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FORMULATION AND EVALUATION OF NANOSPONGES LOADED HYDROGEL OF TIZANIDINE HYDROCHLORIDE

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ABSTRACT

Tizanidine hydrochloride, is centrally acting skeletal muscle relaxant. It undergoes first pass metabolism after oral administration which result in less bioavailability (40%). Tizanidine hydrochloride is mostly used in treatment of multiple sclerosis, back pain or certain injuries to the spine or CNS. The present study was carried out to formulate and evaluate Nanosponges loaded hydrogel of Tizanidine hydrochloride which will overcome of limitation of oral bioavailability. In the present research work nanosponges were prepared by hyper-cross linked β-cyclodexrin method by using diphenyl carbonate as cross-linker. Cyclodextrin based nanosponges are gain more consideration due to their improvement of solubility of poorly water soluble drugs. Four different formulations of hydrogels were prepared by using carbopol 934 with varying concentration of carbopol 934 and various evaluation studies were carried out. It was found that, nanosponges has potential modify drug release rate. The nanosponges loaded hydrogel has good drug release within 12 hours (96.33%), good stability, no irritancy.

KEYWARDS: Tizanidine Hydrochloride, Nanosponges, Hydrogel.

INTRODUCTION

Today, number of drugs are taken orally, but these are not as effective as required. The conventional dosage form are essential to be administered in multiple doses at specific time interval with specific amount required for effective therapy. Drugs which are administered by multiple dosing has various disadvantages such as lack of patient compliance, inconvenient

administration, first pass metabolism, chances of overdose in case of administered prior to time interval, skip of dose by patient, to reduce such problems transdermal drug delivery system are designed.[4,5] Transdermal drug delivery system is the non-invasive delivery of medicament from the surface of skin-the largest and highly accessible organ of human body.[1,3] Transdermal drug delivery system has significant advantages over other conventional delivery system. Transdermal delivery provide constant administration, controlled delivery of the drug and also allows continuous input of drugs having short biological half-lives and eliminates pulsed entry into systemic circulation, which may causes undesirable side effects. The main advantage of the topical delivery system is to avoid first pass metabolism. Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption like presence of enzymes, gastric emptying time, pH changes are another advantage of topical preparations. Nanosponges loaded topical formulations can act as local transformer for rate-limiting membrane barrier for modulation of systemic absorption and sustained drug release and therefore overcome the limitations of topical formulations Tizanidine hydrochloride, is centrally acting skeletal muscle relaxant. It usually undergoes first pass metabolism after oral administration resulting in less bioavailability (40%) therefore there is need to design formulation to overcome limitation of less bioavailability. Thus the main objective of the present study was to formulate and evaluate nanosponges loaded hydrogel of Tizanidine hydrochloride.[6]

MATERIALS AND METHODS

Tizanidine Hydrochloride was gifted by Blue cross, Nashik. Betacyclodextrin, diphenyl carbonate, DMSO, Triethanolamine, carbopol 934, Propylene Glycol and alcohol purchased from modern science, Nashik. All other excipients and solvents used were of the analytical pharmaceutical grade.

Compatibility Studies Using FT-IR Spectroscopy

A compatibility study for Tizanidine hydrochloride was carried out with excipients to determine possibility of any drug-excipients interaction/ incompatibility. Excipients studied included Carbopol 934, β-cyclodextrin physical mixture. These samples were subjected to compatibility studies and stored for 30 days at elevated temperature and humidity conditions of 40 ± 2^0C / 75 ± 5% RH. After 30 days, IR spectra of these stored samples were obtained. IR studies of the drug and excipients were carried out to understand the compatibility between them. The results are shown in figure no 2.

Methodology: Preparation of Nanosponges[9]

Tizanidine Hydrochloride was formulated by hyper-cross linked β-cyclodexrin method. Nanosponges of β-cyclodextrin were prepared by using diphenyl carbonate as cross-linker. β-cyclodexrin and diphenyl carbonate was use in four different molar ratios as 1:2, 1:4, 1:6, 1:8 were dissolve in 50ml dimethyl sulfoxide and it allowed to react at 90^0C for 4 hours. After completion of reaction, cool it and excess of deionized water added to remove the unreacted β-cyclodexrin and diphenyl carbonate. The obtained white powder was dried overnight in oven at 60^0C. NSs were stored at 25^0C. The composition of nanosponge formulation was in Table no.1. The prepared nanosponges were characterised depending upon the entrapment efficacy and particle size.

Preperation of Tizanidine Hydrochloride Loaded Nanosponges[9]

Aqueous suspension (10ml water) of nanosponges was prepared and adequate amount of drug (80mg) was dispersed in it with constant stirring for specific time (30min) required for complexation. After complexation, the uncomplexed (undissolved) drug from complexed drug separated by centrifugation (10min). Then the solid crystals of nanosponges are obtained by solvent evaporation method.

Table. 1: Composition of Nanosponges Formulation.

