

The use of noble, colloidal particles for biosensing applications

The aim of the laboratory experiment is to demonstrate the influence from surface charge on colloidal stability using noble metal (gold) colloidal particles, and how this system can be utilized in construction of a biosensor system.

The detection of colloidal gold and/or silver particle aggregation by uv-vis optical spectroscopy offers a very sensitive detection system, allowing detection down to 10 femtomole of the analyte. In brief, the detection principle relies on the fact that the optical spectrum for a suspension of colloidal gold/silver particles changes when the particles aggregate. The change in optical spectrum is due to a change of the surface plasmon resonance of the particles as they approach each other and reach interparticle distances less than or close to the particle diameter. A very simple way to achieve this is by addition of a salt to the solution, thus reducing the electrostatic repulsion between the colloidal particles. In addition, by keeping the colloidal particles separated in solution, aggregation can also be induced upon specific recognition of biomolecules immobilized on the particles. The principle is illustrated in Fig. 1, where colloidal gold particles have been functionalized with a lipid layer, that contains a special molecule called biotin, which is a vitamin that binds specifically to a protein called streptavidin. In the initial state (before addition of streptavidin) the particles are free in solution. However, upon addition of streptavidin the particles will couple to each other through the biotin-streptavidin binding, thereby forming large aggregates. This is just an example of a functionalization strategy, many other biomolecules can be used for the specific recognition as well, e.g. antigens-antibodies or nucleotides. In the laboratory exercise we will primarily concentrate on colloidal aggregation induced by salt, and discuss the possibility to use this for biosensor applications.

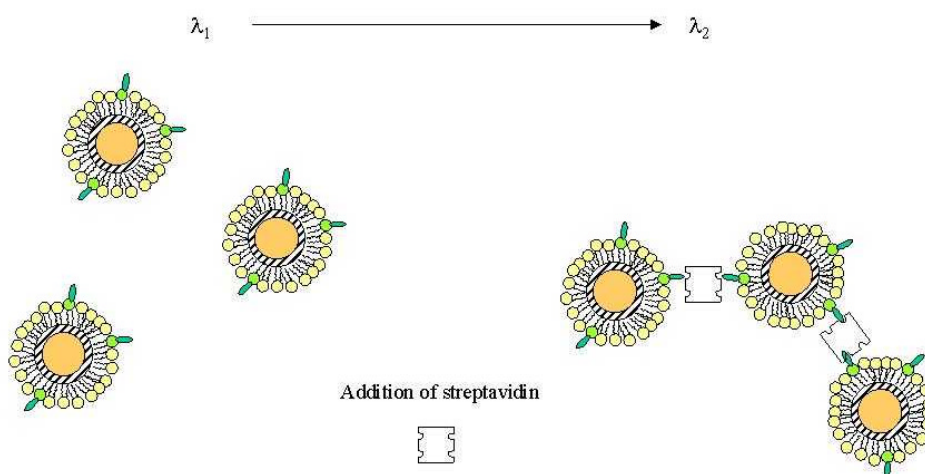


Figure 1 Principle of the detection system

Theory

In order to provide a better understanding, this section gives a brief overview over some central physical aspects of the system, beginning with some terminology. After

that stability of colloidal systems will be discussed as well as the physical origin of the optical properties of colloidal suspensions of gold and silver particles.

Definition of colloidal particles

The size of a colloidal particle is somewhere between 1 and 1000 nm, but most often one talks about colloidal systems rather than giving a formal definition of a colloidal particle. A colloidal system can be defined as a dispersed phase distributed uniformly in a finely divided state in a dispersion medium. Finely divided, in this case, means that the particles have a size range from 1 to 1000 nm. Familiar examples of colloidal systems are fog, smoke, milk and paint. These examples also represent different types of colloidal systems with respect to the three states of matter, gases, liquids and solids. Fog is a dispersion of liquid droplets in a gas, whereas smoke is build up of solid particles in gas. A common term for these cases is aerosols. Milk goes into another category, familiar as emulsions, and consists of fine droplets of fat in an aqueous phase. Paint is build up of solid particles in a liquid phase and colloidal systems of that type are known as sols or suspensions. These are just a few examples of colloidal systems and colloidal system types. Nature shows a great diversity of colloidal systems, but the one considered in this case is of suspension type, i.e. solid particles in a liquid phase.

