

OptiBind[®] Polystyrene and OptiLink[®] Carboxylate-Modified Microparticles

September 2005

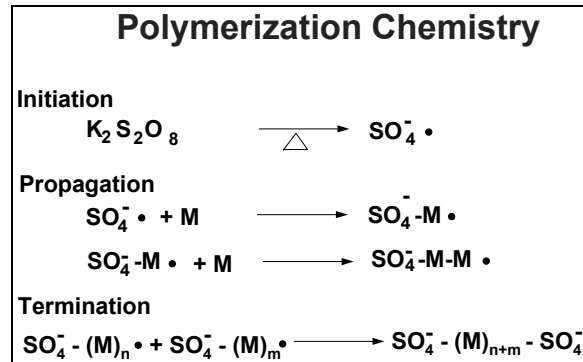
Microparticles The word “microparticle” refers to individual plastic spheres in the size range of 0.03 μM to 2 μM in diameter. A suspension of microparticles has the milky appearance of latex rubber. For this reason, microparticle suspensions have historically been referred to as latex. We prefer to use the term microparticle (MP) in our literature. Other manufacturers use terms such as: microsphere, nanosphere, bead, etc. to describe the same product.

Most OptiBind and OptiLink particles are polystyrene (PS) based with a coefficient of variation (CV) of diameter of less than 2%. Although the individual particles are transparent and colorless, the white color of a MP suspension is caused by light scattering. Iridescent colors (pink, green, blue, etc.) are sometimes seen with MP suspensions. This occurs when the uniform particles line up in regular arrays and act as a diffraction grating. This is not abnormal; it is a property of particle uniformity and most often occurs when MPs have been cleaned or after prolonged storage.

Traditionally called CMs (carboxyl-modified latex), these particles are composed primarily of polystyrene (95-99%). A secondary acidic monomer, typically acrylic acid, is used to add carboxyl groups to the surface. They have the same refractive index as PS-MP. The percent of carboxyl coverage on the surface can be varied from ~1% to 100% depending on the manufacturing formulation. These carboxyl groups provide: a handle for covalent coupling, additional negative charge for colloidal stability (see below), and a hydrophilic surface.

Manufacture Our microparticles are made by emulsion polymerization. For plain polystyrene microparticles (PS-MPs), the ingredients are water, styrene, a buffer, a surfactant, and an initiator. When mixed together, the surfactant emulsifies the styrene into the water phase, where the polymerization begins. Upon heating, the initiator breaks down into primary free radicals.

Manufacture Cont'd



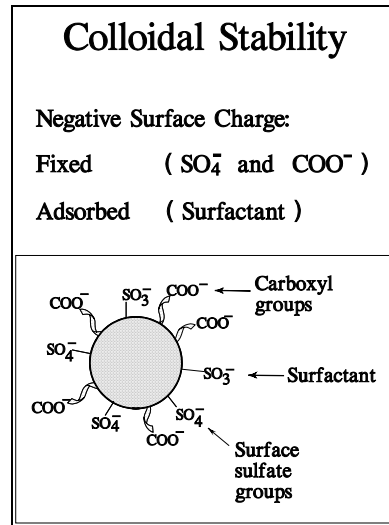
In the initiation step, a sulfate free radical reacts with the vinyl group of styrene forming a sulfated free radical. This reacts with another styrene, to begin chain propagation. Chain termination results from the collision of two growing chains or one growing chain with another primary free radical. Thus, each polystyrene chain has a sulfate group at both ends. The chains fold up as they become insoluble, forming a surface active seed particle. The growing spheres are stabilized in water by the absorption of surfactant and by orienting with the hydrophobic styrene groups inside and the charged sulfate groups on the surface. These seed particles absorb styrene and free radicals from the aqueous phase and continue to grow until the styrene is depleted.

The carboxyl-modified particles (CM-MPs) are made by a process similar to that used in making plain PS-MPs. The one additional ingredient is acrylic acid which is copolymerized with the styrene. The carboxyl groups reside on the surface. CM-MPs can be produced with a wide range of carboxyl content. The carboxyl content of a CM-MP can have dramatic effects on the surface characteristics and colloidal stability. This in turn affects the MP reagent sensitivity and stability.

Colloidal Stability

A suspension of MPs is said to be colloidal stable if they remain suspended and separate. Colloidal particles exhibit Brownian motion due to their small size. This allows the particles to resist the pull of gravity and remain suspended. Electrostatic repulsion arises from electric charge on the surface which prevents the MPs from sticking together. The combined effect of electrostatic repulsion and Brownian motion provides colloidal stability.

