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석사학위 논문

A study on development of triphenylphosphonium modified mesoporous silica nanoparticle for enhanced algicidal efficacy of DP92

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DP92 의 살조 효과 증대를 위한 트리페닐포스포늄을 수식한 다공성 실리카 나노 입자 개발에 관한 연구

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다공성 실리카 나노입자는 약물, DNA, si-RNA, 펩타이드와 같은 다양한 물질의 전달체로서 연구되어 왔다. 다공성 실리카는 표면에 많은 구멍이 있기 때문에 표면적이 크고, 구멍의 크기 조절이 용이하여 표면적을 쉽게 조절할 수 있다. 특히, 다공성 실리카 나노입자의 구멍 내부는 소수성 성질이 강해 소수성 약물의 가용화에 적합한 특성을 갖고 있다. 또한, 다공성 실리카 나노입자 표면은 화학적으로 수식하기에 용이하다.

트리페닐포스포늄은 친유성 양이온이며 세포 내로 약물의 전달을 촉진시킨다. 트리페닐포스포늄의 양이온 성질은 음전하를 띤 세포 표면과 상호 작용할 수 있다. 또한 미토콘드리아와도 상호작용이 가능해 세포내부로의 이행이 용이하다. 따라서 우리는 트리페닐포스포늄이 수식된 다공성 실리카 나노입자를 개발한다면 조류 세포막과 트리페닐포스포늄이 수식된 다공성 실리카 나노입자간 전하 상호 작용 및 조류 내부로의 침투 촉진 효과를 통해 약물 전달 효율을 향상 시킬 수 있을 것으로 예상하였다.

매년 발생하는 적조현상은 해양 환경과 수산업에 심각한 문제를 야기한다. 적조현상을 관리하기 위해 화학 살조제, 점토 응집제, 바이러스 또는 적조의 천적 사용과 같은 여러 방법이 사용되어 왔다. 그중 화학적

살조제가 가장 널리 사용되는 방법이다. 적조현상 문제를 해결하기 위한 살조제로 시클로 헥실-(3,4- 디클로로 벤질) 아민 (DP92)을 새로이 합성하였다. 그러나, DP92 는 소수성 성질이 매우 강해 수상환경에 적용하기가 어렵다. 따라서 DP92 를 가용화하여 DP92 의 살조력을 개선하기 위해 DP92 를 트리페닐포스포늄이 수식된 다공성 실리카 나노입자에 봉입하였다

입자 직경, 제타 전위 및 봉입 효율과 같은 다공성 실리카 나노입자 및 트리페닐포스포늄이 수식된 다공성 실리카 나노입자의 물성평가를 진행하였다. 트리페닐포스포늄이 수식된 다공성 실리카 나노입자의 조류로의 이행을 평가하기 위해 다공성 실리카 나노입자와 트리페닐포스포늄이 수식된 다공성 실리카 나노입자를 쿠마린-6 로 표지하고 조류의 형광 흡광도 분석과 조류 주변의 형광 현미경 관찰을 진행하였다. 살조력을 평가하기 위해, 조류에 DP92 가 봉입된 다공성 실리카 나노입자와 트리페닐포스포늄이 수식된 다공성 실리카 나노입자를 가하고 살조력을 평가하였으며, IC_{50} 값으로 계산하여 살조력을 나타내었다. 마지막으로 DP92 의 조류내부로의 침투 효율을 측정하여, TPP-MSNP 군에서 살조효과 증가가 약물의 조류내부로의 침투 증가로 인한 것임을 확인하였다. 본 연구를 통해 경제적이며 상용화가 가능한 DP92 의 약물 전달 시스템을 개발하였으며, 다양한 난용성 약물에 응용이 가능하리라 생각된다.

2. Introduction

Mesoporous silica nanoparticle (MSNP) has been studied for delivery of various materials such as drugs [1], DNA, si-RNA [2], peptides nucleic acid [3]. MSNP is capable of loading large quantities of drug, due to its large surface area, large pore volume and adjustable pore [4,5]. MSNP can be safely used at the applicable concentration range in living organism [6]. MSNP surface is easy to chemically modify. MSNP is low cost and easy to manufacture. In addition, MSNP is possible to use for the drug delivery of hydrophobic drug, because MSNP pores has highly hydrophobic [7].

Triphenylphosphonium (TPP) is lipophilic cation and material having three phenyl groups. Since TPP has enough hydrophobicity, it can stimulate to penetrate cell membranes that compose of phospholipid bilayers. Furthermore, it is possible to transit to the mitochondria due to charge interaction between positive charged TPP and negative charged mitochondria. Therefore, TPP can be used to promote the transport of chemical materials into and mitochondria [8, 9, 10].

Harmful algal blooms (HABs) cause critical problems in marine environment and fishery industry [11]. To manage HABs, several methods have been used such as chemical algicides, clay flocculants, using virus or natural enemy [12,13]. Chemical algicides are the most widely used method. However, it is limited to use chemicals algicides to marine environment because most of chemical algicides cause toxicity not only to harmful algal blooms but also to the marine life. New chemical algicide, cyclohexyl-(3,4-dichlorobenzyl) amine (DP92), was developed. DP92 is environmentally safe compared to the used compounds, and has the characteristic of selectively algicidal effect. However, since DP92 is very hydrophobic, it is difficult for DP92 to directly use to aquatic environment. Therefore, solubilization of DP92 needs to enhance the algicidal efficacy of DP92 [14].

