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# FORMULATION AND EVALUATION OF NANOSPONGE TABLET

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## ABSTRACT

The aim of the present study is development and characterization of silymarin nanosponges and its tablet formulation targeted for Liver cirrhosis. Nanosponge is a modern category of material and is composed of minute particles with small, nanometer-wide cavities. Different kinds of materials can be used to fill these little cavities. These tiny particles have the potential to transport both hydrophilic and lipophilic drug substances, which can boost the stability of molecules or drugs that are poorly water soluble and also bioavailability. Utilizing a methodical quality by Design approach, silymarin-loaded nanosponge were produced and optimized, first, the Critical quality attributes (CQAs) and Critical material attributes (CMAs) of the nanosponge formulation were determined (CMAs). The present work aimed to design and optimize nanosponge with higher drug entrapped efficiency, Drug content and better stability. The formulation was optimized by 3 factor 3 level Box-Bechken design using Polymer concentration(X1), Stirring time(X2) and Stirring rate(X3) as independent variable (CMAs). Particle size (Y1). Entrapment efficiency (Y2), Percentage practical yield (Y3) and Drug content (Y4) was evaluated as dependent variables. Optimized formulation was then evaluated for FTIR, DSC, XRD, Particle size analysis, Zeta potential, SEM. Formulation was also found stable at different storage condition as dependent CQAs. Optimized formulation was then evaluated for FTIR, Particle size analysis, Zeta potential, SEM & Hepatoprotective activity

**Keywords:** Liver cirrhosis, Silymarin, Nanosponge, QbD, Box Behnken Design.

## INTRODUCTION

Liver is highly susceptible to toxicity caused by chemotherapeutic agents due to its dynamic role in clearing and altering chemicals from systemic circulation. Liver injury may be intensified when more than maximum tolerated dose of certain medicinal agents is taken. Various chemicals that provoke liver injury are termed hepatotoxic agents including chemotherapeutics, aflatoxins, carbon tetrachloride, chlorinated hydrocarbons, and some antibiotics.[1] Silymarin, a polyphenolic substance extracted from milk thistle, is a mixture of four isomeric flavonolignans: silibinin, isosilibinin, silydianin, and silychristin, of which silibinin is the most active

component.[2,3] Additionally, a recent study elaborated the value of silychristin and its dehydroderivative in terms of hepatoprotective action.[4] It has been used in herbal medicines for almost 200 years based on its antioxidant, anti-inflammatory, immunomodulatory, and anti-viral activities.[5,6] Nanosponges are tiny mesh like structure having the size of a virus with an average diameter below 1µm. They can load both hydrophilic and hydrophobic molecules. They have been a proved spherical colloidal nature, reported to have a very high solubilization capacity for BCS class II (poorly soluble drugs) by their inclusion and non-inclusion behavior. These have lately been created and put forth for medicine delivery. It can extend the release of poorly water soluble medicines and increase their bioavailability in addition to solubilizing them. 7-9

In order to decrease dosage frequency or boost medicine effectiveness, sustained release delivery systems are used. Localization at the site of action, a lower dosage requirement, or consistent drug distribution are all ways to accomplish this. The optimal medicine delivery system would need to have two components: a single dose, and the length of the treatment—whether it be for a few days or a week, as with an infection, or for the patient's entire lifetime. It should minimise side effects by delivering the active component to the site of action directly. 10-12

## MATERIALS AND METHODS

### Materials:

Silymarin was purchased from Vital Herbs. Other chemical, reagent and solvents used are Ethyl cellulose, polyvinyl alcohol, Dichloromethane, Ethanol, buffer solution, HPMC, Crospovidone, Mg stearate receives from laboratory.

