

Short Communication**Factors Affecting Sizes of Magnetic Particles Formed by Chemical Co-precipitation**N. Tresilwised,¹ P. Pithayanukul^{1*} and C. Plank²¹Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand;²Institute of Experimental Oncology, Technical University of Munich, Munich, Germany.

Abstract Magnetic particles are used for medical and pharmaceutical purposes such as delivery systems for drugs, genes, and radionuclides. In this study, the technique of chemical co-precipitation in aqueous solution was applied for the synthesis of iron-based magnetic particles. The size and size distribution of magnetic particles were determined by photon correlation spectroscopy. Oxygen-free conditions achieved by vacuum or nitrogen gas bubbling during the process of synthesis, together with the addition of an appropriate coating material (citric acid or linear polyethyleneimine) resulted in a stable dispersion of the magnetic colloid with average hydrodynamic diameter of 217-329 nm and polydispersity index of particle size distribution of 0.13-0.28. ©All right reserved.

Keywords: chemical co-precipitation, magnetic particles, particle sizes

INTRODUCTION

The study of magnetic colloids started in 1965.¹ These magnetic particles are being used for medical and pharmaceutical purposes such as delivery systems for drugs,² genes,^{3,4} and radionuclides.⁵ They are used as contrast agents in magnetic resonance imaging (MRI) which is routinely applied in the field of diagnostic imaging.⁶ Magnetic particles are also attractive for *in vitro* applications in medical diagnostics, such as research in genetics and technologies based on immune magnetic separation (IMS) of cells, proteins, DNA/RNA, bacteria, viruses and other biomolecules.⁷ All these applications require that these nanoparticles exhibit high magnetization values with a narrow size distribution of less than 100 nm; uniformity in their physical and chemical properties permits the optimum results in diagnostics and therapy. Advances in the use of magnetic particles for biomedical applications depend on new synthetic methods with better control of the size distribution and of particle surface

characteristics.⁸ Many methods have been developed to synthesize magnetic nanoparticles such as use of polyols,⁹ microemulsions,^{9,10} laser pyrolysis,¹¹ sonochemical synthesis,¹² and chemical co-precipitation.¹³⁻¹⁵ At present, magnetic nanoparticles are usually synthesized by microemulsion or chemical co-precipitation. The synthesis process for both methods is similar. In this procedure, the iron-based magnetic nanoparticles (Fe₃O₄) are formed at a pH of around 10-11. However, the pH value of the original ferrite solution containing ferrous ion (Fe²⁺) and ferric ion (Fe³⁺) is around 1-2. Thus, base solutions such as potassium hydroxide, sodium hydroxide or ammonium hydroxide must be added to the ferrite solution.¹⁵ To control the reaction kinetics, which is strongly related to the oxidation speed of the iron species, the synthesis of particles must be carried out in an oxygen-free environment by passing nitrogen gas.^{14,16} Nevertheless, the microemulsion method is rather complicated with problems such as the formation of micelles, the difficulty in removing surfactants

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from nanoparticles and only small quantities of iron oxide particles can be produced. Chemical co-precipitation may therefore be the most promising method because of its simplicity, productivity and low cost in the production process.