Various formulations have been studied for solubilization of DP92 including emulsions, liposomes, and polymeric micelles. Those formulations showed improved solubilization and enhanced algicidal effect. However, it is difficult for previously studied formulations to commercialize, because the phospholipids used that formulations are very expensive [14]. Therefore, we selected MSNP as new delivery system for DP92

because MSNPs has less costs than phospholipids used in other formulations (emulsions, liposomes and polymeric micelles) and has high drug loading capacity. Furthermore, we modify MSNP surface with TPP. TPP-MSNP can stimulate to transit DP92 into HABs effectively. Two feature of TPP-MSNP makes to deliver DP92 into HABs possible. DP92 can be applied to aquatic environment due to solubilization of MSNP. And MSNP modified with TPP can target HABs and permeate algal cell membrane due to TPP characteristic of hydrophobicity and positive charge [15]. Thus, We have designed new formulation that DP92 was entrapped by TPP-MSNP .

In this study, the properties of MSNP (as control) and TPP-MSNP such as particle diameter, zeta potential and encapsulation efficiency were evaluated. To evaluate of TPP-MSNP into algae, MSNP and TPP-MSNP labelled with coumarine-6 were used. Fluorescence absorbance analysis in algae and fluorescence microscope observation around algae were performed on *Heterosigma akasiwo* (H.A.) and *Heterocapsa Circularisquama* (H.C.) and the algicidal activity experiment of DP92 in TPP-MSNP and MSNP was carried out against H.C. and H.A. [14].

3. Materials and methods

3-1. Materials

Methanol, ACN was purchased sigma-aldrich. Coumarin-6 was purchased sigma-aldrich. Mesoporous silica and mesoporous silica modified by TPP were fabricated by Professor Ho Jung Kim. DP92 was synthesized by Professor Hoon Cho.

3-2. Synthesis of DP92

3,4-Dichlorobenzaldehyde (0.5 mg) and cyclohexylamine (282 mg) were mixed with stirring for 1 h at room temperature in methanol (30 ml). Sodium borohydride (161 mg) was added to the mixed solution. The mixture was then stirred at 25 ℃ until the starting materials identified with TLC analysis disappeared. The mixture was then extracted with methylene chloride and washed with water. The organic layer was dried over anhydride magnesium sulfate and evaporated to acquire cyclohexyl-(3,4-dichlorobenzyl)amine (DP92). 1H NMR (300 MHz, CDCl3) δ 7.44 (d, J = 1.8 Hz, 1H), δ 7.38 (d, J = 8.0 Hz, 1H), δ 7.17 (dd, J = 8.0 and 1.8 Hz, 1H), δ 3.76 (s, 2H), δ 2.47 (m, 1H), δ 1.91 (m, 10H).

3-3. Preparation of DP92-Loaded MSNP and TPP-MSNP

MSNP (1 mg) or TPP-MSNP (1 mg) were mixed with DP92 (15 mg) in 1 ml of methanol. The mixture was sonicated by bath type sonicator (Power sonic 420, Hwasin tech, Korea) for 4 h followed by centrifugation at 5000 rpm, for 5 min. And the supernatant was collected to analyze concentration of DP92 loaded MSNP or loaded TPP-MSNP The concentration of DP92-loaded MSNP and DP92 loaded TPP-MSNP were measured by UV spectrophotometer (TU-1800, Duksan tech, Korea). To remove unloaded DP92, the sediment was washed with methanol 3 times repeated. To eliminate methanol, water washing was accomplished 2 times.

3-4. Characterization of MSNP and TPP-MSNP

3-4-1. Transmission electron microscopy

Transmission electron microscopy (TEM) was performed to identify the morphology and diameter of MSNP and TPP-MSNP by JEOL-JEM 2010 instrument (JEOL, Japan). The MSNP or TPP-MSNP was diluted with distilled water. The solution was sampled on a copper grid coated with a perforated polymer film. The excess solution was drawn off with a filter paper. Uranyl acetate solution (2%, w/v) was dropped onto the copper grid to stain sample. The copper grid was left overnight to dry. The dried sample was investigated with the TEM instrument operating 120 kV [14].

3-4-2. Particle diameter and zeta potential analysis

The particle diameter and polydispersity index (PI) of MSNP or TPP-MSNP were measured by dynamic light scattering (DLS, Zetasizer ELSZ-2000, Japan). The zeta potential of MSNP or TPP-MSNP was measured by same instrument (DLS, Zetasizer ELSZ-2000, Japan). Before sample was measured, all samples were diluted 10-fold with distilled water.

3-4-3. Determination of encapsulation efficiency of DP92 loaded MSNP by UV analysis

The encapsulation efficiency of DP 92 was analyzed by UV lamp. The detection wavelength was fixed at 237 nm. The supernatant collected during the preparation process was used to analyze the encapsulation efficiency.

$$\text{EE (\%)} = \frac{\text{Amount of DP92 included in MSNP (mg)}}{\text{Amount of initial feeded DP92 (mg)}} \times 100$$

$$= \frac{\text{Initial applied to DP92 weight (mg) - Amount of DP92 in supernatant}}{\text{Initial applied to DP92 weight (mg)}} \times 100$$

3-5. Fluorescence microscopy

To observe migration into algae, fluorescence dye labelled TPP-MSNP and MSNP were prepared. The coumarin-6 labelled MSNPs were prepared in the following method. MSNP (1 mg) or TPP-MSNP (1 mg) was mixed with coumarin-6 (5 mg) in methanol (1 ml). The mixtures were stirred for 24h followed by centrifugation at 5000 rpm, for 5 min. Then, the sediment was washed by the washing method described in "Preparation of DP92 loaded MSNP and TPP-MSNP". H.A. or H.C. (180 μl) were transferred to 96 wells, mixed with coumarin-6 labelled MSNP, TPP-MSNP (20.00 μl) and incubated at algae culture condition for 24h. The incubated algaes were observed by a fluorescence microscope (Olympus IX 71, Olympus, Japan).

