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(54) Benævnelse: **FREMGANGSMÅDE TIL FREMSTILLING AF POLYSACCHARIDKUGLER**

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WO-A1-2012/028623
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DESCRIPTION

Description

Technical field of the invention

[0001] The present invention relates to polysaccharide beads, and more particularly to manufacture of polysaccharide beads by inverse suspension techniques.

Background of the invention

[0002] Crosslinked polysaccharide beads are commonly used as stationary phases for chromatographic separation of proteins and other biomolecules. Such beads were introduced in the early 1960-ies (see e.g. US3208994,) mainly for laboratory separation purposes. Since then their use has grown dramatically and crosslinked polysaccharide beads are now used routinely in large scale manufacturing processes for separation of many biopharmaceuticals such as monoclonal antibodies, plasma components, insulin and various recombinant proteins.

[0003] The most common way to prepare polysaccharide beads is by inverse suspension processes, where an aqueous solution of a polysaccharide is emulsified as a water-in-oil (w/o) emulsion in a continuous oil phase and the emulsion droplets are solidified either by crosslinking or by thermal gelation. Such processes are described in e.g. US3208994, US4794177 and US6602990 which use chlorinated hydrocarbons or aromatic hydrocarbons as the oil phase. An issue here is that large scale use of halogenated hydrocarbons and aromatic hydrocarbons is currently being phased out for environmental reasons.

[0004] Accordingly there is a need for methods to manufacture polysaccharide beads without the use of halogenated or aromatic solvents.

Summary of the invention

[0005] One aspect of the invention is to provide an environmentally acceptable method of manufacturing polysaccharide beads. This is achieved with a method as defined in the claims. One advantage is that the method does not use halogenated or aromatic solvents. Further advantages are that spherical beads with good pore structures and mechanical properties can be obtained.

[0006] Further suitable embodiments of the invention are described in the dependent claims.

Drawings

[0007]

Fig. 1 shows an outline of the method of the invention, a) addition of the water phase to the oil phase, b) water-in-oil emulsion, c) solidified beads dispersed in oil phase.

Fig. 2 shows the crosslinking of dextran with epichlorohydrin according to an embodiment of the invention.

Fig. 3 shows microscope pictures of the swollen particles from a) sample 9090 (2-MCH) and b) sample 5595 (3-MCH).

Fig. 4 shows microscope pictures of the swollen particles from sample 5624 (cyclohexane + 3-MCH).

Fig. 5 shows microscope pictures of the swollen particles from a) sample 6462 (CPME) and b) sample 5182 (CPME + 3-MCH).

Fig. 6 shows microscope pictures of the swollen particles from a) sample 6671 (DIBK) and b) sample 5382 (DIBK + 3-MCH).

Fig. 7 shows microscope pictures of the swollen particles from sample 9355 (MAK).

Detailed description of embodiments

[0008] In one aspect, illustrated by Figs. 1-2, the present invention discloses a method of manufacturing polysaccharide beads, comprising the steps of:

1. i) Providing a water phase 1 comprising an aqueous solution of a polysaccharide. This can be accomplished by dissolving a polysaccharide in water or in water comprising one or more additional components such as salts, buffers, alkali, reducing agents etc. The polysaccharide can e.g. be a native polysaccharide such as dextran, pullulan, starch, alginate, guar gum, locust bean gum, konjac, agar, agarose, carrageenan etc. As an example, the polysaccharide can be dextran, e.g. dextran with a weight average molecular weight, Mw, of 20-4000 kDa, 40-2000 kDa or 100-500 kDa. Alternatively, the polysaccharide can be a derivative of a native polysaccharide, such as a cellulose ether, an agarose ether, a starch ether, DEAE dextran etc. Advantageously, the polysaccharide is water-soluble at room temperature or at elevated temperatures.
2. ii) Providing an oil phase 2 comprising at least one water-immiscible organic solvent and

at least one oil-soluble emulsifier. The water-immiscible organic solvent(s) can suitably have a water solubility of less than 5 vol. %, such as less than 3 vol. % or less than 2 vol. % at 25°C and the solubility of water in the water-immiscible organic solvent(s) can suitably be less than 5 vol. %, such as less than 3 vol. % or less than 2 vol. % at 25°C. The oil-soluble emulsifier(s) can suitably be soluble in the organic solvent(s) and the oil phase can be prepared by dissolving one or more oil-soluble emulsifiers in the water-immiscible organic solvent or a mixture of water-immiscible organic solvents. The concentration of the emulsifier in the oil phase can e.g. be 0.01-0.5 g/ml, such as 0.05-0.3 g/ml. These values can also refer to the total concentration of emulsifiers in the oil phase, in case mixed emulsifiers are used. If a polymeric emulsifier is used, the viscosity of the oil phase may vary with the molecular weight and concentration of the emulsifier.

