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International Journal of Pharmaceutics 336 (2007) 367–375
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Haloperidol-loaded PLGA nanoparticles: Systematic study of particle size and drug content

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Received 8 September 2006; received in revised form 29 November 2006; accepted 30 November 2006
Available online 5 December 2006

Abstract

We have produced haloperidol-loaded PLGA/PLA nanoparticles by using two emulsification-solvent evaporation methods: homogenization and sonication. We have established how five independent processing parameters and two materials characteristics control the particle size and drug content. The interdependencies between processing and materials parameters and the subsequent nanoparticle characteristics are discussed in terms of underlying scientific principles that are broadly applicable to the production of drug-loaded polymer nanoparticles. This level of understanding should quicken the pace of designing protocols for making new drug-PLGA nanoparticles. It was determined that the particle size of haloperidol-loaded PLGA/PLA nanoparticles is effectively controlled by the amount of shear stress transferred from the energy source to the organic phase, which is strongly correlated to the following parameters: type of applied energy, aqueous phase volume, and polymer concentration in the organic solvent. The drug content of these nanoparticles is controlled by reducing the diffusion of the drug from the organic to the aqueous phase during the solvent evaporation stage of the preparation and by increasing the drug–polymer interactions. The following significantly inhibit drug diffusion: large particle size, higher polymer concentration and polymer molecular weight, and reducing the drug solubility in the aqueous phase by adjusting the pH. Specific drug–polymer interactions are engineered by optimizing the lactide to glycolide ratio (L:G ratio) and including specific polymer end groups. When optimized, the drug-loaded PLGA/PLA nanoparticles contain as much as 2.5% haloperidol.

Keywords: Controlled release; Haloperidol; Nanoparticles; PLGA end groups; Drug-delivery

1. Introduction

Controlled drug delivery systems use biodegradable polymers to release pharmaceutical drugs at a controlled rate for days, weeks or months. PLGA microparticles and nanoparticles have been widely studied for extended release of hydrophobic drugs. The controlled release of a model hydrophobic drug from PLGA nanoparticles depends on the characteristics of the particles, including particle size, size distribution, drug content, incorporation and surface morphology (Gabor et al., 1999). The particle size and drug content are particularly important characteristics that determine drug release. These characteristics depend on the specific fabrication parameters employed to make the particles in the system (Seo et al., 2003). To control these characteristics, it is vital to isolate and establish the effect of each processing and materials parameter on particle size and drug content noting that the effects may vary with different drugs.

The aim of this research is to develop a systematic methodology to control particle size and drug content and to employ scientific principles that link the various processing parameters to the nanoparticle characteristics. The broad scientific principles enable both the control of particle size and drug content and the extension of our results from the haloperidol-PLGA system to other hydrophobic drugs encapsulated in PLGA/PLA nanoparticles.

The general emulsification-solvent evaporation method employed to produce nanoparticles involves a number of processing and materials parameters: power and duration of energy applied, aqueous phase volume, pH of the aqueous phase,

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doi:10.1016/j.ijpharm.2006.11.061

polymer and drug concentration in the organic phase, polymer molecular weight, polymer L:G ratio, polymer end groups, solvent volume, and surfactant concentration. Each of these processing and materials parameters influences the size and/or the drug content of the nanoparticles, which can be understood by applying appropriate scientific principles, as summarized here.

In a typical emulsification-solvent evaporation process employed to produce PLGA/PLA nanoparticles, the nanoparticles are formed as a result of shrinkage of the emulsion nanodroplets (containing the polymer and drug dissolved in organic solvent) and the size of the final nanoparticles (formed upon solvent evaporation from the emulsion nanodroplets) correlates with the size of nanodroplets (Desgouilles et al., 2003; Galindo-Rodriguez et al., 2004). The basic scientific principle governing the size of nanodroplets is that the external energy source provides shear stresses to the organic phase, which results in the formation of nanodroplets. The size of the droplets is inversely correlated to the magnitude of shear stresses. Any change in processing or materials parameters that reduces these shear stresses will increase the nanodroplet size. The most direct influence on the shear stresses in the organic phase is exercised by the energy density (external energy applied per unit total volume). Increasing the energy density directly increases the shear stresses and results in more efficient droplet breakdown and hence a reduction in nanodroplet size. The viscous forces in the organic and aqueous phase oppose the shear stresses in the organic phase. Reducing the organic phase viscosity reduces the viscous forces, which results in a net increase in shear stress felt by the organic phase. This decreases the nanodroplet size.

The drug molecules are trapped inside the nanoparticles as a result of solidification of the nanodroplets. Bodmeier and McGinity (1987) have shown that for a PLGA-quinidine base system there is no drug loss once the polymer starts solidifying from the surface to the core. Thus the entire drug loss occurs during the transition of nanodroplets to nanoparticles due to diffusion of drug from the nanodroplets to the surrounding aqueous phase. Any change in processing or polymer parameters that hinders drug diffusion from the nanodroplets to the aqueous phase will result in increased drug content in the nanoparticles. For example, diffusion can be hindered if we (i) reduce the diffusion time of the drug, (ii) increase the diffusional resistance to the drug molecules, and/or (iii) reduce the drug solubility in the aqueous phase. Another principle that can be used to increase the drug content is to increase the drug–polymer interactions. We can use the above principles to understand and methodically vary the size and drug content of the nanoparticles.

