Spherotech, Inc.
27845 Irma Lee Circle, Unit 101, Lake Forest, IL 60045

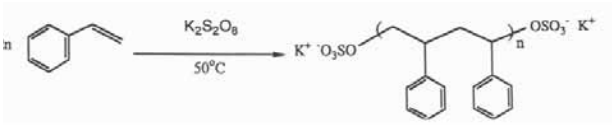
SPHERO™ Polystyrene Particles

- Uniform Shape and Size
- Multi-liter Capabilities
- Available from 0.05 to 200 µm

The SPHERO™ polystyrene particles are prepared by conventional emulsion polymerization with styrene as the monomer and potassium persulfate or benzoyl peroxide as polymerization initiator. In general, microparticles less than 0.5 µm are prepared in one step followed by a cleaning step on mixed bed ion exchange resin to remove detergent and inorganic salts. Larger particles are prepared by step wise growing of smaller particles with the addition of styrene monomer and initiator without any additional detergent. The microparticles are cleaned by repeated centrifugation. Cleaned microparticles are resuspended in deionized water. Sodium azide (0.02%) is added as bacteriostatic. As a result, the **SPHERO**™ microparticles can be coated with proteins without further cleaning.

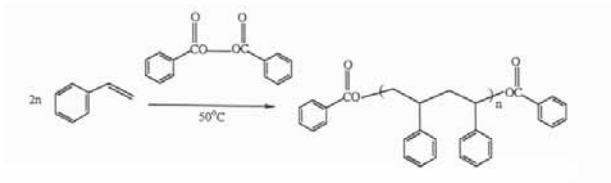
Microparticles made using potassium persulfate as initiator have sulfate groups on their surface. As a result, these particles are negatively charged and are hydrophilic, as shown in equation (1).

(1)



The **SPHERO**™ polystyrene particles greater than 3 µm are usually prepared using benzoyl peroxide as the initiator. These particles are relatively more hydrophobic, as shown in equation (2).

(2)



SPHERO™ polystyrene particles are composed of linear polystyrene without any cross-linking agent. These particles cannot tolerate organic solvents such as toluene, xylene, chloroform, methylene chloride, acetonitrile, dimethyl formamide (DMSO) or acetone. However, SPHERO™ polystyrene particles are stable in the presence of some water miscible solvents such as dimethyl sulfoxide and alcohols. Uniform size cross-linked polystyrene particles that are stable in the presence of organic solvents are also available.

Uniform SPHERO™ polystyrene particles are ideal for use in immunoassays such as latex agglutination, particle base enzyme immunoassays and fluorescence immunoassays. A tight size range of SPHERO™ polystyrene particles is maintained by monitoring size using a NICOMP Laser Particle Sizer (for particles less than 3 µm) and a Scanning Electron Microscope and/or Beckman Coulter Z3 Multisizer™ 3 for larger particles. Although the size measurements are accurate, these particles are not certified for use as calibration standard for size measurements or pore size analysis.

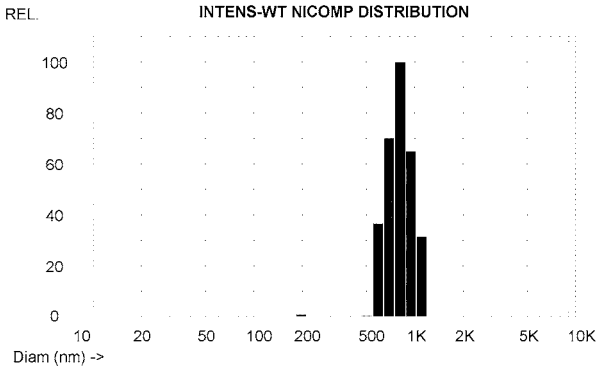


Figure 2 Histogram of SPHERO™ 0.8 µm Polystyrene Particles from the NICOMP Laser Particle Sizer

