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Research Article

FORMULATION DEVELOPMENT AND EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF TOPICAL TENOXICAM NANOGEL ON ANIMAL MODEL

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ABSTRACT

Background: The present study is to increase the transport of tenoxicam through transdermal route, and also to present it as a possible replacement for the oral NSAID therapy for rheumatoid arthritis. **Objective:** The present investigation was to develop a tenoxicam nanogel with reduced particle size in order to improve the bioavailability of the anti-inflammatory drug, Tenoxicam, and evaluates its potential in carrageenan induced rat paw edema. **Methods:** The present study is to formulate nanosizes dispersion of tenoxicam by using Modified emulsification- diffusion Method and incorporation of gelling agent Noveon Polycarbophil AA-1 to produce nanogel. The formulation is characterized by standard procedure like rheology, particle size, drug content, in-vitro diffusion study of nanogel. **Results:** The formulations are characterized for rheological properties and it was found to be significant, the anti-inflammatory activity of tenoxicam nanogel was found comparable with standard Diclofenac Sodium gel and has shown edema inhibition 85% after 4 h of treatment. Particle size 213.1nm, drug content 97.05%, entrapment efficiency 89.30, drug diffusion 95.14% and pH 6.2. **Conclusion:** Formulated tenoxicam nanogel by using noveon polycarbophil AA-was found suitable as a standard topical gel formulation and it can be used safely for treatment of edema and rheumatoid arthritis.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs in modern medicine. NSAIDs are very effective in the alleviation of pain, fever, and inflammation, and millions of patients worldwide have found relief in their use since the discovery of the soothing properties of willow bark more than 3,500 years ago. [1] Oxicam belong to a long-acting class of non-steroidal anti-inflammatory drugs (NSAIDs) which display potent anti-inflammatory and analgesic activity and is effective in the treatment of rheumatoid arthritis, osteoarthritis, and degenerative joint diseases. These drugs are also found to be very good antioxidants. Tenoxicam are the representative drugs belonging to the oxicam group. In recommended doses, tenoxicam appear to be the equivalent of aspirin, indomethacin or naproxen for the long-term treatment of rheumatoid arthritis and osteoarthritis.[2]

Tenoxicam is anti-inflammatory painkiller sometimes called Non-steroidal anti-inflammatory drugs (NSAIDs), or just anti-inflammatories.[3-4] Tenoxicam is used to ease pain and reduce inflammation in rheumatic conditions, and also to treat painful conditions such as sprains and strains, and other muscles or joint. It works by blocking the effect of chemicals in our body, called cyclooxygenase (COX) enzymes. These enzymes help to make other chemicals in the body, Called prostaglandins. Some are produced at site of injury or damage, and cause pain and inflammation. By blocking the effect of COX enzymes, fewer prostaglandins are produced, which means pain and inflammation, are eased. Rheumatoid arthritis is a chronic, progressive, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks the joints.[4-5] Apart from other categories of drugs, non-steroidal anti-

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inflammatory drugs (NSAIDs) remain the most widely used class of drug for its treatment. Tenoxicam is one of the NSAIDs of oxicam class; it is very potent and useful for chronic use in rheumatoid arthritis. But as with other NSAIDs, this drug is also associated with gastrointestinal (GI) side effects, such as nausea, dyspepsia, epigastric pain, indigestion, diarrhoea, vomiting and flatulence. These effects have been reported in 7-17% of patients treated, with nausea and epigastric pain being the most common symptoms. Hence, the side effects associated with the oral tenoxicam strongly support the need for a transdermal formulation of tenoxicam. This route of administration can be devoid of GI side effects as well as associated with other benefits of topical NSAIDs (e.g. site-specific delivery). [5-6]

The most important advantage of transdermal drug delivery is it bypasses hepatic first pass metabolism, enhances therapeutic efficiency and maintains steady plasma level of the drug.[7] Topical administration of tenoxicam has shown its penetration is very high by this route compared to other NSAIDs. The ultra-deformable vesicles for solving the problem of transcutaneous transport of tenoxicam to reduce the side effects and to provide more targeted delivery for the chronic treatment of rheumatoid arthritis.[8] Finally, the objectives of the study also include converting the colloidal suspension of the ultra-deformable vesicular system into a more patient-acceptable and clinically viable formulation by incorporating it into carbopol hydrogel System. [7-8-9]

