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Preparation of poly(methacrylic acid-g-poly(ethylene glycol)) nanospheres from methacrylic monomers for pharmaceutical applications

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Abstract

Nanospheres of poly(methacrylic acid-grafted-poly(ethylene glycol)) were prepared by solution/precipitation polymerization. As colloidal drug delivery carriers, they present unique properties that render them promising candidates for oral protein delivery. The polymerization was carried out in water and the resulting suspension was freeze-dried. As with many colloidal systems, the freeze-dried suspension showed strong agglomeration after drying. The effects of preparation conditions on the particle size and redispersion were investigated using photon correlation spectroscopy. Furthermore, the ability of different types and concentrations of stabilizers (cryoprotectants and steric stabilizers) in preventing this phenomenon was addressed. Pluronics®, block copolymers widely used as nonionic surfactants, were the most effective in stabilizing the particles during the freeze-drying process. Pluronic® P123, however, increased significantly the particle size of the nanospheres. On the other hand, lyophilizates obtained in the presence of Pluronic® F68 had good redispersion properties and no change in particle size was observed.

Keywords: Nanospheres; Drug delivery; Poly(methacrylic acid); Hydrogels; Pluronic®; Lyophilization

1. Introduction

The ever-increasing evolution of the pharmaceutical field, discovery of disease mechanisms and improved understanding of the human body physiology have rendered the need of 'smart' delivery systems more compelling over the last decade. Biosensors, responsive polymeric networks (Lowman and Peppas, 1999c; Peppas et al., 2000a; Kost and Langer, 2001) and controlled drug delivery systems possess great potential for filling the need for better control of drug administration. The common feature of these systems is the attempt to mimic the physiological needs of the body. Pulsatile delivery, controlled release and

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site-specific delivery are among the strategies endeavored (Wise et al., 2000; Siepmann and Peppas, 2001).

The ultimate goal in developing a new drug delivery system is improvement of the efficacy of the active compound administered, attenuation of undesired side effects, and ultimately increase of the patient compliance. Colloidal drug delivery systems are among the carriers used to achieve this objective (Kreuter, 1994; Torres-Lugo and Peppas, 2002; Torres-Lugo et al., 2002a,b). It has been shown that drug properties such as solubility, absorption through biological membranes, bioavailability, as well as carrier properties like residence time in a certain site and site specificity can be improved by decreasing the drug or carrier particle size (Müller et al., 2001). Micronization and lately nanosizing have been investigated as potentially good techniques (Robinson and Peppas, 2002) for targeting the drug directly or close to the site of action. Tarr et al. (1987) demonstrated that microemulsions possess better intestinal absorption characteristics compared to the conventional emulsions. This result was explained in terms of greater surface area of the dosage form, and therefore, increase in the release of the drug and more intimate contact with the intestinal wall. Yet, nanoparticulate systems have attracted more attention in the field. Compared to the micron range systems, they possess a higher surface area that can lead to higher loading efficiency of active ingredients and a more intimate contact with biological tissues.

Colloidal systems that have been investigated over the years range from liposomes and niosomes to microemulsions and nanospheres (De Jaeghere et al., 1999a). Nevertheless, one of the shortcomings is the stability of these carriers. Both chemical and physical stability are challenges that sometimes hamper the development of colloidal drug carriers. A nanoparticulate system, as well as any other carrier designed for drug delivery, should preserve its original physical and chemical properties during the formulation and storage, maintain the content of drug achieved at the end of the loading, and guarantee an effective release of the drug in the body (Schwarz and Mehnert, 1997). However, in the case of nanoparticulate drug delivery systems in the form of either emulsions or solid formulations problems have been encountered such as leaking, flocculation, agglomeration after redispersion. Therefore, preparation conditions as well as storage represent critical stages (Morishita et al., 2002; Sipahigil et al., 2002).

In this contribution, we address the optimization of complexation-based, pH-sensitive nanoparticulate formulations as possible delivery carriers for proteins and peptides. In particular, we have focused on grafted hydrogels composed of poly(ethylene glycol) (PEG) grafted on poly(methacrylic acid) (PMAA), henceforth designated as P(MAA-g-EG) hydrogels.