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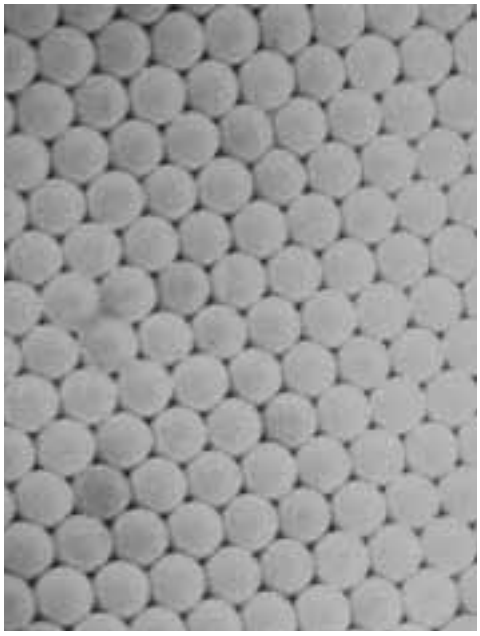
SPHERO™ Polystyrene Particles

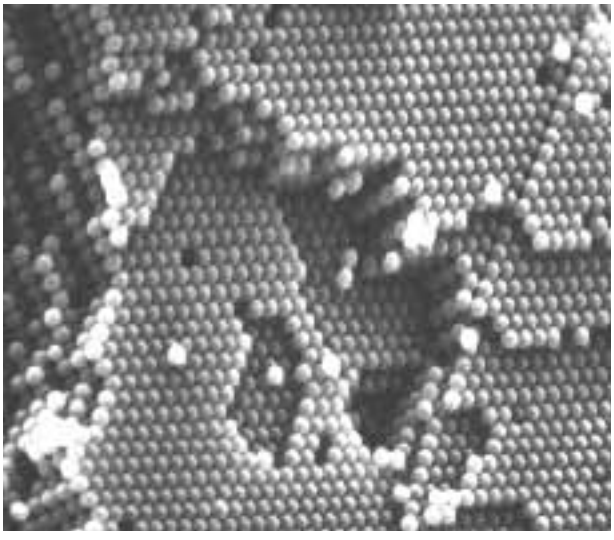
Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Polystyrene	0.05-0.1	5.0	PP-008-10	10 mL
			PP-008-100	100 mL
Polystyrene	0.2-0.3	5.0	PP-025-10	10 mL
			PP-025-100	100 mL
Polystyrene	0.4-0.6	5.0	PP-05-10	10 mL
			PP-05-100	100 mL
Polystyrene	0.7-0.9	5.0	PP-08-10	10 mL
			PP-08-100	100 mL
Polystyrene	1.0-1.4	5.0	PP-10-10	10 mL
			PP-10-100	100 mL
Polystyrene	1.5-1.9	5.0	PP-15-10	10 mL
			PP-15-100	100 mL
Polystyrene	2.0-2.4	5.0	PP-20-10	10 mL
			PP-20-100	100 mL
Polystyrene	2.5-2.9	5.0	PP-25-10	10 mL
			PP-25-100	100 mL
Polystyrene	3.0-3.4	5.0	PP-30-10	10 mL
			PP-30-100	100 mL
Polystyrene	3.5-3.9	5.0	PP-35-10	10 mL
			PP-35-100	100 mL
Polystyrene	4.0-4.4	5.0	PP-40-10	10 mL
			PP-40-100	100 mL
Polystyrene	4.5-4.9	5.0	PP-45-10	10 mL
			PP-45-100	100 mL
Polystyrene	5.0-5.9	5.0	PP-50-10	100 mL
			PP-50-100	10 mL
Polystyrene	6.0-8.0	5.0	PP-60-10	100 mL
			PP-60-100	10 mL
Polystyrene	8.0-12.9	2.5	PP-100-10	10 mL

Figure 3 Scanning Electron Microscope (SEM) photos of polystyrene particles are shown below to illustrate the uniformity of their size. (a) Single sheet of 0.8 µm polystyrene particles. (b) Face-centered-cubic packing of 0.86 µm particles. Theoretically, particles fill ~74% of the space regardless of their size. (c) 3.4 µm polystyrene particles on the surface of a human hair, which is about 100 µm in diameter.

