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Research Article

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Techniques for Surface Modification of Aqueous-Stable Superparamagnetic Iron Oxide Nanoparticles

Abstract

Background: The iron oxide nanoparticles involved in this study are unique in their superparamagnetic properties, defined as their ability to flip the direction of their magnetic field under influence of temperature. This property has a variety of environmental and biomedical uses. Indeed, the exchange of ligands on the surface of these particles enables exploration of such applications. The purpose of this study is to determine an efficient method of ligand exchange in order to standardize the surface modification of these iron oxide nanoparticles (IONPs). Namely, the primary methods of ligand exchange to be evaluated are shaking and sonication of reaction mixtures. As part of this method comparison, the exchange of oleic acid (OA) ligands for 3,4-Dihydroxyphenylacetic acid (DOPAC) ligands serves as a general model for method comparison. When comparing methods, both time and quantity of materials required are considered. The quality of the final product is also considered, assessed by factors such as oxidation state, colloidal stability, and extent of ligand exchange.

Methods: Three methods of ligand exchange are performed, after which their products are compared. The first method involves shaking the mixture overnight for a duration of 18 hr. The second method involves sonication for a duration of 30 min. The third method involves sonication of the reaction mixture for an additional 30 min. (duration of 60 min. in total).

Results: The products were analyzed using Fourier-transform infrared spectroscopy (FT-IR), zeta potential measurements, thermogravimetric analysis (TGA), and x-ray photoelectron spectroscopy. FT-IR measurements indicate that the one-time sonication method leads to the surface of the IONPs bearing the most residual oleic acid, a disadvantageous result. TGA analysis indicates that the twice-sonicated product is more favourable than the once-sonicated product.

Limitations: Larger data sets of FT-IR, TGA, zeta potential, and XPS must be collected before the best method may be confirmed. Zeta potential measurements must be repeated for the shaken product at a concentration that matches that of the other products. As such, a direct comparison may be made. TGA must also be repeated for the shaking product in order to eliminate possible inaccuracies. Namely, these could result from technical difficulties encountered in the measurement discussed above. While zeta potential measurements indicate that the twice-sonicated product has the highest colloidal stability, XPS measurements did not vary significantly enough between methods to suggest a most advantageous method.

Conclusion: According to the TGA and zeta potential measurements, the twice-sonicated product appears to be most favourable in terms of coverage. XPS suggests that all methods are comparable in terms of oxidation of the IONPs' iron.

Introduction

The use of aqueous stable superparamagnetic iron oxide nanoparticles (IONPs) is widespread. Biomedical and bioengineering applications include enhancement of magnetic resonance imaging contrast, tumor hyperthermia (1), drug delivery, tissue repair, and detoxification of biological fluids.(2) The potential for widely varying surface coatings of IONPs allows for widely varying applications. For example, IONPs may bind to proteins, antibodies, or drugs, as well as be redirected to specific tissues using an external magnetic field.(3) Other applications include the development of hybrid organic-inorganic materials.(4) Other applications outside biomedicine include data storage and water treatment.(5) Significantly, the preparation of aqueous-stable IONPs required by such applications generally involves some form of ligand exchange technique. The goal of this process is to optimize magnetic attributes of these nanoparticles, especially to improve hydrophilicity. In fact, this is particularly necessary for biomedical applications.

Such ligand exchange techniques include mechanochemical milling, shaking, and sonication. Mechanochemical milling is useful for circumventing issues such as solvent compatibility limitations, and also eliminates the need for chlorinated solvents.(6) Indeed, it is possible to employ mechanochemical milling to eliminate intermediate substitution steps in making superparamagnetic IONPs soluble in aqueous buffers. For instance, this allows for one-step conversion of monodisperse hydrophobic oleic-acid capped superparamagnetic IONPs to hydrophilic Tiron-capped IONPs. While useful, mechanochemical milling will not be considered in this particular comparison of ligand exchange methods, focusing rather on the shaking and sonication methods.

To this end, the ligand exchange of oleic acid (OA) ligands for 3,4-Dihydroxyphenylacetic acid (DOPAC) will serve as a general model for method comparison (Fig. 1).

