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FORMULATION AND EVALUATION CHARACTERIZATION OF NANOEMULSION CONTAINING *ARTHROSPIRA PLATENSIS* (SPIRULINA) EXTRACT

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ABSTRACT

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. Spirulina platensis research is conducted as follows like immune system modulation; anti-viral activity; cancer preventive properties and cardiovascular benefits. The extract of *Arthrospira platensis* was a poorly aqueous soluble drug, so that to enhance the solubility and penetrability of drug it was formulated into nanoemulsion by constructing pseudo-ternary phase diagrams through aqueous titration method to select the surfactant and co-surfactant, and then nanoemulsion was evaluated for solubility, droplet size. The *Arthrospira platensis* extract containing nanoemulsion was characterized by Drug content, SEM, *In vitro* drug release studies. Zeta potential and mean droplet size studies confirm that the Nanoemulsion droplets are in the nanosized range and also it shows uniform distribution of surface charges throughout the droplets in the formulation which shows the good stability and dispersibility of droplets in the phase. 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: Nanoemulsion, *Arthrospira platensis*, Pseudo ternary phase diagram, Spirulina.

INTRODUCTION

Nanoemulsions are dispersions of nano-scale droplets produced by shear-induced rupturing. Nanoemulsions are defined as oil in water or water in oil emulsion producing a transparent product that has a droplet size from 20-200nm and does not have the tendency to coalesce. Nanoemulsions are more transparent and non creamy emulsion as visibly because the droplets can be much smaller than optical wavelengths of the visible spectrum. So nanoemulsions can appear more transparent in the visible spectrum and show very little scattering (Haritha *et al.,* 2013).

The term 'Nanoemulsion' refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water stabilized by an interfacial film of surfactant molecules. Nanoemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of an oil phase and water phase in combination with a surfactant. The dispersed phase droplet size is about 5 nm-200 nm and should have very low oil/water interfacial tension. Cosurfactant or co solvent is used in many cases in addition to the surfactant, the oil phase and the water phase (Chowdary KPR and Madhavi BLR., 2005).

Arthrospira platensis (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*'). *Spirulina platensis* have following pharmacological activities like immune system modulation, anti-viral activity, cancer preventive properties and cardiovascular benefits. Spirulina is about 60% of complete, highly

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digestible protein; it contains all essential amino acids; Spirulina contains is rich in B vitamins, minerals, trace elements, chlorophyll and enzymes and more β-carotene than any other whole food. It is the best whole food source of gamma linolenic acid (GLA) 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 is to develop nanosized *Arthrospira platensis* extracts containing emulsion, the surface area of droplets increases so that there will be a increase in solubility of drug, the increase in surface area can be achieved by reduce the droplet size into nano sized range. The formulation was optimized by different surfactant concentration and ultrasonication time (Haritha *et al.,* 2013; Chowdary KPR and Madhavi BLR, 2005; Bob C *et al*., 2010)

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

Screening of excipients based on solubility

The choice of excipients is based on the solubility of drug by surfactant and co-surfactant, the solubility of *Arthrospira platensis* crude extract in various oils, surfactants, co-surfactants was determined by dissolving an excess amount of drug in each vial containing 1 g of the selected vehicle. The mixtures were shaken for 2 hrs in a bath sonicator and centrifuged; the supernatant was taken and filtered. The filtrate was suitably diluted and concentration of drug was determined by UV-spectrophotometer.

