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(54) Title: PIGMENT-LOADED SOLID LIPID NANOPARTICLES

(57) Abstract: Solid lipid nanoparticles (SLNs) and suspensions thereof in an aqueous phase are provided, in which the SLNs have high content of oil-soluble pigment and high stability. A food product, such as a beverage, comprising said SLNs is also provided.

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PIGMENT-LOADED SOLID LIPID NANOPARTICLES

TECHNICAL FIELD

Solid lipid nanoparticles (SLNs) are provided, in which said nanoparticles comprise an oil-soluble pigment and a high-melting lipid. A suspension comprising such SLNs in an aqueous phase is also provided.

BACKGROUND

Natural pigments such as carotenoids and chlorophylls are generally unstable in the presence of light, heat or oxygen. Additionally, such pigments tend to be insoluble in water, and stabilizing matrix formulations are typically required when such pigments are used in aqueous environments, e.g. in foodstuffs.

Solid lipid nanoparticles (SLNs) are formulations based on small, spherical particles. SLNs contain an active substance with the lipid core, and one or more surfactants are typically used to promote stability and dispersibility in an aqueous phase.

SLNs which contain carotenes are known from e.g. *Helgason et al.* J. Agric. Food. Chem. 2009, 57, 8033-8040 and *Gutiérrez et al.* Trends in Food Science & Technology, 32 (2013) 73-83.

The light-sensitive nature of pigments means that, increased pigment concentration in an SLN leads to increased uptake and concentration of light energy within the SLN. This can - in turn – lead to degradation of the pigment and/or SLN, see e.g. *Helgason et al.* ibid. Known pigment-containing SLN formulations typically have low pigment concentration.

A particular problem for pigment-containing SLNs is therefore to achieve increased pigment concentration in the SLN, while maintaining stability. Increasing the pigment concentration allows savings in the manufacture and use of the SLNs, as less SLN can be used to achieve the same pigmenting effect in e.g. a foodstuff. Additionally, the SLN itself should form a stable aqueous suspension in an aqueous environment.

The present technology aims to address such problems in pigment-containing SLNs.

SUMMARY OF THE INVENTION

So, in a first aspect, a solid lipid nanoparticle (SLN) is provided which comprises:

a. a core comprising:

i. a lipid having a melting point above 40ºC, and

ii. an oil-soluble pigment

b. a dual surfactant system comprising

i. a polysorbate, and

ii. a phospholipid

wherein the ratio of polysorbate:phospholipid (i:ii) in said dual surfactant system is between 10:1 and 1:10 and the amount of pigment is 5-40% w/w of said SLN.

In a further aspect, a suspension comprising said solid lipid nanoparticles (SLNs) as defined herein in an aqueous phase is provided.

In yet a further aspect, a method for producing a suspension of solid lipid nanoparticles (SLNs) as described herein is provided, said method comprising the steps of:

A. Providing a liquid oil phase comprising (a) a lipid having a melting point above 40ºC; and (b) an oil-soluble pigment, and heating said liquid oil phase so as to dissolve the oil-soluble pigment;

B. Providing an aqueous phase comprising a dual surfactant system (c), which comprises (i) a polysorbate, and (ii) a phospholipid, such that the ratio of polysorbate:phospholipid (i:ii) is between 10:1 and 1:10;

C. Mixing said liquid oil phase from step A. with said aqueous phase from step B. in a high shear mixer to create an emulsion;

D. Passing the emulsified mixture from step C through a homogenizer;

E. Cooling the homogenized mixture from step D, thereby providing a suspension of solid lipid nanoparticles (SLNs).

A suspension is provided comprising first solid lipid nanoparticles (SLN-1) and second solid lipid nanoparticles (SLN-2) in a single aqueous phase, wherein each of SLN-1 and SLN-2 are as described herein, and wherein the oil-soluble pigments in each of SLN-1 and SLN-2 are different.

A food product, preferably a beverage, is provided, comprising solid lipid nanoparticles as described herein.

The use of solid lipid nanoparticles as described herein as a colorant in a food product, such as a beverage is also provided.

DETAILED DISCLOSURE

Solid lipid nanoparticles (SLNs) are provided, which have a particular use in food products such as beverages.

In the context of the present invention SLNs are solid at 25ºC, and preferably have a particle size of less than 300nm. Particle size being defined as the Z-average measured by dynamic light scattering.