3. iii) Emulsifying the water phase in the oil phase to form a water-in-oil (w/o) emulsion. As illustrated in Fig. 1, this can e.g. be done by adding the water phase 1 to the oil phase 2 in an emulsification vessel 3 under agitation provided by an agitator 4, such that the water phase is dispersed as discrete liquid droplets 5 in the continuous oil phase 2, stabilized by the emulsifier. Other techniques known in the art can also be used, e.g. continuous emulsification using static mixers, membrane emulsification etc. Depending on the viscosity of the oil phase, some optimization of the agitation intensity may be required to achieve specific particle sizes of the beads produced. Alternatively (or additionally), the concentration and/or type of the emulsifier may be varied in order to get certain particle sizes.
4. iv) Inducing solidification of the water phase in the w/o emulsion. This means that the liquid droplets 5 are converted to (solid) gel beads 6 by gelation of the polysaccharide. The gelation can be induced e.g. by adding a crosslinking agent to chemically (covalently) crosslink the polysaccharide, as further discussed below, or by lowering the temperature to cause thermal gelation of the polysaccharide, as is also discussed below. Once the water phase droplets have been solidified into gel beads, the beads may be recovered by sedimentation and/or filtration and they may be further washed and processed to provide beads suitable for separation or cell cultivation purposes. The further processing may e.g. include further crosslinking steps and/or derivatisation with reagents to introduce functional groups.

[0009] The at least one organic solvent is an alicyclic ketone defined by Formula II. Alternatively, or additionally, the at least one organic solvent does not contain halogens (i.e. the molecules of the solvent do not contain halogen atoms) and has Hansen solubility parameter values in the ranges of δD = 15.0 - 18.5 $MPa^{1/2}$, δP = 3.5 - 8.5 $MPa^{1/2}$ and δH = 4.0 - 5.5 $MPa^{1/2}$. The oil phase can also comprise a mixture of halogen-free water-immiscible organic solvents, where the mixture has Hansen solubility parameter values in the ranges of 6D = 15.0 - 18.5 $MPa^{1/2}$, δP = 3.5 - 8.5 $MPa^{1/2}$ and δH = 4.0 - 5.5 $MPa^{1/2}$. Suitably, the content of halogenated solvents in the oil phase can be less than 1 mol %, such as less than 0.1% or less than 0.01 %. The Hansen solubility parameters are discussed in detail in C M Hansen: The three dimensional solubility parameter and solvent diffusion coefficient - Their importance in

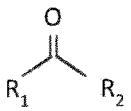
surface coating formulation, Copenhagen 1967. δD is the dispersion force contribution to the solubility parameter (cohesive energy density) of a solvent, while δP is the polar force contribution and δH is the hydrogen bonding force contribution. Tables of Hansen solubility parameters for different solvents can be found e.g. in J Brandrup, E H Immergut Eds. Polymer Handbook, 3rd edition, John Wiley & Sons 1989, pp. VII/540-VII/544.

[0010] In certain embodiments, illustrated by Fig. 2, step iv) comprises crosslinking the polysaccharide. This can be accomplished e.g. by adding a crosslinking agent to the w/o emulsion. The crosslinking agent may e.g. be a compound with two electrophilic functionalities, which can react e.g. with two hydroxyl groups on the polysaccharide and cause crosslinking by the formation of covalently bonded links between polysaccharide chains. The hydroxyl groups are particularly nucleophilic at high pH conditions and it can be advantageous to use a high pH water phase in the method, e.g. by adding NaOH or other suitable alkali (e.g. KOH) to the water phase. The alkali (NaOH or KOH) concentration in the water phase may e.g. be at least 0.1 M, such as 0.1-2 M or 0.5-1 M. Examples of electrophilic crosslinkers include epichlorohydrin, diepoxides and multifunctional epoxides, as well as divinylsulfone and halohydrins like 1,3-dibromo-propanol-2. The crosslinker can suitably be added to the w/o emulsion, such that it dissolves in the oil phase and diffuses into the water phase droplets.

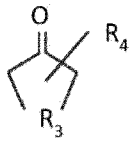
[0011] In some embodiments, step iv) comprises thermal gelation of the polysaccharide. In this case, the polysaccharide can be a hot-water soluble polysaccharide that forms a gel upon cooling. Examples of such polysaccharides are e.g. agar and agarose, which are soluble at temperatures of about 60°C and higher but form solid gels upon cooling to e.g. about 40°C or lower. In this case, steps i)-iii) can be performed at a temperature where the polysaccharide is soluble and in step iv) the temperature is lowered to a temperature below the gel point of the particular polysaccharide used.

[0012] In certain embodiments, at least one emulsifier is a cellulose derivative, such as a cellulose ester or a cellulose ether. Among cellulose esters, cellulose mixed esters, such as cellulose acetate butyrate can be particularly useful. Cellulose acetate butyrate (CAB) of different grades is commercially available, e.g. from Eastman Chemical Company (USA). The molecular weight of the CAB can suitably be 10-100 kDa, such as 15-75 kDa or 16-70 kDa, determined by gel permeation chromatography as the polystyrene equivalent number average molecular weight (Mn). The acetyl and butyryl contents can e.g. be 2-20 wt. % acetyl content and 20-60 wt. % butyryl content, such as a) 10-15 wt.% acetyl content and 30-40 wt.% butyryl content or b) 2-5 wt.% acetyl content and 50-60 wt.% butyryl content or c) 2-15 wt.% acetyl content and 30-60 wt.% butyryl content. An example of a cellulose ether useful as an emulsifier is ethyl cellulose.