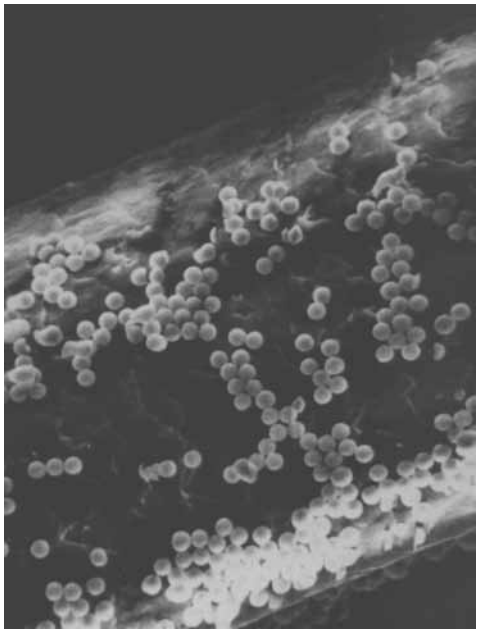
(A)



(B)



(C)



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SPHERO™ Cross-linked Polystyrene Particles

Non-Uniform Crosslinked Particles

- Cost effective alternative if uniform shape is not required
- Uniform size distributions
- Stable in organic solvent

Uniform Crosslinked Particles

- Highly uniform and monodispersed
- Available from 3 micron to 30 micron
- Stable in only aqueous solvent

Spherotech offers a wide range of cross-linked polystyrene particles. Both non-uniform and uniform shape cross-linked polystyrene particles are manufactured at Spherotech. The low cost non-uniform particles are useful when particle shape does not matter. These non-uniform cross-linked polystyrene particles are stable in the presence of organic solvents. Figure 4 shows the differences between the polymeric particles consisting of polystyrene and particles made from copolymers, styrene/divinylbezene.

If perfect spherical monosized polymer particles are needed Spherotech also has cross-linked polystyrene particles that are uniform in size and shape. However, these particles are not stable in the presence of organic solvents. Figures 5 and 8 show Beckman Coulter Multisizer™ 3 histograms for Cat. No. PPX-150-10 (Polystyrene Particles, Crosslinked, 2.5% w/v, 15.2 µm, 10 mL) and Cat. No. CPX-30-10 (Carboxyl Polystyrene Particles, 0.5% DVB Crosslinked, 5% w/v, 3.0-3.4 µm, 10 mL).

SPHERO™ Large Research Grade Cross-linked Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Polystyrene, Cross-linked, Research Grade	38.0-44.0	5.0	PPX-400-10	10 mL
Polystyrene, Cross-linked, Research Grade	53.0-62.0	5.0	PPX-600-10	10 mL
Polystyrene, Cross-linked, Research Grade	90.0-105.0	5.0	PPX-1000-10	10 mL
Polystyrene, Cross-linked, Research Grade,	125.0-149.0	5.0	PPX-1400-10	10 mL
Polystyrene, Cross-linked, Research Grade	212.0-249.0	5.0	PPX-2200-10	10 mL

SPHERO™ Non-Uniform Cross-linked Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Polystyrene, Cross-linked, non-uniform shape	0.4-0.6	5.0	PPX-05-10	10 mL
Polystyrene, Cross-linked, non-uniform shape	0.7-0.9	5.0	PPX-08-10	100 mL
Polystyrene, Cross-linked, non-uniform shape	1.0-1.9	5.0	PPX-10-10	10 mL
Polystyrene, Cross-linked, non-uniform shape	2.0-2.4	5.0	PPX-20-10	10 mL
Polystyrene, Cross-linked, non-uniform shape	2.5-2.9	5.0	PPX-25-10	10 mL

SPHERO™ Uniform Cross-linked Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Polystyrene, Cross-linked	5.0-5.9	5.0	PPX-50-10	10 mL
Polystyrene, Cross-linked	8.0-12.9	2.5	PPX-100-10	10 mL
Polystyrene, Cross-linked	13.0-17.9	2.5	PPX-150-10	10 mL
Polystyrene, Cross-linked	24.0-30.0	2.5	PPX-250-10	10 mL

