Formulation and Characterization In vitro Release of Topical Nanogel from Natural Source in the Management of Psoriasis.

Shivanshu Gangwar*, Md.Semimul Akhtar
S.R.M.S College of Engineering and Technology,Bareilly,U.P.

Abstract
Combination of curcumin with anti-inflammatory drug like caffeine shows augmented antipsoriatic action com-pared to curcumin alone and reduce the time taken for treatment of Psoriasis. The objective of the present study was to develop nanosponge (NS) based topical gel of curcumin (CUR) and caffeine (CFN) combination that acts as a potential system for the treatment of psoriasis. NS composed of dimethyl carbonate (DMC) as crosslinker and beta-cyclodextrin (β- CD) as polymer were prepared by hot melt method and incorporated in topical gels. evaluation studies for NS and nanogels were conducted. Ex vivo animal studies were carried out for optimized formulation using mouse model of imiquimod- induced psoriasis. Results The physical and chemical characteristics exhibited by the prepared NS and gels (F1-F9) were found to be optimal. The optimization resulted in achieving formulation N10 with 69.72% in vitro drug release and 12,329.78cp viscosity. Histopathology studies revealed that prepared nanogel has promising anti- psoriatic activity. The results concluded that CUR and CFN combi-nation has reduced the time required for showing anti-psoriatic activity to 10 days when compared to CUR alone that took around 20 days. Moreover, the nanogel has depicted sustained drug release till 12 h. From the experimental findings it has been concluded that CUR and CFN combination significantly augmented the anti-psoriatic efficacy with respect to individual components and also reduced the time required for onset of effect. Thus, the proposed nanogel would be an imperative drug delivery system for more effective anti-psoriatic therapy.

Keywords Topical drug delivery . Nanosponges . Psoriasis . Curcumin . Caffeine . Topical gel . Imiquimod

Introduction
Psoriasis is a skin disease which is distinguished by massive proliferation, thick inflammatory cell infiltrates, generation of new blood vessels, modifications in lymphatic structure and impaired differentiation of epidermis. It is an autoimmune disorder in which environmental and genetic components plays a major function [1–3].

Majority of drugs available at the moment like Methotrexate, Cyclosporine, Adalimumab, Etanercept (biologics); topical agents like Corticosteroids, Vitamin D-3 derivatives, retinoids, coal tar etc. are been used for treating psoriasis via both systemic and local therapies. Despite the fact that many therapies exist and even more have been proposed, there in treating psoriasis, no single treatment gives complete and satisfactory cure and most of them have ad-verse effects. In recent times phytopharmaceuticals have gained a lot of attention and interest by the researchers, and they are striving hard to develop something more efficient, safe and reliable for anti- psoriatic therapy. In near future diverse new molecules, viz., phytoconstituents (Curcumin, Capsaicin, Silymarin, Quercetin, Berberine, Beta amyrin etc.) could support the therapies which are now in use. These phytoconstituents have better therapeutic value and fewer side effects. Present work has been carried out using curcumin which is one of the major phytoconstituents hav-ing anti psoriatic activity [4, 5].

Curcumin (CRN) is a natural polyphenolic phytochemical, extracted from rhizome of turmeric (Curcuma longa)having many biological and pharmacological activities such as anti-oxidant, antitumor, anti-inflammatory, anti-psoriatic, anti-car- cinogenic, free radical scavenger, to list a few [6]. Its physical properties include some challenging aspects which further makes him a candidate of choice for research and develop-ment. It is poorly water soluble, highly photoreactive agent with rapid metabolism and poor absorption that leads to de-prived bioavailability. To date, numerous reports have pro- posed and proven its significant effect and potential in allevi-ating psoriasis along with properties of numerous receptors to which curcumin binds [7–9]. Furthermore, CRN induced sup-pression of phosphorylase kinase activity makes it a very stur-dy contender for the resolution of human psoriasis [10]. In order to reduce the time needed by CRN to show its activity in treating psoriasis, which greatly impacts on patient'spsy-chological state, herein an attempt has been made by combin-ing CRN with anti inflammatory druglike caffeine.

Caffeine (CFN) is a methylxanthine moiety capable to hin-der the phosphodiesterase (PDE) enzyme; which helps in hy-drolysis of cyclic nucleotides resulting in elevated concentra- tions of intracellular cAMP (cyclic adenosine monophosphate). Cell surface receptors inhibition for adenosine is another pro-posed mechanism [11]. Reduced intracellular cAMP levels are seen in cutaneous leukocytes of patients with psoriasis. Many researchers have proposed that as a phosphodiesterase inhibitor and methylxanthine, CFN, increases intracellular cAMP levels; which consequently suppress inflammatory pathways and pso-riasis progression [12, 13]. Nanotechnology and nanomedicines despite being excep-tionally vast research meadows offers solutions to many un-solved puzzles of drug delivery and therapeutics and so a burgeoning bough of science. In order to improve the solubil- ity and stability of CRN, various studies implying nanocarriers in the form of nanoparticles, lipid-based nano-spheres, nanocrystals, liposomes and polymer-based delivery systems were reported[14–16]. Our exhaustive search has revealed that till date no study has reported in combination use of CRN and CFN as NS for the treatment of psoriasis. In view of this fact, in the present study, an attempt has been made to develop cyclodextrin NS of CRN and CFN in combination as a new- fangled, potential delivery system for combating psoriasis.

Materials and methods

CRN was obtained from Chaitanya Agro Herbals, Mysuru, India, as a gift sample. CFN, dimethyl carbonate (DMC), guar gum and Carbopol-934 were purchased from Loba Chemie, Mumbai, India. β-CD was procured from Himedia, Mumbai, India. All other reagents and chemicals used were of analytical grade. Ultra-purified water was used for all experiments.

Methodology Preformulation studies

i. **Ultraviolet Visible (UV-Visible) Spectroscopy**

The standard solutions of CUR and CFN (10 µg/ml) were separately scanned in 200 to 500 nm range using methanol as blank. Maximum absorbance wavelengths were determined. The overlay spectrum of these drugs was plotted which is shown in Figure 1. From this spectrum an isosbestic point was determined which is the wave length at which spectra of two drugs cross each other (292 nm). Dilutions were prepared and their absorbances were recorded at obtained isosbestic point wavelength and calibration curve was plotted.

ii. **Fourier Transform Infra-Red (FT-IR) spectroscopy**

CUR, CFN, drug mixture and polymer FT-IR spectra were recorded over 4000 to 400 cm^{-1} range by KBr pellet method.

iii. **Differential Scanning Calorimetry (DSC) Analysis**

Thermograms of of CUR, CFN, drug mixture and polymer were recorded at 20 °C /min rate, over 40 to 300 °C range temperature.

