

Encapsulation of magnetic nanoparticles in a pH-sensitive poly(4-vinylpyridine) polymer: a step forward to a multi-responsive system

Ana Arizaga*, Gemma Ibarz, Rafael Piñol and Ainhoa Urtizberea

Departamento de Física de la Materia Condensada, Instituto de Ciencia de Materiales de Aragón, CSIC – Universidad de Zaragoza, Pedro Cerbuna 12, Zaragoza 50009, Spain

A multiresponsive system that consists on pH-responsive polymer microspheres with encapsulated iron oxide magnetic nanoparticles that rendered the core magnetic to enable externally controlled actuation under magnetic induction has been developed. The inorganic nanoparticles were first prepared and, then, further encapsulated in a pH-sensitive poly(4-vinylpyridine). These spheres have been obtained by a modification of the simple, rapid and high-reproducible nanoprecipitation method. Magnetic measurements showed that the iron oxide nanoparticles are superparamagnetic and, therefore, able to undergo a local increase of the temperature when an oscillating magnetic field is applied. Magnetically triggered heating and pH sensitivity can be useful for biomedical applications.

1. Introduction

Encapsulation of magnetic nanoparticles in polymers has been a promising approach to improve the applicability of these inorganic particles in biomedical applications [1]. The systems may have magnetic cores with polymer or inorganic coating which can be functionalised, or may consist of porous polymers that contain magnetic nanoparticles precipitated within the pores [2–4].

Magnetic nanoparticles offer exciting new opportunities to induce remote effects, since it is possible to use their properties to absorb energy and convert it into heat. The use of superparamagnetic nanoparticles for hyperthermia purposes was first introduced by Jordan et al. [5]. From this study, it was proven that a superparamagnetic suspension can absorb the energy of an alternating magnetic field with the high efficiency and convert it into heat. This property can be used to increase locally the temperature and enhance the polymer mobility inducing the release of an encapsulated drug.

The amount of heat released by magnetic nanoparticles under the influence of an alternating magnetic field depends on their shape, magnetic anisotropy, average size and size dispersion. It is known that monodisperse nanoparticles provide the optimum values [6]. We therefore used the organic thermal decomposition method, which permits obtaining highly crystalline and monodisperse nanoparticles with good size control [7]. However, these nanoparticles should be encapsulated into a hydrophilic matrix to achieve high stability in aqueous suspensions in order to open the possibility of their use to biomedical applications. Some of the organic solvents that we employed in this work are non-biocompatible, so this is a first proof of concept in order to achieve a more biocompatible system.

Recently, several groups have achieved the encapsulation of oleic acid-coated magnetite nanoparticles into spheres of polymers such as poly-epsilon-caprolactone, poly(lactide) and poly(D,L-lactide-co-glycolide) and methoxy poly(ethylene-glycol) poly(lactide) copolymer by means of nanoprecipitation method [8–11]. The group of Liu demonstrated that polymer shells consisting of poly(ethyleneoxide)–poly(propylene-oxide)–poly(ethylene-oxide) block copolymers and containing iron oxide nanoparticles that were synthesised by internal precipitation could be magnetically triggered showing a burst-like release of the model drug Vitamin B12 [12]. Besides, it is interesting to obtain and characterise a polymer system able to combine pH and thermomagnetic response with the aim of using these properties to selectively tune the release of any drug encapsulated inside.

It is well known that poly(4-vinylpyridine) (P4VP) is a pH dependent polymer with a critical transition pH value at 4.7 [13,14]. The variation of pH modifies the number of charged groups inside the polymer structure producing changes in the hydrogel particle size because of the charge repulsion. The synthesis of polymer P4VP hydrogel spheres has been accomplished usually using monomer polymerisation or cross-linking [15]. In a recent work, we presented a method in which the fabrication consists on solvent exchange method or nanoprecipitation without help of a cross-linker [16]. The P4VP spheres can change their volume as a function of the pH and ionic strength. The packing density of the formed nanoparticles is much lower than in case of a monomer polymerisation or cross-linking. Moreover, the synthesis without cross-linking permits also a high swelling of the systems.

