METHOD OF PREPARING STABLE SUSPENSIONS OF INSOLUBLE MICROPARTICLES

This invention relates to compositions and procedures that yield sub-micron and micron-size stable particles of water-insoluble or poorly soluble drugs or other industrially useful insoluble compounds. This invention provides for the first time a reliable HLB-based selection criteria for selecting the type and amount of surface modifiers used to obtain sub-micron stable suspensions.

BACKGROUND OF THE INVENTION

Various proposals have been made for preparing formulations of water-insoluble drugs in aqueous solutions using surface modifiers such as phospholipids alone or with one or more surfactants. However, no criteria are set out for selecting the characteristics and quantities U.S. 5,145,684 describes a poorly soluble drug having a non-crosslinked surface modifier adsorbed on its surface. The amount of surface modifier is 0.1% - 90% by weight, and the resulting particle size is less than 400 nm. The use of cloud-point modifiers is described in US 5,298,262, 5,326,552, 5,336,507, 5,340,564 and 5,470,583 in which a poorly-soluble drug or diagnostic agent has adsorbed on its surface both a cloud-point modifier and a non-crosslinked ionic surfactant. The cloud point modifier is said to increase the cloud point of the surfactant such that the resulting nanoparticles are resistant to particle size growth upon heat sterilization at 121° C. These patents provide different examples of specific cloud point modifiers used in conjunction with different surfactants in which the cloud-point modifying surfactants are arbitrarily selected.

WO 98/07414 describes a poorly soluble drug having two surface modifiers adsorbed on its surface; the addition of the second surface modifier provides approximately a 50% reduction in particle size as compared to the use of only one modifier.

EP 0580690B1 describes solubilizing water-insoluble peptides by coating them with a charged phospholipid such that the weight ratio of drug to phospholipid is above a critical number. Poloxamer 188 is also used to prepare the drug particles at concentration from 0.01% - 0.5%. A reduction in the magnitude of the zeta potential is observed as the poloxamer 188 concentration is increased.

US 5,091,187 renders water-insoluble drugs injectable by formulating them as aqueous suspensions of phospholipid-coated microcrystals. The crystalline drug is reduced to 50nm – 10 μm by sonication or other processes inducing high shear in the presence of phospholipid. Phospholipid is described as the sole surface modifier.

US 5,858,410 solubilizes water-insoluble drugs by the addition of a surfactant (synthetic or natural) using a piston-gap homogenizer. The resulting particles are determined by photon correlation microscopy to be in the range of 10nm – 1,000 nm, with less than 0.1% of the population above 5 microns. Again, the surface modifiers are arbitrarily selected.

DESCRIPTION OF THE INVENTION

The compositions prepared according to the method of this invention include, in addition to particles of a water-insoluble ore poorly soluble drug or other industrially useful compound, natural or synthetic phospholipids or surfactant alone, or in combination with each other. According to the procedures of this invention the type and amount of surface modifiers is chosen relative to the drug, such that the system Hydrophile-Lipophile Balance (HLB) value of the system, defined as:

$$\text{System HLB} = \sum_j \frac{(\text{weight of surfactant j})}{\text{weight of drug})} \times (\text{HLB value of surfactant j})$$

is within the range of 4 to 9. When the system HLB is within this range, the resulting formulation has a volume-weighted average particle size that is less than about 1 micron, and exhibits good stability at different temperatures, and stress tests. As used in this specification and claims the term system means the entire composition including drug(s), surface modifiers, carriers, vehicles, diluents and other components customarily present is such compositions.

The Hydrophile-Lipophile Balance (HLB) is a scale that balances between two opposing tendencies present in a surfactants: hydrophilic (that portion which has an affinity towards water) versus lipophilic (that portion which has an affinity towards oil). The more hydrophilic surfactants have high HLB numbers (in excess of 10), while surfactants with HLB numbers from 1-10 are considered to be lipophilic. Preferably the HLB value of the surface modifier or modifiers is between 5 and 35.

