Research

Title:

Full Title: Formulation Development and Evaluation of Sulfasalazine Loaded Nanosponges for Treatment of Inflammatory Bowel Disease**.**

Authors:

Navnath D. Madane, Sucheta S. Bhise, Omkareshwari A. Gaikwad, Rushi K. Ghule, Pratik Y. Dhole. Rajkumar V. Shete.

Affiliations:

1. Department of Pharmaceutics, Rajgad Dnyanpeeth's College of Pharmacy, Bhor Tal. Bhor Dist. Pune 412206
2. Department of Pharmacology, Rajgad Dnyanpeeth's College of Pharmacy, Bhor Tal. Bhor Dist. Pune 412206

***Address Correspondence to:**

Navnath Dhondiram Madane.

Research Scholar,

Rajgad Dnyanpeeth's College of Pharmacy, Bhor

E-mail id- navnathmadane2001@gmail.com

Contact No- + 91 7775929375

Abstract:

Objective: The objective of research is to formulate an Evaluation of Sulfasalazine Loaded Nanosponges for Treatment of Inflammatory Bowel Disease. Sulfasalazine belongs to BCS class IV drug having low solubility, low permeability. This research aims to improve bioavailability of drug by increasing its solubility by encapsulation in Nanosponges. It is an inhibitor of the cyclo-oxygenase pathway, it inhibits the production of prostaglandin E2 in inflamed intestinal specimens. **Material and Method:** The Emulsion Solvent Diffusion method by using 3^2 Full Factorial Design. One factor was evaluated at three levels and concentration of Ethyl Cellulose (X1) and concentration of Polyvinyl Alcohol (X2) were selected as independent variables and using different organic and inorganic solvent mixtures. **Results:** The first performed Preformulation study of drug and excipients Solubility, Melting point, UV Spectroscopy, FTIR, DSC. The

formulated Sulfasalazine nanosponges were characterized by various tests like Production yield, Entrapment efficacy, in vitro drug release, Particle size, Zeta potential, SEM, etc. F5 Batch of Nanosponges out of nine formulations was found to be optimized. The average Particle size of the optimized F5 batch was measured at 164.4 nm, with a zeta potential of 14.98 mV. The entrapment efficiency across 9 batches varied from 23.2% to 96.8. The entrapment efficiency of optimized batch was found to be 96.8%. *In-vitro* drug release for these batches was observed between 23.08% and 31.71% in 8hr. The application of Nanosponges proved the potential for Colon delivery of Sulfasalazine over the conventional Tablet formulations and Colon drug delivery for Sulfasalazine has been successfully developed.

Keywords: Nanosponges, Colon, IBD, Sulfasalazine, Controlled release.

1. **Introduction:** Nanosponges are the recent advances in nanotechnology. In these substances with cavities of nanometer dimension into which broad brand of substance can be encapsulated. These particles can carry both lipophilic and hydrophilic substances and enhance the solubility of poorly water-soluble molecules. (1) They transform the treatment of numerous disease and initial trials indicates that this technology is five times more successful in targeting drugs to breast cancer cells than ordinary methods. (2) Nanosponges are types of nanoparticles that encapsulate the drug within the core made up of polymer and they circulate throughout the body to reach the site of action and release the drug in a predictable manner. (3) Inflammatory bowel disease (IBD) is a chronic relapsing and remitting inflammatory disorder of a small intestine and colon. Ulcerative colitis (UC) and Crohn's disease (CD) are two main types of IBD. (4) Colon targeted drug delivery has been the center of mainly studies in current years due to its potential to progress treatment of local diseases affecting the colon, while minimizing the systemic side effects. Several instances of disease states which impact the colon include CD, UC and irritable bowel syndrome (IBS). (5) A Several regularly used drugs for the treatment of this illness comprise hydrocortisone, metronidazole, sulfasalazine, dexamethasone, prednisolone and others. (6) the delivery of these drug purposely to the colon without being absorbed first in the upper (GI) tract allows for an elevated concentration of the drug to arrive at colon with negligible systemic absorption. (7) The colonic substance has a longer retention time (up to 5 days), and the colonic mucosa is known to make easy the absorption numerous drugs, making this organ a perfect site for drug delivery. (8) Sulfasalazine (SLZ) is the anti-inflammatory drugs used to treat various IBD such as UC and CD due to indication of T-lymphocyte apoptosis modulates inflammatory mediators. It is a poorly absorbed drug with approximately 5-19 hr elimination half-life. Sulfasalazine which are BCS class IV Drug. The absolute bioavailability of SLZ is less than 15%. SLZ is a prodrug of 5-aminosalicylic acid that is covalently linked to the antibiotic sulfapyridine by an azo bond. (9,10)

2. MATERIALS AND METHODS

2.1 Materials:

Sulfasalazine was purchased from yarrow chem products, Mumbai. Ethyl cellulose, Polyvinyl alcohol and Dichloromethane Research lab, Mumbai. All the material used for formulation of Nanosponges are of Analytical grade.

3. Preformulation Studies

3.1 Physical characteristics

By visual examination, the drug was identified for physical characters like color, texture and odour etc.

3.2 Solubility

Solubility studies of Sulfasalazine were carried out in water, Methanol, Ethanol, Dichloromethane, Dimethyl Sulfoxide, Dimethyl formamide, and in buffer solution pH (6.8). 10 mg of drug was dissolved in each solvent separately and solubility was determined qualitatively.

