

CORE-SHELL CAPSULE COMPRISING MASLINIC ACID

FIELD OF THE INVENTION

The present invention relates to a core-shell capsule comprising maslinic acid. In particular,
5 the present invention relates to a core-shell capsule comprising maslinic acid embedded in a hydrophobic core comprising a solid polysorbate with a polyethylene glycol (PEG) moiety, cross-linked with a polyacid to provide a polyethylene glycol-containing shell surrounding the hydrophobic core.

10 BACKGROUND

Maslinic acid derived from olives has been garnering attention as a dietary supplement because it exhibits many health benefits including anti-inflammatory, antioxidant and antineoplastic, hypoglycemic, cardioprotective and antitumoral activity. Maslinic acid is a
15 pentacyclic triterpenoid. Due to its poor solubility (4mg/L) in aqueous solution, the bioavailability of maslinic acid is low, and suitable formulation of maslinic acid is indispensable to improve the bioavailability of maslinic acid as dietary supplement to provide health benefits. However, very limited works on the formulation of maslinic acid have been reported to improve the bioavailability of maslinic acid. So far, encapsulation of maslinic acid
20 using cyclodextrin has been explored which shows some improvements in dispersion in aqueous solution but it might not improve the bioavailability.

One of the methods to improve the bioavailability of aqueous-insoluble dietary supplements is to dissolve the dietary supplements in certain amphiphilic surfactants. US 6,056,971 and
25 US 6,300,377 disclose dissolving water insoluble dietary supplements in solubilizing agents such as polysorbate, and the bioavailability of formulated coenzyme Q10 was improved (US 6,056,971).

US 6,403,116 teaches dissolving an aqueous solution of coenzyme Q10 in polysorbate. This
30 formulation might provide more encapsulation of coenzyme Q10 in polysorbate to improve the bioavailability, however the capsules formed might not be stable during the formulation processes, storage and after administration of the formulation.

US10,105,293 discloses core-shell particles formed by encapsulating an active ingredient in
35 a hydrophobic core of amphiphilic biocompatible polymer with poly(ethylene glycol) (PEG)

shell being cross-linked by formation of hydrogen bonding with poly(acrylic acid). The active ingredient disclosed in this document is liquid silicone oil and its uses liquid surfactant to solubilize the active ingredient.

- 5 It is therefore desirable to provide a capsule comprising maslinic acid that provides sufficient bioavailability and a process for forming the same that seeks to address at least one of the problems described hereinabove, or at least to provide an alternative.

SUMMARY OF INVENTION

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In accordance with a first aspect of this invention, a core-shell capsule comprising maslinic acid is provided. The core-shell capsule comprises maslinic acid embedded in a hydrophobic core comprising a polysorbate with a polyethylene glycol (PEG) moiety, cross-linked with a polyacid or a salt of polyacid to provide a polyethylene glycol-containing hydrophilic shell
15 surrounding the hydrophobic core.

In one embodiment, the polysorbate is a solid ethoxylated sorbitan ester based on a natural fatty acid. In another embodiment, the polysorbate is a solid ethoxylated sorbitan triester based on a natural fatty acid.

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In one embodiment, the polyacid is a polymer with conjugated carboxylic acid groups. In another embodiment, the salt of polyacid is sodium salt of poly(acrylic acid).

- 25 In accordance with a second aspect of this invention, a process for forming a core-shell capsule is provided. The process comprising melting a solid polysorbate with a polyethylene glycol (PEG) moiety with maslinic acid; blending the melted polysorbate and maslinic acid to form a mixture; cooling the mixture; adding deionized water and homogenizing the mixture under high shear condition to obtain an emulsion; and adding a polyacid or a salt of polyacid to the emulsion to cross-link with the polysorbate by forming hydrogen bonding between the
30 PEG moiety of the polysorbate and repeating units of the polyacid to form a core-shell capsule comprising a hydrophobic core with polyethylene glycol (PEG)-containing shell surrounding the hydrophobic core.

- 35 In one embodiment, the process further comprises adding an acid to the emulsion after cross-linking to adjust the pH of the emulsion to 5 or below.