In this study, we developed a method of emulsification-solvent evaporation using sonication, which gives unimodal particle population for a wide range of processing parameters. This provides a constant particle size while probing the effect of various processing parameters on drug content, thereby eliminating interference from changing particle size on drug content. The technique of sonication is first optimized and then utilized to establish the effect of varying the processing parameters and polymer characteristics on particle size and drug content. The characteristics of particles obtained from sonication are also compared with those of particles obtained from homogenization and nanoprecipitation under similar conditions. The technique of nanoprecipitation is used only for comparison purposes because the drug content is too low to be practical. This study demonstrates that sonication effectively produces small (~220 nm) particles with narrow size distributions in which the drug content can be increased by careful manipulation of various parameters including polymer concentration, initial haloperidol concentration, solvent volume and polymer type. These results are discussed in terms of the scientific principles outlined above.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA) 50:50 DL (molecular weight, 7 kDa), 50:50 DL (14 kDa), 50:50 DL (24 kDa), 50:50 DL (48 kDa), 50:50 DL (63 kDa), 65:35 (114 kDa), 75:25 (92 kDa) 100:0 (109 kDa) were purchased from Alkermes, USA. Polyvinyl alcohol (PVA) (MW, 25,000, 88% hydrolyzed) was purchased from Polysciences Inc., USA. Haloperidol, phosphate buffered saline (PBS), ammonium acetate, 1-Piperazineethane sulfonic acid, 4-(2-hydroxyethyl)-monosodium salt (HEPES) were purchased from Sigma, USA. Acetonitrile, dichloromethane (DCM) and acetone were purchased from Fisher scientific. All the solvents were HPLC grade.

2.2. Nanoparticle preparation

Nanoparticles were prepared by using three different methods: emulsification by homogenization-solvent evaporation (homogenization), emulsification by sonication-solvent evaporation (sonication), and nanoprecipitation. Homogenization and sonication involve preparation of an organic phase consisting of polymer (PLGA or PLA, typical concentration, 10 mg/ml) and drug (haloperidol, typical concentration, 0.5 mg/ml) dissolved in DCM (typical volume, 5 ml). This organic phase is added to an aqueous phase containing a surfactant (PVA, typical concentration, 1%, 50 ml) to form an emulsion. This emulsion is broken down into nanodroplets by applying external energy (through a homogenizer or a sonicator) and these nanodroplets form nanoparticles upon evaporation of the highly volatile organic solvent. The solvent is evaporated while magnetic stirring at 300 rpm under atmospheric conditions for 4 h leaving behind a colloidal suspension of PLGA nanoparticles in water. Nanoprecipitation is similar to homogenization and sonication, except the organic solvent is acetone, a water miscible solvent, and there is no application of external energy. Once the colloidal suspension of nanoparticles is prepared using one of the above three methods, the free drug is removed by using our extraction method (Budhian et al., 2005) to obtain the final nanoparticulate suspension containing encapsulated drug.

We studied the effect of various processing parameters and polymer characteristics on mean particle size and drug content particles prepared by homogenization, sonication and nanoprecipitation. The processing parameters include, polymer concentration in the organic phase, initial haloperidol concentration in the organic phase, solvent volume, PVA concentration in

the aqueous phase and the aqueous phase volume. Polymer characteristics include the molecular weight (MW) of the polymer and the lactide to glycolide (L:G) ratio of the polymer.

Unless otherwise mentioned, all the experiments are conducted by varying one of the parameters while keeping all the other processing parameters at a set of standard conditions: 10 mg/ml of PLGA 50:50, MW 51 kDa and 0.5 mg/ml of haloperidol in DCM (for homogenization and sonication) or acetone (for nanoprecipitation) as the organic phase and 50 ml of 1% PVA solution as the aqueous phase. The aqueous to organic ratio and the surfactant to PLGA ratio is 10:1 and polymer to drug ratio is 20:1. Solvent volume is 5 ml. The speed and duration for homogenization is 12,000 rpm and 7 min and sonication is carried out at a power of 7 units for 7 min. In all the figures, each data point represents a batch of nanoparticles and error bars indicate the standard deviation of the mean from multiple measurements (dynamic light scattering) of a single batch. Error bars are omitted when the error is less than 10% of the mean. Hence multiple data points corresponding to a particular value of a variable demonstrate the batch-to-batch reproducibility, while the error bars for a data point demonstrate the reproducibility within a batch.

2.3. Particle size and size distribution in sonication

We have isolated the effect of both the time and duration of sonication on the size and size distribution of our PLGA particles. Increasing the power and/or the duration of sonication reduces the mean diameter of nanoparticles and changes the population distribution from bimodal to unimodal (data not shown). This happens because increasing the power and/or duration of sonication increases the energy causing droplet breakdown, which increases the shear stress resulting in a decrease in particle size. Mainardes and Evangelista (2005) have also reported a decrease in particle diameter with increasing sonication time for a system of PLGA nanoparticles.

