Drug Development and Industrial Pharmacy, 28(3), 317–328 (2002)

RESEARCH PAPER

Gadolinium-Loaded Nanoparticles Engineered from Microemulsion Templates

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ABSTRACT

Microemulsions (oil-in-water) have been used as templates to engineer stable emulsifying wax and Brij 72 (polyoxyl 2 stearyl ether) nanoparticles. The technique is simple, reproducible, and amenable to large-scale production of stable nanoparticles having diameters below 100 nm. Investigation of the process variables showed that the amount of surfactant used in the preparation of microemulsion templates had the greatest influence on the microemulsion window, as well as the properties and stability of the cured nanoparticles. Emulsifying wax and Brij 72 nanoparticles (2 mg/mL) made with 3 mM polyoxyl 20 stearyl ether and 2.3 mM polysorbate 80, respectively, were the most stable based on retention of nanoparticle size over time. Gadolinium acetylacetonate (GdAcAc), a potential anticancer agent for neutron capture therapy (NCT), was entrapped in stable nanoparticles. The apparent water solubility of GdAcAc was increased more than 2000-fold by entrapment into nanoparticles. The entrapment efficiency of GdAcAc was about 100% for emulsifying wax nanoparticles and 86% for Brij 72 nanoparticles, as determined by gel permeation chromatography (GPC). Elution profiles were obtained with light scattering (counts per second) to detect nanoparticles and ultraviolet (UV) absorption of GdAcAc at 288 nm. Challenges of these cured nanoparticles in biologically relevant media such as 10% fetal bovine serum, 10 mM phosphate-buffered saline, 150 mM NaCl, and 10% lactose at 37°C for 60 min demonstrated that these nanoparticles are stable. The ease of preparation of these very small and stable nanoparticles, and the ability to entrap lipophilic drugs such as GdAcAc with high efficiency, suggested that these systems may have potential in cell targeting, especially for specific delivery to tumor cells for NCT.

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Key Words: *Brij 72; Emulsifying wax; Gadolinium acetylacetonate; Neutron capture therapy; Polysorbate*

INTRODUCTION

Nanotechnology is becoming increasingly more important in the pharmaceutical, chemical, and engineering fields. This is primarily due to the fact that particles made at nanoscale have different and in many cases more desirable physical, chemical, and biological properties than larger particles. For example, in the pharmaceutical field, nanoparticles have been used to deliver drugs, genes, diagnostics, and vaccines more efficiently (1–4). Due to their small size, nanoparticles meeting specific criteria may be able to deliver molecules to specific tissues of interest (i.e., liver, brain, and solid tumor) and/or aid in the direct entry of entrapped drug molecules into cells (either nonspecifically or specifically via cell targeting ligands). However, delivering drug molecules efficiently to specific tissues or into specific cells is difficult due to significant physical and biological barriers. Conventional approaches to nanoparticulate carriers, including polymeric nanoparticles and liposomes, have notable disadvantages, such as: (i) the toxicity of components (5), (ii) physical and biological instability (6), (iii) the lack of a suitable method of production that will accommodate a wide range of drugs both on small and large scales (7), and (iv) the necessity for manufacturing processes utilizing polymerization, solvent evaporation, or high-torque mechanical mixing that are expensive, potentially damaging to drugs, and/or may be unscalable for commercialization.

An alternative to polymeric nanoparticles and liposomes is incorporation of drugs into microemulsions. A microemulsion is a biphasic mixture of two immiscible liquids stabilized by a surfactant and usually a co-surfactant. Microemulsions are thermodynamically stable, isotropically clear, and are formed without excessive mixing, with dispersed droplets in the range of 5 nm to 100 nm. Microemulsions have been proposed as drug delivery systems to enhance the absorption of drugs across biological membranes (8–10). Although microemulsions have advantages as delivery systems, they do have important limitations. For example, the dispersed liquid droplets are not stable in biological fluids. As such, microemulsions are not effective in delivering drugs intracellularly or targeting drugs to different cells of the body. Clearly, a significant advancement in the field would be made if one could avoid the problems associated with polymeric nanoparticles and liposomes and instead combine the unique advantages of these systems with microemulsions to engineer tissue and cell-specific nanoparticles using inexpensive, reproducible, and scalable processes.

In this study we have proposed the use of microemulsions as templates from which nanoparticles can be engineered. Nanoparticles are solid, colloidal particles consisting of macromolecular substances with a mean diameter between 1 nm and 1000 nm. At room temperature the particles are in solid state, thereby reducing the rapid loss of incorporated drugs due to diffusion. This aspect is very important for controlled release and cell targeting of drugs (11). The use of microemulsions as templates is based on the "natural" and spontaneous formation of microemulsions that can easily be used as precursors to form nanoparticles. Several groups have described nanoparticles prepared from microemulsion systems by dilution of warm microemulsions (at 70°C) with distilled cold water (2–3°C) under mechanical stirring (12–14). However, dilution of the initial microemulsions has disadvantages. For example, the incorporated molecules (drugs) will be diluted in the process, thereby creating difficulties in handling and/or injecting larger volumes in clinical practice settings. Further, in some cases the dilution process may necessitate freeze-drying of the nanoparticles, which may not be desirable or cost-effective.

