ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 5, 2023

Online - 2455-3891
Print - 0974-2441

Research Article

NANOSPONGE HEALING APPROACH FOR TREATMENT OF STREPTOCOCCAL GANGRENE

TANUSHREE C[1]*, RAJESH VERMA[2], JAYA SHARMA[2], PANKAJ SHARMA[2]

[1]Department of Pharmaceutics, Al-Ameen College of Pharmacy, Bengaluru, Karnataka, India. [2]Department of Pharmaceutics, Apex University, Jaipur, Rajasthan, India. Email: tanu177@rediffmail.com

Received: 10 November 2022, Revised and Accepted: 12 January 2023

ABSTRACT

Objective: The envisaged research work aims to develop a nanosponge loaded gel for the treatment of Streptococcal gangrene.

Methods: In the present study, permeability is enhanced by targeted drug release formulation of topical clindamycin phosphate nanosponges were prepared by emulsion solvent diffusion method using ethyl cellulose as release retardant polymer and PVA as surfactant or emulsifier. Nanosponges were prepared by emulsion solvent diffusion method by changing drug polymer ratio (1:0.05, 1:0.1, and 1:0.15) and process parameters were optimized using 3^2 full factorial central design. CP nanosponges were then incorporated into a hydrogel prepared using Carbopol 934.

Results and Discussion: The drug loaded nanosponges were evaluated for physical appearance, drug content, entrapment efficiency, and particle size. Characterization of CP nanosponges was done by SEM for the formulation. *In vitro* release study indicated that the release of CP varied according to the concentration of matrix forming polymer. The best standardized formulation G5 and G6 were further evaluated for microbiological studies. Microbial studies were done using *Staphylococcus aureus* as the strain organism and the activity of the gel against the organism was evaluated by measuring the zone of inhibition. It was also found to be stable for 2 months during its stability studies.

Conclusion: Thus, it was concluded that CP can be formulated as nanosponge hydrogel that can release the drug up to 24 h with increased permeability and targeted release. Therefore, clindamycin phosphate nanosponges prepared are promising drug delivery for topical application as being more useful than conventional formulation therapy.

Keywords: Clindamycin phosphate, Streptococcal gangrene, Nanosponges, Hydrogel.

© 2023 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/) DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i5.46787. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Streptococcal Gangrene (SG), generally known as flesh-eating disease, is an infection that results in the death of the body's soft tissue. Its a rare bacterial infection of the soft tissue that is part of the connective tissue system that runs throughout the body. It is an unembellished disease of unexpected onset that spreads rapidly. The most commonly infected areas are the limbs and perineum. Classically, the infection enters the body through a disruption in the skin such as a cut or burn. Risk factors include poor immune function such as diabetes, obesity, cancer, alcoholism, intravenous drug use, and peripheral vascular disease. SG is triggered by a bacterium (monomicrobial NF) or numerous bacteria (polymicrobial NF) infecting the tissue just beneath the skin (subcutaneous tissue). On infection, the bacteria or bacterium spreads through the fascia, producing endotoxins (toxins released as the bacteria die and break apart or are lysed) and exotoxins (toxins released by bacteria as waste) that confines blood supply to tissue (tissue ischemia), digestion of cells by enzymes ensuing in a lesion containing of pus and the fluid remains of dead tissue. Because the blood supply to these tissues becomes diminished, neither antibiotics nor the body's self-mechanisms to fight infection are able to reach them. Normally, the treatment requires surgical debridement (surgical removal of dead and infected tissue). *Staphylococcus aureus* bacteria are a common cause of NF. Methicillin-resistant *S. Aureus* (MRSA) is a strain of these bacteria that is a major source of SG. It does not typically spread between people [1,2]. Depending on the infecting organism the disease are further classified into four types of infection, that is, Type 1 infection, Type 2 infection, Type 3 infection, and Type 4 infection [3,4]. The list of causative organism for (SG) along with its types is provided in Table 1.

Drugs of choice used in treating SG are Clindamycin (26.8%), Vancomycin (25.5%), Meropenem (6.1%), Cilastatin (6.1%), Piperacillin (5.6%), Daptomycin (4.8%), Metronidazole (2.6%), Ampicillin (2.2%), and Others (16.9%). Clindamycin is an antibiotic recommended for the treatment of a numerous bacterial infections. It can be used against some cases of MRSA. Clindamycin is used basically for the treatment of anaerobic infections caused by vulnerable anaerobic bacteria, which includes dental infections, and infections of the respiratory tract, skin, and soft tissue. The oral formulation used for the treatment of SG is not as operative and hence leads to gangrene. Hence, the envisaged research aims to develop novel drug delivery system for treating SG. Nanosponge is a nascent and developing technology which offers controlled drug delivery for topical use. Nanosponges are minute sponges with a dimension of a virus with an average diameter below 1 μm. These tiny sponges have the capability to circulate around the body until they encounter the specific target site and stick on the surface and began to release the drug in a controlled and foreseeable manner [5,6]. They provide prolonged release as well as improving drugs bioavailability. It is a tiny mesh-like structure in which a large variety of substances can be encapsulated. Nanosponges release the drug to specific targeted site instead of circulating throughout the body thereby making it more effective for treating SG. As clindamycin is the first choice of drug for the treatment of SG and it has low topical bioavailability of 4–5%; hence, the bioavailability can be increased by giving nanosponges which will have direct targeting for cell eating bacteria and also give sustained release of the drug over a period of time.

METHODS

Clindamycin Phosphate was a gift sample from Mylan laboratory Ltd. Ethyl cellulose(SD Fine Chemicals Ltd., Mumbai), Dichloromethane, Triethanolamine, Di-sodium hydrogen orthophosphate, Glycerine Propylene glycol, and Potassium dihydrogen orthophosphate were procured from SD Fine Chemicals Ltd., Carbopol, Methanol from

HiMedia Laboratory Pvt. Ltd., Mumbai, Sodium hydroxide pellets from Qualigens fine chemicals, Carbopol (HiMedia Laboratory Pvt. Ltd., Mumbai), Sodium hydroxide pellets (Qualigens fine chemicals, Mumbai), Di-sodium hydrogenphosphate (SD Fine Chemicals Ltd., Mumbai), Glycerine (SD Fine Chemicals Ltd., Mumbai), Propylene glycol (SD Fine Chemicals Ltd., Mumbai), and Dialysis Membrane(Hi-Media Ltd., India).

