Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Tridax Procumbens Linn Nanogel as Alternative Treatment for Rheumatoid Arthritis

OMKAR A PATIL*

Department of Pharmaceutics, Rajarambapu College of Pharmacy, Kasegaon, Maharashtra - 415404,

ARTICLE DETAILS

Article history:
Received on 06 June 2018
Modified on 22 June 2018
Accepted on 25 June 2018

Keywords:
Tridex procumbens,
Rheumatoid arthritis,
Nanogel,
Zeta potential,
Particle size.

ABSTRACT

Nanogels are designed to spontaneously incorporate biologically active molecules through the formation of salt bonds, hydrogen bonds, or hydrophobic interactions. Nanogels can be used for the treatment of topical bacterial and fungal infection skin cancer, inflammation and bone regeneration etc. The present research has been undertaken with the aim to formulate and evaluate the antibacterial nanogel of *Tridex procumbens* Linn. Nanogel were prepared by using carbopol 940, carbopol 934, ethanol, methyl paraben, propyl paraben, EDTA (Ethylenesiaminetetraacetic acid), triethanolamine. FTIR (Fourier-transform infrared) analysis and DSC (Differential Scanning Calorimetry) study was performed to ensure the compatibility between plant extract and excipients. The prepared nanogel were evaluated for particle size analysis and zeta potential and other parameter like pH, viscosity, spreadibility, extrudability, drug content also determined. In vitro release of all formulation was found between 75-97.88% formulated by using different carbopol. Optimized batch F4 shows acceptance result for all parameter and having release up to 97.88% within 5 h. Partical size of optimized batch was found to be 246 nm. The results indicated the prepared nanogel is safe and effective to treate rheumatoid arthritis.

INTRODUCTION

Now a day's plant derived novel molecules have ability to replaced vast chemically synthesized broad spectrum antibiotics. Due to incredible ventures, like having no side effects, non narcotic, easily available at affordable prices, so the demand of plant based medicines seems to be increasing. In traditional medicines plants used to treat chronic as well as infectious diseases [1]. In modern pharmacopoeia drugs derived from plant an important segment because sources of biologically active molecules and blue prints for the development of modified derivatives with reduced toxicity, and or enhanced activity [2]. India is a country rich in indigenous herbal resources which grow almost 20,000 plant species, out of which about 2,500 are of medicinal value [3]. In ayurveda plants are also known to relieve various diseases.

Author for Correspondence:
Email: omkarpatil3332@gmail.com

Wild plants supply medicines (antibiotic, antispasmodics, emetics, anti-cancer, antimicrobials etc.), crafts and cosmetics to rural and urban communities [4].

Rheumatoid arthritis is a systemic autoimmune and chronic disorder involving damage to one or more joints. Affecting the synovial joints and typically producing symmetrical arthritis and produce joint destruction, responsible for the deformity and disability [5]. Rheumatoid arthritis affect 0.5-1 % of the population worldwide [6]. The pro inflammatory cytokines, mainly TNF-α, IL-1β and IL-6 produced by monocytes, macrophages and synovial fi broblasts are suggested to play an impor-tant role in the pathogenesis and disease progression of Rheumatoid arthritis [7]. The drugs which are commonly used for treatment for Rheumatoid Arthritis are steroidal, non- steroidal anti-inflammatory, disease modifying ant rheumatic and immunosuppressant drugs that are known to produce various side effects like gastrointestinal disorders, immunodeficiency and humoral disturbances [8].

Tridax procumbens Linn is a native of tropical America and naturalized in tropical Africa, Asia, Australia and India belongs to Compositae family. The plant is a small herb having short, hairy blade like leaves and this plant popularly called "*coat buttonsin*" in english because of the appearance of flowers (yellow corolla) [9]. Plant is a semi prostate, annual, creeper herb. Stem is ascending 30-50cm height, sparsely hairy, rooting at nodes. Leaves are simple, exstipulate, opposite, lanceolate to ovate 3-7cm long irregularly toothed margin, shortly petioled, hairy on both surfaces, base wedge shaped. Flowers of plant are tubular, yellow with hairs, inflorescence capitulum. *Tridax* has two types of flower: ray florets and disc florets with basal palcentation [10]. On the *Tridex procumbens* Linn scientific literatures are enormous and have reported to confer activity against gastritis it is also antioxidant, antidiabetic, antimicrobial, antiseptic, insecticidal and antiparasitic [11].