This model proves to be especially useful because shaking and sonication methods are known to reliably produce IONP-DOPAC products. This exchange is favoured due to the catechol group's higher affinity for iron (III) as compared to that of the carboxylate.(7) The functional groups present

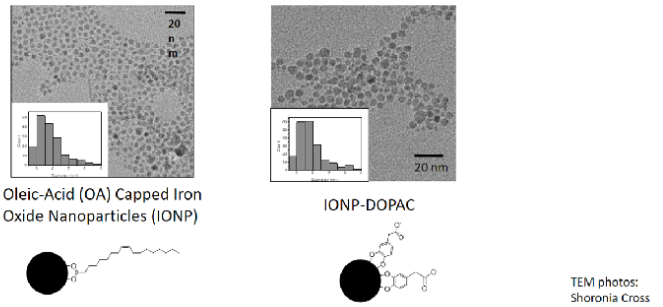

Fig. 1. TEM Photos of Starting Material and Product.

on the surface of the catechol impart aqueous stability. In other words, DOPAC binds through the catechol group, leaving the ionizable carboxyl group exposed to solution, thus making the particles aqueous-stable.

The first method evaluated, the shaking method, involves shaking the mixture overnight for approximately 18 hr.(8) On one hand, the shaking method features a significant benefit in its ability to carry out an almost complete ligand exchange. Nevertheless, a small amount of OA remains on the IONPs regardless of the method used, and the surface of the IONPs will always have OH groups present. Therefore, the surface will never be fully covered with any ligand.

On the other hand, this method disadvantages the process due to the amount of time it takes to reach completion. This could greatly encumber both the synthesis of IONPs functionalized with dopamine derivatives, and the subsequent tests to be performed on these products.

The two other methods to be examined involve sonication of the reaction mixture.(9) Previously it was theorized that the IONPs first undergo partial exchange, forming an intermediate that is covered partially by DOPAC and partially by OA.

In one sonication method, the mixture undergoes sonication for 30 min., which is favorable due to its short reaction time.(10) However, this technique can hinder the exchange process by its production of a compound which may not have optimal DOPAC coverage. Additionally, sonication's more energetic nature poses a greater risk of degradation or oxidation of the IONPs.

In the second method of this kind, the mixture is subjected to 30 min. of sonication, followed by a second addition of DOPAC to the particles, and another 30-min. period of sonication. A potential advantage of this method is an optimized addition of DOPAC to the surface of the IONPs. Still, this technique demands double the amount of ligand, and results in an inevitable loss of IONPs following the second sonication. Indeed, this purification (with washing) comes with a noticeable loss of IONPs. Furthermore, this method's second sonication risks causing more degradation and oxidation of IONPs.

The difference between theoretical products resulting from these methods is illustrated in Fig. 2. Both sonication methods are clearly favourable over shaking due to their comparatively short reaction time.

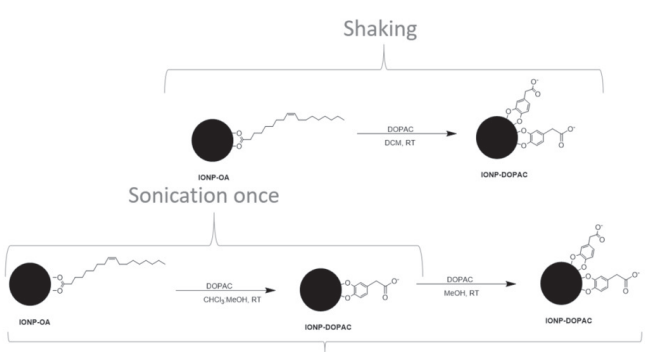

Fig. 2. Methods to be Compared.

Looking towards the future, the consistency resulting from a standardized methodology will enhance the reliability and usefulness of findings related to the various dopamine derivatives, which will be synthesized, in collaboration with the Lumb group, and analyzed. Afterwards, these dopamine derivatives will be used as a model for ligand electronic effects. As the aromatic ring will become more or less electron deficient depending on the ligand involved, the effect of this property on particles' binding behaviour and stability will be explored. For example, an electron-poor ligand may pull electrons off the surface iron, affecting its oxidation state and the crystallinity of the nanoparticle.