The engineering process in this study employed the formation of oil-in-water microemulsions at 55°C that upon cooling in one vessel resulted in the production of stable nanoparticles having diameters less than 100 nm. Emulsifying wax and Brij 72 (polyoxyl 2 stearyl ether) used as the prototype nanoparticle matrix materials are generally regarded as nontoxic and nonirritant materials. Emulsifying wax is a nonionic wax prepared from a combination of cetostearyl alcohol and a polyethylene derivative of fatty acid ester of sorbitan in a molar ratio of about 20 : 1. Brij 72 is a polyoxyethylene alkyl ether

of alcohol. Preparation of nanoparticles through microemulsion templates is expected to have many advantages since: (i) the natural engineering process can easily be adapted to include different types of drugs, (ii) nanoparticles having sizes less than 100 nm can easily be engineered, (iii) no organic solvents were used during the preparation, and (iv) water-insoluble drugs can be solubilized in the dispersed oil phase of the microemulsion, resulting in high entrapment efficiencies in the cured nanoparticles.

Gadolinium acetylacetonate (GdAcAc), a potential agent for neutron capture therapy (NCT), was entrapped into stable nanoparticles intended for tumor-specific delivery. Gadolinium has been proposed as an alternative to boron in NCT (15–16). Earlier studies on the delivery of gadolinium (Gd) applied systems that were untargeted and/or biologically unstable with diameters of 400 nm to several microns and are not ideal for systemic targeting of tumors (17–19). It is expected that nanoparticles having diameters less than 100 nm will be more effective in specific delivery into tumors than larger particles. Studies by several groups using liposomes and other macromolecules have shown that the typical pore size of vascularized tumors is about 200 nm to 500 nm, with some fenestrations as small as 50 nm to 60 nm (20–21). It has also been reported that tumors tend to be more "leaky" than normal tissues, with associated high hydrostatic pressure, thereby hindering the transport of larger particles into some regions of the tumor (i.e., central necrotic regions) as well as forcing particles out of tumors (20–25). Taken as a whole, this study highlights the importance of using small particles (i.e., less than 100 nm, or even 50 nm) in the delivery of cancer therapies. As such, stable gadolinium-loaded nanoparticles having diameters below 100 nm engineered from oil-in-water microemulsion templates may potentially be exploited as a delivery system for neutron capture therapy of tumors.

MATERIALS AND METHODS

Emulsifying wax and polyoxyethylene 20 sorbitan monooleate (Tween 80) were purchased from Spectrum Chemicals (New Brunswick, NJ). Poloxyl 20 stearyl ether (Brij 78) and poloxyl 2 stearyl ether (Brij 72) were obtained from Uniqema (Wilmington, DE). Lactose and Gd(III) acetylacetonate hydrate were purchased from Aldrich Chemical Co. (Milwaukee, WI). Sephadex G-75 and blue dextran were obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation and Characterization of Oil-in-Water Microemulsion Templates

Emulsifying wax nanoparticles were prepared by accurately weighing 2 mg of emulsifying wax (matrix material) into a 7-mL screw-capped glass vial. To the melted emulsifying wax, various amounts of 100 mM Brij 78 solution were added. Finally, filtered water (0.22 μm filter, Nalgene International) was added under magnetic stirring to prepare the microemulsion with a final volume of 1000 μL. Prepared microemulsions were optically transparent systems. The microemulsion droplet size at 55°C was determined using a Coulter N4 Plus Sub-Micron Particle Sizer at 90° (Beckman Coulter Corporation, Miami, FL). To ensure an accurate measurement of the microemulsion droplet size at the desired temperature (55°C), both the cuvette (Coulter Corporation, Miami, FL) and the particle sizer sample holder were maintained at 55°C to prevent the warm microemulsions from cooling. A similar procedure was used to prepare Brij 72 nanoparticles (2 mg/mL) using Tween 80 (10% w/v) as the surfactant and filtered water to prepare microemulsions with a final volume of 1000 μL.

Preparation of Nanoparticles from Microemulsion Templates

The warm oil-in-water microemulsion templates were used to engineer solid emulsifying wax and Brij 72 nanoparticles. Four methods were applied to cure nanoparticles from warm microemulsions, as follows. Method A: cooling the undiluted oil-in-water microemulsions at 55°C to room temperature. Method B: cooling the oil-in-water microemulsions at 55°C by placing the undiluted microemulsions in the freezer. Method C: cooling the oil-in-water microemulsions at 55°C by placing the undiluted microemulsions in a refrigerator at 4°C. Method D: diluting (1 : 10) the oil-in-water microemulsions at 55°C with cold water (4°C). The properties and characteristics of the nanoparticles cured in each preparation were investigated.

Characterization of Nanoparticles

Photon Correlation Spectroscopy (PCS)

The particle sizes of cured solid nanoparticles were determined using a Coulter N4 Plus Sub-Micron Particle Sizer at 20°C by scattering light at 90° (Beckman Coulter Corporation, Miami, FL). Prior to the particle size measurement, the nanoparticles dispersion was diluted (1 : 10) with filtered water (0.2 μm filter, Nalgene International) to ensure that the light scattering signal in particle counts per second (CPS) was within the sensitivity range of the machine. The values for the mean diameter and polydispersity index were the averages of results obtained for two separate preparations (n=5).

