The synthesis and characterization of polymer coated iron oxide microspheres

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For biomedical applications drug carrying polymers are coated around magnetic iron oxide particles to form microspheres. In the present study, the iron oxide powder was ball milled. Microspheres were then synthesized by solvent evaporation, resulting in iron oxide particles encapsulated in a polymer and drug coating. Various parameters, such as the duration of milling and agitation speed as well as the polymer concentration were varied. A milling time of 72 h was found to yield a small size and narrow size distribution of particles; the average particle size was about 600 nm. Measurements of the change in grain size and the magnetic properties of the powder with milling time were performed. It was determined that the size of the microspheres was not sensitive to the initial particle size, but it could be decreased by variation of agitation speed or polymer concentration. The agitation speed and polymer concentration of 400 rpm and 0.04 g poly(l-lactic acid) in 8 g dicholoromethane, respectively, was found to yield small, spherical microspheres with a narrow size distribution. The surface morphology and magnetic properties of the microspheres was also analyzed.

Introduction

There are several scientific and clinical applications of magnetic microspheres. Such microspheres, sometimes called carriers, are magnet-polymer systems. Additionally, in the case of drug delivery, for example, a drug may also be dissolved in the polymer. The purpose of the polymer is to act as a solvent for a chemical, antibody, genetic material or drug that will participate in a useful *in vivo* or *in vitro* reaction. Such polymer–magnet coated composite particles are called microspheres. Applications for such microspheres include cell separation and analysis, molecular biology, rapid detection of bacteria, drug delivery, radionuclide therapy, magnetic resonance imaging (MRI) contrast agents, and hyperthermia [1–9]. The magnetic material used in microspheres is often iron oxide powder that consists of individual particles of magnetic iron oxide. A polymer matrix is used to encapsulate the iron oxide particles.

The present method of synthesis of such microspheres requires elaborate procedures [10–17]. The current methods of making such microspheres are mostly based on chemical synthesis methods following the pioneering work of Ugelstad *et al*. [18–20]. In this method, the microspheres are produced from monosized macroporous polymer spheres and which are magnetized by an *in situ* formation of ferrimagnetic material inside the pores, thus producing the required magnetizable microspheres. However, this method has limitations, for example there is no choice in the type of magnetic particle and it is not easy to vary the magnetic properties of the microspheres.

On the other hand, Rudge *et al*. [21] proposed an alternative method of making a magnetically targeted carrier for liver cancer treatment. Ball milling of iron powder with activated carbon was used to produce carbon-coated iron particles, these particles were then coated with anti cancer drugs. We decided to combine the advantages of the above two methods: by selecting ball milling we have a wide choice of magnetic systems and magnetic properties to choose from; instead of iron used by Rudge *et al*. [21], we use iron oxide which is biodegradable and the magnetic properties were varied by changing the process parameters during ball milling. These changes in parameters will result in a change in size and morphology of the magnetic particles. The process parameters to obtain particles and microspheres with a small average size, spherical morphology and a narrow size distribution were studied. Instead of activated carbon we use polymers as done previously by Ugelstad, but we use solvent evaporation to prepare the microspheres.

The first variable is the size of the magnetic particle. For *in vivo* applications, the magnetic particle will not biodegrade readily, hence we should use the minimum size, this minimum size and the process parameters required to produce this size are reported. The second set of variables is the size, size distribution and morphology of the microspheres. The size should be as small for most

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applications; for a given volume of microspheres, a higher surface area can obtained for the desired reactions. A narrow size distribution and uniform morphology is preferred since it makes the rheological and mechanical properties of the microsphere more uniform and thus easier to control.

The magnetic material chosen is iron oxide because it is ferrimagnetic, readily available, chemically stable, nontoxic and noncarcinogenic. Poly(L-lactic acid) (PLLA) was used to encapsulate the particle because it is biodegradable and dissolves easily in a number of organic solvents. Dichloromethane (DCM) was used as a solvent for PLLA. The drug theophylline (anhydrous 1,3-dimethylxanthine) was incorporated into the polymer coating. Theophylline is one of the most commonly used medications for the treatment of the symptoms of chronic asthma; in this case it is simply used as a model drug to study the processing techniques.

First, ball milling of iron oxide particle was performed to study the effect of milling time on the size of iron oxide particle. Then, iron oxide was encapsulated within the biodegradable polymer and drug coating. The average size, morphology, size distribution of the particle and the microspheres for different process parameters was determined by X-ray diffraction, scanning elecron microscopy (SEM) and the magnetic properties were evaluated by VSM.

Experimental procedure

The experimental procedure can be divided into three sections: mechanical milling of iron oxide particle, encapsulation of iron oxide, and process parameters to alter the size of the microspheres.

