MAGNEFY™ NEW PRODUCT DEVELOPMENT: MAGNETIC PARTICLE CHARACTERIZATION AND QC TESTING

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INTRODUCTION

Many types of magnetic particles are used as the solid phase in bioassays and separations. While their superparamagnetic properties may be similarly amenable to automation and high-throughput workflows, their specific physical and optical characteristics permit the development of highly tailored reagents for specific assay systems, formats, and platforms. For the particle manufacturer, this necessitates the design of a comprehensive characterization and QC plan, both to achieve routine synthesis objectives (defined specifications, reproducibility, stability), and to support use in specific applications.

In the case of our new magnetic microparticle, Magnefy™, we endeavored to manufacture a product with both broad application to assay development, and for focused use in nucleic acid isolation. Characterization of the product line and routine QC of production batches thus needed to encompass fundamental characteristics of the product line, defined specifications for each production batch/Lot, and application-specific performance requirements.

PRODUCT CHARACTERIZATION: GENERAL

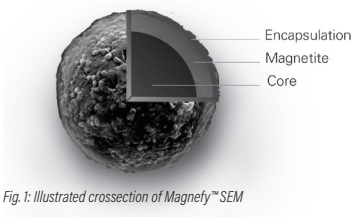


Fig. 1: Illustrated crossection of Magnefy™ SEM

Magnefy™ are ~1µm superparamagnetic microspheres comprised of a proprietary polymer core with an iron oxide layer and carboxylated polymer encapsulation.

Basic characterization of the product line includes fundamental particle traits such as general morphology / surface topography, size profile, carboxyl titration, iron content, and magnetic separation rate.

SEM IMAGING

As discrete physical characteristics of 1µm particles are below the resolution capabilities of standard light microscopy, SEM imaging was conducted to provide general information regarding gross morphology, surface roughness and size distribution. Magnefy demonstrated a regular spherical nature with cooperative fines. Imaging was performed using an FEI Quanta FEG 250 @ 8kv, secondary electron detector. (Fig. 1)

SIZE ANALYSIS

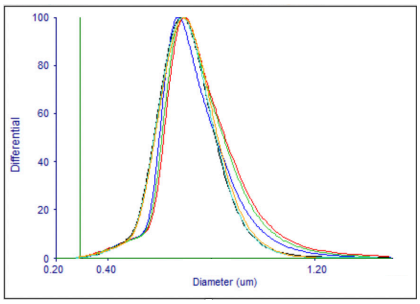


Fig. 2: Sizing Analysis

Size distribution analysis was performed using a BI-DCP (Brookhaven Disc Centrifuge) to capture the full particle population while circumventing low-end noise. Populations demonstrated a mean diameter of ~1µm and Gaussian distribution.

SURFACE COOH TITRATION

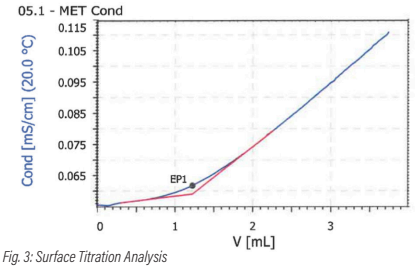


Fig. 3: Surface Titration Analysis

Magnefy COOH particles suspended in NaCl diluent underwent conductometric titration against NaOH under nitrogen (855 SR Ti Analyzer, Metrohm). Prior to titration, particle samples were cleaned using IX resin to remove loosely-associated/residual charged species from their surfaces. Particles demonstrated high levels of functionality, ~500µeq/g.

