Chapter 3

Fluorescent sophorolipid molecular assembly and its magnetic nanoparticle loading: a pulsed laser process

A novel route employing UV laser pulses (KrF Excimer, 248 nm) to irradiated bio-derived sophorolipid molecules is introduced. We report realization of strong green fluorescence in fully biocompatible highly spherical mesoscale molecular assembly of sophorolipid created by pulsed UV laser processing of a water-based dispersion of sophorolipid. We have separately examined the consequences of laser irradiation of glucose and oleic acid components which form the sophorolipid. This fluorescence character appears to be driven by the oleic component while the assembly process is assisted by the glucose component. Importantly the laser synthesized mesostructures can be easily redispersed in aqueous medium after being dried and can also be loaded with magnetic nanoparticles (magnetite) for inducing hyperthermia effect.
3.1 Introduction

Bio-imaging vehicles which can absorb light and facilitate fluorescent or colorimetric detection are of fundamental significance to various medical applications such as photo-thermal or photo-dynamic therapy.\textsuperscript{1-2} Inorganic nanoparticles, especially semiconductor quantum dots (e.g. CdSe), absorb light strongly and possess good luminescence properties, making them suitable vehicles for such applications domains.\textsuperscript{3-7} Yet one does not witness their widespread use in medical applications possibly because of their limited drug loading capability restricted only to the nanoparticles surface and inherent high level of toxicity.\textsuperscript{8}

Another serious problem faced when using such inorganic nanomaterials (e.g. iodine, gadolinium, and radioisotopes) as contrasting agents in magnetic imaging is their unduly long residence time in the body long after the delivery procedure and higher noise to target signal ratio. To overcome these disadvantages, bio-organic nanoparticles are now being extensively used in therapeutics and for diagnostic imaging because of their much higher drug loading capacity, perfect biocompatibility and controlled activation under specific conditions such as pH, temperature etc.\textsuperscript{9} Biosurfactants derived from microbes are an interesting category of bio-organic systems with potential for applicability in biomedicine.\textsuperscript{10-12}

They can be produced from renewable feedstock or waste material by a natural fermentation.\textsuperscript{13-19} Such micro-organism derived biosurfactants are also structurally very diverse. Moreover, they are readily degradable and display low toxicity.\textsuperscript{20-22} These properties are clearly desirable over those of traditional surfactants which can be eco-toxic, susceptible to bio-accumulation and generally averse to biodegradability. Some traditional surfactants with improved environmental performance such as alkyl polyglucosides, alkyl polyglucamides and fatty ester methyl ester ethoxylates are in use. However they are not necessarily made from renewable resources and may involve partial chemical processing.

A number of biosurfactants such as rhamnolipids (\textit{Pseudomonas aeruginosa}), sophorolipids (\textit{Candida bombicola}), trehalose lipids, cellobiose lipids, mannosylerythritol lipids, surfactin (\textit{Bacillus subtilis}) and emulsan (\textit{Acinetobacter calcoaceticus}) have been subjected to different scientific studies. Apart from surfactin
and emulsan, all others are glycolipids which represent easily available important class of biosurfactants. Our work involves the use of sophorolipids because they are easily synthesized by non-pathogenic yeast using very cost effective resources. Sophorolipids (SL) are amphiphilic molecules which contain both hydrophobic (nonpolar) and hydrophilic (polar) groups. This character enables them to reduce the surface and interfacial energies leading to formation of emulsions. The foremost reasons for a high and increasing level of interest in Sophorolipid is due to their biodegradability and low toxicity as well as their unique structures that can facilitate their engineering to suit a specific application domain.23–26

When dissolved in water Sophorolipid molecules can form micelles-like structures. Some literature reports also discuss supramolecular assemblies27–30 of sophorolipid monolayer in the form of vesicles, helical fibers/ribbons/tubules, and even rigid rods. Herein we report pulsed UV laser induced formation of novel self-assembled vesicular mesostructures of biosynthesized Sophorolipid without the addition of any stabilizing agent, or other organic or inorganic additives. Most interestingly these Sophorolipid based mesostructures are highly fluorescent as well, in contrast to the non-fluorescent property of the parent molecules. We have performed several experiments to elucidate the possible origin of such fluorescence.

