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Facile microwave synthesis of uniform magnetic nanoparticles with minimal sample processing

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ABSTRACT

We present a simple and rapid method for the synthesis of small magnetic nanoparticles (diameters in the order of 5–20 nm) and narrow size distributions (CV's of 20–40%). The magnetite nanoparticles were synthesized in green solvents within minutes and the saturation magnetization of the particles was tunable by changes in the reaction conditions. We show that this particle synthesis method requires minimal processing steps and we present the successful coating of the particles with reactive bisphosphonates after synthesis without washing or centrifugation. We found minimal batch-to-batch variability and show the scalability of the particle synthesis method. We present a full characterization of the particle properties and believe that this synthesis method holds great promise for facile and rapid generation of magnetic nanoparticles with defined surface coatings for magnetic targeting applications.

1. Introduction

Drug delivery is an important method in the fight against cancer and other diseases as it increases specificity between the target site and the drug, thereby lowering side effects due to whole body drug exposure. Different vectors that interact with a target site in the body (*e.g.*, an inflamed tissue) can facilitate drug delivery. Magnetic nanoparticles (MNPs) can be used as such vectors and can be guided and enriched at the target site by directed magnetic fields [1]. Magnetic drug targeting is a recent drug delivery method where the drug is bound to the MNPs and, after delivery to the target, is released to induce a therapeutic effect [2]. Different factors can impact the quality of the magnetic drug carrier, which include its size distribution and composition, its cell permeability and especially its protective coating and release characteristics of the bound drug. A major challenge in the development of magnetic drug carriers for clinical therapy has been the poor particle size distribution of MNPs, methods producing large amounts of particles per batch, and the poor mass throughput of methods producing MNPs with excellent size distributions [3]. In addition, the protective coating of the MNPs needs to be multifunctional; that is it needs to be a thin biocompatible coating and it needs to facilitate peptide and antibody conjugation, as well as encapsulate/bind and controllably release a drug.

Conventional methods to synthesize MNPs that have high yields include microemulsion syntheses [4] and wet chemical methods based on iron salt solutions through co-precipitation or thermal decomposition from organometallic solvent solutions [5,6]. The latter method has gained attraction as a fast synthetic strategy to create uniform MNPs, but requires high temperatures, toxic chemicals, and the addition of stabilizing agents [7–10]. Over the past years, solvothermal synthetic strategies have been studied in detail [11], particularly with a focus on low cost precursor materials including iron pentacarbonyl [7], iron oleate [12], iron(II) acetate [9], and iron(III) acetylacetonate [8]. The main advantage of solvothermal synthesis is the high level of control over the reaction conditions. But the use of toxic and combustible solvents (*e.g.*,

Abbreviations used: CV, Coefficient of variation; DSC, Differential Scanning Calorimetry; DLS, Dynamic light scattering; FT-IR, Fourier-Transform Infrared; MNP, Magnetic nanoparticle; M_{sat}, Saturation magnetization; SQUID, Superconducting Quantum Interference Device; TEM, Transmission Electron Microscopy; TEG, Triethylene glycol; VSM, Vibrational sample magnetometry; XRD, X-Ray Diffraction

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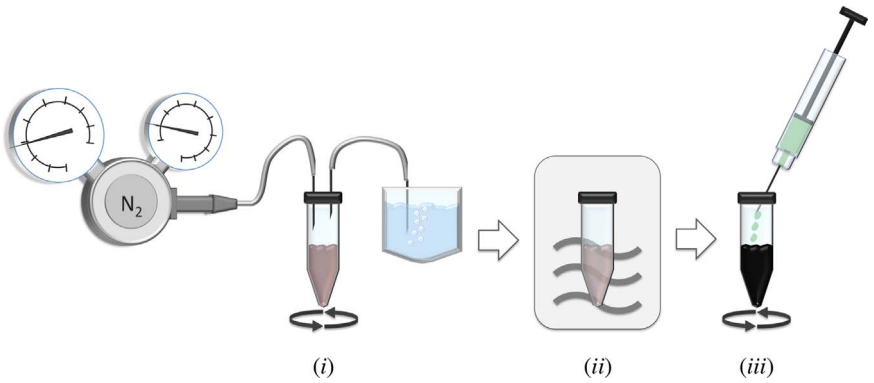

Fig. 1. General process flow in the particle synthesis: (i) The precursor iron salt is dissolved under inert conditions, (ii) undergoes thermal decomposition in a microwave oven resulting in the formation of nanoparticles, (iii) which are coated directly in the reaction vessel (one pot method). Subsequent precipitation and separation/washing using a magnet or centrifuge yields stable water-soluble particles.

benzyl ethers or octadecene) limits the application of particles created by this method. The use of these particles in biological applications such as magnetic drug targeting requires extensive post-processing to separate the MNPs from toxic solvents and residual stabilizing agents, and it often requires further wet chemical methods to create biocompatible and functional coatings. All these steps increase the time required to create functionalized and biocompatible MNPs. In addition, the use of different solvents for MNP synthesis and subsequent coating can create challenges in terms of solubility mismatches between MNPs and coating materials, changes in colloidal stability of the particles, and low yield of the final coated biocompatible MNPs. To overcome these limitations and increase the versatility and applicability of MNPs as magnetic drug carriers in *in vivo* applications, it is desirable to take advantage of the wealth of available methods for particle synthesis and to minimize the overall processing steps (*i.e.*, reduction in centrifugation and washing steps), thereby creating a one-pot synthetic method with high control over the particle quality, high yield, and high mass throughput per particle batch.

