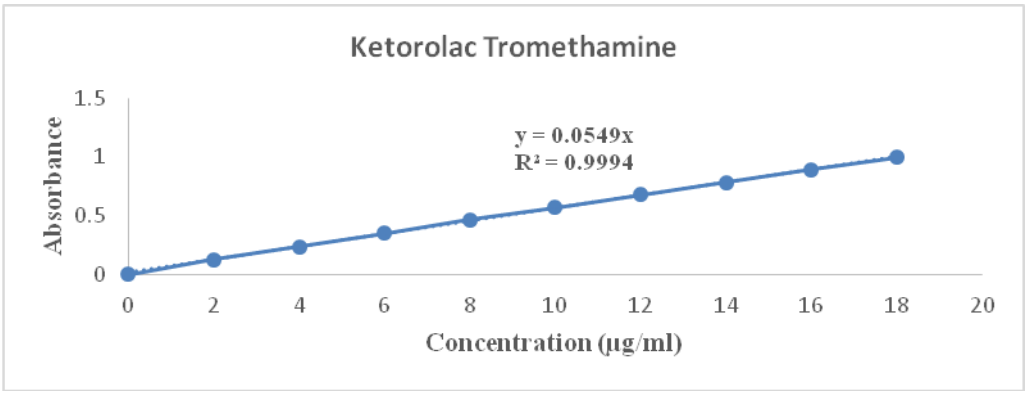

Figure 2: Standard calibration curve of Ketorolac Tromethamine.

Drug-excipients Compatibility

The FT-IR peaks of pure Ketorolac Tromethamine is presented in the Figure No. 3 showed characteristics of –OH stretch at 3354.68cm^{-1}, aromatic asymmetric and symmetric groups at 2926.32cm^{-1} and 2872.10cm^{-1}, C-OH and C-OH stretch at 1057.03cm^{-1} and 1197.83cm^{-1} respectively. The mixture of Ketorolac tromethamine with carbopol 934P is shown in Figure No. 4 and mixture of Ketorolac Tromethamine with ethyl cellulose is shown in Figure No. 5. The observed characteristic peaks are in limit and thus chosen excipients for formulation were found to be compatible and have no chemical interaction with the Ketorolac Tromethamine. Therefore, the FTIR studies ruled out the possibility of any drug polymer interaction and hence contribution of drugs along with excipients can be formulated safely.

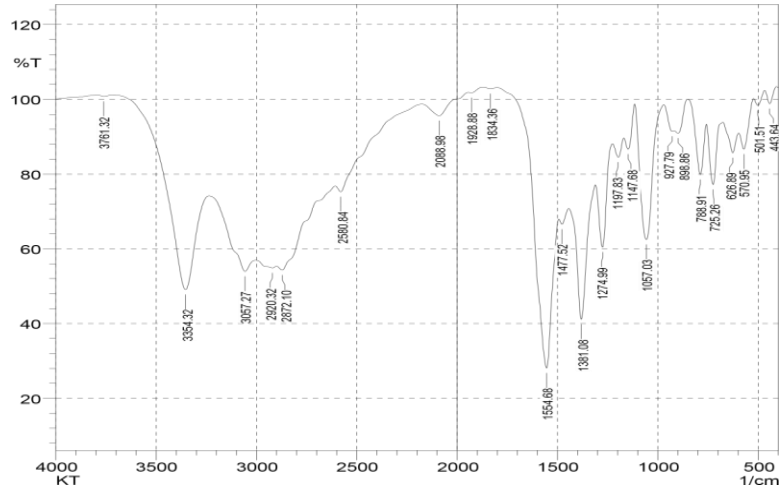

Figure No. 3: FT-IR spectra of Ketorolac Tromethamine.