### Methods:

**Formulation of nanosponge by emulsion solvent diffusion method: [13-15]**

Six batches of nanosponges were prepared using Silymarin (API), Ethyl cellulose (EC), Dichloromethane (DCM) and PVA shown in table no.1; by emulsion solvent diffusion method. In this method, an appropriate quantity of EC was dissolved in 20ml of Dichloromethane and Silymarin was added and using a magnetic stirrer to dissolve. This was considered the organic internal phase. Then internal phase was added drop by drop with the help of syringe using to an aqueous external phase which contained an appropriate quantity of PVA in 100ml distilled water and stirred for 2 hrs using a magnetic stirrer at 1000 rpm. The resultant dispersions were filtered and desiccated for 24 hrs at 40℃. The powder obtained was used for evaluation.

**Table no1: Formulation of Nanosponges**

| Batch | Ingredients | | | |
|---|---|---|---|---|
| | Drug(mg) | EC(mg) | PVA(mg) | DCM(ml) |
| NS1 | 100 | 200 | 300 | 20 |
| NS2 | 100 | 100 | 300 | 20 |
| NS3 | 100 | 300 | 300 | 20 |
| NS4 | 100 | 200 | 300 | 20 |
| NS5 | 100 | 300 | 300 | 20 |
| NS6 | 100 | 100 | 300 | 20 |

### Preparation of Nanosponge Tablet:

From nanosponge batches N6 batch was found to be optimized which is used for preparation of tablet by using direct compression method.

**Formulation table for tablet:**

**Table no2: Formulation Table For Nanosponge Tablets**

| Ingredients | F1 | F2 | F3 | F4 | |
|---|---|---|---|---|---|
| Nanosponge | 100 | 100 | 100 | 100 | Drug |
| Ethyl cellulose | 40 | 50 | - | - | Binder |
| HPMC | - | - | 40 | 50 | Binder |
| Crospovidone | 58 | 48 | 58 | 48 | Disintegrant |
| Mg stearate | 2 | 2 | 2 | 2 | Lubricant |

**Evaluation parameters:**

**A. Drug characterization:**

**1. Physical appearance**

The physical appearance of Silymarin was observed i.e. color, odour, nature etc.

**2. Determination of Total Ash Value:**

Ash values were determined to identify the quality & purity of crude drug.

**3. Determination of melting point**

The sample obtained was characterized for the melting point of the substance. The melting point was determined by introducing a small amount of substance to the capillary attached to thermometer in melting point apparatus and constant heat was supplied. The drug substance was observed for meltingand the melting point was noted.

**4. Estimation of silymarin by UV-spectroscopy method:**

Accurately weighed (10mg) Silymarin was dissolved in minimum quantity of methanol in 100ml volumetric flask. Then the volume was made up to100ml with methanol resulting in standard stock solution (100µg/ml). a set of standard dilutions of 2,4,6,8,10,12 µg/ml was prepared by transferring 0.2,0.4,0.6,1.0,1.2 ml aliquots of stock solution (10µg/ml). to series of 10ml of volumetric flasks and volume was made up with methanol. The absorbance of each dilution was measured in UV spectrophotometer at 287nm. Graph of concentration vs. absorbance was plotted.

**5. Fourier Transforms Infra-red Spectroscopy (FTIR):**

FTIR study was done on drug and drug excipient mixture to find out the compatibility between them. The FTIR study was performed using Perkin Elmer FTIR in the range of 4000-400cm$^{-1}$.

**B. Nanosponge Formulation:**[16-18]

**1. Physical appearance:**

The physical appearance of Silymarin Nanosponge was observed.

**2. % Entrapment Efficiency:**

Drug entrapment efficiency was calculated by centrifugation method, about 5 ml Silymarin loaded nanosponge was taken in tube and furthers it was centrifugated in cooling centrifuge tube at 1200 rpm for 20 min. after centrifugation, the supernatant layer was removed and diluted with appropriate solvent and the absorbance of this solution was recorded at 287nm. The entrapment efficiency was calculated by using the formula.

$$Entrapment\ efficiency = \frac{total\ dtug - free\ drug}{total\ drug} \times 100$$

**3. Percentage Yield:**

The percentage yield of nanosponges was measured by weighing the Nanosponges and the initial excipients used for the preparation of Nanosponges i.e. weight of drug, polymer, and crosslinker employed in the preparation. The percentage yield was calculated using the following formula.

