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DESIGN AND *INVITRO* CHARACTERIZATION OF NANOSUSPENSION CONTAINING *ARTHROSPIRA PLATENSIS* (SPIRULINA) EXTRACT

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ABSTRACT

In this present research poorly water soluble extract of *Arthrospira platensis* was formulated into Nanosuspension by Nanoprecipitation method by using various surfactants and by altering the homogenization speed to decrease the particle size and to enhance the solubility. The physicochemical parameters of *Arthrospira platensis* extract containing nanosuspension were characterized by UV Spectroscopy and FTIR studies. The *Arthrospira platensis* extract containing nanosuspension were characterized by Zeta Sizer, Drug content, Entrapment Efficiency, SEM, *In vitro* drug release studies and stability studies. The preformulation FTIR studies confirm that there is no any incompatibility between the extract and the excipients. Zeta potential and mean particle size studies confirm that the Nanosuspension particles are in the nanosized range and also it shows uniform distribution of surface charges throughout the particles in the formulation which shows the good stability and dispersibility of particles in the phase. By increasing the homogenization time, it shows that there is a decrease in particle size of the nanosuspension. The *In vitro* drug release studies shows that F6 formulation shows maximum and desirable release pattern of drug release for 98.45% at 30 minutes time interval. Based on these results we concluded that the F6 formulation of *Arthrospira platensis* extract containing nanosuspension was the best formulation. The *In vitro* drug release studies shows that F3formulation shows maximum and desirable release pattern of drug release for 96.54% at 30 minutes time interval. Based on these results we concluded that the F3 nanoemulsion containing *Arthrospira platensis* extract was the best formulation.

Key Words:- Nanosuspension, *Arthrospira platensis*, Nanoprecipitation method, Spirulina.

INTRODUCTION

The poor water solubility of drugs is main difficulty on drug formulation. Nanosuspension system is an advanced technology to enhance the poor solubility of drugs. When there is a reduce in particle size of drug particles into nano range, it leads to an important enhancement in the dissolution rate and therefore enhancement of its bioavailability. Poor water soluble drugs solubility was increased by formulating them as nanosuspensions. Nanosuspensions are a submicron colloidal dispersion of pharmaceutical active ingredient and particles in a liquid phase was stabilized by various surfactants and also co surfactants.

A pharmaceutical nanosuspension is define as "very finely dispersed solid drug particles in an aqueous vehicle, surfactants used for stability, moreover oral and topical use or parental and pulmonary administration, with nano particle size, important to an increased is less than 10nm-1000 nm in size (i.e. 1 µm). The particle size division of the hard particles in nanosuspension is usually less than one µm with an average particle size ranging between 100 and 1000 nm. An increase in the dissolution rate of nanosized particles (particle size < 10 µm) is

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related to an increase in the surface area and saturation solubility because of the vapor pressure effect (Ritika SL *et al.,* 2012; Ashish K *et al.,* 2012; Kalpesh S *et al.,* 2011; Geeta V *et al.,* 2012).

Arthrospira platensis (hereafter referred to as 'Spirulina') is a uni-cellular microalgae which grows in fresh water, in salt water, as well as in brackish bodies of water. It grows best in a highly alkaline environment of pH 10-12. The species Arthrospira platensis, previously referenced as 'Spirulina platensis' (commonly referred to as 'Spirulina'). Now a days in four main areas the Spirulina platensis research are conducted as follows like Immune system modulation; anti-viral activity; cancer preventive properties and cardiovascular benefits. Spirulina is about sixty percent complete, highly digestible protein; it contains all essential amino acids; Spirulina contains more beta-carotene than any other whole food; it is the best whole food source of gamma linolenic acid (GLA); it is rich in B vitamins, minerals, trace elements, chlorophyll, and enzymes; and it is abundant in other nutrients, such as carotenoids, sulfolipids, glycolipids, phycocyanin, superoxide dismutase, RNA and DNA (Bob C *et al.,* 2010).

The present investigation was aimed to at the development of *Arthrospira platensis* nanosuspension to increase the solubility of drug, optimize the revolutions and concentrations of surfactants

MATERIALS AND METHODS

Arthrospira platensis was collected from the sea water of Kanyakumari district. Collected, dried and stored in a suitable condition for further use. All chemicals used in the formulation were of analytical reagent grade.