We also show that such mesostructures can be easily loaded with magnetic (magnetite) nanoparticles for easy recovery and potential applicability as RF hyperthermia31 agents. Indeed, iron oxide nanoparticles (Fe₃O₄) based magnetic hyperthermia has been extensively investigated in the field of biomedical and pharmaceuticals science. A major problem has been to achieve a sufficiently high concentration of magnetic nanoparticles for bulk solution heating inside the cells. We demonstrate that the Sophorolipid mesostructures can be easily loaded with high density magnetite nanoparticles conferring on them the capability to easily fuse with the cell membrane. Such mesostructures can permeate cells very effectively at high concentration enabling effective delivery of the load.

Concurrent fluorescence and magnetism further enhances the value to these systems in the context of both bio-imaging, targeted drug delivery and controlled drug release. We would like to further emphasize on the green aspect of this work. Herein no synthetic chemical steps are used for production of sophorolipid mesostructures. The
product formed is completely biodegradable and the end products fatty acid and glucose are non-toxic. Sophorolipid used for nano/meso-assemblies is FDA approved and allowable limit for human uptake is as high as 5 ml/kg wt. For nano/meso assemblies we require only few mgs. Moreover there is no stress on the body system if taken in by any route.

The details of experiments performed and related procedures are given in a later section. Briefly, in our experiments, three different types of sophorolipids crude, acidic and lactonic, dispersed in water were laser irradiated with pulsed excimer laser (λ=248 nm, pulse width 20 ns, energy density~166 mJ/cm²). Microscopy studies and other physical characterizations were performed for all the three types of samples under different conditions. When the crude Sophorolipid was processed with laser irradiation, mixed population of some spherical microstructures with cloudy tube-like structures were obtained. Lactonic sophorolipid formed undefined hazy. However, in the case of the acidic form of sophorolipid, which is diacetate in nature, extremely well-defined and fairly uniform spherical microstructures were seen to form (Figure 3.1). The size range of such sophorolipid mesostructure was 0.5 - 2.5µm which could be reduced to less than 100 nm by optimizing process parameters such as laser irradiation time, energy and stirring solution. Indeed, a ~100 nm size represents an ideal size range for bio-imaging experiment in medical science. Noting that the basic structure of sophorolipid molecule comprises of oleic acid and glucose forms, we also examined the effects of pulsed excimer laser irradiation on these two molecules to elucidate the contributions of these molecular components in assembly and related fluorescence. The reference to Sophorolipid in the following sections implies its acidic form unless specifically stated otherwise.

### 3.2 Experimental

#### 3.2.1 Materials & Equipment’s

**Production of Sophorolipid**

Sophorolipid was prepared by the resting cell method. In the first step adequate cell mass was harvested by growing the *Candida bombicola (ATCC 22214)* in MGYP
Then the cells were re-dispersed in production medium containing 10% glucose. This production medium was supplemented with hydrophobic secondary carbon source i.e. oleic acid in absolute alcohol. Oleic acid Sophorolipid was formed as a brown and viscous liquid which was found to settle at the bottom of the flask after 96 to 120 hrs of incubation. After incubation period the cells were separated from the broth by centrifugation at 5000 rpm, 10ºC for 20 min. The SL formed was extracted from the supernatant with ethyl acetate. To the ethyl acetate phase, anhydrous sodium sulphate was added for removal of residual water, filtered and then ethyl acetate was removed under vacuum. The unconverted fatty acid was removed by washing with n-hexane. Crude sophorolipid was purified by column chromatography which has been given four different type sophorolipid forms. Different forms of sophorolipid were analyzed by LCMS, MALDI TOF, NMR and HPLC.

3.2.2 The Process

Synthesis of Sophorolipid mesostructures by Laser Irradiation

For synthesis of mesostructures, different concentrations range of Sophorolipid (1.0-10 mg/ml) were mixed in distilled water and then sonicated for 3 hours. Sophorolipid water emulsion looked visibly turbid (Figure 3.2) after sonication because of amphiphilic nature of SL which was then irradiated by Laser pulses (wavelength 248 nm, energy density 166 mJ and frequency 10 Hz). Similar experiment was also performed on oleic acid and glucose.