In this article, we introduce the synthesis and characterisation of pH responsive P4VP polymer microspheres and the encapsulation of magnetic nanoparticles that could give rise to multifunctional systems. Indeed this system is able to show pH sensitivity and magnetic heating properties. The synthesis is carried out without help of cross-linking or monomer polymerisation but using a very simple modified nanoprecipitation technique.

2. Materials and methods

2.1. Materials

1,2-Dichlorobenzene (DCB) (99%) was supplied by Panreac. Oleic acid (pure) was purchased from Fluka and iron pentacarbonyl (pure), polyvinylalcohol (PVA) [87–89%, hydrolysed and average Mw 13000–23000], trimethylamine *N*-oxide dihydrate, Copper dichloride (99.999%), methyl 2-chloropropionate (97%) were all products of Sigma Aldrich, Madrid, España. Absolute Ethanol (reagent grade), dichloromethane (DCM) (extrapure) and methanol (min 99.8%) were purchased from Scharlau, Barcelona, España. 4-Vinylpyridine (4VP) (Sigma Aldrich, 95%) was distilled under vacuum and stored at 5°C. Copper chloride (Sigma Aldrich) was purified by stirring in acetic acid for several hours, then filtrating, washing with acetic acid, ethanol and diethyl ether successively and stored under vacuum. *Tris*[(2-pyridyl) methyl] amine (TPMA) was prepared by means of previously reported procedures [17]. Iron oxide nanoparticles used in this study were synthesised according to the published literature [7].

2.2. Synthesis of P4VP

We used Atomic Transfer Radical Polymerisation (ATRP) as polymer synthesis route to obtain narrow molecular weight polymers with established lengths and low-polydispersity values [18]. A 50 mL Schlenk flask was charged with Cu(I)Cl (51.41 mg), Cu(II)Cl (29.90 mg) and TPMA (215.20 mg) under Argon atmosphere. Six millilitres of deoxygenated 2-propanol was introduced via syringe.

After stirring, 6 mL of deoxygenated monomer 4VP (6 mL, 55.64 mmol) was added. Immediately, the mixture was frozen in liquid nitrogen and degassed. The flask was immersed in an oil bath thermostated at 40°C. After 10 min deoxygenated methyl 2-chloropropionate (0.0825 mL, 0.743 mmol) was introduced into the flask. After 7 h of reaction the mixture was cooled to room temperature. The dark green solution was diluted with 2-propanol and poured into diethyl ether. The green precipitated polymer was dissolved in DCM, 2 g of Dowex[®]50WX2-100 ion-exchange resin was added and the mixture was stirred for 90 min. Then the mixture was filtered to remove the resin beads. The resulting polymer solution was washed with water, brine, dried over anhydrous magnesium sulphate and concentrated under vacuum. The polymer was dissolved in a small amount of DCM and precipitated into a large volume of cold diethyl ether. The purified polymer was collected by filtration and dried under vacuum at 50°C for 3 days.

2.3. *Obtention of blank P4VP spheres*

We used a nanoprecipitation method without cross-linking to obtain P4VP nanospheres, because it is well known that the swelling ratio of the spheres decreases as the number of cross-links increases [19,20]. One advantage of this system is that under certain conditions the non-crosslinked polymer can lose its spherical shape and disperse. Following this procedure, we expect to obtain the maximum release when a substance is encapsulated inside the polymer system [16].

In order to obtain the polymer spheres 0.05 g of P4VP was dissolved in a solution of 1 mL of DCM with 1 mL of methanol. This organic solution was then poured under magnetic stirring into a polar phase consisting of 16 mL ethanol: water in proportion (1 : 3) for 20 min. One percent PVA was used as the stabilising and emulsifying agent. A white and stable suspension is obtained.