Construction of pseudo-ternary phase diagrams

Surfactant and co-surfactant mix in each group were mixed in different volume ratios and the stock of 10 mL of each group was prepared. These ratios were chosen in increasing concentration of co-surfactant with respect to surfactant and increasing concentration of surfactant with respect to co-surfactant for detailed study of the phase diagrams for the nanoemulsions formation. The ratio was shown in Table no. 1 and the selected optimized surfactant mix was selected for the nanoemulsion formulation. Selection parameter results was shown in table no.2

Preparation of Nanoemulsion

Nanoemulsion were prepared using castor as the oil, tween80 as the surfactant and PEG-200 as the co-surfactant. In all formulations, the amount of crud drug was kept constant. Accurately weighed crude drug was placed in a beaker and oil, surfactant and co-surfactant were added. The components were mixed by gentle stirring with magnetic stirrer and the resulting mixture was placed in ultra-sonication until it gets homogenous mixture (Kunal J *et al*., 2013; Rajendra C *et al.,* 2011; Patravale BV *et al.,* 2004)

Average droplet size distribution

Droplet size distribution was measured by dynamic light scattering using Zetasizer (zetsizer Malvern 3000 HS (UK) using Malvern Zetasizer version 6.1 software.). The nanoemulsion 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* nanoemulsion was 1.48 (Kunal J *et al*., 2013; Rajendra C *et al.,* 2011; Patravale BV *et al*., 2004; Mukesh G *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 droplet size distribution. Monodisperse samples have a lower PDI value; whereas higher value of PDI indicates a wider droplet size distribution and the Polydispersity nature of the sample (Goel A *et al.,* 2010). PDI can be calculated by the following formula

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

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

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

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

Zeta potential

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 nanoemulsion of mobility (μ) was converted into zeta potential values

using Smouluchowski relation. It is an important parameter to analyze the long term stability of nanoemulsion (Patravale BV *et al.,* 2004; Mukesh G *et al.,* 2014).

Scanning Electron Microscopy (SEM)

The surface morphology of *Arthrospira platensis* nanoemulsion was observed by SEM with an accelerating energy of approximately 1.5-20 kV. The nanoemulsion mounted on stub and coated with gold and carbon prevent charge up of sample and measure the surface morphology of the droplet (Patravale BV *et al.,* 2004; Mukesh G *et al.,* 2014; Shakeel F *et al.,* 2007).

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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 nanoemulsion also diluted for 5 µg/ml and absorbance was determined at same nm. The drug content was calculated by following formula (Patravale BV *et al.,* 2004; Mukesh G *et al.,* 2014; Shakeel F *et al.,* 2007).

$$\text{Drug Content} = \frac{\text{Test Absorbence}}{\text{standard absorbance}} \text{X100}$$

***Invitro* Drug Release Studies**

The *Invitro* drug release studies of *Arthrospira platensis* nanoemulsion 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 nanoemulsion 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 (Kunal J *et al*., 2013; Rajendra C *et al.,* 2011; Patravale BV *et al.,* 2004; Mukesh G *et al.,* 2014; Shakeel F *et al.,* 2007; Shanti B *et al.,* 2013; Kawakami K *et al.,* 2000).

RESULTS AND DISCUSSION

From the preliminary optimization parameters it shows that F3 and F5 nanoemulsion shows better physical characterization, so that these formulations are carried for the further evaluation parameter.

Nanoemulsion droplet analysis

The morphology and size of the nanoemulsion were determined by scanning electron microscopy. The SEM results shown that the morphology of the droplet of *Arthrospira platensis* nanoemulsion was found to be round, spherical and nanosized droplets. And also it shows uniform dispersion of droplets in the continuous phase. The results are shown in Figure 1.The droplet size, zeta potential and polydispersity index of the formulations are shown in the Figure 2 and Table 3.The mean droplet size for formulations was varies surfactant concentration in range of 172.6 ± 6.8 to 266.0 ± 4.2 nm. It means that hydrodynamic diameter of droplet size decrease with increases in surfactant concentration. Zeta potential also an important parameter to analyze the long term stability of the nanoemulsion. Generally higher Zeta potential values (+) or (-) indicate long term stability because of electrostatic repulsions between droplets with same charges avoid aggregation. Zeta potential observed for the prepared nanoemulsion was found to be in the range of -2.4± 0.6 to -12.3± 1.2 mV, in which F3 formulation shows more surface charge distribution which leads to long time stable formulation. The droplet size distribution was narrow as the formulation has less Polydispersity index, which corresponds to a monodisperse system. From these studies the formulation F3 shows that good dispersibility, which confirms the uniform dispersibility of the droplet in the nanoemulsion.