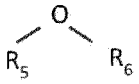
[0013] In some embodiments at least one organic solvent is a C_6-C_{10} alicyclic ketone defined by Formula II,



(I)



(II)



(III)

wherein:

R_1 and R_2 are, independently of each other, C_1 - C_5 alkyl groups;

R_3 is a C_1-C_5 alkylene group;

R_4 is hydrogen or a C_1-C_5 alkyl group attached to any one of the non-carbonyl carbon atoms in the ring structure; and

R_5 and R_6 are, independently of each other, C_1 - C_6 alkyl or cycloalkyl groups.

[0014] In some embodiments, at least one organic solvent is defined by Formula II, with R_3 and R_4 defined as above. R_3 may e.g. be a C_2 alkylene group and R_4 may be a methyl group.

[0015] In certain embodiments, the organic solvent can be selected from the group consisting of 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, cyclohexanone, diisobutyl ketone, methyl n-amyl ketone, methyl isoamyl ketone, methyl isobutyl ketone, cyclopentyl methyl ether and their mixtures. At least one organic solvent can e.g. be selected from the group consisting of 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, cyclohexanone, methyl n-amyl ketone, methyl isoamyl ketone, methyl isobutyl ketone and cyclopentyl methyl ether, such as from the group consisting of 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone and cyclohexanone. In specific cases at least one organic solvent can be 2-methylcyclohexanone.

[0016] A polysaccharide bead, or a plurality of polysaccharide beads, can be prepared by the method of any embodiment disclosed above. Disclosed herein is also a crosslinked polysaccharide bead, or a plurality of crosslinked polysaccharide beads, comprising at least 0.1 ppm, such as 0.1-100 ppm of a C_6-C_{10} aliphatic or alicyclic ketone or ether. This may e.g. constitute solvent residues from a manufacturing process as discussed above. The amount may be measured e.g. by headspace GC or GC analysis of extracts, using e.g. mass spectroscopy or flame ionization as detection method. The C_6-C_{10} aliphatic or alicyclic ketone or ether can be as defined by Formulas I, II or III as discussed above and it can e.g. be selected from the group consisting of 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, cyclohexanone, diisobutylketone, methyl n-amylketone, methyl isoamyl ketone, methyl isobutyl ketone, cyclopentyl methyl ether and their mixtures.

[0017] In some embodiments, the bead or plurality of beads have a diameter of 40-125 µm in dry form. In the case of a plurality of beads, the diameter can be determined as the volume-weighted median diameter, d50v, e.g. by laser light diffraction or by electrozone sensing counting techniques.

[0018] In certain embodiments, the bead or plurality of beads comprise at least 0.1 mmol/g, or 1-6 mmol/g, covalently bonded charged groups, such as carboxymethyl-, sulfopropyl-, diethyl aminoethyl-, and/or diethyl-(2-hydroxy-propyl)aminoethyl- groups. Such groups are useful for ion exchange separations and groups like diethyl aminoethyl groups can also promote the growth of adherent cells when the beads are used as microcarriers in cell cultivation. The derivatisation with charged groups can be accomplished by methods well known in the art.

[0019] One or more polysaccharide beads as described above can be used for separation of a target biomolecule, such as a target protein, from impurities or contaminants.

[0020] One or more polysaccharide beads as described above can be used as microcarriers for cultivation of cells.

Examples

Emulsification method (reference example with 1,2-dichloroethane)

[0021] 165 g dextran of Mw 150-250 kD was dissolved in 390 ml distilled water and simultaneously 22 ml 50% sodium hydroxide (NaOH) was added under stirring. 0.8 g sodium borohydride ($NaBH_4$) was added to the solution.

[0022] 21g cellulose acetate butyrate (CAB) was added into a 1000 ml glass reactor with an overhead agitator. The agitator was started and 350 ml 1,2-dichloroethane (EDC) was added (the CAB concentration was thus 0.060 g/ml EDC). The solution was stirred until the CAB had dissolved. The water phase was then added to the oil phase under agitation at 50°C and the agitation was continued until a suitable droplet size had been reached, as judged from microscopy of small samples withdrawn. When a suitable size was obtained, the emulsion was immediately stabilised by adding 22.4 ml epichlorohydrin (ECH) to crosslink the dextran and thus solidifying the droplets. 20 minutes after the addition of ECH, 50 ml of EDC was added to make the reaction mixture less viscous and easier to agitate.