The presence of ionic moieties in the polymeric network renders these systems responsive to pH changes in the surrounding environment (Lowman et al., 1998a,b; Peppas and Lowman, 1998; Lowman and Peppas, 1999a; Lowman et al., 1999). The unique behavior of this system is the hydrogen bonding between the hydrogen on the carboxylic acid group of the PMAA and the oxygen in the grafted PEG chain. In acidic conditions, at pH lower than the pK_a of the methacrylic acid, the carboxylic acid groups of the PMAA are non-ionized and the system is able to form hydrogen bonds within the network. Therefore, complexes form and the crosslinked network is collapsed (Torres-Lugo and Peppas, 1999; Lowman and Peppas, 1999b; Ichikawa and Peppas, 1999; Lowman and Peppas, 2000; Peppas et al., 2000a). When the pH rises and reaches values in the alkaline region, the complexation reverses and the system swells (Fig. 1). This type of complex formulation is reversible in nature (Lowman et al., 2000). This behavior has been extensively studied in our laboratories. (Drummond et al., 1989; Brannon-Peppas and Peppas, 1989; Klier and Peppas, 1991; Bell and Peppas, 1996; Peppas and Bures, 1999). Furthermore, this system has been shown to be able not only to respond to pH change in the surrounding environment, but has also been proven to inhibit the proteolytic enzymes of the gastrointestinal tract and open the tight junctions present in the intestinal wall and responsible for the poor absorption. Therefore, it is a promising candidate for the delivery of environ-

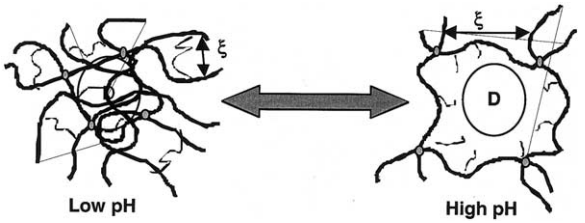

Fig. 1. Network structural changes due to variations on environmental pH. At low pHs (left) interactions between the tethered grafts with the protonized ionic moieties increase. The formation of interpolymer complexes occurs and the system is in the collapsed state. Higher pHs (right) disrupt the interpolymer complexes and ionic moieties deprotonize leading to extensive swelling.

mentally-susceptible bioactive agents characterized by a poor permeation through the intestinal wall (Kim and Peppas, 2002; Huang et al., 2002a,c; Peppas et al., 2000b,c; Kim and Peppas, 2001).

Recently, we have been able to produce P(MAA-g-EG) hydrogels as a monodisperse nanospheres suspension using a solution/precipitation polymerization method in water (Robinson and Peppas, 2002; Foss and Peppas, 2001). Yet, as stated before, it is important to ensure stability of the system both in the form of dispersion and after drying. In the latter case, the solid product obtained has to be able to reconstitute into the original system.

Therefore, the main goal of this research was to investigate the critical parameters in the formulation process in order to have a successful production of pH sensitive nanospheres. In particular, we addressed the redispersion and size preservation of the particles after the drying step.

2. Materials and methods

2.1. Nanospheres preparation and purification

The monomers used were methacrylic acid (MAA, Polysciences, Warrington, PA) and poly(ethylene glycol) monomethylether monomethacrylate (PEGMA, Polysciences, Warrington, PA) with PEG molecular weights of 200, 400, and 1000. Tetraethylene glycol dimethacrylate (TEGDMA, Polysciences, Warrington, PA) was used as a crosslinking agent and 1-hydroxycyclohexyl phenyl ketone (Irgacure® 184, CIBA-GEIGY, Hawthorne, NY) was selected as the photoinitiator. MAA was vacuum distilled at 54 °C/25 mmHg in order to remove the inhibitor, hydroquinone. PEGMA, TEGDMA, and Irgacure® 184 were used as received.

The P(MAA-g-EG) nanospheres were prepared by solution/precipitation polymerization in water. The two monomers, MAA and PEGMA with different chain lengths were mixed in a molar ratio of 1:1 repeating units of MAA to EG. Tetraethylene glycol dimethacrylate (TEGDMA) was added in the amount of 0.75% moles of the total moles of the monomers. The photoinitiator was incorporated in the amount of 0.5% w/w of the monomer mixture. After complete dissolution of the components, the solution, properly sealed, was diluted in deionized water and purged with nitrogen for 20 minutes in order to remove any dissolved oxygen, well known free radical scavenger. The flask was then exposed to UV light for 15 min at an intensity of 100 mW/cm^2. The resulting dispersion of P(MAA-g-EG) nanospheres was washed in a dialysis membrane with different cutoff (cutoff 25 000 MW, SpectraPor 7; cutoff 300 000 MW, SpectraPor 4, Spectrum Laboratories, Rancho Dominguez, CA) to remove any unreacted monomers and sol fraction.