Drug Content and Entrapment Efficiency

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 F3 formulation showing high % drug content of 87.25 ± 2.14. The experimental results indicated that the concentration of surfactant, ultrasonication time had critical effects on the nanoemulsion formation. The little bit reduction in entrapment efficiencies was observed with the varying speed. Among the surfactants and cosurfactant ratio used in the nanoemulsion, 3:1 ratio 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 nanoemulsion was performed in phosphate buffer 6.8 using egg membrane as a dialysis bag, using dissolution apparatus II. The *invitro* drug release profile of nanoemulsion formulations shown in the figure 4. Droplet size has a direct effect on the drug release profile from the formulations. Formulation F3 with a smaller average droplet size of 172.6 ± 6.8 gave fast release pattern of 96.54% at 30 minutes. It shows that smaller droplets have a high surface area compared to their volume, therefore most of the drug will be at or near the droplet surface and can be readily released. From the results it was concluded that increased surfactant concentration with increases drug release. So F3 formulation concluded as best formulation.

Table 1. Formulation of *Arthrospira platensis* Nanoemulsion - Different volumes of Smix (Surfactant and Co-surfactant)

S.No	Volume of Surfactant (mL)	Volume of Co surfactant (mL)	Ratio of Smix
1	10	10	1:1
2	20	10	2:1
3	30	10	3:1
4	10	20	1:2
5	10	30	1:3

Table 2. Optimization of nanoemulsion formulation

S. no	Formulation code	Optimization parameter			
		Phase separation	Physical Stability	Appearance	Type of Emulsion
1	F1	Phase separation	Poor	Dull white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).	Grade D
2	F2	Phase separation	Poor	Dull white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).	Grade D
3	F3	Stable	Good	Rapidly forming (within min) nanoemulsion, having a clear appearance.	Grade A
4	F4	Phase separation	Poor	Dull white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).	Grade D
5	F5	Stable	Good	Rapidly forming (within 1 min) nanoemulsion, having a clear appearance.	Grade A

Table 3. Droplet size analysis of *Arthrospira platensis* nanoemulsion

Formulation	Mean droplet size (nm)	Polydispersity index	Zeta potential (mV)
F3	172.6 ± 6.8	0.987± 0.002	-12.3± 1.2
F5	266.0 ± 4.2	1.048 ± 0.024	-2.4± 0.6

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

Sl No.	Formulation	% Drug Content	% Entrapment Efficiency
1	F_3	87.25 ± 2.14	82.34 ± 2.40
2	F_5	83.66 ± 2.02	76.44 ± 2.42

Fig 1. SEM image of F3 nanoemulsion

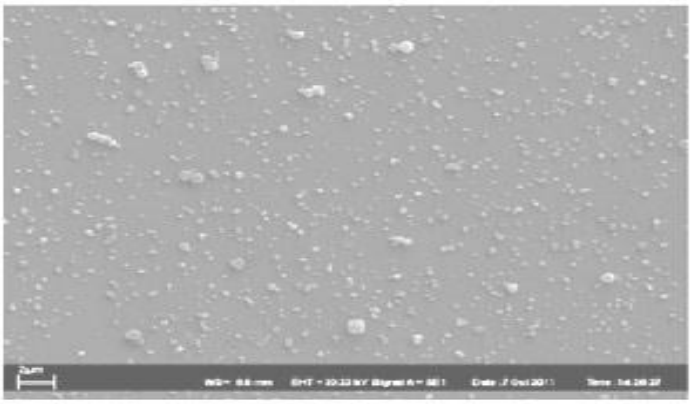

Fig 2. Droplet size and Zeta potential analysis of F3 formulation

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Measurement Results

Date	: Thursday, April 09, 2014
Measurement Type	: Particle Size
Sample Name	: F3
Scattering Angle	: 85
Temperature of the holder	: 25.6°C
T% before meas.	: 17971
Viscosity of the dispersion medium	: 0.896mPa·s
Form Of Distribution	: Standard
Representation of result	: Scattering Light Intensity
Count rate	: 68kCPS

