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Nanogel of Tridax procumbens Linn as a potential in rheumatoid Arthritis therapy

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Abstract

Molecular parts that are biologically active can be added to nanogels on their own by making salt bonds, hydrophobic contacts, or hydrogen bonds. Nanogels could be used in many medical situations to help with things like skin cancer, inflammation, bacterial and fungal diseases, and wound healing. The main goal of this project is to study Linn's antibacterial nanogel. To make the nanogel, ethanol, carbopol 934, methyl paraben, propyl paraben, EDTA, and triethanolamine were mixed together. FTIR and DSC studies were used to see if the plant extract and excipients were compatible. We checked the nanogel's viscosity, extrudability, spreadability, pH, and drug content, as well as its zeta potential and particle size. In vitro tests showed that leakage happened in 75–97.88% of all formulas made with different carbopols. An up to 97.88% release rate in 5 hours and acceptance results for all parameters are shown by the optimized batch B6. The best batch of particles had a size of 246 nm, we found. The results show that the nanogel that was made is a safe and effective way to treat RA.

Keywords: Nanogel, Tridax procumbens, Rheumatoid Arthritis, therapy

Introduction

Nanogels are made up of very small, swollen particles that are made up of flexible networks of hydrophilic or amphiphilic polymers that have been joined together chemically or mechanically. You can also make polymer networks that are anionic or ionic. By changing the nanogels' chemical make-up, size, and shape, different kinds of biomolecules may be able to fit inside them (Chen, *et al.,* 2016; Van, *et al.,* 1995). This makes sure that healing molecules are released into the body in a controlled way. Nanogels can be filled with biological or pharmaceutical molecules of choice by letting the agents accidentally come into contact with the polymer matrix (Wojdasiewicz, *et al.,* 2014). This creates hydrophilic particles that are spread out widely. Pharmaceutical dictionaries today include a lot of drugs that come from plants. This is because plants have a lot of medically active chemicals and can be changed to make new drugs with better or worse activity or toxicity. People in both rural and urban areas use native plants to make crafts, medicines, and beautycare items (Kumar, *et al.,* 2013; Ahire, *et al.,* 2018).

The best design can give the loaded biomolecule the protection it needs to keep it from breaking down. Nanogels, which are made of bendable structures, can be used to release drugs selectively or to package drugs (Makwana, *et al.,* 2016; Deshmukh, *et al.,* 2017). Researchers have shown that nanogels could be used to send drugs precisely to the right places and to create multifunctional nanocarriers for therapeutics. Since the beginning of the decade, they have also been used for systemic drug release. Rheumatoid arthritis is an autoimmune disease that affects one or more joints and lasts for a long time (Saisri, *et al.,* 2021; Natarajan, *et al.,* 2019).

Putting pressure on the synovial joints, which usually leads to symmetrical arthritis and joint breakdown, which causes disability and loss of function. Medications like steroids, non-steroidal anti-inflammatory drugs, disease-modifying drugs, anti-rheumatic drugs, and immunosuppressants are often used to treat rheumatoid arthritis (Namadeva, *et al.,* 2024). There is a history of these drugs having a number of bad effects, including stomach issues, a weakened immune system, and issues with the body's humoral reactions. The grass species Tridax procumbens Linn grows a lot in humid areas. In the past, it was used to treat lung catarrh, dysentery, malaria, stomachaches, diarrhea, and high blood pressure (Sultana, *et al.,* 2013). It is also used to check for bleeding from cuts, scrapes, and accidents and to stop hair loss. It has strong effects on slowing down breathing and is antibacterial, insecticidal, parasiticidal, and protective for the liver. Tridax procumbens Linn is a plant species in the Compositae family that is native to tropical America and has spread to tropical Africa, Asia, Australia, and India (Bhinge, *et al.,* 2017; Thombre, *et al.,* 2022).

The herb looks like a blade and has small, hairy leaves that look like blades. Because the flowers look like coat buttons, the plant is often called "coat buttons" in English. The plant is an annual vine herb that is semiprostatic. With a height of 30 to 50 cm, the stem doesn't have much hair and grows upwards. It also has roots in the nodes (Neamtu, *et al.,* 2017). The leaves are simple, arranged in opposition, lack stipules, and are lance-shaped to oblong in shape with a wedge-shaped base. The edges of the leaves have oddly shaped teeth that are 3–7 cm long. In addition, the leaves have short petioles. In a capitulum inflorescence, the plant has tube, yellow flowers with fine hairs on them. Tridax has two different kinds of flowers: ray

florets and disc florets with a base placentation (Talele, *et al.,* 2021).
A lot of research has been done on Tridex procumbens Linn, and it has been shown to help with gastritis. In addition, it kills parasites, bacteria, germs, infections, bugs, is an antioxidant, and lowers blood sugar.

