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Coating Evaluation and Purification of Monodisperse, Water-Soluble, Magnetic Nanoparticles Using Sucrose Density Gradient Ultracentrifugation

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Supporting Information

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Magnetic nanoparticles have numerous applications in biomedicine and biomedical research.[1] The bioavailability of these nanomedicines is a complex function of several physicochemical and material properties and, as such, requires tight control of various chemical and physical nanoparticle properties that include size, shape, flexibility, surface charge, and surface chemistry.[2] The most widely used magnetic nanoparticles contain iron oxide nanocrystals because the toxicity, metabolism, and pharmacokinetics of intravenously injected iron oxide based nanoparticles have been extensively documented.[3] However, increasing *in vivo* sensitivity and efficiency requires nanocrystals with specific compositions. Thus, recent research has focused on modifying the magnetic properties of iron oxide nanocrystals by doping with transition metal ions such as cobalt, manganese, nickel, and zinc.[4,5]

There are numerous chemical methods for producing magnetic nanocrystals.[6] Solution phase decomposition of organometallic precursors at high temperatures in an organic solution containing a surfactant can produce highly monodisperse nanocrystals on a large scale and with a well-defined size.[7] However, nanocrystals generated by thermal decomposition are stabilized by a hydrophobic surfactant, such as oleic acid, to control crystal growth and prevent particle aggregation, which limits their direct biomedical application. Micellar encapsulation of nanocrystals with PEGylated lipids by hydrating a dry film or by stepwise increases of the solvent polarity are established methods for transferring hydrophobic nanocrystals into aqueous solutions.[8−10] The resulting water-soluble nanoparticles are purified from empty polymer micelles using differential ultracentrifugation. Although this approach is effective in separating encapsulated nanocrystals from empty phospholipid micelles, the resolution of differential centrifugation is limited to particles with large differences in sedimentation coefficients.[11]

The resolving power of differential ultracentrifugation in a homogeneous medium can be increased by using density gradient ultracentrifugation,[12] which is a preparative ultracentrifugation technique routinely used in biochemical and biological sample preparations. The increased resolution of rate-zonal separation by density gradient ultracentrifugation, which separates components within a mixture based primarily on particle size and mass,[13] can be used to isolate monocrystalline nanoparticles from empty micelles and aggregated nanocrystals.

Here, we apply a well-established method based on sucrose density gradient ultracentrifugation, which is routinely used for the purification and separation of biomacromolecules, to evaluate and obtain a highly monodisperse population of water-soluble magnetic nanoparticles suitable for biomedical applications. Cobalt ferrite and manganese ferrite were used as model nanocrystals to illustrate the flexibility of sucrose gradient ultracentrifugation because they are representative examples of two bimetallic ferrites with biomedical applications. Both nanocrystals are superparamagnetic and can be used as contrast agents in MRI, but manganese ferrite has a larger magnetic moment than either cobalt ferrite or iron oxide,[5] which increases the detection sensitivity. Further, cobalt ferrite nanocrystals can mediate the conversion of an alternating magnetic field into heat for applications in cancer therapy using magnetic fluid hyperthermia.[14]

Cobalt ferrite (Figure 1A) nanocrystals with a mean diameter of 6.0 nm ±7% were coated with 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG-2000) by hydrating a dry film containing a mixture nanocrystals stabilized by oleic acid and DSPE-PEG-2000. Sucrose gradient ultracentrifugation was used to qualitatively evaluate the resulting self-assembled, water-soluble nanoparticles based on the differential rate of nanoparticle sedimentation according to the Stokes equation.[13]

The different bands of nanoparticles visible in Figure 1B indicate that coating hydrophobic nanocrystals with DSPE-PEG-2000 using the film hydration method produces a heterogeneous mixture of nanoparticles, which were separated by the sucrose density gradient. The major fraction of nanoparticles with the slowest sedimentation rate were isolated and analyzed by dynamic light scattering. Despite the complexity of the sample prepared by film hydration, nanoparticles could be isolated using sucrose density gradient

