Thesis for the Degree of Master of Science

Administration of Dye-Loaded Polymeric Nanoparticles to the Red Tide Dinoflagellates, *Akashiwo sanguinea* and *Alexandrium pacificum*

by

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적조생물 *Akashiwo sanguinea* 와 *Alexandrium pacificum* 에 대한 염료함유 고분자 나노입자 활용 연구

Advisor: Prof. Minseok Kwak, Ph.D.

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Dedication

This master thesis is dedicated to my husband Fuead Hasan for his endless support and to my respected advisor Prof. Minseok Kwak for giving me the opportunity to work with his research team and improve me as an intellectual scientist also making me what I am right now.

Contents

List of Figures

List of Tables

Administration of Dye-Loaded Polymeric Nanoparticles to the Red Tide Dinoflagellates, *Akashiwo sanguinea* and *Alexandrium pacificum*

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Abstract

Dinoflagellate blooms in water lands have been increasingly reported which produce large amount of toxin compounds. Here reported engineered nanoparticles consist of two different rylene derivatives, naphthalene organic dye Lumogen violet and quarterrylene organic near-infrared dye, IR788, encapsulated in polymeric micelles. In addition, to avoid dissociation of polymer, the core of micelle was stabilized via *semi*-interpenetrating network formation. Nanoparticle uptake and its effects were conducted on the marine red tide dinoflagellates, *Akashiwo sanguinea* and *Alexandrium pacificum* respectively. Infrared laser irradiation was exposed on the nanoparticle treated *Akashiwo sanguinea* under different exposure and nanoparticle conditions, the resulting system showed reduced cell survival due to photothermal effect studies on microalage. The results suggested that the nanoparticle (IR788) can be applied for potential red tide algal elimination further, although it requires to determine the safety assessment of biological toxicity of the nanoparticle.

적조생물 *Akashiwo sanguinea* 와 *Alexandrium pacificum* 에 대한 염료함유 고분자 나노입자 활용 연구

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Abstract

최근 적조현상으로 인한 생물 독성 문제가 지속적으로 보고되고 있다. 이에 우리는 적조의 원인이 되는 미세조류 제거를 위하여 유기 근적외선(NIR) 염료인 루모젠 IR788 를 포함하는 Pluronic 고분자 기반의 미셀 나노입자를 제조하였다. IR788은 근적외선 영역의 빛에 노출되면 열을 발생시키는 쿼터릴렌 광열 물질로서 소수성의 미셀 코어에서 안정화되어 수용액상에 분산된다. 또한 고분자의 농도 및 주변 온도 변화로부터 미셀의 해리를 방지하기 위해, 미셀의 코어 부분은 반-상호 침투 네트워크(sIPN)를 형성하여, 저온에서도 안정적인 구조를 유지할 수 있게 하였다. 나노입자의 미세조류로의 섭취 능력 및 광열 효과를 평가하기 위하여 아카시오 상귀니아(*Akashiwo sanguinea*) 및 알렉산드륨 퍼시피움(*Alexandium pacificum*) 2종의 와편모충류 미세조류를 사용하였다. 나노입자는 형광 이미징 및 대조군으로서 IR788-sIPN 외에 형광물질인 루모젠 바이올렛(LV)를 포함하는 LV-sIPN를 추가적으로 제조하였다. 미세조류로의 섭취 능력은 LV-sIPN를 이용하여 형광이 있는 미세조류를 개수하여 나타냈고, 광열 효과는 808 nm 근적외선에 IR788-

sIPN 및 LV-sIPN을 노출시켰을 때 세포생존율을 통해 평가하였다. 그 결과, 나노입자는 효과적으로 미세조류로 섭취되었으며 IR788-sIPN이 LV-sIPN과 비교하여 우수한 광열 효과를 가진 것을 확인할 수 있었다. 이는 IR788-sIPN 나노입자를 조류 제거에 잠재적으로 이용할 수 있음을 나타내며 또한, 이를 위하여 나노입자의 생물학적 독성 평가가 선행되어야 함을 보여준다.

Ⅰ. Introduction

1. Research Overview

Nanotechnology development has significantly increased in different areas due to the wide application of nanoparticles (NPs). At least one dimension in the range of 1 to 100 nm are defined as the particles size of nanoparticles[1]. Based on their chemical composition nanoparticles (NPs) can be separated into five categories: nano-oxide, carbon nanomaterials, nano-metal, quantum dots and other NPs such as organic polymers[2]. In a variety of conventional products nanoparticles (NPs) have been used for instance cleaning agents, clothes, tableware, children's toys and domestic appliances[3]. Due to their unique capabilities such as their mechanical properties, contact reactivity, optical properties and electrical conductivity Nanoparticles (NPs) have variety of applications in the fields of food packaging, textiles, optoelectronics, biomedicine, cosmetics, energy and catalysis[4-5].

Polymeric micelle composed of amphiphilic block copolymers have been shown to be a promising nano-carrier for tumor imaging and drug delivery applications[6-8]. This nanoparticle attracted much attention because of its easily controlled physicochemical properties (ex: particle, charge surface, degradation rate) and the surface of amphiphilic block copolymer is also

easy to be functionalized with other moieties [9-10]. Above their critical micelle temperature (CMT) or concentration (CMC), the block copolymers can be self-assembled into a supramolecular structure composed of a hydrophobic core for loading hydrophobic molecules and hydrophilic shell which can provide protection from dissociation in the aqueous environment [11-13].

Among those, Pluronic block copolymer consisted of poly (ethylene oxide) and poly(propylene oxide) (PEO-*b*-PPO-*b*-PEO) has been widely studied as a therapeutic agents carrier since its excellent bioavailability [14-16].

In the aqueous environment above its CMT and CMC, Pluronic material can be spontaneously formed nanosized core-shell micelles having a hydrophobic core composed by PPO segments and a shell dominated by PEO segments. The PEO chain of Pluronic can form hydrogen bonds with aqueous molecules resulting in a tight shell around micellar core which prevent the hydrophobic molecules from being removed from the core [17-20].

Although Pluronic has been considered to be a promising drug carrier, these micelles which have soft cores are thermodynamically unstable than other polymeric micelles with solid core (ex: poly(styrene)-poly (ethylene oxide)). In order to stabilize Pluronic micelle, many stabilization methods [21] have been conducted by other groups focused on PEO and PPO functionalization [22-24]. Mostly, reported methods required complicated organic synthesis route and also those were known influence the micellization properties of Pluronic molecules [22]. The innovative method

to stabilize Pluronic micelle has been developed by Petrov *et al* [25] via formation of semiinterpenetrating network (sIPN) in the core of Pluronic F-68 using PETA (pentaerythritol tetraacrylate) as a cross-linker. The interpenetrating networks of poly(PETA) and polyether chains were stabilized Pluronic micelle from degradation below its CMC or CMT.

An alternative to be considered as a photostable dye under intense irradiation is rylene-based dye, a polycyclic dye with exceptional photophysical properties such as high photostability and intense absorption[26]. Rylene dye with high number of naphthalene units are known to be able to absorb wavelength up until 1,000 nm[27]. Process of loading hydrophobic dyes has been widely done using polymeric micelles[28]. Cyanobacterial and Dinoflagellate blooms in water lands have been increasingly reported [29-30]. Accumulated cyanobacterial cells produce large amount of toxin compounds [31] that are poisoning to human being and organisms [32-33]. Moreover, Dinoflagellate blooms induce brevetoxins and ichthyotoxins of the water leading to fish kills and degradation of water recreational value [34]. Several treatments include flocculation, filtration, clay minerals, ultrasonic, biological methods such as cyanophage etc. were applied to Dinoflagellate marine microalgal blooms (red tides). However, most methods are immobile therefore low effective when applied in large scale waters such as lakes and reservoirs. Also Most of the previous studies of nontoxicity thus far conducted have evaluated the toxicity of nanoparticles themselves, but there has been very little study thus far conducted on the toxicity of NPs exposed to IR light or sunlight in a

natural environment.

In this present work, polymeric nanoparticles (NPs) were loaded with two types of quarterrylene dye and stabilized to avoid dissociation of the polymer. Pluronic® F127, a triblock copolymer consisted of hydrophobic poly(propyleneoxide) (PPO) and hydrophilic poly(ethyleneoxide) (PEO) chain (PEO-*b*-PPO-*b*-PEO), was used as a base for the polymeric micelle with Lumogen® IR788 (a quarterrylene dye) and Lumogen Violet as loaded moiety[35-38]. Those photothermal dye-loaded NPs were further stabilized via *semi*-interpenetrating network (sIPN) within the core of the micelle using a crosslinker, PETA (pentaerythritol tetraacrylate). A UV irradiation was used to promote the network formation of crosslinker, holding the PPO core within micelle[25]. The effects of IR788-sIPN NPs and LV-sIPN NPs on two Dinoflagellate species *Akashiwo sanguinea, Alexandrium pacificum* were evaluated after applying NPs in the algal media and later *Akashiwo sanguinea* species was selected for Infrared (808nm) laser irradiation treatment under different exposure conditions. Algal media without NPs were used as a control compared to media contained NPs. Algae were selected as the test species owing to their importance as a primary producer in the aquatic environment. The other objective of this study was to obtain a better understanding of the toxicity difference of photothermal dye-loaded IR788-sIPN NPs and LV-sIPN NPs.

2. Literature Review

2.1. Rylene Dyes

Rylene is a polycyclic hydrocarbon constructed of naphthalene units as a base connected in peri-position. Rylenes in form of rylene diimide (RD), which contains two diimide ring at the terminal naphthalene, are commonly used as pigment, semiconductor, or dye due to their strong fluorescence, large absorption coefficient, and superior thermal and photostability[42,43]. Rylene derivatives have different photophysical and photothermal characteristics depending on the size of its core, as shown from a study of lower-ring rylene compounds[44-46].

One of the derivatives of the rylene diimides is the quarterrylenediimide (QDI) group, which is an expanded-core derivative of RD with an intense absorption at especially 808 nm wavelength, thus making it a promising material for deep-penetrating photothermal material[47]. An example of QDI is the Lumogen IR788, a quarterrylenetetra carboxylic diimide[48]. Despite the superior characteristics, QDI has poor solubility in water and high tendency to form an aggregate because of its rigid structure and nonpolarity[42,49]. Structure of Lumogen violet and IR788 are shown in **Figure 1**.

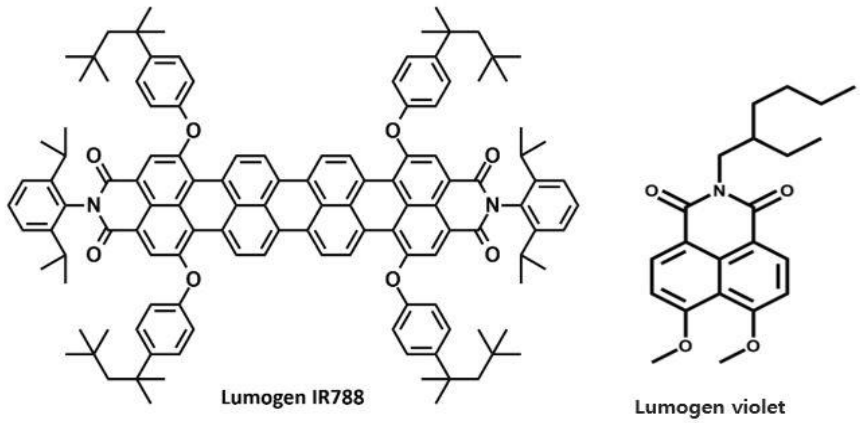


Figure 1. Chemical structure of rylene, Lumogen IR788 and Lumogen violet

2.2. Polymeric Micelles, Nanoparticles and *Semi*-Interpenetrating Network

Nanotechnology development has significantly increased in different areas due to the wide application of nanoparticles (NPs). At least one dimension in the range of 1 to 100 nm are defined as the particles size of nanoparticles[1]. In recent years' polymeric micelles have known to attract huge interest for pharmaceutical applications as gene and drug delivery system, and as a carrier for contrasting agent in drug diagnostic imaging[7,8,39]. The reason for this popularity is due to the core-shell structure of the micelles. A combination of hydrophobic micelle core, suitable for loading or encapsulation for various therapeutic agent, with a hydrophilic micelle shell, ensures improved solubility of the agent, while also gives protection from undesired pharmacokinetics due to the aqueous

environment surrounding it[16,20,39]. This kind of core-structure can be seen in an amphiphilic copolymer. A known type of copolymer with these properties is Pluronic, which consist of hydrophobic propylene oxide (PO) and hydrophilic ethylene oxide (EO) in an arrangement of PEO-*b*-PPO-*b*-PEO (**Figure 2**).