Nanosponges

(I) **Compatibility study**

Between the drug mixture and polymer, chemical compatibility was studied using FT- IR and DSC analysis.

i. **FT-IR analysis**

It was carried out for mixture of drugs and beta cyclodextrin, to check the drug and polymer compatibility. IR spectra of the pure drug mixture and physical mixture of polymer-drug mixture's were compared and compatibility was evaluated to identify shifts, appearance or disappearance of peaks.

ii. **DSC Analysis**

These studies helped in identifying thermal behavior of pure drug mixture, and drug - polymer physical mixture so that their compatibility can be confirmed.

(II) **Experimental method**

i. **Synthesis of β-CD NS (melt method)**

Using β-CD as a polymer and DMC as a cross linker in various ratios, three types of NS formulations were prepared. Excess amount of DMC was melted at about 90 °C and hot melt anhydrous β-CD was added. The reaction mixture was mixed for 5 h with continuous heating by a magnetic stirrer, for occurance of reaction between substrate components. Later, the mixture was allowed to cool, grounded in a mortar and treated with ethanol to remove impurities and excessive unreached DMC. Post purification, NS were stored at 25 °C until

further use.

ii. Loading of drugs into NS

The NS were suspended in water and sonicated for few minutes, to avoid the formation and presence of any aggregates. In this aqueous suspension, excess amount of drug mixture was dispersed. The resultant suspension was maintained under constant stirring for 5 h to allow complexation between CD NS and drugs. The uncomplexed drug was separated from complexed drug after complexation reaction, by performing centrifugation for 10 min at 2000 rpm. The obtained sediment was freeze dried to get solid powder of drug loaded NS . In **Table 1** detail of NS prepared with different ratios of polymer and cross linker are quoted.

Table 1: Details of different batches of NS prepared

Sl. No.	NS formulation code	Drug (mg)	Polymer: Crosslinker (mg)	Polymer: crosslinker (Ratio)
1.	NS1	150	100:200	1:2
2.	NS2	150	100:400	1:4
3.	NS3	150	100:800	1:8

(III) Evaluation of NS

- **Entrapment efficiency (EE)**

Determining the drug amount embedded in NS is of major significance, because it determines the release characteristics and consequently the therapeutic potency. To calculate the EE, exactly weighed quantity of NS (150 mg) was dissolved in methanol, sonicated for 15 min to break the complex and centrifuged. The obtained supernatant was then filtered, diluted suitably using 7.4 pH phosphate buffer solution (PBS), and analyzed by UV-spectrophotometer, at 292 nm (isosbestic point of CUR and CFN). EE was calculated by using the below mentioned formula:

$$Entrapment\ efficiency = C_a\ /\ C_{th}\ \times 100 \qquad (1)$$ Where, C_a: drug content (actual) in NS; C_{th}: drug content (theoretical)

- *In vitro* **drug release studies**

These studies were conducted for all the NS formulations by using a release cell which resembles Franz diffusion cell . In short, a cellophane membrane (wetted) was expanded over the open-ended glass tube end. Using a rubber band it was made watertight. 20 mg NS were placed in the donor compartment (glass tube above the cellophane membrane). In a 100 ml beaker which is controlled thermostatically (50 rpm) at 37°C (i.e. receptor compartment) PBS of 50 ml (pH 7.4, 10 mM) was placed and into this the glass tube was immersed vertically. From the receptor compartment 5 ml samples of the release medium were withdrawn at predetermined time points up to 12 h and sink conditions were maintained. UV spectrophotometer was used to determine drug content at 292 nm. Based on the above results, NS formulation (NS 2) with maximum EE and *in vitro* drug release was selected and further characterization studies were done for it.

(IV) Characterization of NS 2

Characterization studies like Scanning Electron Microscopy (SEM) analysis, particle size analysis (PSA), zeta potential determination, FT-IR and DSC analysis were carried out for NS 2

- **Scanning electron microscopy (SEM)**

  The morphology and surface texture of the optimized NS was observed. Samples were mounted on carbon mount and scanned at15 kV accelerating voltage and suitable magnification at room temperature.

- **Particle size analysis (PSA) and Zeta potential measurement**

  The mean particle size, distribution, and Zeta potential/surface charge of the resulting NS were determined by dynamic light scattering (DLS) technique.

- **FT-IR and DSC analysis**

  FT-IR and DSC studies were carried out for prepared optimized NS formulation

(V) Experimental design for formulations of NS gels

In the present work a 2-factor and 3-level full factorial design (3^2 FFD) was used to obtain statistically significant and optimized formulation ingredients. Design Expert® software, version 11.0, (Stat-Ease Inc., Minneapolis, MN, USA), was used to create design. Using Design of Experiment (DoE), amount of gelling agent (X1) and amount of polymer (X2) which are two independent variables were optimized at 3 different levels: low, medium and high. Viscosity (cP) (R1) and *in vitro* drug release (%) (R2) were selected as response variables. Various parameters of prepared formulations were evaluated and characterized.

(VI) Preparation of NS based gel

Measured amount of Carbopol-934 was soaked in 5 ml of water for 2 h. It was then neutralized with addition of triethanolamine (TEA) *via* continuous stirring. Guar gum and drug loaded NS (equivalent to topical doses of drugs) were dissolved in pre weighed and appropriate amount of propylene glycol. This mixture was then transferred to the carbopol container and mixing was done for further 20 min. The dispersion was kept aside for 60 min, for complete hydration and swelling of gel components. Before performing viscosity studies, all the prepared gel samples were allowed to equilibrate for at least 24 h at room temperature. **Table 2** represents formulation chart of prepared NS based gel formulations.

Table 2: Formulation chart of different NS based gel formulations

Formulation code	Carbopol-934 (%)	Guar gum (%)	Propylene glycol (%)	Propyl paraben (%)	TEA (ml)
N1	0.4	0.4	0.5	0.02	0.1
N2	0.4	0.5	0.5	0.02	0.1
N3	0.4	0.6	0.5	0.02	0.1
N4	0.5	0.4	0.5	0.02	0.1
N5	0.5	0.5	0.5	0.02	0.1
N6	0.5	0.6	0.5	0.02	0.1
N7	0.6	0.4	0.5	0.02	0.1
N8	0.6	0.5	0.5	0.02	0.1
N9	0.6	0.6	0.5	0.02	0.1

(VII) Evaluation of NS based gel

- **Determination of pH**

pH of all prepared gels was determined.

