

# Nanoencapsulation of the sasanquasaponin from *Camellia oleifera*, its photo responsiveness and neuroprotective effects

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**Abstract:** Sasanquasaponin, a bioactive compound isolated from seeds of *Camellia oleifera*, shows central effects in our previous research. In order to investigate its neuroprotective effects, a new kind of nanocapsule with photo responsiveness was designed to deliver sasanquasaponin into the brain and adjusted by red light. The nanocapsule was prepared using sasanquasaponin emulsified with soybean lecithin and cholesterol solution. The natural phaeophorbide from silkworm excrement as a photosensitizer was added in the lipid phase to make the nanocapsules photo responsive. The physicochemical properties of encapsulation efficiency, size distribution, morphology and stability were measured using high-performance liquid chromatography, particle size analyzer, transmission electron microscope, differential scanning calorimetry and thermogravimetry. Photo responsiveness was determined by the sasanquasaponin release in pH 7.5 phosphate buffer under the laser at 670 nm. The neuroprotective effects were evaluated by the expression of tyrosine hydroxylase (TH), decrease of inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  in the brain, and amelioration of kainic acid-induced behavioral disorder in mice. The nanocapsules had higher encapsulation efficiency and stability when the phaeophorbide content was 2% of lecithin weight. The average size was 172.2 nm, distributed in the range of 142–220 nm. The phaeophorbide was scattered sufficiently in the outer lecithin layer of the nanocapsules and increased the drug release after irradiation. TH expression in brain tissues and locomotive activities in mice were reduced by kainic acid, but could be improved by the sasanquasaponin nanocapsules after tail vein injection with 15 minutes of irradiation at the nasal cavity. The sasanquasaponin took effect through inflammatory alleviation in central tissues. The sasanquasaponin nanocapsules with phaeophorbide have photo responsiveness and neuroprotective effects under the irradiation of red light. This preparation presents a new approach to brain neuroprotection, and has potential for clinical application.

**Keywords:** sasanquasaponin, nanocapsule, photo responsiveness, neuroprotection, phaeophorbide, red light, drug delivery

## Introduction

Sasanquasaponin is a kind of saponin isolated from the seeds of *Camellia oleifera*, which is a plant grown in the mountain regions of central China, and in Taiwan.<sup>1</sup> It differs from the tea plant in that its leaves cannot be used in beverages, but its seeds contain 30% oil and are of a quality similar to olive oil.<sup>2</sup> Because of the strong commercial demand for ligneous oil, *C. oleifera* is widely cultivated. Its production has led to a rapid increase in byproducts of its seeds, which provide an excellent source of sasanquasaponin.

Natural saponins have neuroprotective effects relevant to suppression of oxidative stress, elimination of free radicals, and inhibition of inflammation.<sup>3</sup> Our former

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researches disclose that sasanquasaponin has various hypolipidemic, antioxidant, anti-inflammatory, and other pharmacological activities;<sup>4</sup> its analgesic activities reveal its central roles.<sup>5</sup> Sasanquasaponin is a safe compound with an LD<sub>50</sub> of 4.5 g/kg and an accumulation coefficient of 5.3 in rats,<sup>6</sup> thus can be a prodrug candidate for neuroprotection. However, its large structure (molecular weight 1,222 Da) inhibits absorption by the brain. Nanoencapsulation of sasanquasaponin may enhance its delivery into the brain.

There are many methods of drug delivery based on nanotechnology, such as liposomes, micelles, polymeric and lipid-based nanoparticles, dendrimers and carbon nanotubes.<sup>7</sup> Each method has both advantages and disadvantages. For example, carbon nanotubes have gained attention for their high surface area, enhanced cellular uptake, and lasting effectiveness, but easily result in a persistent accumulation in tissue, followed by inflammatory reaction.<sup>8</sup> Moreover, it is difficult to achieve the photo responsive release by carbon nanoparticles. Phospholipid liposome is an advantageous drug delivery system for different drugs, as it possesses the characteristics of biocompatibility, biodegradability, potentiality of targeting nanoparticles to particular cells or tissues, possibilities of controlled release, and protection against degradation of drugs.<sup>9</sup> The loaded nanoparticles are internalized in cells via endocytosis and fluid phase pinocytosis, which helps drugs penetrate cytoplasm.<sup>10</sup> In order to achieve controlled release of nanocapsules, the liposomes are modified in the structure with pH response, thermal response, bioenzymatic response, and photo response.<sup>11</sup> Only photo response can achieve far distance and no harm adjustment. Currently, ultraviolet light is used to destroy the liposome or change its permeability by isomerization of azobenzene in it,<sup>12,13</sup> but ultraviolet light is easily absorbed by tissues and is harmful to cells.

We have designed a photo responsive nanocapsule of the sasanquasaponin. Its release can be adjusted by red light, which has low tissue absorption and deep penetration.<sup>14</sup> The photo responsive liposome is prepared by lecithin mixed with the phaeophorbide from silkworm excrement, and it inserts the liposome as a photosensitive agent excited by 670 nm laser light. The physicochemical properties of the nanocapsule including size distribution, morphology, encapsulation efficiency (EE), differential scanning calorimetry (DSC), and photo controlled release were analyzed, and its neuroprotective effects evaluated, by the expression of tyrosine hydroxylase (TH), inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  in brain, and locomotive activities in mice injured by excitotoxicity of kainic acid. The sasanquasaponin has been successfully

nanoencapsulated in the liposome, and the nanocapsules with phaeophorbide have photo responsiveness and neuroprotective effects under the irradiation of red light.