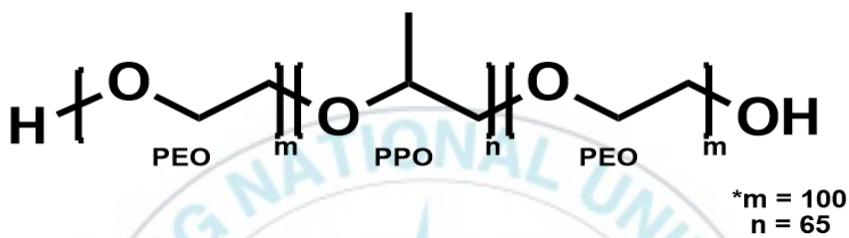


Figure 2. Chemical structure of pluronic F127

Micelle formation from Pluronics in aqueous solution is highly influenced by concentration of the Pluronics and surrounding temperature. These terms are usually addressed as critical micelle temperature (CMT) and critical micelle concentration (CMC)[40]. When both conditions are fulfilled, Pluronic unimer will self-assemble to form a micelle. On the other hand, if at any given time either concentration or temperature drops below the CMC and CMT, it will dissociate back into unimer, releasing any loaded molecule within the core of the micelle[35-38].

Stabilization of Pluronic micelle using *semi*-interpenetrating network (sIPN) have been studied and yielded nice results[25]. In a sIPN, a linear or branched polymer penetrates a network made usually by a crosslinker[41]. One reported a method to stabilize Pluronic micelle using pentaerythritol

tetraacrylate (PETA) shown in **Figure 3** as a crosslinker.

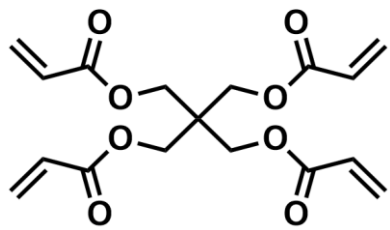


Figure 3. Chemical structure of PETA

PETA is polymerized within the micelle core via UV irradiation at an elevated temperature above 50°C to help promote internalization into the core. The resulting sIPN showed improved stability, able to maintain the micellar structure under CMC and CMT[25]. **Figure 4** shows a simple visualization on how sIPN can help stabilize a polymeric micelle.

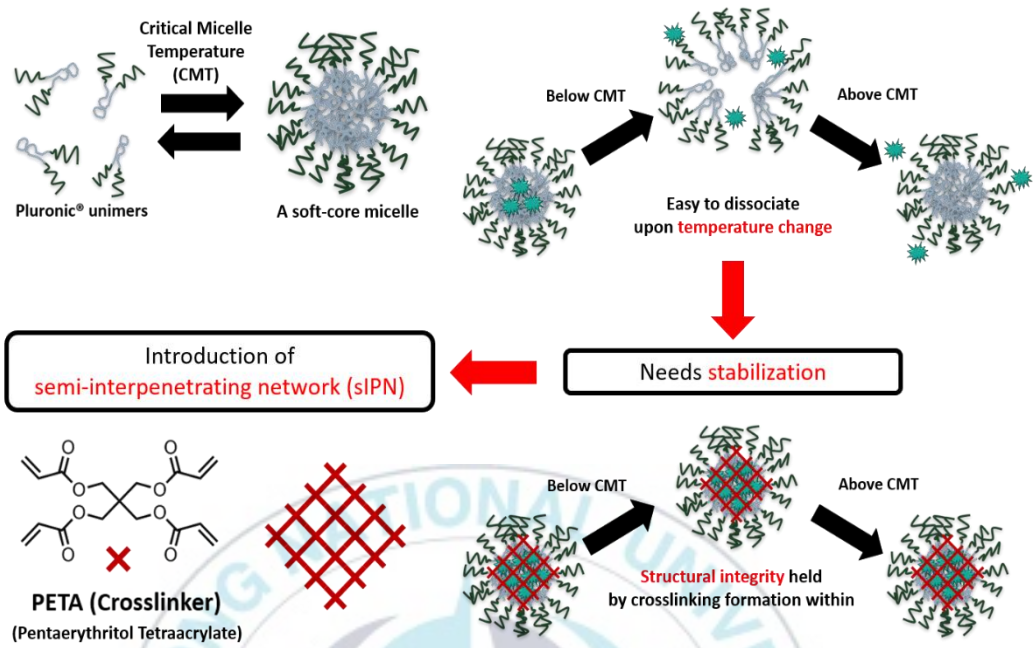


Figure 4. Illustration of sIPN role to stabilize a polymeric micelle

2.3. Heat Conversion Efficiency of Photothermal Material

Heat at a certain level can induce death of both for animal and algae cell specially dinoflagellate. At temperature above 30°C, damage on cell will occur as a result of an unwanted product from chemical reactions specially for dinoflagellate algae cell. These facts lead to a conclusion that heat can be used to eliminate red tides (Occur by dinoflagellate algae). NIR laser irradiation has been studied in order to induce heat and radiation from this source are less harmful since it is less absorbed by biological tissues, but when coupled with a NIR absorber agent, it can generate heat with high enough level to induce heat. Also, the characteristics of NIR

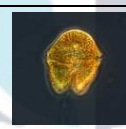
laser ensures deeper penetration into biological tissues[50-53].

2.4. Water Discoloration and Pollution Due to Red Tide

The discoloration in the water happens due to algal pigments, so red pigment containing algae's bloom named as red-tide which is a common name for algal blooms, which are large concentrations of aquatic microorganisms, such as protozoans and unicellular algae (e.g. dinoflagellates and diatoms). The most conspicuous effects of red tides are the associated wildlife mortalities and harmful human exposure. The production of natural toxins such as brevetoxins and ichthyotoxins are harmful to marine life and blooms which can injure animals or the ecology are called Harmful Algal Blooms (HABs)[2-4]. Red tide species can be found in oceans, bays, and estuaries, but they cannot thrive in freshwater environments and certain species of phytoplankton and dinoflagellates found in red tides contain photosynthetic pigments that vary in color from brown to red[54]. When the algae are present in high concentrations, the water may appear to be discolored or murky. The most conspicuous effects of red tides are the associated wildlife mortalities and harmful human exposure and the production of natural toxins such as brevetoxins and ichthyotoxins are harmful to marine life. Blooms which can injure animals or the ecology are called "Harmful Algal Blooms" (HABs)[54]. *Akashiwo sanguinea* and *Alexandrium pacificum* are two species of marine dinoflagellates well known for forming blooms that result in red tides due

to red pigments on their body. The below **Table1** represents some of the characteristics which differentiate red tide dinoflagellates *Akashiwo sanguinea* and *Alexandrium pacificum*.

Table 1. Differentiation of red tide dinoflagellates *Akashiwo sanguinea* and *Alexandrium pacificum*.

Characteristics	***Akashiwo sanguinea***	***Alexandrium pacificum***
Cell shape and outer layer	Oval and unarmed (lack of thick cellulose wall)	Round and covered with ornamented cellulose wall named 'theca'
Favorable temperature	20°C-26°C	20°C-24°C
Figure		
Toxin/Bioactive Compound	Surfactants	Saxitoxins
Human Health Effects or Syndrome	Suspected respiratory irritant	Respiratory paralysis, death (Paralytic Shellfish Poisoning or PSP)
Impacts to wildlife or domestic animals	Migratory bird deaths, including protected species	Fish and shellfish mortality, Marine mammal deaths
Cell uptake ability	High	Low

2.5. Nanoparticles for the Removal of Harmful Algal Species

Toxicities of ZnO, TiO_2 and CuO nanoparticles to microalgae were conducted and algal growth inhibition test taking in account potential shading of light, results showed that the shading effect by nanoparticles was negligible and ZnO nanoparticles were most toxic followed by nano CuO and nano TiO_2 [55]. Several treatments include flocculation, filtration, clay minerals, ultrasonic, biological methods such as cyanophage etc. were applied to Dinoflagellate marine microalgal blooms (red tides). However, most methods are immobile therefore low effective when applied in large scale waters such as lakes and reservoirs.

2.6. Light Irradiation on Algal Species

A study was evaluated to determine the ecotoxic effects of zinc oxide nanoparticles and titanium dioxide nanoparticles to a green alga under visible, UVA, and UVB irradiation conditions and algal growth was found to be inhibited as the nanoparticle concentration increased and ZnO NPs caused destabilization of the cell membranes[56]. Another research was conducted to determine the effects of UV-C irradiation on algal growth and cell integrity were investigated to develop a potential method for preventing cyanobacterial blooms suggested the potential application of sub-lethal UV-C irradiation for cyanobacterial bloom control with a

predictable low ecological risk[57].

2.7. Natural Infrared

Sunlight, at an effective temperature of 5780 kelvins (5510 °C, 9940 °F), is composed of near-thermal-spectrum radiation that is slightly more than half infrared. At solar zenith angle, sunlight provides an irradiance of just over 1 kilowatt per square meter at sea level and of this energy, 527 watts is infrared radiation[58]. Nearly all the infrared radiation in sunlight is near infrared and infrared radiation is popularly known as "heat radiation, but light and electromagnetic waves of any frequency will heat surfaces that absorb them[58]. Infrared light from the Sun accounts for 49%[58] of the heating of Earth. The International Commission on Illumination (CIE) recommended the division of infrared radiation into the following three bands: Infrared-A: 700 nm to 1,400 nm, Infrared-B: 1,400 nm to 3,000 nm, Infrared-C: 3,000 nm to 1 mm. The amount of solar radiation received by Earth is maximum 1413 W/m^2 to minimum 1321 W/m^2 (1 W/m^2 = 0.0001 W/cm^2).

Ⅱ. Lumogen Violet and IR788 sIPN Nanoparticles Formation and Algae Species Collection and Culture

This chapter describes formation of sIPN nanoparticles (NPs) at the core of a polymeric micelle containing Lumogen violet(LV) and IR788 dye, with several experiments to confirm the sIPN NPs formation. Two marine microalgal species i.e. *Akashiwo sanguinea* and *Alexandrium pacificum* which are well known for making red tide on marine water body, there cultures and culturing conditions were also describe in this chapter.

1. Methodology

1.1. Materials

Lumogen violet and IR788 were kindly provided by BASF AG (Germany). Lumogen violet and IR788 were kindly provided by BASF AG (Germany). Pluronic F127 (Mn 12,600) and PETA were purchased from Sigma Aldrich (Korea). Chloroform (99.8%, HPLC grade) was purchased from Samchun (Korea) and Acetone (99.9%) was purchased from DUKSAN (Korea), Mili-Q water was obtaining from NCHM laboratory.

Three (3) species of dinoflagellates were kindly provided by Marine Protistan Ecophysiology Laboratory, Pukyong National University, Busan. Lugol solution applied for cell staining and algae cells were fixed using glutaraldehyde $C_5H_8O_2$. Sterilized sea water (30% salinity) was used for algae cell dilution.