Formulation code	NSs1	NSs 2	NSs 3	NSs 4	NSs 5	NSs 6
β-CD/ cross linker ratio	1:2	1:2	1:4	1:4	1:6	1:6
Drug/NSs ratio	1:1	1:2	1:4	1:6	1:8	1:10
% EE	78.90	81.0	83.5	92.7	87.50	85.8

Preparation of Tizanidine Hydrochloride Nanosponges Loaded Hydrogel[10]

Accurately weighed amount of carbopol 934 was taken and soaked in 100ml water for 24 hours for complete swelling of the polymer. To the weighed amount of carbopol gel base, Tizanidine hydrochloride nanosponges equivalent to 1%w/w were uniformly dispersed. Methyl paraben and propyl paraben were added as a preservative. Glycerine as moistening agent. Triethanolamine was added drop wise with gentle stirring for adjusting the pH.

Table. 2: Composition of Nanosponges Loaded Hydrogel Formulation.

Ingredients	Quantity
Tizanidine hydrochloride nanosponges	1 % w/w
Carbopol 934	0.25% w/v
Methyl paraben (g)	0.1
Propyl paraben (g)	0.05
Methanol (ml)	5

Glycerin (ml)	10
Triethanolamine	q.s.

Evaluation Studies of Prepared Nanosponges

Characterization of Nanosponges

1. Particle size analysis

The particle size of Tizanidine hydrochloride nanosponges was determined using a Malvern Zeta sizer. From this, the mean diameter was measured. Measurements were made at the fixed angle of 90° of the samples. The samples were suitably diluted with distilled water during measurement. The instrumental setting was fixed at a viscosity, temperature and refractive index of 0.887 cp, 25^{o}C and 1.33 respectively.

2. Scanning electron microscopy

Scanning electron microscope was used for the evaluation of the surface morphology of nanosponges.

3. Production yield (%)

For calculating production yield, the theoretical mass was calculated initially by taking the mass of solid ingredients added. All the prepared nanosponges formulations were accurately weighed and the weight was recorded. The production yield of the nanosponges was then determined using by using the following equation,

Production yield (%) = Practical mass of nanosponges x 100 / Theoretical mass (polymer + drug)

4. Entrapment efficiency

The entrapment efficiency of nanosponges were determined by adding 10 ml of phosphate buffer of pH 7.4 and sonicated in a bath sonicator and filtered. 1 ml of filtrate is made up to 10 ml with phosphate buffer and was assayed spectrophotometrically at 318 nm (UV visible spectrophotometer, model UV-2600, Shimadzu). The amount of drug entrapped was calculated from the following equation.

$$\%EE = \frac{\text{Amount of Drug} - \text{Concentration of drug x 100}}{\text{Total amount of drug}}$$

5. Zeta potential: Zeta potential was performed for the nanosponges dispersion to check the stability of dispersion.

6. Differential Scanning Calorimetry (DSC): Thermogram of Tizanidine hydrichloride nanosponge formulation batch F4 was obtained using differential scanning calorimeter. Nanosponge samples were kept in aluminium pan, sealed and heated at heating rate 10^0/min over temperature range of 50 to 400°C. By purging nitrogen with flow rate of 100ml/min.

Evaluation Studies of Prepared Nanosponges Loaded Hydrogel

1. Viscosity determination

The viscosity of prepared hydrogels was measured using Brookfield viscometer. Viscosity was measured at 25^0C at 1 rpm using spindle no.LV- 61.

2. pH determination: The pH of hydrogel formulation was noted using calibrated pH meter. 1gm of Tizanidine hydrochloride nanosponge loaded hydrogel was uniformly dispersed in 100 ml of distilled water and kept for 2 hours at room temperature. Then, pH of the dispersion was measured at 25^oC.

3. Spreadability: 1 gm of gel was weighed and put it on the spreadability apparatus having one glass slide at lower side and another slide at upper side. Weight was tied at upper slide. After putting gel onto the lower slide, upper slide put onto lower slide and counted the time required to slide over both slides from gel. And calculate the spreadability using formula, (Length of slide = 15.9cm).

a. S= M*L/ T

b. S= spread ability

c. M = weight tied to upper slide

d. L = length of glass slide.

e. T = Time taken to separate two slides (sec).

4. Drug content estimation

One gram gel (10 mg drug) was taken into 10ml of volumetric flask and was diluted upto the marks with methanol (1000µg/ml). The solution was filtered through whatmann filter paper no.42. 1ml of above solution was pipetted out in 10ml volumetric flask and diluted to the marks with phosphate buffer pH 7.4 (100µg/ml). From this 1 ml of solution was pipetted out and transferred into 10ml volumetric flask and diluted to the marks with phosphate buffer pH 7.4 (10µg/ml). Absorbance of resulting solution was measured at 318 nm. The concentration of Tizanidine hydrochloride can be obtained as, y= mx+c.