New therapies incorporating nanotechnology and an increased understanding of rheumatoid arthritis, osteoarthritis have brought us closer to the goal of a safe and efficacious treatment of the disease. Novel topical carriers have been evaluated for enhancing skin penetration of tenoxicam (TNX) exemplified by microemulsion, nanogel, niosomes, liposomal hydrogel, deformable liposomes, and solid lipid nanoparticles (SLNs). However, they exhibit limitations of poor drug encapsulation efficiency, expulsion of drug during storage and high-water content in the formulation. Nanogels have been explored largely in topical preparations, which overcome to a large extent the drawbacks associated with other nanocarriers. It is a new-generation nanogel formulation composed of a solid lipid matrix entrapping variable spatially incompatible liquid lipid nano-compartments and stabilized by surfactants. Nanogels have an imperfect crystal structure, which prevents the expulsion of encapsulated drug thereby increasing drug loading. [8-9]

The studies showed that the nanocarrier system gives improved transdermal and dermal delivery properties. This work describes the potential of nanocarrier system in transdermal delivery of tenoxicam, prepared by using pharmaceutically accepted ingredients and using penetration enhancers.

MATERIAL AND METHODS

Materials

Pure tenoxicam (TNX) drug was gift sample from Ramdev Chemicals, Pvt. Ltd. Boisar. Noveon Polycarbophil AA- 1(NP AA-1) gift sample from Lubizol PVT.LTD Mumbai, methyl paraben, propyl paraben, propylene glycol, tween 80 purchased from Unique Biological, Kolhapur and DMSO was purchased from Fine Chemical Ltd., Mumbai.

Nanogel Synthesis

The nanogel of the tenoxicam was prepared by modified emulsification-diffusion method. 30mg of tenoxicam was weighed and dissolved in 10 ml DMSO containing polymer (Noveon polycarbophil AA-1). This organic phase containing drug polymer mixture was added into the 20ml of aqueous phase containing Tween 80 and Propylene glycol, with constant stirring at 5,000- 10,000 rpm using (T-10 basic Ultra Turrax). Addition of organic phase was done with the help of syringe positioned with needle directly into the aqueous stabilizer solution at the rate of 0.5 ml/min. The resulting dispersion was stirred for 6 min at 10,000-25,000 rpm and was subjected to the sonication for 5- 10 min. Then double distilled water was added slowly to the dispersion with subsequent stirring for 1 hour to induce diffusion of organic solvent into the continuous phase and leading to the formation of nanodispersion.[8-34]

Tenoxicam (TNX) nanogel characterization

The various physico-chemical parameters studied to characterize the topical Tenoxicam nanogel are as following:

Homogeneity

The developed TNX nanogel was tested for homogeneity by visual inspection after the nanogel has been set in the container. It was tested for appearance and presence of aggregates. [10]

Grittiness

The formulation was evaluated microscopically for the presence of particles if any. [10]

pH

The pH of formulation was determined by using digital pH meter. 1 g of gel was dissolved in 100 ml of distilled water and stored for 2 h. The pH measurement of formulation was done in triplicate and standard deviation was calculated. The pH of gel must be ideally near to normal pH of the skin to avoid any irritation. [11]

Drug content and Entrapment Efficiency

1gm of nanogel was dissolved in 10 ml of DMSO solvent. Centrifuged at 5,000 rpm for 15 min using Microcentrifuge (Remi). 1 ml supernatant was withdrawn and diluted up to 10 ml in DMSO. Diluted supernatant solution analyzed at 365 nm using UV spectrophotometer (Shimadzu 1800), against blank/control DMSO. Drug content and EE was calculated.[8]

Viscosity study

The measurement of viscosity of the prepared nanogel was done with Brookfield viscometer. The nanogels were rotated at 2.5 rpm using spindle no. C75-1 and the corresponding dial reading were noted.[10]

