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Aqueous nanodispersions prepared by a salting-out process

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Summary

Cellulose acetate phthalate nanodispersions were prepared in the form of a redispersible powder by a new preparation technique based on the salting-out process between two miscible solvents. This method involved the selection of salts producing the salting-out of acetone from water. Poly(vinyl alcohol) was used as protective colloid during the particle formation step and in the finished product. For a pharmaceutical use, the advantage of this new technique is that the process does not require any surfactant or increase in temperature and thus may be useful for biopolymers or heat-sensitive substances. These nanodispersions may also be dried by lyophilization and the particles subsequently redispersed in an aqueous medium before use.

Introduction

Aqueous polymeric dispersions (latexes) have been used in pharmaceutical applications such as film coating, granulating and controlled release (Vanderhoff and El-Aasser, 1988). The presence of residual monomers as well as traces of emulsifiers, initiators or buffers may render dispersions obtained by emulsion polymerization unsuitable for certain pharmaceutical purposes. An alternative is to produce nanodispersions from preformed polymers, the so-called pseudo-latexes (El-Aasser et al., 1977; Vanderhoff et al., 1979). Among the different possible methods, the most common one consists of three basic steps (Bindschaedler, 1985; Gurny et al., 1985): (i) dissolving the polymer in a volatile water-immiscible solvent; (ii) dispersing the polymer solution in an aqueous phase containing surfactants; (iii) removing the organic solvent by vacuum distillation to obtain a water-based polymeric dispersion. In contrast, the technique we have developed makes use of water-miscible solvents instead of water-immiscible solvents and furthermore, a protective colloid is used instead of surfactants (Ibrahim, 1989, Bindschaedler et al., 1990). The finished product is obtained in the form of a redispersible powder and can easily be resuspended in an aqueous medium.

Earlier, an apparently similar method for making microparticles was described in two patents (Sloan and Mann, 1957a,b). The latter does not

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permit the obtaining of nanodispersions and, furthermore, cannot make use of a single water-miscible solvent such as acetone, but always of a mixture of two organic solvents as already described for common nanodispersion preparation methods. The purpose of this work was to develop from pre-existing polymers, such as cellulose derivatives, aqueous nanodispersions which do not require surfactants and which may be dried to produce redispersible powders.

Materials and Methods

Acetone was chosen as the organic water-miscible solvent on the basis of its pharmaceutical acceptability (Bindschaedler et al., 1990) and its physico-chemical properties (Matkovich and Christian, 1973). Cellulose acetate phthalate (CAP, Eastman, Kingsport, U.S.A.) was selected as a model compound (Ibrahim et al., 1991) and poly(vinyl alcohol) (PVAL, polyvinyl alcohol 100 000, Fluka) was used as a hydrocolloid due to its high salt tolerance.

Selection of salting-out agents

Equal volumes of acetone and of highly concentrated aqueous salt solutions were added into a 100 ml glass flask. The mixtures were then stirred by performing twenty 180° rotations of each flask and allowed to stand for 24 h in a water bath at 25°C. The volumes were measured 30 min after a second series of twenty 180° rotations of each flask. The salts tested were either provided by Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland): aluminum chloride ($AlCl_3 \cdot 6H_2O$), aluminum nitrate ($Al(NO_3)_3 \cdot 9H_2O$), aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$), ammonium chloride ($NH_4Cl$), ammonium nitrate ($NH_4NO_3$), ammonium sulfate ($(NH_4)_2SO_4$), calcium chloride ($CaCl_2 \cdot 2H_2O$), calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$), calcium sulfate ($CaSO_4 \cdot 2H_2O$), magnesium chloride ($MgCl_2 \cdot 6H_2O$), magnesium nitrate ($Mg(NO_3)_2 \cdot 6H_2O$), magnesium sulfate ($MgSO_4 \cdot 7H_2O$), potassium chloride ($KCl$), potassium nitrate ($KNO_3$), potassium sulfate ($K_2SO_4$), sodium chloride ($NaCl$), sodium nitrate ($NaNO_3$) and sodium sulfate ($Na_2SO_4$).