Preformulation studies

UV-visible spectrophotometric method

A Shimadzu UV-1700 double beam UV-Visible spectrophotometer was used for all measurements. The absorption spectra were recorded over the wavelength range of nm 400–200 nm, against a solvent blank, in quartz cuvettes with a width of 1cm. The linearity of the calibration curves and the obedience of the method to Beer's law are authenticated by the high value of the correlation coefficient. The absorption maxima (λ_{max}) for pure clindamycin phosphate were found to be 202 nm which is within the specified limit.

Formulation studies-preparation of drug loaded nanosponges

Nanosponges were prepared by emulsion solvent diffusion method using different proportion of ethyl cellulose and polyvinyl alcohol. The dispersed phase containing ethyl cellulose and drug was dissolved in 20 mL dichloromethane and slowly added to polyvinyl alcohol in 100 mL of aqueous continuous phase. The reaction mixture was homogenized using Ultra Turrax followed by sonication using Probe Sonicator for 20 min. The nanosponges formed were then lyophilized and stored in a well closed container [7,8]. To obtain the most satisfactory nanosponge formulation, different formulation parameters such as concentration of retardant material, that is, ethyl cellulose was varied from 0.05, 0.27 to 0.15%, surfactant, that is, polyvinyl alcohol from 0.5, 1.5 to 2.5, volume of external phase from 50, 100, to 150 mL, and internal phase from 10, 20, to 30 were standardized keeping all other parameters constant one at a time in trial and error basis. The effect of process parameters such as stirring speed and time of sonication was also studied. The time for homogenization and sonication was kept constant throughout the study, that is, 30 min and 20 min, respectively. The detailed study of formulation is stated in Table 2.

Optimization using Minitab software (Tables 4,5 and 7)

The optimization of the surfactant concentration and the drug polymer ratio was carried out by Minitab using Factorial Design. The study was carried out incorporating two independent variables surfactant proportion (X_1) and the drug: polymer concentration (X_2) whereas the dependent variables included particle size (Y_1), entrapment efficiency (Y_2), and Poly disperse index (Y_3). Three different concentrations of the surfactant, that is, 0.5%, 1.5%, and 2.5% were selected for the optimization studies. The combination of the drug: polymer ratio was employed in three different proportions 1:0.05, 1:0.1, and 1:0.15. The experiment was carried out at three different levels −1, 0, and +1 wherein −1, 0, and +1 depicts low, medium, and high concentrations, respectively. A set of 13 formulations was generated which included 9 test and 4 control formulations as shown in Table 4.

Physicochemical characterization of clindamycin phosphate loaded nanosponges

Determination of drug content

Nanosponge equivalent to 10 mg of clindamycin phosphate was dissolved and made up to the mark in 50 mL volumetric flask with methanol, further 10 mL was diluted to 100 mL with methanol and the final dilution were made using methanol to get a concentration within Beer's range [9,10]. The absorbance was measured spectrophotometrically at 202 nm using blank nanosponges treated in the same manner as sample. The results obtained were in triplicate.

Drug loading efficiency

The free drug content was determined by washing the nanosponge with methanol and the filtrate was collected and analyzed spectrophotometrically at 202 nm [11,12].

The loading efficiency (%) was calculated in accordance to the following equation.

$$Efficiency\ of\ Loading = \frac{\begin{array}{c}Actual\ Clindamycin\ phosphate\\ content\ in\ nanosponges\end{array}}{\begin{array}{c}Theoretical\ Clindamycin\\ phosphate\ content\end{array}} \times 100$$

Table 1: List of causative organism and types of SG

Types of SG	Etiology	Organisms	Clinical progress	Mortality
Type I (70–80% cases)	Polymicrobial, synergistic, often bowel flora-derived	Mixed anaerobes and aerobes	More indolent, better prognosis, easier to recognize clinically	Variable; depends on underlying co-morbidities
Type II (20–30% cases)	Often monomicrobial, skin- or throat-derived	Usually group A β-hemolytic streptococcus (GAS), occasionally *S. Aureus*	Aggressive, protean presentations, easily missed	>32%, depends if associated with myositis or toxic shock
Type III	Gram-negative, often marine-related organisms	*Vibrio* spp. mainly	Seafood ingestion or water contamination wounds	30–40%
Type IV (fungal)	Usually trauma associated, immunocompetent patients	*Candida* spp. immunocompromised patients. Zygomycetes immunocompetent patients	Aggressive with rapid extension especially if immunocompromised	>47% (higher if immunocompromised)

Table 2: Standardization of formulation parameters

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Clindamycin phosphate (g)	10	10	10	10	10	10	10	101	10	10	1010	10
EC (%)	0.05	0.1	0.15	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Internal phase (mL)	20	20	20	20	20	20	20	20	20	10	20	30
External phase (mL)	100	100	100	100	100	100	50	100	150	100	100	100
PVA (%)	2.5	2.5	2.5	0.5	1.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

Table 3: Effect of formulation parameters on particle size, entrapment efficiency

S. No	Formulation code	Formation of nanosponges	Particle size (nm)	Entrapment efficiency (%)
1	F1	+	72.04	84
2	F2	+	108.4	79
3	F3	+	135.5	73
4	F4	+	384.8	78
5	F5	+	168.3	81
6	F6	+	90.18	83
7	F7	+	296.8	77.87
8	F8	+	216.8	79.02
9	F9	+	385.6	65.4
10	F10	+	249.5	78.4
11	F11	+	186.4	80.56
12	F12	+	378.4	68.98

+sign indicates formation of nanosponges

Table 4: Factorial design batches for the nanosponge formulations

Variables	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28
X1	−1	−1	0	1	0	1	−1	1	−1	0	1	1	−1
X2	0	−1	0	1	−1	0	−1	−1	1	1	1	−1	1

Percentage yield

The percentage yield of the nanosponge was calculated accurately by taking the initial weight of the raw materials and the final weight of the nanosponge obtained [11,12].

$$Percentage\ yield = \frac{Practical\ weight\ of\ nanosponges}{Theoretical\ weight} \times 100$$

Particle size

The mean particle size of nanosponge was measured by Malvern Zeta sizer (Malvern Instrument Ltd). The dispersions were diluted with Millipore-filtered water to an appropriate scattering intensity at 25°C and sample was placed in a disposable sizing cuvette and the particle size was analyzed.

