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(54) Benævnelse: **Indkapslet agglomeration af mikrokapsler og fremgangsmåde til fremstilling deraf**

(56) Fremdragne publikationer:

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DESCRIPTION

Field of the Invention

[0001] This invention relates to microcapsules.

Background of the Invention

[0002] Microcapsules are defined as small particles of solids, or droplets of liquids, inside a thin coating of a shell material such as beeswax, starch, gelatine or polyacrylic acid. They are used, for example, to prepare liquids as free-flowing powders or compressed solids, to separate reactive materials, to reduce toxicity, to protect against oxidation and/or to control the rate of release of a substance such as an enzyme, a flavour, a nutrient, a drug, etc.

[0003] Over the past fifty years, the prior art has concentrated on so-called 'single-core' microcapsules. However, one of the problems with single-core microcapsules is their susceptibility to rupture. To increase the strength of microcapsules, it is known in the art to increase the thickness of the microcapsule wall. However, this leads to a reduction in the loading capacity of the microcapsule. Another approach has been to create so-called "multi-core" microcapsules. For example, United States patent 5,780,056 discloses a "multi-core" microcapsule having gelatine as a shell material. These microcapsules are formed by spray cooling an aqueous emulsion of oil or carotenoid particles such that the gelatine hardens around "cores" of the oil or carotenoid particles. Yoshida et al. (Chemical Abstract 1990:140735 or Japanese patent publication JP 01-148338 published June 9, 1989) discloses a complex coacervation process for the manufacture of microcapsules in which an emulsion of gelatine and paraffin wax is added to an arabic rubber solution and then mixed with a surfactant to form "multi-core" microcapsules. Ijichi et al. (J. Chem. Eng. Jpn. (1997) 30(5):793-798) micoroencapsulated large droplets of biphenyl using a complex coacervation process to form multi-layered mirocapsules. United States patents 4,219,439 and 4,222,891 disclose "multi-nucleus", oil-containing microcapsules having an average diameter of 3-20 µm with an oil droplet size of 1-10 µm for use in pressure-sensitive copying papers and heat sensitive recording papers. While some improvement in the strength of microcapsules may be realized by using methods such as these, there remains a need for microcapsules having good rupture strength and good oxidative barrier to the encapsulated substance, preferably in conjunction with high load volumes. Illustrative of this need is the current lack of commercially available 'multicore' microcapsules.

Summary of the Invention

[0004] There is provided a microcapsule as claimed in claim 1.

[0005] Microcapsules of the present invention may be used to contain a loading substance for a variety of applications.

Brief Description of the Drawings

[0006]

Figure 1 is an optical micrograph (400 X) of encapsulated agglomerations of microcapsules in accordance with the invention.

Figure 2 is a second optical micrograph (400 X) of encapsulated agglomerations of microcapsules in accordance with the invention.

Detailed Description

Composition:

[0007] The loading substance is an oil selected from fish oils, vegetable oils, mineral oils, derivatives thereof or mixtures thereof, or the loading substance in an omega-3 fatty acid selected from α-linolenic acid (18:3n3), octadecatetraenoic acid (18:4n3), eicosapentaenoic acid (20:5n3) (EPA) and docosa-hexaenoic acid (22:6n3) (DHA), and derivatives thereof and mixtures thereof. Many types of derivatives are well known to one skilled in the art Examples of suitable derivatives are esters, such as phytosterol esters, branched or unbranched C_1-C_{30} alkyl esters, branched or unbranched C_2-C_{30} alkenyl esters or branched or unbranched C_3-C_{30} cycloalkyl esters, in particular phytosterol esters and C_1-C_6 alkyl esters. Preferred sources of oils are oils derived from aquatic organisms (e.g. anchovies, capelin, Atlantic cod, Atlantic herring, Atlantic mackerel, Atlantic menhaden, salmonids, sardines, shark, tuna, etc) and plants (e.g. flax, vegetables, algae, etc). While the loading substance may or may not be a biologically active substance, the microcapsules of the present invention are particularly suited for biologically active substances, for example, drugs, nutritional supplements, flavours ormixtures thereof. Particularly preferred loading substances include antioxidants, such as CoQ_{10} and vitamin E.

[0008] The primary shell and the outer shell are each formed from a complex concervate between gelatine type A and polyphosphate. A particularly preferred form of gelatine type A has a Bloom strength of 50-350, more preferably a Bloom strength of 275.

[0009] The molar ratio of gelatine type A; polyphosphate is preferably 8:1 to 12:1.

