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Mobility of manufactured nanoparticles within a natural organic gel

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Chapter 1. Introduction and literature review

1.1. Introduction to nanotechnology and nanomaterials

1.1.1. Definitions

Nanotechnology and nanosciences involve the manipulation of matter on a near atomic scale to produce new structures, devices, and materials (labsafety.gwu.edu/nanotechnology-safety).

There have been multiple efforts to define nanomaterials (NMs), including a focus on defining them for regulatory purposes that enables products containing NMs to be identified and regulated, with limited success to date. Most of these definitions focus solely on size aspects. According to ISO (The International Organization for Standardization), nanomaterial is defined as “material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale” (Boverhof et al. 2015) (Aven 2011) (ISO, 2010) and “nanoparticle (NP)” as a “nano-object with all three external dimensions in the nanoscale” where nanoscale is defined as the size range from approximately 1–100 nm.

At the nanoscale, NPs exhibit specific surface areas that are greater than the corresponding conventional forms. In addition, decreasing the particle size increases the percentage of reactive atoms at the surface often resulting in higher reactivity and altered or new surface properties (Auffan et al. 2009). However, the threshold of 100 nm in the definition of NMs is poorly relevant. Indeed, for metal oxide and metallic NPs, the change of properties more often happens around 30 nm (Auffan et al. 2009).

At the nanoscale, fundamental mechanical, electrical, optical, and other properties can significantly differ from their bulk material counterparts (Morose 2010). As example, nanoscale gold particles appear red or purple and not yellow as their bulk counterparts, and instead of being chemically inert, nanoscale gold shows good catalytic properties (Daniel & Astruc 2004). Based on this statement, a definition of nanoparticles based on their non-bulk size-dependent properties was proposed (Auffan et al. 2009).

Recently, the EU Commission’s definition emphasized on NMs external dimensions, and on properties that are characteristic of the nanoscale such as the large specific surface area of the materials and/or the specific physico-chemical properties that are different from those of the non-nanoform of the same material (EC 2011)a. The definition of nanomaterials also covers natural or manufactured active substance or

non-active substance containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm (EC, 2011a). However, this definition may exclude from materials with an internal structure (*e.g.* porous materials with a relatively large internal surface area) or materials with a surface structure at the nanoscale.

Beyond nanoscale and specific properties inherent to NMs, the definition can consider only intentionally produced NMs in regulatory definitions applied to a specific sector (food, pesticides...) (EC 2011)b or include natural, incidental or manufactured nanomaterials in advisory ones (EC 2011)a.

The complexity associated with implementing a definition of NMs originates from the many core elements featuring NMs: size, agglomerates and aggregates, distributional thresholds, novel properties, solubility, natural versus intentionally-manufactured... Currently, inconsistencies in definitions exist among countries, governments, industry, and standards organizations. A substance may be a nanomaterial under one regulatory scheme, but not another. Moreover, key considerations for safety assessment are absent from several definitions (Boverhof et al. 2015).

1.1.2. Physicochemical properties of nanomaterials

The behavior, fate, and toxicity of ENM clearly depend on their properties, such as composition, chemistry, physical-chemical parameters. Table 1 summarizes several key parameters that characterize NMs, together with several techniques that can be used to characterize these materials.

Table 1.1. Important ENM properties and common methods for characterization

Physicochemical Properties	Common Characterisation Methods^a
Size and size distribution	EM, AFM, DLS, NTA
Shape	EM, AFM, UV-vis (for plasmonic nanoparticles)
Agglomeration or aggregation state	EM, DLS, UV-vis (for plasmonic nanoparticles)

Crystal structure	XRD, ED
Surface chemistry/charge/area	AES, EELS, XPS, solid-state NMR, ζ -potential, BET
Stability over time/dissolution	DLS, UV-vis, ICP-AES, ICP-MS, colorimetric assays
Dosing metric	Variable
Uptake	ICP-AES, ICP-MS, TEM, fluorescence, flow cytometry, NAA

a Abbreviations: EM, electron microscopy; AFM, atomic force microscopy; DLS, dynamic light scattering; NTA, nanoparticle-tracking analysis; UV-vis, UV-visible spectroscopy; XRD, X-ray diffraction; ED, electron diffraction; AES, Auger electron spectroscopy; EELS, electron energy loss spectroscopy; XPS, X-ray photoelectron spectroscopy; NMR, nuclear magnetic resonance; BET, nitrogen adsorption/desorption isotherm; ICP-AES, inductively coupled plasma atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry.

1.1.3. Applications and uses of nanomaterials

Nanomaterials market was valued at \$14,741.6 million in 2015, and is expected to reach \$55,016 million by 2022, even if, factors such as stringent environmental regulations and high price of nanomaterials may hamper the market growth (<https://www.alliedmarketresearch.com/nano-materials-market>).

In 2017, the Project on emerging Technologies (<http://www.nanotechproject.org/>) lists more than 1,600 manufacturer-identified nanotechnology-based consumer products introduced to the market. Among the most highly manufactured nanomaterials in the world, titanium oxide ranks in the top five.

Nanotechnology already has a major impact on electronics, coatings, construction, food technology, the design of new materials such as nanocellulose, telecommunication, environmental technologies, medical technologies and drug development, nano-biocide applications and energy productions as well as new agriculture, water purification systems and the utilization of solar energy among others (www.greenfacts.org/en/nanosafety/l-2/1.htm). Currently, the paints and

coatings, adhesives and sealants, electronics and consumer goods, and personal care segments are the major end-user markets for nanomaterials (<https://www.alliedmarketresearch.com/nano-materials-market>). As example, nano-TiO₂ is mostly used as UV shielders in cosmetics, or self-cleaning materials, hence is present in many consumer goods and in industry (Charitidis et al. 2014).

The possibilities associated with nanotechnology are seemingly unlimited, and the developments seem to be proceeding towards even more complexity (Savolainen et al. 2013). According to Roco et al. (2011), there are 4 generations of nanomaterials developing with time from 2000 to 2020, from simpler ones to more complex (Roco 2011).

The plethora of different types of materials at nano-scale gives almost unlimited technological benefits but it also holds the possibilities for unexpected interactions between these materials and biological systems at the molecular, cellular, organ and organisms levels (Shvedova et al. 2010) (Nel et al. 2006). In addition to their impact on society, also the possible effects of engineered nanomaterials (ENMs) on human health and consequences to the environment are very important. Awareness about the potential hazards of the applications of these materials and technologies are consequently on the development and have to be taken to account during their use (Savolainen et al. 2013).

1.1.4. Life cycle and release of NMs

Nanomaterial life cycle and release can be described in several steps such as production, processing, product and use, recycling and weathering. All these steps can be done in air, soil, sediment, and water (Figure 1.1). End of life activities refer to activities related to reuse or recycling, such as disassembling, and mechanical or thermal processes like crushing, melting, torch cutting; waste treatment, e.g. incineration; and disposal, e.g. landfill. It shows that any release will end up in some compartments of the environment. Consequently, this may then lead to an exposure of the environment, and humans through the environmental compartments.

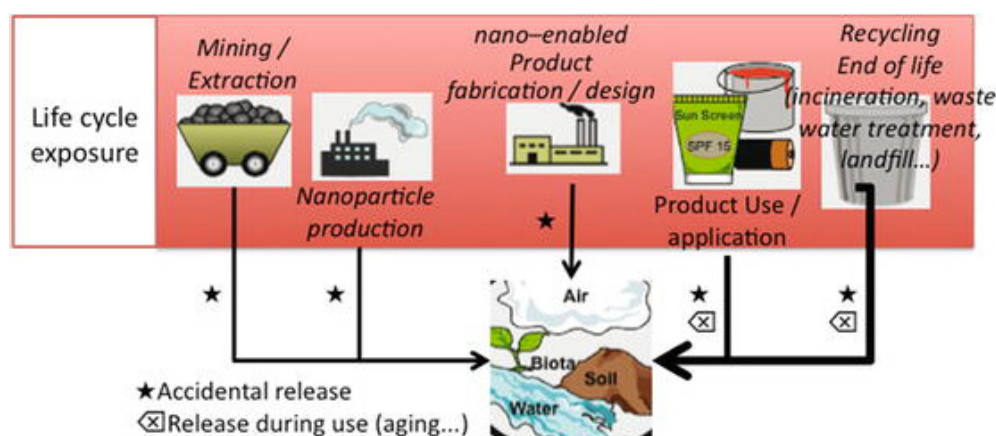


Figure 1.1: Exposure scenarios during the lifecycle of ENMs (Rose et al. 2012)

Environmental behavior and fate determine the transport/mobility of the NMs as well as their transformation. These processes influence also the likelihood of environmental exposure as well as possibly their hazard potential. An example is the environmental and biological degradability of NMs (Nowack et al. 2016).

Through the life cycle of ENMs, i.e. from production of ENMs through their use and final disposal, their environmental and health impacts vary. A main cause of variation is the environmental exposure as a result of release of ENPs. This in turn depends on how ENMs are processed from raw materials and incorporated into a nanoproduct (commercial product containing ENMs) and how this product is affected by the surroundings in its lifetime (from use to disposal stage). The exposure is also a function of how the ENPs behave in the environment, prior to having effects on biota and humans.

1.2. Nanoparticles and nanomaterials interactions with the environment

1.2.1. Environmental transformation and colloidal stability

Fate of ENMs in aqueous environments is controlled by intrinsic properties of NMs and interactions between the NMs and natural and anthropogenic chemicals in the system, biological and abiotic processes.

Fate that can act on NMs in products, and after their release, include photochemical transformation, oxidation and reduction, dissolution, precipitation, adsorption, desorption, combustion, biotransformation, and abrasion, among other biogeochemically driven processes (Nowack & Bucheli 2007) (Wiesner et al. 2006) (Auffan et al. 2010). As shown in Figure 1.2, interactions of NPs with adsorbed environmental (macro)molecules can interfere with many of these transformations (Louie et al. 2016).

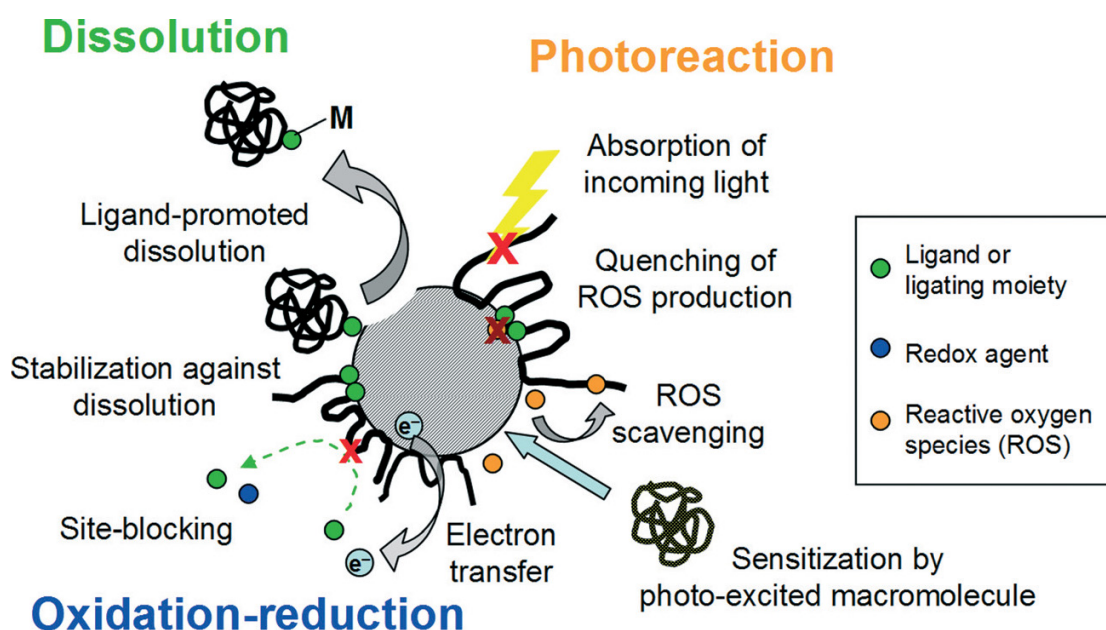


Figure 1.2: Possible effects of adsorbed macromolecules on ENM (Louie 2016)

Water is an important media used to study NMs behavior. The surface-charge and structure properties of NMs are one of the most important factors that govern their stability and mobility as colloidal suspensions or their aggregation into larger particles and deposition in aquatic systems (Wagner 2014). The key environmental factors that control the colloidal stability are pH value, ionic strength, electrolyte valence, and particle coating (Stankus et al. 2011).

Stable colloidal suspensions of NPs are a prerequisite for efficient interactions of NPs with algae, which may lead to uptake or toxic effects. In soils, the mobility of NPs in pore water is an essential condition for interactions with plant roots or fungi hyphae (Navarro 2008). Chemical transformations, redox activity, mobility, chemical interactions, and agglomeration of NMs in water are important features that determine

NMs fate and transport (Figure 1.7) as well as their potential toxicity (Blinova et al. 2010) (Keller et al. 2010).

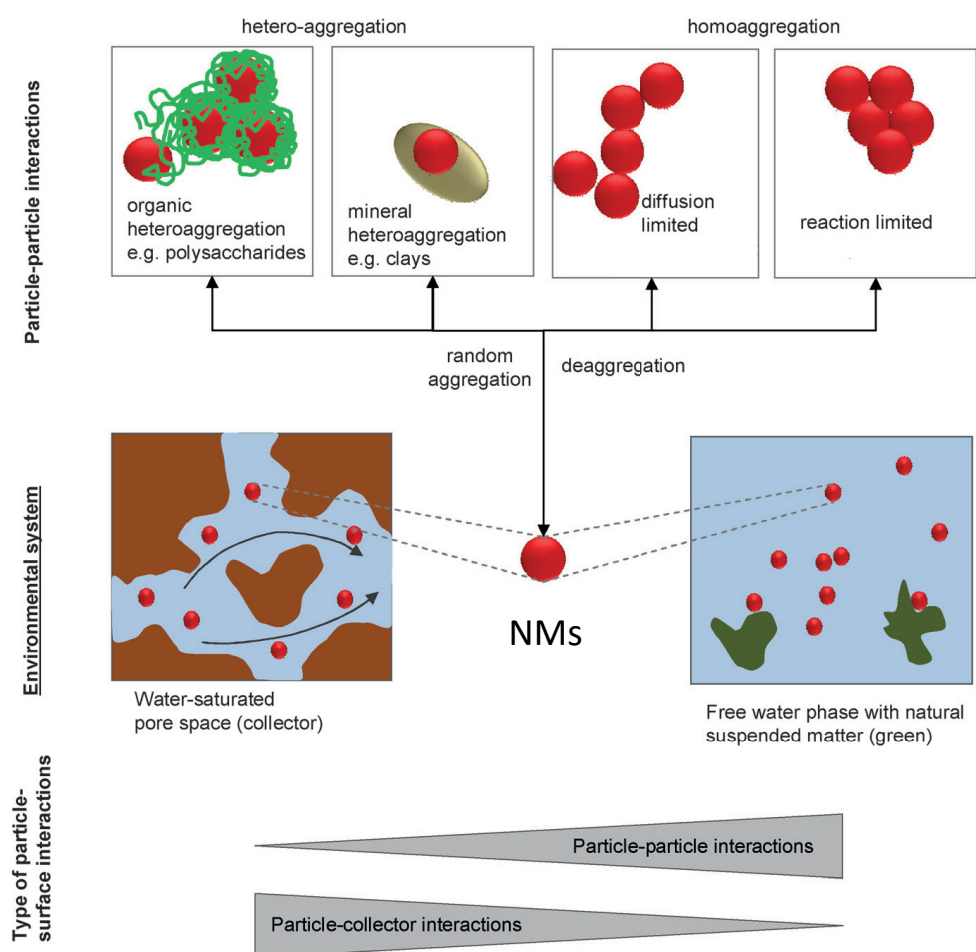


Figure 1.3: Example of particle aggregation processes in aqueous media from Wagner 2014

Stability of ENMs in water is highly influenced by their solubility and dispersion. Dissolution is controlled by particle- and solution-specific properties such as the solubility constant, chemical speciation and the specific surface area and mass-transfer kinetics reviewed in (Wagner et al. 2014). The surface structure, charge, and charge homogeneity of the NPs, which are dependent on dynamic adsorption of environmental molecules, are critical for the dispersion, flocculation, and deposition of nanoparticles in the environment (Wagner et al. 2014).

Agglomeration and settling are common mechanisms to occur when ENMs enter into water systems, potentially affecting their solubility. Some NPs tend to form

agglomerates when they are in contact with aqueous systems and tend to settle with time. The rate of NPs aggregation in solution will depend on the concentration, surface area, and forces involved in collisions like Brownian motion and van der Waals attractions (Handy et al. 2008).

Differences in natural organic compounds, pH, ionic strength, and surfactant presence in fresh, marine, and sea waters will have a significant influence on NPs aggregation and performance. Because of its ionic strength (due to salinity), seawater modifies the surface charge of NMs generating more particle collisions, aggregation, and precipitation. As a consequence, aggregation of NMs is more likely to occur in seawater than in fresh water where the presence of organic matter allows NPs coating and diffusion. In addition, viscosity and surface tension may also contribute toward trapping NMs on the ocean surface (Handy et al. 2008).

Polymeric substances secreted by marine microorganisms may induce agglomeration, stabilize NPs dispersion, or act as chelating agents for inorganic metal binding (Miao et al. 2009). Fullerenes and carbon nanotubes (CNTs) tend to agglomerate due to their structural properties and hydrophobic character. As a result, numerous studies have been conducted in order to stabilize them in aqueous solutions. Capping agents, surfactants, and stabilizers are applied to decrease steric repulsions and form a stable aqueous dispersion (Perez et al. 2009).

Therefore, ENMs aggregation, deposition, and mobilization can help to better predict the NPs fate and stability in water systems.

Hypothetical case studies were used to illustrate how fate and transport processes are likely to act on ENMs as they exist in currently available products. The examples include titanium dioxide in sunscreen and paint, nanoscale silver in textiles, composite structures containing carbon nanotubes, and cerium oxide in the fuel combusted in diesel engines (Botta et al. 2011) (Kaegi et al. 2008) (Al-Kattan et al. 2013) (Pakarinen et al. 2013) (Fouqueray et al. 2012). These products are different in fundamental properties or behaviors critical for assessing the risk of exposure to NMs. These include their solubility (insoluble TiO_2 vs soluble nano-Ag), redox activity (unreactive TiO_2 vs reactive cerium oxide), and product use pathways to the environment (Mitrano et al. 2015).

The major obstacle in studying environmental ENMs release and transformation is the identification of the particles themselves. Discrimination of particles by type (e.g. engineered vs. natural vs. particles produced during the manufacturing process itself)

is of importance when assessing exposures, and in subsequent analyses that interface with health studies.

1.2.2. Environmental fate

The fate of NMs is schematically represented in Figure 1.4 (Perez et al. 2009). This describes all the environmental compartments to which NPs may be released.

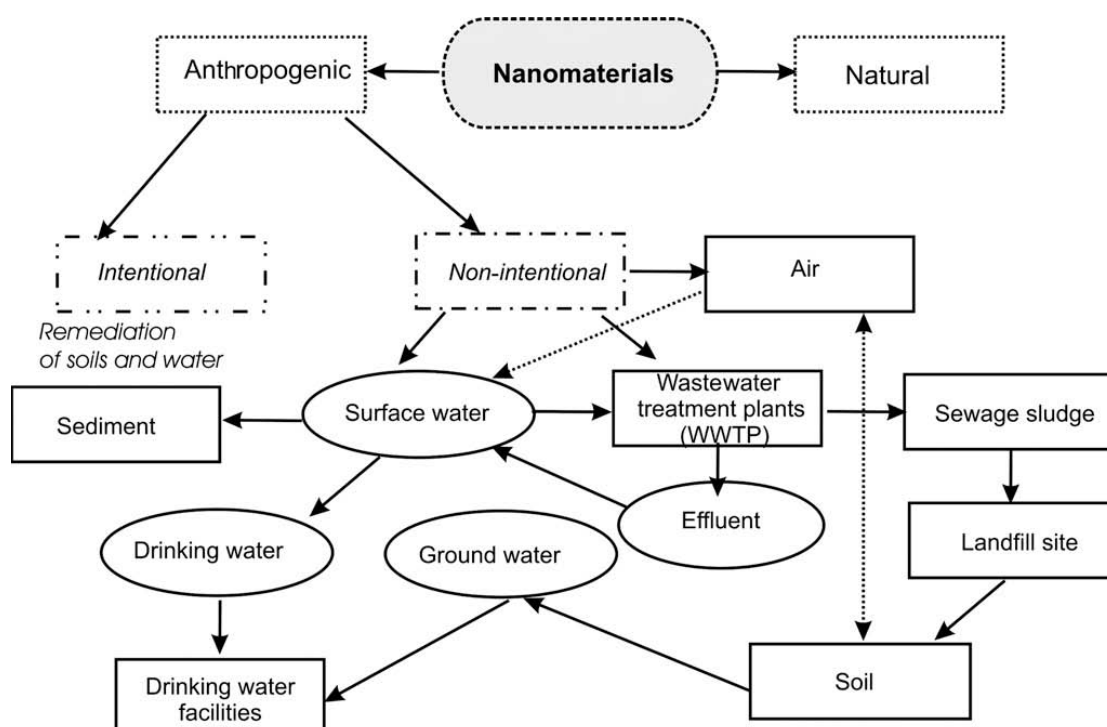


Figure 1.4: The fate of nanomaterials in the environment (Perez et al. 2009)

Understanding the transport and fate of NPs in the environment is a key way to assess the exposure-driven risk (Petosa et al. 2010). In soil and water systems, the fate of NPs is largely determined by transport and deposition mechanisms. Comprehensive understanding of the transport and deposition of engineered nanoparticles (NPs) in subsurface (soil and water) is required to assess their potential negative impact on the environment (Petosa et al. 2010).

An ubiquitous element present at the surface of many environmental media is biofilm. Biofilm is a three-dimensional polymer network that interconnects and transiently immobilizes biofilm cells (Flemming & Wingender 2010). Cells are embedded in a

self-produced matrix of extracellular polymeric substances (EPS) and adhere to a surface (Figure 1.6). Polysaccharides and proteins are main components of these EPS (Flemming et al. 2007).

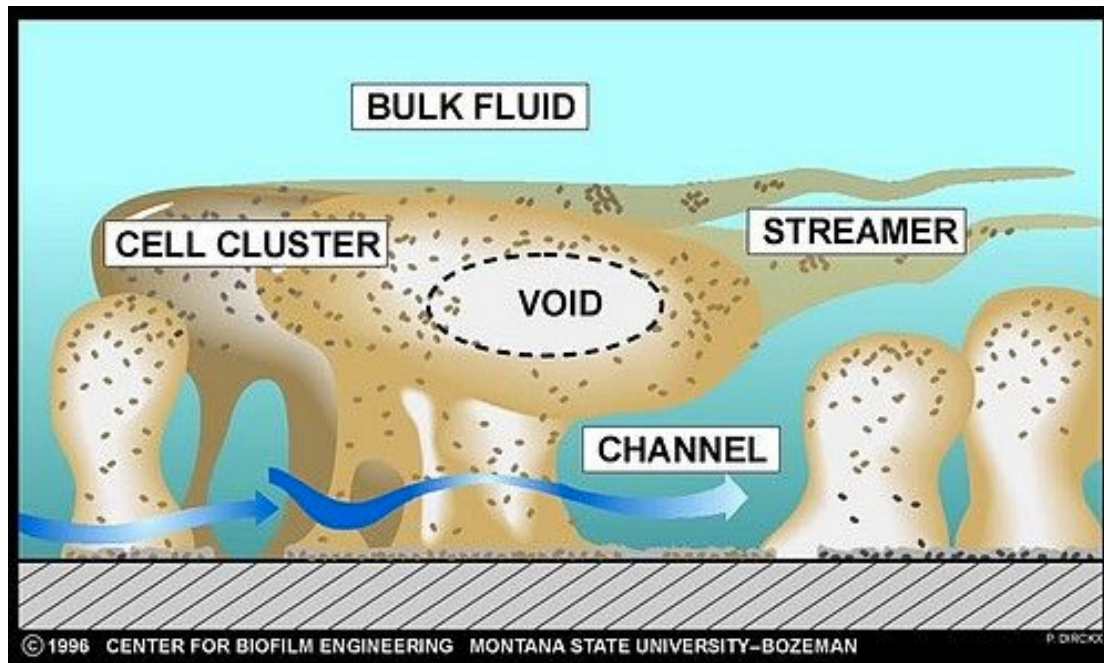


Figure 1.6: Biofilm architecture (From Center for biofilm engineering, Montana State University)

Biofilms are ubiquitous and are found at the surface of soil particles, plant roots and water-sediment interfaces as coatings surround particles. In the environment, biofilms are understood as biological consortia of autotrophs and heterotrophs, coexisting in a matrix of hydrated extracellular polymeric substances (EPS). These two main biological components are respectively mainly algae and cyanobacteria on one side, and bacteria and fungi on the other (Figure 1.7) (Sabater et al. 2016). Micro- and meiofauna also inhabit the biofilm, preying on the organisms and organic particles and contributing to its evolution and dispersal (Majdi et al. 2011).

Biofilms also form on and within the human host. At the pulmonary air-liquid interface, opportunistic bacteria, such as *Pseudomonas Aeruginosa* can develop as biofilms in cystic fibrosis patients. The biofilm architecture protects the bacteria, allowing some escape from antibiotics therapy (Singh et al. 2000). Microbial biofilms can grow on medical devices inserted into the body, e.g. catheters in the blood circulation and urinary tracts, producing severe and chronic infections. The use of medical device is the greatest exogenous predictor of healthcare-associated infection

(Manangan et al. 2002) (Stone 2009).

