PRODUCTION OF MAGNETICALLY AND PH RESPONSIVE BIODEVICES BY DENSE GAS TECHNOLOGY

Un T. Lam, Raffaella Mammucari and Neil R. Foster*

School of Chemical Sciences and Engineering, The University of New South Wales, Sydney, NSW 2052, Australia

* Corresponding author. Tel: +61-2-9385 4341; fax: +61-2-9385 5966.
E-mail address: n.foster@unsw.edu.au

ABSTRACT

The use of superparamagnetic Iron Oxide Nanoparticles (SPIONs) in drug targeting devices and in Magnetic Resonance Imaging (MRI) contrast agents has shown some promising results in recent years. However, when they are administered orally as MRI contrast agent, there is the potential risk of partial dissolution in the gastric fluids. Surface modification of the SPIONs is required to protect them against dissolution and to increase stability against oxidation and enhance biocompatibility. Current coating processes rely on different versions of monomer polymerization, which have major drawbacks such as long processing time and tedious purification. The application of Dense Gas (DG) technology in the encapsulation of nanoparticles is an attractive option as it usually involves a single processing step and is able to produce product with narrow particle size distributions and free from residual solvents. In this study, SPIONs are coated with Eudragit® S100 using the Aerosol Solvent Extraction System (ASES). The ASES process produced magnetically responsive nanocomposites with diameter less than 150nm. The effect of processing parameters on product morphology, particle size, product composition and magnetic properties were investigated.

1. INTRODUCTION

Superparamagnetic iron oxide nanoparticles (SPIONs) have gained great interest among researchers in the biomedical field over the last few decades due to their attractive properties of biocompatibility and their unique magnetic property. Potential applications of SPIONs in the biomedical field range from drug targeting, cancer diagnosis, cancer therapy, to tissue imaging. Among these, using SPIONs as contrast agents in MRI has achieved the greatest success since the first commercialization of SPIONs-based MRI contrast agent. When SPIONs are used as a contrast agent, the diagnostic sensitivity and specificity is improved compared to conventional contrast agents [1, 2] and high quality images can be produced with a better lesion demarcation [3]. SPION-based contrast agents can be administered intravenously or orally. Oral MRI contrast agents are specifically important for gastrointestinal MRI in the diagnosis of ulcerative colitis, and stomach and bowel cancer. Several studies using oral iron oxide contrast agent for gastrointestinal MRI were conducted in humans[4-6] along with the first clinical trial on oral MRI contrast agent in 1990[7]. Oral iron oxide particles have also been employed as magnetic tracer in the monitoring of the movement and activity of the large intestine[8] and the stomach[9]. Magnetite (Fe_3O_4) is the most commonly used form of iron oxide used in MRI, mainly

because if its non-toxicity. When magnetite nanoparticles are used as oral MRI contrast agents, there is the potential risk of partial dissolution in the gastric fluids as iron oxide is soluble in hydrochloric acid[10]. Therefore, a coating material is required to protect the contrast agent from the acidic environment in the stomach thus preventing its dissolution. In addition, coating of the SPIONs brings other advantages such as increased stability against oxidation - thus increased shelf life - and enhanced biocompatibility. A previous study on the production of iron oxide particles coated by a pH-responsive polymer, xylan, was conducted [11]. Major drawbacks include the use of surfactant and toxic solvents such as chloroform and cyclohexane which made product purification time consuming and difficult. Other coating processes for SPIONs involve in-situ polymerization of deposited monomers. In situ-polymerization presents the drawbacks of presence of unreacted chemicals in the final product and need of intensive post-processing. Using Dense Gas (DG) as antisolvent in the manufacturing of polymer composites including iron oxide is a promising approach. Products from DG-based processes have narrow size distribution as well as other unique advantages such as being solvent-free. An additional feature of DG processing is the intrinsically sterile processing environment[12]. A previous study on coating magnetite nanoparticles have been conducted by Chattopadhyay and Gupta using a supercritical fluid antisolvent process[13]. Three types of biocompatible polymer, poly(lactic-*co*-glycolic acid), poly (methyl methacrylate) and Eudragit® RS were selected as the coating materials. An anti-inflammatory drug was used as the model drug. Ren *et al.* also conducted studies using CO_2 as the antisolvent for the co-precipitation of different combination of polymers and drugs around preformed magnetite nanoparticles[14]. However, no quantitative magnetic characterization was performed for both studies. In this study, the use of a DG- based process known as Aerosol Solvent Extraction System (ASES) to coat the SPIONs with a pH-responsive polymer was presented. The effect of processing parameters on product morphology, particle size, product composition and magnetic properties were studied. Eudragit® S100 was selected as the coating material as it is biocompatible and is insoluble in pH< 7 and slowly soluble in pH> 7 and is frequently used in colonic drug delivery. It is a methacrylic anionic copolymer which offers good storage stability.