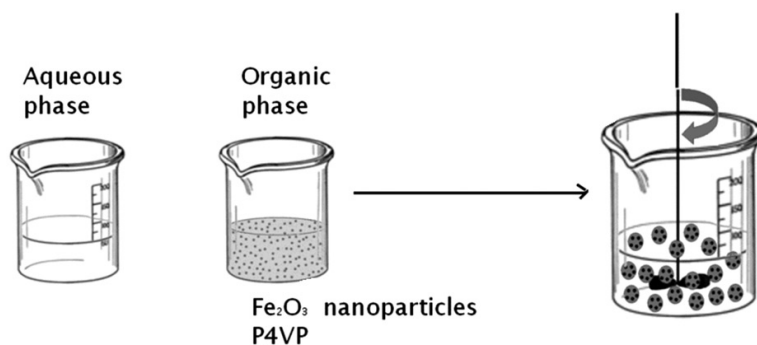
2.4. *Preparation of oleic acid coated magnetic nanoparticles*

The synthesis of highly crystalline and monodisperse maghemite nanoparticles was carried out in organic medium by means of the Hyeon method [7]. This procedure, which allows varying particle size by controlling the experimental parameters, is based on the thermal decomposition of iron pentacarbonyl in the presence of oleic acid. The resulting iron nanoparticles were transformed to monodisperse maghemite nanoparticles by controlled oxidation using trimethylamine oxide as a mild oxidant.

In a typical synthesis for 8 nm iron oxide nanoparticles, a reaction vessel containing 40 mL of 1,2-DCB and 2.2 g of oleic acid was heated under an argon flush to 100°C. An amount of 0.8 mL of Fe (CO)₅ solution was then rapidly injected through a septum into the hot vessel containing the DCB solvent and the oleic acid. This solution was then heated at 180°C for 48 h and vigorously stirred. The nanoparticle dispersion, or ferrofluid, was cooled to room temperature, then 1.36 g of trimethylammonium oxide was added and the temperature was first raised to 130°C during 2 h and then to reflux during 3 h. A dark brown stable solution is obtained.

2.5. *Encapsulation of magnetic nanoparticles into P4VP spheres*

Magnetic nanoparticles have been encapsulated in polyvinylpyridine (PVP) by a variation of the nanoprecipitation procedure. The encapsulation method involved the formation of an oil-in-water emulsion consisting of an organic phase including the polymer and the magnetic nanoparticles and an aqueous phase containing the surfactant. To obtain the polymer spheres with magnetic nanoparticles two solutions were prepared. The aqueous one consists of a solution of the surfactant, PVA, in a mixture of water and ethanol, and the organic phase was prepared by dispersing the ferrofluid and the P4VP in DCM and methanol.



Scheme 1. Synthesis scheme of the encapsulation procedure.

When the organic phase containing the magnetic nanoparticles was poured under moderate stirring to the aqueous phase, the spheres were formed entrapping the nanoparticles inside (Scheme 1).

A typical encapsulation procedure is described as follows. The aqueous phase was prepared dissolving 0.015 g of PVA in 12 mL of hot water. When the solution was cooled, 4 mL of ethanol was added and all was poured into a beaker. The organic phase was prepared dispersing one drop of as-prepared magnetic ferrofluid in 1 mL of DCM, then 1 mL of methanol was added and 0.05 g of PVP was dissolved. The organic phase was then added into the aqueous one under magnetic stirring at 800 rpm for 20 min. A light beige stable suspension is obtained.

2.6. Characterisation

TEM images were recorded on a JEOL JEM-200CX transmission electron microscope at an acceleration voltage of 120 kV, for sample preparation one drop of the sample is placed in a copper-carbon grid and dried in air. SEM was performed with a JEOL JSM 6400 scanning electron microscope. The images were taken at a voltage of 40 kV. Sample preparation was performed by drying a drop of as prepared sample directly at the grid surface. Dynamic light scattering (DLS) results were obtained from a Zetasizer NanoZS ZEN3600 from Malvern Instruments. The crystallite phase was identified by recording XRD of the sample using a Philips PW 1830 diffractometer. The magnetic nanoparticles were washed with acetone by means of magnetic separation for three times and then dried in air. The magnetic properties of a ferrofluid sample that consists of the nanoparticles dispersed in DCB were studied by using a commercial SQUID magnetometer (MPMS, Quantum Design). The original sample was diluted down to a concentration of 1 mg $\text{Fe}_2\text{O}_3/\text{mL}$ in which the influence of the magnetic interaction between the particles can be neglected and then the ferrofluid was poured into a gelatine capsule sealed with vacuum grease.