2.4. Nanoparticle characterization

Nanoparticles were characterized for size and drug content as described previously (Budhian et al., 2005) and briefly summarized here. The size and size distribution were measured by laser dynamic light scattering. The mean diameter of nanoparticles is based on intensity as given by dynamic light scattering. Each diameter is the mean of three readings within a batch and error represents the standard deviation of the mean. The polydispersity index of the particle size can be measured by the instrument and ranges from 0 to 0.3, where 0.3 refers to the most polydisperse population. The polydispersity indexes of these haloperidol-PLGA nanoparticles, particularly those prepared by sonication and nanoprecipitation, are low and show little variability between different batches of particles prepared under various conditions. Unless otherwise mentioned, the polydispersity indexes of unimodal particles prepared by sonication and nanoprecipitation are 0.05–0.07, while those from homogenization are 0.10–0.14.

The haloperidol content and incorporation efficiency were measured by dissolving the nanoparticles and using HPLC. The incorporation efficiency was obtained as the ratio of the amount of haloperidol incorporated in the nanoparticles to the total amount of haloperidol used. Drug content was calculated as the ratio of the mass of drug in the nanoparticles to the total initial mass amount of the polymer.

3. Results and discussion

3.1. Effect of polymer concentration in the organic phase

Fig. 1a shows the effect of polymer concentration in the organic phase on the mean diameter of two batches of nanoparticles produced by sonication. The polymer concentration is varied from 5 to 66.6 mg/ml while keeping other processing parameters at standard conditions. Increasing the polymer concentration leads to a gradual increase in nanoparticle diameter while maintaining a unimodal size distribution. In contrast, our homogenization method at these concentrations produced bimodal size distributions with increasing polymer concentration (Budhian et al., 2005).

Increase in polymer concentration leads to an increase in the viscous forces resisting droplet break down by sonication. The viscous forces oppose the shear stresses in the organic phase and the final size and size distribution of particles depends on the net shear stress available for droplet breakdown. The importance of

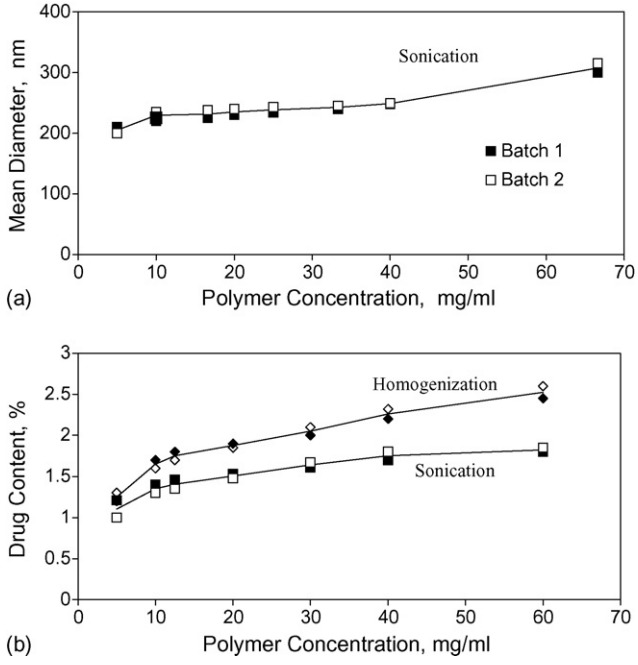

Fig. 1. Effect of polymer concentration in the organic phase on the (a) mean diameter, and (b) drug content of nanoparticles prepared by homogenization (♦, ◊) or sonication (■, □). In this and subsequent figures, each point represents a batch and error bars indicate the standard deviation of the mean reading within a batch. Error bars are omitted when the error is within 10% of the mean reading. Particles were prepared using PLGA 50:50, 3.5A, MW 51 kDa and 0.5 mg/ml of haloperidol in DCM as the organic phase and 50 ml of 1% PVA as the aqueous phase. The aqueous to organic ratio and PVA to PLGA ratio is 10:1 and the polymer to drug ratio is 20:1. Solvent volume is 5 ml.

polymer concentration in controlling the size of particles produced by the general emulsification process has previously been reported for other PLGA/PLA systems (Desgouilles et al., 2003; Zweers et al., 2003). We have also shown the effect of polymer concentration in organic phase on the size and size distribution of particles prepared by homogenization and by nanoprecipitation with acetone (Budhian et al., 2005), where the particles produced by homogenization yield a bimodal size distribution with increasing polymer concentration. However, our aim here is to use the method of sonication to maintain unimodal particle populations with changes in polymer concentrations so that we can monitor the effect of polymer concentration on drug content of particles with minimal interference from the changing size distribution of particles.

Fig. 1b shows the increase in drug content with increasing polymer concentration in the organic phase for two batches of nanoparticles produced by homogenization or sonication. The increase in polymer concentration increases the organic phase viscosity, which increases the diffusional resistance to drug molecules from organic phase to the aqueous phase, thereby entrapping more drug in the polymer nanoparticles. Increasing polymer concentration also increases particle size (Fig. 1a) and drug content is known to increase with particle size in other systems (Gorner et al., 1999). An increase in particle size increases the length of diffusional pathways into the aqueous phase, thereby reducing the drug loss through diffusion and increasing the drug content. Also, the time required for polymer precipitation decreases at higher polymer concentration, so there is less time for drug molecules to diffuse out of nanoparticles, which increases the drug content. The increase in drug content is steeper for the homogenization method because the larger particles of the bimodal distribution (∼900 nm) dominate the drug content.