- **Homogenicity**

After placing the gels in the container, all formulations were tested for homogenicity (aggregates presence and appearance) by inspecting visually.

- **Spreadability studies**

Spreadability signifies the area of affected part or skin on which fomulation spreads easily when applied. This determines the therapeutic efficacy of formulation. Spreadability studies were carried out by placing calculated amount of gel between two slides and applying specific load on them, which leads to spreading of gel. It was represented in expressions of time (in seconds). Superior spreadability is expressed by least time needed for separation of slides. To calculate spreadability, below formula was used.

$$S = ML / T \qquad (2)$$

Where, S is Spreadability, M is Weight (g) tied to upper slide, L is Length (cm) of glass slides, T is Time (sec) taken for complete separation of two slides

Apparatus used was wooden block-glass slide in which approximately 20 g of weight was applied and estimation of time needed for total partition of upper movable slide from lower fixed slide was done.

- **Viscosity studies**

All measurements were carried out by viscometer at 10 rpm and 37 ± 0.5 ºC temperature using spindle No. 6. The rheological properties of the formulated NS based gels were studied at different rpm and the viscosity was calculated in cP.

- ***In vitro* drug diffusion studies**

NS based gels were permeated through an artificial cellophane membrane. 0.5 g of NS based gel was placed in Franz diffusion apparatus donor compartment. The receptor medium consists of pH 7.4 PBS. 37 ± 0.5 ºC temperature was maintained to replicate the human skin state at the time of the experiment. 5 ml of sample aliquots were withdrawn at 0.5, 1, 2, 4, 8 and 12 h and replaced with equivalent volume of fresh receptor media. Collected samples were spectrophotometrical analysis was done at 292 nm for samples collected and the amount of drug released from gel was calculated.

(X) *Ex vivo* permeation studies

Animals were purchased from Adita Biosys Pvt. Ltd., Tumkuru, India. Skins of healthy BALB/c mice were collected by sacrificing them and were used for *ex-vivo* permeation studies. Topical gels containing pure CRN and NS based gel (N10) were prepared and permeated through dorsal skin of mouse. Procedure similar to that of *in-vitro* drug diffusion studies was followed.

7. RESULTS AND DISCUSSION
Preformulation studies
i. Ultraviolet Visible (UV-Visible) Spectroscopy

Maximum absorbance wavelengths (λ max) of CUR and CFN in methanol are shown in **Table 3**. Overlay plot of two drugs is shown in **Figure** 1 From the overlay plot isosbestic point of two drugs was found to be 292 nm.

Table 3: Maximum wavelengths of CUR and CFN mixture

Solvent	Drug	of maximum absorbance, λ max (nm)	
		Observed	**Reported**
Methanol	Curcumin	419	421
	Caffeine	272	273

The calibration curve values and graph of CUR and CFN mixture was prepared in methanol and are shown in **Table 4** and **Figure** 2 respectively. 0.998 was found to be the regression coefficient with 0.0287 as slope value and 0.0064 as Y intercept value. From the results, linear correlation among concentration and absorbance is indicated in the range of 0-30 µg/ml of CUR and CFN mixture in methanol.
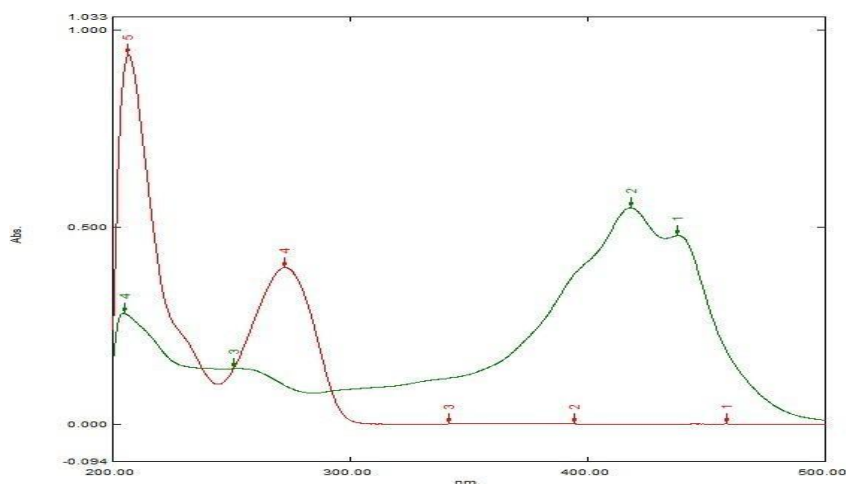

Figure 3: Overlay spectrum of CUR and CFN mixture in Methanol
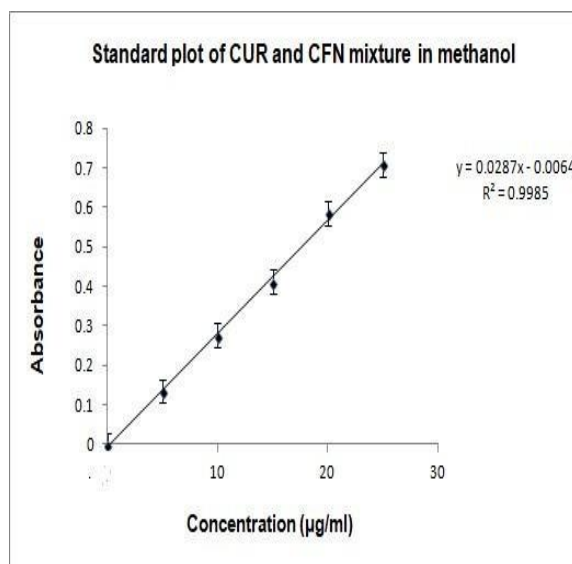
Figure 4: Calibration curve of CUR and CFN mixture

ii. Fourier Transform Infra-Red (FT-IR) spectroscopy

FT-IR Spectrum of the CUR, CFN and drug mixture (CUR and CFN) samples exhibited all the distinctive IR peaks that are given in the literature, signifying presence of functional groups pertaining to CUR and CFN in the drug mixture (141)..

iii. DSC Analysis

CUR and CFN mixture has shown two endotherms in thermogram of DSC as shown in **Figure 4**. The first endotherm that appeared at around 175.37 °C represents the melting point of CUR (183 °C theoretically). The second endotherm at 227.40 °C represents the melting point of CFN (235 °C theoretically). These DSC analysis of drug mixture implied that no significant change was noticed in melting points of both the drugs after combining them.