## Materials and methods

### Chemicals

Sasanquasaponin (purity 95%) was isolated from the seeds of *C. oleifera*, and prepared in our lab with structural identification.<sup>4</sup> Phaeophorbide (purity 97%) was isolated from silkworm excrement and made in our laboratory as reported.<sup>15</sup> Soybean lecithin, cholesterol, and kainic acid were purchased from Guangzhou Qiyun Biotech Company (Guangzhou, People's Republic of China). TNF- $\alpha$ , IL-1 $\beta$  and TH immunoassay kits were bought from Jiancheng Biotech Company, Nanjing, People's Republic of China. Diethyl ether and other chemicals were purchased from Qianhui Reagent Company, Guangzhou, People's Republic of China.

### Animals

The experiments were carried out on male C57BL/6 mice of weight 25 $\pm$ 3 g. The animals were housed under conditions of 25°C $\pm$ 2°C, 50% $\pm$ 10% humidity with a 12-hour light/dark cycle. Food and water were accessible ad libitum. The experiments were performed in accordance with the Chinese guidelines for the use of laboratory animals, and received approval from the experimental animal ethics committee of South China University of Technology. All efforts were made to minimize animal suffering and to reduce the number of animals used.

### Nanoencapsulation of the sasanquasaponin

The sasanquasaponin nanocapsules were prepared using the emulsion evaporation method described in Foco et al.<sup>16</sup> In brief, 500 mg of lecithin, 50 mg of cholesterol and phaeophorbide (0, 1%, 2%, 3%, 5%, and 10% of lecithin weight) were completely dissolved in ethanol at 60°C as the lipid phase. Twenty-five milligrams of sasanquasaponin were dissolved in pH 7.5 phosphate buffer containing 0.9% Tween-80 as the aqueous phase. The lipid phase was emulsified with the aqueous phase at 10,000 rpm and 60°C for 30 minutes. The mixed solution was filtered through the membrane (pore size 1  $\mu$ m) and ultra-centrifuged at 60,000 $\times$  g at 4°C for 10 minutes. The precipitate was lyophilized to obtain the nanoparticles.

### Determination of encapsulation efficiency and drug loading content

Sasanquasaponin in the supernatant of centrifugation was determined by high-performance liquid chromatography

(HPLC) (Agilent Company, Santa Clara, CA, USA) in the following conditions: column: Diamonsil C18 (150×4.6 mm, 5 μm), mobile phase: 80% (v/v) methanol, flow rate: 0.7 mL/min, temperature: 30°C, injection volume: 15 μL, and wavelength 208 nm.

EE and drug loading content (DLC) were important indices for nanocapsule preparation, and calculated as follows:

$$EE\% = \frac{\text{Drug content in nanocapsule}}{\text{Total content of used drug}} \times 100$$

$$DLC\% = \frac{\text{Drug content in nanocapsule}}{\text{Weight of nanocapsule}} \times 100$$

The sasanquasaponin content in nanocapsule was calculated from total content of used sasanquasaponin subtracting the sasanquasaponin content in the supernatant.

### Test of particle size and zeta potential

Particle size and zeta potential used to evaluate the stability of the sasanquasaponin nanocapsules were determined using a Nano-2S MDT-2 Malvern particle size analyzer (Malvern Instruments Limited, Malvern, UK). Before testing, the appropriate sample was dispersed in water with refractive index 1.33. Data were calculated as the average of 5 repetitions.

### Morphological observation of particles

The sasanquasaponin nanocapsules were diluted to 0.5 μg/mL with water, dropped to the copper grid covered with carbon film before drying at 25°C, then dyed with 2% phosphotungstic acid. Specimens were scrutinized under a JEM-2100F high resolution transmission electron microscope (TEM) (JEOL Company, Tokyo, Japan) at 200 kV.

### Differential scanning calorimetric and thermogravimetric analysis

Differential scanning calorimetric (DSC) and thermogravimetric analysis (TG) were performed on an SDT-Q6000 thermogravimetric analyzer (TA Incorporated, New Castle, DE, USA). Four milligrams of sasanquasaponin nanocapsules were weighed and heated from 20°C–200°C under nitrogen flow at a rate of 20°C/min.

### Nanocapsule release measurement

The dialysis diffusion method was employed to measure release of the sasanquasaponin nanocapsules *in vitro*.<sup>17</sup>

Thirty milligrams of sasanquasaponin nanocapsules were dispersed in 3 mL of pH 7.5 phosphate buffer, and put in the dialysis bag (molecular weight cutoff 3,500 Da), which was placed in the container with 50 mL of pH 7.5 phosphate buffer, stirred at 100 rpm, and maintained at 37°C±0.5°C. One milliliter of the solution was consecutively sampled at intervals of 1 hour, and the same volume of pH 7.5 phosphate buffer was complemented at designated intervals. The amount of sasanquasaponin released into the solution was determined in three repetitions of HPLC, and cumulative release was added during the next 12 hours. The photo responsive release was carried out with irradiation by 100 mW laser (Three Top Photoelectric Company, Dongguan, People's Republic of China) at 670 nm.