Stability of colloidal systems

Due to Brownian motion, colloidal particles in a solution will always come in contact with one another to some extent. A central issue when discussing colloidal systems is their stability, i.e. whether they remain in a dispersed state, or tend to aggregate. It is well known from thermodynamics that if a system is kept at a constant temperature, it will tend to change spontaneously in a direction, which will lower its free energy. It is, though, important to notice that the change towards a lower energy only occurs if there is a suitable mechanism present that enables it to take place.

There is always an attractive force between the particles in a solution, the so called “long-range van der Waals force”, which has its origin in the ordinary van der Waals force. However when two colloidal particles approach each other, the atoms in one particle are to some extent able to interact with all atoms in the other particle, and vice versa, and these effects can to a first approximation be treated as additive. As a consequence, the particles can interact over relatively large distances. This force is known as the long-range van der Waals force, as aforementioned, or the Hamaker force. The van der Waals interaction potential between two large ($R > 2$ nm) spheres at small separations (D) can to a first approximation be expressed as.

$$W(D)_{vdW} = -\frac{AR}{12D} \quad (1)$$

where A , the Hamaker constant, is typically $0.5 - 1.0 \cdot 10^{-20}$ J (and usually slightly larger for most solid surfaces).

From a thermodynamic point of view, the free energy will decrease as the particles approach each other. Colloidal particles in a solution may nevertheless stay separated, because most colloidal particles are in fact electrically charged. As a consequence, there will be an excess of oppositely charged ions, called counterions, around them (Fig. 2).

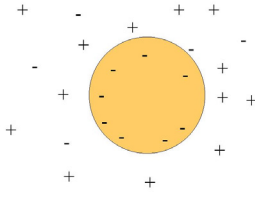


Figure 2 Counterions around a charged colloidal particle

These counterions, also known as the diffuse electric double layer, do not fully neutralize the original charge on the surface, and cause the particles to repel. If the charge balance in the solution is changed, for instance by adding a salt, this diffusive electric double layer will shrink. At sufficiently high salt concentration, the counterions are accumulated so close to the particles that the electrostatic repulsion is no longer strong enough to overcome the long-range van der Waals forces, which, as a consequence, results in aggregation of the particles. The electrical double layer interaction between two equally charged spheres is to a first approximation:

$$W(D)_{DL} \approx 4\pi R \epsilon \epsilon_0 \psi^2 e^{-\kappa D} = C_1 \psi^2 e^{-C_2 \sqrt{[NaCl]} D} \quad (2)$$

where the relation between the surface charge, σ , and the surface potential, ψ , is given by:

$$\sigma \approx \epsilon \epsilon_0 \psi \sqrt{\frac{e^2 [NaCl]}{\epsilon \epsilon_0 k T}} = \epsilon \epsilon_0 \psi \kappa \quad (3)$$

where $1/\kappa$ defines the Debye length, and $[NaCl]$ represents the salt concentration (of any monovalent salt).

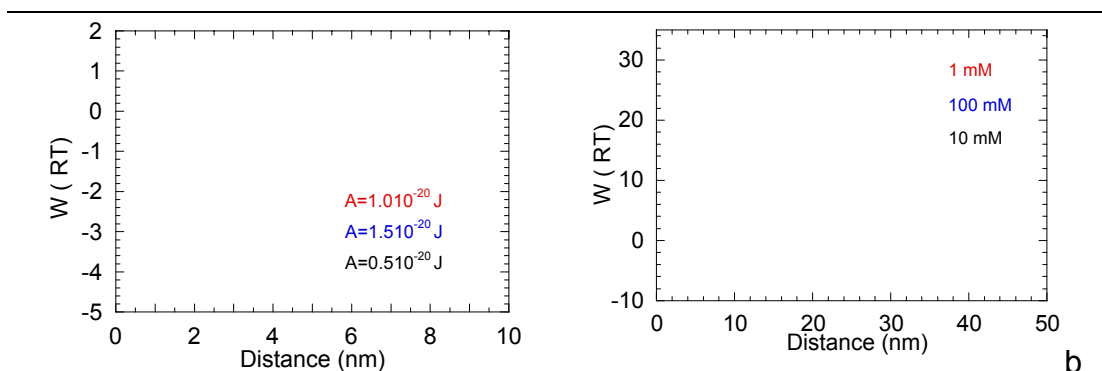
If the charge density of the two surfaces are not equal, ψ^2 in Eq. 2 is replaced by $\psi_1 \psi_2$. If they are of opposite sign this product is negative, and the double layer interaction is attractive. Note that both the Debye length and the surface potential decrease at increased ionic strength. Consequently, both the magnitude *and* the decay length of the interaction decrease at increased ionic strength.