MATERIALS AND METHODS

The complete adult Tridax procumbens Linn plant was obtained from the botanical garden. Research-Lab Fine Chem Industries in India were the providers of carbopol 940 and carbopol 934. However, triethanolamine, methyl paraben, propyl paraben, and polypropylene glycol were acquired from Sigma Aldrich in India. All substances included in the investigation were of analytical grade.

Plant Extracts Preparation

It was carefully cleaned to get rid of any leftover dirt and debris on the Tridax procumbens Linn plant, and then it was left to dry out in a shaded area. Tridax procumbens Linn was ground into a coarse powder using methanol in a soxhlet extractor for 72 hours (Agarwal, *et al.,* 2017).

Nanogel Preparation

The following steps were used to make the nanogel. It takes the preparation of two separate stages in order to make nanogel. Table 1 lists all the necessary amounts. Equal parts of ethanol and propylene glycol should be used to dissolve the extract. In a different step, the right amount of water was added to the carbopol to make a gel-like material. After that, EDTA, propyl paraben, and methyl paraben were added in measured amounts. Ultrasound mixing makes sure that each phase is evenly mixed and goes through sonication on its own. Following this, phase 1 is slowly added to phase 2 while being stirred constantly with a homogenizer. The mixture was mixed together until a gel formed. It was then treated with ultrasonic waves for 20 minutes using an ultrasonication. After that, triethanolamine was added to the gel to change its pH and make it smooth (More, *et al.,* 2016; Jain, *et al.,* 2011).

Table 1: Nanogel Formulation batches

Comp. (%)	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**
Plant Extract	1.0	1.5	2	1	2	1	1.5	2
Carbopol 934	-	-	-	1	2	1	2	3
Carbopol 940	1	2	3	2	-	-	-	-
EDTA	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Ethanol	3	3	3	3	3	3	3	3
Triethanolamine	0.3	0.3	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.	q. s.	q. s.

Compatibility Study

FT-IR and DSC Studies

FT-IR tests were done to see if the drug was breaking down and to see if the excipients were compatible. The Japan FT-IR Jasco 4600 device was used to record the infrared spectra. We scanned the FT-IR spectrum of the plant extract and blend between 4000 and 650 cm−1. The DSC thermogram of a dried plant extract and combination was taken with a TA WS thermal detector. In a temperature range of 40 to 300oC, the samples were heated at a steady rate of 10oC/min while being tightly sealed in aluminum cases. A steady flow of 50 milliliters per minute of nitrogen gas was used to keep the air from moving (Lakshmi, *et al.,* 2011).

Evaluation of the Nanogel

pH

A digital pH meter was used to get three readings that showed the pH. To make sure the glass electrode was completely covered, it was put into the gel system and the average of the three readings was found (Mohale, *et al.,* 2014).

Spreadability

For one minute, about one gram of nanogel was laid flat between two parallel plates. After the gel that was stuck to the top plate was taken off, its width was measured. A set weight of 50 grams held the top plate in place (Ahirrao, *et al.,* 2022).

Viscosity

With a Brookfield viscometer that has spindle number 74, the viscosity readings were done at a temperature of 25°C and rotational speeds between 50 and 250 rpm. The rheological results correctly show many features of the nanogel, such as how it binds, entangles, and crosses-links. How to Measure Particle Size It was found out what size particle it was. As the dispersion medium, methanol is used in the experiment. At 25 degrees Celsius, it has a viscosity of 0.5476 centipoise and a count rate of 2283 kilocounts per second (Mir, *et al.,* 2016).

Zeta Potential

Its conductivity was found to be 0.0321 millisiemens per centimeter (mS/cm), and its viscosity was measured to be 0.5651 centipoise (cP). Its electrovoltage was also recorded as 3.9 volts. It was tested at 25 °C to see what the nanogel's zeta potential was (Goci, *et al.,* 2014).

Estimation of Active Constituents

A 50 mL volumetric flask was filled to its fullest limit with methanol, and then one gram of each formulation was put into it. To make sure that the active ingredients were completely mixed, the flask was shaken very hard. Whatman filter paper was used to screen the solution, which was then extracted. 0.1 mL of the filtered liquid was mixed with 10 mL of methanol. Spectrophotometry with a reference curve set at a wavelength of 246 nm was used to measure the amount of active components (Saini, *et al.,* 2016).