The water insoluble or poorly water soluble compound may be selected from various therapeutic agents, including an antifungal agent, immunosuppressive or

immunoactive agent, antiviral agent, antineoplastic agent, analgesic or anti-inflammatory agent, antibiotic, antiepileptic, anesthetic, hypnotic, sedative, antipsychotic agent, neuroleptic agent, antidepressant, anxiolytic, anticonvulsant agent, antagonist, neuron blocking agent, anticholinergic or cholinomimetic agent, antimuscarinic or muscarinic agent, antiadrenergic, or an antarrhythmic, antihypertensive agent, hormone or a nutrient.

The surface modifiers employed usually fall into two general categories, phospholipids and surfactants. The phospholipid may be any naturally occurring phospholipid or mixtures of phospholipids, sometimes referred to herein as "commercial" phospholipids, such as egg or soybean phospholipid or a combination thereof. The phospholipid may be desalted, hydrogenated or partially hydrogenated or natural, semi-synthetic or synthetic. Examples of commercially available phospholipids include but are not limited to egg phospholipids P123 (Pfanstiehl), Lipoid E80 (Lipoid); and hydrogenated soy phospholipids Phospholipon 90H and 100H (Natterman) and 99% pure egg and soy phosphatidyl choline (Avanti Polar Lipids). The amount of phospholipid present in the composition ranges from 0.01% to 50%, preferably from 0.05% to 20%.

The surfactant, sometimes referred to as a second surface modifier, includes: (a) natural surfactants such as casein, gelatin, tragacanth, waxes, enteric resins, paraffin, acacia, gelatin cholesterol esters and triglycerides (b) nonionic surfactants such as polyoxyethylene fatty alcohol ethers, sorbitan fatty acid esters, polyoxyethylene fatty acid esters, sorbitan esters glycerol monostearate, polyethylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, poloxamers, poloxamines, methylcellulose, hydroxycelllulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose; polyvinyl alcohol, polyvinylpyrrolidone, and synthetic phospholipids, and (c) colloidal clays such as bentonite, veegum and colloidal silica. A detailed description of these surfactants may be found in Remington's Pharmaceutical Sciences, and Theory of Practice of Industrial Pharmacy, Lachman et al 1986.

Specific examples of suitable second surface modifiers include the following: poloxamers, such as Pluronic ™ F68, F108, and F127, which are block copolymers of ethylene oxide and propylene oxide available from BASF, and poloxamines, such as

Tetronic ™ 908, which is a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylene-diamine available from BASF, Triton ™ X-100, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas. Tween 20, 40, 60 and 80, which are polyoxyethylene sorbitan fatty acid esters available from ICI Specialty Chemicals, Carbowax™ 3550 and 934, which are polyethylene glycols available from Union Carbide, hydroxy propylmethylcellulose and polyvinylpyrrolidone.

Preferably the surface modifier is a polyoxyethylene sorbitan fatty acid ester, a block copolymer of ethylene oxide and propylene oxide, polyoxyethylene stearate a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine, an alkyl aryl polyether sulfonate, polyethylene glycol, hydroxy propylmethylcellulose, and polyvinylpyrrolidone.

The surfactant desirably is a polyoxyethylene sorbitan fatty acid ester polyoxyethylene stearate, a block copolymer of ethylene oxide, and propylene oxide, a tetra functional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine, an alkyl aryl polyether sulfonate, polyethylene glycol, hydroxy propylmethylcellulose, and polyvinylpyrrolidone.

The phospholipid may be desalted, hydrogenated or partially hydrogenated or natural, semisynthetic or synthetic and preferably is phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinoistol, phosphatidylglycerol or phosphatidic acid.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

To further illustrate and describe the selection process of the present invention the following experiments were carried out. In the examples that follow a premix was processed at a constant temperature and pressure by using high-pressure equipment that subjects the formulation to shear, cavitation, impact, and attrition, that is in either a Microfluidizer or a homogenizer. Details are given in the following table.