5. *In-vitro* drug release studies

In-vitro drug release study was carried out by using Franze diffusion cell. The Tizanidine hydrochloride nanosponge loaded hydrogels were permeated through an artificial cellophane membrane. The receptor medium was filled with phosphate buffer of pH 7.4. The cellophane membrane was previously soaked overnight in the diffusion medium which was placed between donar and receptor compartment. 0.6 gm of hydrogel was spread uniformly on the membrane, which is in contact with receptor medium. The whole assembly was placed on the thermostatically control magnetic stirrer with continue stirring. During the experiment, temperature was maintained at 37± 0.5^0C to simulate the human skin condition. At specific interval, 1 ml of sample was withdrawn from the receptor compartment and replace with same volume of PBS 7.4. The samples withdrawn were analysed spectrophotometricaly at 318nm. The amount of drug released was calculated and the percentage drug released was plotted against time.

Kinetics of in vitro drug release: The results obtained from in vitro release studies were attempted to be fitted into various mathematical models as follows

1. Cumulative percent drug released Vs. Time (Zero order kinetics)
2. Log cumulative percent drug retained Vs. Time (First order kinetics)
3. Cumulative percent released Vs. The square root of Time (Higuchi model)

Log cumulative percent drug released Vs. Log Time (Korsmeyer-Peppas model) Peppas model, the value of ‘n' show the release mechanism of the drug.

6. Stability studies

Stability testing plays a important role in the drug development process. The objective of stability testing is to provide evidence on how the quality of prepared formulation varies with time under the influence of different environmental factors, such as temperature, humidity, and light to recommended storage conditions and recommend shelf life for the drug product. Stability studies were carried out on the optimized formulation according to ICH guidelines. The optimized formulation was packed in a tightly closed container and was stored in the ICH certified stability chamber maintained at 40 ± 2^0C and 75% ± 5% RH for 60 days. Samples were withdraw at intervals of fifteen days and checked for physical appearance, pH, drug content.

7. Skin irritancy study

Skin irritation study was performed with prior permission of Institutional Animal Ethical committee (IPEC) under the purview of committee for the purpose of control and supervision of Experimental Animals (CPCSEA), on Swiss albino mice (25-30g, 3 male and 3 female) was applied with the hair remover cream, under anaesthesia, 24 hour before the beginning of experiment. About 1 g of final formulation to be tested was applied to the sensitive part of the skin (one site) and other site was kept as control. Animals was observed for 7 days to check the skin reddening and inflammation on animals.

RESULTS AND DISCUSSION

Compatibility studies using FT-IR Spectroscopy

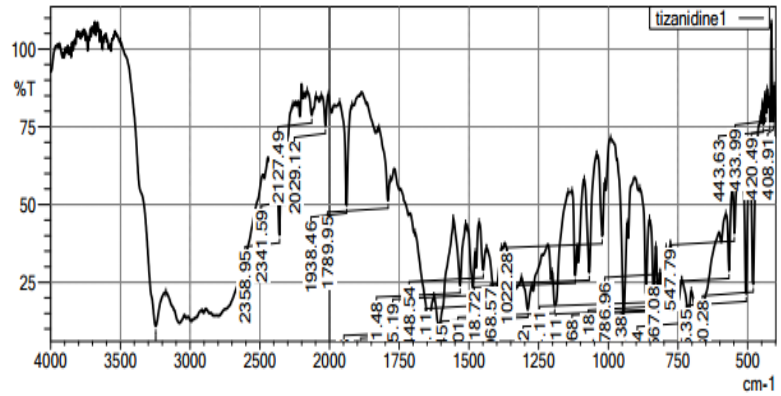


Figure No. 1: FTIR spectra pure drug.

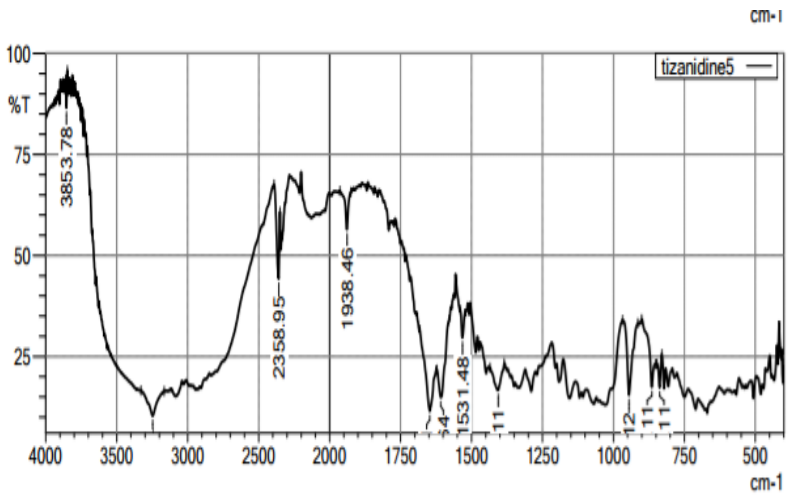


Figure No. 2: FTIR Spectra drug + polymer.

After the compatibility study of Tizanidine hydrochloride with excipients, the IR spectra of pure drug and drug-excipient physical mixture were analyzed. The figure 1 & 2 indicate no

interaction between drug and excipients when compared with spectra of the pure drug as all functional groups were present.

