FORMULATION AND EVALUATION OF TINOSPORA CORDIFOLIA LOADED NANOSPONGES

B.V. Bhagat*[1], S. A. Hapse*[1], A. R. Pawar*[2], S.N. Belhekar[3], M. D. Sonawane[4]

[1]Dr. Vithalrao Vikhe Patil Foundations College of Pharmacy, Ahilyanagar, Maharashtra, India, 414111

[2]Mula Education Society's College of Pharmacy, Sonai, Newasa, Ahilyanagar, Maharashtra, India, 414105

[3]Gourishankar Institute of Pharmaceutical Education and Research, Limb, Satara, Maharashtra, India, 415015

[4]Pravara Rular College of Pharmacy, Loni, Maharashtra, India, 413736

Address for correspondence:

Dr Babasaheb Vasantrao Bhagat

Dr. Vithalrao Vikhe Patil Foundations College of Pharmacy,

Ahilyanagar, Maharashtra, India 414111

ABSTRACT

Background: Tinospora cordifolia, a natural product with numerous uses in traditional ayurvedic medicine, has gained global interest due to its active components and biological role in illness prevention. **Methodology:** This study focuses on the formulation and evaluation of nanosponges loaded with Tinospora cordifolia extract, using an emulsion solvent diffusion method. **Results and Discussion:** SEM images revealed the nanosponges, which were visually inspectable through optical binocular microscopy. The F12 batch showed fine, spherical nanosponges with a maximum percentage yield of 74%. The nanosponges were porous, smooth, and spherical, with a particle size of 192.674 nm. **Conclusion:** Diabetes mellitus may be treated with nanosponges loaded with Tinospora Cordifolia. This may lead to a decrease in insulin resistance and an increase in insulin receptor sensitivity on cells. Furthermore, it could lead to continuous drug delivery, which would lower dosage, frequency of administration, and adverse effects.

KEYWORDS

Tinospora Cordifolia, Nanosponges, Antidiabetic, Polymer, Ethyl cellulose, SEM, TEM, FT-IR, Particle size.

INTRODUCTION

Diabetes mellitus, a prevalent metabolic illness, leads to microvascular consequences like retinopathy, neuropathy, and nephropathy, as well as macrovascular complications like heart attack. The disease is rapidly spreading globally, with 90% to 95% of cases caused by non-insulin-dependent diabetes, which results in higher blood glucose levels [1, 2].

Insulin and antidiabetic drugs are currently used to treat diabetes, but their effectiveness in reducing hypoglycemic effects is being studied. Certain plants have been found to affect hypoglycemia [3]. These plants, rich in glycosides, alkaloids, terpenoids, flavonoids, and carotenoids, have been shown to induce insulin production, prevent glucose absorption, and promote insulin-dependent processes [4]. The development of new antidiabetic drugs from medicinal plants holds great potential for treating diabetes globally [5].

Nanosponges, microscopic particles with a wide variety of components, can transfer lipophilic and hydrophilic chemicals, making less water-soluble molecules more soluble [6]. These tiny structures, resembling meshes, have the potential to revolutionize disease treatment, with a polyester scaffold structure acting as its backbone [7]. Polyester segments are cross-linked with tiny molecules to form a spherical shape, containing medication molecules. These segments are encapsulated in nanoparticles, which can be categorized into encapsulating, complexing, and conjugating nanoparticles based on their interaction with drugs [8].

Thus the aim of this study was to improve taste of Tinospora Cordifolia extract through incorporating into ethyl cellulose nanosponges. Thirteen formulations were designed based on the use of Plant extract- Polymer ratio, aqueous phase, stirring time. The evaluations of Tinospora cordifolia extract nanosponge formulations along with their In vitro drug release pattern are reported.

MATERIAL AND METHOD

Materials

Tinospora Cordifolia plant was collected from rural areas of Nannaj Dumala, Tal. Sangamner, Dist. Ahmednagar, Maharashtra. Ethyl Cellulose, Methanol, Polyvinyl Alcohol, Dichloromethane were obtained from Lobachemie Pvt. Ltd., Mumbai and Acetone was obtained from Molychem, Mumbai.