1.2. Instruments

Shaking of solutions was carried out using Lab Companion SKF 2050 Shaker (Jeio Tech), while for UV irradiation, OmniCure series 2000 (Lumen Dynamics, Canada) was utilized. For CMT test, incubation and centrifugation of solution at low temperature was done using R17 Micro Refrigerated Centrifuge (Hanil Science Industrial). Separation of supernatant and precipitate was performed using 0.2 µm syringe filter (Ministart® Sartorius Stedium). Samples were stored inside a 20-mL glass vial. Absorption spectra was recorded using SpectraMax M2 (Molecular Devices, U.S.A.) in a quartz cuvette with a 10 x 10 mm light path (Hellma Analytics). Sample was put in a 2-mL microcentrifuge tubes and irradiated with 808 nm Laser (Changchun New Industries, China) for photostability evaluation. Laser power were adjusted using a radiometer (R2000 from Omnicure, Canada).

Sterilization of algal culture media were done with Spirit-lamp, algal cell samples were placed in Sedgwick Rafter chamber (SR chamber) for counting under light microscopy (With fluorescence light-DAPI Mode),

irradiation experiment was conducted applying Laser source/machine (PSU-H-LED) (808 nm wavelength) on algae cell.

1.3. Species Collection

Two species of dinoflagellate marine microalgae Akashiwo *sanguinea* (Strain AS-USA) and *Alexandrium pacificum* (Strain AP-LOHABEE04) were obtained by pipetting single cells from seawater samples collected during blooms from Chesapeake Bay (2015), Masan (June 2, 2016) and Yongho (Busan-July 19, 2018) respectively.

1.4. Algal Cell Culturing

Akashiwo sanguinea (Strain AS-USA), *Alexandrium pacificum* (Strain AP-LOHABEE04) isolated single cells were transferred to polystyrene cell culture plates containing sterile f/2-SE culture medium under an inverted microscope. Identification of all clonal isolates of *Akashiwo sanguinea* and *Alexandrium pacificum* have been confirmed with large subunit (LSU) rDNA sequencing. Cells were cultured in sterile f/2-SE medium with a salinity of 27.5 for *Akashiwo sanguinea*, 30 for *Alexandrium pacificum* made with autoclaved and *0.2 μm* filtered seawater. The cultures were maintained at 20°C in an incubator with a 14 h light:10 h dark cycle.

2. Experimental

2.1. Preparation of Lumogen Violet sIPN Nanoparticle

Two stock solution were prepared: (1) Pluronic F127 (100 mg/mL) in $CHCl_3$; (2) PETA (100 mg/mL) in acetone. First, PETA film was prepared by depositing 0.125 mL of the PETA stock solution onto one clean vial and evaporating the solvent at ambient temperature in a fuming hood. In another vial to form micelle loaded with lumogen violet (LV), F127 (5 mL of the stock solution) and lumogen violet (sock liquid, 10 μL for 0.002% lumogen violet (LV) ratio based on F127) were mixed then $CHCl_3$ was evaporated using a rotary evaporator under reduced pressure, hydrated with 4.5 mL ultrapure (18 MΩ) water, and agitated using an orbital shaker (200 rpm) at r.t. overnight. Water color (transparent) on (F127 10 wt%) was added to the PETA film prepared and further agitated under the same condition. Argon gas was added to the solution before UV irradiation. The agitated solution was irradiated using UV illuminator at 1.5 W/cm^2 for 10 min at 50°C to promote crosslinking of PETA in the micelle core. LV sIPN Nanoparticle (NP) was obtained followed by filtration of the solution through a 0.2 μm syringe filter to remove residual impurities. Scheme for the LV sIPN NP preparation is visualized by **Figure 5**.

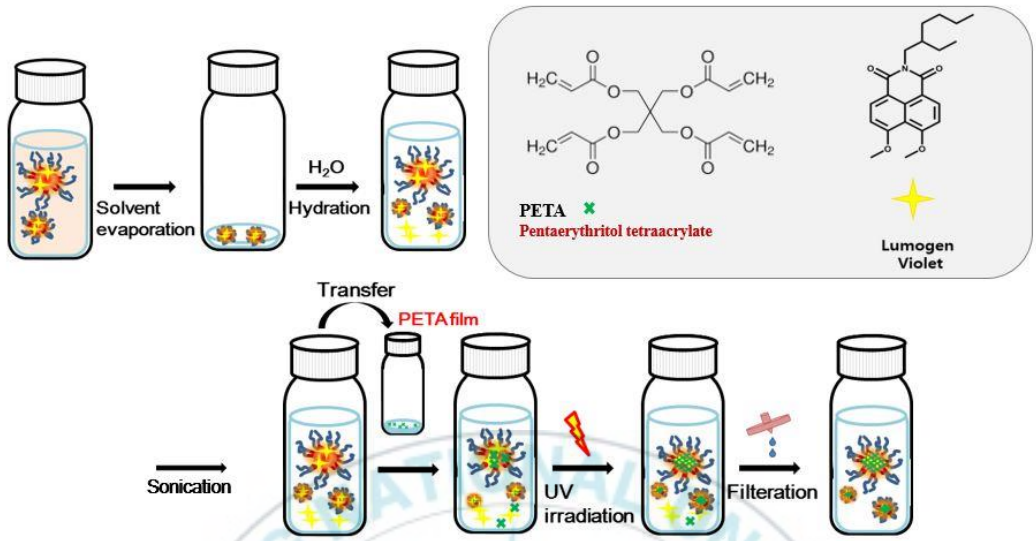


Figure 5. Preparation scheme of Lumogen violet-sIPN nanoparticle

2.2. Preparation of IR788 sIPN Nanoparticle

Two stock solution were prepared: (1) Pluronic F127 (100 mg/mL) in $CHCl_3$; (2) PETA (100 mg/mL) in acetone. First, PETA film was prepared by depositing 0.125 mL of the PETA stock solution onto one clean vial and evaporating the solvent at ambient temperature in a fuming hood. In another vial to form micelle loaded with IR788, F127 (5 mL of the stock solution) and IR788 (solid, 15 mg for 3% IR788 ratio based on F127) were mixed then $CHCl_3$ was evaporated using a rotary evaporator under reduced pressure, hydrated with 4.5 mL ultrapure (18 MΩ) water, and agitated using an orbital shaker (200 rpm) at r.t. overnight. The dark green aqueous solution (F127 10 wt%) was added to the PETA film prepared and further

agitated under the same condition. Argon gas was added to the solution before UV irradiation. The agitated solution was irradiated using UV illuminator at 1.5 W/cm^2 for 10 min at 50°C to promote crosslinking of PETA in the micelle core. IR788 sIPN was obtained followed by filtration of the solution through a 0.2 μm syringe filter to remove residual impurities. Scheme for the IR788 sIPN preparation is visualized by **Figure 6**.

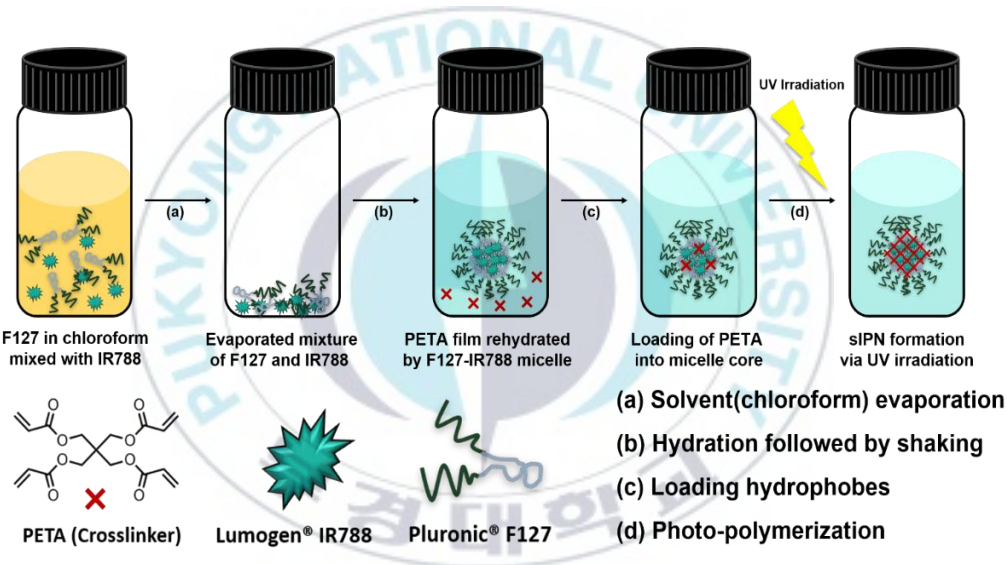


Figure 6. Preparation scheme of IR788 sIPN nanoparticle

2.3. Critical Micelle Temperature Test

Both Lumogen violet and IR788 sIPN nanoparticles were incubated at 4°C, below the CMT (17°C, 10 wt% F127) for overnight, followed by 10-minute centrifugation at 4°C on 13, 475 RCF. The supernatants of LV-sIPN

and IR788-sIPN NPs were separated and the absorption spectra before and after CMT test were recorded at 300-430 nm and 550 – 900 nm respectively. The supernatants of LV-sIPN NP was separated and the fluorescence spectra before and after CMT test were recorded at 375-560 following 360 nm as excitation wavelength.

2.4. Nanoparticle Characterization

The Lumogen Violet-sIPN NPs and IR788-sIPN NPs were prepared in NanoChemistry & Hybrid Materials laboratory and final products were in liquid form of watery and dark-green color respectively. LV-sIPN NPs and IR788-sIPN NPs sizes were 15.21 nm and 25.58 nm, respectively. The particle size distribution of nanoparticle detected by using dynamic light scattering (DLS, Zetasizer Nano User, Malvern).

3. Results and Discussion

3.1. CMT Test to Confirm Lumogen Violet and IR788 sIPN Nanoparticle Formation

The CMT (critical micelle temperature) test was done by incubating of samples (sIPN NPs) at the temperature below its CMT for overnight then followed by centrifugation at 4 °C for 10 min on 14000 rpm. The supernatant was transferred into a clean tube for further characterization. CMT test was done to evaluate the sIPN formation in the core of micelle. The values of CMT varies depending on temperature and concentration of amphiphilic polymers, and the CMT for given concentration of F127 in H_2O (10% wt) was 17°C. Both sample i.e. LV-sIPN and IR788-sIPN NPs were incubated for overnight at 4°C, a temperature way below CMT. **Figure 7** and **Table 2** shows that absorption and fluorescence spectra for 0.002% LV-sIPN NP and absorption spectra for both IR788 3% micelle and IR788 3% sIPN which had a significant difference before and after CMT test.