In a first aspect, a solid lipid nanoparticle (SLN) comprising (a) a core and (b) a dual surfactant system is provided. It has been discovered that SLNs with particular compositions (especially those with a particular dual surfactant system) have improved stability over other compositions.

The core (a) of said SLN comprises (i) a lipid having a melting point above 40ºC, and (ii) an oil-soluble pigment. During production, a mixture of the lipid and the oil-soluble pigment are warmed together, so that the pigment dissolves in the lipid. The pigment thus becomes homogeneously blended in the lipid. The pigment remains non-crystalline upon solidification of the lipid.

The lipid is the major component of the SLN core, and is typically present in an amount of between 30 and 70% w/w of said SLN, preferably between 40 and 65% w/w, more preferably 45 and 65% w/w. The lipid is typically a saturated lipid and may be but are not limited to a fully saturated triglyceride, such as e.g. fully hydrogenated sunflower oil, fully hydrogenated

rapeseed oil fully hydrogenated palm oil or fully hydrogenated soybean oil. The lipid preferably has a melting point above 40ºC, such as above 45ºC, such as above 50ºC. Further the lipid preferably has a melting point below 100ºC, such as below 98ºC, such as below 95ºC, such as below 85ºC, such as below 75ºC.

The oil-soluble pigment is the minor component of the SLN core and is present in an amount of between 5 and 40% w/w of said SLN, such as between 5 and 25% w/w, preferably between 5 and 20% w/w. Oil-soluble pigments useful in the present technology are naturally-occurring oil-soluble pigments, such as e.g. carotenoids or chlorophylls. Particular carotenoids include α-carotene, β-carotene, lycopene, lutein, bixin and norbixin. Of these, β-carotene is preferred.

The dual surfactant system (b) of said SLN is located at the surface of the SLN, and acts both to stabilize the SLN and to ensure its miscibility in aqueous systems. The dual surfactant system (b) is present in an amount of between 20 and 70% w/w, 25 and 75% w/w, 25 and 50% w/w, suitably between 30 and 40% w/w of said SLN.

The dual surfactant system (b) comprises (i) a polysorbate, and (ii) a phospholipid, and the ratio of polysorbate:phospholipid (i:ii) in said dual surfactant system is between 10:1 and 1:10. In a particular embodiment the ratio is between 5:1 and 1:5, such as 4:1 and 1:4, such as 2:1 and 1:2. Suitably, the polysorbate and phospholipid components are present in equal amounts, or an excess of polysorbate is present. Suitably, therefore, the ratio of polysorbate:phospholipid (i:ii) in said dual surfactant system is between 10:1 and 1:1. In a particular embodiment the ratio is between 5:1 and 1:1, such as 4:1 and 1:1, such as 2:1 and 1:1. As an alternative, the ratio of polysorbate:phospholipid (i:ii) in said dual surfactant system is between 1:1 and 1:10. In a particular embodiment the ratio is between 1:1 and 1:5, such as 1:1 and 1:2.

Polysorbates are emulsifiers based on ethoxylated sorbitan esterified with fatty acids. Typically said polysorbate is polysorbate 80, although other polysorbates such as polysorbate 60, polysorbate 40 and polysorbate 20 may be used.

The phospholipid according to one particular embodiment is selected from sunflower lecithin, soy bean lecithin, cotton seed lecithin, rape seed lecithin or egg yolk lecithin.

The SLNs additionally comprise an antioxidant, suitably a tocopherol such as α-tocopherol. The antioxidant may be present in an amount of between 1-10 % (such as around 5%) w/w of said SLN. Such SLNs have improved stability over other SLNs.

The SLN according to this technology suitably have a mean particle size, measured by dynamic light scattering, of 100-150nm. Particle size is determined according to the method defined in the examples, and is provided in terms of Z-average (average particle size) and PDI (polydispersity index).

In a second aspect, a suspension comprising solid lipid nanoparticles (SLNs) as described herein in an aqueous phase is provided.

It has been found that it is possible to prepare a suspension with high lipid and pigment content with the particular SLNs of the invention. In order to use SLN suspensions as food coloring additives, it is necessary to have a high concentration of pigment. If the concentration is <1%, the required addition of suspension to a product to obtain a coloring property would be so high that issues with off-flavor and composition changes in the final product i.e. a food application would arise. With the present invention it is possible to reach a pigment content above 1% w/w in the suspension, and thereby make it interesting for the color industry. At the same time, increasing the net content of pigment in the suspension also limits the net amount of product needed to be produced and transported making production easier and more economically feasible.