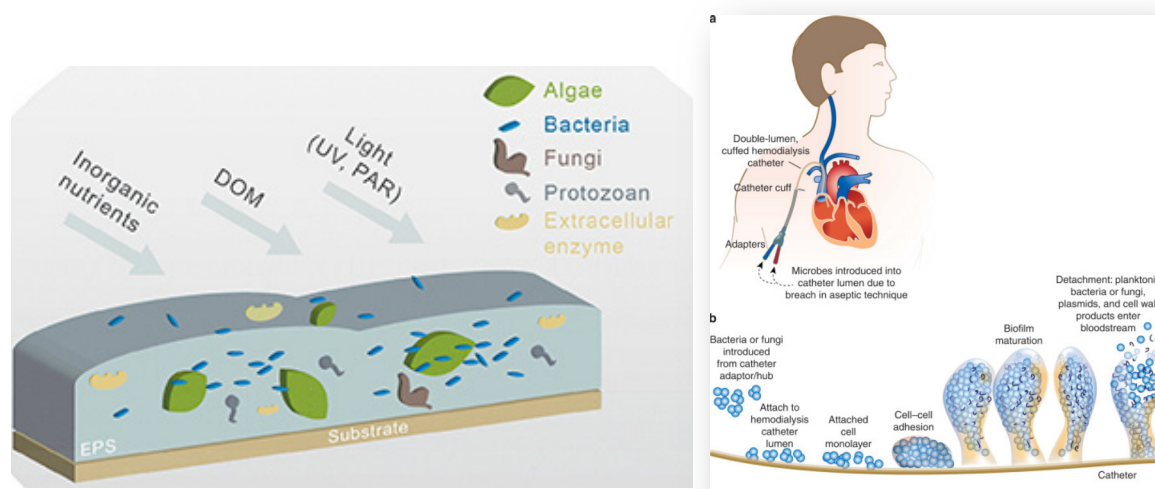


Figure 1.7: Scheme of environmental aquatic stream biofilm (Sabater et al., 2016) and of biofilm formation and infection of human lung through catheter device (Mermel 2014)

Attention has been paid only recently to studying the impact that biofilms may have on the fate and transport of NPs in a biofilm-coated porous media. Biofilm was found to reduce the mobility of NPs saturated porous media (Tripathi et al. 2011) (Tong et al. 2010) (Lerner et al. 2012) (Xiao & Wiesner 2013) (Mitzel & Tufenkji 2014) and subsurface environments (Morales et al. 2007). Reduced mobility of NPs in these systems could not be predicted by the surface charge alone, suggesting that hydrophobic interactions as well as steric and bridging interactions by macromolecules on NPs surfaces were in play (Xiao & Wiesner 2013). Moreover, these biofilms present the likely possibility for unique biological and chemical transformations of NPs. Ultimately, the environmental fate of NPs is influenced by biofilms, and therefore requires a more in-depth understanding of their fundamental properties (Ikuma et al. 2015).

It is very therefore important to study the interactions of nanoparticles in the presence of environmental coating such as components of EPS, since it is dominant in mobility and transport mechanism in environmental sphere. These interactions involve three

steps, transport to the vicinity of the biofilm, deposition, and diffusion into the layer (Figure 1.5).

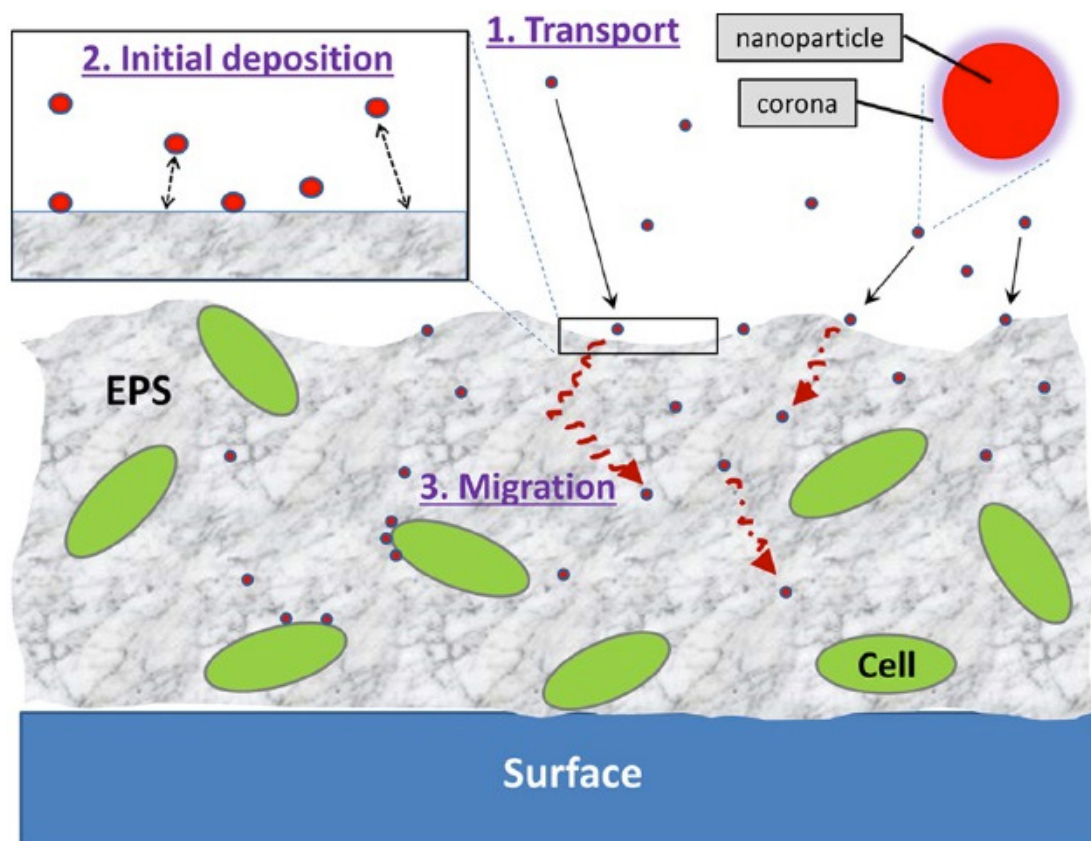


Figure 1.5: Schematic illustration of NPs-biofilm interactions in the environment (Ikuma 2015)

Understanding the micro- and nano-scale complexity within biofilms and the interaction of NPs in environmental matrices is an emerging concern in both the environment and health. NPs–biofilm interactions are crucial to assess the potential risk of exposure for by biota.

The further section of this literature review will thus focus on environmental and human health impacts of nanomaterials, especially in biofilm way of life.

1.3. Environmental implication of nanomaterials and impact on human health

The study of environmental implication of nanomaterials and impact on human health is a very productive field. Hence this section is not intended to be fully exhaustive, but representative of the main trends.

Released engineered nanoparticles will potentially impact organisms on all levels of the food chain, but the most widespread consequences will result from impacts exerted on low trophic level organisms like bacteria, which play critical roles in the ecosystem (Maurer-Jones et al. 2013).

Globally, NPs can impede the formation of biofilms and alter functions. TiO₂ NPs under UV irradiation produce reactive oxygen species and decrease the growth and development of bacteria (Arroyo et al. 2014) (Dhandapani et al. 2012) as biofilms. ZnO NPs suppress biofilm formation of wastewater biofilms through dissolution and release of Zn²⁺ (Hou et al. 2014) and of the opportunistic pathogens (Khan et al. 2014).

Shewanella oneidensis is an aquatic organism, distributed world-wide in a variety of environments. These bacteria are important for geochemical nutrient cycling as a metal reducer, a function which is performed by the secretion of electron shuttling molecules, i.e. flavin. Using quartz crystal microbalance (QCM) and gene expression, (Maurer-Jones et al. 2013) showed that *S. oneidensis* biofilm formation is slowed in the presence of TiO₂ NPs. However, riboflavin secretion, a function related to the *S. oneidensis* metal reducing capacity, is increased significantly in a nanoparticle dose-dependent manner, showing implications for total ecosystem health.

Few studies have evaluated the impact of NPs on biofilms vs planktonic cells (reviewed in (Saleh et al. 2015), most of them on Ag(0) NPs exposure. These studies, generally conclude that biofilms show reduced susceptibility to Ag(0) NPs as compared to planktonic cells (Fabrega et al. 2009) (Choi et al. 2010) (Radzig et al. 2013) (Mohanty et al. 2012).

Biofilm and planktonic *P. aeruginosa* cells susceptibility to Ag NPs differed from one another when cells were pre-exposed to a subinhibitory concentration of Ag NPs.

Biofilm cells with previous exposure to Ag NPs show greater tolerance to the following Ag NPs exposure than planktonic cells. Hence, long-term exposure to low concentrations of NPs could turn opportunistic human pathogens (e.g., *P. aeruginosa*) less susceptible to Ag NP-based antimicrobial agents.

Iron concentrations are extremely low in body fluids. Iron-containing nanoparticles may influence the ability of bacteria to scavenge iron for growth, affect virulence and inhibit antimicrobial peptide (AMP) function. (Borcherding et al. 2014) show that iron oxide NPs (hematite) can increase *P. aeruginosa* bacterial biofilm growth, virulence and impair innate immunity in a particle size dependent way. Dissolution, increase in the amount of bioavailable iron and impairment of AMP function by adsorption onto nanoparticle surfaces can explain the consequences of exposure to iron-containing NPs.

These results emphasize the challenges in understanding NPs–biofilm interactions for risk assessment of nanotechnology. Biofilms are chemical, and physical heterogeneous structures and NPs are inherently complex objects. Simpler model for biofilm uses some components of the EPS matrix such as exopolysaccharides, forming biogels.

The last section will review the mechanisms of NPs mobility, deposition on porous media, and transport of NPs in biofilms and biogels.

1.4 Deposition, mobility – transport, and filtration of NPs

1.4.1. Particle transport processes in saturated porous media

The NPs transport behavior in saturated porous media is governed by several processes such as deposition, dissolution, filtration, aggregation that are summarized in Figure 1.6. These processes are controlled by the properties of the NPs and the chemical conditions of their surroundings (Wagner et al. 2014).

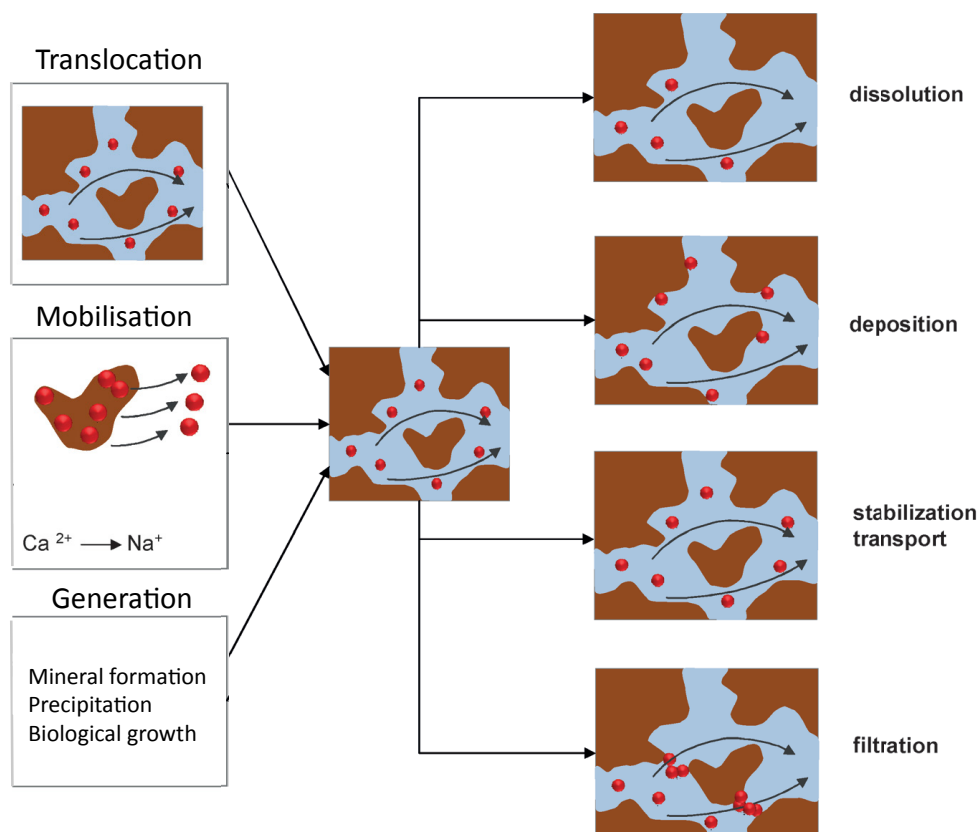


Figure 1.6: Illustration of particle transport processes in aquifers (adapted from Wagner 2014 and (Kretzschmar 1994))

1.4.2. Dissolution

Considering the properties inherent to the particle, size is considered as the primary physicochemical property affecting solubility of NPs with various other parameters such as, surface area, surface morphology, crystallinity and crystal structure, strength of the surface bonds, their spatial arrangements and the presence of impurities (Misra et al. 2012). Dissolution refers to a thermodynamic equilibrium, hence is reversible. The dissolution and re-precipitation of nanomaterials are important processes that influence the transport and bioavailability of metals in the environment and are regulated by the NOM (Wagner et al. 2014). Dissolution is a surface reaction and depends on surface area and mass transfer between the surface and the bulk solution. As dissolution is dependent on the specific surface area, dissolution rates are often reduced by aggregation, attachment, and surface coverage by adsorbed organic

compounds such as engineered coatings, environmental macromolecules (EPS and natural organic matter, NOM) (Wagner et al. 2014).

Apart from the intrinsic properties of the NPs discussed above, characteristics of the surrounding media (pH, ionic strength, water hardness, oxygen...) and presence of organic components (natural organic matter, polysaccharides, proteins) can also affect suspension stability and lead to NPs agglomeration/aggregation, further affecting the exposed surface area and dissolution of the particles (Misra et al. 2012).

The most famous NPs for dissolution are silver NPs, Ag(0). Dissolution of Ag NPs to Ag^+ occurs through oxidation of the surface atoms by dissolved oxygen. Dissolution rates decrease as the dissolved oxygen concentration decrease, by the addition of natural organic matter, a reduction in temperature, or an increase in the pH value (Wagner et al. 2014). This suggested that Ag(0) nanoparticles will not be persistent in realistic environmental compartments containing dissolved oxygen (Liu & Hurt 2010). However, using single particle ICP-MS to track dissolution of Ag(0) NPs at environmentally relevant concentrations (ng L^{-1}), (Mitrano et al. 2014) show the importance of water chemistry including chloride, sulfide, and dissolved organic carbon (DOC), low concentrations (stoichiometric Cl^- , S^{2-} and 2 mg L^{-1} DOC) resulting in negligible Ag ENP dissolution over 24 h (Mitrano et al. 2014).

1.4.3. Aggregation and deposition

Aggregation takes place as a result of particle–particle collision due to Brownian motion. Aggregation between similar surfaces is termed homoaggregation. In natural systems, the wide variety and the high concentration of different colloids drive heteroaggregation, which is the aggregation of different particles (Wagner et al. 2014). Aggregation and deposition are two closely related processes. An increase in size due to particle aggregation impacts particle mobility, hence, the time-scale of particle aggregation is an important consideration when conducting nanoparticle deposition studies.

NPs may aggregate with each other or with other objects in the environment (i.e. organic matters). Aggregation and deposition of NPs in the natural environment is determined by their size, shape, NPs and collector charges, the roughness of the collector surface, and the physical chemistry of the solution (Wiesner et al. 2006).

NPs heteroaggregation with other surface may largely decrease their movement in the media. Thus, PVP-coated Ag NPs rapidly interact with natural colloids in the soil which significantly reduce their mobility in the environment (Cornelis et al. 2013). Aggregation and deposition are also influenced by each other. Thus, for example, aggregation of NPs enhances their deposition on a silica sand (Solovitch et al. 2010). Further movement of NPs in the environment depends on their possible attachment to the different surfaces so called collectors (Lecoanet et al. 2004). Some conditions (i.e. ionic strength) in natural aquatic systems may limit mobility of NPs fullerene-based as they form large aggregates that may settle out of the suspension or deposit on surfaces (Brant et al. 2005).

Deposition is the process of flow and attachment of particles to a substrate and hence it may be considered as a limiting case of heterocoagulation. The kinetics of this process is determined by the rate of transport from the bulk of the suspension and by all types of all-range interactions between particle and collector surfaces. These long-range interaction forces include double layer, Van der Waals and steric interaction. Moreover, deposition is determined by the hydrodynamic interaction between particle and surfaces (Tadros, F., Warzynski, P., Zembala, M. 1992).

The transport and deposition of nanoparticles in saturated granular porous media are generally governed by Brownian diffusion (Tufenkji & Elimelech 2004) (Lecoanet et al. 2004), with negligible contributions from gravitational sedimentation and interception. The importance of Brownian diffusion increases with decreasing particle size, thereby increasing the number of collisions between NPs and collector (e.g., aquifer grain) surfaces. In natural or engineered aquatic environments, the interactions between nanoparticles and collector surfaces are generally described by the DLVO theory of colloidal stability, and can either be attractive or repulsive.

NPs deposition is influenced by many factors such as the particle size, the shape, polydispersity and the physical chemistry of the solution (Wiesner et al. 2006). NPs homoaggregation or heteroaggregation with inorganic colloids will favor their deposition (Solovitch et al. 2010), (Labille et al. 2015). The collector surface roughness may also favor or disfavor the deposition of NPs depending on their size (Wiesner et al. 2007).

Particles attachment to the collector surface may be estimated by sticking efficiency. The particle attachment efficiency is a parameter that relates the particle deposition rate measured under favorable conditions to that measured under unfavorable

conditions (Tufenkji & Elimelech 2004). The ratio of the rate of particle deposition on a collector to the rate of collisions with that collector is referred to as the collision efficiency or attachment efficiency factor, α . The attachment efficiency is a function of numerous phenomena including van der Waals forces, electrical double-layer interactions, steric interactions, hydration forces, and particle-surface hydrophobicity. The value α is unity when there are no barriers to particle deposition and attachment (Lecoanet et al. 2004). Particle transport and attachment are often represented as the product of the attachment efficiency α and the collector efficiency η_0

$$\eta_r = \alpha \eta_0 \quad (\text{eq. 1.1})$$

η_0 is the clean bed single collector efficiency that describes the particle transport to an individual collector before particle accumulation alters the collector geometry.

1.4.3.1. DLVO-theory. Favorable and unfavorable deposition conditions

All natural and engineered solids develop surface charge when immersed in water. This creates states for which deposition may be in favorable or in unfavorable conditions. Surface charge may result from ionic substitution within the crystalline framework of minerals, complexation or ionization of surface functional groups, or specific adsorption of ions to solid surfaces.

When particle and collectors in the aqueous media are oppositely charged, attractive double layer interactions develop as the particles approach the collector surface. In most aquatic environments repulsive electrostatic forces between suspended colloids and stationary mineral grain surfaces inhibit colloid deposition. These repulsive forces are a result of the preponderance of negatively charged surfaces found in aquatic environments that produce unfavorable deposition conditions.

Particle-particle interactions and particle-surface interactions play an important role in controlling the aggregation and deposition behavior of NPs in aquatic environments. These interactions have traditionally been described by the DLVO theory of colloidal stability. However, non-DLVO forces such as steric, magnetic, and hydration forces can also play an important role in the aggregation and deposition of engineered nanomaterials (Petosa et al. 2010). Steric interactions can be particularly important for NPs in natural and engineered aquatic environments, as most particles adsorb natural

organic matter that is known to stabilize colloids (Pelley & Tufenkji 2008) (Franchi 2003) (Phenrat et al. 2010).

The classical DLVO theory (Derjaguin & Landau 1947) (Verwey 1947) of colloidal stability describes the total interaction energy experienced by a nanoparticle when approaching another particle (in the case of aggregation) or a collector surface (in the case of deposition). According to the DLVO theory, the stability of nanoparticles suspended in an aqueous environment can be evaluated as the sum of van der Waals (VDW) and electrical double-layer (EDL) interactions. The resultant interaction energy (VT), the sum of VDW and EDL interactions, determines the particle stability as the two surfaces approach one another. In aquatic environments, when particles approach each other (aggregation) or a surface (deposition), the overlap of the diffuse electric double layers results in electrostatic double layer interactions (Petosa 2010).

The kinetics of particle deposition and aggregation are dependent on the height of the energy barrier (Hamaker 1937). Particles that overcome the energy barrier will deposit on a surface or aggregate with another particle in a deep primary energy minimum. The height of the energy barrier for deposition or aggregation is directly dependent on the size of the interacting particles, with smaller particles exhibiting much lower energy barriers. Therefore, nanoparticles will deposit or aggregate more in the primary minimum than micrometer-sized particles. It has been shown that nanoparticles that deposit in primary energy minima are less likely to be released from the surface following changes in solution chemistry, such as reduction of ionic strength or changes in solution pH (Chen & Elimelech 2006).

Thus, for TiO₂ NPs deposition onto the silica surface at different pH and ionic strength, a deposition rate changes with the conditions change. Under favorable conditions for deposition (*i.e.*, pH 3 and 5), the maximum deposition rate is observed at low ionic strength as a result of strong attractive electrostatic interactions between the nanoparticles and the surface. Increasing ionic strength leads to a decrease in deposition rate when the particles and the surface are oppositely charged. The contrary is observed under unfavorable conditions, where the particles and the silica surface are similarly charged. Under these conditions, low deposition rates are measured at low ionic strength, and particle deposition increases at higher salt concentrations (Fatisson et al. 2009).

At the ionic strength of typical natural waters, the interaction energy of particles greater than about 500 nm interacting with similarly charged particles or surfaces is

characterized by a high energy barrier and a secondary energy minimum (Tufenkji & Elimelech 2005) (Hahn & O'Melia 2004). It has been shown that secondary energy minima play a critical role in the deposition and transport of particles greater than approximately 500 nm. Such particles readily deposit in secondary minima, even under conditions where a significant energy barrier exists, resulting in reduced transport in subsurface environments. The deposition or aggregation in secondary energy minima is reversible, as particles are released or disaggregated while ionic strength is decreased (Tufenkji & Elimelech 2005).

Secondary energy minimum is small for NPs (sizes lower than 100 nm) at typical ionic strength of natural waters. Therefore, NPs generally will not significantly deposit or aggregate in secondary minima and in the presence of an energy barrier will experience greater transport than micrometer-sized particles (Petosa et al. 2010).

The NPs surface functionalization also influences the adhesion to the substrate, often decreasing deposition and increasing transport due to higher colloidal stability (Sirk et al. 2009) (Phenrat et al. 2010). Some studies indicate that the deposition is mostly controlled by electrostatic interactions, but that additional factors at the collector surface scale, such as heterogeneity or the extent of deposited NPs, may play an important role on the deposition mechanism (Ikuma et al. 2014).

1.4.3.2. Experimental approaches for deposition study

There exists two main experimental approaches to evaluate nanoparticle deposition rates onto collector surfaces in aqueous environments. The most commonly used approach is the laboratory-scale packed-bed column experiment (Lecoanet et al. 2004) (Lecoanet & Wiesner 2004) (Li et al. 2008) (Tufenkji & Elimelech 2005) (Doshi et al. 2008).

Laboratory column studies are performed using columns packed with granular materials (e.g., glass beads, sand, soil or sediment) and injecting the particles at a known influent concentration (C_0) for a time period (t_0). Particle retention in the packed-bed is evaluated by measuring the effluent particle concentration (C) as a function of time using techniques such as UV-visible and fluorescence spectrophotometry or flow cytometry. The nanoparticle attachment efficiency is commonly evaluated using colloid filtration theory (CFT) (Yao et al. 1971).

Within this approach, it was shown that deposition of fullerene NPs ($n\text{-C}_{60}$) in porous media increased as ionic strength increased. As a result their mobility was limited due to the aggregation or deposition on the surfaces in aquatic system (Brant et al. 2005). So, factors that will tend to decrease the retention of nanomaterials in porous media include a low ionic strength (Espinasse et al. 2007).

Another experimental technique that has recently been used to study nanoparticle deposition kinetics is the quartz crystal microbalance (QCM) (Fatisson et al. 2009) (Quevedo & Tufenkji 2009). In this technique, particle deposition occurs on a clean or functionalized silica-coated QCM crystal, which is excited to oscillate at its fundamental resonance frequency. As particles deposit onto the crystal, the increase in mass (m) on the collector surface results in a measurable decrease in the crystal's resonance frequency (f). For homogeneous, very thin, or quasi-rigid layers, the frequency shift of the oscillating crystal (Δf) is directly related to the increase in mass per unit area (Δmf) by the Sauerbrey relation (Sauerbrey 1959).

1.4.3.3. Effect of natural organic matter on the colloidal fate of nanoparticles

Adsorbing on mineral surfaces polysaccharides substances, humic material, and other organic compounds may change surface heterogeneities, thereby altering the surface charge characteristics and deposition kinetics of NPs (Philip R. Johnson; Sung & Elimelech 1996).

