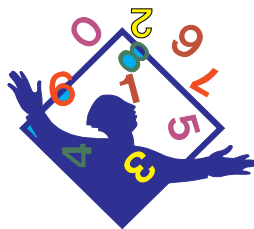


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B E A D S • A B O V E T H E R E S T™



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I. INTRODUCTION

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. Some values (designated with an *) are provided on the Certificates of Analysis and order paperwork that accompanies microsphere shipments.

II. EQUATIONS

A. Solids Content*

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

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

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

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

where: % 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)*

C. # Microspheres/Gram*

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

where: N = # microspheres/gram for dry powders*
 ρ_s = density of solid sphere (g/cm³)*
 d = mean diameter (μm)*

D. # Microspheres/mL*

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

where: N = # microspheres/mL for suspensions in water*
 S = weight % solids (for 10% solids suspension, S=10)*
 ρ_L = density of microsphere suspension (g/mL)*
 ρ_L = 100 • ρ_s / [S (1-ρ_s) + (100 • ρ_s)]
 ρ_s = density of solid sphere (g/cm³)*
 d = mean diameter (μm)*

E. Surface Area / Gram*

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

where: AG = surface area/gram for dry powders (μm²/g)*
 ρ_s = density of solid sphere (g/cm³)*
 d = mean diameter (μm)*

F. Surface Area / mL*

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

where: A = surface area / mL for suspensions in water (μm²/g)*
 S = weight % solids (for 10% solids solution, S=10)*
 ρ_L = density of microsphere suspension (g/mL)*
 ρ_L = 100 • ρ_s / [S (1-ρ_s) + (100 • ρ_s)]
 ρ_s = density of solid sphere (g/cm³)*
 d = mean diameter (μm)*

G. Parking Area (Surface Charge Density)*

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

where: P = parking area (Å²/charge group)*
 D_c = surface charge density or titration value (meq/g)* (provided in µeq/g on COA)
 ρ_s = density of solid sphere (g/cm³)*
 d = mean diameter (µm)*

Notes: a. Surface titer / parking area are not provided for all products.
 b. For a detailed description of parking area, see "The Particle Doctor"® section of our March 2001 issue of *Painless Particles*®, which may be downloaded from our website, www.bangslabs.com.

H. # Charge Groups / Microsphere

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

where: C_M = # charge groups / microsphere
 d = mean diameter (µm)*
 P = parking area (Å²)*

I. Settling Velocity (Specify Medium)

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

where: v = settling velocity (cm / sec)
 d = mean diameter (µm)*
 ρ_s = density of solid sphere (g/cm³)*
 ρ₀ = medium density (g/cm³)*
 h = medium viscosity (poise = g/cm • sec)
 G = gravity (980 cm/sec²)

J. Settling Velocity (In Water)

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

where: 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/cm³)*
 d = mean diameter (µm)*

K. Settling Velocity (In the Centrifuge)

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

where: 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

L. Settling Time

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

where: 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

M. Surface Saturation (Protein)

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

where: S = amount of representative protein required to achieve surface saturation (mg protein/g microspheres)
 ρ_s = density of solid sphere (g/cm³)*
 d = mean diameter (µm)*
 C = capacity of microsphere surface for a given protein (mg protein/m² of sphere surface)

Notes: a. C ~ 3 mg/m² for BSA [MW 65kD],
 C ~ 2.5 mg/m² for bovine IgG [MW 150kd].
 b. See Tech Note 204, *Adsorption to Microspheres*, and TechNote 205, *Covalent Coupling*, for more detailed information.

III. SAMPLE VALUES

Table 1: Sample Values

Diameter (Microns)	Beads per gram	Beads per mL	Surface Area (µm ² /g)	Surface Area (µm ² /mL)	Settling Velocity (cm/sec)
0.052	1.3x10 ¹⁶	1.3x10 ¹⁵	1.1x10 ¹⁴	1.1x10 ¹³	7.4x10 ⁻⁹
0.100	1.8x10 ¹⁵	1.8x10 ¹⁴	5.7x10 ¹³	5.7x10 ¹²	2.7x10 ⁻⁸
0.500	1.5x10 ¹³	1.5x10 ¹²	1.1x10 ¹³	1.1x10 ¹²	6.8x10 ⁻⁷
1.000	1.8x10 ¹²	1.8x10 ¹¹	5.7x10 ¹²	5.7x10 ¹¹	2.7x10 ⁻⁶
2.500	1.2x10 ¹¹	1.1x10 ¹⁰	2.3x10 ¹²	2.3x10 ¹¹	1.7x10 ⁻⁵
10.00	1.8x10 ⁹	1.8x10 ⁸	5.7x10 ¹¹	5.7x10 ¹⁰	2.7x10 ⁻⁴
25.00	1.2x10 ⁸	1.2x10 ⁷	2.3x10 ¹¹	2.3x10 ¹⁰	2.0x10 ⁻³
108.0	1.4x10 ⁶	---	5.3x10 ¹⁰	---	---
500.0	1.4x10 ⁴	---	1.1x10 ¹⁰	---	---

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

IV. REFERENCES

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