Table. 3: Identification of Functional Groups in Ftir SPECTRA of Drug.

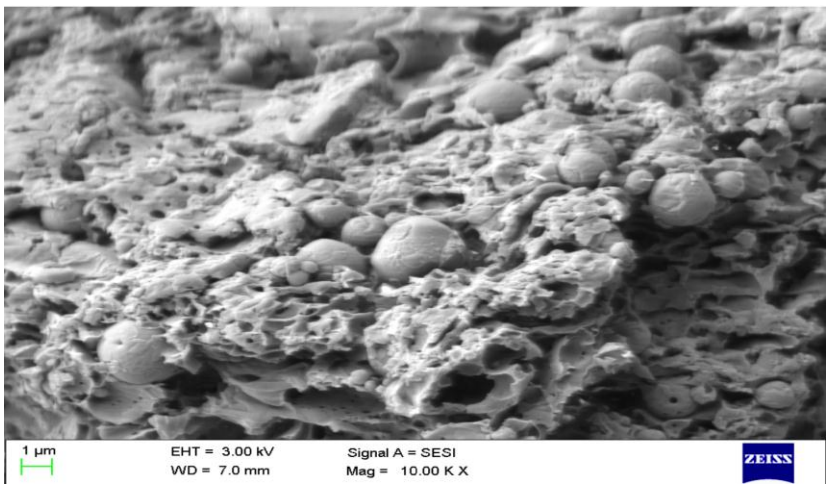
Functional groups	Reported peak frequency (cm^{-1})	Observed peak frequency (cm^{-1})
Aromatic C=C stretch	1400-1600	1608.64
C-H bending (Aromatic)	800-900	837.11
C=N(imines, oxime)	1645-1607	1647.21

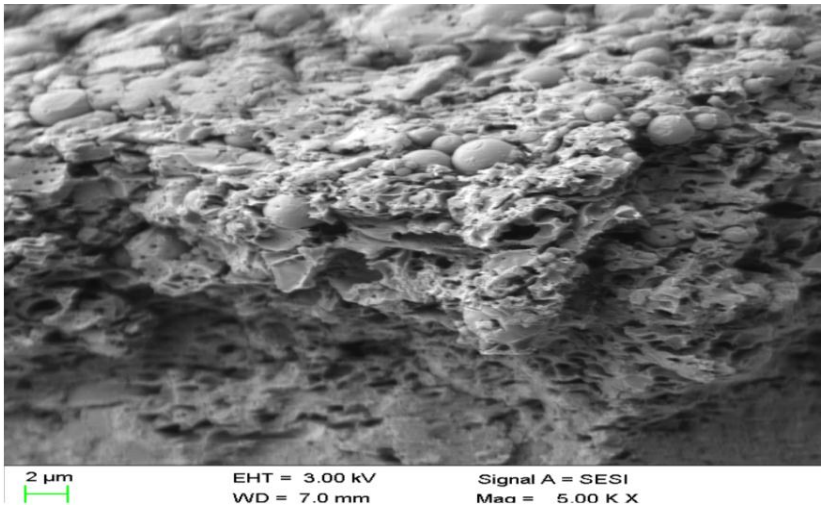
Evaluation of Nanosponges of Tizanidine Hydrochloride

Table. 4: Production Yiled and Entrapment Efficiency of Tizanidine Hydrochloride Nanosponge.

Batch code	Production yield (%)	%EE
F1	37.69	78.90
F2	67.29	81.0
F3	42.14	83.5
F4	44.64	92.7
F5	45.19	87.50
F6	45.81	85.8

1.Scanning electron microscopy: The SEM images showed that the surface of prepared nanosponges was spherical in shape and uniform in size and its surface was porous in nature.





2. Zeta potential

It is the electric potential in interfacial double layer i.e. the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. It is used for the quantification of the magnitude of the charge. Zeta potential is the key indicator of the stability of colloidal dispersion. The magnitude of the zeta potential indicates degree of electrostatic repulsion between similarly charged particles. When zeta potential is small, attractive forces may exceed this repulsion and tend to flocculate. So colloids with high zeta potential negative or positive are electrically stabilized while with low zeta potentials tend to flocculate. As depicted in table no.8.20. The Nanosponges formulation for the optimized batch is having excellent stability.