(l) Compatibility study

FT-IR and DSC analysis were carried out for pure drug mixture and drug & polymer physical mixture to estimate the drug mixture and polymer compatibility.

i. FT-IR analysis

Spectra of pure drug mixture and physical mixture of drugs and polymer were determined. The Functional groups obtained for physical mixture of drugs and polymer was found to be in correlation with pure drug mixture peaks. As shown in **Figure 5** and **Table 12**, the prominent peaks of drug mixture, i.e. 2920.32 cm^{-1} to Akane C-H Stretching; 1358.90 cm^{-1} to Alkene C-H bending; 1285.60 cm^{-1} to Amine C-N-Stretching and 1600.97 cm^{-1} to Amide N-H- bending were noticed in the physical mixture FT-IR spectra. Obtained results state that no interactions between drug mixture and polymer occurred because no change in the peaks was seen. Hence drug mixture (CUR and CFN) and selected polymer were compatible with each other.

ii. DSC Analysis

The DSC Thermograms of drug (CUR and CFN) mixture and drug-polymer physical mixture are shown in **Figure 6**. Drug mixture exhibited endothermic peaks of CUR ad CFN at 171 °C and 225 °C respectively. The physical mixture of drugs and polymer exhibited the endothermic peak of polymer at 99 °C for β-Cyclodextrin. Sharp peaks of drugs are absent in physical mixture indicating the drug mixture is encapsulated in the polymer system, which shows their compatibility with each other.
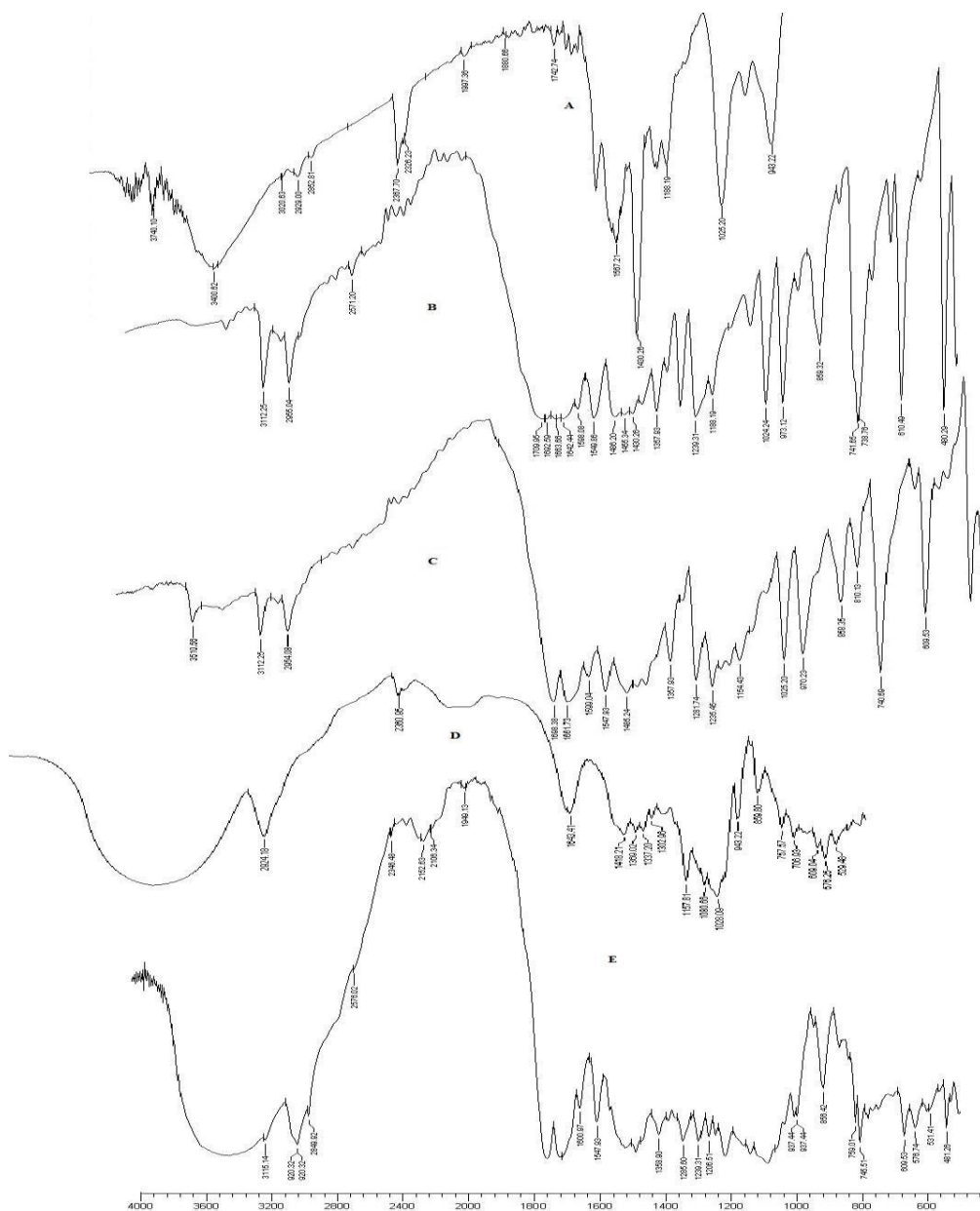
Wave length