Ball milling

The iron oxide powder (Aldrich) is a combination of Fe_2O_3 and Fe_3O_4, with an average particle size of less than 5 μm. Fe_2O_3 is paramagnetic, Fe_3O_4 imparts bulk ferrimagnetic properties [5]. Particles were prepared using high energy ball milling using a planetary mill (Fritsch pulverisette 5). The powder was contained in two stainless steel vials along with 50 stainless steel balls measuring 9.7 mm in diameter. The ball-to-powder weight ratio was 10 : 1. Milling was carried out at a speed of 300 rpm for milling times of 1, 5, 10, 20, 50, 72 and 120 h.

Encapsulation

For the polymer coating around the particle the following procedure was followed: DCM of technical grade (BestChem) was used as a solvent for PLLA. The drug theophylline was purchased from Sigma. A nonsolvent of 0.29% aqueous poly(vinyl alcohol) (PVA) was used to disperse individual polymer/particle microspheres. Iron oxide, which was mechanically milled for 72 h in the previous procedure, was encapsulated in the polymer.

PLLA weighing 0.1 g was dissolved in 8 g of DCM. 25 wt % of iron oxide powder and 0.1 wt % of theophylline was added to the PLLA solution. To homogenize the material, the suspension was put into an ultrasonic bath for 30 min. The suspension was poured into a nonsolvent, 100 ml of PVA, at a speed of 300 rpm and the resulting emulsion was stirred for 90 min. An overhead agitator was used to agitate the emulsion. The magnetic polymer spheres were rinsed with deionized water, dried and stored.

Process parameters

In the first set of experiments, the rotation speed of the agitator was varied. Mechanically milled (72 h milling time) iron oxide, 0.1 g/8 g PLLA/DCM, the concentration of theophylline (0.1 wt %), PVA conc. (0.29 wt %) were fixed in this experiment. The iron oxide powder milled for 72 h was selected, as it was determined to have the smallest mean size. The rotation speed was varied between 300 and 500 rpm.

The viscosity of the PLLA solution has a large impact on the size of the microspheres, less concentrated solutions with lower viscosity resulted in smaller spheres [5]. Hence the concentration of PLLA was varied, with the stirring speed constant at 400 rpm. The above procedure was repeated using iron oxide mechanically milled for 72 h, the mass of PLLA was varied as follows: 0.05, 0.04, 0.02 and 0.01 g of PLLA, each in 8 g of DCM.

Characterization of the powders and microspheres was performed using the SEM, vibrating sample magnetometer (VSM), X-ray diffractometer (XRD) and optical microscope.

Results

Mechanical milling of iron oxide powder

Reduction of size

The size of the iron oxide particle, milled at different times, was measured (Fig. 1). It was observed that the as received particle was irregular and nonuniform in shape. As the milling proceeded, the particle size reduced and became more equiaxed in shape. More than 100 particles from each sample were measured and their mean size calculated. The result is shown in Table I.

A graph of milling time vs. mean particle size was plotted (Fig. 2) which shows that as milling time increased from 0 to 72 h, the size of the particle decreased significantly. However, as the milling time further increased to 120 h, the size of iron oxide particle increases slightly. It was also observed that there was an overall decrease in standard deviation of size and the coefficient of variation with increase in milling duration.

The superimposed size distribution was plotted (Fig. 3). As the milling duration increased, there was a narrowing of the size distribution curves. There was a shift of curves to the left as the mean size gets smaller with longer milling time. Iron oxide powder milled for 72 h gave the smallest mean size with the narrowest distribution of size, therefore this sample was used for the encapsulation experiments.

Grain size

Samples of iron oxide powder were analyzed by using XRD to estimate their grain size. The Scherrer formula