Based on recent information of we can says that nanoparticles has the vast number of their advantages in pharmaceutical and medical field [12]. Nanogel is nano sized particles formed by physically or chemically crosslinked polymer networks that is swell in a good solvent [13].

The size of nanogel ranges from 20-200nm. Nanogels are three dimensional hydrophilic networks that have the tendency to imbibe water or physiological fluid in a large amount, without changing in the internal network structure. They can escape renal clearance and prolonged serum half-life period due to their size [14]. Nanogel can be formulated by a variety of natural polymers, synthetic polymers or a combination thereof, chemically (covalent) crosslinked or physically crosslinked with non-covalent bonds by hydrogen bonds, electrostatic and hydrophobic interactions. The great capacity of absorbing water is attributed to the presence of hydrophilic functional groups, such as –OH, –CONH–, –$CONH_2$– and –SO_3H, along the macromolecular chains in the polymer structure [15].

MATERIALS AND METHODS

Materials

The mature the whole Plant of *Tridax procumbens* Linn were collected from botanical garden, Rajarambapu College of Pharmacy, Kasegaon. Maharashtra, India. The taxonomic identities of plants were confirmed by KRP Kanya Mahavidyalaya, Islampur. Maharashtra, India. Carbopol 940 and carbopol 934 were procured from Research- Lab Fine Chem Industries (Mumbai), India. Whereas Polypropylen glycol, Methyl paraben, Propyl paraben and triethanolamine were obtain from Sigma Aldrich (Mumbai), India. All ingredients used in the research work were of analytical grade.

Preparation of Plant Extracts

The whole plant of *Tridax procumbens* Linn were carefully washed to remove residual materials and earthy matter from the whole plant, then shade dried. Coarse powder of *Tridax procumbens Linn* was extracted with methanol in a soxhlet extractor for 72 h. [16]

Preparation of Nanogel

Following method was used to preparation of nanogel. In the preparation of nanogel two separate phases are prepared as given follow. All required quantities are given in Table 1.

Phase 1: In one part extract dissolved in propylene glycol and ethanol.

Phase 2: In another part carbopol (940 or 934) was added in sufficient water and forms a carbopol gelly in that measured quantity of methyl paraben, propyl paraben and EDTA was added.

Both phases are separately homogenized and sonicated by using ultrasonicator. After that phase 1 is added slowly in phase 2 with continue stirring by using homogenizer. Mixture was homogenize upto gel was formed [upto15min] and sonicate it upto 20min by using ultra sonicator. After that triethanolamine was added for adjusting pH and shiny consistacy to gel [17].

Table 1: Composition of Nanogel

Composition (%)	F1	F2	F3	F4	F5	F6
Extract	1	1.5	2	1	1.5	2
Carbopol 940	1	2	3	-	-	-
Carbopol 934	-	-	-	1	2	3
Ethanol	3	3	3	3	3	3
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02
EDTA	0.03	0.03	0.03	0.03	0.03	0.03
Triethanolamine	0.3	0.3	0.3	0.3	0.3	0.3
water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Compatibility Study

FT-IR Studies

FT-IR studies carried out for perdition of drug and excipients incompatibility The IR spectra were recorded on FT-IR Jasco 4600, Japan. FT-IR spectra of Plant extract and mixture were obtained by scanning in the range between 4000 and 650 cm^{-1} [18].

Differential Scanning Calorimetry (DSC)

DSC thermogram of dried plant extract and mixture was recorded on TA WS thermal analyzer (Shimadzu).The samples were hermetically sealed in aluminum pans and heated at a constant rate of 10°C/min over temperature range of 40 to 300°C. Inert atmosphere was maintained by purging nitrogen gas at flow rate of 50mL/min [19].

EVALUATION OF THE NANOGEL

Measurement of pH

The measurement of pH was carried out in triplicate and the average of the three readings was recorded with the help of digital pH meter by dipping the glass electrode completely into the gel system to cover the electrode [20].