Methods

Plant extraction

Stems of Tinospora cordifolia were cut into small pieces and dried under shade for 7–10 days. After drying, the stems were pulverized using an electric grinder. Firstly, the dried sample was extracted with a solvent of methanol and acetone in a ratio of 70:30 (i.e., 560 mL of methanol and 240 mL of acetone) at 45 °C until a colorless solvent was obtained (for 16 hours) in the Soxhlet apparatus. After the extraction process was completed, the solvent from the round bottom flask was emptied into the beaker. That beaker was put in a water bath for 6 hours for the evaporation of solvent. After that, the extract was put on petri plates under sunlight for 2–3 days [9].

Preparation of Tinospora Cordifolia loaded Nanosponges

Table 1: Formulation of Nanosponges by Emulsion Solvent Diffusion Method.

Batch	Extract	Polymer	Dichloro-methane	Aqu. Phase		Stirring Time	Particle size
				Water	PVA		
F1	1 gm	1 gm	20 mL	100 ml	4 gm	30 min	205.894 nm
F2	1 gm	2 gm	20 mL	100 ml	4 gm	30 min	186.083 nm
F3	1 gm	3 gm	20 mL	100 ml	4 gm	30 min	194.105 nm
F4	1 gm	1 gm	20 mL	100 ml	3 gm	30 min	212.07 nm
F5	1 gm	2 gm	20 mL	100 ml	3 gm	30 min	450 nm
F6	1 gm	3 gm	20 mL	100 ml	3 gm	30 min	448.27 nm
F7	1 gm	1 gm	20 mL	100 ml	3 gm	30 min	1087.12 nm

F8	1 gm	2 gm	20 mL	150 ml	3 gm	30 min	838.599 nm
F9	1 gm	3 gm	20 mL	150 ml	3 gm	30 min	1194.97 nm
F10	1 gm	1 gm	20 mL	150 ml	3 gm	45min	967.661 nm
F11	1 gm	2 gm	20 mL	150 ml	3 gm	45 min	904.735 nm
F12	1 gm	2 gm	20 mL	150 ml	3 gm	60 min	44.807 nm
F13	1 gm	3 gm	20 mL	150 ml	3 gm	60 min	74.613 nm

The emulsion solvent diffusion method was used to formulate Tinospora cordifolia-loaded nanosponges by using ethyl cellulose (Polymer). In this emulsion solvent diffusion method there are two phase Inner phase (Organic phase) and Outer phase (Aqueous phase).The Organic phase consists of a specified amount of drug and polymer that was dissolved in 20 ml of an organic solvent (dichloromethane). Firstly aqueous phase was prepared; in this phase 3 gm of polyvinyl alcohol (PVA) was taken and slowly added into 150 ml distilled water. This was an aqueous phase. After complete dissolution of PVA, this phase was cooled at room temperature, finally aqueous phase is ready. Then 1 gm of drug (extract) and 2 gm of polymer (Ethyl Cellulose) was added in 20 ml of Dichloromethane (solvent) and mixed it properly. This was an organic phase. After that aqueous phase was put on magnetic stirrer at 800-1000 rpm, and organic phase was added in aqueous phase drop wise. This solution was continuously stirred for 50 to 60 minutes, then filtered and washed with distilled water. Nanosponge was dried at room temperature for 24 hours [10].

EVALUATION OF NANOSPONGES

Prepared Nanosponges was evaluated for various parameters, are as follows

Determination of production yield

The production yield of nanosponge was determined by calculating accurately the initial weight of the raw materials and the final weight of the Nanosponge obtained. Following formula was used to calculate production yield [8, 11].