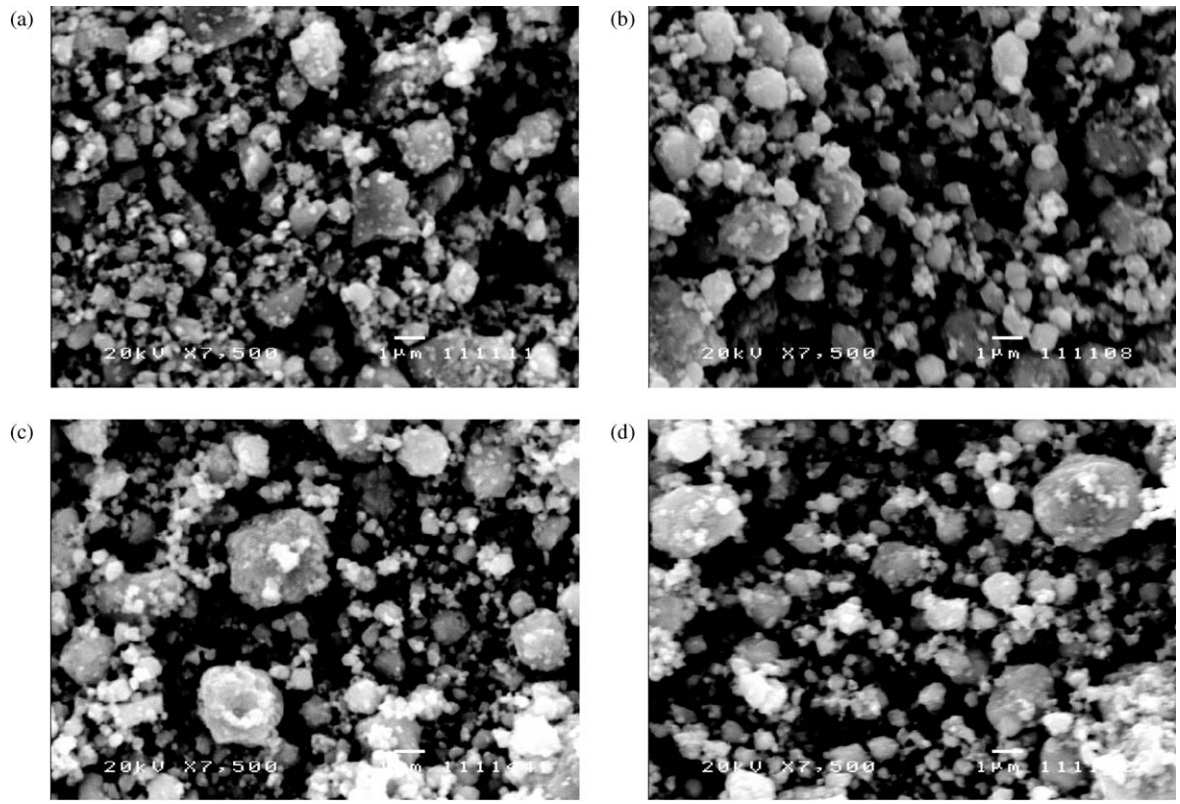

Figure 1 SEM micrographs of iron oxide particles after mechanically milling for (a) 1 h, (b) 20 h, (c) 72 h, (d) 120 h.

below can be used to estimate the grain size from the measured width of the diffraction curves. According to this formula, grain size and diffraction width are inversely proportional. The combined diffraction curves are shown in Fig. 4. It was observed that generally, as the milling time increased, the diffraction peaks was broader. This broadening of peaks suggested a decrease in grain size as milling time increased. Table II shows the relative peak broadening, from which it can be seen that the value of B $(= 0.5[\theta_2 - \theta_1])$ increases with milling time. The grain size was not calculated using the Scherrer's formula because the quantitative determination requires accurate determination of background correction and correction for lattice strains [22, 23].

Magnetic properties

The powders were studied using a VSM. The result of the effect of milling time on the magnetic properties are shown in Table III. Broadly, the saturation magnetization decreased with increase in milling time.

Polymer coating on the iron oxide particle

Effect of particle size on the size of microspheres

After the process of polymer encapsulation of iron oxide particle for the ball-milled samples, the magnetic polymeric microspheres were observed by the SEM for size determination and surface morphology (Fig. 5). The microspheres were spherical and uniform in shape with a narrow size distribution. More than 100 particles from each sample were measured (Table IV). The size of the microspheres was found to be independent of the initial iron oxide particle size (Fig. 6). Thus, to reduce the size of the microspheres, we considered other factors such as increasing the speed of the agitator, or reducing the viscosity of the polymer solution by lowering its concentration.

Fig. 7 can be used to analyze the surface morphology of the microspheres. The surface has round holes and iron oxide particle. There were very few particles on the surface, most of it was encapsulated in the core of the microsphere.

TABLE I Change in particle size with milling time

Milling time (hr)	Mean size (μm)	Std dev	Coeff. variation
0	2.4	1.4	0.6
1	1.7	1.3	0.8
5	1.5	1.4	0.9
10	1.4	0.7	0.5
20	0.8	0.4	0.5
50	0.8	0.5	0.6
72	0.6	0.3	0.5
120	0.7	0.3	0.4

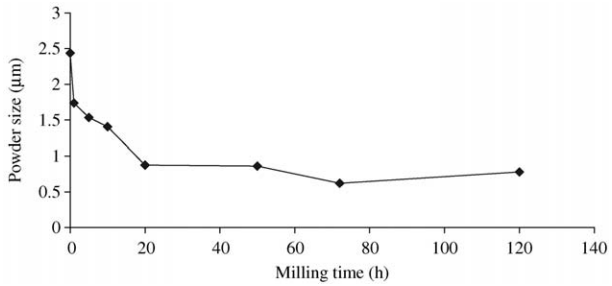

Figure 2 Graph of milling duration vs. particle size.