The total interaction potential of two equally charged spheres (a sphere and a flat surface or two flat surfaces) is given by the sum of W_{vdW} and W_{DL} :

$$W(D)_{TOT} = W(D)_{DL} + W(D)_{vdW} \quad (4)$$

As an illustration, Fig. 3 a shows $W(D)_{tot}$ for two spheres with a radius of 6.25 nm (c.f. e.g. the protein ferritin or a gold nanoparticle) with a common charge of 0.03 C/m^2 (which equals one charge per $\sim 10 \text{ nm}^2$) in 100 mM NaCl using a Hamaker constant of 0.5, 1.0 and $1.5 \cdot 10^{-20} \text{ J}$. In Fig. 3 b, the interaction potential at 1, 10 and

100 mM NaCl solution for the same system using a Hamaker constant of $1 \cdot 10^{-20}$ J is shown.



a
Figure 3 Illustration of the interaction between two spheres with a common charge of 0.03 C/m^2 at (a) 100 mM NaCl and a Hamaker constant of 0.5, 1 and $1.5 \cdot 10^{-20}$ J and (b) at 1, 10 and 100 mM NaCl using a common Hamaker constant of $1.0 \cdot 10^{-20}$ J. (The sphere has the same diameter as ferritin ($\varnothing \sim 12.5$ nm) and the charge corresponds to ~ 2 charges per subunit.) The repulsion between a sphere (ferritin molecule) and a flat surface, assuming the same charge and Hamaker constant, is approximately a factor of two larger.

It is possible to identify a limit where slow aggregation turns into rapid aggregation when adding a salt to the solution. This is called the critical coagulation concentration. This theory, involving only two types of identified forces (electrostatic repulsive and universal attractive) in an electrostatically stabilized solution is also commonly referred to as the DLVO-theory (after Deryaguin, Landau, Verwey and Overbeek).

Another way to achieve stability for colloidal systems is to coat the particles with an adsorbed layer of some kind, known as steric stabilization. Polymer layers are often used for this purpose. The repulsion is in this case due to mainly two factors. Firstly, the layers make it impossible for the particles to approach each other with interparticle distances as small as for the uncoated particles, i.e. the long-range van der Waals force becomes weaker. This is, however, only true as long as the dielectric properties of the coating material is similar to the dispersion medium. If it is, instead, similar to the core material it will only have the effect of increasing the radius of the particle. Secondly, when such particles approach each other, the polymer concentration between the particles will increase, as schematically illustrated in Fig. 4 for two planar surfaces. Due to osmotic effects, the dispersion medium will diffuse into the region between the particles, thereby causing repulsion. When the polymers on two adjacent particles interact, as a result of particles approaching each other, the number of configurations the polymers can adopt will decrease. This will lead to a reduction of entropy and hence an increase in free energy. As previously mentioned, the system tends to lower its free energy, which means that the particles will repel each other.

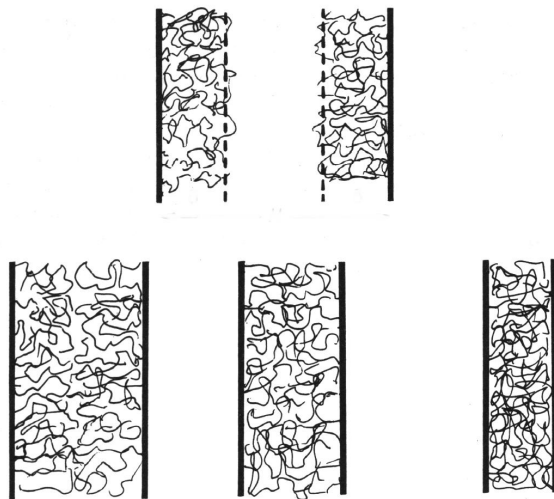


Figure 4 Increased polymer concentration when two surfaces approach

Plasmon resonance of colloidal gold and silver particles

A surface plasmon is an electromagnetic field charge-density oscillation that can exist at an interface between two materials with dielectric constants of opposite signs, for instance a metal-dielectric interface. A dielectric material has electrons that are confined to their orbits, i.e. they cannot be liberated under normal circumstances. A more common word for dielectrics is insulators. Explained in a simple way the plasmon can, for metal colloidal particles, be seen as a transport of conduction electrons in the particles, when they are influenced by an external electric field. Since the positive charges in the particles are assumed to be immobile, a polarization takes place when the negative charges are moved. Fig. 5 illustrates colloidal particles under influence of an electric field that causes them to be time dependent dipoles.