3.2. Effect of organic solvent volume

Fig. 2a shows the effect of organic solvent volume on the mean diameter of two batches of nanoparticles produced by sonication. As the organic solvent volume is increased, the diameter remains constant at ∼225 nm, while the drug content decreases from ∼1.8 to 1% (Fig. 2b). Decreasing the solvent volume decreases the time of solvent evaporation, which decreases the time during which the nanodroplets are in the liquid state (and have not transitioned into nanoparticles). This allows less time for drug diffusion and increases the drug content.

3.3. Effect of PVA concentration in the aqueous phase

Fig. 3a shows the effect of PVA concentration in the aqueous phase on the mean diameter of two batches each of nanoparticles produced by homogenization, sonication, or nanoprecipitation. As the PVA concentration is increased, the mean diameter of nanoparticles first decreases and then gradually increases. A PVA concentration of 0.5% produces bimodal population of particles with sonication or nanoprecipitation (data not shown). The diameters of particles show similar trends for duplicate batches illustrating the control we have with these preparation methods.

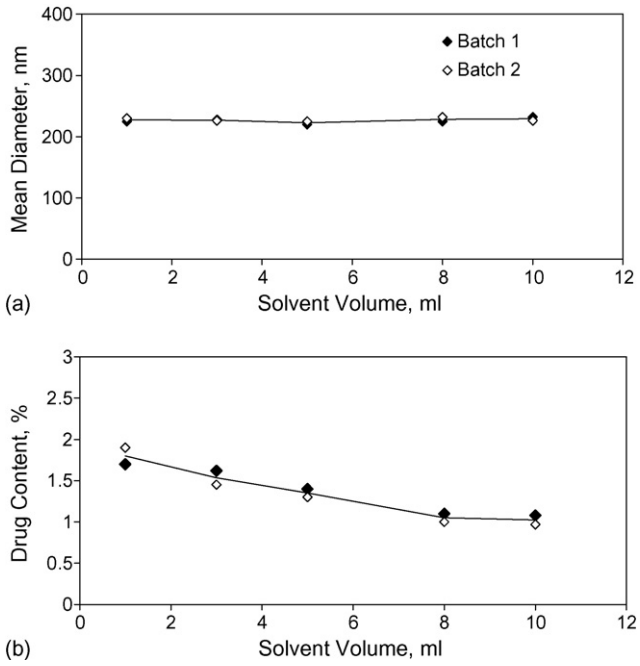

Fig. 2. Effect of solvent volume on (a) mean diameter, and (b) drug content of two batches of nanoparticles (♦, ◊) prepared by sonication. Particles prepared as in Fig. 1, except the solvent volume varies and the polymer concentration is kept constant at 10 mg/ml.

The presence of surfactant molecules stabilizes the emulsion nanodroplets and prevents them from coalescing with each other. For effective stabilization, the surfactant molecules must cover the organic/aqueous interfacial area of all the droplets.

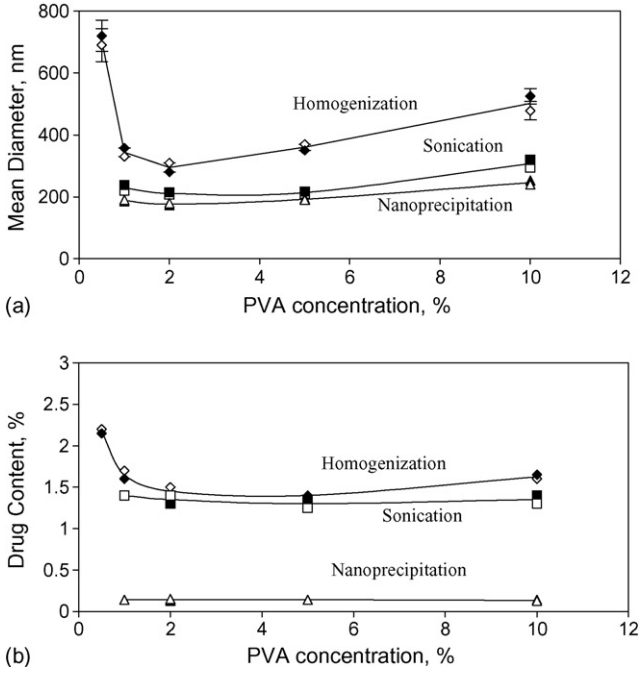

Fig. 3. Effect of PVA concentration in the aqueous phase on the (a) mean diameter, and (b) drug content of nanoparticles prepared by homogenization (♦, ◊), sonication (■, □) or nanoprecipitation (▲, △). Particles prepared as in Fig. 1, except the PVA concentration varies and the polymer concentration is kept constant at 10 mg/ml. Aqueous phase volume is 20 ml for nanoprecipitation.

Hence a minimum number of surfactant molecules are required to achieve small particle size and narrow size distribution. For a given volume ratio of aqueous to organic phase, this translates to a minimum concentration of the surfactant solution. At very low concentrations of PVA (<0.5% for homogenization and <1% for sonication and nanoprecipitation) the total amount of PVA, relative to PLGA, is insufficient to stabilize the emulsion droplets and causes bimodal distributions. The minimum concentration of PVA needed to stabilize the emulsion is higher for sonication and nanoprecipitation than for homogenization, because the smaller particles formed by sonication or nanoprecipitation increase the total interfacial area and consequently the PVA requirement. As the PVA concentration is increased, the size of particles produced by the three methods plateaus and then increases due to the increased viscosity of the aqueous phase; the viscosity increase reduces the net shear stress available for droplet breakdown and is more prominent in homogenization. Zweers et al. (2003) have reported an increase in size of PLGA nanoparticles at high PVA concentrations (5–10%), while Allemann et al. (1992) have reported a continuous decrease in particle size. In order to resolve this contradiction, we propose that there are two competing effects at high PVA concentrations. The size decreases due to enhanced interfacial stabilization while it increases due to increased viscosity of the aqueous phase. The concentration of PVA at which one effect starts dominating over the other depends on the system and processing parameters. For our system of PLGA nanoparticles, the size first decreases due to better stabilization and then increases at higher PVA concentrations due to high aqueous phase viscosity.