Suitably, the suspension consists of SLNs as defined herein in an aqueous phase; i.e. no other components are present.

In terms of the amounts of each component, the suspension may comprise:

i. a lipid having a melting point above 40ºC, in an amount of 10 to 35% w/w of the total suspension; and

ii. an oil-soluble pigment, in an amount of 1 to 20% w/w of the total suspension;

iii. a dual surfactant system, in an amount of 1 to 25% w/w of the total suspension.

The lipid having a melting point above 40ºC is typically present in an amount of 10 to 35% preferably 10 to 20% w/w, more preferably 14 to 18% w/w of the total suspension.

The phospholipid is typically present in an amount of 0.2 to 10%, 0.5 to 8%, 1 to 7%, 1 to 6% w/w, preferably 1 to 5% w/w of the suspension.

The polysorbate is typically present in an amount of 1 to 20% w/w, such as 2 to 15% such as 5 to 10%.

The oil-soluble pigment is typically present in an amount of 1 to 20% w/w, 1 to 10% preferably 1 to 5% w/w of said suspension.

The amounts of each component in the suspension are given in w/w % of the total suspension. The remaining weight of the suspension – after all components are included – is made up by water.

The aqueous phase of said suspension preferably comprises a pH adjustment agent such that the pH of the suspension 5 or less, preferably 3 or less. The addition of pH adjustment agent also improves microbiological stability.

The aqueous phase may also comprise a food preservative such as a sorbate salt, particularly potassium sorbate. Other additives may be carbohydrates such as glucose syrup, dextrose or sugar, or organic acids, antioxidants and antimicrobials.

The SLNs provided herein may be used in admixtures with one or more additional pigments. Such additional pigments may be in crystalline form. In one particular aspect, a coloring composition may comprise an SLN suspension as defined herein, and a suspension of carotenoid crystals with a mean particle size of below 3 microns stabilized with emulsifiers or hydrocolloids. If the pigment in the SLN suspension defined herein is a carotene the suspension would have a yellow to orange shade. If the crystalline pigment is also a carotene, the crystals will have an orange to reddish shade. By mixing the SLN suspension with the crystals it is possible to yield any orange shade. In a particular embodiment the carotene is a beta-carotene.

The SLNs provided herein may be used in admixtures, in which different SLNs in the same aqueous phase comprise different pigments. In a third aspect, therefore a suspension comprising first solid lipid nanoparticles (SLN-1) and second solid lipid nanoparticles (SLN-2) in a single aqueous phase is provided. Each of SLN-1 and SLN-2 are as defined herein; however, the oil-soluble pigments in each of SLN-1 and SLN-2 are different. Two pigments are termed "different" if their visible absorption spectra differ. In this manner, SLNs with different pigments can be used as building-blocks to provide a desired pigment in admixture.

A food product, an animal feed, a cosmetic or a pharmaceutical product, comprising solid lipid nanoparticles as defined herein is also provided. The SLNs of this technology are particularly suited to liquid food products, preferably beverages.

A further aspect of the present technology is the use of solid lipid nanoparticles as described herein as a colorant in a food product (such as a beverage), an animal feed, a cosmetic or a pharmaceutical product.

A method for producing a suspension of solid lipid nanoparticles (SLNs) is also provided. The method comprises the general steps of:

A. Providing a liquid oil phase comprising (a) a lipid having a melting point above 40ºC; and (b) an oil-soluble pigment; and heating said liquid oil phase so as to dissolve the oil-soluble pigment

B. Providing an aqueous phase comprising a dual surfactant system (c), which comprises (i) a polysorbate, and (ii) a phospholipid, such that the ratio of polysorbate:phospholipid (i:ii) is between 10:1 – 1:10;

C. Mixing said liquid oil phase from step A. with said aqueous phase from step B. in a high shear mixer to create an emulsion;

D. Passing the emulsified mixture from step C through a homogenizer;

E. Cooling the homogenized mixture from step D, thereby providing a suspension of solid lipid nanoparticles (SLNs).

EXAMPLES

Method for measuring particle size:

The lipid particle size was determined using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments Ltd, UK) using 10 mm polystyrene cuvettes. Calculations were conducted based on a refractive index of 1.59. The Z-average (Z-ave) refers to average particle size and the PDI refers to the polydispersity index.