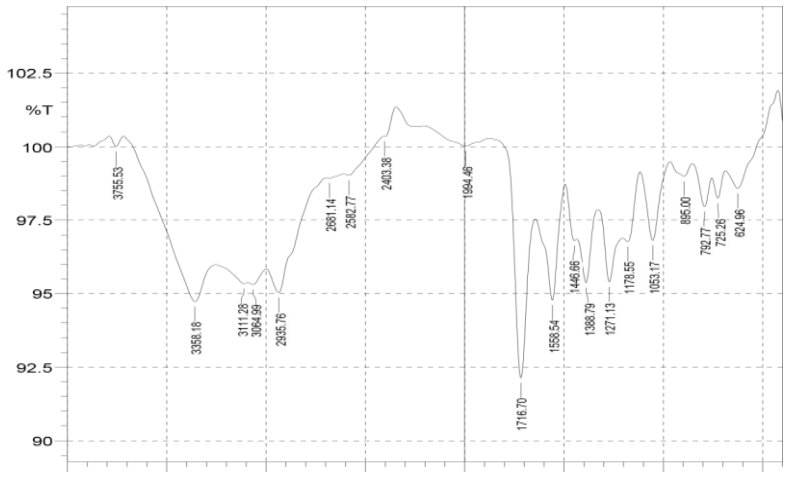

Figure No. 4: FT-IR spectra of Ketorolac Tromethamine + carbopol 934P.

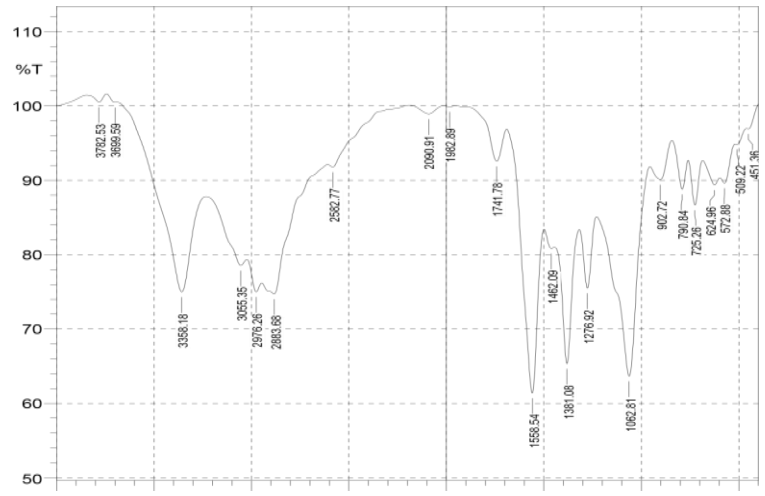

Figure No. 5: FR-IR spectra of Ketorolac Tromethamine + ethyl cellulose.

Differential Scanning Calorimetric (DSC)

Differential Scanning colorimetry was conducted on Ketorolac Tromethamine pure drug and in combination with carbopol 934P and ethyl cellulose. The thermogram of pure drug Ketorolac Tromethamine with carbopol 934P exhibited an endothermic peak at 146.97^{0}C (Figure No. 6) corresponding to its melting point, where as in case of Ketorolac Tromethamine with ethyl cellulose exhibited an endothermic peak at 143.70^{0}C (Figure No. 7). The combined mixture of pure drug Ketorolac Tromethamine with both carbopol 934P and ethyl cellulose exhibited endothermic peak at 163.62^{0}C (Figure No. 8) corresponding to its melting point. Based on results of compatibility studies it may be concluded that the excipients used were found to be compatible with Ketorolac Tromethamine.