Fig. 3b shows the effect of PVA concentration on the drug content for two batches each of nanoparticles produced by homogenization, sonication and nanoprecipitation. As the PVA concentration is increased, the drug content first decreases and then plateaus with a slight increase for particles made by homogenization. Drug content is not shown for PVA concentration of 0.5% for sonication and nanoprecipitation, because a bimodal population is formed. The changes in drug content with PVA concentration are predominantly a result of changing particle size. As discussed above, larger nanoparticles have higher drug content, because fewer drug molecules have sufficient time to diffuse into the aqueous phase. Hence, the change in drug content is most appreciable for homogenization where the particle size changes the most and the drug content follows the same trend as the particle size, such that larger particles contain more drug.

3.4. Effect of aqueous phase volume

Zweers et al. (2003) used the method of salting-out along with mechanical stirring at high speeds to obtain PLA/PLGA nanoparticles with acetone. They have reported that the particle size increases with the increase in aqueous to organic phase ratio. On the other hand, Fonseca et al. (2002) used the nanoprecipitation method to produce PLGA nanoparticles and have reported that doubling the aqueous phase volume led to a significant decrease in nanoparticle size. Here we explore the effect of

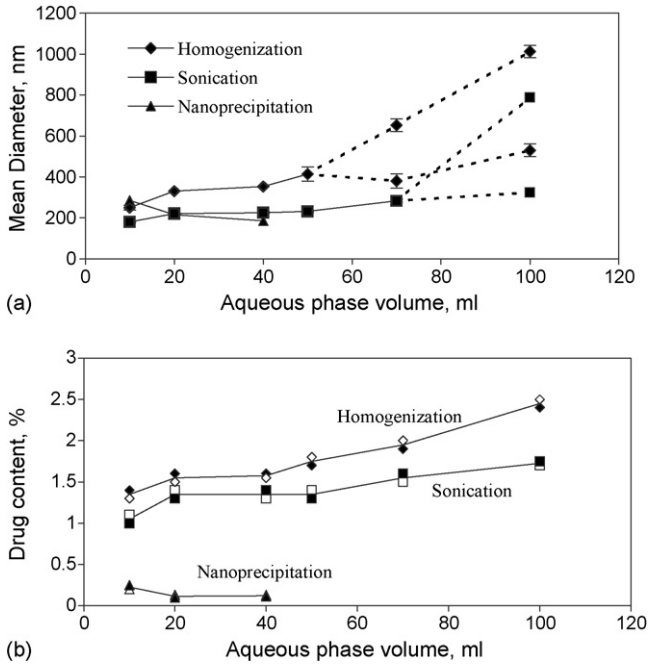

Fig. 4. Effect of aqueous phase volume on the (a) mean diameter, and (b) drug content of nanoparticles prepared by homogenization (♦,◊) sonication (■, □) or nanoprecipitation (▲, △). Particles prepared as in Fig. 1, except the aqueous phase volume varies and the polymer concentration is kept constant at 10 mg/ml. Solid lines indicate unimodal population while dotted lines indicate bimodal population.

aqueous phase volume on particle diameter for our specific methods of preparing drug-loaded PLGA nanoparticles. Increasing the aqueous phase volume in our homogenization and sonication methods increases the mean diameter, after which the population becomes bimodal (Fig. 4a). Similar trends are observed for multiple batches of nanoparticles (not shown).

These trends occur because the external energy (and thus the shear stress) causing droplet breakdown is constant while the volume of the system increases with increasing the aqueous phase volume. The same amount of energy must now be distributed in a larger volume leading to less droplet breakdown and larger nanoparticles. This effect becomes more evident at higher aqueous volumes and the population becomes bimodal. The onset of bimodality in particle population comes earlier for particles prepared by homogenization than sonication since the energy applied through homogenization is less than sonication.

The size profile of nanoparticles prepared with nanoprecipitation shows the opposite trend: a gradual decrease in size of the unimodal particle population followed by the failure to form particles due to immediate polymer precipitation. With nanoprecipitation, the particles are formed due to rapid solvent migration to the aqueous phase (Quintanar-Guerrero et al., 1997). As the volume of the aqueous phase increases, the particle size decreases due to the increased diffusion of the water-soluble solvent (acetone) in the aqueous phase. At a certain point, this diffusion of acetone to the aqueous phase becomes so rapid that the polymer immediately precipitates before agglomerating into particles.