Method for determination of content of beta-carotene:

The content of β-carotene in the particles was determined as follows: The mixture was diluted with water to a β-carotene concentration around 0.06 mg/mL. This solution was then diluted by a factor 50 in acetone. The sample concentration was measured using a conventional Vis spectrophotometer (VWF V-3000PC). The content of β-carotene was calculated using the extension coefficient, $E^{1\%}_{1cm}$, 2559 at 454 nm

Example 1:

In beaker A, 80.0 g of fully hydrogenated sunflower oil and 5.0 g α-tocopherol was mixed and heated to 165°C. 15.0 g crystalline β-carotene was added and dissolved.
In beaker B, 40.0 g of polysorbate 80 and 10.0 g of sunflower lecithin were dissolved in 348.0 g demineralized water and heated to 80°C. Beaker A and B were mixed using a high-shear mixer at 20.000 RPM. The mixture was then passed through a two-valve homogenizer 6 times at a pressure of 800 bar while maintained at 80°C. The homogenized mixture was then cooled to 25°C under stirring (150 rpm). 0.50 g potassium sorbate was added and pH was regulated below 3.00 with citric acid.

Particle size was determined using dynamic light scattering. Z-ave = 144.9 nm, PDI = 0.183. The particle size was stable over a two-month period.

Table 1 - Particle size over time for example 1

Day 0		After 2 weeks		After 1 month		After 2 months	
Z-ave	PDI	Z-ave	PDI	Z-ave	PDI	Z-ave	PDI
144.9 nm ±0.557 nm	0.183	144.1 nm ±1.17 nm	0.191	146.1 nm ±1.78	0.207	144.5 nm ±1.18 nm	0.200

Concentration was determined to be 2.41 %w/w.

Example 2:

In beaker A, 70.0 g of fully hydrogenated sunflower oil and 5.0 g α-tocopherol was mixed and heated to 165°C. 25.0 g crystalline β-carotene was added and dissolved. In beaker B, 40.0 g of polysorbate 80 and 10.0 g of sunflower lecithin were dissolved in 348.0 g demineralized water and heated to 80°C. Beaker A and B were mixed using a high-shear mixer at 20.000 RPM. The mixture was then passed through a two-valve homogenizer 6 times at a pressure of 800 bar while maintained at 80°C. The homogenized mixture was then cooled to 25°C under stirring (150 rpm). 0.50 g potassium sorbate was added and pH was regulated below 3.00 with citric acid.

Particle size was determined using dynamic light scattering. Z-average = 167.6 nm, PDI = 0.212. The particle size was stable over a two-month period.

Table 2 - Particle size over time for example 2

Day 0		After 2 weeks		After 1 month		After 2 months	
Z-ave	PDI	Z-ave	PDI	Z-ave	PDI	Z-ave	PDI
167.6 nm ±0.520 nm	0.212	170.5 nm ±1.92 nm	0.221	174.3 nm ±3.48 nm	0.206	169.7 nm ±0.950 nm	0.221

The beta-carotene concentration was determined to be 4.30% w/w.

Example 3:

In beaker A, 90.0 g of fully hydrogenated sunflower oil and 5.0 g α-tocopherol was mixed and heated to 165°C. 5.0 g crystalline β-carotene was added and dissolved. In beaker B, 40.0 g of polysorbate 80 and 10.0 g of sunflower lecithin were dissolved in 348.0 g demineralized water and heated to 80°C. Beaker A and B were mixed using a high-shear mixer at 20.000 RPM. The mixture was then passed through a two-valve homogenizer 6 times at a pressure of 800 bar while maintained at 80°C. The homogenized mixture was then cooled to 25°C under stirring (150 rpm). 0.50 g potassium sorbate was added and pH was regulated below 3.00 with citric acid.

Particle size was determined using dynamic light scattering. Z-ave = 122.8 nm, PDI = 0.171. The particle size was stable over a two-month period.

