Strategy for Selection of Methods for Separation of Bioparticles From Particle Mixtures

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Received 2 March 2005; accepted 10 January 2006

DOI: 10.1002/bit.20885

Abstract: The desired product of bioprocesses is often produced in particulate form, either as an inclusion body (IB) or as a crystal. Particle harvesting is then a crucial and attractive form of product recovery. Because the liquid phase often contains other bioparticles, such as cell debris, whole cells, particulate biocatalysts or particulate by-products, the recovery of product particles is a complex process. In most cases, the particulate product is purified using selective solubilization or extraction. However, if selective particle recovery is possible, the already high purity of the particles makes this downstream process more favorable. This work gives an overview of typical bioparticle mixtures that are encountered in industrial biotechnology and the various driving forces that may be used for particle–particle separation, such as the centrifugal force, the magnetic force, the electric force, and forces related to interfaces. By coupling these driving forces to the resisting forces, the limitations of using these driving forces with respect to particle size are calculated. It shows that centrifugation is not a general solution for particle–particle separation in biotechnology because the particle sizes of product and contaminating particles are often very small, thus, causing their settling velocities to be too low for efficient separation by centrifugation. Examples of such separation problems are the recovery of IBs or virus-like particles (VLPs) from (microbial) cell debris. In these cases, separation processes that use electrical forces or fluid–fluid interfaces show to have a large potential for particle–particle separation. These methods are not yet commonly applied for large-scale particle–particle separation in biotechnology and more research is required on the separation techniques and on particle characterization to facilitate successful application of these methods in industry.

Keywords: particle–particle separation; bioparticle; inclusion body; cell debris; recovery

INTRODUCTION

There is a large and growing market for compounds that are produced with biotechnological processes. Some of these compounds obtain a particulate form during their production. Examples of bulk compounds are β-carotene with a production of ≈500 metric tons per year (Chemical Week, April 19, 1995) and a turnover of ≈$320 million per year (www.chemicalmarketreporter.com), vitamin E with a production of ≈65,000 metric tons per year and a turnover of approximately $2,000 million per year (Chemical Week, March 21, 2001 and May 21, 1997), and L-tryptophan with a production of ≈750 metric tons per year and a turnover of approximately $38 million per year (Chemical Week, November 5, 1997). Alternative processes for the production of these compounds may avoid the formation of a particulate product, but are not necessarily more favorable. Dealing with particulate products thus is an important issue in biotechnology.

Bioparticles can be formed by aggregation of molecules or by active accumulation of these molecules in specific compartments inside cells. Active accumulation involves active transport of components to specific locations inside the cells where they may aggregate or where they are included in polymers. The latter process does not necessarily yield solid particles, whereas aggregation does. Aggregation requires supersaturation of the liquid phase. A particulate product will therefore only be formed by aggregation when the saturation concentration is exceeded.

The saturation concentration of a compound is related to its interactions with all of the surrounding molecules. If these interactions are energetically favorable, the compound has a high saturation concentration and vice versa. Figure 1 depicts the octanol–water partitioning coefficient ($K_{ow}$), which is a measure for polarity, against the molar mass of various organic compounds. The shaded areas indicate the solubility of these compounds in water. The graph shows that a decrease in solubility corresponds to an increase in molar mass and $K_{ow}$. Thus, small polar molecules like many amino acids remain dissolved in water up to concentrations of 100 g/L or higher, but with somewhat larger and less polar molecules, the solubility is already exceeded at lower concentrations. Most products in biotechnological processes consist of large complex molecules with high molar mass. These products are likely to be produced above their saturation concentration and will thus form particles during the production process. Moreover, it has been shown in many enzymatic conversion processes that precipitation or crystallization of the product(s) has a favorable effect on the conversion rate (Uljin
BIOTECHNOLOGICAL PROCESSES

OVERVIEW OF PARTICLE MIXTURES IN BIOTECHNOLOGICAL PROCESSES

In this section, an overview is given of reported processes in which bioparticles are formed in the presence of other particles. These bioparticles may be intracellular particulate microbial products, extracellular particulate microbial products, VLPs, or solid products in biocatalysis. In Tables I and II, references are

Table I. References on biotechnological processes that yield particle mixtures.

<table>
<thead>
<tr>
<th>Category</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular particulate products produced with microorganisms</td>
<td>(Beards et al., 1993; Buque-Taboada et al., 2004; Kometani et al., 1997; Kumagai, 1999; Leueneberger, 1985; Matsumae et al., 1995; Michielsen et al., 2000a,b; Miller, 1985; Nakayama, 1985; Spassov et al., 1996; Takahashi, 2003)</td>
</tr>
<tr>
<td>Extracellular particulate products produced with microorganisms</td>
<td></td>
</tr>
<tr>
<td>VLPs produced produced with microorganisms</td>
<td>(Andrews et al., 1995; Cruz et al., 2000; Kitano et al., 1987; Kaimper et al., 2002; Meijer et al., 1996; Moran, 1999; Tsoka et al., 2000)</td>
</tr>
<tr>
<td>Solid products produced with immobilized catalysts</td>
<td>(Takahashi, 2003; Davison et al., 1997; Kasche and Galunske, 1995; Kuhl et al., 1990; Lee et al., 1999a; Zmitrowicz et al., 1991)</td>
</tr>
<tr>
<td>Production of solid products and solid by-products with enzymes</td>
<td>(Blacker and Holt, 1997; Youshko et al., 2002)</td>
</tr>
</tbody>
</table>

Table II

<table>
<thead>
<tr>
<th>Category</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. The relation between solubility, polarity, and molar mass for various organic compounds. The shaded areas mark the solubility range of the compounds. $K_{ow}$ is the octanol–water partitioning coefficient, which is a measure for the polarity of the compounds. Compounds (Weast et al., 1922; http://www.mdbio.com.tw/AM/am-trimethoprim.htm; http://chemfinder.cambridgesoft.com/result.asp; Sangster, 1997): (1) ampicillin, (2) trimethoprim, (3) alanine, (4) glycine, (5) methionine, (6) phenylalanine, (7) serine, (8) tryptophan, (9) DL-valine, (10) codein, (11) phenylbutazone, (12) quinidine, (13) phenytoin, (14) cimetidine, (15) chlorothiazide, (16) theophylline, (17) ethacrynic acid, (18) furosemide, (19) phenobarbitol, (20) caffeine, (21) diazepam, (22) meprobamate. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]
<table>
<thead>
<tr>
<th>Type of inclusion body</th>
<th>Size (µm)</th>
<th>Characteristics*</th>
<th>Organism</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>0.05–2.25</td>
<td>--, variable shape</td>
<td>Many (mainly recombinant) microorganisms</td>
<td>Aggregation, incorrect folding, lacking the ability to excrete</td>
<td>(Bowden et al., 1991; Fischer et al., 1992; Fischer et al., 1995; Gram et al., 1994; Hellebust et al., 1989; Hoess et al., 1988; Honda et al., 2000; Koller et al., 1995; LaVallie et al., 1993; Mitraki and King, 1989; Rinas et al., 1992; Valax and Georgiou, 1993; Wangsa-Wirawan et al., 2001b; Wetzel et al., 1991; Wong et al., 1997a)</td>
</tr>
<tr>
<td>Polyphosphate granules</td>
<td>0.048–1</td>
<td>--, amorphous, spherical</td>
<td>Many m.o.’s</td>
<td>Phosphate storage and regulation, energy storage, accumulation of unwanted (toxic) metals or metals used in the metabolism</td>
<td>(Bode et al., 1993; Lins and Farina, 1999; Shively, 2003)</td>
</tr>
<tr>
<td>Starch granules</td>
<td>1–100</td>
<td>--, variable shape</td>
<td>Plant cells</td>
<td>Energy storage</td>
<td>(Jane et al., 1994)</td>
</tr>
<tr>
<td>Cyanophycin</td>
<td>~&gt;0.5</td>
<td>--, variable shape</td>
<td>Cyanobacteria, recombinant <em>E. coli</em></td>
<td>Nitrogen storage</td>
<td>(Oppermann-Sanio and Steinbüchel, 2002; Shively, 2003)</td>
</tr>
<tr>
<td>Glycogen granules</td>
<td>0.02–0.3</td>
<td>+/- variable shape</td>
<td><em>Many prokaryotes</em></td>
<td>Hypothesis: energy/carbon storage</td>
<td>(Shively, 2003)</td>
</tr>
<tr>
<td>Polyhydroxyalkanoate granules</td>
<td>0.1–0.8</td>
<td>+, spherical</td>
<td>Bacteria, algae, etc.</td>
<td>Energy/carbon reserve</td>
<td>(Shively, 2003)</td>
</tr>
<tr>
<td>Sulfur globules</td>
<td>0.1–1</td>
<td>+</td>
<td><em>Thiorhodaceae</em> and other apochlorotic sulfur bacteria</td>
<td>Hypothesis: sulfur storage</td>
<td>(Lins and Farina, 1999; Shively, 2003)</td>
</tr>
<tr>
<td>Magnetosomes</td>
<td>0.04–0.1</td>
<td>+, contains Fe₃O₄ or iron sulphides</td>
<td>Magnetotactic bacteria</td>
<td>Helps in search for nutrients due to magnetism</td>
<td>(Dunin-Borkowski et al., 1998; Lins and Farina, 1999; Proksch et al., 1995)</td>
</tr>
<tr>
<td>Carboxysomes</td>
<td>0.09–0.5</td>
<td>+</td>
<td>Blue–green algae, many nitrifying bacteria and thiobacilli</td>
<td>Hypothesis: storage of ribulose-1,5-diphosphate carboxylase</td>
<td>(Shively, 2003)</td>
</tr>
<tr>
<td>Other crystals</td>
<td>1–15</td>
<td>--, variable shape</td>
<td>Various m.o.’s</td>
<td>Due to increased production</td>
<td>(CN1294191; Eonseon et al., 2003; Jeoong et al., 1999)</td>
</tr>
</tbody>
</table>

*, + indicates that the inclusion body is surrounded by a membrane and – indicates that there is no membrane.
listed of the various categories of particle mixtures that are described in this section.