Extraction process by using Soxhlet extraction process

Sample of *Arthrospira platensis* (100.0 g) was extracted by Dichloromethane (1000 mL) using Soxhlet apparatus. After 6 cycles of the extract, the solvent was evaporated under vacuum and the obtained extract was dried under vacuum (50^{o} C, 24 hours), and the resulting viscous oily liquid was concentrated and dried by rotary flash evaporator at 55 ^{o}C for half an hour, dried and stored in a suitable condition for further use

Preparation of *Arthrospira platensis* Nanosuspension

Arthrospira platensis nanosuspension is prepared by Nanoprecipitation method. The weighed amount of *Arthrospira platensis* extract was dissolved in acetone. The stabilizer PVP K 30 and surfactants were dissolved in water. The water solution was placed under homogenizer probe at a fixed rpm at a fixed time as per the formulation. Acetone solution is added drop by drop to the formulation using a needle. After this process over at a particular time interval, the formulation was placed under ultra sonicator for 15 mins. Then formulation was stored at room temperature and evaluated further. The formulations are tabulated in Table 1 (Priti M *et al.,* 2012; Jana P *et al.,* 2011; Venkatesha T *et al.,* 2011; Hitesh R *et al.,* 2009; Mothilal M *et al.,* 2012)

Optimization of *Arthrospira platensis* nanosuspension

To increase the solubility of *Arthrospira platensis* the nanosuspension, it was optimized by using different ratios of surfactants and different RPM speed of homogenizer. By optimizing these characters like revolution time and different surfactant concentrations, it will lead to decreases the particle size and increases the solubility of extract in dosage form. The Optimization of formulation by physical appearance and sedimentation rate are tabulated in Table 2.From these results F3,F5,F6,F9 are selected as optimized formulation for the further evaluation parameters (Priti M *et al.,* 2012; Jana P *et al.,* 2011; Venkatesha T *et al.,* 2011; Hitesh R *et al.,* 2009; Mothilal M *et al.,* 2012)

Average particle size distribution

Particle size distribution was measured by dynamic light scattering using Zetasizer (zetsizer Malvern 3000 HS (UK) using Malvern Zetasizer version 6.1 software.). The nanosuspension formulation was diluted with triplicate distilled water for the dynamic lighter scattering analysis. Measurements were made in triplicate at 25 ± 1 ^{o}C.Optical properties of the sample were defined as follows. Refractive index of *Arthrospira platensis* nanosuspension was 1.44 (Debjit B *et al.,* 2012; Ajay JY *et al.,* 2012; Brito RS *et al.,* 2015; Vijay K *et al.,* 2014)

Polydispersity Index

The Polydispersity index can also be measured from Dynamic light scattering Instruments.PDI is an index of width or spread or variation within the particle size distribution. Monodisperse samples have a lower PDI value; whereas higher value of PDI indicates a wider particle size distribution and the Polydispersity nature of the sample (Ajay JY *et al.,* 2012; Brito RS *et al.,* 2015; Vijay K *et al.,* 2014). PDI can be calculated by the following formula

Polydispersity index = $\boldsymbol{D(0.9) - \frac{D(0.1)}{D(0.5)}}$

Where' D (0.9) = corresponds to particle size immediately above 90% of the sample

D (0.5) =corresponds to particle size immediately above 50% of the sample.

D (0.1) = corresponds to particle size immediately above 10% of the sample.

Zetapotential

Zeta potential was measured by Laser Doppler Anemometry by using zetasizer nano zetsizer Malvern 3000 HS (UK) using Malvern Zetasizer version 6.1 software. Electrophoretic nobilities of the nanosuspension of mobility (μ) was converted into zeta potential values using Smouluchowski relation. It is an important parameter to analyze the long term stability of nanosuspensions (Ajay JY *et al.,* 2012; Brito RS *et al.,* 2015; Vijay K *et al.,* 2014)

Scanning Electron Microscopy (SEM)

The surface morphology of *Arthrospira platensis* Nanosuspension were observed by SEM with an accelerating energy of approximately 1.5-20 kV. The nanosuspension mounted on stub and coated with gold and carbon prevent charge up of sample and measure the surface morphology of the particle (Ajay JY *et al.,* 2012; Brito RS *et al.,* 2015; Vijay K *et al.,* 2014)

Drug content

10 mg of pure *Arthrospira platensis* extract is dissolved in 10 ml of suitable organic solvent. The solvent is suitably diluted as 5 μ gm/ml and absorbance was determined by UV Spectrophotometer . The prepared nanosuspension also diluted for 5 μg/ml and absorbance was determined at same nm. The drug content was calculated by following formula (Ajay JY *et al.,* 2012)

$$\text{Drug Content} = \frac{\text{Test Absorbence}}{\text{standard absorbance}} * 100$$

