International Journal of Pharmaceutics, 87 (1992) 247–253

IJP 02946

Preparation of aqueous polymeric nanodispersions by a reversible salting-out process: influence of process parameters on particle size

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(Received 16 March 1992)
(Accepted 14 June 1992)

Key words: Nanoparticle; Latex; Polymeric aqueous dispersion; Salting-out; Methacrylic acid copolymer; Poly(dl-lactic acid); Ethylcellulose

Summary

Polymeric nanoparticles were prepared as aqueous dispersions using an emulsion technique involving a salting-out process which avoids surfactants and chlorinated solvents. The preparation method consists of adding an electrolyte-saturated or a nonelectrolyte-saturated aqueous solution containing poly(vinyl alcohol) (PVAL) as a viscosity-increasing agent and stabilizer to an acetone solution of polymer under continuous stirring. The saturated aqueous solution prevents acetone from mixing with water by a salting-out process. After the preparation of an oil-in-water emulsion, water is added in a sufficient amount to allow complete diffusion of acetone into the aqueous phase, inducing the formation of nanospheres. Several manufacturing parameters were varied: stirring rate, internal/external phase ratio, concentration of polymer in the acetone phase, type of electrolyte, concentration and type of PVAL in the aqueous phase.

Introduction

Aqueous suspensions of polymeric nanoparticles (latexes) have been used in pharmaceutical applications such as film coating (Bindschaedler et al., 1986), controlled release (Gumowski et al., 1987) and drug targeting (Vanderhoff and El-Aasser, 1988). These aqueous dispersions can be divided in two main categories, according to their method of preparation. They can be produced either by polymerization reactions or by dispersion of preformed polymers (pseudo-latexes) (Gurny, 1991). The presence of residual monomers as well as of surfactant residues and sometimes of initiators may render the former techniques unsuitable for certain pharmaceutical preparations such as injectable dosage forms (Oppenheim, 1981). The latter techniques rely on the emulsification of water-immiscible organic solutions of preformed polymers in aqueous phases containing surfactants, followed by the removal of the solvents under reduced pressure. An impor-

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tant drawback of these methods for pharmaceutical applications is the remaining surfactants.

The aim of this work was to control and optimize, regarding mean particle size, a technique originally developed in our laboratories (Ibrahim, 1989; Bindschaedler et al., 1990; Ibrahim et al., 1992). In this procedure, a polymer dissolved in a water-miscible solvent is emulsified in an aqueous phase containing no surfactant. A two phase system is formed due to the presence of a salting-out agent in the aqueous phase.

Several manufacturing parameters were varied: homogenization, stirring rate, internal/external phase ratio, concentration and type of polymer in the organic phase, type of salting-out agent, concentration and type of stabilizing agent in the aqueous phase. By modulating these parameters, it was possible to produce particles with an average size ranging from 170 to 900 nm, with monomodal distributions.

Finally, other polymers, salting-out agents and solvents were tested to demonstrate the capabilities of this new manufacturing technique.

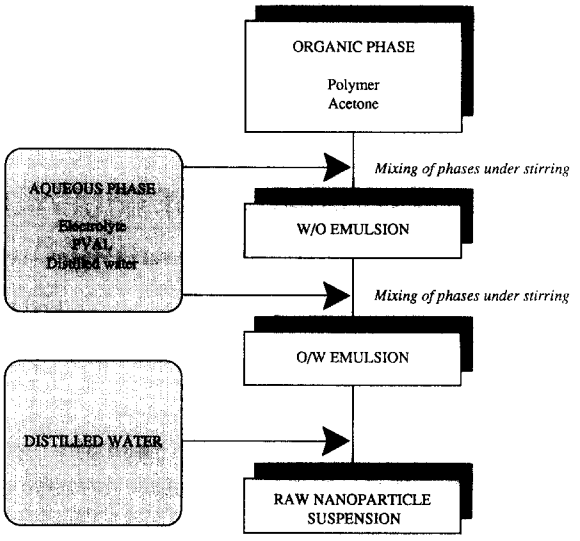

Fig. 1. Preparation of polymeric nanoparticles produced by a salting-out process.