**Intracellular Particulate Microbial Products**

Non-excreted microbial products accumulate inside the microorganism and often cause the formation of crystals or IBs that have size in the range of 0.05–1.2 μm. Crystals are formed when the product is produced above its saturation concentration, such as in the case of β-carotene production by *Blakeslea trispora* (Jeong et al., 1999) and the production of xanthophylls by microorganisms or microalgae (Eonseon et al., 2003). IBs are formed when the product is accumulated in a specific compartment inside the cells, such as with polyhydroxyalkanoates (Lee et al., 1999b), or when it aggregates inside the microorganism, which often happens with peptides and proteins that are folded incorrectly due to chaperones that are lacking in the organism (Buchner et al., 1992; Honda et al., 2000; Wong et al., 1997a). Besides protein IBs, crystals, and PHA granules, many other forms of IBs can be found in nature, such as phosphate granules, starch granules, sulfur globules, carboxysomes, magnetosomes, glycogen inclusions, and cyanophycin inclusions. In Table II, an overview is given of the characteristics of these IBs. It is important to note that not all of these IBs are (yet) of economical interest, but the overview gives a good impression of what microorganisms are capable of.

The structure and characteristics of IBs may depend on the cell compartment where the compound is accumulated. For instance, β-lactamase IBs in *E. coli* were found to be amorphous when produced in the periplasmic space and highly regular-shaped when produced in the cytoplasm (Bowden et al., 1991). It is difficult to predict the IB properties and its location inside the cell just by looking at its chemical composition. In addition, the surface properties of the IBs may be influenced by intracellular dissolved compounds that adsorb onto the IBs. This phenomenon makes prediction of the IB properties even more difficult.

The separation of particulate intracellular microbial products from biomass is generally performed by product release through homogenization in combination with centrifugation. The IB enriched fraction is subsequently dissolved and extracted with chemicals and, if required, the product is refolded in a proper refolding buffer (Fischer et al., 1992; Gram et al., 1994; Hellebust et al., 1989; Honda et al., 2000). Since most of the IBs contain the product at high purity, dissolution and extraction does not seem to be the most efficient separation operation and direct recovery by particle–particle separation may increase the separation efficiency and reduce the number of process steps.

**Extracellular Particulate Microbial Products**

A reasonable number of cases have been reported where an excreted microbial product forms crystals (5–100 μm) due to supersaturation of the liquid phase (Beards et al., 1993; Buque-Taboada et al., 2004; Michielsen et al., 2000a,b; Spassov et al., 1996). For instance, both the substrate and product are crystals in the conversion of Ca-maleate to Ca-D-malate by permeabilized *Pseudomonas pseudoalcaligenes* (Michielsen et al., 2000a,b). These processes yield mixtures of microbial cells and particulate product(s), again making particle–particle separation a key step in downstream processing.

Currently, many of these products are separated from other particles by dissolution in a second (organic) liquid phase followed by crystallization (Buque-Taboada et al., 2004). Particle–particle separation is only considered in those cases where the density and/or size differences between the product particles and the other particles are large enough for separation by centrifugation.

**Virus-Like Particles (VLPs)**

Viruses and VLPs are particles with sizes between 20 and 200 nm that are produced intracellularly. The particles often consist of an inactivated virus or a surface antigen of a virus that is produced by a genetically modified microorganism. For recovery of these intracellular VLPs, the cells have to be disrupted, thus creating a mixture of cell debris and VLPs. When the virus is still active, however, the cells may be lysed spontaneously. Again it is clear that the production of these bioparticles gives rise to a particle–particle separation problem. Purification of VLPs is currently performed with a wide variety of separation techniques, such as centrifugation (Cruz et al., 2000; Tsoka et al., 2000), filtration (Cruz et al., 2000; Kuiper et al., 2002; Tsoka et al., 2000), extraction without dissolution (Andrews et al., 1995; Kitano et al., 1987), and chromatography (Cruz et al., 2000; Kuiper et al., 2002; Tsoka et al., 2000) that are used in a large train of separation operations. Direct VLP isolation by particle–particle separation would reduce the number of process steps and might thus lower the downstream processing costs.

**Particles in Enzyme Catalysis and Biotransformation**

Solid-to-solid conversion in enzymatic synthesis is thermodynamically possible if the apparent equilibrium constant is larger than the solubility ratio of the product(s) and substrate(s) (Diender et al., 1998). Besides the advantages of conventional enzymatic synthesis, such as high regio- and stereoselectivity, absence of racemization and reduced need for side-chain protection, solid-to-solid conversion has additional advantages. The reaction yields may increase by product precipitation in water (Ulijn et al., 2003), which eliminates the use of organic solvents to shift the thermodynamic equilibrium toward synthesis. Using water instead of solvents is of course favorable for environmental reasons, but more importantly, it may also avoid inactivation of the biocatalyst by the solvent (Eichhorn et al., 1997).

Enzymatic reactions yielding solid products that have a typical size of 5–100 μm require particle–particle separation in subsequent process steps when there are other particles
present. These other particles may be particulate biocatalysts, particulate by-products or crystals of unconverted enantiomer in case of conversion of a mixture of racemic crystals into chiral crystals (Straathof et al., 1998). When an immobilized catalyst is the only other particle present in the mixture, separation may be very easy by careful selection of the catalyst carrier material with respect to size, density, and structure, for example catalytic membranes (Kasche and Galunsky, 1995) and catalytic expanded bed media (Van der Wiel et al., 1990, 1996, 1997). With other particle mixtures in enzymatic conversion processes particle–particle separation may be a key step in the purification process. Currently, product purification is performed in most cases with (selective) extraction and (selective) crystallization of the product (Kometani et al., 1997; Kuhl et al., 1990; Matsumae et al., 1995; Miller, 1985; Yan et al., 1999).

**PROPERTIES OF TYPICAL BIOPARTICLES AND THEIR SUSPENSIONS**

In order to determine which separation process is suitable for the recovery of a particulate bioproduct from a particle mixture, the particle properties as well as the liquid phase properties should be known. In this section, properties of bioparticles and their suspensions are reviewed.

**Particle Properties**

**Morphology, Shape, and Composition**

Bioparticles have a large variation in shape, composition, and morphology. Most extracellular particulate microbial products and bioparticles that are formed in biocatalysis are produced as highly pure crystals with varying shape. IBs, on the other hand, may be crystalline or amorphous even when they are composed of the same material but produced in different compartments of a microbial cell (Bowden et al., 1991). In addition, IBs composed of the same material may have a surrounding membrane in one organism, while in another organism it lacks this membrane (Shively, 2003). It is, therefore, impossible to give a general impression of the morphology, shape, and composition of intracellular particulate microbial products.

**Particle Size Distribution**

The particle size distribution is a key parameter for almost all particle–particle separation processes and it will be treated in more detail in Sections “Forces in Particle–Particle Separation” and “Optimization and Control of Driving Forces.” The overview of bioparticle properties presented in Table III shows that the particle size ranges from 0.05 to 100 μm and that there is a large probability for the particle size distributions of two types of bioparticles to overlap.

**Particle Charge**

Bioparticles have a surface charge density that is dependent on their surface chemistry and the composition of the fluid phase. In many cases, the surfaces of bioparticles contain carbonyl, amino, and hydroxide groups. All of these groups can exchange OH⁻ or H⁺ with the surrounding medium causing a change in surface charge. When a charged particle is in solution, it attracts ions with opposite charge and repels ions with like charge. This phenomenon, in combination with the thermal motion of the ions surrounding the particle, causes the formation of an electrical double layer around the particle. The thickness of this electrical double layer is dependent on the ionic concentration of the liquid, which is typically in the range of 0.01 mol/L to 1 mol/L in biological systems, the valency of the ions and their thermal motion.