II. MATERIALS AND METHODS

Magnetite (Fe_3O_4) nanoparticles were kindly supplied by NanoMaterials Technology Pte Ltd. Eudragit® S100 was kindly supplied by Evonik Degussa. Ethanol (HPLC grade) was purchased from Scharlau Chemie. Carbon dioxide (≥ 99.5% purity) was purchased from Linde Australia.

A schematic diagram of the experimental setup is presented in Figure 1. The high pressure precipitation chamber (Jerguson sight gauge no.32 fitted with a 5 μm frit) was immersed in a water bath to maintain the system temperature. The temperature of the water bath was controlled by a recirculation heater (Thermoline Unistat). Magnetite nanoparticles were supplied as a suspension in ammonia solution. They were washed and re-dispersed in ethanol without further surface modification. An appropriate amount of polymer was added into the SPIONs suspension. The suspension was then loaded into the injection chamber.

In the ASES process, the precipitation chamber is pre-pressurised with CO_2 at the required temperature and pressure. Fresh CO_2 is continuously delivered into the system by a

reciprocating piston pump (Thar P-50). The flow rate was maintained at 15 g/min by adjusting the metering valve (V3). The SPIONs suspension was delivered co-currently with the CO_2 into the precipitation vessel through a stainless steel capillary nozzle (175 μm i.d.). The flow rate was maintained at a rate of 0.2 mL/min by a HPLC pump (Waters 515). Removal of residual solvent was achieved by passing fresh CO_2 into the precipitation chamber for 1 hour. Experimental conditions are presented in Table 1.

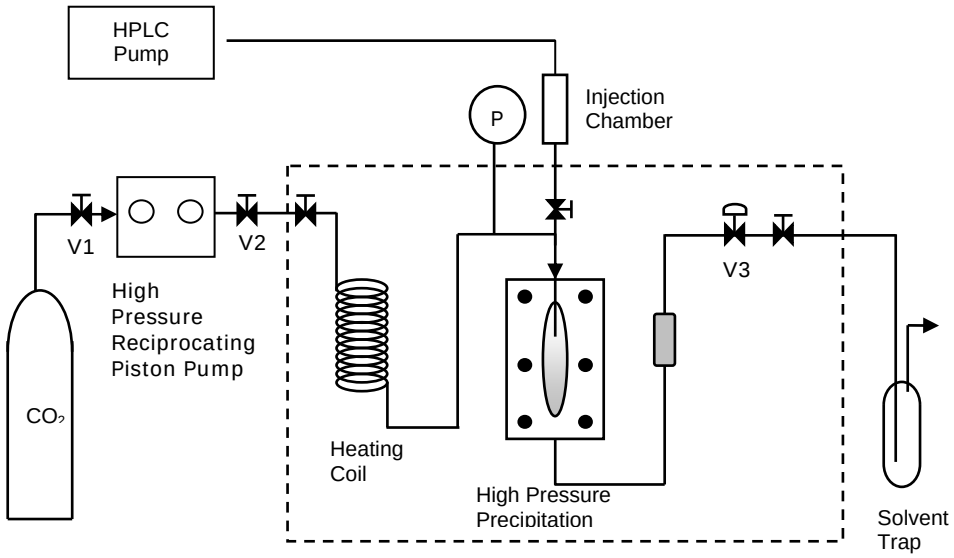

Figure 1 Schematic diagram of the ASES apparatus.

Table 1 Experimental variables for the ASES process with CO_2 flow rate at 15 g/min, 160 bar, solvent flow rate 0.2mL/min, nozzle ID 175μm

Sample Code	Temperature (^oC)	Polymer Concentration (mg/mL)	Polymer: Fe_3O_4 Ratio (by mass)
S1	20	10	10:1
S2	25	10	10:1
S3	15	10	10:1
S4	15	10	15:1
S5	15	10	5:1
S6	15	7.5	10:1
S7	15	5	10:1

Particle morphology of the magnetic nanocomposites was examined using scanning electron microscopy (SEM) (Hitachi S900). Average particle size was estimated from the SEM images by measuring the diameter of 100 particles. Magnetite content was determined by thermogravimetric analysis (Hi-Res Modulated TGA 2950, TA Instruments). The heating rate was 20^oC/min and the samples were heated from room temperature to 800^oC under a constant flow of nitrogen. Magnetic characterization of the product was conducted by a vibrating sample magnetometer (Riken Denshi BHV-35H). All of the characterization was performed on dry samples.