[0023] The crosslinking reaction was terminated after 20±4h by adding 500 ml acetone. The reaction mixture was then transferred to a 3000 ml glass reactor containing 1250 ml acetone. The solution was stirred for 30 min and then the gel was subsequently allowed to sediment. The supernatant was decanted and the washing procedure was repeated 5 times with acetone and then 7 times with 60% aqueous ethanol and 4 times with 95% ethanol. The gel was then

dried under vacuum at 60°C for 48 h and the resulting powder was sieved between 40 and 100 µm sieves.

[0024] The results from five repetitions of this experiment are shown in Table 2, as samples 3077, 3088, 3092, 3081 and 1175.

SEC analysis

[0025] Approximately 3-4 grams of powder was weighed in a plastic bottle to which roughly 100 ml of either a 9 mg/ml NaCl solution or the running buffer (0.15M NaCl+ 0.05 M phosphate buffer, pH 7.0) was added. The gel was left to swell and sediment for at least 16 hours. It was then washed several times and diluted in the running buffer to generate a 50-60% gel slurry. Columns of 10 mm diameter and 30 cm height (GE Healthcare HR10/30) were packed with the initial flow rate of either 1.0 or 1.2 ml/min, and the final flow rate was either 1.2 or 1.7 ml/min. The packed columns were then tested in an effectiveness test and evaluated through selectivity tests with dextran standards and proteins as described in L Hagel: pp. 51-87 in J C Janson (Ed): Protein Purification: Principles, High Resolution Methods, and Applications, 3rd edition, Wiley 2011. The test proteins were alpha chymotrypsinogen type II, bovine (Mw 26 kDa), ribonuclease A, bovine (Mw 13.7 kDa) and lysozyme, chicken (Mw 14.3 kDa), with 0.15 M NaCl, 0.05M Sodium Phosphate, pH 7.0 as running buffer. K_D data (i.e. the fraction of the bead volume accessible for a probe molecule of a particular size) for these proteins on the prototypes are shown in Table 3.

Water regain

[0026] Water regain (Wr) is the amount of water taken up inside the beads for 1 g dry beads. A high Wr value indicates that the swollen gel is less dense and can separate high Mw target molecules. 15 g of dry beads were equilibrated with 500 ml water for 24 h. A 3.3 x 11.5 cm weighed centrifuge tube with a 10 µm bottom filter was filled with the gel slurry and centrifuged at 1800 rpm for 10 min. After determining the wet weight of the tube, it was dried over night at 105 °C and the dry weight was determined. The water regain was calculated as the drying weight loss (as ml water) per g dry gel.

Particle size distribution

[0027] The particle size distribution was measured in a Coulter Multisizer (Beckman Coulter), using the electrozone sensing technique.

Microscopic examination

[0028] After crosslinking, the beads were examined in a light microscope with phase contrast optics and the presence of surface dimples, inclusions, aggregates etc. was noted.

Emulsifications with different solvents

Solvent abbreviations

[0029]

CPME - Cyclopentyl methyl ether

DIBK - Diisobutyl ketone (2,6-dimethyl-4-heptanone)

EDC - 1,2-dichloroethane

MAK - Methyl n-amyl ketone

2-MCH - 2-methylcyclohexanone

3-MCH - 3-methylcyclohexanone

4-MCH - 4-methylcyclohexanone

[0030] Emulsifications were carried out according to the reference example above, with other solvents replacing EDC, and in some cases with different types and/or concentrations of CAB. The CAB types used are specified in Table 1. The beads were characterized as described above and the results are collated in Tables 2 and 3 and in the discussion below.

Table 1. CAB types used (all from Eastman Chemical Company and with data according to the supplier's data sheets)

CAB type	Molecular weight*, kDa	Viscosity**, poise	Butyryl content, wt %	Acetyl content, wt %
381-0.5	30	1.9	37	13
381-20	70	76	37	13.5
500-5	57	19	51	4
551-0.01	16	0.038	53	2

* Polystyrene equivalent number average molecular weight (Mn), as determined by gel permeation chromatography
** Viscosity determined by ASTM Method D 1343. Results converted to poises (ASTM Method D 1343) using the solution density for Formula A as stated in ASTM Method D 817 (20% Cellulose ester, 72% acetone, 8% ethyl alcohol).

Table 2. Results from emulsifications with different solvents and solvent combinations