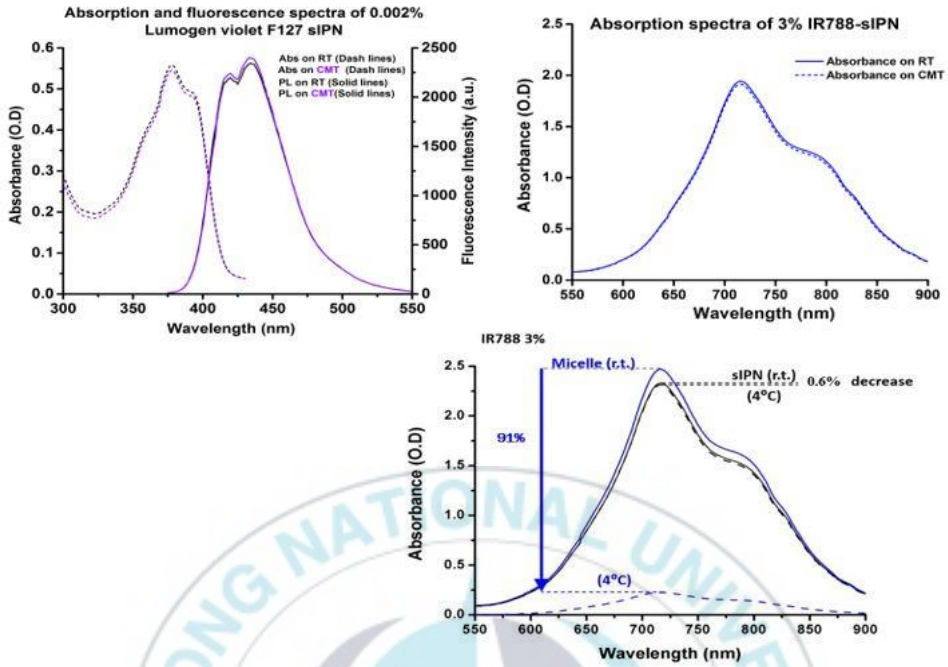


Figure 7. Absorption and fluorescence spectra of 0.002% Lumogen violet-sIPN NP, absorption spectra of IR788 sIPN NP and IR788 micelle at 3% IR788 concentration before and after CMT test.

The formation of sIPN NPs in the core of micelle was evaluated by stability test at a temperature below its CMT. At the given concentration (10%), CMT of Pluronic F127 is 17 °C. Before the CMT test we can observe that IR788 3% micelle has 25% higher maximum O.D. value at the peak compared to IR788 3% sIPN. This is due to the shrinking of the micelle core in IR788 3% sIPN as an effect of *semi*-interpenetrating network formation, causing reduced loading capacity of the micelle. After CMT test, though, it became apparent that the O.D. value of IR788 3% micelle drastically decreased up to 91% because of the dissociation of

micelle into unimers under CMT, releasing most of the loaded dyes in the process. This release of dye was not observed in IR788 3% sIPN, as proven by an O.D. decrease of only 0.6%, owing to the structurally fixed core of the sIPN. As written in the **Table 2**, after CMT test, the obtained result revealed that both F127 sIPN nanoparticles i.e. LV-sIPN and IR788-sIPN NPs can maintain their micellar structure (not dissociated to unimers) at temperature below the CMT. The absorption and fluorescence intensity of LV-sIPN nanoparticle and absorption spectra of IR788-sIPN nanoparticle sIPN are depicted in the Figure 7 and the related data is presented in the **Table 2**.

Table 2. Absorbance of Lumogen violet-sIPN and IR788-sIPN nanoparticle samples before and after CMT test

Sample	**Absorbance of sample stored at**		**Ratio intensity**
	25°C (RT)	**4°C(CMT)**	**(4°C/ 25°C)**
0.002% Lumogen violet-sIPN NP Micelle	0.5569 (377 nm)	0.5454(378nm)	0.979
IR788 3% sIPN NP	2.3330 (716nm)	2.2490 (716nm)	0.964

3.2. Particle Size Distribution of Nanoparticles

Lumogen Violet-sIPN NPs and IR788-sIPN NPs sizes were 20.21 nm and 25.58 nm, respectively. Polydispersity Index (Pdi) for LV-sIPN NPs was 0.683 and IR788-sIPN NPs was 0.462. **Figure 8** showed the size distribution of both LV-sIPN NPs and IR788-sIPN NPs. The particle size for IR788-sIPN nanoparticles were comparatively bigger than LV-sIPN nanoparticles because IR788 dye from IR788-sIPN have high tendency to form an aggregate because of its rigid structure and nonpolarity.

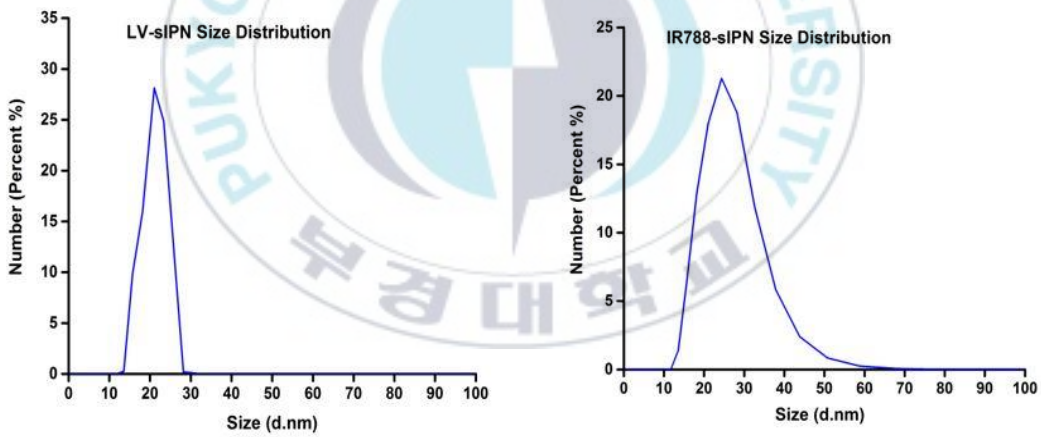


Figure 8. Particle size distribution of Lumogen violet-sIPN and IR788-sIPN nanoparticles

Ⅲ. Nanoparticles Uptake of Red Tide Dinoflagellate Species and Laser Irradiation with Near-Infrared Light

This chapter describes the thorough experimental and analysis of LV-sIPN and IR788 sIPN nanoparticle as uptake material for dinoflagellate marine microalgal species which are specially well known for producing red-tide on marine water, which mainly focused on the uptake of fluorescence dye based nanoparticle i.e. LV-sIPN NP by two red-tide species i.e. *Akashiwo sanguinea* and *Alexandrium pacificum* effect of IR788 sIPN NP on a specified red-tide species i.e. *Akashiwo sanguinea* when irradiation was exposed with NIR-808 on IR788-sIPN NP uptake cells with various sets of irradiation parameters and time.

1. Methodology

1.1. Materials

All materials used in this chapter were the same with materials used for earlier sets of experiments done in chapter II.

1.2. Instruments

Instruments used in this chapter for nanoparticles preparation and algal cell counting were the same with instruments used for earlier sets of experiments done in chapter II. Irradiation was carried out using 808 nm Laser (Changchun New Industries, Korea) with the sample put inside a 1-mL microcentrifuge tubes. Laser power were adjusted using a radiometer (R2000 from Omnicure, Canada). Irradiation applying times were counted using stop watch.

2. Experimental

2.1. Settling Assay of Nanoparticles Uptake by Dinoflagellate Species

Select dinoflagellate marine red-tide species i.e. *Akashiwo sanguinea, Alexandrium pacificum* bloom containing culture media and open part of media was sterilizing with spirit-lamp flame also make sure zero contamination of species containing media. Each 1 ml of sample from red-tide species bloom containing culture media transfer to 1.5 ml tube then 10μL of Lugol (red color) was add with each 1 ml sample. Three (3) Sedgwick Rafter chamber (SR chamber) were prepared and place each Lugol solution mix 1 ml sample to SR chamber. Then 5 line from each SR

chamber was counted under light microscopy (10x). Right after counting 8 ml of solution containing red-tide species and sea water together (depend on cells initial counting) (2500 cells / ml) were added to each 6 well plate. 2µL of nanoparticle (LV-sIPN) containing each 3 plates of 6 well-plate marked as experimental plate and other 3 plate without nanoparticles considered as control plate. After settling well plates, at time 0 hour 1ml cells sample from each experimental and control plates placed in SR chamber and glutaraldehyde was used to fixed algae cells for cell counting at 0 hour. Then the 6-well plate was set to dark for 24 h after cell counting at 0 hour. At time 24 hours same method was followed for cell counting as like 0-hour cell counting. For uptake confirmation of nanoparticles, cells were observed under fluorescence light while number of alive cells were counted under light microscopy.

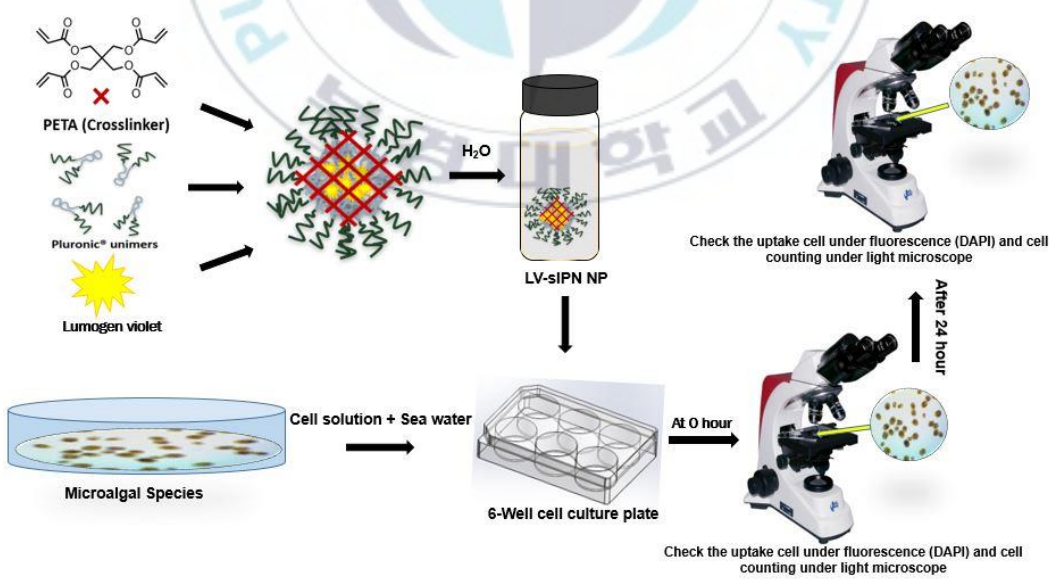


Figure 9. General scheme of nanoparticles uptake experiment

2.2. Laser Irradiation Under Near-Infrared Light

6-Well plate set up after initial counting and counting within time all were the same with the methods used for earlier sets of experiments done in sub-chapter 2.1. under chapter III. Both LV-sIPN and IR788-sIPN nanoparticles were selected as NP material and only one kind of dinoflagellate marine microalgal species i.e. *Akashiwo sanguinea* was used as algae cell sample. Irradiation experiment was conducted applying Laser source/machine (PSU-H-LED) (808 nm wavelength) on algae (*Akashiwo sanguinea*) cell.

2.2.1. Laser Power Variation

Different Laser power were performed for the treatment of nanoparticles uptake algae cells with fixed solution. LV-sIPN and 3% IR788 sIPN nanoparticle (2μL NP in 8ml algae solution) were used while the laser power was 1.5 W/cm^2, again only IR788 sIPN nanoparticle (2μL NP in 8ml algae solution) was applied for the treatment of nanoparticles uptake algae cells while varying the laser power i.e. 1.0 and 0.5 W/cm^2 as because our future goal is to expose nanoparticle uptake algae cell (*Akashiwo sanguinea*) under sunlight for natural irradiation. 1.5 W/cm^2 power is too intense, so 1.0 and 0.5 W/cm^2 power are two better option for comparison if in future we expose nanoparticle uptake algae cell (*Akashiwo sanguinea*) under sunlight.

2.2.2. Irradiation Time Variation

LV-sIPN and 3% IR788-sIPN nanoparticle uptake algae samples were irradiated using an 808 nm laser when laser power was 1.5 W/cm^2 and again only IR788 sIPN nanoparticle uptake algae samples were irradiated using an 808 nm laser with 1.0 and 0.5 W/cm^2 laser power. Irradiation was done inside a makeshift cold chamber, and irradiation times were set depends on laser power i.e. for 1.5 W/cm^2 irradiation times were 15, 20, 30 minute, for both 1.0 W/cm^2 and 0.5 W/cm^2 laser power irradiation times were 10, 30, 45, 60 minute.