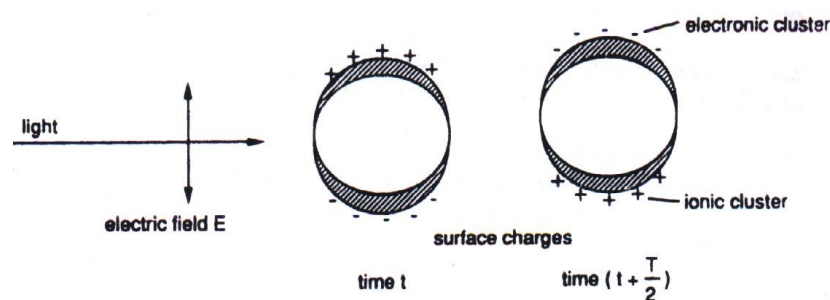


Figure 5 Dipole resonance of colloidal particles

The resonance frequencies for the particles (that give rise to a certain color of the solution, if the corresponding wavelength is in the visible area), are dependent of the particle size, the surrounding medium and the cluster formation, if the latter occurs. If the particles have aggregated, they will influence each other so there will be a number of resonance frequencies that yields for the whole system. The fact that there will be

several resonance frequencies are due to that the cluster is not symmetrical. Hence, there are many ways for the particles to influence one another.

In order, to estimate the resonance frequency, the following discussion can be used. The model adopted is a free electron model, but since the particles used in this case are noble metals, the free electron model will give a quite good estimation. The polarization of a sphere as a whole, due to an external electrical field, E_0 , is calculated. It is assumed that $r \ll \lambda$, where r is the particle radius and λ in the wavelength of the incident radiation. It is also assumed that the particles are perfectly spherical. The internal field, E_i , thus becomes:

$$E_i = E_0 \frac{3\varepsilon_m}{\varepsilon + 2\varepsilon_m} \quad (5)$$

where ε is the dielectrical constant for the particles and ε_m is the dielectrical constant for the surrounding medium. The internal electrical field (Eq. 5) shows a resonance behavior when $|\varepsilon + 2\varepsilon_m|$ has a minimum. However the dielectrical constants are in fact complex numbers that have frequency dependence. Usually ε_m is taken to be real and constant in the visible area, but $\varepsilon = \varepsilon_1(\omega) + i\varepsilon_2(\omega)$. The condition for resonance behavior then becomes

$$[\varepsilon_1(\omega) + 2\varepsilon_m]^2 + [\varepsilon_2(\omega)]^2 = \text{minimum} \quad (6)$$

One requirement is that $\varepsilon_1(\omega)$ is a negative number. If $|\varepsilon_1(\omega)| = 2\varepsilon_m$ and $\varepsilon_2(\omega) = 0$, the result will be an infinitely high resonance peak for just one certain frequency.

The real part of the dielectrical constant can further be written as:

$$\varepsilon_1(\omega) = 1 - \frac{\omega_p^2}{\omega^2} \quad (7)$$

where ω_p is the Drude plasma frequency. It is further assumed that the relaxation constant $\Gamma \ll \omega$, where Γ is defined as Fermi velocity divided by the electron mean free path. Eq. 7 can be rewritten as

$$\omega = \sqrt{\frac{\omega_p^2}{1 - \varepsilon_1(\omega)}} \quad (8)$$

From Eq. 8 it is possible to make a prediction of the plasmon resonance frequency and it is also possible to qualitative discuss what will happen if the surrounding media changes. It is also known that the imaginary part of the dielectrical constant, $\varepsilon_2(\omega)$, increases with increasing wavelength. It means that $\varepsilon_2(\omega)$, that works as a damping factor (see Eq. 6), will contribute more and more with increasing wavelengths. Regarding the optical spectrum the peaks will be broader at longer wavelengths.

Task for the laboratory experiment

1) The first step is to manufacture a colloidal system to work with, in this case a suspension of gold particles, having a diameter of ~ 40 nm, in water. The particles are prepared by reduction of gold chloride, $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, with sodium citrate, $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$. The experimental details will be given by the time for the laboratory experiment.

2) Biological functionalization of colloidal systems is a difficult process that also requires a lot of time. So, for that reason it will not be possible to do such advanced experiments within the limits of this course. However there are simpler ways to illustrate the principles of the system. Instead of using biorecognition-induced aggregation, a salt can be used to induce the aggregation between the particles (according to the discussion in the theory section). Optical spectroscopy will be used for measurements on a non-aggregated and an aggregated system respectively. The results will be compared and discussed according to the theory.