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Original Article

LOXOPROFEN NANOSPONGES: FORMULATION, CHARACTERIZATION AND EX-VIVO STUDY

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ABSTRACT

Objective: The objective of this study is to optimize a nanosponge formulation for Loxoprofen and then incorporating it into a gel formulation offering a controlled drug release, enhanced skin permeation and thus better bioavailability.

Methods: Loxoprofen nanosponges were prepared using the emulsion solvent diffusion method and formulated using Polyvinyl alcohol, Ethylcellulose and Dichloromethane. The effect of the different formulation variables like ethyl cellulose: polyvinyl alcohol ratio, drug: ethyl cellulose ratio, stirring time, stirring speed, internal phase volume and external phase volume on the particle size, entrapment efficiency, production yield, polydispersity index and Zeta potential was investigated. The optimized nanosponge formulation was incorporated into a gel. The loaded gel was evaluated by *in vitro* release and permeation studies and the results were compared to that of a marketed formulation (Loxonin® gel).

Results: The optimized formulation showed 67.29±1.19 % entrapment efficiency, 239.8±16.95 nm particle size and-8.32±0.87 mV Zeta potential. The drug was released slowly from the nanosponge-loaded gel where the cumulative percentage of drug released was only 77.71±0.42 % in 8 h where it was incorporated in the entrapped form while it was 99.31±0.64% from Loxonin® gel where it was in the unentrapped form. The cumulative percent of drug permeated through the skin from the nanosponge-loaded gel was 98.66±0.14% for 24 h while it was only 60.38±0.18% from Loxonin® gel.

Conclusion: The nanosponge-loaded gel showed more sustained drug release and a better drug permeation when compared to a marketed gel (Loxonin® gel).

Keywords: Loxoprofen sodium, Nanosponges, Ethylcellulose, Gel, Carbopol 934, Emulsion solvent diffusion method

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INTRODUCTION

Osteoarthritis is a leading cause of disability among older adults worldwide. Treatment aims are to alleviate inflammatory pain and improve physical function through non-pharmacological and pharmacological interventions. Non-steroidal anti-inflammatory drugs (NSAIDs) are recommended as first-line therapy [1]. NSAIDs are among the most frequently prescribed drug groups. These drugs are used locally or systemically in the treatment of various chronic inflammatory conditions, relieving pain, reducing fever, and preventing local inflammation [2], NSAIDs lead to unfavorable effects on the stomach as a result of inhibition of prostaglandins, which play a role in the protection of the gastric mucosa. Furthermore, the acidic character of NSAIDs may lead to local irritation on the gastrointestinal mucosa which is known as NSAIDs gastropathy [3]. Therefore, some NSAIDs are administered transdermally to achieve local or systemic effect as an alternative to oral and parenteral administration [4]. Loxoprofen sodium is a phenyl propionic acid type non-steroidal anti-inflammatory drug with excellent efficacy in treating inflammatory rheumatoid diseases and relieving acute pain. Conventional transdermal delivery systems such as ointments and creams are associated with side effects due to the uncontrolled drug release from the formulation. Therefore, attention is shifted towards the development of particulate carrier systems such as nanosponges for drug-controlled delivery [5]. In recent years, more focus has been drawn towards nanoparticulate systems e. g. nanosponges, as they offer more precise control of drug release [6]. Nanosponges are a class of polymer-based colloidal structures having nanosized cavities. A wide variety of topical agents can be safely incorporated into nanosponges for getting the benefits of these systems [7]. They are non-irritating, non-mutagenic, non-allergenic, non-toxic [8] and can serve as a local depot for sustained drug release, facilitate drug permeation across the skin, improve the bioavailability, increase the stability, reduce drug toxicity and irritation, decrease adverse effects and improve the patient compliance by prolonging the dosage intervals [9], and thus overcoming the limitations of conventional transdermal delivery systems. Several topical dosage forms are used to deliver NSAIDs. Many widely used topical agents such as ointments, creams and lotions have many disadvantages. They are sticky in nature causing uneasiness to the patient when applied and have low spreadability and they may also exhibit stability problems. The use of gels has emerged both in cosmetics and pharmaceutical preparations because of their unique array of features in terms of use and patient acceptability. Gel is a dosage form formed by the entrapment of large amounts of aqueous or hydroalcoholic liquid in a network of colloidal solid particles, which may consist of inorganic substances, such as aluminum salts or organic polymers of natural or synthetic origin. The constitution of a high aqueous component permits greater dissolution of drugs, and also permits easy migration of the drug through a vehicle that is essentially a liquid, compared with either the ointment or cream bases. Moreover, using hydrogel topical formulation as a delivery system can reduce irritation and improve retention on the skin compared to the other topical formulations [10]. It was reported that hydrogel increased drug skin absorption and permeation 10 times higher than oil-based formulations [11]. Also, hydrogel unique property (porosity) provides beneficial sustained and controlled drug delivery of hydrophobic drugs via a suitable release mechanism. The objective of the present study is to assess the applicability of formulating nanosponges of Loxoprofen sodium and incorporating them into a transdermal gel and comparing this nanosponge-loaded gel to conventional gels to clarify the advantages of the nanosponges technology.

MATERIALS AND METHODS

Materials

Loxoprofen sodium dihydrate was obtained as a gift sample from Egyptian Group for Pharmaceutical Industries (EGPI) (Cairo, Egypt), Ethylcellulose, dialysis bag (100KD cut off) and Dichloromethane were obtained from Sigma-AlDrich (St. Louis, USA), Polyvinyl alcohol was procured from LOBA chemie PVT. LTD (Mumbai, India), Propylene glycol and Triethanolamine were purchased from El Nasr pharmaceutical chemicals (Cairo, Egypt), Carbopol 934 was

procured from Techno pharm Chem (India). Distilled water was used throughout the study. All the other chemicals were of analytical grade and were utilized without any further purification.

Animals

This study was conducted after approval from the ethical committee of animal care of the faculty of pharmacy, Cairo University, Egypt (Approval number: serial no. of the protocol: PI (2118)). Nine male albino Wistar rats weighing (200-250 g) were obtained from and acclimatized at the central animal house of the national organization for drug control and research (NODCAR). The animals were kept in individual cages under well-defined and standardized conditions (humidity and temperature-controlled room; 12-h light and 12-h dark cycle) and they were fed with standard dry food and water ad libitum. The skin of the rats was collected and used in the ex-vivo permeation study.

Method

Preparation and optimization of Loxoprofen sodium nanosponges

Emulsion solvent diffusion method

In this method, different proportions of ethyl cellulose (EC) and polyvinyl alcohol (PVA) are used to prepare the nanosponges. Two phases are used in this method; the dispersed is organic and the continuous is aqueous. The dispersed phase consists of the drug and EC dissolved in 20 ml of dichloromethane and the required amount of PVA is added to 150 ml of distilled water (the continuous phase). The organic phase was slowly added to the aqueous phase and the mixture was stirred for 2 h at 1000 rpm using a magnetic stirrer [12]. The resultant nanosponge dispersion was centrifuged at 6000 rpm and 25 °C for 60 min and the excess solvent was decanted. The separated nanosponges were air dried at room temperature then packed in airtight vials for evaluation.