Sample	Solvent 1	Solvent 2	CAB type	CAB conc. g/ml	Wr (ml/g)	d50v (µm)
3077	EDC	-	381-20	0.060	4.93	70.5
3088	EDC	-	381-20	0.060	5.03	67.8
3092	EDC	-	381-20	0.060	4.96	70.7
3081	EDC	-	381-20	0.060	5.06	72.0
1175	EDC	-	381-20	0.060	4.90	70.9
9090	2-MCH	-	381-20	0.060	4.74	33.1
5133	3-MCH	-	381-20	0.060	4.77	42.4
5505	3-MCH	-	381-20	0.054	4.60	50.7
5595	3-MCH	-	381-0.5	0.060	4.94	72.7
5892	3-MCH	-	381-0.5	0.090	5.17	73.6
5928	3-MCH	-	500-5	0.060	5.07	57.0
4904	4-MCH	-	381-20	0.060	4.62	44.5
5624	Cyclohexane (20 vol%)	3-MCH (80 vol%)	381-20	0.060	4.71	42.3
5806	Cyclohexane (75 vol%)	3-MCH (25 vol%)	551-0.01	0.21	5.15	68.9
6462	CPME	-	500-5	0.12	4.85	68.1
6724	CPME	-	551-0.01	0.31	5.93	66.6
5182	CPME (75 vol%)	3-MCH (25 vol%)	381-20	0.060	4.83	68.3
5443	CPME (75 vol%)	3-MCH (25 vol%)	381-20	0.060	5.07	52.8
5868	CPME (75 vol%)	3-MCH (25 vol%)	381-20	0.060	4.97	70.0
6551	DIBK	-	500-5	0.080	4.78	69.2
6671	DIBK	-	500-5	0.080	4.94	71.9
6672	DIBK	-	551-0.01	0.23	5.50	61.2
5108	DIBK (75 vol%)	3-MCH (25 vol%)	381-20	0.060	4.56	39.4
5382	DIBK (75 vol%)	3-MCH (25 vol%)	381-20	0.060	4.77	45.0
6725	DIBK (75 vol%)	Cyclohexa none (25 vol%)	381-20	0.047	4.59	50.2
9355	MAK	-	381-20	0.060	4.86	65.8
11854	DIBK (75 vol%)	2-MCH (25 vol%)	381-20	0.036	5.04	

Table 3. K_D data for three test proteins in gel filtration experiments performed with prototypes packed in columns

Sample	Alpha chymotrypsinogen	Ribonuclease	Lysozyme
5133	0.077	0.224	0.409
5505	0.056	0.229	0.418
5595	0.102	0.281	0.457
5892	0.120	0.294	0.472
5928	0.105	0.279	0.468
4904	0.059	0.341	0.510
5624	0.072	0.213	0.395
5806	0.112	0.293	0.466
6462	0.106	0.257	0.435
6724	0.171	0.330	0.508
5182	0.081	0.248	0.425
5443	0.130	0.277	0.462
5868	0.115	0.283	0.461
6551	0.111	0.265	0.448
6671	0.099	0.257	0.441
6672	0.153	0.303	0.487
5108	0.017	0.130	0.337
5382	0.078	0.258	0.435
6725	0	0.167	0.403
9355	0.101	0.251	0.432
11854	0.099	0.259	0.453

[0031] The solvents used were also analysed by NMR spectroscopy before and after emulsification model experiments performed in the absence of dextran and the emulsifier. The spectra before and after were essentially identical, showing that no degradation occurred during the reaction conditions used. This was in contrast to an ester solvent, t-butyl acetate, which although being a sterically hindered ester, was completely hydrolysed under the strongly alkaline conditions used.

Discussion

[0032] The solvents evaluated produce beads that can be used for chromatography, as

evidenced e.g. by their performance in the SEC analysis. The measured properties are in the same range as for the reference prototypes, which shows that the new solvents perform well. Due to the different interactions between the solvents and the CAB emulsifier, the CAB type and concentration had to be varied in order to get suitable oil phase viscosities. The beads were generally spherical (Figs 7-11), but with the solvents 3-MCH and 4-MCH (particularly when used alone), some inclusions and dimples occurred on the beads.

[0033] This written description uses examples to disclose the invention, including the best mode, and also to enable any person skilled in the art to practice the invention, including making and using any devices or systems and performing any incorporated methods. The patentable scope of the invention is defined by the claims.

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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- **L HAGEL**Protein Purification: Principles, High Resolution Methods, and ApplicationsWiley2011000051-87 [0025]

Patentkrav

1. Fremgangsmåde til fremstilling af polysaccharidkugler, omfattende trinnene:

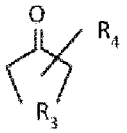
i) tilvejebringelse af en vandfase omfattende en vandig opløsning af et polysaccharid;

ii) tilvejebringelse af en oliefase omfattende mindst et organisk opløsningsmiddel som ikke kan blandes med vand og mindst et olieopløseligt emulgeringsmiddel;

iii) emulering af vandfasen i oliefasen for at danne en vand-i-olie- (v/o) emulsion; og

iv) fremkaldelse af størkning af vandfasen i v/o-emulsionen,

hvor det mindst ene organiske opløsningsmiddel er en alicyklisk keton defineret af formel II,



(II)

hvor:

R_3 er en C_1-C_5-alkylengruppe; og

R_4 er hydrogen eller en C_1-C_5-alkylgruppe.