Polyol synthesis of Fe$_3$O$_4$ Nanoparticles

For the synthesis of super paramagnetic Fe$_3$O$_4$ nanoparticles, 1 mM of iron acetylacetonate was mixed in 30 ml triethylene glycol and sonicated for 5 minutes in the presence of argon gas. A round bottom flask was kept in silicon oil bath and the temperature was raised (2ºC/min) to 278ºC. After 30 minutes at constant temp (278ºC) the product was cooled to room temperature and then thoroughly washed with ethyl acetate and separated by magnet. It was dried overnight in an oven at 50ºC.
Figure 3.1 Figurative depiction of the experimental process

Figure 3.2 Actual image of Appearance of (A) water (B) unirradiated sophorolipid (C) irradiated sophorolipid (D) magnetic sophorolipid mesostructure

Synthesis of Fe$_3$O$_4$ impregnated Sophorolipid mesostructures

For synthesis Fe$_3$O$_4$ encapsulated Sophorolipid mesostructures, different concentrations of Sophorolipid (1-10 mg/ml) and Fe$_3$O$_4$ (10-100 µl, 20 mg/ml) nanoparticles were mixed in double distilled water and sonicated for 3 hours. After sonication Sophorolipid and Fe$_3$O$_4$ solution appeared light brown and turbid, which was then irradiated by UV laser pulses (wavelength 248 nm, energy 150 mJ and frequency 10 Hz). It was found that 5:1 ratio of Sophorolipid to Fe$_3$O$_4$ nanoparticles
gave highly reproducible result. Sample were collected after different time intervals and analyzed by different techniques stated above.

3.3 Characterization

Scanning electron microscopy with Energy-dispersive X-ray spectroscopy (EDX) (FEI Quanta 200 3D) was used for the determination of morphology and elemental composition. X-ray photoelectron spectroscopy (XPS) (ESCA-3000, VG Scientific Ltd. UK, with a 9 channeltron CLAM4 analyser under vacuum better than $1 \times 10^{-8}$ Torr, Al Ka radiation (1486.6 eV) and a constant pass energy of 50 eV) was employed to study the chemical state of carbon in the materials respectively. X-ray Diffraction (XRD, Philips X’Pert PRO) and Raman spectroscopy (a confocal micro-Raman spectrometer LabRAM ARAMIS Horiba JobinYvon, with laser excitation wavelength of 532 nm) were also used to examine the characteristics of the carbonaceous compounds. Thermo gravimetric analysis (TGA) was performed to determine the thermal stability. We also obtained images of the graphitic CNSs by techniques of Field Emission Scanning Electron Microscopy (FESEM, Hitachi S-4200) and High Resolution-Transmission Electron Microscopy (HR-TEM, FEI Tecnai 300). Conductivity measurement was carried out on a pellet by a two probe method.

3.4 Results and Discussion

Three different types of sophorolipids crude, acidic and lactonic, dispersed in water were laser irradiated with pulsed excimer laser ($\lambda=248$ nm, pulse width 20 ns, energy density~166 mJ/cm$^2$). Microscopy studies and other physical characterizations were done for all the three types of samples under different conditions. When the crude Sophorolipid was processed with laser irradiation, mixed population of some spherical microstructures with cloudy tube-like structures were obtained. Lactonic sophorolipid formed undefined hazy structure, where only undefined structures were seen to form. However in the case of acidic form of sophorolipid, which is diacetate in nature, extremely well-defined and fairly uniform spherical microstructures were formed (Figure 3.3).
Size range of sophorolipid microstructure was 0.5 - 2.5µm which could be reduced to less than 100 nm by optimizing process parameters such as laser irradiation time, energy and stirring solution. 100 nm sizes represent ideal size range for bio-imaging experiment in medical science. Noting that the basic structure of sophorolipid molecule comprises of oleic acid and glucose forms, we examined the effects of pulsed excimer laser irradiation on these two molecules as well to elucidate the contributions of these molecular components in assembly and related fluorescence.