Twenty formulations were prepared using six varying formulation factors; polymer surfactant ratio (EC PVA ratio), drug polymer ratio, stirring time, stirring speed, the volume of internal organic phase and volume of the external aqueous phase. The effect of these variables on the production yield, particle size, polydispersity index (PDI), Zeta potential, and entrapment efficiency was studied. Initially, six nanosponge formulations were prepared. EC was used as entrapping agent, Dichloromethane as cross-linking agent and PVA as an emulsifying agent. Nanosponges were prepared using different EC: PVA ratios; 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6 (F1 to F6 respectively) while the other variables were kept constant; distilled water volume was 150 ml, drug: EC ratio 1:2, stirring time 2 h, stirring speed 1000 rpm and Dichloromethane volume 20 ml.

Another six nanosponge formulations were prepared using the chosen EC: PVA ratio 1:6 and different drug: EC ratios; 1:0.5 (F12), 1:1 (F7), 1:3 (F8), 1:4 (F9), 1:5 (F10) and 1:6 (F11) using distilled water volume of 150 ml, stirring time 2 h, stirring speed of 1000 rpm and Dichloromethane volume of 20 ml.

To find out the optimum stirring time, two formulations were prepared using stirring time 3 h (F13) and 1 h (F14) using distilled water volume of 150 ml, EC: PVA ratio 1:6, a drug: EC ratio 1:5, a stirring speed of 1000 rpm and a dichloromethane volume of 20 ml. Two more formulations were prepared using stirring speeds of 800 (F15) and 900 rpm (F16) using stirring time of 3 h, the distilled water volume of 150 ml, EC: PVA ratio 1:6, a drug: EC ratio 1:5 and dichloromethane volume of 20 ml, another two formulations were prepared using dichloromethane volume of 10 ml (F17) and 30 ml (F18) using EC: PVA ratio 1:6, a drug: EC ratio 1:5, stirring time of 3 h, stirring speed 900 rpm and distilled water volume of 150 ml. Two final formulations were prepared using distilled water volume of 100 ml (F19) and 200 ml (F20) using EC: PVA ratio 1:6, a drug: EC ratio 1:5, stirring time 3 h, stirring speed of 900 rpm and Dichloromethane volume 20 ml.

Evaluation of Loxoprofen soduim-loaded nanosponges

Determination of production yield

It was calculated using the weight of the formed nanosponges after drying and the initial total weight of the drug and polymer used for the preparation of nanosponges [13].

Production yield % was calculated by using the following equation:

$$\text{Production yield (\%)} = (\text{Practical mass of nanosponges}/\text{Theoretical mass [polymer+drug]}) \times 100 \quad \ldots\ldots (1)$$

Measurement of particle size, zeta potential and polydispersity index

The Particle size, PDI and Zeta potential of the prepared nanosponge formulations were measured using a Zeta sizer (Malvern Zeta sizer Nano series Ver. 7.11, Serial Number: MAL1044595) [14]. For this, aqueous dispersions of nanosponges were diluted to an appropriate scattering intensity (100 µl of the dispersions diluted with 20 ml distilled water) [15].

Entrapment efficiency

A UV spectrophotometric method was used to calculate the entrapment efficiency of the nanosponge formulations. UV-visible spectrophotometer (Unicam, England) was used to determine the maximum absorbance (λmax) of Loxoprofen sodium. A calibration curve was plotted in distilled water at the determined (λmax). Accurately weighed 10 mg of the formulation was added to 60 ml distilled water and stirred using a magnetic stirrer (Magnetic Stirrer Hot Plate: Prolabo: France) at 1000 rpm for 15 min then filtered. The absorbance of the filtrate was measured spectrophotometrically at the predetermined λmax and the concentration was calculated using the constructed calibration curve [16].

% Entrapment efficiency was calculated using the following equation

$$\text{\% Entrapment efficiency} = (\text{Actual drug content in the nanosponges}/\text{Theoretical drug content}) \times 100 \quad \ldots\ldots (2)$$

The optimized nanosponge formulation was selected for further studies.

Surface morphological studies

Surface morphology of the optimized nanosponge formulation was studied using scanning electron microscopy (SEM) [17], scanning electron microscope (Quanta FEG 250) operated at an acceleration voltage of 20 kV was used.

The optimized nanosponge formulation was selected for formulating a gel using Carbopol 934 as a gelling agent. Drug-excipient compatibility studies (Differential scanning calorimetric (DSC) studies and Fourier Transform Infrared (FTIR) analysis) were performed before formulation.

Differential scanning calorimetric studies

The melting point of the pure drug was compared to the melting point of the drug in the physical mixture of the gel-forming polymer Carbopol 934 and the selected optimized nanosponge formulation (DSC-60, Shimadzu Corporation, Japan) was used after calibration with Indium and lead standards, samples (3-5 mg) were heated (range 25-400 ^{0}C, 10 ^{0}C/min) in crimped Aluminum pans under a nitrogen atmosphere at a flow rate of 10 ml/min [18]. The recorded thermograms were analyzed for any interaction between the drug and the excipients.

Fourier transform Infrared analysis

Fourier transform infrared analysis was used to check any kind of chemical interaction between Loxoprofen sodium and the excipients that would be used in the formulation of the nanosponge-loaded gel. Schimadzu FT-IR Affinity-1 Spectrometer was used. Samples of pure Loxoprofen sodium and the physical mixture of the gel-forming polymer Carbopol 934 and the selected optimized nanosponge formulation were mixed with IR grade KBr. Samples were scanned in the range from 4000 to 400 cm^{-1} and carbon black reference. The detector was purged carefully by clean, dry helium gas to increase the signal level and reduce moisture [19].

Design and preparation of Loxoprofen-loaded nanosponge gel

The optimized nanosponge formulation was selected for formulating hydrogel using Carbopol 934 as a gelling agent, propylene glycol (PG) as a permeation enhancer and Triethanolamine (TEA) as a pH neutralizer, a conventional loxoprofen sodium 1% gel was also prepared [20].

Preparation of Loxoprofen-loaded nanosponge gel

Accurately weighed amount of Loxoprofen sodium nanosponge powder and the required quantity of Carbopol 934 polymer were dispersed in part of the calculated amount of distilled water then PG was added and they were homogenized, TEA was slowly added with constant stirring for neutralization till a viscous gel was formed, the final weight was completed by distilled water and final homogenization was made [14].

Preparation of the conventional gel

Carbopol 934 was dispersed in the solution of Loxoprofen sodium in part of the required amount of distilled water, then PG was added and they were homogenized, TEA was added slowly with constant stirring till a viscous gel was formed, the final weight was completed by distilled water and final homogenization was made.

The prepared gels were stored in tightly closed screw-capped plastic jars at 5 °C for further investigations.

