Using Magnetic Microparticles in Molecular and Cellular Isolations

a report by
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Introduction to Magnetic Microparticles

In the past five decades, major advances have been achieved in molecular diagnostic testing for infectious diseases. Many of the significant advances are attributed to the use of magnetic microparticles in research laboratories as a solid phase support. Their ability to isolate or absorb various molecules in applications such as ribonucleic acid (RNA) purification, magnetic cell separation, magnetic particle enzyme immunoassay (EIA) and many other applications makes them beneficial to molecular and cellular isolations.

During immunoseparations, magnetic microparticles target and separate cells from whole blood, bone marrow, core blood and prepared samples such as buffy coat, mononuclear cells and tissue digests. These particles are very useful for the isolation of T cells, B cells, stem cells, stromal cells, cancer cells, as well as proteins and nucleic acids. However, there are endless applications waiting to be discovered.

Magnetic microparticles supplied by Spherotech, Inc. are prepaired using uniform, monodispersed, polystyrene particles as the core. The polystyrene particles used are prepared using emulsion polymerization. Due to the quality of the polystyrene seed particle, the magnetic particles are also uniform in size and shape. This ensures that the physical and chemical properties of the particle are consistent. As a result, the binding between the particle and its intended target is rapid and efficient. Magnetic microparticles are available uncoated, fluorescent, functionalized and precoated with antibodies or ligands in a variety of sizes. The bead's activity is determined by the molecular chemistry coated on to its surface and size.

The Characteristics and Uses of Paramagnetic Microparticles

One type of magnetic microparticles manufactured by Spherotech is paramagnetic. They are prepared by coating a layer of iron oxide and polystyrene onto monodispersed, polystyrene core particles. As a result, the magnetic particles are spherical in shape, uniform in size, and paramagnetic in nature. Their uniformity is shown in **Figure 1.** The iron content of these magnetic particles can be adjusted. However, in general, iron represents about 10% to 15% of the particle. Due to their iron content they are easily separated from a suspension using a SPHERO™ Magnetic Separator. In addition, they resuspend when removed from a magnet since no detectable magnetism is retained even after repeated magnetic field exposure. Their magnetism is utilized as a rapid, efficient means for separating the beads from the supernatant without centrifugation.

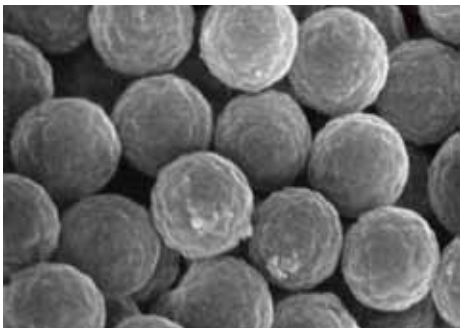
Figure 1 SEM Photo of Spherotech Cat. No. CM-30-10 (Carboxyl Magnetic Particles, 2.5% w/v, 3.36µm, 10 mL). at 5000x.

Surfaces of magnetic polystyrene particles can be modified with functional groups to suit various applications. These paramagnetic particles, ranging in size from 0.25µm to 200.0µm, are manufactured with surface functional groups such as carboxyl or amino. As a result, covalent coupling of proteins, antibodies or antigens are possible. Carboxylated functionalized magnetic microparticles are used for protein coupling with carbodimides. The amino functionalized magnetic particles contain amino groups for glutaraldehyde or carbodimide coupling to proteins. After coating, magnetic particles are used for cell separation, affinity purification, DNA probe assays, magnetic particle EIA, etc. The paramagnetic particles are available in four formats: smooth surface, cross-linked, high iron, and fluorescent.

Smooth Surface Magnetic Microparticles

The first form of paramagnetic particles is the smooth surface magnetic particles. These particles have a thick polymer layer to encapsulating its iron oxide layer. As a result, the surface is iron oxide-free. This eliminates any interferences with enzyme activities or undesirable effects caused by exposed magnetite. In addition, iron will not leach from the particles in aqueous solutions. An example of a smooth surface magnetic bead is shown in **Figure 2**

Figure 2 SEM photo of SPHERO™ Smooth Surface Magnetic Particles, CMS-40-10 (Carboxyl Magnetic Particles, Smooth, 2.5% w/v, 4.6µm, 10 mL) at 5000x.



Cross-linked Magnetic Microparticles

The second form of paramagnetic particles is the cross-linked magnetic particles. These particles are coated with cross-linked polymer on the surfaces of iron oxide crystals. This renders them resistant to common organic solvents such as acetone, acetonitrile, dimethylformamide (DMF) and chloroform. The size of this type of particles ranges from 1–15µm. Cross-linked magnetic particles have significantly greater surface area and iron oxide content compared with the 4µm linear polystyrene magnetic particles. The cross-linked magnetic particles are the ideal solid phase for cell separation, affinity purification applications, and the magnetic removal of micro-organisms, viruses, and cross reactants in serum.