Preparation method for CAP nanodispersions

This new preparation method consists of the addition of a water-soluble polymer (PVAL) to a highly concentrated salt solution, so as to obtain a viscous gel. Separately, CAP is solubilized in a water-miscible organic solvent. Although this organic solvent is miscible in all proportions with pure water, a two liquid-liquid phase system is formed corresponding to a water-in-oil (w/o) emulsion, when a portion of the gel is added to the organic solution. Such a system is obtained when the salt dissolved in the aqueous phase is capable of producing the salting-out of the organic solvent. Upon further addition of the gel to the organic phase under vigorous stirring, an oil-in-water (o/w) emulsion is formed, at room temperature, after phase inversion has occurred. Pure water is then added to the resulting emulsion in a sufficient quantity, so that all the organic solvent diffuses into the aqueous phase, inducing the formation of nanoparticles suspended in the resulting organic-aqueous medium. The resulting particles are then collected after several washings performed by centrifugation, discarding of the supernatant phase and resuspension of the particles in pure water. Drying by lyophilization and the use of a protective colloid ensure a satisfactory redispersion of the powdered product. The preparation scheme for a CAP nanodispersion as a dry redispersible powder is shown in Fig. 1.

A typical formula (Table 1) consisted of two phases prepared separately: an aqueous phase (250 g), containing 3.7% (w/w) PVAL and 60.5% (w/w) magnesium chloride hexahydrate, and an organic phase (170 g), corresponding to a 17.6% (w/w) solution of CAP in acetone. The aqueous gel was then progressively incorporated, under vigorous mechanical stirring (350 rpm) into the organic phase to form an o/w emulsion. This operation was performed at ambient temperature for a volume ratio organic phase/aqueous phase of 0.7. Distilled water (250 ml) was then added to the resulting emulsion to allow the complete diffusion of the acetone into the aqueous phase. The resulting dispersion was centrifuged for 15 min at 18 000 rpm. The supernatant phase was discarded and the sediment was resuspended in distilled water. This operation was repeated until

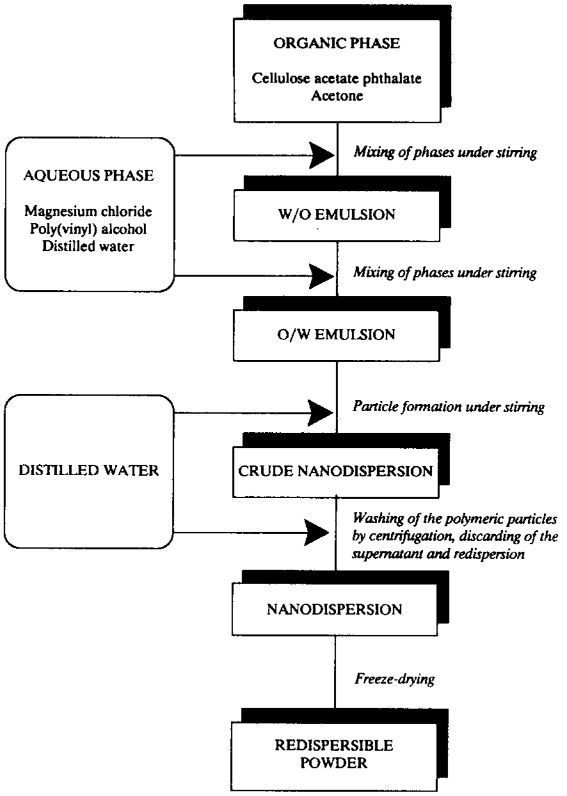

Fig. 1. Preparation of CAP nanodispersions produced by a salting-out process of acetone from water.

no trace of chloride was detected by a chloride limit test (USP XXII). A nanodispersion was obtained by simply suspending the nanoparticles in an appropriate aqueous phase after the last washing. To produce a redispersible powder, the nanoparticles of polymer were dried by lyophilization (cooling temperature, −28°C; sublimation and secondary drying, 25°C; duration of the total process, 15 h) once the washing operation was completed.

TABLE 1

Typical formula for the preparation of a CAP nanodispersion

Emulsion step	
Organic phase	170.0 g
Cellulose acetate phthalate	30.0 g
Acetone	140.0 g
Aqueous phase	250.0 g
Poly(vinyl alcohol)	9.2 g
Magnesium chloride hexahydrate	151.2 g
Distilled water	89.6 g
Particle formation step	
Addition of distilled water	250 g

TABLE 2

Classification of compounds tested as salting-out agents of acetone from water

Substance	Sulfate	Chloride	Nitrate
Aluminum	C	D	B
Calcium	A	D	B
Magnesium	A	D	B
Ammonium	C	C	A
Potassium	A	A	A
Sodium	A	C	A

Only compounds of class D and, in some cases, of class C are suitable for the salting-out of acetone from water in the preparation of nanoparticles.