Table 1: The composition of Loxoprofen-loaded nanosponge hydrogel and loxoprofen sodium conventional hydrogel

Component	Loxoprofen-loaded nanosponge hydrogel	Loxoprofen sodium conventional hydrogel
Carbopol 934	2 g	2 g
Loxoprofen sodium dihydrate nanosponge	2 g	-
Loxoprofen sodium dihydrate	-	1 g
PG	20 ml	20 ml
TEA	5 ml	3 ml
Distilled water (q. s)	To 100 g	To 100 g

Evaluation of gels

The prepared formulations and a marketed reference formulation (Loxonin®) gel were evaluated.

Physical examination

The prepared formulations and the reference formulation were first inspected visually for their appearance, clarity, color and homogeneity [7].

Viscosity determination

The viscosity of the prepared hydrogels and the reference formulation was measured using (Brookfield DVIII cone and plate viscometer) with spindle no. 52 [21]. Viscosity was recorded at 25 °C, at 10 and 100 rpm.

pH determination

The pH was measured using a calibrated pH meter (HANNA Instruments, Portugal), 0.5 g gel was diluted with 4.5 ml distilled water then the pH was measured at 25 °C [14].

***In vitro* release studies**

In vitro drug release studies for the pure drug, the marketed product Loxonin® gel, the conventional hydrogel, the optimized formulation nanosponge loaded gel and the optimized nanosponge formulation were performed. 100 mg of samples were suspended in 2 ml phosphate buffer pH 5.5 and added to a dialysis bag (100KD cut off) [22]. The dialysis bag was sealed properly from both the top and the bottom and inserted into 30 ml dissolution medium (phosphate buffer pH 5.5) in a 50 ml falcon tube. The whole system was fixed in a shaking water bath (Fischer Scientific, USA), rotating at 100 rpm with temperature adjusted to 37±1 °C Temperature and pH were chosen to simulate that of human skin. At specified time intervals, 1 ml medium sample was withdrawn and immediately replaced with another 1 ml of equally warmed fresh buffer to maintain sink conditions for 8 h. Withdrawn samples were filtered and assayed at each time interval for the drug released at the predetermined λ max [23] using UV-Visible spectrophotometer (Unicam, England) and phosphate buffer pH 5.5 as blank. A calibration curve of serial drug dilutions was constructed to enable the calculation of the amount of released drug in the sample. From this percentage drug released was calculated and values of cumulative % drug released were plotted versus time.

Kinetic analysis of drug release

To study the drug release mechanism from the optimized formulation and the nanosponge-loaded hydrogel formulation, the release data was fitted to zero-order, first-order, second-order, Higuchi, Hixson-Crowell and Baker Lonsdale kinetic models [24]. The kinetic model with the highest coefficient of correlation (R^2) value was considered to be the best fit model for describing the drug release.

Ex-vivo skin permeation studies

A comparative study was carried out on the Loxoprofen-loaded nanosponges gel and a marketed reference formulation Loxonin® gel. Shaved rat skin was mounted between the donor and receptor compartments of a vertical Franz diffusion cell [25], with the stratum corneum side facing the donor compartment and the dermal side facing the receptor compartment, which was 11 ml phosphate-buffered saline (PBS) pH 7.4, stirred by a magnetic bar at 100 rpm and a temperature of 37 °C±1 °C maintained by water circulation throughout the experiment. 250 mg of each gel formulation was applied uniformly to the dorsal side of the rat skin in the donor compartment. Available surface area for permeation was 1.23 cm^2, 1 ml samples were withdrawn from the sampling port of the receptor compartment at predetermined time intervals and immediately replaced with another 1 ml of equally warmed fresh buffer for 24 h [26]. Samples were analyzed using a validated UV method and the concentration of the permeated drug was determined at the predetermined λ max. A graph was constructed between the cumulative percentage drug permeated versus time [27]. The data obtained were also subjected to mathematical models like zero-order, first-order, second-order, Higuchi, Hixson-Crowell and Baker Lonsdale kinetic models [28].

N. B. all measurements in this study were in triplicates and average values were calculated and noted.

RESULTS AND DISCUSSION

Preparation and optimization of Loxoprofen sodium nanosponge formulations

Effect of formulation variables on the physicochemical characteristics of nanosponge formulations

In order to assess the effect of EC: PVA ratio on the physicochemical characteristics of nanosponges, six formulations with different EC: PVA ratios were prepared. All formulations resulted in the formation of nanosponges. The entrapment efficiency of the prepared nanosponges was in the range of 7.99±2.11 to 33.9±3.28 %. The highest value was found to be with formulation F4. The concentration of the surfactant needs to be optimized to avoid foaming, particle aggregation and a decrease in entrapment efficiency. With respect to PVA, entrapment efficiency decreased when the EC/PVA ratio exceeded 1:4, which was found to be optimum concerning the entrapment efficiency. The most uniform size distribution was obtained with EC/PVA ratio 1:1 in F1 which showed the lowest PDI of 0.348±0.02. The surface charge of nanosponges was determined by Zeta potential and it was found to be in the range of-9.85±2.56 to-40.7±0.87 mV. The formulation F5 with EC/PVA ratio 1:5 showed Zeta potential value of-40.7±0.87 mV. At this high potential, nanosponge particles would exhibit a great repulsion with each other, which will significantly prevent their agglomeration. It was observed that the above 1:5 ratio increasing

the concentration of PVA decreased the production yield. This may be due to the increased foaming during the nanosponge preparation with the increase in the amount of PVA hindering the formation of the nanosponges [29]. Particle size did not follow any particular pattern. Smallest particle size was obtained with F6 with EC/PVA ratio 1:6. Particles of size range 100-200 nm are favored for topical formulations [30]; that is why this ratio was chosen for further optimization.

The effect of the drug to polymer ratio on the physicochemical characteristics of the formulated nanosponges was examined for various ratios from 1:0.5 to 1:6. Nanosponges were successfully formed with all formulations except F9 and F11 having drug to polymer ratios 1:4 and 1:6 respectively, they showed particle sizes out of the nano range (>1 µm) and hence did not meet the requirements to be characterized as nanoparticles [12], for this reason they were discarded from further studies. The entrapment efficiency of the valid nanosponges was in the range of 12.15±1.76 to 49.45±3.21 %. Maximum value was achieved with 1:5 ratio. The valid nanosponges mean particle size ranged from 62.89±7.69 to 533.2±18.12 nm. The mean particle size was found to increase with an increase in polymer amount [31]. This may be due to the availability of a large quantity of polymer. It was observed that as the polymer amount decreases, the particle size decreases. This may be due to the fact that at low polymer concentration and relatively higher drug content, the amount of polymer available per nanosponge to encapsulate the drug becomes less. The thickness of polymer wall is thereby reduced leading to small-sized nanosponges [14]; moreover, large quantities of EC increased the viscosity of the system which created hindrances in the formation of smaller droplets [32]. At low EC quantities, diffusion of the internal phase (dichloromethane) into the external phase (aqueous phase) was improved, reducing the time for droplet formation, which resulted in smaller particle sizes. It was observed that as the polymer amount in the formulation increases, the production yield also increases till drug: EC ratio 1:2 above which the production yield decreased. This may be due to that larger amounts of polymer increased the wall thickness of the nanosponge as indicated by a larger particle size resulting in the formation of lesser number of nanoparticles [29]. F12 formulation with drug to polymer ratio 1:0.5 showed the least PDI value. Formulation F10 with drug to polymer ratio 1:5 showed the best values concerning entrapment efficiency and Zeta potential and this ratio was selected for further optimization.