3-6. Fluorescence absorbance measurement

To evaluate transition into algae, coumarin-6 labeled MSNP or TPP-MSNP were used. Coumarin-6 labeled TPP-MSNP or MSNP were prepared in the same method described in "Fluorescence microscopy". Coumarin-6 labeled TPP-MSNP or MSNP (500 μl) was mixed with algae (4.5 ml) in conical tube. Coumarin-6-labeled TPP-MSNP, or MSNP applied algae was incubated in an incubator for 24 h. To evaluate penetration degree of algae, coumarin-6 inside algae was isolated with the following isolation method. The mixture was centrifuged at 800 g, for 5 min, and then supernatant was removed to separate algae from TPP-MSNP and MSNP labelled with coumarin-6 that did not transit into the algae. After adding the new culture medium, the mixture was

sonicated with probe sonicator (VCX 500, Sonics & Materials, INC, USA) for 5min to rupture of algae cell wall. The sonicated solution was centrifuged at 800 g, for 5 min. Then, fluorescence absorbance of supernatant was measured with microplate analysis system (Spectramax M3, Molecular devices, USA).

3-7. Algicidal activity of DP92-loaded MSNP or TPP-MSNP

To evaluate the algicidal activity of DP 92-loaded TPP-MSNP, MSNP and DP92 dissolved DMSO, H.A. and H.C. were used as algae models. The last concentration were adjusted to 1, 0.5, 0.4, 0.2, 0.1, 0.05 μM in H.A. and 2, 1, 0.5, 0.4, 0.2, 0.1 μM in H.C. To adjust this concentration, F/2 medium was used by dilution solution. At 96 wells, 180 μl of algae was taken. And 20 μl of DP92 loaded MSNP was given to 180 μl of algae. After Incubation of algae applicated DP92 loaded TPP-MSNP or MSNP for 24h, the number of alive algae was checked by optical microscopy with 400 magnification (Olympus, Tokyo. Japan)

$$\text{Algicidal activity (\%)} = \frac{\text{The number of alive algae (After incubation 24h)}}{\text{The number of alive algae (First point)}} \times 100$$

3-8. HPLC analysis for transition efficiency of DP92

The HPLC system consisted of pumps (P 6.1L), an autosampler (AS 6.1L), and a UV detector (DAD 2.1L) (Azura, Germany). A C18 column (Luna C18, 4.6 mm × 150 mm, 5 μm; Phenomenex, Torrance, CA, USA) was used. And columne was heated by column oven to 40 ℃. DP92 was eluted with a mobile phase consisting of 10 mM ammonium acetate/ACN (30:70, v/v%), and the detection wavelength was fixed at 237 nm. The flow rate was 1.0 ml/min. The injection volume was 10.00 μl.

3-9. Transition efficiency of DP92-loaded MSNP or TPP-MSNP

To evaluate transition efficiency into algae, DP92 loaded MSNP or TPP-MSNP was used. DP92 loaded MSNP or TPP-MSNP was mixed with HABs (H.A. or H.C.) in conical tube. The mixtures (10 ml) were adjusted to 1 μM and left for two hour for transition of DP92 into algae. Then, DP92 was isolated from the algae to use method described in “Fluorescence absorbance measurement”. The isolated sample was lyophilized for concentration. Lyophilized samples were dissolved in methanol (1 ml). Then, DP92 concentrations were measured with HPLC system (Azura, Germany).

4. Results and discussion

4-1 Characterization of MSNP and TPP-MSNP

The TEM image was taken to identify the morphology and particle diameter of MSNP and TPP-MSNP. Fig 2 and 3 were the morphology of MSNP and TPP-MSNP, subsequently. In the Fig 2 and 3, the shapes of both MSNP and TPP-MSNP were similarly spherical, and the particle diameters of them were around 100 nm. The similar shape of MSNP and TPP-MSNP suggested that the modification of TPP onto MSNP did not affect the shape of plain MSNP. The results of dynamic light scattering analysis were similar with the results of TEM image. As described Table 1, the particle diameters of MSNP or TPP-MSNP were 126.43 ± 9.46 nm and 104.27 ± 2.05 nm, subsequently. MSNP was slightly larger than TPP-MSNP. However, the particle diameters were similar not to affect other physical properties. PI values of MSNP or TPP-MSNP were 0.25 ± 0.01 and 0.20 ± 0.02. The result suggested that the fabricated MSNP or TPP-MSNP was homogeneous.

Due to cationic property of TPP, we expected for TPP-MSNP to induce positive surface charge. As expected, TPP-MSNP surface was positively charged at 25.41 ± 1.14 mV, while the MSNP was negatively charged at -31.67 ± 1.53 mV. The results suggested that TPP was well-modified on the MSNP surface and TPP-MSNP interacted with algae cell surface that was negatively charged.