Formulation type	Processing Machine	Total Passes at Operating Pressure	Average Pressure (kPsi)	Average Temperature (C)
Cyclosporine	Avestin C-50 homogenizer	200	18	10
Ursodiol	Avestin C-5 homogenizer	100	18	13
Fenofibrate	Microfluidizer M11O EH	50	18	5

A "pass" is defined as one cycle of the formulation through the different elements of the processing machine. The "pass" or cycle for each machine is as follows: Avestin C-50 and C-5: Formulation is placed in inlet reservoir then passes to the homogenization valve, next a heat exchanger then back to the inlet reservoir. It is the homogenization valve that subjects the formulation to the forces of shear, cavitation, impact and attrition. M110 EH: The formulation is first put through 20 passes of the bypass loop, defined as follows: inlet reservoir to auxiliary processing module to heat exchanger then back to inlet reservoir. The resulting formulation is then put through the interaction chamber loop, defined as follows: inlet reservoir to auxiliary processing module to interaction chamber to heat exchanger then back to inlet reservoir. It is in the interaction chamber where the formulation is subject to the forces of shear, cavitation, impact and attrition. Followed by processing, each formulation was collected and placed in vials, capped with rubber stoppers and sealed with an aluminum cap, for stability testing. Acceptable particles are those microparticles falling within the range of 0.05 to 10 microns.

In the examples that follow the following materials are employed.

List of Abbreviations of Surface Modifiers	
Full Name	Abbreviation
Lipoid E-80	LipE80
Phospholipon 100H	Ph 100H
Myrj 52	Myrj 52
Tween 80	Tw 80
Pluronic F68 (also known as Poloxomer 188)	PF68
Pluronic F108 (also known as Poloxomer 338)	PF108
Pluronic F127 (also known as Poloxamer 407)	PF127
Tetronic 908	T908

List of Suppliers	
Name	Supplier/ Location
Cyclosporine	North China Pharmaceutical Company, China
Ursodiol	Tokyo Tanabe, Tokyo, Japan
Fenofibrate	Laboratorio Chimico Internazionale s.p.a., Milan, Italy
Lipoid E-80	Lipoid GMBH, Ludwigshafen, Germany
Phospholipon 100H	American Lecithin Company Natterman Phospholipids, Oxford, Connecticut, USA
Myrj 52	ICI, Wilmington, Delaware, USA
Tween 80	ICI, Wilmington, Delaware, USA
Tetronic and Pluronic Block Polymers	BASF, Mount Olive, New Jersey, USA

The five different tests were used to evaluate the stability of the formulations.

Stability Test	Description
4°C	Sample stored at 4°C (temperature controlled)
25°C	Sample stored at 25°C (temperature controlled, 60% relative humidity)
40°C	Sample stored at 40°C (temperature controlled)
Shaking	Sample laid down on its side on a shaking table at ambient room temperature. The shaking speed was at 100 rpm-110 rpm.
Thermal Cycling	One cycle defined as follows: sample stored at 4°C for 1-2 days, then at 40°C for 1-2 days.

A formulation is regarded as being stable if at least two of the following conditions are satisfied:

(1) The average particle size is less than 1.5 μm at 4°C over a period of four weeks.

(2) The average particle size is less than 1.5 μm at 25°C over a period of four weeks.

(3) The average particle size is less than 2.5 μm at 40°C over a period of one week.

(4) The average particle size is less than 1.5 μm following 7-day shaking.

(5) The average particle size is less than 1.5 μm following 3 cycles of thermal cycling.

Example A

In this example the effect of system HLB on particle size and stability of cyclosporine microparticles were assessed. We found that when the combination of phospholipid plus one surface modifier are chosen such that the system HLB value is above 9, the resulting formulation is unstable. However, if a combination is chosen such that the resulting system HLB value is less than 9 (but greater than 0), the resulting formulation is sub-micron size and stable. The control experiment without surface modifier is included as a reference.

TABLE 1.1 CYCLOSPORINE 5% w/w

Ex	Surface Modifier #1			Surface Modifier #2			Size (µm)	# of Passes	System HLB
	Type	% w/w	HLB	Type	% w/w	HLB			
1	-	-	0	-	-	0	8.33	138	0
2	LipE-80	10	7	-	-	-	2.86	187	14
3	LipE-80	9	7	PF68	1	29	1.77	177	18.4
4	Ph 100H	2	6	Tw80	2	15	1.04	180	8.4

The above formulations were prepared in 200 gram batches on the Avestin C-50 at an operating pressure of 18,000 psi. Prior to homogenization, 5.5% w/w mannitol was added along with 1N NaOH to adjust the pH in the range 7-8. Particle size is a volume-weighted average, measured on the Malvern Mastersizer. Example 1 exhibited an average particle size in the range of 7 µm - 9 µm during homogenization. The extrapolation of data indicates that the particle remains in this range even after 180 passes.