Scanning electron microscopy

Photographs of polymer particles after freeze-drying were obtained by electron microscopy.

Results and Discussion

Selection of the salting-out agents

18 salts, composed of six different cations and three different anions, were tested in mixtures of equal volumes of acetone and water. These salts were categorized into four classes according to the behavior of their saturated or highly concentrated aqueous solution in contact with acetone (Table 2):

Class A: No liquid-liquid phase separation but precipitation of the solute in the organic-aqueous medium.
Class B: No liquid-liquid phase separation, however high the acetone-water volume ratio may be.
Class C: Liquid-liquid phase separation accompanied by a partial precipitation of the salting-out agent in the medium.
Class D: Liquid-liquid phase separation without formation of a precipitate.

The volume percentage of acetone recovered was dependent on: (i) the ratio of the solvent volumes (water and acetone); (ii) the type of the solute dissolved in the aqueous phase; (iii) the concentration of the solute in the aqueous phase.

Eight salts behaved as compounds of class A (calcium sulfate, magnesium sulfate, ammonium nitrate, potassium sulfate, potassium chloride, potassium nitrate, sodium sulfate and sodium nitrate). Precipitation occurred almost immediately and mainly because of the change in solubility of the salt from the water medium to the water-acetone medium.

Three salts behaved as compounds of class B (aluminum nitrate, calcium nitrate and magnesium nitrate). The solutes were soluble in the organic-aqueous phase but did not produce the salting-out of acetone from water.

Four salts behaved as compounds of class C (aluminum sulfate, ammonium sulfate, ammonium chloride and sodium chloride). An upper acetone phase and a precipitate were observed. For some solutes like sodium chloride, it should be noted that lower concentrations effected phase separation without precipitation. Thus, the classification of some compounds of class C may be changed to class D, when lower salt concentrations are considered. Aluminum sulfate and ammonium sulfate produced liquid-liquid phase separation but the initial volume of the upper phase was larger than the acetone volume introduced into the mixture and furthermore, this volume decreased by increasing salt concentration (Ibrahim, 1989).

Three salts behaved as compounds of class D (aluminum chloride, calcium chloride and magnesium chloride). Five compounds out of 18, all from class C and D, showed potential properties for producing the salting-out of acetone from water. They were all chloride derivatives and their physico-chemical properties are listed in Table 3.

Effect of type of solute on acetone volume recovery

The acetone volume recovery was studied for the chloride derivatives which were capable of causing a solvent separation phenomenon (aluminum chloride, calcium chloride, magnesium chloride, ammonium chloride and sodium chloride). Various solutions of decreasing salt concentration were prepared and mixed with equal volumes of acetone, according to the procedure described in the preceding section.

The amount of salt dissolved in the aqueous phase was first expressed as a salt concentration ratio, which was calculated from the amount of salt dissolved, divided by its concentration at saturation at 25°C. The advantage of this method resides in the diagrammatic representation of the volume of acetone recovered vs the salt concentration ratio, which takes into account the difference in solubility of the salts involved. Fig. 2 shows that the maximal volume that can be recovered is reached before saturation. The maximal volume of acetone recovered depends on the type of solute and its order was: sodium chloride > magnesium chloride > calcium chloride > aluminum chloride > ammonium chloride.