DP92-loaded MSNP and TPP-MSNP was prepared at various feeding concentrations (10, 15, 20 mg) and the encapsulation efficiencies of them were measured. The purpose of these test were identifying the change of encapsulation efficiency as the feeding drug concentration was changed, and finding the concentration that showed the optimal encapsulation efficiency while feeding a small amount of DP92. DP92 in MSNP and TPP-MSNP displayed high encapsulation efficiency (EE>70%). The encapsulation efficiencies were almost constant regardless of the drug concentration, except for TPP-MSNP group with 20 mg of feeding drug concentration. The encapsulation efficiencies of MSNP and TPP-MSNP were most reproducible and optimal at 15mg of feeding drug concentration. Therefore, 15 mg of feeding drug concentration was chosen for preparation of DP92 in MSNP or TPP MSNP [16].

4-2. Algae-targeting of TPP-MSNP

In order to investigate of algae-targeting of TPP-MSNP, fluorescence microscopy was performed. Two species of HABs (H.A. and H.C.) were employed to identify algae targeting and algicidal activity. H.A. and H.C. were treated with TPP-MSNP and MSNP (as control) labeled with coumarin-6, and then were incubated at algae culture condition for 24h. In TPP-MSNP group, green fluorescence was observed around and inside of the H.A. and H.C.. Because green fluorescence represented the coumarin-6 labelled TPP-MSNP, TPP-MSNP was also existed around and inside of the H.A. and H.C.. The result suggested that TPP-MSNP was successfully targeted at HABs. However, in the MSNP group, no fluorescence appeared except for the red fluorescence of the algae itself. It showed that TPP targeting effect did not occur.

Fluorescence absorbance measurement was performed to quantitatively measure the degree of transition into the algae. Similar to fluorescence microscopy, TPP-MSNP and MSNP were labelled with coumarin-6 and treated at H.A. and H.C., and then incubated at algae culture condition for 24 h. In addition, the amount of coumarin-6 that has isolated into the algae was measured at H.A. and H.C.. The fluorescence absorbance values of MSNP and TPP-MSNP at H.A. were 120.49 and 171.46, subsequently. The values of MSNP and TPP-MSNP at H.C. were 177.69 and 230.64, subsequently. The degree of fluorescence absorbance in the TPP-MSNP group was higher than in the MSNP group at both HABs. This result also suggested that TPP-MSNP was successfully targeted at HABs. In both MSNP and TPP-MSNP, fluorescence absorbance value at H.C. has higher than the value at H.A.. This result suggested that H.C. was considered to be more sensitive to foreign materials than H.A..

The green fluorescence of coumarin-6 labeled TPP-MSNP around and inside HABs and the higher fluorescence absorbance of coumarin-6 labeled TPP-MSNP suggested that TPP-MSNP was efficiently transferred by the electrostatic attraction and cell targeting effect. Therefore, transition of DP92 with TPP-MSNP into HABs is also expected to be good, and algicidal activity of DP92 with TPP-MSNP is expected to be improved.

4-3. Algicidal activity of DP92 loaded MSNP or TPP-MSNP

To perform algicidal activity experiment, H.A. and H.C. were treated with DP92 loaded TPP-MSNP or MSNP and with DP92 in DMSO (as control), and incubated in algae culture condition. After 24 h of incubation, alive algaes were counted. At HA, the IC_{50} values of TPP-MSNP and MSNP were 0.03 ± 0.01 and 0.16 ± 0.03 μM, subsequently. The IC_{50} value of the control was 0.27 ± 0.02 μM (Fig 5 and Table 4). The IC_{50} value of control was 7 and 1.6 times higher than the IC_{50} value of TPP-MSNP and MSNP, subsequently. The reason for the high IC_{50} value in the control was explained as the difficulty of transition into the algae due to insufficient solubilization. However, in MSNP or TPP-MSNP group, sufficient solubilization was occurred and DP92 was well transferred into algae. Furthermore, IC_{50} value of TPP-MSNP was 4 times higher than the IC_{50} value of MSNP. The result suggested that TPP-MSNP effectively targeted algaes and enhanced the algicidal activity compared to MSNP.

At H.C., the IC_{50} value of TPP-MSNP and MSNP were 0.10 ± 0.02 and 0.29 ± 0.02 μM, subsequently. The IC_{50} value of the control was 1.90 ± 0.09 μM (Fig 6 and Table 4). IC_{50} value of control was 19 and 6 times higher than the IC_{50} value of TPP-MSNP and MSNP, subsequently. Similar to H.A., H.C. also had lower algicidal activity in the control due to insufficient solubilization. IC_{50} value of TPP-MSNP was 3 times higher than the IC_{50} value of MSNP. As with H.A., we identified the effect of TPP on algae targeting and the enhanced algicidal activity at H.C..

Additionally, IC_{50} value of TPP-MSNP was three times higher than MSNP at H.C.. But, IC_{50} of TPP-MSNP was four times higher than MSNP at H.A.. The enhancement of algicidal acitivty with TPP was greater in H.A.. The results suggested that H.C. sensitively interacted with TPP contrary to H.A..

Due to solubilization of DP92 by MSNP, the algicidal activity of DP92 was enhanced up to 1.6-fold over both HABs as compared to the control. Furthermore, the electrostatic interaction between the postively charged TPP-MSNP and the cell wall of HABs and the stimulation of penetration by TPP-MSNP might further enhance delivery of DP92 compared to TPP-unmodified MSNP group, despite of the algae rapid movement in the aqueous environment. In fact, the TPP-MSNP group showed more than

3-fold higher algicidal activity than the MSNP group in both HABs [14, 17]. Our results suggested that TPP-MSNP maximized the algicidal activity of DP92. Moreover TPP-MSNP is easily fabricated and cost-effective compared to substance (phospholipids) used in previously studied formulation (emulsions, liposomes and polymeric micelles) for solubilization. Therefore, DP92 loaded TPP-MSNP is a suitable formulation for managing the HABs because it is possible to commercialize and mass-use DP92 loaded TPP-MSNP with the enhanced algicidal effect [18].