Materials and Methods

Materials

Acetone was chosen as the organic water-miscible solvent on the basis of previous work (Bindschaedler et al., 1990), physico-chemical properties (Matkovich and Christian, 1973) and pharmaceutical acceptability with regard to toxicity (Rabiant, 1991). Tetrahydrofuran (Merck, Darmstadt, Germany), ethyl acetate (Merck) and isopropyl alcohol (Merck) were also used. The methacrylic acid copolymers Eudragit® S and Eudragit® E (Röhm Pharma, Weiterstadt, Germany), poly(dl-lactic acid) (PLA) (Medisorb ® 100 DL, Medisorb Technologies International L.P., Cincinnati, OH, U.S.A.) and ethyl cellulose N10 (Hercules, Wilmington, DE, U.S.A.) were used as polymers. Eudragit® S is an anionic copolymer based on methacrylic acid and methyl methacrylate, soluble from pH 7 upwards. Eudragit® E is a copolymer of cationic nature, based on dimethylaminoethyl methacrylate and neutral methacrylic esters. It is soluble below a pH of 5. Magnesium chloride hexahydrate (Fluka, Buchs, Switzerland), magnesium acetate tetrahydrate (Merck) and sucrose (Siegfried, Zofingen, Switzerland) were used as salting out agents. Poly(vinyl alcohol) (PVAL) 100 000 (Fluka), Mowiol® 4–88, 8–88 (Hoechst, Frankfurt am Main, Germany) and PVAL 22 000 (BDH, Poole, U.K.) were selected as stabilizing colloids.

Nanoparticle preparation

The preparation method consists of adding a water-soluble polymer (PVAL) to a highly concentrated solution of an electrolyte or nonelectrolyte, so as to obtain a viscous gel (aqueous phase). Eudragit® S, Eudragit® E, PLA or ethyl cellulose are dissolved separately in acetone or another suitable solvent (organic phase). Although this organic solvent is miscible in all proportions with pure water, a liquid-liquid phase system is formed when a portion of the gel is added to the organic solution (Fig. 1). Such a system is obtained when the salt dissolved in the aqueous phase is capable of producing salting-out of the organic solvent. Upon further addition of the gel to the organic phase under vigorous mechanical stirring, an oil-in-water emulsion is formed at room temperature. Pure water is then

added to the resulting emulsion in a sufficient quantity for the acetone to diffuse into the aqueous phase, thus inducing the formation of nanoparticles suspended in the resulting organic-aqueous medium (Ibrahim et al., 1992).

Particle size determination

Nanoparticle sizes were measured by photon correlation spectroscopy (PCS) using a Coulter® Nanosizer® (Coulter Electronics, Harpenden, Hertfordshire, U.K.) and a Zeta-sizer® III (Malvern, Worcestershire, U.K.). Each value given is the average of three measurements. Polydispersity values are given for Nanosizer® measurements. Values of 0 or 1 describe essentially mono-sized particle suspensions, whereas higher values indicate that the size distribution is larger.

Results and Discussion

Variation of process parameters

With this manufacturing technique, polymeric nanoparticles produced with PVAL 100000 as stabilizing agent, under mild agitation, as described in an earlier work (Ibrahim et al., 1992), have a mean diameter close to 1 μm. In order to reduce the size, the influence of homogenization on particle size was first investigated with the Polytron® Kinematica® (Littau, Switzerland) using a PTDA 3020/2 rotor at 10000 rpm and the Büchi® high pressure homogenizer 196 (Flawil, Switzerland). Table 1 shows that it is possible to reduce the mean diameter to 427 nm, using the Polytron® before adding the dilution water, but it should be noted that the temperature rises up to 50°C during this 2 min process. The process with the Büchi® homogenizer was not feasible before adding half of the dilution water because of the excessive viscosity of the emulsion. Homogenization after the addition of a part of the dilution water does not significantly reduce the mean sizes of the particles, since they are probably already partially formed at this step.