In one embodiment, the process further comprises freeze-drying the emulsion after the pH of the emulsion is adjusted.

BRIEF DESCRIPTION OF THE DRAWINGS

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The above advantages and features of a core-shell capsule in accordance with this invention are described in the following detailed description and are shown in the drawings:

Figure 1A shows a ^1H NMR spectra of Tween-65/maslinic acid micelles (Tween-65/ maslinic acid with a weight ratio of 4:1, 2.5 wt% in deuterium oxide (D_2O) with dimethylformamide (DMF) as standard) without cross-linking. Figure 1B shows the spectra of a pure poly(acrylic acid) (PAA) (with a molecular weight of about 5,000 Dalton) before mixing with the Tween-65/maslinic acid emulsion. Figure 1C shows the crosslinked Tween-65/maslinic acid micelles after mixing with poly(acrylic acid) at a PAA/PEG molar ratio of 1:1. The * indicates the D_2O residual solvent peak. A little DMF was added in D_2O as standard.

Figure 2 shows the measurements of particle sizes using dynamic light scattering (DLS) technique for the Tween-65/maslinic acid emulsion mentioned in Example 2 and the Tween-65/maslinic acid with poly(acrylic acid) complexes mentioned in Example 7.

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Figure 3 shows the measurements of particle sizes using DLS technique for a series of Tween-65/maslinic acid emulsions with varied Tween-65/maslinic acid weight ratio at the concentration of 1 mg/ml.

Figure 4 shows the ^1H NMR spectra of (A) a pure sodium salt of poly(acrylic acid) (PAA) (with a molecular weight of about 1,200,000 Dalton) before mixing with Tween 65/maslinic acid emulsion (with a solution concentration of 0.25 wt% and pH value adjusted to 5 using hydrochloric acid solution); and (B) crosslinked Tween 65/maslinic acid micelles (with a weight ratio of 4:1, 1.25 wt% in D_2O) after mixing with sodium salt of PAA at a PAA/PEG molar ratio of 0.25:1 and with the pH of the solution adjusted to 5 using hydrochloric acid solution. The * indicates the D_2O residual solvent peak. A little DMF was added in D_2O as standard.

Figure 5 shows the measurements of particle sizes using DLS technique for the Tween 65/maslinic acid-PAA complexes mentioned in Example 10.

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Figure 6 shows the measurements of particle sizes of in situ formed crosslinked capsules and re-dispersed crosslinked capsules after freeze-drying for two formulations: (A) Tween 65/maslinic acid with a weight ratio of 4:1; and (B) Tween 65/maslinic acid with a weight ratio of 1:4. Both formulations are crosslinked using sodium salt of PAA with a cross-linking degree of approximately 10% under pH 5. The concentration used for DLS test was 1 mg/ml.

DETAILED DESCRIPTION

The present invention relates to a core-shell capsule comprising maslinic acid embedded in a hydrophobic core comprising a polysorbate with a polyethylene glycol (PEG) moiety, cross-linked with a polyacid to provide a polyethylene glycol-containing hydrophilic shell surrounding the hydrophobic core. The polysorbate is cross-linked with the polyacid by forming hydrogen bonding with the polyacid.

Suitable polysorbate that can be used in the present invention includes ethoxylated sorbitan ester based on a natural fatty acid such as Tween 60 (or polysorbate 60); and ethoxylated sorbitan triester based on a natural fatty acid (stearic acid) such as Tween 65 (or polysorbate 65). In one embodiment, the polysorbate is a solid ethoxylated sorbitan ester based on a natural fatty acid. In another embodiment, the polysorbate is a solid ethoxylated sorbitan triester based on a natural fatty acid. In yet another embodiment, the polysorbate is Tween 65 (or polysorbate 65). The weight ratio of the polysorbate to the maslinic acid is in a range from 10:1 to 1:10.