4-4. Transition efficiency of DP92 loaded MSNP or TPP-MSNP

To identify that the enhanced algicidal activity in TPP-MSNP group was due to the increase in the transition of DP92 into the algae, transition efficiencies were measured. At H.A., transition efficiency of TPP-MSNP and MSNP were 83.28 ± 6.00 and 53.00 ± 6.01 %, subsequently. Transition efficiency in TPP-MSNP was 1.5 times higher than in MSNP. Similarly, at H.C., transition efficiency of TPP-MSNP and MSNP were 50.94 ± 6.81 and 36.12 ± 4.24 %, subsequently. Transition efficiency in TPP-MSNP was 1.4 times higher than in MSNP. The results suggested that enhanced algicidal activity of DP92 with TPP-MSNP was due to the increase of transition into algae with TPP-MSNP.

Because TPP is a material that targets mitochondria of living cells, TPP is possible to promote transition into living cells. In fact, we have confirmed that the DP92 loaded TPP-MSNP was transferred into the algae cell by transition efficiency experiment. This experiment sufficiently showed that enhanced algicidal activity of DP92 with TPP-MSNP was due to the increase of transition into algae with TPP-MSNP [19].

5. Conclusion

TPP-MSNP was developed for targeting of algae and entrapment of DP92. Through fluorescence microscopy and fluorescence absorption measurement, we identified toTPP-MSNP successfully targets HABs. In algicidal activity experiments, DP92 in TPP-MSNP showed a higher algicidal activity than DP92 in MSNP. Enhanced algicidal activity of DP92 was demonstrated by solubilization of DP92, inducement of charge interaction between TPP-MSNP and HABs surface and accelerating penetration of DP92 into HABs. Finally, we successfully developed DP92 in TPP-MSNP with low costs and enhancement of algicidal activity.

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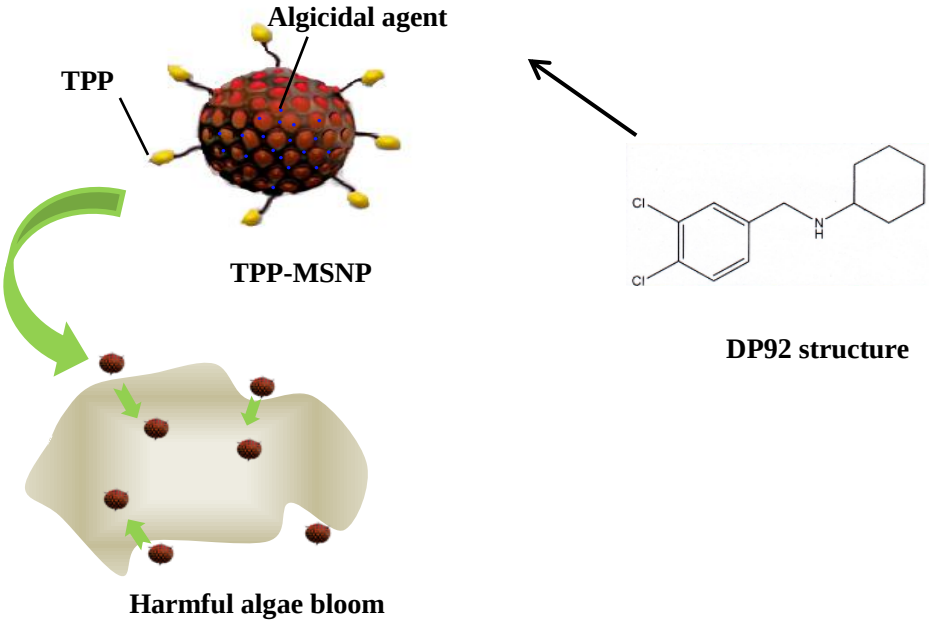

Figure 1. Schematic illustration of the delivery of algicidal agent (DP92) in TPP-MSNP on Harmful algae bloom