Table 3 - Particle size over time for example 3

Day 0		After 2 weeks		After 1 month		After 2 months	
Z-ave	PDI	Z-ave	PDI	Z-ave	PDI	Z-ave	PDI
122.8 nm ±0.702 nm	0.171	124.0 nm ±0.560 nm	0.150	123.6 nm ±1.60 nm	0.168	123.6 nm ±1.27 nm	0.148

The beta-carotene concentration was determined to be 0.92 % w/w.

Example 4:

In beaker A, 80.0 g of fully hydrogenated sunflower oil and 5.0 g α-tocopherol was mixed and heated to 165°C. 15.0 g crystalline β-carotene was added and dissolved. In beaker B, 25.0 g of polysorbate 80 and 25.0 g of sunflower lecithin were dissolved in 348.0 g demineralized water and heated to 80°C. Beaker A and B were mixed using a high-shear mixer at 20.000 RPM. The mixture was then passed through a two-valve homogenizer 6 times at a pressure of 800 bar while maintained at 80°C. The homogenized mixture was then cooled to 25°C under stirring (150 rpm). 0.50 g potassium sorbate was added and pH was regulated below 3.00 with citric acid.

Particle size was determined using dynamic light scattering. Z-ave = 153.3 nm ± 1.65, PDI = 0.192.

Concentration was determined to be 2.84% w/w.

Example 5:

In beaker A, 92.5 g of fully hydrogenated sunflower oil and 5.0 g α-tocopherol was mixed and heated to 165°C. 2.50 g crystalline β-carotene was added and dissolved. In beaker B, 40.0 g of polysorbate 80 and 10.0 g of sunflower lecithin were dissolved in 348.0 g demineralized water and heated to 80°C. Beaker A and B were mixed using a high-shear mixer at 20.000 RPM. The mixture was then passed through a two-valve homogenizer 6 times at a pressure of 800 bar while maintained at 80°C. The homogenized mixture was then cooled to 25°C under stirring (150 rpm). 0.50 g potassium sorbate was added and pH was regulated below 3.00 with citric acid.

Particle size was determined using dynamic light scattering. Z-ave = 119.9 nm, PDI = 0.150. The particle size was stable over a two-month period.

Table 4 - Particle size over time for example 5

Day 0		After 2 weeks		After 1 month		After 2 months	
Z-ave	PDI	Z-ave	PDI	Z-ave	PDI	Z-ave	PDI
119.9 nm ±0.681 nm	0.150	121.0 nm ±0.660 nm	0.143	123.5 nm ±1.27 nm	0.140	119.9 nm ±0.839 nm	0.156

Concentration was determined to be 0.449% w/w.

Example of application:

The product from example 1 was tested in a soft drink medium. Product was dispersed in soft-drink medium to a total β-carotene concentration of approximately 0.2 g/L. The product was tested for heat stability by exposing the soft-drink to 92°C for 40 seconds. The soft drink was also tested for ring formation in plastic bottles. Test bottles were left to stand or lay for 4 weeks after which they were analyzed for ring formation on the plastic. The product passed both application tests. Table 5 shows the results of the heating test:

Table 5 – Application data from heat stability study

		Before heat treatment	After heat treatment
Datacolor	L*	67.21	68.06
	C*	117.64	118.59
	h*	74.46	75.07
	DE2000	REF	0.93

As can be seen from the heat stability study, the products show low color difference (DE2000) before and after heat treatment.

Comparative example 1:

A preparation was made without adding a phospholipid. In beaker A, 80.0 g of fully hydrogenated sunflower oil and 5.0 g α-tocopherol was mixed and heated to 165°C. 15.0 g crystalline β-carotene was added and dissolved. In beaker B, 50.0 g of polysorbate 80 were dissolved in 348.0 g demineralized water and heated to 80°C. Beaker A and B were mixed using a high-shear mixer at 20.000 RPM. The mixture was then passed through a two-valve homogenizer 6 times at a pressure of 800 bar while maintained at 80°C. The homogenized mixture was then cooled to 25°C under stirring (150 rpm). 0.50 g potassium sorbate was added and pH was regulated below 3.00 with citric acid buffer.

The sample was unstable to moderate shaking/stirring, leading to irreversible agglomeration of the sample. The agglomerated product was not dispersible in water and could as such not be used. Particle size or color could as such not be determined in application.

Although the technology has been described with reference to a number of aspects and examples, the skilled person will be able to combine features from different aspects and examples. Further details of the technology are evident from the appended set of claims.