Fig. 4b shows the effect of aqueous phase volume on the drug content for two batches each of nanoparticles produced

by homogenization, sonication and nanoprecipitation. As the aqueous phase volume is increased, the drug content of particles produced by homogenization and sonication increases monotonically. The drug content of particles produced by nanoprecipitation decreases slightly and after that the particles are not formed due to polymer precipitation. On increasing the aqueous phase volume the amount of drug that can dissolve in the aqueous phase increases, which increases the drug loss into the aqueous phase. However this loss is insignificant for these changes in the aqueous phase volume where the loss is overcompensated by the simultaneous gain in drug content associated with larger particles. As was observed for the PVA concentration, the aqueous phase volume impacts the particle size that in turn controls the drug content.

3.5. Effect of initial haloperidol concentration in the organic phase

Fig. 5a shows that the mean diameter is independent of the initial haloperidol concentration in the organic phase for two batches of nanoparticles produced by sonication. As the initial haloperidol concentration is increased, the drug content first increases, then plateaus and finally decreases (Fig. 5b), while the drug incorporation efficiency remains constant and then decreases (Fig. 5c). Similar trends are observed for both batches of nanoparticles indicating good batch-to-batch reproducibility.

The drug content in the nanoparticles is affected by the drug–polymer interactions and the drug miscibility in the polymer. The importance of drug miscibility in the polymer has been discussed by Panyam et al. (2004) for a hydrophobic drug–polymer system of dexamethasone or flutamide-loaded PLGA/PLA nanoparticles. They have reported that higher drug–polymer miscibility leads to higher drug incorporation. We have also reported the effect of drug–polymer interaction/miscibility in our previous publication (Budhian et al., 2005), where we established that increasing the drug–polymer interaction, by introducing more acid end groups in the polymer chain, increases the drug content and drug incorporation in the range tested.

Here we explore the effect of changing the initial drug concentration over a wider range on the drug content of nanoparticles. As the initial drug concentration or the drug input to the system increases, the drug incorporation process operates at its maximum efficiency (∼82%, owing to drug losses during preparation) and the drug content increases until it reaches its limiting value determined by the drug miscibility in the polymer at the given processing conditions and polymer characteristics, where it plateaus. Increasing the initial drug concentration beyond the limit of drug miscibility leads to constant drug content and a decrease in drug incorporation efficiency due to fixed amount of drug going into the particles in spite of increasing drug input. Further increase in initial haloperidol concentration above 2.5 mg/ml causes the drug content to decrease as the haloperidol molecules within the polymer matrix are attracted towards the haloperidol molecules in the aqueous phase and migrate to the aqueous phase and nucleate haloperidol crystals. This results in the appearance of needle-shaped crystals of haloperidol in the aqueous phase at high initial drug concentrations. Optimal nanoparticle fabrication methods want both maximum drug content and maximum drug incorporation, which we observe at the intermediate drug concentrations of 1 mg/ml.

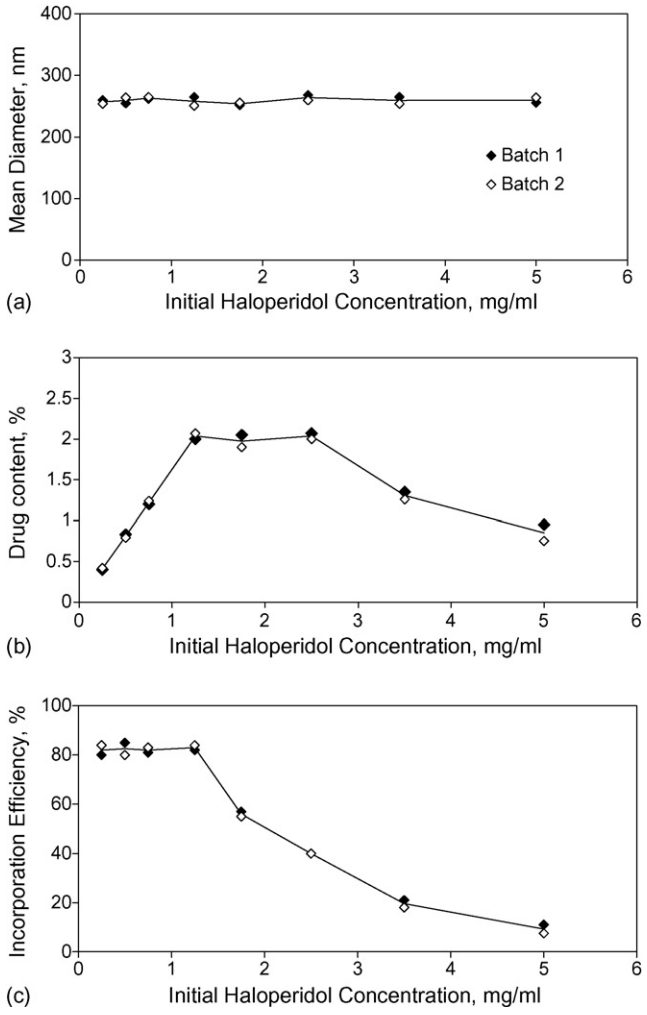

Fig. 5. Effect of initial haloperidol concentration in the organic phase on the (a) diameter, (b) drug content and (c) drug incorporation efficiency of two batches of nanoparticles (♦, ◊) prepared by sonication. Particles are prepared using 50 mg/ml of PLGA 50:50, MW 51 kDa in DCM as the organic phase and 20 ml of PVA as aqueous phase. The aqueous to organic ratio and the PVA to PLGA ratio is 10:1. Solvent volume is 2 ml.