3.3 Melting Point

A Small quantity of drug was placed into a fusion tube. That tube was placed in digital melting point apparatus. Then the melting point was recorded.

3.4 Determination of λ max of sulfasalazine

Accurately weighed 100 mg of drug was dissolved in 100 ml of pH 6.8 buffer solution to form 1000µg/ml stock solutions (stock solution I). From stock solution I, 1ml solution was withdrawn and diluted to 10 ml to get a solution of concentration 100µg/ml (stock solution II), which then served as standard solution.

From this stock solution II, aliquots of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml were pipetted out into a series of 10 ml volumetric flask and diluted up to 10 ml with pH 6.8 phosphate buffer to get solutions with concentrations of 2µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml and 10 µg/ml. Absorbances of the resulting solutions was measured at λ_{max} 359 nm using UV spectrophotometer against respective parent solvent as a blank. The standard calibration curve was obtained by plotting absorbance V/s. concentration in µg/ml.

3.5 FTIR Spectroscopy

The aim of FT-IR study is to determine purity of drug and study drug-excipient compatibility. FTIR spectroscopy can be used to investigate and predict any possible physiochemical interaction between different components in a formulation and therefore it can be applied for the selection of suitable compatible excipients while selecting the ingredients, we would choose those which are stable, compatible, cosmetically and therapeutically acceptable. The aim of the present study was

to find out the possible interaction between Ethyl Cellulose, Poly vinyl Alcohol and the drug Sulfasalazine and to identify the compatibility between the drug and other excipients. 10 mg of the sample put in FTIR plate surface and touch senser using OPUS Software in Bruker ALPHA II FT-IR Spectrophotometer (11).

3.6 Differential scanning calorimetry (DSC)

The DSC studies carried out to observe the thermal behavior of pure drug were carried out using differential scanning calorimeter (STAR SW 12.10). Sample of about 5 mg was placed in a 50µl perforated aluminum pan and sealed. Heat runs for each sample were set from 5°C to 300°C using nitrogen as purging gas and samples were analyzed**.**

4. Characterization of Nanosponges

4.1 Preparation of sulfasalazine nanosponges

Sulfasalazine nanosponges were prepared by emulsion solvent diffusion method. In this method, the two phases used are aqueous and organic. Aqueous phase consists of polyvinyl alcohol and organic phase include sulfasalazine and ethyl cellulose dissolve organic solvent dichloromethane. And this phase added slowly to the aqueous phase and stirred for 2hr. After stirring they filter in using Whatman (0.45mm) pore size filter paper. After filter they dried in hot air oven 40°C for 24hr. (12)

4.2 Experimental Design

Optimization of the Nanosponges preparation procedure was done by using 3^2 full factorial design. Concentration of Ethyl Cellulose (X1) and Polyvinyl alcohol (X2) were selected as independent variables. % Entrapment efficiency (Y1) and Invitro drug release (Y2) was selected as dependent variables.

Table 2. Composition of Sulfasalazine loaded Nanosponges by 3^2 factorial designs.

Batch. no	Sulfasalazine(mg)	Ethyl cellulose(mg)	Polyvinyl alcohol (mg)	Dichloromethane (ml)	Distilled water(ml)
F1	100	100	300	20	100
F2	100	100	100	20	100
F3	100	200	100	20	100
F4	100	200	200	20	100
F5	100	300	200	20	100
F6	100	100	200	20	100
F7	100	300	100	20	100
F8	100	300	300	20	100
F9	100	200	300	20	100

5. Characterization of Sulfasalazine Nanosponges

5.1 Percentage Yield (%)

The dried nanosponges were weighed and the percentage yield was calculated by using the following formula, where actual weight of the nanosponges was divided by the total amount of material that was used for the preparation of the Nanosponges.

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug + polymer}} * 100$$

5.2 Entrapment efficacy

Sulfasalazine Nanosponges equivalent to 10 mg were weighed and suspended in 10 ml pH 6.8 phosphate buffer for 30 min. The suspension was then stirred on Magnetic Stirrer at 1000 rpm. The drug content was determined by measuring absorbance at 359 nm after appropriate dilutions with pH 6.8 phosphate buffer. The entrapment efficiency was calculated by using formula as follows-:

$$\text{Entrapment efficacy} = \frac{\text{Initial amount of drug in nanosponges-Free drug}}{\text{Initial amount of drug in nanosponges}} * 100$$

5.3 FT-IR Spectral Analysis

The aim of FT-IR study is to determine purity of drug and study drug-excipient compatibility. FTIR spectroscopy can be used to investigate and predict any possible physiochemical interaction between different components in a formulation and therefore it can be applied for the selection of suitable compatible excipients while selecting the ingredients, we would choose those which are stable, compatible, cosmetically and therapeutically acceptable. The aim of the present study was to find out the possible interaction between Ethyl Cellulose, Poly vinyl Alcohol and the drug Sulfasalazine and to identify the compatibility between the drug and other excipients. 10 mg of the sample put in FTIR plate surface and touch senser using OPUS Software in Bruker ALPHA II FT-IR Spectrophotometer

5.4 Particle size

The average mean width and particle sizes of loaded nanosponges were investigated employing the Horiba SZ-100 instrument and the Dynamic Light Scattering method. To obtain the correct light scattering intensity for Sulfasalazine nanosponges, the dried nanosponges were distributed in distilled water.