Spreadability study

One of the criteria for a gel to meet the ideal qualities is that it should possess good spread ability. It is the term expressed to denote the extent of area which gel readily spreads on application to the skin or affected part. The therapeutic efficacy of a formulation also depends upon its spread ability value. The Spread ability of the gel was determined by measuring the spreading diameter of gel (1 g) between two horizontal plates

of 20 cm × 20 cm after one min of time. The standard weight of 125 g was applied on the upper plate to determine spread ability. The diameter of spreaded circle was measured in cm and the result obtained is average of three determinations.[10]

Particle size

Particle size of prepared TNX nanogel was determined by photon correlation spectroscopy (PCS) that analyzes the fluctuations in light scattering due to Brownian motion of the droplets using a zetasizer (Ver. 6.20 Malvern Instruments Ltd.). The formulation (0.1 ml) was dispersed in 50 ml of DMSO in a volumetric flask, mixed thoroughly with vigorous shaking and light scattering was monitored at 25 °C a 90° angle. The droplet size distribution of the optimized TNX nanogel was determined by Malvern zetasizer (Ver. 6.20 Malvern Instruments Ltd.). All measurements were performed in triplicate at 25 ºC. [15-16]

Zeta potential

The zeta potential of the selected formulation was measured by using a Zetasizer (Ver. 6.20 Malvern Instruments Ltd.). [15-16]

FTIR spectra study

To study any possible interaction between Drug and excipients, FT-IR spectrum of both pure TNX and TNX nanogel formulation were taken and compared. The nanogel excipients should be compatible with drug for a formulation. [11]

Skin irritation study

To determine effect of nanogel on skin, taking 2 rabbit of either sex in the weight range 1.5-2.0 kg was used. The entire undamaged skin was used and hairs were removed three days before the experiment. In test group, topical TNX nanogel was applied and in control group, gel base was applied on the back of animal. The gel was applied daily up to seven days and then the treated skin was visually examined for any type of skin irritation and score for skin irritation was assigned as 0 (no irritation), 1 (no erythema but definite dryness), 2 (Moderate erythema), 3 (moderate to severe erythema with slight edema) and 4 (moderate to severe erythema with severe edema extending beyond the marked area).[31-32]

In vitro permeation study (By using Franz Diffusion)

The in vitro release was carried out with the artificial cellophane membrane (molecular weight cut off 1000 Da) by using Franz diffusion cell. The cell consists of two chambers, the donar and the receptor compartment. The donar compartment was open at the top and was exposed to atmosphere. The temperature was maintained at 37±0.5˚C and receptor compartment was provided with sampling port. The diffusion medium used was phosphate buffer pH 7.5. The drug containing nanogel gel with a support of backing membrane was kept in the donar compartment and was separated from the receptor compartment by cellophane membrane. Cellophane membrane was previously soaked for 24hr phosphate buffer pH 7.5. The donar and receptor compartments were held together using a clamp. The receptor compartment with 30 ml phosphate buffer pH 7.5 of was maintained at 37±0.5˚C and stirred with magnetic stirrer, to prevent the formation of concentrated drug solution layer below the cellophane membrane. Samples of 1ml were collected at predetermined time intervals and replaced with fresh media. The concentration of drug was determined by UV spectrophotometer at 365nm. [13]

Assessment of Anti-inflammatory activity Animals

The Albino Wistar rats (150-300 gm BW male/female) were used for the present study. These were maintained under standard environment conditions and were feed with standard pellet diet. The animals were given a time of seven days before experiment to get acclimatized with laboratory conditions. They were deprived of from food before 18 h of experiment. After this they were taken for experiment. The experimental protocol was subjected to the scrutinization of Institutional Animal Ethical Committee (IAEC) of TKCP College of Pharmacy, Warananagar and was approved under the Protocol No. IAEC/TKCP/ 2014/15 (Reg. No. 1090/PO/Re/S/CPCSEA, New Delhi.) and animals were kept with proper care according to the guidelines of CPCSEA.