### Neurodegenerative animal model induced by kainic acid

The neurodegenerative animal model was induced by administration of kainic acid according to Zhang and Zhu,<sup>18</sup> with some modification. Forty-eight male mice were randomly divided into six groups (eight mice for each group): normal group, kainic acid group, and other four groups medicated with the sasanquasaponin nanocapsule (high dose with irradiation, low dose with irradiation, high dose without irradiation, and low dose without irradiation). Medicated groups of mice were injected to tail vein once a day with the sasanquasaponin nanocapsule at 5 mg/kg (low dose) and 25 mg/kg (high dose), respectively, for 6 days, accompanied by diethyl ether anesthesia and nasal cavity irradiation with 100 mW laser at 670 nm for 15 minutes, and other groups were injected with normal saline instead. On the seventh day of administration, the mice except normal group were intranasally administered 45 mg/kg kainic acid in 40 μL solution to prepare the neurodegenerative mouse model. The mice were reared for an additional 7 days and decapitated after behavioral tests, and half the brain was quickly separated to determine the levels of TNF-α and IL-1β using the enzyme-linked immunosorbent assay (ELISA) as described in the immunoassay kits used. The other brain half was kept for immunohistochemical analysis.

### Behavioral test

The locomotive activities of mice were monitored by a ZZ-6 spontaneous activity apparatus (Chengdu Taimeng Software Company, Limited, Chengdu, People's Republic of China). Mouse motion counts were collected by computer with the infrared-sensitive motion detection system.<sup>19</sup> Each mouse was placed in the testing chamber for a ten-minute

adaptation period, which was followed by a ten-minute recording period.

## Assay of TH expression

The test was carried out on the basis of protocol used by Zhou et al.<sup>20</sup> The half brains of mice were sunk in paraformaldehyde solution (pH 7.4, 40 g/L) containing 300 g/L sucrose for 48 hours, and cryostat-sectioned to slices (60  $\mu$ m thickness). Brain slices were incubated in TH antibody with dilution of 1:5,000, stained by diaminobenzidine (DAB), and mounted to slides for photography. Optical density of striatum in the photographs was analyzed by Image-Pro Plus 6.0 software (Media Cybernetics Incorporated, Rockville, MD, USA).

## Statistical analysis

Data were expressed as mean  $\pm$  standard deviation, and analyzed with SPSS version 13.0 software (IBM, Armonk, NY, USA). Significant tests among the groups were based on one-way analysis of variance (ANOVA) and Student–Newman–Keuls method tests.

## Results and discussion

### Characteristics of the sasanquasaponin nanocapsule

Nanocapsules can be prepared by different methods of emulsion diffusion, nanoprecipitation, emulsion evaporation, dialysis, and so forth.<sup>21</sup> Among these methods, emulsion evaporation avoids toxic solvents and offers an effective approach for manufacturing nanoparticles. With the advantages of safety, biocompatibility and biodegradability, lecithin and cholesterol are traditionally applied as the shell of liposome,<sup>22</sup> but red light responsive liposome has seldom been reported. Therefore, the formability of sasanquasaponin nanocapsules added with phaeophorbide was investigated. Our results showed DLC and EE increased until the phaeophorbide content reached up to 2% of lecithin weight, but decreased thereafter (Table 1). The best DLC and EE

were 3.8% $\pm$ 0.2% and 84.2% $\pm$ 5.3%, respectively, indicating better drug loading and encapsulation at that content of phaeophorbide.