Table 1.2 – Stability of Cyclosporine Microparticles Example 4
Formulation processed for 211 passes; terminal particle size was 1.00 µm

Temperature (°C)	Initial size (microns)	Final size (microns)	Days
4	1.00	0.81	56
25	1.00	0.80	82

From the above data examples 2 and 3 in Table 1.2 show that the combination of Lipoid E-80 with Pluronic F68, such that the total w/w% of the surface modifiers is 10% does not lead to a stable sub-micron formulation, given that the system HLB value of these formulations is greater than 9. Example 4 illustrates the effect of reducing the system HLB value to 8.4 using a suitable combination of phospholipid and surface modifier, which leads to a micron-sized, stable formulation.

Example B

Next the effect of system HLB on particle size and stability of ursodiol microparticles was studied. These experiments, prepared in 50 gram batches with 5.5% w/w mannitol, illustrate that when the combination of phospholipid plus one or more surface modifiers are chosen such that the system HLB value is above 9 or less than 4, the resulting formulation is unstable. However, if a combination is chosen such that the system HLB value is between 4 and 9, the resulting formulation is sub-micron size and stable. The control experiment without surface modifiers is included as a reference.

TABLE 2.1 – URSODIOL 10% w/w + 2 Surface Modifiers

Ex	Surface Modifier #1			Surface Modifier #2						Stable
	Type	% w/w	HLB	Type	%w/w	HLB	Size (µm)	# of Passes	System HLB	
1	-	-	0	-	-	0	12.61	0*	0	No
2	Lip E80	2.4	7	-	-	-	1.40	105	1.7	No
3	Lip E80	6	7	-	-	-	0.99	104	4.2	Yes
4	Lip E80	6	7	PF68	2	29	1.31	107	10	No
5	Lip E80	3.8	7	PF68	2	29	0.99	106	8.5	Yes
6	Lip E80	1.6	7	T908	0.8	31	1.15	107	3.6	No

Ursodiol 10% w/w + 3 Surface Modifiers

Ex	Surf. Mod. 1		Surf. Mod. 2		Surf. Mod. 3					
	Type, % w/w	HLB	Type, % w/w	HLB	Type, %w/w	HLB	Size (µm)	Passes	System HLB	Stable
7	Lip E80, 6.1	7	Myrj 52, 2	16.9	PF68, 1.1	29	1.35	102	11.6	No

* In absence of surface modifiers, mixing is quite difficult, excessive foam is generated, and the formulation cannot be processed.

Table 2.2 - Stability of IDD-P™ Ursodiol

Ex	Size (micr)	4C stability		22C stability		40C stability		7-day Shaking	3-cycle Therm
		Days	Size	Days	Size	Days	Size		
3	0.99	28	1.03	28	1.05	7	1.07	1.05	1.07
5	0.99	28	1.02	28	1.03	7	1.06	1.04	1.09

Results for Tables 2.1 and 2.2 show the following important conclusions:

Examples 1,2 and 3 in Table 2.1 illustrate the effect of increasing the phospholipid concentration from 0%, 2.4% w/w and 6% w/w such that the system HLB values are 0, 1.7, and 4.2 respectively. In case of example 1 where there are no surface modifiers, mixing of the drug and water is difficult, and the formulation cannot be homogenized. The formulation with the system HLB above 4 is sub-micron size and stable, whereas the others are not.

Examples 3 and 4 illustrate the effect of increasing the PF 68 concentration from 0% to 2%, at a fixed phospholipid concentration of 6%, such that the system HLB values are 4.2 and 10 respectively. The formulation with the system HLB between 4 - 9 is sub-micron size and stable, whereas the other formulation is not.