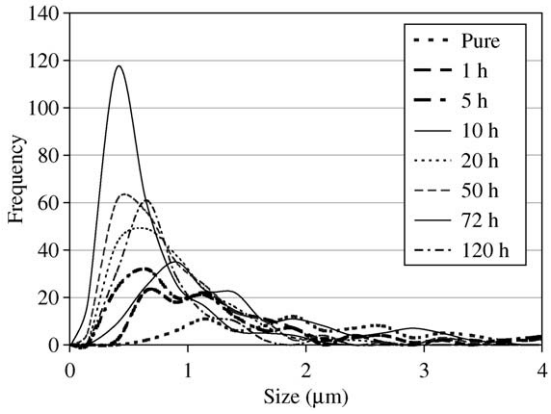

Figure 3 Size distribution of all iron oxide samples.

Effect on magnetic properties

The magnetic properties of iron oxide were expected to change after polymer encapsulation. The magnetic properties of the microspheres and the powders were determined. These results show that the coercivity of the uncoated powders was 301 Oer, while the magnetization was 34.2 emu/g. After coating, the coercivity increased to 325 Oer, while the magnetization decreased to 15.0 emu/g with the addition of the polymer coating.

Variation in parameters

Variation in speed of agitator

The size of the microspheres was expected to change with the increase in the speed of the agitator from 300 to 500 rpm, keeping the initial powder size and the concentration of polymer constant (Table V). These results were also compared to those prepared with a speed of 300 rpm. By increasing the agitation speed, there was a significant decrease in the size of the microspheres. The standard deviation of the size distribution decreased dramatically with the increase in speed. Fig. 8 shows the SEM micrograph. It was

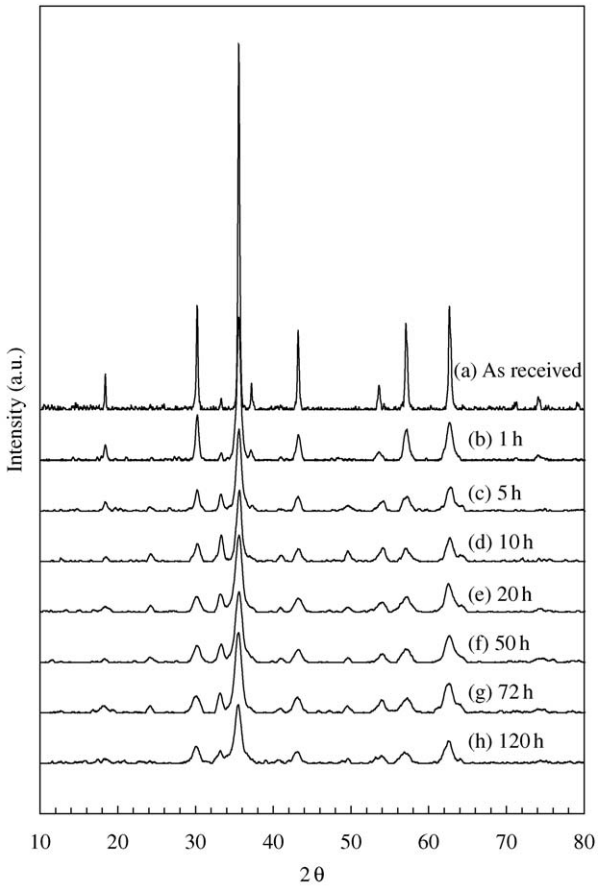

Figure 4 Combined diffraction curves for as milled iron oxide samples.

observed that although a speed of 500 rpm generated much smaller microspheres, the particles lost their spherical shape. The surface was much rougher than those generated using an agitation speed of 400 rpm. This is the reason for the selection of 400 rpm as the speed of agitation for the procedure of reduction of size through changing polymer concentration.

Effect of polymer concentration

The sizes of microspheres synthesized from using different polymer concentration were measured. The results were tabulated (Table VI), and compared with the original concentration of 0.1 g/8 g of PLLA/DCM.