A measure for the electrokinetic behavior of a particle is given by its electrokinetic potential or zeta-potential, which is the potential at the surface of shear between the charged surface and the electrolyte solution. The relation between pH, ionic strength, and the zeta-potential of bioparticles, such as microbial cells and IBs, has been studied to a reasonable extent (Egorova, 1994; Van der Wal et al., 1997; Wangsa-Wirawan et al., 2001a,b; Yan et al., 1992). The typical zeta-potential for bioparticles lies in the range of −100 mV to 30 mV (Van der Wal et al., 1997; Yan et al., 1992).

**Suspension Characteristics**

**Density of suspension.** The density of a suspension is dependent on the density and concentration of the particles and the density of the liquid. Table III shows that the bioparticle density is in the range of 900 to 1,540 kg/m³. Since most biotechnological processes are carried out in aqueous systems with densities ranging from 1,000 to 1,050 kg/m³, the density of the suspension will be in between 900 and 1,540 kg/m³. In this work, the density of water will be used for all particle suspensions because most products are produced in water.

**Viscosity of suspension.** In most biotechnological processes, water is the main liquid phase. The viscosity of the liquid phase may, however, be much higher than the viscosity of water due to dissolved compounds. For example, in fermentative processes that produce an intracellular product, cells have to be disrupted in order to release the product. As cells are ruptured, DNA and other molecules are released causing a viscosity increase. This viscosity increase can be limited by making use of cell disintegration methods that hydrolyze these molecules. Nevertheless, the liquid phase in fermentative processes is very likely to show non-Newtonian behavior with viscosities exceeding the viscosity of water.

Besides dissolved molecules, the particle volume fraction and the particle shape influence on the viscosity. Einstein (Investigations on the theory of Brownian movement, 1926) obtained the following theoretical relation for the viscosity of identical non-interacting rigid spherical particle suspensions at low particle volume fractions:

\[ \eta = \eta_0 \cdot (1 + 2.5c) \]
Table III. An overview of bioparticle characteristics.

<table>
<thead>
<tr>
<th>Particle</th>
<th>Diameter (μm)</th>
<th>Density (kg/m$^3$)</th>
<th>Iso-electric point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole cells</td>
<td>0.5–5.0 (Agerkvist and Enfors, 1990; Bowden, 1985; Harrison, 1991; Hayashi et al., 2001b; Kula et al., 1990)</td>
<td>1,080–1,120 (Erbeldinger et al., 1998; Wong et al., 1997b)</td>
<td>3.0–5.0 (Hayashi et al., 2001a,b)*</td>
</tr>
<tr>
<td>Yeast</td>
<td>2.0–10 (Bowden, 1985; Harrison, 1991; Kula et al., 1990; Siddiqi et al., 1996)</td>
<td>~1.040 (Lipschutz et al., 2000; Nikolai and Hu, 1992)</td>
<td>—</td>
</tr>
<tr>
<td>Fungi and algae</td>
<td>40–70 (Harrison, 1991)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mammalian</td>
<td>5–40 (Bowden, 1985; Lipschutz et al., 2000; Nikolai and Hu, 1992)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Plant</td>
<td>50–100 (Bowden, 1985)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cell debris</td>
<td>0.05–3.0 (Agerkvist and Enfors, 1990; Bowden, 1985; Kula et al., 1990; Wong et al., 1997a; Wong et al., 1997b)</td>
<td>1,061–1,090 (Wong et al., 1997b)</td>
<td>~2.8 (Wangsa-Wirawan et al., 2001b)*</td>
</tr>
<tr>
<td>Yeast</td>
<td>0.05–8.0 (Kula et al., 1990; Siddiqi et al., 1996)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Inclusion bodies</td>
<td>0.05–1.2 (Taylor et al., 1986; Walker and Lyddiatt, 1999; Wangsa-Wirawan et al., 2001b; Wong et al., 1997b)</td>
<td>900-1,260 (Preusting et al., 1993; Taylor et al., 1986; Wong et al., 1997a,b)</td>
<td>~5.0 (Wangsa-Wirawan et al., 2001b)*</td>
</tr>
<tr>
<td>Crystals</td>
<td>1.0–100 (Bell et al., 1982, 1983; Jauregi et al., 2001; Spassov et al., 1996; Wolff et al., 1997)</td>
<td>1,000-1,540 (Absolom et al., 1986; Bell et al., 1983; Jauregi et al., 2001; Leung et al., 1999; Matthews, 1974)</td>
<td>3–11 (Weast et al., 1964; Chang, 1981)**</td>
</tr>
<tr>
<td>VLPs</td>
<td>0.02–0.20 (Andrews et al., 1995; Cruz et al., 2000; Kiian et al., 1987; Tsoka et al., 2000)</td>
<td>1,140–1,190 (Cruz et al., 2000)</td>
<td>—</td>
</tr>
</tbody>
</table>

*These iso-electric points are available in literature but for other bioparticles different values may be obtained.

**If the iso-electric point of crystal is equal to the molecules of which it is composed.
where \( c \) is the particle volume fraction, \( \eta \) the apparent viscosity, and \( \eta_0 \) the viscosity of the suspending medium. This equation is valid for \( c \ll 0.1 \). In the concentration range, where this equation is applicable, the influence of particle concentration is small. At higher particle concentrations, the influence can be much larger (Bird et al., 2002). In general, it can be said that the viscosity of bioparticle suspensions may vary from the viscosity of water to very high values (Agerkvist and Enfors, 1990; Mosquera et al., 1981; Wong et al., 1997a). In this work, the viscosity of water will be used, as it can be regarded as the common lower limit in bioprocesses, which can be approached by dilution and chemical or enzymatic treatment.

**FORCES IN PARTICLE–PARTICLE SEPARATION**

The forces acting on a particle moving in a fluid are dependent on the presence of force fields, the particle properties, the properties of the fluid phase(s), the properties of other particles in the suspension and the mutual movement of each. The resultant of these forces may be the driving force for particle–particle separation. In this section, various forces are estimated using the particle (and liquid phase) properties that were discussed in “Properties of Typical Bioparticles and Their Suspensions.” The particle shape is assumed to be spherical to avoid complex calculations, even though shape differences may be the basis for particle–particle separation.

**External Field Forces**

**Centrifugal Force**

The centrifugal force, which is often applied in biotechnology, can be used for particle–particle separation if particles in a mixture show different settling velocities under influence of this external field. In the equation below, the net resultant of the centrifugal force on a particle in a fluid phase is shown.

\[
F_C = a \cdot \Delta \rho \cdot V
\]

where \( a \) is the centrifugal acceleration (m/s²), \( \Delta \rho \) the density difference between the particle and the suspension (kg/m³), and \( V \) the particle volume (m³). The difference in \( F_C \) for two types of particles depends on their differences in density and volume. An increase in centrifugal acceleration may cause an increase in the absolute force difference between the particles. The typical upper limit of the centrifugal acceleration that can be achieved with industrial scale centrifuges is 20,000g. Therefore, the maximum \( F_C \) that can be achieved in bioparticle mixtures is approximately \( 5.5 \times 10^{-5} \) N when the particles have a maximum size and density of 100 \( \mu \)m and 1,540 kg/m³ respectively. The force decreases rapidly with a decreasing particle diameter due to its dependence on the particle volume and thus on the cubical particle diameter.

**Electric Force**

The electric force is proportional to the electric field strength and the particle charge. The effective charge of a particle can be described as a function of its zeta-potential and the Debye decay length (Van de Ven, 1989) that is a measure for the thickness of its electrical double layer around the particle. The reciprocal of the Debye decay length, \( \frac{1}{\kappa} \), can be calculated with the following equation (Schulze, 1984):

\[
\kappa = \sqrt{\frac{\varepsilon^2 \cdot N_a \cdot \vartheta \cdot k \cdot T}{e \cdot \sum (C_i \cdot z_i^2)}}
\]

where \( 1/\kappa \) is the Debye decay length (m), \( e \) the elementary charge (C), \( N_a \) the Avagadro number, \( \varepsilon \) the dielectric permittivity of the fluid (F/m), \( k \) the Boltzmann constant (J/K), \( T \) the temperature (K), \( C_i \) the ionic concentration of the solution (mol/m³), and \( z_i \) the ion valency (−). In the equation below, the influence of the Debye decay length on the electric force exerted onto a particle is shown (Schulze, 1984).

\[
F_e = q \cdot E = 2 \cdot \pi \cdot d_p \cdot \left( 1 + \frac{1}{2} \cdot \kappa \cdot d_p \right) \cdot \varepsilon \cdot \zeta_p \cdot E
\]

where \( q \) is the particle charge (C), \( E \) the electrical field strength (V/m), \( d_p \) the particle diameter (m), and \( \zeta_p \) the zeta-potential of the particle (V). Equation 4 shows that the difference in \( F_e \) for two types of particles is governed by their radius and zeta-potential differences. The absolute force difference increases with an increase of the electrical field strength.

