Formulation and Evaluation of Solid Lipid Nanoparticles of Wheatgrass (Triticum Aestivum) Extract

Neha Minocha[1]*, Nidhi Sharma[2], Parijat Pandey[3], Sangita Saini[4]

ABSTRACT:

Objective: The development of nanoparticles for drug delivery is gaining popularity swiftly, especially for anticancer drugs as it holds the possibility of addressing a number of issues with these medications. The rationale of current research-work was to formulate wheatgrass loaded solid lipid nanoparticles by means of hot-homogenization method. Specifically, we investigated the effects of chemical that stabilizes the surface and time for sonication on the amount of wheatgrass extract that maximally and efficiently entrapped in solid lipid nanoparticles.

Method: Under the methodology section, three factors were assessed i.e., concentration of sodium alginate (2-10 mg/ml), concentration of chitosan (2.5-12.5 mg/ml) and time required for sonication (5 - 20 min). Different batches of solid lipid nanoparticles, were evaluated for their size of particles in formulation, entrapment efficiency, polydispersity index, percentage yield and loading capacity. The optimized batch was characterized for its compatibility studies by Fourier Transform Infrared Spectroscopy (FTIR), zeta potential for stability and Scanning Electron Microscopy for morphology.

Results: Characterization and evaluation of prepared SLNs showed that batch NM-3 (formulated using chitosan and sodium alginate having amount of 7.5mg and 5mg, respectively while sonicating the formulation for 15 minutes) having a maximum entrapment efficiency 49.75±0.13 % (w/w), a maximum drug loading capacity 54.78±0.19 % (w/w) observe smallest particle size of 375.5nm among all fifteen batches (NM-1 to NM-15). FTIR spectra interpretation of NM-3 indicated the compatibility of drug and the polymer as, comparison of the interpretations for both the FTIR showed no significant changes.

Conclusion: Wheatgrass loaded nanoparticles are developed by analyzing the nano-effects caused by biologically acceptable and compatible chitosan and the time required for sonication.

Keywords: Wheatgrass, Solid lipid nanoparticles, Chitosan, Hot homogenization, FTIR, Characterization, Nanoparticles.

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INTRODUCTION:

Recently, the use of nanotechnology has been implemented to create a drug deliver system that is more efficient, regulated, controlled and reliable. In the past two decades, extensive focus has been made on the advancements in the novel drug delivery system (NDDS) [1]. NDDS delivers the active ingredient at the target site without any potential side effect to body organs and without any immunogenicity reactions. Manufacturing technique use to formulate newer drug delivery systems enhances the penetrability, stability and solubility of drug, which ultimately aid in their acceptability towards patient compliance [2].

Nanostructured materials like liposomes [3], emulgel [4-5], solid lipid nanoparticles [6], nanocrystals [7] and etc. have gained great

***Corresponding Author:** Neha Minocha
Address: [1*, 4] Shri Baba Mastnath Institute of Pharmaceutical Sciences and Research, Baba Mastnath University, Rohtak 124001, Haryana, India. [1]E-mail: neha.minocha.bmu@gmail.com
[2]Dr. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar-201204, Uttar Pradesh, India.
[3]Department of Pharmaceutical Sciences, Gurugram University, Gurugram 122018, Haryana, India.
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interest [8-9]. With the developments in nanotechnology, we have several approaches to administer lipophilic as well as hydrophilic drugs with improved bioavailability and solubility profiles. Drug delivery systems involving nanotechnology have evolved as an interesting approach to improve the formulations' most desirable properties. The objective of devising novel drug delivery systems is to augment the scientific efficiency via recent medications by refining the process of drug bio-distribution, volume, and dose duration [10].

Owing to small size of drug and formulated particles, it delivers the active ingredient to the target site in a firm manner. The active ingredient is dissolved, encapsulated, entrapped, or linked to a matrix of nanoparticles. The important part when creating a delivery mechanism for nanoparticles, is to modulate the release, surface chemistry, and to regulate the particle size to achieve the drug's site-specific effect at the optimal rate and optimal dosage for the treatment [11].

Chitosan is a biocompatible material that has a great deal of potential for building nanoparticulate drug delivery systems since it can control drug release. Chitosan has a wide range of uses in controlled drug delivery systems and is soluble in dilute acidic solutions. Nanoparticles having chitosan as an ingredient possess several benefits, primarily for the designing of novel nanoparticulates [12-13].