Production yield= Practical mass nanosponges ÷ Theoretical mass (polymer + Drug) × 100

FTIR

The Shimadzu Spectrophotometer equipment was utilized to perform FTIR analysis and identify the characteristic peaks and their functional groups. Fourier Transform or FT-IR Perhaps the most precise method for identifying the many types of chemical bonding (functional groups) found in compounds is infrared spectrophotometry. The annotated spectrum shows the wavelength of light absorbed that distinguishes the chemical bond. Chemical bonding inside a molecule can be used to resolve the spectrum's infrared absorption. The FTIR analysis was conducted using the extract's plant components. Condensed plant extract (100 mg) in KBr pellet was used to create translucent sample discs [12].

Scanning electron microscopy (SEM)

SEM analysis was performed to determine the microscopic characters (shape and morphology) of prepared Tinospora Cordifolia-loaded nanosponges. Nanosponges were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy (Hitachi X650, Tokyo, Japan) at different magnifications. Samples were placed on a glass slide kept under vacuum, and then, using a sputter coater unit, samples were coated with a thin gold layer. The unit operated at a 15 kV acceleration voltage [13].

Particle size

The particle size of nanosponge is a key factor in their performance, as it significantly impacts the rate and extent of drug release and consequently its absorption. A smaller particle size improves drug release as it offers a larger interfacial area for diffusion of the drug. Particle size analysis of prepared nanosponges was carried out using a zeta sizer (Particulate System Nano Plus) [14].

In-vitro study

Dissolution studies on powder samples of pure Tinospora Cordifolia extract and Nanosponges formulations were carried out using USP type II apparatus (Erweka DT 700). Pure drug (100 mg) and NS (equivalent to 100 mg of drug) were accurately weighed and filled in hard gelatin capsules. Dissolution was carried out at 37 ± 0.5 ^{o}C with a paddle speed of 50 rpm using 900 mL of phosphate buffer (pH 6.8) as a dissolution medium. Samples (5 mL) were withdrawn at

predetermined time intervals and replenished with the same amount of fresh, preheated (37 ± 0.5°C) dissolution medium in order to maintain the sink condition. The samples were filtered (pore size 0.45 µm membrane filter) and analyzed using a calibration curve with an R2 value of 0.998 at λmax 229 nm. All the studies were performed in triplicate.Correlation Coefficient (R2) values were used to explain the release kinetics of the drug [14, 15].

Transmission electron microscopy (TEM)

TEM was used at transmissions of 60 kV where approximately 10 µL of nanosponge (NS) sample was diluted with Milli-Q water to 100 µL. In order to visualize the sample, 5 µL of the watery mixture, the sample was held on a network, One drop of formulation suspension was deposited on a carbon-coated copper grid and allowed to dry for contrast enhancement which was then held on glass plate for microscope and then microscopically observed [8, 16].

RESULTS AND DISCUSSION

In the study, Tinospora Cordifolia extract was entrapped in Ethyl Cellulose nanosponges using dichloromethane as a solvent. Thirteen formulations were designed based on based on the use of Plant extract- Polymer ratio, aqueous phase, stirring time. Production yield, FT-IR, SEM, Particle Size Measurement, TEM of the selected formulation were studied. In vitro drug release was investigated for selected formulation.

Determination of production yield

By precisely quantifying the initial weight of the raw materials and the end weight of the nanosponges produced, the production yield of the nanosponges was ascertained**.**

Table 2: Production yield (%)

Batch	% Yield	Batch	% Yield
F1	72%	F8	67%
F2	58%	F9	64%
F3	53%	F10	71%
F4	54%	F11	69%
F5	70%	F12	74%
F6	59%	F13	63%
F7	63%	-	-

The percentage yield was minimum for formulation F3 (53%) and maximum for formulation F12 (74%). From the results it was concluded that as the drug and polymer used in ratio 1:2, and

aqueous phase containing PVA (3gm) in 150 mL distilled water with stirring time 60 min obtained higher yield.

FTIR

The compound's functional groups were examined using FTIR. There were four main peaks seen. The first peak had a peak value of 2948.73 and revealed an aromatic ring (C-H). The other peaks showed primary, secondary amines, and amides (NH) with peak values of 2310.86 and 2335.80, and α, β-unsaturated aldehydes and ketones (C = O) with a peak value of 1051.20. The final peak value, which had values of 2850.68 and 2948.73, showed the existence of alkyl halides (C-H). After analyzing the primary peaks and functional groups of the dynamic compound groupings, the findings were contrasted with a typical infrared chart.