$$\%Yield = \frac{Weight\ of\ nanosponge}{Weight\ of\ drug + Weight\ of\ polymer + Weight\ of\ cross\ linker} \times 100$$

**4. Drug content:**

Drug Content in nanosponge is determined on UV spectroscopy. Nanosponge (10mg) were dissolved in the mixture of methanol by shaking manually for 2 min. one ml of the resultant solution was taken and diluted with PBS 7.4 upto 10 ml and then absorbance was recorded at 359 nm using spectrophotometer and then concentration was obtained by using equation of standard calibration curve.

$$Drug\ content = \frac{Sample\ absorbance}{Standard\ absorbance} \times 100$$

**5. *In-vitro* drug release study:**

In vitro drug release from nanosponge formulation was carried out by using dialysis membrane employing in two sided open ended cylinder. Nanosponge (1gm) of drug was placed in dialysis membrane previously soaked overnight. The two side open cylinder was placed in 100ml of buffer solution and stirred with the help of magnetic stirrer at 1500 rpm/min. Aliquots (5ml) of release medium were withdrawn at different time interval and the sample was replaced with fresh buffer solution to maintain constant volume. The sample was analyzed using a UV spectrophotometer.

**6. Particle size:**

Instruments based on dynamic light scattering and the photon correlation principle are mainly used to determine the particle size.

**7. Zeta potential:**

Zeta potential based on electrophoretic mobility of optimized nanosponge formulation was determined by using zetasizer.

**8. Scanning emission microscopy:**

Surface morphology and size of optimized nanosponge was determined by using scanning emission microscopy.

**C. Nanosponge Tablet Formulation:**[19-21]

**1. Tablet thickness and size:**

The thickness and diameter of tablets were important for uniformity of tablet size. Thickness and diameter were measured using Vernier Calipers.

**2. Tablet Hardness:**

The hardness of tablets of each formulation was measured by a Monsanto Hardness tester. The hardness was measured in terms of Kg/cm2.

**3. Friability;**

Ten tablets were weighed accurately and placed in the tumbling apparatus that revolves at 25rpm dropping the tablets through a distance of six inches with each revolution. After 4min, the tables were weighed and the percentage loss in tablets weight was determined.

$$Friability(\%) = 100 \times (W1 - W2)/W1$$

Where, W1- Initial weight

W2- Final weight

**4. Weight variation:**

Ten tablets were selected randomly and the average weight was calculated. Weight variation was calculated and compared with I.P. standards.

**5. In-vitro dissolution study:** The dissolution test was conducted using 900 ml of phosphate buffer pH 6.8, at 37 0.5°C and 50 rpm. Five ml of the sample solution was removed from the dissolving equipment every five, ten, twenty, twenty-five, and thirty minutes. To keep the washbasin condition, samples were substituted with an equivalent volume of the dissolving media. Whatmann filter paper was used to filter the sample, and a UV spectrophotometer was used to evaluate the solutions at 279 nm (SHIMADZU, UV-2450, Japan). The overall drug release % was determined.

**6. *In-vitro* hepatoprotective activity:** [22-24]

In-vitro hepatoprotective activity was assessed by the ability of a sample N3 extract to preclude the oxidative stress induced by D-GalN in HepG2 cells.

**7. Stability studies:**

Optimized formulation was subjected to stability as per ICH guidelines at the following conditions (ICH 2003). Samples were kept in stability chamber at 40± 2 and 75±5 RH and Room temperature for up to 90 days. The stability parameter such as Hardness, Thickness, In-vitro drug release was determined as function of the storage time.