Scanning electron microscopy (SEM)

SEM is an electron optical imaging procedure that provides exact photographic images and essential information. SEM is useful for illustrating the morphology and size of microscopic samples with particle size as low as 10–12 g. The sample was located in an evacuated chamber and scanned in a controlled manner by an electron beam. Interaction of the electron beam with the sample produces a diversity of physical phenomena that, when detected, are used to form images and provide elemental data about the specimens. SEM (JSM 840 A) was used to study the surface morphology of the nanosponges of optimized formulation [11,12]. The samples were examined after they were gold sputtered by means of 25 nm gold film thickness.

In vitro drug release and its kinetics

Diffusion studies were carried out using Franz diffusion cells with 38 ml of receptor cell volume and 5 mL donor compartment. The receptor compartments were filled with phosphate buffered pH 7.4 which simulated the physiological pH. Study was carried out using treated cellophane membrane. The barrier was equilibrated for 2 h, and then air bubbles were removed. The entire setup was placed on a thermostatic magnetic stirrer and the temperature was maintained at 37°±0.5°C throughout the study. Donor compartments were filled with 5 ml of 0.4 g of NS equivalent to 0.1 g of clindamycin phosphate

Table 5: Coded values and actual values for independent variables

Coded values	Actual values	
	X1 (%)	X2
-1	0.5	1:0.05
0	1.5	1:0.1
1	2.5	1:0.15

in phosphate buffer pH 6.8 solutions and covered with aluminum foil to prevent evaporation of vehicle. At regular time intervals; 0.5, 1, 2…24 h, samples were withdrawn followed by replacement with fresh receptor solution. The drug content in the sample was quantified by UV-Spectrophotometric method. To analyze the drug release mechanism, release kinetics was also investigated the outcomes of *in vitro* drug loaded nanosponges were computed using various kinetic models viz. The zero-order rate Eq. C=kot, where C is the concentration of the drug at time (t) and ko is the zero-order release rate constant. The first order Equation Log C=logC0-kt/2.303 is used to evaluate the mechanism of drug release, facts for the first 60% of drug release were plotted into the Korsmeyer peppas equation $Mt/M=K_t^n$, Where (Mt/M) is the fractional solute release, (t) is the release time, (K) is a kinetic constant, which is characteristic of the drug/polymer system, and (n) is an exponent that personifies the mechanism of release of tracers. Model fitting was accomplished using the PCP DISSO software [13,14].

Preparation of nanosponges based hydrogel

To obtain a suitable topical formulation for application, nanosponges (F22) were incorporated into a gel base. After the preliminary tests for the selection of suitable polymer for gel, Carbopol was found to be ideal. Carbopol was soaked overnight with minimum quantity of water and was allowed to swell up. The Carbopol dispersion was stirred using continuous mechanical stirring for 15 min. The pH of the resulting dispersion was adjusted with Triethanolamine to form a translucent gel. 0.3 mL IPM and glycerol-water mixture with the drug loaded nanosponges was added to the gel under stirring to prepare 30 g of gel. Clindamycin phosphate nanosponges equivalent to 2.5% w/w of drug were dispersed into the gel base. Permeation enhancer was also added [15,16]. With a view to incorporate clindamycin phosphate nanosponges suitable for topical application, different formulation using Carbopol ranging from 0.5 to 1.5 % were coded as G1-G3, G4-G6 were prepared containing different permeation enhancer (propylene Glycol) to investigate the effect on the permeation characteristics of clindamycin phosphate nanosponges. Formulations G1 to G6 were evaluated for their physical appearance and consistency the results are reported in Table 6.

Evaluation of nanosponges loaded gel formulations

Homogeneity

The prepared hydrogels were visually inspected for clarity, colour and transparency. The prepared nanosponges loaded CP gels were also assessed for the presence of any particles. Smears of gels were prepared on glass slide and detected under the microscope for the presence of any particulate matter or grittiness [17,18].

pH of the gels

The pH range for a perfect gel for topical use is 4.5–7. If the pH of the gel goes beyond 7.2, it reaches to alkaline state and the gels irritate the skin. One gram of each blank formulation was dispersed in 30 ml of distilled water and the pH was measured using a Micropro Gradmate digital pH meter. Average of three determinations was recorded [17,18].

Spreadability

Spreadability experiment was performed with the help of glass slides and a wooden block, which was provided by a pulley at one end. By this

Table 6: Formulation of nanosponges loaded hydrogel

Formulation code	Carbopol 934 concentration (%)	Triethanolamine (w/v) (%)	Clindamycin phosphate nanosponges (w/w) (%)	Propylene glycol
G1	0.5	0.5	1	--------
G2	1.0	0.5	1	--------
G3	1.5	0.5	1	---------
G4	1	0.5	1	5
G5	1	0.5	1	10
G6	1	0.5	1	15

Table 7: 3^2 Full factorial design runs with actual values of particle size, entrapment efficiency, P.D.I:

Formulation code	Run	Factor 1 PVA (%)	Factor 2 Clindamycin Phosphate: EC ratio	Response 1 Particle size (nm)	Response 2 P.D.I	Response 3 Entraptment efficiency (%)
F16	1	0.5	1:0.05	384.80	0.129	78.98
F17	2	0.5	1:0.1	496.20	0.487	65.11
F18	3	0.5	1:0.15	532.80	0.689	54.23
F19	4	1.5	1:0.05	168.30	0.586	81.34
F20	5	1.5	1:0.1	232.40	0.346	72.23
F21	6	1.5	1:0.15	286.80	0.984	69.35
F22	7	2.5	1:0.05	72.04	0.137	84.13
F23	8	2.5	1:0.1	108.40	0.786	79.26
F24	9	2.5	1:0.15	135.50	0.982	73.17
F25	10	2.5	1:0.05	90.18	0.123	83.14
F26	11	2.5	1:0.1	115.6	0.324	74.37
F27	12	2.5	1:0.15	167.9	0.276	78.23
F28	13	2.5	1:0.05	105.34	0.195	80.11

method, spreadability was estimated on the basis of "Slip" and "Drag" characteristics of gels. A bottom glass slide was fixed on this block. An excess of gel (about 1 g) of different formulations was placed on the ground slide. The gel was then squeezed in between this slide and another glass slide having the dimension of fixed ground slide. Surplus of the gel was removed off from the edges. The top plate was then subjected to pull of 20 g, lesser the time taken for parting of two slides better was the spreadability [17,18].