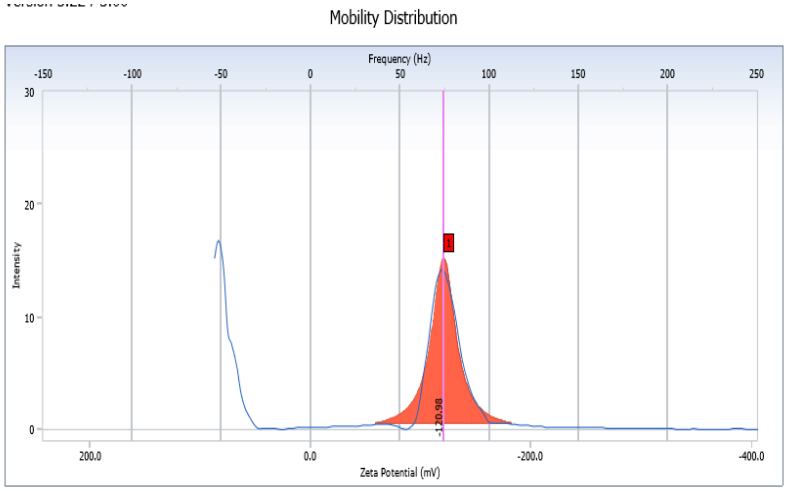


Fig. 3: Zeta Potential of Tizanidine Hydrochloride Nanosponges Batch F4.

Table. 5: Zeta Potential Study of Optimized Nanosponges Batch F4.

Peak	Zeta potential (mV)	Mobility (cm^2/Vs)
1	-120.98	-9.435e-004
2	- mV	- cm^2/Vs
3	- mV	- cm^2/Vs
Mean	-120.98	-9.435e-004

Table. 6: Zeta Potential Determination of Optimized Batch F4.

Determination of optimized batch Standard for Zeta potential (mV)	Stability behavior	Obtained results of nanosponges batch F4 (mV)
From 0 to ±5	Rapid coagulation or flocculation	
From 10 to ±30	Incipient instability	
From 30 to ±40	Moderate stability	
From 40 to ±60	Good stability	
More than ±61	Excellent stability	-120.98

3. Paticle size and polydispersity index

Particle size was done by zeta sizer of optimized batch F4. The particle size was found to be 382.3nm which is less than 500nm hence preferred for Nanosponges preparation. Graph was observed, in which the particle size ranges from 200 to 800nm which is in increasing order due to increase in concentration of polymer but after certain concentration the ratio of drug to polymer was increased the particle size decrease.

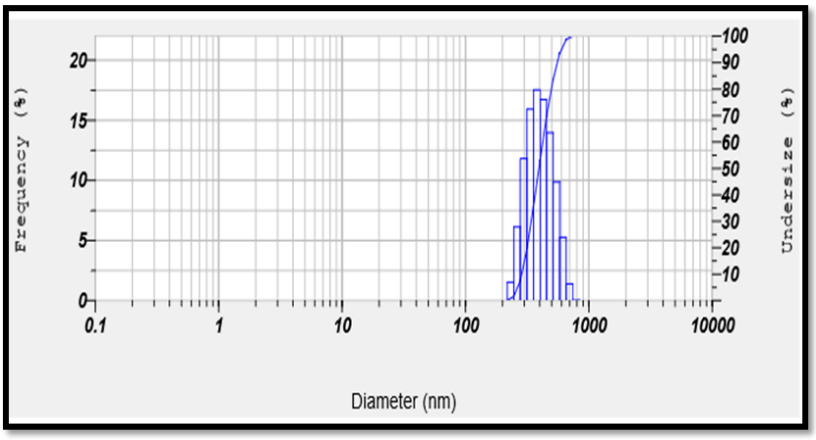


Fig. 4: Particle Size of Tizanidine Hydrochloride Nanosponges Batch F4.

Peak No.	S.P. Area Ratio	`S.D	Mean
1	1.00	46.7nm	382.3nm
2	---	---nm	---nm
3	---	---nm	---nm
Total	1.00	46.7nm	382.3nm

PDI

PDI is an index of width or spread or variation within the particle size distribution. Monodisperse sample have lower PDI value, whereas PDI of higher value indicates a wider particle size distribution and polydisperse nature of sample. PDI can be calculated by the following equation,

$PDI=\Delta d/d_{avg}$

PDI= 46.7/382.3, =0.122

Where, D is the width of distribution denoted by SD.

d_{avg} is the average particle size denoted by MV(nm)in particle size data sheet.

As depicted in table no.7. The Nature of Nanosponges formulation for the optimized batch shows mid-range of monodispersiblity.

Table. 7: Polydispersibility Index According to Its Type of Dispersion.

Polydispersibility index	Type of dispersity
0-0.05	Monodisperse standard
0.05- 0.08	Nearly Monodisperse
0.08-0.7	Mid-range Monodisperse
>0.7	Very polydisperse

Evaluation of Nanosponges Loaded Hydrogel

Table. 8: Evaluation Parameters of Nanosponges Loaded Hydrogel Formulation.

Batch code	pH	% drug content	Viscosity (cp)
F1	7.1	95.4	10471
F2	7.0	94.2	9541
F3	6.83	93.3	8641
F4	6.88	92.3	6981

4. *In Vitro* Drug Release Study

The in vitro release studies were carried out in phosphate buffer of pH 7.4 using cellophane membrane in a Franz diffusion cell apparatus. Amongst all formulations, F1 formulation showed highest drug release compared to other formulations. The F1 formulation showed drug release of 96.13% at the end of 12 hour. The in vitro release data were plotted in Figure.