Carrageenan-induced rat paw edema method

Albino Wistar Rats were divided in three groups (two animals in each, n= 6) as positive control, standard treatment group and test group. The positive control group was compared with standard i.e. Diclofenac Sodium gel 1.0% (Standard group) and prepared nanogel formulation of tenoxicam. Tenoxicam nanogel (Test group). Edema was induced by injection of Carrageenan (0.1 ml, 1% w/v in normal saline) into the sub-plantar tissue of the right hind paw in all groups animal. The linear paw circumference was measured using digital Plethysmometer. The paw circumference measurements were made before induction of edema and at hly intervals for 4 h after induction. No any treatment was given to control group. Both standard and test gel was applied to the sub-plantar tissue of the right hind paw of animal in 1 g quantity by gently rubbing with index finger for 50 times. The percentage value of edema inhibition was calculated by the following formula-

% inhibition= 1- (y-x / b-a) × 100

Where,
x= Initial paw thickness of test group animal,
y= Paw thickness of test group animal after treatment,
a= Initial paw thickness of control group animal,
b= Paw thickness of control group animal after treatment. [31- 33]

RESULTS AND DISCUSSION

Evaluation of topical gel formulation

The observations of various evaluation parameters for topical tenoxicam nanogel are given in Table no 2. The TNX nanogel was found transparent, smooth, reduced particle size and having good spread ability. The drug content in nanogel was determined with the help of standard calibration curve of tenoxicam in DMSO, Figure no 1. Absorbance for nanogel sample (1mg/ml). The drug content and EE was calculated using straight line equation of standard curve for observed absorbance using the following formula-

$$\text{Drug content (\%)} = \frac{\text{Total amount of Nanogel} \times \text{Amount of drug in 0.5 gms}}{\text{Amount of nanogel in gms}} \times 100 \quad \text{---------- (1)}^{8}$$

$$\text{Entrapment Efficiency (\%)} = \frac{\text{W initial drug} - \text{W free drug}}{\text{W initial drug}} \times 100 \quad \text{---------- (2)}^{8}$$

The comparison of FTIR spectrum study of TNX nanogel and tenoxicam confirmed no any excipients interaction. The

excipients were found compatible with tenoxicam in (Fig 2) and (Fig 3). *In-vitro* diffusion study of topical tenoxicam nanogel through artificial cellophane membrane has shown that 95.14% % drug release after 8 h and it was increasing with time (Table 3, Fig 5). The diffusion of nanogel can be more increased with addition of penetration enhancers.

Table no 1 Nanogel composition for 30 gm of topical Tenoxicam nanogel

Sr.No	Drug/Excipients	Amount
1	Tenoxicam	30 mg
2	Noveon polycarbophil AA-1	700 mg
3	Propyl Paraben	30mg
4	Methyl Paraben	20mg
5	Polyethylene glycol	5 ml
6	Tween 80	2 ml
7	DMSO	10ml
8	Dist. Water	qs
	Total Weight	**30 gm**

Table no 2 Evaluation parameters for topical Tenoxicam nanogel.

Sr.No	Evaluation parameters	Results
1	Gel appearance	Transparent yellow
2	Homogeneity	Good
3	Grittiness	Absent
4	Viscosity	0.3678 Pa.s
5	pH	6.2±0.5
6	Spread ability	8.37±07g.cm/sec
7	Drug content	97.05 %
8	Entrapment efficiency	89.30 %
9	% Drug release	95.14 %
10	Particle size	213.1 nm
11	Zeta Potential	-9.73 to -15.33 mV

Table no 3 Score of irritation and edema after application of test sample tenoxicam nanogel and marketed gel sample Sod. Diclofenac 0.1%.

Rabbit no.	Reaction	Score for skin reaction							
		Tenoxicam nanogel (test nanogel sample)				Sod. Diclofenac 0.1% (marketed gel sample)			
		30 min	1 hr	12 hr	24hr	30 min	1 hr	12 hr	24hr
1	Erythema	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Edema	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	Erythema	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Edema	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table no 4 Effect of topical Tenoxicam nanogel on Carrageenan Induced Paw Edema in rats.