3. Results and discussion

Blank polymer nanospheres were synthesised with a content of 1% in PVA. In order to study their shape and size, we used the DLS technique because scanning electron microscopy (SEM) or transmission electron microscopy (TEM) provide a nonrealistic image due to the dry process. The polymer spheres can change their shape and size during this process owing to the irregular water content lost. To probe the hydrogel dependence with the pH, two P4VP spheres dispersions of the same 1% PVA sample were measured by DLS, one at pH 4.1 and the other at pH 6.8. As it can be observed at Figure 1 the diameters of the nanospheres measured by means of DLS, increased by a factor three from an average diameter of 230 nm at pH 6.8 until 660 nm at pH 4.1, so the pH dependence of the polymer spheres sizes was probed.

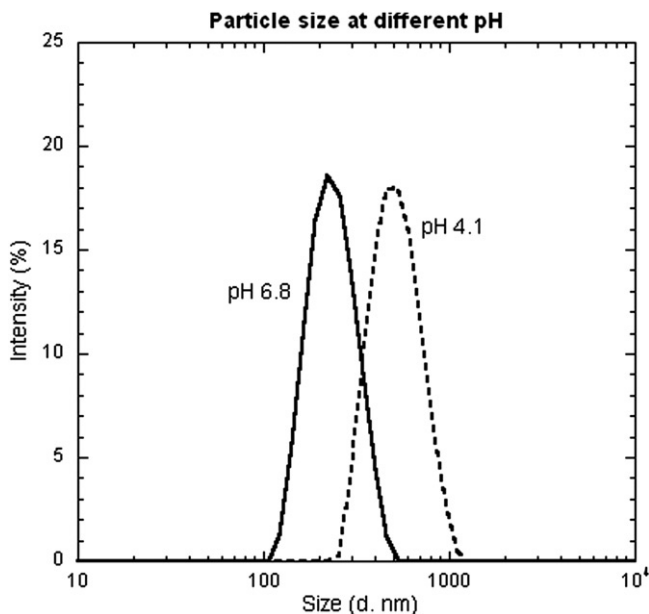


Figure 1. Hydrodynamic diameter of P4VP nanospheres at different pH.

When the pH was under 4.1, the polymer spheres lost their shape and the polymer dispersed into the solution. From TEM micrographs (data not shown), no aggregation can be observed.

The main challenge in the synthesis of the polymer spheres was to encapsulate well-defined superparamagnetic nanoparticles in order to obtain a material with the ability of increasing its temperature by the application of an alternating magnetic field. For this purpose, we used a ferrofluid based on iron oxide particles in organic medium, with high crystallinity, homogeneous morphology, monodispersity and superparamagnetic properties. Figure 2(a) shows the TEM micrograph of the bare magnetic nanoparticles. The sample shows nearly uniform distribution of particle size with high particle density and regular spherical shape with an average diameter of 6 nm.

The iron oxide phase was identified from the XRD pattern (Figure 3). Diffraction peaks at (111), (220), (311), (400), (442), (511) and (440) can be observed. The peaks are consistent with the standard data of an inverse spinel structure, meaning the formation of either magnetite (Fe_3O_4) or maghemite ($\gamma\text{-Fe}_2\text{O}_3$). As the two oxides are isostructural, the XRD technique cannot clearly differentiate between them, especially in the nanoscale because the characteristic reflections are broad and appear almost at the same 2 θ positions. In any case, both crystalline phases are known to be non-toxic and are currently used for human administration, although maghemite is more stable than magnetite [21]. An additional method which permits identifying the iron oxide phase was the measurement of the saturation magnetisation of the sample.