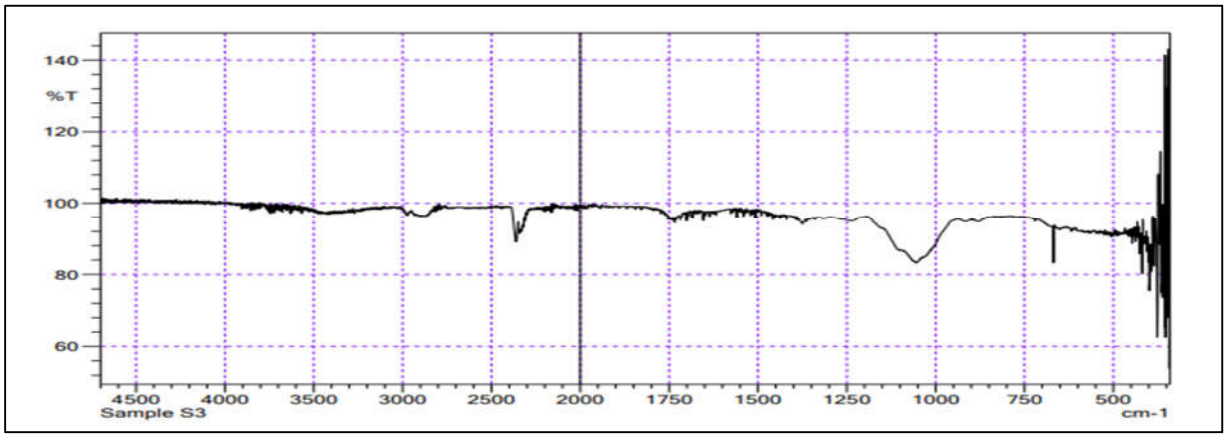


Figure 1: FTIR Spectrum of Nanosponges loaded with Tinospora Cordifolia extract

Scanning electron microscopy (SEM)

SEM analysis of the formulated Tinospora Cordifolia loaded nanosponges was performed to evaluate the surface morphology of nanosponges. The SEM images of formulation F12 are shown in Figure 2. SEM images showed the nanosponges was porous with a smooth surface morphology and spherical in shape. The spongy and porous nature of the nanosponges can be seen in the below figure 2. The presence of pores was due to the impression of diffusion of the solvent dichloromethane.