Bioparticles have sizes up to 100 \( \mu \)m and zeta-potentials as low as \(-100\) mV. They are generally suspended in water (\( \varepsilon = 7.12 \times 10^{-10} \) F/m) with ionic concentrations ranging from 0.01 mol/L to 1 mol/L. This causes the maximum \( F_e \) that can be reached in biological systems with a typical maximum electrical field of \( 6 \times 10^4 \) V/m (Perry et al., 1998), to be in the order of \( 3.8 \times 10^{-7} \) N. To our knowledge, the electric force has not yet been applied for large-scale particle–particle separation in biotechnology, though it is used in the mineral industry for the recovery of minerals. In biotechnology, the electric force has been mainly used for particle surface charge determination as well as the recovery/purification of bioparticles and biomolecules on analytical scale by electrophoresis using membranes, gels, and capillaries (Henskens and Dieijen-Visser, 2000; Jones et al., 1983; Micale et al., 1980; Netz, 2003; Preece and Luckman, 1981; Young, 1976), and by dielectrophoresis (WO200196857; Washizu et al., 2000). Besides these small-scale applications, there are indications that the force has potential for large-scale applications as well (Douglas et al., 1995; Ivory, 1993; Morgan et al., 1997).

**Magnetic Force**

Some bioparticles contain compounds that are (para)magnetic. Examples of these bioparticles are magnetotactic
bacteria and red blood cells. Magnetotactic bacteria use magnetosomes, which are intracellular magnetic particles, to find the optimal oxygen concentration and/or redox potential in water and at sediment–water interfaces (Dunin-Borkowski et al., 1998). Magnetosomes consist of iron oxide magnetite crystals or iron sulphate crystals, which are enveloped by a membrane (Lins and Farina, 1999). Red blood cells contain hemoglobin, which gives them paramagnetic properties (Melville et al., 1975). Besides bioparticles with natural magnetic properties, non-magnetic bioparticles can be magnetized. Microorganisms and microbial products can be highly efficient bioaccumulators of soluble and particulate forms of metals (Lins and Farina, 1999). Because the metals are (para)magnetic, the bioparticles will obtain magnetic properties as well. The extent to which the bioparticles become magnetic depends on the absorption time and metal concentration (Bahaj et al., 1989). Adsorption of magnetically labeled antibodies onto cells or other bioparticles can have a similar effect (McCloskey et al., 2003). This process is very selective because of the specific interactions between the antibodies and their binding sites.

Magnetized bioparticles can move in a magnetic field, where the magnetic force exerted onto the particle can be described with the following equation:

\[
F_M = \chi \cdot H \cdot V \frac{dB}{dx} \tag{5}
\]

where \(\chi\) is the magnetic susceptibility of the particle (\(\sim\)), \(H\) the magnetic field intensity (A/m), \(\frac{dB}{dx}\) the magnetic field gradient (T/m). Equation 5 shows that the magnetic force difference is proportional to the differences in particle volume and particle magnetization, which is the product of susceptibility and magnetic field intensity.

The magnetic susceptibility is the intensity of magnetization of a particle when it is placed in a uniform magnetic field. For microorganisms with absorbed metal ions, this susceptibility is in the range of 0–28.7 \(\times 10^{-5}\) (SI units) (Bahaj et al., 1989), but other conditions may yield higher susceptibilities. The magnetization of magnetosomes in magnetotactic bacteria has been reported to be close to that of bulk magnetite (Dunin-Borkowski et al., 1998; Proksch et al., 1995), which has a saturation magnetization of 4.8 \(\times 10^{5}\) A/m with a susceptibility of about 3.1 (SI units) (Heider et al., 1996). These high values will, however, only hold for particles containing a very large fraction of magnetite. Therefore, the data for magnetized microorganisms, as presented in Bahaj et al. (1989), will be used for the estimation of the maximal magnetic force in this work.

The magnetic field gradient, which has a typical maximum value of 2.5 \(\times 10^{7}\) T/m in an industrial-scale superconducting electromagnet (Perry et al., 1998), influences the absolute force difference between the particles. The maximum magnetic field intensity that can be obtained in such a device is 3.98 \(\times 10^{6}\) A/m. Using this value for the magnetic field intensity may give an overestimation of the magnetic force since the saturation magnetization of the bioparticles is probably lower than this magnetic field intensity. Nevertheless, if the saturation magnetization is neglected and the maximum magnetic field gradient can be applied, the maximal \(F_M\) that can be reached in biological systems is in the order of 1.5 \(\times 10^{-4}\) N for 100 \(\mu m\) particles. Even though this force can possibly exceed the centrifugal force, it has been applied in biotechnology mostly on a small scale for the concentration and separation of cells (Comella et al., 2001; Cullison and Jaykus, 2002; McCloskey et al., 2003; Melville et al., 1975; Owen, 1978; Radbruch et al., 1994; Zborowski et al., 2003) and the purification of molecules by their adsorption onto magnetic adsorbents followed by recovery of the magnetic adsorbents (Heeboll-Nielsen et al., 2003; Hubbuch and Thomas, 2003).

### Interaction Forces

Besides the external field forces, there are forces acting between particles and objects, such as other particles, part of the separation device or fluid–fluid interfaces. The magnitude of these forces depends on the particle properties and the properties of the object/interface that the particle is interacting with. Here, only spherical and planar geometries are considered.

#### Van der Waals Interaction

The Van der Waals force results from interactions between atoms that are dipoles and/or induced dipoles. In most cases, this force is attractive since the free energy of a system is lower when the dipoles and/or induced dipoles are aligned and thus attract one another. In Equation 6, an approximation of the interaction force between two particles is given (Schulze, 1984):

\[
F_{vdW} = -\frac{A}{12 \cdot h^2} \cdot \left(\frac{d_p \cdot d}{d_p + d}\right) \quad \text{if} \quad \left(\frac{d_p \cdot d}{d_p + d}\right) >> h \tag{6}
\]

where \(A\) is the Hamaker constant (J), \(h\) the distances between the surfaces of the two particles, and \(d\) the diameter of the other sphere (m).

For the interaction between a flat surface and a spherical particle at close distances, the diameter of the other sphere, \(d\), can be chosen much larger than the particle diameter \(d_p\), which results in the following equation (Schulze, 1984):

\[
F_{vdW} = -\frac{A \cdot d_p}{12 \cdot h^2} \quad \text{for} \quad h << d_p << d \tag{7}
\]

The parameters determining the selectivity of particle–particle separation with the Van der Waals force are the particle diameter and the Hamaker constant. The latter typically lies between 10^{-21} and 10^{-19} J (Fielden, 1996; Han, 2002; Hayashi et al., 2001b; Koliadima, 1999; Okada et al., 1990b; Shaw, 1966).

#### Electrostatic Interaction

For two spherical particles with zeta-potentials between −60 mV and 60 mV (Hogg et al., 1965) the electrostatic
interaction force can be approximated with the following equation (Hogg et al., 1965; Okada et al., 1990a):

\[ F_E = 2 \cdot \pi \cdot \varepsilon \cdot d_p \cdot \frac{d_p \cdot d}{d_p + d} \cdot \kappa \cdot \frac{\zeta_p \cdot \zeta}{1 + e^{-\kappa h}} \left[ e^{-\kappa h} - \frac{(\zeta_p - \zeta)^2}{2 \cdot \zeta_p \cdot \zeta \cdot (1 - e^{-2\kappa h})} \right] \]

(8)

for \( \frac{d_p \cdot d}{d_p + d} \gg h \) and \( \kappa \cdot d_p >> 1 \)

where \( \zeta \) is the zeta-potential of the surface interacting with the particle (V). In order to calculate the interaction force between a surface and a spherical particle at close distances the diameter of one of the two spheres can again be chosen much larger than the other. This results in the following approximation (Hogg et al., 1965; Okada et al., 1990a):

\[ F_E = 2 \cdot \pi \cdot \varepsilon \cdot d_p \cdot \kappa \cdot \frac{\zeta_p \cdot \zeta}{1 + e^{-\kappa h}} \left[ e^{-\kappa h} \right] \]

for \( h << d_p \ll d, \kappa \cdot d_p >> 1 \) and \( \frac{(\zeta_p - \zeta)^2}{\zeta_p \cdot \zeta} << 1 \)

(9)

The selectivity of the electrostatic interaction force for particle–particle separation is determined by the differences in radius and zeta-potential of the particles that need to be separated. The absolute force difference can be increased by an increase of the zeta-potential of the surface the particles are interacting with.

The Van der Waals force and the electrostatic interaction force are both dependent on the distance between the particle and the interacting surface. The resultant of these two forces is called the DLVO force. In Figure 2, a typical force–distance curve is presented for the DLVO force, the Van der Waals force, and a repulsive electrostatic interaction force. Forces larger than zero represent repulsive forces and negative forces are attractive. When two types of particles interact with the same object or fluid–fluid interface, their DLVO force–distance curves will be different. Particles with a repulsive DLVO force will move away from the surface and particles with an attractive DLVO force will move towards the surface. This phenomenon has been applied for separation in potential barrier field-flow fractionation (Hansen and Giddings, 1989; Koliadima and Karaiskakis, 1990), a method in which the particle suspension flows parallel to a (charged) surface. Another example of the use of the DLVO force for bioparticle separation was given by Hayashi et al. (2001a) in a study that showed that various microorganisms having different zeta-potentials can be separated on the basis of these differences by adsorption onto a charged surface.