High Iron Paramagnetic Particles

A new product line Spherotech is proud to introduce is our innovative High Iron Paramagnetic Particles. These have a higher percentage of iron content than our regular paramagnetic particles. As a result, the separation time of the beads from the supernatent is reduced. The High Iron particles are supplied as amino or carboxylated functionalized nanoparticles which have the potential to be coated with protein. As a customer requested product, Streptavidin Coated Yellow Fluorescent Magnetic Particles were engineered in our High Iron products line for target separation from whole blood. Currently, Spherotech is offering only selected types of High Iron particles; however, custom particles can be manufactured in our Research & Development department.

Fluorescent Magnetic Microparticles

The last form of the paramagnetic particles are the fluorescent magnetic particles. These are magnetic particles that are also fluorescent at different excitation wavelengths. They are prepared by either staining the polystyrene particles with a solution of appropriate fluorophore or by polymerizing a layer of fluorophore in styrene onto the polystyrene core particles. The fluorophores used during preparation are water insoluble. As a result, they are very stable once incorporated into the particles. These fluorophores do not leach. In addition, their color and fluorescence remains stable for long periods of time under proper storage conditions. They are available in amino and carboxylated functionality in six different excitation/emmision wavelengths. Please refer to Sphero Fluorescent Particles catalog pages at http://www.spherotech.com/product%20detail%20files/fluorescent%20beads.pdf for the spectra of the fluorophores used by Spherotech. These particles are chosen for their uniformity, diameter and fluorescent dye content.

Paramagnetic Microparticles Coated with Antibodies and Proteins

Another benefit of paramagnetic particles is they can be coated with antibodies and proteins. The antibodies and ligand coated on the magnetic particles intended for cell separation include Goat anti-Mouse IgG (H&L), Goat anti-Mouse IgG (Fc), Goat anti-Mouse IgG (H&L cross-absorbed), Goat anti- Rabbit IgG (H&L), Goat anti-Mouse IgG (Fc), Goat anti-Human IgG (H&L), Sheep anti-Rat IgG (H&L), biotin, avidin and streptavidin. Likewise, Protein A and Protein G-coated magnetic particles are used for binding various species of IgG from human, mouse and rabbit serum. In addition, Spherotech also manufactures fluorescent coated paramagnetic particles. These beads are available coated with Goat anti-Mouse IgG (Fc), Streptavidin, and Biotin. These coated fluorescent magnetic particles typically contain Spherotech's Yellow, Nile Red, or Pink fluorophore. As a result, the assay target is detected via fluorescence based instrumentation after magnetic separation from the biological samples. Nonetheless, coated magnetic beads offer a wide range of chemical and surface properties that are used for cell separation and modification.

Ferromagnetic Microparticles

In addition to paramagnetic particles with iron oxide, Spherotech also manufactures ferromagnetic particles with chromium dioxide. Ferromagnetic particles retain magnetism once exposed to a magnetic field. As a result, these particles are useful for studying mechanotransduction across cell surfaces and through the cytoskeleton. This is performed by binding particles to a cell surface receptor and applying mechanical stress directly using a device to twist the particle. This application is possible since these particles are provided functionalized and can be coated so that they are specific to a cell surface receptor. Functionalized ferromagnetic particles are prepared by coating a thin layer of functionalized polymer onto the surface. Afterwards, proteins can be covalently coupled to the particle's functional groups. Currently, Goat anti-Mouse IgG (H&L) and Streptavidin coated ferromangtic beads are also available from Spherotech. However, other polyclonal antibodies, proteins and ligands can also be coupled to ferromagnetic particles as custom products.

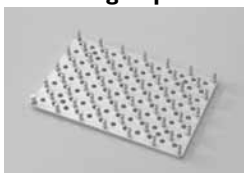
Magnetic Separators

Spherotech offers magnetic separators to easily separate paramagnetic and ferromagnetic particles from a suspension. A variety of designs and sizes are available to accommodate different working volumes. For example, the FlexiMag Magnetic Separator contains interchangeable tube holders to secure different sizes of tubes and bottles. The FlexiMag Magnetic Separator Jr. separator uses 1.5mL microfuge tubes, 5mL cryovials, 10x75mm or 12x75mm tubes. In addition, our HandiMag separator can also be used with various containers such as microfuge tubes, test tubes or centrifuge tubes since it only requires secure placement by hand or rubber band. Spherotech also offer magnetic separators for particular tests and plates. For instance, the MiniTube Mag Separator can be used with sixteen 1.5mL tubes. For 96-well plates, the MicroMag, UltraMag, and UltraMag Deep Well (DW) Separators are available. See http://www.spherotech.com/mag_sep.htm for more details.

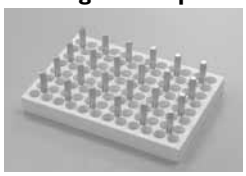
MicroMag Separator

FlexiMag Separator Jr.

UltraMag Separator



UltraMag DW Separator



Conclusion

The characteristics and functionality of magnetic microparticles and their applications as a novel solid phase separation technique have been discussed. Microparticles are synthesized to contain chemically active groups to perform selective separations. Designed to produce a particle-target complex, they can be isolated easily from the sample matrix using a SPHERO™ Magnetic Separator. The particle-target complex is then separated from the sample matrix by removing the supernatant. The magnetic microparticle function is determined by its size, coating properties, type of magnetic material, and particle structure. Nonetheless, SPHERO™ magnetic microparticles are used in small scale research as well as with automated high-throughput diagnostic analyzers around the world.