Colloidal Stability



The negative surface charge of our PS-MPs is derived from sulfate groups and adsorbed surfactant. CM-MPs additionally have negative charge from carboxyl groups. ***Anything that changes the net charge on the surface will affect the colloidal stability.*** This concept is very important in processing MPs.

Microparticles with diameters greater than about $0.4 \mu\text{M}$ settle at an appreciable rate because the pull of gravity overcomes the Brownian motion of the particles. If particles are settled, resuspend by swirling, rolling, or sonication. Avoid producing foam. PS-MP particles with diameters greater than $0.6 \mu\text{M}$ should be resuspended monthly to prevent irreversible aggregation.

Storage and Handling

The PS-MP are made with deionized water under carefully controlled conditions. After dilution and bottling, MPs are pasteurized and sealed to prevent contamination. We recommend storing MPs at $2 - 8^\circ \text{C}$. Once opened, additional precautions are necessary to prevent contamination. These include:

- Pour from the bottle instead of repeatedly pipetting
- Make a stock solution
- Prepare working aliquots
- Keep the bottle closed when not in use
- Keep refrigerated

Proprietary Surfactant

Seradyn MPs can be used *as is* for most applications. The anionic surfactant ***does not*** interfere with the binding of proteins and ***does not*** cause proteins to desorb from MP surfaces (Seradyn, unpublished studies). The common surfactants or detergents used in biological applications (SDS, Tween 20, Triton X-100) can remove protein from MP surfaces, but are ***not used*** in Seradyn MP production.

Cleaning

The PS-MP are designed to be used without further treatment—*they do not need to be cleaned for most applications*. Some buffer salts, soluble polymer, and free detergent are present in the aqueous phase after polymerization. Surfactant molecules adsorbed onto the surface of the particles contribute to colloidal stability. PS-MP may be cleaned by ion exchange, membrane filtration, or centrifugation. However, removing the adsorbed surfactant may cause aggregation of PS-MP. In-house data demonstrates that our proprietary surfactant does not interfere with protein binding. For these reasons, we *do not* recommend cleaning PS-MPs.

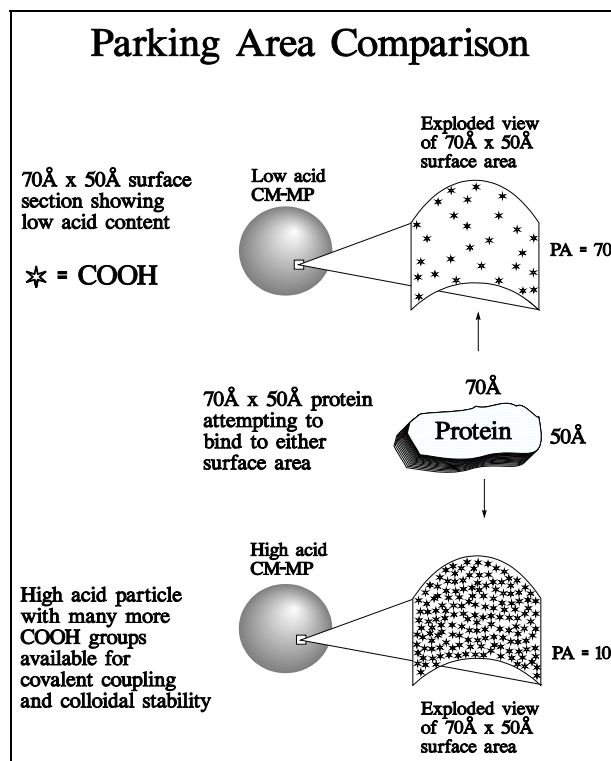
The CM-MPs may be used *as is* for most applications. Soluble polymer (short chain polyacrylic acid) is present in CM-MPs and the amount of soluble polymer increases with the carboxyl content. In general, soluble polymer does not appear to interfere with protein binding or with covalent coupling reactions (Seradyn, Inc., unpublished data). While we do not routinely recommend cleaning CM-MPs, this should be decided on a case-by-case basis.

Surface Characteristics

PS-MP surfaces are very hydrophobic and adsorb proteins almost instantaneously. Sulfate content is determined by conductometric titration with NaOH of ion exchange resin cleaned microparticles. Sulfate content is reported in milliequivalents of sulfate per gram solids of PS-MPs (meq/g), and Parking Area (PA). PA is the calculated surface area per functional group. Note that a high sulfate PA number denotes a low surface density of sulfate groups. PA is given in units of angstroms squared, Å².

