IJPSR (2020), Volume 11, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 October 2019; received in revised form, 06 February 2020; accepted, 12 March 2020; published 01 October 2020

FORMULATION OPTIMIZATION OF BENZYDAMINE HYDROCHLORIDE NANOSPONGES LOADED HYDROGEL FOR TREATMENT AGAINST MOUTH ULCERS

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Keywords:

Encapsulation, Controlled release, Site specificity, Dosing frequency

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ABSTRACT: This study is aimed at encapsulating Benzydamine Hydrochloride into nanosponges that involve different crosslinkers to polymers in the ratio of 1:1, 1:2, 2:3, *etc*., which are used, in order to protect these structures, to control their release and maintain their site-specificity. Emulsion Solvent Diffusion technique was applied for the formation of nanosponges, which were incorporated into carbopol hydrogel to give controlled release of the drug so that it adheres for a longer duration to the buccal mucosa and would gradually release the drug to give the desired release. The pre-formulatory DSC and FTIR study indicated there were no interactions of Benzydamine Hydrochloride (BZH) with excipients (Poly Vinyl Alcohol and Poloxamer 188). The formation of these nanosponges is confirmed through DSC, FTIR, FESEM, and TLC studies. BZH was loaded up to 88%, 85%, 72% w/w. Particle sizes of the loaded NS formulations were between 128 and 250 nm with low polydispersity indices of 0.6. Zeta potential was -17 to -150 mV, which gave a stable colloidal nanosuspension. Release studies were optimized by evaluating through different membranes such as the dialysis membrane, cellulose acetate membrane, and excised porcine mucosa. The release of the final formulation was evaluated by the use of permeation enhancers. Obtained nanogel showed controlled release over the conventional preparation to prolonged-release and to decreased the dosing frequency.

INTRODUCTION: Nanotechnology has been gaining tremendous importance due to the smaller particle size, increased bioavailability, and site-specificity. Nanosponges are the epitome of nanotechnology, their utility in pharmaceutical and non-pharmaceutical areas has been remarkable in changing times.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(10).4945-56

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(10).4945-56

Nanosponges are a part of nanotechnology involving cross-linking between linkers and cross linkers forming inclusion complexes with drug entities; they form tiny void like structures with an internal diameter in the range of 10-1000 nm [1]. Their porous structures help in the release of drug molecules in a controlled manner; they form site-specific molecules that adhere to the biological membranes like oral cavity and cause permeation through it.

Nanosponge structures have an inner particle size in the nanometer scale in x-, y- and z- dimensions and the outer dimensions in the range of nanometers or micrometer [1].

Minor recurrent aphthous stomatitis is round, clearly defined, small, painful ulcers that heal in 10–14 days without leaving a scar. In major recurrent aphthous stomatitis (Sutton's disease), lesions are larger greater than 1.0 cm, can last for 6 weeks, and frequently scar. The third variety of recurrent aphthous stomatitis is herpetiform aphthous ulcers, which presents as multiple clusters of pinpoint lesions that can coalesce to form large irregular ulcers and last 7–10 days possibly because of an immune defect (*e.g.*, HIV, infections) or nutritional disorders (*e.g.*, vitamin deficiencies) [2].

Benzydamine Hydrochloride (BZH) is a non-steroidal anti-inflammatory drug used conventionally to treat mouth ulcers. Properties of BZH such as molecular weight of 345.91 daltons, the solubility of 0.0491 mg/ml, having less than 5 condensed ring structures, and boiling point less than 250 ℃ makes them ideal for forming nanosponge complexes. Formation of cross-linking bonds with polymers and crosslinkers makes stable nanostructures when loaded with active [3]. The particle sizes of these nanostructures enable them to cross multiple barriers as the pore size of the oral cavity is 2 µm, particles that are less than this pore size can cross the mucus membrane [4].

This work is focused on the development of new formulations for BZH, which consist of encapsulation into nanosponges for increasing their shelf-life and making them more target specific for the release of the drug [5]. These nanosponges may solubilize BZH and form inclusion complexes obtained from the binding of amine group with polymer due to its high inclusion abilities, thereby increasing the drug stability.

MATERIALS AND METHODS:

Materials: Benzydamine Hydrochloride was obtained from Bal Pharma Limited (Bangalore, India) as a gift sample. Poly Vinyl Alcohol (Hot Water Solube-Pharma Grade) obtained from West coast Laboratory (Mumbai, India). Dichloro-methane obtained from SRL Pvt. Ltd. (Mumbai, India) was of analytical grade. Freshly Distilled water was used throughout the studies.

Method of Preparation: [6, 7] A series of nanosponges in different ratios of excipients were prepared by emulsion solvent diffusion technique using poloxamer F188 and polyvinylalcohol (PVA). PVA was dissolved in distilled water to form an aqueous phase. Weighed quantities of Poloxamer with BZH were dissolved in Dichloromethane (DCM), forming an organic phase which, was added dropwise to the solution of PVA with water. The organic phase was added dropwise to the aqueous phase with continuous stirring using a Magnetic Stirrer (1MLH, Remi) optimized to 1250 rpm for 3 h. The temperature of aqueous phase was maintained at 40 ℃ to obtain uniformity of PVA solution. Poloxamer 188 forms stable complexes with PVA and DCM, which is an organic solvent and acts like a crosslinker. The excipients used were in ratios as shown in **Table 1**.

The formulations were quoted from F1-F7.

TABLE 1: GIVES THE RATIO FOR FORMULATION AND THEIR %ENTRAPMENT EFFICIENCY

Formulation	Ratio	Poloxamer 188 (mg)	Poly Vinyl Alcohol (mg)	Entrapment efficiency %
F1	1:1	50	50	70
F2	1:1	100	100	88
F3	1:2	50	100	85
F4	2:3	70	100	72
F5	1:8	12.5	100	62
F6	1:5	20	100	71
F7	1:4.	25	100	51

Histological Studies: The histological studies were conducted as per approval from Institutional Animal Ethics Committee on Albino Wistar Rats for testing the effect of gel.

Physicochemical Characterization of BZH Loaded Nanosponges:

Fourier Transform Infrared Spectroscopy (FTIR): [6, 7] 1 mg Benzydamine Hydrochloride was grounded and intermittently mixed with 99mg of Potassium Bromide. Grinding and mixing were done with motor and pestle. The mixture was then passed into a disc in an evaluable die. The entire operation was conducted under controlled humidity, and then the sample was scanned using Bruker Model Alpha T, Opus software, BrukerOptics, Germany.