Table. 9: % Drug Release Study of All Batches.

Time in hrs	Batch 1	Batch 2	Batch 3	Batch 4
	% DR	% DR	% DR	% DR
1	1.86	5.38	2.33	0.3015
1.3	5.93	7.66	8.16	4.10
2	13.46	14.5	14.66	9.65
2.3	15.63	18.83	22.83	17.5
3	19.66	24.16	26.5	22.83
3.3	24.96	27.66	30.66	30.23
4	30.76	31.33	36.5	34.71
5	37.06	36.66	41.5	39.27
6	43.83	46	46.16	44.04
7	56.76	53	51	49.25
8	60.8	61.16	56.83	55.08
9	63.7	71.5	64.83	62.15
10	81.2	78.33	71.16	68.81
11	88.2	86.0	79.66	79.48
12	96.13	94.66	90.66	86.9

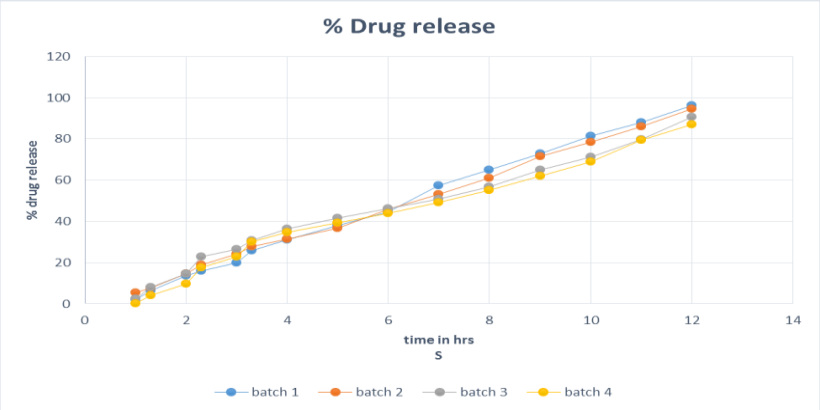


Fig. 5: Comparison of percentage drug release profile of formulations.

Kinetics of in vitro drug release: The in vitro drug release data of all the Tizanidine hydrochloride nanosponge loaded hydrogel formulations was subjected to the goodness of fit test by linear regression analysis according first orders and zero order kinetic equations, Higuchi's and Korsmeyer–Peppas models to ascertain mechanism of drug release.

Table. 10: Kinetic study of nanosponges based hydrogel formulations.

Formulation code	Zero order	First order	Higuchi model	Korsmeyer-Peppas model
	R^2	R^2	R^2	R^2
HF1	0.94	0.85	0.98	0.95
HF2	0.97	0.83	0.98	0.98
HF3	0.86	0.69	0.91	0.90
HF4	0.92	0.56	0.90	0.79

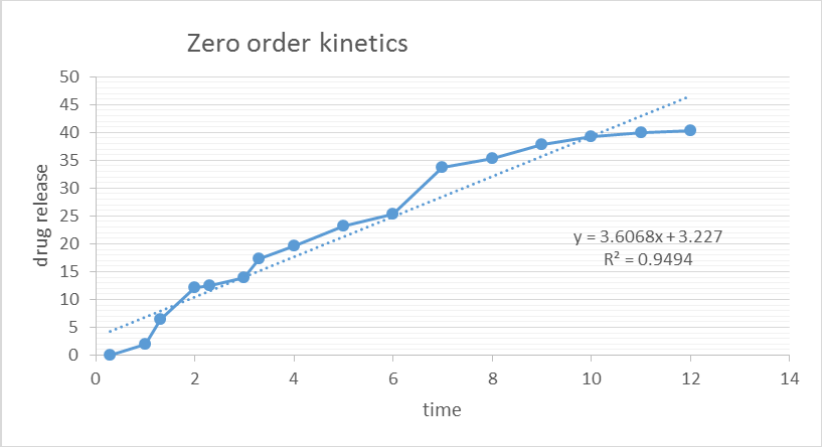


Fig. 6: Zero-Order Release Kinetics Profile of Optimized Formulation F1.

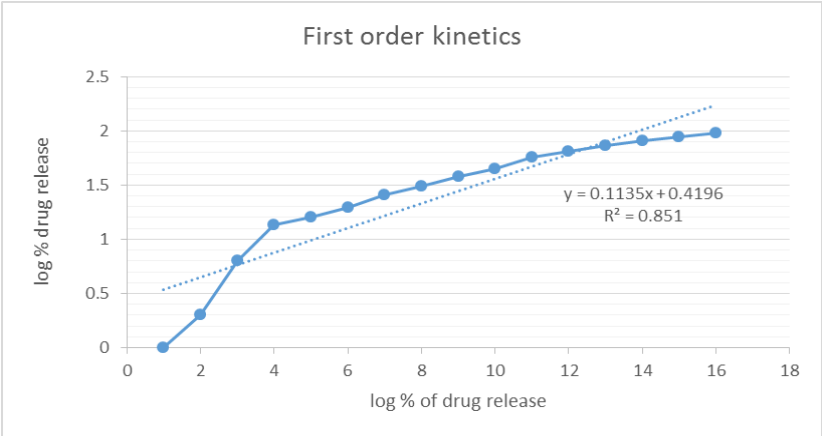


Fig. 7: First Order Release Kinetics Profile of Optimized Formulation F1.