Spreadability was then calculated using the following formula:

S = M × L/T

Where, S = is the Spreadability,
M = is the weight in the pan (tied to the upper slide),
L = is the length moved by the glass slide,
T = represents the time taken to separate the slide completely from each other.

Extrudability study

A locked collapsible tube comprising above 20 g of the gel was hard-pressed at the crimped end and a clamp was applied to avert any rollback. The cap was removed and the gel was extruded till the pressure was dissipated.

Viscosity

The viscosity of gels is dependent on type and concentration of polymer used. The ideal viscosity of gels varies from 2000 to 6000 cps. It is an important parameter in evaluation of physical parameters of gel. As the viscosity reduces, spreadability and extrudability also reduces. It also affects the stability of gels. The viscosity of different mucoadhesive gel formulations was determined using a Model DV- III + Programmable Rheometer Brook field viscometer using spindle #7 at 100 rpm with torque ranging from 10 to 100%. Average of three determinations was recorded.

Percentage yield of the gel

Weight of all the ingredients used was added up theoretically. Total prepared gel was weighed which was the practical yield. The percentage yield was calculated according to the formulae [19].

% yield= Practical yield/Theoretical yield *100

Drug content

One gram of clindamycin phosphate gel was accurately weighed dissolved using methanol, sonicated for a period of 10–15 min and made up to the mark in 10 mL volumetric flask with methanol. From this, 1 mL was pipette out and diluted to 10 mL with methanol. From this, 0.5 mL was pipette and finally diluted to 10 mL to get a concentration within Beer's range. The absorbance was measured spectrophotometrically at 202 nm against blank gel treated in the same manner as sample. Average of three readings was recorded [20].

In vitro release studies (Tables 11 and 12)

Diffusion studies were carried out using Franz diffusion cells with 38 mL of receptor cell volume and 5 ml donor compartment. The receptor compartments were filled with phosphate buffered pH 7.4 which simulated the physiological pH. Study was carried out using treated cellophane membrane. The entire setup was placed on a thermostatic magnetic stirrer and the temperature was maintained at 37°±0.5°C throughout the study. Donor compartments were filled with 5 mL of 0.4 g of gel equivalent to 01 g of clindamycin phosphate in phosphate buffer pH 6.8 solutions and covered with aluminum foil to prevent evaporation of vehicle. At regular time intervals; 0.5, 1, 2…24 h, samples were withdrawn followed by replacement with fresh receptor solution. The drug content in the sample was quantified by UV-spectrophotometric method as mentioned above [21].

Microbiological studies

The standardized final formulation of nanosponge of Clindamycin Phosphate by emulsion solvent diffusion method, that is, F22 and also the standardized gel formulation, that is, G5 and G6 were selected for

the microbial studies. The primary objective was to compare anti-bacterial activity of the developed formulations with that of the saline water solution. Mueller Hinton agar was the nutrient medium used for the study. After complete sterilization, the medium was kept aside at room temperature. 0.05 ml diluted suspension culture of *S. Aureus* in NaCl 0.9% was added to 100 ml of medium at 47±2°C and used as inoculated layer. The medium (30 mL) was poured into a sterilized Petri dish to give a depth of 3–4 mm, and was assured that the layer of medium is uniform in thickness by placing Petri dish on a leveled surface. After hardening the medium by solidification at room temperature, with the help of a sterile cork borer, cups of each 6 mm diameter were pierced and scooped out from the Petri dish. Using sterile pipettes sample solutions (0.5 g, 1 g) of different formulation equivalent to 10 mcg of the drug were fed into the cup. The Petri dish was then incubated for 48 h at 37°C. After incubation, the zone of inhibition was measured [22].

RESULTS AND DISCUSSION

Preformulation studies

UV-visible spectrophotometric method

λmax of clindamycin phosphate was determined in pH 6.75 and pH 7.4 in UV spectrophotometer (Shimadzu UV-1700 double beam spectrophotometer) by scanning in a wavelength range of 400–200 nm. λmax of clindamycin phosphate was found to be 202 nm. A simple reproducible method of estimation was standardized against the blank. The standard graph obtained was linear, with regression coefficient of 0.998 and 0.997 which is mentioned in Fig. 1.

Formulation of nanosponges by emulsion solvent diffusion method

In emulsion solvent diffusion method, the formation of the nanosponge is by the rapid diffusion of dichloromethane into the aqueous medium. The instant mixing of the dichloromethane and water (aqueous medium to the polymer medium) induced precipitation of the polymer at the interface of the droplets, thus forming a shell enclosing the dichloromethane and the dissolved drug. The finely dispersed droplets of the drug-polymer solution were solidified in the aqueous phase via diffusion of the solvent. Effect of different formulation parameters was tested through trial and error method to get optimized formulation. The polymer used in the formulation of emulsion solvent diffusion method was ethyl cellulose which acted as a retardant material in the internal phase, Polyvinyl alcohol was the surfactant, dichloromethane solvent and water as a vehicle the formed. By varying the concentration of the retardant material from 0.05% to 0.15%, the particle size also varied from 72.04 nm to 135.5 nm. The minimum concentration of surfactant required to bring about the formation of uniform nanosponges was found to be 0.05% v/v of internal phase which resulted in the particle size of 72.04 nm. By varying the concentration of surfactant from 0.5% to 2.5% v/v, the particle size also varied from 532.8 nm to 72.04 nm. Nanosponge did not form in the absence of surfactant. The results of this envisaged research work showed that the particle size ranged from 216 to 385 nm while the entrapped drug concentration ranged from 65.4% to 79%, when the volume of external phase varied from 50 mL to 150 mL. It was found that 100 mL was the optimum external phase volume required which resulted in the particle size range of 216.8 nm with % entrapment of 79.02 and free drug concentration of 3.17%. The optimum external phase volume of 20 mL was found to be of optimal range for formation of nanosponges. It was observed that stirring at 15,000 rpm speed using ultra TURRAX homogenizer (Fig. 2) resulted in better nanosponges with uniform particle size. This reduced particle size with increase in stirring speed may be due break down of larger emulsion droplets into small particles. The formed nanosponges were evaluated for their physical characteristics, particle size, and free drug content.