TABLE 1

Influence of the type of homogenization on the mean diameter of Eudragit® S nanoparticles (organic phase: Eudragit® S 17.7%, 70 g; aqueous phase: PVAL 3% and $MgCl_2$ 60%, 100 g)

Type of treatment	Mean size (nm)	Polydispersity
Without homogenization	938	2
Polytron® (2 min before adding water)	427	4
Polytron® (5 min after adding half of the dilution water)	646	2
Büchi® (15 cycles after adding half of the dilution water)	707	3

TABLE 2

Influence of the PVAL type on the mean diameter of the nanoparticles (organic phase: Eudragit® S 17.7%, 70 g; aqueous phase: PVAL and $MgCl_2$ 60%, 100 g; stirring rate, 900 rpm)

Type of PVAL	Molecular weight	Concentration % (w/w)	Mean size (nm)	Polydispersity
PVAL	100000	3	938	2
PVAL BDH	22000	10	1117	0
Mowiol® 8-88	49000	8	248	2
Mowiol® 4-88	26000	11	243	1

The influence of PVAL type was then evaluated. Four products were used as stabilizing and viscosifying agents (Table 2). The concentration of the solutions depended on both the type and the molecular weight of the PVAL in order to obtain a viscosity of the aqueous phase ranging from 4 to 5 Pa s (shear rate: 10 s^{-1}). Table 2 shows that Mowiol® 4-88 (molecular weight M_w 26000) stabilizes the emulsion well and allows a significant reduction in the mean size of the nanoparticles (243 nm). All the PVALs used are partially hydrolyzed grades, except BDH 22000, which is a 96% hydrolyzed grade.

Based on these results, Mowiol 4-88 was chosen for all subsequent steps. The effects of the percentage of PVAL in the external phase (Fig. 2), stirring rate (Fig. 3), percentage of Eudragit® S in the internal phase (Fig. 4), and internal/external phase ratio (Fig. 5) were evaluated. The results clearly show that by varying the concentration of Mowiol® 4-88 in the external phase, the size of the nanoparticles can be controlled within a wide range (186-1130 nm). PVAL appears to exert a steric stabilizing action on the dispersed droplets of the internal phase (Tadros, 1983) and

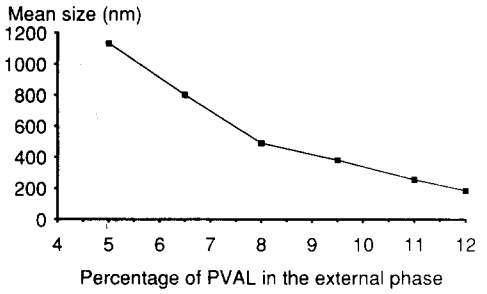

Fig. 2. Influence of the percentage of Mowiol® 4–88 in the external phase on the mean size of the nanoparticles. External phase: Mowiol® 4–88 at different concentrations and $MgCl_2$ 60%, 100 g; internal phase: Eudragit® S 17.7%, 70 g.

avoids the need for a surfactant in the emulsification process. It is then assumed that the smaller the droplets of the internal phase of the emulsion, the smaller the nanoparticles after the dilution step with distilled water.

An increase in stirring rate allows a slight decrease in the particle size. To a first approximation, Fig. 3 shows that the Hinze-Clay relation used in emulsion technology may be employed to predict the size of nanoparticles as a function of stirring rate:

$$x = C\nu^{-5/6}$$

where x is the diameter of the droplets, C represents a constant and ν is the stirring rate (Walstra, 1983).

The mean size of the particles can also be adjusted within the range from 200 to 500 nm by

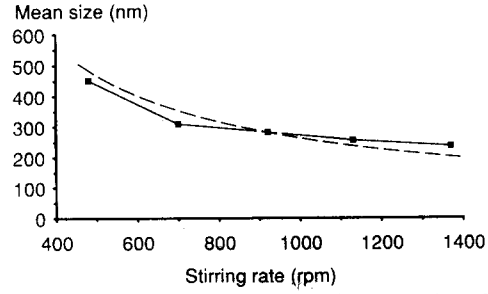

Fig. 3. Influence of the stirring rate on the mean size of the nanoparticles, comparison of experimental data with Hinze Clay relation (dashed line). External phase: Mowiol® 4–88 at different concentrations and $MgCl_2$ 60%, 100 g; internal phase: Eudragit® S 17.7%, 70 g.

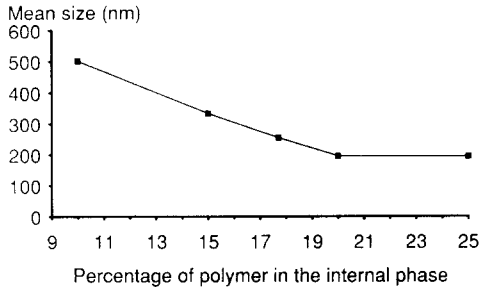

Fig. 4. Influence of the amount of Eudragit® S in the internal phase on the mean size of the nanoparticles. External phase: Mowiol® 4–88 at different concentrations and $MgCl_2$ 60%, 100 g; internal phase: Eudragit® S at different concentrations 70 g.

varying the polymer concentration in the internal phase, or the internal/external phase ratio (Figs 4 and 5).