Extrudability of Nanogel

After 20g of gel was tightly packed into a collapsible tube and the end was shut by crimping it, a clamp was used to stop any movement backward. After the cap was taken off, the gel came out. During the weighing process, the amount of stretched gel that was collected was measured. The amount of the gel that was pushed out was found (Saini, *et al.*, 2016; Ahire, *et al.*, 2023).

Skin Test

Ten physically fit people between the ages of twenty and thirty-five were picked at random. Before the study, each person filled out a permission form. Participants who had serious skin diseases or bronchial asthma were not allowed to take part in the study. Each volunteer's wrists were used for a patch test, which involved putting 0.5g of base on the skin's surface in a 0.6 cm circle. Formulations B1 through B8 were given to each person separately to see if they had any skin reactions. The test spots were picked out and stuck in place with tape. The patches were put on and left there for 48 hours so that the area where they were put on would not get clean. After 48 hours, the patches were taken off, and readings were taken an hour later. The face was looked at for any signs of redness, swelling, or spots. The clear symptoms and any itching or pain that comes with them make it clear that the product is having a problem. As long as the skin doesn't show any of the above signs, the product is safe to use (Aslani, *et al.*, 2016; Saini, *et al.*, 2016).

In-vitro Release Study

In a controlled lab setting, experiments were done using the Franz-type diffusion method to look into how the nanogel released. To make sure the sink conditions were right, a receptor fluid made of a phosphate buffer and 25% ethanol was used. Kept at 37 degrees Celsius plus or minus 0.5 degrees Celsius and stirred all the time at 600 spins per minute. In order to keep the sink state, 1 ml samples were taken from the receiver compartment and, at set times, replaced with the same amount of new receptor fluid. Spectrophotometry was used to measure the amount of medicine that was released through the cellophane membrane at a frequency of 249 nm.

RESULTS AND DISCUSSION

Compatibility Study

Finding out more about Fourier Transform Infrared Spectroscopy (FT-IR) The IR spectrum of the plant extract and the FTIR spectrum of the mixture were used to check how well the drug and excipient worked together. By looking at the spectra, we can see that the wave numbers of the medicine and the drug-excipient mix haven't changed much. So, figures 1 and 2 show that the medicine and excipients are compatible.