Determination of Spreadability

About 1g of nanogel was placed (sandwiched) between 2 horizontal plates (20 X 20 cm^2) for 1 minute. The upper plate was then removed and the diameter of the gel adhering to it was measured. On the upper plate tied standardized weight was 50 gm [21]. Spreadability was then calculated by using the formula:

$$S = M \cdot L/t$$

Where,

M is the weight (g) tied to the upper glass slide; ***L*** is the length (cm) moved on the glass slide

And ***t*** is time to separate the slide (s).

In this present experiment, *M* = 50 g, '*S*' is recorded.

Viscosity Studies

The viscosity determinations were carried out on Brookfield viscometer with spindle number 74 at 25°C and 50-250rpm. The rheological measurements are determined to signify various properties of nanogel like association, entanglement and cross-linking [22].

Particle Size Measurement

Particle size was measured on (Zeta Perkin Malvern Zetasizer, UK). At temperature 25°C, viscosity of dispersion 0.5476 cP, methanol is used as disperant medium at count rate 2283kCps [23].

Zeta Potential Measurement

Zeta potential of nanogel was measured on (Zeta Perkin Malvern Zetasizer, UK) at temperature 25°C and viscosity of dispersion medium 0.5476cP at conductivity 0.0342 mS/cm and electrovoltage 3.9v [24].

Estimation of Active Constituents in Gel Formulation (Drug Content)

1 gram from each formulation was taken in a 50mL volumetric flask and made up to volume with methanol up to mark and shaken well to dissolve the active constituents in methanol. The resulting solution was filtered through whatman filter paper and 0.1 mL of the filtrate was pipetted out and diluted to 10mL with methanol. The content of active constituents was estimated spectrophotometrically by using standard curve plotted at 246 nm (λ_{max} of active constituents in the extracts) [25].

Extrudability of Nanogel

20g of gel was filled in collapsible tube and pressed firmly at the crimped end and a clamp was applied to prevent any roll back. The cap was removed and the gel was extruded. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated [26].

Homogeneity and Appearance

Physical appearance and homogeneity of the prepared nanogel were evaluated by visual perception [27].

Nanogel Evaluation on Skin (Patch Test)

Ten healthy volunteers were randomly selected between ages 20 and 35 years. Prior to the study, consent form was filled by each of them. Volunteers having serious skin diseases, asthma were excluded from the study. Patch test was per-formed on the forearms of each volunteer by applying 0.5g of base to cover radius of 0.6cm on skin surface, formulation F1-F6 was applied separately to determine any possible reactions on the skin. Adhesive tape (Sperobliss Medicare, Pune India) was used to fix them in place and the test sites were marked. The patches were left in place for 48h, during which care was taken not to wash the applied area. After 48h, the patches were removed and reading was taken one hour later.

Table 2: Evaluation Parameters for Nanogel Formulation

Batch	pH	Viscosity *(Cps)*	Spreadability g cm/sec	Extrudability	Drug content (%)	Physical appearance
F1	6.57	7954	9.5	Good	99.7	Dark green, smooth, Homogenous
F2	6.55	8112	9.6	Excellent	99.3	Dark green, smooth, homogenous,
F3	6.64	8120	9.9	Good	99.5	Dark green, smooth, homogenous
F4	6.70	7930	8.6	Excellent	99.8	Dark green, smooth, Homogenous
F5	6.50	8110	8.6	Excellent	99.8	Dark green, smooth, homogenous,
F6	7.10	8121	8.9	Good	99.4	Dark green, smooth, homogenous,