Treatment	Rat paw edema volume (ml), Time in hr				
	0.0 min	0.30 min	1 hr	2 hr	4 hr
Gel base	0.58	0.84	0.96	1.00	1.10
Test group	0.50	0.78	1.19	1.25	1.40
Std group	0.60	0.85	1.26	1.30	1.65

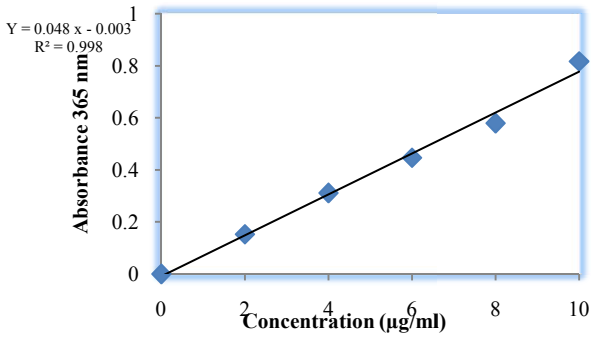


Figure No 1 Determination of drug content in TNX nanogel.

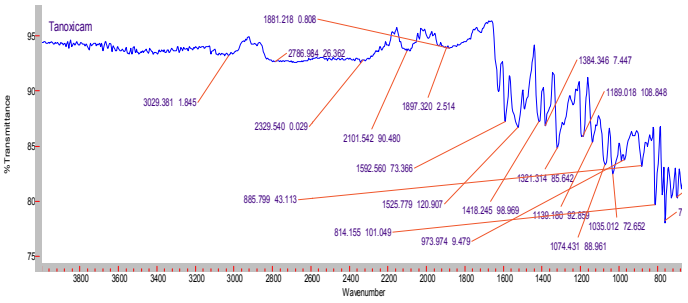


Figure No 2 FTIR spectra of pure Tenoxicam drug.

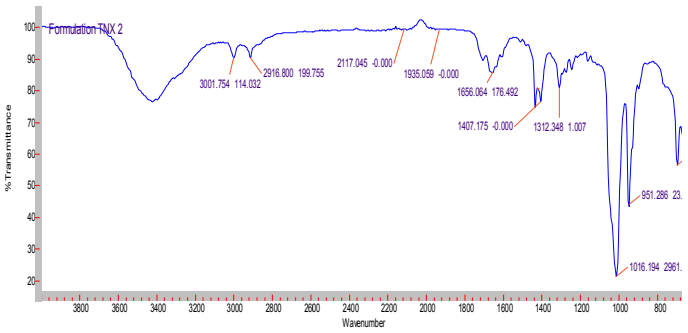
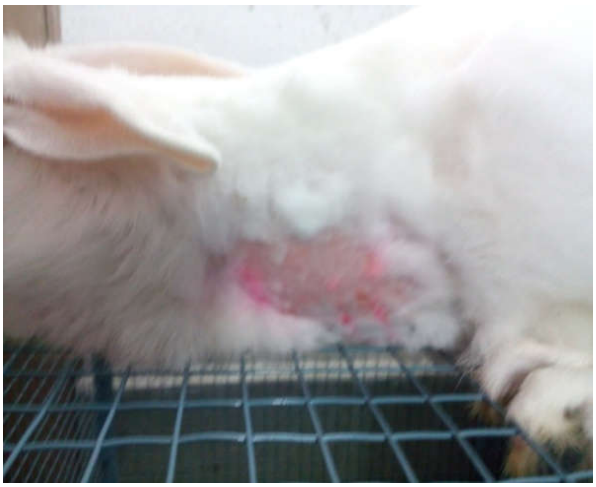
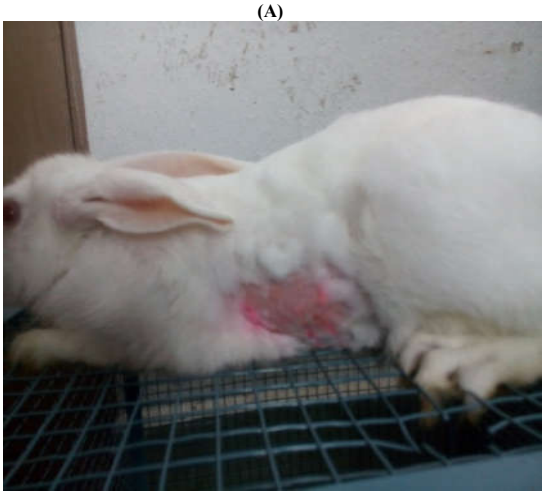


Figure No 3 FTIR spectra of topical tenoxicam Nanogel.