SPHERO™ Functionalized Cross-linked Polystyrene Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Carboxyl-polystyrene, Cross-linked	3.0-3.4	5.0	CPX-30-10	10 mL
Carboxyl-polystyrene, Cross-linked	5.0-5.9	2.5	CPX-50-10	10 mL
Carboxyl-polystyrene, Cross-linked	6.0-6.9	2.5	CPX-60-10	10 mL
Carboxyl-polystyrene, Cross-linked	8.0-12.9	2.5	CPX-100-10	10 mL
Carboxyl-polystyrene, Cross-linked	13.0-17.9	2.5	CPX-150-10	10 mL
Amino-polystyrene, Cross-linked	2.0-2.9	1.0	APX-20-10	10 mL
Amino-polystyrene, Cross-linked	3.0-3.4	2.5	APX-30-10	10 mL
Amino-polystyrene, Cross-linked	6.0-6.9	2.5	APX-60-10	10 mL

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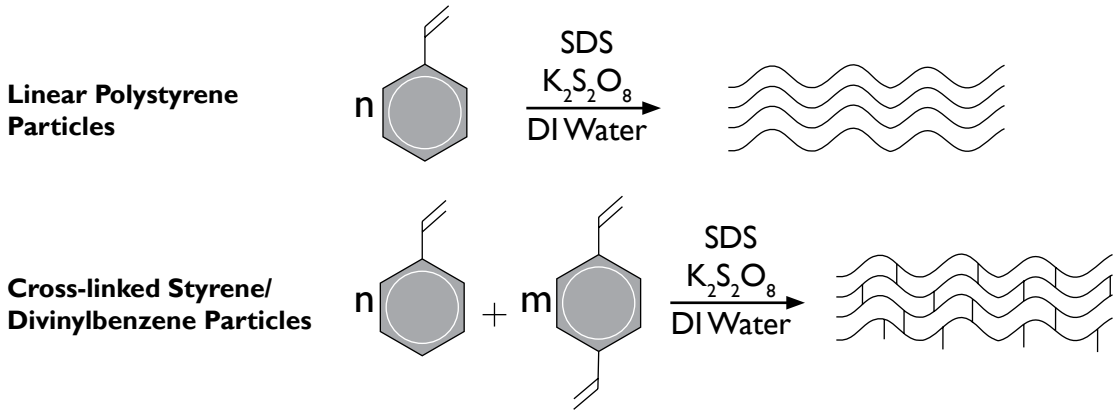

Figure 4 Differences between linear polystyrene particles and cross-linked copolymers made of styrene/ divinylbenzene

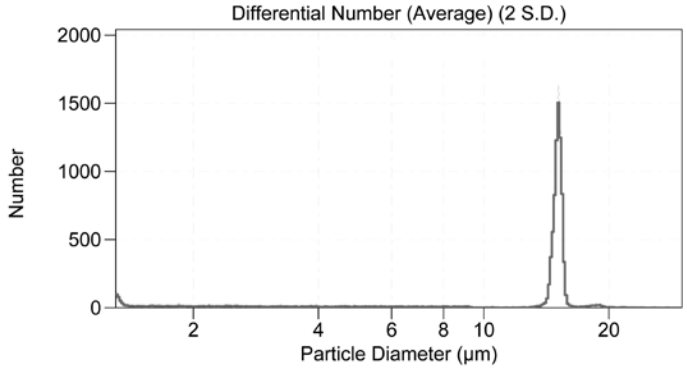

Figure 5 Histogram of SPHERO™ Cat. No. PPX-150-10, 15.2 µm Polystyrene Cross-linked Particles from a Beckman Coulter Multisizer™ 3 Coulter Counter