Cancer is a disease that is life-threatening and comes from a family of complex diseases. Though chemotherapy might effectively treat cancer, the noxious side effects and discomfort linked with chemotherapies may depreciate a patient's wellbeing. Free radicals are formed as an outcome of natural metabolism, causing oxidative stress and eventually leading to the expansion of several diseases. For cancer patients with prostate, breast, and colorectal cancers, functional diets and herbal therapies are complementary medicines. To be on safer side, one needs to include anti-oxidants in their diet. For cancer patients, functional diets and herbal therapies are complementary medicines. The accessibility of targeted treatments has turned out to be a very convenient method in treatment of breast cancer [14].

Natural anti-oxidants found in wheatgrass (*Triticum aestivum*) have been shown to be effective at preventing cancer. Numerous vitamins, β-carotene, and enzymes included in wheatgrass have anti-oxidative and free radical-scavenging properties. It contains an antioxidant enzyme super oxide dismutase, which is able to convert freely reactive species of oxygen in radical form into hydrogen peroxide and a molecule of oxygen, killing cancerous cells in the process [15-16].

The purpose of current research was to formulate wheatgrass (*Triticum aestivum*) loaded solid lipid nanoparticles by means of hot homogenization method. Specifically, we investigated the effects of chemical that stabilizes the surface and time for sonication on the amount of wheatgrass extract that maximally and efficiently entrapped in solid lipid nanoparticles.

MATERIALS AND METHODS:

Materials: Raw material was collected, standardized and authenticated prior to use by CSIR- National Institute of Science Communication and Information Resources, New Delhi vide ref. no. NISCAIR/RHMD/Consult/2021/3785-86. The plant was identified as Wheatgrass (*Triticum aestivum*) belonging to the family Gramineae. Chitosan having molecular weight of 40-80 kDa was bought from Fluka Chemie, Germany. Wheatgrass (*Triticum aestivum Linn.*) Leaves were selected for the present study. Gallic acid, Potassium bromide, Sodium alginate, Calcium chloride were bought from Central Drug House, New Delhi. Methanol was purchased from Merck India Ltd., Mumbai, India and Dimethyl sulphoxide (DMSO) was purchased from Fisher Scientific Pvt. Ltd., Mumbai, India. Rest of the ingredients were used as provided by the manufacturer.

Methods:

Extraction of wheatgrass: The powder was created by crushing the shade-dried leaves of the wheatgrass plant. The soxhlet extraction method was used to defat wheatgrass powder, which weighed 30 grams, using petroleum ether. To find the most effective solvent for extraction, the defatted extract was dried before being extracted with various solvents, including water, methanol, ethanol, and

chloroform in different soxhlet apparatus. The extracts obtained from each solvent were filtered, concentrated to dryness at 40°C under decreased pressure, and then kept at a temperature of less than 4°C for future research. Methonolic extract was discovered to be the most suitable after analyzing the yield values of various solvents' extracts [17-18].

Preparation of Wheatgrass (*Triticum aestivum*) loaded Solid lipid Nanoparticles:

Hot homogenization technique was used to prepare *Triticum aestivum* extract loaded solid lipid nanoparticles (SLNs). In this method, sodium alginate solution (Solution A) was prepared in 10 ml of deionized water (2–5 mg/ml). Solution B of calcium chloride in deionized water (10 mg/ml) has been prepared. Chitosan solution (solution C) was prepared using 1% v/v acetic acid (5–10 mg/ml as per DOE) for 10 ml. The drug solution was prepared by dissolving wheatgrass extract (*Triticum aestivum*) and chitosan solution at a strength of 10 mg/ml, naming this mixture solution D. The chitosan and drug solution were together mixed to form a homogeneous solution using a magnetic stirrer having 700–1000 revolutions per minute upto 4 hours. With the help of a syringe, 10 ml of sodium alginate solution was added to 1 ml solution of calcium chloride and kept under constant stirring. Resultant combination of calcium chloride and sodium alginate was named solution E. While magnetic stirring, wheatgrass (*Triticum aestivum*) extract and chitosan mixture (solution D) were added to solution E drop by drop at a rate of 1 drop per sec during stirring. The solution obtained was sonicated by means of a probe sonicator at variable times (6 to 15 minutes), and the resulted wheatgrass extract loaded nanoparticle dispersion was centrifuged at a rate of 26000 revolutions per minute for 30 minutes. Upper layer of the dispersion was segregated, and solid lipid nanoparticles were subjected to lyophilized with the help of freeze-drying technique. Solid lipid nanoparticles of *Triticum aestivum* extract were formulated to analyze the effects on the amount of sodium alginate, amount of chitosan, and sonication time on the drug's capacity of loading into formulation, percentage yield, entrapment efficiency, particle size, and polydispersity index of wheatgrass (*Triticum aestivum*) [19-21]. In this research work, three factors were assessed and these were: X1 = Concentration of sodium alginate; X2 = Concentration of chitosan and X3 = Sonication Time. The preparation of wheatgrass loaded solid lipid nanoparticles were designed as per Table 1.