To understand the likely fate and behavior of NPs in the environment, it is essential to understand their interaction with natural water components under a variety of physicochemical conditions, such as pH as well as type and concentration of natural organic macromolecules at environmentally relevant conditions. Natural organic matter (NOM) represents one of the major parts of natural colloids with humic substances making up 50 to 80 % of the NOM in freshwater. The presence of NOM, composed of thousands of organic compounds (e.g. organic acids, sugars and others carbohydrates, cellulosic materials, alginate, proteins, lipids, etc.), in all natural aqueous matrices has a profound effect on the NPs stability and thus on their mobility and deposition behavior (Chen & Elimelech 2008) (Phenrat et al. 2010) (Sani-Kast N. et al. 2017). The interaction of natural organic matter (NOM) with NPs, and its effects on the NPs fate, was studied extensively and was shown to depend on many

physicochemical factors. Thus a presence of humic acid significantly hinders TiO_2 deposition onto silica surfaces via steric and electrostatic repulsion even under high ionic strength which has an important implication for the mobility of these NPs (Thio et al. 2011). Surface coating of NPs by NOM likely affects aggregation behavior, resulting in reduced aggregation and steric stabilization mechanisms. Reduced aggregation due to steric or electrostatic stabilization mechanisms is often observed (Phenrat et al. 2010). NPs coating was shown to influence their adhesion on natural organic matter (humic acid) (Sirk et al. 2009). Polyelectrolyte coating decreases the deposition and enhances the NPs mobility. NPs coated with NOM were also observed more mobile. NPs may be coated with hydrophilic material, functional groups, or biomolecules (e.g., proteins, polysaccharides) at their surface that can have significant amounts of bound water that may lead to additional repulsive hydrophilic interaction (Petosa et al. 2010). For example, fulvic and humic acids enhance NPs transport by sorbing to the surfaces of particles and reducing attachment efficiencies through steric stabilization and increase hydrophilicity of the particle surface (Lecoanet et al. 2004). The presence of NOM in the environment is a controlling factor that determines stability of NPs among others (Stankus et al. 2011). However, in most of these cases, NOM was used as initially free molecules in solution, which strongly favours interaction with NPs. This is not the case in biofilms where NOM is rather immobile on the colonized collector surface. In these conditions, NOM reformation is very limited and the interaction with NPs may be altered.

1.4.4. Transport in porous media, filtration

As in the nature and the environment porous media are frequently encountered the transport of mobile colloids through porous media is an important process in both natural and engineered systems. Understanding colloidal transport and their role on contaminant transport in porous media is of major importance for the exposure study as mobility of colloidal particles have a great impact on the environmental contamination such as in soil and ground water. Soil, sand, lungs are few examples of a large variety of natural and artificial porous materials.

The transport of colloids in porous media and the colloid facilitated transport of contaminants have been studied in the past (McGechan & Lewis 2002) (Sen & Khilar

2006). Several studies have investigated the transport of a wide range of engineered NPs through porous media (Lecoanet et al. 2004) (Lecoanet & Wiesner 2004) (Dunphy Guzman et al. 2006).

Particles transport in filtration is analogous to transport in flocculation processes. A particle size with a minimum contact opportunity exists. Approach that can be used to describe filtration process (the removal of suspended particles within a filter) involves two separate and distinct steps. First, the transport of suspended particles to the immediate vicinity of the solid-liquid interface (i.e., filter, grain of the porous medium, another particle already on the surface); and second, the attachment of the particles to this surface.

It was previously predicted by Smoluchowski that in water the transport of particles larger than about 1 micrometer is accomplished by velocity gradients, fluid motion, interception and settling, while for smaller particles, Brownian diffusion is effective (Yao et al. 1971) (McGechan & Lewis 2002) (Yao et al. 1971).

Porous media can be visualized as a network of flow chambers, having random size and flow conductivity, connected together by openings of smaller size (Perkins, T.K. Johnston 1963). Therein, the apparent diffusion coefficient D is less than the molecular diffusion coefficient D_0 , as measured in the absence of a porous medium.

The movement of colloids and therefore also of NPs in soil and other porous media is impeded by two processes: straining or physical filtration where the particle is larger than the pore and is trapped, and true filtration where the particle is removed from solution by interception, diffusion and sedimentation (McGechan & Lewis 2002).

The particles entrapment depends on different conditions. Two important ones are pores constriction and connectivity (Sen & Khilar 2006). The size of particles or the ratio of particles size to that of pore constriction is a crucial parameter in determining entrapment phenomenon. Particles smaller than 100 nm are predicted to have very high efficiencies of transport to collector surfaces (which can retain or repel NPs) due to Brownian diffusion. If all particles collector contacts were to result in particle attachment to the collector, these small particles would be retained to a large extent by the porous medium.

Additional restrictions on particle movement include sorption and various physico-chemical interfacial reactions such as electrostatic, Van der Waals or hydrophobic forces.

However, particles removed from solution by such processes can readily become

resuspended upon changes in the chemical or physical conditions (e.g. changes in pH, ionic strength, flow rate) (Sen & Khilar 2006). The particles adhering from the pore surfaces of the porous media can be released or detached. The parameters that influence on these processes are the ionic strength, pH, and the composition of the permeating liquid. Studies on the transport and deposition of engineered nanoparticles in porous media, different types of sands at various solution ionic strengths (IS) (Torkzaban et al. 2010) showed that the solution IS determines the extent of the mass transfer of NPs on the solid surface and the amount of deposition on the favorable sites.

Surface charge heterogeneities are also important for controlling the transport and mobility of colloids in granular porous media. Subsurface aquatic environments are generally unfavorable settings for particle deposition due to the charge similarity between colloids and mineral grain surfaces, small amounts of favorably charged sites can have a profound effect on colloidal mobility (Philip R. Johnson; Sung & Elimelech 1996). Polysaccharide substances, humic material and other organic compounds will alter the surface charge characteristics and deposition kinetics. Surface charge heterogeneity on the sand grains is shown to increase collision efficiency and deposition for fullerene NPs (Li et al. 2008).

The three processes of particles mobilization, deposition and aggregation occur simultaneously in general. However, relative importance of each of these processes strongly depends on the chemical and physical conditions of the system.

1.4.5. Diffusion of NPs in biofilm

It is known that NPs deposition depends also on the presence of organic molecules in the medium (Fatisson et al. 2010). The presence of biofilms has an effect of NPs transport. For zerovalent iron NPs higher retention was measured at the collector surface in the presence of biofilm (Lerner et al. 2012).

The permeability of the biogel (that is a main part of biofilm) for NPs determines in great extent the NPs mobility and exposure (Peulen & Wilkinson 2011), (Ikuma et al. 2015). This gel acts as a filtrating layer between NPs and living organisms (Lieleg & Ribbeck 2011). Biofilms properties such as porosity, surface charge, and reactivity

have an impact on NPs mobility, and thus need to be taken into account to understand the NPs transport through the biofilm.

1.4.5.1. Biofilm properties

Many bacteria secrete and surround themselves with extracellular polymeric substances (EPS), a mix of polysaccharides, proteins, lipids, nucleic acids, and humic materials (Flemming & Wingender 2010) (Whitchurch et al. 2002). EPS matrix is usually highly hydrated and most of the water is not bound to EPS but is localized in pore spaces between adjacent polymer chains (Schmitt et al. 1995). A community of bacteria embedded in extracellular polymers is named a biofilm. In the aqueous environment such as soil and surface water, most of the surfaces can be colonized by such bacterial biofilms (Ikuma et al. 2015). These biofilms may vary in chemical and physical properties but they mainly consist of 80 % of polysaccharide, which determine the gel structure, i.e. density and porosity (De Beer et al. 1994). EPS determines the mechanical stability of the biofilm. Some of these polysaccharides are neutral and some polyanionic.

Biofilms are thought to play an important role as diffusive barriers in environmental systems and human health (Lieleg & Ribbeck 2011), resulting in increased resistance to disinfectants and antibiotics. The density, thickness and stickiness of the EPS depend largely on the strain of bacteria and the conditions under which they are grown. Since mass transport in a biofilm is primarily due to molecular diffusion, it is critical to understand the main parameters influencing diffusive fluxes in a biofilm.

Biofilms include water channels in their volume that makes their structure heterogeneous (Ivleva et al. 2009), (Stewart & Franklin 2008). Biofilm (consists of polysaccharides) was characterized as a viscoelastic material having the structure of a hydrogel (Hall-Stoodley et al. 2004) (Garrett et al. 2008). It has swelling and deswelling basic property of gels. The biofilm depth can vary from a few micrometers up to centimeters depending on the strain and the environment. The physicochemical and structural natures of EPS influence the bacterial physiology (Bohrer et al. 1984) (Lawrence et al. 1994). The nature and organization of the EPS in a biofilm are responsible, in large part, for its porosity and diffusive properties.

1.4.5.2. Diffusion of NPs in biofilm

The fate and transport of engineered NPs in the natural environment is influenced by the presence of biofilm. In the presence of dense biofilms, NPs mobility in the environment is significantly hindered. NPs larger than 50 nm have a decreased mobility in biofilms when compared to smaller ones (Peulen & Wilkinson 2011). In the range of environmental conditions biofilm enhances deposition of NPs (Tong et al. 2010). The biofilm biochemical and physical characteristic have a great influence on NPs deposition.

The sorption rate can be controlled by several factors including: diffusion NPs from the solution to the boundary layer surrounding the biofilm; diffusion from the boundary layer to the surface of the biofilm; pore diffusion from the surface of the biofilm to the internal sites; and uptake which involves of physico-chemical sorption (Sahle-Demessie & Tadesse 2011).

Diffusion of nanoparticles is the dominant transport mechanism in biofilms. The mobility and bioavailability of nanoparticles will largely depend upon their diffusion coefficients that are related to their size and physicochemical characteristics and the biofilm nature. Structure, composition, variable biofilm density and particles charge, and chemical composition of the nanoparticle were found to be the important parameters controlling the diffusion of nanoparticles. In order to study the diffusion through biofilm, hydrogel may be used as a simple model of constrained medium (Peulen & Wilkinson 2011).

The transport of particles in the pores of hydrogels and biofilms can be characterized in terms of Brownian motion (reflected by a self-diffusion coefficient). Generally, diffusion in biofilm consists of 2 main parts: diffusion into the biofilm and interaction with the organisms in biofilm (Golmohamadi 2012 thesis).

Limitations of diffusion process were studied also in biological hydrogels such as mucus and the extracellular matrix (ECM). It was already observed that diffusion coefficient in biofilm is smaller than in water solutions (Stewart 1998) due to the presence of microbial cells, extracellular polymers and other materials trapped in the biofilm.

Mobility of NPs within biofilm was shown to depend on steric constraint and affinity

with the biofilm components (Fatin-Rouge et al. 2003) (Labille et al. 2007). It is influenced by many factors such as NPs charge (Golmohamadi et al. 2013), aggregation (Stewart 1998) (Choi et al. 2010) and biofilm hydrophobicity (Xiao & Wiesner 2013) and structure (Labille et al. 2007).

Limitations in mass transport are mainly dependent on the biofilm structure (density and porosity of the EPS) and on the biofilm thickness (De Beer et al. 1994). Thickness of the biofilm was shown to reduce diffusion for small particles (Peulen & Wilkinson 2011). Generally, the diffusion of small nanoparticles was reduced by 60-80 % in comparison with water (Golmohamadi et al. 2013).

Diffusion in biofilm is influenced by hydrophobicity of the surrounding environment (Habimana et al. 2011). Hydrophobicity of the biofilm increases deposition of hydrophobic NPs to the EPS. Polysaccharide, as the main constituent of biofilm matrix was shown to strongly control the diffusion of solutes in biofilms and the limitations to diffusion in biofilms are primarily caused by the EPS of the biofilm matrix (Zhang et al. 2011) and influence the interaction with NPs (Lawrence et al. 2007).

Moreover, recent studies (Kurlanda-Witek et al. 2015) showed that NPs mobility is dependent on the binding capacity of biofilms rather than on the type of porous media. Biofilm-coated porous media retained more NPs than those without biofilm. It may have impact on the surface charge of the porous medium eliminating porous-medium-specific effects. It was previously shown that environmental biofilm can be binding matrices for NPs (Battin et al. 2009) (Ferry et al. 2009) (Kroll et al. 2014) as well as extracellular polymeric substances (EPS) (Flemming & Wingender 2010) (Nevius et al. 2012). The mechanisms regulating particles uptake by biofilm in the aquatic environment was shown strongly dependent on the NPs surface ionization (functionalization/charge) and not the core composition of the NPs.

It was shown that simple concept of size filtering is not sufficient to describe the complex permeability properties of hydrogels. Interactions between traversing particles and the hydrogel may also play an important role for filtering. Strongly interacting particles are retained in the mucus hydrogel whereas weakly interacting particles are allowed to pass (Lieleg et al. 2010).

Interaction of NPs and biofilm was shown to be based on many forces such as electrostatic, steric, and hydrophobic (Golmohamadi et al. 2013) (Lerner et al. 2012) (Habimana et al. 2011). The interaction between NPs and the biofilm can be reviewed

as a process that consists of 3 steps: transport of NPs to the vicinity of the biofilm; attachment to the biofilm surface; migration within the biofilm (Ikuma et al. 2015). These steps are determined by NPs characteristics, the physicochemical and biological structure of the biofilm matrix, and environmental conditions such as water chemistry, flow, and temperature (Petosa et al. 2010) (Levard et al. 2012). Also it was shown that environmental conditions influence biofilm itself (pore sizes change with different ionic strength) (Sutherland 2001) (Karatan & Watnik 2009).

If NPs have attractive interactions with the polysaccharide, their diffusion will be limited and they may not pass through the gel. So, the affinity of NPs for the EPS appears as a key parameter determining the fate and exposure of NPs.

In some studies it was shown that diffusion coefficients in biofilm decreased with increasing negative charge of the solute (Zhang et al. 2011). But further studies done to determine influence of charge upon the diffusion in model environmental biofilm indicated that electrostatic interactions were not a major factor affecting diffusion and for most biofilms in the environment, the diffusion of solutes and NPs will be primarily controlled by obstruction rather than electrostatic interactions. The latter has a limited effect on the particles diffusion coefficients and the nature or charge of the surface coatings did not play a significant role on particle diffusion in the biofilm. It was observed a little effect due to charge on the diffusion measurements in the biofilms (Golmohamadi et al. 2013). Ionic strength of the solution did not have influence on the diffusion coefficient in the biofilm both for negatively and positively charged particles. The small effect of the electrostatic interactions on the diffusion in biofilm can be due to its complexity, the chemical heterogeneity of the matrix (composed of polysaccharides, proteins, lipids, bacteria, etc.) and local structure of the biofilm. In contrast, the self-diffusion coefficients of NPs with sizes more than 10 nm greatly decreased in the biofilms in comparison with those ones in water. Furthermore, much larger NPs (> 66 nm) appeared to be completely excluded from the biofilms. This indicates that most nanomaterials will become significantly less mobile as they increase in size beyond 10 nm.

Solution pH was shown to influence on NPs and biofilm chemistry when the biofilm nanoTiO₂ uptake at neutral pH was enhanced over acidic conditions. Hydroxylation of TiO₂ NPs, dependent on pH and the salinity of the solution, influences the stability of colloids, the sorption kinetics via the nature of limiting phases: diffusion through the boundary layer or intrabiofilm mass transfer and the sorption mechanism (Sahle-

Demessie & Tadesse 2011). The adsorption is irreversible because biofilm limits diffusion of particles out of the pores and the complexation binding active sites on the surface hydrated biofilm to the hydrophilic TiO₂ NPs.

The EPS was shown to be the effective electrostatic filter suppressing the diffusion motion of both positively and negatively charged objects. This mechanism allows uncharged particles easily diffuse through the matrix, while charged particles are effectively trapped (Lieleg et al. 2009). Charged particles can only diffuse within EPS matrix as long as their surface charge does not exceed a well-defined window. The mobility of highly charged particles is drastically reduced to zero even though the particles are significantly smaller than the mesh size of the EPS hydrogel.

The electrostatic interactions influence diffusion in biofilm and are regulated by the polymer charge, particle surface and environmental conditions. Charge states are sensitive to pH because they depend on the protonation levels of the amino acids and linked sugar moieties may add additional charges, so pH has an influence on a diffusion of charged particles.

Thus, electrostatic interactions were shown to be a key parameter that determines a microscopic permeability of the mucin hydrogel. They are influenced by buffer conditions such as pH and ionic strength and particle surface charge and mucin density. The higher the particle surface potential is the stronger the particle mobility is suppressed. Higher concentration of salt, i.e. ionic strength increases the mobility of charged particles. In buffer, the surface charge of particles is partially shielded by solubilized ions that form shells of counter charges around the surface of the molecule. This Debye screening becomes more pronounced with increasing salt concentrations. As a consequence, the strength of the attractive or repulsive forces between diffusing particles and the hydrogel will depend on the salt content. Thus, higher salt concentration appears to increase the mobility of charged particles predominantly by weakening their interactions with the mucin polymers rather than by reorganizing the mucin network. Thus, the mobility of neutral particles is not affected by changes in the salt concentration. Higher pH values were shown to increase particles mobility that is pronounced more for neutral than for the charged ones. At pH 3 a mucin gel forms a more effective barrier toward particle diffusion and is also differently impacting toward the surface charge of translocating particles than a mucin hydrogel at pH 7 (Lieleg et al. 2010).

Due to the interaction between negatively charged biofilm biopolymers and positively charged diffusing molecules small charged molecules are sometimes less mobile than larger neutral solutes (Stewart 1998). This mainly concerns bacterial exopolysaccharides such as alginate or gellan gum (Kumon 1994). Due to electrostatic interaction transport of NPs and liposomes can be limited in biofilms as NPs are immobilized in it (Messiaen et al. 2013).

Diffusion in biofilm is also influenced by the temperature and viscosity (Converti et al. 1999). The research done for model virus particles (bacteriophages) showed that different density and viscosity of biofilms and its interaction with them do not influence highly their diffusion inside the bacterial biofilm. Their diffusion can be described as anomalous (Lacroix-Gueu et al. 2005).

Surface modification of NPs such as ‘protein corona’ that is a temporarily evolving collection of organic molecules that associate with NPs influence on their interaction with an environment (Cedervall et al. 2007) (Walczyk et al. 2010). This ‘corona’ may also include other biomolecules such as proteins (Monopoli et al. 2012).

It was shown that surface charge heterogeneity resulted in varying degrees of NPs deposition due to changes in electrostatic interactions (Liang et al. 2007) and electrostatic forces are important mechanism for deposition of TiO₂ NPs onto synthetic biofilms (Sahle-Demessie & Tadesse 2011) and for fullerene NPs onto surfaces coated with EPS (Tong et al. 2010).

Size of charged particles was shown to influence of their sorption onto the biofilm. Negatively charged polystyrene NPs with SO⁻⁴ groups showed greater sorption onto *Alteromonas macleodii* biofilms compared to NPs functionalized with amine (-NH) or carboxyl groups (Nevius et al. 2012).

For charged silver NPs it was observed a decrease in their self-diffusion coefficients with their size increase higher than 50 nm and with negative charge increase in comparison with neutral and positively charged NPs (Peulen & Wilkinson 2011). The effect of NPs size is also dependent on the density of the biofilm, diffusion of bigger NPs larger 50 nm becomes limited only in dense biofilms.

Also soluble extracellular polymeric substances (EPS) produced by freshwater and marine algae was shown to influence on the fate and surface properties of TiO₂ NPs with different coatings in the environment (Adeleye & Keller 2016). Adsorption of EPS on the TiO₂ NPs depends on particle surface area, surface charge and hydrophobicity. Interaction between EPS and NPs is mainly driven by electrostatic

interactions and chemical bonding (bridge-coordination) between the COO^- group of EPS and TiO_2 .

To conclude, different factors influence the particle movement within biofilm. In particular, the binding capacity of biofilms has been shown to initially limit the NPs mobility (Kurlanda-Witek et al. 2015). Moreover, polysaccharides, as the main constituent of the biofilm matrix, were shown to strongly control the diffusion of solutes (Zhang et al. 2011) and to influence the interaction with NPs (Lawrence et al. 2007) (Ikuma et al. 2014) (Miller et al. 2013). Hydrogen bonding, ionic interactions and dehydration of polar groups were shown to be important factors which influence NPs and polysaccharide interactions (Zeng et al. 2012). Attractive interactions between NPs and polysaccharides tend to limit the NPs diffusion in biofilms, while repulsive interactions may favour mobility if steric constraints allow it. The affinity of NPs for polysaccharides thus appears to be a key parameter to evaluate in order to assess the fate and exposure of NPs. That is why a study of affinity released NPs in the environment to the biofilm components will clarify overall transport within a biofilm.

1.4.6. Diffusion of particles in gels porous media and in aqueous polymer systems

Environmental biofilms are heterogenous systems, chemically, biologically and physically. To decrease the level of complexity, model polysaccharides can be used to mimick simpler model of biofilms in order to study deposition and diffusion of particles. These three-dimensional network matrix based only on polysaccharide are called biogels.

Diffusion in gels is an important component in many environmental, pharmaceutical, and biological applications of gels (Hoffman 1995) (DeRossi 1991). Hydrogels are hydrophilic polymer networks that may absorb from 10-20 % up to thousands of times their dry weight in water. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve. They are ‘reversible’ or ‘physical’ gels when the networks are held together by molecular entanglements, and/or secondary forces including ionic, H-bonding, or hydrophobic forces (Hoffman 2012). Hydrogels are ‘permanent’ or ‘chemical’ gels when they are covalently cross-linked networks. Chemical hydrogels may also be generated by cross-linking of water-

soluble polymers, or by conversion of hydrophobic polymers to hydrophilic polymers plus cross-linking to form a network. Like physical hydrogels, chemical hydrogels are not homogeneous. They usually contain regions of low water swelling and higher cross-link density, called ‘clusters’, that are dispersed within regions of high swelling, and low cross-link density.

In the study of particles diffusion in aqueous polymer systems such as gels, different behaviors have been observed. It is known that the diffusion of the particles is linked to the physical properties of the polymer network and the interactions between the polymer and the solvent itself.

Generally, diffusion in hydrogels depends on physical and chemical interactions between the diffusing particles and mainly the carbohydrate matrix of the hydrogel (Lieg & Ribbeck 2011). These are three main mechanisms that govern diffusion in porous media: steric (physical obstruction), electrostatic and chemical interactions between NPs and gels (Fatin-Rouge et al. 2003). Binding of the particles to a gel was shown to be a major factor in this process. For homogeneous gels alteration of solvent properties influences on the diffusion and for heterogeneous gels the obstruction effect is prevalent (Muhr & Blanshard 1982). It was shown by Muhr that diffusion of particles in the more heterogeneous gels (e.g. agar/water) is only slightly slower than in the solvent. A greater reduction in their diffusion rate seems to occur for the more homogeneous gels (e.g. polyacrylamide/water). This reduction appears to be a result of different factors such as obstruction effect, hydrodynamic drag and specific particle-polymer interaction.

Generally, in polysaccharide hydrogels it is well established that molecular diffusion is often simply limited by the obstruction of the polymer network, with reduced diffusion observed for larger particles or higher gel densities (Lawrence et al. 1994).

Numerous types of environmental stimuli (physical or chemical such as pH, temperature, electric fields, light) can induce this volume change, which is utilized in many applications of gels, such as drug delivery devices, actuators, etc. (Hoffman 1995). Increase in the volume fraction of macropores was found to result in the increase in the diffusion coefficients of the solution flowing through gels. The previous diffusion study in mucus showed a decrease of particle mobility with increasing particle size. The viscoelasticity of mucus also reduces the diffusion rates (Dawson et al. 2003). Gel structure (swelling, compression) was shown in alginate hydrogel to have influence on diffusion (Golmohamadi & Wilkinson 2013).

Increasing the density of the calcium alginate gel leads to decrease its pore sizes and increase obstruction effects, especially for the larger particles (Favre et al. 2001) (Liu et al. 2002) (Golmohamadi & Wilkinson 2013). In turn, the swelling factor is largely influenced by the ionic strength of the solution. Greater gel charge also leads to increase diffusion fluxes (Golmohamadi et al. 2012). A large number of papers which have been published on the diffusion of solutes in agar gels (Sebti et al. 2004) (Gutenwik et al. 2004) show that the rate of diffusion of molecules in agar gels is low. Diffusivity is dependent on structural properties and kinetics parameters, such as gel architecture (pore size and strand thickness) and viscosity (Yamamoto et al. 2002). The architecture of the agar gel is modified by the agar concentration and so the rate of diffusion decreases with increasing gel concentration. Agarose content between 3 to 6 % does not have any effect on nisin (polypeptide) diffusion within it. However, agarose content of 8 % significantly reduces diffusion in agarose gel (Sebti et al. 2004).

If there is no possibility of particles binding to the gel then their movement in the gel may be restricted by physical obstruction, by increasing the hydrodynamic drag on the particle, by reducing available free volume of the solvent (Amsden 1998). Obstruction effects refer to the loss of free volume due to the presence of the polymer matrix and the resulting decrease in mean free path for the diffusing particles.

The dominating effect in this case will be determined by particles size, the volume fraction of polymer present and the composition of polymer. The underlying of models based on the obstruction theory is that the presence of impenetrable polymer chains causes an increase in the path length for diffusive transport. Thus, the polymer chains act as a sieve, allowing particles passage only when it can pass between the polymer chains. In models based on this theory, the polymer chains are considered to be immobile and randomly embedded in a uniformly permeable continuous phase (Amsden 1998).

Free volume theory considers that particles move within the liquid phase i.e. free volumes (Amsden 1998). Based on this theory it is developed a model of particles transport through swollen gel membranes (Yasuda et al. 1969). It assumes that the particles diffuse through the water phase only, that the polymer and water molecules are randomly mixed, the particle can diffuse through the gel only if it finds a sequence of free volumes larger than the particle molecule, that the effective free volume available is essentially the free volume of water in the gel. The presence of the

hydrogel's polymer chains reduces the amount of free volume of the aqueous phase available for the particle transport (Yasuda et al. 1969). For highly swollen gels, and especially for macroporous heterogeneous gels, the free volume of the water within the gel available to the particle is likely to be the same as the free volume of the bulk water surrounding the gel. This is because heterogeneous hydrogels have very stiff polymer chain segments and will not compete effectively with the particle for the free volume formed within the water phase. Due to this considerations the free volumes contributions to the particles diffusivity may be considered negligible.