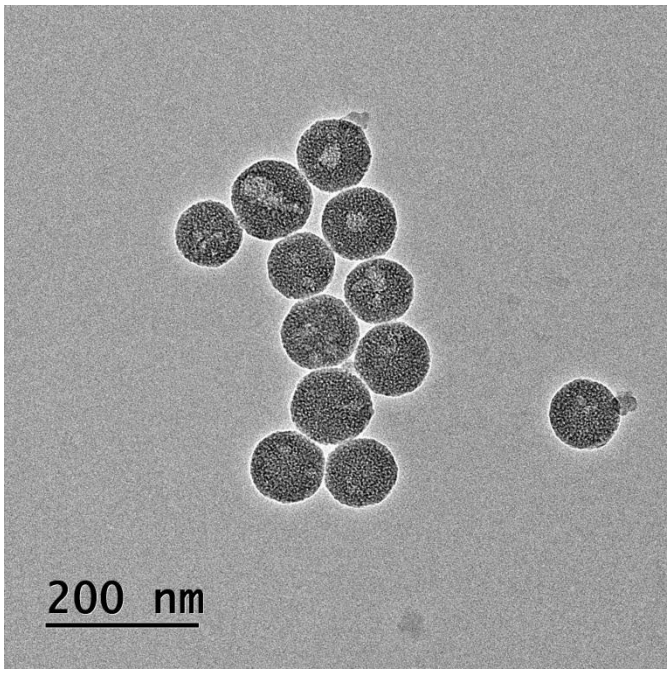

Figure 2. The TEM image of MSNP

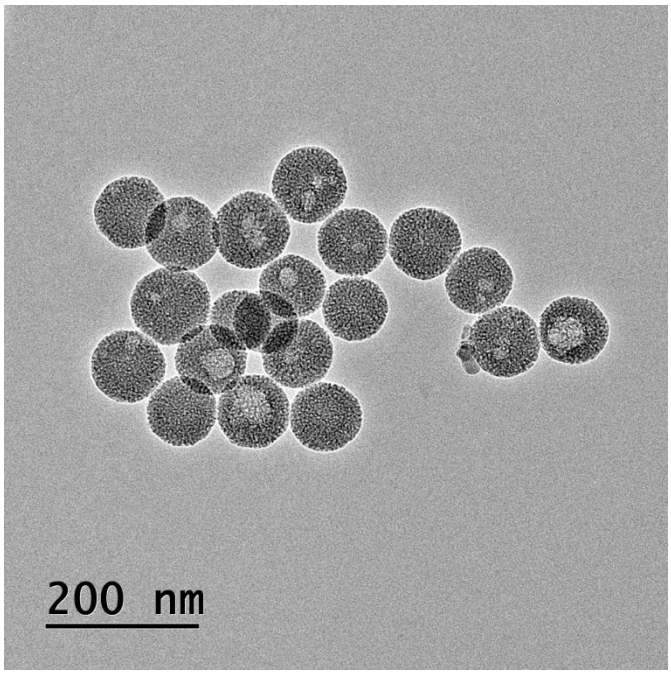

Figure 3. The TEM image of TPP-MSNP

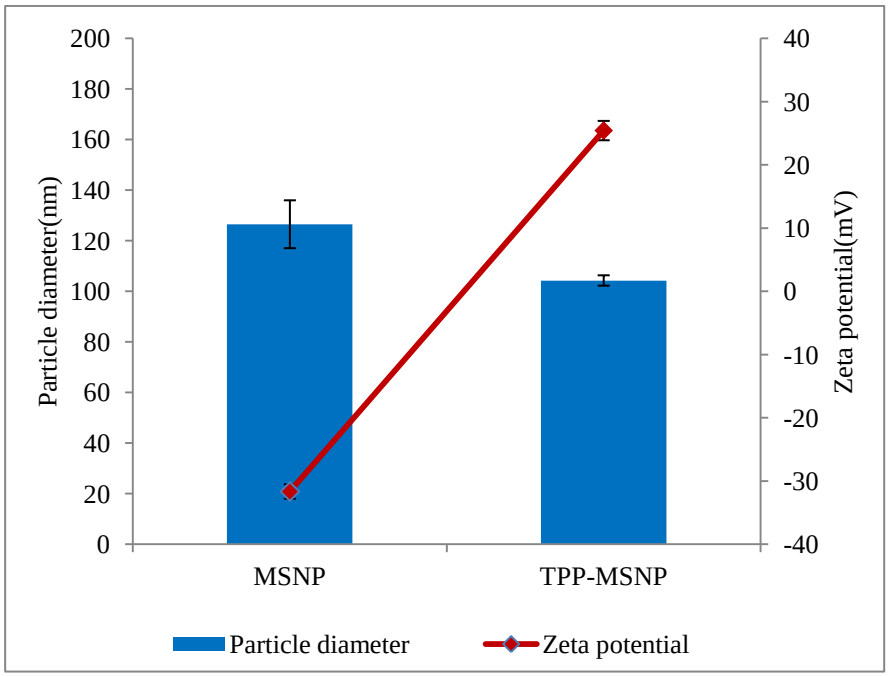

Figure 4. The characterization of MSNP or TPP-MSNP

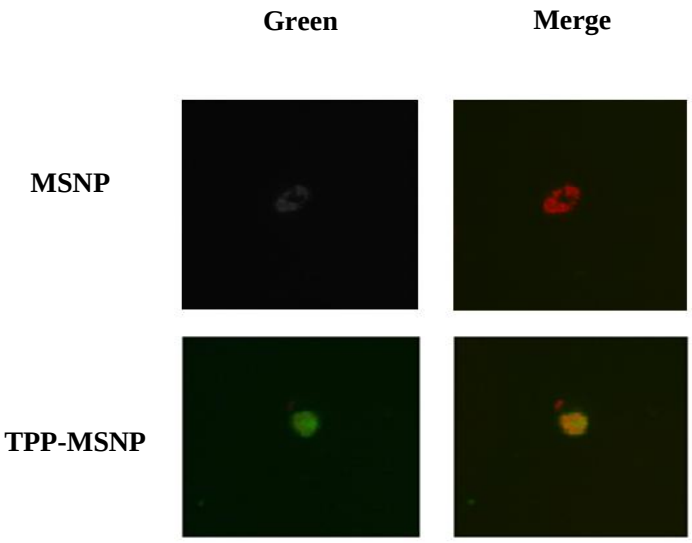

Figure 5. Fluorescence image of coumarin-6 labeled MSNP or TPP-MSNP on *H.Akasiwo*

