

ICONN 2015 [4<sup>th</sup> -6<sup>th</sup> Feb 2015]  
International Conference on Nanoscience and Nanotechnology-2015  
SRM University, Chennai, India

## To Study the Potential of Microbial Lipid Based Nanostructured Lipid Carriers for Topical Drug Delivery Applications

Dhanalakshmi. J<sup>1</sup>, Divakar.R<sup>1</sup>, Amy Magdalene Paul\*

Department of Physics and Nanotechnology, SRM University, Chennai, India

**Abstract:** Lipid Nano carriers show great potential as topical carriers due to their permeability and Lipophilic nature. In order to overcome the flaws faced with the previous generations of Lipid nanocarriers, a new class of carriers were developed called as Nanostructured Lipid carriers(NLC). In this work, we have synthesized NLCs using Microalgal based lipids extracted from *Spirulina platensis*. The average size of the NLCs were found to be around 400nm using Scattering techniques. The morphology of the particles were analyzed using Scanning electron microscopy and were found to be present in clumps which can be separated into individual particles by ultrasonication. The particles were found to be very stable and cationic in nature with a high zeta potential of around 80mV. The permeability was tested *in vitro* using Franz diffusion cell with goat skin which showed better penetration through the skin. This can be enhanced by decreasing the size of the particles by subjecting it to high pressure homogenization. These results indicate that these Microlipid based NLCs can offer greater potential as a topical carrier to treat a wide variety of diseases like actinic keratosis, arthritis etc.

### Introduction

Liposomes, Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are the three major classes of Lipid nanocarriers which are in current use for topical/transdermal drug delivery applications. SLN are prepared by using solid lipis which form particles similar to oil in water emulsion concept. To overcome the major challenges associated with SLN and Liposomes, such as poor drug loading, drug leakage etc, NLC was developed. A combination of spatially different lipids enables higher drug loading in NLC formulation. Solid lipid nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) are the new generation of nanoparticulate active substance vehicles and are attracting major attention as novel colloidal drug carriers for topical use. Compared with polymeric nanoparticles, lipid nanoparticle has lower toxicity because of the absence of solvents in the production process and also relatively low cost of the excipients. NLC combine their advantages such as controlled release, biodegradable and protection of active compounds. Especially, NLC can favor drug penetration into the skins, maintain a sustained release to avoid systemic absorption, act as a UV sunscreen reduces irritation<sup>1,2</sup>. SLN represents a particulate system, which can be produced by one of the following techniques, namely, high pressure homogenization<sup>3</sup>, microemulsion template technique<sup>4</sup>, solvent emulsification

evaporation technique<sup>5</sup>, solvent displacement technique<sup>6</sup>, solvent emulsification diffusion method<sup>7</sup>, phase inversion<sup>8</sup> and a very recently introduced membrane contractor technique<sup>9</sup>. The choice of lipids is an important parameter in determining the drug loading characteristics of the carrier and hence it is always advantageous to use a combination of lipids. Various lipids from natural sources are being used in recent days like coconut oil, olive oil etc. A special class of lipids which has a very high nutritional value and medicinal value is that which is extracted from microalgal species namely *Spirulina platensis*<sup>10,11</sup>. In this study we have extracted the microalgal lipids and used it to prepare NLC formulations with a sample drug Diclofenac Sodium and have tested its potential for usage in topical formulations.

## Materials and methods

### Fatty acids methyl ester analysis

Lipids were obtained from the lyophilized biomass sample according to the method of Folch, *i.e.* lipids were extracted with chloroform/methanol (2:1 v/v), purified in methanol/water (2:1 v/v) containing 9 g/l NaCl (to remove sugars, salts and proteins) and concentrated in a rotary evaporator, residual solvent was evaporated with nitrogen. Fatty acid methyl esters (FAME) were prepared from the lipid samples using the method of Metcalfe and Schmitz, gas chromatography was used to determine the fatty acid profile.

### Preparation of NLCs

A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase is obtained by a Ultra-Turrax high speed stirrer. The Lipid phase was melted with the drug Diclofenac Sodium was dispersed into hot surfactant mixture maintained at the same temperature and was subjected to high speed homogenization for around 30 minutes followed by ultrasonication for an hour to get evenly dispersed Lipid nanoparticles.

