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Formulation development, *in vitro* and *in vivo* assessment of Diclofenac diethylamine nanosponge loaded topical formulation

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Abstract---Background and Objectives: The present work was performed with the objective of novel topical formulation development of poorly water-soluble drug Diclofenac diethylamine by loading the drug-containing nanosponge into the topical formulation. The nanosponges are polymeric nanoparticles that entrap the drug. The drug-loaded nanosponge has different absorption characteristics in comparison to the pure drug in the topical drug delivery system. Methods: The drug was first loaded in the ethylcellulose nanosponge using the emulsion solvent diffusion method. Then Diclofenac diethylamine nanosponge-loaded topical gel formulations were developed by using Carbopol 940 as a gel-forming polymer. Result: The Diclofenac diethylamine-loaded nanosponges were evaluated for various evaluation parameters such as particle size distribution, surface morphology, drug entrapment efficiency, polydispersity index, etc. The topical gel formulations were evaluated for pH, *in vitro* drug release, viscosity, *in vivo* performance and compared with the marketed formulation. The average size of prepared nanosponge formulations was found in the range of 102.8 nm to 475.5 nm. Scanning electron microscopy photographs of developed formulations reveal the spongy and porous nature of particles along with spherical

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shape. The polydispersity index (PI) was found in the range of 0.756 to 0.954. Conclusion: It was found that formulation F3 with a drug-to-polymer ratio of 1:3 has the highest drug loading. Further nanosponges were characterized for different parameters and results revealed acceptable characteristics of F3 formulation. These prepared nanosponges were loaded in topical Carbopol gel (Formulation A3) and evaluated for *in vitro* and *in vivo* performance. The data were fitted to different kinetic models which reveal typical zero order i*n vitro* drug diffusion with prolonged drug release. The developed formulation generated better analgesic and anti-inflammatory responses compared to the standard marketed formulation.

Keywords---nanosponge, water-insoluble drug, polymer, and topical formulation

Introduction

Diclofenac is a well-known nonsteroidal anti-inflammatory medication(Altman et al., 2015) that is commonly used for symptomatic pain and inflammation relief in musculoskeletal problems, toothaches, arthritis, dysmenorrhea, and other conditions. The diclofenac diethylamine is commonly utilized for topical treatments because of its high permeability and low aqueous solubility (Ong et al., 2007). The rapid growth of nanotechnology combined with the imperative to administer drugs in a precise and targeted manner led to the conception of the most recent method of drug delivery, which is known as the nanosponge formulation (Gangadharappa et al., 2017). The improved penetrability of nanocarriers in topical delivery systems and their passive accumulation at the target site contribute to the greater manifestation of these carriers (Vyas et al., 2014). The acronym TDDS stands for topical drug delivery systems. These systems can be easily made into liquid, solid, or semisolid dosage forms. Their primary purpose is to deliver a therapeutically effective concentration of medication in the skin or mucosal layers (Ruela et al., 2016). A minimal chance of inevitable adverse reaction owing to systemic drug distribution is one of the benefits that topical formulations have over oral or parenteral dosage forms (Benson et al., 2019). Patient compliance is another advantage that topical formulations have.

Methods

Preparation of nanosponge

Diclofenac diethylamine nanosponges were created using the emulsion solvent evaporation technique. Nanosponge formulations were created using the ethylcellulose (EC) polymer (Kumar et al., 2021). The internal phase, which included medicine and polymer in 20 ml of dichloromethane (DCM), was slowly introduced to a 0.3 percent w/v Poly Vinyl Alcohol in 100 ml of aqueous exterior phase using a magnetic stirrer for 3 hours at 1000 rpm. The prepared nanosponges were collected by filtration and air-dried in an oven at 45ºC for 24

hours and then packed in vials. The composition of nanosponges was described in table 1.