2. Fremgangsmåde til fremstilling af polysaccharidkugler, omfattende trinnene:

i) tilvejebringelse af en vandfase omfattende en vandig opløsning af et polysaccharid;

ii) tilvejebringelse af en oliefase omfattende mindst et organisk opløsningsmiddel som ikke kan blandes med vand og mindst et olieopløseligt emulgeringsmiddel;

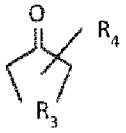
iii) emulgering af vandfasen i oliefasen for at fremstille en vand-i-olie- (v/o) emulsion; og

iv) fremkaldelse af størkning af vandfasen i v/o-emulsionen,

hvor det mindst ene organiske opløsningsmiddel ikke indeholder halogener og har Hansens opløselighedsparameterværdier i områderne på $\delta D = 15,0 - 18,5\ MPa^{1/2}$, $\delta P = 3,5 - 8,5\ MPa^{1/2}$ og $\delta H = 4,0 - 5,5\ MPa^{1/2}$, eller hvor oliefasen omfatter en blanding af halogenfrie organiske opløsningsmidler som ikke kan blandes med vand, idet blandingen har Hansens opløselighedsparameterværdier i

områderne på 6D = 15,0 – 18,5 $MPa^{1/2}$, δP = 3,5 – 8,5 $MPa^{1/2}$ og δH = 4,0 – 5,5 $MPa^{1/2}$,

såsom hvor det mindst ene organiske opløsningsmiddel er en alicyklisk keton defineret med formel II,



(II)

hvor:

R_3 er en C_1-C_5-alkylengruppe; og

R_4 er hydrogen eller en C_1-C_5-alkylgruppe.

3. Fremgangsmåden ifølge et hvilket som helst foregående krav, hvor trin iv) omfatter tværbinding af polysaccharidet, og hvor eventuelt:

a) i trin iv) et tværbindingsmiddel, såsom et tværbindingsmiddel omfattende epichlorhydrin eller et diepoxid, tilsættes til v/o-emulsionen; og/eller

b) tværbindingen udføres under alkaliske betingelser.

4. Fremgangsmåden ifølge et hvilket som helst foregående krav, hvor polysaccharidet omfatter dextran, såsom hvor dextranen har en vægtgennemsnitlig molekylevægt, Mw, på 20-4000 kDa.

5. Fremgangsmåden ifølge et hvilket som helst foregående krav, hvor mindst et emulgeringsmiddel er et cellulosederivat, såsom en celluloseester.

6. Fremgangsmåden ifølge krav 5, hvor det mindst ene emulgeringsmiddel er en celluloseblandet ester, såsom celluloseacetatbutyrat, og hvor eventuelt:

a) det mindst ene emulgeringsmiddel er celluloseacetatbutyrat med en gennemsnitlig molekylevægt, Mn, på 10-100 kDa, såsom 15-75 kDa; og/eller

b) det mindst ene emulgeringsmiddel er celluloseacetatbutyrat med 2-20 vægt-% acetylindhold og 20-60 vægt-% butyrylindhold.

7. Fremgangsmåden ifølge et hvilket som helst foregående krav, hvor det mindst ene organiske opløsningsmiddel:

a) er valgt fra gruppen bestående af 2-methylcyclohexanon, 3-methylcyclohexanon, 4-methylcyclohexanon, cyclohexanon og deres blandinger;

b) er valgt fra gruppen bestående af 2-methylcyclohexanon, 3-methylcyclohexanon, 4-methylcyclohexanon og cyclohexanon; eller

c) er 2-methylcyclohexanon.