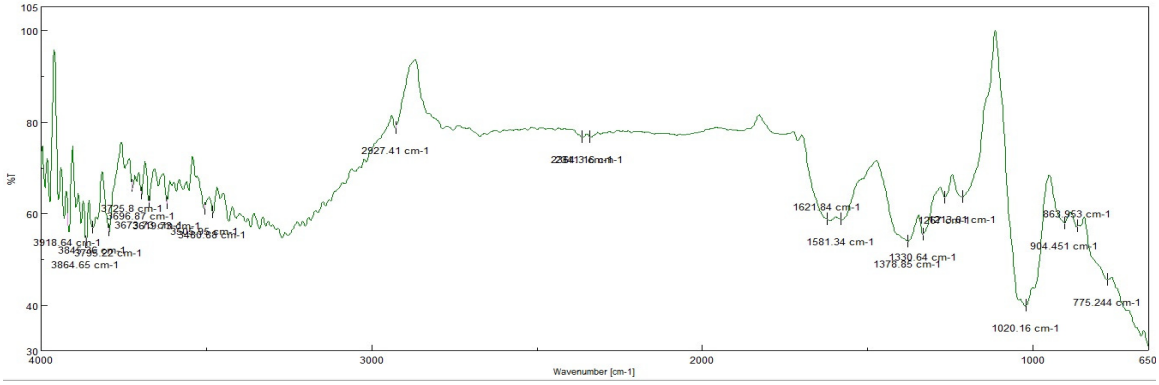

Figure 1: FT-IR spectra of plant extract

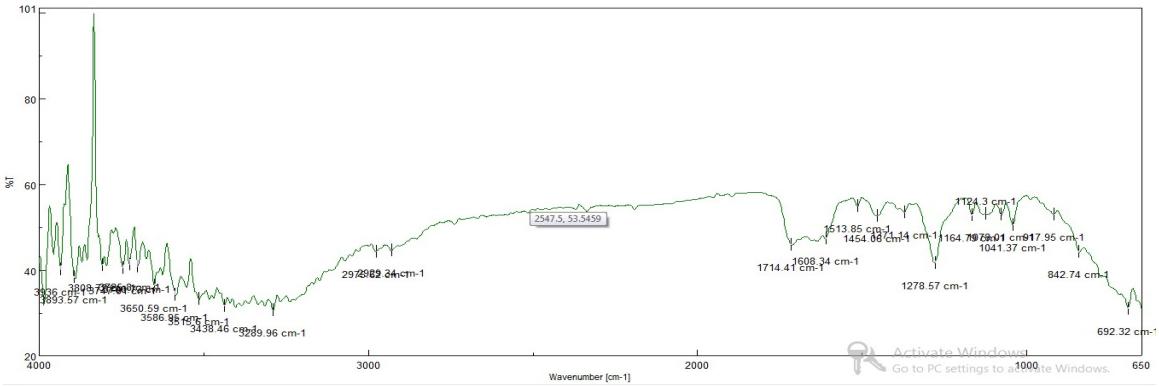

Figure 2: FT-IR spectrum of mixture

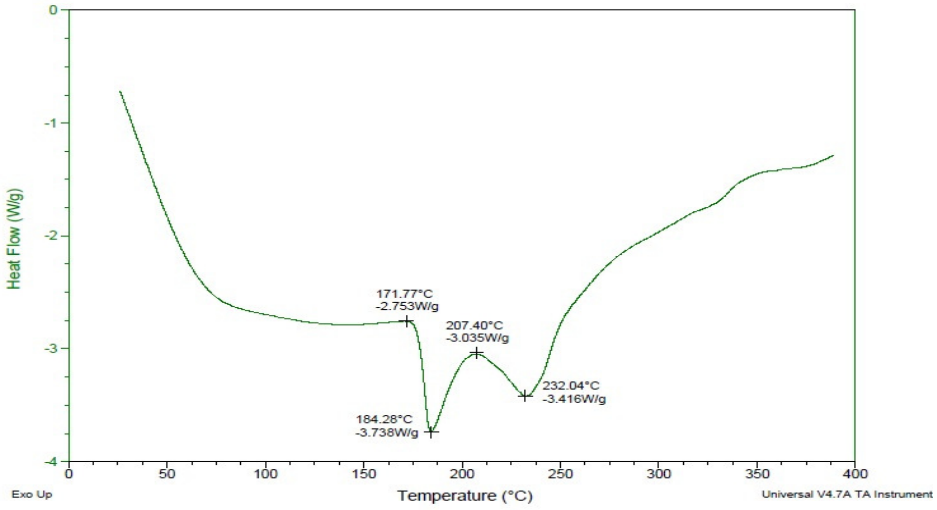

Figure 3: DSC for *Tridax procumbens* plant extract

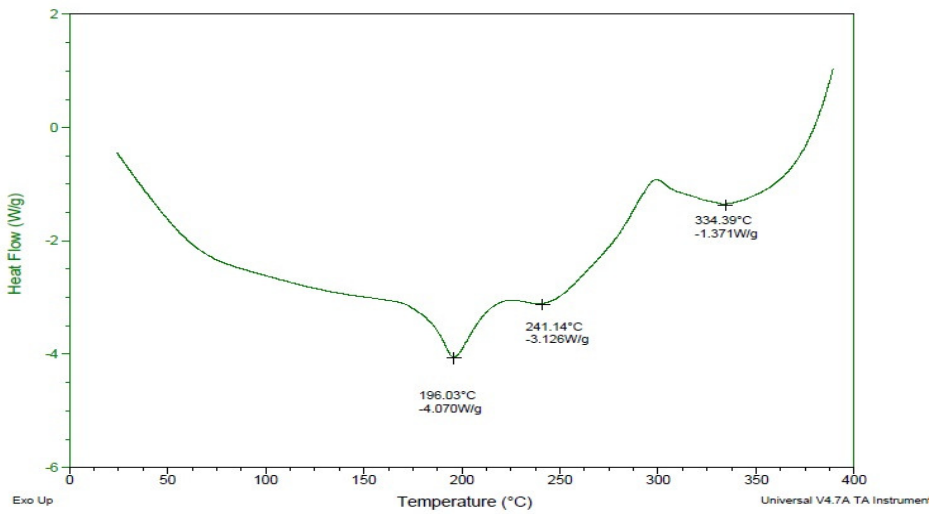

Figure 4: DSC for mixture of excipients and extract

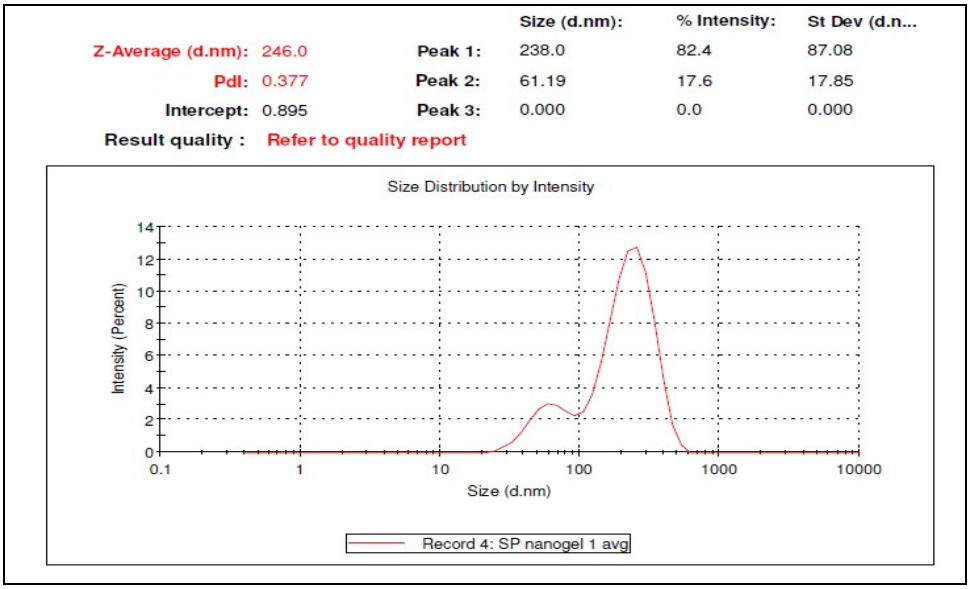

Figure 5: Particle size distribution graph of optimized batch F4

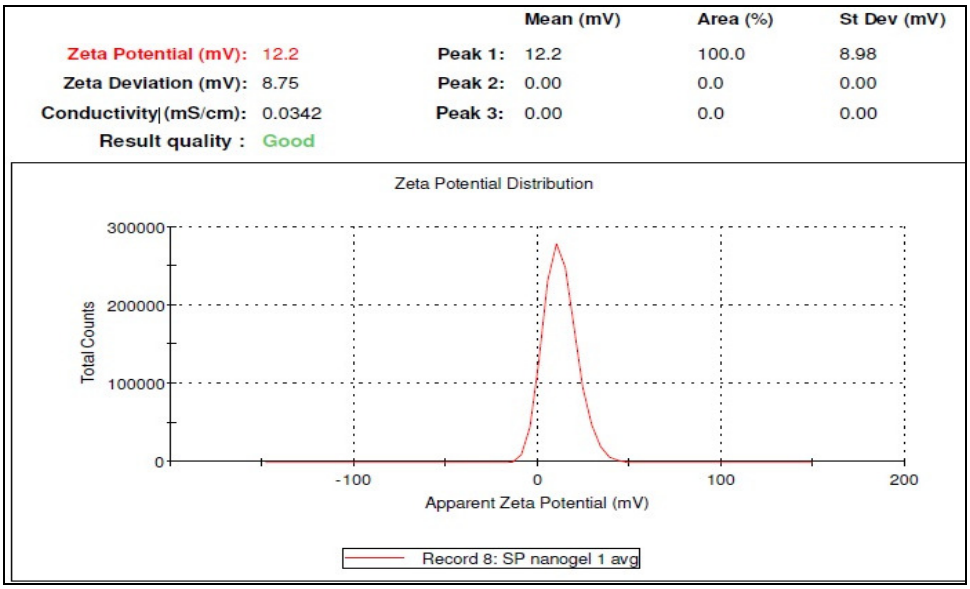

Figure 6: Zeta potential of optimized batch F4

Skin was examined for any redness, itching or blemishes. These visible signs along with any itchy or irritable sensations indicated that there is something wrong with the product. Clear skin devoid of aforesaid visible signs indicated that the product is safe to use [28].

In vitro Release Studies

In vitro release studies of nanogel was carried out by using Franz-type diffusion. The receptor fluid was selected as phosphate buffer (pH 7.4) containing 25% (v/v) ethanol to maintain sink conditions. Maintained at 37 ± 0.5C and continuously stirred at 600rpm. At certain time intervals (15min, 30min,..., 5 h), 1ml samples were withdrawn from the receiver compartment and replaced with an equal volume of fresh receptor fluid to maintain sink condition. The amount of the drug released through the cellophane membrane was analyzed spectrophotometrically at 249 nm. [29].