The magnetisation curves (Figure 4) indicate a superparamagnetic behaviour at 200 K, with no hysteresis, and a blocked magnetic moment at 5 K, with coercive field of 240 Oe. The saturation magnetisation $M_S \sim 75 \text{ emu g}^{-1} \text{ Fe}_2\text{O}_3$ was in agreement with the typical values for maghemite [22].

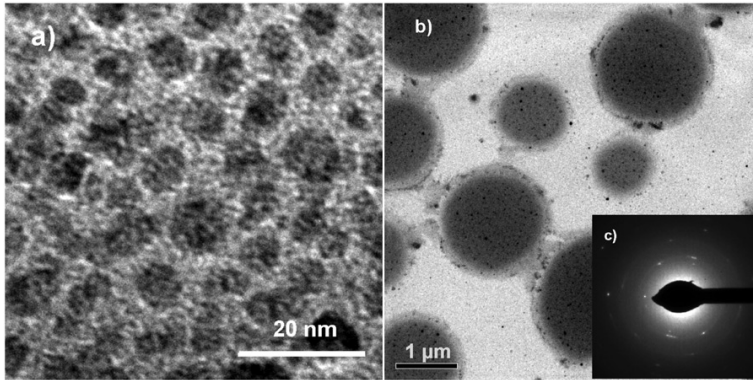


Figure 2. TEM micrographs showing (a) Iron oxide magnetic nanoparticles and (b) P4VP polymer spheres with encapsulated magnetic nanoparticles and c) image of ED pattern of the inner part of a loaded sphere.

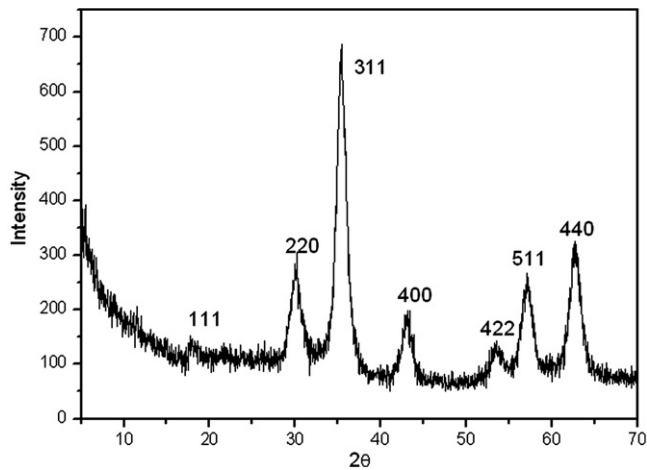


Figure 3. XRD pattern of oleic acid coated iron oxide nanoparticles.

The anisotropy constant was estimated from the analysis of the ac susceptibility measurements. The out-of-phase susceptibility component χ'' (not shown) presented a maximum at the average blocking temperature T_B (χ'') [23]. The dependence of T_B with the excitation frequency of the alternating field was well described by the Arrhenius law. The fitting to the expression $T_B(\chi'') = U/k_B \ln(1/2\pi f\tau_0)$ provided the activation energy U that, as a first approximation, was proportional to the average particle volume, $U = K_{\text{eff}}V$.

The effective anisotropy constant $K_{\text{eff}} = 1.7 \times 10^5 \text{ erg cm}^{-3}$ ($1.7 \times 10^4 \text{ J m}^{-3}$) was in the range expected for maghemite nanoparticles [24]. The specific absorption rate of a maghemite-based ferrofluid prepared by the same method and containing non-agglomerated, highly crystalline and monodisperse nanoparticles with an average size of 11.6 nm and a concentration of 1.89 mg mL^{-1} , measured at 315 K, 3 kA m^{-1} and 109 kHz was 0.89 W g^{-1} [25]. This indicates that this kind of magnetic nanoparticles have the capability of heating under the influence of a magnetic field.

