5 · 2001

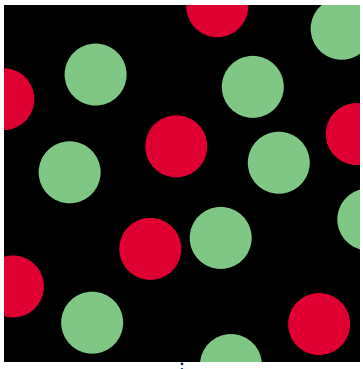
Analytix

Advances in Analytical Chemistry

SIGMA-ALDRICH

New Types of Polymer Particles

The New Generation – Melamine Resin Microparticles



Fluka is pleased to introduce a new product series consisting of monodisperse polymer microspheres. Most of them are based on melamine resin (MF) or poly(methyl methacrylate) (PMMA). Melamine resin particles are considered a new generation of microspheres having many advantages over conventional polymer particles and possessing excellent physical and chemical properties. They are available as white or fluorescent species with either bare or carboxylated particle surfaces. White PMMA beads are offered with high monodispersity over the entire size range from 1 to 100 micron.

Synthesis

Melamine resin microspheres are manufactured by hydrothermal acid-catalyzed polycondensation of methylol melamines in the temperature range 70-100°C without any surfactants. By varying the pH-value, the concentration of methylol melamine, or the reaction temperature, monodisperse particles with predictable size in the range from 0.5 to 15 micron can be produced in a one-pot synthesis.

MF particles possess many functional groups on the surface (methylol groups, amino groups), which can be used for a covalent attachment of other ligands. Because of the high density of polar triazine-amino and -imino groups, MF particles have a hydrophilic, charged surface. For special applications, the MF particles can be fluorescently labeled and/or modified by incorporation of other functionalities, like carboxyl groups, making the particle surface accessible to further chemical derivatization reactions.

Physical and Chemical Properties

Melamine resin particles are characterized by the following:

- Density: 1.51 g/cm^3
- Refractive index: 1.68
- Excellent monodispersity (C.V. ≤3%) and highly uniform spherical shape (figure 1)
- Hydrophilic surface
- High cross-linking density
- High temperature stability up to 300°C
- Superior mechanical strength
- Stable and insoluble in acids and bases
- Extremely high stability in organic solvents, no swelling or shrinking upon contact with organic solvents
- Outstanding long-term stability in dispersions, no additives or stabilizers required

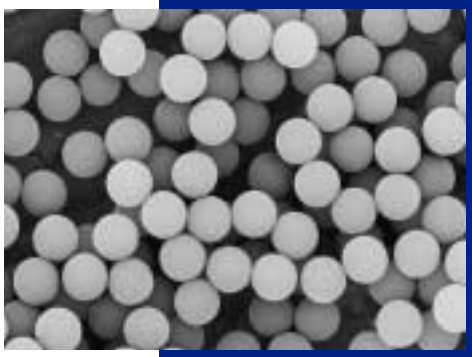


Figure 1:
Scanning electron microscopy image of MF particles

Contents:

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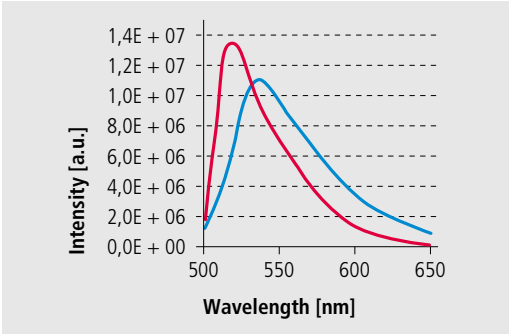

Figure 2:
Fluorescence emission spectra of free FITC (red) and FITC-labeled MF particles (blue)

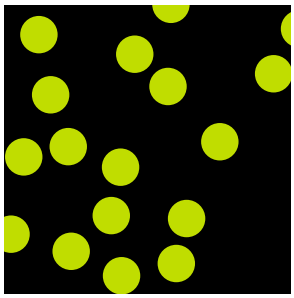

Figure 3:
Fluorescence microscopy image of FITC-labeled MF particles

- Freeze - thaw stability in water
- Particles can be dried directly from their aqueous dispersions
- Free flowing powders of dried particles can be redispersed in any dispersing agent without agglomeration