The magnitude of the maximum DLVO force difference for two particles can be approximated by calculating the force difference at the Debye decay length for a particle with the largest Hamaker constant and maximal electrostatic attraction and another particle with the lowest Hamaker constant and maximal electrostatic repulsion. As mentioned before, the Hamaker constant for bioparticles lies in the range of \( 10^{-21} - 10^{-19} \) J and their zeta-potentials are in the range of \( -100 \) mV to \( 30 \) mV. Thus, the maximal driving force for particle–particle separation of 100 \( \mu \)m particles using the DLVO force is approximately \( 3.8 \times 10^{-7} \) N. In this estimation, the zeta-potential limits for Equations 8 and 9 are ignored. The magnitude of the DLVO is thus reasonable compared to the centrifugal force but it has not yet been applied for large-scale particle–particle separation in biotechnology.

There is another interaction force that depends on the distance between the interacting objects, that is, the hydrophobic interaction force. This force will not be discussed because there is a lack of literature data for bioparticles, which makes estimation of the magnitude of this force impossible. It is worth mentioning, however, that an expression similar to that for the Van der Waals force has been proposed to quantify the hydrophobic force with a constant that is different from the Hamaker constant (Rabinovich and Yoon, 1994).

**Interfacial Tension Force**

The interfacial tension between two fluid phases can be lowered by adsorption of particles at the fluid–fluid interface. The energy reduction that is related to this process can be calculated using (Binks, 2002):

\[ E_I = \pi / 4 \cdot d^2 \cdot \gamma \cdot (1 - \cos \theta)^2 \]

(10)

where \( \gamma \) is the interfacial tension (N/m) and \( \theta \) is the equilibrium contact angle between the particle–fluid interface and the fluid–fluid interface measured through the fluid.
where the particle is pulled into. The increase in energy of the system that is caused by removal of the particle from the interface gives rise to a force that pulls the particle back into the interface. This force is related to the static and dynamic fluid–fluid interfacial tension, the particle diameter, and the velocity of the particle during its removal from the interface. Exact calculation of the interfacial tension force would require a detailed description of the particle removal process (Nguyen, 2003), but it is approximated with the derivative of Equation 10 (Clarke and Wilson, 1983) that is shown in Equation 11. This equation only takes into account the energy difference between the initial state when the particle is at its equilibrium position in the interface and the final state when the particle is fully immersed into one of the fluid phases and neglects the phenomena at intermediate particle positions.

\[
F_1 \approx \frac{\pi/4 \cdot d_p^2 \cdot \gamma \cdot (1 - \cos \theta)^2}{\partial \left( \frac{d_p^2}{2} \cdot (1 - \cos \theta) \right)} = \pi \cdot d_p \cdot \gamma \cdot (1 - \cos \theta)
\]

(11)

where \( \frac{1}{2} \cdot d_p \cdot (1 - \cos \theta) \) is the immersion depth of the particle into the fluid where it will be pulled out of, which is equal to the distance the particle has to be transported for its removal from the interface. Equation 11 shows that the selectivity in particle–particle separation using the interfacial tension force is dependent on differences in contact angle, particle diameter, and the fluid–fluid interfacial tension.

Contact angles of bioparticles depend on the properties of the two fluid phases at the interface. Theoretically, this contact angle can be between 0° and 180° but in practice, less extreme values are encountered, such as the air–water contact angle for bacterial cells that ranges from 5° to 107° (Absolom et al., 1986; Busscher et al., 2000; Chattopadhyay et al., 1995). The large variation in these contact angles is caused by variation of the surface properties of the bacterial cells, as well as the variation in composition of the aqueous phase. For polyhydroxybutyrate, air–water contact angles have been measured with a capillary rise technique that vary from 21° to 90° (Marchessault et al., 2001). The large variation in these values is due to the use of various film preparation techniques. When these contact angles are used, the maximum interfacial tension force that can be encountered for bioparticles of 100 µm diameter is in the order of 5.8 \times 10^{-5} \, \text{N} when adsorbed to an air–water interface (\( \gamma = 0.072 \, \text{N/m} \)). The force thus has a large potential for particle–particle separation in biotechnology.

Particle–particle separation using the interfacial tension force has only been studied to a small extent (Boucher, 1989; Jauregi et al., 2001; Winitzer, 1973a,b). In biotechnology, interfaces have been used for the recovery and/or separation of bioparticles and solutes from mixtures. In these applications, aqueous two-phase systems (Andrews et al., 1995; Asenjo et al., 1991; Guereca et al., 1994; Heywood-Waddington et al., 1986; Kula, 1986, 1993; Walker and Lyddiatt, 1999) and other liquid–liquid systems (Borbas et al., 2001; Dennison and Lovrien, 1997; Hoeben et al., 2004; Jauregi et al., 2001, 2002; Kiss et al., 1998; Pike and Denisson, 1989; Tan and Lovrien, 1972) were used to capture the desired products in the interface between the two liquids. In addition, air flotation which makes use of the adsorption of particles and/or molecules to air bubbles that rise in the liquid phase due to buoyancy, has been applied for the recovery of whole cells (Bahr and Schügerl, 1992; De Dousa et al., 2003; Gahr and Schügerl, 1992; Gaudin, 1975; Grieve and Wang, 1966, 1967; Kalyuzhnii et al., 1965; Palmieri et al., 1996; Sadowski and Golab, 1991; Tybussek et al., 1994; Vlaski et al., 1996; Wang et al., 1994). In all of these examples, interfaces are used to capture the product, but this does not necessarily mean that separation is accomplished solely with the interfacial tension force.

The use of the interfacial tension force for complete particle–particle separation in a single stage requires one particle to adsorb into the interface while the other particle remains in one of the fluid phases. When this is accomplished, the adsorbed particle can be selectively removed from the system by separating the interface from the fluid phase that contains the other particle. There are two methods that can be applied for obtaining a fluid–fluid interface that is enriched with one of the two particles that are present in the system. One method is to create conditions that allow both particles to adsorb but forces one of the two particles to detach from the interface. In this case, detachment can be accomplished by using another force such as the drag force and the centrifugal force, or by competition between the two particles for adsorption to the interface as was reported by Jauregi et al. (2001, 2002). Another method is to create conditions that prevent one of the types of particles from adsorbing into the interface. This can be accomplished by using fluid phases that make it energetically unfavorable for one of the particles to adsorb at the interface or by creating an energy barrier for one of the particles that prevents the particle from approaching the interface close enough for adsorption to take place. This energy barrier could be related to the DLVO force or the hydrodynamic conditions in the system. It is clear that both methods require careful selection of the fluid phases and the hydrodynamic conditions in the separation device. There are many possible fluid phase combinations that have a wide variety of interfacial tensions. For instance, the interfacial tension for aqueous two-phase systems is in the order of 0.3 \times 10^{-3} \, \text{N/m}, while for an air–water interface, it is in the order of 0.072 \, \text{N/m}. This indicates that the range of possibilities for particle–particle separation using the interfacial tension force is comprehensive.

**Dynamic Interactions Between Particle and Fluid**

Forces resulting from the dynamic interactions between particles and fluids depend on particle movement and the change in particle movement relative to the fluid phase. This movement is governed by all forces that act on the particle. The forces discussed in this section are therefore in many
cases dependent on other forces and can only be calculated when these other forces are known. Moreover, the dynamic interactions between particles and fluids are often only applicable in combination with other forces. For this reason, they end up in the force balance on the opposite side of the other forces, which from now on will be regarded as driving forces, as is shown in Equation 12.

\[ \sum_i F_i = - \sum_j F_j \quad (12) \]

where \( F_i \) are the driving forces and \( F_j \) are the dynamic particle–fluid interaction forces. Furthermore the dynamic particle–fluid interaction forces will be regarded as inevitable forces that influence separation using the driving forces even though they can be useful for separation. Their applicability for particle separation will not be discussed in this work.

There are three dynamic particle–fluid interaction forces that are frequently mentioned in literature. These are the drag force, the inertia force and the force resulting from the virtual mass of a particle. Besides these forces, many other forces exist such as the Magnus force that results from a spinning motion of a moving particle and the Kutta-Joukowski lift force that results from the fluid-flow profiles around a particle. These forces are not often applied for particle–particle separation on a large scale but they have been shown to be applicable on a small-scale through for instance field-flow fractionation (Giddings et al., 1976). In field-flow fractionation (FFF), a force field acts perpendicular to a flowing liquid that has a certain flow profile. This force field can be the gravitational force or one of the other field forces that were discussed in Section “External Field Forces.” The applied force field causes the particles to move through the flow profile in the flowing liquid. This causes a differentiation of the particle velocities in the separation device that is the basis for their separation. This process is influenced by the dynamic particle–fluid interaction forces and Brownian motion (see Section “Other Phenomena: Brownian Motion”). In flow FFF, a cross-flow liquid stream is applied perpendicular to a flowing suspension (Giddings et al., 1976; Wahlund and Litzen, 1989). In this process, the particles are distributed on the basis of the dynamic particle–fluid interaction forces, the gravitational force and Brownian motion. Separation on the basis of the lift force is feasible in FFF when it is large enough in comparison to the applied force field (Giddings, 2000).