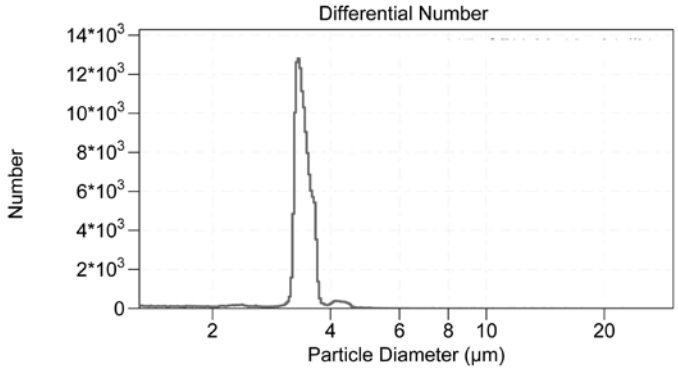

Figure 6 Histogram of SPHERO™ Cat. No. CPX-30-10, 3.3 µm Carboxyl-Polystyrene Cross-linked Particles from a Beckman Coulter Multisizer™ 3 Coulter Counter

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SPHERO™ Functionalized Polystyrene Particles

Specific particle surface chemistry enables a broad range of coating and binding applications. SPHERO™ functionalized polystyrene particles provide reactive groups on uniform microparticles for consistent and repeatable coating and binding. There are several ways to prepare particles with functionalized surfaces.

- Polymerization initiator selection
- Functionalized monomer grafting

A variety of functional groups can be provided on the microparticle's exterior surface by selecting the appropriate polymerization initiator. For instance, if potassium persulfate is used as an initiator for polymerization the particle will have sulfate groups. Similarly, other functional groups can be introduced on the surface of the particles by using other functionalized initiators.

Another method for providing surface functional groups is by grafting functionalized monomer after the polymerization process. This type of functionalized polystyrene particles are prepared by coating a thin layer of functionalized monomer onto the surface of plain particles. As a result, all of the functional groups are on the surface of the particles. The functional groups are attached to the surface of the particles by alkyl chains of two to eight carbons in length depending upon the type of functionalized monomer used.

Below are the surface charge densities for 0.8 µm carboxyl and amino particles:

- 0.8 µm SPHERO™ carboxyl polystyrene particles typically contain ~ 50 µeq/g of carboxyl groups on their surface.
- 0.8 µm SPHERO™ amino polystyrene particles typically contain ~ 15 to 20 µeq/g of amino groups on their surface.

Nonetheless, the choice of particle size and type is dependent upon the intended application. For instance, particles with size of 0.4 to 2.0 µm are suitable for latex agglutination assay, solid phase enzyme immunoassay or solid phase fluorescence immunoassay. Particles with size of 2.0 µm or larger are preferred for flow cytometry applications.

SPHERO™ Carboxyl Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Carboxyl-polystyrene	0.05-0.1	2.5	CP-008-20	20 mL
			CP-008-200	200 mL
Carboxyl-polystyrene	0.2-0.3	5.0	CP-025-10	10 mL
			CP-025-100	100 mL
Carboxyl-polystyrene	0.4-0.6	5.0	CP-05-10	10 mL
			CP-05-100	100 mL
Carboxyl-polystyrene	0.7-0.9	5.0	CP-08-10	10 mL
			CP-08-100	100 mL
Carboxyl-polystyrene	1.0-1.4	5.0	CP-10-10	10 mL
			CP-10-100	100 mL
Carboxyl-polystyrene	1.5-1.9	5.0	CP-15-10	10 mL
			CP-15-100	100 mL
Carboxyl-polystyrene	2.0-2.4	5.0	CP-20-10	10 mL
			CP-20-100	100 mL
Carboxyl-polystyrene	2.5-2.9	5.0	CP-25-10	10 mL
			CP-25-100	100 mL
Carboxyl-polystyrene	3.0-3.4	5.0	CP-30-10	10 mL
			CP-30-100	100 mL
Carboxyl-polystyrene	3.5-3.9	5.0	CP-35-10	10 mL
			CP-35-100	100 mL
Carboxyl-polystyrene	4.0-4.4	5.0	CP-40-10	10 mL
			CP-40-100	100 mL
Carboxyl-polystyrene	4.5-4.9	5.0	CP-45-10	10 mL
			CP-45-100	100 mL
Carboxyl-polystyrene	5.0-5.9	5.0	CP-50-10	10 mL
			CP-50-100	100 mL
Carboxyl-polystyrene	6.0-8.0	5.0	CP-60-10	10 mL
			CP-60-100	100 mL