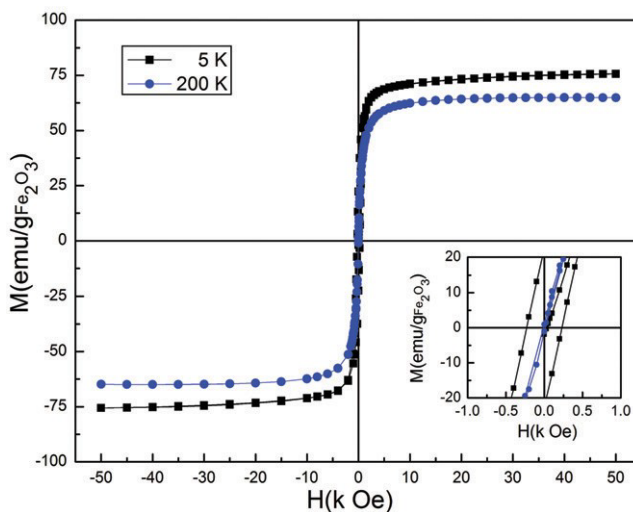


Figure 4. Iron oxide magnetisation curves at 200 and 5 K.

The P4VP spheres with encapsulated magnetic nanoparticles were successfully prepared by the nanoprecipitation method. In order to prove the encapsulation, we can observe the TEM micrograph of the as prepared sample (Figure 2b), where it can be noticed that mainly the iron oxide nanoparticles are inside the polymer spheres. But it also can be observed from TEM that the polymer spheres range in size between 1 and 3 μm . DLS shows a main diameter of 3 μm ranging between 2 and 3.5 μm (data not shown) at pH 6.8, but at pH 4.1 the measure is out of range. These spheres are considerably larger than the spheres obtained without encapsulated magnetic nanoparticles. In fact, both, the polymer spheres polydispersity and average size were increased with the magnetic loading. A possible origin is the incorporation of DCB, which is the magnetic nanoparticles dispersant. The DCB may remain inside the colloid when the particle is formed in a hydrophilic environment, thus increasing the volume of the sphere. A light crown around the polymer spheres can be observed in the TEM micrograph, possibly arising from the evaporation of the solvent, which takes place before the measurement. The sample was observed as prepared, without further purification, in order to check the effectiveness of magnetic nanoparticles internalisation. Some non-encapsulated iron oxide nanoparticles can be observed, but the concentration within the polymer spheres is much higher than outside, revealing a high-encapsulation yield. This little population of magnetic nanoparticles outside the spheres can be easily removed by centrifugation. The inset Figure 2(c) shows the electron diffraction (ED) pattern of a selected portion of the inside of a sphere, revealing the presence of crystalline iron oxide nanoparticles inside the hydrogel microspheres.

4. Conclusions

We have described a straightforward chemical synthesis path to prepare multi-responsive P4VP microspheres, based on the nanoprecipitation method. The polymer used was synthesised by atom transfer radical polymerisation in order to obtain a narrow molecular weight distribution. The obtained P4VP spheres show pH sensitivity and increase their diameter about three times in the range from 6.8 to 4.1. Subsequently, we achieved a hybrid (organic–inorganic) material by encapsulating oleic acid-coated iron oxide nanoparticles with sizes of the order of 6 nm in a P4VP spheres with sizes between 1 and 3 μm . Magnetisation measurements show that the nanoparticles behave as superparamagnets and, therefore, they would be able to increase the nanospheres temperature by the application of alternating magnetic field.

Experiments are in progress in order to characterise the heating capacity of the polymer spheres with magnetic nanoparticles. In next steps, the change of DCM and methanol by biocompatible solvents will be studied and also the encapsulation of a model drug and the study of its release by changing pH and inducing magnetic heating are planned.

Acknowledgements

This work has been supported by the Spanish Ministerio de Ciencia e Innovación (MICINN) and FEDER, Project MAT 2007-61621 and Consolider-Ingenio en Nanociencia Molecular ref. CSD 2007-00010. GI gratefully acknowledges Juan de la Cierva program. AA acknowledges the grant from the FPI program of the MICINN of Spain, project Mat2004-03395-C02-01. AU thanks the European Network MAGMANet for her scholarship. The authors would like to acknowledge Dr. Olivier Stéphane Roubeau for critical reading of the manuscript.

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