Preparation of drug loaded nanosponges

Optimization by 3^2 full factorial design

To optimize drug: polymer ratio and concentration of surfactant, factorial design was adopted. To learn all the probable combinations of both factors at all levels, a two factor, three level full factorial designs were constructed and conducted in a fully randomized manner.

Minitab software was used to apply full factorial design to study response surface of 3 level factorial design with 13 runs in a quadratic model. The formulations were made-up according to a 3^2 full factorial design, letting the concurrent evaluation of two formulation variables and their interaction. Based on the preliminary studies, the two independent variables included were surfactant concentration and drug: polymer ratio. The two variables were compared over 3 levels, +1(High), 0(Medium) and −1(Low). The effect of the two factors on response of dependent variable, that is, Y_1 (Particle Size), Y_2 (Entrapment Efficiency), and Y_3 (P.D.I) was studied by polynomial equation.

Physicochemical characterization of clindamycin phosphate loaded nanosponges

Determination of drug content

The different batches of the drug loaded nanosponges were exposed for drug content analysis. The powdered nanosponges (10 mg equivalent) were dissolved in adequate quantity (100 mL) of phosphate buffer PH 6.8 then filter. The UV absorbance of the filtrate was measured using a UV spectrophotometer at 202 nm. The drug content of different formulation was found to be in the range of 72.46±1.56 to 88.76±0.58 as shown in below Table 8.

Drug loading efficiency

The loading efficiency (% entrapment) of clindamycin phosphate nanosponge formulations are given in Table 8. The loading efficiency calculated for all nanosponges ranged from 54.23 to 84.13%. The

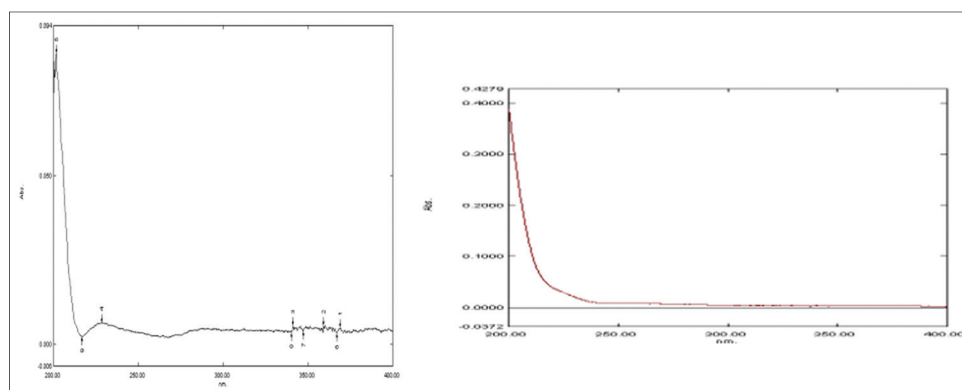

Fig. 1: UV spectrum of clindamycin phosphate of clindamycin phosphate in Phosphate buffer pH 6.75 and 7.4 atλmax was found to be 202 nm

Table 8: Physicochemical properties of prepared nanosponges

S. No	Formulation code	Particle size	P.D.I	% Yield	Drug content	Free drug	Entraptment efficiency
1	F16	384.80	0.129	88.90±0.55	84.89±1.28	8.9	78.98
2	F17	496.20	0.487	88.43±1.11	72.46±1.56	14.9	65.11
3	F18	532.80	0.689	85.81±0.67	72.39±2.64	13.09	54.23
4	F19	168.30	0.586	91.82±1.77	85.48±1.99	9.65	81.34
5	F20	232.40	0.346	89.11±1.90	79.38±2.18	17.9	72.23
6	F21	286.80	0.984	87.38±0.50	75.46±1.67	18.0	69.35
7	F22	72.04	0.137	98.76±2.11	88.76±0.58	7.09	84.13
8	F23	108.40	0.786	96.31±0.32	83.29±1.28	9.19	79.26
9	F24	135.50	0.982	94.30±0.55	79.86±2.42	18.9	73.17
10	F25	90.18	0.123	96.89±2.16	85.17±1.04	8.78	83.14
11	F26	115.6	0.324	95.99±0.77	79.02±0.04	8.99	74.37
12	F27	167.9	0.276	91.76±1.34	77.45±1.78	9.73	78.23
13	F28	105.34	0.195	96.54±1.11	82.23±0.04	8.02	80.11

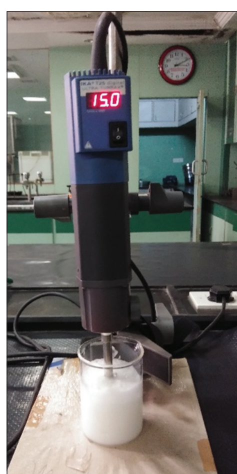

Fig. 2: Preparation of formulation of nanosponges which has been placed in ultra TURRAX homogenizer

highest loading efficiency was found for the F22 formulation to be 84%, where a greater amount of drug was encapsulated. The highest loading efficiency, greater the amount of drug was entrapment.

Percentage yield

The percentage yield of clindamycin phosphate nanosponge formulation is given in Table 8. Production yield calculated for all nanosponges ranged from 88.43% to 98.76%. The production yield was found the highest for formulation F22, that is, 98.76%, respectively. From the production yields of clindamycin phosphate nanosponge formulation, it was indicated that increasing the drug: polymer ratio increased the production yield.

Particle size and PDI

Particle size analyses of all the formulation were carried out using Malvern particle size analyzer. All the formulated NSs were in nano-size range with narrow particle size distribution as per PDI. Out of all the factorial formulations (F16-F28), F22 showed the least particle size of 72.04 nm (Fig. 3), with P.D.I of 0.137 and showed highest entrapment efficiency. The average particle size of nanosponges can be highly influenced by drug: Polymer ratio. The low concentration of polymer selected improves the diffusion of dichloromethane (internal phase) into aqueous phase (external phase) hence giving less time for the droplet formation and so it decreases the particle size. The F22 formulation is the same as F1 formulation which was done in the Preformulation trials.