TABLE 1: BATCHES & VARIABLE CONCENTRATION IN WHEATGRASS LOADED SOLID LIPID NANOPARTICLES

Formulation Batch Code	Concentration of Sodium alginate (mg) (X_1)	Concentration of Chitosan (mg) (X_2)	Sonication Time (min) (X_3)
NM-1	2	2.5	5
NM-2	3.5	5	10
NM-3	5	7.5	15
NM-4	7.5	10	20
NM-5	10	12.5	15
NM-6	2	5	20
NM-7	3.5	7.5	10
NM-8	5	10	5
NM-9	7.5	12.5	10
NM-10	10	2.5	20
NM-11	2	7.5	5
NM-12	3.5	10	15
NM-13	5	2.5	5
NM-14	7.5	5	10
NM-15	10	12.5	15

Characterization of Solid Lipid Nanoparticles:

With the purpose of obtaining the most accurate results for the formulations prepared, the following characterizations were performed: particle size, entrapment efficiency, loading capacity, polydispersity index (PDI), percentage yield, and Fourier Transform Infrared Spectroscopy.

1. **Particle size:** Size of particles in formulation and zeta potential of the diluted samples were analysed using the Zetasizer nano series Nano-ZS90 (Malvern Instruments, Malvern, UK) fitted out with the Hydro dispersing unit. 2 mg of the sample was dissolved in 5 ml of distilled water. A polystyrene cuvette filled with diluted sample was placed in the hydro dispensing unit, and the scan was carried out at 64 runs per sample. This average diameter/scan was calculated after the scan was completed for all 64 runs, and this number is recorded as the Z-average.
2. **Poly Dispersity Index:** A dynamic light scattering technique can be used to determine the polydispersity index by determining the particle size distribution of the dispersion. Value of PDI may range between 0 and 1.0, with value 1.0 representing a dispersion having polydisperse particles while 0 value (zero) representing a system that is solely

monodisperse. Based on the following formula, the polydispersity index computes the extensiveness of the molecular weight distribution of a polymer:

PDI (Polydispersity index) = $\frac{Mw}{Mn}$.....eq (1)

Where, Mw is weight average molecular weight and Mn is number average molecular weight.

3. **Loading Capacity, Percentage Yield and Entrapment Efficiency:** The capacity of drug's loading, percentage yield and entrapment efficiency were find out using wheatgrass extract loaded solid lipid nanoparticles by analysing the dispersion sample followed by centrifugation using the sonication technique. Amount of drug (in percentage) included in the formulation, generated relative to the original amount of drug employed for their manufacture is known as drug entrapment efficiency (E.E.). UV spectroscopy was used to make this calculation. The following equations were used to determine entrapment effectiveness, drug loading, and percentage yield for the sonication process. Each sample was assayed in triplicate.

Loading Capacity (%) = $\frac{\text{Total drug} - \text{Free drug}}{\text{Nanoparticles weight}} \times 100$.....eq (2)

Entrapment Efficiency (%) = $\frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$.....eq (3)

Percentage Yield (%) = $\frac{Total\ nanoparticles\ weight}{\text{Total solid weight}} \times 100$.....eq (4)

4. **Fourier Transform Infrared (FT-IR) spectro-photometry:** By utilizing an interferometer, FT-IR produces infrared spectra by analyzing an interferogram of a sample signal, and then extracting a spectrum from the interferogram. FTIR technique was used to check any chemical interaction of extract loaded solid lipid nanoparticles by the Potassium bromide (KBr) pellet method. An Infrared spectrum was recorded in the 4000-400 cm^{-1} region.