The term "polyacid" as used herein refers to a polymer having a plurality of conjugated carboxylic acid groups containing a polyacrylate backbone. Suitable polyacid includes poly(methacrylic acid), copolymers of methacrylic, poly(acrylic acid), copolymers of acrylic acid and their salts thereof. In various embodiments, the polyacid is poly(acrylic acid). In some embodiments, salt of polyacid is used and this includes sodium polyacrylate. The molecular weight of the polyacrylate is from 500 Dalton to 10,000,000 Dalton. The molar ratio of the carboxylic acid groups (or repeating units) of the polyacid to the PEG unit of the polysorbate (PAA/PEG) is in a range from 2:1 to 0.01:1. In the present invention, the polysorbate is cross-linked with the polyacid by forming hydrogen bonding between the PEG moiety and the carboxylic acid groups from the polyacid.

The core-shell capsule may have a particle size in the nano-size range or the micro-size range. The particle size is dependent upon the concentration of the emulsion. The higher the concentration of the emulsion, the bigger the particle size. The higher the weight ratio of the

maslinic acid to polysorbate, the bigger the particle size due to an increase in the amount of maslinic acid embedded in the hydrophobic core of polysorbate. The core-shell capsule may have a particle size ranging from 100 to 1500 nm, preferably 300 to 900nm.

- 5 The core-shell capsule of the present invention is formulated using food grade materials to improve the bioavailability of maslinic acid. The process employed in forming the core-shell capsule is a straight-forward process and it is suitable for scaling up to produce core-shell capsule of maslinic acid due to the commercially available food grade raw materials and the promising results achieved by the core-shell capsule of the present invention. The core-shell capsule of maslinic acid of the present invention has improved bioavailability and the technology developed in the present invention can be applied to develop new products such as nutrient, medicine, cosmetics and functional food products. Maslinic acid is a very effective anti-inflammation natural ingredient which can be applied for use in joint care and skin care products. Water dispersible maslinic acid provides more opportunities for the applications in functional beverages and holds a high potential to have improved bioavailability.

- In a second aspect of the invention, a process for forming a core-shell capsule is provided. In one embodiment, the process comprises melting a solid polysorbate with a polyethylene glycol (PEG) moiety with maslinic acid, and blending the solid polysorbate and maslinic acid to form a mixture; cooling the mixture and adding deionized water and homogenizing the mixture under high shear condition to obtain an emulsion; and adding a polyacid or a salt of polyacid to the emulsion to cross-link with the polysorbate by forming hydrogen bonding between the PEG moiety of the polysorbate and repeating units of the polyacid to form a core-shell capsule comprising a hydrophobic core with polyethylene glycol (PEG)-containing hydrophilic shell surrounding the hydrophobic core.

- In one embodiment, the solid polysorbate and the maslinic acid are melted together at a temperature ranging from 85 to 95°C, preferably 90°C. The solid polysorbate and the maslinic acid can be melted together or maslinic acid can be added into melted polysorbate for blending.

- The solid polysorbate can be anyone selected from the group consisting of ethoxylated sorbitan ester based on a natural fatty acid and ethoxylated sorbitan triester based on a natural fatty acid (stearic acid). In one embodiment, the polysorbate is a solid ethoxylated sorbitan ester based on a natural fatty acid. In another embodiment, the polysorbate is a solid ethoxylated sorbitan triester based on a natural fatty acid. In yet another embodiment,

the polysorbate is Tween 65 (or polysorbate 65). The weight ratio of the polysorbate to the maslinic acid is in a range from 10:1 to 1:10, preferably 4:1 to 1:4.

5 The polyacid or a salt of polyacid can be anyone selected from the group consisting of poly(methacrylic acid), copolymers of methacrylic, poly(acrylic acid), copolymers of acrylic acid and their salts thereof. In various embodiments, the polyacid is a water soluble polyacid, such as poly(acrylic acid). In some embodiments, salt of polyacid is used and this includes sodium salt of poly(acrylic acid), such as sodium polyacrylate.

10 In another embodiment, the process comprises adding a high molecular weight sodium salt of poly(acrylic acid) to the emulsion followed by adjusting the pH of the mixture to 5 or below.

The pH is adjusted by adding a suitable acid to the mixture. Such acid includes, but is not limited to, citric acid, trisodium citrate, hydrochloric acid, acetic acid, fumaric acid and malic acid.
15 acid.