Table 1
Composition of Nano sponge formulations

Formulation Code	Drug: Polymer Ratio
F1	1:1
F2	1:2
F3	1:3
F4	1:4
F5	1:5
F6	1:6

Preparation of nanosponge loaded topical formulation

The Diclofenac diethylamine nanosponge-loaded gel was prepared by adding 1 gm of carbopol 934 to freshly prepared nanosponge suspension and neutralizing the pH by using the triethanolamine with continuous stirring by using a glass rod (Sharma & Pathak, 2011). The composition of Nano sponge-loaded topical gel formulations is described in table 2.

Table 2
Composition of Nano sponge loaded topical gel formulations

FORMULATION CODE	INGREDIENTS				
	Drug Concentration	Carbopol 934	Distilled water (ml)	Triethanola-mine (ml)	Propylene glycol (ml)
A1	1.16%	1.0%	10	0.8	0.4
A2	1.16%	1.0%	10	0.8	0.4
A3	1.16%	1.0%	10	0.8	0.4
A4	1.16%	1.0%	10	0.8	0.4
A5	1.16%	1.0%	10	0.8	0.4
A6	1.16%	1.0%	10	0.8	0.4

Characterization and Evaluation of Nano sponges
Particle size and Polydispersity

Particle size (z-average diameter) and polydispersity index of Diclofenac diethylamine acid-loaded Nanosponge dispersion were estimated by using a Malvern Zeta sizer (Shringirishi et al., 2014) at 25°C.

Morphology of Nanosponges by Scanning Electron Microscopy

The surface morphology of Nanosponges was examined using scanning electron microscopy. Scanning electron microscopy was used to examine nanosponges to evaluate particle size, shape, and surface morphology. Images were obtained at

various magnifications using a scanning electron microscope at a 10 kV acceleration voltage after the sample was stuck to the SEM sample holder with double-sided sticking tape (Kumar Satpathy et al., 2022).

Drug entrapment efficiency

Nanosponges' drug entrapment effectiveness was measured spectrophotometrically. A sample of Diclofenac diethylamine Nanosponge was combined in methanol and diluted to 100 ml with phosphate buffer (pH 6.8) before being maintained overnight. The drug content in Nanosponges was assessed and expressed as actual drug content.

Evaluation Parameters of Nanosponge Loaded Gel
Determination of pH

The pH of formulated topical gel formulations was evaluated with the help of a digital pH meter. 0.5 gm of prepared gel was dispersed in 50 ml of distilled water. It was kept aside at 25^0 C for 3 hours. After that pH was checked by pH meter by dipping the electrode into the solution.

Drug content

The Diclofenac diethylamine content in nanosponge gel was measured by dissolving 1000 mg of gel in 10 ml solvent (methanol) by sonication. The solution was passed through the Whatman filter paper no. 42 and filtered. Absorbance was measured after suitable dilution at 284 nm in UV - 1800 spectrophotometer.

Homogeneity

It was determined by visual inspection for the appearance of gel and the presence of any aggregates.

Extrudability

Pfizer hardness tester was used to study the extrudability. 20gm of gel was filled in an aluminum tube. The plunger was set up in such a way that the tube was securely held in place. For 30 seconds, a pressure of $1kg/cm^2$ was applied. The amount of gel extruded was measured and weighed. The procedure was repeated at three equidistance places of the tube.

Spreadability

By measuring the spreading diameter of 0.5g of gel between 20 x 20 cm glass plates after 1 min, the spreadability of the formulated gel was determined. The mass of the upper plate was standardized at 500g.

$$S = (m) X (l) X (t)$$

Where S is spreadability, m is weight or mass applied to the glass slide, l is the length of the glass slide and t is time in seconds (Ahmed et al., 2021).

Determination of viscosity

The viscosity of nanosponge-loaded gel was measured at different RPMs by using T spindle no. 1 and the heldal unit of Fungi Lab rotational viscometer.

***In-vitro* drug release studies (dialysis membrane)**

The Franze diffusion cell was selected for *in-vitro* diffusion studies by using a dialysis membrane. The diffusion cell was fabricated by a local vendor. Receptor compartment volume was made to hold 60 ml of diffusion media. The dialysis membrane was fixed between the donor and receptor chamber of the Franz diffusion cell. 0.5 gm of gel was spread homogeneously over the dialysis membrane. Dialysis membrane was tied over receptor chamber which was filled with phosphate buffer pH 7.4. The temperature was kept at 37^0C. The media was stirred with the magnetic bead at 150 RPM. 2 ml sample was withdrawn at a periodic time interval and sink condition was maintained by replacing the same with fresh media. The sample was filtered and absorbance was taken by UV double beam spectrophotometer.