Fig. 9 shows that the size of the microspheres decreases with decreasing quantity of PLLA until a mass of 0.04 g, below which there is very little further reduction in size. Besides a decrease in size, the standard

TABLE II Analysis of X-ray diffraction curves ($\lambda_{Cu} = 1.5418$Å)

Milling time (h)	Intensity (CPS)		2θ			cosθ	B
	Max	Half	2θ	$2\theta_1$	$2\theta_2$		
0	722	361	35.55	35.70	35.45	0.9523	0.12
1	283	141.5	35.55	35.85	35.30	0.9523	0.27
5	161	80.5	35.60	35.95	35.20	0.9521	0.37
10	141	70.5	35.65	36.00	35.20	0.9520	0.4
20	152	76	35.60	36.05	35.05	0.9521	0.5
50	140	70	35.60	36.10	35.05	0.9521	0.52
72	159	79.5	35.55	36.00	35.00	0.9523	0.5
120	116	58	35.50	36.00	35.00	0.9524	0.5

TABLE III Magnetic properties of milled iron oxide for various duration

Milling time (h)	Magnetization (emu/g)
0	50.0
5	42.5
10	48.3
20	47.2
50	40.4
120	34.2

deviation decreases as well, thus a narrower size distribution could be obtained.

Fig. 10 shows that as the size of the microsphere gets smaller, more iron oxide particle was deposited on the surface of the spheres. When PLLA concentration decreased to 0.02 g for 8 g DCM, the microspheres were no longer spherical. Fig. 10(b) shows that very little polymer can be seen on the surface, and that the surface was coated with iron oxide particles.

Discussion

Mechanical milling

Powder size analysis

In mechanical milling, collision between the stainless steel balls and the iron oxide particles occurs. Kinetic energy from the balls was imparted to the powder, iron oxide being brittle fractures into smaller pieces; hence the size of iron oxide powder decreases with increasing duration of milling. For the increase in size when iron oxide was milled further to 120 h, it has been suggested that the powder has reached a limit of comminution [15]. This is the limit where further fracturing stops due to the extremely small particles deforming plastically rather than fracture. In addition, as the particle size decreases, surface energy increases, therefore the particles agglomerate, lowering their energy to increase stability.