CLAIMS

1. A solid lipid nanoparticle (SLN) comprising

   a. a core comprising:

      i. a lipid having a melting point above 40ºC, and

      ii. an oil-soluble pigment

   b. a dual surfactant system comprising

      i. a polysorbate, and

      ii. a phospholipid

   wherein the ratio of polysorbate:phospholipid (i:ii) in said dual surfactant system is between 10:1 and 1:10 and the amount of pigment is 5-40% w/w of said SLN.

2. The SLN according to any one of the preceding claims, wherein said SLN additionally comprises an antioxidant, suitably a tocopherol such as α-tocopherol.

3. The SLN according to any one of the preceding claims, wherein said antioxidant is present in an amount of between 1 and 10% w/w of said SLN.

4. The SLN according to any one of the preceding claims, wherein said lipid is present in an amount of between 30 and 70% w/w of said SLN.

5. The SLN according to claim 4, wherein said lipid is a fully saturated triglyceride, such as e.g. fully hydrogenated sunflower oil, fully hydrogenated rapeseed oil fully hydrogenated palm oil or fully hydrogenated soybean oil.

6. The SLN according to any one of the preceding claims, wherein said oil-soluble pigment is a carotenoid or a chlorophyll.

7. The SLN according to claim 6, wherein the carotenoid is a lycopene or a beta-carotene.

8. The SLN according to any one of the preceding claims, wherein said dual surfactant system is present in an amount of between 20 and 75% w/w of said SLN.

9. The SLN according to any one of the preceding claims, wherein the ratio of polysorbate:phospholipid (i:ii) in said dual surfactant system is between 10:1 and 1:1.

10. The SLN according to any one of the preceding claims, wherein said polysorbate is polysorbate 80.

11. The SLN according to any one of the preceding claims, wherein said phospholipid is selected from sunflower lecithin, soy bean lecithin, cotton seed lecithin, rape seed lecithin or egg yolk lecithin.

12. The SLN according to any one of the preceding claims, having a mean Z-average particle size, measured by dynamic light scattering, of less than 500nm, preferably less than 300nm, more preferably between 100 and 150nm.

13. A suspension comprising solid lipid nanoparticles (SLNs) according to any one of the preceding claims in an aqueous phase.

14. The suspension according to claim 13, wherein said suspension comprises:

i. a lipid having a melting point above 40ºC, in an amount of 10-35% w/w of the total suspension; and

ii. an oil-soluble pigment, in an amount of 1-20% w/w of the total suspension;

iii. a dual surfactant system, in an amount of 1-25% w/w of the total suspension.

15. The suspension according to any one of claims 13-14, comprising said lipid having a melting point above 40ºC in an amount of 5-30% w/w, preferably 10-20% w/w more preferably 14-18 % w/w of the total suspension.

16. The suspension according to any one of claims 13-15, comprising said phospholipid in an amount of 2-10% w/w, preferably 2-5% w/w of the suspension.

17. The suspension according to any one of claims 13-16, comprising said polysorbate in an amount of 5-10% w/w.

18. The suspension according to any one of claims 13-17, wherein said oil-soluble pigment is present in an amount of 0.5-20% w/w, preferably 1-5% w/w of said suspension.

19. The suspension according to any one of claims 13-18, wherein said aqueous phase comprises a pH buffer such that the pH of the suspension 5 or less, preferably 3 or less.

20. The suspension according to any one of claims 13-19, wherein said aqueous phase comprises a food preservative such as a sorbate salt, particularly potassium sorbate.

21. A suspension comprising first solid lipid nanoparticles (SLN-1) and second solid lipid nanoparticles (SLN-2) in a single aqueous phase, wherein each of SLN-1 and SLN-2 are defined as per any one of claims 1-13, and wherein the oil-soluble pigments in each of SLN-1 and SLN-2 are different.

22. A food product, preferably a beverage, comprising solid lipid nanoparticles according to any one of claims 1-12, or a suspension according to any one of claims 13-21.

23. The use of solid lipid nanoparticles according to any one of claims 1-12 or a suspension according to any one of claims 13-21 as a colorant in a food product, such as a beverage.