[0010] Processing aids may be included in the shell material. Processing aids may be used for a variety of reasons. For example, they may be used to promote agglomeration of the primary microcapsules, control microcapsule size and/or to act as an antioxidant. Antioxidant properties are useful both during the process (e.g. during coacervation and/or spray drying) and in the microcapsules after they are formed (i.e. to extend shelf-life, etc). Preferably a small number of processing aids that perform a large number of functions is used. For example, ascorbic acid or a salt thereof may be used to promote agglomeration of the primary microcapsules, to control microcapsule size and to act as an antioxidant. The ascorbic acid or salt thereof is preferably used in an amount of about 100 ppm to about 12,000 ppm, more preferably about 1000 ppm to about 5000 ppm. A salt of ascorbic acid, such as sodium or potassium ascorbate, is particularly preferred In this capacity.

[0011] The structure of encapsulated agglomerations of microcapsules in accordance with the present invention may be seen in Figures 1 and 2, which show that smaller (primary) microcapsules have agglomerated together and that the agglomeration is surrounded by shell material to form a larger microcapsule. Each individual primary microcapsule has its own distinct shell called the primary shell. Furthermore, any space that there may be between the smaller microcapsules is filled with more shell material to hold and surround the smaller microcapsules thereby providing an extremely strong outer shell of the larger microcapsule in addition to the primary shell that forms the smaller microcapsules within the larger microcapsule. In one sense, the encapsulated agglomeration of microcapsules may be viewed as an agglomeration of walled bubbles suspended in a matrix of shell material, i.e. a "foam-like" structure. Such an encapsulated agglomeration of microcapsules provides a stronger, more rupture-resistant structure than is previously known in the art, in conjunction with achieving high loads of loading substance.

[0012] The primary microcapsules (primary shells) typically have an average diameter of about 40 nm to about 10 µm, more particularly from about 0.1 µm to about 5 µm, even more particularly about 1 µm. The encapsulated agglomerations (outer shells) may have an average diameter of from about 1 µm to about 2000 µm, more typically from about 20 µm to about 1000 µm, more particularly from about 20 µm to about 100 µm, even more particularly from about 50 µm to about 100 µm.

[0013] The encapsulated agglomerations of microcapsules typically have a combination of payload and structural strength that are better than mufti-core microcapsules of the prior art. For example, payloads of loading substance can be as high as about 70% by weight in microcapsules of the present invention having an average size of about 50 µm for the outer shells and an average size of about 1 µm for the primary shells.

Process:

[0014] The microcapsule according to the invention may be prepared by the following process. In this process, an aqueous mixture of a loading substance, the first polymer component of the shell material and the second polymer component of the shell material is formed. The aqueous mixture may be a mechanical mixture, a suspension or an emulsion. When a liquid loading material is used, particularly a hydrophobic liquid, the aqueous mixture is preferably an emulsion of the loading material and the polymer components.

[0015] In a more preferred aspect, the first polymer component is provided in aqueous solution, preferably together with processing aids, such as antioxidants. A loading substance may then be dispersed into the aqueous mixture, for example, by using a homogenizer. If the loading substance is a hydrophobic liquid, an emulsion is formed in which a fraction of the first polymer component begins to deposit around individual droplets of loading substance to begin the formation of primary shells. If the loading substance is a solid particle, a suspension is formed in which a fraction of the first polymer component begins to deposit around individual particles to begin the formation of primary shells. At this point, another aqueous solution of the second polymer component may be added to the aqueous mixture.

[0016] Droplets or particles of the loading substance in the aqueous mixture preferably have an average diameter of less than 100 μm, more preferably less than 50 μm, even more preferably less than 25 μm. Droplets or particles of the loading substance having an average diameter less than 10 μm or less than 5 μm or less than 3 μm or less than 1 μm may be used. Particle size may be measured using any typical equipment known in the art, for example, a Coulter™ LS230 Particle Size Analyzer, Miami, Florida, USA.

[0017] The amount of the polymer components of the shell material provided in the aqueous mixture is typically sufficient to form both the primary shells and the outer shells of the encapsulated agglomeration of microcapsules. Preferably, the loading substance is provided in an amount of from about 1% to about 15% by weight of the aqueous mixture, more preferably from about 3% to about 8% by weight, and even more preferably about 6% by weight.