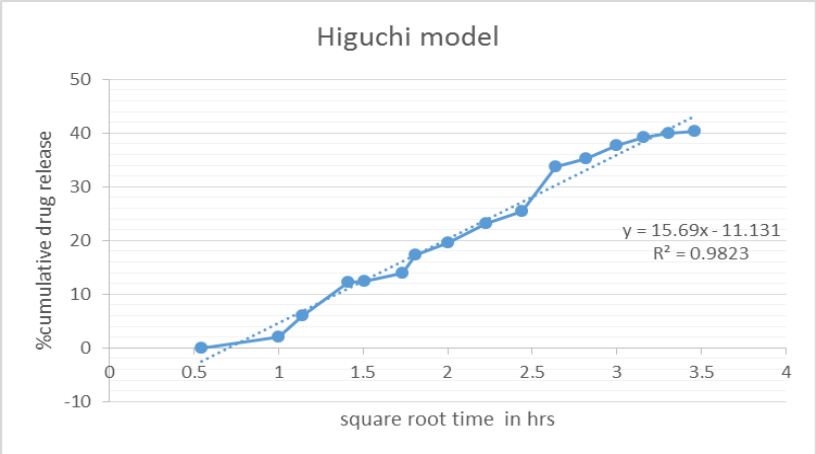


Fig. 8: Higuchi Release Kinetics Profile of Optimized Formulation F1.

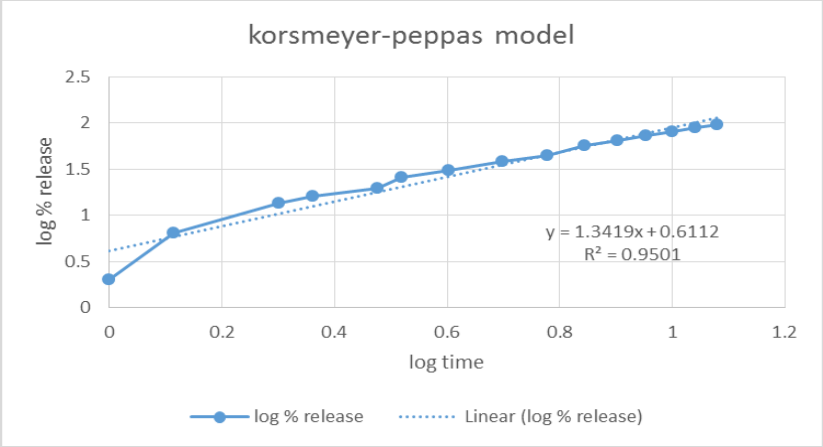


Fig. 9: Korsmeryer-Peppas Release Kinetics Profile of Optimized Formulation F1.

5. Stability Studies

Stability studies were carried out on optimized formulation F1 for a period of 2 month. The comparison of the parameters at predetermined time interval studies was represented in Table 10.

Table. 10: Evaluation parameters of hydrogel formulations before, during and after stability.

Parameters	Before stability study	Stability study (after15 days)	Stability study (after15 days)
pH	7.1	6.9	7
% drug content	95.4	94.8	95.2
Appearance	White colored nanosponges suspended in a transparent gel base	white colored nanosponges suspended in a transparent gel base	white colored nanosponges suspended in a transparent gel base