***Invitro* Drug Release Studies**

The *invitro* drug release studies of *Arthrospira platensis* nanosuspension was performed by dialysis bag method. Egg membrane was used as a dialysis bag. The dialysis method is carried out using dissolutions apparatus II (Paddle). The dissolution apparatus filled with 500 ml of distilled water and stirred at 50 rpm and maintained at 37°C ± 0.5°C. The prepared nanosuspension was poured into the dialysis bag and the bag was tied to paddle apparatus. The sample were withdrawn from the apparatus at a various time intervals and replaced through distilled water to maintain the sink condition. The absorbance of withdrawn samples was measured using UV-Visible spectrophotometer. The percentage of drug release was calculated and tabulated (Brito RS *et al.,* 2015; Vijay K *et al.,* 2014)

RESULTS AND DISCUSSION

SEM results of Particle in Nanosuspension

The morphology and size of the nanosuspension were determined by scanning electron microscopy. The SEM results shown that the particle sizes of *Arthrospira platensis* nanosuspension were found to be round, spherical and nanosized particles. The results are shown in Figure no 1.

Particle size analysis

The particle size, zeta potential and polydispersity index of the formulations are shown in the Figure 2 and Table 3.The mean particle size for formulations was varies from 158.6 ± 2.2 to 382.7± 4.2 nm based on the concentration of surfactant. It means that hydrodynamic diameter of particle decreased with increase in concentration of the surfactant. Zeta potential plays an important parameter to analyze the long term stability of the nanosuspension. Generally higher Zeta potential values (+) or (-) indicate long term stability because of electrostatic repulsions between particles with same charges avoid aggregation. Zeta potential observed for the prepared nanosuspension was found to be in the range of -1.6± 0.2 mV to -14.1± 0.4 mV, in which F6 formulation shows more surface charge distribution which leads to long term stable formulation. The particle size distribution was narrow as the formulation has Polydispersity index nearer to 0.1, which corresponds to a monodisperse system. In the Present research Polydispersity values ranges between 0.176 ± 0.024 to 0.948 ± 0.024. Formulation F6 shows that good polydispersity index of 0.176, which confirms the uniform dispersibility of the particle in the nanosuspension (Malakar J *et al.,* 2012; Dhaval J *et al.,* 2010; Shah A *et al.,* 2013; Prabhakar CH *et al.,* 2011)

Drug Content and Entrapment Efficiency

Nanosuspension formulation

The experimental results shown that by increasing the concentration surfactants used for formulations results in high drug entrapment efficacy. From these results it was concluded that F6 formulation showing high % drug content of 96.62 %. The experimental results indicated that the concentration of surfactant, homogenization speed had critical effects on the entrapment and drug content of nanosuspension formation. Entrapment efficiency results shows that on increase in concentration of surfactant there was an increase in entrapment efficiency of drug in the formulation. The little bit reduction in entrapment efficiencies was observed with the homogenization varying speed. Among the surfactants used in the

nanosuspension, poloxamer 188 shows high entrapment efficacy. The results are shown in table 4 and figure 3.

In–Vitro Drug Release Studies

In vitro drug release from the nanosuspension was performed in distilled water using egg membrane as a dialysis bag in dissolution test apparatus II. The *invitro* drug release profile of nanosuspension formulations shown in the figure 3. Particle size has a direct effect on the drug release profile from the formulations. Formulation F6 with a smaller average particle size of 158.6 nm gave large initial burst release of 98.64% drug release with in half hour. It shows that smaller particles have a high surface area compared to their volume, therefore most of the drug will be at or near the particle surface and can be readily released. From the results it was concluded that increased surfactant concentration increases drug release. So F6 formulation concluded as best formulation.