2.2. Freeze-drying process

D(+)Trehalose (Sigma, St. Louis, MO), D(+)Glucose, PEG average M_n 3400, PEG average M_n 8000, PVP 10 000, PVP 40 000, Pluronic®, P123, average M_n 5800 (Aldrich, Milwakee, WI), and Pluronic® F68 solution (10%) (Sigma, St. Louis, MO) were tested as cryoprotectants and stabilizers during the drying process.

Aliquots of the nanospheres suspension were transferred in Cryule® vials (Wheaton, Millville, NJ) of 5 ml capacity and added to various amounts of cryoprotectant and steric surfactant solutions before freeze-drying.

In order to investigate the effect of the freezing rate upon the particle size preservation and the reconstitution ability of the dried system, both slow and rapid cooling were used. Slow freezing

was performed in a conventional freezer. Rapid freezing of the samples was carried out by immersing the vial in liquid nitrogen, or by adding dropwise the nanospheres to liquid nitrogen. The frozen nanospheres were then lyophilized using a LabConco freeze-dry system (model 77500, Kansas City, MO) for 36 h at a temperature of −53 °C and maximum vacuum. The yield of the suspensions was determined by weighing the dried samples recovered.

2.3. Characterization

Particle size and size distribution measurements of the bulk suspension were performed using photon correlation spectroscopy (PCS). A Coulter® N4 Plus submicron particle sizer (Coulter, Miami, FL) was used to determine the mean particle diameter and the polydispersity index (PI). It must be noted that the PI of a Coulter particle size is defined differently than the PI of a molecular weight distribution.

Each sample was properly diluted with water or buffer solutions, according to the need, in order to maintain the count per second between 5×10^4 and 1×10^6. The diluent was filtered with a 0.45 μm filter to remove any impurities that could affect the scattering of the light. Each sample was measured six times for at least 6 min at 20 °C and at an angle of 90°. Both unimodal and size distribution processor (SDP) analysis were performed.

3. Results

3.1. Polymerization and nanospheres purification

Nanospheres of P(MAA-g-EG) were synthesized by a UV initiated free radical solution/precipitation polymerization in water. The kinetics of this reaction has been reported in the literature before (Scott and Peppas, 1999; Ward and Peppas, 2000; Scott et al., 2000; Ward and Peppas, 2001; Ward et al., 2002). After exposure to UV light, the reaction mixture, initially in the form of a clear solution, turned into a milky suspension of nanospheres. The effect of the washing process on the particle size distribution was probed. Photon correlation spectroscopy, also known as dynamic light scattering, was used. The sample was illuminated by a laser beam and the particles undergoing Brownian motion were detected. Light scattered by these particles was received by a fiber-optic cable placed at a particular angle and the fluctuations in scattering intensity were analyzed.

The polydispersity index (PI) is a very important parameter that gives an idea about the reliability of the data obtained with PCS analysis. In correlation spectroscopy, PI is a dimensionless number extrapolated from the autocorrelation function. It ranges from values of 0.010 for monodispersed polystyrene standard latex particles up to values around 0.5–0.7. Values greater than 0.7 are characteristic of samples with a very broad size distribution. In this case, the sample is either not suitable for dynamic light scattering, or presents agglomeration problems.

It has been shown that the best reproducibility and accuracy occurs for submicron size particles (Finsy, 1994). For this reason, the preparation of the sample is a crucial step for reproducibility. Samples must consist of well-dispersed suspension with a size ranging usually from 3 nm to 3 μm. The concentration is also an important factor. At high sample concentration, multiple scattering occurs and no reliable particle diameters can be collected. In order to avoid this phenomenon, the count per second was maintained between 5×10^4 and 1×10^6.