5.5 Zeta potential

The nature and composition of the environment, as well as the surface charge of the particle, anything adsorbed to the interface and nature and composition of the surroundings, all influence the zeta potential. A zeta sizer containing zeta cells and gold-plated polycarbonate cells can be used to calculate the surface charge of the nanosponges. The zeta potential is crucial in determining the stability of nanosponges.

5.6 Scanning Electron Microscopy (SEM)

The particle size observed by SEM was consistent with that obtained from microscope and (FEI Nova NanoSEM 450) laser particle size analyzer. The section of nanosponges showed an internal dense structure with occasional small pore (Wenjia Guo A et al., 2015). Prior to examination, samples were mounted on an aluminum stub using a double-sided adhesive tape and making it electrically conductive by coating with a thin layer of gold (approximately 20 nm) in vacuum. The scanning electron microscope was operated at an acceleration voltage of 5 kV and resolution of 4000.

5.7 In-vitro drug release study

In-Vitro Drug Release studies were performed by using USP Dissolution Test Apparatus II (Paddle method). Accurately weighed amounts (100 mg) of Nanosponges were introduced into 900 mL of PBS (phosphate buffer saline, pH 6.8) and stirred with 100 rpm at (37.0±0.5) °C. Five ml samples were withdrawn at selected time intervals, filtered and analyzed spectrophotometrically at 359 nm. (Jasco UV-530).

6. RESULTS AND DISCUSSION

6.1 Melting Point:

Reported Melting Point of Sulfasalazine -: 248-250^0C

Observed Melting Point of Sulfasalazine-: 250^0C.

6.2 Solubility Study:

Solubility studies of Sulfasalazine were carried out in water, Methanol, Ethanol, Dichloromethane, Dimethyl Sulfoxide, Dimethyl formamide, and in buffer solution pH 6.8. Solubility was determined qualitatively. The results are as shown in Table 8.1.

Table 3. Solubility Studies in different solvents

Sr. No.	Name of Solvent	Solubility data
1.	Water	Insoluble
2.	Methanol	Slightly soluble
3.	Ethanol	Slightly soluble
4.	Dichloromethane	Slightly soluble
5.	Dimethyl Sulfoxide	Soluble
6.	Dimethyl Formamide	Soluble
7.	pH 6.8 buffer	Soluble

6.3 Determination of λ_{max} and calibration curve:

The absorbance of the sample solution of Sulfasalazine shows maximum absorption at wavelength 359 nm in phosphate buffer pH 6.8 After the graphical representation of the absorbance we can calculate the regressions coefficient of the Sulfasalazine that gives the linearity of the absorbance.

Table 4. Calibration curve data for Sulfasalazine in pH 6.8 Phosphate buffer at 359 nm.

Sr. No.	Concentration (µg/ml)	Absorbance
1	2	0.055
2	4	0.102
3	6	0.153
4	8	0.199
5	10	0.253

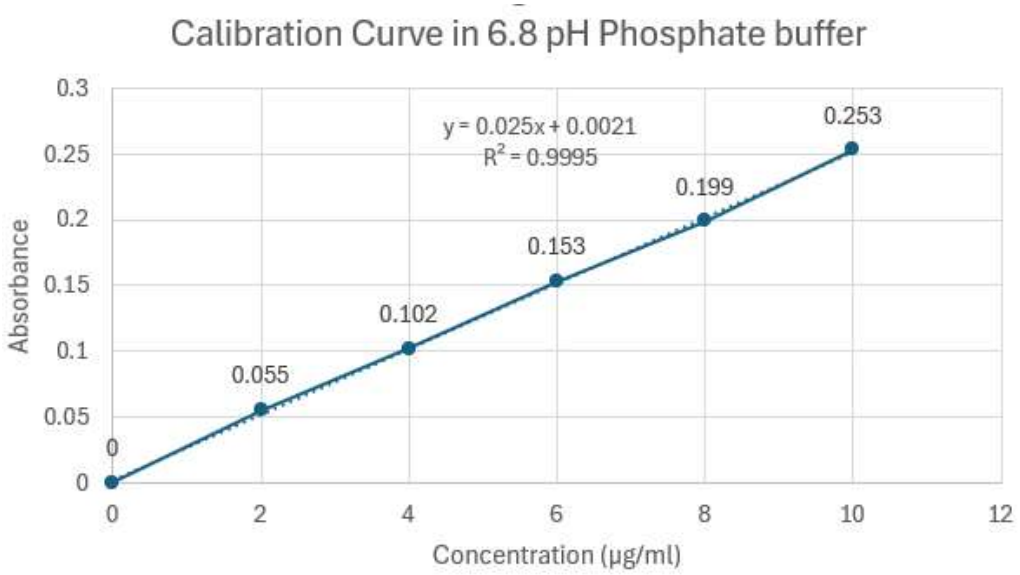

Figure 1 Calibration curve of Sulfasalazine in Phosphate buffer pH 6.8

6.4 FT-IR Analysis-

The FT-IR spectra of Sulfasalazine have shown asymmetric stretching of SO2 peak at 1358.26 cm^{-1}, C=O stretch at 1677.02 cm^{-1}, COO symmetric stretching at 1261.41 cm^{-1}, N=N stretching at 1394.18 cm^{-1}, C-C ring stretching at 1197.27 cm^{-1}, OH bending at 1394.18 cm^{-1}.