The representation of acetone recovery vs concentration of salts expressed in mol per l (Fig. 3) shows that phase separation starts at different molarity, depending on the type of salt and is in

TABLE 3

Physico-chemical properties of the salting-out agents of acetone from water

Compound	Formula	Relative molecular weight	Concentration at saturation		Acetone recovery (%)
			(g/l)	(mol/l)	
Aluminum chloride	$AlCl_3 \cdot 6H_2O$	241.44	750.0	3.11	28.6
Calcium chloride	$CaCl_2 \cdot 2H_2O$	147.02	455.0	3.18	28.9
Magnesium chloride	$MgCl_2 \cdot 6H_2O$	203.33	790.0	3.89	32.0
Ammonium chloride	NH_4Cl	53.50	283.0	5.29	19.2
Sodium chloride	NaCl	58.45	317.0	5.42	37.1

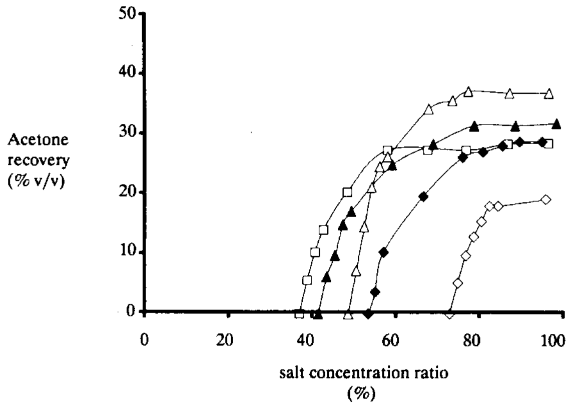

Fig. 2. Recovery of the acetone phase as a function of the salt concentration ratio calculated as: amount of salt dissolved, divided by its concentration at saturation at the temperature of 25°C. (□) Aluminum chloride; (◆) calcium chloride; (▲) magnesium chloride; (◇) ammonium chloride; (△) sodium chloride.

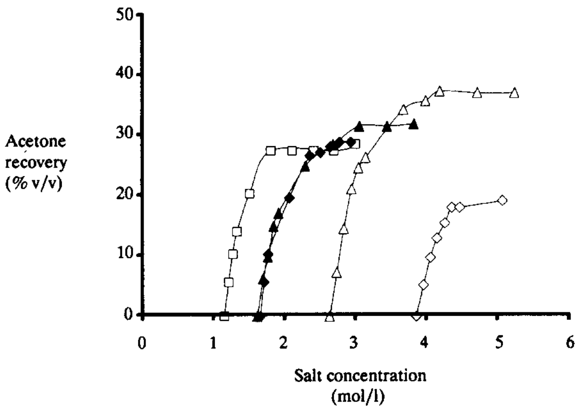

Fig. 3. Recovery of the acetone phase as a function of the molarity of the salts dissolved in the aqueous phase. (□) Aluminum chloride; (◆) calcium chloride; (▲) magnesium chloride; (◇) ammonium chloride; (△) sodium chloride.

the following order: aluminum chloride (1.19 mol/l) < magnesium chloride (1.68 mol/l) < calcium chloride (1.69 mol/l) < sodium chloride (2.68 mol/l) < ammonium chloride (3.94 mol/l). This sequence is identical to that obtained by ordering the salts according to decreasing free energies of hydration, or even according to enthalpies, since the entropic contribution to hydration is relatively minor (Table 4). An aluminum chloride molecule is able to retain a much larger number of water molecules than an ammonium chloride molecule. This may explain why, based on a molar scale, aluminum chloride is more effective than ammonium chloride in initiating salting-out of acetone. Table 4 also shows that polyvalent cations make a more important contri-

TABLE 4

Thermodynamic properties of chlorides vs salt concentration at incipient phase separation

		$-\Delta G_h^\circ$ [a] (kJ mol^{-1})	$-\Delta H_h^\circ$ [b] (kJ mol^{-1})	z^2 [c] / $(r_x + 0.25)$	z^2 [c] / $(r_x - 0.25)$	Salt concentration (mol l^{-1}) [d]
Cation	NH_4^+	-	307	0.58		
	Na^+	382	409	0.83		
	Ca^{2+}	1515	1577	3.23		
	Mg^{2+}	1836	1921	4.44		
	Al^{3+}	4625	4665	12.00		
Anion	Cl^-	352	381		0.64	
Salt	NH_4Cl	-	688			3.94
	NaCl	734	790			2.68
	$CaCl_2$	2219	2339			1.69
	$MgCl_2$	2540	2683			1.68
	$AlCl_3$	5681	5808			1.19

[a] Free energy of hydration: $-\Delta G_{298\,K}^\circ$ for $M^{z\pm}$ (g) → $M^{z\pm}$ (aq.) (Bernard and Busnot, 1984).