In one embodiment, the process further comprises freeze-drying the emulsion prior to adding polyacid or a salt of polyacid to the emulsion. In another embodiment, the process further comprises freeze-drying after the polyacid is added and the pH of the mixture is adjusted to
20 5 or below.

The process of the present invention eliminates the use of solvent in the process, including the use of liquid surfactant/emulsifier. The emulsifier used in the process of the present invention is a solid polysorbate and it has most hydrophobic properties than other
25 polysorbates. It is common knowledge to add more hydrophilic emulsifier to balance the hydrophobic molecules to achieve water dispersity/solubility. Especially, as taught in the prior art, it should be liquid when used as a solvent/solubilizer. However, in the present invention, hydrophobic solid polysorbate is used. The use of this unconventional strategy by incorporating more hydrophobic solid polysorbate into forming a core-shell capsule produces
30 unexpected results. In addition, the melting point of the solid polysorbate is around 40.6°C but the inventors have found and selected a special temperature process condition of 85 to 95°C, preferably 90°C to achieve the technical effect, which is unexpected. Further, the invention also discloses an advantageous composition of maslinic acid combined with a solid polysorbate only, where the polyacid is added as a separate step. The PEG moiety present
35 in the polysorbate allows hydrogen bonding between the PEG moiety and the carboxylic groups from the polyacid to be formed without the need to add a separate PEG in the process.

To facilitate a better understanding of the present invention, the following examples of specific embodiments are given. In no way should the following examples be read to limit or define the entire scope of the invention. One skilled in the art will recognize that the examples set out below are not an exhaustive list of the embodiments of this invention.

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EXAMPLES

Materials

In the examples that follow, the following materials were used.

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Tween 65 (Guangdong Runhua Chemistry Co. Ltd, China); Maslinic acid (Nutris, Spain); Poly(acrylic acid) (PAA) (molecular weight of about 5,000 Dalton, 50% solution in water; Polysciences, Inc.); Trisodium citrate (Singma-Aldrich Pte Ltd); Sodium polyacrylate (molecular weight of about 1,200,000 Dalton); Citric acid (Singma-Aldrich Pte Ltd).

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Example 1

2.14g of Tween 65 and 0.36g of maslinic acid were melted and blended at 90°C. After cooling down to room temperature, a yellowish solid was obtained. 25 mL of deionized water was added to the yellowish solid and the mixture were homogenized using a high shear mixer (Ultra Turrax T18 basic Homogenizer) until a fine homogeneous emulsion was obtained. The emulsion was further freeze-dried to obtain light yellowish powder.

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Example 2

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2g of Tween 65 and 0.5g of maslinic acid were melted and blended at 90°C. After cooling down to room temperature, a yellowish solid was obtained. 25 mL of deionized water was added to the yellowish solid and the mixture were homogenized using a high shear mixer (Ultra Turrax T18 basic Homogenizer) until a fine homogeneous emulsion was obtained. The emulsion was further freeze-dried to obtain light yellowish powder.

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Example 3

1.67g of Tween 65 and 0.83g of maslinic acid were melted and blended at 90°C. After cooling down to room temperature, a yellowish solid was obtained. 25 mL of deionized water was added to the yellowish solid and the mixture were homogenized using a high shear mixer

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(Ultra Turrax T18 basic Homogenizer) until a fine homogeneous emulsion was obtained. The emulsion was further freeze-dried to obtain light yellowish powder.

Example 4

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1.25g of Tween 65 and 1.25g of maslinic acid were melted and blended at 90°C. After cooling down to room temperature, a yellowish solid was obtained. 25 mL of deionized water was added to the yellowish solid and the mixture were homogenized using a high shear mixer (Ultra Turrax T18 basic Homogenizer) until a fine homogeneous emulsion was obtained. The emulsion was further freeze-dried to obtain light yellowish powder.

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Example 5

0.5g of Tween 65 and 2.0g of maslinic acid were melted and blended at 90°C. After cooling down to room temperature, a yellowish solid was obtained. 25 mL deionized water was added to the yellowish solid and the mixture were homogenized using a high shear mixer (Ultra Turrax T18 basic Homogenizer) until a fine homogeneous emulsion was obtained. The emulsion was further freeze-dried to obtain light yellowish powder.