Calculation Results

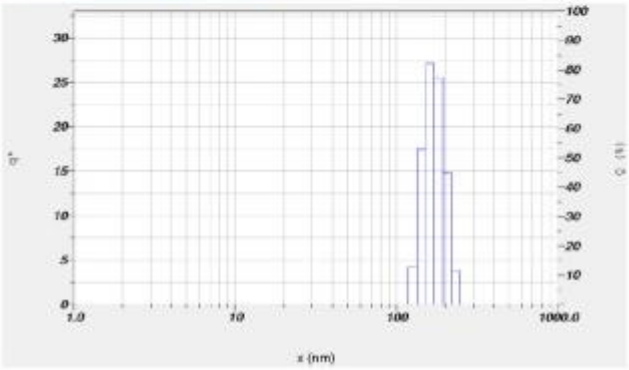
Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	172.6 nm	24.9 nm	163.6 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	172.6 nm	24.9 nm	163.6 nm

Cumulant Operations

Z-Average	: 452.2 nm
PI	: 0.987

Molecular weight measurement

Molecular weight	: ---
Mark-Houwink-Sakurada parameters	: ---



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201510111245015.nzt

Measurement Results

Date	: Thursday, April 09, 2014 12:25:33
Measurement Type	: Zeta Potential
Sample Name	: F3
Temperature of the holder	: 25.0°C
Viscosity of the dispersion medium	: 0.894 mPa·s
Conductivity	: 2.081mS/cm
Electrode Voltage	: 2.8 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-12.3 mV	-0.0000109cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	: -12.3 mV
Electrophoretic Mobility mean	: -0.0000109cm2/Vs

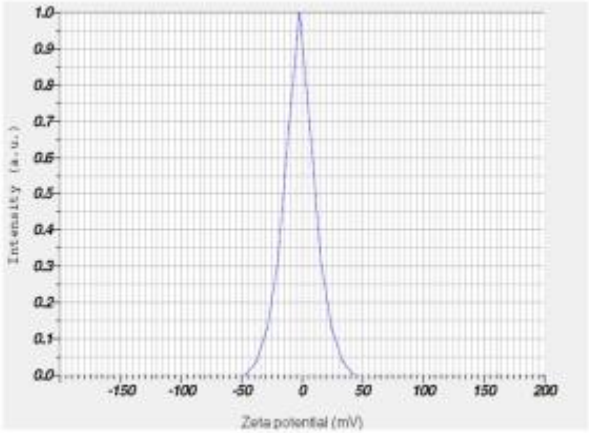


Fig 3. Drug content and entrapment efficiency in graphical representation.