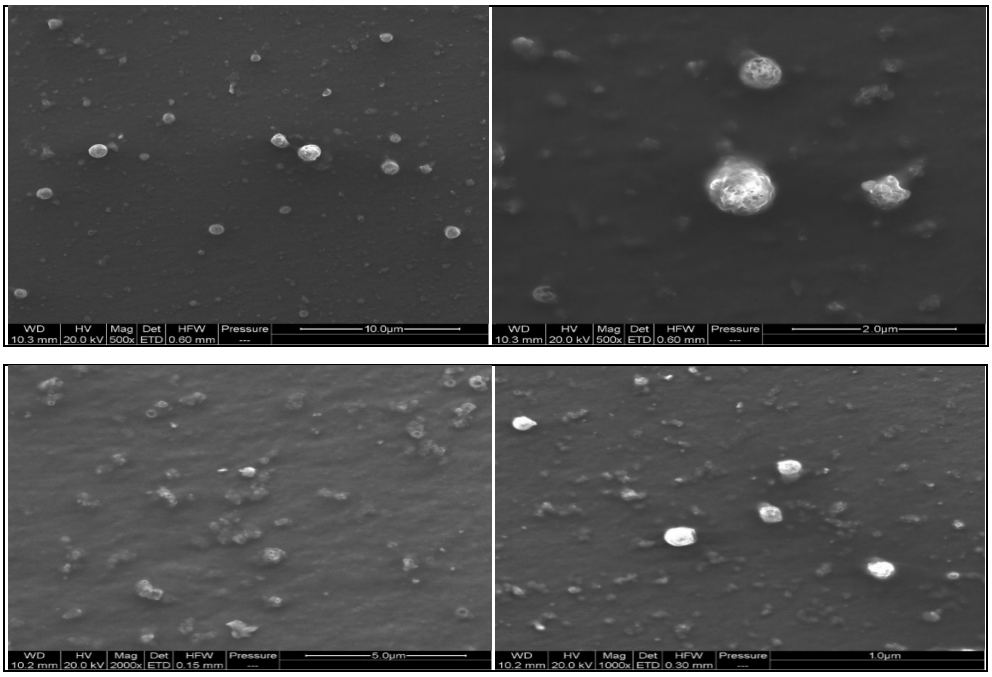
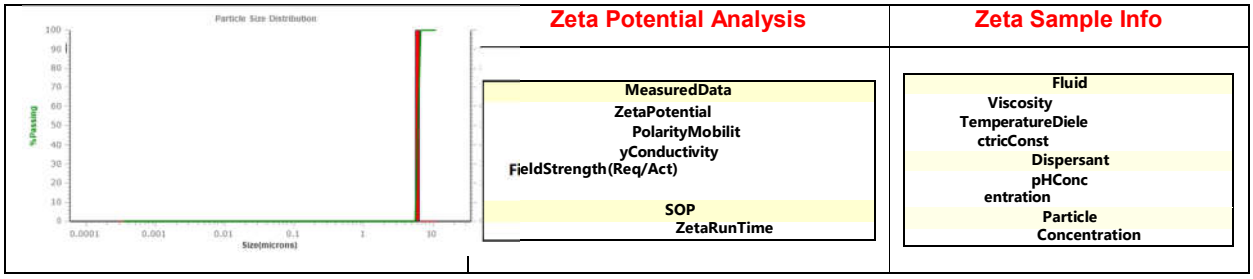


Figure 2: SEM image of Nanosponges loaded with Tinospora Cordifolia extract (F12)

Particle size

Using a Nano Plus zeta sizer, the average particle size of the prepared Tinospora Cordifolia loaded nanosponges was determined. The average particle size of the Tinospora Cordifolia loaded nanosponges made with ethyl cellulose (F12) were determined by particle size analysis to be 192.674 nm, or less than 1 um, or 1000 nm. Figure 3 shows the zeta size distribution of ethyl cellulose –Tinospora Cordifolia nanosponges.