Transmission Electron Microscopy (TEM)

The size and morphology of nanoparticles cured from microemulsion templates were observed using a JEOL electron microscope in the Electron Microscopy Facility at the University of Kentucky. A carbon-coated 200-mesh copper specimen grid was glow-discharged for 1.5 min. One drop of the nanoparticles dispersion was deposited on the grid and left to stand for 1.5 min, after which time any excess fluid was removed with filter paper. The grid was stained with one drop of 1% uranyl acetate (0.2 μm filtered) for 30 sec and any excess uranyl was removed with filter paper. The grids were allowed to dry for a further 10 min and examined with the electron microscope.

Stability of Nanoparticles Under Biological Conditions

To assess the stability of the nanoparticles in biological media, Brij 72 and emulsifying wax nanoparticles were diluted (1 : 10) with either water, 10% fetal bovine serum (FBS), 10 mM phosphate-buffered saline (pH 7.4), 150 mM NaCl, or 10% lactose. The particle size of nanoparticles in each medium was monitored for 60 min at 37°C using a Coulter N4 Plus Sub-Micron Particle Sizer by scattering light at 90°.

Incorporation of GdAcAc into Nanoparticles

Various amounts (0.1 mg to 1 mg) of GdAcAc were added to 2 mg of melted nanoparticles matrix materials contained in a 7-mL screw-capped glass vial on a hot plate at 55°C. Gadolinium-loaded emulsifying wax and Brij 72 nanoparticles were produced using the method described above. The effect of GdAcAc on the formation of oil-in-water microemulsions and nanoparticles was studied. The entrapment efficiencies of GdAcAc in nanoparticles were determined by gel permeation chromatography (GPC) (Sephadex G75; 30 cm by 0.5 cm column) with water as the mobile phase. One hundred microliters of nanoparticles containing GdAcAc was eluted down the column, and a total of 10 fractions (1 mL each) were collected. Each fraction (1 mL) was monitored using both light scattering (CPS) to detect nanoparticles and ultraviolet (UV) absorption to detect GdAcAc (at 288 nm) with a Hitachi spectrophotometer U-2000.

RESULTS AND DISCUSSION

Solid nanoparticles have been engineered directly from oil-in-water microemulsion templates. The engineering process involved the preparation of microemulsion templates with emulsifying wax and Brij 72 as nanoparticle matrix materials, and Brij 78 (polyoxyl 20 stearyl ether) and Tween 80 (polyoxylethylene sorbitan monooleate) as surfactants. Both the matrix materials and surfactants have properties that are suitable for the formation of oil-in-water microemulsions. Ideally, the amphiphatic matrix materials combined with surfactants having moderate hydrophilic–lipophilic balance (HLB) values (i.e., 14–16) promoted the formation of very small and stable nanoparticles. Various combinations of the microemulsion components (melted oil, surfactant, and water) were studied with the aim of determining the optimal combinations of each ingredient. Special attention was paid to the ternary systems that produced nanoparticles having diameters below 100 nm.

For reasons that remain unknown, the combination of Brij 72 (2 mg/mL) and Brij 78 (surfactant) resulted in nanoparticles having diameters above 100 nm. For example, 2 mg of Brij 72 with 3.0 mM or 6.0 mM of Brij 78 in the final volume of 1000 μL with water produced nanoparticles having diameters of 160 nm (±7.4) and 178 nm (±0.8), respectively. In contrast, 3.0 mM Brij 78 and emulsifying wax matrix systems (2 mg/mL), produced nanoparticles having sizes below 100 nm. Tween 80 was found to be a suitable surfactant for Brij 72 systems

(2 mg/mL), since nanoparticles having diameters below 100 nm were produced with a final Tween 80 concentration as low as 2.3 mM.

All the preparations were optimized to ensure that the lowest concentrations of surfactants were used to achieve stable nanoparticles (having diameters less than 100 nm). The results showed that the final amounts of surfactants used in preparing microemulsion templates influenced the stability of the cured emulsifying wax and Brij 72 nanoparticles. For example, as shown in Fig. 1, the final Brij 78 concentration used to prepare emulsifying wax nanoparticles (2 mg/mL) had a pronounced effect on the size of the cured nanoparticles. Finally, Brij 78 in the concentration range of 3 mM to 22 mM produced an apparent microemulsion window from which nanoparticles having diameters less than 100 nm were cured (Fig. 1). In addition, the results indicated that concentrations as low as 2–3 mM of Brij 78 were effective in the preparation of emulsifying wax nanoparticles (2 mg/mL). The particle sizes of systems with no surfactant added could not be determined since they were turbid. The droplet size of the oil phase in the microemulsion templates made with a final surfactant concentration of 10 mM (Fig. 1) was measured at 55°C and found to be 11±3 nm, which demonstrates that nanoparticles can be engineered directly from microemulsion templates.

Results from TEM of the nanoparticles supported the data obtained from laser light scattering. As shown in the TEM micrograph of Fig. 2, very spherical emulsifying wax nanoparticles having diameters from 50 to 120 nm were indeed produced from microemulsion templates. Additional studies were performed to ascertain the stability of emulsifying wax nanoparticles over a period of 24 hr using final Brij 78 concentrations from 3 mM to 15 mM. Particular attention was paid to the apparent effect of the final surfactant concentration on the stability of the cured nanoparticles. Brij 78 concentrations from 3 mM to 15 mM were used to prepare emulsifying wax nanoparticles (2 mg/mL) and the particle size of the cured nanoparticles was measured at both 10 min and 24 hr after curing. As shown in Fig. 3, a final surfactant Brij 78 concentration of 3 mM produced stable 50 nm nanoparticles over 24 hr, whereas those nanoparticles made with >3 mM surfactant, although initially having <100 nm diameters, agglomerated over 24 hr to produce 600–700 nm particles. A similar trend was observed with Brij 72 nanoparticles (2 mg/mL), whereby nanoparticles prepared with a final Tween 80 concentration of 2.3 mM were more stable than nanoparticles prepared with higher amounts of Tween 80 (i.e., 9 mM). The final surfactant concentration was thus a critical parameter greatly influencing the preparation of microemulsions, the curing of small nanoparticles, and the retention of nanoparticle size over time.