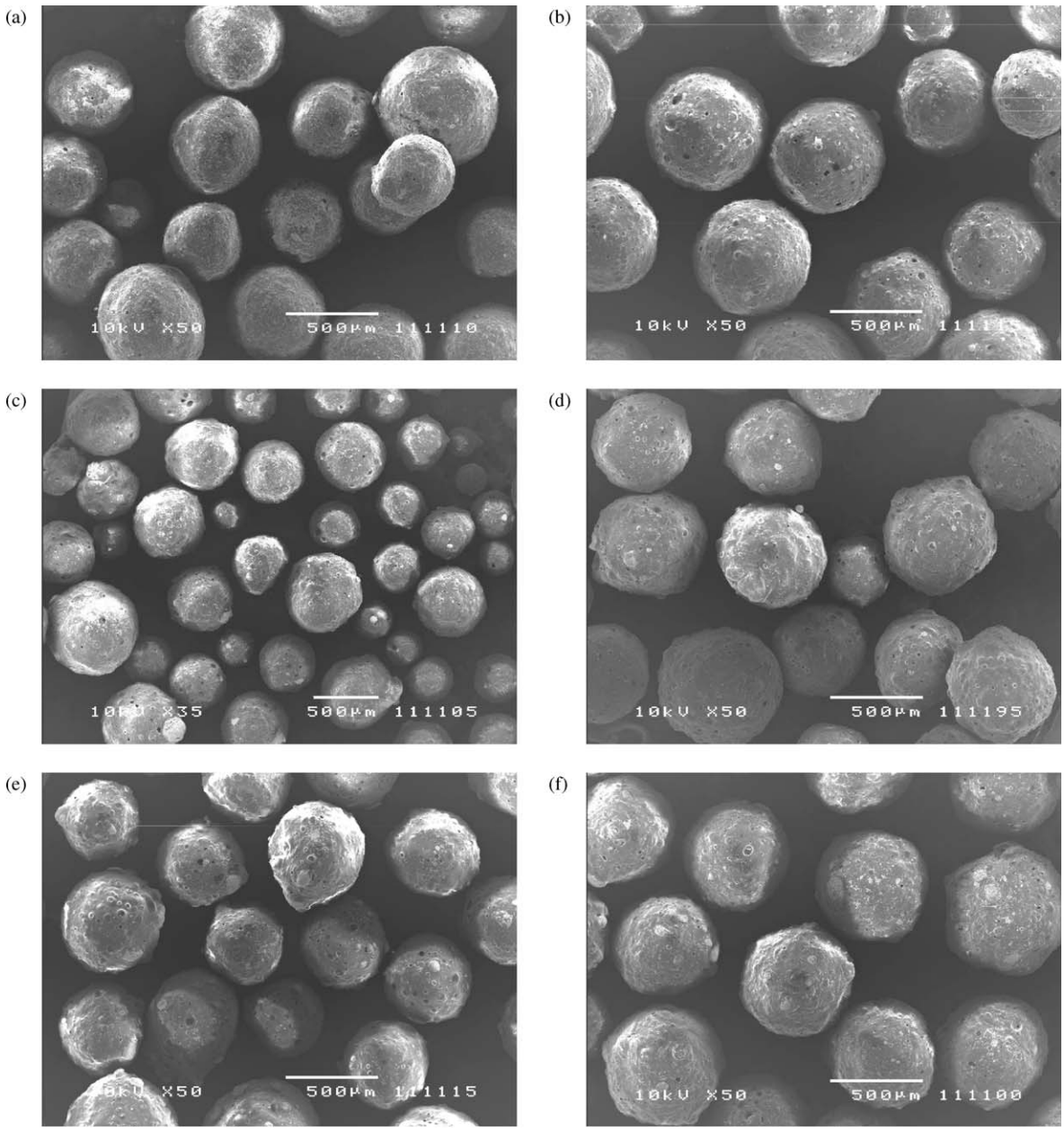

Figure 5 SEM micrographs of microspheres of different milled iron oxide of time (a) 0 hr, (b) 5 h, (c) 20 h, (d) 50 h, (e) 72 h, (f) 120 h.

TABLE IV Results of sizes of microspheres

Milling time (h)	After polymer coating (μm) Average	Std Dev	Coeff. variation
0	511	88.5	0.17
1	557	80.4	0.14
5	521	113.9	0.21
10	467	99.8	0.21
20	496	128.7	0.25
50	500	116.0	0.23
72	472	89.2	0.18
120	546	106.2	0.19

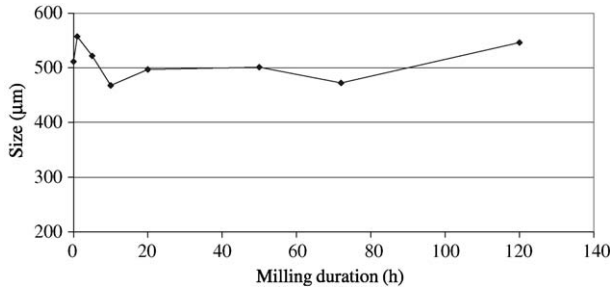

Figure 6 Effect of milling time of initial magnetite on final size of microsphere.

Grain size

It was observed that with milling the peaks were broadened suggesting that a large amount of defects were introduced into the samples by mechanical attrition. The broadening of the experimental diffraction curves was caused by the small size of the diffracting grains, the lattice strain and the instrumental broadening. Grain size decreased with mechanical milling for the first 20 h, due to the increase in the number of defects within the particle. Beyond 20 h, the reduction in grain size appeared to stop. This is perhaps because as more grain boundaries were created, there is strengthening of the material. This result of grain size reduction with milling duration until saturation is similar to those of Zhao [17]. Although the material used by Zhao was iron, he found that a steady-state grain size can be reached when the powders were milled for a certain period of time. An extension of milling time did not decrease the grain size further.

Polymer coating

Size of microspheres and surface morphology

The size of the microspheres was independent of the average size of iron oxide powder. This is because the microsphere does not consist of only one iron oxide particle in it; a bigger microsphere would just have a higher volume of iron oxide particle embedded in it. The average size of the microspheres was about 509 μm. Hertzog obtained microspheres of size 160 μm, the smaller size could be due to difference of stirring speed, which was not stated in the paper [5]. There were small patches of particles randomly distributed on the surface of the microspheres, along with holes. Since iron oxide was not soluble in DCM or PLLA, the dry particles form small ''bubbles'' in the viscous solution, as shown in Fig. 11. If these bubbles migrate to the surface of the sphere, they will burst, exposing a small quantity of iron oxide.

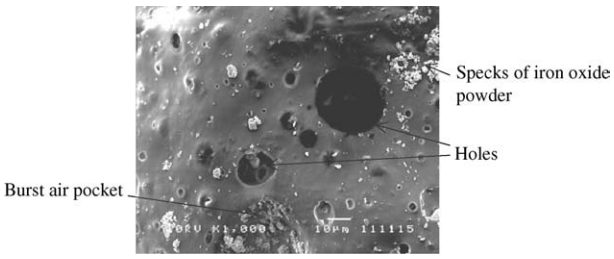

Figure 7 SEM micrograph of the surface of a microsphere.

Magnetic properties after polymer coating

PLLA is not a magnetic material, and by encapsulating the magnetic iron oxide, it acts as a barrier to magnetization, hence the magnetization of the microspheres is smaller than the uncoated iron oxide.