After 24 hr of Market formulation

(A)



After 24 hr of test formulation

(B)

Figure No 4 A & B Skin Irritation Study

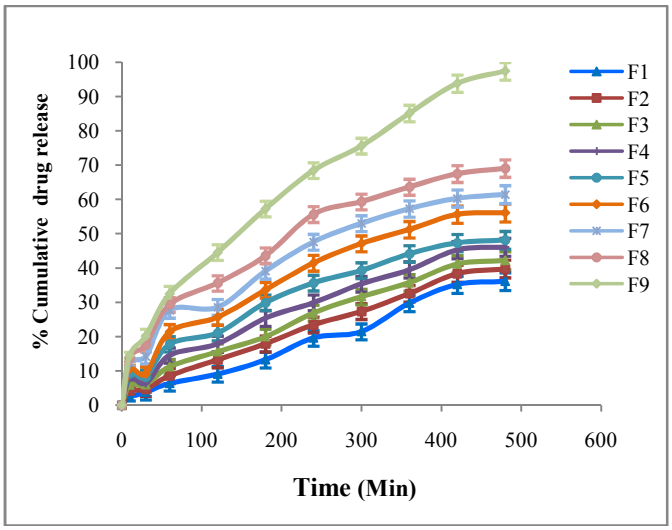


Figure No 5 *In-vitro* diffusion study of topical batches F1 to F9 of Tenoxicam Nanogel.

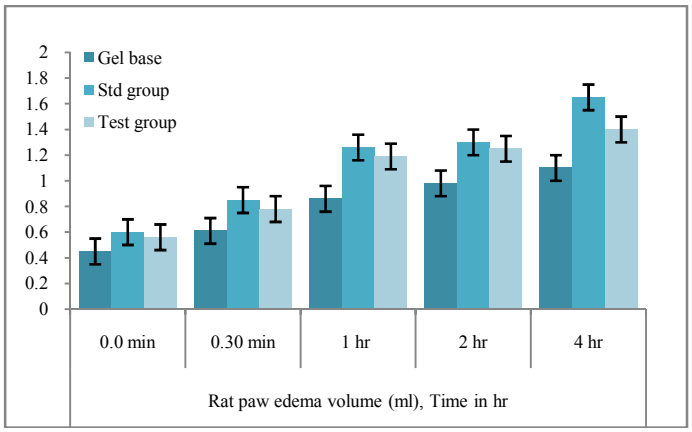


Figure No 4 Anti-inflammatory activity study of topical Tenoxicam nanogel.

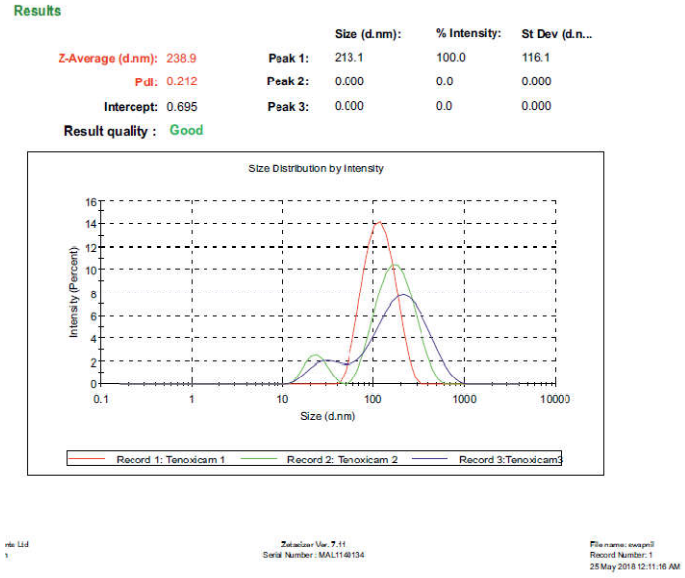


Figure No 5 Particle size of tenoxicam nanogel.