## RESULT AND DISCUSSION:

### A. Drug characterization:

Drug obtained from Vital Herbs was found off white yellow, characteristic odour, and fine powder. Melting point of drug was found 158°C. Silymarin is freely Soluble in ethanol and methanol. Ash value found to be 1.30%

The UV absorbance of Silymarin standard solution in the range of 2-12 µg/ml of drug in methanol showed linearity at λmax 287nm. The linearity was plotted for absorbance (A) against concentration (C) with $R^2$ value 0.9974 and with the slope equation y=0.0585x+0.0068. The absorbance values and standard curve were in below fig.no.1

The FTIR frequencies of Silymarin are in reported range which indicate that obtained sample was pure, fig.no.2

**Table no3: Absorbance value of silymarin**

| Solvent | Concentration (ug/ml) | Absorbance (nm) |
|---|---|---|
| Methanol | 2 | 0.126 |
| | 4 | 0.245 |
| | 6 | 0.354 |
| | 8 | 0.461 |
| | 10 | 0.603 |

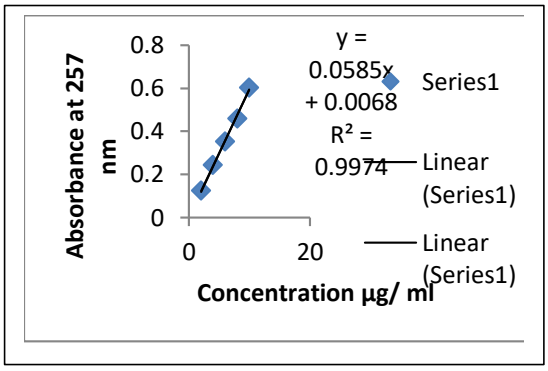


**Fig no1: Calibration curve of Silymarin**

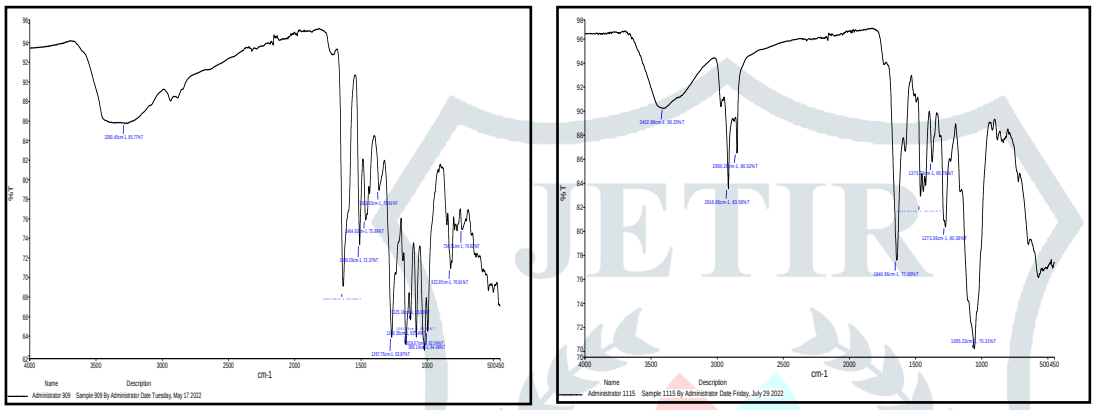

**Fig no2: FTIR spectra of a) drug and b) Nanosponge Tablet**

**B. Evaluation of Nanosponges:**

Entrapment efficiency of N6 batch was high i.e. 88.12%, % yield was 74.23% and drug content was 89.56% (table 3). The in vitro release of Silymarin nanosponges were performed and measure the release at 1hr, 2hr, 3hr, up to 12hrs. The formulation NS6 shows highest in vitro drug release i.e. 80.02%(table 4) & fig no. 3. From the all nanosponge formulations, NS6 shows high entrapment efficiency, drug content, %yield and in vitro drug release and on the basis of that it was optimized for nanosponge tablet formulations. Particle size of nanosponge formulation was shown in fig no.4 Zeta potential was shown in fig. no.5 Zeta potential values in the range of -30mV to +30mV of either charge characterize stable formulation. SEM images are shown in fig no.6