Differential Scanning Calorimetry (DSC): [8] The DSC analysis was carried out to study the purity of Benzydamine Hydrochloride and interaction with an excipient and of the final formulation. The thermograms were obtained using Thermal Analysis System DSC 6220 High Sensitivity Muce,

Woodland USA, by heating the sample at 10 °C /min. A dry purge of nitrogen gas (20 ml/min) was used for all runs. Samples were heated from 35 ° - 400 °C.

Particle Size and Polydispersity Index: [11] Particle size of BZH nanosponges was determined by using Nanosight NS500 3.1 (Malvern Instruments Ltd., Worcestershire, UK) with controlled motorized stage with a charge-couple device (CCD) that allows visualization and tracking of laser-illuminated particles undergoing Brownian motion in suspension. The samples were directly introduced into the edge between the glass slide and specially designed coverslips, which were analyzed at 685nm red laser at 25° by nanoparticle tracking analysis (NTA), Viton Fluor elastomer O ring analytical software version.

Particle size results were calculated from the determined translational diffusion coefficients using a modified version of the Stokes-Einstein formula,

$$(x, y)^2 /4t = Dx, y = kBT/3\pi\eta d$$

Where $(x, y)^2$ is the mean-square displacement in two dimensions, Dx, y is the translational diffusion coefficient in two dimensions, kB is Boltzmann's constant, T is the sample temperature, t is the time, η is the viscosity of the dispersion medium and d is the sphere-equivalent hydrodynamic diameter.

The polydispersity index of the formulation was found out by

$$PDI = \Delta d / davg$$

Where d is the width of distribution denoted by SD and davg is the average particle size

Zeta Potential Analysis: [11] Nanoparticle Tracking Analysis (NTA) using Nanosight NS500 version 3.1 (Malvern Instruments Ltd., Worcestershire, UK) was used for zeta potential measurements by using an automatic mode to which prime fluidics were performed. The surface charge was determined by applying potential through positive and negative electrode. Applied voltage was set at 24v, and dielectric constant at 80. The temperature was maintained at 25°.

Field Emission Scanning Electron Microscopy: [11] A FESEM 300 ZEISS Germany Field Emission Transmission Electron microscope was used, and the particle size was measured, also the surface and internal morphology of nanosponges was observed.

Thin Layer Chromatography: [10] This adsorptive chromatography was performed to determine and define the formation of nanosponge. The formation of complex with nanoponges decrease their refractive index from the pure drug as they are complexed. The solvent system optimized was made of ethyl acetate: methanol: strong ammonium solution (30%). They were analyzed in UV-Visible light.

Entrapment Efficiency of Nanosponges: [10] BZH loading into the nanosponges was expressed as percentage of BZH in the produced nanosponges with respect to the initial amount of BZH that was used for synthesizing the nanosponges .The entrapment efficiency of the formulations was found out by centrifuging the formulation at 12000 rpm for 45 min to separate the formed nanosponges from the free drug .The supernatant was withdrawn and analyzed by UV-Visible spectrophotometer at 305.6 nm.

Entrapment efficiency (EE) = (Amount of drug added during preparation – amount of drug in the supernatant) × 100 / Amount of drug added during preparation

***In-vitro* Release of Benzydamine Hydrochloride from Nanosponge Formulations:** [10] The *in-vitro* release of F2 was carried out using a dialysis membrane Hi-Media, cut-off 12,000 Da. The donor phase consisted of formulations containing a fixed amount of BZH in phosphate buffer at pH 6.8 and pH 7. The receiving phase consisted of phosphate buffer, pH 6.8 and pH 7 added to maintain proper sink conditions. The receiving phase was completely withdrawn and replaced with fresh medium after fixed time intervals, suitably diluted and analyzed using the UV-Visible spectro-photometric method.

Stability Determination of Benzydamine Hydrochloride: [10] BZH and nanosponge formulations (BZH-NS) were subjected to short-time stability studies 24 h in PBS solution pH 6.8 at 37 °C. Formulations containing an equal amount of 200 µg/ml of BZH were dispersed and were mixed uniformly using a vortex. An aliquot of each suspension was taken, and the amount of BZH was determined using the UV-Visible spectro-

photometric method at 305.6 nm. After 24 h, BZH was expressed in % w/w in each formulation in comparison with non-complexed BZH.

Nanosponges samples were subjected to long term stability at temperatures 25 ± 2 °C and 8 ± 2 °C wherein they were intermediately analyzed for particles size and zeta potential.

Equilibrium Swelling Study: [10] Swelling behavior of the hydrogel was determined by placing 0.2 g of the hydrogel in a previously weighed tea bag. It was performed by measuring the weight gain as a function of immersion time in 10 mL of pH 6.8 Phosphate buffer at 37 ± 2 °C. Measurements were taken until equilibrium hydration degree was reached when three consecutive determinations gave the same weight. Before measurement, a tea bag was hung up to 15 min in order to remove the excess media. The equilibrium swelling (ES) was calculated by

Equilibrium Swelling = Weight of swollen gel-Weight of dried hydrogel / Weight of dried hydrogel

Formulation of Nanosponge-Loaded Hydrogels: [5, 10, 12] The gel-forming polymer was soaked in water overnight and then dispersed by agitation at approximately 600 rpm with the aid of magnetic stirrer to get a smooth dispersion. The stirring was stopped, and dispersion was allowed to stand for 15min so that any entrained air could escape. To this aqueous solution, triethanolamine was added at a slow speed. At this stage, nanosponges and permeation enhancers were incorporated into the prepared base. Benzydamine Hydrochloride being weakly acidic drug required higher concentrations of 2% v/v triethanolamine for neutralization.

***In-vitro* Release:** [5, 10] The gel was analyzed for release through cellulose acetate membrane, which had a pore size of 0.45µm. 0.5 g of the gel was placed on the membrane assembled on a Franz Diffusion cell. The assembly was previously simulated to sink conditions, using Phosphate Buffer pH 6.8. The temperature of the assembly was maintained at 37 ± 2 °C. Samples were withdrawn at predetermined time intervals and were analyzed by UV-vis Spectrophotometer at 305.6 nm. Carbopol 971 NF[13] and Carbopol Ultrez 10 were used as the two gelling agents, the release of which was then optimized.

Drug Content Analysis: [12] Drug content of the gels was determined by dissolving 1g gel in 60 ml in Phosphate Buffer pH 6.8. Quantitatively transferred to volumetric flasks of these solutions and dilutions of these solutions were made with the same buffer solution. The resulting solutions were then filtered from 0.45µm membrane filters before subjecting the solution to spectrophotometric analysis for BZH at 305.6 nm[5,] and concentration of BZH in each sample was determined using spectrophotometry. Samples from drug-free gel were used as a blank solution during analysis.