RESULTS AND DISCUSSION:

In the experimental work, total 15 formulations of wheatgrass loaded solid lipid nanoparticles were formulated by optimizing three parameters – amount of sodium alginate (X1) in the range of 2mg, 3.5mg, 5mg, 7.5 mg and 10 mg, Concentration of chitosan (X2) in the range of 2.5mg, 5mg, 7.5mg, 10mg and 12.5mg and Sonication Time (X3) in the range of 5 min, 10 min, 15 min and 20 min. The results are shown in Table 2, which shows the loading capacity, entrapment efficiency, percentage yield, particle size, and polydispersity index for each formulation. Looking at the above 15 formulations, batch code NM-3 was found to be finest suitable because of maximum loading capacity of 54.78±0.19 (w/w), maximum entrapment efficiency of 49.75±0.13 (w/w) and minimum particle size of 375.5nm by using sodium alginate as surface stabilizer at sonication time ~ 20 min and having maximum percentage yield of 44.12%

Loading Capacity, Percentage Yield and Entrapment Efficiency: Prepared solid lipid nanoparticles having wheatgrass as an active ingredient were evaluated for loading capacity, entrapment efficiency and percentage yield and are tabulated in Table 2.

TABLE 2: EVALUATION OF WHEATGRASS LOADED SOLID LIPID NANOPARTICLES

Formulation Batch Code	Particle size (nm)	PDI	Loading Capacity (%)	Entrapment Efficiency (%)	Percentage Yield (%)
NM-1	565.1	0.725	33.21±0.13	29.79±0.12	38.50
NM-2	645.8	0.860	40.99±0.11	38.50±0.11	39.75
NM-3	375.5	0.627	54.78±0.19	49.75±0.13	44.12
NM-4	725.4	1.000	45.54±0.25	42.07±0.25	34.23
NM-5	631.9	0.722	43.67±0.36	40.88±0.16	31.79
NM-6	489.9	0.777	42.81±0.12	39.77±0.38	36.67
NM-7	460	0.682	52.77±0.17	48.11±0.56	41.30
NM-8	401.4	0.810	53.05±0.19	48.50±0.24	40.95
NM-9	568.6	1.000	40.67±0.23	38.22±0.12	30.99
NM-10	650.2	1.000	42.13±0.24	38.88±0.13	30.54
NM-11	424.2	0.677	50.79±0.18	47.28±0.14	42.11
NM-12	379.5	0.820	51.72±0.20	47.11±0.16	41.67
NM-13	806.7	0.998	40.22±0.15	37.21±0.17	32.28
NM-14	787.8	1.000	38.51±0.19	35.55±0.19	33.79
NM-15	643.5	0.795	37.54±0.18	34.26±0.25	33.02

Graphical representation of Paricle size (Figure 1), Entrapment Efficiency (Figure 2), Polydispersity index (Figure 3), loading capacity (Figure 4) and percentage yield (Figure 5)of all the formulations are shown below as figure 1 to 5.