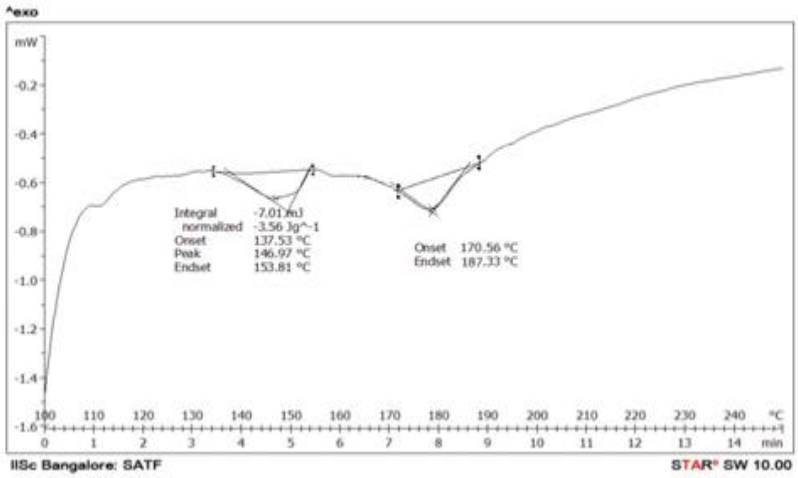

Figure No. 6: DSC thermogram of Ketorolac Tromethamine + carbopol 934P.

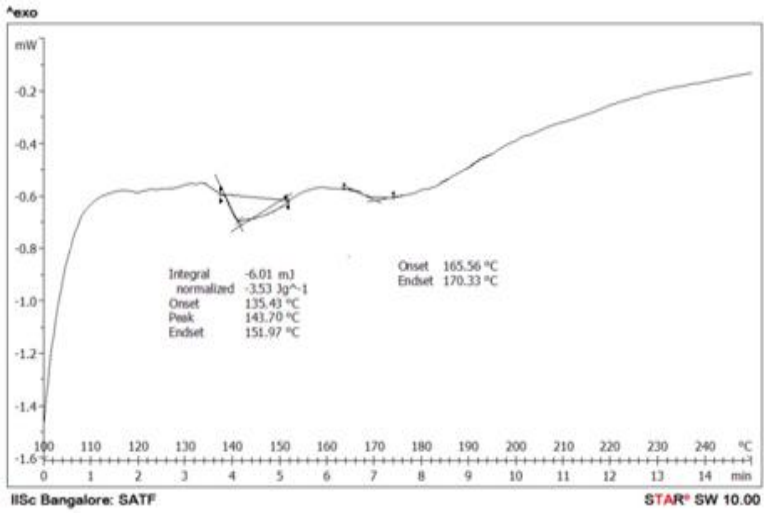

Figure No. 7: DSC thermogram of Ketorolac Tromethamine + ethyl cellulose.

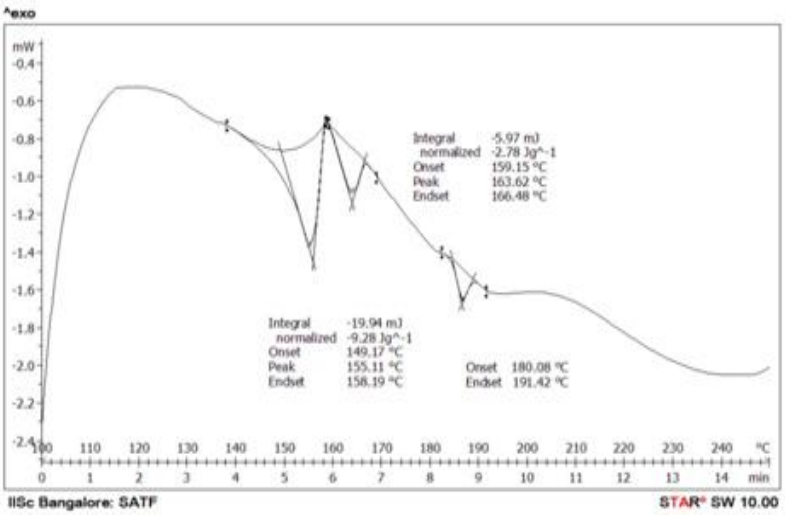

Figure No. 8: DSC thermogram of Ketorolac Tromethamine + carbopol 934P + ethyl cellulose.