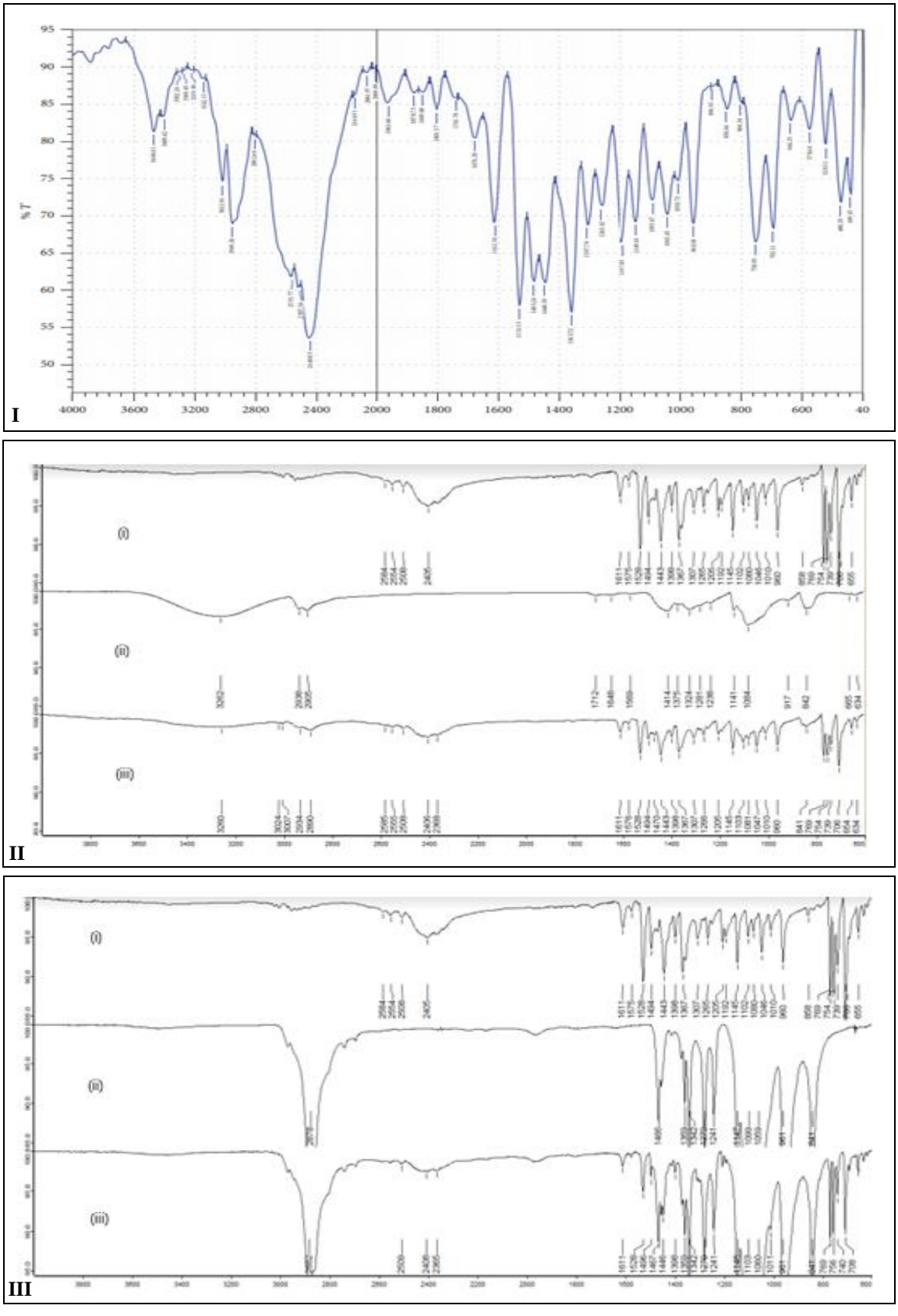
Texture Analysis: [10] Texture analysis of the hydrogel was done in compression mode using Texture Pro CT V1.8 Build 31, pre-test speed of probe 1.0 mm/s, test speed of 1.0 mm/s. The probe TA3/100 was depressed a distance of 14.92 mm, with load cell capacity 10 kg. The force-distance curve was recorded, and peak values in the negative and positive areas were interpreted for adhesiveness and firmness, for the standard and formulated gel.

RESULTS AND DISCUSSION: Benzydamine Hydrochloride has solubility less than 200 µg/ml; it has been conventionally used to treat mouth ulcers in humans for a long time. The enhanced release improved bioavailability. FESEM studies showed that the regular spherical shape and sizes of nanosponges are unaffected even after drug encapsulation FESEM measurements revealed a mean particle size of about 128 nm to 250 nm for BZH nanosponges.

FTIR: As shown in **Fig. 1**, FTIR of the plain drug shows characteristic peaks of amines and benzyl group which disappear in the peaks of final formulations, the disappearance of the peaks shows the interaction of the excipients indicating formation of complexes. N-H bending band at 1640-1560 cm^{-1} indicate primary amine, 1150-1085 cm^{-1} having C-O stretching indicates presence of ether group.

C-N stretching occurs at 1350-1000 cm^{-1}, 3261 cm^{-1} is a peak shown by poly vinyl alcohol indicative of O-H stretching. Two distinct absorption bands occurring at 2950 and 2905 cm^{-1} resulted from CH_2-CH_2 groups. The band at 919 cm^{-1} indicate (CH_2) rocking vibration.

The bands at 854 and 611 cm^{-1} are assigned to (C-C) stretching vibration and OH bending respectively. The formulation shows shift in peak from 2404 to 2437 cm^{-1} for BZH and 2878 to 2881 cm^{-1} and 3261 to 3259 cm^{-1} for poloxamer 188 this shows formation of benzydamine hydro-chloride nanosponges.