DRAWINGS

Drawing

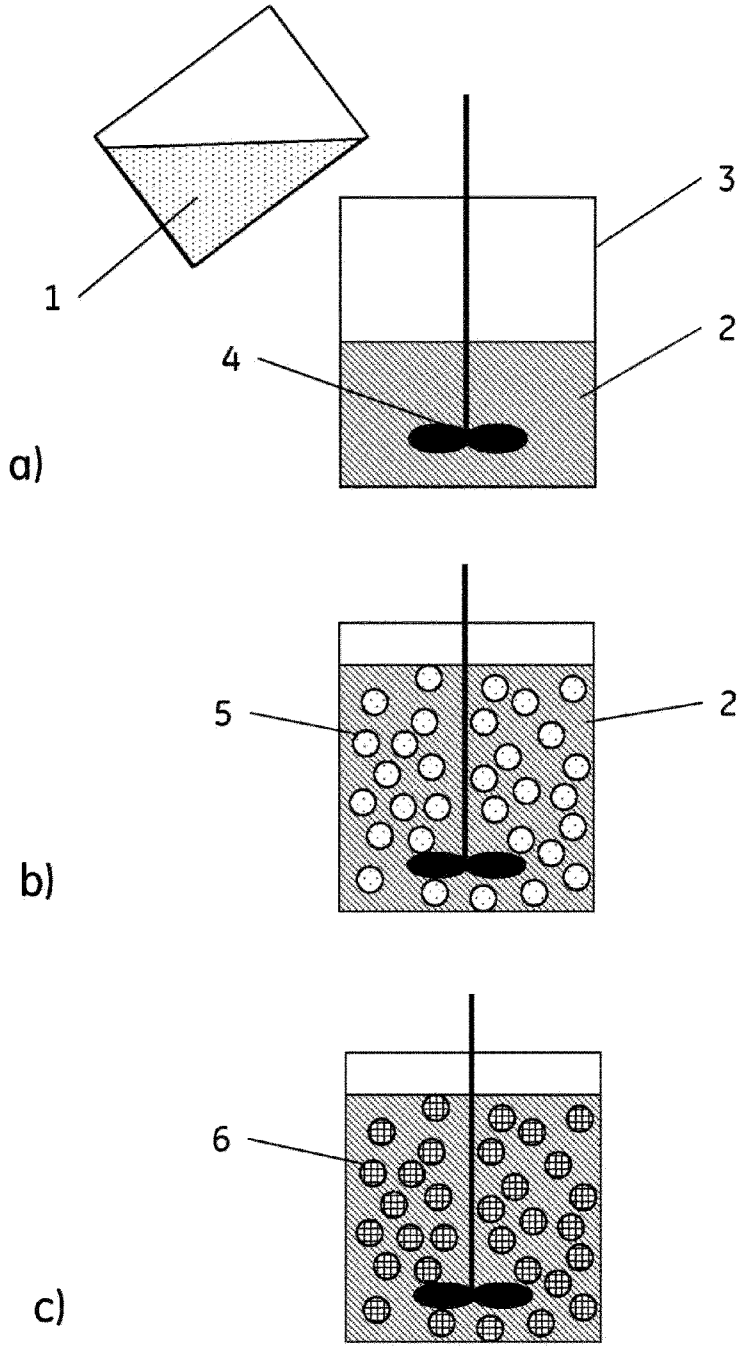


Fig. 1.

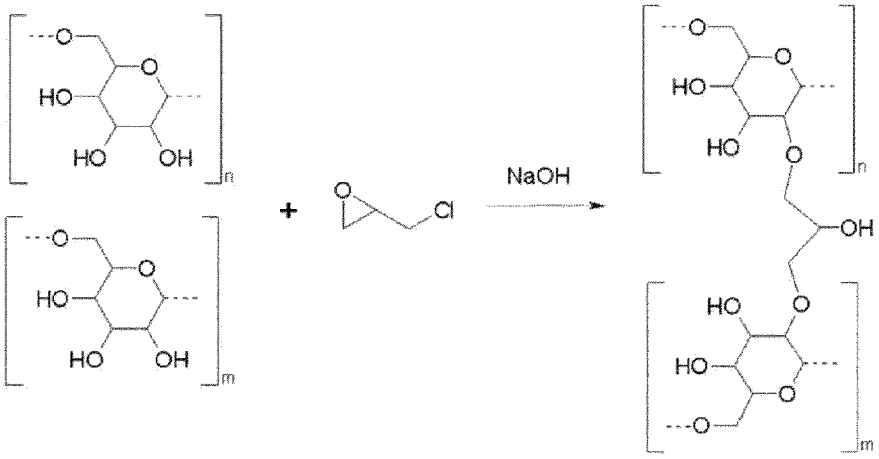
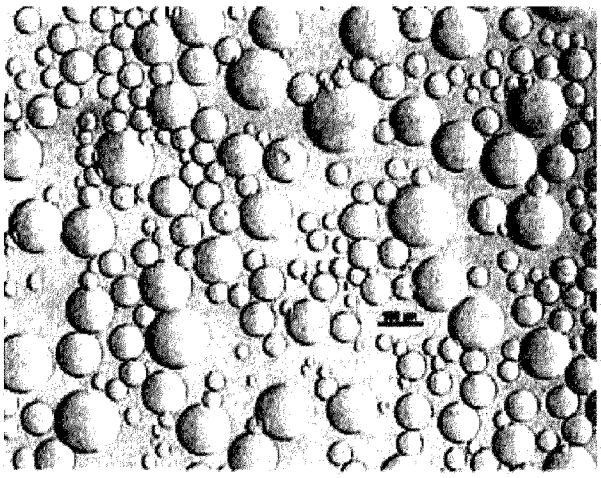


Fig. 2.

a)



b)



Fig. 3.