EVALUATION OF KETOROLAC TROMETHAMINE GEL

Visual examination revealed that all the systems were transparent. The addition of liquid excipients improved the consistency of gels. The results for evaluation of gel parameters such as Viscosity, pH, drug content uniformity, spreadability, extrudability and viscosity are given in Table No. 4. The pH values of all developed formulae was in range 6-7 which is considered acceptable to avoid the risk of irritation upon application to the skin. The values of spreadability indicated that the polymer used gave gels spread by small amount of shear. The formulation F8 resulted in drug content 99%±0.36, spreadability 9±0.50gm-cm/sec along with viscosity of 13420cps was selected as a best batch.

Table No. 4: Drug content uniformity of Ketorolac Tromethamine gel:

Formulation code	Drug content (%)	pH	Spreadability (gm-cm/sec)	Extrudability	Viscosity (CPS)
F1	95%±0.60	6.4±0.52	9±0.26	Good	12550±5.56
F2	76%±0.36	6.6±0.20	11±0.36	Better	14378±8.54
F3	82%±0.45	6.9±0.26	8±0.50	Poor	15895±3.60
F4	107%±0.10	7.4±0.26	9±0.43	Poor	17210±11.13
F5	103%±0.10	7.2±0.10	8±0.36	Good	13412±11.13
F6	102%±0.26	7.4±0.45	10±0.30	Better	14951±1.0
F7	98%±0.60	7.1±0.36	10±0.10	Better	12980±8.0
F8	99%±0.36	7.0±0.36	9±0.50	Good	13450±6.24

Preservation efficacy test for Ketorolac Tromethamine gel

The Zone of Inhibition of Ketorolac Tromethamine gel containing 0.005%, 0.008%, 0.01% and 0.02% of Benzalkonium chloride was calculated as shown in Table No. 5 and Figure No. 9. Since the ZOI was maximum in 0.008% of BKC, this concentration was selected for the further formulations.

Table No. 5: Preservative efficiency by Zone of Inhibition Method.

Time (Hour)	Concentration of BKC (%)	Zone of Inhibition (cm)
24	0.005	1.1
	0.008	1.4
	0.01	1.2
	0.02	1.3
48	0.005	1.1
	0.008	1.5
	0.01	1.3
	0.02	1.3
72	0.005	1.1
	0.008	1.7
	0.01	1.3
	0.02	1.3

Figure No. 9: Effect of preservative efficiency after 72 hrs.

***In-vitro* diffusion study of gel**

The results of *in-vitro* diffusion studies showed the cumulative drug release of the prepared gel for the formulations F1 to F8 as shown in Table No. 6 and Figure No. 10. The percentage cumulative drug release of F1 to F8 was 36.44±0.23, 43.86±0.12, 47.14±0.54, 37.70±0.68, 41.2±32, 39.50±0.51, 43.40±0.50 and 47.37±0.39% respectively. Among the eight batches, F8 showed the maximum release of 47.37±0.39% over a period of 5 hrs.

Table No. 6: *In-vitro* diffusion study of gel (F1 to F8).