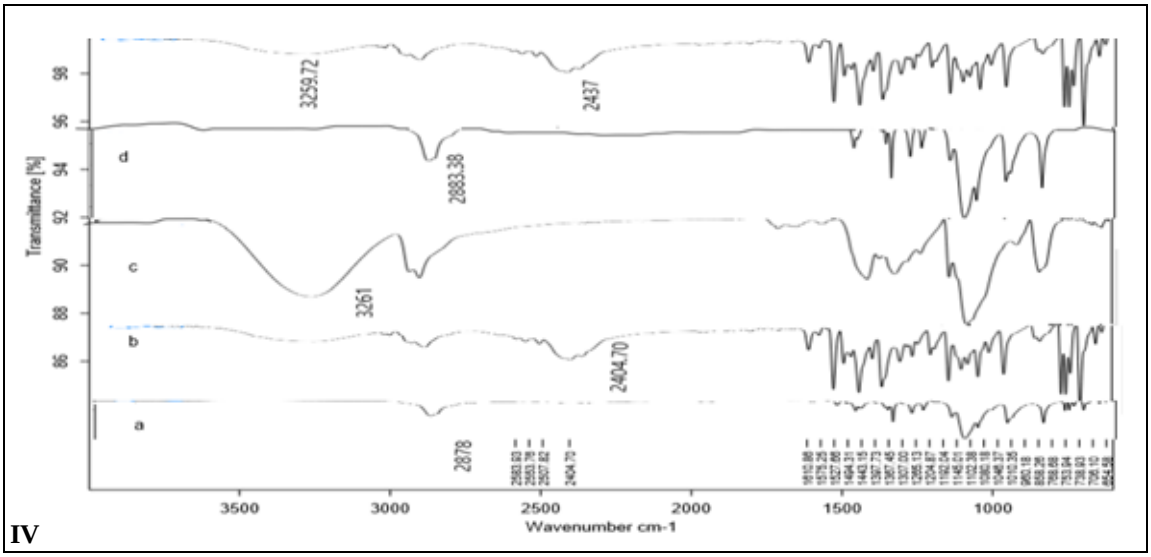


FIG. 1: FTIR SPECTRA. I: SHOWS PEAK OF BENZYDAMINE HYDROCHLORIDE, II: (I) INDICATES PEAK OF BENZYDAMINEHYDROCHLORIDE, (II) SHOWS PEAK OF POLYVINYL ALCOHOL. (III) INDICATES PEAK OF PHYSICAL MIXTURE BENZYDAMINE HYDROCHLORIDE WITH PVA. III: (I) INDICATES PEAK OF BENZYDAMINEHYDROCHLORIDE, (II) SHOWS PEAK OF POLOXAMER 188. (III) INDICATES PEAK OF PHYSICAL MIXTURE BENZYDAMINE HYDROCHLORIDE WITH POLOXAMER 188. IV: A: INDICATES PEAK OF POLY VINYL ALCOHOL B: SHOWS PEAK OF BENZYDAMINE HYDROCHLORIDE. C: PEAK INDICATING POLOXAMER 188 D: INDICATES PEAK OF POLOXAMER WITH BZH. E: THE PEAK OF BZH NANOSPONGE FORMULATION

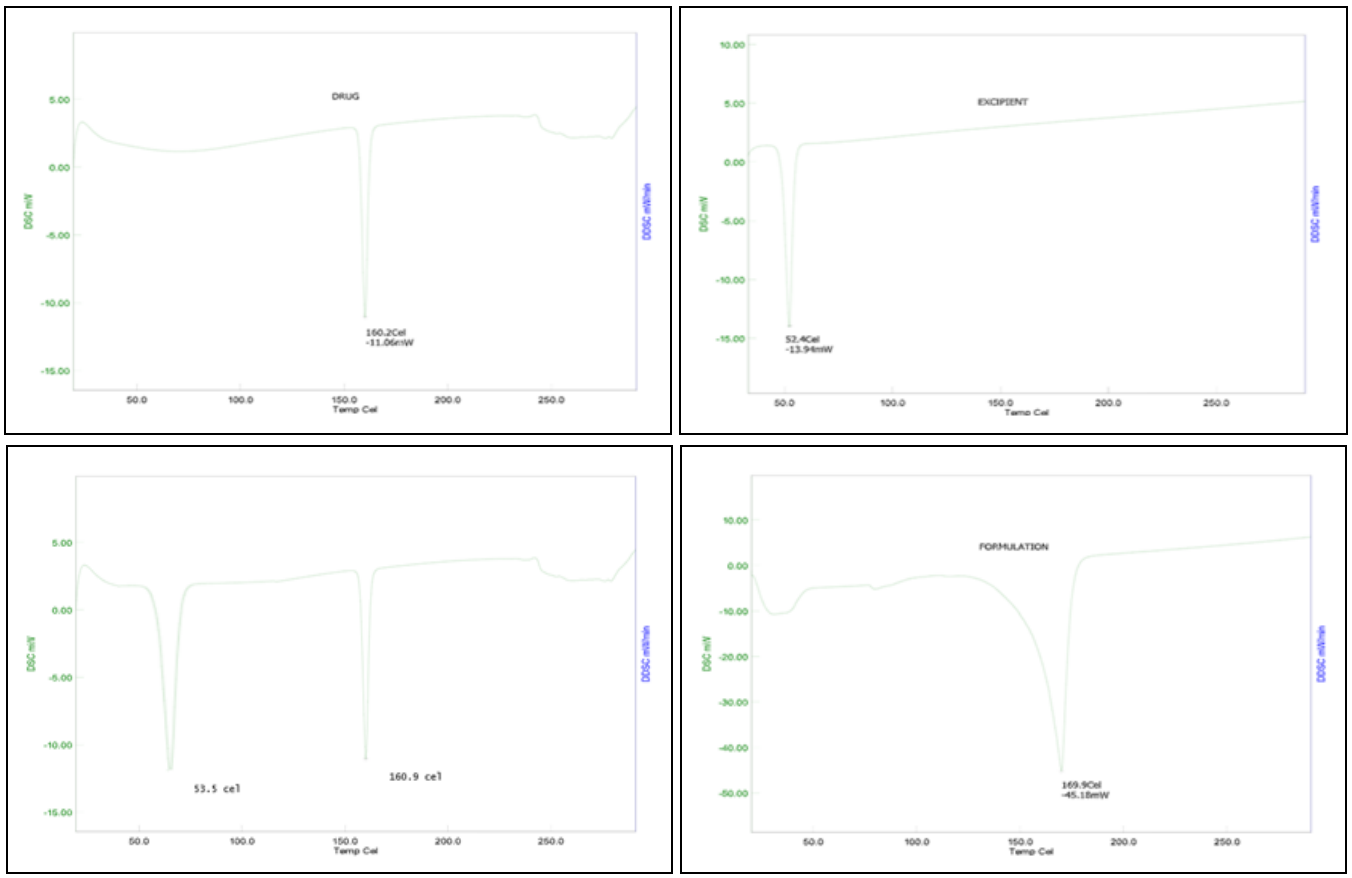


FIG. 2: PEAKS FOR DSC ANALYSIS

Differential Scanning Microscopy: As shown in **Fig. 2a**, DSC shows peaks at 52.2 °C for excipient, (b) 160 °C for BZH. DSC spectra are indicative of drug-polymer interaction that probably plays a role in modulating drug release; they were recorded towards higher melting temperatures. The physical

mixture in DSC spectra shown in **Fig. 2c** has no shift from 52.2 to 53, and a shift of peak from 160.2 to 160.9 shows there is no drug excipient incompatibility between the polymer and drug. The peaks in the possible cause for the increase in temperature in nanosponge formulation is the interaction between the polymer and the drug molecule that can form hydrogen bonding with hydroxyl groups of the polymer. The final formulation is shown in **Fig. 2d** a peak at 169 °C, which shows an enlargement of the peak due to encapsulation of the drug in the final formulation.

