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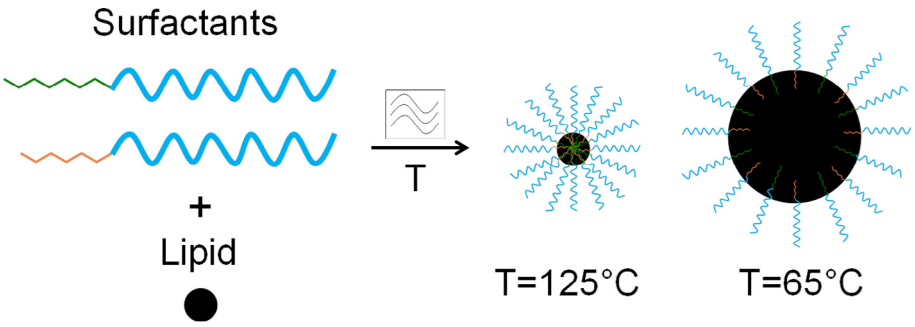
Rapid microwave-assisted synthesis of sub-30 nm lipid nanoparticles

Stuart S. Dunn [a,b], Denis R. Beckford Vera [a,b], S. Rahima Benhabbour [c], Matthew C. Parrott [a,b,d,*]

[a] Biomedical Research Imaging Center, University of North Carolina at Chapel Hill, NC, United States
[b] Department of Radiology, University of North Carolina at Chapel Hill, NC, United States
[c] Center for Nanotechnology in Drug Delivery, Division of Molecular Pharmaceutics, UNC Eshelman School of Pharmacy, United States
[d] The Carolina Institute for Nanomedicine, University of North Carolina at Chapel Hill, NC, United States

GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: Accessing the phase inversion temperature by microwave heating may enable the rapid synthesis of small lipid nanoparticles.
Experiments: Nanoparticle formulations consisted of surfactants Brij 78 and Vitamin E TPGS, and trilaurin, trimyristin, or miglyol 812 as nanoparticle lipid cores. Each formulation was placed in water and heated by microwave irradiation at temperatures ranging from 65 °C to 245 °C. We observed a phase inversion temperature (PIT) for these formulations based on a dramatic decrease in particle Z-average diameters. Subsequently, nanoparticles were manufactured above and below the PIT and studied for (a) stability toward dilution, (b) stability over time, (c) fabrication as a function of reaction time, and (d) transmittance of lipid nanoparticle dispersions.
Findings: Lipid-based nanoparticles with distinct sizes down to 20–30 nm and low polydispersity could be attained by a simple, one-pot microwave synthesis. This was carried out by accessing the phase inversion temperature using microwave heating. Nanoparticles could be synthesized in just one minute and select compositions demonstrated high stability. The notable stability of these particles may be explained by the combination of van der Waals interactions and steric repulsion. 20–30 nm nanoparticles were found to be optically transparent.

Abbreviations: NPs, Nanoparticles; T_m, melting temperature; **BTM, BTL, or BTMy**, **B**rij 78, vitamin E **T**PGS, and **M**iglyol 812, tri**L**aurin, or tri**M**yristin; delta-10 (Δ_{10}), the point at which particle size increased by 10%; $BTM_{65/125}$, BTM NPs fabricated at 65 °C or 125 °C; $BTL_{65/125}$, BTL NPs fabricated at 65 °C or 125 °C; D_z, Z-average diameter; PDI, polydispersity index.

* Corresponding author at: University of North Carolina at Chapel Hill, 2203 Marsico Building, 125 Mason Farm Rd., Chapel Hill, NC 27599-7513, United States
E-mail addresses: ssdunn@unc.edu (S.S. Dunn), denisrbv@email.unc.edu (D.R. Beckford Vera), benhabs@email.unc.edu (S.R. Benhabbour), parrotmc@email.unc.edu (M.C. Parrott).

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1. Introduction

The unique and wide-ranging properties of nanoparticles (NPs) have advanced numerous technologies. Ideal nanoparticle properties include low polydispersity for homogeneity, optical transparency for aesthetics, simple fabrication for scale-up, and physical stability for long-term application [1–3]. In addition, physical stability toward dilution is a critical property in drug delivery and cosmetics where NPs encounter notable changes in concentration. Nanoparticle size is a key property in determining behavior. For example, in sensing and analytical detection [4,5], the light absorption and scattering properties of nanoparticles depend on size. Moreover, in nano-therapeutics, the size of NPs can influence blood circulation time, tissue targeting, drug release rate, and corresponding efficacy [6–9]. In cosmetics, skin hydration and absorption of active ingredients is influenced by nanoparticle size [10]. For food products, the color and appearance are crucial characteristics to consumers and depend on NP size [11,12]. The importance of nanoparticle size can also be noted for catalysis [13,14], pesticides [15,16], and energy [17,18] industries.