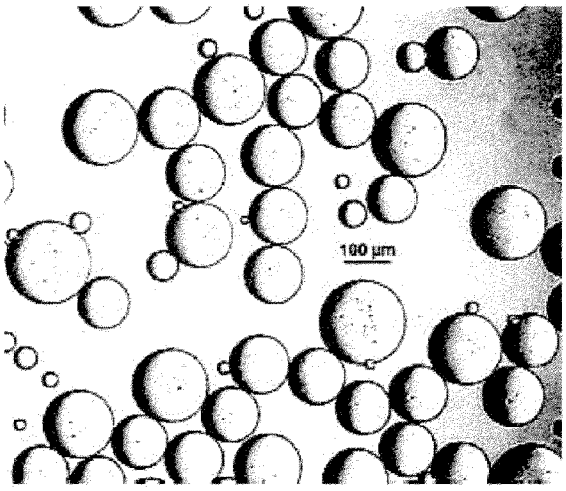
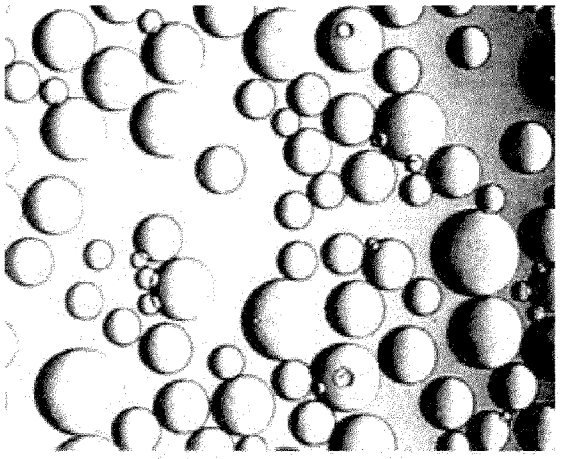


Fig. 4.

a)



b)

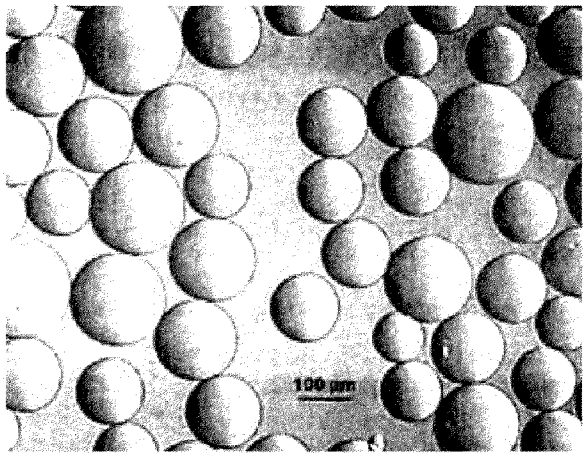
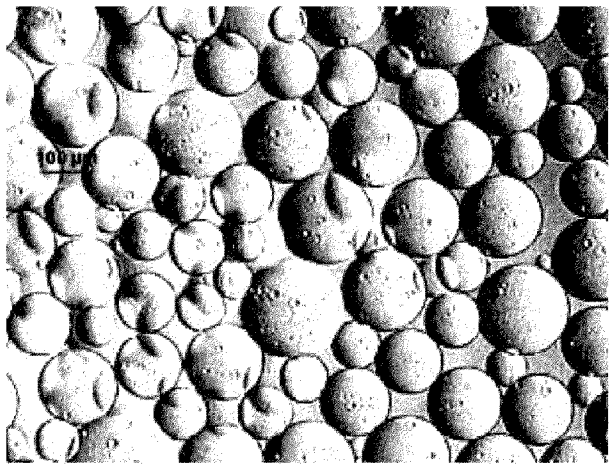


Fig. 5.

a)



b)

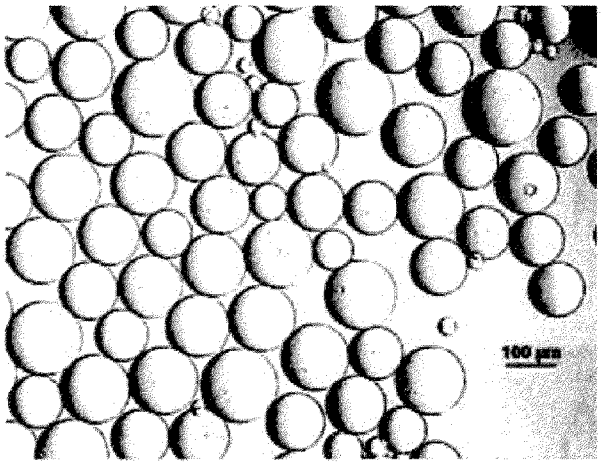


Fig. 6.

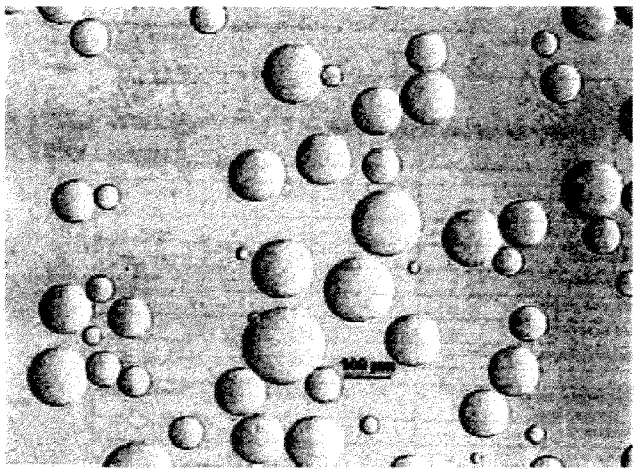


Fig. 7.