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Characterization of the adhesion force between magnetic microscale particles and the influence of surface-bound protein

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ABSTRACT

The adhesion force between microscale particles depends primarily on the surface structure, charge density, and their material. In the case of magnetic particles, adhesion force additionally depends on their magnetization. A miniaturized experimental setup was designed to measure and characterize the interactions between the particles in a particle sediment. The influence of the magnetic field and the protein bound on the particle surface on the adhesion force was studied.

It was shown that the particle sediment expands reversibly, if the separation does not exceed the adhesion force between the particles. In the presence of bound protein, higher forces are necessary to separate the particles from each other.

1. Introduction

Nowadays, advances in biotechnology allow for the production and usage of a wide range of proteins. They can be used in food industry or for medical applications, an example being cancer treatment with antibodies. For the latter, it is crucial to achieve an extremely high product purity. The production is done with microorganisms or mammalian cells in a biosuspension. The achieved titers of the target protein are small, between 1 and 10 mg per liter biosuspension. Other biological compounds, e.g. other proteins, with characteristics similar to those of the target protein are also part of the suspension. The task to be solved is a quick enhancement of the product titer, combined with the removal of the impurities. One approach suited for this purpose is high-gradient magnetic separation (HGMS). The concept is based on the application of protein-specific functionalized magnetic particles. The target proteins bind reversibly, e.g. by changing the salt content and/or pH, to the particles and can be removed in a magnetic filter device, while the impurities are washed out [1]. The resulting particle cake has to be removed completely from the device in order to achieve a cost-efficient process (recycling of magnetic particles) and to reduce the risk of biofouling in the filter device.

The adhesion force which has to be overcome to remove the filter cake is the sum of interparticle forces, such as the van der Waals force, electrostatic interactions, and the magnetic force. In order to measure the adhesion force between the particles in a filter cake, a modification of the centrifuge method [2] is used, as will be explained below. The measurements are performed in two steps. First, a particle sediment has to be built up. In the second step, the sediment is placed in a centrifuge, so that the centrifugal force acts opposite to the adhesion forces between the particles. The integrated optics allows for the direct measurement of the phase boundary of the particles and the supernatant. The adhesion forces of the first particle layer of the sediment can be calculated by a force balance.

The particles used for protein purification mainly vary in size, shape, and magnetization. Depending on their surface functionalization, they can bind different proteins. The study reported here was aimed at determining the influence of the parameters mentioned on the adhesion between the particles.

2. Theory

Total adhesion force between particles consists of different force components. The dominant forces in the given system are electrostatic interactions, van der Waals and magnetic forces.

2.1. Van der Waals forces

The van Waals forces have to be taken into account for microscale particles. Important basic research was done by Lifshitz [3] and by Hamaker [4]. In their first studies, neither deformations in the contact area surface nor roughnesses were considered. Derjaguin–Muller–Toporov and Johnson–Kendall–Robert considered the influence of particle deformation on adhesion [5,6]. Rumpf and Rabinovich implemented a roughness correction term based on the work of Hamaker. In the case

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of two spheres with the radius R and the Hamaker constant A_H, the van der Waals force $F_{v.d.W.,\ sph}$ can be calculated as

$$F_{v.d.W.,\ sph} = \frac{A_H * R}{12 * a_0^2} * \left(\frac{1}{1 + \frac{R}{1.485 * rms}} + \frac{1}{\left(1 + \frac{1.485 * rms}{a_0}\right)^2} \right) \quad (1)$$

with the minimal intermolecular distance a_0 and *rms* as the root mean square roughness of the spheres [7]. The approximation is limited to nanoscale roughnesses.

Due to the different influencing factors, such as deformation and roughness, it is difficult to predict the adhesion of particles regardless of all theoretical advances. Another difficulty is the accurate determination of the Hamaker constant, which depends on the materials in contact but also on the surrounding liquid [4].Therefore, diverse experimental setups were developed to measure the adhesion forces. The most common methods are atomic force microscopy [8–10] (AFM), the centrifuge technique [11,12], measurements in a stream channel [13,14], and, recently, the vibration method [15]. With all these techniques, adhesion forces between particles and plane substrates can be measured. However, direct measurement of the adhesion between particles is difficult. Usually, the AFM is applied in that case. As in particle wall measurements, AFM considers only one particle contact at the same time.

In order to obtain adhesion information of a particle collective, a modification of the centrifuge method was realized.