It is an assumption that within a gel there are two types of water: free and bound. Bound water is chemically attached to the gel structure while free water is similar to water in bulk solution. The amount of water in a hydrogel, i.e. the volume fraction of water and its free vs. bound water will determine the diffusion of the particles through the hydrogel (Hoffman 2001) (Zhang & Davison 1999). Probably, the particles diffuse within free water more readily than in the bound one. Swollen gels are more likely to have a high proportion of free water. This facilitates particles diffusion.

Cross-linking may have different effect depending on particles diffusing. Thus, diffusion of humic substances in agarose cross-linked acrylamide is more impeded than their diffusion in the agarose gel while there is no significant difference for metal ions. Hereby diffusion is not simply related to pore size but also reflects the compatibility of the gel and molecular structures. Molecular weight is also important for the diffusion. Diffusion coefficients decrease with increasing molecular weight from fulvic acid to humic substances (Zhang & Davison 1999).

Crosslinking of the hydrogel together with the particle size have an influence on particles movement within it. Thus increasing crosslinking degree and the particle size decrease both particles permeability and partition coefficient (Matsuyama et al. 1997). In this case polymer concentration is also important for the transport.

Independently on the particle size it may occur an interaction filtering due to particles binding interaction with gel and based on their surface properties. Binding interactions increases with charge density (Kreuger et al. 2006) (Jastrebova et al. 2006).

For NPs with polymer coatings (Lerner et al. 2012), or NPs coated with an organic corona, it was shown that steric interactions are important in the aquatic environment due to the polymeric natures of biofilms.

Particles may also diffuse differently into the gel due to their surface properties such as hydrophilicity. Thus, the more hydrophilic particles ribonuclease showed higher permeability and partition coefficient than the less hydrophilic lactalbumin probably due to the interaction with hydrophilic poly(vinyl alcohol) hydrogel (Matsuyama et al. 1997). Chemical interactions, or specific binding to the gel itself, can occur as well and apparently reduce the average rate of diffusion through the polymer matrix. Nonetheless, for some hydrogels electrostatic interactions have been shown to play a key role with respect to the mobility of charged solutes. It was shown that self-diffusion coefficients in the biofilm decreased with an increasing negative charge of the fluorescent probes (Zhang et al. 2011).

Metal ion activities in the polymer gel phase also differ drastically from those in the bulk medium to an extent that strongly depends on ionic strength of the solution (Kalis et al. 2009). For charged rhodamine particles self-diffusion in agarose hydrogel is shown to be increase from 50 to 90 % with increased ionic strength. The diffusion of positively charged substrates decreased as the pH was decreased (Golmohamadi et al. 2012). The physicochemistry of the bulk solution and the nature of the gel will play an important role on particles diffusion, with strong effects observed due to ionic strength and pH variations.

To summarize, the interactions between gel and diffusing particles will depend on the physicochemical properties of the hydrogel and particles: their size, shape or surface properties such as charge, its hydrophilic and hydrophobic character, their hydration and solution conditions such as the ionic strength and the pH of the bulk solution as they may change a charge, swelling of a gel and water content of it (volume fraction) (Yasuda et al. 1969) the extent of gel crosslinking (Amsden 1998), flexibility and concentration of the particles (Hermansson 1982), pore size and the pore size distribution determine the ‘tortuosity’ of the particles displacement in the gel. In addition, hydrodynamic drag (the proximity of solute to the polymer-solvent interface) has an impact on the mobility of the solutes (Cukier 1984). Polymer concentration, flexibility and volume fraction have an impact on the diffusion process (Amsden 2001). For a charged gel, the “effective proximity” can be modified by the ionic strength of the bulk solution, which affects the thickness of the Debye layer. It is shown by some studies that diffusion in the charged hydrogels is influenced by both the pH and ionic strength of the bulk solution (Narita 1997) (Narita et al. 1998) (Kalis

et al. 2009). Structural changes of polymer with temperature also will have an influence on the particle movement (Converti et al. 1999).

Generally, the mobility of NPs within the biogel was shown to depend on steric constraints, i.e. porosity and their affinity with the gel components (Fatin-Rouge et al. 2003) (Golmohamadi et al. 2013). In addition, the mobility is influenced by many factors such as the NPs charge (Golmohamadi et al. 2013) and aggregation (Stewart 1998) (Choi et al. 2010), and gel hydrophobicity (Xiao & Wiesner 2013) and structure (Labille et al. 2007).

As reviewed above, most studies on diffusion in biogels use agarose or alginate as component of the gel matrix. Agarose is produced seaweeds and based on alternating D-galactose and 3,6-anhydro-L-galactopyranose. Alginate is a polysaccharide consisting of mannuronic acid and guluronic acid monomers, produced by algae and by the bacterium *Pseudomonas aeruginosa*. *P. aeruginosa* is, an environmental bacteria found in water and soils, which is an opportunistic human pathogen associated to chronic respiratory infections in cystic fibrosis patients. In biofilms of *P. aeruginosa*, alginate contributes to the architecture of the biofilm in the lungs of patients. However, nonmucoid *P. aeruginosa* strains are the predominant environmental phenotype (Wozniak 2003). Thus, if agarose and alginate constitute well-documented biogels for diffusion studies, they appear poorly relevant as environmental « biogel » models.

Many microbial polysaccharides are used as thickening, gelling, flocculating and moisturising agents (Paul et al. 1986) (Sutherland 1998). Among them, we focused on a polysaccharide called “Soligel” produced by Agro-Recherche et Developpement (ARD, Pômacle, France) from *Rhizobium alarii*, a bacterium isolated from sunflower roots (Alami et al. 2000).

1.4.7. Properties of biogel made from EPS YAS 34

‘Soligel’ is a new polysaccharide produced by *Rhizobium alarii* YAS34. This strain is a gram-negative, aerobic catalase-positive and oxidase-negative bacterium. It is a mobile rod bacterium, which forms elastic, translucent, white colonies (Figure 1.11) on an RCV-glucose medium (20 g/l).



Figure 1.7: Muroid colonies of *Rhizobium alarii* YAS34 (from BRESIN et al. 2008)

The strain YAS 34 biosynthesizes polysaccharide by fermentation in the presence of a culture medium containing a source of preferably assimilated carbon (Alami et al. 2000). Production of YAS 34 from bacterial strain and its further purification is described by (Crompin et al. 2002). They showed that the EPS has gelifying properties, and is stable in a broad range of pH, it does not have a big influence on gel viscosity with time. EPS shows a very important cohesiveness and very good healing after the applications of important strains. During storage, the gelling properties are enforced, indicating a renaturation phenomenon; no hysteresis is observed.

The chemical structure of this exopolysaccharide implied six neutral sugars (two of them in a side chain), one uronic acid in the main chain and one pyruvyl substituent per repeat unit, as well as acetyl substituents not exactly located at that time. Due to the presence of one glucuronic acid in the main chain and one pyruvyl substituent per repeat unit it has polyelectrolytic character.

In dilute solution, under the native conformation, the polysaccharide shows a tendency to interchain interactions. The native sodium and acid conformations are double helices, or helical dimers, formed from two chains; the conformational transition leads to the dissociation of these double helices. Polysaccharide under sodium form has a lower degree of helicity and form physical gels (Villain-Simonnet et al. 2000a). YAS34 exhibits a conformational transition under thermal treatment from its native conformation. The conformational transition is reversible and cooperative for the native polymer under its acid form and corresponds to the change between a double helix formed with two chains to a double helix formed by a single chain folded onto itself and the deacetylated chain is always a coil. The formation of a renaturated double helix (single chain folded over itself) especially in the acid form is a reversible process due to a cooperative disorder-order transition.

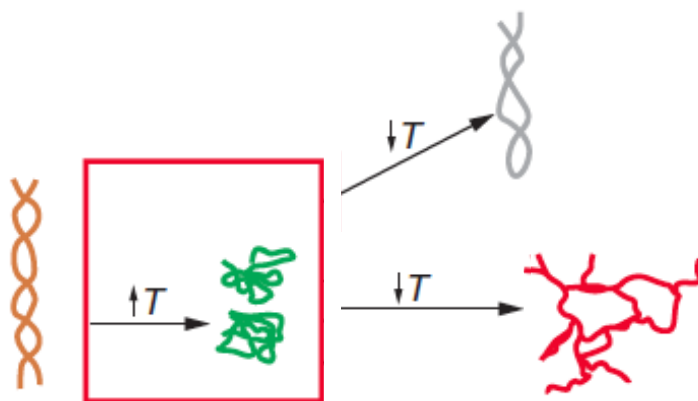


Figure 1.10: Conformational changes of the polymer as a function of the temperature and duration of treatment (Villain Simonet 2000).

The increase in the temperature (T) leads to a destructuration of the double helix. During cooling, the polymer chains rearrange between them according to the severity and duration of treatment. The partially denatured conformation (in red) has gelling properties (adapted from Bresin 2008).

For a concentration 10 g/l at 10^{-1} M NaCl it has gel properties (Dargelas 2002). This temperature for conformational transition is between 60 °C and 80 °C. On the opposite, under the sodium salt form, it occurs irreversible denaturation. This is interpreted due to a change in the arrangement of acetyl substituents.

After cooling, the polyelectrolyte properties and the stiffness of the chain are in a double helical conformation obtained by a chain folding back onto itself. There was not observed any modification of molecular weight. It gives highly viscous aqueous solutions in relation with its conformation in double helices.

After the formation of elastic gel, it is observed a slow evolution of the mechanical properties in relation with a slow stabilization of interactions between residual short helical segments.

The rheological properties of the YAS34 polysaccharide are directly related to its conformation. It changes after heating, also degree of modification in acetyl groups increases. pH conditions influence on acetyl group and on the solution properties (Dargelas 2002). Acetyl groups are needed for gelation. The thermal stability of the obtained gel after cooling depends on the time of thermal treatments, which means on the degree of the helicity. The gelation and gel stability is related to the acetyl group arrangement and to the length of helical segments, degree of helicity. The gel strength

depends directly on the polymer concentration and external salt concentration. For YAS34, the gel strength and its thermal stability remain nearly unchanged for different salt concentrations and nature of salt that is an advantage for the use of this polysaccharide.

The rheological properties and the structure of the polysaccharide are very dependent on the production and purification processes (Dargelas, thesis, 2002).

The EPS34 of *R. alarii* is environmentally produced. Indeed, *R. alarii* colonizes the root of canola as biofilms and produces the YAS34 polysaccharide in the biofilm matrix as evidenced by selective binding of a fluorescent labeled lectin.

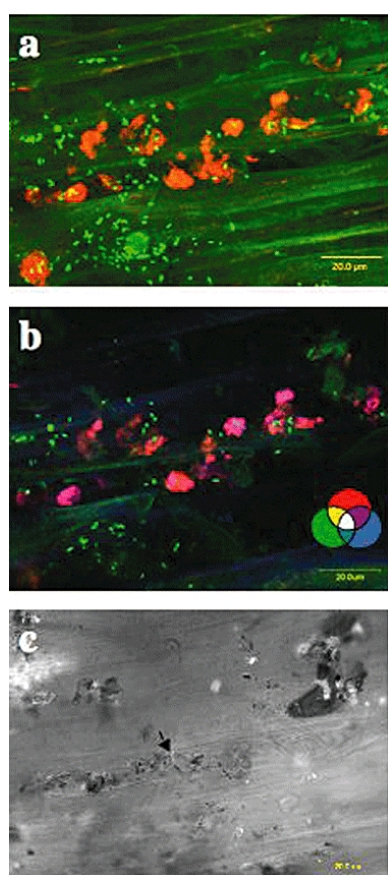


Figure 1.11: A. Localization of green fluorescent protein-expressing bacteria and autofluorescence of root tissues in green and EPS YA34 bound to Alexa660-lectin in red. Some Protozoa were found grazing GFP-tagged bacteria. Scale bar = 20.0μm. B. Imaging of the autofluorescent organic matter (in blue). Superimposition of artificial colours is given in overlapping circles. Scale bar = 20 μm. C. Observation of the same zone in transmission mode at z = 6 μm. Mineral particles (indicated by an arrow) were stacked to the root tissues and embedded into micro-aggregates. Scale bar = 20 μm (from Santaella 2008)

The polysaccharide production by bacterial cells in the rhisosphere contributes to stick soil aggregates, modifies the aggregation of root-adhering soil (Santaella et al. 2008) and eventually improves plant growth (Alami et al. 2000).

Thus, the polymer produced by *R. alarii* appears as a relevant EPS for studying interactions of EPS with NPs.

1.5. Conclusions of the literature review and objectives of the project

Ultimately, the environmental fate of NPs is influenced by biofilms, and therefore requires a more in-depth understanding of their fundamental properties.

Although there have been numerous studies of the diffusion of molecules in hydrogels, as reviewed, and some ones in the biofilms, there are few precise measurements relevant to the diffusion of nanoparticles in biological gel in humid environment and pulmonary gel in human organism. It has to be clarified the transport and deposition processes which perform in these systems when NPs are introduced to them.

Among the wide field of research that constitutes the risk assessment of nanotechnology, this thesis project deals with the exposure aspect, focusing on the diffusion motion of manufactured nanoparticles through biogels, as models of biofilms. The physical behavior of ENPs largely determines their bio-accessibility, following a large number of physico-chemical interactions that depend on both the ENP surface properties and the system composition. Biological gels constitute a natural selective barrier to ENPs exposure. These are e.g. exopolymeric substances produced by bacteria on most of non-sterile surfaces in wet environment (i.e. biofilms). This is also pulmonary gel, or mucus, coating the respiratory apparatus walls. The permeability of the biogel to ENPs largely determines their mobility and exposure to the hosting living organisms. In addition, the surface functionalization of the ENPs, according to their related nano-enabled products, is expected to alter their behavior within the gel.

Measuring and characterizing these phenomena constitute the main focus of this research project.

The aim of this project is to study the diffusion of a selection of engineered nanoparticles within such biogel typically encountered in the environment, in order to better estimate their exposure. A model biogel made of pure bacterial polysaccharide, EPS YAS34, with well-known composition will be used in order to better constrain and identify the physicochemical interactions driving the diffusion or sinking of the ENPs in the pore network.

TiO₂ nanoparticles will be used due to their wide use in many applications. Different surface functionalizations will be compared.

Our work plan consists in 4 chapters that are schematically shown in Figure 1.12. Chapter 1 reviews sunscreen release to the environment and their further behavior in the aquatic system. In the Chapter 2 we studied the interaction of NPs with EPS in aqueous suspension as a function of their concentration ratio and of the solution conditions. In the Chapter 3, we considered the scenario where the NPs collide with a collector in the aqueous environment. We studied the NPs affinity for the collector surface, comparing a collector coated with polysaccharide and a bare SiO₂ mineral collector. In the Chapter 4, we studied the NPs transfer and mobility through the polysaccharide gel. Part of the work presented in this manuscript is published as a scientific paper in the NanoImpact journal and is presented in the Annex of this manuscript.

Scheme of the approach followed in the PhD

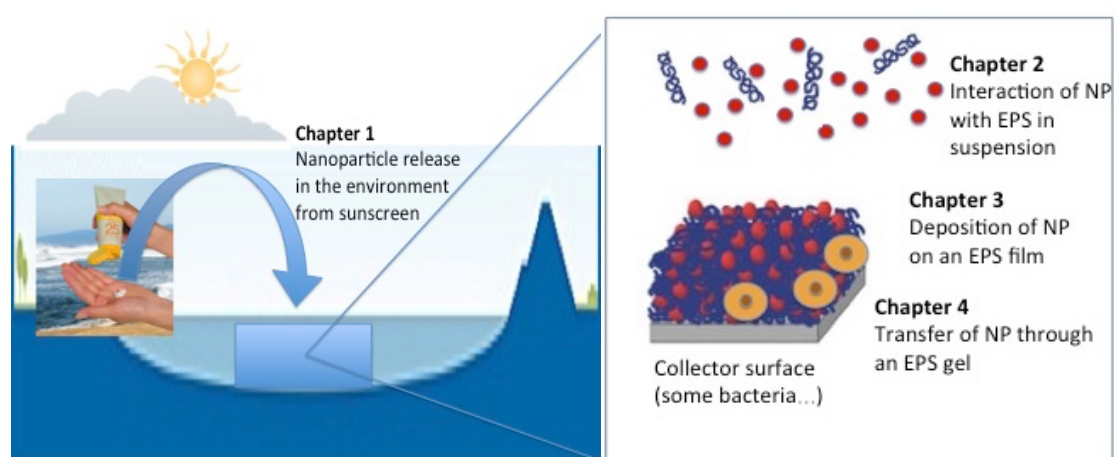


Fig. 1.12. Scheme of the approach followed in the PhD

Chapter 2. Materials and Methods

concentrations (for salt concentrations larger than 0.04 – 0.05 N) and various types of salt (Na^+ , Ca^{2+}) (Villain-Simonet et al. 2000).

2.1.1.1. Proton Titration of the polysaccharide

Potentiometric titration of EPS was done in order to get an insight into the role of pH on the degree of ionization of YAS34, and to determine the point of zero charge (PZC). EPS solutions at 5 g/l in NaCl 10^{-3} – 10^{-1} M was used. The batch volume was 20 ml.

The titration set-up was composed of a pH-meter, an auto-burette, and a titration cell, kept under nitrogen gaz. The pH measurements were made with a combination electrode. Automatic titration was used and small doses of titrant were added continuously. All solutions were prepared with Milli-Q water. Samples were first acidified to pH 3 by HCl addition. The titrant was NaOH (10^{-2} M), and the procedure was stopped when pH 10 was reached. 3 experimental potentiometric titration curves of the gel were realized at three different NaCl concentrations, 10^{-1} , 10^{-2} and 10^{-3} M. The ionisation of the polysaccharide according to pH was calculated by subtracting the free OH^- concentration in solution from the total OH^- titrated to the polysaccharide solution. The $\text{PZC} = 2.9$ was determined as the intersection of the 3 titration curves (Fig. 2.2).

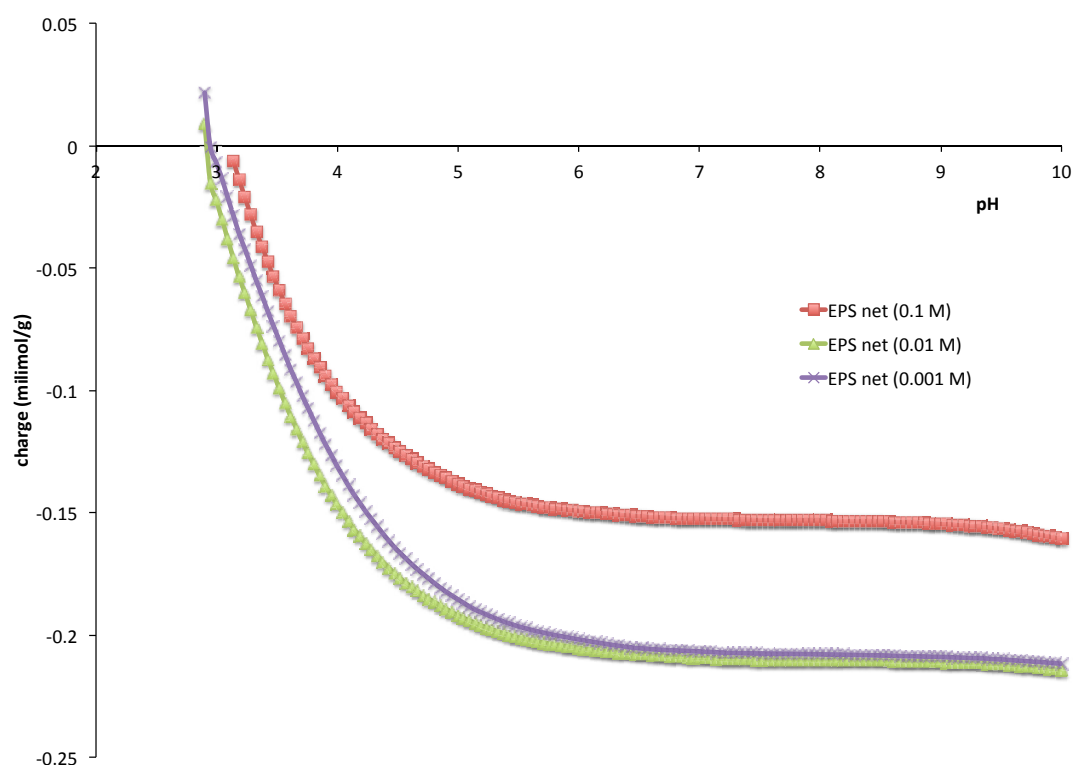


Fig. 2.2. Proton titration curves of the YAS34 exopolysaccharide as a function of the NaCl concentration in the dispersing medium

The dispersion stability of the polysaccharide macromolecule was tested also by DLS as a function of the solution type and ionic strength. Size distribution and Zeta potential were measured in NaCl solution with IS from 10^{-5} to 10^{-1} M and CaCl_2 solution from 10^{-6} M to 10^{-2} M, with EPS concentration of 1 g/l.

The EPS size increased from 500 nm in 10^{-6} M CaCl_2 or 10^{-5} M NaCl to 2000 - 3000 nm in 10^{-2} M NaCl or 10^{-2} M CaCl_2 . This reveals the EPS homoaggregation tendency when at high ionic strength. The zeta potential absolute values decrease with increasing IS: from -40 mV at 10^{-5} M NaCl and -30 mV at 10^{-6} M CaCl_2 to -17 mV at 10^{-1} M NaCl and 10^{-2} M CaCl_2 . This is in agreement with the size measured. The EPS remains stable and dispersed in solution at low IS because the charged moieties favour electrostatic repulsions.

For the deposition studies in Chapter 5, prior to coating of the collector, the polysaccharide solution (1 g/l in water at pH 5.6) was filtered through cellulose nitrate membranes with a pore size of $0.7 \mu\text{m}$ to remove larger aggregates.

2.1.2. TiO₂ nanoparticles

TiO₂ NPs were selected in this work since they are widely used in different applications such as sunscreens and paints. In consequence, they are widely released in the environment. Manufactured NPs are often functionalized in surface in order to match the application requirements. For example, polyacrylate is used as a coating for TiO₂ NPs used in sunscreen to favour hydrophilic dispersion (Labille et al. 2010), (Botta et al. 2011), (Smijjs & Pavel 2011). Pure TiO₂ NPs as well as PAA-coated TiO₂ NPs were selected as a case studies in this work.

Pure TiO₂ nanoparticles were obtained from NanoAmor (anatase, with an average particle size of 5-30 nm). The TiO₂ NPs were obtained as an aqueous 170 g/l dispersion with pH = 1. The average elementary size distribution of the dispersed NPs, measured by dynamic light scattering (Malvern Zetasizer Nano ZS), was centred at 12 nm (Fig. 2.3). The z dependence with pH, which was measured using phase analysis light scattering (Malvern Zetasizer Nano ZS), revealed an isoelectric point $\text{pH}_{\text{IEP}} = 4.9$ (Fig. 2.4). Maximum absorbance in UV-Vis spectroscopy for TiO₂ NPs is at wavelength 270 nm.

Aqueous TiO₂ suspensions were prepared at a working concentration of 100 mg/l for both QCM-D and AFM analyses and various concentrations for TiO₂/EPS suspensions. Ultrapure water (18.2 MΩ, Millipore Milli-Q purification system), NaCl (10^{-5} - 10^{-2} M) or CaCl₂ (10^{-5} - 10^{-4} M) was used as the dispersing medium. For experiments carried out at pH = 3 and 4, the pH was adjusted by adding a constant amount of HCl solution to the dispersing medium, pH 8 was adjusted by adding NaOH solution to the dispersing medium, pH 5.6 was obtained by diluting the NPs suspension with the dispersing medium in Milli-Q water. All reagents used were of high purity.