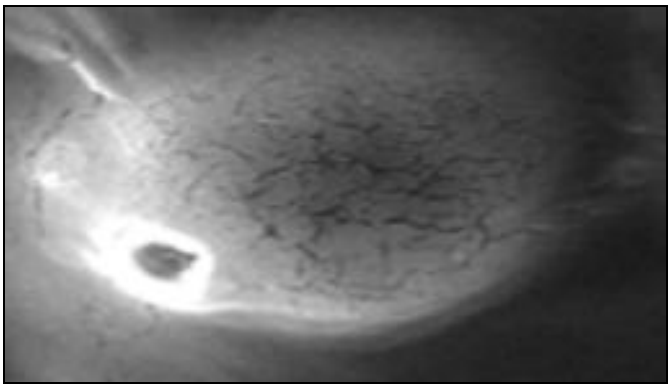


FIG. 3: SINGLE BZH-NS UNDER FESEM

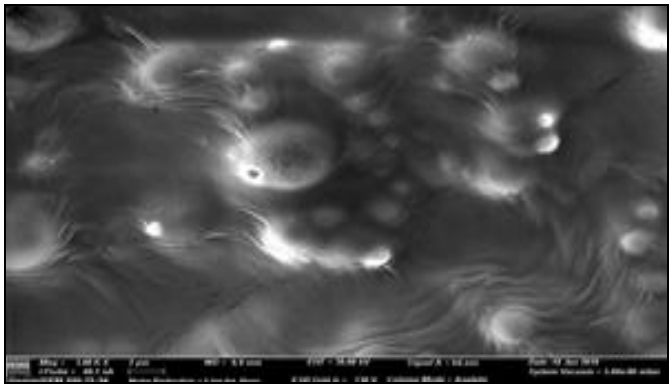


FIG. 4: MULTIPLE BZH-NS UNDER FESEM

Field Emission Scanning Electron Microscopy: The FESEM analysis of the formulation depicts spherical particles with pores and crevices on the outer edge, indicating the formation of nano-sponges, the internal morphology of the structures is porous as indicated in **Fig. 3** and **4**.

Particle Size and Polydispersity Index: The zeta potential of the batches was found to be between -17 to -150 mV as shown in **Table 2**. The particle size of the formulation was found to be between 128 nm to 250 nm; the optimized batch had a particle size of 128 nm.

The polydispersity of the formulation was between 0.3-0.7, and that of the optimized batch was 0.69, which indicates nearly unidisperse formulation.

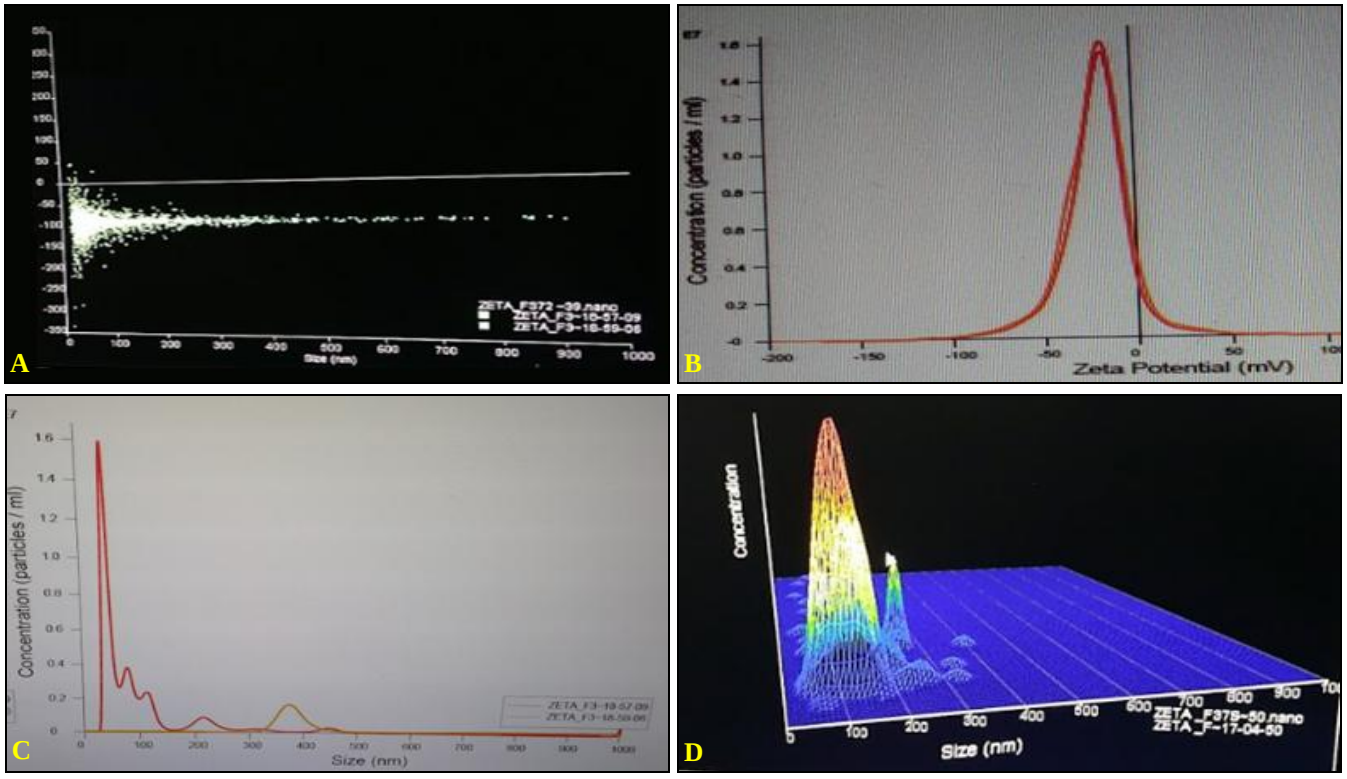


FIG. 5: (A) AND (B) SHOW ZETA POTENTIAL *vs*. CONCENTRATION OF F2 (C) AND (D) SHOW PARTICLE SIZE

TABLE 2: SHOWS PARTICLE SIZE ZETA POTENTIAL AND POLYDISPERSITY INDEX OF THE FORMULATIONS

Formulation	Ratio	Particle Size	Zeta Potential	Polydispersity Index
F1	1:1	141	-25mV	0.3
F2	1:1	166	-17mV	0.6
F3	1:2	128	-21mV	0.6
F4	2:3	148	-90mV	0.5
F5	1:8	210	-131mV	0.7
F6	1:5	191	-150mV	0.7
F7	1:4	250	-141mV	0.6

Entrapment Efficiency: The entrapment efficiency calculated of batches F1-F7 are indicated in **Table 1**; they were found to be between 72-88%.