Fluorescently Labeled and Carboxylate-modified Species

Novel manufacturing techniques allow the preparation of a wide variety of fluorescent microspheres ranging in size, type of fluorochrome, and surface functional groups.
Typical dyes used for MF fluorescent particles are:

- **FITC** (green fluorescence; Excitation/Emission = 506/529 nm)
- **Nile Blue A** (red fluorescence; Excitation/Emission = 636/686 nm)
- **Rhodamine B** (orange fluorescence; Excitation/Emission = 560/584 nm).

Outstanding characteristics of fluorescent MF particles are their narrow size distribution and an intense color and fluorescence (figure 2 and 3). The homogeneously stained particles do not exhibit any leaching of the internally incorporated fluorochromes. Like white MF particles they show high stability in organic solvents. Fluorescent MF beads are also available with COOH-functionalized surfaces with a high density of functional groups (> 0.1 mmole per gram resin).

Applications

MF particles find wide applications as model systems in medicine, biochemistry, colloid chemistry, and aerosol research. They are also suitable as separation phases for chromatography, as a support for immobilized enzymes, and as spacers in liquid crystal displays (LCD).
The fluorescent particles can be used as standards (e.g. in flow cytometry, confocal laser scanning microscopy, light scattering instruments) as well as tracers in environmental science, flow measurements in gases and liquids like Laser Doppler Anemometry (LDA), Particle Dynamics Analysis (PDA), and Particle Image Velocimetry (PIV).

Handling and Storage

Aqueous dispersions of melamine resin particles have excellent stability. Storage at room temperature is possible without bacterial growth. Particles can be washed with alcohol, air dried and autoclaved. Dried particles can be redispersed in water without any agglomeration. Dispersions of MF particles can be frozen. All products are for research use only and are not intended for use in humans or for in vitro diagnostic use.

Poly(methyl methacrylate) Particles

Physical and Chemical Properties

Important properties of the PMMA particles are the following:

- Density: 1.19 g/cm^3
- Refractive index: 1.48
- Hydrophilic anionic surface
- Individually tunable sizes of the particles dependent on the desired application
- Extremely narrow size distribution (figure 4)
- Excellent biocompatibility
- Good mechanical stability
- Soluble in organic solvents like acetone, benzene, or halogenated hydrocarbons
- Reduced non-specific protein binding activity

Applications

The striking advantages of PMMA microspheres are their monodispersity as well as their good biocompatibility. The biocompatibility allows the particles to be used in many medical and biochemical applications. The high monodispersity makes PMMA beads suitable for 2D and 3D particle assemblies and for investigations in the colloidal crystal field. Other possible applications lie in the field of one- and two-component toners for copying machines.
PMMA is reported to have a reduced non-specific protein binding activity. The higher density of 1.19 g/cm^3 provides a heavier particle than polystyrene and allows more rapid separation.

Handling and Storage

Aqueous dispersions of PMMA particles should not be frozen. Particles can also be used as dry powders. Refrigerator storage of the aqueous dispersions at 4°C is recommended. All products are for research use only and are not intended for use in humans or for in vitro diagnostic use.

Certified Polystyrene Particle Size Standard

The increasing need for validation and quality assurance demands accurate and precise tools for calibration. In the range of 100 nm - 30 µm, our calibration standard grade polystyrene particles are among the best characterized particle size standards available.

Physical and Chemical Properties of Polystyrene Particles

Important general properties of our PS-particles are the following:

- Density: 1.05g/cm³
- Refractive index: 1.59
- High monodispersity and uniform spherical shape (figure 5)
- Hydrophobic surface
- Non-specific adsorption of proteins
- Low temperature resistance up to 100°C
- Soluble in organic solvents (dependent on the degree of cross-linking)
- Swellable in organic solvents
- Coefficient of Variation (C.V. value) ≤ 2% for particle size standards

Certified Particle Size Standards

The diameter of these particles is determined by methods described by the National Institute of Standards and Technology (NIST, USA), like Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), optical microscopy, and COULTER COUNTER™ particle size measurement. Devices for particle characterization are calibrated using BCR (Bureau Communautaire de Reference) and NIST-traceable standards (BCR: No. RM 165, 166 or 167; NIST: No. SRM 1690, 1691 or 1692; BCR reference standards are also available from Fluka).