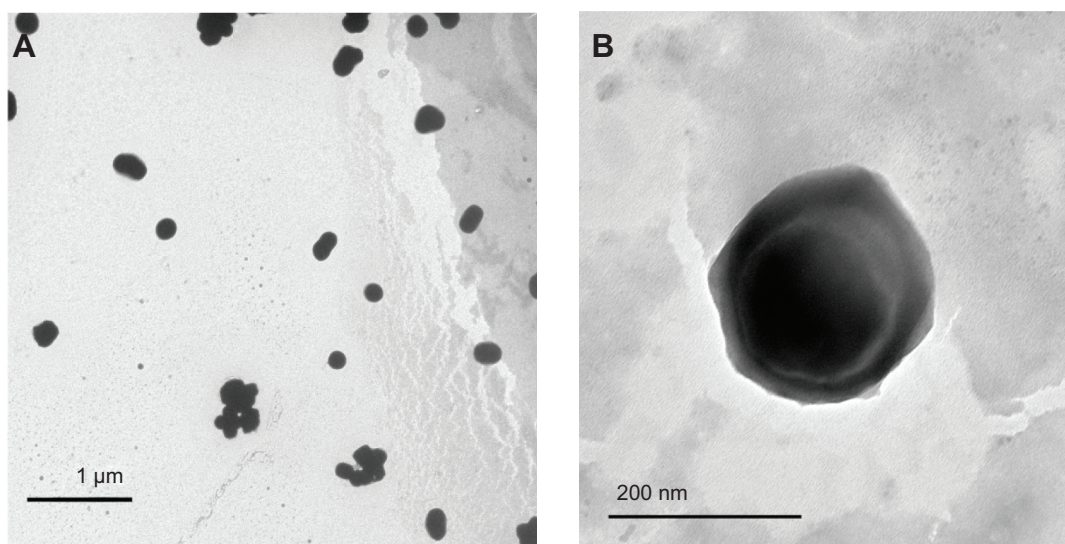
The phaeophorbide is hydrophobic, with a single hydrophilic carboxyl group in its structure. A certain amount of the phaeophorbide can enhance the stability of hydrophobic block in the liposome, but excess phaeophorbide impedes tight junction of lecithin and reduces the DLC and EE. The average particle size and zeta potential of the sasanquasaponin nanocapsules both increased with the phaeophorbide content (Table 1), further confirming that excess phaeophorbide is detrimental to the stability of nanocapsules. The average nanoparticle size and zeta potential were, respectively, 172.2 nm and  $-60.2$  mV at 2% lecithin weight of phaeophorbide content, and the size distributed in the range of 142–220 nm, suggesting that it is proper for the nanocapsule preparation, and the nanocapsules are homogeneous and stable because of proper negative charge repulsion. Morphology of the nanocapsules was observed and photographed with TEM, which is a profitable apparatus to identify internal structures of nanoparticles. TEM images of sasanquasaponin nanocapsules display good dispersity (Figure 1A) and sphericity with clear borderline (Figure 1B). It indicates that the sasanquasaponin is encapsulated by an outer layer of lecithin and cholesterol, and the nanoparticles have not congregated.

In order to further determine the dispersity of polymer in the nanocapsules, DSC and TG are applied as a simple method to verify it.<sup>23</sup> DSC and TG curves of the sasanquasaponin nanoparticles are presented in Figure 2. The nanocapsules exhibited a single endothermic melting peak at 57.7°C. Thermogravimetric patterns of the nanocapsules showed an inflection point at this temperature interval because of free water evaporation, but no reflection in the melting point of phaeophorbide (215°C). These characteristics confirm that the nanocapsules are stable for clinical

**Table 1** Properties of sasanquasaponin nanocapsules with different concentrations of phaeophorbide

Phaeophorbide concentration (% lecithin weight)	Encapsulation efficiency (%)	Drug loading content (%)	Average particle size (nm)	Zeta potential (mV)
0	72.3 $\pm$ 3.4	3.3 $\pm$ 0.1	138.2 $\pm$ 9.3	$-65.0\pm1.2$
1	76.9 $\pm$ 3.1	3.5 $\pm$ 0.1	158.4 $\pm$ 8.1	$-62.5\pm1.6$
2	84.2 $\pm$ 5.3	3.8 $\pm$ 0.2	172.2 $\pm$ 9.7	$-60.2\pm1.5$
3	72.7 $\pm$ 3.8	3.2 $\pm$ 0.2	209.6 $\pm$ 15.1	$-48.1\pm1.2$
5	64.8 $\pm$ 4.3	2.8 $\pm$ 0.2	250.2 $\pm$ 23.1	$-44.2\pm1.8$
10	50.7 $\pm$ 6.2	2.1 $\pm$ 0.3	314.3 $\pm$ 33.3	$-37.0\pm2.3$

**Notes:** Data are presented as mean  $\pm$  standard deviation (n=5). All data show significant differences between treatments ( $P<0.05$ ).



**Figure 1** Transmission electron micrograph of sasanquasaponin nanocapsules at (A) 1,000 $\times$  amplification, and (B) 20,000 $\times$  amplification.

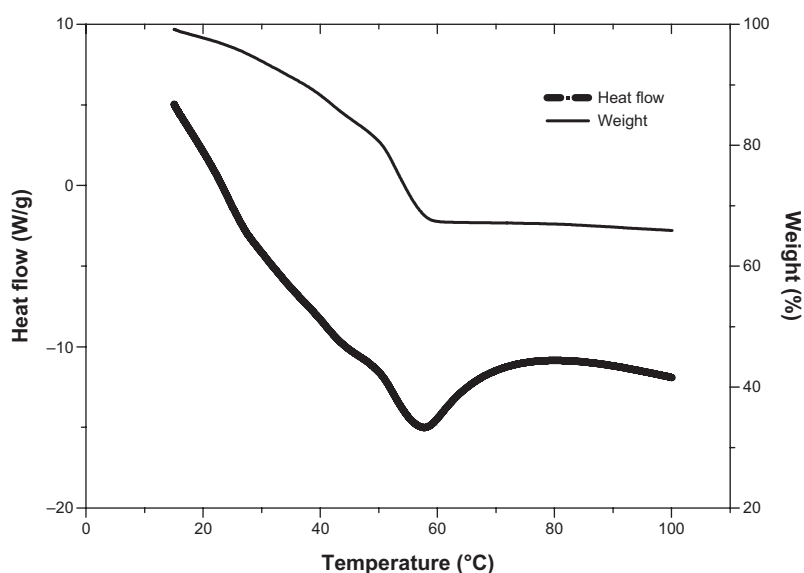
use, the phaeophorbide is scattered sufficiently in lipid phase, and water soluble sasanquasaponin is encapsulated inside the lipid phase.