Skin irritation test (patch test)

The experiment was carried out on a group of eight rats. The emulgel was applied to the rat's freshly shaved skin. Unwanted skin alterations, such as colour changes and changes in skin morphology, were monitored over 24 hours (Murthy & Hiremath, 2001).

***In vivo* anti-inflammatory activity**
Experimental design

Carrageenan injected subplantarly at a concentration of 1 percent (w/v) resulted in the development of edoema in the left hind paw of the rats. Both the F3 formulation and the standard formulation (diclofenac sodium gel) were administered thirty minutes prior to the administration of carrageenan. By employing the mercury displacement method with a plethysmometer, the volume of the paw was determined at intervals of 30, 60, 90, and 120 minutes (CRUNKHORN & MEACOCK, 1971).

- Group 1 (Control group): Carrageenan (1%) was administered in the plantar surface of the rat.
- Group 2 (Standard group): Carrageenan in addition to topically marketed diclofenac gel.
- Group 3 (Test): Formulations F3 and Carrageenan were included in Group 3 of the test.

A comparison was made between the drug-treated group and the carrageenan control group, and the percent inhibition of paw edoema in the drug-treated group was computed using the following formula:

$$\%\text{Inhibition of drug} = Vc\text{-}Vt/Vc\times100$$

where V_c is the inflammatory increase in paw volume control group and V_t the inflammatory increase in paw volume in (drug + carrageenan) treated animals.

In vivo analgesic activity

The hot plate technique was utilised in order to accomplish the analgesic activity. The rats were separated into the following groups, and the amount of time it took for them to react to the hot plate was determined (Kou et al., 2005).

- Group 1 (Control Group): No topical treatment was given and the latency period was calculated.
- Group 2 (Standard Group): The rats were treated with marketed diclofenac gel and its latency period was calculated.
- Group 3 (Test Group): The rats were treated with test formulations, i.e., F3, and the latency period was calculated.

Stability studies

The topical gel was packed in aluminium collapsible tubes (5 g) and tested for 3 months at 5 °C, 25 °C/60 percent RH, 30 °C/65 percent RH, and 40 °C/75 percent RH. At 15-day intervals, samples were extracted and examined for physical appearance, pH, rheological characteristics, and drug concentration.

Result and Discussion

Particle size and Polydispersity

The particle size distribution of prepared nanosponge formulations was estimated by the Malvern particle size analyzer. The average size of prepared nanosponge formulations was found in the range of 102.8 nm to 475.5 nm. The particle size of different formulations was tabulated in table 3. The polydispersity index (PI) was found in the range of 0.756 to 0.954. The polydispersity index is an estimation of heterogeneity of the size of particles in a mixture. As per International Standards Organization (ISO), the polydispersity index value less than 0.05 reveals monodisperse samples, while values greater than 0.7 are common to a broad size range or polydisperse samples.

Table 3
Particle size distribution study of Nano sponge formulations

Formulation Code	Particle Size (nm)	Polydispersity Index
F1	450.7	0.767
F2	475.5	0.954
F3	102.8	0.856
F4	201.2	0.867
F5	350.9	0.756
F6	405.5	0.856

Morphology of Nano sponges by Scanning Electron Microscopy

The surface morphology of developed nanosponge formulations was studied by using scanning electron microscopy (SEM). The representative scanning electron microscopy photographs of the nanosponge formulation were shown in figure 1. Scanning electron microscopy photographs of developed formulations reveal the spongy and porous nature of particles along with spherical shape.