The control of the size, shape and composition of magnetic nanoparticles depends on the type of iron salts used (e.g., chlorides, sulfates, nitrates, perchlorates, etc.), Fe²⁺/Fe³⁺ ratio,¹⁶ presence or absence of oxygen gas, reaction rate and temperature.^{17,18} Kim *et al.*¹⁴ have demonstrated that flowing nitrogen gas through the solution not only protects critical oxidation of the magnetite but also reduces the particle size when compared with methods which do not remove oxygen. For example, the particle size was reduced from 80 Å (in air) to 60 Å (in nitrogen gas). Tailoring of particle size is also obtained by controlling the pH and ionic strength of the precipitation medium. Vayssières *et al.*¹⁹ have reported that, at constant ionic strength imposed by NaNO₃ (or at constant pH), the particle size decreases with increasing pH (or ionic strength). Additionally, if the pH of precipitation is higher than the pH of a given ionic strength, the surface is always completely charged during the formation of particles and the particle mean size does not evolve on aging. A thermodynamic equilibrium state can be reached without kinetic restrictions. If the pH of the suspension is lower than the pH of a given ionic strength, the particles spontaneously grow by an Ostwald ripening process in order to reach a new equilibrium state. The size and size distribution of magnetic particles are extremely important for medical and pharmaceutical applications *in vivo*, particularly intravenous administration. Magnetic particles with larger sizes and/or aggregations of small particles may be trapped and cause emboli within the capillary bed of the lungs. The objective of this preliminary study was to evaluate two factors: type of oxygen-free environment (under a vacuum system or nitrogen gas bubbling) and type of coating material (citric acid or linear polyethyleneimine) that affect the size and size distribution of the magnetic particles obtained by chemical co-precipitation. The results should assist in the development of small

magnetic particles with a narrow size distribution that are safe and effective as carriers or in combination with other carriers, such as liposomes and microspheres, for drug targeting and delivery to specific organs or tissues via intravenous administration *in vivo*.

MATERIALS AND METHODS

Materials

All the chemicals were of reagent grade and were used without further purification. The chemicals used were ferric chloride hexahydrate (FeCl₃·6H₂O > 99%, Merck, Germany), ferrous chloride tetrahydrate (FeCl₂·4H₂O > 99%, Riedel-deHaën, Germany), ammonium hydroxide (29.6 % by weight NH₃ in water, Mallinckrodt, USA), sodium hydroxide (NaOH 98%, Univar, Australia and New Zealand), hydrochloric acid (HCl 37% by volume, Merck KGaA, Germany), citric acid monohydrate (C₆H₈O₇·H₂O, Fisher Chemicals, UK) and linear polyethyleneimine (50 kDa) (linear PEI 50% w/v, Sigma, St. Louis, MO, USA). Water used throughout the study was passed through deionization process and followed by double distillation (Fistream Cyclon system, Sanyo Gallenkamp, England).

Synthesis of Magnetic Particles

The method of Kang *et al.*²⁰ was followed with slight modification. Ferrous chloride tetrahydrate (2.485 g) was dissolved in hydrochloric acid solution (2 M) to a total volume of 50 ml and then filtered through a membrane filter (0.2 µm) to obtain ferrous chloride solution (0.25 M). Ferric chloride solution (0.50 M) was prepared in a separate flask by dissolving ferric chloride hexahydrate (13.515 g) in deionized distilled water to a total volume of 100 ml, and then filtered through a membrane filter (0.2 µm). Deionized distilled water was used as a solvent in order to avoid the production of impurities in the final product.^{21,22} The two solutions, ferrous chloride (39 ml) and ferric chloride (64 ml), were mixed vigorously with a magnetic stirrer in a round-bottomed flask on an ice bath (2-4 °C) under vacuum for 30 minutes. Ammonium hydroxide solution (31 ml; 29.6% by weight) was added drop-wise with vigorous stirring, then the vacuum

system and the ice-bath were removed. Stirring continued at room temperature (30°C) for 2 hours, then the mixture was adjusted to pH 11 with sodium hydroxide (10 M). The precipitate of magnetic particles in the mixture was transferred to a dialysis membrane (molecular weight cut-off 12-14 kDa) and was washed extensively with water by changing water five times per day for 4 days to remove any excess of the unreacted ion salts. The final suspension of magnetic particles was kept in a light-protected glass bottle at 15°C until use.

In order to compare with the effect of vacuum on the particle size and size distribution of the magnetic particles, N₂-gas bubbling was used in place of the vacuum system during the synthesis; all other procedures remained unchanged.