3.6. Effect of polymer molecular weight and L:G ratio

To this point we have varied processing parameters, but now we explore the polymer parameters. The polymer molecular weight is varied from 7 to 63 kDa for PLGA 50:50 and the L:G ratio is varied from 50:50 to 100:0, while keeping other processing parameters at our standard conditions (Fig. 6a). As the PLGA 50:50 molecular weight increases, the mean diameter of particles increases slightly (homogenization) or remains constant (sonication, nanoprecipitation). This is due to the slight increase in viscosity of the organic phase, which reduces the net shear stress available for droplet breakdown in case of homogenization, while it is insignificant for sonication. This effect

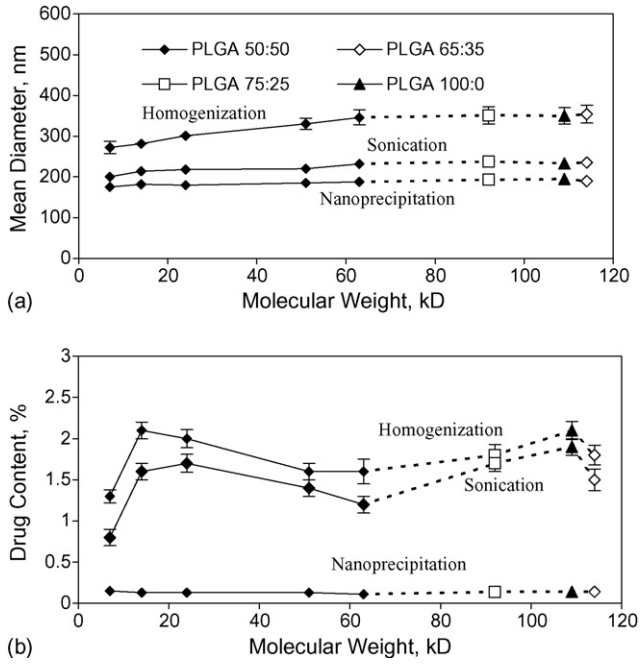

Fig. 6. Effect of polymer molecular weight and L:G ratio on the (a) mean diameter, and (b) drug content of nanoparticles prepared by homogenization, sonication or nanoprecipitation using PLGA 50:50 (♦), PLGA 65:35 (◊) PLGA 75:25 (□) and PLGA 100:0 (▲) Particles prepared as in Fig. 1, except the polymer molecular weight and L:G ratio varies and the polymer concentration is kept constant at 10 mg/ml. Aqueous phase volume is 20 ml for nanoprecipitation.

is prominent only at lower molecular weights (<63 kDa). The mean diameters of particles formed from PLGA 50:50 (63 kDa), PLGA 65:35 (114 kDa), PLGA 75:25 (92 kDa) and PLGA 100:0 (109 kDa) are constant within each preparation method indicating no substantial influence of copolymer composition. Similar results are obtained for multiple batches (data not shown).

Fig. 6b shows the effect of polymer molecular weight and L:G ratio on the drug content of nanoparticles produced by homogenization, sonication and nanoprecipitation. Similar results are obtained for multiple batches (data not shown). As the PLGA 50:50 molecular weight increases from 14 to 63 kDa, the drug content decreases (homogenization, sonication) due to decrease in the number of free carboxylic acid end groups available for hydrogen bonding with haloperidol. This reduces the drug–polymer interaction leading to a reduction in drug content. This is contrary to the result of Seo et al. (2003), who reported an increase in drug content with increase in molecular weight for a hydrophobic drug–polymer system of fentanyl-PLGA microparticles. This is because in our case the drug content is most strongly influenced by the presence of free carboxylic acid groups due to hydrogen bonding with haloperidol (Budhian et al., 2005), while there are no hydrogen bonding opportunities in case of fentanyl-PLGA system. The drug content is much lower for PLGA 50:50 molecular weight of 7 kDa than 14 kDa because at very low molecular weights (7 kDa) the particles do not form effectively due to polymer precipitation. As the L:G ratio is increased from 50:50 to 100:0, the drug content and incorporation increases slightly (homogenization, sonication). This happens due to increased hydrophobicity of polymer at higher L:G ratios, which increases the hydrophobic interaction of haloperidol with PLGA.

The drug content remains constant for nanoparticles produced by nanoprecipitation because the effects of molecular weight and L:G ratio are dominated by the strong interaction between drug and acetone, which carries away most of the drug into the aqueous phase. Small amounts of drug leakage of a crystalline hydrophobic drug from PLGA nanoparticles prepared using acetone as the organic phase solvent has been reported by Layre et al. (2005). However, in our case the drug leakage is substantial due to increased drug–solvent affinity and poor drug–polymer affinity.

3.7. Comparison of fabrication methods

Hydrophobic drugs are usually encapsulated in biodegradable polymers using one of the three methods: homogenization, sonication, or nanoprecipitation. An ideal method would produce nanoparticles with the following characteristics: narrow size distribution, ability to control nanoparticle size in the range 100–1000 nm with unimodal population, high drug incorporation, ability to control drug content effectively over a wide range, use of non-toxic solvent, and relative ease of particle production.