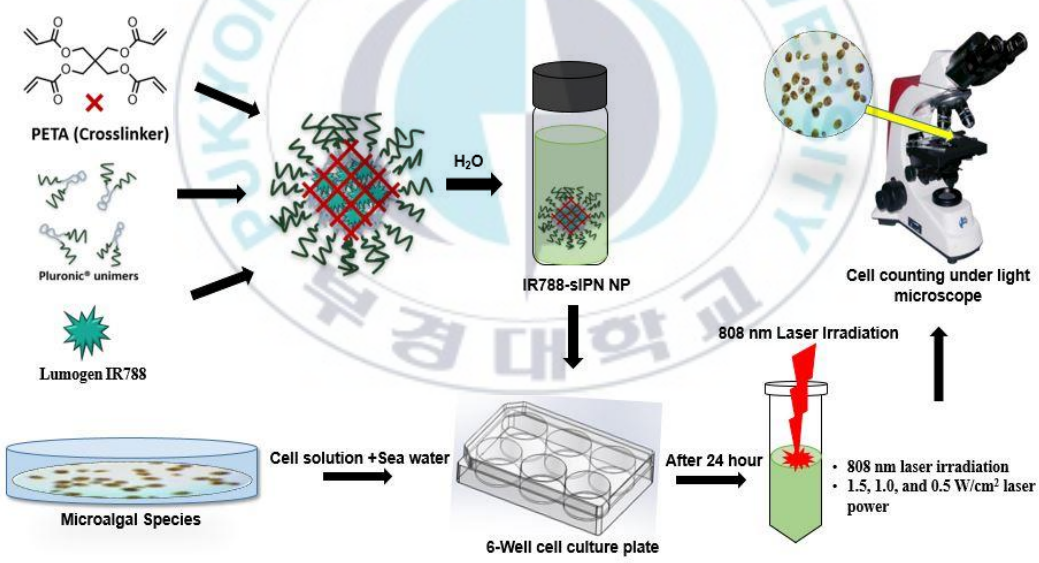


Figure 10. General scheme of laser irradiation experiment

3. Results and Discussion

3.1. Nanoparticles Uptake by Dinoflagellate Species

Uptake of Lumogen violet-sIPN nanoparticle by i.e. *Akashiwo sanguinea* and *Alexandrium pacificum* were observed with light microscope under fluorescence condition after kept LV-sIPN NP (2µL for each 8 ml solution) containing all algal solution i.e. experimental plate under 24-hour light-dark condition. Control plate cells were used as representative of live cell condition after 24-hour light–dark condition. As three (3) Sedgwick Rafter chamber (SR chamber) were prepared and then 5 line from each SR chamber was counted under light microscopy (10x), the absolute cell number for each SR chamber was range from 200 to 400 cells per slide.

3.1.1. Lumogen Violet-sIPN Nanoparticle Uptake of *Akashiwo sanguinea*

Uptake of LV-sIPN nanoparticle by *Akashiwo sanguinea* was observed with light microscope under fluorescence, UV excitation (wavelength) condition (40x magnification). LV-sIPN NP was uptake by almost 95% *Akashiwo sanguinea* cell. Algae cell number also decreased after 24h period of time compares to cell number at 0 hour and the number was counted using light microscope under optical light condition (10x magnification). **Figure 11** Shows *Akashiwo sanguinea* image on different conditions. *Akashiwo sanguinea* cell number decreased and at 0-hour total

0% LV-sIPN NP was on uptake by the algae (*Akashiwo sanguinea*) cell while after 24-hour 95% *Akashiwo sanguinea* cell uptake LV-sIPN NP. **Figure 12** representing the mean cell number of *Akashiwo sanguinea* during treated with LV-sIPN nanoparticle at 0-hour and 24-hour under light-dark condition.

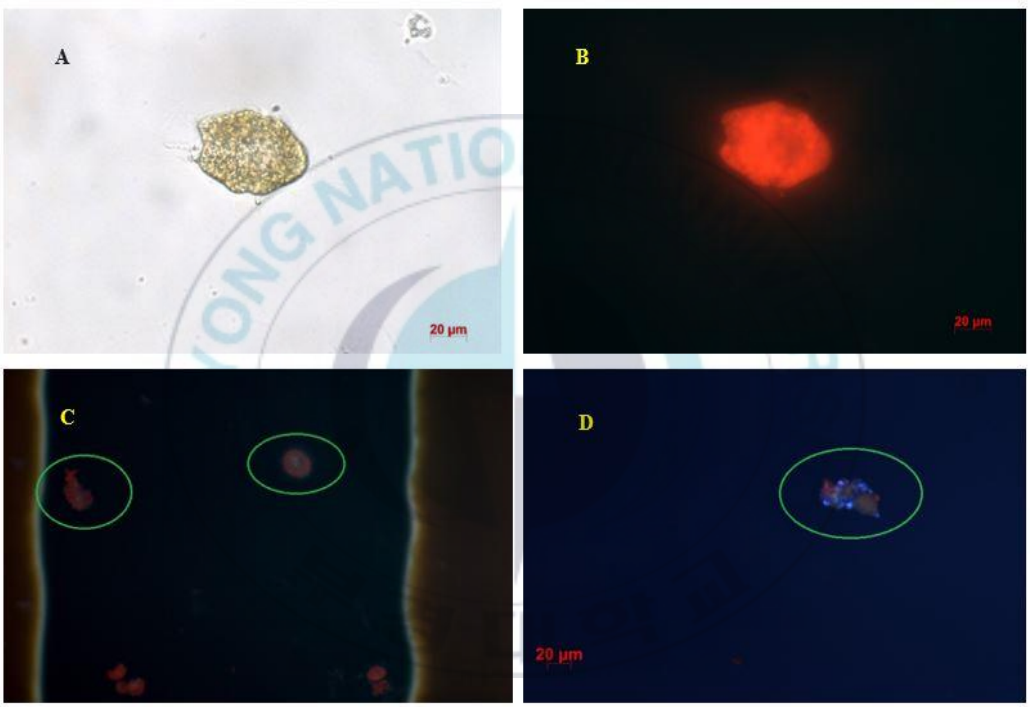


Figure 11. *Akashiwo sanguinea* image on different conditions, (A) normal cell under optical condition, (B) normal cell under fluorescence condition, (C) fluorescence image of uptake cell and (D) fluorescence image after cell blast

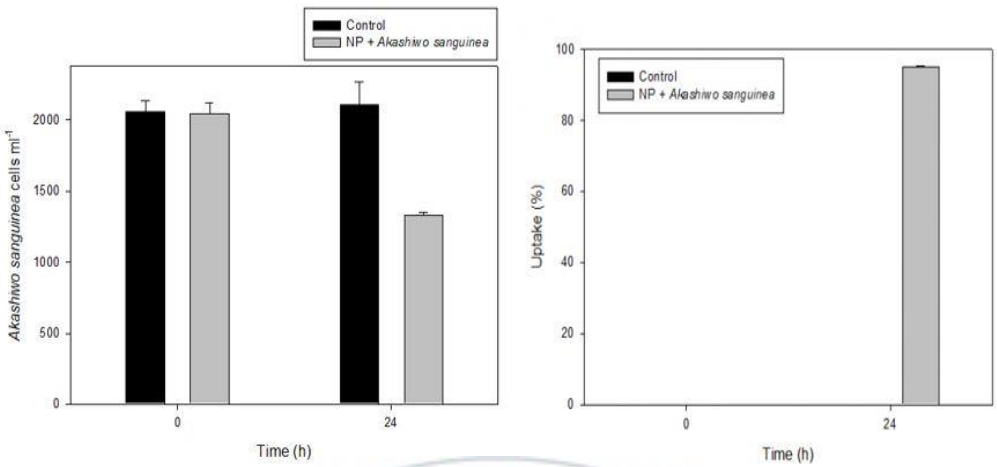


Figure 12. Mean cell number of *Akashiwo sanguinea* against time and nanoparticle uptake percentage (%) against time

3.1.2. Lumogen Violet-sIPN Nanoparticle Uptake of *Alexandrium pacificum*

Uptake of LV-sIPN nanoparticle by *Alexandrium pacificum* was observed under the same condition used for earlier sets of method done in sub chapter 3.1.1 with *Akashiwo sanguinea*. Where 7% of *Alexandrium pacificum* cell uptake nanoparticle though uptake percentage was lower but algae cell number decreased after 24h period of time compares to cell number at 0 hour. **Figure 13** Shows *Alexandrium pacificum* image on different conditions. *Alexandrium pacificum* cell number decrease and at 0-hour total 0% LV-sIPN NP was uptake by algae (*Alexandrium pacificum*) cell while after 24-hour 7% *Alexandrium pacificum* cell uptake LV-sIPN

NP. **Figure 14** representing the mean cell number of *Alexandrium pacificum* during treated with LV-sIPN nanoparticle at 0-hour and 24-hour under light-dark condition.

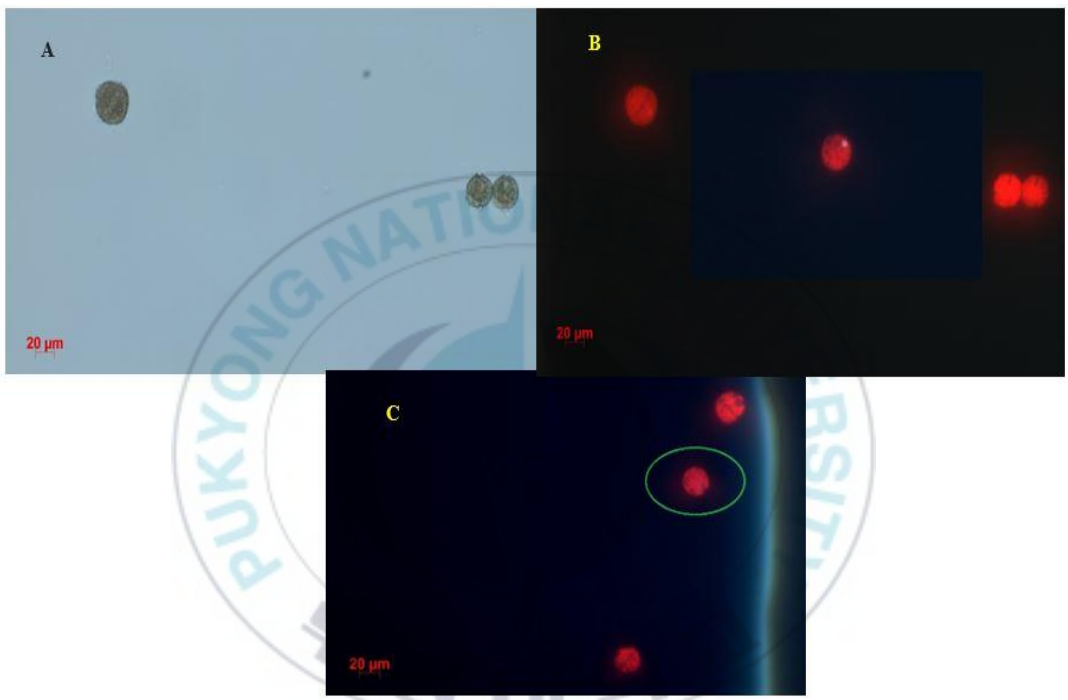


Figure 13. *Alexandrium pacificum* image on different conditions, (A) normal cell under optical condition, (B) normal cell under fluorescence condition and (C) fluorescence image after NP uptake by cell