Variation in parameters

Variation in agitation speed

With an increase in agitation speed, more eddies were created in the emulsion, and as a result the microspheres broke up into smaller particles. However, if the speed was too high, the polymer failed to forms spherical particles as the volatile methyl chloride may have evaporated before the microspheres can be fully formed. As a result, small but distorted microspheres were formed at a agitation speed of 500 rpm.

Variation in PLLA concentration

A lower PLLA concentration results in a solution of lower viscosity. Therefore, the polymer is able to flow more easily and is not so resistant to breaking up when the emulsion is agitated. In addition, as there was a smaller amount of polymer in the emulsion to coat on each microsphere, the thickness of the polymer coating would be decreased. Hence the average size and standard deviation of microspheres decreased with polymer concentration.

As the microsphere size was reduced, more patches of iron oxide particles were seen on the surface. This could be because the air ''bubbles'' were relatively larger now in a smaller microsphere, thus the same amount of powder carried by them would now cover a larger surface area over the smaller microsphere's surface. In addition, the microspheres were found to be less spherical when the polymer concentration decreases.

TABLE V Size analysis of the reduction in microspheres due to change in speed

Speed (rpm)	Mean size (μm)	Std dev	Coeff. variation
300	546	106	0.19
400	397	52	0.13
500	58	10	0.17

Conclusions

The synthesis of the drug-polymer-iron oxide system for biomedical applications was studied. A magnet–polymer composite microsphere was prepared using ball milling of the powder followed by the solvent evaporation technique.

1. The iron oxide powder was produced through mechanical milling. It was determined that a milling time of 72 h produced the smallest particle size of around 0.6 μm with the narrowest size distribution.
2. In the solvent evaporation experiment, it was found that the size of the microspheres was independent of the initial size of the iron oxide particle. Instead, the size-determining parameters were found to be the agitation speed and the polymer concentration. Optimum parameter for the agitation speed was established to be 400 rpm, where the size of microspheres is approximately 400 μm. A further increase of agitation speed to 500 rpm resulted in a loss of spherical shape and a decrease of size to 58 μm.
3. By variation of polymer concentration, the size of

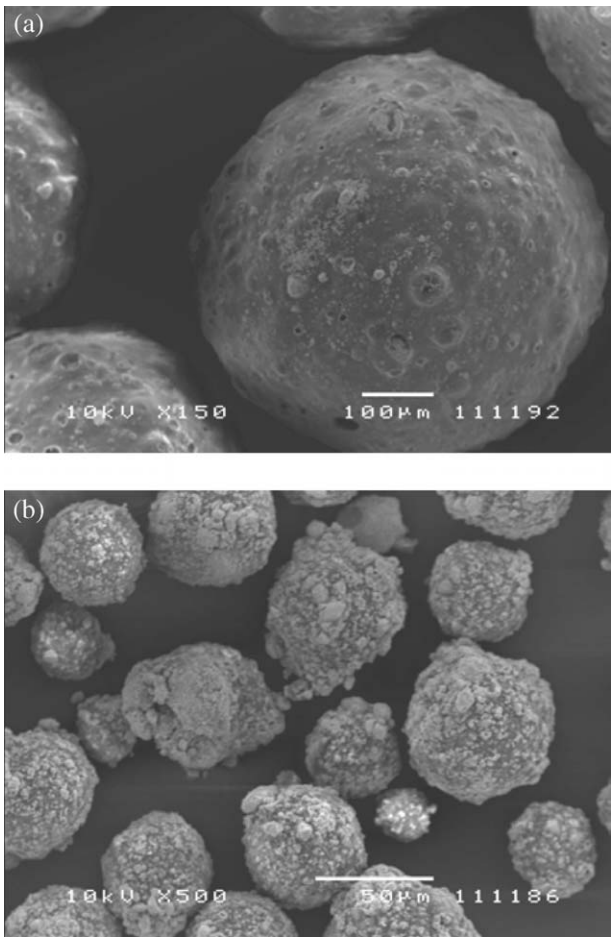

Figure 8 SEM micrographs of microspheres generated from speed of (a) 400 rpm and (b) 500 rpm.

TABLE VI Size analysis of microspheres with respect to changes in polymer concentration

Concentration PLLA/8 g DCM	Mean size (μm)	Std dev	Coeff. variation
0.10	397	52	0.13
0.05	171	30	0.17
0.04	68	8	0.11
0.02	57	10	0.17
0.01	52	10	0.19

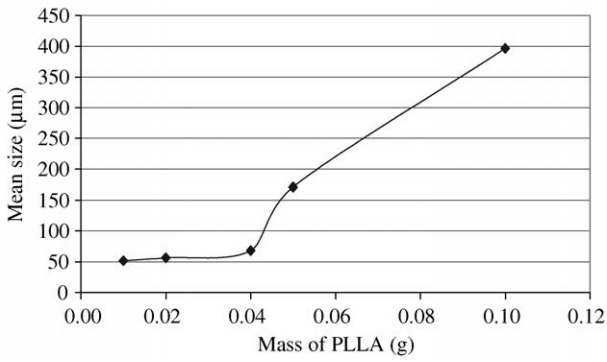

Figure 9 Effect of polymer concentration on microsphere size.