[b] Enthalpy of hydration: $-\Delta H_{298\,K}^\circ$ for $M^{z\pm}$ (g) → $M^{z\pm}$ (aq.) (Bernard and Busnot, 1984).

[c] Parameters affecting the strength of attraction between the water molecules and the ions; z, ionic charge; r_x, ionic crystallographic radius.

[d] Salt concentration at incipient phase separation.

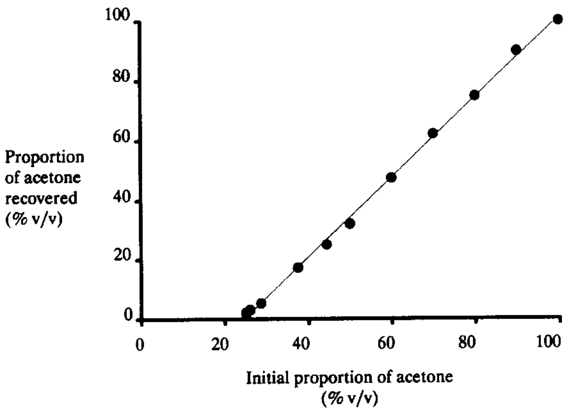

Fig. 4. Recovery of acetone phase as a function of the initial proportion of acetone in the acetone-water-magnesium chloride medium.

bution to the free energy of hydration than chloride does or than polyvalent anions would do. This reflects the fact that most cations have a smaller crystallographic radius, r_x, than anions. The above reasoning can be repeated by considering, instead of the free energy, the function $(z^2/(r_x + 0.25))$ for cations or $(z^2/(r_x - 0.25))$ for anions which reflects the strength with which water molecules are retained by a given cation (Dytnerskii et al., 1972). This expression shows that water binding is proportional to the square of the ionic charge, z^2, and diminishes when the cation radius increases. In Fig. 3, acetone recovery occurred first with salts having a trivalent cation (Al^{3+}), followed by those with a bivalent cation (Mg^{2+} and Ca^{2+}) and finally those with a monovalent cation (NH_4^+ and Na^+). However, it should be noted that sodium chloride produced a higher maximal acetone phase recovery (37.1% v/v) than magnesium chloride did (32.0% v/v) (Table 3), but precipitation of sodium chloride occurred when the salt concentration ratio reached 80% (Fig. 2). No precipitation occurred with magnesium chloride and thus, this salt was chosen for the production of nanodispersions (Fig. 1).

Effect of volume ratio on phase separation

The influence of the volume ratio was illustrated for magnesium chloride. Saturated magnesium chloride solutions and acetone were mixed

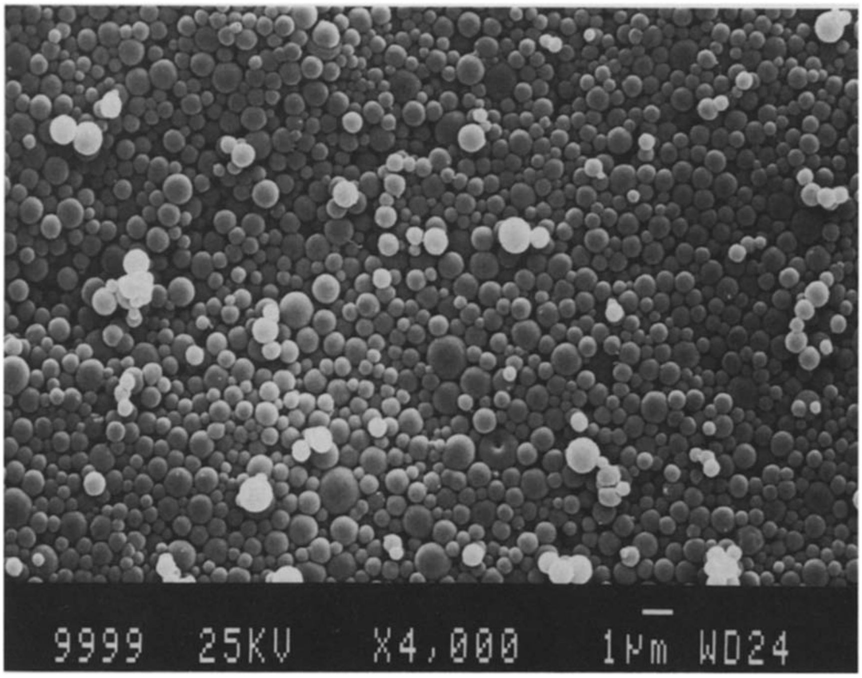

Fig. 5. Freeze-dried CAP nanodispersion visualized by scanning electron microscopy.