IRON OXIDE CONTENT

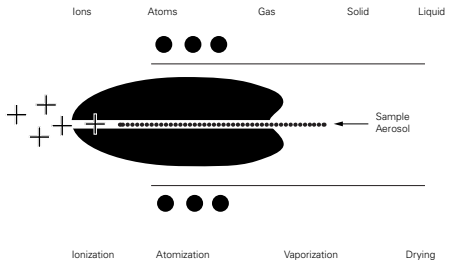
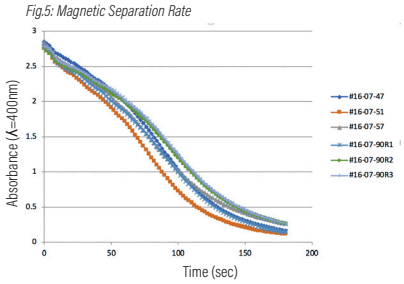


Fig. 4: ICP-MS Concept

Elemental iron determination was performed by an independent laboratory using inductively coupled plasma mass spectrometry (ICP-MS). Iron (Fe) content was found to correspond to ~40% iron oxide (Fe_3O_4).

MAGNETIC SEPARATION RATE

Fig.5: Magnetic Separation Rate



Magnetic separation rate was determined by measuring absorbance over time (Gensys 10S UV-Vis Spectrophotometer, λ = 400nm). Magnefy populations separated rapidly and uniformly.

PRODUCT CHARACTERIZATION: APPLICATION SPECIFIC TESTING

In addition to traditional immunoassay development, we anticipated significant use of Magnefy in molecular applications, including assay development and nucleic acid isolation. Unlike immunoassays, which are typically conducted under protein- and cell-friendly conditions (near-neutral pH, ambient temperatures, aqueous buffers), molecular applications often feature harsh / extreme conditions. Magnefy particles were monitored under a range of such conditions with respect to [Fe] leaching, magnetic separation, and size distribution to assess stability and define limitations / serve as an indicator for suitability. Conditions tested included pH extremes, high salt, chaotrope exposure, temperature cycling, and rigorous washing.

pH Range (pH 1 – pH 14, 15 days)

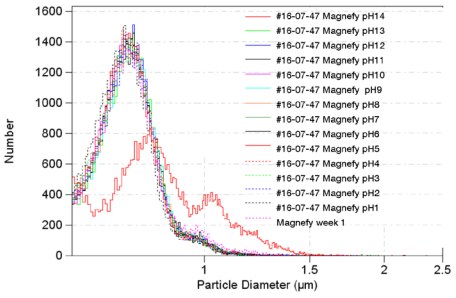


Fig. 6: Sizing under various pH conditions

As evidenced through particle sizing (Coulter M3), Magnefy remained dispersed in solutions from pH 1 – pH 13. Particles formed doublets / triplets at pH 14 (Day 15). Formation of doublets/triplets resulted in accelerated magnetic separation. (Fig.7a)

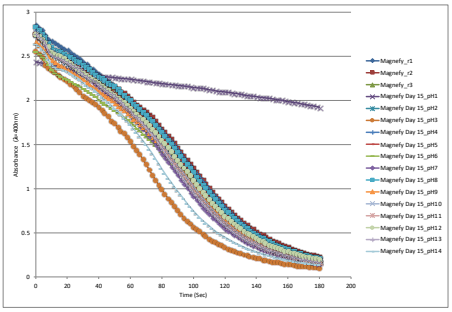


Fig. 7a: Separation under various pH conditions

Dispersion Medium	pH	Day (s)	Supernatant [Fe] (µg/mL)	Supernatant Color
0.1 M HCl	1	15	139.40	clear
0.01 M HCl	2	15	7.65	clear
0.001 M HCl	3	15	3.25	clear
0.0001 M HCl	4	15	-0.03	clear
10 mM MES buffer	5	15	-0.07	clear
10 mM MES buffer	6	15	-0.09	clear
10 mM MES buffer	7	15	-0.09	clear
10 mM Tris buffer	8	15	-0.04	clear
10 mM Tris buffer	9	15	-0.07	clear
0.0001 M NaOH	10	15	-0.09	clear
0.001 M NaOH	11	15	-0.09	clear
0.01 M NaOH	12	15	-0.09	clear
0.1 M NaOH	13	15	-0.16	clear
1 M NaOH	14	15	0.00	clear

Fig. 7b: Separation under various pH conditions

Particle integrity was fully maintained in solutions of pH 4 – pH 14 (15 days). Catastrophic degradation via acid etching of iron oxide was observed with prolonged exposure to strong acid (0.1M HCl pH 1, 15 days), as evidenced by [Fe] leaching (abs λ562 nm) and loss of magnetic responsiveness (Fig. 7a). Prolonged exposure to pH 2 and pH 3 resulted in some [Fe] leaching, though this did not significantly impact the magnetic separation profile

High Salt (1M – 5M NaCl, 15 days)

Magnefy remained singly dispersed and did not leach [Fe] when suspended in solutions of 1M, 2M and 5M NaCl for 15 days. Sizing and magnetic separation profiles were as expected.