Variation of polymer, solvent and salting-out agent

All previous results concerned Eudragit® S dissolved in acetone and emulsified in a magnesium chloride solution, however, the new technique described herein can be varied by using several other polymers, solvents and salting-out agents (Table 3). Mowiol® 4–88 was used as stabilizing colloid in all subsequent examples.

The methacrylic acid copolymer Eudragit® E was examined. Since it is soluble in acidic media, magnesium chloride cannot be used as the electrolyte (concentrated magnesium chloride imparts a pH of 6 to the medium). Neither can a strong base (e.g., sodium hydroxide) be added to modify the pH without resulting the precipitation of

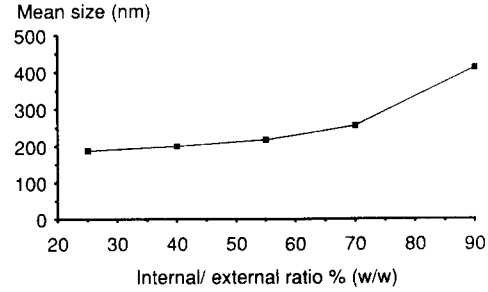

Fig. 5. Influence of the internal/external phase ratio on the mean size of the nanoparticles. External phase: Mowiol® 4–88 at different concentrations and $MgCl_2$ 60%; internal phase: Eudragit® S 17.7%.

TABLE 3

Examples of polymeric nanodispersions produced by the salting-out process

Example No.	Polymer	Solvent	Salting-out agent	Mean size (nm)
1	Eudragit® E	acetone	Mg acetate	172
2	PLA	acetone	Mg acetate	247
3	PLA	acetone	Mg chloride	228
4	Ethyl cellulose N10	tetrahydrofuran	Mg chloride	215
5	Ethyl cellulose N10	ethyl acetate	Mg chloride	480
6	Ethyl cellulose N10	isopropanol	sucrose	1006

magnesium hydroxide. On the basis of previous work, magnesium acetate was selected as salting-out agent (Ibrahim, 1989). The resulting pH of 8 in the external phase allows the formation of nanoparticles having a mean size of 172 nm (internal phase: Eudragit® E 17.7%, 40 g; external phase: magnesium acetate 35% and Mowiol® 4–88 12%, 100 g; stirring rate, 1200 rpm).

PLA nanoparticles were produced by using either an acidic aqueous phase ($MgCl_2$) or a basic aqueous phase (magnesium acetate) (Table 3). For these particles measurements were per-

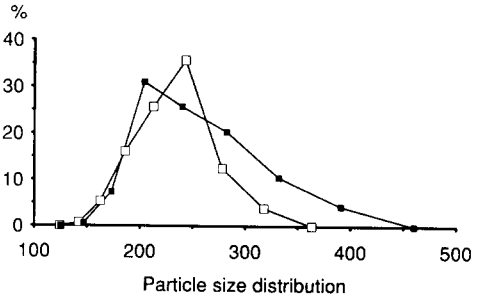

Fig. 6. Particle size distribution of PLA nanoparticles produced with either magnesium chloride (□) or magnesium acetate (■) as salting-out agent.

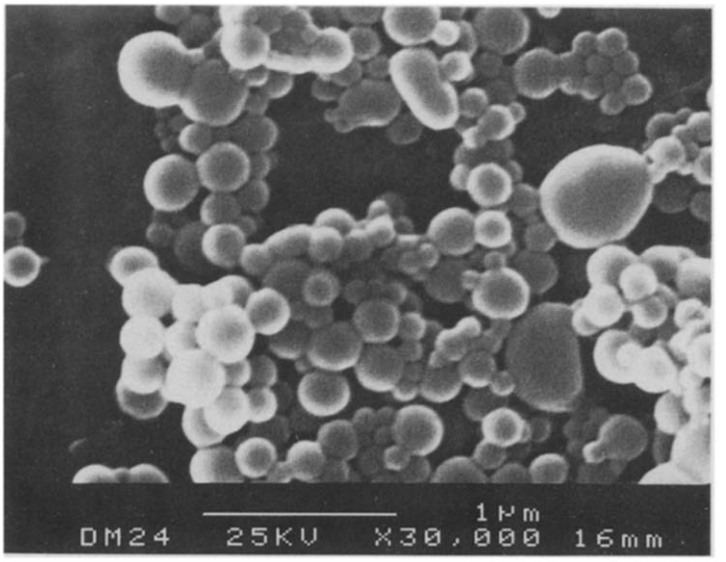

Fig. 7. Scanning electron micrograph of PLA nanoparticles produced with magnesium chloride as salting-out agent.