**Table no 4:** Evaluation of nanosponge

| Formulation | %EE | % Yield | Drug Content |
|---|---|---|---|
| NS 1 | 87.16% | 41% | 84.21% |
| NS 2 | 86.65% | 42% | 83.29% |
| NS 3 | 87.55% | 63.15% | 85.13% |
| NS 4 | 87.89% | 71% | 85.46% |
| NS 5 | 87.55% | 54% | 84.56% |
| **NS 6** | **88.12%** | **74.23%** | **89.56%** |

**Table no 5:** % in vitro drug release of nanosponge

| Time | N1 | N2 | N3 | N4 | N5 | N6 |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 2.42 | 4.5 | 2.88 | 3.8 | 4.99 | 5.2 |
| 2 | 4.17 | 5.6 | 4.37 | 6.77 | 7.22 | 8.25 |
| 3 | 12.45 | 10.86 | 13.08 | 14.21 | 13.21 | 15.13 |
| 4 | 15.84 | 17.09 | 19.27 | 18.27 | 15.03 | 20.6 |
| 5 | 21.58 | 22.57 | 22.47 | 23.08 | 20.3 | 25.9 |
| 6 | 32.48 | 28.67 | 30.56 | 30.93 | 30.22 | 33.14 |
| 7 | 35.85 | 32.84 | 37.76 | 35.48 | 35.24 | 39.25 |
| 8 | 42.85 | 38.04 | 42.57 | 42.24 | 44.75 | 45.13 |
| 9 | 52.84 | 40.28 | 50.85 | 48.67 | 50.73 | 54.06 |
| 10 | 55.89 | 48.21 | 62.54 | 55.27 | 55.08 | 62.39 |
| 11 | 62.58 | 55.27 | 68.28 | 62.87 | 59.06 | 71.16 |
| 12 | 70.85 | 65.24 | 75.21 | 72.82 | 62.29 | 80.02 |

