

Values obtained through use of the following equations should be considered estimates, as the equations are based upon a number of theoretical assumptions. Values that are determined empirically or through use of analytical techniques are expected to differ to an extent. **Many values (such as % solids, diameter, density & surface area) are provided on the Certificate of Analysis and order paperwork that accompanies microsphere shipments.**

Solids Content

$$S = \frac{m \cdot 100}{V_s} \quad \text{OR} \quad m = \frac{S \cdot V_s}{100}$$

S = weight/volume % solids (e.g., $S = 10$ for 5mL suspension, 0.5g microspheres)
 V_s = volume of suspension (mL)
 m = mass of microspheres (g)

% Coefficient of Variation (Size Distribution of the Microsphere Population)

$$\% CV = \frac{SD}{d} \times 100$$

$\% CV$ = % coefficient of variation (size distribution of the microsphere population)
 SD = standard deviation (μm) (Note: Standard deviation is not provided for all products.)
 d = mean diameter (μm)

Microspheres/Gram

$$N = \frac{6 \times 10^{12}}{\pi \cdot \rho_s \cdot d^3}$$

N = # microspheres/gram for dry powders
 ρ_s = density of solid sphere (g/cm^3)
 d = mean diameter (μm)

Microspheres/mL

$$N = \frac{6 \times 10^{10} \cdot S \cdot \rho_L}{\pi \cdot \rho_s \cdot d^3}$$

N = # microspheres/mL for suspensions in water
 S = weight % solids (for 10% solids suspension, $S=10$)
 ρ_L = density of microsphere suspension (g/mL)
 $\rho_L = 100 \cdot \rho_s / [S(1-\rho_s) + (100 \cdot \rho_s)]$
 ρ_s = density of solid sphere (g/cm^3)
 d = mean diameter (μm)

Surface Area / Gram

$$AG = \frac{6 \times 10^{12}}{\rho_s \cdot d}$$

AG = surface area/gram for dry powders ($\mu\text{m}^2/\text{g}$)
 ρ_s = density of solid sphere (g/cm^3)
 d = mean diameter (μm)

Surface Area / mL

$$A = \frac{6 \times 10^{10} \cdot S \cdot \rho_L}{\rho_s \cdot d}$$

A = surface area / mL for suspensions in water ($\mu\text{m}^2/\text{g}$)
 S = weight % solids (for 10% solids solution, $S=10$)
 ρ_L = density of microsphere suspension (g/mL)
 $\rho_L = 100 \cdot \rho_s / [S(1-\rho_s) + (100 \cdot \rho_s)]$
 ρ_s = density of solid sphere (g/cm^3)
 d = mean diameter (μm)

Parking Area (Surface Charge Density)

$$P = \frac{1}{1.004 \cdot D_c \cdot \rho_s \cdot d}$$

P = parking area ($\text{\AA}^2/\text{charge group}$)
 DC = surface charge density or titration value (meq/g) (provided in $\mu\text{eq}/\text{g}$ on COA)
 ρ_s = density of solid sphere (g/cm^3)
 d = mean diameter (μm)
 Notes: Surface titer / parking area are not provided for all products.

Charge Groups / Microsphere

$$C_M = \frac{\pi \cdot d^2 \cdot 10^8}{P}$$

C_M = # charge groups / microsphere
 d = mean diameter (μm)
 P = parking area (\AA^2)

Settling Velocity (Specify Medium)

$$v = \frac{1}{18} d^2 (\rho_s - \rho_0) \frac{10^{-6}}{h} G$$

v = settling velocity (cm / sec)
 d = mean diameter (μm)
 ρ_s = density of solid sphere (g/cm^3)
 ρ_0 = medium density (g/cm^3)
 h = medium viscosity (poise = $\text{g}/\text{cm} \cdot \text{sec}$)
 G = gravity ($980 \text{ cm}/\text{sec}^2$)