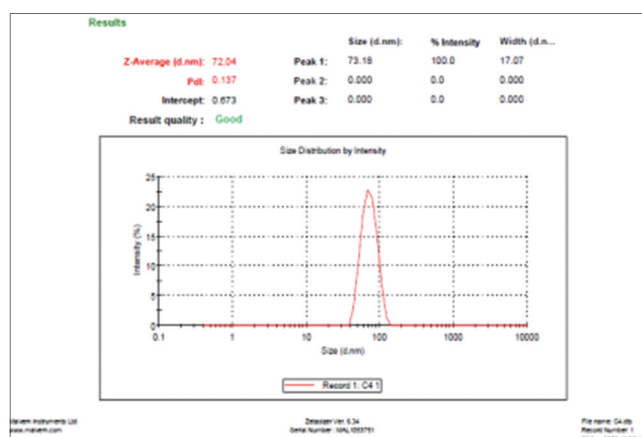

Fig. 3: Particle size and PDI of F22 formulation

SEM

The morphology of the optimized nanosponge prepared by emulsion solvent diffusion method F22 was investigated by SEM. The representative SEM photographs of the nanosponges are shown in Fig. 4. It represents the spherical shape of nanosponges with nanosize range. It was projected that the in-ward diffusion of DCM on the EC polymeric surface contributed to the porus spongy nature of the nanosponges. Furthermore, the micrographs (Fig. 5) revealed that the EC matrix was properly coated over the cp (clindamycin phosphate) and spherical also glazed by PVA responsible for anti-adhesiveness between the particles and smooth surface of nanosponges.

Diffusion studies were carried out using Franz diffusion cells with 38 mL of receptor cell volume and 5 mL donor compartment. The receptor compartments were filled with phosphate buffered pH 7.4 which simulated the physiological pH. The % release of the two optimized formulations (F22 and F25) was studied for 24 h (Table 9 and Fig. 5). It was found that formulation containing polymer and retardant material in 1:0.5 ratio (F28) has shown maximum *in vitro* drug release as compared to other formulations. The drug release was found to be highest for F22, that is, 83.5%. This could be attributed to the highest degree of the encapsulation of drugs in the inner structure of the NS. Fig. 6 shows the comparison drug release studies of F22, F23.

In addition, release profiles of all the formulations have been subjected to diverse release kinetics mathematical models; the interpretation of data of the drug showed that maximum linearity (highest R2 value) was found with Higuchi classical model of diffusion; (R2=0.977) as shown in Table 10. This manifests that the drug release mechanism is by diffusion

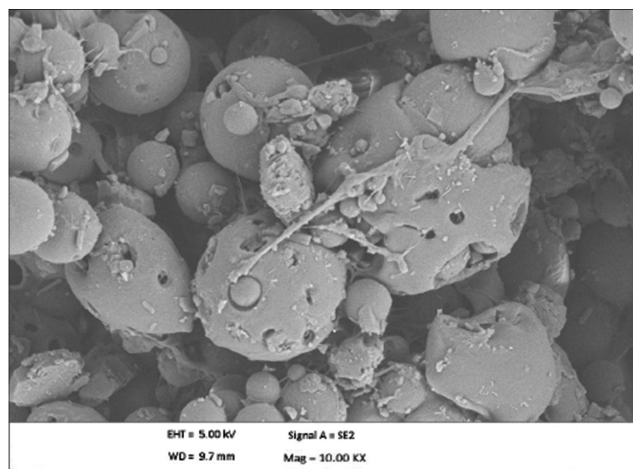

Fig. 4: SEM of optimized formulation

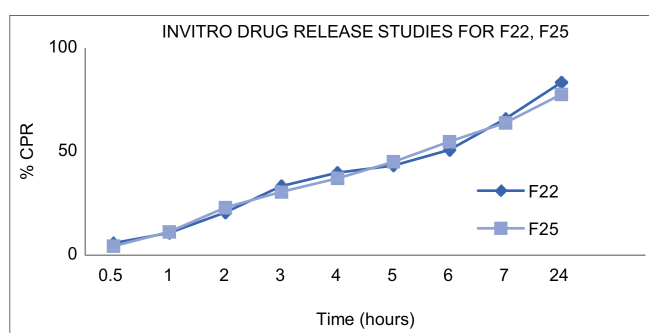

Fig. 5: Comparison drug release studies of F22, F23

process. To determine whether this diffusion process is Fickian or non Fickian, the Korsmeyer–Peppas model was applied to 60% of the release data. The results obtained after the application of Korsmeyer–Peppas model showed exponent ($0.45<n<0.89$) "n" values to be <0.5 which suggests that the diffusion process is obeying Fick's law of diffusion, which revealed that Korsmeyer–Peppas model is the best fit model for F22 with a regression coefficient (r^2) value of 0.997. Moreover, the Korsmeyer–Peppas model suggested all nanosponges represented non swellable matrix diffusion drug release mechanism attributed to porosity in these nanosponges formulations. Based on the above results, F22 formulation was found to have maximum EE and *in vitro* drug release. Henceforth, further characterization studies were performed on this formulation.

Gel loaded nanosponge

Based on the particle size, % entrapment and % release F22 nanosponge formulation was selected for further incorporation into a topical gel. Gels were formulated using Carbopol 934 as the gelling agent. It was found that 1% Carbopol 934 was ideal for the formation of effective and satisfactory gel. Lower concentrations of (0.5%) or higher concentration (1.5%) of Carbopol resulted in a non-consistent gel. As our objective was to achieve greater permeability hence formulation G5 and G6 was finalized which had permeation enhancers incorporated in them. The formed hydrogels were evaluated for physicochemcial properties.

Homogeneity

Homogeneity is the consistency and uniformity of gel. It is required to maintain homogenous nature. The prepared gel formulations G5 and G6 were found to be translucent and homogenous. It did not give any gritty feeling on applying on the skin surface.

Table 9: % Drug Release studies of the nanosponges

S. No	Time	CDR%	
		F22	F25
1	0	0	0
2	0.5	5.87±1.28	4.36±0.12
3	1	10.76±1.21	11.32±0.21
4	2	20.68±0.19	23.05±0.14
5	3	33.41±0.12	30.56±0.16
6	4	39.86±0.09	37.12±0.16
7	5	43.34±0.11	45.13±0.26
8	6	50.86±0.34	54.83±0.24
9	7	65.89±0.12	63.92±0.20
10	24	83.5±0.12	77.7±0.21

pH

In gel formulations pH is maintained to simulate the neutral condition of the skin. The ideal pH range for ideal gel for topical use is 4.5–7. If the pH of the gel goes beyond 7.2, it reaches to alkaline state and the gels starts irritating to the skin. The pH of the prepared gel formulations G5 and G6 was found to be in the range of pH 6.52–6.79.