Fig 1. SEM image of F6 formulation

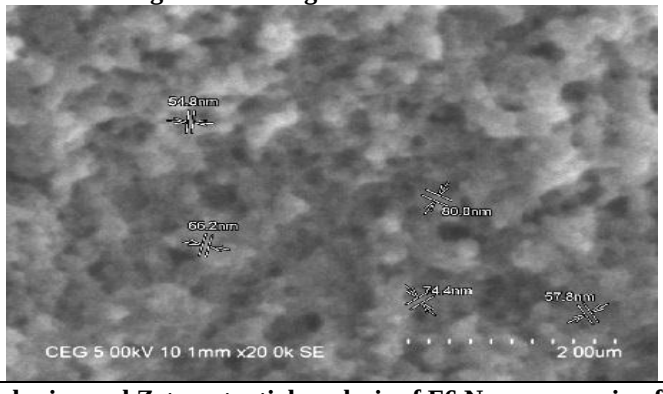
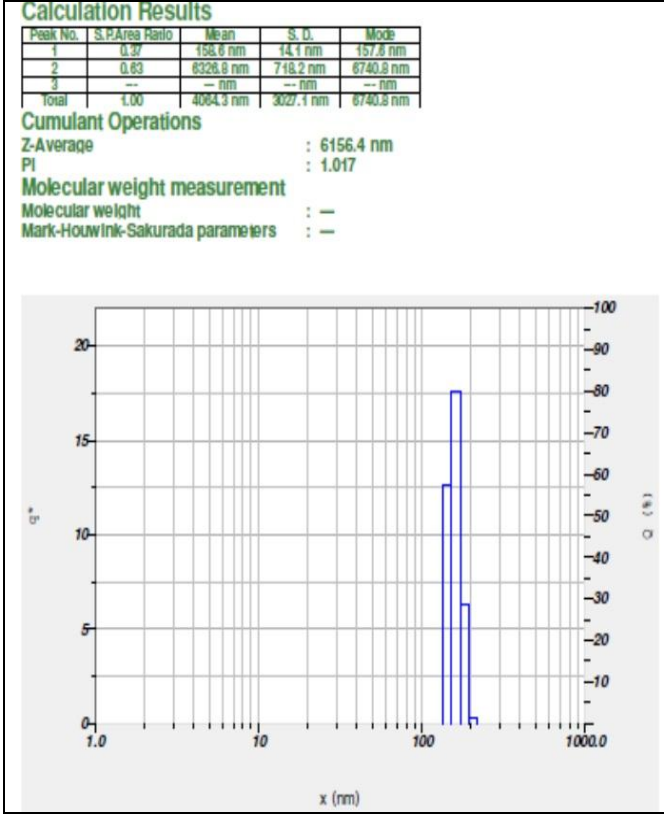


Fig 2. Particle size and Zeta potential analysis of F6 Nanosuspension formulation



Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.37	158.6 nm	14.1 nm	157.8 nm
2	0.63	6326.8 nm	718.2 nm	6740.8 nm
3	---	--- nm	--- nm	--- nm
Total	1.00	4064.3 nm	3027.1 nm	6740.8 nm

Cumulant Operations

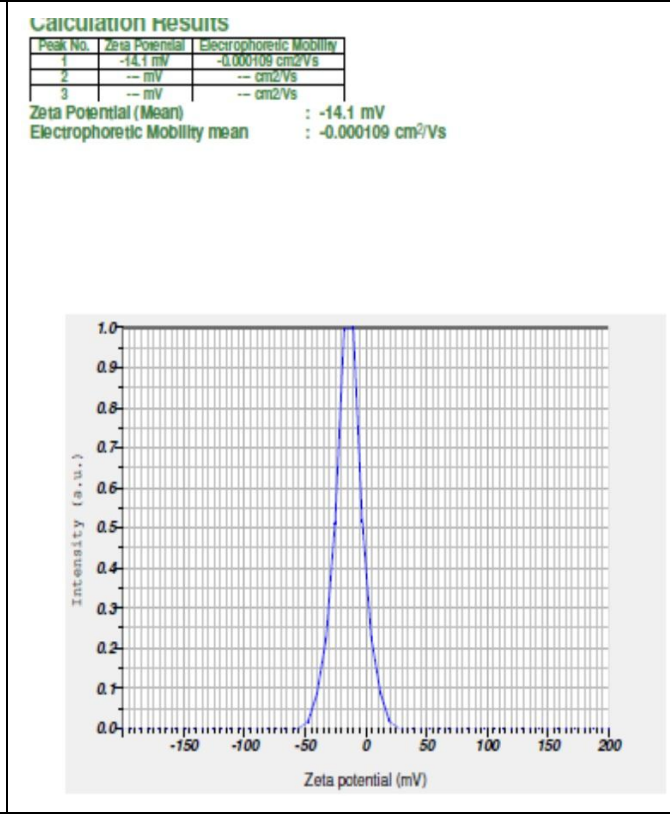
Z-Average : 6156.4 nm

PI : 1.017

Molecular weight measurement

Molecular weight : —

Mark-Houwink-Sakurada parameters : —



Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-14.1 mV	-0.000109 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -14.1 mV