### 3.4.1 Optical properties

The unirradiated Sophorolipid (SL) and the laser irradiated Sophorolipid (SLIR) show a marked difference in their appearance to the naked eye (Figure 3.2). While the unirradiated SL appears milky, the irradiated one appears transparent and yellowish. The unirradiated Sophorolipid solution shows UV absorption peak at 228 nm, while after laser irradiation the peak exhibits a 50 nm red shift to 278 nm, as clearly seen in Figure 3.4. We will return to this observation later in the discussion. For the study of photoluminescence of the unirradiated and irradiated sophorolipid; these samples were excited at the same wavelength of 330 nm. In the case of the unirradiated sophorolipid sample a very weak excitonic emission is observed in UV at 370 nm, while in the case of the irradiated sample a strong emission is seen to occur in the visible at 510 nm (Figure 3.4 1B) Further study by fluorescence microscopy analyses also confirm the appearance of strong green fluorescence in SLIR, while no fluorescence is observed in unirradiated SL (Figure 3.4 1C, D). Polarized microscopy study was performed to explore the morphologies of vesicular mesostructures formed by the laser processing of the acidic form of sophorolipid.
Figure 3.4 (A) UV–Visible spectra measurement of unirradiated and irradiated Sophorolipid solution. Unirradiated SL solution shows absorbance at $\lambda=228$ nm while laser irradiation shifts absorption toward visible region $\lambda=278$ nm. (B) Photoluminescence study of laser irradiated Sophorolipid mesostructures. (C) Fluorescence image of unirradiated SL and (D) laser irradiated SL.

According to Yapei Wang, Xi Zhang, UV light can help vesicles formation from micelles structure. In their work, they have mentioned that upon UV irradiation, the weak interaction between a-Cyclodextrin (a-CD) and cis azobenzene may drive some of the a-CD to slide onto the alkyl chain, and thus the self-organization of AzoC10 complexes with a-CD could form different vesicle-like aggregates. In our case non-thermal photochemical process seems to be responsible for the formation of mesoscopic spherical structures since no ambient heating of the solution takes place under laser exposure.
3.4.2 Time evolution of morphology

To study the process of pulsed UV laser induced self-assembly of sophorolipid, time evolution study was performed. Samples were collected at every 10 minutes interval to check for the changes happening due to pulsed laser irradiation (Figure 3.5 A-F). After initial 10 minutes sheet-like structure of sophorolipid could be seen by local shrinking and formation of some defined globule like structures which are seen to be prominent over the hazy mass present. Antonietti and Forster\textsuperscript{33} have explained the energy conservation in vesicles formation stating that when lipid sheet spreads, it forms vesicle-like structure to minimize its bending energy. We hypothesize that our mesostructure formation follows similar reaction mechanism. The Reaction mechanism kinetics in our study reveals that during the initial 10 minutes of laser irradiation sophorolipid forms sheet-like structure because of laser energy input (equation 1) which further increases during the next 20 minutes.
Figure 3.5 TEM images of Sophorolipid mesostructures evolution with laser irradiation time: (A) sample collected after 10 minutes of laser irradiation; (B) 20 minutes laser irradiation; (C) 30 minutes laser irradiation, sheet like structure begin to form; (D) 40 minutes laser irradiation, sheets start transforming into well-defined structures; (E) 50 minutes laser irradiation, spherical structures begin to form; (F) 60 minutes, complete mesostructures get formed (Scale Bar A -C: 2µm; D-F: 1µm)

After 40 minutes the lipid sheet starts to form some defined structures and these gets converted into spherical mesostructures in the 50 minutes of laser irradiation. Mesostructures start forming when sheet like structures are so large and energy loss due to surface tension is excessive. Laser irradiation for one hour provides the conditions required (equation 2) for the SL mixture to form fully developed spherical mesostructures.

\[ E_{\text{disk}} = 2\pi R \gamma \]  \hspace{1cm} (Equation 1)

\[ E_{\text{bend}} = 8\pi k \]  \hspace{1cm} (Equation 2)
Also it is seen that these structures do not form by providing heat energy from any other source and that laser energy is crucial for the formation of these sophorolipid mesostructures. By fine tuning the laser energy, time and proportion of Sophorolipid, nanoparticles in size domain of ~100 nm could be obtained.