Coating of Magnetic Particles

In order to prevent the aggregation of the magnetic particles after their synthesis under the controlled condition of N₂-gas bubbling, citric acid or linear PEI was used as a coating material for the magnetic particles. Citric acid monohydrate (1.094 g) was dissolved in water (15 ml), the solution was added to the suspension of magnetic particles (15 ml) and the mixture was sonicated for 30 minutes. The particles were then washed extensively in a dialysis membrane (cut-off 12-14 kDa) with water for 3 days. The dispersion of coated magnetic particles was kept for further characterization of its size and size distribution.

A similar procedure was applied for coating the magnetic particles with linear PEI. Linear PEI (cut-off 50 kDa) 1.5 g was dissolved in water (15 ml) and the coating solution was added to the suspension of magnetic particles (15 ml) with sonication for 15 minutes to create the PEI-coated particles. The mixture was washed extensively in a dialysis membrane with water for 3 days before the magnetic dispersion was centrifuged at 10,000×g for 30 minutes. The centrifugation process was repeated four times and finally the dispersion of magnetic particles was sonicated for 1 hour to resuspend the pellets and to produce a stable suspension before characterization of its size and size distribution.

All of the magnetic particles, both uncoated and those coated with citric acid or linear PEI, were synthesized in duplicate.

Analysis of Particle Size and Size Distribution

The magnetic dispersion was diluted with water and then sonicated for 30 minutes. The particle size and size distribution were determined by photon correlation spectroscopy with a Coulter[®] MODEL N4MD at 25°C. The analysis of particle size and size distribution was performed in triplicate for each sample and the result was expressed as the average hydrodynamic diameter (nm) and polydispersity index, respectively.

Statistics

All data are expressed as mean ± standard deviation. Student's *t*-test was performed for all comparisons. For paired observation, the paired *t*-test was used. An error probability level of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Iron-based magnetic particles obtained by chemical co-precipitation of ferric and ferrous chlorides with the addition of alkali solutions in a controlled oxygen-free environment achieved either by the vacuum system or by nitrogen gas bubbling were black in color and were attracted by a magnet (i.e., they had magnetic properties). The black color of the acquired particles indicates that there was no oxidation of the particles during synthesis under the oxygen-free environment. In the presence of dissolved oxygen molecules, the resultant magnetic colloids are usually reddish-brown, a color that indicates contamination with other iron oxides in the colloids as a result of strong oxidation of magnetite.¹⁸ Hence, oxygen gas would also critically affect the physical and chemical properties of the nanosized magnetic particles. The magnetic particles tended to aggregate and precipitate quickly after formation. Figure 1 demonstrates that, without sonication, there is agglomeration of uncoated magnetic particles of almost 10,000 nm in hydrodynamic diameter. After sonication, the average hydrodynamic diameter of the particles was significantly ($p < 0.05$) reduced to 547 nm (under vacuum) or 319 nm (with

N₂-gas bubbling) (Figure 1 and Table 1). This indicates that sonication can disaggregate and redispersed the pseudo-aggregation of the particles. Additionally, sonication is an important technique for sample preparation before size measurement of magnetic particles. There was no significant difference in average hydrodynamic diameter and polydispersity index of the magnetic particles synthesized under a controlled vacuum and those synthesized under N₂-gas bubbling ($p > 0.05$). This implies that the vacuum and N₂-gas bubbling are equally effective in removing the dissolved oxygen from the iron salt solution. However, N₂-gas bubbling is a convenient method for generating an oxygen-free environment when compared with the vacuum system.