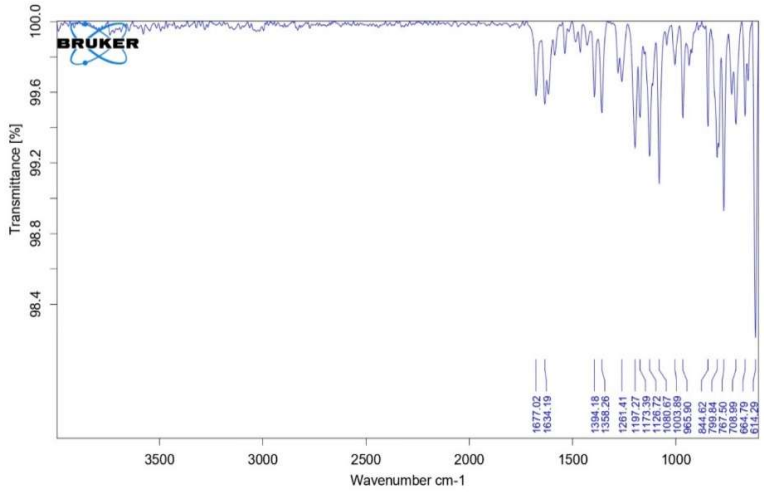

Figure 2 FTIR Spectra of Sulfasalazine

6.5 Differential Scanning Colorimetry (DSC)

The DSC studies carried out to observe the thermal behavior of drug. The characteristic endothermic peak of Sulfasalazine pure drug appeared at 250.38 °C which was within a range of melting point. Thermo-gram of the drug shows thermal stability of the drug. A characteristic peak change in DSC thermo-gram confirmed that Sulfasalazine had experienced chemical interaction and had been entrapped into the Nanosponges.

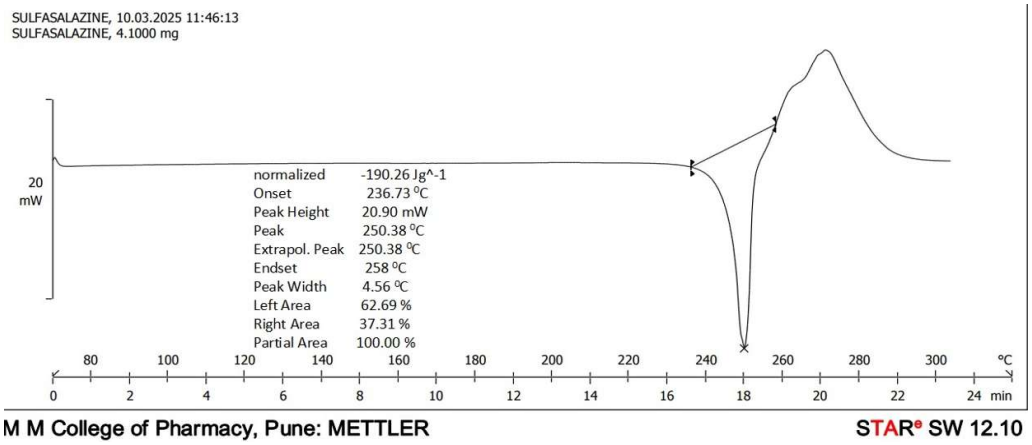

Figure. 4: DSC Thermogram of Pure Sulfasalazine

6.6 Percentage Yield of Nanosponges

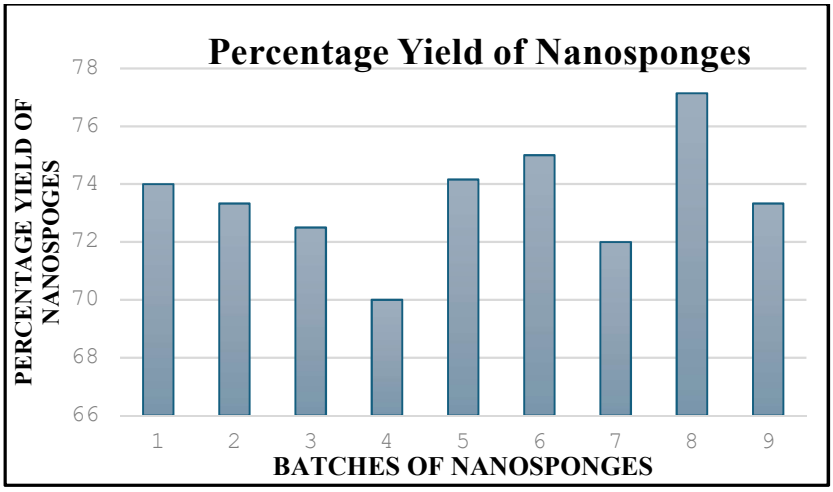

Figure 5 Percentage Yield of Nanosponges

6.7 Scanning Electron Microscopy (SEM):

A scanning electron microscope (SEM) is a type of Electron Microscope that images a sample by scanning it with a high-energy beam of Electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample make signals that contain information related to the sample's surface topography, composition, and other properties such as electrical conductivity etc. The particles were kept on a gold-coated plate and maintained for at least 1 hour at room temperature in a desiccator for complete dryness of the sample. The dried sample was covered with a thin layer of gold and the particle size was evaluated using scanning electron Microscope. The F5 formulation of the Sulfasalazine Nanosponges revealed that the surface morphology found to be spherical and rough due to higher concentration of drug as shown in Figure 4.