Figure 5: FT-IR spectra of (A) Curcumin; (B) Caffeine; (C) Drug mixture; (D) Polymer; (E) Drug+polymer physical mixture
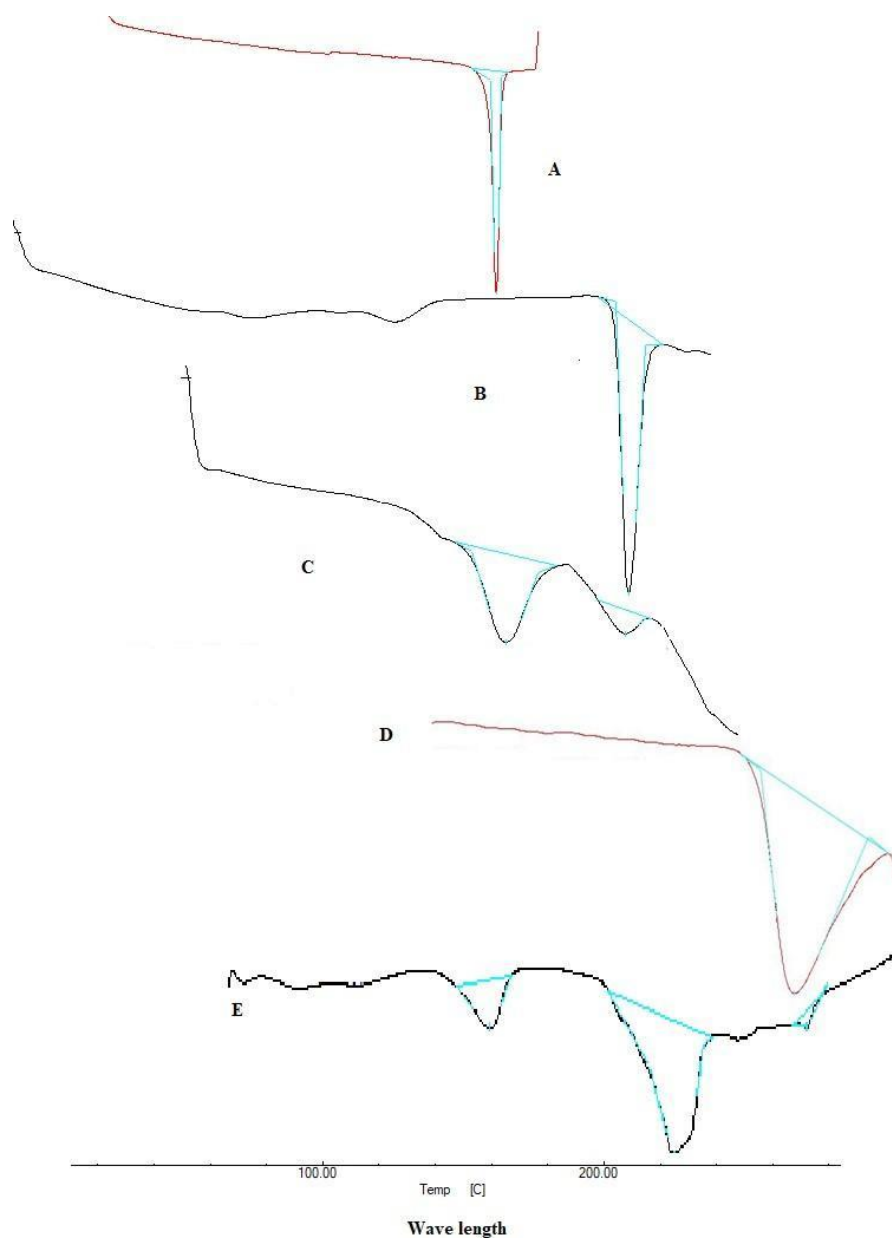
Figure 6: DSC thermograms of (A) Curcumin; (B) Caffeine; (C) Drug mixture; (D) Polymer; (E) Drug+polymer physical mixture

(II) Evaluation of NS

i. Entrapment efficiency (EE)

The drug entrapment efficiencies noted for different NS formulations are given in **Table 7**. It is the amount of drug entrapped in the CD NS cage and was calculated for all the NS formulations. EE of NS batches ranged from 50.26 % to
61.14 %. It was clearly evident that EE has changed significantly when drug and polymer ratio

changed, and it was chiefly dependent on the crosslinker used in the formulation. The highest EE was found for the NS 2 formulation; where larger quantity of drug was encapsulated due to optimum cross linking in the polymeric cage (14). From the drug encapsulation studies, it was also established that DMC acts as an efficient crosslinker for the formulation of NS.

Table 7: Entrapment efficiencies of prepared NS

Sl. No.	Formulation code	Entrapment efficiency (%, Mean±SD*)
1	NS1	50.26±0.053
2	NS2	61.14±0.028
3	NS3	57.65±0.015

*SD- Standard deviation, $n=3$

ii. *In vitro* drug release studies

The NS formulations were subjected to *in vitro* release studies. The drug release profiles obtained for formulations NS1 to NS3 are presented in **Figure 7**. It was found that formulation containing polymer and crosslinker in 1:4 ratio (NS 2) has shown maximum *in vitro* drug release as compared to other formulations. This could be because of lesser extent of inclusion and non-inclusion complexation seen in NS 1 due to insufficient nanochannels or nanopores formation. Whereas in case of NS 3 formulation quite higher extent of crosslinking resulted in inability of drug to enter into the nanochannels (15).
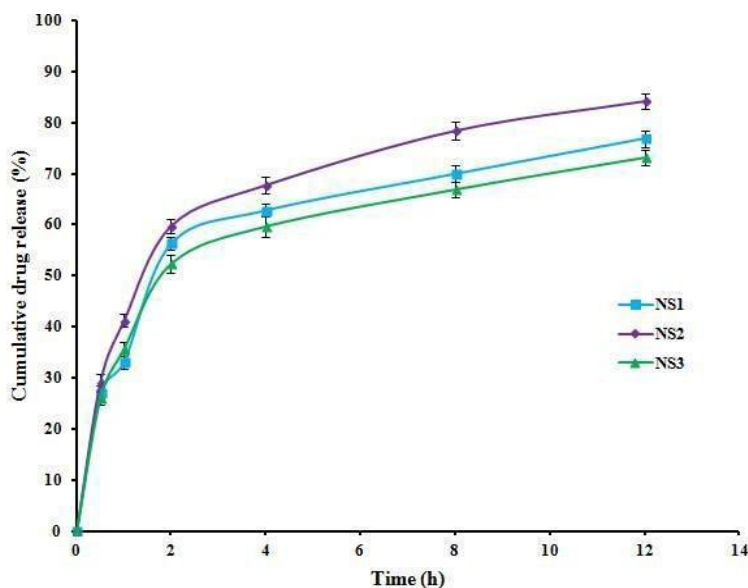

Figure 7: *In vitro* drug release profiles of different NS formulations

Based on the above results, NS 2 formulation was found to have maximum entrapment efficiency and *in vitro* drug release. Hence further characterization studies were carried out on thisformulation.