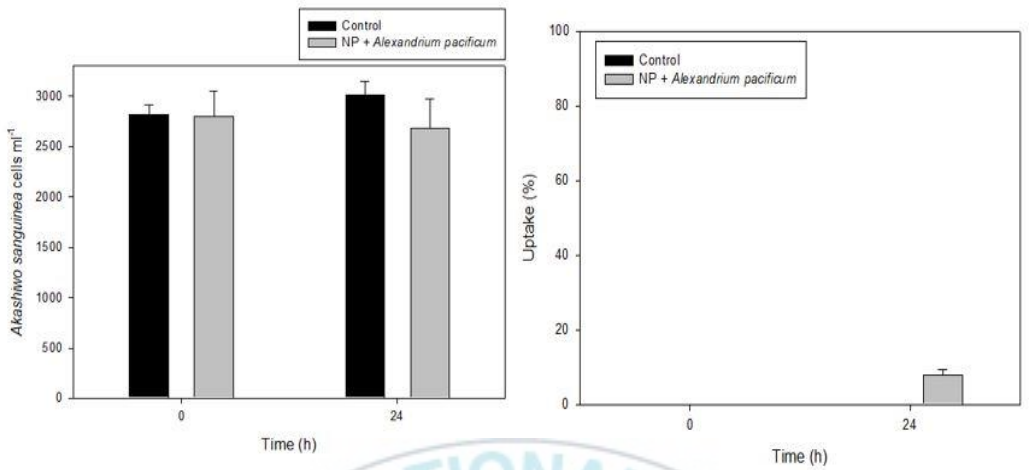


Figure 14. Mean cell number of *Alexandrium pacificum* against time and nanoparticle uptake percentage (%) against time

3.1.3. Nanoparticle Uptake Comparison of Dinoflagellate Species *Akashiwo sanguinea* and *Alexandrium pacificum*

The changing in cell number for *Akashiwo sanguinea* and *Alexandrium pacificum* and cell total NPs uptake percentage were showed in **Figure 15**. Control plates cell number increased but LV-sIPN nanoparticle contain plates showed decreased in number with time **Figure 15** and *Akashiwo sanguinea* experimental plate have higher cell decrease compare to *Alexandrium pacificum* experimental plates. Under fluorescence *Akashiwo sanguinea* Showed higher uptake ability 95.06% whereas *Alexandrium pacificum* cells have lower NP (LV-sIPN NP) uptake 7.93% due to ornamented theca on cell outer layer.

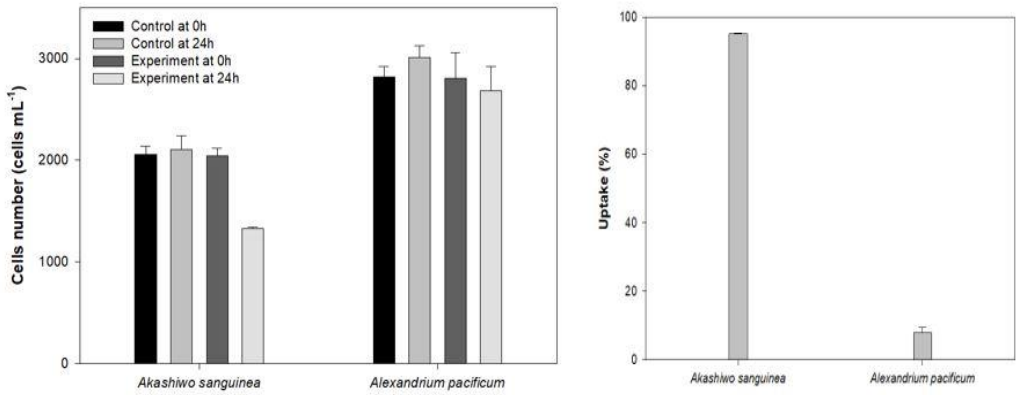


Figure 15. Changes of cell number against time and nanoparticle uptake comparison of dinoflagellate species *Akashiwo sanguinea* and *Alexandrium pacificum*

From **Table 3** the observed results showed that *Akashiwo sanguinea* experimental plate have higher cell decrease compare to *Alexandrium pacificum*. After 24-hour with NP under light-dark condition 64% *Akashiwo sanguinea* cells were alive while *Alexandrium pacificum* alive cells were 94%, whereas uptake ability for both sample species were 95.06% and 7.93% respectively.

Table 3. Changes in cell number against time and nanoparticle uptake percentage of athecate dinoflagellate (*Akashiwo sanguinea)* and thecate dinoflagellate (*Alexandrium pacificum*)

Microalgae	**Number of alive cell (%)**		**NP uptake (%) by algae (at 24 hour of light-dark)**
	0 hour	**24 hour**	
Akashiwo sanguinea	100%	64%	95.06%
Alexandrium pacificum	100%	94%	7.93%

Cell uptake experiments on various (2 species) red-tide microalgae were carried out with Lumogen violet-sIPN nanoparticle to investigate whether cell uptake the nanoparticle or not. LV-sIPN was used as an ideal component for uptake experiment due to easy detection under fluorescence light. The main goal of this experiment was to confirm the uptake of nanoparticle by red-tide algal cell and investigate which red-tide algae have higher nanoparticle(NP) uptake ability, so that further experiment (laser treatment) can be carry-out. Why we check NP uptake of red-tide algae because, if the cell uptake LV-sIPN (NP) then that surely will happen with other type of NP i.e. IR788-sIPN (Infra-red dye loaded sIPN or NP). According to this experiment results 2 types red-tide microalgal species NP uptake ability was 95.06% and 7.93% for *Akashiwo sanguinea* and *Alexandrium pacificum* respectively. We select *Akashiwo sanguinea* for further laser treatment investigation due to higher NP uptake ability.

3.2. Irradiation Effect of Near-Infrared Laser Light on Dinoflagellate Species

3.2.1. Irradiation of Nanoparticles Uptake Algae Under 1.5 W/cm^2 Power at Different Irradiation Time

After finding out that *Akashiwo sanguinea* has better NP uptake ability, LV-sIPN and IR788-sIPN NPs were applied on *Akashiwo sanguinea* and exposed for 15 min, 20 min, 30 min laser (Infrared-808) irradiation under 1.5 W/cm^2 laser power. In all cases, algal cell number decreased with increasing laser light exposed time. **Figure 16** showed growth rate of nanoparticle uptake and control (without NP) Akashiwo *sanguinea* cell and effect of laser irradiation on *Akashiwo sanguinea* including cell decrease percentage at different irradiation time. **Table 4** showed decrease percentage at different irradiation time. Cell number decreased with increasing irradiation time up to 30 min under same cell concentration. Significant differences in algal cell numbers were observed for irradiation conditions and times with different NPs uptake cell.

The highest number of cell decreased for control plate (without NP) was 7%, spotted with 30 min of irradiation time while for 15 and 20 min irradiation decrease percentage were only 3% and 4% respectively. Irradiation had no significant effect on control plate cell due to NPs absence. NPs have toxicity and control cells were free from NP uptake, so compare to NPs uptake cell control plate cells were enough potential to survive under infrared laser.

When 1.5 W/cm² laser irradiation exposed to LV-sIPN nanoparticle uptake Akashiwo *sanguinea* the highest number of cell decreased was 14%, spotted with 30 min of irradiation time while for 15 and 20 min irradiation decrease percentage were 7% and 9% respectively. LV-sIPN NP may had toxicity, so after NPs accumulation and due to light-dark condition (24-hour) algal cells were infirm against infrared laser light.

IR788-sIPN NPs showed highest number of cell decreased compare to both control and LV-sIPN nanoparticle which was 32%, spotted with 30 min of irradiation time, even for 15 and 20 min irradiation decrease percentage were also higher i.e. 16% and 22% respectively compare to both control and LV-sIPN nanoparticle. IR788 dye is an infrared dye which generate heat when it exposes to light also IR788-sIPN NP may had toxic effect on cell, after NP uptake and due to light-dark condition algal cells were infirm and when cell exposed to infrared (808nm) laser light IR788 increased heat inside and on the outer layer of the cell resulted cell blast.

Compare to LV-sIPN nanoparticle, IR788-sIPN nanoparticle uptake algae cell blast occurred with increasing laser irradiation exposed time. Nanoparticles toxicity difference correlates with the particle size, structure and NP dispersion preparation method. IR788 is a near infrared (NIR) dye with heat generation ability in the presence of light. Significant differences in algal growth inhibition were observed, regardless of irradiation exposed time conditions.

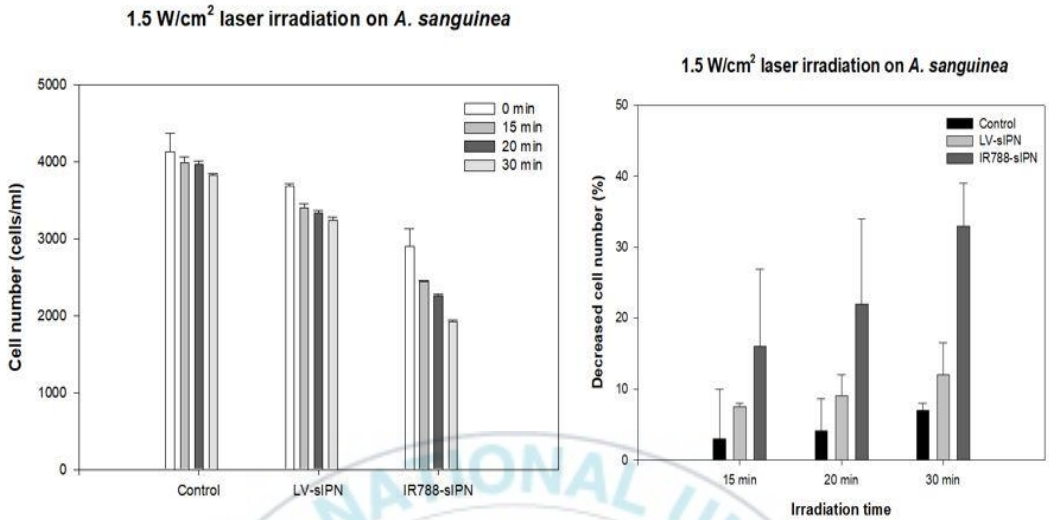


Figure 16. Effect of laser irradiation (1.5 W/cm²) on *Akashiwo sanguinea* and cell decrease percentage at different irradiation time

Table 4. Akashiwo *sanguinea* cell decrease percentage after laser irradiation (1.5 W/cm²) at different irradiation time

Experiment	Irradiation Time	Cell decrease percentage
Control (Without NP)	15min	3%
	20min	4%
	30min	7%
LV-sIPN NP	15min	7%
	20min	9%
	30min	12%
IR788-sIPN NP	15 min	16%
	20min	22%
	30min	33%

3.2.2. Irradiation of IR788-sIPN Nanoparticle Uptake Algae Under 1.0 W/cm^2 and 0.5 W/cm^2 Power at Different Irradiation Time

After finding out, compare to LV-sIPN nanoparticle IR788-sIPN nanoparticle had effectively higher impact on *Akashiwo sanguinea* cell at different laser irradiation time, so IR788-sIPN NP was applied on *Akashiwo sanguinea* for further findings. IR788-sIPN NP uptake *Akashiwo sanguinea* cells were exposed for 10 min, 30 min, 45 min, 60 min laser (Infrared-808) irradiation under 1.0 W/cm^2 and 0.5 W/cm^2 laser power separately. In all cases, algal cell number decreased with increasing laser light exposed time gradually i.e. 0 min > 10 min > 30 min > 45 min > 60

min, afterwards for both IR788-sIPN nanoparticle uptake and control (without nanoparticle) Akashiwo *sanguinea* cell under both 1.0 W/cm^2 and 0.5 W/cm^2 laser power. **Figure 17** showed the effect of laser irradiation under 1.0 W/cm^2 power on both nanoparticle uptake and control (without NP) Akashiwo *sanguinea* cell including cell decrease percentage at different irradiation time. **Table 5** showed decrease percentage at different irradiation time.