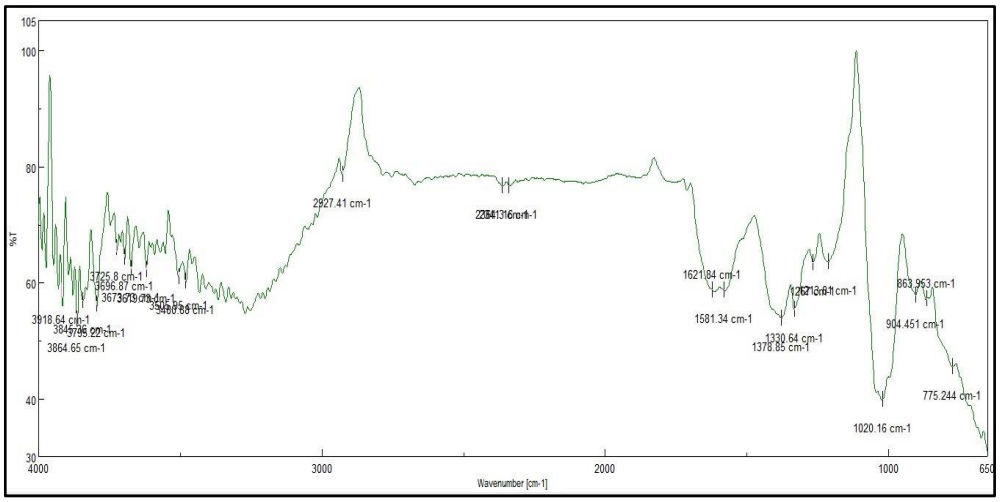

Figure 1: Plant extract FT-IR spectra

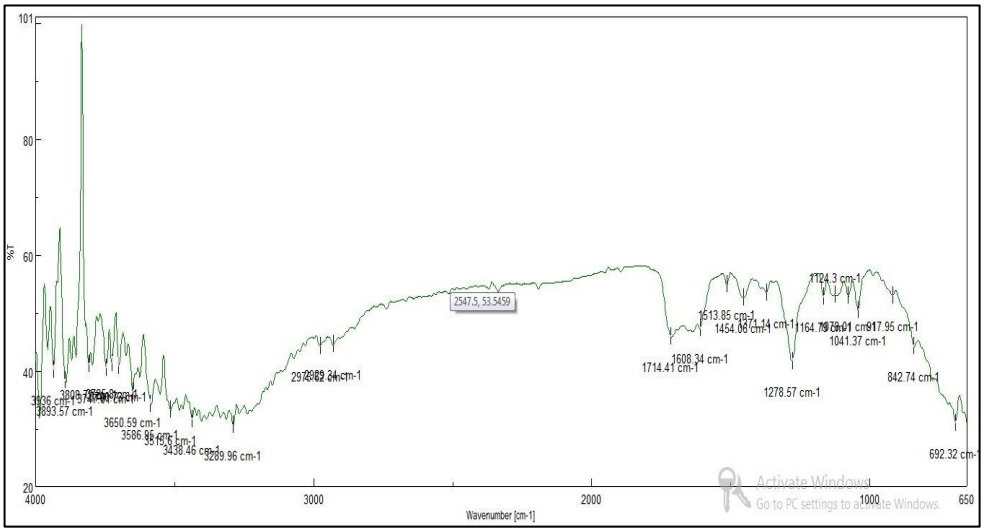

Figure 2: Mixture FT-IR spectrum

Scientists use differential thermophotometry to find out how the amount of heat radiation a material gives off changes as its temperature changes. DSC was used to test the Tridax procumbens plant preparation along with a mixture of other substances. At a temperature of 184.280°C, the pure plant extract showed a clear endothermic peak. At a temperature of 196.030 °C, exothermic peaks were found in the mixture. The most significant point on the line in Figure 3 has shrunk, and no new most significant point has appeared.

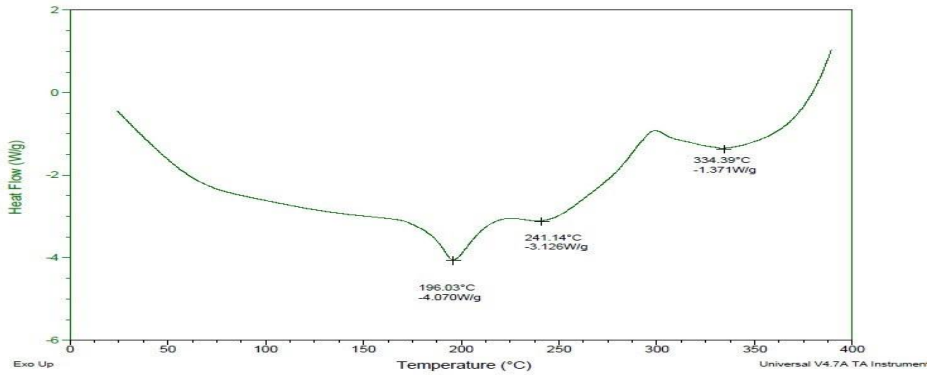

Figure 3: DSC for extract and excipient combination

The small change in the drug's melting point could be because the polymer and medicine mixed together, making each part less pure. However, it doesn't always mean that they might not get along.

Assessment Criteria for Nanogel Preparation

We tested nanogel forms B1 through B8 made with carbopol polymers to see how they behaved physically, including their pH level, viscosity, ability to spread, medicine content, and ability to be extruded. The results of the study, which can be seen in Table 2, were within the acceptable range.

Table 2: Assessment Criteria for Nanogel Preparation

Batch	pH	Viscosity *(Cps)*	Spreadability (g cm/sec)	Extrudability	Drug content (%)	Physical appearance
B1	6.12	7841	9.4	Good	99.8	Dark green and Homogenous
B2	6.23	8003	9.5	Excellent	99.4	Dark green and Homogenous
B3	6.57	8214	9.8	Good	99.6	Dark green and Homogenous
B4	6.69	7940	8.5	Good	99.7	Dark green and Homogenous
B5	6.68	7841	8.5	Good	99.5	Dark green and Homogenous
B6	6.67	7852	8.5	Excellent	99.7	Dark green and Homogenous
B7	6.61	8032	8.5	Excellent	99.6	Dark green and Homogenous
B8	7.33	8212	8.8	Good	99.5	Dark green and Homogenous

With the methanolic extract of Tridax procubens, the gel naturally has a dark green color. The pH of the gel mixtures was between 6.50 and 7.10, which is close to the pH range of skin. The viscosities of the gel mixtures range from 7930 to 8121 centipoise (cps).Based on the numbers, it looks like the gel can spread without much shear force. The high amount of spreadability is shown by the 8.6 to 9.9 g cm/sec range. Over 99% of the medicine was found to be concentrated in Nanogel forms. It was found that the nanogel could be pushed out easily.