### Photo responsiveness of the nanocapsules

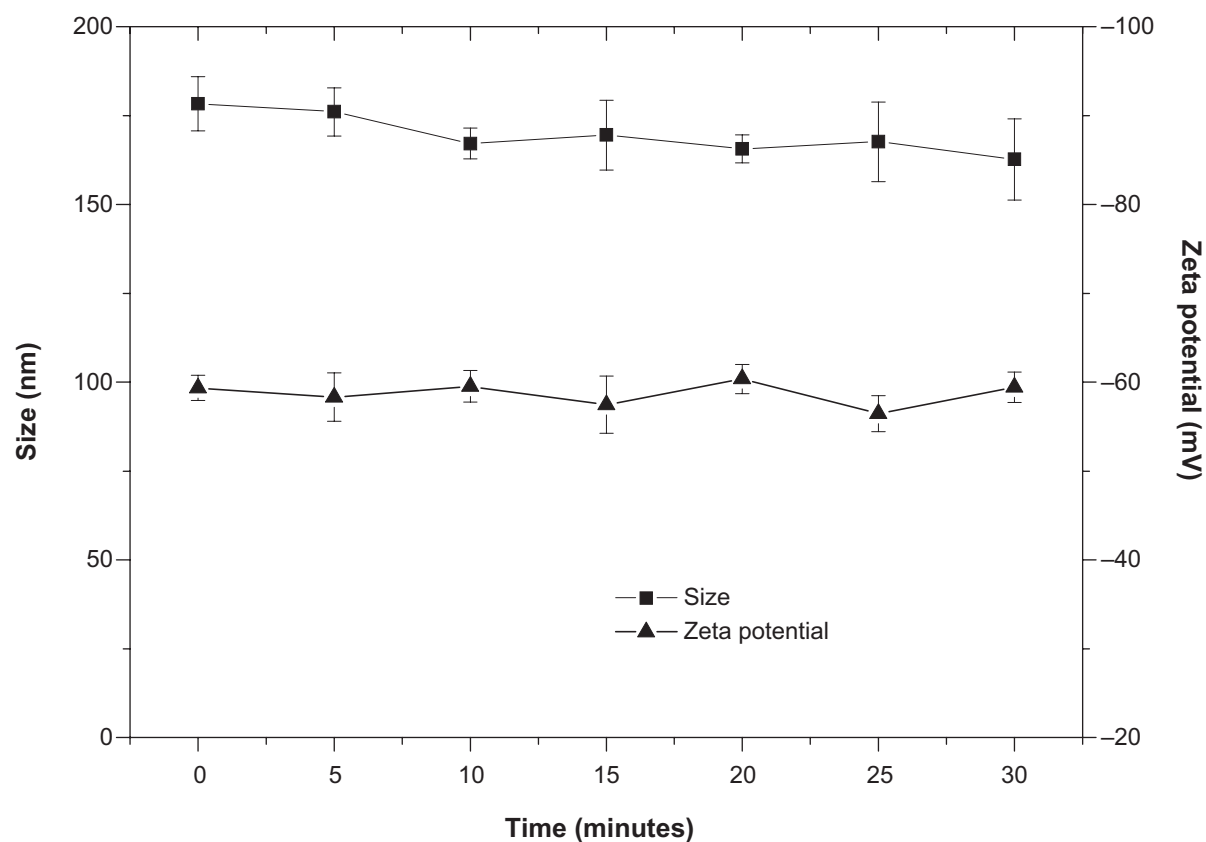
Particle size and zeta potential of the sasanquasaponin nanocapsules had no obvious change after 0–30 minutes of irradiation at 670 nm (Figure 3), suggesting no significant change in basic shape of the nanocapsules. However, the release rate was different in illuminating time. It quickly increased up to 69% and 87% at the interval of 7 hours, respectively, in

15 minutes and 30 minutes irradiation, but only increased to 30% at the same time without irradiation (Figure 4). It demonstrates that the nanocapsules have photo responsiveness, and 670 nm irradiation dramatically improves sasanquasaponin release from the nanocapsules.

A photosensitizer is a generator of singlet oxygen or oxygen radicals which is mainly used in photodynamic therapy to kill cancer cells.<sup>24</sup> The main mechanism of its cytotoxicity is the damage of plasma membrane integrity leading to cytoplasmic leakage.<sup>25</sup> Cell membrane is composed of phospholipids linked by phosphodiester bonds, which can be broken by reactive oxygen species.<sup>26,27</sup> Phaeophorbide, an