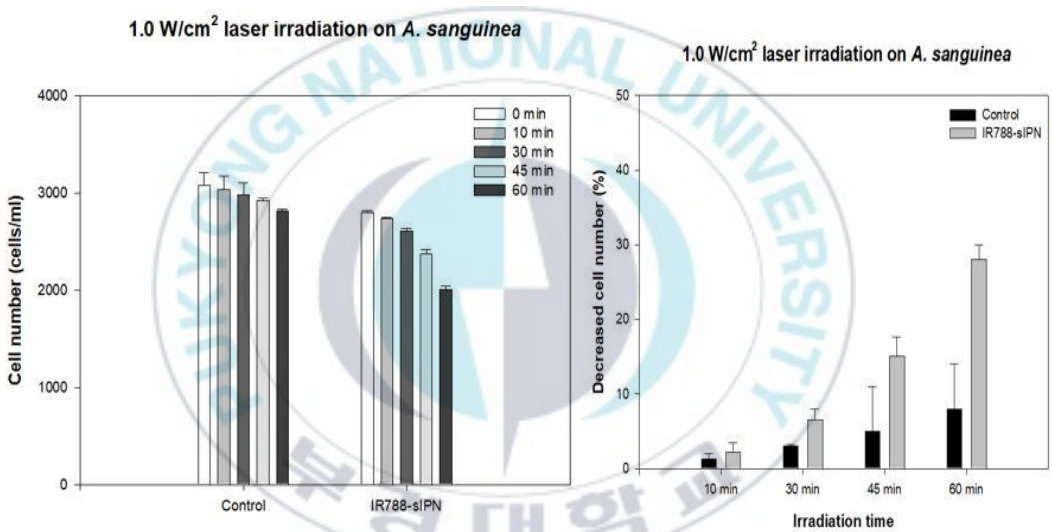


Figure 17. Effect of laser irradiation (1.0 W/cm^2) on *Akashiwo sanguinea* and cell decrease percentage at different irradiation time

The highest number of cell decreased for control plate (without NP) was 8%, spotted with 60 min (1 hour) of irradiation time while for 10, 30 and 60 min irradiation decrease percentage were only 1%, 3% and 5% respectively. Irradiation had no significant effect on control plate cell due

to NPs absence. NPs have toxicity and control cells were free from NP uptake, so compare to NPs uptake cell control plate cells were enough potential to survive under infrared laser.

IR788-sIPN NPs showed highest number of cell decreased compare to control which was 28%, spotted with 60 min (1 hour) of irradiation time, even for 45 min cell decrease was 15% which was also higher then control plate, afterwards even for 10 and 30 min irradiation decrease percentage were also higher i.e. 2% and 6% respectively compare to control. IR788 dye is an infrared dye which generate heat when it exposes to light also IR788-sIPN NP may had toxic effect on cell, after NP uptake and due to light-dark condition algal cells were infirm and when cell exposed to infrared (808nm) laser light IR788 increased heat inside and on the outer layer of the cell resulted cell blast.

Table 5. Akashiwo *sanguinea* cell decrease percentage after laser irradiation (1.0 W/cm²) at different irradiation time

Experiment	Irradiation Time	Cell decrease percentage
Control (Without NP)	10 min	1%
	30min	3%
	45 min	5%
	60min	8%
IR788-sIPN NP	10 min	2%
	30min	6%
	45 min	15%
	60min	28%

Figure 18 showed the effect of laser irradiation under 0.5 W/cm^2 power on both nanoparticle uptake and control (without NP) Akashiwo *sanguinea* cell including cell decrease percentage at different irradiation time. **Table 6** showed decrease percentage at different irradiation time.

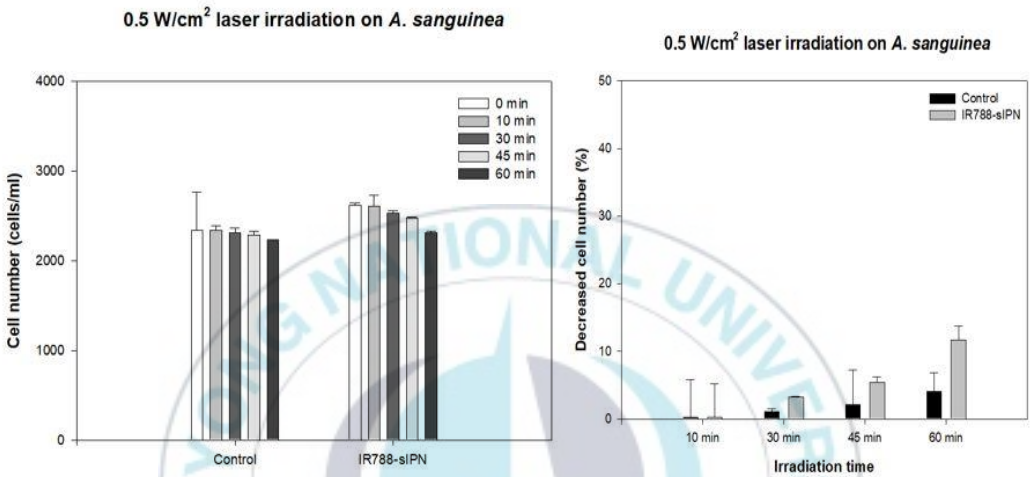


Figure 18. Effect of laser irradiation (0.5 W/cm²) on *Akashiwo sanguinea* and cell decrease percentage at different irradiation time

The highest number of cell decreased for control plate (without NP) was 4%, spotted with 60 min (1 hour) of irradiation time while for 10, 30 and 60 min irradiation decrease percentage were only 0.2%, 1% and 2% respectively. Irradiation had no significant effect on control plate cell due to NPs absence. NPs have toxicity and control cells were free from NP uptake, so compare to NPs uptake cell control plate cells were enough potential to survive under infrared laser.

IR788-sIPN NPs showed highest number of cell decreased compare to control which was 11%, spotted with 60 min (1 hour) of irradiation time, even for 45 min cell decrease was 5% which was also higher then control plate, afterwards even for 10 and 30 min irradiation decrease percentage were also higher i.e. 0.3% and 3% respectively compare to control. IR788 dye is an infrared dye which generate heat when it exposes to light also IR788-sIPN NP may had toxic effect on cell, after NP uptake and due to light-dark condition algal cells were infirm and when cell exposed to infrared (808nm) laser light IR788 increased heat inside and on the outer layer of the cell resulted cell blast.

Table 6. Akashiwo *sanguinea* cell decrease percentage after laser irradiation (0.5 W/cm²) at different irradiation time

Experiment	Irradiation Time	Cell decrease percentage
Control (Without NP)	10 min	0.2%
	30min	1%
	45 min	2%
	60min	4%
IR788-sIPN NP	10 min	0.3%
	30min	3%
	45 min	5%
	60min	11%

Now on the basis of above concepts, the effects of IR788 dye loaded nanoparticles have higher effect on microalgae cells when laser irradiation apply after nanoparticle uptake, as because IR788 dye from IR788-sIPN nanoparticle is an infrared dye which generate heat when it exposes to light. As most of the *Akashiwo sanguinea* cells uptake nanoparticles inside so when IR788-sIPN nanoparticle uptake cell expose to laser irradiation after a certain time of irradiation IR788 increased heat inside and outer layer of the cell resulted cell blast which already showed under **chapter III** in **Figure 16, 17,18** and **Table 4 5 6** with cell decrease percentage**.** According to **Figure 16, 17,18** and **Table 4 5 6,** in all cases algal cell number decreased with increasing laser light exposed time gradually even with lower laser power when irradiation times were higher cell decreases were higher.

Ⅳ. Conclusions

Lumogen violet and IR788 sIPN nanoparticles were, core-stabilized polymeric nanoparticle loaded with quarterrylene dyes, were successfully prepared via self-assembly followed by sIPN formation. It has improved structural integrity and stability as shown by CMT test. Herein, we assessed the uptake ability and effect of LV-sIPN and IR788-sIPN NPs on dinoflagellate marine microalgae *Akashiwo sanguinea* and *Alexandrium pacificum*. Nanoparticles uptake by *Akashiwo sanguinea* was found to be higher compare to *Alexandrium pacificum*, confirmed by fluorescence (DAPI) image after 24-hour light–dark condition.

IR788-sIPN NPs is photoreactive substances that evidence UV light absorption properties in NPs accumulated cells, so UV light absorption was conducted with both IR788-sIPN and LV-sIPN NPs uptake algae cells. LV-sIPN and IR788-sIPN NPs were applied on *Akashiwo sanguinea* and exposed for 15 min, 20 min, 30 min laser (Infrared-808) irradiation under 1.5 W/cm^2 laser power. In all cases, algal cell number decreased with increasing laser light exposed time with no significant differences in results among LV-sIPN and control conditions. Compare to LV-sIPN nanoparticle IR788-sIPN nanoparticle had effectively higher impact on *Akashiwo sanguinea* cell at different laser irradiation time, later on this, IR788-sIPN NP uptake *Akashiwo sanguinea* cells were exposed for 10 min,

30 min, 45 min, 60 min laser (Infrared-808) irradiation under 1.0 W/cm^2 and 0.5 W/cm^2 laser power separately. In all cases, algal cell number decreased with increasing laser light exposed time gradually. IR788 dye is an infrared dye which generate heat when it exposes light or laser light, due to this IR788 dye from IR788-sIPN nanoparticle increased heat inside and on the outer layer of the cell resulted cell blast. We observed evidence of increased algal toxicity of IR788-sIPN nanoparticles under laser light irradiation. In addition, we observed that algal toxicity could be solely due to the IR788 dye from IR788-sIPN nanoparticle. IR788-sIPN nanoparticle can be used for red tide algal elimination further.

As a new kind of material, nanomaterials have been widely used in various fields even though there exists a certain degree of threat to the safety of aquatic organisms. Studies on the toxicity of NPs on algae can not only explore the toxic mechanism of NPs on algae, but also provide a theoretical basis for the safety assessment of biological toxicity of nanomaterials. However, the exposure dose of NPs in the natural environment is usually low, and exposure time can be much longer It is not clear how the environmental factors would influence the transmission of NPs in the food chain. Therefore, it is necessary to establish a more comprehensive aquatic biological system, including primary producers and consumers at different trophic levels, to investigate the transmission and biological effects of NPs along the food chain. For this purpose, great efforts have been made in this area and more work will be conducted in future.

Ⅴ. References

1. Rotello V.M. Nanoparticles: Building Blocks for *Nanotechnology (1st Ed.), Springer, New York* (2003).
2. Yon Ju-Nam *et al.* Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications. *Science of The Total Environment,* Volume **400**,1–3,396-414 (2008).
3. Marambio-Jones, C., Hoek, E.M.V. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J Nanopart Res **12,*** 1531–1551 (2010).
4. Anastasia Georgantzopoulou *et al.* Ag nanoparticles: size- and surface-dependent effects on model aquatic organisms and uptake evaluation with NanoSIMS. *Nanotoxicology*, 7:7, 1168-1178 (2012).
5. Rudramurthy, G.R., Swamy, M.K. Potential applications of engineered nanoparticles in medicine and biology: an update. *J Biol Inorg Chem* **23,** 1185–1204 (2018).
6. Kabanov, A. V, Nazarova, I. R., Astafieva, I. V, Batrakova, E. V, Alakhov, V. Y., Yaroslavov, A, & Kabanov, V. Micelle Formation and Solubilization of Fluorescence Probes in Solution. 1995. *Macromolecules*, *28*, 2303–2314.
7. Torchilin, V. P. Micellar Nanocarriers: Pharmaceutical

Perspectives. 2006. *Pharmaceutical Research*, *24*(1), 1–16.