[0018] The pH, temperature, concentration, mixing speed or a combination thereof is then adjusted to accelerate the formation of the primary shells around the droplets or particles of the loading substance. Complex coacervation will occur between the components to form a coacervate, which further deposits around the loading substance to form primary shells of shell material. The pH may be adjusted to a value from 3.5-5.0, preferably from 4.0-5.0. If the pH of the mixture starts in the desired range, then little or no pH adjustment is required. The initial temperature of the aqueous mixture is preferably set to a value of from about 40°C to about 60°C, more preferably at about 50°C. Mixing is preferably adjusted so that there is good mixing without breaking the microcapsules as they form. Particular mixing parameters depend on the type of equipment being used. Any of a variety of types of mixing equipment known in the art may be used. Particularly useful is an axial flow impeller, such as Lightnin™ A310 or A510.

[0019] The aqueous mixture may then be cooled under controlled cooling rate and mixing parameters to permit agglomeration of the primary shells to form encapsulated agglomerations of primary shells. The encapsulated agglomerations are discrete particles themselves. It Is advantageous to control the formation of the encapsulated agglomerations at a temperature above the gel point of the shell material, and to let excess shell material form a thicker outer shell. It is also possible at this stage to add more polymer components, in order to thicken the outer shell. The temperature is preferably lowered at a rate of 1 °C/10 minutes until it reaches a temperature of from about 5°C to about 10°C, preferably about 5°C. The outer shell encapsulates the agglomeration of primary shells to form a rigid encapsulated agglomeration of microcapsules.

[0020] At this stage, a cross-linker may be added to further increase the rigidity of the microcapsules by crosslinking the shell material in both the outer and primary shells and to make the shells insoluble in both aqueous and oily media. Any suitable cross-linker may be used. Preferred cross-linkers are enzymatic cross-linkers (e.g. transglutaminase), aldehydes (e.g. formaldehyde or gluteraldehyde), tannic acid, alum or a mixture thereof. When the microcapsules are to be used to deliver a biologically active substance to an organism, the cross-linkers are preferably non-toxic or of sufficiently low toxicity. The amount of cross-linker used may be adjusted to provide more or less structural rigidity as desired. For example, the cross-linker may be conveniently used in an amount of about 1.0% to about 5.0%, preferably about 2.5%, by weight of the gelatine type A. In general, one skilled in the art may routinely determine the desired amount in any given case by simple experimentation.

[0021] Finally, the microcapsules may be washed with water and/or dried to provide a free-flowing powder. Drying may be accomplished by a number of methods known in the art, such as freeze drying, drying with ethanol or spray drying. Spray drying is a particularly preferred method for drying the microcapsules. Spray drying techniques are disclosed in "Spray Drying Handbook", K. Masters, 5th edition, Longman Scientific Technical UK, 1991 , the disclosure of which is hereby incorporated by reference.

Uses:

[0022] The microcapsules may be used to prepare liquids as free-flowing powders or compressed solids, to store a substance, to separate reactive substances, to reduce toxicity of a substance, to protect a substance against oxidation, to deliver a substance to a specified environment and/or to control the rate of release of a substance. In particular, the microcapsules may be used to deliver a biologically active substance to an organism for nutritional or medical purposes. The biologically active substance may be, for example, a nutritional supplement, a flavour or a drug. The organism is preferably a mammal, more preferably a human. Microcapsules containing the biologically active substance may be included, for example, in foods or beverages or in drug delivery systems. Use of the microcapsules of the present invention for formulating a nutritional supplement into human food is particularly preferred.

[0023] Microcapsules of the present invention have good rupture strength to help reduce or prevent breaking of the microcapsules during incorporation into food or other formulations. Furthermore, the microcapsule's shells are insoluble in both aqueous and oily media, and help reduce or prevent oxidation and/or deterioration of the loading substance during preparation of the microcapsules, during long-term storage, and/or during incorporation of the microcapsules into a formulation vehicle, for example, into foods, beverages, nutraceutical formulations or pharmaceutical formulations.

<u>Examples</u>

Example 1:

[0024] 54.5 grams gelatine 275 Bloom type A (isoelectric point of about 9) was mixed with 600 grams of deionized water containing 0.5% sodium ascorbate under agitation at 50°C until completely dissolved. 5.45 grams of sodium polyphosphate was dissolved in 104 grams of deionized water containing 0.5% sodium ascorbate. 90 grams of a fish oil concentrate containing 30% eicosapentaenoic acid ethyl ester (EPA) and 20% docosahexaenoic acid ethyl ester (DHA) (available from Ocean Nutrition Canada Ltd.) was dispersed with 1.0% of an antioxidant (blend of natural flavour, tocopherols and citric acid available as Duralox™ from Kalsec™) into the gelatine solution with a high speed Polytron™ homogenizer. An oil-in-water emulsion was formed. The oil droplet size had a narrow distribution with an average size of about 1 μm measured by Coulter™ LS230 Particle Size Analyzer. The emulsion was diluted with 700 grams of deionized water containing 0.5% sodium ascorbate at 50°C. The sodium polyphosphate solution was then added into the emulsion and mixed with a LIghtnin™ agitator at 600 rpm. The pH was then adjusted to 4.5 with a 10% aqueous acetic acid solution. During pH adjustment and the cooling step that followed pH adjustment, a coacervate formed from the gelatine and polyphosphate coated onto the oil droplets to form primary microcapsules. Cooling was carried out to above the gel point of the gelatine and polyphosphate and the primary microcapsules started to agglomerate to form lumps under agitation. Upon further cooling of the mixture, polymer remaining in the aqueous phase further coated the lumps of primary microcapsules to form an encapsulated agglomeration of microcapsules having an outer shell and having an average size of 50 μm Once the temperature had been cooled to 5°C, 2.7 grams of 50% gluteraldehyde was added into the mixture to further strengthen the shell. The mixture was then warmed to room temperature and kept stirring for 12 hours. Finally, the microcapsule suspension washed with water. The washed suspension was then spray dried to obtain a free-flowing powder. A payload of 60% was obtained.

Example 2:

[0025] Encapsulated agglomerations of microcapsules were formed in accordance with the method of Example 1 except that 0.25% sodium ascorbate was used. A payload of 60% was obtained.

Example 3:

[0026] Encapsulated agglomerations of microcapsules were formed in accordance with the method of Example 1 except that no ascorbate was used. A payload of 60% was obtained.

Example 4:

[0027] Encapsulated agglomerations of microcapsules were formed in accordance with the method of Example 1 except that 105 grams of fish oil concentrate was used and a payload of 70% was obtained.

Example 5:

[0028] Encapsulated agglomerations of microcapsules were formed in accordance with the method of Example 1 except that it was applied to triglyceride (TG) fish oil (available from Ocean Nutrition Canada Ltd.) rather than ethyl ester fish oil.

Reference Example 6:

[0029] Encapsulated agglomerations of microcapsules were formed in accordance with the method of Example 1 except that gelatine (type A) and gum arabic were used as polymer components of the shell material.

Example 7:

[0030] Encapsulated agglomerations of microcapsules were formed in accordance with the method of Example 1 except that 150 Bloom gelatine (type A) and polyphosphate were used as polymer components of the shell material and 105 grams of fish oil concentrate was used to obtain a payload of 70%.

Example 8:

[0031] Encapsulated agglomerations of microcapsules were formed in accordance with the method of Example 1 except that transglutaminase was used to cross-link the shell material.

Example 9: Evaluation of microcapsules

[0032] The microcapsules of Examples 1-8 were evaluated for mechanical strength, encapsulated oil quality and oxidative stability.

[0033] Microcapsule shell strength was evaluated by centrifuging a given amount of the prepared microcapsule powders from each of the Examples 1-8 at 34,541 g at 25°C for 30 minutes in a Sorvalf™ SuperT-21 centrifuge. The original and the centrifuged powders were washed with hexane to extract oil released from the microcapsules due to shell breakage under centrifuge force. The ratio of percent free oil of the centrifuged powders to that of the original powders is used as an indicator of the shell strength. The lower the ratio, the stronger is the microcapsule's shell.

[0034] Oil quality in microcapsules was evaluated by crushing the shells of the prepared microcapsule powders from each of Examples 1-8 with a grinder. The encapsulated oil was then extracted with hexane. Peroxide Value (PV) was analyzed with American Oil Chemist Society Method (AOCS Official Method Cd 8-53: Peroxide value). A high PV indicates a higher concentration of primary oxidation products in the encapsulated oil.

[0035] Accelerated oxidative stability was evaluated by placing the prepared microcapsule powders from each of Examples 1-8 in an oxygen bomb (Oxipres™, MIKROLAB AARHUS A/S, Denmark) with an initial oxygen pressure of 5 bar at a constant temperature of 65°C. When the encapsulated fish oil started to oxidize, the oxygen pressure dropped. The time at which the oxygen pressure started to drop is called Induction Period. A longer Induction Period means that the contents of the microcapsules are better protected towards oxidation.