The third studied parameter was stirring time. It varied from 1 h to 3 h. In our study we have found that the stirring time of 3 h yielded nanosponges with the highest entrapment efficiency and production yield with the least particle size which is favored but with the least Zeta potential and highest PDI, which means the least stable formulation and since 3 h resulted in nanosponges with the highest entrapment efficiency and production yield with the least particle size; therefore this stirring time was selected for further optimization.

The effect of the stirring speed on the physicochemical characteristics of the formulated nanosponges was examined. The stirring speed varied from 800 rpm to 1000 rpm. Results indicated that as the speed increased, the particle size and production yield increased. It was also observed that as the speed exceeds 900 rpm, entrapment efficiency is decreased. Our results have also shown that the formulation F16 with 900 rpm stirring speed showed the least PDI value and the highest Zeta potential, which means that it was the most stable formulation. Therefore, this speed was selected for further optimization.

Dichloromethane as internal phase varied in volume from 10 to 30 ml and the impact was studied. As the volume of internal phase increased, particle size and entrapment efficiency did not follow any particular pattern. 20 ml was found to be the optimum volume as it yielded the most ideal formulation F16 showing promising results in all parameters; a favorable least particle size, highest entrapment efficiency and production yield, least PDI value and highest Zeta potential which means the most stable formulation. Therefore, this volume was used for further optimization.

As the volume of distilled water was increased, entrapment efficiency was increased. Although F16 with 150 ml distilled water showed the least particle size, the least PDI value and the highest Zeta potential, which means the most stable formulation. The formulation having maximum entrapment efficiency and production yield was chosen to formulate a hydrogel. The entrapment efficiency of F20 formulation was found to be 67.29±1.19 %. Hence, the formulation F20 was considered the optimized Loxoprofen sodium nanosponge formulation and it was selected for further studies.

Table 2: Physicochemical characteristics of the valid nanosponge formulations

Formulation	Particle size±SD* (nm)	Zeta potential±SD* (mV)	PDI±SD*	Production yield (%)±SD*	Entrapment efficiency (%)±SD*
F1	380.7±5.65	-20.3±4.73	0.348±0.02	17.27±1.43	7.99±2.11
F 2	237.8±11.85	-9.85±2.56	1	21.6±4.51	9.97±5.21
F 3	231.9±8.52	-10.7±1.07	0.54±0.040	22.99±4.89	10.78±3.78
F 4	465.7±9.13	-13.15±0.97	0.453±0.025	25.18±6.32	33.9±3.28
F 5	248.7±7.48	-40.7±0.87	0.392±0.016	38.9±1.54	28.7±3.56
F 6	202.1±5.67	-15.85±1.32	0.494±0.04	34.12±4.21	15.75±2.54
F 7	75.6±3.78	-13±1.17	1	32.41±3.21	12.15±1.76
F 8	219.7±14.76	-8.18±0.38	0.666±0.04	31.38±5.11	44.88±3.65
F 10	533.2±18.12	-28.75±0.87	0.351±0.033	21.1±2.54	49.45±3.21
F12	62.89±7.69	-16.75±0.34	0.3475±0.032	25.57±2.77	30.23±6.32
F13	248±17.89	-7.2±0.77	0.962±0.054	46.2±1.34	58.97±6.55
F14	292.2±10.76	-13.2±0.55	0.813±0.043	41.4±1.89	50.26±6.72
F15	16.35±10	-14.1±0.88	1	33.72±0.67	60.9±0.95
F16	53.42±20.26	-20.2±0.89	0.667±0.042	43.56±1.12	61.82±1.36
F17	191.6±25.19	-10.7±1.15	0.71±0.04	18.84±1.32	51.18±0.82
F18	288.4±28.93	-13.5±0.66	0.854±0.01	41.28±0.98	60.16±0.52
F19	542.5±30.09	-6.27±1.54	0.805±0.02	47.1±6.20	59.96±2.14
F20	239.8±16.95	-8.32±0.87	0.787±0.08	69.55±8.07	67.29±1.19

*Values are expressed as mean±SD, SD: Standard deviation, n=3.

Surface morphology

The morphology of the selected optimized formulation F20 nanosponges was investigated by SEM. The representative SEM photograph of the nanosponges is shown in fig. 1. It was observed that the nanosponges were nanosized particles uniformly spherical in shape with a spongy and porous nature. The pores are tunneled inwards, which may be due to the diffusion of Dichloromethane from the surface of the nanosponges during preparation.

The optimized Nanosponge formulation (F20) was selected for formulating a hydrogel using carbopol 934 as a gelling agent, PG as a permeation enhancer and TEA as pH neutralizer. Drug-excipient compatibility studies (IR, DSC) were performed before formulation.

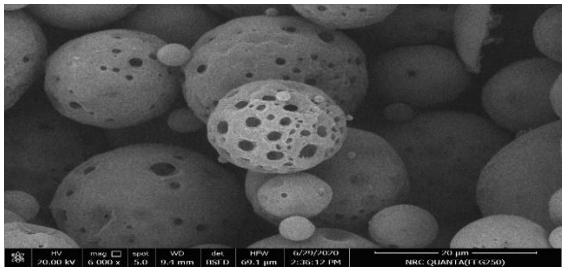
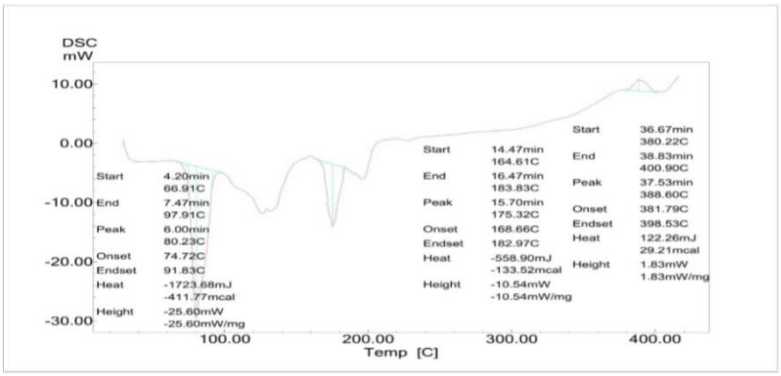