Further studies were carried out to determine if even smaller (less than 50 nm) nanoparticles could be engineered from oil-in-water microemulsions either by modifying the type/amount of surfactant

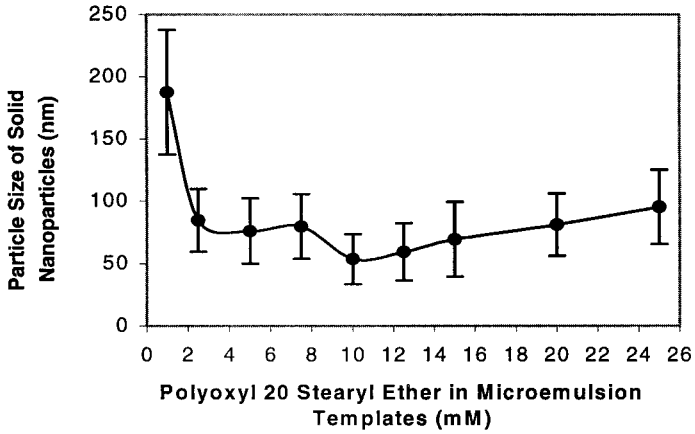

Figure 1. Particle sizes (nm) of emulsifying wax nanoparticles (2 mg/mL) prepared with polyoxyl 20 stearyl ether (Brij 78) (1 mM to 25 mM) as the surfactant. The warm microemulsion templates were cooled for 10 min at room temperature to cure the nanoparticles. Each preparation was later diluted with 0.2 μm filtered water (1 : 10) before particle size measurement.