Particle Size and Zeta Potential

The nanogel's particles were found to be 246 nm on average, which shows that gel particles at the nanoscale level were formed successfully. The nanogel's polydispersity index was found

to be 0.377, which means that it has a uniform makeup and a lot of different globules. Figure 4 shows a graph that shows how the particles are spread out evenly. It was found that the nanogel had a zeta potential of 12.2 mV. When a nanogel's zeta potential number is around ±30 mV, it is said to be stable. As you can see in Figure 5, the number we came up with is 12.2 ± 0.42, which meets the criteria for stability.

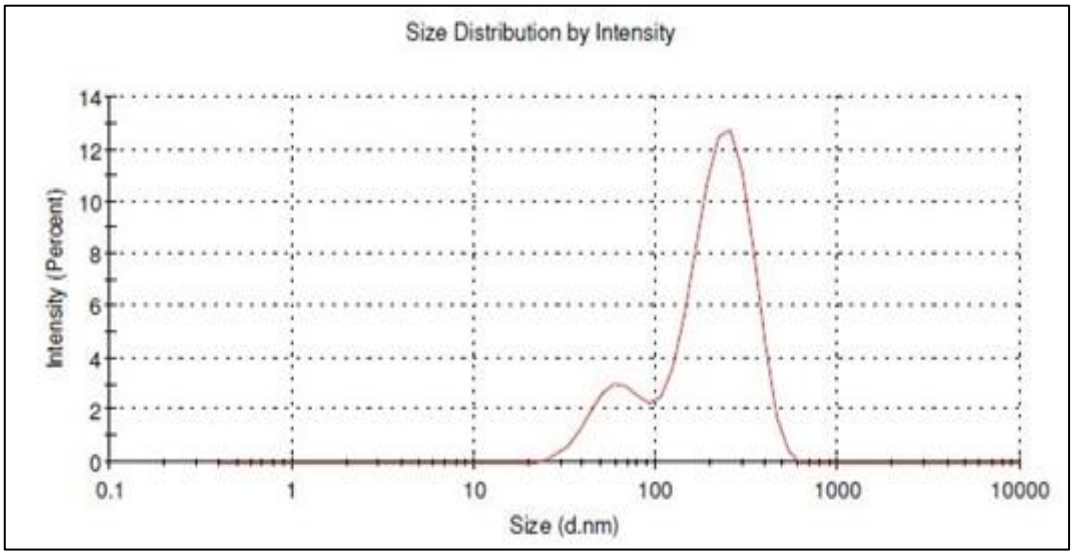

Figure 4: Particle size distribution of batch B6

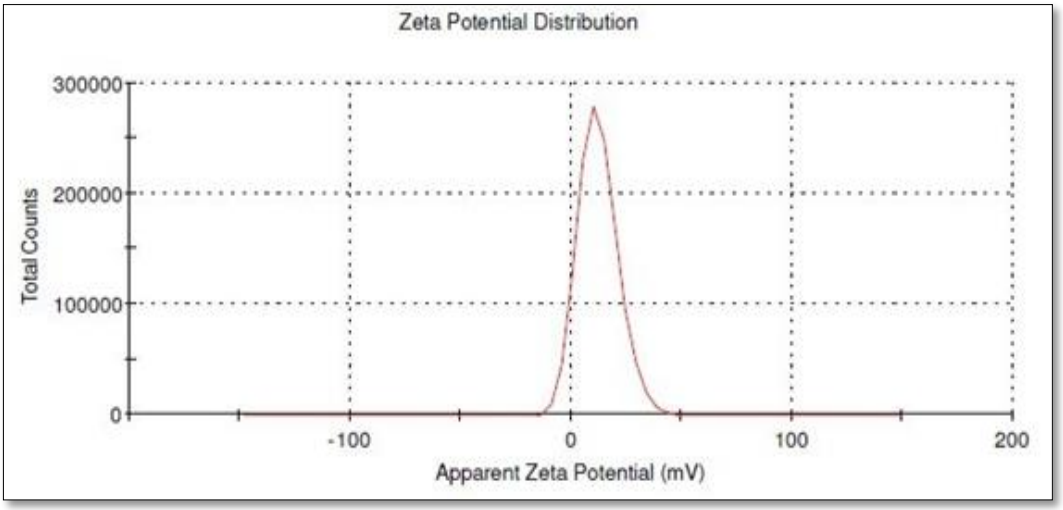

Figure 5: Zeta potential of optimized batch B6

Skin Test

Nanogel review, a skin test, was done to make sure that nanogels were safe to use on human skin. The formulations that were made were put on the volunteers' elbows and left there for 48 hours. After 48 hours, it was seen that the formulations B5–B7 worked very well when put on the volunteers' wrists. They were easy to spread, easy to apply, and felt good to the touch right away and over time. Different from the other versions, these ones caused less irritation and a feeling of greater softness. According to the paired sample t-test, formulations B5–B7

had a big effect on all of the Patch test characteristics. When the formulated solutions B1–B8 were put on the subjects, the Patch test showed that they did not feel any redness or irritation.

In-vitro Release Study