Methods

Ligand exchange via single sonication:(8) A room temperature 2 mL aliquot of a stock solution of 2.5 mg/mL IONP-OA in hexane was dried under nitrogen flow. The particles were then re-dispersed in 14 mL of chloroform by mild vortexing. 10 µL of DI water were added to 44.4 mg of DOPAC dissolved in 1 mL of methanol. This solution was added in 0.5 mL aliquots to the nanoparticle solution in chloroform. The mixture was then sonicated for 30 min. at room temperature.

Ligand exchange via double sonication: After the first 30 min. sonication, the mixture was pelleted by centrifugation at 4000 xg for 30 min. at 4°C. The supernatant was decanted, the pellet was re-dispersed in a solution of 44.4 mg of DOPAC in 10 mL of methanol, and the mixture was sonicated a second time for 30 min. at room temperature.

Ligand exchange via overnight shaking:(7) A 2 mL aliquot of a room temperature 2.5 mg/mL stock solution of IONP-OA in dry DCM was added to 44.4 mg of DOPAC dissolved in 13 mL of dry DCM. 10 µL of DI water were added to this reaction mixture, and the mixture was shaken for 18 hr at room temperature by vortex.

Purification of IONP-DOPAC products: All IONP-DOPAC products were purified identically, regardless of preparation. The reaction mixture was centrifuged at 4000 xg for 30 min. at 8°C. Supernatants were removed by pipet and pellets were re-dispersed in methanol. The mixture was again centrifuged at 4000 xg for 30 min. at 8°C. The supernatants were removed and the particles were dried under nitrogen flow. For FT-IR analysis, 20 µL of methanol was added to create a slurry for plating.

Fourier-Transform Infrared Spectroscopy (FT-IR): ATR-FTIR spectra were collected using a Spectrum Two FT-IR spectrometer equipped with a diamond ATR accessory and processed using Spectrum FT-IR software (PerkinElmer Inc. Waltham, MA, USA). All spectra were recorded between 4000 and 400 cm^{-1}, with 4 cm^{-1} resolution, averaged over 16 scans. A 1 mL aliquot of sample was centrifuged for 30 min. at 4400 rpm, supernatant was removed and methanol was added. The resulting mixture was plated directly onto the FT-IR as a film.

Zeta Potential: Before performing zeta potential measurements, it was necessary to determine the iron concentration via an established procedure, on an aliquot of each IONP solution in buffer, and to then dilute the sample to 0.05 mg/mL. Measurements were performed on a ZetaPlus zeta potential analyzer using Zeta Analysis software (Brookhaven Instruments Corporation, Holtsville, NY, USA). Measurements were performed in 30 mM MES buffer with a pH of 5.99.

Thermogravimetric Analysis (TGA): TGA was performed in a TA Instruments TGA Q-500 thermogravimetric analyzer, using Advantage for Q series v2.5.0.256 and Thermal Advantage v5.4.0 software (New Castle, DE). The temperature was ramped under nitrogen atmosphere at a rate of 10 °C/min from room temperature to 600 °C (700 °C for IONP-OA), with air being introduced at 550 °C.

X-ray photoelectron spectroscopy (XPS): A silicon wafer was washed with acetone, methanol, and isopropyl alcohol. The purified pellet of IONP-DOPAC was re-suspended and added in small amounts to the wafer, which was then dried under nitrogen. XPS measurements were performed on a Thermo Scientific K-Alpha X-ray photoelectron spectrometer, using Thermo Advantage v5.962 software (Waltham, MA). The X-ray

was Al-Kα (1486.7 eV), at a spot size of 400 µm. The plate was washed and the sample was plated in 20 µL aliquots. High resolution Fe spectra were collected at 150 keV pass energy and 50 ms dwell time over 3 scans.

Results

In addition to considerations of time and quantity of material produced, the most important factors in determining the best method for ligand exchange are oxidation state, colloidal stability, particle coverage, and nature of ligand exchange. To inform this process, FT-IR (Fourier-transform infrared spectroscopy) evaluates the nature of coverage, while TGA (thermogravimetric analysis) and zeta potential measurements assess the extent of coverage. Similarly, XPS (x-ray photoelectron spectroscopy) examines the oxidation states.