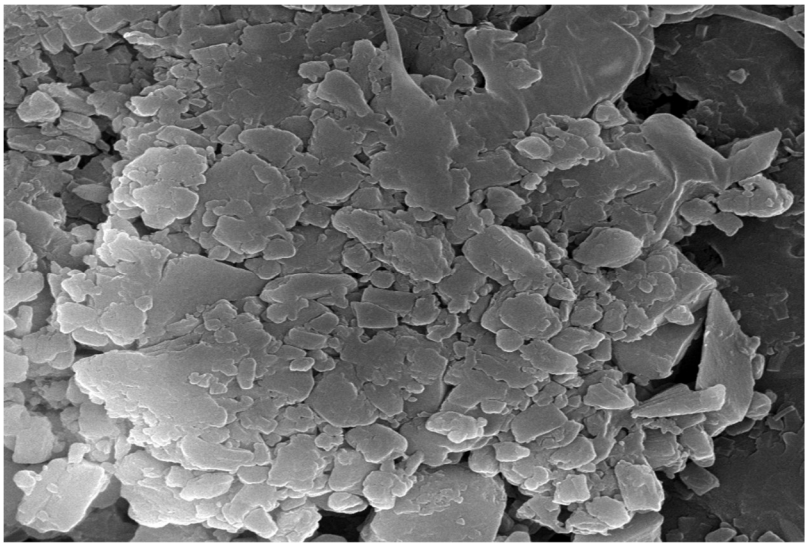

Figure 6: SEM image of Nanosponges Optimized batch F5

6.8 Drug Entrapment Efficiency:

It was observed that an increase in polymer concentration resulted information of bigger Nanosponges entrapping larger amount of drug. The efficiency of encapsulation is significantly increased with the increase in core: coat ratio. When polymer concentration was increased, drug entrapment was found to have increased significantly. Entrapment Efficiency of nanosponges is as shown in Table.

Table 4: Entrapment Efficacy

Batch. no	Entrapment Efficacy
1	53.8
2	23.2
3	25.2
4	87.8
5	96.8
6	48.6
7	92.8
8	93.4
9	95.6

6.9 Mean Particle Size:

The mean particle size of the nanosponges of Sulfasalazine was as shown in table 8.4. The mean particle of F5 batch was found to be 164.4 nm.

Z-Average (nm)	: 2783	Polydispersity Index (PI)	: 1
Peak 1 Mean by Intensity ordered by area (nm)	: 164.4	Run Duration (s)	: 1.68
Peak 2 Mean by Intensity ordered by area (nm)	:	Number Of Runs	: 15
Peak 3 Mean by Intensity ordered by area (nm)	:	Derived Mean Count Rate (kcps)	: 362.6
Di (10)	: 143.3	Attenuator	: 11
Di (50)	: 164.4	Cuvette Position (mm)	: 4.64
Di (90)	: 190.4	Detector Angle (°)	: 90
		Software Version	: 3.2.1.11

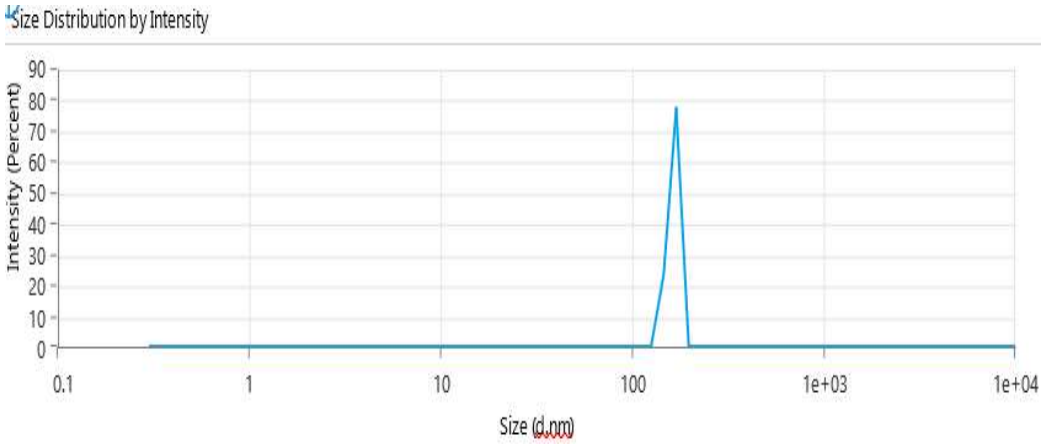

Figure 7: Particle Size of F5 batch of Nanosponges

6.10 Zeta potential:

The zeta potential of nanosponge formulation was as shown in table 8.5 The zeta potential of F5 batch was found to be 14.98 mV. The zeta potential analysis showed that the charge present on nanosponge vesicle surface was stable.