### Particle size

The average diameter and polydispersity index (PDI) of NLC were calculated with the Malvern software using photon correlation spectroscopy (PCS) (Zetasizer Nano ZS, Malvern, UK).

### Zeta potential

Laser doppler electrophoresis technique was applied to measure particle electrostatic charge and stability. The analysis was done with Zetasizer Nano ZS (Malvern, UK) and the results were expressed as zeta potential (ZP)

### Scanning Electron Microscopy

A drop of diluted NLC was placed on to the sample holder dried completely and probed with Scanning Electron Microscopy to analyze the morphology of the particles.

### Differential Scanning Colorimetry

Differential scanning calorimetry (DSC) analysis was done with Mettler DSC 822e (Mettler Toledo, Greifensee, Switzerland). Approximately 10 mg of bulk lipid and NLC were placed in aluminium pans. The pan was heated and the thermograms were recorded at temperature range of 25 to 70°C at a heating rate of 5°C/min. An empty aluminum pan was used as a reference.

### X-Ray Diffractometry

The crystallinity of NLCs were determined by using a powder X-ray diffractometer, equipped with Cu K $\alpha$  radiation.

### Skin permeation tests

The *ex-vivo* permeation through goat skin was evaluated using Franz diffusion cell. A small quantity (0.1g) of the concentrated NLC formulation was applied to the skin surface. At the end of 24 h, the skin was cut homogenized and extracted with methanol and suitably diluted and analyzed spectrophotometrically.

## Results and discussion

### Fatty acid profile

The percentages of the major FAMES in the *Spirulina* cultivated by us were in accordance with previous works by other authors, the principal fatty acids present were palmitic, gamma-linolenic and linoleic acid (Table I).

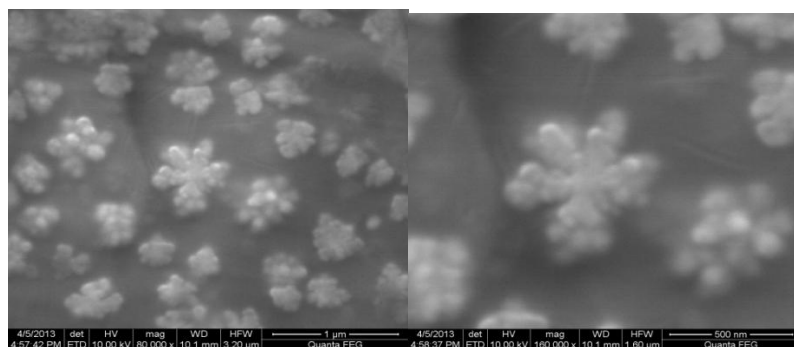
**Table 1: Summary of the composition of lipid mixture extracted from *Spirulina platensis***

| Area % | Composition                             |
|--------|---|
| 0.81   | 2,4-di-tert-butylphenol(ANTIOXIDANT 33) |
| 0.36   | Myristic acid                           |
| 6.15   | Palmitoleic acid                        |
| 29.92  | PALMITIC ACID                           |
| 14.22  | GAMMALINOLEIC ACID                      |
| 26.00  | LINOLEIC ACID                           |
| 1.71   | STEARIC ACID                            |
| 0.49   | VITAMIN E                               |
| 0.92   | ICOSATRIENOIC ACID, LINOLENIC ACID      |

The GCMS results show that the crude lipid extract showed significant compounds like Gamma-Linolenic acid which has potent anti cancer properties, Palmitoleic acid which suppresses inflammation, Vitamin E which has an anti-oxidant and prevents free radicals to create oxidative damage in cells. Moreover it is an essential compound of topical formulations. Other than this, essential fatty acids, essential fatty acids were present like Linoleic acid etc which has high nutritional value and dietary significance. And anti-bacterial compounds were found to be present making the extract anti-bacterial in nature. Hence this lipid mixture serves as an effective component which can have a significant synergistic effect in NLC formulations.

### Morphology

The particle morphology was analyzed using Scanning Electron Microscopy images. The particles were found to be more monodisperse in nature and were found to be in clusters which can be reversed by subjecting it to ultrasonication. The size of the particles were found to be in the size range of 400-450 nanometres.



**Figure 1. SEM images of the lipid nanoparticles at different magnifications**

### Particle size

The particles were found have an average particle size of 400 nm using Dynamic Light Scattering which is in agreement with the SEM images. Further reduction in size can be achieved by using High pressure homogenization during the emulsification phase.