Thin Layer Chromatography: The formation of nanosponges is indicated through the reduction of Refractive Index from the pure drug, the decrease in Rf shows entrapment of drug in molecules.

R_f value 0f 0.833 showed by plain drug BZH was more than the nanosponge formulation having R_f value of 0.476, indicating the formation of complexes as shown in **Fig. 6**.

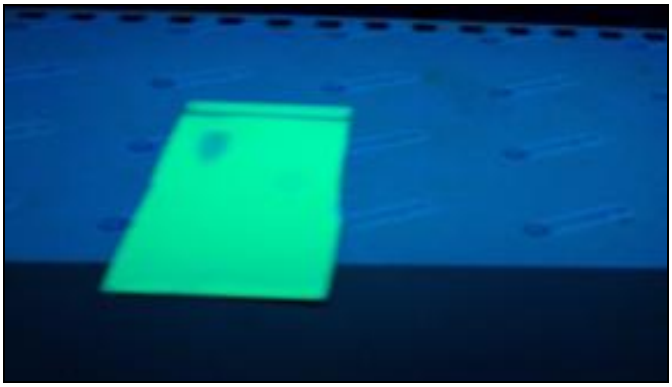


FIG. 6: TLC OF PLAIN DRUG IN COMPARISON WITH BZH-NS

Stability Determination: Short term stability shows that the nanosponges were stable physically and chemically.

The sample was analyzed at 25 ± 2°C and 65% RH, they were analyzed for Particle Size, Zeta Potential and physically for the clarity and appearance of the formulation, at time intervals of 0 days, 30 days and 180 days as shown in **Table 3**.

TABLE 3: STABILITY DATA OF THE F2 AT 25 ± 2 °C AND 65% RH

Test	0 day	30 days	180 days
Visual Appearance	Clear with low turbidity	Clear with turbidity	Turbid
Particle Size	128nm	144	251nm
Zeta potential	-17mV	-91mV	-166Mv

TABLE 3: STABILITY DATA AT 8 ± 2 °C

Test	0 day	30 days	180 days
Visual Appearance	Clear with low turbidity	Clear with low turbidity	Clear with low turbidity
Particle Size	128nm	131nm	133nm
Zeta potential	-17mV	-21mV	-31mV

Drug Release from Nanosponge Formulation: The *in-vitro* release profiles of the formulated nanosponges was done at pH 6.8 and pH 7. They were carried out using the Hi-Media 12,000Da dialysis membrane. The donor phase consisted of formulations containing a fixed amount of BZH in phosphate buffer pH 6.8. The drug release through the stable formulation was higher in pH 6.8, simulated pH of the oral cavity which was 94% when compared with the release in pH 7; simulated physiological pH found to be 81.8% due to higher solubility of a drug in phosphate buffer pH 6.8 the drug release shown by the formulations were for pH 7. Among all the prepared nanosponges, made with suspension, F2 exhibited the highest drug release. Drug release from nanosponges decreased as the total amount of polymer also increased when the ratio between crosslinker and polymer increases [10]. Ideally, the pattern shown by drug release is decreased as the ratio between cross-linker and polymer increases. When the concentration of the polymer is increased, the other factor involving the drug release is the particle size of the sponges. Higher release required lower particle size; also the dissolution behavior of nanosponges is greatly influenced by their surface area porosity and particle size distribution [10]. Finally, on the basis of physicochemical properties and particle size, F2 was selected for incorporation into the topical hydrogel. Carbopol 971 NF and Carbopol Ultrez were used for incorporation Benzydamine loaded nanosponge. The release is as shown in **Table 4**.

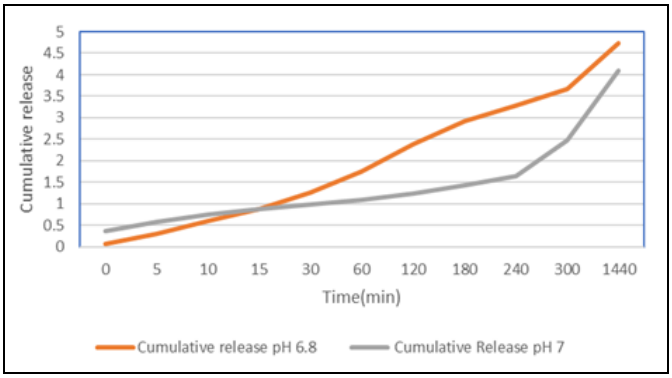


FIG. 7: COMPARATIVE RELEASE OF TWO GELS

TABLE 4: RELEASE OF F2 IN pH 6.8 AND 7

pH	Higuchi R^2	KorseMayer R^2	Zero Order R^2
6.8	0.864	0.941	0.5654
7	0.927	0.931	0.8863

Equilibrium Swelling Study: The equilibrium swelling obtained after 3 h and was found to be 0.94 g/g was G1 and 0.93 g/g for G2.

***In-vitro* Permeation of the Gel:** Gel was prepared as per **Table 5**. Franz Diffusion cell was used for carrying out *in-vitro* diffusion study for 24 h receptor compartment had a volume of 10ml and surface area of 3.14 cm^2. Receptor media used was phosphate buffer pH 6.8. The cellulose acetate membrane was carefully mounted on a diffusion cell. 0.5g of the gel was applied to the cellulose acetate membrane in the donor compartment. Air bubbles were eliminated from the receptor compartment. Contents were stirred using a magnetic bead, and the temperature was maintained by an external jacket to 37 ℃. 1ml aliquots were withdrawn at a periodic time interval. They were analyzed spectrophotometrically at 305.6 nm by UV-visible Spectrophotometer. The cumulative amount of drug was measured at 12 and 24 h. The release from G1 and G2 was calculated and was found that the release fromG1 was found to be 79.33% more than G2, which is 76%. Hence, G1 was used for further studies. Studies were further carried out replacing cellulose acetate membrane with natural membrane; porcine mucosa. The porcine membrane had the epithelial layer towards the donor compartment and connective tissue towards receptor membrane [13]. The release shown by G1 was less through porcine mucosa, and the flux obtained was 1192 µg/cm^2/h; thus, permeation enhancers such as propylene glycol were incorporated in order to improve permeability and hence the drug flux. The flux rate J was determined using the formula of flux

$$J = 1/A^* (dQ /dt) = DKC/ h$$

Where, Jss is the permeation rate (flux) at steady-state (mg/$cm^{2/}$h), A is the area of membrane (cm^2) through which the permeation of the drug takes place; (dQ/dt) ss is the amount of drug passing through the skin per unit time at steady-state (mg/h), C is the drug concentration in the vehicle (mg/ml), K is the partition coefficient of the drug (membrane/vehicle), h is the effective path length (cm), P is the permeability coefficient for the drug (cm/h).