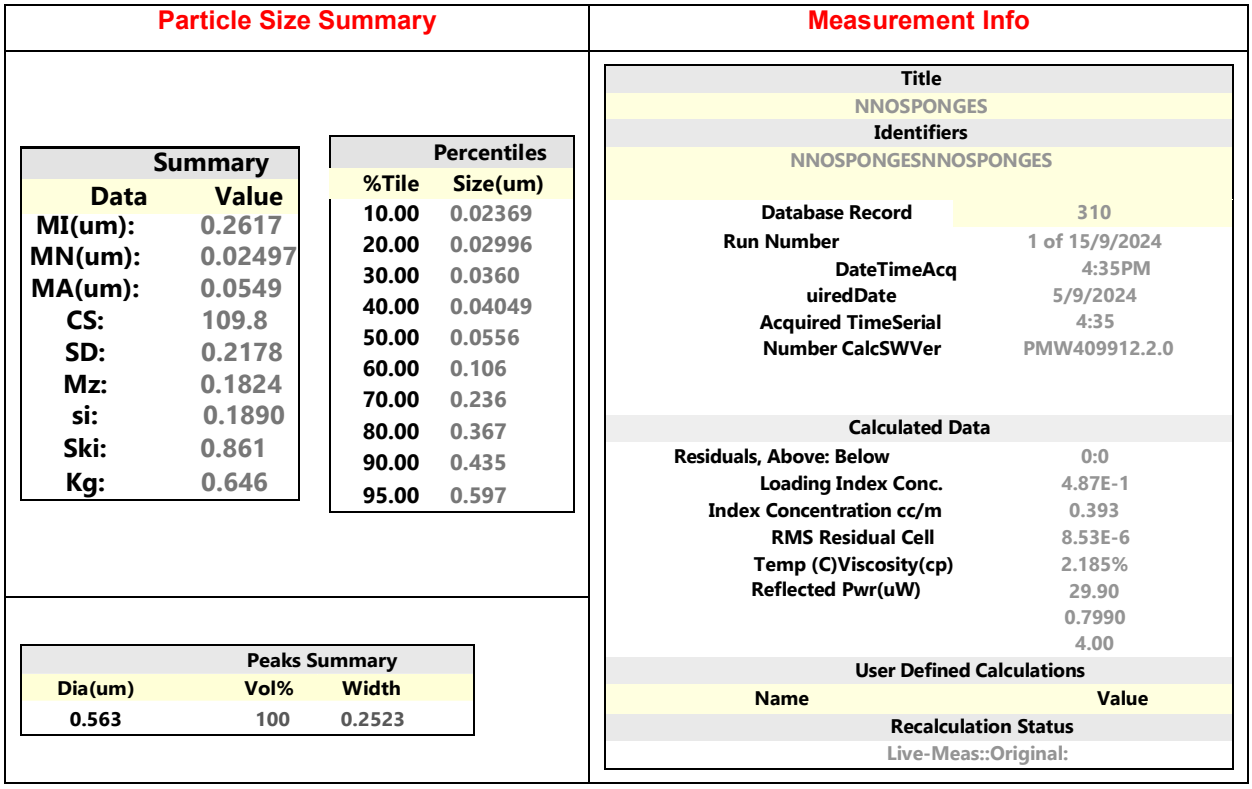


Figure 3: Particle Size Summary of Nanosponges

The Diameter of ethyl cellulose –Tinospora Cordifolia nanosponges was analyzed 0.563 µm which is lesser than 1µm.

In-vitro study

Using a USP Dissolution type II equipment, an in vitro drug release analysis of the prepared Tinospora Cordifolia loaded nanosponges was conducted. The amount of medication discharged at various intervals was noted. Table 3 and 4 presents the in vitro drug release profile data of nanosponges loaded with Tinosporacordifolia and containing ethyl cellulose.

Table 3: In vitro drug release profile of Tinospora Cordifolia loaded nanosponges (F-1 to F7)

Sr. No.	Time (Hrs)	Cumulative percentage drug release (%)						
		F-1	F-2	F-3	F-4	F-5	F-6	F-7
1	0	0	0	0	0	0	0	0
2	1	7.32	7.89	9.22	10.9	8.38	8.85	8.23
3	2	8.96	8.34	10.36	13.67	10.75	9.22	9.51

4	3	9.89	9.03	13.73	15.30	19.43	13.31	10.03
5	4	10.56	10.94	16.95	17.54	21.12	16.75	12.76
6	5	11.45	11.64	20.56	21.16	24.89	23.22	14.90
7	6	14.98	13.94	24.74	24.61	29.00	26.39	16.14
8	7	16.16	17.38	28.86	28.30	36.87	32.38	18.16
9	8	20.54	21.37	32.91	30.41	44.45	37.89	21.28
10	10	23.54	28.84	37.89	32.14	49.35	43.86	24.54
11	12	28.81	35.85	40.00	34.45	52.26	49.93	36.40
12	24	33.10	41.48	43.67	38.61	59.34	54.84	40.16
13	32	34.13	53.37	49.39	40.11	65.89	59.78	42.89
14	36	47.95	60.26	54.54	45.12	72.14	63.60	46.17
15	48	59.11	72.68	59.95	51.59	78.03	67.33	59.95

Table 4: In vitro drug release profile of Tinospora Cordifolia loaded nanosponges (F-8 to F13)