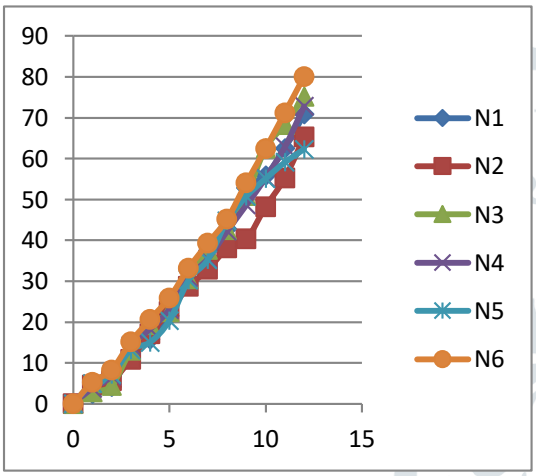


**Fig no3:** In vitro drug release study

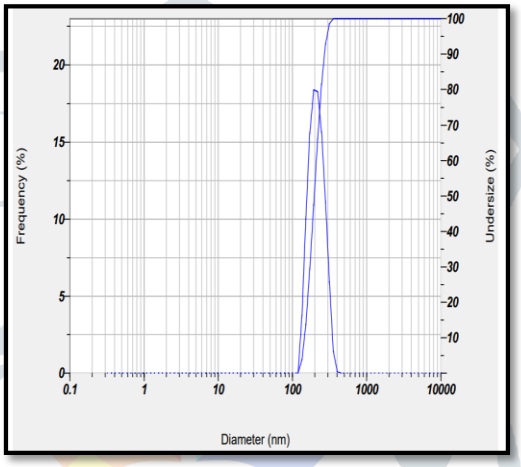


**Fig no4:** Particle size of nanosponge

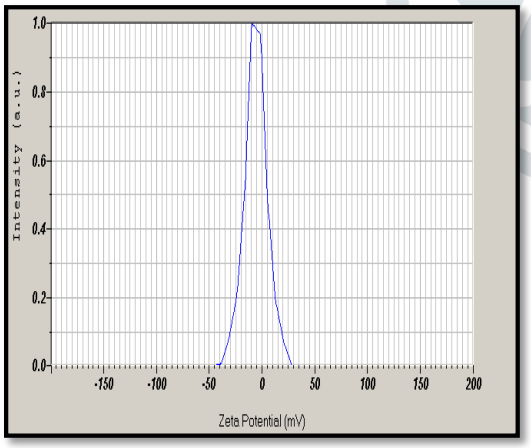


**Fig no5:** Zeta potential

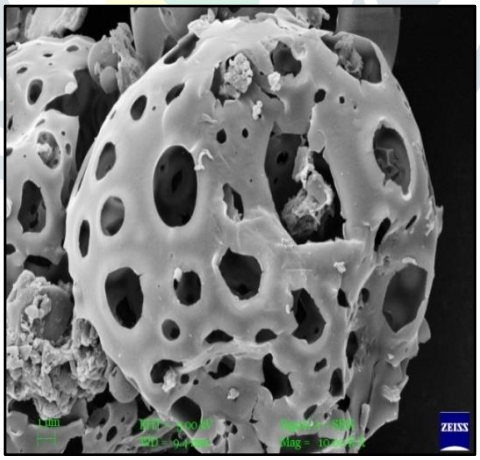


**Fig no 6:** SEM image

**C. Evaluation of Nanosponge Tablet:**

N6 batch was found to be optimized which is used for preparation of tablet from optimized nanosponges. The thicknesses were found to be 3.2-4.6, hardness of tablet was found between 3.2-3.7, the percent friability was found to be 0.13-0.19, weight variation of tablets was found to between 201-203(table no 6). The in vitro dissolution studies of tablet were performed and measure the release at 1hr, 2hr, 3hr, up to 12hrs. The formulation T3 shows highest in vitro drug release i.e. 88.82%(table 7) & fig no.7, in vitro hepatoprotective study At the dose of 10, 40, and 100 µg/ml of sample N3 formulation extract were used for testing the protective effect on D-GalN

induced oxidative damage in the HepG2 hepatic cell line results are shown in table no. 8, and the stability study shown in table no.9

**Table no 6:** Evaluation (post compression) parameter for all formulation

| Formulation | Hardness ($Kg/Cm^2$) | Thickness (mm) | Friability (%) | Weight Variation (mg) |
|---|---|---|---|---|
| T1 | 3.5 | 3.2 | 0.19 | 202 |
| T2 | 3.7 | 3.9 | 0.15 | 203 |
| T3 | 3.2 | 4.4 | 0.13 | 201 |
| T4 | 2.4 | 4.6 | 0.16 | 203 |

**Table no 7:** In vitro dissolution study of tablet

| Time (hr) | %Drug Release | | | |
|---|---|---|---|---|
| | T1 | T2 | T3 | T4 |
| 0 | 0 | 0 | 0 | 0 |
| 1 | 14.25 | 12.58 | 17.44 | 15.24 |
| 2 | 15.74 | 18.57 | 22.57 | 21.48 |
| 3 | 19.75 | 21.75 | 25.85 | 23.59 |
| 4 | 24.08 | 24.58 | 29.39 | 25.48 |
| 5 | 27.89 | 29.85 | 34.2 | 32.58 |
| 6 | 32 | 35.87 | 45.32 | 38.75 |
| 7 | 39.64 | 42.85 | 47.84 | 41.58 |
| 8 | 44.85 | 51.87 | 52.63 | 47.68 |
| 9 | 52.78 | 55.28 | 61.02 | 56.87 |
| 10 | 65.89 | 62.18 | 70.9 | 62.78 |
| 11 | 72.85 | 64.08 | 81.59 | 69.78 |
| 12 | 82.75 | 69.87 | 88.82 | 72.58 |