The release shown by G1 was less through porcine mucosa, and the flux obtained was 1192 µg/cm^2/h; thus, permeation enhancers such as propylene glycol were incorporated in order to improve permeability and hence the drug flux. The flux obtained after the addition of propylene glycol was 1450 µg/cm^2/h; the steady-state flux and permeability coefficient of the hydrogel were significantly higher after the addition of permeation enhancer resulting in increased fluidity of mucosa and thereby improving drug partitioning to the mucosal layer.

Probably, the mucoadhesive properties for Carbopol 971 were better than Carbopol Ultrez 10.

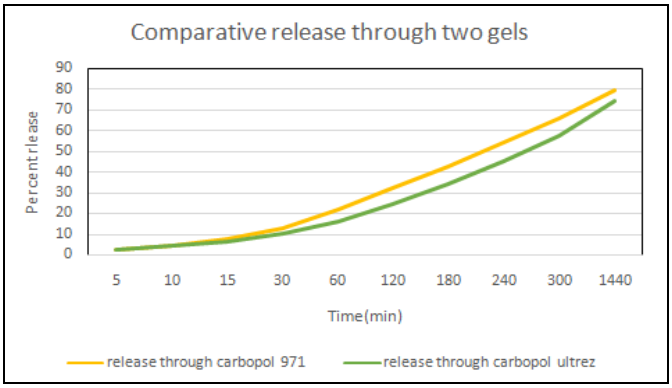


FIG. 8: COMPARATIVE RELEASE THROUGH CARBOPOL 971 AND CARBOPOL ULTREZ 10

TABLE 5: COMPOSITION OF GEL

S. no.	Composition of G1	Composition of G2	Quantity %
1	Benzydamine Hydrochloride NS	Benzydamine Hydrochloride NS	0.15
2	Carbopol 971 NF	Carbopol Ultrez 10	1.5
3	Propylene glycol	Propylene glycol	5
4	Methyl Paraben	Methyl Paraben	0.2
5	Glycerine	Glycerine	8
6	Distilled Water	Distilled Water	Q.S
7	Triethanolamine	Triethanolamine	Q.S to pH 6.8

The controlled release of Benzydamine hydrochloride from nanosponge-loaded hydrogel to the mucosa is due to the retention of drug-loaded nanosponges on the mucosal surface that gradually releases their drug contents over time.

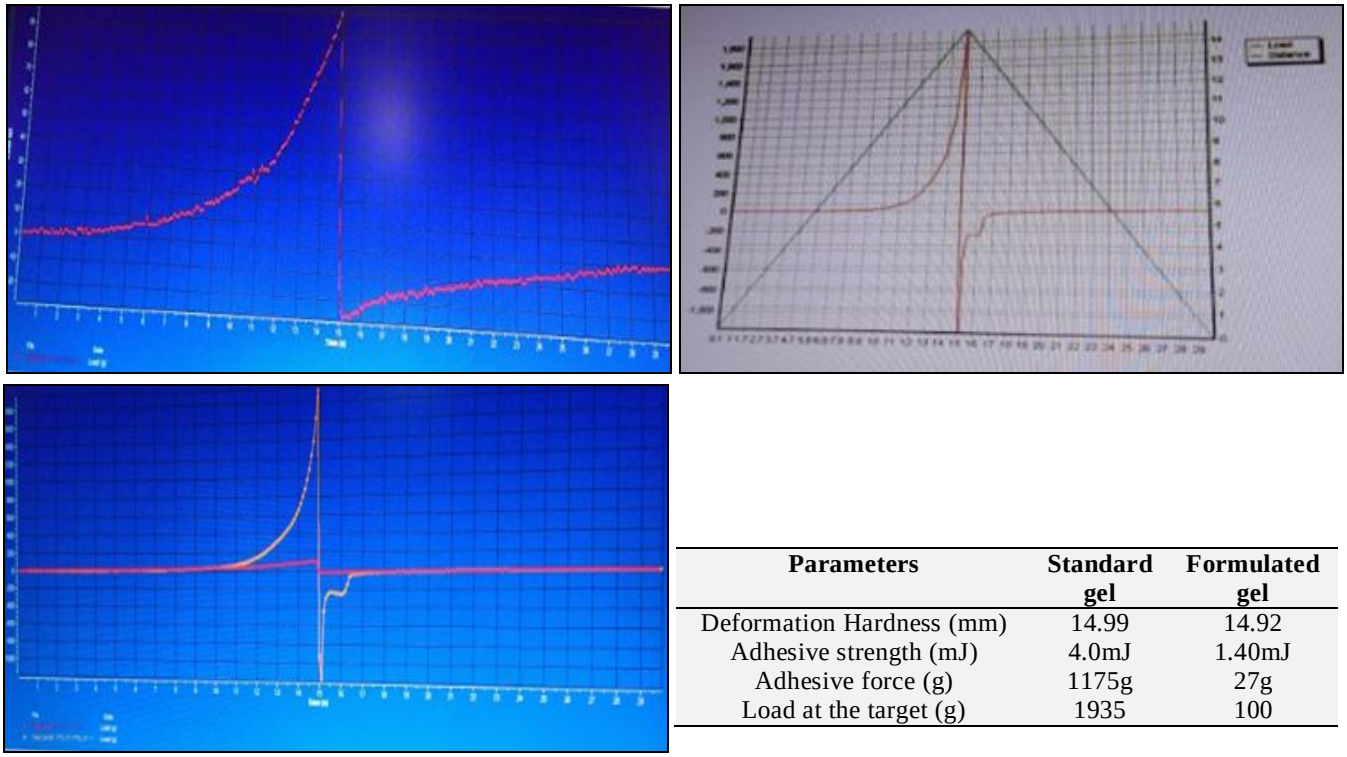
The formulation of a gel consisting of propylene glycol is formulated according to **Table 9**. Incorporation of BZH-NS into hydrogel delayed BZH release as entrapped into a three-dimensional network of Carbopol hydrogel, and the release rate from the gel matrix governs the mucosal penetration.