Figs. 2 and 3 show the different distribution before and after washing the same batch of particles. After polymerization, the dispersion possessed a characteristic pH around 3. The particles were in the collapsed state, as discussed previously. The PCS data (Fig. 2) showed that the average diameter of the suspension lay around 300 nm with a slightly broad distribution. Samples of particles from the same batch, taken after the washing process, showed (Fig. 3) a more narrow distribution in particle size with a decrease in the PI, as expected. The washing process was not only necessary for removing unreacted compounds but also led to a more monodispersed suspension.

We also studied the influence of the dialysis membrane cutoff on the particles size distribution.

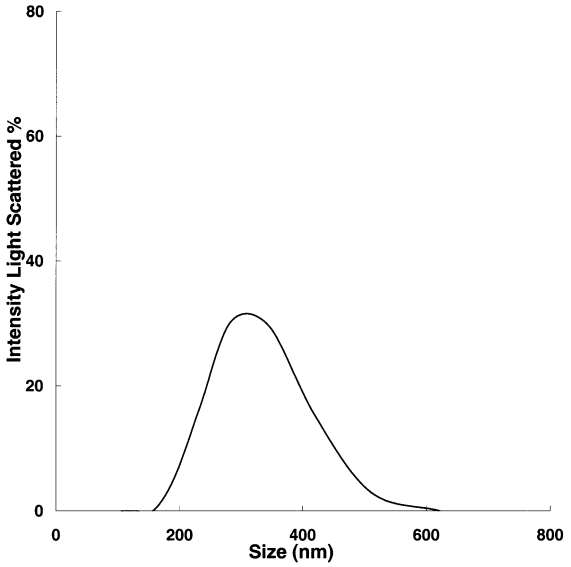

Fig. 2. Size distribution curve obtained from size distribution processor (SDP) analysis of P(MAA-g-EG) nanospheres 1:1 molar ratio. The sample was analyzed using PCS at 20 °C after the polymerization reaction, before washing.

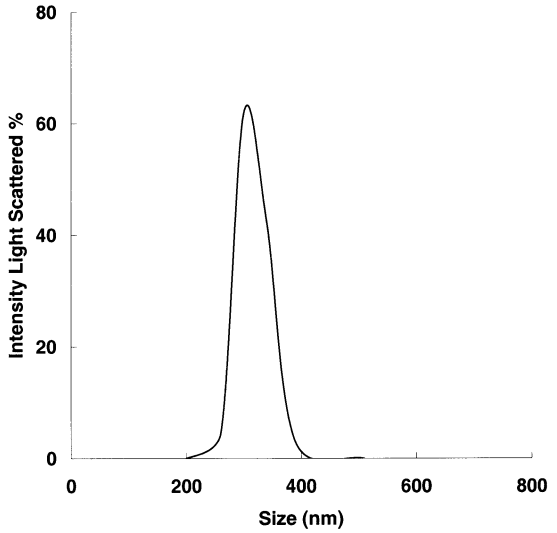

Fig. 3. Size distribution curve obtained from size distribution processor (SDP) analysis of P(MAA-g-EG) nanospheres 1:1 molar ratio. The sample produced by solution/precipitation polymerization was washed with dialysis membrane with a cutoff of 25 000 MW for 5 days and then analyzed without further treatments.

Both 25 000 and 300 000 MW cutoff membranes dialysis were used and sample particles were analyzed with PCS. No significant differences in particle size distribution were detected. Therefore, because of the easiest handling procedure, we decided to use the membrane with the lowest cutoff.

In previous work, Robinson and Peppas (2002) presented the effect of parameters such as the monomers concentration, the amount of cross-linking agent and initiators added to the monomer mixture. Another important factor that might affect the particle size is the length of the PEG chain. We prepared nanospheres using PEG with a nominal weight of 200, 400, and 1000 MW. A ratio of 1:1 of EG units in respect to the repeating units of MAA was used. The lowest PEG length used produced particles with the highest average diameter (Fig. 4). However, the system was not well monodispersed and agglomeration was observed. The particles produced with PEGMA 1000 produced the best suspension (see also Torres-Lugo and Peppas, 2000; Byrne et al., 2002; Huang et al., 2002a,b).