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	14.98	-	-	14.98	14.98
Zeta Peak 1 Mean (mV)	6.766	-	-	6.766	6.766
Zeta Peak 2 Mean (mV)	35.43	-	-	35.43	35.43
Conductivity (mS/cm)	0.01558	-	-	0.01558	0.01558
Wall Zeta Potential (mV)	0	-	-	0	0
Zeta Deviation (mV)	19.28	-	-	19.28	19.28
Derived Mean Count Rate (kcps)	3335	-	-	3335	3335
Reference Beam Count Rate (kcps)	1817	-	-	1817	1817
Quality Factor	1.248	-	-	1.248	1.248

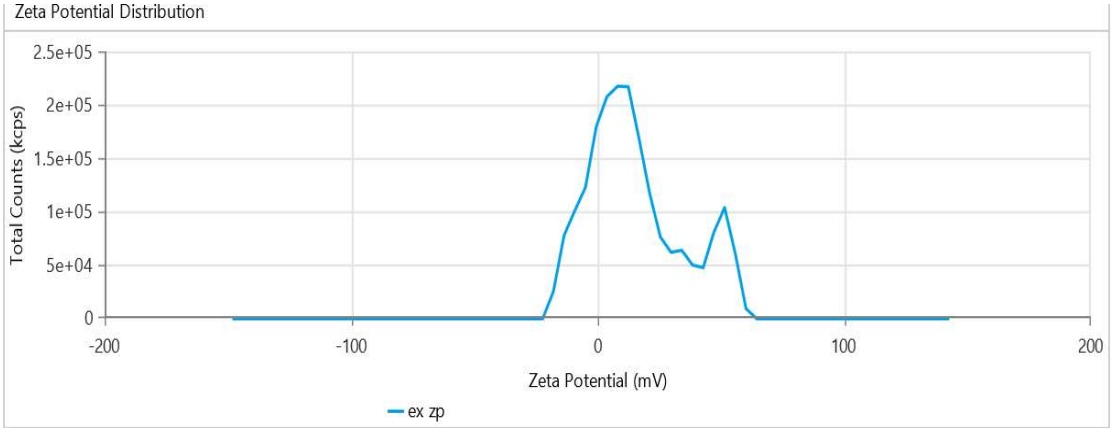

Figure. 8: Zeta Potential of F5 Batch of nanosponges

6.11 In- Vitro Drug Release Studies

The Drug Release studies were carried out in the phosphate buffer pH 6.8. The drug has shown release at alkaline pH. Also, Ethyl cellulose used as release modifier helps to retard the release of the drug in acidic medium and overall controlled release pattern is maintained. *In-Vitro* Drug release studies were performed by using USP dissolution test apparatus II (Paddle method) using 900 mL of PBS (phosphate buffer saline, pH 6.8) and stirring speed of 50 rpm at temperature 37.0±0.5°C. Five mL samples were withdrawn, replaced with fresh media to maintain sink conition, filtered and analyzed spectrophotometrically at 359 nm. The *in vitro* release patterns of nanosponges indicated that the rate of drug release was much higher as compared to pure drug. Also optimized batch F5 has shown prominent drug release of31.71 % in 8 hrs.

Table 5: In-Vitro Drug Release of Nanosponges

TIME	F1%	F2%	F3%	F4%	F5%	F6%	F7%	F8%	F9%
30	1.44	1.08	2.16	1.44	2.52	1.44	1.80	2.52	1.44
60	2.09	2.39	4.27	3.02	4.63	3.02	4.17	5.43	4.34
120	3.12	3.42	7.14	4.13	7.50	4.34	7.81	8.51	7.66
180	5.10	5.40	10.39	5.63	10.25	5.53	11.06	12.02	11.07
240	9.09	9.34	14.22	8.22	14.29	7.51	14.89	17.11	14.61
300	13.02	13.06	17.81	12.52	18.48	11.25	18.40	20.25	18.22
360	16.85	16.73	21.55	16.87	22.78	15.03	22.06	23.60	21.97
420	20.52	20.21	25.04	21.34	27.19	18.93	25.47	26.64	25.43
480	24.08	23.61	27.70	25.85	31.71	23.08	28.43	30.89	29.04