The surface characteristics of CM-MPs are highly dependent upon the amount of carboxylic acid incorporated. Below are some classifications based on carboxyl content. Acid *content* refers to the titrated milliequivalents per gram (mEq/g) and is weight-based. However, the surface area per gram of an MP suspension varies inversely with particle diameter. Therefore, one cannot compare particles of different diameters easily using only the acid content. PA does relate surface carboxyl concentration between MPs of different diameters.

Parking Area



Two particles of the same diameter which have different parking areas will “look” different to a protein molecule. The protein molecule will “park” on different numbers of carboxyl groups on either particle and have a disproportionate opportunity for covalent coupling. Greater covalent bond density will occur with the high acid surface. Our reagent development strategy utilizes acid content to optimize sensitivity and stability. Both acid content and parking area data are available for all lots of CM-MPs.

Parking Area Values (CM-MP)

Acid Content	Parking Area	Properties
Low	60 – 85	Hydrophobic surface, more colloiddally stable than polystyrene.
Medium	35 – 59	Hydrophobic and hydrophilic areas; good colloidal stability and good covalent coupling.
High	10 – 34	Hydrophilic surface; excellent colloidal stability; excellent covalent coupling.

The surface area per gram of a microparticle varies inversely with the particle diameter. Acid content refers to the titrated milliequivalents pergram (meq/g) and is weight based. Parking Area combines acid content and surface area to give you the surface acid distribution which is useful for assay optimization.

Benefits

We believe CM-MP particles to be the best overall choice for immunoassay reagent development. The acid content of a carboxylated particle is a very important criterion in particle selection. Implicit in this statement is the belief that covalent coupling is preferable to adsorption alone for binding proteins to particles. The reasons for this are that **carboxylated particles**:

1. **Adsorb protein more efficiently** than plain polystyrene particles (adsorption precedes covalent coupling to particle).
2. **Give you a choice of surface acid content**, which allows you to match your protein to the best particle for that protein (both in terms of total adsorption and colloidal stability).
3. **Permit adjustment of the colloidal properties** through surface charge. Carboxyl groups can be neutralized, converted to a positive charge, or reacted to form covalent bonds to protein. If left as carboxyl groups, the charge can be adjusted by changing the pH of the storage or reaction buffer.
4. **Insure little or no leaching of bound protein** with:
 - Extended time
 - Detergents
 - Changes in pH
 - Changes in ionic strength
5. **Greater flexibility**:
Covalent coupling with carboxyl-modified microparticles gives greater flexibility in the formulation of storage and reaction buffers.

**Ordering
Opti-Bind
and
Opti-Link
Microparticles**

OptiBind Polystyrene and Opti-Link Carboxylate Microparticles
(Nominal diameter - 10% solids concentration, pasteurized)
Standard Product listed. Ask for custom particles or packaging
availability. Carboxylate-modified products are sorted by parking area.

OptiBind Polystyrene		Catalog No.
0.1 μ M	PS	8100-0397
0.2 μ M	PS	8100-0597
0.3 μ M	PS	8100-0797
0.4 μ M	PS	8100-0997
0.5 μ M	PS	8100-1197
0.6 μ M	PS	8100-1397
0.8 μ M	PS	8100-1797
1.0 μ M	PS	8100-2197

OptiLink Carboxylate-Modified		Catalog No.
0.2 μ M	CM PA=20	8300-0520
0.3 μ M	CM PA=20	8300-0720
0.4 μ M	CM PA=20	8300-0920
0.8 μ M	CM PA=20	8300-1720
1.0 μ M	CM PA=20	8300-2120

OptiLink Carboxylate-Modified		Catalog No.
0.1 μ M	CM PA=50	8300-0350
0.2 μ M	CM PA=50	8300-0550
0.3 μ M	CM PA=50	8300-0750
0.4 μ M	CM PA=50	8300-0950
0.8 μ M	CM PA=50	8300-1750

OptiLink Carboxylate-Modified		Catalog No.
0.1 μ M	CM PA=70	8300-0370
0.2 μ M	CM PA=70	8300-0570
0.3 μ M	CM PA=70	8300-0770
0.4 μ M	CM PA=70	8300-0970
0.8 μ M	CM PA=70	8300-1770

Please advise us of desired package size and preservatives, if required.

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Other Products

You are cordially invited to visit our facilities any time you are in the Indianapolis area. We are just minutes from the Indianapolis International Airport. Please inquire about our other exceptional microparticle products. We have a complete range of microparticles to suit your individual requirements.