Viscosity

Based on the viscosity of the polymer, the ideal viscosity of gels varies from 2000 to 6000 cps. If the gel, has low viscosity it affects the stability of gels. As the viscosity decreases gel loses its spreadability and shows poor extrudability. The viscosity of prepared gel formulations G5 and G6 was evaluated using Brooke filed viscometer and their viscosity varied from 4500 cps–5356 cps.

Spreadability

Spreadability is expressed to represent the extent of area to which the gel readily spreads on application to skin or the affected area. The therapeutic efficiency of the formation also depends on its spread ability values. Therefore, determination of spreadability is significant in assessing gel characteristics. The prepared gel formulations G5 and G6 were evaluated for spreadability and the results showed that spreadability varied from 8.28 g.cm/s to 10.56 g.cm/s, respectively.

Extrudability

It is a typical empirical test used to measure the force essential to extrude the material from tube. The prepared gel formulations G5 and G6 were evaluated for extrudability. G6 showed good extrudability behavior.

Drug content

The prepared gel formulations G5 and G6 were evaluated for drug content. The drug content of G5 was found to be 85.79% and G6 to be 91.8%, respectively.

In vitro release studies of gels

The % releases of the standardized nanosponge gel G5 and G6 were studied for 24 h [23-26]. The gels showed sustained release for the period of 24 h. % release for the 1st h was 9.39% and 10.48% and for the 7th h was 64.86% and 70.89% and for 24th h was 84.45% and 88.96% (Table 12 and Fig. 7).

Microbiological studies

Microbiological studies were carried out for the saline solution with the optimized gels loaded with nanosponges. Mueller-Hinton agar was the nutrient medium and *S. Aureus* was the strain organism used for the study. After incubation for about 24 h, zone of inhibition were measured for the standard and gels. The zones of inhibition was seen the highest, that is, 40.4 mm for the gel formulation (G6). The formulated

Table 10: Kinetic data model release for nanosponges

Code	Zero-order		First-order		Korsmeyer–Peppas		Matrix		Best fit model
	R^2	K	R^2	K	R^2	K	R^2	K	-
F22	0.984	2.536	0.959	2.273	0.997	0.435	0.977	3.412	Korsmeyer Peppas

Table 11: Evaluation of standardized nanosponge gels

S. No	1	2
Formulation code	G5	G6
Appearance and homogeneity	Gel like consistency, very good homogeneity, Translucent	Gel like consistency, very good homogeneity, Translucent
pH±SD	6.52	6.79
Viscosity±SD (cps)	4500±0.2449	5356±0.1476
Spreadability (g.cm/s)	8.28	10.56
Extrudability	Good flow	Excellent flow
% Yield±SD	92.63	93.63
Drug content (%)±SD	85.79±0.0147	91.8±0.0121

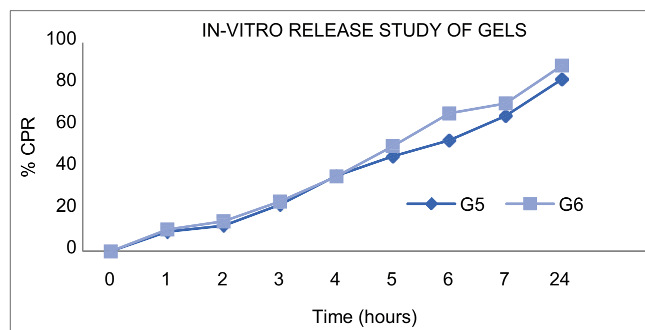

Fig. 7: *In vitro* release study of gels

Table 12: *In vitro* release study of the formulated nanosponge loaded gels

S. No	Time	%CDR	
		G5	G6
1	0	0	0
2	1	9.39±0.27	10.48±0.20
3	2	12.31±0.098	14.38±0.3710
4	3	22.36±0.231	23.92±0.102
5	4	36.1±0.231	35.89±0.278
6	5	45.47±0.156	50.38±0.2298
7	6	53.13±0.121	66.1±0.288
8	7	64.86±0.202	70.89±0.222
9	24	82.45±0.512	88.96±0.212

Fig. 8: Depicting the zone of inhibition for sample clindamycin phosphate which contains 0.5 g, 1 g of gel which has 0.05 mL of inoculums

Table 13: Zone of inhibitions of nanosponges loaded gels of G5 and G6

S. No	Formulation code	Inoculums	Weight of the gel (gm)	Zone of inhibition (nm)
1	G5	0.05	0.5	38.6
2	G6	0.05	1	40.4
3	G5	0.1	0.5	33.9
4	G6	0.1	1	35.1

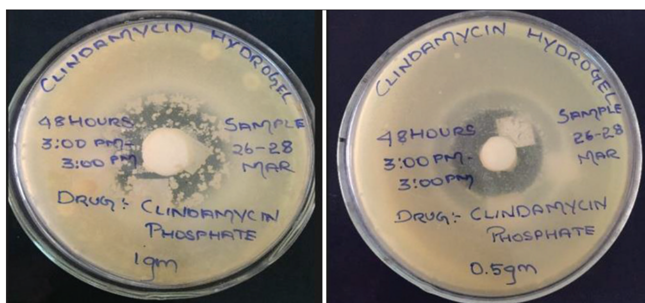

Fig. 9: Depicting the zone of inhibition for sample clindamycin phosphate which contains 0.5, 1 g of gel which has 0.1 mL of inoculums

nanosponge gels G6 showed antibacterial activity which is mentioned in Table 13, Fig. 8 and 9.

CONCLUSION

The present study was an effort to design, develop, and evaluate the clindamycin phosphate loaded nanosponges incorporated in gel to achieve topical drug delivery of the drug for sustained release and increased bioavailability. Clindamycin phosphate is a broad-spectrum antibiotic drug belonging to BCS Class III, exhibiting low permeability and high solubility. The topical bioavailability of the drug is only 4–5%. Hence, the main objective of the envisage research work was to improve the permeability to achieve greater bioavailability. In addition, it was decided to formulate a drug delivery system which would achieve a prolonged release of the drug. These are miniature sponges with a size of a virus. These nanosponges circulate around the body till they meet the specific target site, then stick onto the surface and start to release the drug in a controlled and foreseeable manner. The best standardized formulation obtained by the emulsion solvent diffusion method, that is, F22 showed good loading efficiency of 84.13%, production yield of 98.76%, particle size of 72.04 nm, and drug content was 85.17%.

