Technical Note:
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Selecting Microsphere Surface Properties for Diagnostic Applications

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Key Words:

- Lateral Flow Assays
- Diagnostics
- Surface Properties
- Parking Area
- Reactivity
- pH Stability
- Adsorption
- Covalent Coupling

Our strength is in offering you a complete particle technology. We give you simple protocols for working with particles. We provide you concrete data, backed by years of applications research in our own labs.

1. INTRODUCTION

Polymer microspheres are used in diagnostics for lateral flow chromatographic strip tests, latex agglutination assays, suspension array tests and nephelometric assays. When changes in microsphere properties are suspected as a root cause of reagent batch failures, it is important to gain a better understanding of the microsphere selection process. There are wide variations in microsphere composition, surface properties and size control that can affect the performance of a diagnostic reagent.

THE IMPORTANCE OF MICROSPHERE SURFACE PROPERTIES

The surface of microspheres is one of the most important properties for particles used in diagnostic tests and other applications where proteins and other biomolecules are bound to particles. Residual surfactants, monomers and microbial contamination can interfere with the successful conjugation to the particles. These contaminants are often the cause of batch-to-batch non-reproducibility of the conjugation reactions, and these variations can interfere with the production process for diagnostic tests. Careful control of particle diameter is also very important since the surface area changes exponentially with changing diameter. Variations in surface area can cause apparent changes in sensitivity and can contribute to false positive and negative results. These potential problems can result in a manufacturing dilemma—should a new batch of particles that doesn't pass incoming product tests be used and risk an assay that doesn't work. Consistency in particle manufacturing and quality control assures that these problems will not occur.

The functional groups available on the surface of the microspheres control the chemistry of the conjugation process. Selecting microspheres with the appropriate surface and quality characteristics is the key to developing stable, reproducible diagnostic tests. Following is a discussion of how surface properties effect two broad categories of biomolecular conjugation.

PROPERTIES AFFECTING HYDROPHOBIC ADSORPTION

Microspheres with sulfate and carboxyl groups are designed for hydrophobic (passive) adsorption. The particle surface is very hydrophobic, with a low density of negatively charged surface ions to provide charge stabilization. These microspheres will bind to any molecules that are characteristically hydrophobic, including proteins, peptides and small hydrophobic molecules. The binding affinity tends to increase as molecular weight increases, and can result in the preferential binding of higher molecular weight proteins in mixtures. Specific adsorption of substances such as antibodies is easily accomplished by mixing the microspheres and the protein together at an optimal pH and then separating the unbound protein from the solid phase, usually by centrifugation, dialysis or cross-flow filtration. The charge groups on these microspheres are derived from the initiators used in the synthesis of the particles, resulting in either sulfate or carboxyl ionic groups on the particle surface. The main difference between these two types of hydrophobic microspheres is their pH stability. Sulfate microspheres are stable above pH 3, while carboxyl microspheres are stable above pH 6. There are other, more subtle differences, and these come into play when one or the other particle types give a superior result when antibody is bound to its surface. These differences are not well understood and are not predictable, so it is necessary to experiment to determine which of the two hydrophobic surfaces is best for a particular application.

PROPERTIES AFFECTING COVALENT COUPLING

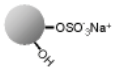
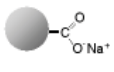
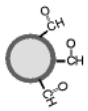
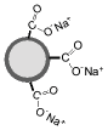
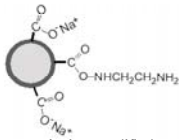
Carboxylate-modified (CML) and aldehyde-modified microspheres are designed for covalent attachment by reaction with amines. The modified microspheres are made from sulfate microspheres by grafting a copolymer containing the desired chemical group onto the surface, producing a thin, relatively hydrophilic polymeric layer. This results in a high density of carboxyl or aldehyde surface groups that can be