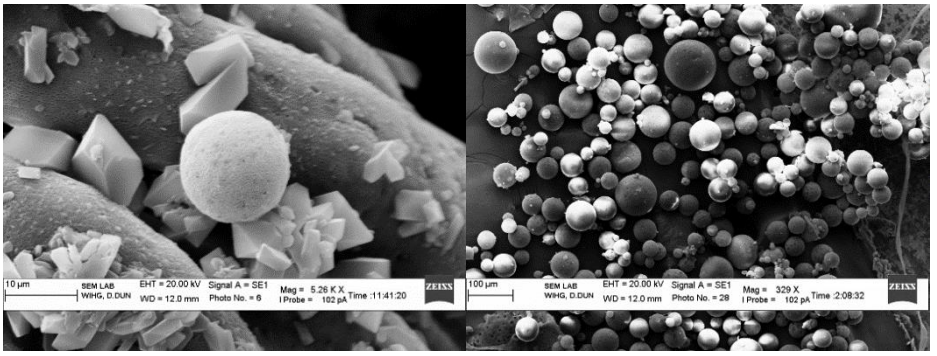

Figure 1. SEM images of prepared nanosponges

Drug entrapment efficiency

Drug entrapment efficiency was measured for all developed formulations. The entrapment efficiency was maximum in the F3 formulation having a 1:3 Drug: Polymer ratio. Random % of drug entrapment was found when polymer concentration was increased. No correlation can be established between polymer concentration and drug entrapment.

Table 4
Drug entrapment efficiency of different Nano sponge formulations

Formulation Code	% Drug entrapment
F1	60.34±1.20 %
F2	52.40±1.82 %
F3	78.50±2.50 %
F4	68.60±1.50 %
F5	58.60±2.20 %
F6	52.50±1.80 %

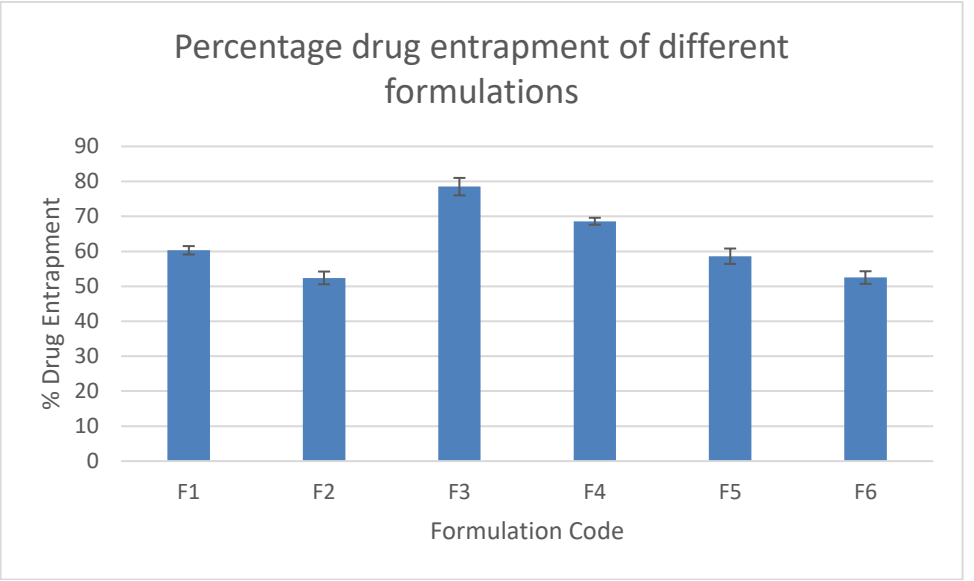

Figure 2. Percentage drug entrapment of different formulations

Estimated parameter of nanosponge based topical gels

The nanosponge-loaded gel was evaluated for various parameters like pH, homogeneity, spreadability, % drug content, and extrudability. The results were reported in Table 5. Prepared gel formulations revealed a pH range between 6.5 to 6.9. All gel formulations showed good homogeneity. % Assay of gel formulations was found in the range of 85.6±1.4% to 94.6±0.86%. Extrudability was found in an acceptable range. Spreadability was found in the range of 18.24 to 26.06 gm-cm/sec.