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Example 6

0.23g of Tween 65 and 2.27g of maslinic acid were melted and blended at 90°C. After cooling down to room temperature, a yellowish solid was obtained. 25 mL of deionized water was added to the yellowish solid and the mixture were homogenized using a high shear mixer (Ultra Turrax T18 basic Homogenizer) until a fine homogeneous emulsion was obtained. The emulsion was further freeze-dried to obtain light yellowish powder.

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Example 7

2g of Tween 65 and 0.5g of maslinic acid were melted and blended at 90°C. After cooling down to room temperature, a yellowish solid was obtained. 25 mL of deionized water was added to the yellowish solid and the mixture were homogenized using a high shear mixer (Ultra Turrax T18 basic Homogenizer) until a fine homogeneous emulsion was obtained. 0.31g of poly(acrylic acid) (PAA) was added to the emulsion and the emulsion was emulsified for about 5 min.

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Trisodium citrate was dissolved in the emulsion while stirring to achieve an emulsion with pH of 5. The final emulsion was further freeze-dried to obtain a white powder. The cross-linking degree was calculated to be about 10% according to the molar ratio of feeding PAA/PEG at 0.1:1.

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Example 8

A known amount of sodium polyacrylate was weighed into clean vial (20 mL). After that, 4 mL of deionized water was added and the mixture was stirred at room temperature for 2 days. By following the same procedures, a series of sodium polyacrylate solutions in water with varied concentrations were prepared. The state of the resultant mixture and its flow ability were checked by tilting the vial to an angle of 90° and keeping the vial in that position for 30 seconds.

Example 9

0.0625g of sodium polyacrylate was added to the fine homogenous emulsion fabricated in Example 2 (25 mL) to obtain a concentration of 0.25 wt% of sodium polyacrylate in the emulsion system. The emulsion was emulsified for about 5 min. The final emulsion was further freeze-dried to obtain white powder.

Example 10

The fine homogeneous emulsion fabricated in Example 2 was further diluted into 3.1 wt% using deionized water. 0.0625g of sodium polyacrylate was added to the diluted emulsion (25 mL) to achieve a concentration of 0.25 wt% of sodium polyacrylate in the emulsion system. The emulsion was emulsified for about 5 min. Citric acid was dissolved in the emulsion while stirring to achieve an emulsion with a pH 5. The final emulsion was further freeze-dried to obtain white powder. The cross-linking degree was calculated to be about 10% according to the molar ratio of the feeding sodium salt of PAA/PEG at 0.1:1.

Example 11

0.0504g of sodium polyacrylate was added to the fine homogeneous emulsion fabricated in Example 5 (25 mL) to achieve a concentration of 0.20 wt% of sodium polyacrylate in the emulsion system. The emulsion was emulsified for about 5 min. Citric acid was dissolved in the emulsion while stirring to achieve an emulsion with a pH 5. The final emulsion was further

freeze-dried to obtain white powder. The cross-linking degree was calculated to be about 10% according to the molar ratio of feeding sodium salt of PAA/PEG at 0.1:1.

Example 12

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Proton nuclear magnetic resonance (^1H NMR) spectroscopy

Proton nuclear magnetic resonance (^1H NMR) spectroscopy was applied for analysis of the samples. It was employed to trace the change of the peaks of poly(acrylic acid) before and after mixing with the emulsion. ^1H NMR spectra were recorded at room temperature on a
10 JEOL ECA 500 MHz NMR spectrometer operating at 500 MHz. Chemical shifts were reported in parts per million (ppm) on the δ scale and were referenced to residual protonated solvent peaks: D_2O spectra were referenced to HDO at δH 4.70.