(III) Characterization of NS 2

i. Scanning electron microscopy (SEM)

SEM analysis was done for the prepared NS to check the morphology and surface texture of the same. NS were roughly spherical in shape with uneven surface and spongy nature (**Figure 8**). The SEM micrographs revealed that formed NS were having several fine surface voids; most likely as a result of solvent diffusion. Moreover, no residual, intact crystals of drugs were seen on NS surface, indicating formation of NS matrix by drug-CD.
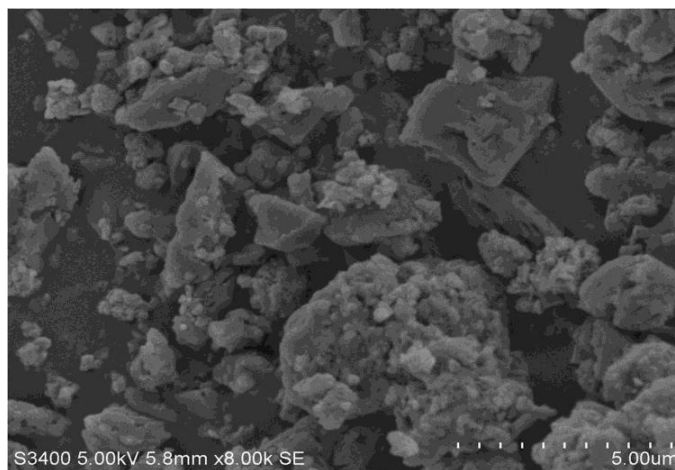

Figure 8: SEM micrograph of NS 2 formulation

ii. Particle size analysis (PSA), Zeta potential measurement

Size of a particle is a very important parameter in NS performance, because drug release rate, extent and drug absorption are majorly affected by it. As the particle size decreases, interfacial area available for drug diffusion increases and thus improve in drug release can be seen. The average particle size of prepared NS formulations was found to be in the range of 170 nm to 200 nm with a PDI range of 0.291±0.073 to 0.395±0.026 representing uniformity in distribution of particle size. The Ostwald ripening probability was conquered because of narrow size distribution (142). Particle size distribution pattern of optimized NS formulation is depicted in **Figure 9**.

Zeta potential plays a vital function in the interaction of formulation with biological system and it has been also reported in various studies. It shows the charge type that is present on the NS surface and also provides idea of stability of the prepared formulation. Zeta potential of NS formulations was in 14.6±1.1 mV to -

28.35 mV range and it increased with an increase in the concentration of crosslinker. This could be credited to carbonate groups presence in its structure that provides physical stability among NS particles by electrostatic repulsion, and thus avoiding aggregations. Also the decrease in particle size leads to increased surface area and as a result to higher Zeta potential.
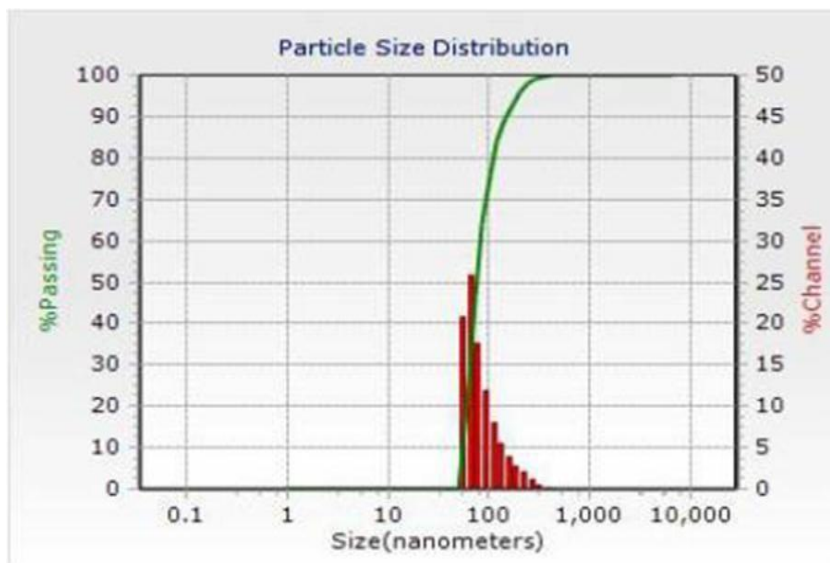
Figure 9: Particle size distribution pattern of the NS 2 formulation

iii.FT-IR analysis of NS

FT-IR spectroscopy of NS2 formulation was carried out for drug mixture entrapment confirmation in the NS. As shown in **Figure 10**, the major peaks of drug mix, i.e. phenolic OH group stretch (3510.56 cm^{-1}), Amine N-H stretch (3112.26 cm^{-1}) and Alkene =C-H bonding (740.69 cm^{-1}) were absent in NS 2 formulation spectra. Thus drug mixture entrapment in the polymer system of NS can be confirmed.

Also other characteristic peaks like Alkane C-H stretch, Alkene C-H bending, Amine C-N-stretch and Amide N-H bending which are present in the pure drug mix and drug and polymer physical mixture were found in the NS 2 formulation. This indicates the presence of drug in the NS.
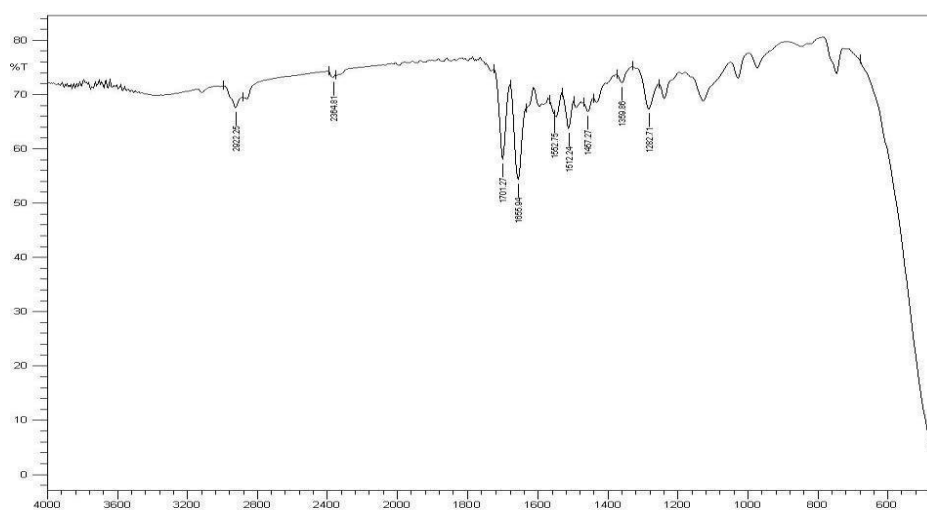