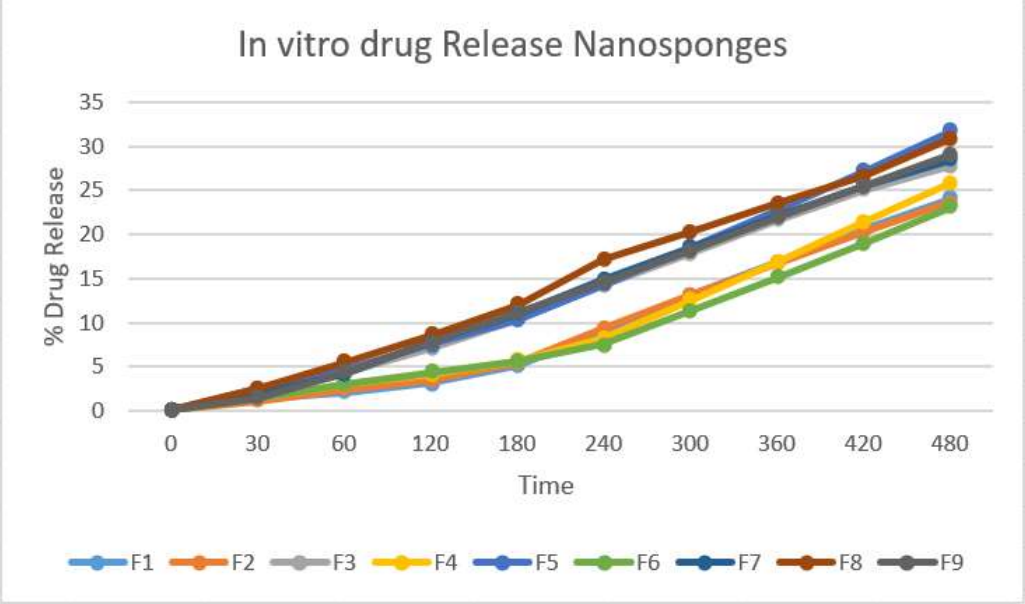

Figure. 9*: In-Vitro* Drug Release in Nanosponges

Table. 8.6: Comparison Pure drug Marketed tablet And Optimized Batch Nanosponges.

Time (hr.)	Pure Drug (%)	Marketed Tablet (%)	F5 Batch Nanosponges (%)
30	2.16	1.80	2.52
60	5.32	4. 70	4.63
120	8.62	10.03	7.50
180	13,06	15.60	10.25
240	17.07	21.33	14.29
300	24.51	27.21	18.48
360	32.48	33.26	22.78
420	41.51	39.88	27.19
480	52.25	47.90	31.71

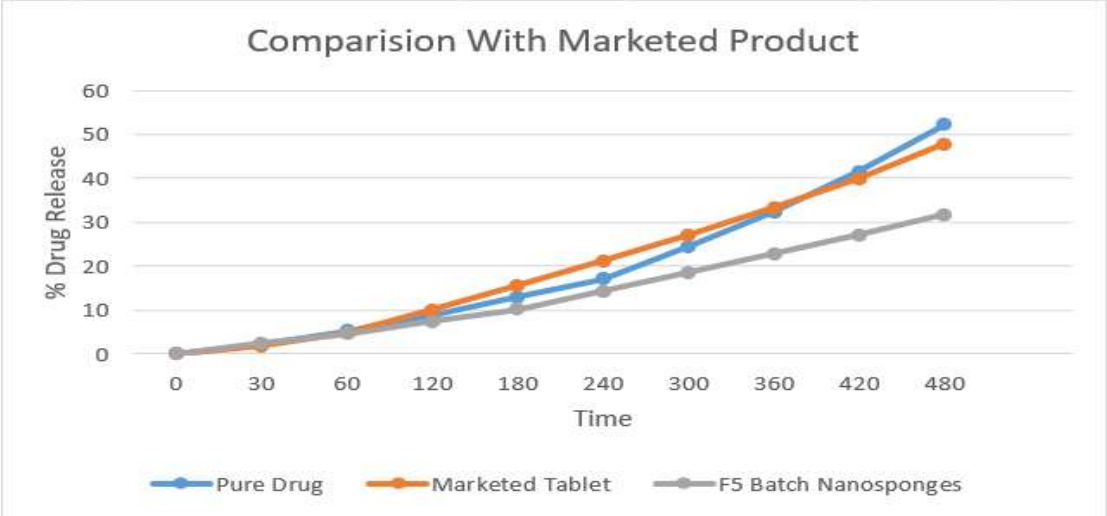

Figure.10: Comparison of Pure drug, Marketed tablet And Optimized Batch Nanosponges.

7. Conclusion:

Sulfasalazine nanosponges are formulated by Emulsion solvent diffusion technique. The ratio of drug and polymer plays a vital role in a formulation that optimizes final formulation. Also, ethyl Cellulose is playing important role as a release modifier that help to retard the drug release. Ethyl cellulose nanosponges have proven to be effective for controlling the release of a drug within the intestinal part of the GIT. Combination of Ethyl cellulose and Polyvinyl alcohol has substantially retarded the drug release in controlled manner up to 24 hours. The optimized batch F5 was characterized by the drug release pattern, SEM, UV Visible Analysis, FT-IR, Percentage Yield, Drug Entrapment Efficiency, Zeta Potential, and Particle Size. The results have confirmed the formation of a stable and efficacious drug delivery system. The smaller particle size of nanosponges occupies the larger surface area in GIT, resulting in increased absorption and higher bioavailability. The controlled release pattern of drug also helps with the delayed therapeutic response and reduced dosing frequency and enhanced patient compliance.

Acknowledgment

The author express gratitude to his guide Prof. **Bhise. S. D.** and co-guide Prof. **Gaikwad. O.A**. Faculty of Pharmacy, of Rajgad Dnyanpeeths College of pharmacy Bhor, Pune, SPPU University for his kind support.