Below the drag force, the inertia force and virtual mass are discussed.

**Drag Force**

The drag force results from friction between the fluid and the particle (friction drag) and the displacement of the fluid phase by the particle (form drag). In the equation below, the general form of the drag force is given.

\[ F_D = C_D \cdot A \cdot \frac{1}{2} \cdot \rho_l \cdot v^2 \quad (13) \]

where \( C_D \) is the drag coefficient, \( A \) is the cross-sectional area of the particle (m²) and \( v \) (m/s) is the particle velocity relative to the fluid phase. The drag coefficient is dependent on the shape of the particle, on its orientation in the fluid and on its relative velocity. The drag coefficient for spherical particles at Reynolds numbers below 0.1 is given by Stokes’ law:

\[ C_D = \frac{24}{Re} \quad \text{for } Re < 0.1. \]

This relation can also be used for Reynolds numbers between 0.1 and 1 but then the drag force is underestimated with about 10%. For Reynolds numbers between 0.5 and \( 6 \times 10^3 \), the drag coefficient can be calculated with the following expression (Bird et al., 2002):

\[ C_D = \left( \frac{24}{Re} + 0.5407 \right)^2 \quad \text{for } 0.5 < Re < 6 \times 10^3. \]

At Reynolds numbers between \( 5 \times 10^2 \) and \( 10^5 \) the drag coefficient obtains a constant value of about 0.44 (Bird et al., 2002).

**Inertia Force**

An expression for the inertial force is given below:

\[ F_{in} = V \cdot \frac{\rho_p \cdot \frac{dV}{dt}}{} \quad (14) \]

where \( \rho_p \) is the particle density (kg/m³). If the inertia force is included in the force balance, particle acceleration becomes a function of particle density, particle size, and the other forces. This causes a differentiation in acceleration of particles with different driving forces, sizes, and/or densities, which could be the basis for particle–particle separation. Application of this principle on an industrial scale will be very difficult. In addition, including the inertia force in the force balance requires time iterations for calculation of the relative particle velocities and their derivative to time, making simple estimation of the particle–particle separation efficiency impossible. Therefore, the inertia force will not be considered in any force calculations in this work. For small particles, the inertia force generally is very small because these particles tend to follow the liquid streamlines (Caullet et al., 1996), which makes this a proper assumption. With larger particles, the inertia force may reduce or even prevent particle acceleration. This can prevent the particles from reaching their terminal velocity that was calculated on the basis of the drag force and the driving forces.

**Virtual Mass**

The virtual mass of a particle accounts for the resistance of the fluid against changes in particle velocity relative to that of the fluid. For spherical particles in a constant fluid flow
profile, the virtual mass is equal to half of the particle volume (Zhang et al., 2000), so that

\[
F_{VM} = \frac{1}{2} V \cdot \rho_l \frac{d\nu}{dt}
\]

where \(\rho_l\) is the liquid density (kg/m\(^3\)) and \(d\nu/dt\) is the acceleration (m/s\(^2\)). The same argument holds for this force as for the inertia force with respect to its influence on the relative particle velocity and its applicability for particle–particle separation. Therefore this force will be excluded from the force balance as well.

**Other Phenomena: Brownian Motion**

The time-average kinetic energy of a particle, regardless of its size, is equal to the average thermal energy of the surrounding molecules, which is 3/2 kT (1/2 kT in one dimension). The kinetic energy of a particle may fluctuate in time and may thus differ from the time-average value. This makes estimation of the microscopic effects of the Brownian motion very difficult. The time-average value will, therefore, be used for estimation of the macroscopic effect of Brownian motion. Equation 16 shows the relation between the one-dimensional concentration gradient and the Brownian force (Van de Ven, 1989).

\[
F_B = -k \cdot T \cdot \frac{dn}{dx}
\]

where \(dn/dx\) is the particle concentration gradient (mol/m\(^4\)). Particles can thus move from a high particle concentration to a low concentration as a result of the Brownian force. In addition, particle motion occurs in homogeneous suspensions where \(dn/dx\) is zero, but will be random. This random motion does not cause a net particle transport and is therefore not useful for particle–particle separation by itself. However, it can act as a dominant transport mechanism and facilitate other processes such as particle adsorption at interfaces. Brownian motion has been used in biotechnology mainly for the separation of molecules by making use of the differences in their transport through membranes and in matrices. Transport in these systems depends on the thermal motion of the compounds, their partitioning in the media and the hindrance they experience within the matrix or membrane. In addition, Brownian motion can play an important role in particle separation using field-flow fractionation (Giddings, 2000).

For estimation of the maximum Brownian force in bioparticle mixtures, the maximum obtainable particle concentration gradient is required. If it is assumed that the highest particle concentration is reached at 60 %v/v particles (dense packing of spheres) and decreases to 0 %v/v over a distance of 10\(^{-3}\) m, then \(1/n \cdot dn/dx\) is approximately \(2 \times 10^3]\)m at an average value of \(n\) resulting in a Brownian force of \(8.1 \times 10^{-18}\) N at a temperature of 298 K. Although this estimation may be off by a couple of orders of magnitude, we can readily see that the Brownian force acting on 100 \(\mu\)m particles is very small compared to the other forces that were discussed in this section. For very small particles the Brownian force becomes more important because it is independent of particle size while the other forces decrease with particle size.

**Overview of Driving Force Differences Between Particles**

The upper limit of the driving force difference for two bioparticles with similar sizes and different properties is estimated for bioparticles and liquid phases with properties that are presented in the Section "Properties of Typical Bioparticles and Their Suspensions" and Section "Forces in Particle–Particle Separation." The result of this estimation is depicted in Figure 3 for the whole range of particle sizes that may be encountered in biotechnology. The graph shows that for particle diameters between 20 nm and 100 \(\mu\)m, the interfacial tension force has the largest potential for separation of particles with the same size. For particle diameters below 0.5 \(\mu\)m, the electrical force also has large potential. The centrifugal force has a much lower potential than these two forces and the graph confirms that it may even be inapplicable for the separation of small particles. Whether or not the interfacial tension force and the electric force are the largest driving forces for particle–particle separation in practice depends on the liquid phase properties and the particle properties for each specific separation problem. These properties can be manipulated to some extent to increase the selectivity for many of the driving forces (Section "Optimization and Control of Driving Forces").

Direct comparison of the driving forces (Fig. 3) can help to determine the forces and processes that should be investigated in more detail. After this initial screening, the limitation to the applicability of the driving forces has to be
taken into consideration. This is done in Section “Selection of a Particle–Particle Separation Technique.”

Before discussing the optimization of driving forces, a small comment has to be made with respect to combining driving forces in particle–particle separation. In many cases, we are forced to deal with combinations of forces because some forces cannot be excluded from the process. Examples of these forces are the Brownian force, the gravitational force and many of the forces that are related to the dynamic interactions between particles and fluids. It is therefore essential to take into account the influences of these forces when designing a process. Furthermore, combinations of driving forces may give additional options in particle–particle separation. Force estimations like those presented in Figure 3 can aid the selection process. Evaluation of these combinations is only valuable when a specific separation problem is regarded because of the larger number of possible combinations and the difficulty of combining some of the forces. Therefore this topic will not be dealt with in this work.

In literature, this strategy is discussed to some extent. For instance, the thesis of Tils (Tils and Tels, 1992) deals with particle separation by flotation in a centrifugal force field, and in field-flow fractionation dynamic particle-fluid interaction forces are often combined with field forces and Brownian motion (see section “Dynamic Interactions Between Particle and Fluid”).

OPTIMIZATION AND CONTROL OF DRIVING FORCES

Particle properties can be manipulated by changing their surface chemistry, which influences the electrical force, the magnetic force, the DLVO force, the interfacial tension force, and possibly the aggregate size.

The surface properties of particles can be controlled by adsorption of surfactants. This technique is commonly applied in flotation and may change the zeta-potential of particles and their contact angle with the fluid–fluid interface (Fraunholcz and Dalmijn, 1998; Hanumantha Rao and Forssberg, 1997). Polymer or polyelectrolyte adsorption may have a similar effect as the surfactants that are used in flotation.

The particle size distribution can be manipulated using aggregation. This process only has a positive effect on particle–particle separation if it is selective for one kind of particle or if the particles aggregate specifically with their own kind resulting in aggregate sizes that yield a larger force difference compared to the force difference for the primary particles. It is therefore very important that heteroaggregation and entrapment of particles in aggregates of other particles is prevented. The aggregation rate of particles in a suspension may be increased by reducing the electrostatic repulsion between the particles, by adding polymers or polyelectrolytes, with shear, by changing the temperature, with high pressure treatment or by enzymatic action (renneting) (Dickinson, 2003). The first two methods will be briefly discussed.