### Zeta potential

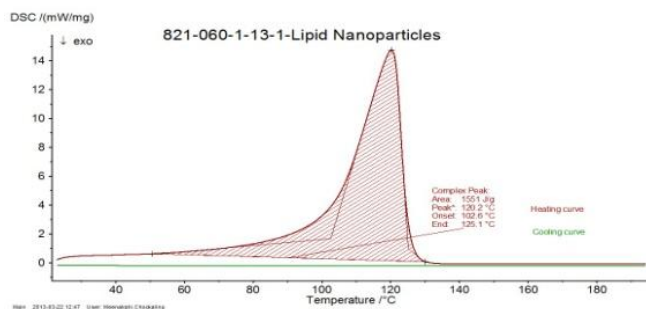
The particles were found to be cationic in nature with a high zeta potential of about 80mV which shows that it can penetrate the tissues more due to its cationic nature and also that the particles are more stable.

## X-Ray Diffraction

The particles showed amorphous nature which is concluded from the XRD results and signifies the presence of more liquid lipids and a higher drug loading capacity which was further confirmed by calculating the entrapment efficiency which was around 98%.

## Differential scanning calorimetry

The DSC results showed that the melting point of the lipids were found to be 120.2°C which shows that the formulation has excellent colloidal stability at the physiological temperature.



**Figure 2: Differential Scanning Colorimetry image of NLC formulation**

## Skin permeation tests

A major concentration of the drug were found to be localized in the skin after being completely absorbed from the surface with a less significant diffusion into the buffer which proves that this formulation can be very ideal for topical applications. Decreasing the size can offer deeper penetration.

## Conclusion

In conclusion, the microalgal lipid based nanocarriers being more stable and prepared from an edible microalgae offers great advantages among current compositions. The penetration can be altered by altering the size of the particles by subjecting it to high pressure based on our requirements. As a greater quantity of drug remained localized in the skin with lesser amount penetrating into the receptor compartment ex vivo as compared with conventional gel it enables specific drug targeting to skin.

## References

1. Sylvia A., Muller R.H., Wissing S.A., Cosmetic applications for solid lipid nanoparticles (SLN). *Int. J. Pharm.*, 254:65–68, (2003)
2. Wissing S.A., Muller R.H., The influence of the crystallinity of lipid nanoparticles on their occlusive properties. *Int. J. Pharm*, 242: 377–379, (2002).
3. Dingler, S. Gohla A., Production of solid lipid nanoparticles (SLN): scaling up feasibilities. *J. Microencapsul* 19 (1):11–16, (2002).
4. M.R. Gasco. Method for producing solid lipid microspheres having a narrow size distribution, US Patent 5 250 236(1993).
5. Sjoström B., Bergenstahl B., Preparation of submicron drug particles in lecithin stabilized o/w emulsions. I. Model studies of the precipitation of cholesteryl acetate. *Int. J. Pharm*, 88:53–62, (1992).
6. Hu, F.Q., Yuan, H., Zhang, H.H., Fang, M., Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. *Int. J. Pharm*, 239:121–128, (2002).
7. Trotta, M., Debernardi, F., Caputo, O., Preparation of solid lipid nanoparticles by a solvent emulsification–diffusion technique. *Int. J. Pharm*, 257:153–160, (2003).
8. Heurtault B., Saulnier P., Pech B., Proust J. E., Benoit J.P., A novel phase inversion-based process for the preparation of lipid nanocarriers. *Pharm. Res.*, 19: 875–880, (2002).

9. Charcosset C., El-Harati A., Fessi H., Preparation of solid lipid nanoparticles using membrane contractor. *J. Controlled Release*, 108: 112–120,(2005).
10. Uner M., Wissing S.A., Yener G., Muller R.H., Skin moisturizing effect and skin penetration of ascorbyl palmitate entrapped in solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) incorporated into hydrogel. *Pharmazie*, 60: 751–755, (2005).
11. Luciane Maria Collaa, Telma Elita Bertolina, and Jorge Alberto Vieira Costab,\*- Fatty Acids Profile of *Spirulina platensis* Grown Under Different Temperatures and Nitrogen Concentrations *Z. Naturforsch* 55-59 (2004).

\*\*\*\*\*