Examples 4 and 5 illustrate the effect of decreasing the phospholipid concentration from 6% to 3.8%, at a fixed PF 68 concentration of 2%, such that the system HLB values are 10 and 8.5 respectively. The formulation with the system HLB between 4 - 9 is sub-micron size and stable, whereas the other formulation is not.

Examples 6 and 7 illustrate the effect of the system HLB value outside the range of 3.9 – 9: particle size greater than 1 micron, and unstable formulations. In particular, example 5 has an system HLB of less than 3.9, whereas example 6 has an system HLB value of greater than 9.

Example C

The example studies the effect of system HLB on fenofibrate particle size and stability. These experiments show that when the combination of phospholipid plus one

or more surface modifiers are chosen such that the system HLB value is less than 4, the resulting formulation is unstable. However, if a combination is chosen such that the resulting system HLB value is between 4 to 9, the resulting formulation is sub-micron size and stable. The control experiment of no surface modifier is included as a reference.

TABLE 3.1 –FENOFIBRATE 10% w/w (+ 5.5% w/w Mannitol)

Ex	Surface Modifier #1			Surface Modifier #2						Stable
	Type	% w/w	HLB	Type	%w/w	HLB	Size (µm)	# of Passes	System HLB	
1	-	-	0	-	-	0	65	0*	0	No
2	Lip E80	3	7	-	-	0	1.06	70	2.1	No
3	Lip E80	4	7	-	-	0	0.95	70	2.8	No
4	Lip E80	3	7	PF127	1	29	0.86	70	5.0	Yes
5**	Ph100H	0.83	6	PF108	1.67	29	0.85	84	5.3	Yes
6**	Ph100H	1.33	6	PF108	0.67	29	0.83	70	2.7	No
				FENOFIBRATE 5% w/w (+5.5% w/w Mannitol)						
7	LipE-80	2	7	PF127	0.5	29	.88	70	5.7	Yes
8	LipE-80	2.3	7	PF127	0.2	29	0.91	70	4.4	Yes

* In absence of surface modifiers, mixing is quite difficult, the drug floats on top of aqueous phase, and the formulation cannot be processed.
**No Mannitol present

The formulations given in Table 3.1 were prepared in 200 gram batches on the M110 EH at an operating pressure of 18,000 psi. Prior to homogenization, 1N NaOH was added to adjust the pH in the range 6-8. Particle size is a volume-weighted average, measured on the Malvern Mastersizer.

Table 3.2 - Stability of Microparticles of Fenofibrate

Ex	Size (micr)	4 C stability		22C stability		40 C stability		7-day shaking
		Days	Size	Days	Size	Days	Size	
4	0.86	33	1.10	29	1.32	8	2.31	1.27
5	0.91	26	1.1	26	1.29	7	1.68	1.16
7	0.88	29	1.01	29	1.18	12	2.47	1.09
8	0.91	35	1.12	35	1.25	7	1.4	1.04

The above examples 2 and 4 in Table 3.1 illustrate the effect of increasing the PF 127 concentration from 0% to 1% w/w such that the system HLB values are 2.1 and 5, respectively. The formulation with the system HLB above 4 is sub-micron size and stable, whereas the other formulation is not. Examples 3 and 4 illustrate the effect of changing the relative amounts of Lip E80 and PF 127 such that the total surface modifier concentration is 4% w/w. The formulation with a system HLB value > 4 (example 4) is stable, whereas the formulation with a system HLB value of < 4 (example 3) is not stable.

Examples 5 and 6 illustrate the effect of changing the relative amounts of Phospholipon 100H and PF 108; the formulation with a system HLB value > 4 (example 5) is stable, whereas the formulation with a system HLB value of < 4 (example 6) is not stable.

Examples 7 and 8 are stable, sub-micron size formulations with total surface modifier concentration of 2.5% w/w, such that the system HLB value of each formulation is between 4 and 9. In both formulations, different combinations of Lipoid E80 and PF 127 are used.

Examples 3 and 7 illustrate the effect of increasing the PF 127 weight ratio relative to the drug from 0 to 1, while maintaining the Lip E80 weight ratio at 4. The

system HLB values are 2.8 and 5.7, respectively. The formulation with the system HLB above 4 is sub-micron size and stable, whereas the other formulation is not stable.