### 3.4.3 Mechanism

**Understanding the origin of fluorescence of the laser induced molecular assembly**

In order to understand the origin of the observed intriguing green fluorescence property and its possible connection with the laser induced reorganization, stitching and assembly of sophorolipid molecules, we performed additional experiments. Noting that the basic structure of sophorolipid molecule comprises of oleic acid and glucose forms (Figure 3.6), we examined the effects of pulsed excimer laser irradiation on these two molecules as well.

![Basic structure of SL produced by yeast C. bombicola](image)

*Figure 3.6 Basic structure of SL produced by yeast C. bombicola*

Interestingly the corresponding data also bring forth the fact that oleic acid molecules upon laser treatment show similar fluorescence property as the sophorolipid molecules (Figure 3.7 A); however the glucose molecules do not. The fluorescence of laser treated oleic acid induced system is weaker and it does not show a well-defined assembly (Figure 3.7 B) as with laser treated sophorolipid molecules.
Thus the broad conclusion is that the glucose part in sophorolipid serves to develop proper molecular assembly in the form of mesosphere, but the main fluorescence features emanate from transformations occurring in the oleic acid type molecular component in the lipid (Figure 3.8 A).

Solution based spectroscopic characterization of self-assembled organic molecules are quite difficult because of highly insoluble aggregates. The assembly formed by SL after continuous 1 hr. irradiation of laser was insoluble in most the organic solvents. This could be the reason that we are not able to take NMR spectra after complete assembly formation.

In order to at least understand and elucidate the initial stages of formation of the sophorolipid mesostructures upon laser treatment, we examined the consequences of
laser irradiation in the product obtained up to 20 minutes by techniques of Nuclear Magnetic Resonance (NMR) and MALDI (Matrix Assisted Laser Desorption/Ionization). The detailed NMR data for various cases of interest are presented in electronic supplementary information (Figure 3.9).

Figure 3.9 $^1$H NMR spectrum of the sophorolipid, confirming the presence of one olefin group in its structure at 5.34 ppm

Figure 3.10 $^1$H NMR spectrum of the sophorolipids, confirming the appearance of one extra olefinic group at 5.37 ppm
In Figure 3.9 we present $^1$H NMR spectra for the unirradiated samples, which form the focus of the following discussion. The NMR spectrum for SL sample matches with the reported data.\textsuperscript{34} The triplet signature (CDCl\textsubscript{3}, TMS, 400 Hz, $\delta$ (ppm); 5.34) marked in Figure 3.9 corresponds to olefinic C=C bond. In the laser irradiated case (Figure 3.10) an extra triplet state is noted (CDCl\textsubscript{3}, TMS, 400 Hz, $\delta$(ppm); 5.37) and its position implies that it represents downfield C=C bond. Moreover, since we do not see a doublet of doublet, this C=C bond is not conjugated with the bond already present in SL.

The downfield shift implies the location of the bond close to the carboxylic group. In Figure 3.11 we present $^{13}$C NMR spectra for the unirradiated samples. Laser irradiated SL $^{13}$C NMR spectroscopy data (Figure 3.12) also show emergence of new peaks at 130.24 and 130.36 ppm which are characteristic of this second C=C bond. This extra C=C clearly enhances the $\pi$- character in SL.

**Figure 3.11** $^{13}$C NMR spectrum of the sophorolipids, confirming the presence of one olefin group in its structure at 129.78, 129.91 ppm.
Figure 3.12 $^{13}$C NMR spectrum of the sophorolipids after laser irradiation, confirming the appearance of one extra olefin group at 130.24, 130.36 ppm.

The MALDI data\(^{35}\) shown in Figure 3.13 also confirm the loss of two hydrogen consistent with the formation of an extra C=C bond.