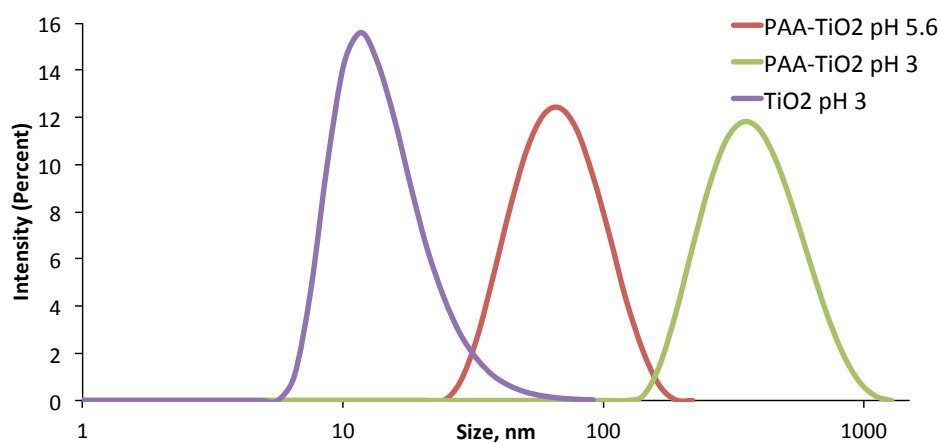


Fig. 2.3. Size distribution of TiO₂ and PAA-TiO₂ at pH 3 and 5.6 measured by dynamic light scattering

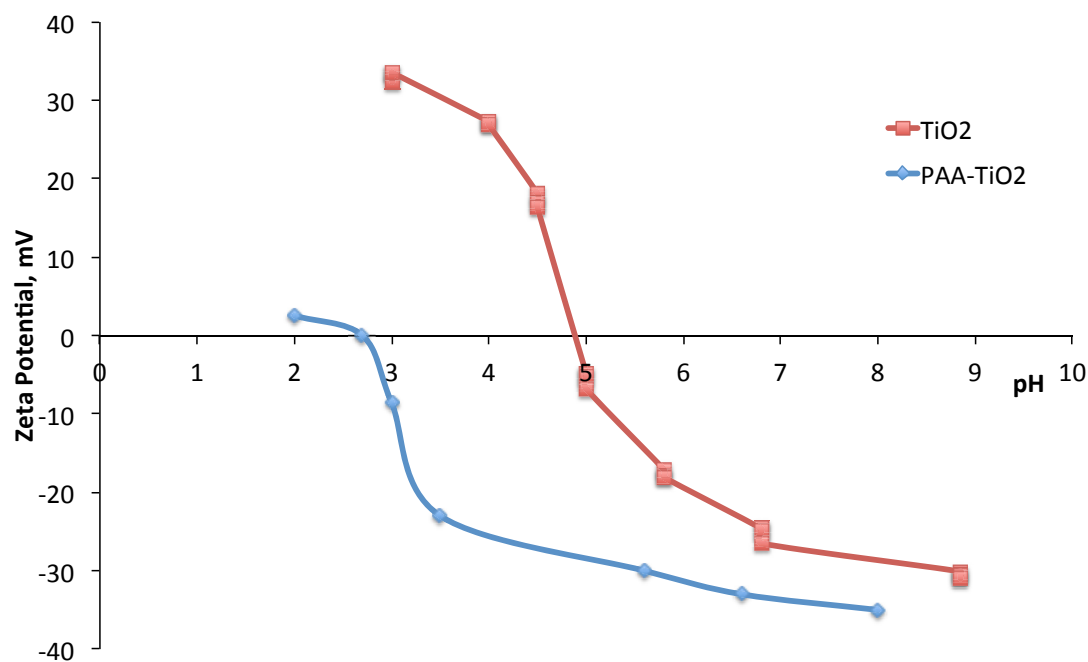


Fig. 2.4. Zeta Potential of TiO₂ and PAA-TiO₂ vs pH, measured by light scattering

PAA-coated TiO₂ was obtained from Sensient Company (Saint-Ouen L'Aumône, France) under the trade name of Covascreen WTI. Covascreen is a TiO₂-based nanocomposite used in the formulation of Sunscreen (trade name TiO₂ Rutile-Covascreen WTI). It is in the form of a hydrophilic gel, ready to integrate the aqueous phase of the sunscreen, as a UV filter. According to the manufacturer's data, the PAA-

coated TiO_2 consists of a nano- TiO_2 core coated with sodium polyacrylate that provides a hydrophilic and negatively charged surface in order to promote dispersion at the nanometric scale in an aqueous medium (Fig. 2.5). Polyacrylate coating is used for stabilizing and dispersing TiO_2 NPs in sunscreens. So, these NPs are a good analogue of the sunscreen residues that may be released in the aqueous environment through the product lifecycle.

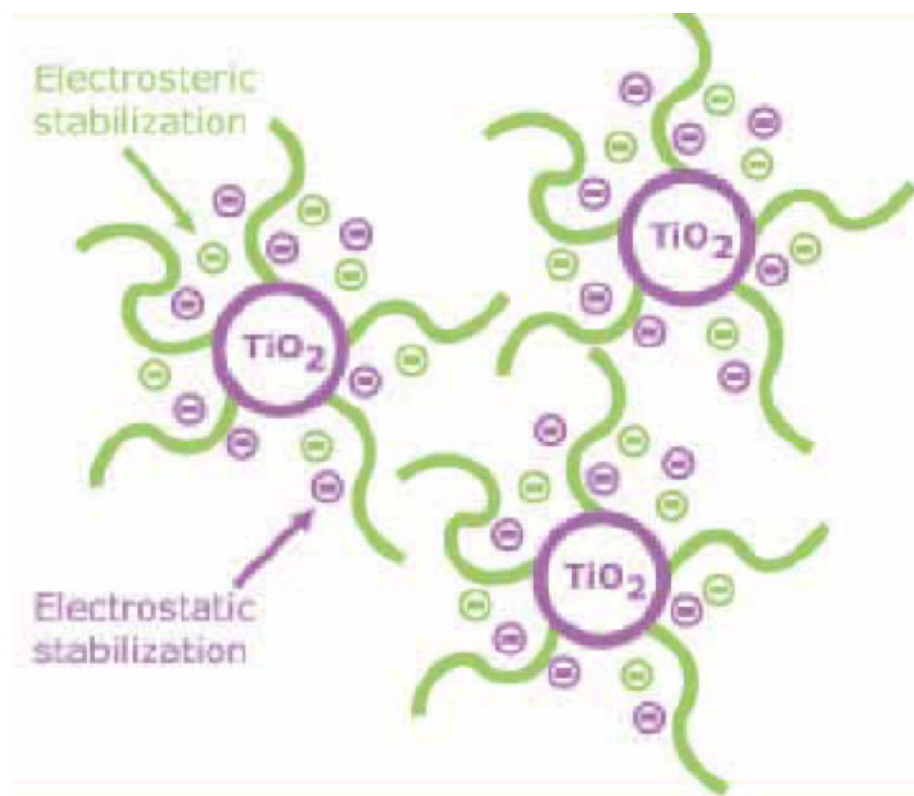


Fig. 2.5. Cross-sectional diagram of the Covascreen nanocomposite (PAA- TiO_2), composed of a core of TiO_2 Nanometric coating and a coating of sodium polyacrylate

This commercial product is obtained as an aqueous gel concentrated at 40 wt.%. Nanoparticle dispersions were prepared by diluting and stirring the commercial product in ultrapure water at 10 g/l for 24 h. Then, the mixture was left to settle out in the dark for 1 month and the supernatant was recovered. The NPs were diluted at a 10 mg/l working concentration in NaCl (10^{-3} M), and the pH was adjusted to 3 by adding HCl solution or left at pH 5.6. The pH_{IEP} of the PAA-coated TiO_2 was measured at 2.7 (Fig. 2.4), so the NPs tend to aggregate at $\text{pH} = 3$, which results in an intensity

weighted distribution centred at 340 nm. The elementary hydrodynamic size of the dispersed NPs centred at 70 nm was measured at pH 5.6 (Fig. 2.3).

2.1.3. *Au nanoparticles*

Au nanoparticles (with an average particle size of 5, 12, 20, 30, 50 nm) **coated with citrate, bPEI** were obtained from Nanocomposix **or silica** were obtained from Sigma Aldrich respectively. The Au NPs are obtained as an aqueous 0,05 mg/ml dispersion. Au NPs coated with branched polyethylenimine, bPEI-Au NPs are positively charged and Au NPs coated with Sodium citrate, cit-Au NPs and silica, SiO₂-Au NPs, are negatively charged. The respective size distributions and UV/vis absorbance spectra given by the manufacturer are shown in Figs. 2.6 - 2.17.

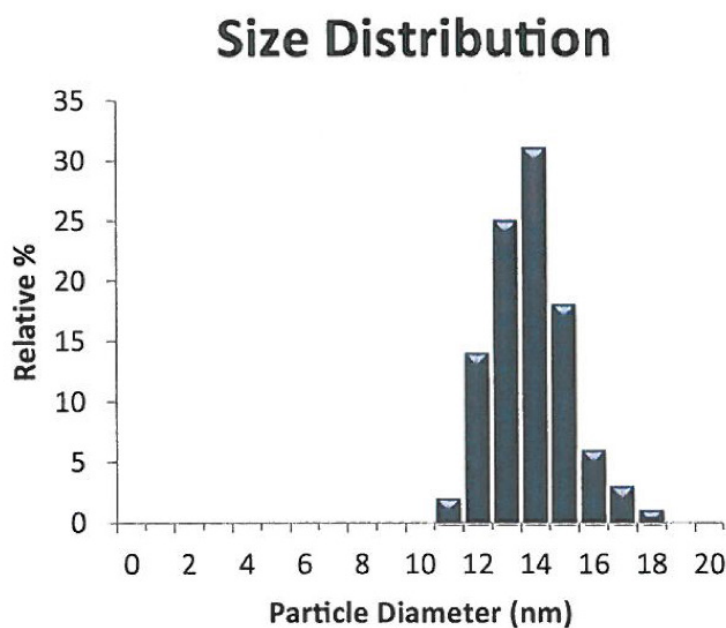


Fig. 2.6. Size distribution of Au NPs 12 nm with citrate coating

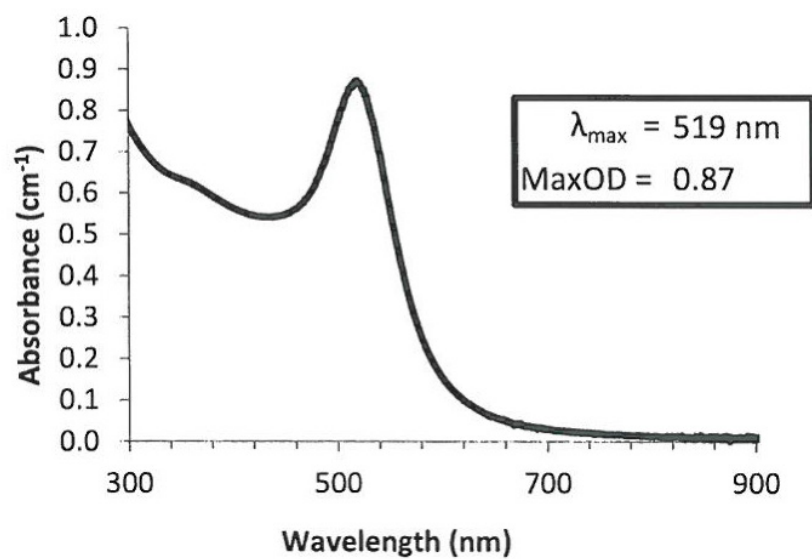


Fig. 2.7. Optical properties of Au NPs 12 nm with citrate coating

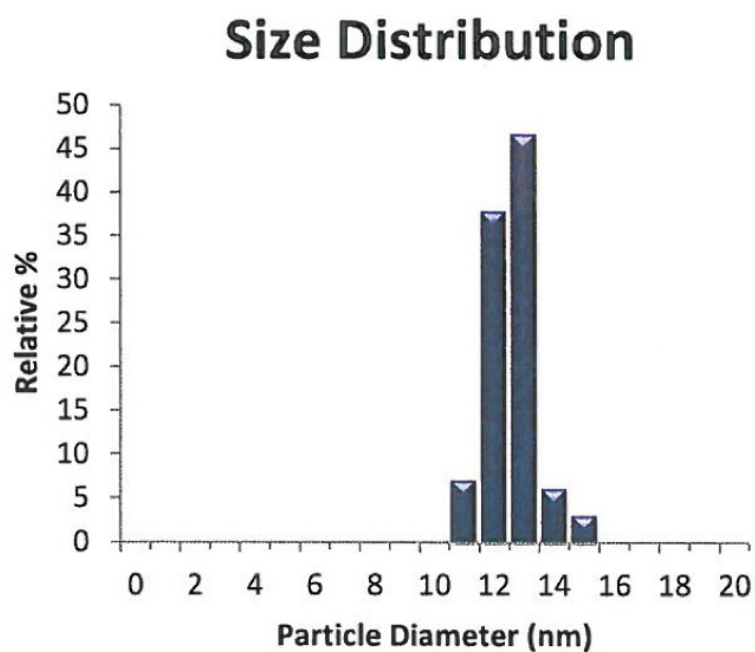


Fig. 2.8. Size distribution of Au NPs 12 nm with bPEI coating

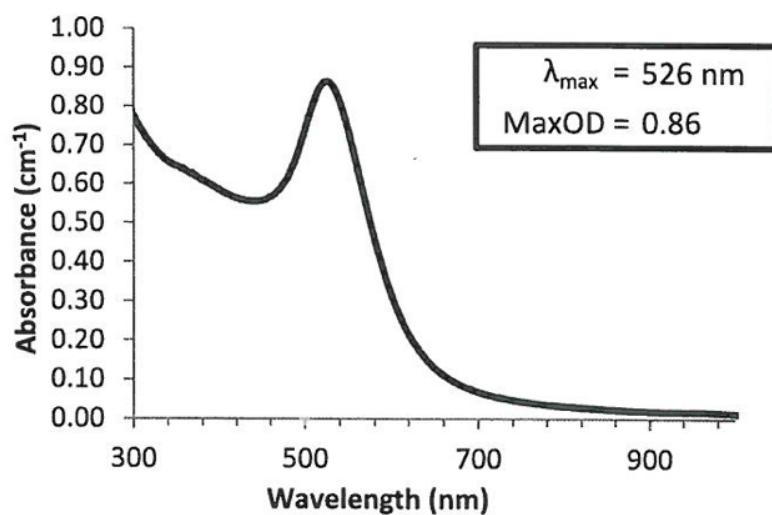


Fig. 2.9. Optical properties of Au NPs 12 nm with bPEI coating

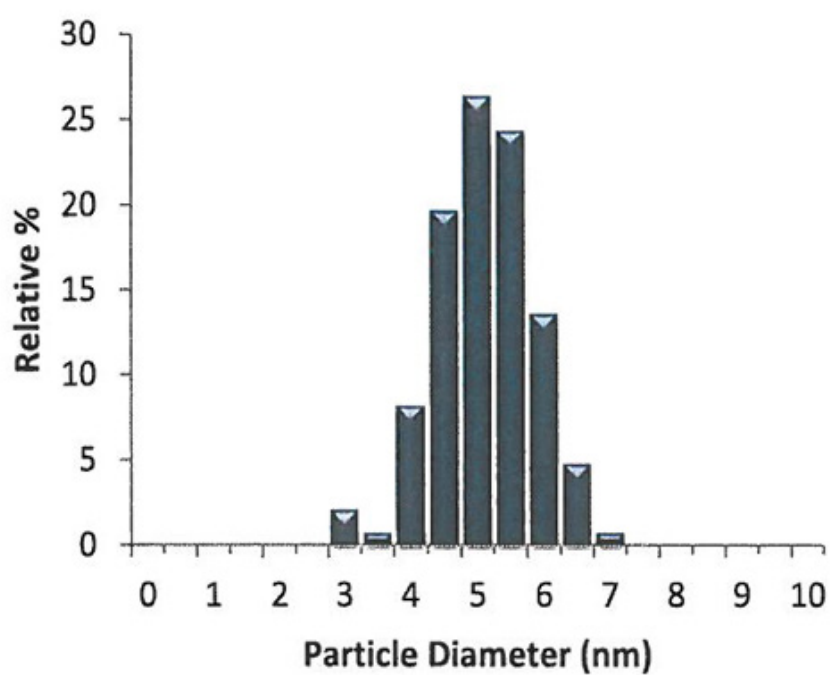


Fig. 2.10. Size distribution of Au NPs 5 nm with citrate coating

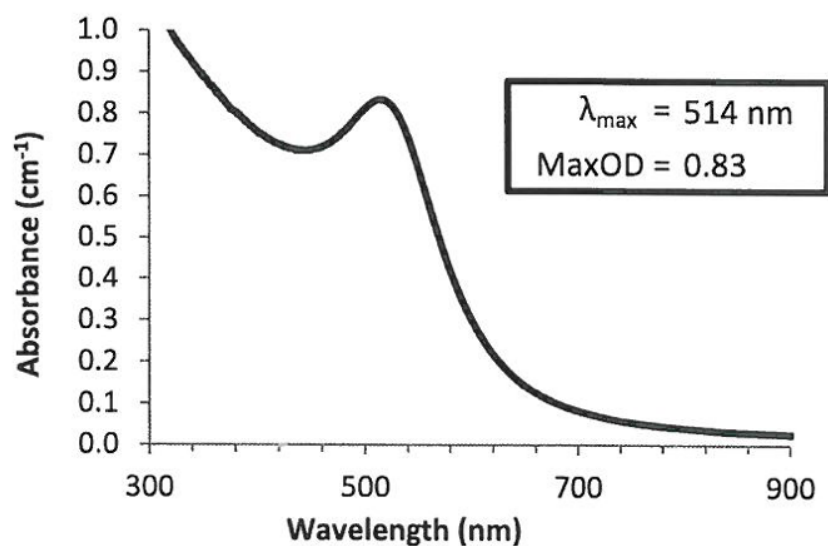


Fig. 2.11. Optical properties of Au NPs 5 nm with citrate coating

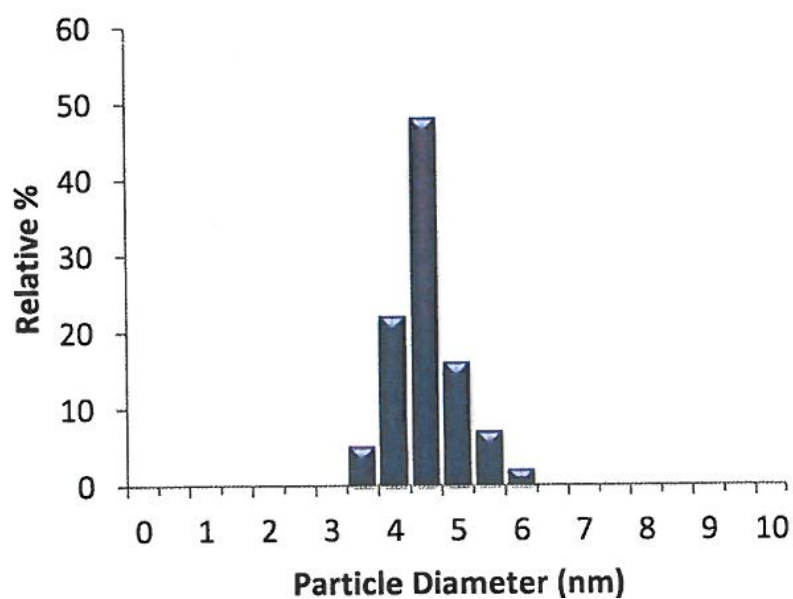


Fig. 2.12. Size distribution of Au NPs 5 nm with bPEI coating

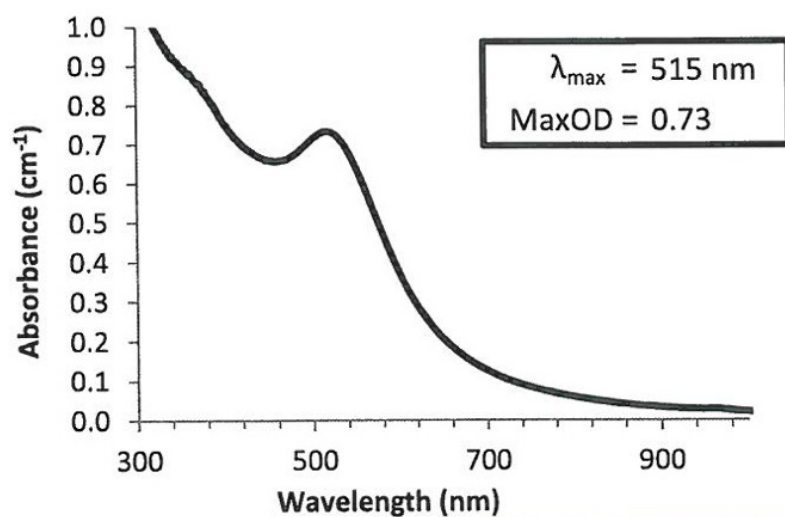


Fig. 2.13. Optical properties of Au NPs 5 nm with bPEI coating

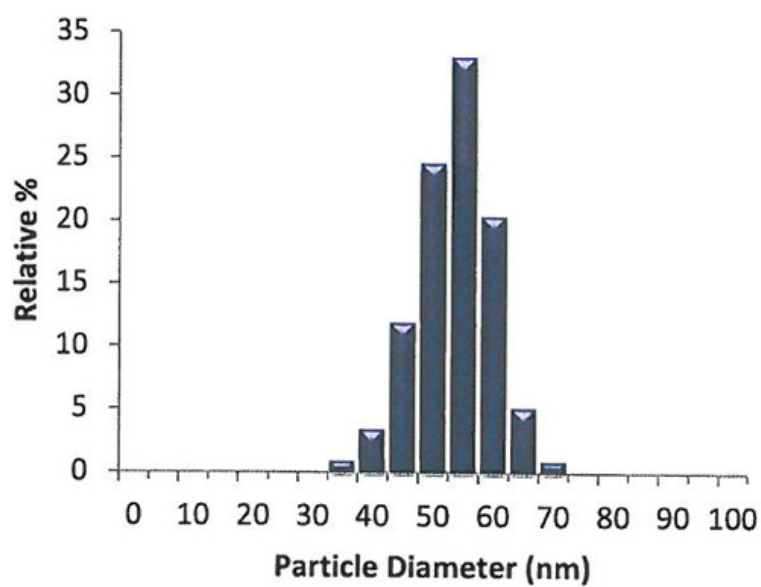


Fig. 2.14. Size distribution of Au NPs 50 nm with citrate coating

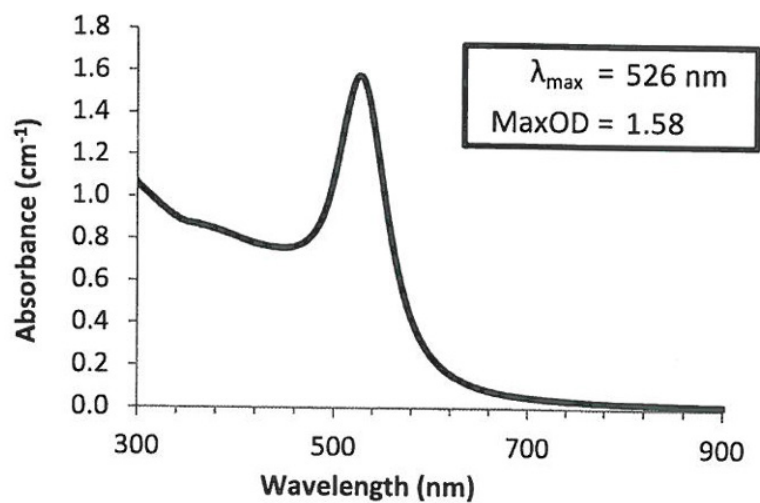


Fig. 2.15. Optical properties of Au NPs 50 nm with citrate coating

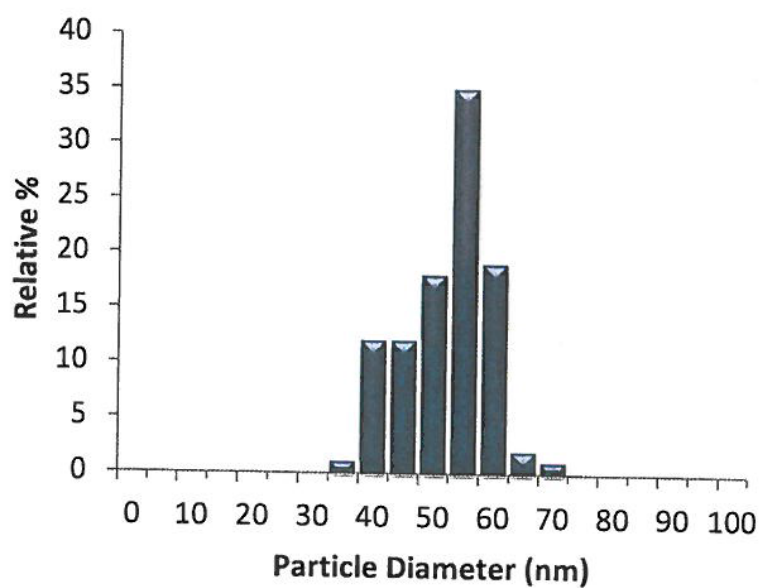


Fig. 2.16. Size distribution of Au NPs 50 nm with bPEI coating

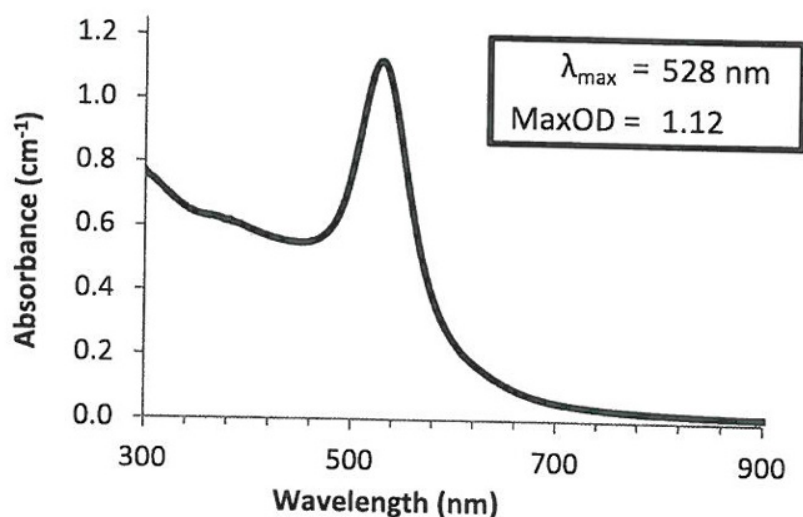


Fig. 2.17. Optical properties of Au NPs 50 nm with bPEI coating

2.2. Methods

2.2.1 Size measurements by dynamic light scattering

2.2.1.1. Objectives

Nanoparticles interaction with EPS was first studied in suspension. For this purpose, NPs and EPS size were measured separately and mixed together.

Particle-size distribution (PSD) plays a fundamental role in controlling the properties of nanomaterials. Particle size determines NPs diffusivity, and the ability of NPs to permeate biological gel and membranes. Size characterization is a key step in the control of NPs and their ultimate release into the environment, and allows monitoring of NPs aggregation (Brar et al. 2010). PSD and Zeta Potential were measured by Dynamic Light Scattering.