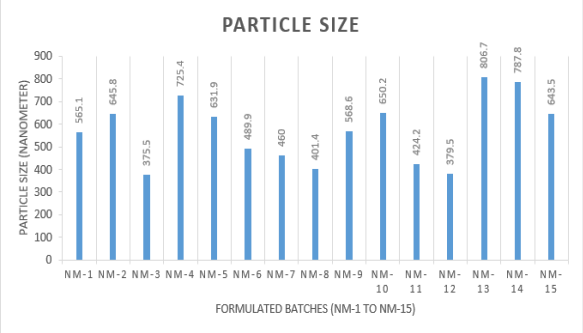

Figure 1: PARTICLE SIZE OF FORMULATIONS

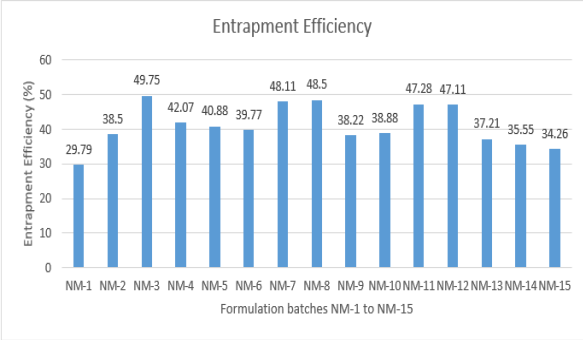

Figure 2: ENTRAPMENT EFFICIENCY OF FORMULATIONS

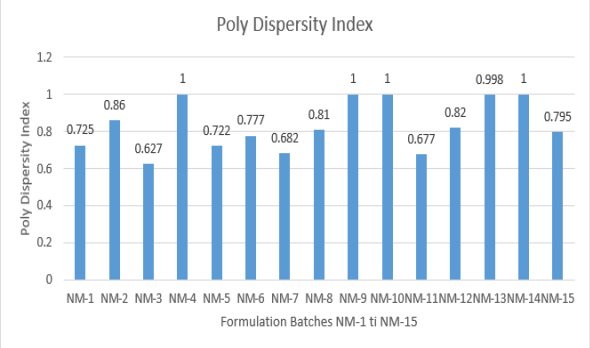

Figure 3: POLYDISPERSITY INDEX OF FORMULATIONS

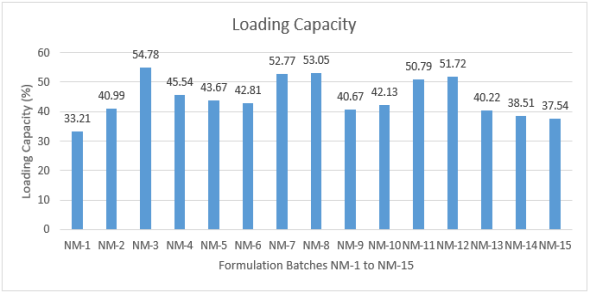

Figure 4: LOADING CAPACITY OF FORMULATIONS

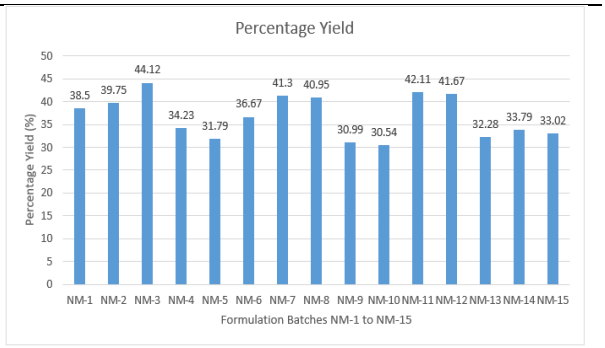

Figure 5: PERCENTAGE YIELD OF FORMULATIONS

Particle Size and Polydispersity Index: All formulations were analyzed for particle size, which ranged from 375.5 to 806.7 nm, and for polydispersity index, which ranged from 0.627 to 1.000. For particle size analysis, the optimized formulation batch NM-3 was selected because it has lower particle size among all the formulations and having polydispersity index value of 0.627, the particle size is illustrated in figure 6.

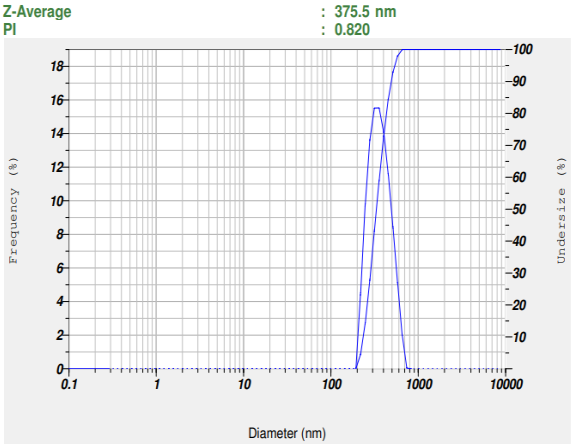

Figure 6: PARTICLE SIZE

Fourier Transform Infrared (FTIR) Spectro-photometry: Spectrum of wheatgrass extract and NM-3 spectrum are illustrated in Figure 7 and 8, respectively, while comparing the peak ranges of wheatgrass extract with NM-3 can be found in Table 3. Both the FTIR spectrum (wheatgrass extract and NM-3) showed no significant alteration. When relating the spectra obtained with reference spectra, no significant shifting of functional peaks, no overlapping of characteristic peaks, and no fresh peaks were observed. The outcomes showed stability of drug throughout the entrapment procedure. The FTIR interpretation indicated that no molecular connections occurred that may perhaps have altered the drug's chemical

arrangement. Consequently, no chemical interaction between the polymer and functional groups of drug exists.