To begin, comparing the FT-IR spectra for each method allows for an initial distinction to be made between the nature of the coverage of the IONP products. Particularly interesting are the two peaks at 2850 cm^{-1} and 2920 cm^{-1}, whose intensities vary between methods.

The peak associated with Fe-O bonds also varies between methods. The FT-IR spectrum of the twice-sonicated IONPs shifts to 573 cm^{-1}. Meanwhile, similar peaks present in the spectra of the twice-sonicated product and the shaking product both appear at 571 cm^{-1} (Fig. 3).

The aromatic C-H peaks at about 1150 cm^{-1} and 1117 cm^{-1} stem from the DOPAC ligand, qualitatively confirming ligand exchange. While these peaks show significant variation between preparations, their intensities and shapes do not vary. Their location changes most commonly between trials of the twice-sonicated IONPs.

Next, examining the TGA (thermogravimetric analysis) results will shed light on the extent of the IONPs' coverage. To begin with, organic material comprises 19.79% of the starting material, IONP-OAs. Following ligand exchange, the IONPs that undergo shaking have 9.043% organic material, the once-sonicated IONPs have 6.880%, and the twice-sonicated IONPs have 7.597% (Fig. 4).

Next, zeta potential measurements allow the evaluation of each method's colloidal stability. The zeta potential measurements are performed in 30 mM MES buffer with a pH of 5.99 and the results are displayed in Table 1.

Finally, the oxidation state of the iron within the nanoparticles is observed through XPS (x-ray photoelectron spectroscopy) (Fig. 5). The Fe 2p-3/2 peak values for each method are compared with each other and with the original IONP-OA (Fig. 5) (Fe 2p-3/2 refers to the specific state of iron that is relevant for these particular reactions). The Fe 2p-3/2's binding energy for IONP-OA peaks at 711.3 eV, while the once-sonicated product, the twice-sonicated product, and the shaken product peak at 711.6 eV, 711.5 eV, and 711.5 eV, respectively.

Discussion

The FT-IR results are significant in their qualitative confirmation of successful ligand exchange. Each method – sonication once, sonication twice, and shaking – is confirmed by FT-IR to have resulted in successful ligand exchange. With this initial confirmation that OA and DOPAC have indeed been exchanged, the relative success of each method can be determined. However, the variations in peak intensities and fingerprint regions between each method require further investigation to explain. Therefore, FT-IR does not indicate a most successful method, but rather it confirms that ligand exchange has occurred as expected.

The two peaks at 2850 cm^{-1} and 2920 cm^{-1} (Fig. 3) are associated with the C-H stretching vibrations of the OA. These peaks vary in intensity between methods, and are most intense in the one-time-sonicated IONPs' spectrum. More intense peaks in this region are associated with residual OA on the surface of the IONPs. The spectrum of the twice-sonicated IONPs is shifted to 573 cm^{-1}, while the other two are at 571 cm^{-1}. This may indicate oxidation of the iron, as oxidation leads to a more positive charge,

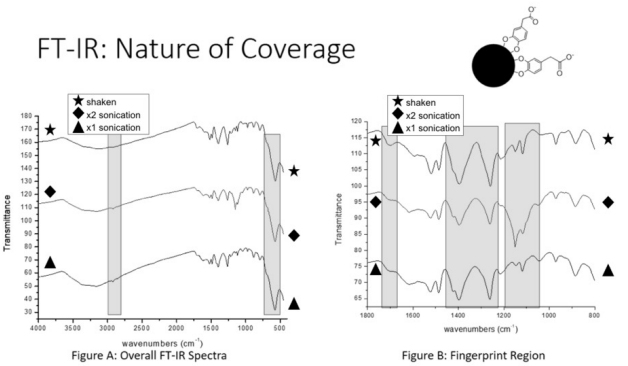

Fig. 3. FT-IR Comparison of Ligand Exchange Methods.