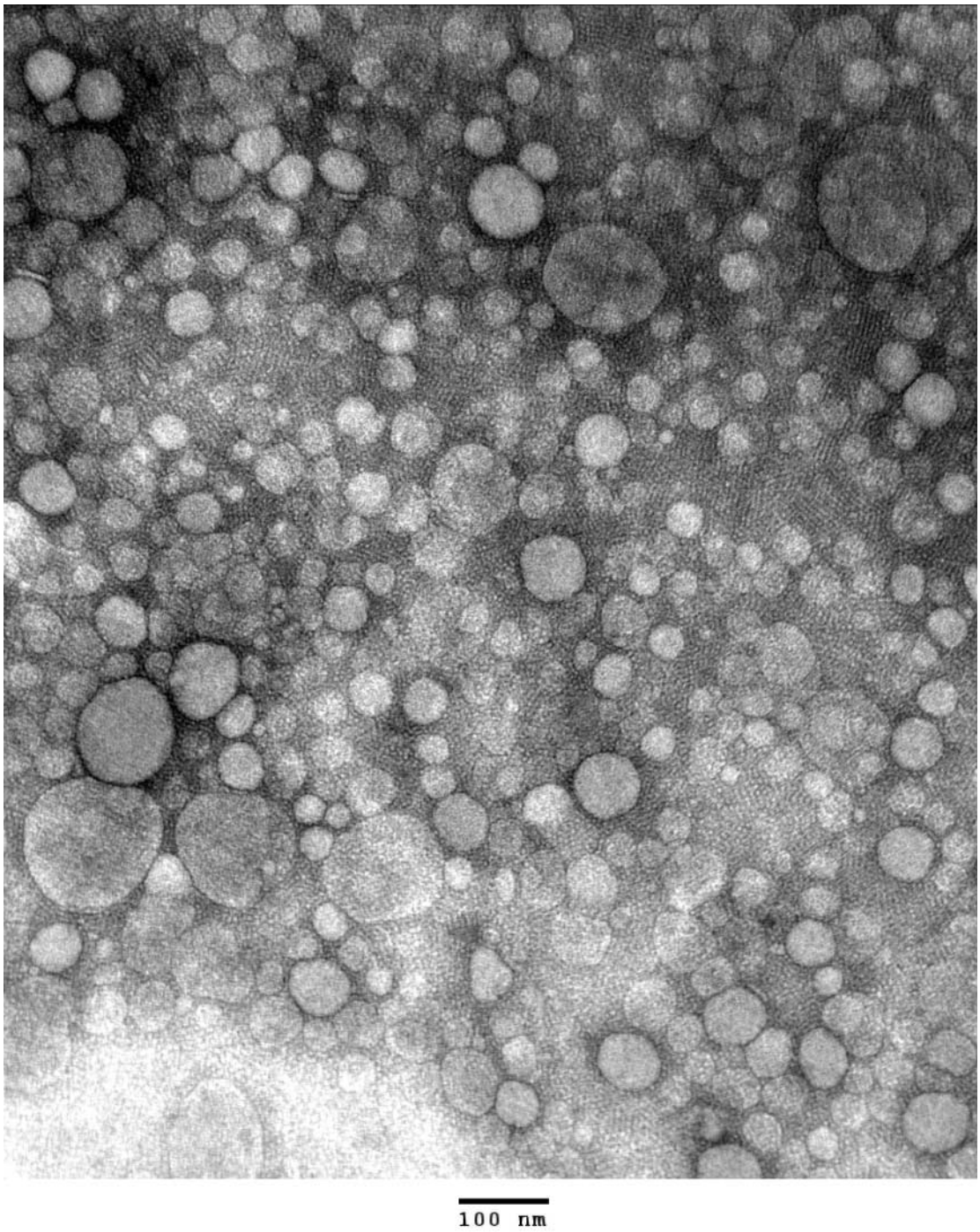

Figure 2. Transmission electron micrograph showing the size and morphology of emulsifying wax (2 mg/mL) nanoparticles engineered from microemulsion templates.

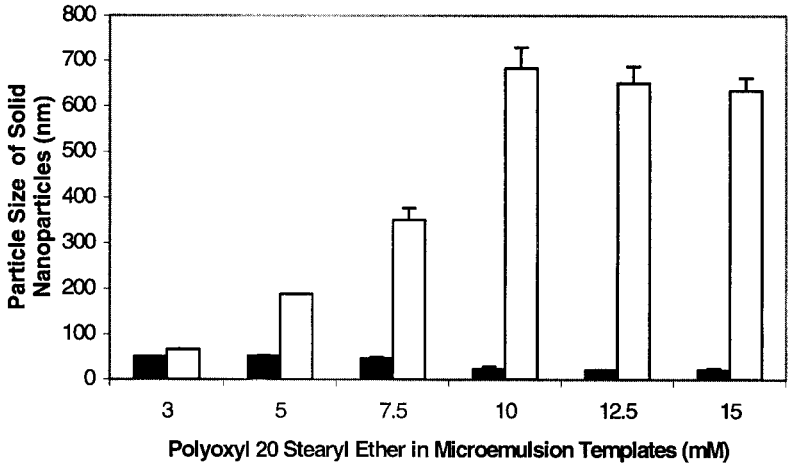

Figure 3. Stability of solid nanoparticle sizes prepared with 2 mg emulsifying wax, 3 to 15 mM polyoxyl 20 stearyl ether (Brij 78) and filtered water to make the final volume of 1 mL. The particle size of each sample, after dilution (1 : 10) with filtered water, was determined at 10 min (black bars) and 24 hr (white bars) after preparation.

used or modifying the component ratios in the microemulsion pseudo-phase diagrams. As shown in Fig. 4A, for nanoparticle systems made with emulsifying wax (0.5 mg/mL to 2.0 mg/mL), there was a direct correlation between microemulsion droplet size at 55°C and the diameter of the cured nanoparticles at 25°C. Above a final emulsifying wax concentration of 1.5 mg/mL the resulting particle size reached an apparent plateau (Fig. 4A). As observed in this study, very small nanoparticles (having diameters less than 50 nm) were obtained when low concentrations of the matrix materials were used. The data obtained in Fig. 4A were presented in a different form in Fig. 4B to verify a direct linear relationship between the droplet size at 55°C and the particle size of cured nanoparticles at 25°C. As shown (Fig. 4B), for samples having a final emulsifying wax concentration from 0.5 mg/mL to 1.5 mg/mL, there was a direct linear relationship between the droplet size at 55°C and the cured nanoparticle size at 25°C. This relationship confirmed that nanoparticles having diameters as small as 5–10 nm could be engineered if the microemulsion templates had very small droplet sizes.

Based on the general trend of the results, emulsifying wax and Brij 72 systems (2 mg/mL) prepared with 3.0 mM Brij 78 and 2.3 mM Tween 80, respectively, were selected as optimal systems. Additional studies were then carried out to determine whether the nanoparticle size was maintained (below 100 nm) when the process variables were varied. For example, the stirring and cooling time of the warm microemulsions are important variables that could affect the size of the cured nanoparticles. Microemulsion templates were stirred at 55°C for 2 to 30 min before they were cooled for 10 min. Also, to determine the effect of cooling time, the stirring time was fixed at 10 min and warm microemulsions were cooled for 2 to 30 min before particle size determination. It was observed that the particle size of emulsifying wax and Brij 72 nanoparticles was stable (below 100 nm) irrespective of the cooling and stirring times of the warm microemulsions. The extent of stirring of microemulsions did not interfere with either the formation of microemulsions or the properties of the cured nanoparticles. The trend is in agreement with the fact that microemulsions form spontaneously.

