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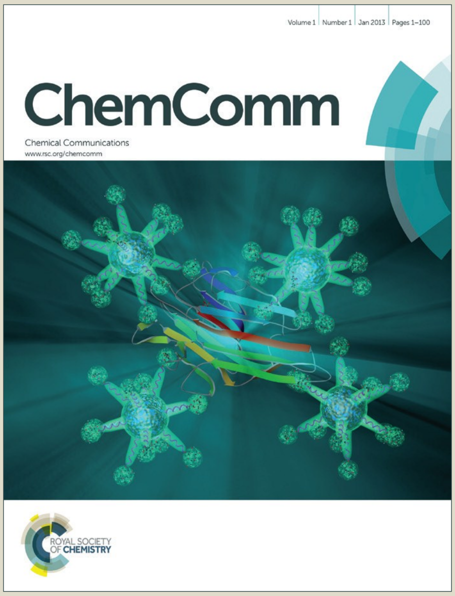

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Self-assembly of a superparamagnetic raspberry-like silica/iron oxide nanocomposite using epoxy-amine coupling chemistry

Manuel Cano and Guillermo de la Cueva-Méndez*

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The fabrication of colloidal nanocomposites would benefit from controlled hetero-assembly of ready-made particles through covalent bonding. Here we used epoxy-amine coupling chemistry to promote self-assembly of superparamagnetic raspberry-like nanocomposites. This adaptable method induced covalent attachment of iron oxide nanoparticles sparsely coated with amine groups onto epoxylated silica cores in the absence of other reactants.

The fabrication of composite nanoparticles (i.e. nanocomposites) constitutes a growing area of research in nanotechnology.[1] Thus, the development of facile and efficient approaches to make this type of nanomaterials is important for the advance of the field. Nanocomposites have unique chemical and physical properties conferred by the individual nanoparticles (NPs) used to make them.[1] This enables the generation of nanomaterials designed "a-la-carte" for specific applications.[1] One example is provided by magnetic nanocomposites,[2] which have potential uses in catalysis,[3] bio-separation[4] and bio-sensing.[5] For many of these applications, relatively large particles sizes are required. Yet, a direct relationship between superparamagnetism and particle size exists, with the smallest NPs being the most superparamagnetic.[6] One way of producing nanomaterials that are both large and superparamagnetic is to attach small iron oxide NPs to the surface of larger diamagnetic (e.g. silica) core particles. This approach would produce raspberry-like nanocomposites with the pursued properties.

Adsorption of magnetic NPs onto the surface of larger core silica particles has been used to produce nanocomposites like the above.[7,8] However, a major problem of this type of composites is that their constituent NPs are not covalently linked to each other, and this compromises their physical integrity under certain conditions (e.g. changes in temperature, pH, ionic strength). Conferring appropriate stability to these nanocomposites under those conditions requires burying the adsorpted magnetic NPs under a dense silica[7] or gold[8] layer. A more straightforward way to produce stable nanocomposites is to induce covalent bonds between their constituent NPs. In line with this, *Griffete et al.* have recently reported a method that enables crosslinking of hydroxyl groups present on the surface of both SPIONs and silica core particles, using diazonium salts.[9]

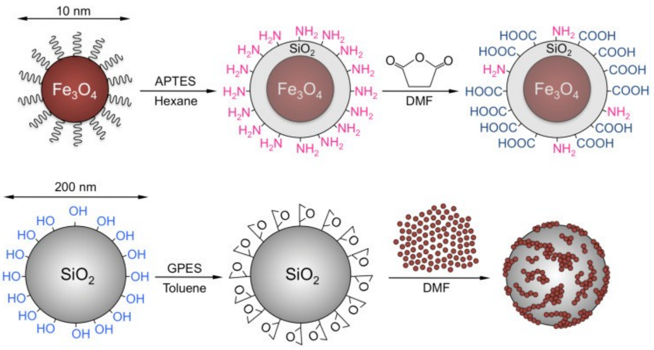

Fig. 1. Scheme depicting the fabrication steps of the raspberry-like superparamagnetic composite (bottom right) and its constituent SPION (top) and silica (bottom left and middle) NPs.