in various proportions. The proportion of acetone recovered was measured according to the procedure described under Selection of the salting-out agents. The results are shown in Fig. 4 for acetone recovery from a 3.81 mol/l magnesium chloride solution at 25°C. Acetone recovery appeared linearly dependent on the initial volume of acetone, once the solubility of the organic solvent in the aqueous phase was exceeded. The initial portion of the graph represents complete miscibility of acetone in the aqueous phase, the intersection of the rising portion with the x-axis corresponding to the solubility of acetone in the aqueous phase (25% v/v). From the properties of magnesium chloride solutions mixed with acetone, it can be seen that acetone diffusion into the aqueous phase is promoted by: (i) decreasing the concentration of salt in the aqueous phase (Figs 2 and 3); (ii) increasing the water/acetone volume ratio (Fig. 4). These two effects will be produced by a same operation, which is the addition of pure water to any magnesium chloride solution-acetone system.

Preparation of CAP nanodispersions

Aqueous dispersions of CAP were prepared by using a polymer partially neutralized by the addition of a sodium bicarbonate solution to the polyelectrolyte dissolved in acetone (Ibrahim et al., 1990). The partial neutralization of the polyelectrolyte can only be performed before the addition of the 3.7% PVAL gel to the organic phase (Ibrahim, 1989). This is because at the beginning of the process, sodium bicarbonate may react with the magnesium salt contained in the gel and, at the final step, it may interact with the polymer particles. The salting-out process is thus used at two preparation stages (Fig. 1). Firstly, to produce the salting out of acetone from the aqueous phase which enables the formation of an emulsion. Secondly, in a reversible manner, to induce the particle formation by diffusion of the organic solvent in the aqueous phase. Photographs were obtained by scanning electron microscopy after freeze-drying of the aqueous dispersion (Fig. 5).

Conclusion

The organic solvent (acetone) used for the preparation of aqueous dispersions from pre-existing polymers by a reversible salting-out process should be selected on the basis of its volatility and low toxicity, especially in view of the pharmaceutical applications of the resulting product. The condition of complete miscibility between the solvent (acetone) and water is not essential, but contributes advantageously to the proper execution of the process. If it is not the case, there is a need for a greater water/organic solvent volume ratio during the formation of the particles.

The selection of the optimal solute is as critical as that of the solvent, and all combinations resulting in the precipitation of the solute are unsuitable. Magnesium chloride was used for the salting-out of acetone from water, but it is also possible to use electrolytes such as sodium chloride, calcium chloride (Ibrahim, 1989) or even non-electrolytes such as sucrose (Matkovich and Christian, 1973; Bindschaedler et al., 1990). The quantity of solute dissolved will preferably be such that the aqueous solution is saturated or practically saturated, or sufficiently concentrated in solute to produce a phase separation phenomenon at the volume ratio used.

PVAL was selected to act as a protective colloid at both the preparation stage (emulsion, formation of particles) and the final stage (lyophilization, redispersion of the particles). The protective colloid must not be excessively soluble in acetone, otherwise the system tends to form a w/o emulsion during the process. Increasing the concentration of hydrocolloid is another suitable means for reducing particle size.

It has been observed, that by varying the stirring speed during the mixing of the aqueous phase (PVAL and $MgCl_2 \cdot 6H_2O$ in water) with the organic phase (CAP in acetone) or by modifying the concentration of the polymer solution, it is also possible to obtain particles of different sizes.

For a pharmaceutical use, the advantages of this new nanodispersion preparation method are:

(1) the process does not require an increase in temperature and therefore may be useful when biopolymers are used or when heat-sensitive substances have to be processed; (2) the process does not require any surfactants; (3) the final product can easily be redispersed before use; (4) a single solvent such as acetone, soluble in all proportions with water can be used; (5) polymer particles can be produced with an average size less than 1 μm; (6) a redispersible powder can be produced after freeze-drying to ensure the stability of the polymer.

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