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SPHERO™ Amino Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Amino-polystyrene	0.2-0.3	2.5	AP-025-10	10 mL
			AP-025-100	100 mL
Amino-polystyrene	0.4-0.6	5.0	AP-05-10	10 mL
Amino-polystyrene	0.7-0.9	5.0	AP-08-10	10 mL
			AP-08-100	100 mL
Amino-polystyrene	1.0-1.4	5.0	AP-10-10	10 mL
			AP-10-100	100 mL
Amino-polystyrene	2.0-2.4	5.0	AP-20-10	10 mL
			AP-20-100	100 mL
Amino-polystyrene	2.5-2.9	5.0	AP-25-10	10 mL
			AP-25-100	100 mL
Amino-polystyrene	3.0-3.4	5.0	AP-30-10	10 mL
			AP-30-100	100 mL
Amino-polystyrene	3.5-3.9	5.0	AP-35-10	10 mL
			AP-35-100	100 mL
Amino-polystyrene	6.0-8.0	5.0	AP-60-10	10 mL
			AP-60-100	100 mL
Amino-polystyrene	8.0-12.9	1.0	AP-100-10	10 mL

SPHERO™ Sulfonate Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Sulfonate-polystyrene	0.7-0.9	5.0	SP-08-10	10 mL
			SP-08-100	100 mL

SPHERO™ Hydroxy Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Hydroxy-polystyrene	0.7-0.9	5.0	HP-08-10	10 mL
			HP-08-100	100 mL

SPHERO™ Dimethylamino Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Dimethylamino-polystyrnene	0.7-0.9	5.0	DP-08-10	10 mL
			DP-08-100	100 mL

SPHERO™ Functionalized Particle Application Examples and Recommendations:

- Carboxyl or Amino functionalized particles are very useful for covalent coupling of proteins, ligands, antibodies or antigens to the surface of the microparticles using water soluble carbodiimide as the coupling agent. Figure 7 is a diagram of antibody and protein coating of carboxyl and amino particles using EDC coupling.
- Varying particle surface charges can be obtained using functionalized particles such as hydroxyl, sulfate and dimethylamino. These particles are used to manipulate the orientation of the coated material by passive adsorption.
- Polyclonal antibodies can be coated to polystyrene particles by passive adsorption. According to our experience, the optimal amount of antibody to particles ratio is ~100 µg of antibody per mL of 0.5% w/v (5 mg solid per mL) of 0.8 µm polystyrene. Since the total surface area of the particles is inversely proportional to the diameter of the particles, the amount of antibody to particles ratio needs to be adjusted accordingly.
- The washing of polystyrene particles to remove unbound proteins or ligands during coating is accomplished by centrifugation or tangential flow filtration for particles with size of 0.4 µm or larger. For smaller size particles gel filtration, dialysis, or tangential flow filtration should be used.

Please refer to SPHERO™ STN-1 for the recommended coating procedures for more information

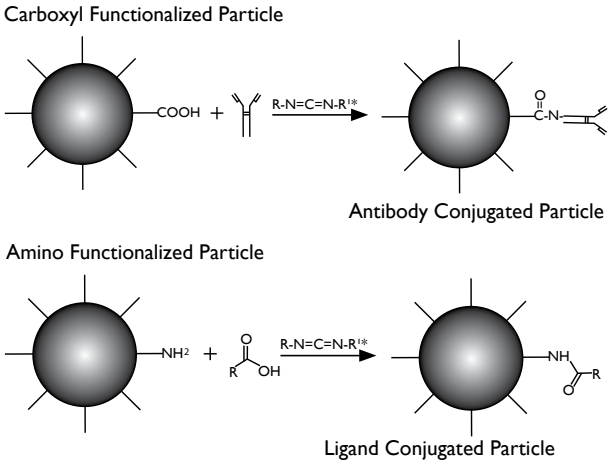

Figure 7 Examples of carbodiimide-mediated coupling processes