To determine the suitability of cured nanoparticles as carriers for sterile parenterals, the systems were filtered though 0.22 μm filters at two different stages of the production of nanoparticles, i.e., by filtering the warm microemulsions at 55°C (before cooling) and filtering the nanoparticle dispersion (after cooling the warm microemulsions at room temperature). The results showed that the particle size of nanoparticles was unchanged after both filtration treatments (data not shown), suggesting that the engineering method may be amenable to preparation of sterile parenterals, and that the

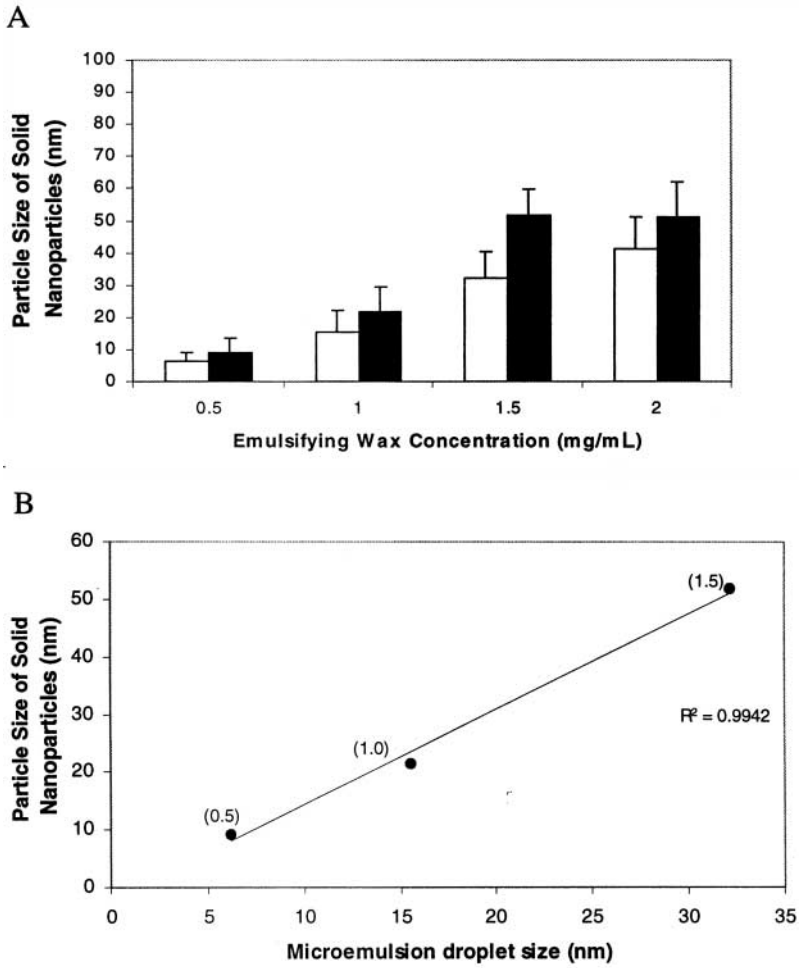

Figure 4. (A) Direct correlation between microemulsion droplet size at 55°C (white bars) and cured nanoparticle size at 25°C (black bars) as a function of emulsifying wax concentration. All systems were made with a final Brij 78 concentration of 10 mM. (B) Correlation between microemulsion droplet size at 55°C and cured nanoparticle size at 25°C made with 10 mM Brij 78 and final emulsifying wax concentrations of 0.5 mg/mL, 1.0 mg/mL, and 1.5 mg/mL.

process is potentially scalable. In fact, to date, we have demonstrated that the process can be scaled from 1 mL up to 100 mL.

Various cooling methods to cure nanoparticles from warm microemulsions were also studied, since it has been demonstrated in this study that nanoparticles can be cured from warm microemulsions by simply cooling the microemulsions at 55°C to room temperature. Nanoparticles cured after various cooling methods retained diameters below 100 nm (Fig. 5). Ideally, as shown in Fig. 5, stable emulsifying wax and Brij 72 nanoparticles (2 mg/mL) could be prepared by simply cooling the templates to room temperature. Earlier reported methods (12,26) required the dilution of the initial microemulsions with cold water to produce nanoparticles. Thus, the engineering process in this study offered some advantages over previously reported methods, since solid nanoparticles could be cured by simply cooling the templates in one vessel.

The in vitro stability of nanoparticles challenged with biologically relevant media has relevance to the potential performance of the nanoparticles as drug delivery systems (27–28). As such, nanoparticles were assessed based on the retention of size over time in various biological media, such as 10% FBS, 10 mM phosphate-buffered saline (pH 7.4), 150 mM

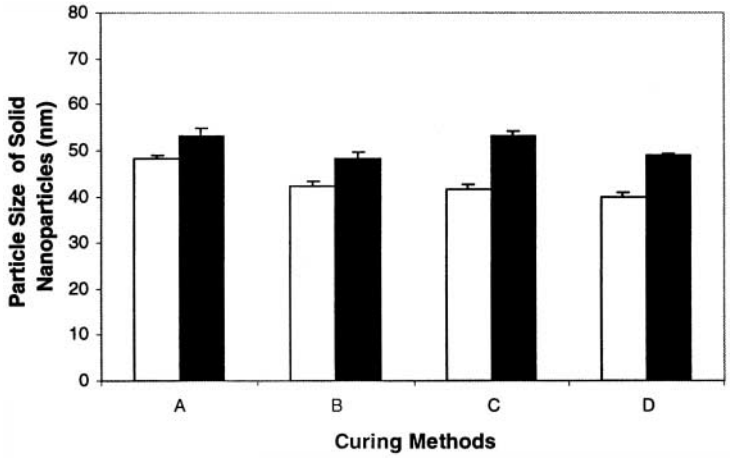

Figure 5. The effect of four curing methods on the particle size of emulsifying wax (white bars) and Brij 72 nanoparticles (black bars) engineered from warm oil-in-water microemulsion templates. Emulsifying wax and Brij 72 microemulsions were prepared with 3 mM Brij 78 and 2.3 mM Tween 80 respectively. In each case, the warm microemulsion was stirred for 10 min at 55°C. After cooling for 10 min, the particle size of cured solid nanoparticles (2 mg/mL) was determined. Method A: cooling the undiluted oil-in-water microemulsion at 55°C to room temperature. Method B: cooling the oil-in-water microemulsion at 55°C by placing undiluted microemulsion in a freezer. Method C: cooling the oil-in-water microemulsion at 55°C by placing undiluted microemulsion in a refrigerator at 4°C. Method D: diluting (1 : 10) the oil-in-water microemulsion at 55°C (1 : 10) with cold water (4°C).