Figure 10: FT-IR spectra of NS 2 formulation

iv. DSC analysis of NS

The DSC thermogram of optimized NS formulation (NS 2) is shown in **Figure 11**. An endothermic peak at 290 ºC was seen which indicates β-Cyclodextrin presence. No peaks pertaining to drugs were noticed which shows that drug mixture is encapsulated in the polymer system, which shows their compatibility with each other.
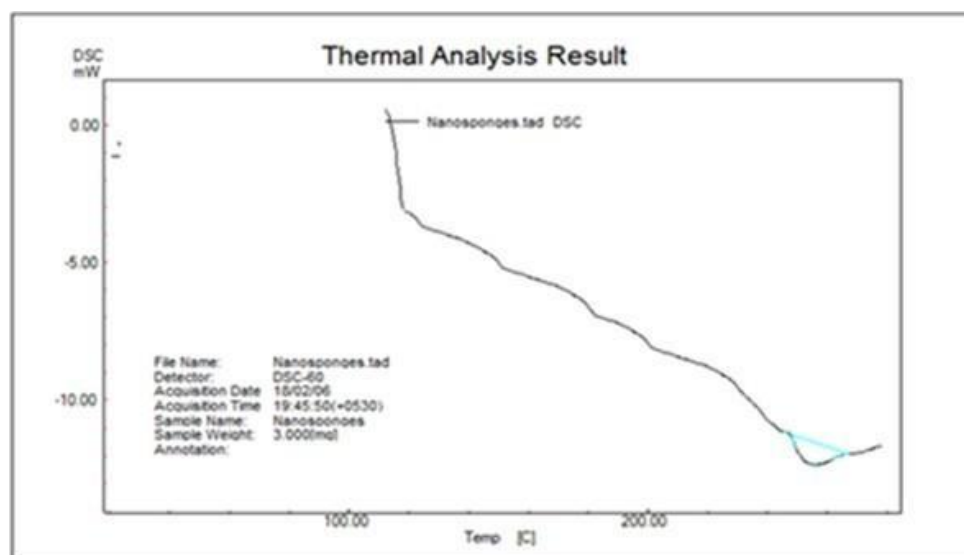

Figure 11: DSC Thermogram of NS 2 formulation

(IV) Evaluation of NS based gels

From the software, nine formulation runs were obtained. Evaluation studies like pH, homogenicity, viscosity, spreadability and *in vitro* drug release studies were performed for NS based gels and the results obtained are represented in **Table 14**. By fixing gelling agent concentration and polymer concentration as independent variables, viscosity and *in vitro* drug release were taken as two responses for experimental design approach (3^2 FFD) using Design Expert® 11 software to attain an optimized formulation. It was further subjected for *in vivo* and *ex vivo* permeation studies.

i. pH determination

pH values of all gels were in the range of 5.2-7.5; which indicated that gel pH were well within the safety range and near to that of skin pH. Notable increase or decrease in the pH values may lead to skin irritation, however, in present study such probability was ruled out by recorded results.

ii. Homogenicity

All the prepared NS based gels were visually inspected and evaluated for homogenicity. The prepared gels were observed to be clear, translucent and homogenized, without any lumps or aggregates.

iii. Spreadability studies

Spreadability is one of the vital characters for an ideal gel. The findings of spreadability studies explaind that prepared formulations easily spreaded on applying small amount. The spreadability was noted to be inversely proportional to the viscosity of gels and it got decreased as the carbopol-934 and guar gum amounts increased. All the prepared NS based gel formulations have shown good spreadability.

iv. Viscosity studies

The viscosity of all the NS based gel formulations was noted in range of 9000- 13000 cP and it was found to be dependent on polymeric concentration in NS based gel formulations.

v. *In vitro* drug diffusion studies

These studies provide essential data about imitative action of the formulation during *in vivo* application. The results indicated that a sustained drug release was offered by formulations. All formulations exhibited initial burst release which might be due to unentrapped drugs in initial hours in the gel matrix, followed by prolonged release of entrapped drugs from NS core over extended period of time. Due to steady erosion of NS and continuous drugs diffusion into the external polymer matrix, drug release from NS occurred. Optimum drug concentration required for controlling symptoms immediately was provided by initial burst release which is followed by prolonged release that helps in maintaining the therapeutic concentration required for overall psoriasis treatment.

(VIII) *Ex vivo* permeation study

Marketed CUR gel has shown drug release for 8 h and after that no release was noticed; whereas N10 formulation has shown drug release till 12 h (max 65.48%). The percent drug release of marketed formulation was noted to be around 63.74% at the end of 8 h and after that no release was seen. Thus, a sustained release of drugs was established from the developed NS based topical gel formulation. The drug release profiles obtained for *ex-vivo* permeation studies are presented in **Figure 18**.
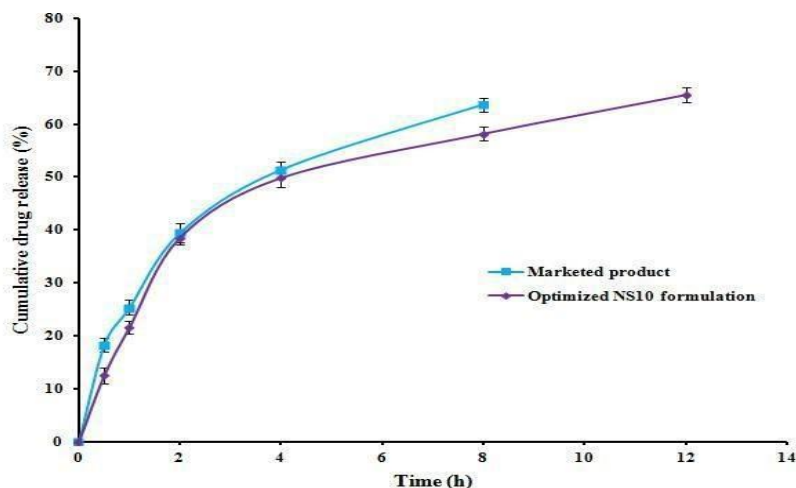