Fig. 1: SEM image of F20 nanosponge formulation with Mag.6 KX

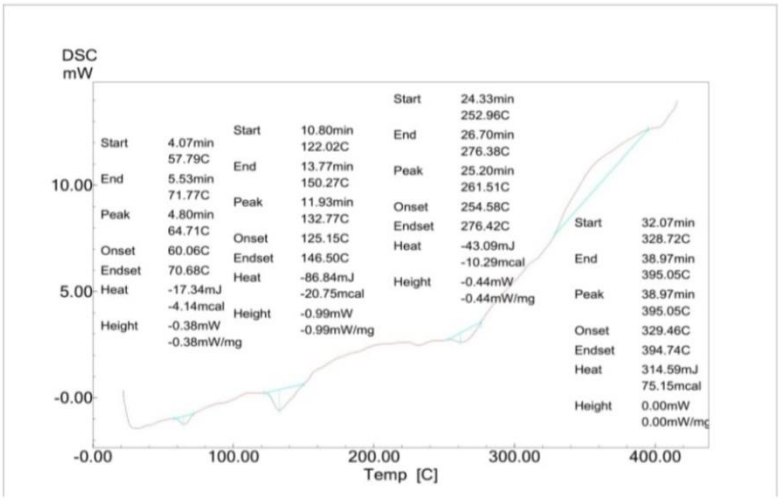
Drug-excipient compatibility studies

Differential scanning calorimetric studies

DSC thermograms of pure Loxoprofen sodium dihydrate and the physical mixture of Carbopol 934 polymer and the optimized nanosponge formulation (F20) are shown in fig. 2. DSC curve of pure Loxoprofen sodium showed a sharp endothermic peak at 80.23 °C corresponding to its melting point, while DSC curve of the physical mixture showed the absence of the drug melting peak indicating the successful encapsulation of the drug within the nanosponge cavities imparting higher thermal stability to the formulation when compared to the pure unentrapped drug [33].



DSC thermogram of pure Loxoprofen sodium dihydrate



DSC thermogram of Loxoprofen sodium F20 nanosponge formulation - Carbopol 934 mixture

Fig. 2: DSC thermograms of pure loxoprofen sodium dihydrate and Loxoprofen sodium F20 nanosponge formulation-Carbopol 934 mixture

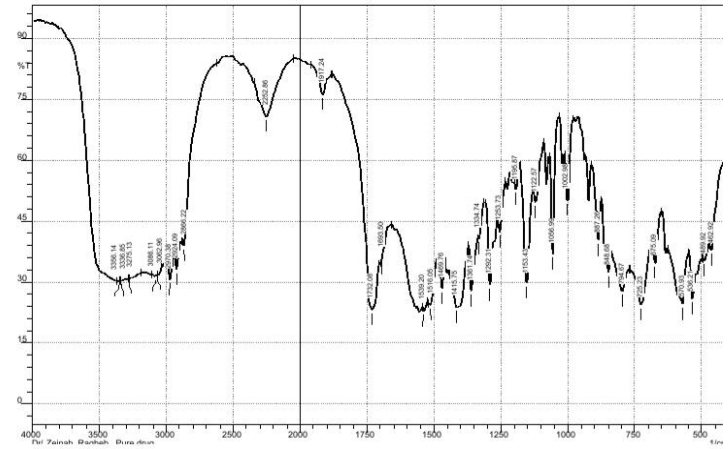
Fourier transform infrared analysis

FTIR spectroscopy was used to check any possible chemical interaction between the drug and the excipients which would be used in the formulation. By comparing FT-IR spectra of the pure drug with that of a physical mixture of nanosponge F20 formulation with Carbopol 934 polymer, it was found that all the characteristic peaks of Loxoprofen sodium appeared at their respective wave number ranges in the FTIR spectrum of the physical mixture which means the absence of any change in the chemical integrity of the drug during preparation and thus the drug stability in the formulation was confirmed [34]. Results are shown in fig. 3.

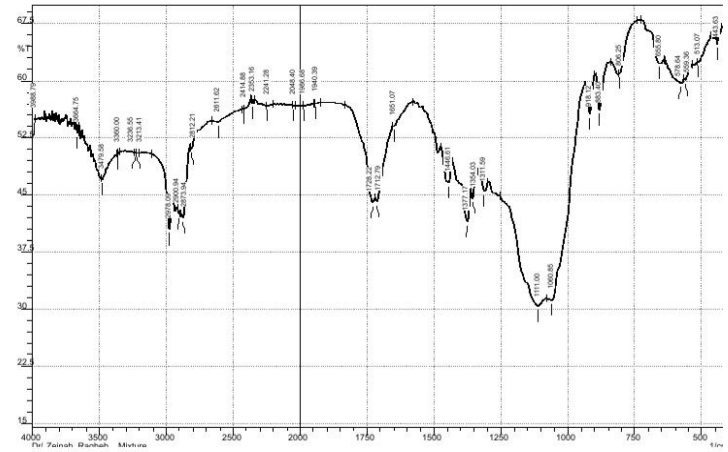
Gel formulations were prepared using Carbopol 934 as a gelling agent and evaluation studies were carried out.

Evaluation of gels

The physical properties of the prepared hydrogels and the marketed formulation Loxonin® gel were evaluated. All the hydrogels were smooth and homogenous. The physicochemical properties like viscosity and pH were determined and tabulated (table 3). The viscosity affects the spreadability, extrudability and drug release. The gels with high viscosity may not extrude from the tube easily, whereas low viscosity gels may flow quickly. Hence, there should be an optimum viscosity. The viscosity values of the prepared formulations were near that of the marketed formulation at different rates of shear. The hydrogel pH values were considered acceptable so there is no risk of skin irritation upon application [35].



FTIR spectrum of pure Loxoprofen sodium dihydrate



FTIR spectrum of Loxoprofen sodium F20 nanosponge formulation - Carbopol 934 mixture

Fig. 3: FTIR spectrum of pure loxoprofen sodium dihydrate and Loxoprofen sodium F20 nanosponge formulation-Carbopol 934 mixture

Table 3: Physical evaluation of hydrogels

Gel formulation	Clarity and color	Viscosity (cPs) at 10 rpm±SD*	Viscosity (cPs) at 100 rpm±SD*	pH±SD*
Loxonin® gel	Transparent white	22146±2.5	3310±3.0	6.45±0.19
Conventional gel	Opalescent white	36982±2.0	6299±1.1	7.77±0.05
Nanosponge-loaded gel	Opaque white	39218±3.0	6070±1.0	8.28±0.02

*Values are expressed as mean±SD, SD: Standard deviation, n=3.

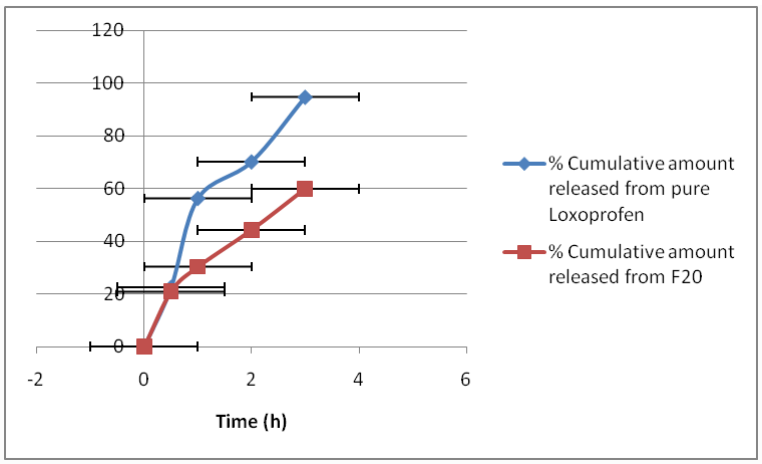


Fig. 4: The cumulative percentage drug release profile of loxoprofen sodium from nanosponge F20 formulation and pure Loxoprofen sodium in phosphate buffer pH 5.5 (Values are expressed as mean±SD, SD: Standard deviation, n=3)

In vitro release studies

The percentage cumulative drug released from the pure drug was compared with the optimized nanosponge formulations F20, where the percentage drug released, was 94.67±0.61 % in 6 h while the pure drug dissolved almost completely after 3 h due to its solubility in phosphate buffer pH 5.5. The matrix of the nanosponges held the drug and controlled its release over a longer period of time as shown in fig. 4. The *in vitro* release profile of Loxoprofen sodium from the optimized formulation F20 in fig. 4 showed a bi-phasic pattern with an initial burst release for the initial 0.5 h followed by a slow and sustained release. Initial burst release may be due to the unentrapped adsorbed drug on the surface, which was released at a faster rate compared to the entrapped drug inside the nanosponges core [36].