RESULTS AND DISCUSSION

Compatibility Study

FT-IR Studies

The drug-excipients compatibility was assessed by IR spectra of plant extract, and mixture by FT-IR spectra. From the interpretation of spectra it is found that there is no worth change in the wave numbers of the drug and drug-excipients combination. Hence the drug and excipients are compatible with each other Fig. 1 and 2.

Differential Scanning Calorimetry (DSC)

Plant extract of *Tridax procumbens* and mixture of excipients and extract characterized for DSC. The pure plant extract showed a sharp endothermic peak at 184.28^{0}c. In mixture endothermic peaks were observed at 196.03^{0}c and peak is reduced and there is no appearance of new peak observed in graph show in Fig. 3 and Fig. 4. This minor change in the melting endotherm of drug could be due to the mixing of drug and polymer, which lowered the purity of each component in the mixture and may not necessarily indicate potential incompatibility.

Evaluation Parameters for Nanogel Formulation

Nanogel formulations F1 to F6 prepared using carbopol polymers were evaluated for physical appearance, pH, viscosity, spreadability, drug content, extrudability. Results of the study were in acceptable limits the details of the same are recorded in Table 2.

From results as shown in Table 2 it is concluded that all formulations show good appearance and homogeneity. The physical appearance of the gel dark green in nature due to dark green color of methanolic extract of *Tridax procubens.* The pH of the gel formulations was in range of 6.50 to 7.10, which lies between normal ranges of skin pH. The viscosity of the gel formulations ranges between 7930 to 8121*cps.*The values of Spreadability indicate that gel is easily spreadable by small amount of shear. Spreadability between 8.6 to 9.9 g cm/sec show good spreadability. Drug content of nanogel formulations were found above 99%. Extrudability of nanogel was observed good and excellent.

Particle Size of the Formulation

The average particle size of nanogel was found to be 246 nm which confirm that formation of nano sized gel particles The polydispersity index (PDI) of the nanogel was found to be 0.377; which indicates the broad distribution of globules and its homogencity, graph depict that it has a homogeneous distribution of particles Fig. 5.

Zeta Potential Measurement

The zeta potential of nanogel was found to be 12.2mV. In general, the zeta potential value of ±30 mV is sufficient for the stability of a nanogel. In our formulation, it is 12.2 ± 0.42 which means it complies with the requirement of the zeta potential for stability Fig. 6.