TABLE 6: COMPOSITION OF GELS

S. no.	Composition of NS gel	Composition of standard	Quantity
1	Benzydamine Hydrochloride NS	Benzydamine Hydrochloride	0.15
2	Carbopol 971 NF	Carbopol 971 NF	1.5
3	Propylene glycol	Propylene glycol	5
4	Methyl Paraben	Methyl Paraben	0.2
5	Glycerine	Glycerine	8
6	Distilled water	Distilled water	Q.S to 10 ml
7	Triethanolamine	Triethanolamine	Q.S to pH 6.8

The standard gel shows higher hardness and cohesive strength then the formulated gel, but due to the encapsulation of drug into the gelling matrix, the release for the gel is delayed-release obtained for formulated gel was slower than the standard.

Texture Analysis: The hardness expresses the applicability of the gel to the desired site. The gels should have low hardness value to be administered to the buccal mucosa easily. The compressibility expresses taking the prepared gel from the container and the simplicity of the spreadability on the application site. The smaller hardness value of the formulated gel indicates ease of application at the desired site. Also, the low adhesive strength and low adhesive force indicate ease of spreading of the gel. As shown in **Fig. 9**.



Parameters	Standard gel	Formulated gel
Deformation Hardness (mm)	14.99	14.92
Adhesive strength (mJ)	4.0mJ	1.40mJ
Adhesive force (g)	1175g	27g
Load at the target (g)	1935	100

FIG. 9: LOAD *vs.* TIME

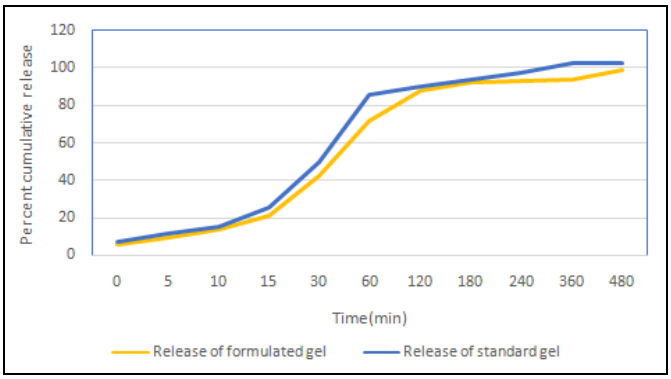


FIG. 10: COMPARATIVE RELEASE BETWEEN FORMULATED GEL AND STANDARD GEL

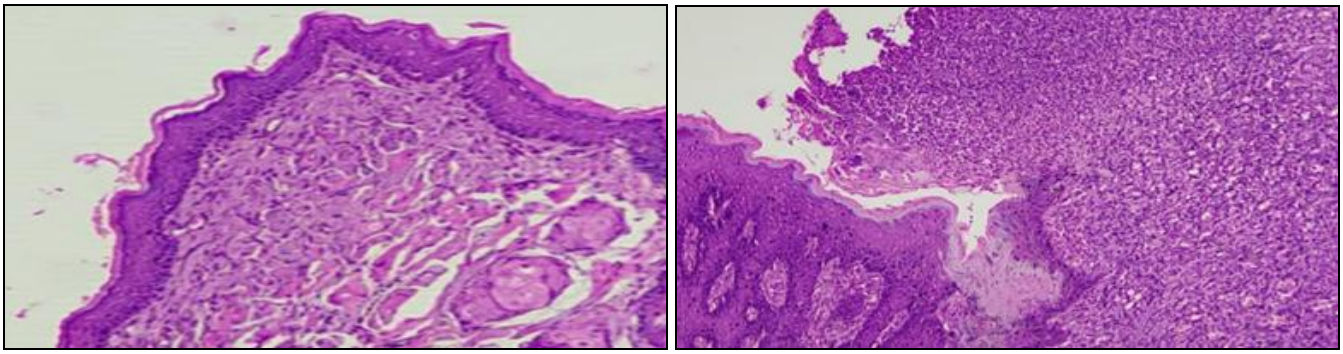


FIG. 11: I. MUCOSAL LAYER BEFORE II. MUCOSAL LAYER AFTER APPLICATION BZH -NS LOADED GEL

Application of BZH-NS loaded Gel: The application of gel is done for a longer duration of time, for the determination of histological studies. The gel was applied to the Albino Wistar Rats as approved by the Institutional Animal Ethics Committee (KMKCPIAEC 181914) by use of cotton bud on the left side of the buccal mucosa. It showed no damage to the mucosal layer, as indicated in **Fig. 11i** and **11ii** before and after the application of gel**.**

CONCLUSION: Nanosponges are incorporated in hydrogels to increase their permeability through the buccal mucosa on application to the oral cavity, to improve the release characteristic, decrease the dosing frequency and hence to increase their bioavailability and to reduce the toxicity. The hypothesis states that these nanosponges form channeled structures that help the passage of drugs across the barrier. Differential Scanning Calorimetric analysis was carried out to verify the effect of pre-formulatory conditions on the state of the drug while it was entrapped in nanosponges. The thermogram of pure drug Benzydamine Hydrochloride showed a sharp exothermic peak at 160 ℃, and the excipients were also analyzed by the same to determine drug excipient interaction.

The disappearance of the peak was due to the entrapment of drug in the nanosponge complex and the interaction between the polymer and the drug molecule that can form hydrogen bonding with hydroxyl groups of the polymer. They were analyzed for formation as they were loaded in the hydrogel, the formed nanosponges were confirmed through DSC, FESEM, and TLC analysis. On incorporation into Carbopol hydrogels, the release given by Carbopol 971 was better than Carbopol Ultrez, forming polymer of choice. The formulated topical drug delivery system containing Benzydamine Hydrochloride loaded nanosponges was thus identified as a system that has the potential to induce prolonged drug release and could, therefore, produce some advantages such as reduction in total dose, frequency of dosing and dose-related systemic side-effects and hence increased bioavailability.

ACKNOWLEDGEMENT: I would like to acknowledge Bal Pharma Pvt. Ltd., for providing Benzydamine Hydrochloride.

CONFLICTS OF INTEREST: There is no conflict of interest between the authors.

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How to cite this article:
Shaji J and Vaswani T: Formulation optimization of benzydamine hydrochloride nanosponges loaded hydrogel for treatment against mouth ulcers. Int J Pharm Sci & Res 2020; 11(10): 4945-56. doi: 10.13040/IJPSR.0975-8232.11(10).4945-56.