Time	Percentage cumulative release							
(min)	F1	F2	F3	F4	F5	F6	F7	F8
30	9.47±0.23	9.21±0.36	10.36±0.38	4.67±0.31	8.82±0.63	6.60±0.32	8.25±0.52	8.08±0.28
60	12.42±0.30	12.21±0.28	11.30±0.51	5.32±0.63	11.56±0.35	6.80±0.64	9.02±0.41	10.4±0.46
90	15.4±0.10	14.36±0.61	15.29±0.90	6.65±0.84	14.34±0.43	10.00±0.52	13.59±0.62	13.13±0.95
120	20.56±0.51	17.71±0.19	18.53±0.47	9.15±0.73	18.17±0.96	13.51±0.75	17.68±0.74	16.32±0.62
150	17.97±0.62	17.05±0.28	21.80±0.80	12.89±0.91	14.74±0.73	15.93±0.35	22.81±0.38	20.98±0.38
180	19.07±0.38	21.50±0.34	26.97±0.82	16.14±0.64	20.54±0.83	19.38±0.42	23.00±0.47	23.31±0.47
210	25.89±0.90	29.15±0.72	29.26±0.31	21.30±0.51	25.92±0.41	23.85±0.61	32.00±0.58	24.30±0.68
240	30.69±0.27	34.94±0.24	31.58±0.61	26.63±0.73	31.45±0.29	30.93±0.83	32.52±0.68	29.93±0.41
270	34.56±0.51	40.90±0.28	39.63±0.31	33.17±0.34	35.25±0.84	37.38±0.34	39.61±0.79	37.20±0.97
300	36.44±0.23	43.86±0.12	47.14±0.54	37.70±0.68	41.2±0.30	39.50±0.51	43.40±0.50	47.37±0.39

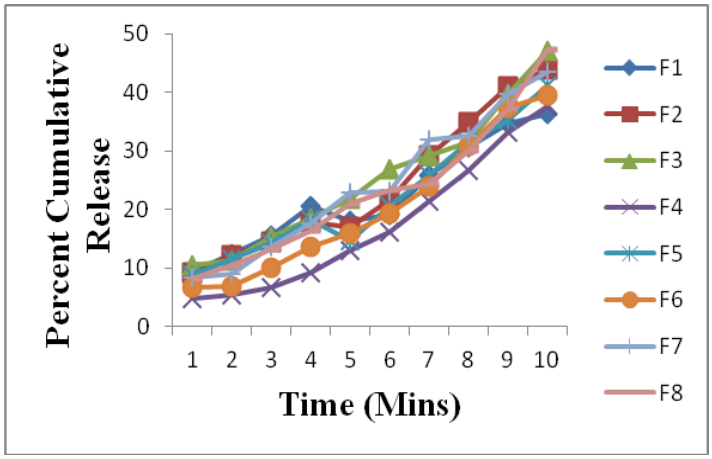

Figure No. 10: *In-vitro* diffusion study of gel (F1 to F8)

EVALUATION OF KETOROLAC TROMETHAMINE MICROSPHERES

Particle size analysis

The resulting microspheres formulated by solvent evaporation method were found to be spherical and free flowing in nature. The mean particle size of microspheres ranged from 96.18±0.04µm to97.33±0.07µm as shown in Table No 7.

Table No. 7: Particle size of microspheres:

Formulation code	Mean particle size (µm)
M1	97.33±0.07
M2	94.84±0.03
M3	96.18±0.04

Scanning Electron Microscopy

SEM analysis of Ketorolac Tromethamine microspheres revealed that all microspheres prepared were spherical in shape and is having porous outer skins as shown in Figure No. 11.

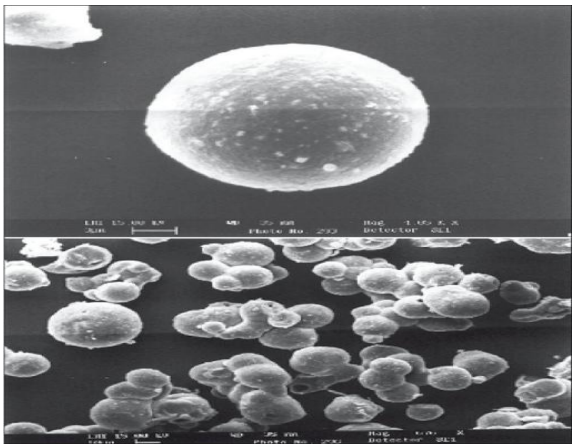

Figure No. 11: SEM of Ketorolac Tromethamine microspheres.

Drug entrapment efficiency

The drug entrapment efficacy of all the formulation was in the range of 81.0 to 95.9%. The drug entrapment efficacy of microspheres was noted to increases with increase in concentration of polymers. Amongst formulations (M1, M2 and M3) M3 have shown good entrapment efficiency as shown in Table No. 8.