Figure 1B shows the ^1H NMR spectrum of pure poly(acrylic acid) (PAA) before mixing with the emulsion. Figure 1C shows the ^1H NMR spectrum of crosslinked Tween 65/ maslinic acid micelles after mixing with PAA at a PAA/PEG molar ratio of 1:1. In this formulation, the weight ratio of Tween 65/maslinic acid is 4:1, and the solid content of the emulsion is about 2.5 wt%. A little DMF was added in D_2O as standard in order to monitor the change in the integral
15 intensity of peaks of PAA before and after crosslinking. For the pure PAA (Figure 1B), the ratio of the integral intensity of the peaks at 1.4-2.6 ppm attributed to PAA to the peak of the internal reference DMF at 7.8 ppm was 12.24. This value dropped to 7.21 after PAA was mixed with the emulsion at a PAA/PEG molar ratio of 1:1. These results reflect that the mobility of PAA is reduced due to the formation of complex between PAA and PEG via the
20 hydrogen bonding. According to the drop of the integral intensity of PAA signals, the cross-linking degree was calculated to be 41.4% at the PAA/PEG molar ratio of 1:1.

In Examples 7 and 10-11, cross-linking degree was calculated. The cross-linking degree is described by the molar ratio of the repeating units of poly(acrylic acid) to the polyethylene glycol-containing PEG. In Example 7, poly(acrylic acid) was used and in Examples 10 and
30 11, sodium salt of poly(acrylic acid) were used to illustrate several embodiments. For the sodium salt of poly(acrylic acid), the concentration in aqueous solution was kept at 0.25 wt% and below to get the fluidity as described in Example 8.

In Examples 10 to 11 where sodium salt of poly(acrylic acid) (sodium polyacrylate) was used, the cross-linking also occurred smoothly after the system was adjusted to acidic condition of pH 5. Citric acid was used for this purpose, but not limited as such. It should be noted that
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the cross-linking will not occur without adjusting the system to acidic condition. Figure 4A shows the ^1H NMR spectrum of pure sodium salt of poly(acrylic acid) (PAA) before mixing with the emulsion with a pH of 5. Figure 4B shows the ^1H NMR spectrum of the crosslinked Tween 65/maslinic acid micelles after mixing with the sodium salt of poly(acrylic acid) at a PAA/PEG molar ratio of 0.25:1 under an acidic condition of pH 5. In this embodiment, the weight ratio of Tween 65/maslinic acid is 4:1, and the solid content of the emulsion is 1.25 wt%. A little DMF was added in D_2O as standard in order to monitor the integral intensity change of PAA signals before and after cross-linking. For the pure sodium salt of PAA (Figure 4A), most carboxylate groups were converted into carboxylic acid groups under the acidic condition of pH 5. The ratio of the integral intensity of the peaks at 1.2 to 2.5 ppm attributed to PAA to the peak of the internal reference DMF at 7.8 ppm was 1.84. This value dropped to 0.76 after PAA was mixed with the emulsion at the PAA/PEG molar ratio of 0.25:1 under acidic condition at pH 5. According to the drop of the integral intensity of PAA signals, the cross-linking degree was calculated to be 14.7% at the PAA/PEG molar ratio of 0.25:1 under an acidic condition of pH 5.

Dynamic light scattering (DLS) measurements

Dynamic light scattering (DLS) measurements were performed on the samples using a Zetasizer Nano ZS (Malvern Instruments, Southborough, MA) with a laser light wavelength of 633 nm at a 173° scattering angle. All measurements were taken after the sample solutions were ultrasonicated for 10 min and equilibrated at room temperature for 10 min.

In Example 7, when poly(acrylic acid) was used with a cross-linking degree of about 10%, the crosslinked core-shell capsule becomes bigger in size than the corresponding non-crosslinked Tween 65/maslinic acid micelles according to DLS test (see Figure 2). The diameter of the crosslinked capsule is ca. 250 nm at the emulsion concentration of 100mg/ml. Upon dilution, the trend of size change for the crosslinked capsules looks similar to that of the non-crosslinked Tween 65/maslinic acid micelles. Typically, the size of crosslinked capsules is ca. 640 nm at 1mg/ml, and ca. 380 nm at 1ug/ml.