6. FTIR Study

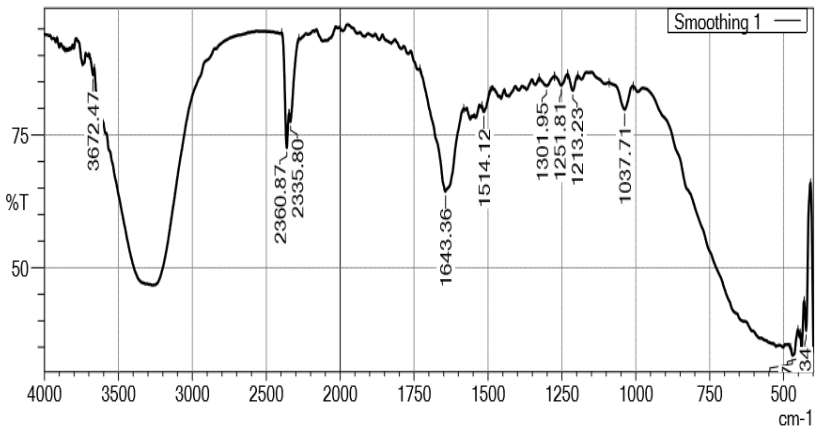
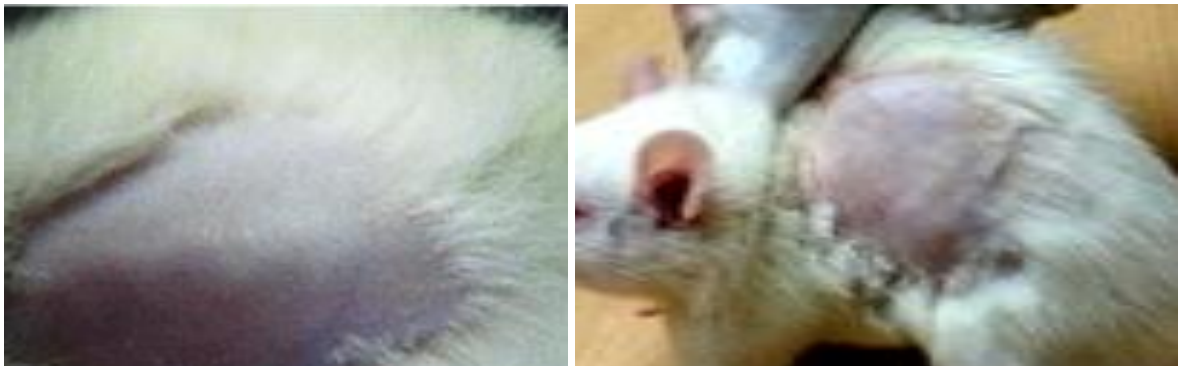


Fig. 10: FTIR Spectra Tizanidine Hydrochloride Nanosponges Hydrogel.

7. Skin Irritation Study

Skin irritation test was performed on Swice Albino mice for optimized formulation. The formulation did not indicate any evidence of skin irritation such as redness of skin or any change in skin observed for 72 hrs after the application of gel. Thus it may be concluded that formulation does not have skin irritation potential and is safe for topical application.



a. **b.**

Fig.11: Skin irritation test.

a. Control

b. After the application of medicated gel to mice

Table. 11: Skin irritation study.

Formulation	Presence of edema 24 hrs	Presence of edema 48 hrs	Presence of edema 72 hrs
Control	0	0	0
Medicated hydrogel	0	0	0

CONCLUSION

The prepared nanosponges was successfully incorporated into topical hydrogel. It can be concluded that, due to their very small particles and its porous structure improve the solubility and bioavailability of poorly soluble drugs. Nanosponges technique offers entrapment of ingredients and site specific delivery, reduce side effects, increase elegance and also nanosponges permit the insoluble drugs and prevent physicochemical degradation of active drug. The mean particle size of all nanosponge formulations was found in the range of 200-800 nm. FT-IR studies confirmed no drug polymer interaction. In-vitro drug release study has shown drug release 96.33% within 12hrs. It has shown no irritancy in skin irritation study. There was no significant changes in the physical parameter when stored temperature and humidity condition of 40 ± 2^{0}C/75±5% RH in the stability study carried out as per ICH guidelines. The nanosponges based formulation showed good stability and better drug

release. The nanosponge system was found to have better penetration of drug through the skin and hence we can speculate that Tizanidine hydrochloride nanosponge loaded hydrogel formulation is a good candidate for topical drug delivery.

REFERENCES

1. Dheeraj T. Baviskar, Jain Dinesh K., Novel Drug Delivery Systems, Nirali Prakashan, Third Edition, 2016; 6: 1-6.6.
2. G. Jilsha, Vidya Viswanad, Nanosponges loaded hydrogel of Cephalexin for topical delivery, International Journal of Pharmaceutical Science and Research, 2015; 6(7): 2781-2787.
3. Anjali S. Kumar et al, Formulation and evaluation of antifungal nanosponges loaded hydrogel for topical delivery, International Journal of Pharmacy and Pharmaceutical Research, 2018; 13(1): 362-378.
4. Bahiram Monika et al, Transdermal drug delivery system with formulation and evaluation aspect", 2012; 1168-11726
5. Nikhil Sharma et al, A Review: Transdermal drug delivery system: A tool for Novel Drug Delivery System, International Journal of Drug Development and Research, 2011; 3(3): 70-72.
6. Suvarna Katti et al, Formulation And Development of Transdermal patches of Tizanidine Hydrochloride, Asian Journal of Research in Chemistry and Pharmaceutical Sciences, 2017; 69-75.
7. Mujoriya Rajesh Z. et al, A Review on Transdermal Drug Delivery, Indo-Global Research Journal of Pharmaceutical Sciences, 2011; 1(2): 52-54.
8. Madhuri Shringirishi et al, Fabrication and Characterization of Nifidipine loaded β-cyclodextrin nanospsonges: An in vitro and in vivo evaluation, Accepted Manuscript, 2-11.
9. Uday B. Bolmal et all, Recent Advances in Nanosponges as Drug Delivery System, Volume-6; Issue-1, Review Article, 2013; 1934-1938.
10. Gauri Ashok Phadtare et al, Formulation and Evaluation of Topical Antifungal Gel containing Fluconazole, Research Article, 2016; 365-368.