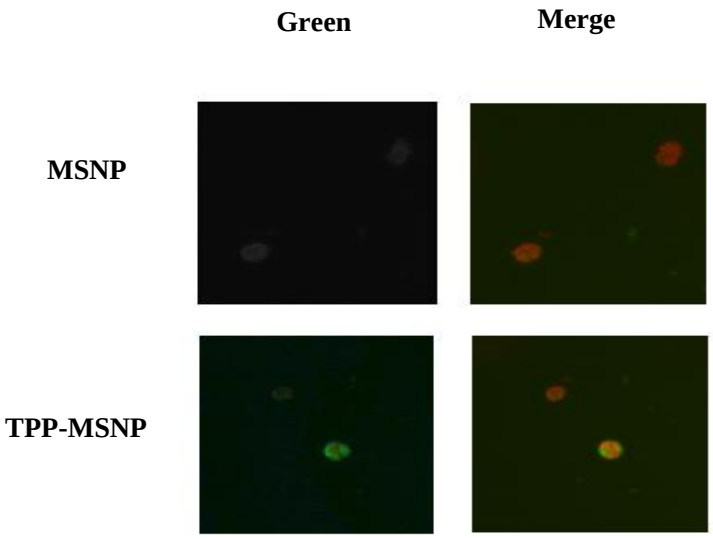

Figure 6. Fluorescence image of coumarin-6 labeled MSNP or TPP-MSNP on *H.Circularisquama*

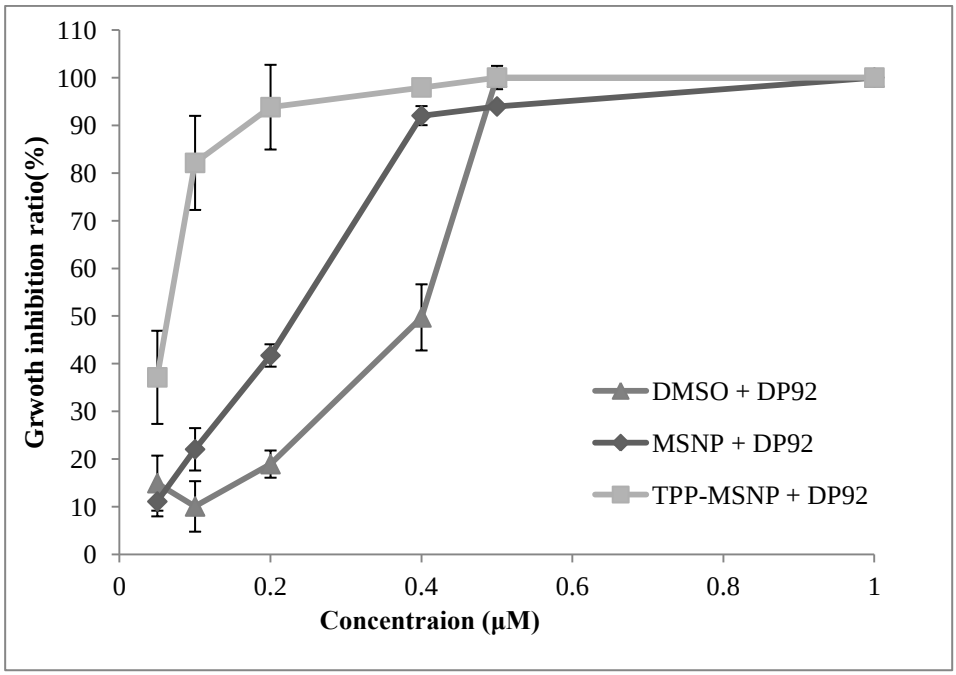

Figure 7. Algicidal activity of DP92 in MSNP and TPP-MSNP against *H.Akasiwo*

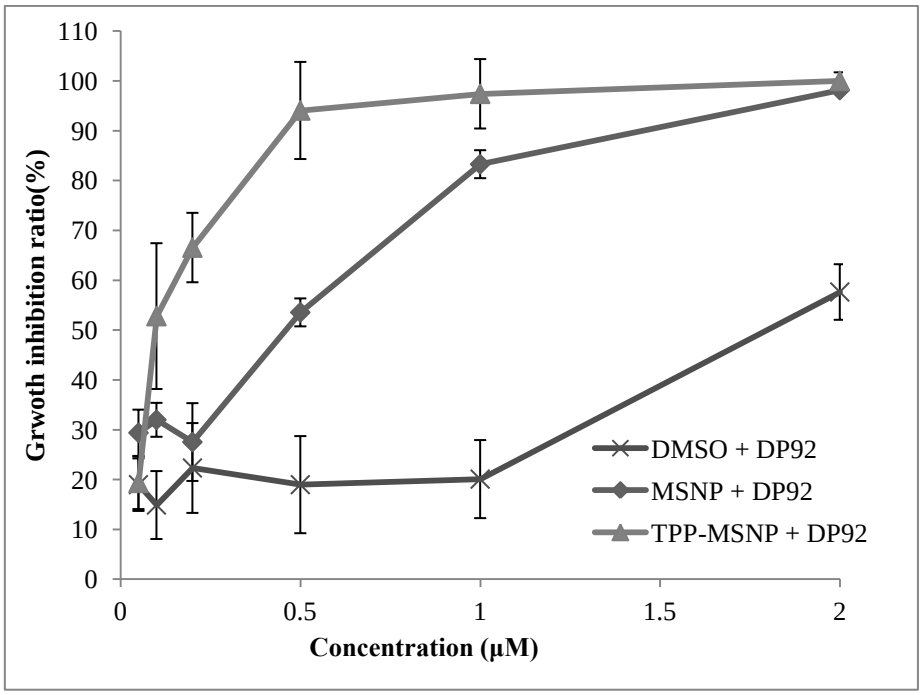

Figure 8. Algicidal activity of DP92 in MSNP and TPP-MSNP against *H.Circularisquama*