EXAMPLE D

The formulation of this example as set out in Table 4.1 was prepared as a 200 gram batch (120 passes at 22°C) on the M110EH at an operating pressure of 18 kpsi. Particle size is a volume-weighted average, measured on the Malvern Mastersizer.

TABLE .4.1 – VEX 5%

Ex	Surface Modifier #1			Surface Modifier #2						Stable
	Type	% w/w	HLB	Type	%w/w	HLB	Size (µm)	# of Passes	System HLB	
1	LIP E80	0.5	7	PF 108	1.0	29	0.34	120	6.5	Yes

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After 4 wks at 25°, the particle size is 0.34 microns, identical to the starting size, hence the particles were stable.

The above example in Table 4.1, with a system HLB within the ranage of 4-9, exhibits good stability at room temperature (four weeks at 25°C). The lyophilized drug (with 5% w/w PVP) reconstituted to 0.37 microns, almost identical to the starting size. In addition, this formulation showed significant bioavailability in dogs and rats. Bioavailability in dogs was 27% and in rats gave 33%.

WHAT IS CLAIMED IS:

1. A method of preparing stable micron or sub-micron size suspensions of a water-insoluble or poorly soluble compound suspended in an aqueous medium containing at least one surface modifier, the method comprising selecting the surface modifier or modifiers such that the hydrophile-lipophile balance (HLB) of the composition , defined as:

$$\text{System HLB} = \sum_j \frac{(\text{weight of surfactant j})}{\text{weight of drug})} \times (\text{HLB value of surfactant j})$$

is between 4 and 9.

2. A method of preparing a stable micron or sub-micron size suspension of a water-insoluble or poorly soluble compound in an aqueous medium containing a phospholipid and at least one surfactant, the method comprising selecting the surfactant or surfactants such that the HLB of the composition , defined as:

$$\text{System HLB} = \sum_j \frac{(\text{weight of surfactant j})}{\text{weight of drug})} \times (\text{HLB value of surfactant j})$$

is between 4 and 9.

3. The method of claim 1 wherein the HLB value of the surface modifier or modifiers is between 5 and 35.

4. The method of claim 2 wherein the HLB of the surfactant is between 5 and 35.

5. The method of claim 1 wherein the surface modifier is a polyoxyethylene sorbitan fatty acid ester, a block copolymer of ethylene oxide and propylene oxide, polyoxyethylene stearate a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine, an alkyl aryl polyether sulfonate, polyethylene glycol, hydroxy propylmethylcellulose, polyvinylpyrrolidone and polyvinyl alcohol.

6. The method of claim 2 wherein the surfactant is a polyoxyethylene sorbitan fatty acid ester polyoxyethylene stearate, a block copolymer of ethylene oxide, and propylene oxide, a tetra functional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine, an alkyl aryl polyether sulfonate, polyethylene glycol, hydroxy propylmethylcellulose, polyvinylpyrrolidone and polyvinyl alcohol.

* C L M S *

7. The method of claim 2 wherein the phospholipid is desalted, hydrogenated or partially hydrogenated or natural, semisynthetic or synthetic.

8. The method of claim 7 wherein the phospholipid is phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinoistol, phosphatidylglycerol, phosphatidic acid lysophospholipids, egg or soybean phospholipid or a combination thereof.

9. The method of claim 1 or claim 2 wherein the water insoluble or poorly water soluble compound is an antifungal agent, immunosuppressive or immunoactive agent, antiviral agent, antineoplastic agent, analgesic or anti-inflammatory agent, antibiotic, antiepileptic, anesthetic, hypnotic, sedative, antipsychotic agent, neuroleptic agent, antidepressant, anxiolytic, anticonvulsant agent, antagonist, neuron blocking agent, anticholinergic or cholinomimetic agent, antimuscarinic or muscarinic agent, antiadrenergic, and antarrhythmic, antihypertensive agent hormone or a nutrient.

10. A drug composition prepared by the method of claim 1 comprising a phospholipid and a surfactant wherein the HLB value of the composition is between 4 and 9 and which following lyophilization the reconstitution maintains substantially the same particle size.