In Examples 10 and 11, when sodium polyacrylate was used with the cross-linking degree of about 10% according to the molar ratio of feeding sodium salt of PAA/PEG, the crosslinked capsules were bigger in size than the corresponding non-crosslinked Tween 65/maslinic acid micelles and the crosslinked capsules using PAA based on DLS results (see Figure 5). Typically, the size of crosslinked capsules using sodium polyacrylate under acidic condition of pH 5 is ca. 780 nm at 1mg/ml, and ca. 480 nm at 1ug/ml.

The powder of the capsules with PEG-containing shell crosslinked using sodium polyacrylate was obtained using freeze-drying. The powder obtained could be re-dispersed in aqueous solution, and the hydrodynamic size of the re-dispersed samples was measured using DLS.

- 5 Figure 6 shows the DLS results of in situ capsules and re-dispersed capsules from two samples with the weight ratio of Tween 65/maslinic acid to be 4:1 and 1:4, respectively, with the cross-linking degree being about 10% under pH 5. For the sample with the weight ratio of Tween 65/maslinic acid of 4:1, the in situ capsules have a hydrodynamic size of ca. 780 nm, while the re-dispersed sample has a hydrodynamic size of ca. 560 nm. For the sample
10 with the weight ratio of Tween 65/maslinic acid of 1:4, the in situ capsules and re-dispersed capsules have almost the same values of size, of ca. 890 nm. These results show that the freeze-dried capsules have dispersion behaviour similar to in situ capsules in aqueous solutions.

15 **Example 13**

Test to confirm maslinic acid is embedded into the micelles of polysorbate.

- Here Tween 65 was adopted. Tween 65 and maslinic acid were mixed well at 90°C. The weight ratio of Tween 65 to maslinic acid was varied from 6:1 to 1:10 (Examples 1 to 6). After
20 cooling down to room temperature, the mixtures were dispersed in water to form emulsion. The emulsions with a concentration of 10 wt% of the mixture of Tween 65 and maslinic acid were stable for at least 1 month at room temperature when the weight ratio of Tween to maslinic acid was in the range from 6:1 to 1:4. However, when the Tween 65/maslinic acid weight ratio was further reduced to 1:10, the emulsion was stable for 3 to 5 days and then
25 phase separation occurred.

- As a typical example, Figure 1A shows ^1H NMR spectrum of the mixture of maslinic acid and Tween 65 at a weight ratio of 1:4 in D_2O . Only the peaks of protons attributed to PEG around 3.5 ppm are observed. The peaks attributed to maslinic acid cannot be observed totally. This
30 reflects that maslinic acid is embedded in the hydrophobic core of Tween 65 micelles, and the hydrophobic core has very low access to water. Therefore, it can be concluded that the core-shell capsules are formed with PEG as shells and aliphatic segments and maslinic acid as the cores.

- 35 The size of maslinic acid loaded Tween 65 emulsion in aqueous solution was determined using dynamic light scattering (DLS), and Figure 2A shows the dependence of the size on the concentration of emulsion of Tween 65/maslinic acid with a weight ratio of 4:1. An

emulsion with a concentration of 10 wt% was used as a stock solution for dilution to the targeted concentration.

5 The diameter of the maslinic acid loaded Tween 65 emulsion is ca. 80 nm at the emulsion concentration of 100mg/ml. It may not be the actual size of maslinic acid loaded Tween-65 micelles because the emulsion at this concentration is very opaque and the laser beam for DLS test (633 nm) was not easy to pass through the emulsion during the test. Once the original emulsion was diluted to 10 mg/ml, multiple peaks were found during the test with a relative stable peak at ca. 260 nm and another peak usually bigger than 1000 nm. This
10 observation indicated that under such high concentration, the individual micelles get bigger due to micelles merging co-exist in the emulsion system. With the concentration further diluted to 1 mg/ml and below, well resolved single peak was observed, indicating a stable micelles system. The size of micelles is ca. 400 nm at 1mg/ml, and it keeps relative stable until 1ug/ml (ca. 300 nm).

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The above experiment clearly shows that the Tween-65/maslinic acid emulsion system is stable upon dilution.