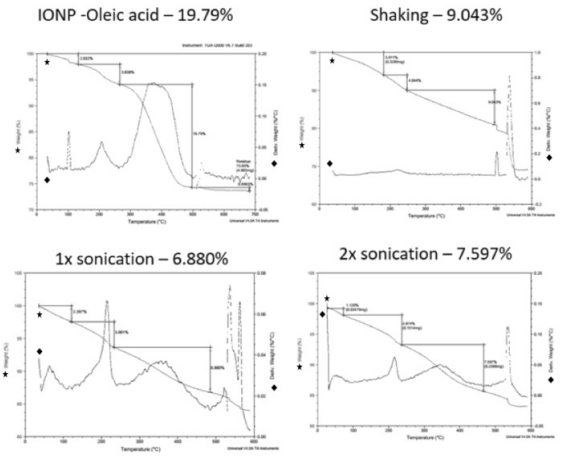

Fig. 4. Results of TGA Analysis Indicating Percentage of Organic Material on Surface.

Technique	Mobility (10^{-8} m^2 / s*V)	Zeta Potential (mV)
Sonication x1 (0.05 mg/mL)	-1.93	-24.75
Sonication x2 (0.05 mg/mL)	-2.49	-31.87
Shaking (0.15 mg/mL)	-2.42	-30.94

Done in 30 mM MES buffer pH 5.99

Table 1. Zeta Potential Measurements.

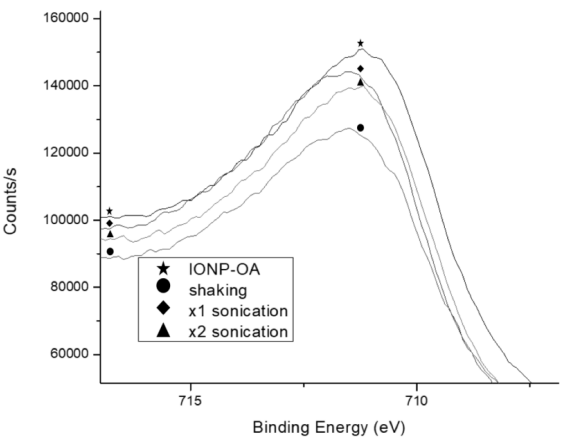

Fig. 5. XPS Comparison of Ligand Exchange Methods.

which results in higher vibrational frequency due to stronger iron-oxygen bonds. This could explain a shift to a higher wavenumber.

While this data confirms that each method results in some ligand exchange, the presence of OA peaks on each spectrum indicates that no method results in complete exchange. The once-sonicated sample has the most intense OA peaks, showing that it results in the least amount of ligand exchange. As such, the once-sonicated method may be the least successful of the three in terms of DOPAC coverage. The possibility of IONP oxidation for the twice-sonicated method, indicated by the shift from 571 cm^{-1} to 572 cm^{-1}, is further investigated through XPS.

Moving on, TGA determines the percentage of DOPAC on the surface of the IONPs, a significant factor in evaluating which method is most useful. The higher the percentage of DOPAC on the surface of the IONPs, the more effective is the method.

Upon initial examination, it appears that the shaking exchange results in the highest percentage of DOPAC on the surface. However, the sudden drop at around 500°C (Fig. 4) is the result of a technical difficulty. The shaking result cannot be relied upon and must therefore be redone.

It can, however, be concluded that the twice-sonicated IONPs are composed of a higher percentage of DOPAC than the once-sonicated IONPs. This indicates the possibility that the double sonication method is superior to the single sonication method.

Now that the extent of coverage has been analyzed through FT-IR and TGA, the nature of coverage is analyzed through zeta potential measurements. These measurements reveal the relative colloidal stability of each method. Colloidal stability is related to the charge of the surface of the nanoparticles, which is indicative of their aqueous stability. Aqueous stability is a highly significant characteristic of IONPs, enabling biomedical and other applications[2].