2.2.1.2. Principle of the method

Particles in suspension are in constant random motion (Brownian motion) due to interaction with the molecules of the suspending fluid. Light is scattered off the particles in suspension. Since the particles cause localized changes in the refractive index, intensity variations are produced by the particles and evaluated using the

second order normalized autocorrelation function (Hoo et al. 2008). DLS measures the time-dependent fluctuations in scattering intensity caused by the Brownian movements of the NPs within a sample. Through application of the autocorrelation function and subsequent calculation of the exponential decay, the diffusion coefficient of the particles can be calculated. Then it relates this movement to an equivalent hydrodynamic diameter according to the Stokes-Einstein theory of Brownian motion. This theory relates particle motion to the suspending fluid viscosity, the temperature, electrical charge, electrical mobility and the size of the particle (Brar & Verma 2011). This technique is extremely accurate when there is a simple distribution of decay times, e.g. for scattering from spherical nanoparticles with a narrow distribution of sizes about a single mean, although issues arise in accurately determining the size distribution. Sample polydispersity can distort the results, and the real populations of particles may not be seen because big particles present in the sample can screen smaller ones.

The challenge with most of the nanomaterials in natural environments is that they have a tendency to self-assemble into aggregates of substantial size. The primary limitation of DLS is that it is often difficult to quantify accurately the amounts of any aggregates that may be present, so the size fractions cannot be correlated with a specific composition (Bootz et al. 2004). Moreover, the particles are considered spherical by DLS techniques, so that the actual size of the particles in the dispersion can be underestimated. Also, data obtained from samples containing particles with heterogeneous size distributions are difficult to interpret, as is often the case in environmental applications. Fig. 2.18 is a representation of a typical set-up for measurement of NPs by light-scattering techniques (Brar & Verma 2011).

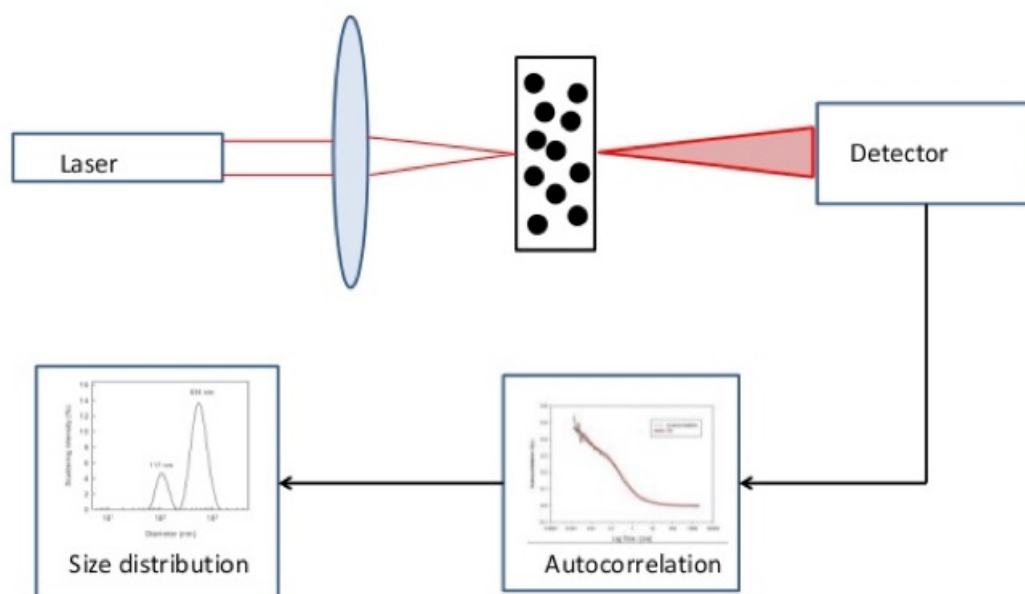


Fig. 2.18. Scheme of the dynamic light-scattering technique for measuring size of NPs

The scattered intensity depends on the following parameters: mass or size of the scattering particles; solute-particle concentration, which should be as small as possible; refractive-index difference of solvent and solute particles.

Derived count rates measured by DLS are related to the particles amount, concentration in the suspension and their size. The lower it is the more diluted suspension is measured. Or the larger it is the larger particles are in the suspension. So, decrease in count rates reveals that the general amount of the measured particles in the suspension is decreased in comparison with a start point.

Also, the analysis of the DLS results obtained for polydisperse colloids reveals that bigger NPs could screen completely the presence of smaller ones.

DLS and AFM are complementary techniques and important tools to characterize nanoparticles. However, when there are DLS and AFM data of polydisperse samples, large particles scatter a significant amount of light so the diameter given by DLS intensity distributions will be higher than that one obtained by AFM even if there is only a small amount of big particles (Zanetti-Ramos et al. 2009).

2.2.1.3. Experimental design and data analysis

In this work, a Malvern Zetasizer Nano ZS from Malver Instruments was used. Each recorded value resulted from the average of three separated measurements.

The size measurements of pure TiO₂, PAA-TiO₂ and EPS were taken separately or in their mixture in 10⁻³ M NaCl. These studies were performed under conditions favorable or unfavorable for the interaction. Favorable conditions were fixed at pH 4.3, giving positively charged TiO₂ NPs and negatively charged polysaccharide. Unfavorable conditions were studied at pH 8 for PAA-TiO₂ NPs as they are negatively charged at pH > 3. Different NPs/EPS ratios were tested. Final TiO₂ concentration in the mixture was 10 mg/l. The EPS concentration in the mixture was varied at 1, 10 or 100 mg/l. After the solution preparation it was placed in the DLS measurement cell for the initial measurements and 1 h later, after agitation and centrifugation steps.

2.2.1.4. Zeta potential measurements

Zeta potential of the analyzed NPs was also measured with Malvern Zetasizer Nano ZS from Malver Instruments using phase analysis light scattering. The z dependence with pH was measured for pure TiO₂ and PAA-TiO₂ NPs in order to determine their isoelectric points. The sample was introduced into a disposable folded capillary cell for these measurements.

2.2.2. Deposition measurements of NPs by Atomic Force Microscopy

2.2.2.1 Approaches

We studied the NPs deposition on two different collector substrates under favourable and unfavourable conditions. These contrasted conditions were obtained by controlling the pH and salt concentration of the NPs dispersing medium.

For pure TiO₂ NPs with an IEP of 4.9, favourable conditions for deposition were obtained by adjusting the pH to 3. Although this condition is not environmentally relevant, our aim here was to obtain contrasting conditions that enable us to better

assess the deposition mechanism. At this pH, TiO₂ NPs are positively charged and stable in suspension, which favours a high affinity for the negatively charged substrate due to electrostatic attractions. However, the TiO₂ NPs repel each other when they are highly charged (Labille et al., 2015), which may limit the deposition density on the substrate. This effect was studied by varying the ionic strength of the solution and altering the type of electrolyte used; either NaCl or CaCl₂ electrolyte was used.

The PAA-coated TiO₂ has an IEP of 2.7. At pH 3, the PAA coating, as well as the EPS and silica substrate surfaces, is weakly charged. This may favour weak hydrophobic interactions. On the other hand, at the natural pH = 5.6, unfavourable conditions for deposition are expected due to the negative charges, which result in electrostatic repulsions between NPs and the substrate.

2.2.2.2. Principle of the method

Atomic force microscopy (AFM) is most often used to obtain topographical information about a surface. AFM is based on the van der Waals forces between the NPs and the AFM tip. A substrate is placed on the sample stand and a reflective cantilever containing a tip is brought near the surface (Giessibl 2003) (Oliveira et al. 2012).

An AFM typically utilizes a cantilever with a sharp probe to scan a sample surface. The cantilever beam is built-in at one end of a piezoelectric displacement actuator controlled by AFM. At the other end of the cantilever is a probe tip that interacts with the surface. At close proximity to the surface, the probe experiences a force (attractive or repulsive) due to surface interactions, which imposes a bending moment on the cantilever. In response to this moment the cantilever deflects and this deflection is measured using a laser beam that is reflected from a mirrored surface on the back side of the cantilever onto a split photodiode (Grobelyny et al. 2011). A schematic diagram of the system is shown in Fig. 2.19.

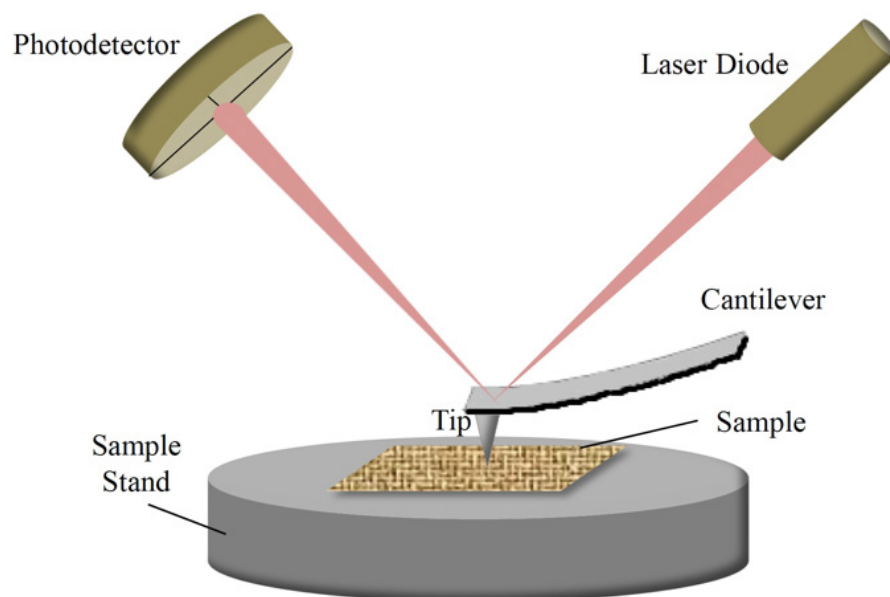


Fig. 2.19. Schematic diagram of the main components of an AFM

AFM is a non-destructive technique capable of operation in a variety of environments, including insulated, fluid, and high vacuum conditions. Additionally, the only requirements for the substrate are a clean, flat, near defect-free surface. Commonly used substrates are gold, silicon, mica, graphite, and glass (Colton 1998). The dimensions of the AFM tip affect the resolution of the AFM image. Sharper tips with a small radius of curvature yield higher resolution images because the tip dimensions are typically smaller than the features on the surface. The large radius of curvature will limit the resolution of height images (Francius et al. 2008) (Birdi 2003). Typical resolutions for lateral (x, y) and height (z) measurements are $\sim 5\text{-}10\text{ nm}$ and better than 0.1 nm , respectively.

Depending on the type and size of surface being imaged and based on the nature of the probe-surface interaction (attractive or repulsive), several different AFM operating modes are possible: contact mode, intermittent contact mode, or non-contact mode (Colton 1998) (Bonnell 2001) (Raposo et al. 2007).

In contact mode the interaction between the tip and surface is repulsive and the tip literally contacts the surface. In the opposite case, non-contact mode, the tip interacts with the surface via long-range surface force interactions. In both cases, changes in the force of interaction between tip and surface result in changes in the deflected laser light. However, as noncontact mode primarily depends upon relatively weak van der

Waals forces of interaction between the tip and surface, surfaces imaged in noncontact mode typically have a low resolution. Further, contact mode often leads to damaged tips and surfaces.

Intermittent contact mode or tapping mode avoids some of the drawbacks of contact and noncontact modes. In tapping mode, the cantilever is oscillated close to its resonance frequency (50-500 kHz) perpendicular to the sample surface, generating an amplitude of 20-100 nm and causing the tip to alternately touch and lift off the surface, at separations closer to the sample than in non-contact mode. As the oscillating probe is brought into proximity with the surface, the probe-surface interactions vary from long-range attraction to weak repulsion and. As a consequence, the amplitude and phase of the cantilever oscillation varies. During a typical imposed 100 nm amplitude oscillation, for a short duration of time, the tip extends into the repulsive region close to the surface and thereby reducing the amplitude. This mode provides the high resolution of contact mode while avoiding the excessive tracking force that often damages both the tip and sample Intermittent contact mode has the advantage of being able to image soft surfaces or particles weakly adhered to the surface and is preferred for nanoparticles characterization measurements (Grobelny et al. 2011) (Hoo et al. 2008) (Colton 1998) (Bonnell 2001). It is shown schematically in Fig. 2.20. where with height profile data is possible to obtain NPs size on the surface.

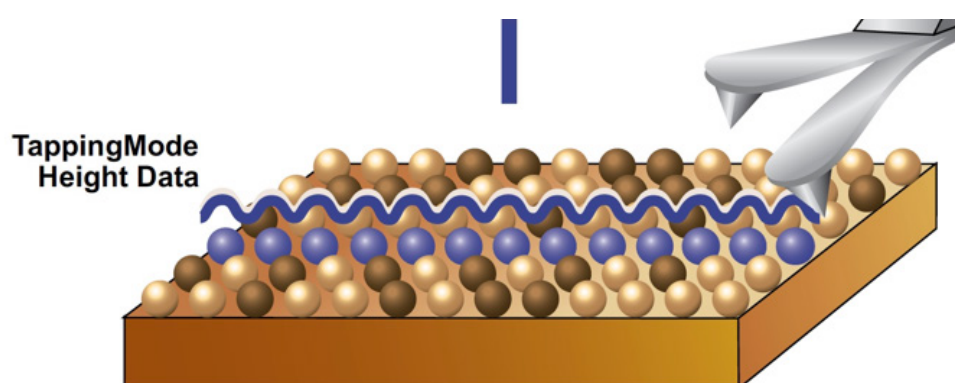


Fig. 2.20. Scheme of the AFM tapping mode

Because AFM can yield high resolution images in a number of different environments and using a variety of underlying substrates, AFM measurement has been applied to a wide range of synthetic and biopolymer films (Jagtap & Ambre 2005) (Almeida et al.

2004) (Lobo et al. 1999) (Francius et al. 2008) (Abe et al. 2012) (Francius et al. 2011) and also of nanoparticles and their interactions (Rao et al. 2007) (Grobelyny et al. 2011) (Pleticapic et al. 2012).

2.2.2.3. Experimental design of AFM measurements and data analysis

The surface morphology of the NPs deposits on the flat substrate was studied by AFM (Innova AFM, Bruker). Silicon wafers (MicroMasch) were used as the flat support. They were first activated with Piranha solution $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ (7:3, v/v), rinsed thoroughly with Milli-Q water, and dried with a stream of nitrogen. The piranha treatment is aimed at activating hydroxyl groups (-OH) on the silicon surface by a hydroxylation process, which results in a highly hydrophilic surface. The wafer was either used as grown to obtain the so-called mineral substrate for AFM, or it was coated with EPS. The EPS substrate was prepared by bilayer formation with PAH adsorption. First, the silicon wafer was immersed in PAH solution (at 4 g/l) and then in the EPS solution (at 1 g/l). Each immersion step lasted for 5 min and was followed by rinsing in pure water (in two manual baths) and then by drying under a nitrogen stream.

The NPs deposition on both EPS and silica substrates was studied by dipping the substrate in 20 ml of the working suspension of TiO_2 NPs for 30 sec. Different electrolytes were tested.

The images were collected in tapping mode under ambient air conditions using a monolithic silicon tip (MicroMasch, Bruker; radius = 8 nm, height = 15 μm , angle = 40° , force constant = 40 Nm^{-1} , frequency = 325 kHz). The reported root mean square (RMS) roughnesses were determined from 2 μm x 2 μm scan areas. Image processing was performed with the Nanoscope, ImageJ and Gwyddion software applications. The analysed areas were 3 μm x 3 μm . To investigate the thickness of the NPs deposit on the substrate, the surface was manually scratched with a knife and the height profile of the scratched area was analysed in at least three different places.

2.2.3. Deposition measurements of NPs by Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D)

2.2.3.1. Approach

Deposition of NPs on the substrate under favourable and unfavourable conditions was measured by QCM-D. These conditions were obtained by the same approach as for the AFM measurements.

2.2.3.2. Principle of the method

A quartz crystal microbalance (QCM) crystal is composed of a thin, circular disk of quartz plated with metal electrodes typically composed of gold or platinum as it can be seen in Fig. 2.21.

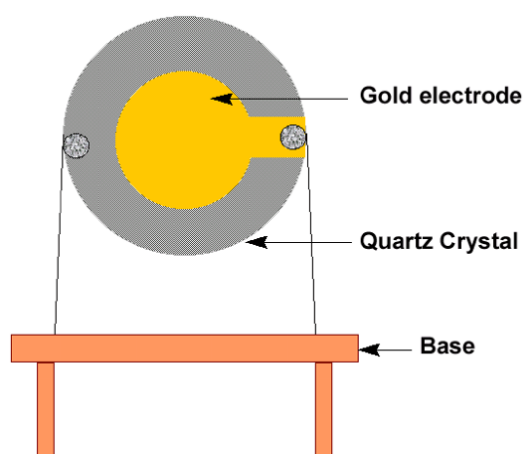


Fig. 2.21. Scheme of a typical piezoelectric crystal

The QCM crystal is typically mounted within a temperature controlled flow cell. The piezoelectricity, or the mechanical stress caused by application of electrical current, of quartz is the basis of the QCM. Application of an alternating potential across the electrodes generates a vibrational wave with an amplitude parallel to the crystal surface (Fig. 2.22A). A transverse, standing acoustic wave is created when the wavelength caused by the oscillating crystal is two times the crystal thickness. Under

conditions in which a standing wave is generated, the crystal is oscillating at its resonant frequency. An applied stress, such as a change in the mass on the crystal or the medium contacting the crystal surface, leads to a change in the resonant frequency of the oscillating crystal. Consequently, the QCM is a sensitive mass balance capable of detecting changes at sub-nanogram limits (O'Sullivan & Guilbault 1999).

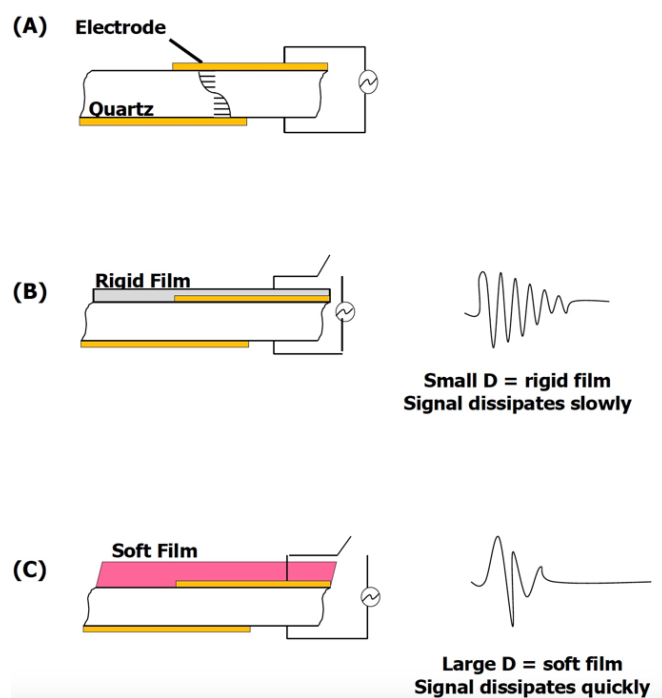


Fig. 2.22. Schematic depiction of a quartz crystal coated with gold electrodes (A) oscillating in the gas phase without adsorption and the resonant, transverse acoustic wave is shown travelling through the thickness of the quartz crystal, (B) coated with a thin, rigid film where the dissipation of stored energy is slow, and (C) coated with a floppy, viscous film where the frictional forces of the viscous medium lead to fast dissipation of stored energy (from Kittle 2012)

When a thin film with a given mass is deposited onto the crystal surface, the wave travels into the film (Fig. 2.22B). The thin film can be treated as an incremental increase in the thickness of the quartz by making three assumptions: the density and shear modulus of the thin film is the same as that of quartz; the film is rigidly attached to the surface; the frequency shift does not depend on radial distance. Thus, this incremental increase in thickness translates into an incremental increase in the distance the resonant wave must traverse, which leads to a decreased resonant

frequency. With these assumptions, the Sauerbrey equation can be derived (Sauerbrey 1959).

Additionally, a QCM equipped with dissipation monitoring (QCM-D) can be used to characterize the adsorption of a soft, viscous layer (e.g. polymer or gel) (Fig. 2.22C). A QCM-D essentially cycles voltage on and off while measuring the decay of the resonating crystal, thereby providing information about the rigidity of the film (Höök et al. 1998). The change in dissipation is,

$$\Delta D = E_{dissipated} / 2\pi E_{stored} \quad (\text{eq. 2.1})$$

where E_{stored} is the energy stored in the sensor crystal and $E_{dissipated}$ is the energy dissipated by the viscous nature of the surrounding medium. Increases in the energy dissipated per oscillation provide qualitative information such as the validity of the Sauerbrey equation, swelling of the film, or structural changes at the surface. An increase in ΔD indicates the addition of a more viscous medium (e.g. swelling), whereas a decrease indicates a change to a more rigid environment (e.g. from networking or release of solvent) (Höök et al. 1998). An absolute shift of $\Delta D \times 10^6$ greater than 5 % of the absolute scaled frequency shift ($\Delta f/n$) signifies that the Sauerbrey equation may be invalid. Additionally, spreading of the overtones during adsorption typically indicates the adsorption of a soft viscous layer.

A typical plot of a material adsorbing from solution is shown in Fig. 2.23.

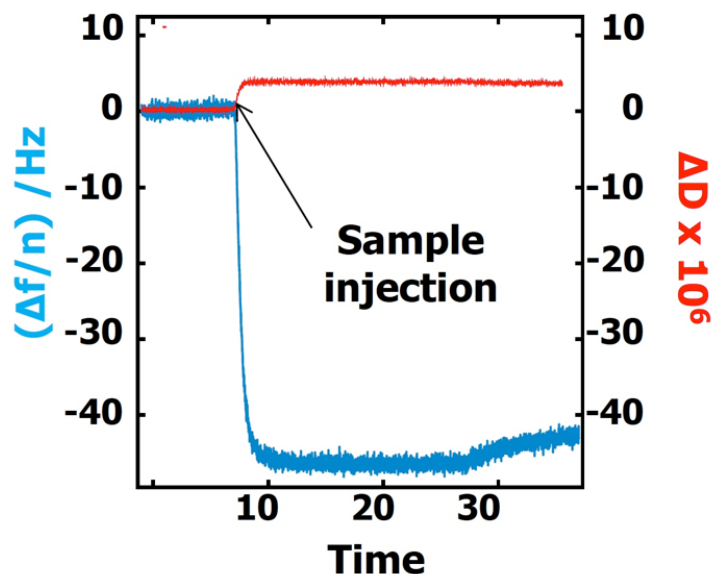


Fig. 2.23. Representative data from a QCM-D measurement

Initially, the system (e.g. a bare gold surface or thin film on the gold sensor) is equilibrated with the solvent. At $t \sim 10$ min, a solution containing an adsorbate is introduced into the flow cell and material adsorbs onto the surface, causing a decrease in the measured resonant frequency. At $t \sim 30$ min, the solvent is again flushed through the flow cell and some reversibly bound adsorbate is washed away (frequency increases slightly). Dissipation increases upon adsorption, but the Sauerbrey equation is likely still valid in this case because the magnitude of $\Delta D \times 10^6$ is less than 5 % of the magnitude of Δf .

2.2.3.3. Experimental design of QCM-D measurements and data analysis

QCM-D is a powerful technique to study the deposition kinetics of NPs on various surfaces (Chen & Elimelech 2008), (Quevedo & Tufenkji 2009), (Fatisson et al. 2010). The QCM-D measurements were carried out using an E4 instrument from Q-Sense (AB, Sweden) (shown in Fig. 2.24) that was simultaneously able to measure changes in the quartz crystal resonance frequency, Δf , and in energy dissipation, ΔD , at seven different frequencies, which are the fundamental frequency ($f_0 \sim 5$ MHz) and at odd overtones up to the 13th overtone.



Fig. 2.24. Scheme of E4 QCM-D system adjusted for flow mode

The resonance frequency of an oscillating quartz crystal is related to the mass of the crystal and thus any mass added to or removed from the electrode induces a frequency shift, Δf , which is related to the change in the sensed mass, Δm . The Sauerbrey equation (eq. 2.2) gives a linear relationship between the measured changes in the frequency and sensed mass for thin, rigid, and evenly distributed surface films:

$$\Delta m = -C \Delta f / n \quad (\text{eq. 2.2})$$

where n ($n=1,3,5,\dots,13$) is the overtone number and C is the mass-sensitivity constant that depends on the quartz crystal (Sauerbrey 1959). In this work, $C = 0.177 \text{ mg m}^{-2} \text{ Hz}^{-1}$ at $f_0=5 \text{ MHz}$. The mass calculated from the Sauerbrey equation using the third overtone ($n=3$) is referred to as the Sauerbrey sensed mass, Δm (Fig. 2.25).

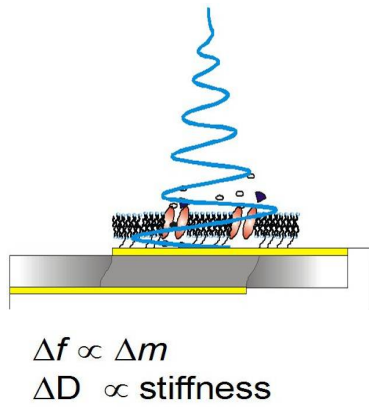


Fig. 2.25. Scheme of the QCM-D principles

The deposition rate and affinity of NPs on the crystal were obtained according to the theory of deposition for a parallel-plate geometry (Adamczyk & Van De Ven 1981) that is a valid approximation for the E4 QCM flow chamber. To compare experimental values to the theoretical particle deposition rate, the latter was evaluated using the Smoluchowski-Levich approximation (Quevedo & Tufenkji 2009):

$$r_d^{SL} = 0.538 \frac{D_{\infty} C_b}{a_p} \left(\frac{Pe * h}{x} \right)^{\frac{1}{3}} \quad (\text{eq. 2.3})$$

where r_d^{SL} is the theoretical particle deposition rate that is only limited by the NPs transport, D is the diffusion coefficient, C_b is the NPs bulk concentration (0.1 g/l), a_p is the NPs radius (6 nm) based on number-weighted DLS measurements, Pe is the dimensional Peclet number for the particles, h is the height of the QCM flow chamber and x is the distance along the flow from the inlet.