Table 1.The physical properties of MSNP or TPP-MSNP

	Diameter (nm)	PI	Zeta potential (mV)
MSNP	126.43±9.46	0.253	-31.67±1.14
TPP-MSNP	104.27±2.05	0.199	25.41±1.53

Table 2. Encapsulation efficiency (EE, %) of DP92 in MSNP or TPP-MSNP

	Encapsulation efficiency (%)	
Drug Conc.(feeding)	MSNP	TPP-MSNP
10 mg	86.67±7.12	90.46±8.24
15 mg	85.19±4.32	88.7±7.25
20 mg	82.31±2.28	73.78±3.47

Table 3. Fluorescence absorbance of coumarin-6 labeled MSNP or TPP-MSNP on HABs

	H.Akasiwo	*H.Circularisquama*
MSNP	120.49	177.69
TPP-MSNP	171.46	230.64

Table 4. IC_{50} values of DP92 loaded MSNP or TPP-MSNP on HABs

IC_{50} (μM)	*H.Akasiwo*	*H.Circularisquama*
Control	0.27 ± 0.02	1.90 ± 0.09
MSNP	0.16 ± 0.03	0.29 ± 0.02
TPP-MSNP	0.04 ± 0.01	0.10 ± 0.02

Table 5. Transition efficiency of DP92 loaded MSNP or TPP-MSNP on HABs

Transition efficiency (%)	*H.Akasiwo*	*H.Circularisquama*
MSNP	53.00 ± 6.01	36.12 ± 4.24
TPP-MSNP	83.28 ± 6.00	50.94 ± 6.81

ABSTRACT

A study on development of triphenylphosphonium modified mesoporous silica nanoparticle for enhanced algicidal efficacy of DP92

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Mesoporous silica nanoparticle (MSNP) has been studied for delivery of various materials such as drugs, DNA, si-RNA, peptides. MSNP has large surface area because of a lot of pores on the surface of MSNP and surface area can be easily adjusted by control of size of the pores. Particularly, the pore is hydrophobic and is suitable for entrapment of hydrophobic drug. MSNP is a material suitable for the solubilization of hydrophobic drugs. Furthermore, MSNP surface is easy to chemically formulate. Triphenylphosphonium (TPP) is lipophilic cation and stimulates penetration of drugs into cells. Its cationic property can interact with cell surface that is negatively charged. Therefore, we designed MSNP modified with TPP for enhancement of drug delivery efficiency by charge interaction between TPP-MSNP and cell wall, and stimulated penetration of TPP-MSNP. Harmful algal blooms (HABs) cause critical problems in marine environment and fishery industry. To manage HABs, several methods have been used such as chemical algicides, clay flocculants, using virus or natural enemy. Chemical algicides are the most widely used method. We was synthesized cyclohexyl-(3,4-dichlorobenzyl) amine (DP92) as a algicidal agent to manage the HABs. However, it is difficult to apply DP92 to water environment due to its low solubility. Therefore,

DP92 was selected as a model drug and encapsulated into TPP-MSNP for solubilization and enhanced algicidal activity.

The properties of MSNP (as control) and TPP-MSNP such as particle diameter, zeta potential and encapsulation efficiency were evaluated. In order to investigate transition of TPP-MSNP into algae, MSNP and TPP-MSNP were labelled with coumarine-6 and fluorescence absorbance analysis in algae and fluorescence microscope observation around algae were performed. To evaluate the algicidal efficacy of MSNP and TPP-MSNP, IC50 of formulation on *Heterosigma Akasiwo* (H.A.) and *Heterocapsa Circularisquama* (H.C.) was evaluated. The particle diameter of MSNP and TPP-MSNP were similar around 150 nm, but zeta potential were -18 mV and +20 mV, subsequently. More than 80% of DP92 was encapsulated into MSNP and TPP-MSNP. The fluorescence microscope image showed that TPP-MSNP was observed around and inside of the algae in contrast to MSNP. Higher fluorescence absorbance was analyzed in algae treated with coumarine-6 labelled TPP-MSNP than control. At H.A, The IC_{50} value of TPP-MSNP was 0.04 ± 0.01 μM and that of MSNP was 0.16 ± 0.03 μM. The IC_{50} value of MSNP and TPP-MSNP were 7 and 1.6 times higher than IC_{50} of DP92 in DMSO, subsequently. At H.C, The IC_{50} value of TPP-MSNP was 0.29 ± 0.02 μM and that of MSNP was 0.10 ± 0.02 μM. The IC_{50} value of MSNP and TPP-MSNP were 19 and 6 times higher than IC_{50} of DP92 in DMSO, subsequently. Transition efficiencies of TPP-MSNP higher were higher than that of MSNP. The results suggest that TPP-MSNP efficiently enhanced algicidal activity of DP92 by charge interaction between TPP-MSNP and algae wall, solubilization of DP92 and stimulated penetration of DP92 into algae

Keyword: Mesoporous silica nanoparticle, triphenylphosphonium, algicidal activity, Harmful algae blooms