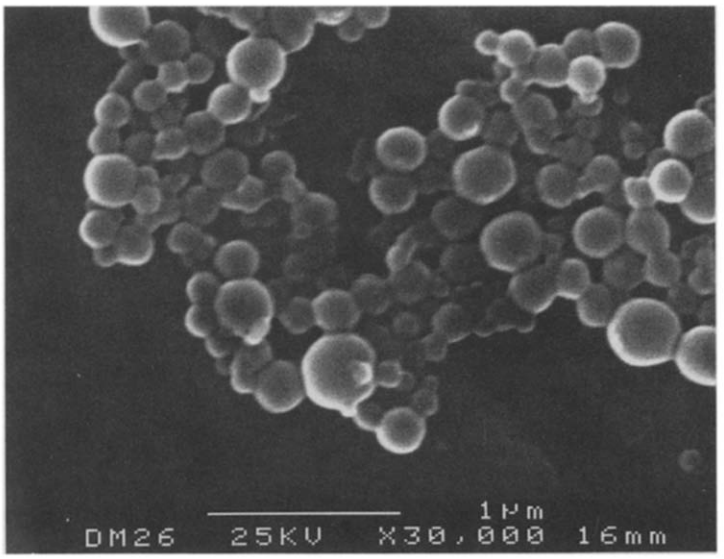

Fig. 8. Scanning electron micrograph of PLA nanoparticles produced with magnesium acetate as salting-out agent.

formed on the Zeta Sizer® which gives intensity vs size data. Fig. 6 demonstrates that the size distributions of PLA nanoparticles are monomodal. The particles produced with $MgCl_2$ as salting-out agent have a mean size of 228 nm (S.D. 37 nm) and those in the case of magnesium acetate have a mean size of 247 nm (S.D. 54 nm). Scanning electron micrographs show that these particles are spherical (Figs 7 and 8). The possibility of producing PLA nanoparticles while using either an aqueous acidic phase or basic phase is very important in the case where one intends to load these particles with a drug, the solubility of which is function of the pH of the medium.

The last three examples of Table 3 indicate that the solvent for the organic phase can also be varied. For example, the use of tetrahydrofuran, ethyl acetate and isopropyl alcohol led to the formation of nanoparticles having mean diameters of 215, 480 and 1006 nm, respectively. It should also be noted that, in the last example, the salting-out agent was sucrose. The use of a non-electrolyte as salting-out agent is interesting, since it offers the possibility of changing the pH of the aqueous phase with acids and bases, in contrast to the case of electrolyte-saturated solutions, which have a fixed pH.

These examples demonstrate that it is possible to carry out this method of manufacture successfully with various polymers, solvents and salting-out agents, the only condition which should be met being that a two-phase system must be obtained in the presence of the salting-out agent at high concentration, which is converted into a single-phase system upon dilution with water.

Conclusions

It has been demonstrated, both in previous results obtained with cellulose acetate and cellulose acetate phthalate (Bindschaedler et al., 1990; Ibrahim et al., 1992) and in the present study carried out with Eudragit® S, Eudragit® E, PLA and ethyl cellulose using the same technique, that this method can be employed with several lipophilic polymers, while avoiding the need for a surfactant. It has been shown that salting-out agents and solvents can be varied according to the pharmaceutical applications for which the particles are intended. This should allow the use of our technique in future studies encompassing several pharmaceutical fields, such as controlled release dosage forms and aqueous film coating.

Moreover, the results with the biocompatible poly(dl-lactic acid) should permit the use of this process for injectable sustained release dosage forms and for drug targeting.

In a subsequent paper, the possibilities of purifying the raw nanoparticle suspensions will be described.

Acknowledgement

One of us (E.A.) wishes to acknowledge a grant and support of this work by Ciba-Geigy, Switzerland.

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