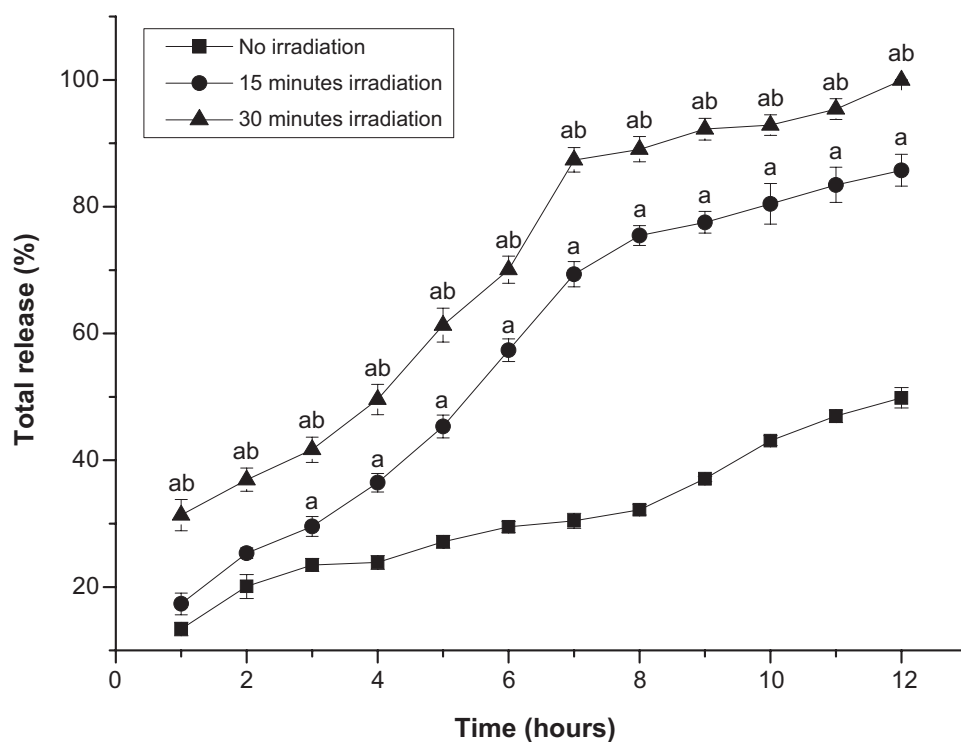


**Figure 2** Differential scanning calorimetric and thermogravimetric chart of the sasanquasaponin nanocapsules.



**Figure 3** Size and zeta potential of the sasanquasaponin nanocapsules at different illuminating times at 670 nm.

**Note:** Data are presented as mean  $\pm$  standard deviation in five repetitions.



**Figure 4** Total release ratio of the sasanquasaponin nanocapsules at different times of irradiation at 670 nm.

**Notes:** Data were presented as mean  $\pm$  standard deviation in three repetitions. a,  $P < 0.01$ , compared with group of no irradiation. ab,  $P < 0.01$ , compared with group of no irradiation and group of 15 minutes irradiation.

effective photosensitizer, can generate singlet oxygen while excited by 670 nm laser.<sup>28</sup> Therefore, the photoactivated release mechanism of sasanquasaponin from the nanocapsules is attributed to damage of the phosphodiester bonds in lecithin of the nanocapsules by phaeophorbide-produced singlet oxygen. This mechanism is illustrated in Figure 5 and is consistent with findings of Bisby et al.<sup>29</sup>

## Protection of TH by the nanocapsules

TH is a key enzyme in the synthesis of monoamine neurotransmitters such as dopamine and noradrenaline in neurons, and its expression is lowered in brain tissues of patients with neurodegenerative diseases.<sup>30</sup> The expression of TH can be reflected by immunostaining intensity of brain slices in immunohistochemistry. Kainic acid-treated mice had less staining in the striatum of brain slices as compared with normal mice, indicating that kainic acid depressed the expression of TH.

Immunohistochemistry of striatum sections in mice with different treatments is shown in Figure 6, and optical density of the striatum is listed in Table 2. The optical intensity has slightly increased from low dose to high dose of the nanocapsules, but obviously increased with the nanocapsules in dose dependence after irradiation at nasal cavity. It proves that 670 nm irradiation increases release of the sasanquasaponin in brain and exerts its protective effects on neurons.

## Effects of the nanocapsules on mouse behavior

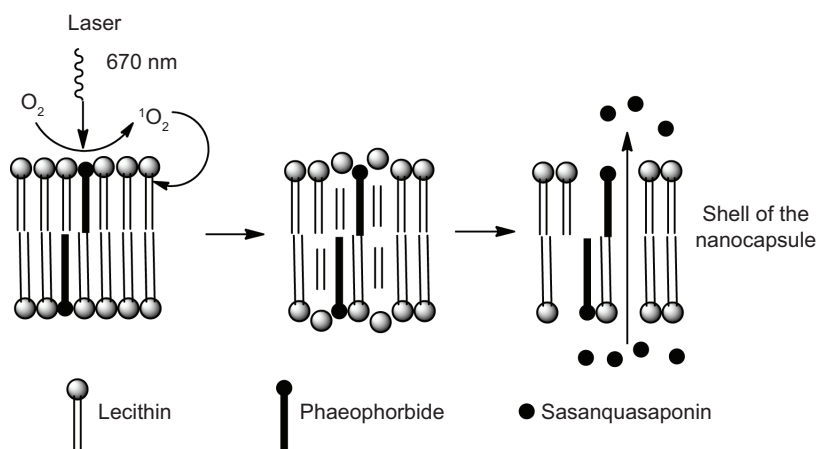
Human patients with neurodegeneration not only evidence neurotransmitter loss in brain, but may also experience motor dysfunction, which can be imitated by animal tests. Spontaneous activities of mice were determined by locomotion test. The

results showed that kainic acid significantly ( $P < 0.01$ ) lessened mouse spontaneous activity, thus suggesting movement disorder. Locomotion counts were increased significantly ( $P < 0.01$ ) after the administration of the nanocapsules with 670 nm irradiation, indicating that the photo responsive nanocapsules improved the spontaneous behavior of mice with kainic acid-induced behavioral disorder. Results are shown in Table 2.