Table No. 8: Drug entrapment efficiency of microspheres:

Formulation code	Ratio of Ketorolac Tromethamine :Polymer	Drug content (%)
M1	1:2	81.0%±0.79
M2	1:3	94.0%±1.30
M3	1:4	95.9%±0.79

***In-vitro* drug release of Microspheres**

In-vitro dissolution profile showed the cumulative drug release of all the three batches M1, M2 and M3 as shown in Table No. 9 and Figure No. 11. The percentage cumulative drug release of M1, M2 and M3 was found to be 28.26±0.27, 35.29±0.16 and 36.66±0.24 respectively. Among the three batches, M3 showed the maximum release of 36.66%±0.24 over a period of 5 hrs.

Table No. 9: *In-vitro* dissolution study of microspheres (M1 to M3).

Time (min)	% cumulative release		
	M1	M2	M3
0	10.13±0.23	10.84±0.35	11.25±0.52
60	12.56±0.96	12.55±0.67	14.88±0.24
90	14.40±0.64	15.28±0.86	16.60±0.38
120	16.24±0.52	18.72±0.95	20.41±0.75
150	19.51±0.84	21.10±0.24	23.80±0.25
180	20.70±0.54	24.67±0.38	25.78±0.19
210	21.21±0.34	27.19±0.46	28.54±0.64
240	23.35±0.24	29.22±0.29	32.45±0.38
270	25.99±0.57	32.39±0.34	34.52±0.29
300	28.26±0.27	35.29±0.16	36.66±0.24

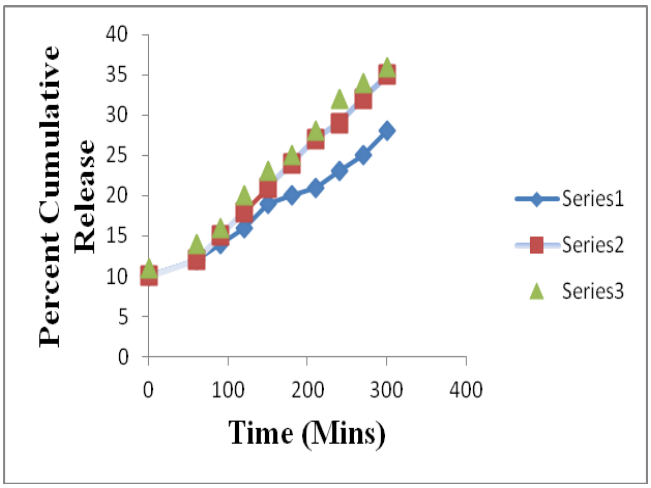

Figure No. 11: *In-vitro* dissolution study of microspheres (M1 to M3).

PREPARATION OF KETOROLAC TROMETHAMINE GEL CONTAINING MICROSPHERES

The formulation F5 to F8 was selected as the best formulation of gel containing Ketorolac Tromethamine. The formulation M3 was selected as the best formulation of microspheres containing Ketorolac Tromethamine. M3 formulation of microspheres was incorporated into gel formulations F5 to F8. The resultant gel containing microspheres of formulations F9 to F12 were transparent.

EVALUATION OF KETOROLAC TROMETHAMINE GEL CONTAINING MICROSPHERES

Visual examination revealed that all the systems were transparent. The results of pH, drug content, pH, spreadability, extrudability and viscosity are shown in Table No. 10. The pH values of all developed formulae were in range 6.9 – 7.2, which is considered acceptable to avoid the risk of irritation upon application to the skin. The values of spreadability indicated that the polymer used gave gels spread by small amount of shear. The formulation F11 resulted in drug content 100±0.52%, spreadability 9±0.50gm-cm/sec along with viscosity of 16610±7.81cps was selected as a best batch.