Due to the above results, the formulations F22 were selected and incorporated into standardized Carbopol based gels (G5 and G6). Evaluation of the formulated gels G5 and G6 showed a production yield of 93.53% and 94.63% and drug content of 85.79% and 91.8%, respectively. The gels G5 and G6 showed an extended release up to 24 h. Microbial studies were carried out using the best formulated gels loaded nanosponge which showed antibacterial activity when

zone of inhibitions was measured. The zone of inhibition was more for the gel loaded nanosponges formulation when compared to that of standard formulation. The smaller zones obtained for the nanosponges may be due to the extended release of the drug from the nanosponge into the medium. Thus it was concluded that the selected antibiotic drug, clindamycin phosphate developed into nanosponge, which was dispersed into Carbopol 934 gel for topical application as a novel approach G6 was the best formulation. The nanosponge based formulation G 6 showed better drug release and good have better penetration of drug through the skin and hence we can speculate that clindamycin phosphate nanosponge loaded hydrogel formulation is a good candidate for topical drug delivery in the treatment of SG.

AUTHOR CONTRIBUTION

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

CONFLICT OF INTEREST

The authors declare that no conflict of interest is associated with this work.

REFERENCES

1. Davoudian P, Flint NJ. Streptococcal gangrene. Contin Educ Anaesth Crit Care Pain 2012;12:245-50. doi: 10.1093/bjaceaccp/mks033
2. Available from: https://www.streptococcal.gangrene.een.wikipedia.org; https://www.en.wikipedia.org/wiki/Necrotizing_fasciitis [Last accessed on 2019 Apr 14].
3. Emedicine. Streptococcal Gangrene: Background, Pathophysiology, Etiology; 2019. Available from: https://www.emedicine.medscape.com/article/2051157-overview [Last accessed on 2019 Apr 14].
4. Clindamycin; 2019. Available from: https://www.en.wikipedia.org/wiki/clindamycin [Last accessed on 2019 Apr 14 2019].
5. Clindamycin-an-Overview; 2019. Available from: https://www.uptodate.com/contents/clindamycin-an-overview [Last accessed on 2019 Apr 14].
6. Manyam N. Formulation and *in-vitro* evaluation of nanosponge loaded extended release tablets of trimethoprim. UPI-J Phama Med Health Sci 2018;1:78-86.
7. Streptococcal Gangrene. NORD National Organization for Rare Disorders. Available from: https://rarediseases.org/rare-diseases/necrotizing-fasciitis [Last accessed on 2019 Apr 14].
8. Hakkarainen TW, Kopari NM, Pham TN, Evans HL. Necrotizing soft tissue infections: Review and current concepts in treatment, systems of care, and outcomes. Curr Probl Surg 2014;51:344-62. doi: 10.1067/j.cpsurg.2014.06.001, PMID 25069713
9. Vishwakarma A, Nikam P, Mogal R, Talele S. Review on nanosponges: A BeneficiationFor novel drug delivery. Int J PharmTech Res 2014;6:11-20.
10. Bachkar AB. Nanosponges: A potential nanocarrier for targeted drug delivery. World J Pharm Res 2014;4:751-62.
11. Mitragotri SD. Designer biomaterials for nanomedicine. Adv Funct Mater 2009;19:3843-54.
12. Hamidi M, Azadi A, Rafiei P. Hydrogel nanoparticles in drug delivery. Adv Drug Deliv Rev 2008;60:1638-49. doi: 10.1016/j.addr.2008.08.002, PMID 18840488
13. Sajja HK, East MP, Mao H, Wang YA, Nie S, Yang L. Development of multifunctional nanoparticles for targeted drug delivery and non-invasive imaging of therapeutic effect. Curr Drug Discov Technol 2009;6:43-51. doi: 10.2174/157016309787581066, PMID 19275541
14. Swaminathan S, Vavia PR, Trotta F, Torne S. Formulation of beta cyclodextrin based nanosponges of itraconazole. J Incl phenom Macro Chem 2007;57:89-94.
15. Kumari P, Mishra SK. A comprehensive review on novel microsponges drug delivery approach. Asian J Pharm Clin Res 2016;9:25-30.
16. Farsana P, Sivakumar R, Haribabu Y. Hydrogel based nanosponges drug delivery for topical applications-a updated review. Res J Pharm Technol 2021;14:527-30. doi: 10.5958/0974-360X.2021.00096.2
17. Maanvizhi S. Design development and evaluation of ibuprofen loaded nanosponges for topical application. Int J Chem Tech Res 2018;11:218-27.
18. Shoaib Q, Abbas N, Irfan M, Hussain A, Arshad MS, Hussain SZ, *et al*. Development and evaluation of scaffold-based nanosponge formulation for controlled drug delivery of naproxen and ibuprofen. Trop J Pharm Res 2018;17:1465-74. doi: 10.4314/tjpr.v17i8.2
19. Abbas N. Formulation and evaluation of fluconazole loaded nanosponges for improved topical drug delivery. B J Pharm 2017;2:41-2.
20. Dubey P, Sharma HK, Shah S, Tyagi CK, Chandekar AR, Jadon RS. Formulations and evaluation of cyclodextrin complexed ceadroxil loaded nanosponges. Int J Drug Deliv 2017;9:84-100. doi: 10.5138/09750215.2180
21. Viswanad V. Nanosponge loaded hydrogel of cephalexin for topical delivery. Int J Pharm Sci Res 2017;3:2781-9.
22. Penjuri SCB, Ravouru N, Damineni S, Bns S, Poreddy SR. Formulation and evaluation of lansoprazole loaded nanosponges. Turk J Pharm Sci 2016;13:304-10. doi: 10.4274/tjps.2016.04
23. Subhash P, Mohite SK. Formulation design and development of artesunate nanosponge. Eur J Pharm Med Res 2016;3:206-11.
24. Kumar P. Design and characterization of miconazole nitrate loaded nanosponges containing vaginal gels. Int J Pharm Anal Res 2016;5:410-7.
25. Badr-Eldin SM, Aldawsari H, Labib G, El-Kamel A. 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;10:893-902. doi: 10.2147/IJN.S74771
26. Srinivas P, Reddy A. Formulation and evaluation of isoniazid loaded nanosponges for topical delivery. Pharm Nanotechnol 2015;3:68-76. doi: 10.2174/2211738503666150501003906