Many industries utilize lipid-based nanoparticles due to their biocompatibility, scalability, cheap raw materials, tunable cargo release, and the use of aqueous solvents in synthesis. Notable lipid-based nanoparticles include nanoemulsions and solid-lipid nanoparticles (SLNs). Lipid-based NPs consist of a core, liquid or solid, that is stabilized by surfactants. This yields stable dispersions with small sizes, large surface areas, and distinct core morphologies. Lipid NPs are typically made by high pressure homogenization, high shear homogenization, and ultrasonication [10,19]. More recently, SLNs have been prepared by a microemulsion-based technique using a Taguchi model of experimental design [20]. A follow-up study on this work involved the fabrication of these SLNs with microwave heating [21]. Compared to conventional heating, microwave heating yielded lower polydispersity particles with smaller sizes and a core-shell structure in a possible lamellar arrangement [22].

Microwave irradiation has also been utilized to synthesize nanoemulsions. For example, surfactant free emulsion polymerization was carried out for poly(methyl methacrylate) particles using microwave methodology [23]. Sub-50 nm crosslinked particles were obtained in a one-step process with good polydispersity. Another example involves the microwave-assisted synthesis of poly(ethylene glycol)-*block*-poly(styrene) emulsion nanoparticles, which assumed smaller size and lower polydispersity when compared to conventional heating [24].

Smaller nanoparticles with lower polydispersity indices may be obtained by microwave synthesis on account of efficient localized heating and lower temperature gradients throughout the sample. Furthermore, microwave synthesis generally provides increased rates of reaction and product yields due to fast, homogeneous, and efficient dielectric heating [25]. In conventional heating, the vessel walls are heated first and then the heat diffuses into the reaction mixture. Conversely, microwave heating allows for higher temperatures to be reached rapidly by homogeneous heating of the entire reaction volume.

Here, we harness the advantages of microwave synthesis to influence the size of nanoparticles with a lipid-triglyceride core, both liquid and solid. Specifically, microwave heating was used to reduce lipid NP size. Small nanoparticles were tested for stability and scalability. We found an approach that is scalable and offers rapid synthesis of nanoparticles using biocompatible excipients. Furthermore, this approach provides a one-pot synthesis yielding purified nanoparticles directly from the microwave reactor. Nanoparticles with a lipid-triglyceride core, both liquid and solid, were rapidly fabricated using microwave heating. These nanoparticles had (1) small sizes depending on microwave conditions, (2) low polydispersity, (3) physical stability, and (4) optical transparency.

2. Experimental section

2.1. Materials

Polyoxyl 20-stearyl ether (Brij 78) was obtained from Uniqema (Wilmington, DE), d-alpha tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS) was acquired from Eastman Chemicals (Kingsport, TN), and Miglyol 812 was purchased from Sasol (Witten, Germany) Trilaurin and trimyristin were purchased from Sigma Aldrich. Solvents were obtained from Thermo Fisher Scientific, Inc. Accessories for particle fabrication and analysis were obtained from Biotage and Thermo Fisher Scientific, Inc. The magnetic stir bars were made of ferrite and coated with poly(tetrafluoroethylene), and had a V-shape to fit the microwave vials. NP fabrication occurred in a Biotage® Initiator Classic microwave synthesizer. Dynamic light scattering occurred in a Zetasizer Nano ZS Particle Analyzer (Malvern Instruments, Inc.). % Transmittance was calculated from measurements on a SpectraMax M5 Plate Reader (Molecular Devices).

2.2. Synthesis of lipid-based particles

The formulation of nanoparticles was selected based on previous work [26,27]. In a typical experiment, Brij 78, Vitamin E TPGS, and trilaurin were dissolved separately in chloroform to make stock solutions in 1.5 mL Eppendorf tubes. Aliquots were taken from the stock solutions containing 1.75 mg of Brij 78, 0.75 mg of Vitamin E TPGS, and 1.25 mg of trilaurin, which were added to a 0.5–2.0 mL microwave reaction vial and mixed by pipetting. The solvent was evaporated, yielding a film of the components. Filtered, deionized water (0.5 mL) was then added to the film to provide a 7.5 mg/mL concentration. The vial was charged with a magnetic stir bar and sealed with a microwave cap. The vial was then loaded into the microwave synthesizer for a given time at a particular temperature using the very high absorption setting and fixed hold time (countdown starts after reaching the target temperature). All particles were synthesized in triplicate.

2.3. Characterization of lipid-based particles

The size of lipid-based particles was evaluated using Zetasizer Nano ZS Particle Analyzer (Malvern Instruments, Inc.). For the analysis of particles synthesized as a function of temperature and time, 0.5 mL of the particle dispersion in the microwave vial was directly aliquoted into a disposable microcuvette. Three measurements were taken for each particle sample using automatic measurement duration and 173° backscatter general purpose analysis model at 25 °C in water. We collected D_z, PDI, PDI width, and ζ-potential. The ζ-potential was measured on samples in 10 mM NaCl using a clear disposable zeta cell with automatic duration time, three measurements, and the Smoluchowski approximation.

2.4. Characterization of particle dilution

For the dilution studies, 0.5 mL of particles was diluted from 7.5 mg/mL to 2.5 mg/mL with water for the initial 3-fold dilution measurement. 100 μL of this initial solution was added to 900 μL water in a disposal cuvette to provide 30-fold dilution. For 150-fold dilution, 200 μL of the initial solution was added to 800 μL water. This was repeated out to a maximum of 30,000-fold