Recent advances in novel microwave-based synthetic methods [13–15] provide the foundation to design simplified synthetic strategies that take advantage of isochoric reaction conditions and so called greener solvents. Greener solvents are less toxic, produce fewer harmful byproducts, and are favorable in synthetic chemical methods aimed at biological applications. Among the greener solvents are dihydroxy alcohols, including triethylene glycol (TEG), whose physicochemical properties are favorable for solvothermal decomposition methods (*i.e.*, high viscosities and boiling points). TEG is typically used as a plasticizer [16] as well as a dehydration solvent in the gas industry [17]. It is also known to be a mild disinfectant and shows no known toxicities unlike the related di (ethylene glycol) [18,19]. In addition, its high solubility in water is ideal to simplify sample post-processing steps. Other alcohols suitable for solvothermal decomposition due to their high viscosities and boiling points include benzyl alcohol, which is immiscible in water. Synthesis of MNPs with benzyl alcohol and triethylene glycol as solvents has been shown to yield uniform MNPs in autoclaves [20–23] and by microwave-based methods [24,25]. However, all these methods required several processing steps followed by multiple washing and (often) centrifugation steps to separate the MNPs from the solvents. Microwave based MNP synthesis was also shown from aqueous Fe^{2+} and Fe^{3+}, resulting in γ-Fe_2O_3 and α-Fe_2O_3 MNPs [26,27], which exhibit lower saturation magnetization than Fe_3O_4.

Here we present a fast method to produce large quantities of functionalized MNPs for the use as magnetic drug carrier precursors with excellent size distributions by a combination of facile microwave synthesis and a bisphosphonate coating strategy. The bisphosphonate coating provides an active amine group for conventional amine-reactive crosslinker chemistry [28], which is ideal for functional bioconjugation to cell permeable proteins, antibodies, and drugs. The particles created by this one-pot synthetic method are uniform in size and shape, water soluble, and contain mainly magnetite, as supported by full particle characterization.

2. Materials and methods

2.1. Particle synthesis and coating

Microwave synthesis experiments were conducted in an Initiator$^+$ (Biotage Sweden AB, Uppsala, Sweden). All experiments were conducted with a solution of iron acetylacetonate ($Fe(acac)_3$; $Fe(C_5H_7O_2)_3$; cat. F300; Sigma Aldrich Ltd., Oakville, ON, Canada) in tri(ethylene glycol) (cat. #T59455, Sigma Aldrich) under inert conditions. Prior to the synthesis, the $Fe(acac)_3$ was mixed in Biotage microwave vials together with the solvent and sealed with vial caps. Typical reaction volumes were 2 mL, but larger reaction volumes (up to 20 mL) were used in scale-up studies.

A customized N_2 – flood system was used to blanket the solution with N_2 for 30 min (Fig. 1). The $Fe(acac)_3$ was then converted into magnetic nanoparticles through thermal decomposition in the Initiator$^+$ by first heating to 200 °C for up to 24 h (t_h) followed by reflux heating to 250 °C for up to 24 h (t_{rf}). The total reaction time is given as the sum of t_h, t_{rf}, and the time used for heating and cooling to the pre-set temperatures (typically 5–10 min).

The particles were coated directly in the microwave vials by injecting alendronic acid (cat. #A2120, 98% pure; TCI America, Portland, OR, USA) through the vial cap's occulum. The alendronic acid solution was prepared fresh the same day of coating in ultrapurified water (MilliQ, Millipore Corp., Milford, MA) at a concentration of 5 mg/mL aided by sonication for 60 min. The particles were then separated and prepared for analysis by precipitation in ethyl acetonate, followed by centrifugation at 8200*g* for 10 min. This process was repeated and the particles redispersed in water or dried under nitrogen for analysis.

2.2. Particle bioconjugation

Different stock solutions were prepared on the day of the conjugation. A stock solution of 0.5 M MES buffer (2-(N-morpholino) ethanesulfonic acid (MES); cat. # M3671; Sigma Aldrich Ltd.) at pH 3–4 was prepared in distilled water. The conjugation buffer consisted of 6 mg/mL EDC (N-(3-dimethylamino-propyl)-N′-ethylcarbodiimide hydrochloride; cat. # E7750; Sigma Aldrich Ltd.) and 12 mg/mL NHS (N-hydroxy-succinimide; cat. # 130,672;