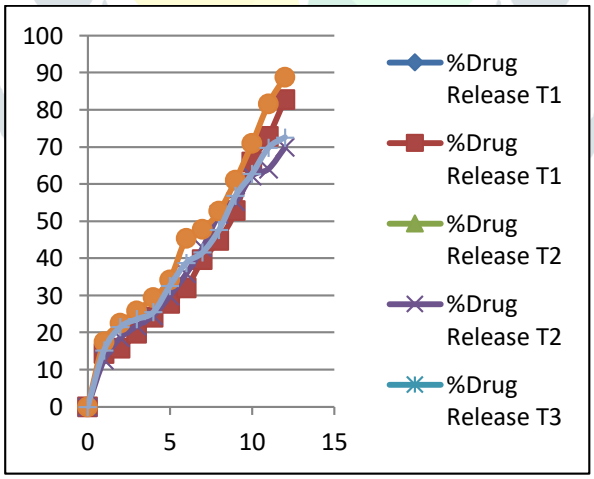


**Fig no7:** In vitro study of nanosponge tablet

**Table no 8:** In vitro hepatoprotective activity

| Sr. no | Concentration | Conc. (µg/ml) | OD | OD | OD | Mean | Percentage of cell viability |
|---|---|---|---|---|---|---|---|
| 1. | Control | | 0.416 | 0.476 | 0.438 | 0.443 | |
| 2. | Silymarin | 10 | 0.372 | 0.341 | 0.361 | 0.358 | 80.81 |
| 3. | Silymarin | 50 | 0.380 | 0.383 | 0.367 | 0.376 | 84.87 |
| 4. | Silymarin | 100 | 0.400 | 0.464 | 0.433 | 0.432 | 97.52 |
| 5. | Sample N3 | 10 | 0.208 | 0.211 | 0.326 | 0.248 | 55.99 |
| 6. | Sample N3 | 50 | 0.280 | 0.267 | 0.271 | 0.272 | 61.39 |
| 7. | Sample N3 | 100 | 0.381 | 0.361 | 0.391 | 0.377 | 85.10 |

**Table no 9:** Stability study

| Sr. No. | No. of Days | Hardness (kg/cm) | Thickness (mm) | In-vitro drug release |
|---|---|---|---|---|
| 1 | Initial | 3.2 | 4.4 | 88.82 |
| 2 | 15 | 3.18 | 4.36 | 88.78 |
| 3 | 30 | 3.15 | 4.32 | 88.75 |
| 4 | 45 | 3.10 | 4.3 | 88.71 |
| 5 | 60 | 3.07 | 4.26 | 87.76 |
| 6 | 75 | 3.1 | 4.19 | 87.70 |
| 7 | 90 | 3 | 4.1 | 87.54 |

## CONCLUSION:

The emulsion solvent diffusion method was used to create the nanosponges. The measured values of the improved nanosponge's entrapment effectiveness, zeta potential, and particle size. The spherical, porous character of the optimised nanosponges was evident. The Silymarin-loaded nanosponge tablets were made utilising the direct compression method and several polymer grades. All four tablets—T1, T2, T3, and T4—had pre- and post-compression characteristics that were found to be under the I.P. limit for sustained release tablets. Through in-vitro Study it can be concluded that T3 tablets shows better release. The optimized NS6 formulation showed maximum drug content of 89.56 % and when compared to other formulations. The optimized NS6 tablet remained stable during the entire period of study when stored at different temperatures and humidity conditions. Hence, it can be concluded that the nanosponge tablet loaded with silymarin can be an effective drug delivery system for Liver Cirrhosis.

## REFERANCES:


1. Suva M.A. A Brief Review on Liver Cirrhosis: Epidemiology, Etiology, Pathophysiology, Symptoms, Diagnosis and Its Management. Inventi Rapid: Molecular Pharmacology. 2014 Mar 20;2:1-5.
2. Fraschini F, Demartini G, Esposti D. Pharmacology of silymarin. Clinical drug investigation. 2002 Jan;22(1):51-65.
3. ŠIManek VI, Kren V, Ulrichová J, Vicar J, Cvak L. Silymarin: what is in the name…? An appeal for a change of editorial policy. Hepatology. 2000 Aug;32(2):442-4.