As shown in fig. 5, Loxoprofen sodium nanosponge-based gel formulation sustained the release for more duration when compared to the other formulations where the drug was in the free unentrapped form. Hence, it was clear that drug was released slowly from the dosage form when it was incorporated in the entrapped form rather than that in the unentrapped form [30]. This could be due to the thickness of the nanosponges' matrix wall which could lead to a longer diffusional path, and consequently controlled drug release. It is assumed that the possible mechanism behind the drug release is a combination of dissolution, diffusion and erosion where the decrease in release pattern may be due to the difficulty of the diffusion of the drug through the hydrophobic core.

When comparing the cumulative percent of released Loxoprofen sodium from the conventional gel and pure Loxoprofen sodium and also from the nanosponge F20 and the nanosponge F20 loaded gel, it was observed that the drug release decreased with the presence of the polymer as shown in fig. 6 and fig. 7. This may be due to the fact that the release of drug from the polymer matrix takes place after the complete swelling of the polymer.

Kinetic data modeling studies

Different kinetic models were applied to find out the release behavior from the optimized formulation F20 and the nanosponge loaded hydrogel. The interpretation of data showed that maximum linearity (highest R^2 value) was found with Hixon Crowell model and Baker Lonsdale model, respectively. The Baker and Lonsdale model indicates that the structure of the releasing matrix is spherical, because Baker and Lonsdale model is based on the Higuchi diffusion model, this proves that the matrix is porous, and with channels and the drug can diffuse out of these pores and channels [37]. This diffusion is the principal mechanism of drug release which may be controlled by the porosity of the nanosponges.

Ex-vivo skin permeation studies

The Loxoprofen-loaded nanosponges gel formulation showed higher drug permeation through the skin within 24 h where the cumulative percent of drug permeated through the skin was found to be 98.66±0.14 % for 24 h while it was only 60.38±0.18 % for Loxonin® gel. The nanosponges being in nanosize can easily permeate the skin without any need to permeation enhancers [38], in addition to the huge surface area available for dissolution, nanosponges are nano-sized colloidal bearer, so they easily pierce into the skin [39], that explains why the ex-vivo skin permeation from the Loxoprofen loaded nanosponges gel is higher. All these factors synergize and improve the drug bioavailability. A graph was constructed by plotting the cumulative percent drug permeated versus time (fig. 8).

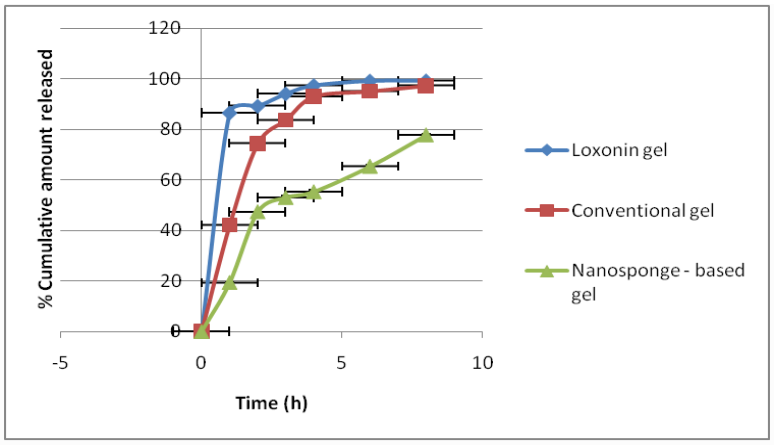


Fig. 5: Comparative release profiles of the different gel formulations in phosphate buffer pH 5.5 (Values are expressed as mean±SD, SD: Standard deviation, n=3)

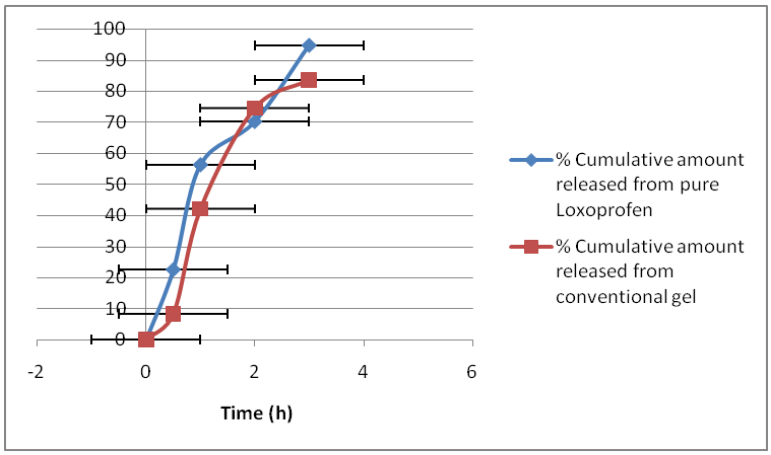


Fig. 6: Comparative release profiles of the conventional gel and pure loxoprofen sodium in phosphate buffer pH 5.5 (Values are expressed as mean±SD, SD: Standard deviation, n=3)

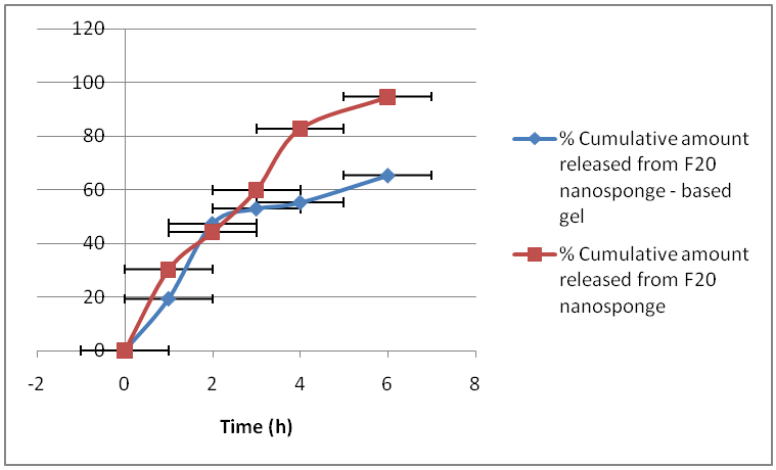


Fig. 7: Comparative release profiles of the nanosponge F20 and the nanosponge F20 based gel in phosphate buffer pH 5.5 (Values are expressed as mean±SD, SD: Standard deviation, n=3)