Table 5
Characterization of Nano sponge loaded topical gel formulations

Formulation code	pH data	Homogeneity	Spreadability (gm-cm/sec)	% drug content	Extrudability
A1	6.6	Good	26.06±0.85	90.5±1.5	**
A2	6.9	Good	20.64±0.90	85.6±1.4	**
A3	6.5	Good	22.50±1.5	94.6±.86	***
A4	6.7	Good	18.24±0.56	94.2±1.2	**
A5	6.8	Good	22.24±0.86	93.5±1.5	**
A6	6.5	Good	25.24±1.2	90.8±1.2	**

Symbol: (*) Acceptable, (**) Good, (***) Excellent.

The viscosity of optimized formulations (F3)

The viscosity of nanosponge-loaded gel was measured at different RPMs by using T spindle no. 1 and the heldal unit of Fungi Lab rotational viscometer. The viscosity reading was tabulated in table 6 and graphically represented in figure 3.

Table 6
Viscosity of Nano sponge loaded topical gel formulations

Formulation code	Viscosity (Centipoise)
A1	1205.5±15.75
A2	1310.8±14.25
A3	1291.6±20.52
A4	1308.5±25.20
A5	1350.95±30.5
A6	1408±28.5

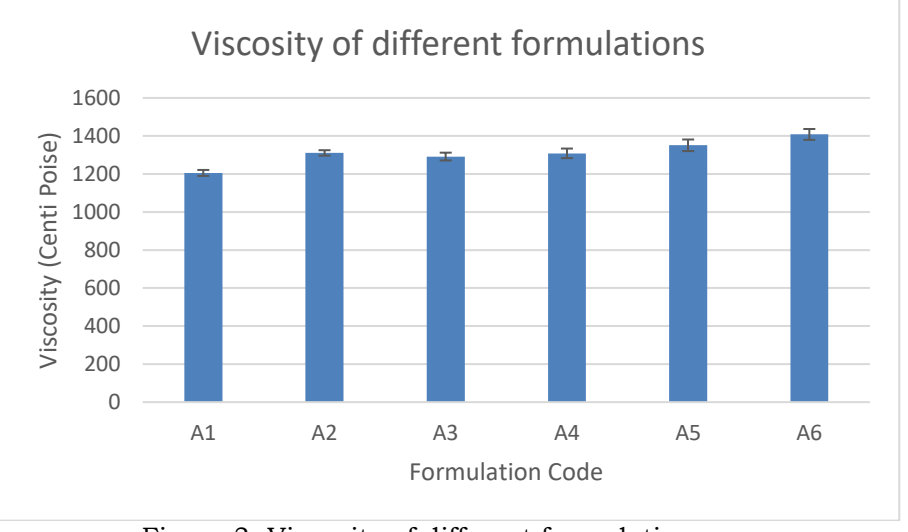

Figure 3. Viscosity of different formulations

***In-vitro* diffusion studies**

In-vitro diffusion study was performed by using Franz diffusion cell. *In-vitro* diffusion data were tabulated in table 7 and graphically represented in figure 4. *In-vitro* diffusion data revealed that % drug release was decreased as the concentration of polymer increased in initial hours.

Table 7
In-vitro diffusion study of Nano sponge loaded topical gel formulations

Time (Hours)	% Drug release of formulation A1	% Drug release of formulation A2	% Drug release of formulation A3	% Drug release of formulation A4	% Drug release of formulation A5	% Drug release of formulation A6
0	0±0	0±0	0±0	0±0	0±0	0±0
1	20.05±0.55	18.5±1.5	15.95±1.25	13.52±0.21	11.02±2.7	8.85±1.56
2	35.45±1.25	32.2±1.2	30.15±0.85	27.72±2.16	22.58±2.4	15.82±2.5
3	65.15±1.55	62.18±1.8	59.9±1.26	57.66±0.45	46.4±1.56	39.5±2.7