chemically activated to give a reactive intermediate that will couple with amines on proteins and other biomolecules. Carboxylate-modified microspheres differ from the hydrophobic carboxyl microspheres in that the surface is somewhat porous, more hydrophilic and has a relatively high charge density of 10-125 Å2 /carboxyl. They are more stable in the presence of high concentrations of electrolytes (up to 1 M univalent salt). Unlike the hydrophobic carboxyl microspheres, the high density and better availability of the carboxyl groups on these microspheres facilitate reaction with protein amines after conversion to active esters with carbodiimide reagents. Aldehyde-modified microspheres have aldehyde groups grafted to the surface and can react with protein amines through Schiff base formation. Since the aldehyde-modified microspheres do not require chemical activation and thus offer a convenient one-step method of covalent attachment, they should be considered as a simpler alternative to carboxylate-modified microspheres. Amine-modified microspheres are prepared from carboxylate-modified microspheres by converting some of the carboxyl groups to amine groups. The resulting amine-modified particles still retain a net negative charge to ensure good charge stability, and can easily be coupled to antibodies and other proteins using a variety of bi-functional linkers. This conjugation approach offers a different way of attaching molecules to the particle surface, and often results in better activity of the conjugate.

MICROSPHERE MANUFACTURING QUALITY

With so many microsphere variables affecting the reagent-making process, it is essential that all phases of microsphere design and production be tightly controlled in a reproducible environment. This is a strong contribution to reagent batch repeatability.

Microsphere Surface Properties*

More Hydrophobic					More Hydrophillic
Surface Group	 Sulfate	 Carboxyl	 Aldehyde	 Carboxylate-modified (CML)	 Amine-modified
Diameter	0.10 µm - 1.2 µm	0.10 µm - 0.39 µm	0.10 µm - 0.80 µm	0.04 µm - 5 µm	3 µm - 5 µm
Reactivity	Non-reactive	Non-reactive	Reactive with Amines	Reactive with Amines	Nucleophilic *(Attracted to centers of positive charge)*
Description	Hydrophobic, plain polystyrene microspheres -SO4 groups result from choice of initiator	Hydrophobic -COOH groups result from choice of initiator	Hydrophobic -CHO from grafted copolymer surface layer, also has -SO4 groups	Hydrophillic -COOH from grafted copolymer surface layer or PS-MAA copolymer	Hydrophillic Prepared from CML by converting -COOH to NH2 groups
pH Stability	> pH 3	> pH 6	> pH 3	> pH 6	Amphoteric
pKa	Approximately 2	Approximately 5	Approximately 2	Approximately 5	N/A
Parking Area (A^2 / group)**	N/A	102 - 175	80 - 227	9 - 112	N/A
CONJUGATION	For additional information on conjugation methods see Technical Note 027 or the Microparticle Reagent Optimization Manual***				
Hydrophobic Adsorption (Passive)	Yes			Less strongly, attach covalently	Less strongly, attach covalently
Covalent Coupling (Carobodiimide)	No	Not recommended	No	Yes. Use two step Method	No
Covalent Coupling (Schiff Base)	No	No	Yes. Use one step Method	No	No
Bifunctional Linkers	No	No	Yes	Yes	Yes
Compatible Ligands	Hydrophobic microspheres bind to any molecule with hydrophobic character including proteins, peptides and small hydrophobic molecules. Binding affinity tends to increase with increasing molecular weight.		Proteins, peptides, oligonucleotides		
			Biomolecules containing amine groups under midl conditions (pH 7 - 9)		
Comments	Primary difference between sulfate and carboxyl microspheres is pH stability. Subtle differences in binding preferences not well understood/ require experimentation.		Similar applications to CML yet simpler alternative (conjugation does not require activation). Also offers alternate conjugation methods.	Differences between CML and carboxyl microspheres: CML carboxyl groups are hydrophilic and designed to be reactive toward amines using carbodiimide. It has a somewhat soft surface, higher charge density, more –COOH groups.	Some COOH groups are converted to amines, resulting in a reactive amine surface. Retains a net negative surface charge.

* Most available in undyed, colored or Firefli™ Fluorescent dyes. Diameters shown are commonly available sizes, custom products available.

** Parking area will vary by size and batch. See product Specification Sheets for more details.

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