Sr. No.	Time (Hrs)	Cumulative percentage drug release (%)					
		F-8	F-9	F-10	F-11	F-12	F-13
1	0	0	0	0	0	0	0
2	1	9.32	9.16	10.52	10.88	11.92	9.9
3	2	10.10	11.46	13.13	15.26	20.62	11.10
4	3	13.17	15.93	16.38	22.54	24.90	13.17
5	4	19.79	20.46	19.46	30.24	31.24	20.79
6	5	23.57	24.83	23.84	36.56	38.89	24.57
7	6	27.94	29.57	29.47	41.14	43.27	28.94
8	7	33.56	35.67	35.89	46.83	47.53	35.56
9	8	39.67	39.47	40.34	50.85	52.69	41.67
10	10	45.93	46.93	46.89	53.94	54.16	46.93
11	12	48.94	49.54	52.43	56.99	57.61	51.94

12	24	51.18	53.57	59.99	61.50	63.35	57.18
13	32	58.6	59.87	65.98	67.39	70.58	64.6
14	36	63.78	65.05	72.59	75.83	73.72	76.78
15	48	69.37	71.45	83.57	81.88	91.19	88.65

Tinospora Cordifolia extract - ethyl cellulose nanosponges formulation F12 demonstrated the best release of 91.19% at the end of 48 hours, according to the in vitro release data. The ratio of medication to polymer has an impact on the release rate. A rise in drug release was noted in relation to the drug:polymer ratio. For every formulation, it was shown that as the amount of polymer increased, the drug release dropped. This could be because the medication is released from the polymer matrix only after the polymer has completely swelled, and the time needed for the polymer to swell rises with the amount of polymer in the formulation.

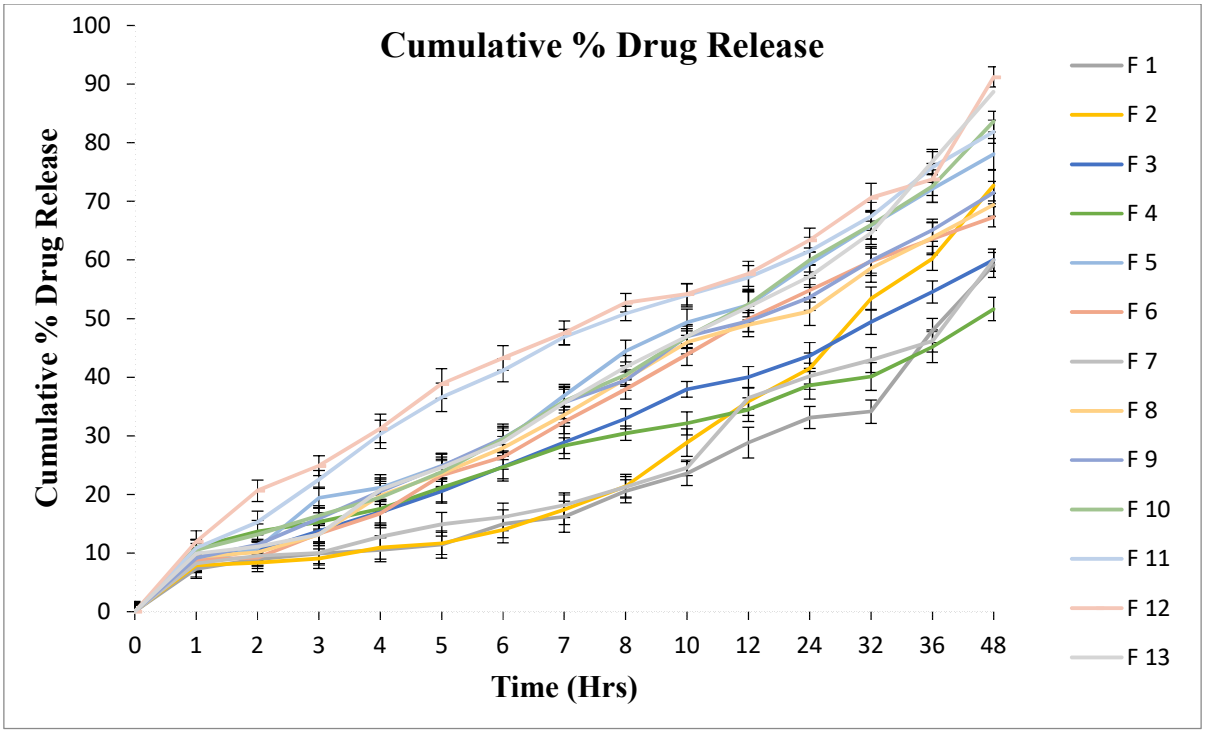


Figure 4: In vitro drug release profile of Tinospora Cordifolia loaded nanosponges