III. RESULTS AND DISCUSSIONS

Precipitation of magnetic nanocomposites by the ASES was successful with spherical nanoparticles obtained from all the experimental conditions. The effect of polymer solution concentration was studied at 15^{o}C by using polymer concentrations between 5mg/mL and 10 mg/mL. An increase in particle size with increasing polymer concentration was observed. Particle diameter increased from 69.1 nm with polymer concentration of 5 mg/mL to 102.4nm at polymer concentration of 10 mg/mL. Particles obtained from solutions of lower concentration appear to be more discrete than those produced from more concentrated solutions. The increase in size and agglomeration could result from the higher viscosity of concentrated solutions. Increments in solution viscosity can delay jet development, resulting in larger droplet formation during atomization, hence less efficient solvent extraction.

No general trend was observed for the effect of increasing temperature at constant pressure and the increasing amount of SPIONs. As the ratio between polymer and SPION is relatively high, the SPIONs have a higher chance to be fully encapsulated within the polymer coating. Hence particle morphology is unaffected.

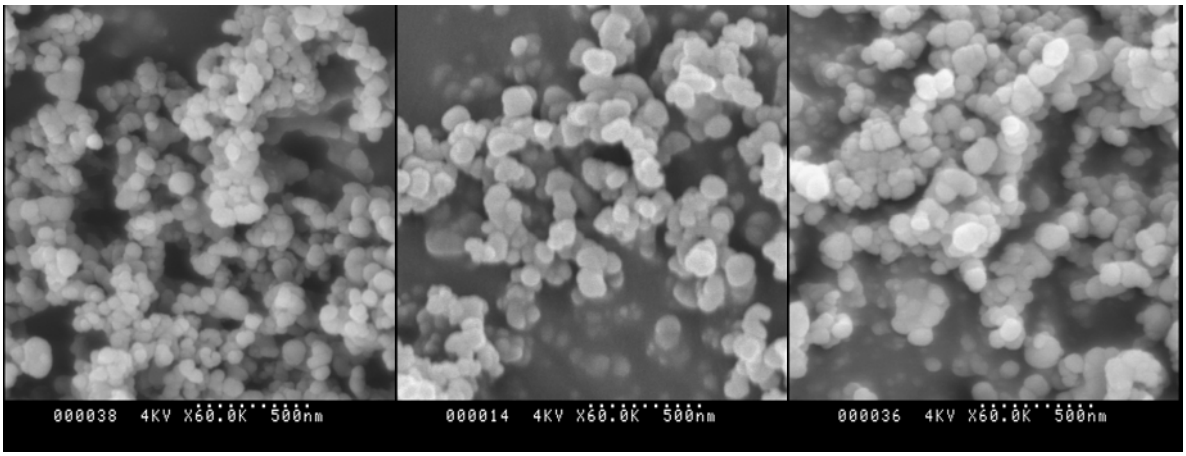

Figure 2 Eudragit-SPION nanocomposite produced by ASES from polymer solutions of (left) 5 mg/mL, (middle) 7.5 mg/mL, (right) 10 mg/mL. Experimental conditions: 160 bar, 15^{o}C, polymer: SPIONs mass ratio 10: 1.

Magnetite weight percentage obtained from TGA is determined by measuring the residual weight of the nanocomposites at 800^{o}C. Results are listed in Table 2. The final weight percentage correlated with the initial SPION loading very well. The final composition of the nanocomposite can be easily tailored by changing the initial SPION loading.

The magnetic response of the nanocomposites was characterized by measuring the magnetization curve in a magnetic field ranging from -10000 Oe to 10000 Oe. Saturation magnetization (Ms) is determined from the magnetization curve and is defined as the maximum induced magnetic moment after the saturation magnetic field is reached. The saturation magnetization is commonly expressed on a weight basis and is about 74-76 emu/g for bulk magnetite. As expected, the saturation magnetization of the coated magnetite would be diluted according to the weight percentage of magnetite presence. The magnetization can be normalized against the weight percentage of SPIONs in the composite.

The normalized curves for all the composites overlap with each other (Figure 3), which is an indication that there is no change in the magnetic susceptibility of the SPIONs after processing. Also, the lack of hysteresis for all the magnetization curves indicates that the superparamagnetic properties are persevered as there is no remnant magnetization at zero magnetic field.

Table 2 Summary of product characterization of the SPIONs/Eudragit nanocomposites.