The development of alternative methods, using functional groups other than hydroxyl, to produce nanocomposites through covalent bonding of their constituent NPs would be of great value. Particularly interesting would be to use a chemistry that avoids the formation of homo-associations (which are likely to occurs when bonding is induced between identical functional groups), as this would maximise the yield and purity of the pursued nanocomposite. Here, we describe a facile method to produce raspberry-like superparamagnetic nanocomposites by covalently linking silica core NPs to a large number of monodisperse superparamagnetic iron oxide NPs (SPION), using epoxy-amine coupling chemistry. The procedure is summarized in Figure 1. First, monodisperse 10 nm SPIONs coated with oleic acid were synthesized in octadecene by thermal decomposition of an iron(III) oleate precursor, as described in *Park et al.*[10] and appropriately characterized (Fig. S1-S3 in ESI). The resulting hydrophobic SPIONs were converted to hydrophilic ones through a ligand exchange reaction using (3-aminopropyl)triethoxysilane (APTES).[11] The increase in water solubility of our particles observed as the latter reaction progressed was an indication of the success of the silane ligand exchange. This procedure coats SPIONs with a large number of amine groups, conferring them a net positive charge on the surface. The efficiency of this reaction was determined by measuring their zeta-potential (SPION-APTES; Fig. 2a). To counteract the tendency of positively charged SPION-APTES to aggregate in aqueous media, we carried out a partial conversion of amine groups into carboxylic acid groups using succinic anhydride (SA) in N,N-Dimethylformamide (DMF) for 2 hours.[12] This treatment reduced largely the number of amine groups on the surface of these particles, as revealed by the dramatic change of their zeta-potential values once the reaction was

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*Synthetic Biology and Smart Therapeutic Systems Laboratory, Andalusian Centre for Nanomedicine and Biotechnology, BIONAND, Severo Ochoa 35, 29590 Campanillas, Málaga, Spain.
E-mail:gdelacueva@bionand.es; Tel:+34 952367611;
Fax: +34 952367610*

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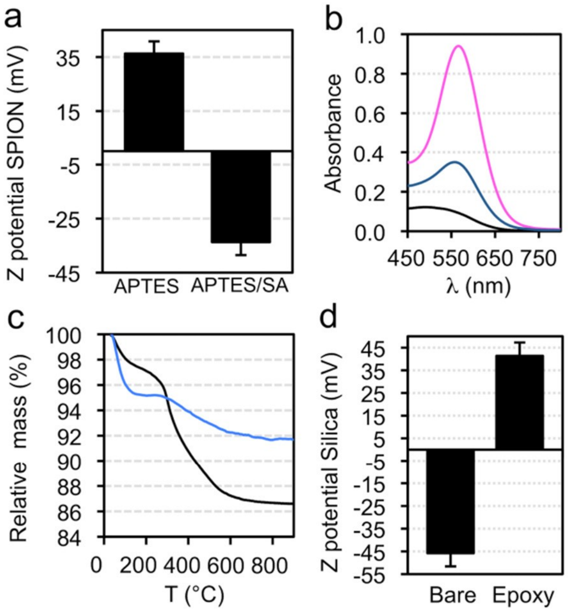

Fig. 2. Monitorization of the changes on surface chemistry during the fabrication of constituents SPIONs and Silica NPs. (a) Zeta-potential values of SPION-APTES and SPION-APTES/SA. (b) UV-visible spectra of SPION-APTES (pink), SPION-APTES/SA (blue) and a blank control sample (black) treated with nynhidrin. (c) TGA curves of bare (blue) and epoxysilane- modified (black) silica NPs. (d) Zeta-potential values of the silica NPs sample analyzed in (c).

completed (compare values for SPION/APTES and SPION-APTES/SA in Fig. 2a). In order to confirm that surface amine groups were still present on the surface of these NPs (which would be required latter on to stablish covalent bonds with the core silica NPs) we used the nynhidrin method[13] (Fig. 2b).