Transmission electron microscopy (TEM)

The consistent spherical form and sizes of the nanosponges were revealed by TEM examinations. TEM measurements showed that the average particle size was around 156.25 nm. The drug-loaded NS's diameters were shown by laser light scattering to be between 50 and 500 nm, which was consistent with the findings of the TEM investigations.

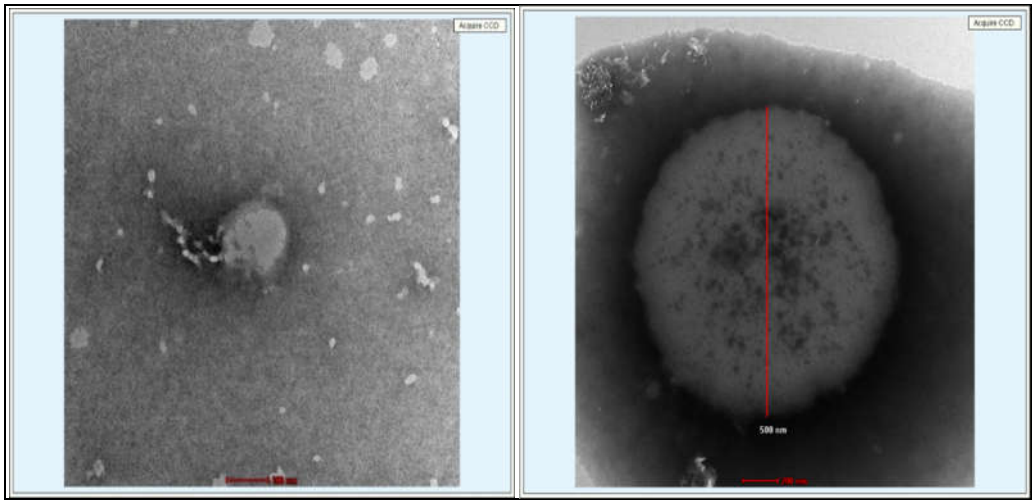


Figure 5: TEM image of Nanosponges loaded with Tinospora Cordifolia extract

SUMMARY AND CONCLUSION

The study aimed to create nanosponges loaded with Tinospora cordifolia extract to target diabetes mellitus and release medication in a regulated manner. FTIR studies confirmed the presence of alkaloids and flavonoids in the extracts. SEM pictures made it evident that the nanosponges were porous and spongy. The emulsion solvent diffusion method was used for formulation, and the nanosponges were found to be spherical, porous, and smooth. The nanoscale range of the prepared sample was verified by the particle size analysis, with the formulation F12 yielding an average particle size of 192.67 nm. Tinospora Cordifolia loaded nanosponges can be made quickly and cheaply using an emulsion solvent diffusion method with hydrophobic polymers such as ethyl cellulose. Tinospora Cordifolia-loaded nanosponges may be used as a treatment for diabetes mellitus. Insulin receptor sensitivity on cells may rise as a result, and insulin resistance may decrease. Additionally, it might result in continuous drug delivery, which would reduce side effects, dose and frequency of administration.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Anil Pawar, Babasaheb Bhagat and Sandip Hapse collected plant and others excipients. Also carried out extraction and formulation of nanosponges loaded with Tinospora cordifolia extract. Santosh Belhekar conducted evaluation of prepared formulation for various parameters. Manisha Sonawane wrote the first draft of the manuscript and all authors reviewed and revised previous versions. All authors contributed to the study's conception and design and gave final approval.

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