Figure 1 shows the results of an in vitro release study that used the Franz diffusion cell to look into how nanogel B1 through B8 versions released. To study release, a phosphate buffer solution with a pH of 7.4 is used as the medium. Because they are naturally thicker, batches B1, B2, and B3 (which were made with carbopol 940) have a mild drug release of 60 to 75%. But batches B4, B5, B6, B7, and B8 made with carbopol 934 have a higher drug release rate, between 90 and 97%. There is a difference in how drugs are released from nanogel because carbopol 940 has a higher viscosity than carbopol 934. It takes 5 hours for batch B6 to hit 97.88% drug release, which is the highest rate of all the batches. Carbopol 934 is a low viscosity polymer that is used in Figure 6 to make the best batch. This polymer made it possible for the batch to release more medicine than other versions. The drug release patterns of different nanogel mixtures showed that low viscosity polymers help the drug release more, while high viscosity polymers stop the drug release.

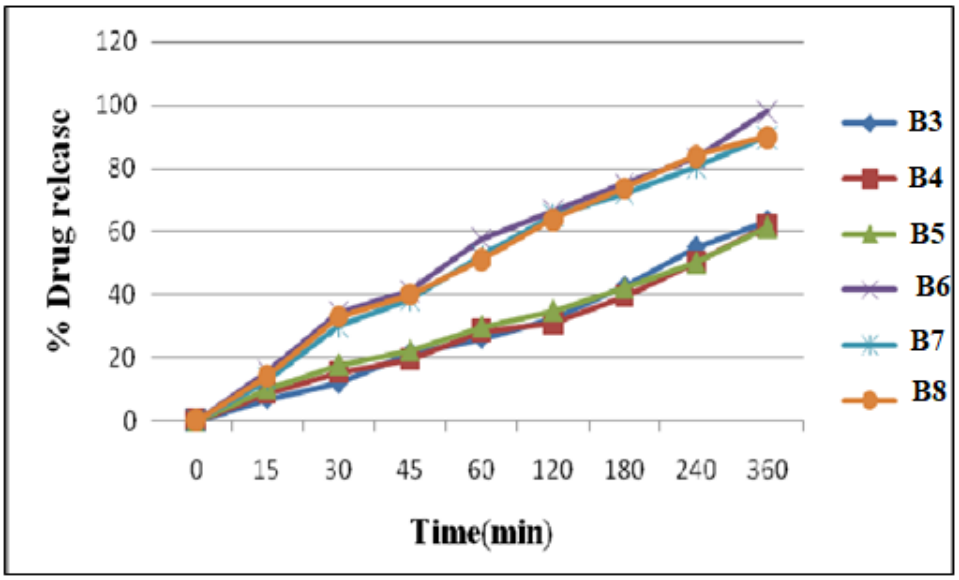

Figure 6: An *in-vitro* study of the formulation's release

CONCLUSION

A plant called Tridax procumbens Linn was used to make nanogel, which was then tested for its zeta potential, drug content, pH, ability to spread, viscosity, extrudability, particle size, and in vitro release. Based on the results, carbopol 934 seems to be a great material for making nanogels. The optimized batch B6 showed the best results of all the formulations. Results should be within the accepted range for all parameters, nanoparticle size should be within the acceptable range for zeta potential, and there should be no signs of skin irritation.

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