Experiments with the silica substrate were made using a piezoelectric AT-cut quartz crystal covered by a 50-nm-thick silica layer (QSX303, Q-Sense AB, Sweden) that is hereafter referred to as the silica QCMD substrate. To activate the surface, the crystals were cleaned in piranha solution H_2SO_4/H_2O_2 (7:3, v/v), rinsed thoroughly with Milli-Q water, and dried with a stream of nitrogen. Then, the crystals were subjected to a plasma etching device (Harrick Plasma).

The EPS substrate was prepared as follows. Piezoelectric AT-cut quartz crystal coated on each side with gold electrodes (QSX301) were used.

PAH (polyallylamine hydrochloride) ($M_w = 120 - 200,000$ g/mol, Polysciences) was used as a cationic anchor to support the EPS polyanion on the substrate. Aqueous PAH was prepared in ultrapure water at 1 g/l for QCM-D and 4 g/l for AFM. A drop of PAH solution (1 g/l for QCM-D) was deposited onto the crystal for 5 min, thoroughly rinsed with pure water and dried with nitrogen stream. Then, a drop of EPS solution (1 g/l) was deposited onto the cationic anchor with the same rinsing and drying approach. After that the crystal, hereafter called the EPS substrate, was set in the E4 QCM-D system.

All measurements were carried out at 20 °C using the QCM flow cell modules. A baseline was first established by continuously flowing an electrolyte solution on the quartz crystal surface at a flow rate of 0.1 ml min⁻¹. At time t_0 , the continuous flow of the NPs suspension was started until a frequency plateau was reached and signal dissipation was observed. To control the reversibility of the NPs deposition, a rinsing step was then performed by flowing a solution of the same electrolyte without NPs. When a plateau was reached again, a second NPs injection was performed to verify if surface saturation was achieved after the first NPs injection. QCM-D experiments were conducted in replicate.

Some control experiments were conducted under the same experimental conditions to verify that TiO₂ NPs deposition did not occur on the bare gold crystal or the gold crystal coated with only PAH.

To quantify and compare the results obtained by AFM and QCM-D, a final surface coverage ratio, θ , was calculated for both types of experiments. It shows the percentage surface coverage of the NPs with respect to the total surface of the substrate. With AFM, θ_{AFM} was calculated by image analysis using ImageJ software, by counting the surface area corresponding to a topography above a 2.5 nm threshold. The total number of nanoparticles counted was approximately 150 000. With QCM-D, the NPs mass deposited by surface area was converted into surface coverage θ_{QCM-D} by considering spherical NPs of 12 nm in diameter and 3.78 g/cm³ in density.

2.2.4. NPs transfer through the polysaccharide gel using filtration unit and UV-spectroscopy

2.2.4.1. Approach

In order to study the NPs transfer through the gel, the suspension was passed through the gel layer utilizing a filtration unit. Time resolved measurements of the NPs concentration in the filtrate were realized using UV-spectroscopy.

Au NPs were used because their functionalization favors the dispersion stability in a large pH range.

2.2.4.2. Ultraviolet-visible (UV-Vis) spectroscopy

Ultraviolet-visible spectroscopy or spectrophotometry (UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The absorption or reflectance directly affects the perceived color of the chemicals involved. The molecules undergo electronic transitions (Faust 1997). When a sample is exposed to light energy that matches the energy difference between a possible electronic transition within the molecule, a fraction of the light energy would be absorbed by the molecule and the electrons would be promoted to the high energy state orbital. A spectrometer records the degree of absorption by a sample at different wavelength and the resulting plot of absorbance (A) versus wavelength (l) is known as a spectrum. A scheme of the UV-Vis spectrometer is shown in Fig. 2.26.

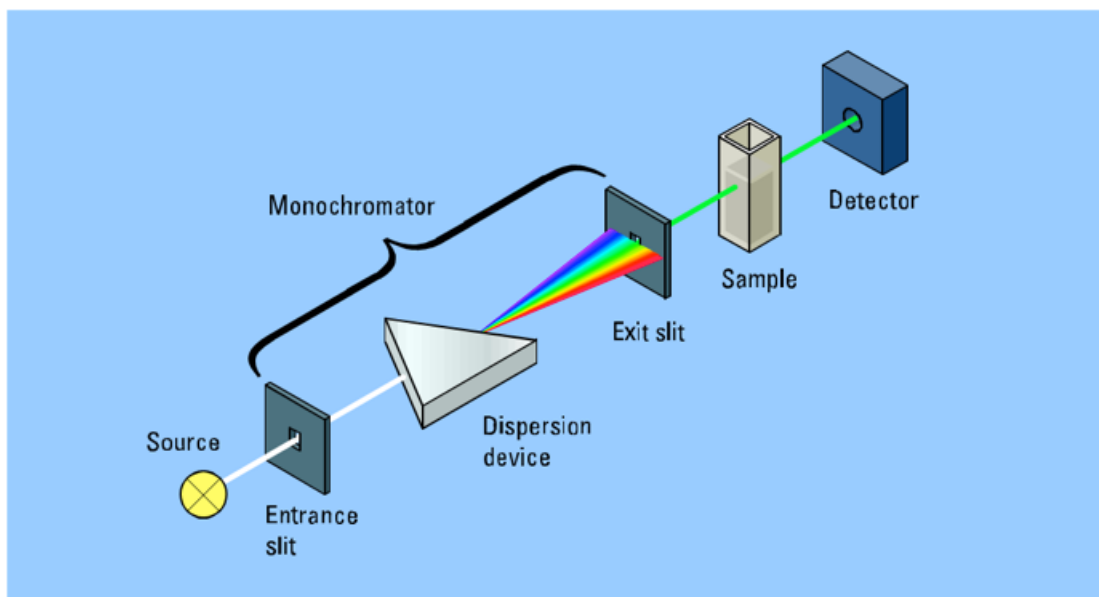


Fig. 2.26. Schematic representation of the UV-Vis spectrometer

2.2.4.3 Experimental design of the percolation experiments and data analysis

Transport of Au NPs through the polysaccharide gel was studied by passing NPs suspension through a gel layer using a filtration unit under vacuum or connected to a syringe pump working at a constant flow rate. Cellulose nitrate ($0.45\ \mu\text{m}$) membrane was chosen as a support for the polysaccharide layer. This type of membrane was selected among some others tested according to the lowest retention of Au NPs by the membrane only under our working conditions. Cellulose nitrate displayed the lowest affinity to Au NPs according to UV measurements data.

The gel layer on the membrane was prepared as follows. 1.5 ml of 15 g/l (or 10 g/l) hot solution was poured on the membrane in the filtration unit, and passed through at 1 ml/min rate. The polysaccharide macromolecules remained in the retentate forming the gel. Then it was let cooling down and gelifying within 5 min. Afterwards, 2.5 ml of NPs suspension (1 mg/l) were poured and passed through the gel in the filtration unit under vacuum at 1 ml/h rate, or 5 ml of NPs suspension (1 or 5 mg/l) were introduced to the syringe system and passed through the gel at 1 ml/h rate.

NPs retention by the gel layer was calculated by measuring the maximum absorbance of NPs suspension in the filtrate. UV-Vis spectroscopy was used for this purpose. Maximum absorbance wavelength for Au NPs as a function of the coating and size is shown in Figs. 2.6 – 2.17. A series of batch experiments employing gold NPs were

performed to examine the retention of NPs on the gel surface. UV spectroscopy measurements were performed during all the filtration experiment in order to quantify the amount of Au NPs passing through the gel layer along with time and volume.

The influence of different parameters on the NPs breakthrough was studied. The polysaccharide concentration used to form the gel layer was 10 or 15 g/l. The NPs dispersing medium was salt-free or 10^{-3} M NaCl solution. The flow rate for NPs injection was 0.5 or 1 ml/h. Different Au NPs were also tested and compared, having different size or coatings (5 or 12 nm, bPEI or citrate coating).

2.2.5. NPs transfer study by Diffusion cell set

2.2.5.1. Approach

Free diffusion of NPs through a gel layer without pressure was studied using a diffusion cell. The set up enabled to measure the NPs concentration passing from a donor solution to an acceptor solution through a gel layer by simple random diffusion.

2.2.5.2. Diffusion cells set

Diffusion cell set consists of 2 compartments: donor and acceptor compartments with 8 ml volume each (Fig. 2.27). An O-ring PTFE spacer (thickness 1.5 mm) was intercalated between the compartments in sandwich between 2 membranes. A gel volume was placed in the cavity of the O-ring, kept flat on each side thank to the membranes support. NPs were introduced at time 0 into the donor compartment.

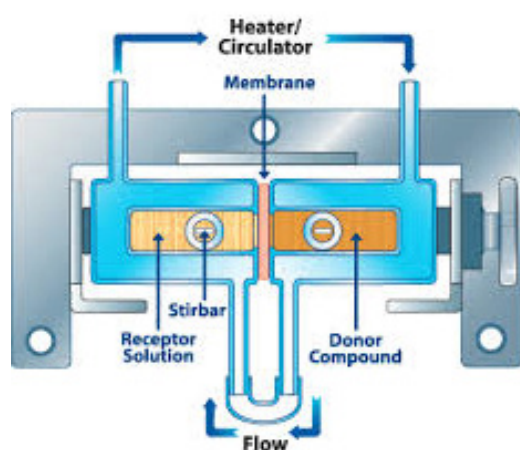




Fig. 2.27. Scheme of the diffusion cells set

2.2.5.3. Experimental design

The diffusion of TiO₂ NPs through the gel was studied utilizing the diffusion cell set. UV-Vis spectroscopy was used for quantification NPs concentration in the acceptor compartment as a function of time. In line flow cell for constant measurement as well as punctual measurement in regular quartz cuvette were tested.

In order to select the membrane supporting the gel, which had the lowest influence on diffusion process, different types were tested. The membranes affinity for the TiO₂ NPs was compared. TiO₂ suspension (10 mg/l) was filtrated through the different membranes and quantified in the filtrate by UV absorbance.

Membranes made of nylon with pore size 20 or 1.2 μm, nitrocellulose with pore size 1.2 μm, and polycarbonate with pore size 1.2 μm were tested. The lowest affinity for NPs was obtained with nylon membranes, which were then selected.

Diffusion conditions: TiO₂ NPs suspension (50 mg/l) was prepared in 10⁻³ M NaCl at pH 8. NPs size was 50 nm, zeta potential -24 mV. Temperature was maintained at 25 °C. Concentration of TiO₂ particles was measured by UV-Vis spectroscopy at wavelength 270 nm.

Diffusion conditions were chosen at pH 8 compatible with environmental conditions. The gel charge is negative as well as the TiO₂ NPs surface. Electrostatic repulsions are thus expected between the NPs and the gel fibers.

EPS gel was prepared from hot solution prepared at 10 g/l and stirred 1 h at 60 °C. It was pored in the spacer on a membrane, cooled down and covered with the second membrane.

Quantification of the polysaccharide in solution by Dubois method

Quantification of the EPS in solution after interaction with the NPs was tested using the Dubois method. This method determines the concentration of sugars and related substances in solution, via a colorimetric.

The free EPS to quantify in solution was first separated from the NPs by ultracentrifugation. A calibration curve for the EPS was properly measured.

2 ml of sample was put in a colorimetric tube. 1 ml of 80 % phenol and 5 ml of 95.5 % sulfuric acid were added, and the sample was left for 10 min. The mixture was shaken during 10 min and left before measurements in a water bath at 25-30 °C for 20 min. Then the measurements were performed in 20 min. Yellow-orange coloration of the mixture was quantified by UV absorbance at 490 nm. In this reaction, sulfuric acid breaks down any polysaccharides to monosaccharides, pentoses (5-carbon compounds) are dehydrated to furfural, and hexoses (6-carbon compounds) to hydroxymethyl furfural. They react with phenol to produce a yellow-gold color.

Chapter 3. TiO_2 release from sunscreen during aging in water

3.1. Introduction

Sunscreens are one of the most commercialized nanotechnology-based products (Som et al. 2010). They are complex formulations often based on water in oil emulsion, containing mixture of organic/inorganic and lipophilic/hydrophilic compounds (Lazar et al. 1997) (Sambandan & Ratner 2011). Formulation used in the sunscreen enhances TiO_2 aggregation that changes their penetration properties. Coating the NPs in sunscreens reduces the toxic effects especially when silica-base coatings are used but cannot completely prevent these effects (Smijs & Pavel 2011).

Titanium dioxide TiO_2 is the most used inorganic filter due to its effective UV reflection and absorption capacities (Smijs & Pavel 2011) (Newman et al. 2009).

The TiO_2 core in the sunscreens is usually coated with a layer of the aluminium oxide, which is used to inhibit photosensitivity and cancel any photocatalytic effect during UV excitation. The $[\text{Al}(\text{OH})_3]$ also enhances grafting of an organic and amphiphilic outer coating, which favors dispersion of the particles part in the organic cream. A special inert coating material (dimethicone) is applied to the surface of minerals particles to improve dispersion in sunscreen products and resist the formation of large particle agglomerations. Nano-sized TiO_2 is mainly present in the sunscreens in comparison with micro-sized. In the filter mainly nano-sized TiO_2 particles from 10 to 100 nm are used for transparency and efficacy (Serpone et al. 2007) (Nohynek et al. 2007).

When sunscreens are washed from the skin they penetrate into the aquatic environment that may induce their aging and creation of new residues with new surface properties for which the fate and impact are unknown. The sunscreen residues containing TiO_2 particles during and after their release to the aquatic systems may alternate and create a new hazard and impact on the aquatic organisms (Santaella et al. 2014). The residues may include particles and organic substances with the properties and colloidal behavior different from the materials or bare NPs that were initially in the cream depending on the aging processes that happen during the materials release. The size, surface properties and environmental impacts of the residues depend on the type of physical and chemical reactions that nanomaterial is exposed to during aging. The aging steps may forward the residues from the first step with large size to the release of products with nano-size. They certainly have different

properties and environmental behavior causing a different impact on the environment. It is very important to know the characteristics of the residues at different steps of their environmental aging.

Nano-TiO₂ is one of the most manufactured nanoscale materials, with an annual probability distribution of the production in the EU of 10 000 t per year, with major use in cosmetics (Sun et al. 2014). Based on flows, from production and use through release, the predicted environmental concentration of nano-TiO₂ is in the 0.4 - 1.4 mg/l range in surface water (Sun et al. 2014). In South Australia, a modelled mass flow of 0.34 t/year reaches seawater due to sunscreen use on beach in South Australia (Sun et al. 2015). In this Chapter we studied the release of TiO₂ from two sunscreens formulated initially either with nano- or with micro-sized TiO₂ particles, and how the TiO₂ particle size influences their fate under contrasted aqueous conditions. A base formulation free of TiO₂ particles was also studied as a reference. The creams were submitted to an artificial aging simulating environmental conditions (light, dark, water, stirring). The residues released from the creams were then characterized in term of size distribution and Ti concentration. The release and dispersion stability was also tested in water under various salinities (pure and seawater).

3.2. Materials

The sunscreen formulations used in this work were designed by Ethical Skin Care Company (Neuchatel, Switzerland) for the purpose of this project. The three formulations studied were based on the same composition except regarding the incorporation of TiO₂ nano or micro. A dispersion of fine TiO₂ in cyclopentaciloxane is designed for use in sunscreens. According to the manufacturer, the sunscreen formulation consists of a TiO₂ core of nano- or microsize.

3.2.1. *Micron-sized TiO₂ particles*

The micron-sized TiO₂ particles used are commercialized by Merck Company under the trade name UV-TITAN M161. There is no exact concentration of TiO₂ but based on the literature 75 % of coated filter is TiO₂, [Al(OH)₃] and stearic is mineral fraction of the filter of 10 % and 15 % respectively. 12.6 % of micro-sized filter is in the base formulation.

3.2.2. Nanosized TiO₂ particles

The nanosized TiO₂ is commercialized as SPD-T5 by ShinEtsu company. Fine Titanium Dioxide coated is in the cream in 40 wt%, cyclopentasiloxane 50 wt% and silicone dispersant 10 wt%. The TiO₂ core is coated with a layer of aluminium oxide [Al(OH)₃] which inhibits a photoactivation of the TiO₂. A polyglyceryl-3 polydimethylsiloxiethyl dimethicone (PDMS) also called methicone/dimethicone copolymer as a trade name, cyclopentasiloxane and stearic acid are used here. The copolymer consists of [Si-O(CH₃)₂]_n repeat units. 40 % of TiO₂ with the mineral fraction [Al(OH)₃] and stearic acid is in SPD-T5 filter and 40 % of SPD-T5 is in the base formulation.

3.2.3. Base formulation

These two TiO₂ filters were dispersed in the base formulation which consists of water (solvent), Pentylene Glycol (Humectant, Preservative), Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer (Thickening Agent and Emulsifier).

3.3. Methods

3.3.1. Titanium quantification in the samples

Titanium quantification in the initial creams, as well as in the aged sampled, was carried out by ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectroscopy) measurement. Cream samples were submitted to acid digestion in closed 30 ml poly-tetrafluoroethylene (PTFE) vials: 0.1 g of the cream was introduced into the vial with 1 ml of concentrated ultrapure 69 % HNO₃ (Sigma Aldrich) and 2 ml of 30 % H₂O₂ (Fisher scientific). The mixture was let 2 h on a heating plate at 120 °C. Excess acid was evaporated during 24 h at 120 °C. After cooling, 0.5 ml of ultrapure 40 % HF (VWR) was added in each vial and the mixture was closed and heated at 65 °C for 1 h. The excess of HF was immediately neutralized by 0.4 g of analytical grade boric acid (VWR). The final digest was diluted by 25 ml of 5% HNO₃ and analyzed using a Jobin-Yvon Horiba ICP-AES (Ultima C). The titanium

analytical line was 334.941 nm. External calibration was carried out with standards (SCP Science) prepared in 5 % HNO₃ with concentration range 1-200 mg/l.

3.3.2. Aging protocol of sunscreens

The aging of the TiO₂ was studied by introducing a sunscreen into a glass beaker containing pure or seawater and submitting it to prolonged agitation.

Seawater was obtained by preparing 35 g/l NaCl solution. An exact mass of 0.6 g of cream was introduced into 100 ml water. Aging was simulated by submitting this mixture to magnetic stirring at 690 rpm. Solutions were agitated during 30 min, 24 h or 48 h in order to follow the processes and dispersion of the sunscreen during the different steps of aging. The effect of sunlight was also studied by exposing the sunscreen under aging to a white lamp. Natural sunlight was simulated using a sodium discharged 400 W lamp, located 30 cm from the open reactor. To study the aging of TiO₂ samples in the absence of light, the reactor was covered and capped with aluminum foil. These samples were subjected to the same procedure under the same time and temperature conditions. During all aging experiments, the solution pH was not modified and measured before and after the procedure. No significant evolution was observed from the initial value of pH 6.

3.3.3. Physical-chemical characterization of the stable suspension

After agitation simulating aging, the mixtures were allowed to settle in the dark for 24 h in order to separate the colloidal phase from settleable aggregates. Then, the mixtures got organized in two phases: a stable colloidal suspension and a sticky precipitate settled down. These two phases were fractionated and the colloidal suspension (supernatant) was further characterized in term of size distribution.

Size measurements in the stable colloidal suspension were carried out by DLS (Malvern Nano ZS, Malvern Instrument, UK) and laser diffraction (Malvern Mastersizer 3000, Malvern Instrument, UK). The samples were introduced without prior dilution into the respective measuring cells. Each recorded value resulted from the average of three distinct measurements.

The TiO_2 nanoparticles involved in the stable colloidal suspension were quantified by Ti analysis with ICP-AES after acid digestion as previously described. Exactly 20 ml of supernatant, were submitted to acid digestion in closed 30 ml PTFE vials. External calibration was done with standards prepared in 5 % HNO_3 with concentration range 1-200 mg/l.

3.4. Results and discussion

After settling down a transparent solution was observed in the base formulation solutions. Sediments and turbid supernatant were observed with sunscreen containing nano- TiO_2 aged in pure water, and sediment and clear supernatant with nano- and micro- TiO_2 formulations in seawater (Fig. 3.1)

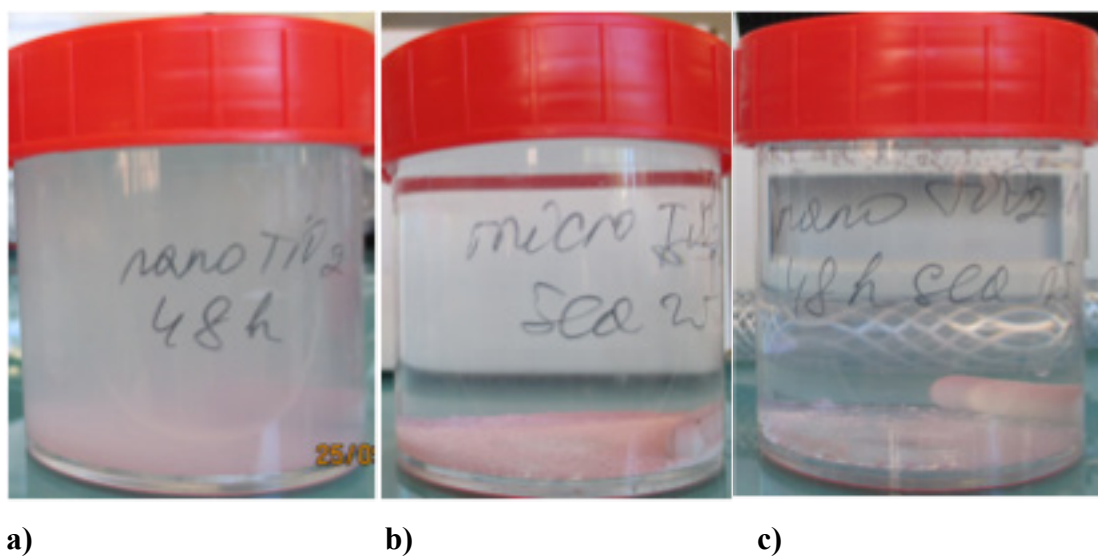


Fig. 3.1. a) Nano- TiO_2 in pure water, sediment and turbid supernatant; b) Micro- TiO_2 in seawater (SW), sediment and clear supernatant; c) Nano- TiO_2 in seawater, sediment and clear supernatant

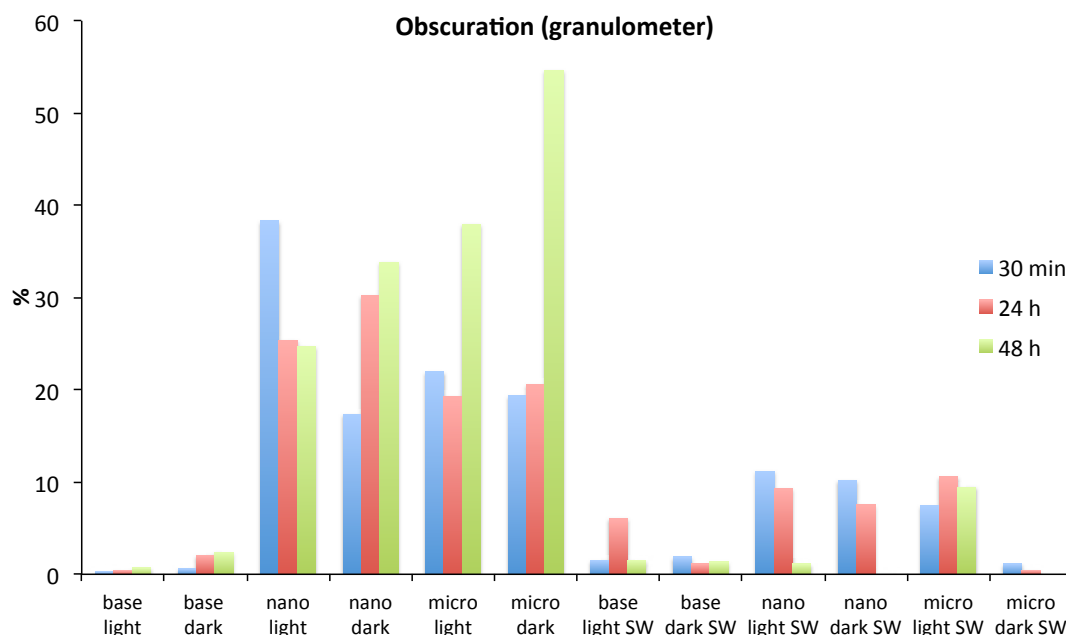


Fig. 3.2. Obscuration values (obtained by laser diffraction). Base formulation, Nano-TiO₂ and Micro-TiO₂ in pure and seawater. Agitation time 30 min, 24 h and 48 h.

The obscuration values obtained by laser diffraction are related to both the concentration and size of particles in suspension. They are higher in nano-TiO₂ supernatant aged in dark conditions than in nano-TiO₂ supernatant aged under light conditions (30 % vs 25 %). This suggests that the NPs are either more concentrated or larger in size in the supernatant aged in dark.

For micro-TiO₂ supernatant aged in pure water, no effect of light was observed on the obscuration values. This suggests that the release of micro-TiO₂ to the supernatant does not depend on the light condition in pure water.

But for micro-TiO₂ supernatant aged in seawater there is a difference in obscuration values between light and dark conditions, 10 vs 1 %. This indicates that the release of micro-TiO₂ in seawater is enhanced under light condition.

The obscuration values obtained for the base formulations are around 1.5 % both in pure and seawater under light and dark conditions. This agrees with the absence of TiO₂ particle in these samples. In Tables 3.1, 3.2, 3.3 are summarized the obscuration values measured for the different conditions studied.