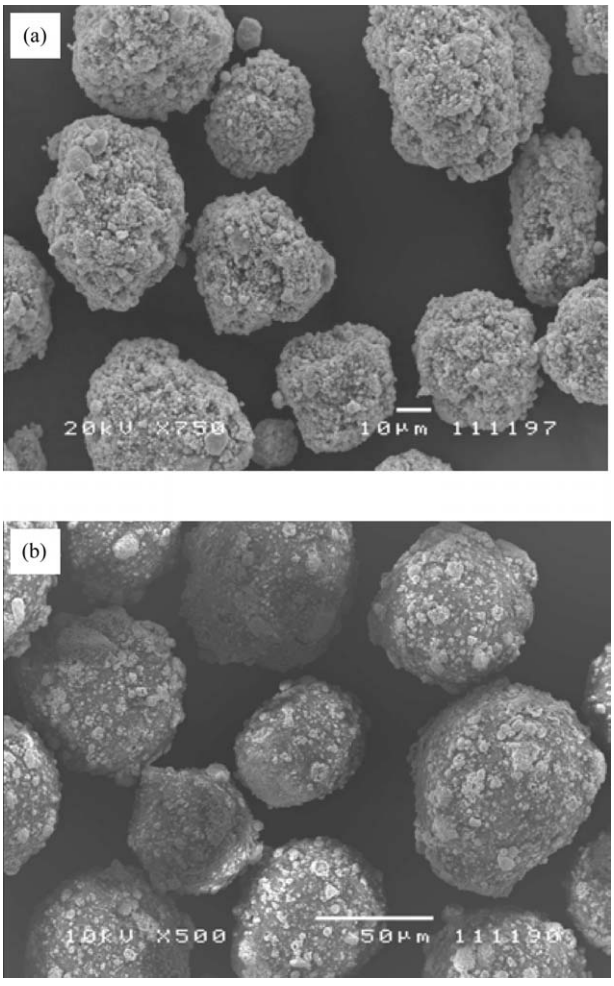

Figure 10 SEM micrographs of microspheres of PLL weighing (a) 0.04 g (b) 0.01 g in 8 g of DCM.

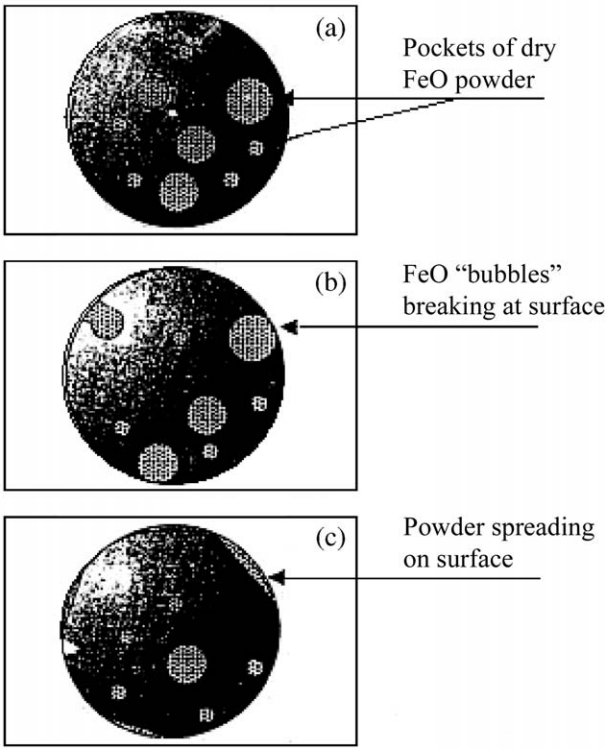

Figure 11 Explanation of why holes and small patches of particle were found on microsphere's surface.

microsphere was found to decrease with polymer concentration, up to 0.04 g of PLLA in 8 g of DCM, which formed microspheres of around 55 μm. Although smaller sized microspheres was achievable with further decrease in polymer concentration, the particles were no longer spherical, and there was considerable iron oxide on the surface.

4. Magnetization decreased after the polymer encapsulation of iron oxide particle compared with the uncoated particle. In addition, magnetization decreases with increasing thickness of the polymer coating.

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