Chaotrope (1M – 6M Guanidine Thiocyanate)

Magnefy (1mg / mL) were tested in 1M – 6M guanidine thiocyanate in 10mM Tris at pH 6.4 and pH 8. Particles were tested for iron leaching at 1hr, 4hr, 7hr, and 24hr time points. No leaching was observed. Dispersion and magnetic responsiveness were also tested at 24hr; particle sizing and magnetic separation were unaffected by chaotrope exposure, and yielded expected profiles.

Temperature Cycling (95°C 5min / RT 5 min x 50 cycles)

Magnefy (1mg/mL particles in 10mM Tris pH 9) underwent temperature cycling (95°C 5min / RT 5min) for 50 cycles. Sizing and magnetic separation were unaffected by temperature cycling, and yielded expected profiles.

Rigorous Washing (10 x Washing)

Magnefy (1mg/mL) were washed with de-ionized water / magnetic separation 10 times. Particles were re-dispersed by pipetting only. Particle size and magnetic separation profiles indicated the fidelity of the dispersions (no evidence of aggregation or stickiness), and no [Fe] was observed in the supernatant.

APPLICATION-SPECIFIC TESTING: DNA ISOLATION

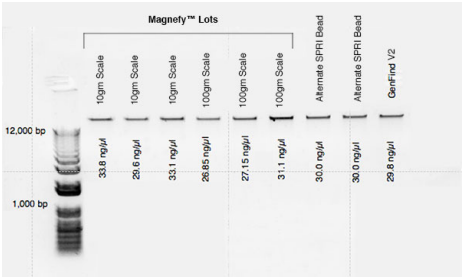


Fig. 8: DNA Isolation

One intended application for Magnefy included nucleic acid isolation, most notably, their use as a solid phase for SPRI (solid phase reversible immobilization). SPRI features the binding of DNA or RNA to a carboxylated magnetic support under conditions of high salt and PEG.

SPRI-based isolation of genomic DNA from mammalian blood (goat) was used as a model system. Magnefy bound gDNA from lysed whole blood under conditions of 2.5M NaCl / PEG 8000 for the preparation of PCR-ready DNA.

DISCUSSION

Magnefy underwent extensive product characterization, including SEM imaging, sizing, surface titration, iron content determination, and magnetic separation rate to demonstrate general suitability for assay development. Application-based stress testing (pH extremes, high salt, chaotrope exposure, temperature cycling, rigorous washing) and functional testing (SPRI-based gDNA isolation) were undertaken to support use in molecular applications, most notably nucleic acid isolation.

Initial product characterization also provided a framework for the establishment of formal manufacturing and QC specifications. Analyses that are featured in routine QC for Magnefy include sizing, surface titration, magnetic separation rate, and gDNA isolation.

CONCLUSIONS

In particle manufacturing, product characterization and QC programs should be comprehensive, speaking to basic characteristics for general use, defined manufacturing and QC specifications, and any application-specific requirements. In the case of Magnefy, initial characterization and routine QC evidence basic characteristics and quality that support use in assay development, (e.g. high surface titer for ligand coating, size and magnetic separation profiles that are suitable for automated analyzers, etc). Specialized stress testing and functional testing (gDNA isolation) support use in molecular applications, and SPRI-based DNA isolation in particular.

A manufacturer's particle characterization and QC cannot guarantee optimal performance for every application or assay, but they aid in the identification of promising candidates for particle screening, and provide a foundation for microparticle reagent development efforts.

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