Table No. 10: Drug content uniformity in Ketorolac Tromethamine gel containing microspheres:

Formulation	pH	Drug content (%)	Spreadability (gm-cm/sec)	Extrudability	Viscosity (CPS)
F9	6.9±0.36	97%±0.45	10±0.62	Better	16245±5.56
F10	7.2±0.10	80%±1.22	10±0.50	Good	15310±2.64
F11	6.9±0.30	100%±0.52	9±0.50	Good	16610±7.81
F12	7.1±0.10	98%±0.17	8±0.52	Good	16120±3.0

***In-Vitro* diffusion studies of Ketorolac Tromethamine gel containing microspheres:**

The *in-vitro* diffusion profile of the designed formulations, F9-F12 was studied by using Franz diffusion cell under specified conditions. The formulations were subjected to diffusion in the media of phosphate buffer of pH 7.4 for 5hrs. In first 30 minutes of diffusion studies, all the formulations exhibited less than 10% of cumulative drug release. But in further case of diffusion studies, variations in drug release profile are observed and it was found to be depended upon the amount of excipients used (polymer plasticizer ratio of microspheres). At the end of diffusion studies for 5hrs none of the formulations of gel containing microspheres showed drug release more than 45% and F11 showed 43.97±0.23% (Table No. 11). After thorough comparison of evaluation parameters, F11 was found to show sustained release of drug and fulfilled needed criteria of topical gel containing microspheres.

Table No. 11: *In-vitro* diffusion study of microspheres (F9 to F12).

Time (min)	% cumulative release			
	F9	F10	F11	F12
0	6.18±0.23	6.8±0.56	7.14±0.63	7.65±0.52
60	9.52±0.35	10.9±0.27	8.95±0.57	9.48±0.17
90	11.13±0.68	14.1±0.39	10.61±0.85	11.18±0.28
120	13.75±0.29	16.57±0.42	13.67±0.54	12.70±0.24
150	13.71±0.64	15.87±0.85	17.71±0.29	15.81±0.78
180	16.08±0.41	20.00±0.96	22.32±0.46	19.85±0.49
210	22.06±0.27	23.00±0.75	22.00±0.57	25.48±0.54
240	27.09±0.57	29.37±0.54	27.83±0.38	29.77±0.38
270	33.13±0.48	35.32±0.34	32.79±0.63	34.47±0.64
300	40.51±0.23	40.15±0.31	43.97±0.23	37.53±0.52

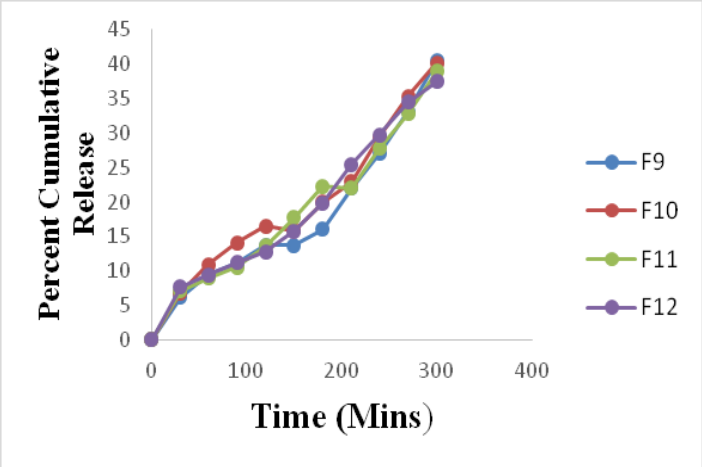

Figure No. 12: Comparison of *In-vitro* Diffusion Profiles F9-F12.

Stability studies:

The evaluation parameters showed satisfactory results and by comparison with previous formulation, it showed no degradation formulation. Thus it can be concluded that stability studies on F11 formulation was within the specification and stable (Table No. 12).

Table No. 12: Stability Studies of Formulation F11.