The certificate of analysis of each batch includes particle diameter and standard deviation as well as data like solids content, particle number per ml, specific weight, and refractive index.

Applications of Polystyrene Particles

Polystyrene particle size standards are typically used for calibration of flow cytometers, particle and hematology analyzers, confocal laser scanning microscopes and zeta-potential measuring instruments.

In general polystyrene particles are also very common for immunoassays (latex agglutination test, FACS). Many of the tests are based on non-covalent binding of specific antibodies, antigens or cells to the hydrophobic particle surface. Covalent attachment of proteins to surface modified particles is possible. Due to their solubility in organic solvents like THF, polystyrene particles can be used as templates for preparing hollow capsules by self-organized deposition of nanoparticles. The high monodispersity makes PS beads suitable for 2D and 3D particle assemblies and for investigations in the colloidal crystal field. For applications other than calibration, we also offer research grade polystyrene particles.

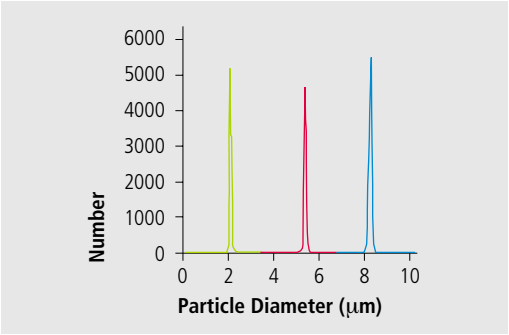

Figure 5:
Size distribution curves of three different calibration standard particles measured on the COULTER COUNTER™; 2.0 µm (green), 5.5 µm (red), 8.1 µm (blue)

Handling and Storage

Aqueous dispersions of PS particles should not be frozen and dried. Refrigerator storage at 4°C is recommended. To avoid microbial growth, a small amount of a preservative (commonly 0,02% sodium azide) is added. All products are for research use only and are not intended for use in humans or for in vitro diagnostic use.

Additional Information and Customized Microparticles

Please find below an overview of microparticles available from stock, (figure 6). For individual specifications and local prices see our new Fluka and Riedel-de Haën catalog 2001/02. In case you did not receive it, please contact us to get your free copy.

Although we already offer a large range of products, you might have special needs regarding size, functionalization or other criteria. We welcome the opportunity to design and develop particles tailored to your needs.

For additional information about our current range of products, or for inquiries for customized products, please contact Pierre Nording at pnording@sial.com or send a fax +41 81 756 54 49.

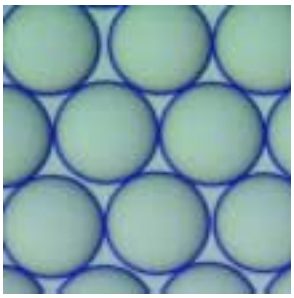

Figure 4:
Light microscopy image of 60 µm PMMA particles

Figure 6:
Available particle sizes from stock

Resin	Modification, special quality	Particle sizes [µm]
Melamine		2, 3, 4, 5, 6, 7, 8, 9, 10, 12
Melamine	FITC labeled	1, 2, 3, 4, 5, 6, 7, 8, 10
Melamine	Nile Blue A labeled	1, 3, 4, 6, 8, 9, 10
Melamine	Rhodamine B labeled	1, 2, 4, 5, 6, 7, 9, 10
Melamine	Carboxylate, FITC labeled	1, 3, 6, 10
Melamine	Carboxylate, Nile Blue A labeled	1, 3, 6, 10
Melamine	Carboxylate, Rhodamine B labeled	1, 3, 6, 10
PMMA		1, 3, 4, 6, 8, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100
Polystyrene		1, 2, 3, 5, 7, 8, 10, 15, 20, 25, 30
Polystyrene	Size Standard	0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 20.0, 30.0

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