4	79.55±1.85	76.2±1.56	74.55±1.5	70.62±1.26	65.5±1.23	62.42±1.5
5	92.3±2.05	90.5±0.9	88.4±0.66	85.8±1.2	80.52±1.8	71.55±1.2
6	98.95±0.5	96.2±0.66	95.8±0.92	94.92±0.9	89.8±2.2	79.8±1.5

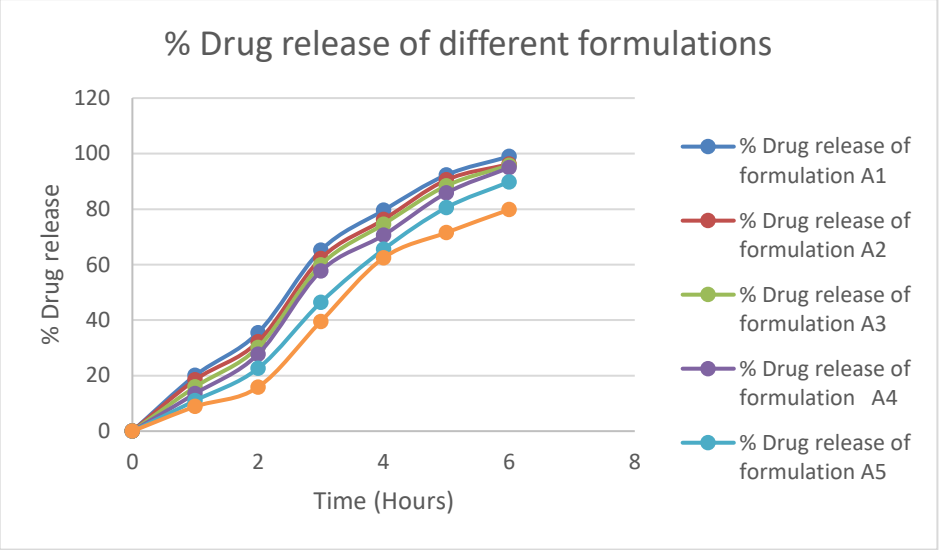

Figure 4. % Drug Release of different gel formulations loaded with Diclofenac diethylamine nanosponge

Skin Irritation test

No allergic symptoms like inflammation, redness, irritation appeared on rats up to 24 h.

Anti-inflammatory activity

Formulation A3's anti-inflammatory activity was estimated and compared to diclofenac marketed preparation. The percent inhibition of the commercial formulation and A3 was discovered to be 62.35 and 65.82 percent, respectively. This demonstrated that the formulations were just as effective as the commercially available product.

Analgesic activity

The lapse time for the formulas increased. Diclofenac gel and A3 formulations were compared (marketed preparation). Diclofenac gel and A3 had 5.6 and 5 second lapse times, respectively.

Stability study

After 3 months of storage, all of the generated nanosponge-loaded gel formulations were determined to be stable, with no changes in their physical appearance, pH, rheological properties, or drug concentration.

Conclusion

The nanosponge of poorly water-soluble drug Diclofenac diethylamine was successfully prepared with ethyl cellulose as a polymer. Different ratios of drug and polymer were tried to achieve maximum drug loading and It was found that formulation F3 with a drug-to-polymer ratio of 1:3 has the highest drug loading. Further nanosponges were characterized for different parameters and results revealed acceptable characteristics of F3 formulation. These prepared nanosponges were loaded in topical Carbopol gel and evaluated for *in vitro* release. The data were fitted to different kinetic models which reveal typical zero order i*n vitro* drug diffusion with prolonged drug release and therefore it can be effectively used in the treatment of pain. The nanosponge-based topical gel formulation is ideal for effective pain management and anti-inflammatory treatment since the nanocarrier could permeate the drug deeper into the skin layer where other topical semisolid preparations may not reach. Diclofenac diethylamine nanosponge-loaded gels were found homogeneous. pH was found near to 7. All prepared topical gels have good spreadability and acceptable extrudability. *In vivo* anti-inflammatory and analgesic activity of developed formulation was compared with a marketed formulation which reveals comparable analgesic and anti-inflammatory effect of optimized formulation.

Conflict of interest:

The authors have no conflicts of interest regarding this investigation.

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