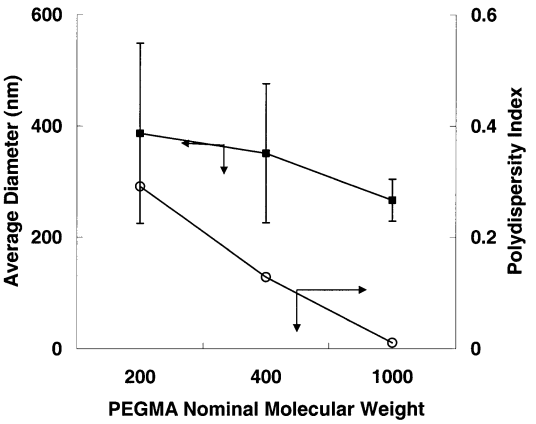

Fig. 4. The effect of different PEG chain lengths on the nanospheres average size (■) and the polydispersity index (PI) (○) of P(MAA-g-EG) nanospheres obtained from 1:1 molar ratio. Samples were washed in dialysis membrane with a cutoff of 25 000 MW for 5 days and then analyzed with both unimodal and size distribution processor analysis.

3.2. The need of an adjuvant in the freeze-drying step

As mentioned before, at the end of the polymerization reaction, the resulting suspension consisted of monodispersed nanospheres with an average diameter of 300 nm. If samples of this suspension were freeze-dried without the addition of any type of adjuvant (cryoprotectant or stabilizers), the redispersion in acidic medium could not be achieved, even using sonication. A strong type of aggregation was observed. On the other hand, if samples from the same batch were resuspended in alkaline media, this problem was not encountered.

At low pH values, i.e. in acidic redispersion media, as previously described, the carboxylic acid groups of the PMAA are protonated and therefore able to establish hydrogen bonds within the network (Madsen and Peppas, 1999). During the freeze-drying process, the system underwent a drastic transition. The medium of the suspension, in this case water, was crystallized at low temperatures and thereafter sublimated. The sample from an aqueous and very diluted state reached a dried and highly concentrated state. In other words, the nanospheres became in intimate contact with each other. When we attempted to resuspend them in acid, the system was under ideal conditions for hydrogen bonding complexation that, we believe, occurred not only within the polymer networks, but also between particles in intimate contact, as they resulted after the drying step.

On the other hand, at higher pH values, the same carboxylic groups were ionized, and electrostatic repulsion occurred. Therefore, the dried nanospheres resuspended in alkaline media did not agglomerate.

One of the important goals of freeze drying is to produce a dried sample that can easily reconstitute the original suspension (Oetjen, 1999). The need of a stabilizer during this step of the formulation was obvious. Different excipients were taken under investigation. Trehalose and Glucose were tested as cryoprotectants in concentrations that ranged from 1 to 5% (w/w). Amphiphilic excipients such as PEG 3400, PEG 8000, PVP 10 000 and PVP 40 000 and nonionic surfactants like Pluronic® P123 and Pluronic® F68 were tested as well in concentrations ranging from 0.1 to 2% (w/w). The goal of this experiment was to screen the potential ability of these excipients in decreasing the physical instability of the dispersion.

Samples of the dispersion were added with NaOH 0.1 M in order to swell the network. When the particle size was reaching value of 500–600 nm, the excipient in study was added. The pH of the system was then lowered by adding proper volumes of HCl in order to reach a pH close to the original suspension (circa 3). The system collapsed and redispersability was investigated via PCS.

Only steric stabilizers such as Pluronic® were able to clearly avoid the strong agglomeration phenomenon, observed in all the other cases.

Pluronic® are nonionic surfactants block copolymers of propylene oxide and ethylene oxide widely used in pharmaceutical formulations as protein stabilizers, coating agents, and steric stabilizers of nanoparticulate systems (Izutsu et al., 1995; De Jaeghere et al., 1999a,b; Mehnert and Mader, 2001; Redhead et al., 2001). In our case, Pluronic® were able to stabilize the nanospheres by steric hindrance and by shielding the surface of the particles decreasing, therefore, hydrogen bonding between particles.