Table 3: Partical Size and Zeta Potential Results of Optimized Batch F4

Particle size (nm)	Polydispersity ındex	Zeta potential (mV)
246.0	0.377	12.2

Nanogel valuation on Skin (Patch Test)

Patch test was performed to check the safety of the nanogels on human skin. The prepared formulations were applied on the forearms of volunteers and kept for 48h. After 48h it was observed that the parameters namely; ease of spreadability, application, sense just after application and on long term, irritation as well as sense on softness on application of formulations F4-F6 over forearms of volunteers, were quite good as compared other formulations. With paired sample t-test, it was evident that the effects of formulations F1-F6 were highly significant ($p < 0.001$) regarding all parameters of Patch test. Volunteers reported that there was no irritation and redness after application of the

prepared all formulations F1-F6 Results of the Patch test are shown in Figure 7.

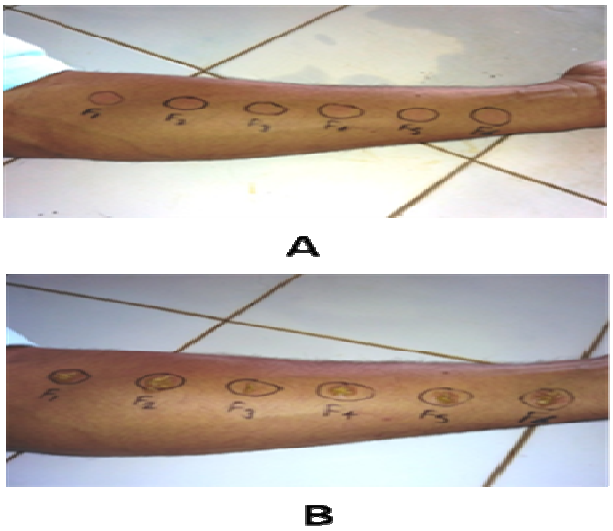

Figure 7: (A) Formulations F1-F6 application at time = 0 h; **(B)** Effect of formulations after 48h application on forearms of volunteers.

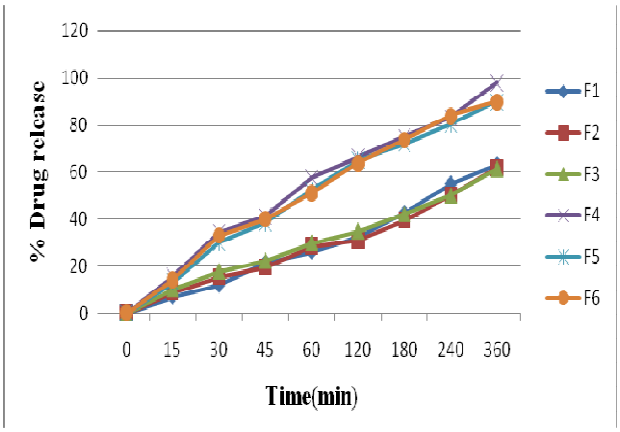

Figure 8: *In vitro* release study of nanogel

In vitro Release Studies

In vitro release study of nanogel F1 to F6 formulations are recorded by using franz diffusion cell in Fig. 1. For release study phosphate buffer pH 7.4 is used as medium. Batch F1, F2, F3 prepared by using carbopol 940 shows low drug release between 60-75%, due to higher viscous in nature while batch F4, F5, F6 prepared by using carbopol 934 show higher releases up to 90-97%, due to less viscous in nature. Carbopol 940 is highly viscous than Carbopol 934 which alter drug release from nanogel. From all batches F4 show high drug release 97.88 % within 5 h Show in Figure 8, this optimized batch formulated by using low viscous polymer Carbopol 934 so it release high drug concentration compared with other formulation. From drug release pattern of various nanogel formulation it concluded that high viscous polymer retard drug release and low viscous polymer facilitate higher drug release.

CONCLUSION

Nanogel containing *Tridax procumbens* Linn was prepared and evaluated for various test like pH, Spreadability, viscosity, extrudability, particle size analysis, zeta potential, drug content, patch test and *In vitro* release. From these result it can be concluded that Carbopol 934 is excellent polymers for preparation of nanogel. Optimized batch F4 was the best formulation among all the formulations. Show results within range for all parameter tests and nanosized particle size with zeta potential within range and no signs of skin irritation.

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