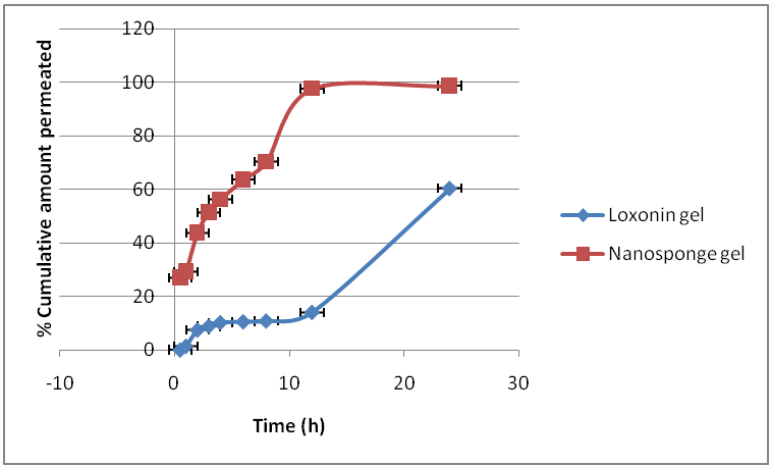


Fig. 8: The cumulative percent drug permeated versus time for Loxoprofen-loaded nanosponges gel and Loxonin® gel (Values are expressed as mean±SD, SD: Standard deviation, n=3)

The data obtained were subjected to the kinetic data modeling to find out the permeation mechanism from the Loxoprofen loaded nanosponges gel and it was found that it follows Higuchi model, which indicates diffusion mechanism.

CONCLUSION

A nanosponge-based hydrogel formulation of Loxoprofen sodium was successfully prepared. The nanosponge-loaded gel showed more sustained drug release when compared to a conventional gel and better drug permeation when compared to a marketed gel offering additional benefits such as reduction in dose, dosing frequency and thus related systemic side effects. Therefore it can be beneficial for use in the treatment of various chronic inflammatory conditions.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Magni A, Agostoni P, Bonezzi C, Massazza G, Mene P, Savarino V, Fornasari D. Management of osteoarthritis: expert opinion on NSAIDs. Pain Ther. 2021;10(2):783-808. doi: 10.1007/s40122-021-00260-1, PMID 33876393.
2. Lionberger DR, Joussellin E, Lanzarotti A, Yanchick J, Magelli M. Diclofenac epolamine topical patch relieves pain associated with ankle sprain. J Pain Res. 2011 Mar 7;4:47-53. doi: 10.2147/JPR.S15380, PMID 21559350.
3. Heyneman CA, Lawless Liday C, Wall GC. Oral versus topical NSAIDs in rheumatic diseases: a comparison. Drugs. 2000 Sep;60(3):555-74. doi: 10.2165/00003495-200060030-00004, PMID 11030467.
4. Hooper L, Brown TJ, Elliott R, Payne K, Roberts C, Symmons D. The effectiveness of five strategies for the prevention of gastrointestinal toxicity induced by non-steroidal anti-inflammatory drugs: a systematic review. BMJ. 2004 Oct 23;329(7472):948. doi: 10.1136/bmj.38232.680567.EB, PMID 15475342.
5. Ghanbarzadeh S, Arami S. Enhanced transdermal delivery of diclofenac sodium via conventional liposomes, ethosomes, and transfersomes. BioMed Res Int. 2013;2013:616810. doi: 10.1155/2013/616810.
6. Jain K, Gupta U, Jain NK. Dendronized nanoconjugates of lysine and folate for treatment of cancer. Eur J Pharm Biopharm. 2014 Aug;87(3):500-9. doi: 10.1016/j.ejpb.2014.03.015, PMID 24698808.