The electrostatic repulsion between particles can be reduced by changing the nature and concentration of the ions in solution (Eq. 3), by reducing their zeta-potential through pH change (Eq. 8) or by specific adsorption of surfactants or ions on their surface (Chang and Hshieh, 1991). The latter strategy has been shown to work successfully for air–water interfaces with aluminium (Li and Somasundaran, 1992) and magnesium ions (Li and Somasundaran, 1991) as well as for polystyrene particles with surfactants (Okada et al., 1990b). Addition of polymers or polyelectrolytes can also lower the electrostatic repulsion, but more importantly these compounds may form bridges between the particles by adsorbing onto the particle surface. Bridging causes an increase in particle aggregation rate due to more effective particle collisions and a decrease in aggregate break-up rate. Examples of chemicals that can be used for particle size manipulation are borax, which is an effective flocculation agent for cell debris, as reported by Tsoka et al. (2000) and calcium ions that can be used to flocculate microorganisms by forming metal-polymer complexes with the polymers on the surface of the microorganism, as reported by Sanin and Vesilind (1996).

SELECTION OF A PARTICLE–PARTICLE SEPARATION TECHNIQUE

In the following sections, a method for creating a window of operation for the driving forces is presented and the selection procedure for suitable separation methods is discussed for typical particle mixtures that are encountered in biotechnology.

Window of Operation for the Driving Forces in Particle–Particle Separation

The window of operation for the field forces depends on the particle properties and the minimum velocity difference that is required for their separation. The velocity of a particle can be calculated by solving the force balance between the driving forces and the dynamic particle–fluid interaction forces (Eq. 12). From the dynamic particle–fluid interaction forces only the drag force will be taken into account for reasons that were discussed in Section “Overview of Driving Force Differences Between Particles.” The driving forces for separation of bioparticles will often be small near their separation limits causing particle velocities to be small as well. Stokes’ law can therefore be applied for the drag force in most cases. As an example, in Equation 17 Stokes’ drag is included in the force balance of Equation 12 in case the centrifugal force is the only driving force.

\[ F_C = F_t \to a \cdot \Delta \rho \cdot \pi \cdot d^3 P \]

\[ = 3 \cdot \pi \cdot \eta \cdot d \cdot v \to d_{p,lim} \]

\[ = \sqrt{\frac{18 \cdot \eta \cdot v_{min}}{a \cdot \Delta \rho}} \]
The limiting particle diameter that can be separated with the driving force is a function of the particle properties, the fluid properties, the centrifugal acceleration, and the minimum required terminal particle velocity. By subtracting the minimum required terminal particle velocities of all particles in a mixture, the minimum particle diameters that are required for the separation of the mixture can be calculated. The relation between the minimum particle diameters and the minimum required velocity difference in a binary particle mixture is shown below for the field forces in case of a direction perpendicular to the liquid flow. In order to make this theory applicable for particle–particle separation instead of particle–liquid separation, we have to allow for different apparatus designs dependent on the direction and magnitude of the particle velocities. In Figure 4, two possible designs are presented (Giddings, 1991). These designs show that for complete separation in devices with similar dimensions that are used for processing equal volume fluxes, the minimum required velocity difference between the particles should be in the same order as the terminal velocity of a single particle that is required for particle–liquid separation. With a feed suspension, the vectors indicate the direction of particle movement relative to the liquid phase. The distribution will become an issue in this design. The vectors indicate the direction of particle movement parallel to the liquid flow. In case of particle movement parallel to the liquid flow, the velocity difference between particle 1 and particle 2 is in opposite direction of the liquid flow ($v_1 < v_2$), while the net velocity of Particle 2 is in the direction of the liquid flow ($v_2 > v_1$). The window of operation of the Brownian force will not be discussed because estimation of the particle concentration gradient is arbitrary and will therefore not give an accurate selection criterion. Nevertheless, the Brownian force may be applicable if the differences in particle transport rates through membranes, in matrices, and in field-flow fractionation are large enough.

Estimation of the minimum required velocity difference requires knowledge of the feed flux, particle concentration in the feed, and the area available for separation. All of these parameters depend on the design of the separation device. To make comparison of the driving forces possible, we will limit ourselves to using the minimum particle velocity that is required for centrifugal separation. This velocity can be calculated with the Sigma theory (Ambler, 1961), which states that separation of a particle from the liquid phase with an efficiency of 100% requires a minimum particle velocity perpendicular to the liquid flow equal to the ratio of the volumetric liquid flux and the available settling area perpendicular to the liquid flow. In order to make this theory applicable for particle–particle separation instead of particle–liquid separation, we have to allow for different apparatus designs dependent on the direction and magnitude of the particle velocities. In Figure 4, two possible designs are presented (Giddings, 1991). These designs show that for complete separation in devices with similar dimensions that are used for processing equal volume fluxes, the minimum required velocity difference between the particles should be in the same order as the terminal velocity of a single particle that is required for particle–liquid separation. With a feed suspension, the vectors indicate the direction of particle movement relative to the liquid phase.

\[
\Delta v_{\text{min}} = \frac{a}{18 \cdot \eta} \cdot \left| \Delta \rho \cdot d^2_{\text{p,lim,1}} - \Delta \rho \cdot d^2_{\text{p,lim,2}} \right|
\]

(18)

\[
\Delta v_{\text{min}} = \frac{2 \cdot \varepsilon \cdot E}{3 \cdot \eta} \cdot \left| (1 + 0.5 \cdot \kappa \cdot d_{\text{p,lim,1}}) \cdot \zeta_1 - (1 + 0.5 \cdot \kappa \cdot d_{\text{p,lim,2}}) \cdot \zeta_2 \right|
\]

(19)

\[
\Delta v_{\text{min}} = \frac{H}{18 \cdot \eta} \cdot \frac{dB}{dx} \cdot \left| \chi_1 \cdot d^2_{\text{p,lim,1}} - \chi_2 \cdot d^2_{\text{p,lim,2}} \right|
\]

(20)

The window of operation for the interfacial tension force cannot be constructed in a similar manner as for the field forces and the DLVO force because it completely depends on the design of the separation device, as was discussed in Section “Dynamic Interactions Between Particle and Fluid.” In Section “Selection of a Driving Force for Particle–Particle Separation,” this design will be treated in more detail to facilitate construction of the operation window for the interfacial tension force. The operation window of the Brownian force will not be discussed because estimation of the particle concentration gradient is arbitrary and will therefore not give an accurate selection criterion. Nevertheless, the Brownian force may be applicable if the differences in particle transport rates through membranes, in matrices, and in field-flow fractionation are large enough.

\[
\Delta v_{\text{min}} = \frac{2 \cdot \varepsilon \cdot \kappa \cdot \zeta}{3 \cdot \eta} \cdot \frac{e^{-\kappa h}}{1 + e^{-\kappa h}} \cdot (\zeta_1 - \zeta_2) + \frac{A_2 - A_1}{36 \cdot \pi \cdot \eta \cdot h^2}
\]

(21)
flux of 1 m³/h that is reasonable for large-scale processes and an equivalent settling area of 2 × 10⁴ m² (Perry et al., 1998), which is approximately the upper limit for industrial centrifugation at high gravitational acceleration (11,500g), the minimum required particle velocity difference is approximately \((1 \times 11,500)/(3,600 \times 2 \times 10⁴) = 1.6 \times 10^{-3}\) m/s. For the magnetic and electric force, this minimum required velocity difference is in the same order when industrial-scale electric and magnetic separators having a separation section with an approximate length, width, and height of 3, 1, and 0.05 m (dimensions are estimated from handbook data (Perry et al., 1998)) are considered for treating the same feed. Therefore, the same velocity difference will be applied in all calculations.

In the following section, a window of operation is created for each of the driving forces when applied to typical particle mixtures in biotechnology.

Selection of a Driving Force for Particle–Particle Separation

The overview of particle mixtures that was presented in section “Overview of Particle Mixtures in Biotechnological Processes” shows that there are different classes of separation problems that may require different purification strategies. In most of these separation problems product purification requires removal of whole cells or cell debris. Construction of the operation window for the centrifugal force requires information on particle size and density, which is available in literature for most particle mixtures. Estimation of the electric, and magnetic force, on the other hand, requires the magnetic susceptibility and particle charge, which are scarcely reported in literature. These particle properties are therefore estimated on the basis of the data that were presented in Sections “Electric Force” and “Magnetic Force.” In Figure 5, the relation between the limiting particle diameters for the centrifugal, electric and magnetic force are shown for extreme cases of IB purification (Fig. 5A and B), VLP purification (Fig. 5C), and extracellular particulate microbial product purification (Fig. 5D and E) that may be encountered in industry. In the calculation of these limits, the liquid phase density is assumed to be 1,000 kg/m³, since most biotechnological processes are carried out in water. Higher liquid densities may give larger velocity differences in centrifugation and may thus lower separation limits, but possibilities for density manipulation depend on each specific separation problem and will not be considered here. Furthermore, the density of whole cells and cell debris is assumed to be 1,085 kg/m³ (Wong et al., 1997b).