Reference:

1. Selvamuthukumar Subramanian, Anandam Singireddy, Kannan Krishnamoorthy and Manavalan Rajappan, Nanosponges: A Novel Class of Drug Delivery System - Review, Journal of Pharmacy and Pharmaceutical Sciences, Jan, 2012; 15(1): 103-111.
2. Trotta F, Cavalli R, Tumiatti W, Zerbinati O, Rogero C, Vallero R. Ultrasound-assisted synthesis of Cyclodextrin-based nanosponges. EP1 786 841 B1, 2007.
3. Gupta M, Agrawal U, Vyas SP. Nanocarrier-based topical drug delivery for the treatment of skin diseases. Expert Opin Drug Deliv. 2012;9(7):783-804. doi: 10.1517/17425247.2012.686490, PMID 22559240.
4. Hanauer SB. Inflammatory bowel disease: Epidemiology, pathogenesis, and therapeutic opportunities. Inflamm Bowel Dis. 2006; 12: S3-S9. https://doi.org/10.1097/01.MIB.0000195385.19268.68
5. Das S, Deshmukh R, Jha A. Role of natural polymers in the development of multiparticulate systems for colon drug targeting. Syst Rev Pharmacy 2010; 1(1):79-85. https://doi.org/10.4103/0975-8453.59516.
6. Leuva VR, Patel BG, Chaudhary DJ, Patel JN, Modasiya MMK. Oral colon-specific drug delivery system. J Pharm Res 2012; 5(4):2293-7.
7. Kumar M, Ali A, Kaldhone P, Shirode A, Kadam VJ. Report on pharmaceutical approaches to colon targeted drug delivery systems. J Pharm Res 2010; 3(3):157-159.
8. Philip AK, Philip B. Colon targeted drug delivery systems: a review on primary and novel approaches. Oman Med J 2010; 25(2):79-87. https://doi.org/10.5001/omj.2010.24
9. Zheng W, Winter SM, Mayersohn M, Bishop JB, Sipes IG. Toxicokinetics of sulfasalazine (salicylazosulfapyridine) and itsmetabolites in B6C3F1 mice. Drug Metab Dispos 1993; 21(6):1091-1097.
10. Ramezani Z, Dibaee N. Determination of sulfasalazine in sulfasalazine tablets using silver nanoparticles. Iranian J of Pharm Sci 2012; 8(2):129-134.
11. Mehmood Y, et al. UV-Visible Spectrophotometric Method Development and Validation of Assay of Iron Sucrose Injection. Int J Pure App Biosci. 2015;3(2):41-53.
12. Kurhe A, Prakash K, Pande V. Scaffold based drug delivery system: A special emphasis on nanosponges. IJPDA. 2015;3(4).
13. Ashnil V, Sukhdev S, Rupinder K. Topical gel as drug delivery system: A review. Int J Pharm Sci. 2013;23(2):374-82.
14. Su YJ, Chen TH, Hsu CY, Chiu WT, Lin YS, Chi CC. Safety of metformin in psoriasis patients with diabetes mellitus: A 17-year population-based real-world cohort study. J Clin Endocrinol Metab. 2019;104(8):3279-86. doi: 10.1210/jc.2018-02526, PMID 30779846.
15. Gupta M, Agrawal U, Vyas SP. Nanocarrier-based topical drug delivery for the treatment of skin diseases. Expert Opin Drug Deliv. 2012:9(7):783-804. doi: 10.1517/17425247.2012.686490, PMID 22559240.

16. Rena G, Pearson ER, Sakamoto K. Molecular mechanism of action of metformin: Old or new Insights? Diabetologia. 2013;56(9):1898-906, doi: 10.1007/s00125-013-2991-0, PMID 23835523.
17. Ip W, Kirchhof MG. Glycemic control in the treatment of psoriasis. Dermatology. 2017;233(1):23-9. doi: 10.1159/000472149, PMID 28538228.
18. Shringirishi M, Prajapati SK, Mahor A, Alok S. Yadav P. Verma A. Nanosponges. A potential nenocarrier for novel drug delivery-a review. Asian Pac J Trop Dis. 2014; 4:5519-26. doi: 10.1016/S2222-1808(14)60667-8.
19. Donnelly RF, Singh TRR, Gartand MJ, Migalska K, Majithiya R, McCrudden CM, et al. Hydrogel-forming microneedle arrays for enhanced transdermal drug delivery. Adv Funct Mater. 2012;22(23):4879-90. doi: 10.1002/adfm.201200864, PMID 23606824.
20. Patel EK, Oswal RJ. Nanosponges and microsponges: A novel drug delivery system. Int J Res Pharm Chem. 2012;2(2):237-44.
21. Balasaheb MT, Moreshwar PP. Nanosponges: An emerging drug delivery system. Int J Inst Pharm Life Sci. 2015;5(6):160-74.
22. Shaikh AN, Pawar AY. Formulation and evaluation nanosponges loaded hydrogel of luliconazole. IJSDR.ISSN: 2455-2631, 2020;5(8):215-27.
23. Ozoude CH, Azubuike CP, Ologunagba MO, Tonuewa SS, Igwilo CI. Formulation and development of metformin-loaded microspheres using Khaya senegalensis (Meliaceae) gum as co-polymer. Futur J Pharm Sci. 2020;6(1):120. doi: 10.1186/s43094-020-00139-6.
24. Gangadharappa HV, Sarat M. Formulation and evaluation of Celocoxin nanosponges Hydrogel for Topical Application. Int J Drug Deliv Sci Technol. 2017:1-48.
25. Jilsha V, Viswanad V. Nanosponges loaded hydrogel of cephalexin for topical drug delivery. Int J Pharm Sci Res. 2015;6(7):2781-9.