Figure 3 shows the hydrodynamic size of the formed micelles from the mixture of Tween 65
20 and maslinic acid of different weight ratio at the concentration of 1 mg/ml. For pure Tween 65, two peaks at 38nm and 160nm are observed. When the weight ratio of Tween-65/maslinic acid is changed from 6:1 to 1:4, the size of the micelles increases from ca. 440 nm to ca. 920 nm. This is reasonable because more maslinic acid is embedded in the hydrophobic cores of Tween 65.

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Although embodiments of the present invention have been shown and described, the present invention is not limited to the described embodiments. Instead, it would be appreciated by those skilled in the art that changes may be made to the embodiments without departing from the scope of the invention, the scope of which is set forth in the following claims.

CLAIMS

1. A core-shell capsule comprising maslinic acid embedded in a hydrophobic core comprising a polysorbate with a polyethylene glycol (PEG) moiety, cross-linked with a polyacid or a salt of polyacid to provide a polyethylene glycol-containing hydrophilic shell surrounding the hydrophobic core.
2. The core-shell capsule according to claim 1, wherein the polysorbate is a solid ethoxylated sorbitan ester based on a natural fatty acid.
3. The core-shell capsule according to claim 1, wherein the polysorbate is a solid ethoxylated sorbitan triester based on a natural fatty acid.
4. The core-shell capsule according to claim 3, wherein the polysorbate is Tween 65.
5. The core-shell capsule according to claim 1, wherein the polyacid is a polymer with conjugated carboxylic acid groups.
6. The core-shell capsule according to claim 1, wherein the polyacid is a polymer with conjugated carboxylic acid groups with polyacrylate as the backbone.
7. The core-shell capsule according to claim 6, wherein the polyacid is selected from the group consisting of poly(methacrylic acid), copolymers of methacrylic acid, poly(acrylic acid), copolymers of acrylic acid or their salts thereof.
8. The core-shell capsule according to claim 1, wherein the molar ratio of the repeating unit of the polyacid to the polyethylene glycol (PEG) unit of the polysorbate is in the range from 2:1 to 0.01:1.
9. The core-shell capsule according to claim 1, wherein weight ratio of polysorbate to maslinic acid is in a range from 10:1 to 1:10.
10. The core-shell capsule according to claim 1, wherein the core-shell capsule has a particle size ranging from 100 to 1500 nm.
11. A process for forming a core-shell capsule, the process comprising:
melting a solid polysorbate with a polyethylene glycol (PEG) moiety with maslinic acid;

blending the melted polysorbate and maslinic acid to form a mixture;
cooling the mixture;
adding deionized water and homogenizing the mixture under high shear condition to
obtain an emulsion; and

5 adding a polyacid or a salt of polyacid to the emulsion to cross-link with the
polysorbate by forming hydrogen bonding between the PEG moiety of the polysorbate and
repeating units of the polyacid to form a core-shell capsule comprising a hydrophobic core
with polyethylene glycol (PEG)-containing hydrophilic shell surrounding the hydrophobic
core.

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12. The process according to claim 11, wherein the solid polysorbate and the maslinic
acid are melted together at a temperature ranging from 85°C to 95°C.

13. The process according to claim 11, further comprising:

15 adding an acid to the emulsion after cross-linking to adjust the pH of the emulsion to
5 or below.

14. The process according to claim 11, wherein the solid polysorbate is a solid
ethoxylated sorbitan ester based on a natural fatty acid or a solid ethoxylated sorbitan triester
20 based on a natural fatty acid (stearic acid).

15. The process according to claim 14, wherein the solid polysorbate is Tween 65.

16. The process according to claim 11, wherein the weight ratio of the polysorbate to the
25 maslinic acid is in a range from 10:1 to 1:10.

17. The process according to claim 11, wherein the polyacid is selected from the group
consisting of poly(methacrylic acid), copolymers of methacrylic acid, poly(acrylic acid),
copolymers of acrylic acid or their salts thereof.

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18. The process according to claim 17, wherein the salt of polyacid is sodium polyacrylate.

19. The process according to claim 11, wherein the molar ratio of the repeating units of
the polyacid to the polyethylene glycol (PEG) unit of the polysorbate is in the range from 2:1
35 to 0.01:1.

20. The process according to claim 13, further comprising freeze-drying the emulsion after the pH of the emulsion is adjusted.