Here, the products of all the tested methods display zeta potential measurements surpassing the 20 mV threshold, which indicates particle stability (Table 1).(11) To be more specific, the twice-sonicated product has the highest zeta potential, followed by the shaking product, followed by the once-sonicated product. However, as the measurement of the shaking product is performed at a different concentration from the sonication products, a reliable, direct comparison cannot be made. Therefore, between -30.94 mV for the shaking and -31.87 mV for the double sonication, it is unclear which is truly more stable. Comparison between the measurements of the sonicated products, both of which are conducted at the same concentration, indicates that the twice-sonicated product is more aqueous stable. This finding, combined with the results of the TGA, suggests that the twice-sonicated product may be preferable over the once-sonicated product.

Now that coverage and colloidal stability have been compared, the last factor to evaluate is the iron's oxidation state in the IONPs. As the IONPs are meant to be involved in subsequent tests and reactions, it is important to evaluate the oxidation state of the iron, as it will affect the IONPs' behaviour.(12)

According to the results of the XPS, the surfaces of the exchange products of each method are oxidized with respect to the starting IONP-OA. The peak values of the products are all greater than that of the oleic acid, which indicates a higher oxidation state; while the oleic acid peak appears at 711.3 eV, the once-sonicated and shaking products appear at 711.5 eV, and the twice-sonicated product appears at 711.5 eV. It is unclear, however, whether any particular method causes significantly more oxidation of the particles' surfaces. While there is a shift in binding energy before and after the ligand exchange, as evidenced by the difference in binding energy between the oleic acid sample and the rest of the samples, the peaks of the products of each method do not significantly vary from each other at 711.5 eV and 711.6 eV (Fig. 5). Therefore, the XPS results do not indicate whether any exchange technique causes more IONP oxidation than the others. Further trials and peak deconvolution are required to obtain a better understanding of the oxidation state of the iron in the XPS spectra.

Conclusion

Previous studies have investigated the relationships between ligands' chemical structures and the nature of their binding on magnetic IONPs. (9) This study further explores possibilities in ligand-design and ligand-exchange strategies, seeking to determine a standardized method for ligand exchange. The standardization of such a method will allow for reliable production and comparison of custom-built IONPs.

According to the FT-IR measurements, all methods result in ligand exchange. TGA measurements indicate that the twice-sonicated product appears to be the most favourable in terms of DOPAC coverage. According to the XPS measurements, all methods are comparable in terms of oxidation of the iron within the IONPs. It is important to underline that the shaking sample cannot be directly compared to the sonication methods due to inaccuracies of TGA and zeta potential measurements. Additionally, zeta potential measurements should be combined with sizing data, through dynamic light scattering or nanoparticle tracking analysis; this will enable confirmation of colloidal stability.

Further study to determine the best method of ligand exchange would benefit from larger data sets of FT-IR, TGA, zeta potential, and XPS. Zeta potential measurements must be repeated for the shaken product at a concentration that matches the other products, so that a direct comparison may be made. TGA must also be repeated for the shaking product to eliminate possible inaccuracies resulting from technical difficulties encountered in the measurement discussed above. It would additionally be useful in future to repeat the zeta potential measurements in different buffers to explore whether the nanoparticles' colloidal stability varies with the buffer in which they are suspended. Similarly, it would be useful to perform a comparison of these methods as they apply to ligands other than DOPAC, such as dopamine. This will allow for a conclusion that would apply to a wider variety of ligands. Additional testing should also include investigation of the oxidation states of the iron in each method's product. It may be useful to fit the $Fe^{3+/2+}$ octahedral and tetrahedral XPS peaks, in order to examine the ratio. This may provide information about changes in the crystal structure, magnetic properties, and oxidation. Furthermore, use of selected area electron diffraction (SAED), combined with transmission electron microscopy (TEM), may provide useful information about the crystallinity of the various products. It may also provide useful information relating to sizing and morphology of the nanoparticles. Lastly, further study should include comparison of supernatants, as well as FT-IR spectra of twice-sonicated products in methanol and other solvents. This may be useful for exploring the reason for loss of IONPs in the washing step following centrifugation after the second DOPAC addition. It is possible that this loss of product may be minimized or eliminated if a different solvent is used.

Through comparison of various methods of ligand exchange for superparamagnetic aqueous stable IONPs, this study comes one step closer to the drug discoveries and water treatments of the future. Through the standardization of a method of ligand exchange, the enormous potential for biomedical and other advancements can begin to be actualized.

Acknowledgements

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