Even though the uncoated magnetic particles were redispersed by sonication, they aggregated and precipitated again. To stabilize the particles after their synthesis, citric acid or linear PEI was used as the effective coating material for the magnetic particles. The average hydrodynamic diameter and particle size distribution with polydispersity index of the magnetic particles coated with citric acid were significantly ($p < 0.05$) smaller (217 ± 13 nm) and narrower (0.28 ± 0.03) than those of the uncoated magnetic particles (319 ± 23 nm and 0.46 ± 0.08 , respectively) (Table 1). Small molecules such as citric acid can be adsorbed on the surface of the magnetic particles and can act as a low molecular weight stabilizer. The mechanism is based on ion-exchange, in which the ionizable sites of the magnetic particles can be replaced by citrate anions that form a complex with the surface iron atoms.²³ This induced electrosteric

stabilization forces on the surface of the magnetic particles due to the negatively charged citrate ions, and resulted in smaller particle size as well as stabilization.

Although there was no significant difference ($p > 0.05$) between the average particle sizes of linear PEI-coated particles (329 ± 18 nm) and uncoated particles (319 ± 23 nm), the polydispersity index of the particle size distribution of the linear PEI-coated particles was significantly ($p < 0.05$) narrower (0.13 ± 0.06) in comparison with the uncoated particles (0.46 ± 0.08). This could be due to the polymeric nature and the adsorption behavior of the linear PEI, thereby influencing their size. The surfaces of the PEI-coated magnetic particles were saturated with the adsorbed linear PEI, and this induced both hydration forces and electrosteric stabilization forces.

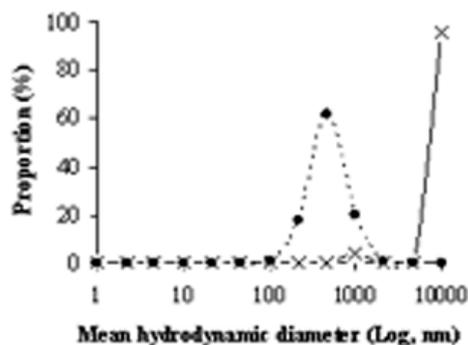


Figure 1. Mean hydrodynamic diameter of uncoated magnetic particles synthesized under a vacuum system without (x) and with sonication (•).

Table 1. Physicochemical characteristics of the magnetic particles synthesized under an oxygen-free environment with different coating materials

Oxygen-free environment	Coating material	Mean hydrodynamic diameter of particles (\pm S.D.) (nm)	Mean polydispersity index of particles (\pm S.D.)
Under vacuum	No	547 ± 165	0.54 ± 0.03
N ₂ -gas bubbling	No	319 ± 23	0.46 ± 0.08
N ₂ -gas bubbling	Citric acid	217 ± 13	0.28 ± 0.03
N ₂ -gas bubbling	Linear PEI	329 ± 18	0.13 ± 0.06

PEI = polyethyleneimine

The hydration forces were related to the hydrophilic character of the linear PEI. The electrosteric stabilization force was due to the presence of charged polyelectrolyte loops and tails when surface saturation was reached.²⁴ The polymeric nature and high molecular weight of the linear PEI coating led to larger particles than the citrate-coated particles. Nevertheless, the polydispersity index of particle size distribution between magnetic particles coated with linear PEI and those coated with citric acid was not significantly different ($p > 0.05$). In contrast to the uncoated magnetic particles, magnetic colloids of both the linear PEI-coated particles and the citrate-coated particles were stable without precipitation during the first week of observation and after that, even though a small amount of precipitation occurred, it could easily be redispersed by shaking.

In conclusion, the effectively oxygen-free environment (under vacuum or N₂-gas bubbling) and the type of coating material were considered important factors in the synthesis of small magnetic particles with narrow size distribution. Since the magnetic particles are used as a carrier in combination with other carriers (e.g., liposomes, microspheres) for drug targeting and delivery to specific organs or tissues via intravenous administration *in vivo*, the magnetic particles should be less than 100 nm. Other factors in their synthesis that may affect particle size should be investigated further, e.g. type of iron salts used (e.g., sulfates, nitrates, perchlorates, etc.), Fe²⁺/Fe³⁺ ratio, reaction rate, temperature and different coating materials.

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