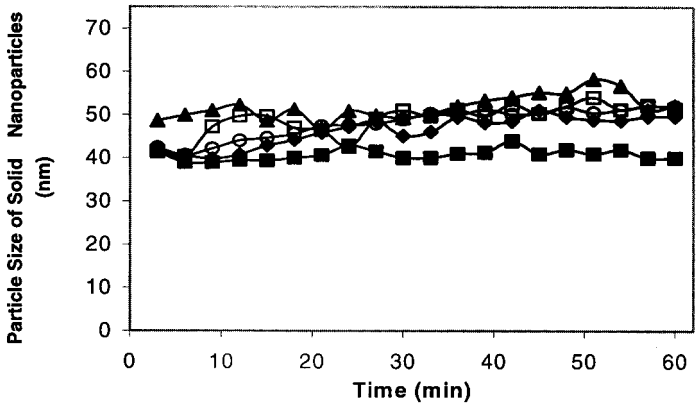

Figure 6. The stability of Brij 72 nanoparticles challenged with various media at 37°C for 60 min. Brij 72 nanoparticles were engineered with 2 mg Brij 72, 2.3 mM Tween 80, and filtered water to make a final volume of 1 mL. The microemulsion was stirred for 10 min at 55°C and cooled for 10 min at room temperature. The nanoparticles were challenged by diluting (1 : 10) with different biological media at 37°C: (◆) water, (▲) 10 mM PBS, (■) 10% FBS, (○) 150 mM NaCl, and (□) 10% lactose.

NaCl, and 10% lactose. Lactose (10%) was included in this study as an example of a potential cryoprotectant that may be used for lyophilization of the nanoparticles. As shown in Fig. 6, cured Brij 72 nanoparticles "challenged" with various biological media at 37°C were found to be stable over 60 min. A similar result was obtained with emulsifying wax nanoparticles (data not shown). These data suggested that engineered nanoparticles did not undergo thermal transitions at 37°C that would have resulted in changes in particle size.

The application of the nanoparticles in drug entrapment was investigated using GdAcAc. This is very poorly water soluble ($< 0.5\,\mu g/mL$) and was selected with the expectation of achieving high entrapment efficiencies in nanoparticles using the

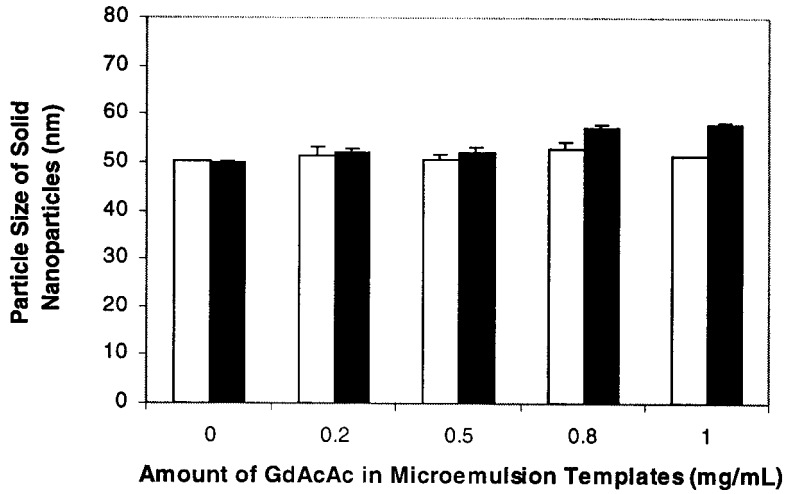

Figure 7. The effect of the incorporation of gadolinium acetylacetonate (GdAcAc) in emulsifying wax (white bars) and Brij 72 nanoparticles (black bars) using 3 mM Brij 78 and 2.3 mM Tween 80 respectively. The warm microemulsion was stirred for 10 min and later cooled for 10 min at room temperature to cure the nanoparticles (2 mg/mL). The nanoparticles were diluted 1 : 10 with filtered water before particle size measurement.

oil-in-water microemulsion templates. In fact, 1 mg of GdAcAc was found to be soluble in 2 mg of melted emulsifying wax or Brij 72. The incorporation of GdAcAc (from 0.2 mg to 1 mg) in both emulsifying wax and Brij 72 nanoparticles (2 mg/mL) had little or no effect on the resulting particle sizes of the cured nanoparticles, as shown in Fig. 7. It is noteworthy that up to 1 mg of GdAcAc could be incorporated in these nanoparticle systems (2 mg/mL), resulting in an apparent 2000-fold enhancement of GdAcAc solubility using this nanotemplate process.