Electrophoretic Mobility mean : -0.000109 cm^2/Vs

Fig 3. Drug content and entrapment efficiency of *Arthrospira platensis*

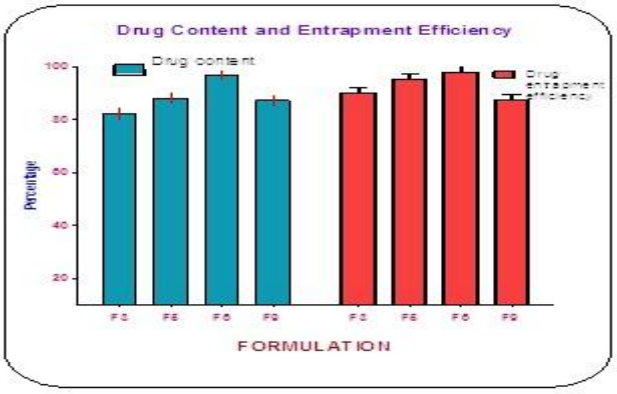


Fig 4. Cumulative % Drug Release Profile of *Arthrospira platensis* Nanosuspension

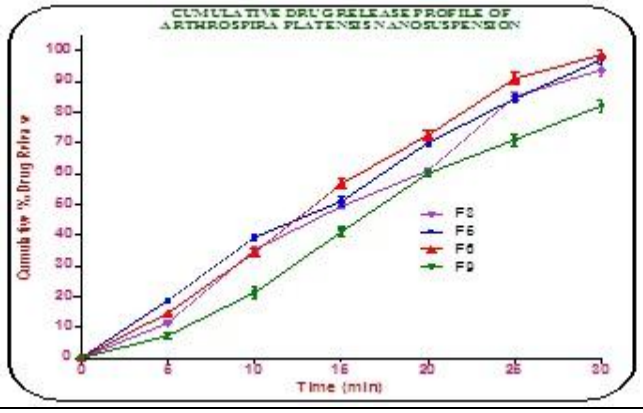


Table 1. Formulation of *Arthrospira platensis* Nanosuspension

Ingredients	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Arthrospira platensis(mgs)	10	10	10	10	10	10	10	10	10
PVP K 30 (mgs)	30	30	30	30	30	30	30	30	30
SLS (mgs)	10	20	30	-	-	-	-	-	-
Poloxamer 188 (mgs)	-	-	-	10	20	30	-	-	-
Tween 20 (ml)	-	-	-	-	-	-	5	10	15
Acetone (ml)	2	2	2	2	2	2	2	2	2
Water (ml)	40	40	40	40	40	40	40	40	40
Polyethylene glycol	1	1	1	1	1	1	1	1	1
Homogenization (RPM)	3000	5000	8000	3000	5000	8000	3000	5000	8000
Homogenization time (hrs)	2	4	6	2	4	6	2	4	6

Table 2. Optimization of formulation by physical appearance and sedimentation rate

S. no	Formulation code	Optimization parameter		
		Sedimentation rate	Appearance	Physical Stability
1	F1	1.542 ± 0.002	Sedimentation more	Poor
2	F2	1.428 ± 0.004	Sedimentation more	Poor
3	F3	0.012 ± 0.002	No sedimentation	Good
4	F4	1.654 ± 0.002	Sedimentation more	Poor
5	F5	0.102 ± 0.004	No sedimentation	Good
6	F6	0.142 ± 0.002	No sedimentation	Good
7	F7	1.634 ± 0.004	Sedimentation more	Poor
8	F8	1.580 ± 0.002	Sedimentation more	Poor
9	F9	0.040 ± 0.002	No sedimentation	Good

Table 3. Particle size analysis of *Arthrospira platensis* nanosuspension

Formulation	Mean particle size (nm)	Polydispersity index	Zeta potential (mV)
F3	362.8 ± 4.2	0.948 ± 0.024	-5.4 ± 0.2
F5	382.7 ± 3.4	0.753 ± 0.022	-7.2 ± 0.4
F6	158.6 ± 2.2	0.176 ± 0.024	-14.1 ± 0.4
F9	250.2 ± 3.2	0.202 ± 0.022	-1.6 ± 0.2

Table 4. Drug Content and Entrapment Efficiency of *Arthrospira platensis* nanosuspension

Sl No	Formulation	% Drug Content	% Entrapment Efficiency
1	F_3	82.21	90.15
2	F_5	88.25	95.15
3	F_6	96.62	98.12
4	F_9	87.30	87.57

CONCLUSION

Arthrospira platensis extract was a poor water soluble drug; its solubility was increased by formulating it as nanosuspension. In the present research the nanosuspension was formulated by nanoprecipitation method by using different surfactants. In the present research it has been concluded that the F6 formulation was the best formulation. Thus it was concluded that the Nanoprecipitation method along with poloxamer 188 surfactant and homogenization time plays a major role in decreasing the particle size of nanosuspension by increasing the solubility and penetrability of drug in the by nanosuspension through GIT.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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