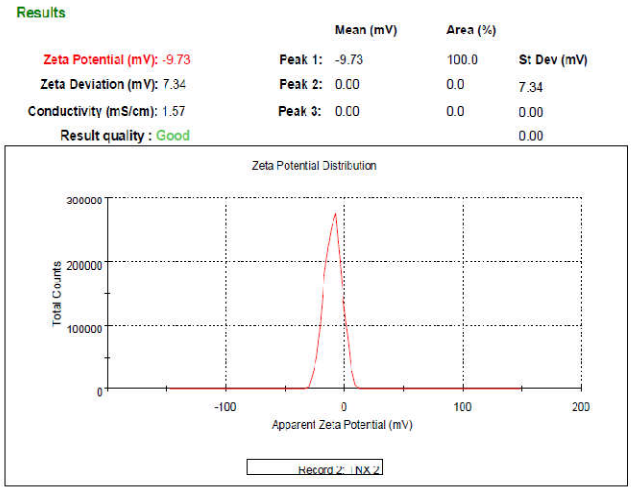


Figure No 6 Zeta potential of tenoxicam Nanogel.

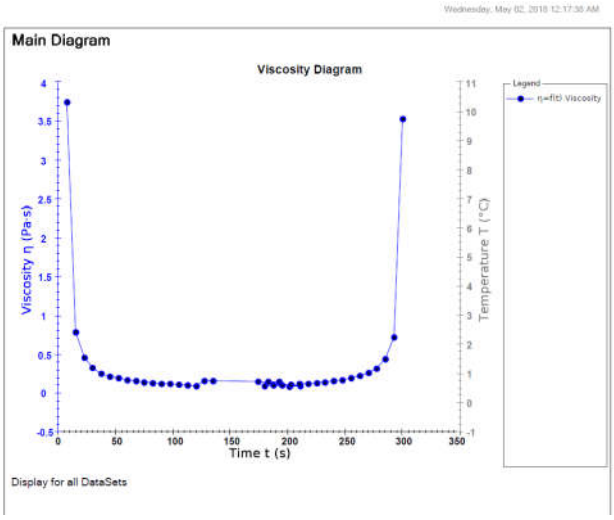


Figure No 8 Viscosity flow of tenoxicam Nanogel.

DISCUSSION

Tenoxicam nanogel is the semi-solid system of drug delivery and constitutes a well reputation among various pharmaceutical dosage forms. Now a day's nanogels are becoming popular due to their safe and effective use and higher penetration rate. So, the study was designed to formulate topical tenoxicam nanogel.

CONCLUSION

The novel nanogel form represents an effective and better carrier for the transdermal/topical preparations. The prepared tenoxicam nanogel formulation showed the better penetration on the skin may be due to the enhanced contact between the drug and the skin resulting from more surface area and hydration. The formulation showed stability over the study period and showed substantial increase in the efficacy. The prepared formulation proves to be the better alternative for the oral administration of tenoxicam (NSAID) and eliminates the limitations of the drug like gastric disturbances, low bioavailability; short half live and first pass effect. The production of the formulation is also proved to be better and cost effective in comparison with oral dosage forms.

It can be concluded that Tenoxicam nanogel is an ideal and effective formulation. It can be used safely for treatment of

Rheumatoid arthritis and inflammatory conditions. This study also supports the activities of this tenoxicam nanogel for treatment of pain, inflammation and pyrexia. Further, determination of exact mechanism of action and lead active constituent for anti-inflammatory activity of TNX nanogel will be the new target of study.

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Conflict of Interest

The authors have no any conflict of interest.

Abbreviation Used

TNX: Tenoxicam; nm: Nanometer; gm: Gram; ml: Milliliter; kg: Kilogram; rpm: Revolutions per minute; sec.: Second; µg/ml: Microgram per mililiter; g.cm/s: Gram centimeter per second; EE: Entrapment efficiency, CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals, IAEC: Institutional Animal Ethical committee.

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