Additionally, 200 nm diameter SiO$_2$ NPs were prepared by the classical Stöber method[14] and characterized appropriately (Fig. S5). These silica NPs were subsequently epoxysilane modified with 3-(3-Glycidyloxypropyl)trimethoxysilane (GPES) using the toluene method (Fig. 1).[15] To confirm the epoxy-silanization of these silica NPs we carried out thermogravimetric (TGA) and zeta-potential analyses, which revealed an increment of weight loss of around 5.1% (Fig. 2c), and a surface charge change from −43 to +41 mV (Fig. 2d), in agreement with what had been previously reported.[16] Last, the particles above were used to produce a raspberry-like silica-SPION nanocomposite (Fig. 1). For this, we mixed the epoxylated silica NPs with the amine-carboxylic acid SPIONs (1:4000 ratio, w/w) in DMF, and incubated the mixture at 65 ºC for 48 hours under gentle stirring.[17] Then, the resulting nanocomposite was separated from free constituent particles using three sequential cycles of centrifugation (9300 xg; 0.5 min) and re-suspension in water.

Scanning (SEM) and transmission (TEM) electron microscopy images were obtained for all relevant samples throughout the procedure (Fig. 3). Figure 3a shows a TEM image of the oleic-acid-stabilized SPIONs (SPION-OA), highlighting their monodispersity, spherical shape, and average diameter of 10.6 ± 1.2 nm (calculated by averaging a minimum of 30 NPs per sample). As expected from a procedure that only leads to a thin coating, silane ligand exchange reaction of the SPION-OA with APTES did not induce any significant morphological changes in the resulting SPIONs (SPION-APTES).[11] However, these positively charged SPIONs tend to form multi-particulate aggregates (Fig. 3b). Treatment of SPION-APTES with SA to reduce the number of amine groups on their surface did not change the size or shape of the resulting particles (SPION-APTES/SA) but greatly reduced their aggregation (Fig. 3c). Similarly, our bare SiO$_2$ NPs preparations were imaged by SEM (Fig. 3d) and TEM (Fig. 3e). Neither the size (200 nm average diameter) nor the spherical shape of these silica particles varied upon their epoxy-silane modification (Fig. 3f).[15] Cross-linking SPION-APTES/SA to these epoxylated silica NPs produced raspberry-like nanocomposites with an homogeneous distribution of SPIONs onto the surface of the core particles, as shown by TEM (Fig. 3g and Fig. S6).