Settling Velocity (In Water)

$$V_m = 5.448 \times 10^{-5} \cdot (\rho_s - 1) \cdot d^2$$

V_m = maximum settling velocity (cm/sec) for a single microsphere settling in water at room temperature under the influence of normal gravitational force (1G)
 ρ_s = density of solid sphere (g/cm^3)
 d = mean diameter (μm)

Settling Velocity (In the Centrifuge)

$$V_h^{5\%} = \frac{2}{3} \cdot V_m \cdot G$$

$V_h^{5\%}$ = true settling velocity or hindered velocity (cm/sec) for a 5% w/w suspension of microspheres settling in water at room temperature
 V_m = maximum settling velocity
 G = multiples of earth gravitation constant, G forces

Settling Time

$$t = \frac{h}{V_h^{5\%}} \text{ OR } \frac{h}{V_{ch}^{5\%}}$$

t = settling time (sec)
 h = distance from the top of the liquid to the bottom layer of settled solids (cm)
 $V_h^{5\%}$ = true settling velocity or hindered velocity (cm/sec) for a 5% w/w suspension of microspheres settling in water at room temperature under the influence of normal gravitational force (1G)
 $V_{ch}^{5\%}$ = hindered settling velocity in the centrifuge (cm/sec) for a 5% w/w suspension of microspheres settling in water at room temperature

Surface Saturation (Protein)

$$S = \frac{6}{\rho_s d} \cdot C$$

S = amount of representative protein required to achieve surface saturation (mg protein/g microspheres)
 ρ_s = density of solid sphere (g/cm^3)
 d = mean diameter (μm)
 C = capacity of microsphere surface for a given protein (mg protein/ m^2 of sphere surface)

Notes: $C \sim 3 \text{ mg}/\text{m}^2$ for BSA [MW 65kD], $C \sim 2.5 \text{ mg}/\text{m}^2$ for bovine IgG [MW 150kd].¹
 See Tech Note 204, *Adsorption to Microspheres*, and TechNote 205, *Covalent Coupling*, for more detailed information.

SAMPLE VALUES

Diameter (Microns)	Beads per gram	Beads per mL	Surface Area ($\mu\text{m}^2/\text{g}$)	Surface Area ($\mu\text{m}^2/\text{mL}$)	Settling Velocity (cm/sec)
0.052	1.3×10^{16}	1.3×10^{15}	1.1×10^{14}	1.1×10^{13}	7.4×10^{-9}
0.100	1.8×10^{15}	1.8×10^{14}	5.7×10^{13}	5.7×10^{12}	2.7×10^{-8}
0.500	1.5×10^{13}	1.5×10^{12}	1.1×10^{13}	1.1×10^{12}	6.8×10^{-7}
1.000	1.8×10^{12}	1.8×10^{11}	5.7×10^{12}	5.7×10^{11}	2.7×10^{-6}
2.500	1.2×10^{11}	1.1×10^{10}	2.3×10^{12}	2.3×10^{11}	1.7×10^{-5}
10.00	1.8×10^9	1.8×10^8	5.7×10^{11}	5.7×10^{10}	2.7×10^{-4}
25.00	1.2×10^8	1.2×10^7	2.3×10^{11}	2.3×10^{10}	2.0×10^{-3}
108.0	1.4×10^6	---	5.3×10^{10}	---	---
500.0	1.4×10^4	---	1.1×10^{10}	---	---

Notes: Calculations for 0.052-25.0 μm are based on a suspension of polystyrene microspheres (density = 1.05 g/cm³) at 10% solids (w/v). 108 and 500 μm diameter microsphere calculations compositions are based on compositions of poly(styrene/2% divinylbenzene), density = 1.06 g/cm³, and the calculations are based on dry presentation.

REFERENCES

1. Cantarero, L.A., J.E. Butler, J.W. Osborne. 1980. *The adsorptive characteristics of protein for polystyrene and their significance for solid-phase immunoassays*. Analytical Biochemistry, 105: 375-382.
2. Bangs, L.B. 1987. *Uniform latex particles*. Indianapolis: Seragen Diagnostics, Inc.

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