24. A method for producing a suspension of solid lipid nanoparticles (SLNs) according to any one of claims 13-21, said method comprising the steps of:

A. Providing a liquid oil phase comprising (a.) a lipid having a melting point above 40ºC; and (b.) an oil-soluble pigment, and heating said liquid oil phase so as to dissolve the oil-soluble pigment;
B. Providing an aqueous phase comprising a dual surfactant system (c), which comprises (i) a polysorbate, and (ii) a phospholipid, such that the ratio of polysorbate:phospholipid (i:ii) is between 10:1 and 1:10;
C. Mixing said liquid oil phase from step A. with said aqueous phase from step B. in a high shear mixer to create an emulsion;
D. Passing the emulsified mixture from step C through a homogenizer;
E. Cooling the homogenized mixture from step D, thereby providing a suspension of solid lipid nanoparticles (SLNs).

25. A method for coloring a food, a beverage, a dietary supplement or a pharmaceutical product with the use of the SLN suspension according to any of claims 13-21.

26. A coloring composition comprising the SLN suspension according to any of claims 13-21 and a suspension of carotene crystals with a mean particle size of below 3 microns stabilized with emulsifiers or hydrocolloids or a combination

27. Use of a coloring composition according to claim 26 for coloring of a food, a beverage, a dietary supplement or a pharmaceutical product.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/059773

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23L2/58 A23D7/005 A23D7/01 C09B61/00 C09B47/00
C09B67/00 A23L29/10 A23L5/44 A23L5/47 C09B67/08
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23L A23D C09B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AKHOOND ZARDINI ALI ET AL: "Production and characterization of nanostructured lipid carriers and solid lipid nanoparticles containing lycopene for food fortification", JOURNAL OF FOOD SCIENCE AND TECHNOLOGY, SPRINGER (INDIA) PRIVATE LTD, INDIA, vol. 55, no. 1, 30 October 2017 (2017-10-30), pages 287-298, XP036414384, ISSN: 0022-1155, DOI: 10.1007/S13197-017-2937-5 [retrieved on 2017-10-30] page 288, paragraph Methods - page 297, paragraph Food application of developed nanoparticles; figure 4A; tables 1,2	1-25

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 June 2019	21/08/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Munteanu, I

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Harald F. Krug: "Nanomaterial, Dokumentkennung RD-14-02336", R?mpp Online, Version 3.26, 1 April 2009 (2009-04-01), XP055035458, Retrieved from the Internet: URL:http://www.roempp.com/prod/roempp.php [retrieved on 2012-08-14] the whole document	1,12
A	US 9 907 758 B2 (PANJAB UNIVERSITY DEPARTMENT OF BIOTECHNOLOGY (DBT), PUNJAB (IN)) 6 March 2018 (2018-03-06) column 6, line 12 - column 8, line 33; claims 1-20; examples 1,2,3	1-25
A	US 9 925 149 B2 (NANOSPHERE HEALTH SCIENCES LLC [US]) 27 March 2018 (2018-03-27) examples 1-10	1-24
A	IOANA LACATUSU ET AL: "Lipid nanocarriers based on natural compounds: An evolving role in plant extract delivery : Lipid nanocarriers based on natural compounds", EUROPEAN JOURNAL OF LIPID SCIENCE AND TECHNOLOGY., vol. 116, no. 12, 1 August 2014 (2014-08-01), pages 1708-1717, XP055516879, DE ISSN: 1438-7697, DOI: 10.1002/ejlt.201300488 page 1709, paragraph 2 Materials and methods - page 1710, paragraph 2.3; table 1	1-24
A	RO 128 703 A2 (UNIV POLITEHNICA DIN BUCURESTI [RO]) 30 August 2013 (2013-08-30) page 3, paragraph 4 - page 6, paragraph 1; claims 1-8; examples 2,3	1-24

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2019/059773

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-25

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-25

   Solid lipid nanoparticles loaded with pigments

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2. claims: 26, 27

   Coloring composition comprising a solid lipid nanoparticles suspension and a suspension of carotenoid crystals

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/059773

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 9907758 B2	06-03-2018	US 2014348938 A1	27-11-2014
		WO 2013105026 A1	18-07-2013
US 9925149 B2	27-03-2018	AU 2014337519 A1	05-05-2016
		CA 2926797 A1	23-04-2015
		CA 3002989 A1	23-04-2015
		EP 3057604 A1	24-08-2016
		US 2016263047 A1	15-09-2016
		WO 2015057751 A1	23-04-2015
RO 128703 A2	30-08-2013	NONE	