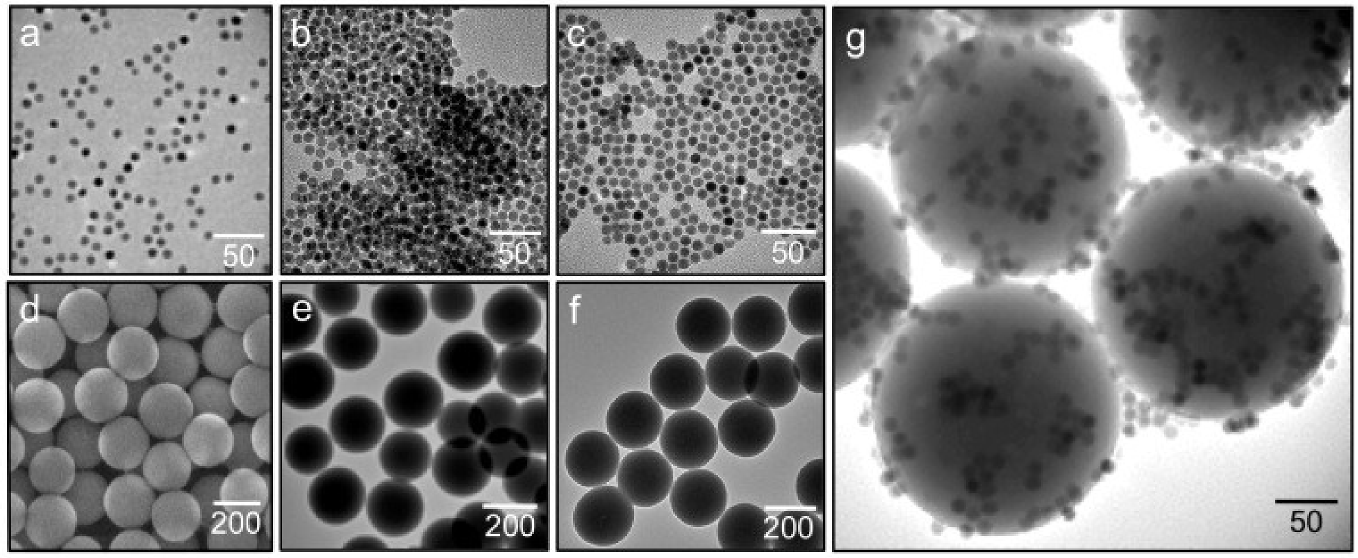

Fig. 3. Morphological analysis of the raspberry-like nanocomposite and of its constituent NPs and their precursors. TEM images of SPION-OA (a), SPION-APTES (b), and SPION-APTES/SA (c). SEM (d) and TEM (e) images of bare silica NPs. (f) TEM image epoxysilane-modified silica NPs. (g) TEM image of raspberry-like composite NPs.

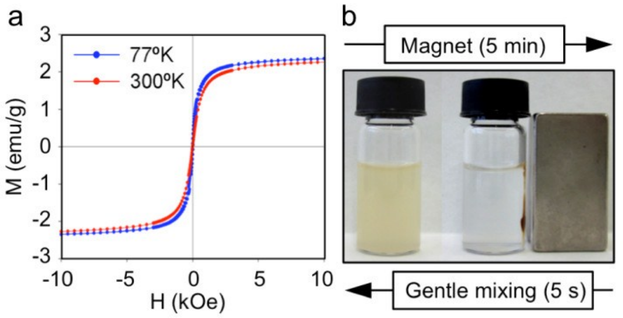

Fig. 4. Analysis of the superparamagnetic properties of the raspberry-like nanocomposites. (a) Magnetization curves of the nanocomposite shown in Fig. 3g at the indicated temperatures. (b) Attraction and re-dispersion of the nanocomposite in solution in the presence (right) or absence (left) of a magnet.

Finally, we used a Vibrating Sample Magnetometer (VSM) to test whether the superparamagnetic properties of the isolated SPIONs (Fig. S3a) were conferred to the nanocomposite. Magnetization plots showed absence of coercivity and remanence in our composites at two different temperatures 77 and 300 ºK, and a saturation magnetization value of 2.4 emu·g^{-1} at room temperature, which confirmed their superparamagnetic nature (Fig. 4a). This is close to the value obtained for similar nanocomposites produced by physisorption of SPIONs to silica cores used as DNA biosensors,[8] and much higher than those observed for a nanocomposite with a single SPION embedded in a silica shell used as an MRI contrast agent.[18] The magnetic character of our raspberry-like particles was evident macroscopically, using a colloidal suspension of the latter in PBS. In these conditions, the composite was attracted towards a neodymium magnet in a few minutes and could be easily re-dispersed by gentle tapping (Fig. 4b).

Our work here demonstrates the feasibility of using epoxy-amine coupling chemistry for the facile and effective construction of raspberry-like superparamagnetic nanocomposites. Our method uses constituent monodisperse NPs (which, in the case of SPION, is an important requisite for the construction of sensitive and reliable magnetic nanodevices[19]) that can be easily synthetized, in large scale and at low cost, and covalently linked to each other without using microemulsions, copolymers, surfactants and/or coupling agents. Amine and epoxy groups were incorporated on the surface of SPION and silica particles, respectively, using two different silanization methods that our results reveal as efficient approaches for NP modification. The epoxy-amine chemistry used here could be applied to attach moieties different from SPIONs to the silica core particles, and therefore to tailor nanocomposites to a broader range of specific applications. Yet, the validation of our approach using SPIONs is particularly useful, helping us to demonstrate not only a facile fabrication of superparamagnetic nanocomposites, but also a homogeneous surface functionalization of core NPs, as SPIONs can be easily observed using TEM. This superparamagnetic composite could be used as a chassis for more complex multifunctional NP with potential applications in fields like magnetic isolation of cells,[20] proteins[21] or nucleic acids,[8] and magnetic resonance imaging,[18] to name only a few.

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