4. Yang Z, Zhuang L, Lu Y, Xu Q, Chen X. Effects and tolerance of silymarin (milk thistle) in chronic hepatitis C virus infection patients: a meta-analysis of randomized controlled trials. BioMed research international. 2014 Oct;2014.
5. Hardy T, Mann DA. Epigenetics in liver disease: from biology to therapeutics. Gut. 2016 Nov 1;65(11):1895-905.
6. Gillessen A, Schmidt HH. Silymarin as supportive treatment in liver diseases: A narrative review. Advances in therapy. 2020 Apr;37(4):1279-301.
7. Osmani RA, Thirumaleshwar S, Bhosale RR, Kulkarni PK. Nanosponges: the spanking accession in drug delivery-an updated comprehensive review. Der Pharm Lett. 2014;5:7-21.
8. Kundlas J. Nautiyal., Jassal M. Nanosponges: As Originated Form For Targeted Drug Delivery. International Journal of Recent Advances In Pharmaceutical Research. 2015;5(2):75-81.
9. Jilsha G, Viswanad V. Nanosponges: A novel approach of drug delivery system. Int. J. Pharm. Sci. Rev. Res. 2013;19(2):119-23.
10. Kaur S, Kumar S, Nanosponges; An innovative drug delivery system, Asian journal of pharmaceutical & clinical research, vol.12, (2019).
11. Panda S, Vijayalakshmi SV, Pattnaik S, Swain RP. Nanosponges: A novel carrier for targeted drug delivery. Int J PharmTech Res. 2015;8(7):213-24.
12. Selvamuthukumar S, Anandam S, Krishnamoorthy K, Rajappan M. Nanosponges: A novel class of drug delivery system-review. Journal of Pharmacy & Pharmaceutical Sciences. 2012 Jan 17;15(1):103-11.
13. Satpathy TK, Chaubey N, Brahma CK, Maheshwari M. Formulation and Evaluation of Lamotrigine Loaded Nanosponges. Research Journal of Pharmacy and Technology. 2022;15(1):229-35.
14. Satpathy TK, Chaubey N, Brahma CK, Maheshwari M. Formulation and Evaluation of Lamotrigine Loaded Nanosponges. Research Journal of Pharmacy and Technology. 2022;15(1):229-35.
15. Omar SM, Ibrahim F, Ismail A. Formulation and evaluation of cyclodextrinbased nanosponges of griseofulvin as pediatric oral liquid dosage form for enhancing bioavailability and masking bitter taste. Saudi Pharmaceutical Journal. 2020 Mar 1;28(3):349-61.
16. Cianchino VA, Favier LS, Ortega CA, Peralta C, Cifuente DA. Formulation development and evaluation of Silybummarianum tablets. Rodriguésia. 2020 Jul 13;71.
17. K. Ravi Shankar, formulation and evaluation of sustained release matrix tablets of baclofen, International Journal of Pharmaceutical Sciences and Research, September(2018).
18. Subhash PB, Mohite SK. Formulation design & development of ArtesunateNanosponge. Eur J Pharm Med Res 2016; 3 (5): 206. 2016;211.
19. Raja CH, Kumar GK, Anusha K. Fabrication and evaluation of ciprofloxacin loaded nanosponges for sustained release. Int J Res Pharm Nano Sci. 2013; 2:1-9.
20. British pharmacopoeia, (2014), Page no. 1405-1406.
21. Narwate BM. Sustained release dosage form: a concise review. International journal of pharmaceutics and drug analysis. 2017 May 2:153-60.

**22.** Practical pharmacognosy techniques and excipients by Dr. K. R. Khandelwal and Dr.Vrunda sethi, Niraliprakashan, Page No.25.1-25.

**23.** Barnett R. Liver cirrhosis. The Lancet. 2018 Jul 28;392(10144):275.Wiegand J, Berg T, The etiology diagnosis and prevention of liver cirrhosis, 110(6)85- 91, 2013

**24.** European Liver Transplant Registry. 2012.

**25.** Bircher J, Benhamou JP, McIntyre N, Rizzetto M, Rodés J. Oxford textbook of clinical hepatology.