In each of these nanoparticle systems that contained from 0 to 1.0 mg of GdAcAc, GPC was carried out to determine the entrapment efficiency of GdAcAc. Analysis of GPC profiles obtained for emulsifying wax nanoparticles confirmed that GdAcAc co-eluted with nanoparticles and that the entrapment efficiency of GdAcAc was approximately 100% (see Fig. 8). In each of the GPC experiments, the possible effects of surfactant on the elution profile of free GdAcAc were monitored. It was observed that incorporation of 1 mg of GdAcAc in optimized Brij 72 systems produced a lower entrapment efficiency of 86%, which was attributed to micellar solubilization of a small amount of GdAcAc by Tween 80. This effect was not observed with Brij 78. The critical micelle concentrations (CMC) for Tween 80 and Brij 78 at 25°C in water are 0.1 mM and 0.75 mM, respectively. Importantly, we also demonstrated by laser light scattering using PCS that the CMC values of both Tween 80 and Brij 78 remained essentially unchanged at both 37°C and 55°C. Thus, the slightly lower GdAcAc entrapment efficiencies for Brij 72 nanoparticles using Tween 80 as the surfactant could be explained by micellar solubilization of GdAcAc by excess Tween 80. Even after GPC elution of nanoparticles in 2.3 mM Tween 80, the resulting 10-fold dilution would still leave the final Tween 80 concentration at or slightly above its CMC value.

In order to assess the feasibility of the nanoparticles as a possible gadolinium delivery system for NCT, we have made estimates of the number of target atoms of ^{157}Gd that can be achieved based on the entrapment efficiency of GdAcAc. For example, in a formulation with 1 mg of GdAcAc in 2 mg/mL nanoparticles, if we are able to achieve 10% delivery efficiency of nanoparticles to a 2 cm diameter tumor after a systemic administration, the number of ^{157}Gd target atoms in the tumor will be about 3.13×10^{16} (based on 15.7% natural abundance of ^{157}Gd). Upon a single irradiation for 60 min with a neutron fluence of 6.0×10^{9} n/cm^2, the number of ^{158}Gd atoms that will be produced due to $^{157}Gd(n,\gamma)^{158}Gd$ in the tumor will be approximately 4.79×10^{7}, producing a tumor dose of

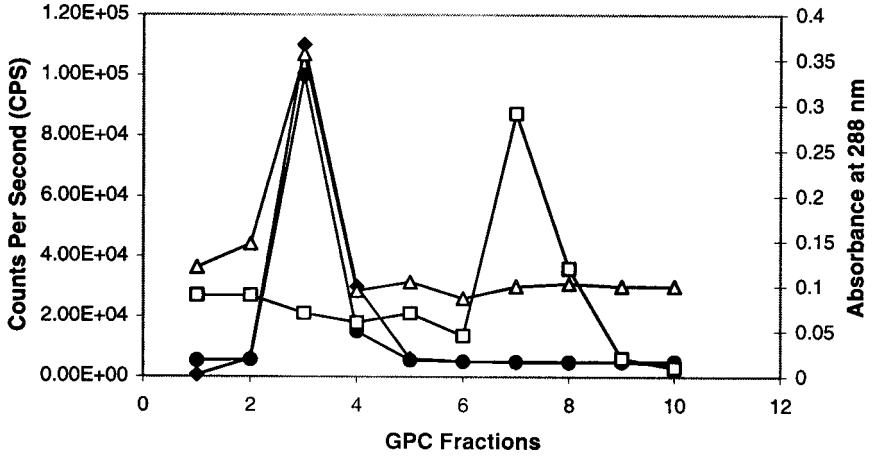

Figure 8. Entrapment efficiency of 0.5 mg gadolinium acetylacetonate (GdAcAc) in emulsifying wax nanoparticles (2 mg/mL) using gel permeation chromatography (GPC) elution profiles. The GPC fractions with nanoparticles were detected by laser light scattering (CPS) and the GdAcAc was measured by UV absorbance at 288 nm: (●) CPS for emulsifying wax nanoparticles, (◆) CPS for GdAcAc-loaded emulsifying wax nanoparticles, (□) absorbance of GdAcAc in water alone, (△) absorbance of emulsifying wax nanoparticles containing GdAcAc.

10–60 Gy. In previous human and animal studies (17,29,30) using gadolinium NCT, an approximate dose of 10 to 60 Gy was effective in reducing tumor growth. Present studies are focusing on the inclusion of a tumor-specific ligand on the surface of gadolinium-loaded nanoparticles, as well as determining the biodistribution of the tumor-targeted nanoparticles containing GdAcAc in a mouse tumor model.

CONCLUSIONS

These studies showed that nanoparticles (2 mg/mL) having diameters less than 100 nm could be engineered from oil-in-water microemulsion templates. The process is simple, scalable, and can be optimized to produce nanoparticles that are stable physically as well as in biological fluids. The entrapment efficiency of GdAcAc was nearly 100% in emulsifying wax nanoparticles and 86% in Brij 72 nanoparticles. The apparent water solubility of GdAcAc was increased more than 2000-fold by entrapment into nanoparticles. It is expected that gadolinium-loaded nanoparticles may be effective as carriers for the direct delivery of high amounts of gadolinium into tumor cells for NCT.

ACKNOWLEDGMENTS

This work was supported, in part, by NSF grant BES-9986441, and by the AFPE and the Burroughs Wellcome Fund through the AACP New Investigators Program for Pharmacy Faculty. The authors would like to acknowledge Dr. Michael Jay (Professor of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky) for useful discussions, and Mr. Zhengrong Cui for the TEM images.

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