Parameters	F11
pH	6.9±0.1
Viscosity	16610 cps±5.29
Drug content	98.86%±0.07
In-vitro Diffusion studies	38.22%±0.09

CONCLUSION

From the result obtained formulation F1-F8, we can conclude that carbopol 934P 2% and 1.5% and propylene glycol concentration of 15% and 25% (F5-F8) were found to be more acceptable for the development of topical gel containing Ketorolac Tromethamine microspheres and among the KT gel formulations F1-F8, F8 showed better sustained release profile of 47.37±0.39 for 5 hrs along with satisfactory pH, drug content, spreadability and viscosity compared to other formulations. Microspheres of 3 different concentrations of drug and polymer were prepared with 1:2(M1), 1:3(M2) and 1:4(M3) ratios of KT and ethyl cellulose. Microspheres ratio of 1:4 (M3) showed better drug entrapment of 95.9% and release profile of 36.66% over 5hrs in comparison to 1:2 and 1:3. SEM of optimized M3 formulation were discrete, spherical with nearly smooth surface. The best formulation of microspheres M3 was incorporated into selected gel formulations F9-F12. The formulations F9-F12 were further evaluated for drug content, pH, spreadability, extrudability, viscosity and *in-vitro* diffusion studies to select the best sustained release formulation. After thorough evaluation of F9-F12 formulation, F11 showed the satisfactory sustained release profile of 43.97±0.23% after 5 hrs with suitable drug content, pH, spreadability and viscosity. From the stability studies result, it was concluded that the F11 formulation containing microspheres was successfully formulated and evaluated to sustain the release of KT over a period of time.

Further research can be done regarding its *in-vivo* result. This research can be considered as one illustration of topical NSAIDs, which combined with new drug delivery system (microparticulate). Therefore, it can be applicable for some other NSAIDs as well.

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Conflict of interest:

The authors declare that there are no conflict of interest.

LIST OF ABBREVIATIONS:

ABBREVIATIONS	FULLFORMS
ABS	Absorbance
amps	Ampere second
BP	British pharmacopeia
CAP	Cellulose acetate phthalate
CDR	Cumulative drug release
CLA	Cumulative loss amount
Cm	Centimeter
cm^2	Centimeter square
°C	Degree Celsius
Cmc	carboxymethylcellulose
COX	Cyclooxygenase
Conc.	Concentration
Cps	Centi poise
DEE	Drug entrapment efficiency
DSC	Differential scanning colorimeter
EC	Ethylcellulose
FT-IR	Fourier transform infrared spectroscopy
gm	Gram
HPC	Hydroxypropylcellulose
HPLC	High pressure liquid chromatography
HPMC	hydroxypropylmethylcellulose
HPTLC	High pressure thin layer chromatography
hrs	Hour
ICH	International council of harmonisation
JP	Japan pharmacopeia
KT	Ketorolac tromethamine

KV	Kilovolt
mg	milligram
Mps	Mean particle size
µg/ml	Microgram per milliliter
µm	Micrometer
ml	Milliliter
mm	Millimeter
Min	Minute
mol	Mole
MP	Melting point
N	Normality
NaoH	Sodium hydroxide
Na-cmc	Sodium carboxymethylcellulose
NDDS	Novel drug delivery system
nm	Nanometer
NSAID	Non-steroidal anti-inflammatory drug
PVA	Poly vinyl alcohol
RH	Relative humidity
rpm	Rotation per minute
Sec	Seconds
SEM	Scanned electron microscopy
s.aureus	Staphylococcus aureus
TRA	Trans-retinoic acid
USPNF	United states pharmacopeia and national formulary
USP	United states pharmacopeia
UV	Ultraviolet
w/w	Weight by weight
w/0/0	Water in oil,oil in oil
XRD	X-ray diffraction
pH	Power of hydrogen ion concentration
%	Percentage
λ_{max}	Absorption maxima
α	Alpha
β	Beta
γ	Gamma

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