2.2. Magnetic force

Magnetism can be induced by two sources: Moving electric charges within a conductor or fluctuations of the electron position on an atomic scale, causing a permanent magnetic character of the body. In the given setup, the magnetic field is created by permanent magnets. The resulting magnetic field $\boldsymbol{H}$ is conservative (no work is done on a closed loop)

$$\nabla \times \boldsymbol{H} = 0 \quad (2)$$

and the magnetic flux $\boldsymbol{B}$ is divergenceless (the field runs in closed loops)

$$\nabla \cdot \boldsymbol{B} = 0\,. \quad (3)$$

In a non-magnetic medium, both fields are correlated by

$$\boldsymbol{B} = \mu_0(\boldsymbol{H} + \boldsymbol{M}) \quad (4)$$

with μ_0 being the permeability of vacuum and $\boldsymbol{M}$ the magnetization of the medium [16]. The magnetic force F_M on a particle with the volume V_P and the magnetization M_P within a magnetic field is given by [1]

$$F_M = \mu_0 \cdot V_P \cdot M_P \cdot \nabla \boldsymbol{H}\,. \quad (5)$$

In the case of two dipoles μ_i and μ_j at the distance r, the resulting force between them is given by

$$\boldsymbol{F} = \nabla\left(\mu_j \cdot \boldsymbol{B_i}\right) = \frac{3 \cdot \mu_0}{4 \cdot \pi \cdot r^5} \left((\mu_i \cdot \boldsymbol{r})\mu_j + \left(\mu_j \cdot \boldsymbol{r}\right)\mu_i + \left(\mu_i \cdot \mu_j\right)\boldsymbol{r} - \frac{5(\mu_i \cdot \boldsymbol{r})\left(\mu_j \cdot \boldsymbol{r}\right)}{r^2}\boldsymbol{r} \right) \quad (6)$$

with $\boldsymbol{r}$ being the vector pointing from i to j [17]. Eq. (6) only applies to permanent dipoles. For induced dipoles which influence each other, the problem is more complex. In the case of superparamagnetic particles, each particle constitutes an induced dipole and no analytical solution is available. It can only be approximated by simulation.

2.3. Electrostatics

Electrostatic forces occur between two charged bodies. If both bodies carry the same charge type, they repel each other. If the charge is opposite, they attract each other. Solids usually carry negative charge when dissolved in water. As a result, a layer with positively charged ions forms around the solids. A diffuse ion layer follows.

For two spherical particles i and j with diameters d_i and d_j at the distance r, the electrostatic potential E_{el} can be described by the Derjaguin approximation. The electrostatic force F_{el} is then given by its gradient

$$F_{el} = \nabla E_{el} = \frac{128\pi \cdot N_A \cdot c_{ion} \cdot k_B \cdot T}{\kappa^2} \frac{d_i \cdot d_j}{4r} \gamma_i \gamma_j \exp\left[-\kappa\left(r - \frac{d_i + d_j}{2}\right)\right] \times \left(\frac{1}{r} + \kappa\right) \quad (7)$$

where N_A is Avogadro's constant, c_{ion} is ion concentration, k_B is Boltzmann's constant, T is temperature and κ is the Debye–Hückel parameter. The Debye–Hückel parameter is given by

$$\kappa = \left(\frac{\epsilon \cdot k_B \cdot T}{\sum_i (c_{ion} \cdot z_i^2) \cdot N_A \cdot e_0^2} \right)^{-\frac{1}{2}} \quad (8)$$

with ε being the permittivity, z the charge of the ion I, and e_0 the electron charge [18]. The parameter γ depends on the zeta potential ζ and is given by

$$\gamma = \tanh\left(\frac{z \cdot e_0 \cdot \zeta}{4 \cdot k_B \cdot T}\right)\,. \quad (9)$$

3. Methods and material

3.1. Material

3.1.1. Particles

Superparamagnetic particles of different providers were used for the experiments. They consist of nanoscale magnetite colloids (d < 5 nm) which are embedded in a polymer or silica matrix. The particle sizes ranged from 0.5 to 20 μm. Due to the small size of the individual magnetite core, the entire particle becomes superparamagnetic. Superparamagnetic means that the particles magnetize in an external magnetic field, but lose their magnetization once the external field is removed. Table 1 gives an overview of the used particles and their characteristic values.

The MagPrep particles have anion exchange groups (tri-methyl-ammonium-propyl) on their surface and are able to bind proteins there.

3.1.2. Proteins

The proteins *Bovine Serum Albumin* (*BSA*) (Carl Roth, Germany) and *Catalase* (Sigma-Aldrich, Germany) were used. Both are commonly applied as model proteins and, hence, well-characterized and inexpensive. *BSA* is a small protein with 583 amino acids and a molecular weight of 66.5 kDa. Its isoelectric point (pI) is 4.7. The molecular weight of *Catalase* is 250 kDa with 526 amino acids and it has a pI of 5.4.

3.1.3. Magnets

The magnetic fields were generated by permanent magnets (BR Technik Kontor GmbH, Germany). Due to the different experimental setup, two magnet geometries were used, magnets of cubic geometry with an edge length of 10 mm and magnets of cylindrical geometry with a diameter of 7 mm and a height of 7 mm, which are magnetized in axial direction. Both geometries were available for the magnet