Figure 3.13 MALDI-TOFMS mass spectra of the acidic sophorolipids. (A) sample collected at 0 min. (B) sample collected at 20 min. Assignments for the [M+Na]\(^+\) molecular adduct ions.
Figure 3.14 A shows the FTIR spectra of the sophorolipid prior to and after laser irradiation for 1 Hr. The ‘before irradiation’ case reveals a broad band at 3350 cm\(^{-1}\) corresponding to the O–H stretch frequency in the glucose moiety of the molecule. The asymmetrical and symmetrical stretch modes of methylene (CH\(_2\)) groups occur at 2928 and 2854 cm\(^{-1}\), respectively. Sophorolipid has two strong absorption bands arising from C–O and C–O stretching; the C–O absorption band at 1,744 cm\(^{-1}\) includes contributions from these groups. Moreover, sugar C–O stretch of C–O–H groups is found at 1,048 cm\(^{-1}\) and the band at 1,452 cm\(^{-1}\) corresponds to the C–O–H in-plane bending of carboxylic acid (–COOH) in the structure of the product. All these structural details are in conformity with the literature reports\(^{36}\). The laser irradiated sophorolipid brings out some interesting aspects. Most of the peaks that occur in FTIR for this case are similar to the ‘before irradiation’ case demonstrating the fact that the general molecular structure of the sophorolipid is retained after the photochemical process as well. However a specific significant difference arises post-irradiation. FTIR analysis clearly shows appearance of an intense peak at 1630 cm\(^{-1}\) which can be attributed to an increase in the C=C bond (\(\pi\) character).\(^{37}\) The 20 minutes treated sample also shows this signature. Intriguingly similar to the sophorolipid case a new peak at 1630 cm\(^{-1}\) is also seen to emerge in the case of laser irradiated Oleic acid (Figure 3.14 B), which also exhibits fluorescence, as stated earlier.

![Figure 3.13 (A, B)](image)

**Figure 3.13 (A, B)** FTIR data of unirradiated and laser irradiated SL and oleic acid respectively. An extra peak at 1630 cm\(^{-1}\) in both SL and oleic acid corresponds to increase alkene (-C=C-) in oleic acid and SL molecular structure.
Clearly the fluorescence character is tied to the appearance of this extra C=C bond in these structures post-irradiation. It may be remembered that the discussion pertaining to the appearance and the downfield location of the C=C bond from NMR and MALDI refers to the 20 minutes irradiated sample. Further irradiation leading to fully evolved spherical assemblies could have additional modifications which cannot be ascertained easily. The emergent assembly and interactions of the π - π structures induced by laser treatment could explain the strong fluorescence observed in SL case. It may be further noted that Oleic acid does not show equally strong fluorescence (although exhibits the same basic weak fluorescence and molecular changes) because it does not lead to the complex assembly. Thus, our analyses suggest that the elements of fluorescence are induced in the system via laser induced extra C=C bond character, however enhancement of fluorescence originates from the assembly character.

3.5 Magnetic SL structure by embedding Fe₃O₄ nanoparticles

Iron oxide (Magnetite, Fe₃O₄ or Maghemite, gamma-Fe₂O₃) nanoparticles have long been known to be useful in drug delivery and nano-medicinal applications. However their intrinsic surface area is not that high. We therefore undertook the task of exploring the possibility of loading them on the highly spherical laser synthesized sophorolipid mesostructures. Needless to mention that this can render them even more biocompatible, especially in so far as their interaction with cells is concerned. Indeed sophorolipids could help facilitate the entry of the iron oxide nanoparticles inside cells since lipids can easily fuse with the cell membrane. Moreover iron oxide impregnated SL particles can be directed easily to a specific site under the influence of an external magnetic field favoring the applicability of this bio-inorganic composite system to cancer hyperthermia. Super-paramagnetic Fe₃O₄ nanoparticles were synthesized by polyol method. These monodispersed nanoparticles were then mixed with Sophorolipid solution and irradiated with UV laser pulses. Complex structures embedded with Iron oxide nanoparticles in SL were thus obtained which could be easily separable using a magnet. Studies were also performed by adding Fe₃O₄ nanoparticles during synthesis of spherical mesostructures at intervals.
Figure 3.14 TEM images of (A) Fe₃O₄ Nanoparticles (NP), and (B-E) Fe₃O₄ NP loaded SL assemblies formed by laser irradiation. The images B, C, D and E are for cases when the NPs were added to the SL solution after initial laser irradiation for 0, 20, 30 and 40 mins, respectively. The Fe₃O₄ content and the total irradiation time of 60 minutes were the same in all cases. The picture in (F) shows the appearance of magnetic NP loaded SL mesostructures dispersion for case (B) without (left) and with (right) magnetic field. It is seen that the entire dispersion is pulled by the magnet.