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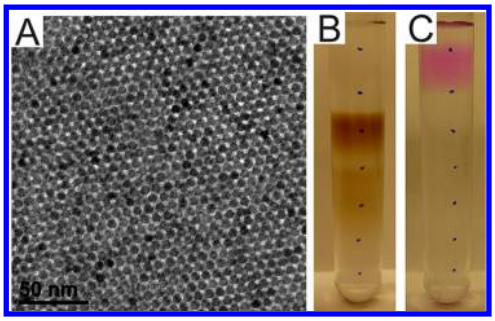

Figure 1. Water-soluble nanoparticles prepared by dry-film hydration. (A) Transmission electron microscopy image of oleic acid stabilized cobalt ferrite nanocrystals. Digital camera images of (B) nanoparticles prepared by the dry-film protocol and (C) empty phospholipid micelles labeled with PKH26 after separation by sucrose density gradient ultracentrifugation. The markings in B and C indicate the approximate position of the density gradient steps (water, 5, 30, 40, 50, 60, 70, and 80% sucrose).

ultracentrifugation that had a hydrodynamic diameter of 22.14 ± 0.08 nm and a polydispersity index of 0.08 ± 0.01. Smaller nanoparticles with a hydrodynamic diameter of 18.78 ± 0.07 nm and a polydispersity index of 0.10 ± 0.01 could be isolated following encapsulation with DSPE-PEG-1000 using film hydration. Isolation of particles of this quality from the heterogeneous mixture of nanoparticles produced by film hydration would be difficult with differential ultracentrifugation, which has a limited capability to separate particles with similar sizes. The estimated hydrodynamic diameters are expected to increase for nanocrystals coated with DSPE-PEG-1000 and DSPE-PEG-2000 by approximately 3.4 nm,[10,15] which corresponds to the experimentally measured increase in hydrodynamic diameter. Separation of nanoparticles from empty phospholipid micelles was confirmed by sucrose density gradient ultracentrifugation of phospholipid micelles labeled with a lipophilic dye (Figure 1C). The micelles stayed within the 5% sucrose density step, which provides effective separation from the micelle encapsulated cobalt ferrite nanocrystals that were located near the 30−40% sucrose boundary.

Solvent exchange using stepwise increases in solvent polarity to self-assemble water-soluble nanoparticles was recently described as a method for micellar encapsulation of hydrophobic nanocrystals that overcomes the heterogeneity of samples prepared by dry film hydration.[9] Nanoparticles prepared by solvent exchange sedimented as one major band within the sucrose density gradient (Figure 2A). These nanoparticles had a hydrodynamic diameter of 26.2 ± 0.1 nm and a polydispersity index of 0.09 ± 0.01. Routine translation of small scale analytical separations to larger scale bulk sample preparation is an advantage of centrifugation as a preparative technique. To illustrate this advantage, nanoparticles were isolated with a hydrodynamic diameter of 26.5 ± 0.2 nm and a polydispersity index of 0.08 ± 0.01 after scaling up the rate-zonal ultracentrifugation protocol by approximately 2.5 fold (Figure 2B). In addition to the band of nanoparticles visible in Figure 2B, density gradient ultracentrifugation provides a convenient method to remove a population of water-soluble nanoparticles with a large sedimentation rate that pelleted on the bottom of the centrifuge tube (Figure 2C). The increased homogeneity of the nanoparticles prepared by solvent exchange is an advantage of this protocol compared to the film hydration method because the overall yield will be improved, which

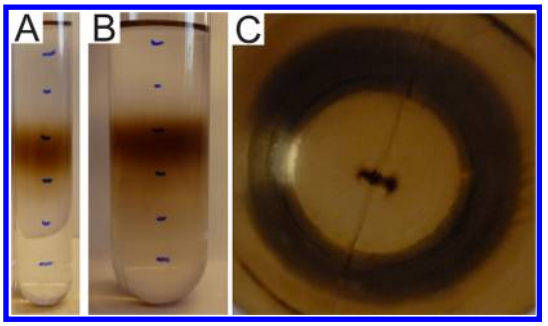

Figure 2. Water-soluble nanoparticles prepared by solvent exchange. Digital camera images of sucrose density gradient ultracentrifugation separation of nanoparticle samples containing either (A) 175 μg or (B and C) 500 μg of total iron. C is a top view of B. The markings in B indicate the approximate position of the density gradient steps (water, 30, 40, 50, 60, 70, and 80% sucrose).

allows more efficient and cost-effective scale-up for preparing nanoparticles for preclinical and clinical applications.