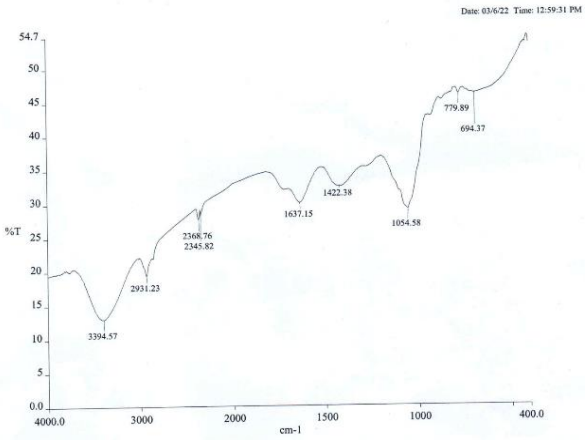

Figure 7: FTIR SPECTRA OF PURE DRUG EXTRACT-WHEATGRASS (*Triticum aestivum*)

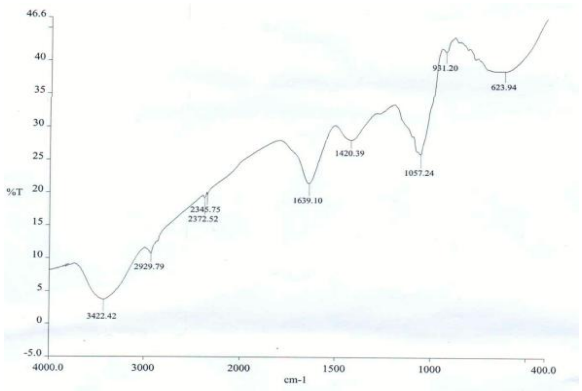

Figure 8: FTIR SPECTRA OF NM-3

TABLE 3: PEAK VALUES IN FTIR OF WHEATGRASS EXTRACT AND SLN-

Reference peaks	Functional group	Name of functional group	Drug	SLN
3500–3200	O–H stretch	Alcohols	3394.57	3422.42
3000–2850	C–H stretch	Alkanes	2931.23	2929.79
1500–1400	C–C stretch	Aromatics	1422.38	1420.39
1320–1000	C–O stretch	Ethers	1054.58	1057.24
950–910	O–H bend	Carboxylic acids	-	931.20
850–550	C–Cl stretch	Alkyl halides	779.89	623.94
2400-2000	O=C=O stretching	Carbon dioxide	2345.82	2368.76
1680–1640	–C=C– stretch	Alkenes	1637.15	1639.10

Scanning Electron Microscopy:

To observe the surface morphology of prepared nanoparticles, the optimized formulation was subjected to scanning electron microscopy. The illustration of image is shown in Figure 9.

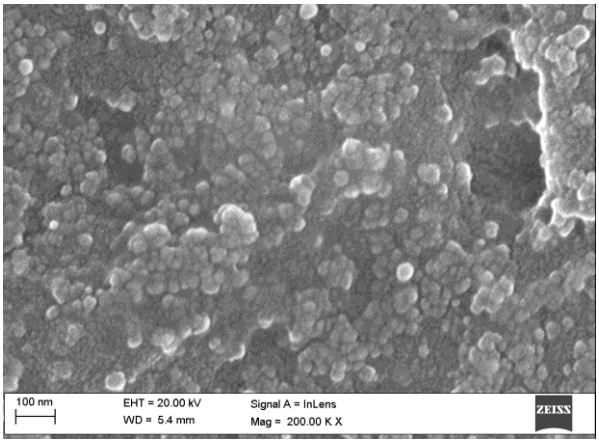

Figure 9: SCANNING ELECTRON MICROSCOPY OF OPTIMIZED FORMULATION BATCH NM-3.

CONCLUSION:

Hot homogenization method and sonication method has been effectively functionalized to formulate wheatgrass extract loaded solid lipid nanoparticles. On wheatgrass loaded chitosan nanoparticles, formulation parameters such as surface stabilizers, surface stabilizer amount, and sonication time were evaluated for their effects on entrapment efficiency, loading capacity, percentage yield, and particle size. The NM-3 was observed as the optimized batch as it has the maximum loading capacity, greater entrapment efficiency with the smallest particle size. In the current study, it was shown that hot homogenization and sonication may be used to create wheatgrass loaded solid lipid nanoparticles and the formulation parameters, such as surface stabilizers, and sonication time, all have an effect on nanoparticle formulation.

CONFLICT OF INTEREST: None

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