7. Abbas N, Parveen K, Hussain A, Latif S, Uz Zaman SU, Shah PA, Ahsan M. Nanosponge-based hydrogel preparation of fluconazole for improved topical delivery. Trop J Pharm Res. 2019 Feb 1;18(2):215-22. doi: 10.4314/tjpr.v18i2.1.
8. Wakure BS, Salunke MA, Mane PT, Awale SR, Shinde RD, Eklinge SS. Nanosponges as novel carrier for topical delivery of luliconazole-an antifungal drug. Int J Pharm Sci Res. 2022;12(10):5570.
9. Jenning V, Schafer Korting M, Gohla S. Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. J Control Release. 2000 May 15;66(2-3):115-26. doi: 10.1016/s0168-3659(99)00223-0, PMID 10742573.
10. Aldawsari HM, Badr-Eldin SM, Labib GS, El-Kamel AH. Design and formulation of a topical hydrogel integrating lemongrass-loaded nanosponges with an enhanced antifungal effect: *in vitro*/*in vivo* evaluation. Int J Nanomedicine. 2015 Jan 29;10:893-902. doi: 10.2147/IJN.S74771, PMID 25673986.
11. Haltner Ukomadu E, Sacha M, Richter A, Hussein K. Hydrogel increases diclofenac skin permeation and absorption. Biopharm Drug Dispos. 2019 Jul;40(7):217-24. doi: 10.1002/bdd.2194, PMID 31242332.
12. Amer RI, El-Osaily GH, Gad SS. Design and optimization of topical terbinafine hydrochloride nanosponges: application of full factorial design, *in vitro* and *in vivo* evaluation. J Adv Pharm Technol Res. 2020 Jan–Mar;11(1):13-9. doi: 10.4103/japtr.JAPTR_85_19, PMID 32154153.
13. Solunke RS, Borge UR, Murthy K, Deshmukh MT, Shete RV. Formulation and evaluation of gliclazide nanosponges. Int J App Pharm. 2019 Nov 1;11(6):181-9. doi: 10.22159/ijap.2019v11i6.35006.
14. Ahmed MM, Fatima F, Anwer MK, Ibnouf EO, Kalam MA, Alshamsan A, Aldawsari MF, Alalaiwe A, Ansari MJ. Formulation and *in vitro* evaluation of topical nanosponge-based gel containing butenafine for the treatment of fungal skin infection. Saudi Pharm J. 2021 May 1;29(5):467-77. doi: 10.1016/j.jsps.2021.04.010, PMID 34135673.
15. Rao BN, Reddy KR, Fathima SR, Preethi P. Design, development and evaluation of diltiazem hydrochloride loaded nanosponges for oral delivery. Int J Curr Pharm Sci. 2020 Sep 17:116-22. doi: 10.22159/ijcpr.2020v12i5.39784.
16. Sujitha YS, Muzib YI. Formulation and optimization of quercetin loaded nanosponges topical gel: ex vivo, pharmacodynamic and pharmacokinetic studies. Int J App Pharm. 2019 Sep 1;11(5):156-65. doi: 10.22159/ijap.2019v11i5.32850.
17. Swaminathan S, Pastero L, Serpe L, Trotta F, Vavia P, Aquilano D, Trotta M, Zara G, Cavalli R. Cyclodextrin-based nanosponges encapsulating camptothecin: physicochemical characterization, stability and cytotoxicity. Eur J Pharm Biopharm. 2010 Feb;74(2):193-201. doi: 10.1016/j.ejpb.2009.11.003, PMID 19900544.
18. Dubey P, Sharma HK, Shah S, Tyagi CK, Chandekar AR, Jadon RS. Formulations and evaluation of cyclodextrin complexed ceadroxil loaded nanosponges. IJDD. 2017 Oct 31;9(3):84. doi: 10.5138/09750215.2180.
19. Penjuri SCB, Ravouru N, Damineni S, Bns S, Poreddy SR. Formulation and evaluation of lansoprazole loaded nanosponges. TJPS. 2016 Dec 1;13(3):304-10. doi: 10.4274/tjps.2016.04.
20. Iriventi P, Gupta NV. Development and evaluation of nanosponge loaded topical herbal gel of wrightia tinctoria. Int J App Pharm. 2020 Jan 1;12(1):89-95. doi: 10.22159/ijap.2020v12i1.31198.
21. Aggarwal G, Nagpal M, Kaur G. Development and comparison of nanosponge and niosome based gel for the topical delivery of tazarotene. Pharm Nanotechnol. 2016 Sep 28;4(3):213-28. doi: 10.2174/2211738504666160804154213, PMID 29052500.
22. Shah NV, Seth AK, Balaraman R, Aundhia CJ, Maheshwari RA, Parmar GR. Nanostructured lipid carriers for oral bioavailability enhancement of raloxifene: design and *in vivo* study. J Adv Res. 2016 May 1;7(3):423-34. doi: 10.1016/j.jare.2016.03.002, PMID 27222747.
23. Omar SM, Ibrahim F, Ismail A. Formulation and evaluation of cyclodextrin-based nanosponges of griseofulvin as pediatric oral liquid dosage form for enhancing bioavailability and masking bitter taste. Saudi Pharm J. 2020 Mar 1;28(3):349-61. doi: 10.1016/j.jsps.2020.01.016, PMID 32194337.
24. Higuchi T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci. 1963 Dec;52:1145-9. doi: 10.1002/jps.2600521210, PMID 14088963.
25. El- Leithy ES, Makky AM, Khattab AM, Hussein DG. Nanoemulsion gel of nutraceutical co-enzyme Q10 as an alternative to conventional topical delivery system to enhance skin permeability and anti-wrinkle efficiency. Int J Pharm Pharm Sci. 2017 Nov 1;9(10):207. doi: 10.22159/ijpps.2017v9i11.21751.
26. Ansari KA, Vavia PR, Trotta F, Cavalli R. Cyclodextrin-based nanosponges for delivery of resveratrol: *in vitro* characterisation, stability, cytotoxicity and permeation study. AAPS PharmSciTech. 2011 Mar;12(1):279-86. doi: 10.1208/s12249-011-9584-3, PMID 21240574.
27. Shabbir M, Sajid A, Hamid I, Sharif A, Akhtar MF, Raza M, Ahmed S, Peerzada S, Amin MU. Influence of different formulation variables on the performance of transdermal drug delivery system containing tizanidine hydrochloride: *in vitro* and ex vivo evaluations. Braz J Pharm Sci. 2018;54(4). doi: 10.1590/s2175-97902018000400130.
28. Pawar AP, Gholap AP, Kuchekar AB, Bothiraja C, Mali AJ. Formulation and evaluation of optimized oxybenzone microsponge gel for topical delivery. J Drug Deliv. 2015 Feb 18;2015:261068. doi: 10.1155/2015/261068, PMID 25789176.
29. Hussain A, Latif S, Abbas N, Irfan M, Arshad MS, Bukhari NI. Hydroxypropyl cellulose-based orally disintegrating films of promethazine HCl for the treatment of motion sickness. Trop J Pharm Res. 2018 Jun 1;17(6):991-6. doi: 10.4314/tjpr.v17i6.2.
30. Ahmed RZ, Patil G, Zaheer Z. Nanosponges- a completely new nano-horizon: pharmaceutical applications and recent advances. Drug Dev Ind Pharm. 2013 Sep;39(9):1263-72. doi: 10.3109/03639045.2012.694610, PMID 22681585.
31. El-Assal MI. Nano-sponge novel drug delivery system as carrier of anti-hypertensive drug. Int J Pharm Pharm Sci. 2019 Oct 1:47-63. doi: 10.22159/ijpps.2019v11i10.34812.
32. Shoaib QUA, Abbas N, Irfan M, Hussain A, Arshad MS, Hussain SZ, Latif S, Bukhari NI. Development and evaluation of scaffold-based nanosponge formulation for controlled drug delivery of naproxen and ibuprofen. Trop J Pharm Res. 2018 Aug 1;17(8):1465-74. doi: 10.4314/tjpr.v17i8.2.
33. Moin A, Deb TK, Osmani RA, Bhosale RR, Hani U. Fabrication, characterization, and evaluation of microsponge delivery system for facilitated fungal therapy. J Basic Clin Pharm. 2016 Mar;7(2):39-48. doi: 10.4103/0976-0105.177705, PMID 27057125.
34. Almutairy BK, Alshetaili A, Alali AS, Ahmed MM, Anwer MK, Aboudzadeh MA. Design of olmesartan medoxomil-loaded nanosponges for hypertension and lung cancer treatments. Polymers (Basel). 2021 Jul 11;13(14):2272. doi: 10.3390/polym13142272, PMID 34301030.
35. El-Housiny S, Shams Eldeen MA, El-Attar YA, Salem HA, Attia D, Bendas ER, El-Nabarawi MA. Fluconazole-loaded solid lipid nanoparticles topical gel for treatment of pityriasis versicolor: formulation and clinical study. Drug Deliv. 2018 Nov;25(1):78-90. doi: 10.1080/10717544.2017.1413444, PMID 29239242.
36. Pandya KD, Shah NV, Gohil DY, Seth AK, Aundhia CJ, Patel SS. Development of risedronate sodium-loaded nanosponges by experimental design: optimization and *in vitro* characterization. Pharmaceutical Sciences. 2019;81(2):309-16. doi: 10.36468/pharmaceutical-sciences.512.
37. Ramteke KH, Dighe PA, Kharat AR, Patil SV. Mathematical models of drug dissolution: a review. Sch Acad J Pharmacol. 2014;3(5):388-96.
38. Ghasemiyeh P, Mohammadi Samani S. Potential of nanoparticles as permeation enhancers and targeted delivery options for skin: advantages and disadvantages. Drug Des Dev Ther. 2020 Aug 12;14:3271-89. doi: 10.2147/DDDT.S264648, PMID 32848366.
39. Simranjot Kaur, Sandeep Kumar. The nanosponges: an innovative drug delivery system. Asian J Pharm Clin Res. 2019 May 30:60-7. doi: 10.22159/ajpcr.2019.v12i7.33879.