Gel electrophoresis is a routine technique for analysis of proteins and nucleic acids that can be applied to qualitatively assess the coating of water-soluble nanoparticles.[9,10,16] Agarose gel electrophoresis uses a gel matrix composed of physically cross-linked chains of agarose, a nonionic linear polysaccharide, to separate molecules based on size and charge in an applied electric field based on molecular sieving. To evaluate agarose gel electrophoresis as a method for qualitative interpretation of nanoparticle preparations, nanoparticles were prepared using solvent exchange at an iron to phospholipid weight ratio of 1:5, 1:10, and 1:20 and separated using agarose gel electrophoresis (Figure 3A) or sucrose density gradient ultracentrifugation

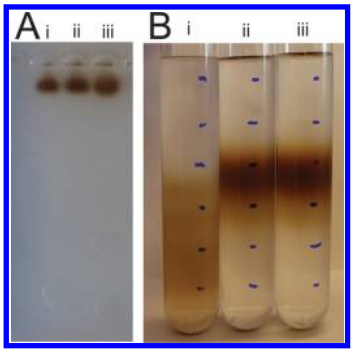

Figure 3. Qualitative evaluation of nanoparticle preparations. Samples were initially prepared at an iron to DSPE-PEG-2000 weight ratio of (i) 1:5, (ii) 1:10, and (iii) 1:20 by solvent exchange. (A) Samples were analyzed by agarose gel electrophoresis (0.6%, 100 V, 60 min) prior to (B) sucrose gradient ultracentrifugation. The markings in B indicate the approximate position of the density gradient steps (water, 30, 40, 50, 60, 70, and 80% sucrose).

(Figure 3B). The different nanoparticle preparations have a similar electrophoretic mobility through a 0.6% agarose gel, with no indication of sample differences with respect to nanocrystal coating. However, after separation through a sucrose density gradient, the sample prepared at a 1:5 weight ratio contains a visibly more heterogeneous population of water-soluble nanocrystals than the samples prepared at a 1:10 and 1:20 weight ratio. This illustrates the advantage of rate-zonal ultracentrifugation for qualitative evaluation of nanoparticle preparations compared to agarose gel electrophoresis.

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The modularity of sucrose density gradient ultracentrifugation for rate-zonal separation of water-soluble nanoparticles was demonstrated by isolating micelle encapsulated manganese ferrite nanocrystals, which can be used to increase the sensitivity compared to iron oxide nanocrystals for contrast enhanced MRI. Manganese ferrite nanocrystals were coated with DSPE-PEG-2000 and separated by sucrose density gradient ultracentrifugation (Figure S1, Supporting Information). The nanocrystal cores had an average diameter of 5.9 nm ±7%, and the water-soluble nanoparticles had a hydrodynamic diameter of 23.8 ± 0.1 nm with a polydispersity index of 0.086 ± 0.009.

In conclusion, sucrose density gradient ultracentrifugation is a cost-effective and easily scalable preparative technique that allows sensitive evaluation and isolation of homogeneous, water-soluble nanoparticles. As expected, we found that rate-zonal separation using sucrose density gradients is a more effective method for evaluating nanoparticles than agarose gel electrophoresis, which can have pore diameters larger than 100 nm.[17] From here, we will use cobalt as a nonradioactive tracer to analyze the pharmacokinetics and biodistribution of cobalt ferrite nanocrystals coated with PEGylated phospholipids because cobalt is an endogenous essential dietary nutrient that is found at low levels in the human body.[18] Additional resolution of similar sized nanoparticles or separation of other metallic and inorganic nanocrystals is possible by decreasing the sucrose gradient step sizes, allowing partial diffusion of the gradients to produce a more linear gradient, using a gradient maker to create linear gradients, or using small molecule alternatives to sucrose such as iohexol or iodixanol with a lower viscosity than sucrose because sedimentation rate varies inversely with the solvent viscosity.[13]

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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