Nanoprecipitation produces particles with narrow size distribution over a wide range of processing parameters (Figs. 3a, 4a and 6a). The size control is achieved mainly by varying the polymer concentration and particles of size 200–500 nm, with narrow distribution were produced. The drug content of particles produced by nanoprecipitation is consistently very low (<0.2%) across a wide range of parameters (Figs. 3b, 4b and 6b). Nanoprecipitation uses acetone, which is considerably less toxic than DCM utilized by homogenization and sonication. If the toxicity of the solvent is a big concern, then nanoprecipitation method is better than the other two methods. Nanoprecipitation does not require a source of external energy and would be easy to scale-up relative to homogenization and sonication, which require an energy source. Hence, nanoprecipitation is highly recommended to produce small size nanoparticles (<500 nm diameter) with narrow size distributions when very low drug content is desirable. It must be noted that the low drug content is due to strong haloperidol–acetone interaction and this might not be the case for other hydrophobic drugs with weaker solvent–drug interactions.

Sonication and homogenization methods are two variations of the emulsification-solvent evaporation method. These methods give particles with drug content (0.5–2.5%) much higher than the nanoprecipitation method and the drug content is effectively controlled (Figs. 3b, 4b and 6b). Sonication and homogenization produce unimodal or bimodal particle populations of a wide size range by varying processing parameters including power, time, PVA concentration, polymer concentration, and aqueous phase volume (Figs. 1a, 3a and 4a). However, these methods suffer from the drawback of using a toxic solvent and being more cumbersome than the nanoprecipitation method. Sonication or homogenization methods are recommended when higher drug loading and/or a broad range of particle size with wider size distributions are desired. The drug content and sizes of particles

Table 1
Relationship between scientific principles and processing and materials parameters to control the particle size and drug content for nanoparticles prepared by emulsification-solvent evaporation method

	Governing scientific principles	Pertinent processing and materials parameters
Decrease particle size	Increase shear stress during emulsification	Increase applied energy during emulsification (power, duration) Decrease relative volume of aqueous phase Decrease polymer concentration in organic phase Intermediate surfactant concentration in aqueous phase Decrease polymer molecular weight
Increase drug content	Inhibit drug diffusion during organic solvent evaporation √ Longer diffusion path length √ Shorter diffusion times √ Increase resistance to diffusion √ Reduce drug solubility is aqueous phase	Increase particle size Decrease relative volume of organic solvent Increase polymer concentration in organic phase Intermediate polymer molecular weight Select organic solvent with intermediate drug–solvent interactions Reduce drug solubility in the aqueous phase (alter pH)
	Increase drug–polymer interaction	Include specific interactions between drug and polymer end groups Optimize polymer L:G ratio Sufficient initial drug content for specific interactions

produced by homogenization are consistently higher than sonication, (Figs. 3a, 4a and 6a). The size distribution of particles produced by sonication is narrower than the particles produced by homogenization. Hence sonication is better suited to produce small size nanoparticles (<300 nm diameter) with narrow size distribution and homogenization is recommended when larger size (>300 nm) particles are needed, the size distribution is not very critical and the objective is to maximize the drug content. The three fabrication methods compared here do not present an exhaustive list of methods of producing hydrophobic-drug-loaded PLGA nanoparticles and other methods including high-pressure homogenization and spray drying were not considered for this study. The three methods discussed here are commonly used methods and they were used to provide nanoparticles with properties that lead to a better understanding of the scientific concepts.

4. Conclusions

We used homogenization or sonication in our emulsification-solvent evaporation method to produce haloperidol-loaded PLGA/PLA nanoparticles and compared the results with the nanoprecipitation method. Particles produced by nanoprecipitation had small size (∼185 nm) but the technique was not pursued because of very low drug content (∼0.15%). For nanoparticles produced by homogenization or sonication, we examined the scientific principles controlling particle size and drug content. Table 1 enumerates the principles governing particle size and drug content and links each principle to the pertinent processing and materials parameters. Increase in polymer concentration is the most effective parameter to increase the size of particles. The drug content of particles produced by homogenization and sonication was increased up to 2.5% by optimizing the initial haloperidol concentration (∼1.2–2.6 mg/ml), reducing the solvent volume (5–1 ml), increasing the L:G ratio (50:50–100:0), and/or reducing the molecular weight of PLGA 50:50 (63–14 kDa). Based on these results, we recommend the sonication method to produce unimodal particles of diameters <300 nm with narrow size distribution and fairly high drug content, and homogenization method to produce particles of diameters in the range of 300–1000 nm, with broader size distributions and higher drug content. Since the drug content is inversely related to the diameter of particles, small nanoparticles (diameter <200 nm) can be judiciously used to deliver highly potent drugs that require low drug amount to achieve desired therapeutic results, or to deliver drugs involved in short-duration therapies.

The principles summarized in Table 1 can be applied to any hydrophobic-drug-polymer system produced by the emulsification-solvent evaporation technique to interpret both the general and system-specific results. By way of example, these principles were applied rationally to produce unimodal particles of various mean sizes (220–1000 nm) and drug content (0.2–2.5%) that allows us to tailor the drug release profiles according to the given objectives as described elsewhere (Budhian, 2006).

Acknowledgements

This research was supported by financial assistance from the Commonwealth of Pennsylvania through the Nanotechnology Institute. We are thankful to Prof. Anthony Lowman for providing laboratory facilities and to Meredith Hans (Drexel University) and Yuling Liang (University of Pennsylvania) for their invaluable help with the experiments.

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