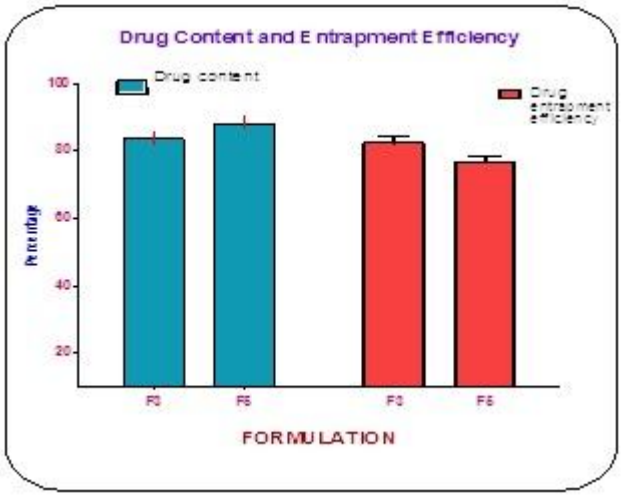
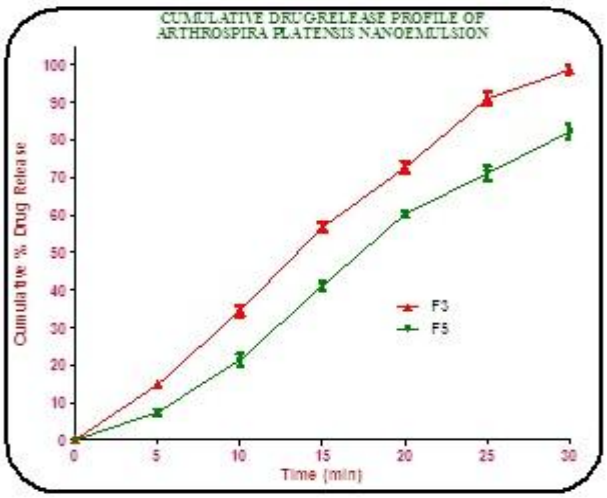


Fig 4. Cumulative percentage drug release profile of *Arthrospira platensis* Nanoemulsion



CONCLUSION

Arthrospira platensis extract was a poor water soluble drug; its solubility was increased by formulating it as nanoemulsion. In the present research the nanoemulsion was formulated by pseudo ternary phase diagram by using different surfactants. In the present research it has been concluded that the F3 formulation was the best formulation. Thus it was concluded that the nanoemulsion with *Arthrospira platensis* extract was increasing the solubility and will be increases the drug penetrability through GIT.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

REFERENCES

Bob C, Gerald R, Cysewski. Potential health benefits of spirulina microalgae. *Nutra foods*, 9(2), 2010, 19-26.

Chowdary KPR, Madhavi BLR. Novel drug delivery technologies for insoluble drugs. *Ind.Drugs*., 42(9), 2005, 557-563.

Goel A, *et al.* 3-Acetyl-11-keto-beta-boswellic acid loadedpolymeric nanomicelles for topical anti-inflammatory and anti-arthritic activity. *J. Pharm. Pharmacol.*, 62, 2010, 273-278.

Haritha, *et al.* A brief introduction to methods of preparation, applications and characterization of nanoemulsion drug delivery systems. *Indian Journal of Research in Pharmacy and Biotechnology*, 1(1), 2013, 25-28.

Kawakami K and Yoshikawa T. Microemulsion's formulation for enhanced absorption of poorly soluble drugs prescription design. *J. Control. Release*, 81, 2000, 65–74.

Kunal J. Enhanced Oral Bioavailability of Atorvastatin via Oil-in-Water Nanoemulsion using Aqueous Titration Method. *J. Pharm. Sci. & Res*, 5(1), 2013, 18 – 25.

Mukesh G, *et al.* Development and Optimization of Plant Extract Loaded Nanoemulsion Mixtures for the Treatment of Inflammatory Disorder. *Current Research in Drug Discovery*, 1(2), 2014, 29-38.

Patravale VB, *et al.* Nanosuspensions: a promising drug delivery strategy. *Journal of Pharmacy and Pharmacology*, 56, 2004, 827-840.

Rajendra C, Anand K. Development and bioavailability studies of atorvastatin nanoemulsion. *Int. J. of Pharm. & Life Sci.*, 2 (8), 2011, 982-988.

Shakeel F, Baboota S, Ahuja A, Ali J and Aqil M. Nanoemulsions as vehicles for transdermal delivery of aceclofenac. *AAPS PharmSciTech*, 8, 2007, 191-199.

Shanti BM, *et al.* Nanosuspension of Phyllanthus amarus extract for improving oral bioavailability and prevention of paracetamol induced hepatotoxicity in Sprague–Dawley rats. *Adv. Nat. Sci.: Nanosci. Nanotechnol*, 4, 2013, 1-7.