It was observed that when Fe₃O₄ nanoparticles were added during synthesis at the beginning itself, almost all of the particles got embedded in the core of these mesostructures. Fe₃O₄ nanoparticles which were added after 20 and 40 minutes during the synthesis of the SL mesostructures were more peripheral in their location. These embedded SL structures were smaller and compact in nature when compared to pure SL vesicular mesostructures. EDAX analysis of laser irradiated sophorolipid wherein Fe₃O₄ was added right at the beginning is shown in Table 1. It brings out the percentage of iron oxide nanoparticles in the sophorolipid mesostructures. It is important to point out that depending upon the time of addition of Fe₃O₄ nanoparticles; we can define the location of these nanoparticles in Sophorolipid assemblies.
Table 1 EDAX Analysis of Laser Irradiated magnetic SL mesostructures with Fe$_3$O$_4$ embedded nanoparticles (Figure 3.14 B-E)

<table>
<thead>
<tr>
<th>Element</th>
<th>wt %</th>
<th>wt %</th>
<th>% K-ratio</th>
<th>Z</th>
<th>A</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>C K</td>
<td>79.15</td>
<td>86.32</td>
<td>0.5136</td>
<td>1.009</td>
<td>0.6425</td>
<td>1.0001</td>
</tr>
<tr>
<td>O K</td>
<td>15.05</td>
<td>12.32</td>
<td>0.0210</td>
<td>0.9945</td>
<td>0.1402</td>
<td>1.0002</td>
</tr>
<tr>
<td>Fe L</td>
<td>5.80</td>
<td>1.36</td>
<td>0.0171</td>
<td>0.8617</td>
<td>0.3415</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

To get a particular location of Fe$_3$O$_4$ nanoparticles (center, periphery and outer surface in sophorolipid mesostructures requisite amounts of Fe$_3$O$_4$ in Sophorolipid solution with various combinations were tried as described in materials in methods.

Cyto-toxicity and hyperthermia experiment

Cyto-toxicity analysis of the laser-synthesized sophorolipid mesostructures was done using MTT assay. For this a HeLa derived cell line was used. Spherical sophorolipid sample, which had been dried to powder form, was resuspended in sterile distilled water before the assay. Then appropriate concentration of the sample was added to the wells of a 96 well plate and the plate was incubated for 2 days.

*Figure 3.15 (A) Cytotoxicity of Sophorolipid mesostructures performed on HeLa derived cell line on 96 well plates; the plate was incubated for 2 days and optical density was measured at 540 nm; (B) Temperature rise of water dispersions of SL and magnetic NP loaded SL mesostructures under RF excitation as a function of time*
The viability of cells was then checked by their ability to reduce the tetrazolium salt (MTT) to bluish purple colored Formazan crystals, which can be solublized by acidified propanol and optical density measured at 540 nm. This assay proved to us that the SL mesostructures are not detrimental to the viability of eukaryotic cells even at a concentration of 50 µg/ml (Figure 3.15A).

Hyperthermia experiment was also done using a Radio Frequency source. Magnetite loaded SL mesostructures (5 mg/ml Fe₃O₄ nanoparticles) was used for this experiment. The data were collected at 0 min, 4 min, 10 min and 15 minutes for both the unirradiated SL sample and the magnetite-loaded SL mesostructures sample formed by laser irradiation. The data shown in Figure 3.15A clearly bring out that the biocompatible Sophorolipid mesostructures loaded with Fe₃O₄ nanoparticles can serve as effective hyperthermia agents.

3.6 Conclusions

Highly spherical mesoscale Sophorolipid molecular assemblies are synthesized using a simple one step method which involves irradiating a water solution of acidic sophorolipid with UV laser (Excimer, 248 nm) pulses. Remarkably, the laser assembled mesostructures exhibit strong green fluorescence, while the original sophorolipid molecules do not show any. Cyto-toxicity assay shows that these are non-toxic to living cells even at 50µg/ml. It is further shown that such mesostructures can be easily impregnated with super-paramagnetic iron oxide nanoparticles, and the corresponding synthesis protocol concurrently leads to a reduction in the assembly size down to 100 nm scales. Importantly, the fluorescence property is retained and the magnetite loaded assembly can be heated by RF excitation for hyperthermia application.

References


Pradeep Kumar Singh

Savitribai Phule Pune University