[0036] Results are shown in Table 1. The results indicate that the agglomerated microcapsules prepared in accordance with the present invention have excellent strength and resistance to oxidation of the encapsulated loading substance.

Table 1

run #	load (%)	ascorbate (%)	Induction period (hr)	PV value	free oil ratio	notes
1	60	0.50	38	3.0	2.0	
2	60	0.25	34	4.1	1.5	

run #	load (%)	ascorbate (%)	Induction period (hr)	PV value	free oil ratio	notes
3	60	0.0	26	7.8	1.5	
4	70	0.50	38	3.2	1.7	
5	60	0.50	37	0.28	3.0	TG oil
Ref. 6	60	0.50	30	3.4	1.5	gum arabic
7	70	0.50	38	4.4	2.2	150 bloom gelatin
8	60	0.50	33	3.2	1.1	enzymatic cross linking

[0037] Other advantages which are obvious and which are inherent to the invention will be evident to one skilled in the art. Since many possible embodiments may be made of the invention without departing from the scope of the claims, it is to be understood that all matter herein set forth or shown in the accompanying drawings Is to be interpreted as illustrative and not in a limiting sense.

REFERENCES CITED IN THE DESCRIPTION

Patent documents cited in the description

- US5780056A **[0003]**
- JP1148338A **[0003]**
- US4219439A **[0003]**
- US4222891A **[0003]**

Non-patent literature cited in the description

- CHEMICAL ABSTRACTS, 140735 **[0003]**
- **LJICHI et al.**J. Chem. Eng. Jpn., 1997, vol. 30, 5793-798 **[0003]**
- **K. MASTERS**Spray Drying HandbookLongman Scientific Technical19910000 **[0021]**

P A T E N T K R A V

1. Mikrokapsel, omfattende en agglomeration af primære mikrokapsler, hvor hver enkelt primær mikrokapsel har en primær skal, der indkapsler en substans til ifyldning, og hvilken agglomerationen er indkapslet af en ydre skal, hvor den primære skal og den ydre skal hver er dannet fra et komplekst coacervat mellem to polymerkomponenter, hvor én polymerkomponent er gelatine type A, og den anden er polyphosphat, og hvor:

(a) substansen til ifyldning er en olie, hvor olien er valgt blandt fiskeolier, vegetabilske olier, mineralske olier, derivater deraf eller blanding deraf; eller

(b) substansen til ifyldning er en omega-3-fedtsyre, hvor fedtsyren er valgt blandt α-linolensyre, octadecatetraensyre, eicosapentaensyre og docosahexaensyre og derivater deraf og blandinger deraf; og

substansen til ifyldning kan omfatte en antioxidant valgt blandt CoQ_{10} og vitamin E.

2. Mikrokapsel ifølge krav 1, hvor derivaterne er valgt blandt phytosterolestere, forgrenede eller uforgrenede C_1-C_{30}-alkylestere, forgrenede eller uforgrenede C_2-C_{30}-alkenylestere eller forgrenede eller uforgrenede C_3-C_{30}-cycloalkylestere.

3. Mikrokapsel ifølge krav 1, hvor substansen til ifyldning er en olie afledt fra ansjoser, lodde, atlantisk torsk, atlantisk sild, atlantisk makrel, atlantisk menhaden, salmonider, sardiner, haj eller tun.

4. Mikrokapsel ifølge krav 1, hvor substansen til ifyldning er en olie afledt fra hør eller grøntsager.

5. Mikrokapsel ifølge krav 1, hvor substansen til ifyldning er en olie afledt fra alger.

6. Mikrokapsel ifølge krav 1, hvor mikrokapslen indeholder ascorbinsyre eller et salt deraf i skallerne i en mængde på 100 ppm til 12.000 ppm.

7. Mikrokapsel ifølge krav 1, hvor polymerkomponenterne er gelatine type A og polyphosphat i et molforhold på fra 8:1 til 12:1.

DRAWINGS

Fig. 1

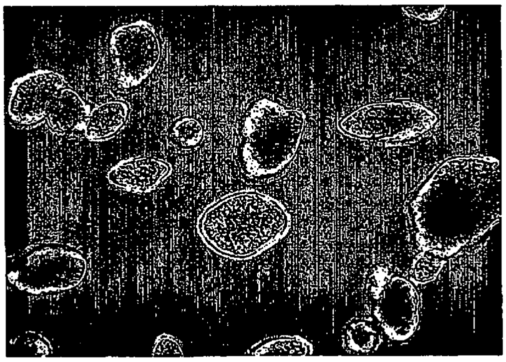

Fig. 2