However, an interesting phenomenon was observed. When Pluronic® P123 was added to the system in increasing amount (Fig. 5), the particle size and the PI of the nanospheres increased. We interpreted these data as indicative of a coating phenomenon. However, the coating layer that can be calculated subtracting the normal size of the particle to the particles coated was too thick and could affect the release of the bioactive eventually loaded in the system. For this reason, the use of Pluronic® P123 was abandoned. Pluronic® F68, on the other hand, was able not only to produce a dried resuspendable sample but also was possible to achieve a good stability of the system after the adjunct of acid and no increase in particles size was detected as shown in Fig. 6. We proceeded with this excipient as the best stabilizer and we tried the resuspension after the drying process. As Fig. 7 reported, increasing the amount of Pluronic® F68 in the suspension before freeze-drying, was decreasing the agglomeration phe-

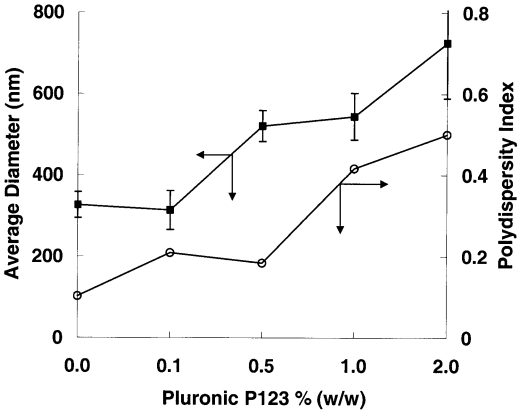

Fig. 5. Average nanospheres size (nm) and polydispersity index (PI) of P(MAA-g-EG) nanospheres as a function of the concentration of Pluronic® P123. Samples of the nanospheres suspension were added with different concentrations of surfactant (% w/w) and PCS analysis was performed without further treatment.

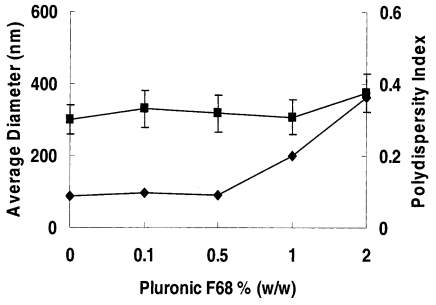

Fig. 6. Average nanospheres size (nm) and polydispersity index (PI) of P(MAA-g-EG) nanospheres as a function of the concentration of Pluronic® F68 (% w/w). Samples of the particles suspension were added with different amounts of stabilizer and data were collected before freeze-drying.

nomenon of the nanospheres when resuspending them. However, concentration higher than 0.5% w/w was increasing the PI. This was probably due to slight agglomeration or micelles formation of the surfactant. A total of 0.5% w/w was a concentration high enough to guarantee a good redispersion of the dried system, without increasing the average diameter, and a good stability over time.

The influence of freezing velocity on quality of reconstituted polymeric particles was also addressed. Both slow and fast freezing were studied.

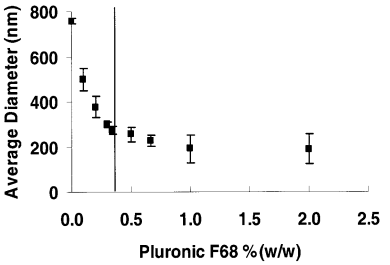

Fig. 7. Average nanospheres size (nm) and polydispersity index (PI) of P(MAA-g-EG) nanospheres lyophilized and reconstituted as a function of the concentration of Pluronic® F68. The stabilizer was added to the particle suspension before freeze-drying. Redispersion by manual shaking. From 0 to 0.34% (w/w) (vertical line) strong agglomeration was observed.

Slow freezing was performed placing the sample in a conventional freezer until complete freezing. Fast freezing was carried out using liquid nitrogen as aid. The vials containing the suspension were either cooled by plunging them directly into liquid nitrogen or by adding the suspension dropwise to liquid nitrogen.

The dried product obtained with either the two fast methods proved to have a better redispersability, compared to the one obtained with slow freezing. In the latter case, a slight sonication was necessary to achieve a good redispersion.

4. Conclusions

P(MAA-g-EG) nanospheres present very promising characteristics for oral delivery of proteins. However, the small size range, and the sensitivity to pH changes in the surrounding environment render the drying step and the resuspension challenging tasks. Preparation conditions and the drying method have to be tailored in order to maintain a narrow particle size distribution and guarantee a good redispersability. This study has showed that optimizing the conditions during the preparation leads to suspension with very narrow particle size distribution over time. Moreover, the addition of a steric stabilizer was crucial for the production of lyophilized nanospheres with good redispersion properties.

Acknowledgements

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