## Inhibition of inflammation in brain

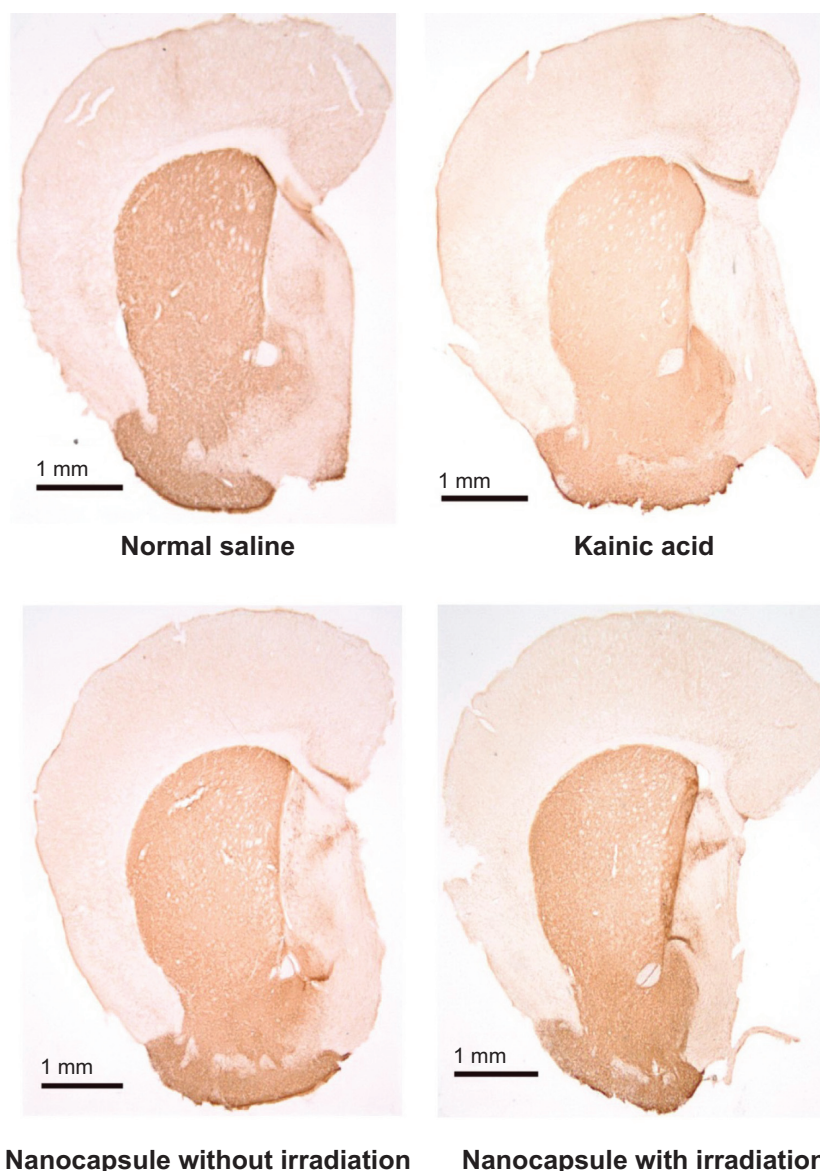
Kainic acid can induce neuroinflammation through an increase in the expression of TNF- $\alpha$  and IL-1 $\beta$  in brain.<sup>31</sup> The anti-inflammatory effects of the sasanquasaponin nanocapsule on brain tissues were investigated. The results showed that TNF- $\alpha$  and IL-1 $\beta$  were significantly ( $P < 0.01$ ) increased in mice brains within the kainic acid-treated group, but decreased in the groups treated with the sasanquasaponin nanocapsule accompanied by 670 nm irradiation. There were significant differences ( $P < 0.01$ ) between the irradiation groups and no irradiation groups receiving the same dose, suggesting that the irradiation improved sasanquasaponin release to control inflammation in brain. Results are illustrated in Table 2.

TNF- $\alpha$  and IL-1 $\beta$  had obvious neurotoxicity on neurons.<sup>32</sup> High level expression of TNF- $\alpha$  could induce great loss of neurons, accompanied by the proliferation of glial, monocytes and macrophages.<sup>33</sup> TNF- $\alpha$  and IL-1 $\beta$  could also cause phosphorylation and degradation of IKK- $\beta$  kinase, leading to translocation of NF- $\kappa$ B into the nucleus to promote expression of inflammatory genes.<sup>34</sup> This experiment shows that kainic acid increases the expression of TNF- $\alpha$  and IL-1 $\beta$  in brain, triggering inflammation and leading to neuron loss. Sasanquasaponin inhibits the expression of TNF- $\alpha$  and IL-1 $\beta$  in the mouse brain induced by kainic acid,



**Figure 5** Mechanism of sasanquasaponin release from the nanocapsules with a laser at 670 nm.

**Note:** Irradiation excites phaeophorbide to produce a singlet oxygen, damaging the lecithin to let sasanquasaponin pass through the outer shell of the nanocapsules.