Figure 18: Drug release profiles of marketed CUR formulation and optimized NS based gel formulation obtained during *ex vivo* permeation studies

Table 8: Results of evaluation parameters for NS based gels

Formulation code	pH	Homogenicity	Spreadability#	scosity (cP)	In vitro drug release (%)
N1	6.2	Clear, translucent	+++	9747±0.22	76.63±1.12
N2	5.9	Clear, translucent	+++	9958±0.13	75.24±0.98
N3	5.3	Clear, translucent	+++	10246±0.18	73.82±0.93
N4	6.1	Clear, translucent	+++	11785±0.23	70.45±1.03
N5	7.5	Clear, translucent	+++	12316±0.11	69.32±0.76
N6	5. 7	Clear, translucent	+++	12954±0.16	68.51±0.94
N7	5.8	Clear, translucent	+++	13625±0.27	66.42±0.57
N8	6.0	Clear, translucent	+++	14128±0.21	65.14±0.34
N9	6.1	Clear, translucent	+++	14934±0.19	63.95±1.07

#+- poor; ++- intermediate; +++- good

CONCLUSION:

Nanosponges (NS) containing Curcumin (CUR) and Caffeine (CFN) mixture developed as potential systems in treating Psoriasis. In NS preparation, β-CD was used as polymer and DMC as crosslinker. By altering their ratios, various NS formulations were developed by melt method. Evaluation studies were carried out for these NS. NS2 with higher entrapment efficiency and *in vitro* drug release was considered for further studies. Further studies like *ex vivo*, *in vivo* and stability studies were carried out for this optimized formulation. The obtained topical gels NS which contained combination of CUR and CFN have produced anti psoriatic effect within 7 days, whereas CUR alone produced the activity in 14 days and above indicating quick action of former. the drug mixture produced sustained release till 12 h. This helps in reduction of drug dose, dosing frequency and other limitations pertaining to classic dosage forms and drugs used. Hence it was proved that CUR and CFN combination can be used in treating psoriasis effectively.

REFERENCES

1. Michelle AL, Anne MB, James GK. Pathogenesis and therapy of psoriasis. Nature. 2007;445(22):866–72.

2. Rendon A, Schäkel K. Psoriasis Pathogenesis and Treatment. Int J Mol Sci. 2019;20(6):1475. Published 2019 Mar 23. https://doi.org/ 10.3390/ijms20061475.

3. Boehncke WH. Etiology and pathogenesis of psoriasis. Rheum Dis Clin N Am. 2015;41(4):665–75. https://doi.org/10.1016/j.rdc.2015. 07.013.

4. Khanna NM. Turmeric - Nature's precious gift. Curr Sci. 1999;76: 1351–6.

5. Golnaz S, Minoo A, Parvin M, Jinous A, Kosar R, Mehdi R. Topical turmeric Microemulgel in the Management of Plaque Psoriasis; a clinical evaluation. Iran J Pharm Res. 2015;14(3): 865– 76.

6. Yunus P, Omid F, Stephen LA, Muhammed M, Alexandra EB, Thomas PJ, et al. Evidence of curcumin and curcumin analogue effects in skin diseases: a narrative review. J Cell Physiol. 2018;234(2):1165–78.

7. Vollono L, Falconi M, Gaziano R, et al. Potential of Curcumin in Skin Disorders. Nutrients. 2019;11(9):2169. Published 2019 Sep

10. https://doi.org/10.3390/nu11092169.

8. Nardo VD, Gianfaldoni S, Tchernev G, et al. Use of Curcumin in Psoriasis. Open Access Maced. J Med Sci. 2018;6(1):218–20. Published 2018 Jan 21. https://doi.org/10.3889/oamjms.2018.055.

9. Varma SR, Sivaprakasam TO, Mishra A, Prabhu S, Rangesh RM. Imiquimod-induced psoriasis-like inflammation in differentiated Human keratinocytes: Its evaluation using curcumin. Eur J Pharmacol. 2017;813:33–41. https://doi.org/10.1016/j.ejphar. 2017.07.040.

10. Baden HP, Gifford AM. Isometric contraction of epidermis and stratum corneum with heating. J Investig Dermatol. 1970;54(4): 298–3.

11. Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, HenryE,etal.Chemopreventive and therapeutic effects of curcumin. Cancer Lett. 2005;223:181– 90.

12. Boushey HA. Bronchodilators and other agents used in asthma. In: Katzung BG, editor. Basic and clinical pharmacology. 7th ed. Los Altos (CA): Appleton & Lange; 1998. p. 330–7.

13. Wilken R, Veena MS, Wang MB, Srivatsan ES. Curcumin: a review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. Mol Cancer.10; 2011.

14. Vali A, Asilian A, Khalesi E, Khoddami L, Shahtalebi M, Mohammady M. Evaluation of the efficacy of topical caffeine in the treatment of psoriasis vulgaris. J Dermatolog Treat. 2005;16(4): 234â€"7. https://doi.org/10.1080/09546630510011801.

15. Yang HB, Song W, Chen LY. Differential expression and regulation of prohibition during curcumin-induced apoptosis of immortalized human epidermal HaCaT cells. Int J Mol Med. 2014;33(3):507–14.

16. Alashqar M, Goldstein N. Caffeine in the treatment of atopic der-matitis and psoriasis: a review. From Gene to Clinic International Congress, London: Psoriasis; 2017.

17. Osmani RAM, Hani U, Bhosale RR, Kulkarni PK, Shanmughanathan S. Nanosponge carriers- an archetype swing in cancer therapy: a comprehensive review. Curr Drug Targets. 2017;18(1):108–18.

18.   Osmani RAM, Kulkarni PK, Shanmuganathan S, Hani U, Srivastava A, Prerana M, et al. A 32 full factorial design for devel-opment and characterization of a nanosponge-based intravaginal in situ gelling system for vulvovaginal candidiasis. RSC Adv. 2016;6: 18737–50.

19.   Sai VC, Priti PP, Kisan RJ, Gajendra G, Vilasrao JK. Cyclodextrin-based nanosponges: a propitious platform for enhancing drug de-livery. Expert Opin Drug Deliv. 2014;11:111–20.

20.   Venkatalakshmi R, Jason Y. Development and evaluation of mouth dissolving tablets using natural super Disintegrants. J Young Pharm. 2017;9(3):332–5.