In Figure 5, the area marked with horizontal dotted lines represents all particle diameter combinations that cannot be separated with the magnetic force and the area marked with vertical dotted lines represents the combinations that cannot be separated with the centrifugal force. The electric force only fails to give separation for particle diameter combinations exactly on the boundary line that is depicted in Figure 5. If larger velocity differences are required for the electric force the two particle diameter limits will move apart in a similar manner as with the magnetic force. Furthermore, the DLVO force may be applied to all particle diameter combinations since the minimum particle velocity difference is exceeded in all cases. However, applicability of this force will depend very much on process design. The limits of the interfacial tension force are not depicted for reasons that were discussed in Section “Window of Operation for the Driving Forces in Particle–Particle Separation.”

In each of the graphs depicted in Figure 5, the area that is marked with dashed lines confines the range of particle sizes that are encountered in biotechnology. These areas show that separation of extracellular particulate microbial products from whole cells can be performed with centrifugation in most cases regardless of the density of the product. Purification of IBs and VLPs by centrifugation, on the other hand, will be difficult or even impossible in many cases because many particle size combinations lie outside of the working area for centrifugation. In addition, cell debris may have a broad particle size distribution causing part of the cell debris particle size to be below the separation limit for centrifugal separation in many cases. This inevitably leads to incomplete separation. When the centrifugal force fails, one of the other driving forces must be selected.

The magnetic force, the interfacial tension force (in combination with the DLVO force), and the electric force are used in the paper and/or mineral industry, which may make application of these forces in biotechnology possible within a reasonable time span. The electric force and the interfacial tension force are most favorable because in theory there are very few separation problems that cannot be resolved with these forces. Even though the actual zeta-potentials may be different from those that were assumed for constructing Figure 5, a small difference in zeta-potential is sufficient for particle separation with the electric force \(\Delta_{\text{min}}\) still exceeded. Since the zeta-potential can be easily manipulated in biological systems by varying the pH, nearly all particles with a different relation between pH and zeta-potential can be separated with the electric force. It is much more difficult to indicate what particle mixtures can be separated with the interfacial tension force (in combination with the DLVO force) because the mechanisms of flotation and interfacial partitioning are not fully understood yet. In addition, the mixtures that need to be treated in biotechnology are often very complex due to the large number of components that are present, which makes prediction of the particle behavior very difficult. Nevertheless, an attempt will be made to construct a window of operation for the interfacial tension force. As indicated in Section “Dynamic Interactions Between Particle and Fluid,” there is a number of strategies for the use of the interfacial tension force for particle–particle separation. These strategies have in common that one type of particles needs to remain adsorbed at the interface while the other particles remain in the continuous phase, for instance, due to an energy barrier that prevents adsorption of these particles at the interface or due to competition between the particles for adsorption at the interface. In most biotechnological
Figure 5. An overview of the particle–particle separation limits for the centrifugal force, the electric force, and the magnetic force for extreme particle–particle separation problems that are encountered in biotechnology. Particle 1 represents cell material with a magnetic susceptibility of $2.5 \times 10^{-4}$ and a zeta-potential of $-0.030$ V. Particle 2 represents the particulate product with a magnetic susceptibility of $1.0 \times 10^{-4}$ and a zeta-potential of $-0.010$ V. The vertical lines indicate the conditions where centrifugation is not applicable for separation and the horizontal lines indicate the area where the magnetic force is not applicable for separation. The electric force can be applied for all particle size combinations that are not on the line that indicates the separation limit. The area confined by the dashed lines indicates all particle size combinations that are encountered in biotechnology for each class of separation problems. A: Separation of IBs with a high density (1,260 kg/m$^3$) from microbial cell debris. B: Separation of IBs with a low density (900 kg/m$^3$) from microbial cell debris. C: Separation of VLPs with a density of 1,190 kg/m$^3$ from microbial cell debris. D: Separation of extracellular particulate microbial products with a high density (1,540 kg/m$^3$) from whole cells. E: Separation of extracellular particulate microbial products with a low density (1,000 kg/m$^3$) from whole cells.
processes, the density of the continuous (often aqueous) phase will be higher than the density of the dispersed phase. This density difference can be utilized in the process by making the dispersed phase rise through the continuous phase under influence of the gravitational force or centrifugal force. The interfacial tension force acting on the particle that is adsorbed at the interface should therefore exceed the gravitational (and shear) force(s) that acts on the particle to prevent detachment from the interface. In Figure 6, the relation is given between contact angle, interfacial tension, and the maximum particle diameter that still allows particles to remain at the interface under gravity. For construction of this graph, the particle density and continuous phase density are assumed to be 1,540 and 1,000 kg/m³, respectively and the contact angles are assumed to be in the range that was reported in section “Forces in Particle–Particle Separation.” The operation window shows that the interfacial tension force can be applied for separation of particle sizes ranging from molecular scale up to approximately 0.01 m regardless of the differences in particle densities as long as particle adsorption at the interface is selective for one of the particles in the mixture. The process of creating selectivity can be very complex, however. It depends on the hydrodynamics near the interface, the interaction forces (Section “Interaction Forces”) and competition effects. Thorough discussions on the application of interfaces for particle separation lie outside of the scope of this work and can be found elsewhere (Clarke and Wilson, 1983; Finch and Dobby, 1990; Gaudin, 1975; Schulze, 1984).

CONCLUSION
Selection of a particle–particle separation technique requires knowledge of the particle properties, such as density, magnetic susceptibility, zeta-potential, and hydrophobicity. When these properties are known, the optimal particle–particle separation technique can be chosen by solving the force balance between the drag force and the driving forces. Force estimations reported in this work show that the interfacial tension force (in combination with the DLVO force) and the electrical force, in whatever way applicable, have large potential for particle–particle separation in biotechnology. The electrical force requires very small differences in zeta-potential to make complete separation possible and the window of operation of the interfacial tension force indicates its applicability to a very broad range of particle sizes (up to 0.01 m). The use of the electrical force and the interfacial tension force becomes desirable when centrifugation cannot be applied for particle–particle separation. This is generally the case when particle sizes are small (≈1 μm) and/or when the density differences between the particles are small. Examples of such separation problems are the separation of IBs and VLPs from cell debris. Currently, only limited use is made of these alternative particle–particle separation techniques due to a lack of knowledge. Therefore R&D efforts should increase in this area to extend the downstream processing toolbox that is currently available to the biotech industry.

NOMENCLATURE
\(a\) centrifugal acceleration (m/s²)
\(A\) Hamaker constant (J)
\(A_\text{c}\) cross-sectional area of particle (m²)
\(B\) magnetic field (T)
\(c\) particle volume fraction (-)
\(C_D\) drag coefficient (-)
\(C_i\) ionic concentration (mol/m³)
\( d \) diameter of sphere (m)
\( d_p \) particle diameter (m)
\( d_{p,\text{lim}} \) limiting particle diameter (m)
\( e \) elementary charge (C)
\( E \) electric field (V/m)
\( E_1 \) energy required to pull a particle out of a fluid-fluid interface (J)
\( F_B \) Brownian force (N)
\( F_e \) electric force (N)
\( F_{\text{el}} \) electrostatic interaction force (N)
\( F_F \) drag force (N)
\( F_C \) centrifugal force (N)
\( F_I \) general expression for the driving forces (N)
\( F_T \) interfacial tension force (N)
\( F_{\text{in}} \) inertia force (N)
\( F_J \) general expression for the dynamic particle-fluid interaction forces (N)
\( F_{\text{SM}} \) magnetic force (N)
\( F_{\text{sw}} \) Van der Waals force (N)
\( F_{\text{VM}} \) force caused by virtual mass of particle (N)
\( h \) distance between the surfaces of a particle and an object (m)
\( H \) magnetic field intensity (A/m)
\( k \) Boltzmann constant (J/K)
\( n \) particle concentration (mol/m³)
\( N_{\text{Av}} \) Avogadro number (#/mol)
\( \ell \) time (s)
\( T \) temperature (K)
\( q \) particle charge (C)
\( n \) Reynolds number (–)
\( \nu \) relative velocity between particle and fluid (m/s)
\( \nu_{\text{min}} \) minimum required particle velocity (m/s)
\( V \) particle volume (m³)
\( x \) distance (m)
\( z_i \) ion valency (–)
\( \chi \) magnetic susceptibility (–)
\( \epsilon \) dielectric permittivity of the fluid (F/m)
\( \gamma \) interfacial tension (N/m)
\( \eta \) apparent viscosity (Pa s)
\( \eta_0 \) liquid viscosity (Pa s)
\( \kappa \) Debye decay length (m)
\( \theta \) contact angle between particle and fluid-fluid interface (°)
\( \rho_1 \) liquid phase density (kg/m³)
\( \rho_p \) particle density (kg/m³)
\( \zeta \) zeta-potential of a surface (V)
\( \zeta_p \) particle zeta-potential (V)

References


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