8. Arranja, A., Schroder, A., Schmutz, M., Waton, G., Schosseler, F., & Mendes, E. Cytotoxicity and Internalization of Pluronic Micelles Stabilized by Core Cross-linking. 2014. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, ***196***, 87–95.
9. Wei, Z., Hao, J., Yuan, S., Li, Y., Juan, W., Sha, X., & Fang, X. Paclitaxel-loaded Pluronic P123/F127 mixed polymeric micelles: Formulation, optimization and *in vitro* characterization. 2009. *International Journal of Pharmaceutics*, 376(1-2), 176–185.
10. Oh, K. T., Bronich, T. K., & Kabanov, A. V. Micellar formulations for drug delivery based on mixtures of hydrophobic and hydrophilic Pluronic® block copolymers. 2004. *Journal of Controlled Release*, *94*(2-3), 411–422.
11. Rösler, a, Vandermeulen, G. W., & Klok, H. a. Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. 2001. *Advanced Drug Delivery Reviews*, *53*(1), 95–108
12. Panyam, J., & Labhasetwar, V. Dynamics of endocytosis and exocytosis of poly(D,L-lactide-co-glycolide) nanoparticles in vascular smooth muscle cells. 2003. *Pharmaceutical Research*, *20*(2), 212–220.
13. Nguyen, D. H., Bae, J. W., Choi, J. H., Lee, J. S., & Park, K. D. Bioreducible cross-linked Pluronic micelles: pH-triggered release of doxorubicin and folate-mediated cellular uptake. 2013. *Journal of*

Bioactive and Compatible Polymers, *28*(4), 341–354.

14. Yang, L., & Alexandridis, P. Physicochemical aspects of drugdelivery and release from polymer-based colloids. 2000 *Current Opinion in Colloid and Interface Science*, *5*(1-2), 132–143.
15. Choi, K. Y., Liu, G., Lee, S., & Chen, X. Theranostic nanoplatforms for simultaneous cancer imaging and therapy: current approaches and future perspectives. 2012. *Nanoscale*, *4*(2), 330–342.
16. Kataoka, K., Harada, a, & Nagasaki, Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. 2001. *Advanced Drug Delivery Reviews*, ***47*(1)**, 113–131
17. Kwon, G. S., Naito, M., Kataoka, K., Yokoyama, M., Sakurai, Y., Okano, T. Block copolymer micelles as vehicles for hydrophobic drugs. 1994. *Colloids and Surfaces B: Biointerfaces*, *2*(4), 429–434
18. Ferrari, M. Nanovector therapeutics. 2005. *Current Opinion in Chemical Biology*, *9*, 343–346.
19. Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R., & Langer, R. Nanocarriers as an emerging platform for cancer therapy. 2007. *Nature Nanotechnology*, 2(12), 751–760.
20. Ryu, J. H., Lee, S., Son, S., Kim, S. H., Leary, J. F., Choi, K., Kwon, I. C. Theranostic nanoparticles for future personalized medicine. 2014. *Journal of Controlled Release*, **190**, 477–484.
21. Ishida, O., Maruyama, K., Sasaki, K., & Iwatsuru, M. (1999). Size-dependent extravasation and interstitial localization of

polyethyleneglycol liposomes in solid tumor-bearing mice. *International Journal of Pharmaceutics*, *190*(1), 49–56.

22. Micelles by Polymerization ofN-Vinyl-2-pyrrolidone. 2002. *Macromolecular Rapid Communications*, *23*(1), 26–31.
23. Abdullah-Al-Nahain, Lee, H., Lee, Y. S., Lee, K. D., & Park, S.Y. Development of Disulfide Core-Crosslinked Pluronic Nanoparticles as an Effective Anticancer-Drug-Delivery System. 2011. *Macromolecular Bioscience*, *11*, 1264–1271.
24. Pruitt, J. D., Husseini, G., Rapoport, N., & Pitt, W. G. Stabilization of Pluronic P-105 Micelles with an Interpenetrating Network of N, N -Diethylacrylamide. 2000. *Macromolecules*, *33*(25), 9306–9309.
25. Petrov, P., Bozukov, M. & Tsvetanov, C. B. Innovative approach for stabilizing poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) micelles by forming nano-sized networks in the micelle. *Journal of Materials Chemistry* **15**, 1481–1486 (2005).
26. Avlasevich, Y. & Müllen, K. An Efficient Synthesis of Quaterrylenedicarboximide NIR Dyes. *The Journal of Organic Chemistry* **72**, 10243–10246 (2007).
27. Avlasevich, Y., Li, C. & Müllen, K. Synthesis and applications of core-enlarged perylene dyes. *Journal of Materials Chemistry* **20**, 3814 (2010).
28. Rapoport, N. Physical stimuli-responsive polymeric micelles for anti-cancer drug delivery. *Progress in Polymer Science* **32**, 962–990 (2007).

29. Guo, L., 2007. Ecology-doing battle with the green monster of Taihu Lake. Science 317, 1166.

30. Jin, X.C., Xu, Q.J., Huang, C.Z., 2005. Current status and future tendency of lake eutrophication in China. Sci. China Ser. C 48, 948–954.

31. Falconer, I., Bartram, J., Chorus, I., Goodman, T.K., Utkilen, H., Burch, M., Codd, G.A., 1999. Safe levels and safe practices. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences Monitoring and Management. WHO, London, pp. 161–182.

32. van Apeldoorn, M.E., van Egmond, H.P., Speijers, G.J.A., Bakker, G.J.I., 2007. Toxins of cyanobacteria. Mol. Nutr. Food Res. 51,7–60.

33. Zhang, H., Zhang, J., Zhu, Y., 2009. Identification of microcystins in waters used for daily life by people who live on Tai Lake during a serious cyanobacteria dominated bloom with risk analysis to human health. Environ. Toxicol. 24, 82–86.

34. Oliver, R.L., Ganf, G.G., 2000. Freshwater blooms. In: Whitton, B.A. (Ed.), The Ecology of Cyanobacteria – their Diversity in Time and Space. Kluwer Academic Publishers, The Netherlands, pp. 149–194.

35. Alexandridis, P., Nivaggioli, T. & Hatton, T. A. Temperature Effects on Structural Properties of Pluronic P104 and F108 PEO-PPO-PEO Block Copolymer Solutions. *Langmuir* **11**, 1468–1476 (1995).

36. Rapoport, N. Stabilization and activation of Pluronic micelles for

tumor-targeted drug delivery. *Colloids and Surfaces B: Biointerfaces* **16**, 93–111 (1999).

37. Kabanov, A. V., Batrakova, E. V. & Alakhov, V. Y. Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery. *Journal of Controlled Release* **82**, 189–212 (2002).

38. Nguyen, V. P., Kim, H., Kang, M., Kwak, M. & Kang, H. W. Application of organic IR788-loaded *semi*-interpenetrating network dyes for photoacoustic imaging. *Japanese Journal of Applied Physics* **56**, 07JF12 (2017).

39. Kabanov, A. V., Batrakova, E. V. & Alakhov, V. Y. Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery. Journal of Controlled Release 82, 189–212 (2002).

40. Stolnik, S., Illum, L. & Davis, S. S. Long circulating microparticulate drug carriers. *Advanced Drug Delivery Reviews* **64**, 290–301 (2012).

41. Doytcheva, M. *et al.* Ultraviolet-induced crosslinking of solid poly (ethylene oxide). *Journal of Applied Polymer Science* **64**, 2299–2307 (1997).

42. Chen, L., Li, C. & Müllen, K. Beyond perylene diimides: synthesis, assembly and function of higher rylene chromophores. *J. Mater. Chem. C* **2**, 1938–1956 (2014).

43. Weil, T., Vosch, T., Hofkens, J., Peneva, K. & Müllen, K. The Rylene Colorant Family-Tailored Nanoemitters for Photonics Research and Applications. *Angewandte Chemie International Edition* **49**, 9068–

9093 (2010).

44. Zhao, X., Xiong, Y., Ma, J. & Yuan, Z. Rylene and Rylene Diimides: Comparison of Theoretical and Experimental Results and Prediction for High-Rylene Derivatives. *The Journal of Physical Chemistry A* **120**, 7554–7560 (2016).

45. Nolde, F., Pisula, W., Müller, S., Kohl, C. & Müllen, K. Synthesis and Self-Organization of Core-Extended Perylene Tetracarboxdiimides with Branched Alkyl Substituents. *Chemistry of Materials* **18**, 3715–3725 (2006).

46. Pschirer, N. G., Kohl, C., Nolde, F., Qu, J. & Müllen, K. Pentarylene- and Hexarylenebis(dicarboximide)s: Near-Infrared-Absorbing Polyaromatic Dyes. *Angewandte Chemie International Edition* **45**, 1401–1404 (2006).

47. Yang, G. *et al.* A Single 808 nm Near-Infrared Light-Mediated Multiple Imaging and Photodynamic Therapy Based on Titania Coupled Upconversion Nanoparticles. *Chemistry of Materials* **27**, 7957–7968 (2015).

48. Guo, L.-X. *et al.* A calamitic mesogenic near-infrared absorbing croconaine dye/liquid crystalline elastomer composite. *Chemical Science* **7**, 4400–4406 (2016).

49. Liu, C. *et al.* A Water-Soluble, NIR-Absorbing Quaterrylenediimide Chromophore for Photoacoustic Imaging and Efficient Photothermal Cancer Therapy. *Angewandte Chemie International Edition* (2019) doi:10.1002/anie.201810541.

50. Chen, Z., Zhang, L., Sun, Y., Hu, J. & Wang, D. 980-nm Laser-Driven Photovoltaic Cells Based on Rare-Earth Up-Converting Phosphors for Biomedical Applications. *Advanced Functional Materials* **19**, 3815–3820 (2009).

51. Chen, W. R., Adams, R. L., Carubelli, R. & Nordquist, R. E. Laser-photosensitizer assisted immunotherapy: a novel modality for cancer treatment. *Cancer Letters* **115**, 25–30 (1997).

52. Chen, W. R., Adams, R. L., Higgins, A. K., Bartels, K. E. & Nordquist, R. E. Photothermal effects on murine mammary tumors using indocyanine green and an 808-nm diode laser: an in vivo efficacy study. *Cancer Letters* **98**, 169–173 (1996).

53. Savchuk, Ol. A., Carvajal, J. J., Massons, J., Aguiló, M. & Díaz, F. Determination of photothermal conversion efficiency of graphene and graphene oxide through an integrating sphere method. *Carbon* **103**, 134–141 (2016).

54. Agricultural and biological sciences (Red Tide-An overview). *Science Direct*. *Handbook of Toxicology of Chemical Warfare Agents (Second Edition)*, 2015.

55. Aruoja, V., Dubourguier, H.-C., Kasemets, K., Kahru, A., 2009. Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. Sci. Total Environ. **407**, 1461–1468.

56. Woo-Mi Lee, Youn-Joo An., Effects of zinc oxide and titanium dioxide nanoparticles on green algae under visible, UVA, and UVB

irradiations: No evidence of enhanced algal toxicity under UV pre-irradiation. *Chemosphere*, **91** (2013) 536–544.

57. Yi Tao, Xihui Zhang, Doris W.T. Au, Xianzhong Mao, Kan Yuan., The effects of sub-lethal UV-C irradiation on growth and cell integrity of cyanobacteria and green algae. *Chemosphere* **78** (2010) 541–547.

58. Introduction to Solar Energy. *Passive Solar Heating & Cooling Manual*. Rodale Press, Inc. 1980. Archived from the original (DOC) on 2009-03-18. Retrieved 2007-08-12

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