**Figure 6** Tyrosine hydroxylase (TH) immunohistochemistry of striatum sections in mice with different treatments.

**Notes:** The mice received sasanquasaponin nanocapsules (25 mg/kg tail vein injection), accompanied by nasal cavity irradiation with a 100 mW laser at 670 nm for 15 minutes; kainic acid (45 mg/kg intranasal administration). Half brains were sectioned for determination of TH expression by immunohistochemistry. Photographed by camera at 50× magnification.

**Table 2** Effects of the sasanquasaponin nanocapsules on the expression of tyrosine hydroxylase (TH), inflammatory cytokines, and the kainic acid-induced behavioral disorder in mice

Groups	Dose (mg/kg)	Optical density	Locomotive count	TNF- $\alpha$ (pg/mg)	IL-1 $\beta$ (pg/mg)
Normal saline	/	0.119 $\pm$ 0.009 <sup>a</sup>	164.2 $\pm$ 21.5 <sup>a</sup>	34.7 $\pm$ 10.8 <sup>a</sup>	44.3 $\pm$ 8.8 <sup>a</sup>
Kainic acid	45	0.052 $\pm$ 0.006	62.8 $\pm$ 17.3	174.1 $\pm$ 44.6	259.5 $\pm$ 39.2
Low dose of the nanocapsule	5	0.064 $\pm$ 0.007 <sup>a</sup>	79.2 $\pm$ 17.0	102.0 $\pm$ 25.6 <sup>a</sup>	148.0 $\pm$ 35.7 <sup>a</sup>
High dose of the nanocapsule	25	0.067 $\pm$ 0.004 <sup>a</sup>	92.9 $\pm$ 15.1 <sup>a</sup>	80.0 $\pm$ 15.6 <sup>a</sup>	102.4 $\pm$ 28.0 <sup>a</sup>
Low dose of the nanocapsule, plus irradiation	5	0.076 $\pm$ 0.004 <sup>a,b</sup>	104.4 $\pm$ 16.0 <sup>a,b</sup>	71.2 $\pm$ 13.4 <sup>a,b</sup>	93.6 $\pm$ 12.1 <sup>a,b</sup>
High dose of the nanocapsule, plus irradiation	25	0.094 $\pm$ 0.014 <sup>a,b</sup>	124.4 $\pm$ 22.9 <sup>a,b</sup>	46.4 $\pm$ 14.6 <sup>a,b</sup>	58.9 $\pm$ 13.0 <sup>a,b</sup>

**Notes:** Mice were administered nanocapsules by tail vein injection once a day for 6 days, accompanied by nasal cavity irradiation with 100 mW laser at 670 nm for 15 minutes after administration. Kainic acid was intranasally administered on the seventh day. Seven days later, mice were decapitated after behavioral testing. Optical density of the striatum in three brain slices of each mouse was measured after TH immunohistochemistry; locomotive counts of 10-minute duration were recorded by computer with an infrared-sensitive motion-detection system; TNF- $\alpha$  and IL-1 $\beta$  were determined using ELISA. Data are presented as mean  $\pm$  standard deviation (n=8). Values annotated with <sup>a</sup>( $P<0.01$ ) are as compared to kainic acid group; values annotated with <sup>b</sup>( $P<0.01$ ) are as compared to the no irradiation group with corresponding dose.

**Abbreviations:** TNF- $\alpha$ , tumor necrosis factor alpha; IL-1 $\beta$ , interleukin-1 beta; ELISA, enzyme-linked immunosorbent assay.

and 670 nm irradiation promotes drug release of the nanocapsules. Through this process of anti-neuroinflammation, neurons in brain are protected from degeneration.

## Conclusion

Sasanquasaponin, a bioactive compound isolated from the seeds of *C. oleifera*, can be nanoencapsulated in liposome of soybean lecithin and cholesterol. Phaeophorbide, a natural photosensitizer, can be inserted in the outer shell of the liposome at a certain concentration to form the stable photo responsive nanocapsules. The properties of the nanocapsules are characterized by size distribution, DLC, EE, DSC, TG and TEM, confirming that the phaeophorbide is well-scattered in the lipid phase, and sasanquasaponin is encapsulated in the nanocapsules. The release of sasanquasaponin from the nanocapsules is improved by irradiation at 670 nm, which excites the phaeophorbide to produce singlet oxygen leading to breakdown of lecithin and leakage of the sasanquasaponin nanocapsules. The neuroprotective effects of the nanocapsules are demonstrated by improvement of kainic acid-induced neurodegenerative symptoms in mice. Laser light irradiation of 670 nm on the mouse nasal cavity does little harm, but increases sasanquasaponin release from the nanocapsules and plays an important role in TH expression in mouse brain and behavior amelioration. It is mainly attributed to inflammatory control by the sasanquasaponin. This research provides a nanoencapsulation method of sasanquasaponin with photo responsiveness at red light, which is effective in prevention of mouse brain from neurodegeneration, and holds promise for human clinical application.

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## Disclosure

The authors report no conflicts of interest in this work.

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