Sample Code	Particle Size (nm) (*based on SEM)	Starting SPIONs loading (Wt %)	SPION (Wt %) (by TGA)	Recovery Yield (%)	Saturation Magnetization Ms (emu/g)
S1	138.3	9.1	10.9	77.8	8.2
S2	127.3	9.1	9.4	83.4	8.0
S3	102.4	9.1	11.5	68.3	7.7
S4	122.9	6.3	4.6	93.6	3.6
S5	127.1	16.7	19.0	82.8	14.4
S6	92.8	9.1	10.0	79.1	9.3
S7	69.1	9.1	9.9	74.2	7.7

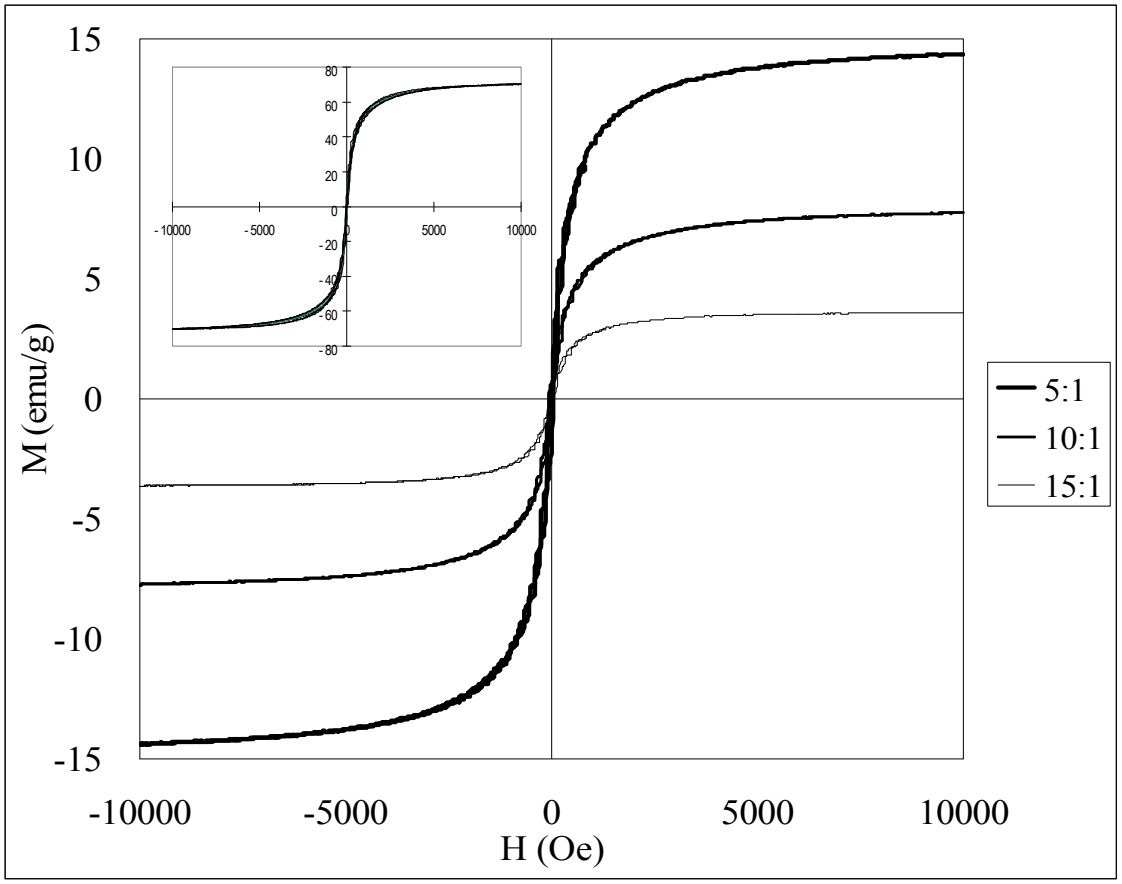

Figure 3 Magnetization curve of magnetic nanocomposites with different polymer:SPIONs mass ratio: 5:1; 10:1;15:1. Experimental conditions: 160 bar, 15^{o}C, polymer concentration 7.5 mg/mL, nozzle size 175 µm. The insert is the normalized magnetization based on the SPIONs weight percentage.

IV. CONCLUSIONS

Formation of pH and magnetically responsive nanocomposites of particle size less than 150 nm were successfully produced by the ASES process. The use of toxic solvent and surfactant was eliminated. Within the range of conditions investigated, there was no significant effect of the temperature and the SPIONs loading on the particle morphology. The superparamagnetic properties were preserved after the processing. The magnetic nanocomposites can be potentially employed as oral MRI contrast agents and as drug carrier for colon-specific drug delivery.

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