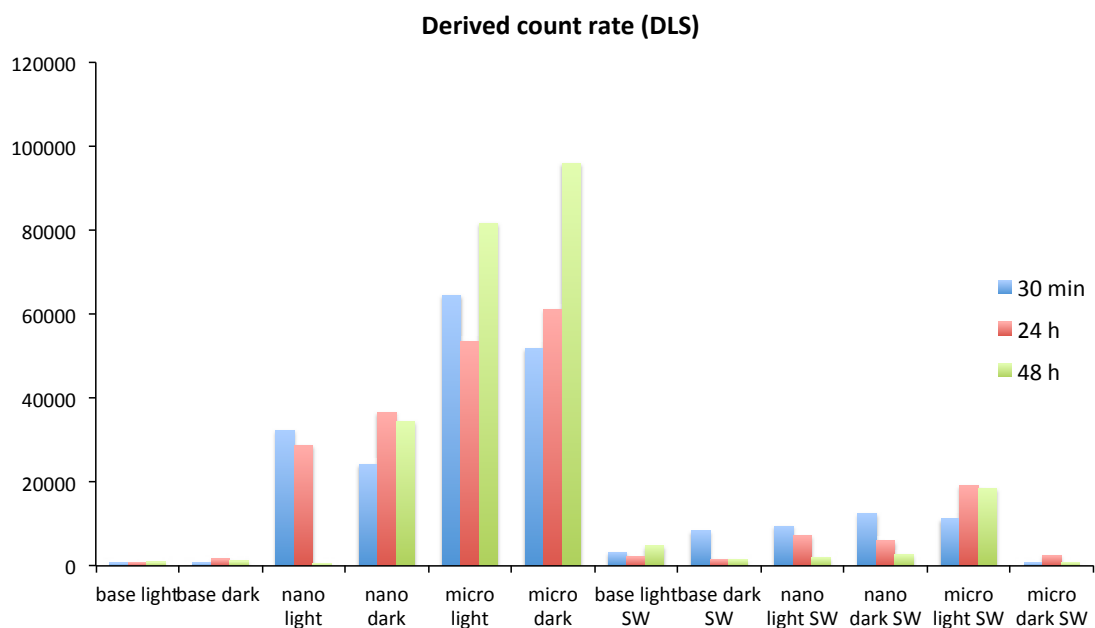


Fig. 3.3. Derived count rate (obtained by DLS). Base formulation, Nano-TiO₂ and Micro-TiO₂ in pure and seawater. Agitation time 30 min, 24 h and 48 h.

The derived count rates measured by DLS are related to the particle concentration and size in suspension. The results depicted in Fig. 3.3 are generally in good agreement with the obscuration values measured with the laser diffraction almost for all samples.

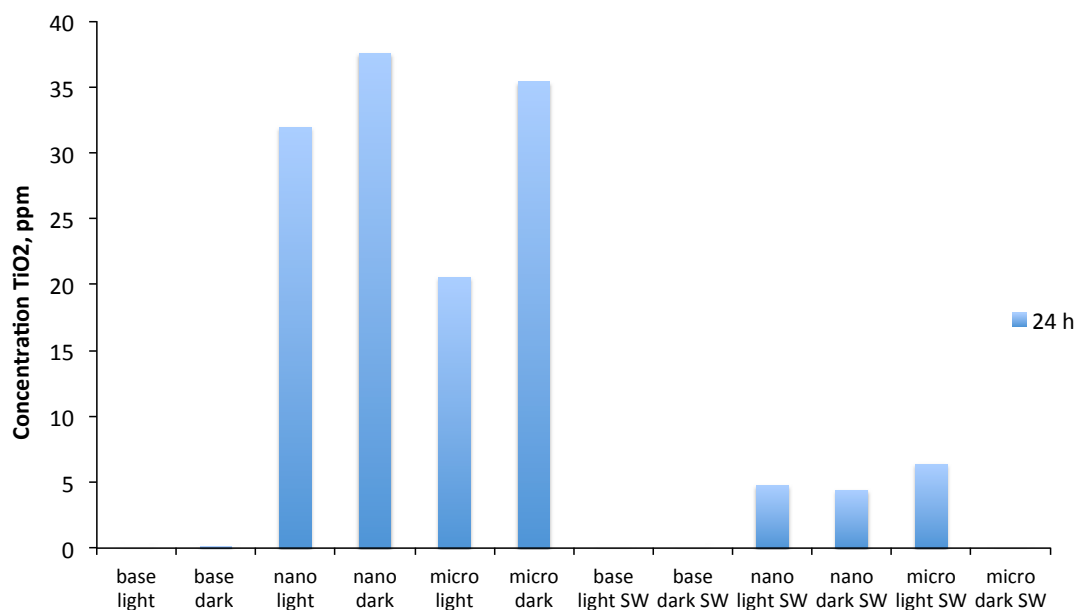


Fig. 3.4a. TiO₂ Concentration (obtained by ICP-AES). Base formulation, Nano-TiO₂ and Micro-TiO₂ in pure and seawater. Agitation time 24 h.

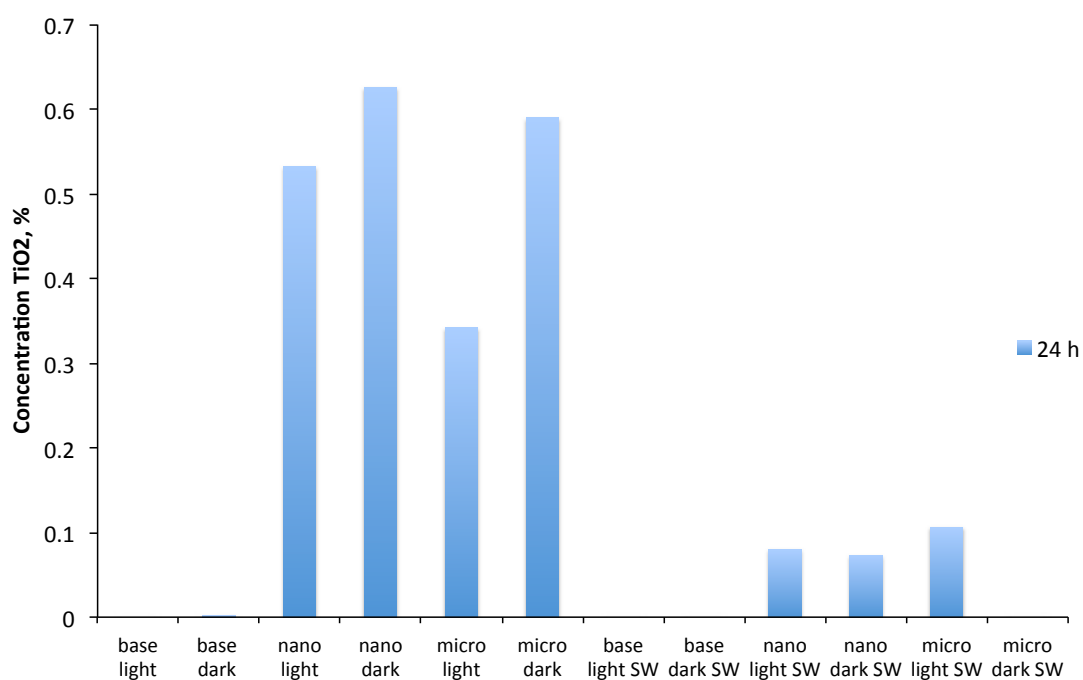


Fig. 3.4b. TiO₂ Concentration in % from the initial agitated amount (obtained by ICP-AES). Base formulation, Nano-TiO₂ and Micro-TiO₂ in pure and seawater. Agitation time 24 h.

ICP-AES analyses were used to quantify the amount of TiO_2 in the alteration solution after 24 h of aging. Nano- TiO_2 concentration under light and dark conditions in pure water was 32 and 37 mg/l respectively, micro- TiO_2 concentration – 20 and 35 mg/l. In seawater the concentration was much lower in comparison with pure water – around 5 mg/l for nano- TiO_2 both under light and dark condition and for micro- TiO_2 under light condition. For the base samples, as expected, no TiO_2 was detected in the suspension. These values are in good agreement with the obscuration and count rate values measured previously (Figs. 3.2 and 3.3).

Concentration of TiO_2 obtained in ppm was converted in wt% with regard to the amount initially agitated 0.6 g of the cream (Fig. 3.4b).

3.4.1. Time evolution along with aging

The time evolution of the nano- TiO_2 particles size distribution under aging in light or dark conditions is plotted in figures 3.5 and 3.6.

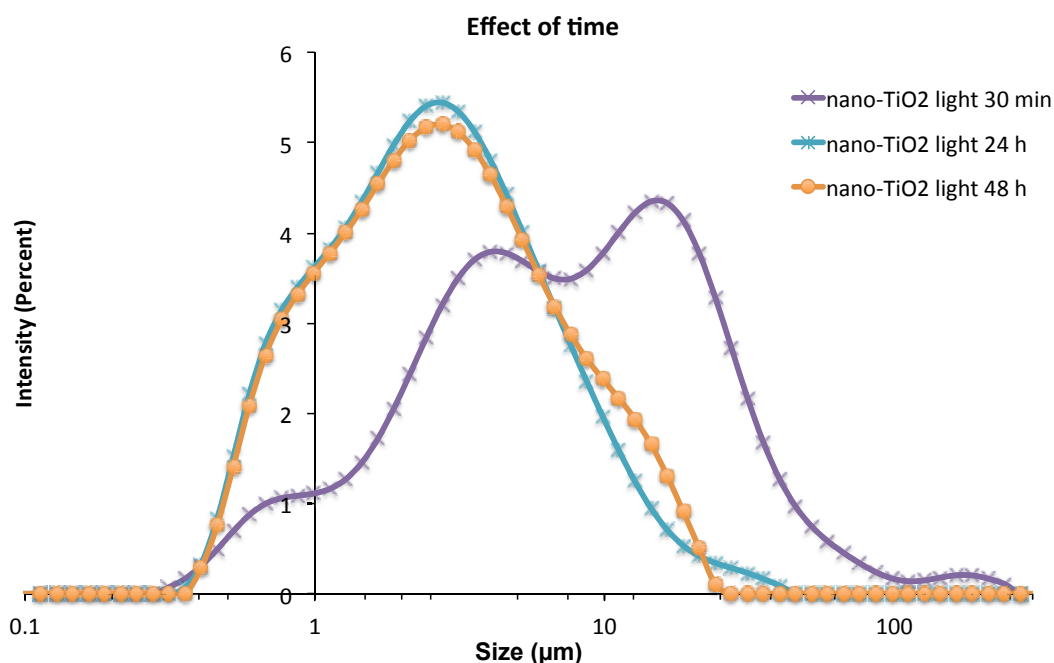


Fig. 3.5. Size distribution of Nano- TiO_2 under light conditions in pure water. Agitation time 30 min, 24 h, 48 h.

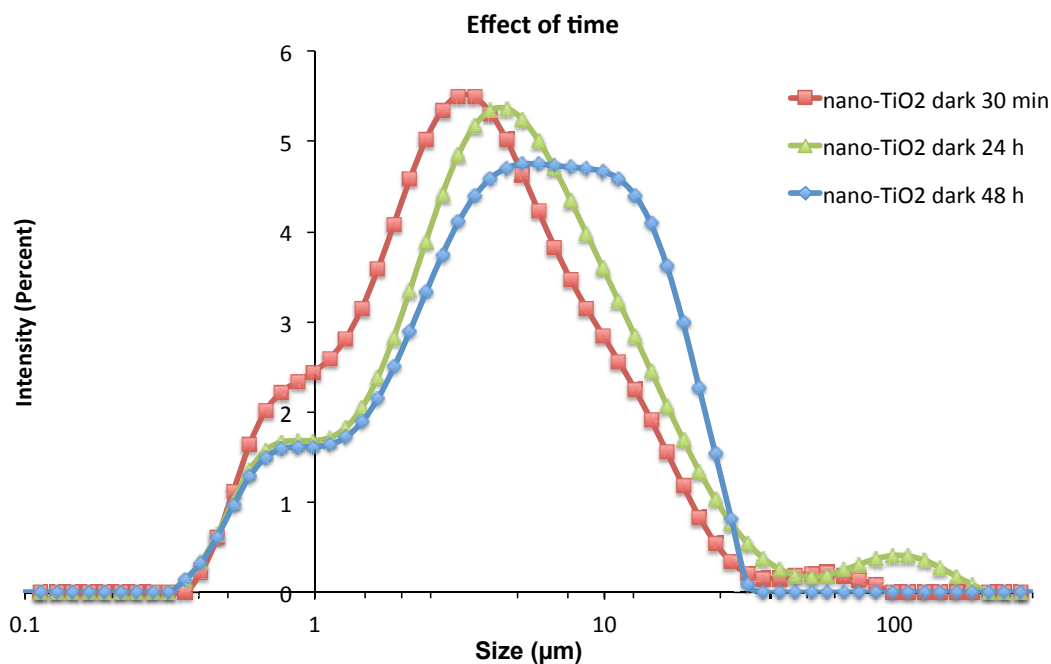


Fig. 3.6. Size distribution of Nano-TiO₂ under dark conditions in pure water. Agitation time 30 min, 24 h, 48 h.

Two size classes typically constitute the distribution, centered at 0.6 μm and 5 μm. However, it should be noted that the size distribution of the base formulation also contains 2 peaks (Fig. 3.7). So, these peaks with larger sizes may be partly due to the presence of oil droplets from the emulsion. For this reason, the size distribution should be analyzed in perspective with the particle concentrations indicated in figures 3.2-3.4.

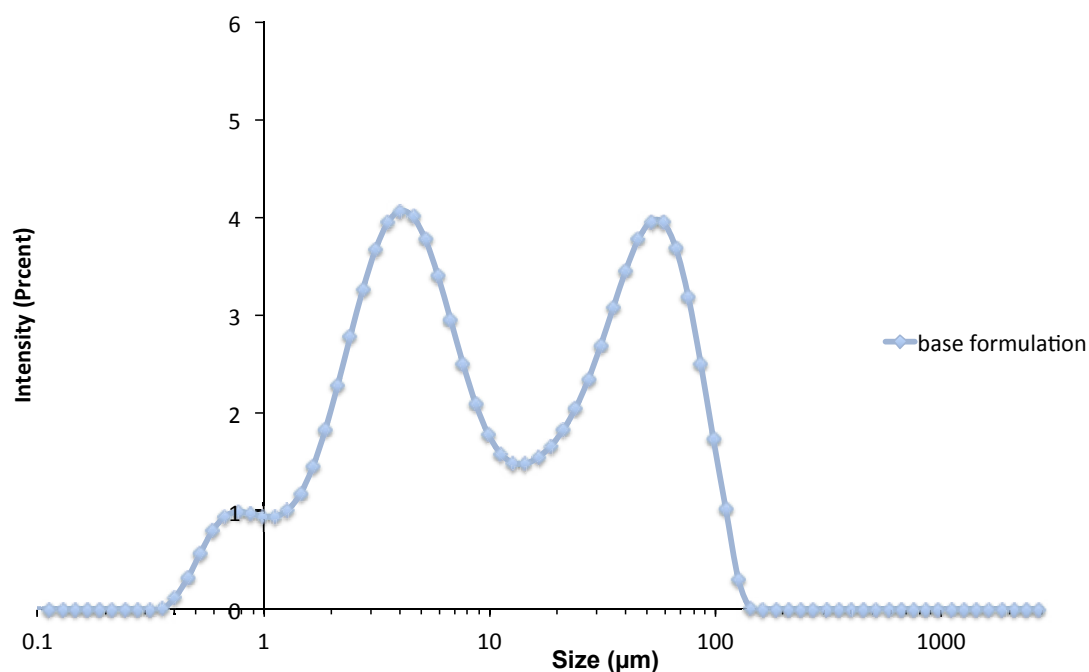


Fig. 3.7. Size distribution of the base formulation in pure water. Agitation time 24 h.

In the dark, both the nanoparticle size and concentration tend to increase with aging time. This suggests that NPs aggregate and remain stable in suspension. Indeed, when NPs are released in water, the dilution effect can decrease the NPs effective surface charge, which favors a size increase (Follens et al. 2008).

In contrast, the trend observed in the case of light condition suggests the rapid aggregation of the nanoparticles in the first 30 min, followed by sedimentation. The size distribution and particle concentration are indeed larger at 30 min of aging than at longer time. This could be due to a light-dependent alteration of the organic matrix embedding the TiO₂ particles or constituting the formulation. The photodegradation of these organic molecules such as dimethicone copolymer, cyclopentasiloxane and stearic acid involved in the large aggregates favors the TiO₂ particles dispersion in the aqueous medium.

Our hypothesis is that the nanoparticle coating undergoes alteration and desorption in the aqueous media. This enables the nanoparticle reorganization in the sunscreen residues toward a more hydrophilic character.

3.4.2. Effect of microsize particles vs nanoparticles

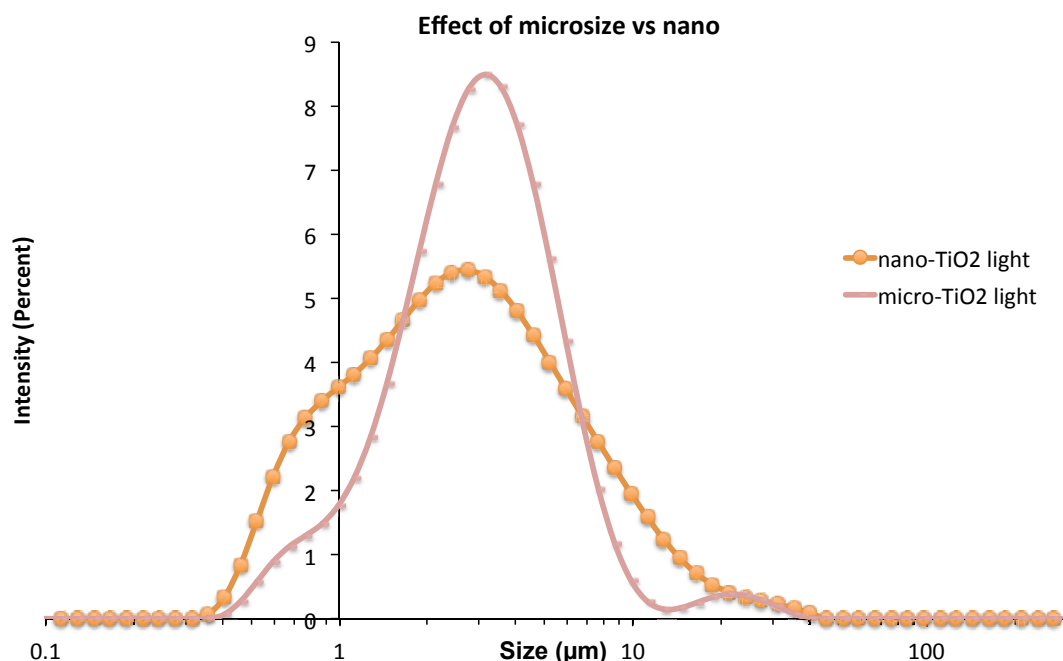


Fig. 3.8. Size distribution of Nano- and Micro-TiO₂ under light conditions in pure water. Agitation time 24 h.

To determine the effect of microsize vs nano for TiO₂ particles aged under light and dark conditions in pure and seawater they were compared in terms of size distribution. Surprisingly, in pure water, the size distribution of micro-TiO₂ is not shifted to larger values than that of nano-TiO₂. The peaks of their size distribution was almost the same under light conditions and slightly shifted to the larger size for micro-TiO₂ under dark conditions in pure water. 2 peaks at 0.6 μm and around 6 μm are measured for both nano- and micro-TiO₂. This suggests that both types of particles are relatively aggregated in the present conditions.

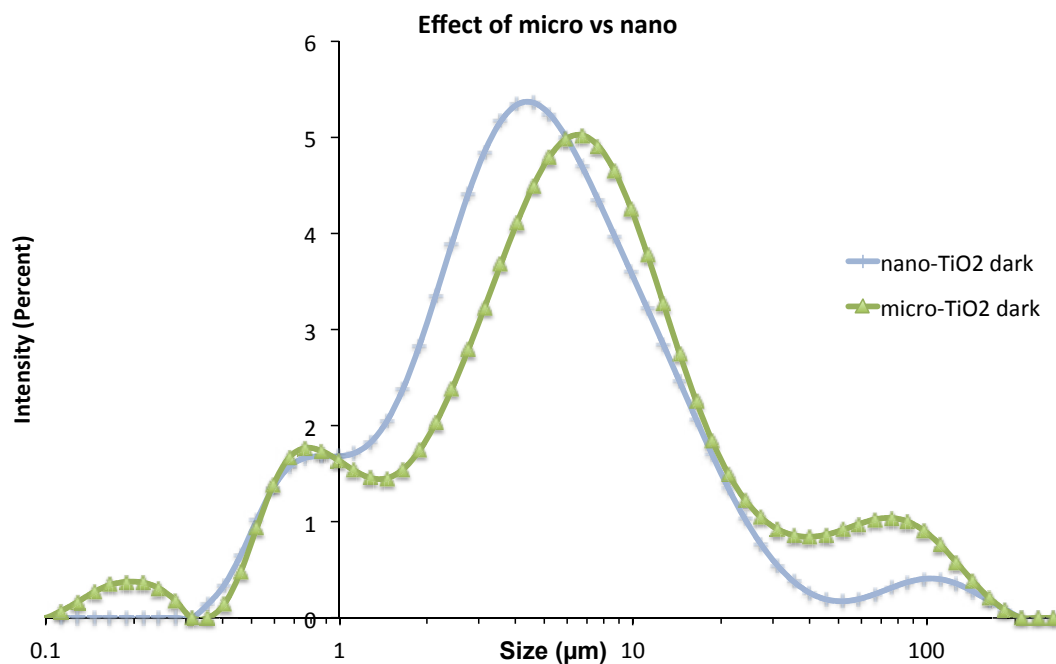


Fig. 3.9. Size distribution of Nano- and Micro-TiO₂ under dark conditions in pure water. Agitation time 24 h.

Under dark conditions peaks around 100 nm are observed for both micro- and nano-sized particles. Probably this size distribution corresponds to the droplet dispersion.

In seawater a tendency to aggregation is observed for both nano- and micro-TiO₂ particles (Fig. 3.10). A peak of size distribution for nanoparticles is shifted to 15 μm in comparison with 0.6 and 2.4 μm in pure water under light conditions (Fig. 3.13) and from 3 to 5 μm for microsized particles (Fig. 3.14).

Aging in dark in seawater does not have any effect on the size distribution of nano-TiO₂. Size distribution of smaller population remains the same at 0.6 μm and is slightly shifted from 10 to 14 μm for the larger one (Fig 3.15).

3.4.3. Effect of light

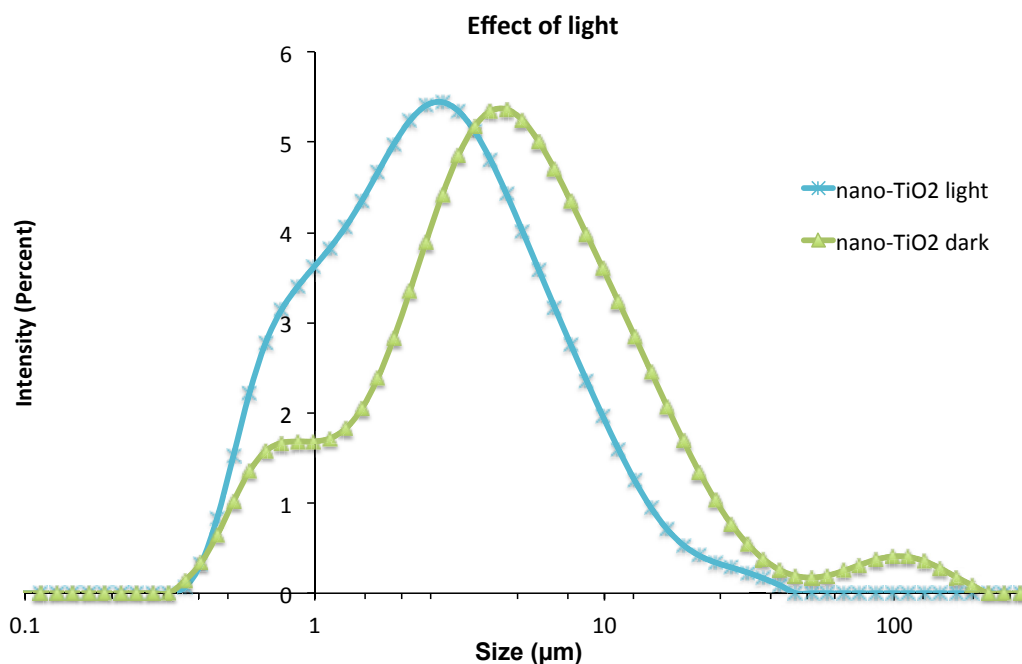


Fig. 3.11. Size distribution of Nano-TiO₂ under light and dark conditions in pure water. Agitation time 24 h.

To better compare the effect of light vs. dark condition during aging, nanosized TiO₂ particles aged under light and dark conditions are plotted together in term of size distribution in Fig. 3.11.

For nano-TiO₂ in pure water, there is a minor trend toward larger size in dark condition. This size increase is accompanied by an increase in obscuration and in TiO₂ concentration. This suggests that the TiO₂ aggregates remain more suspended in the dark than in light condition, as already discussed.

For the micro-TiO₂ formulation aged in pure water, the size distribution under dark conditions is larger than that under light conditions. This suggests that in the dark, larger NPs aggregates are present in the supernatant. The corresponding concentration values indicate an increase in TiO₂ particles concentration remaining in suspension in dark compared to light condition. This suggests that the large aggregates measured here and remaining in suspension contain the TiO₂.

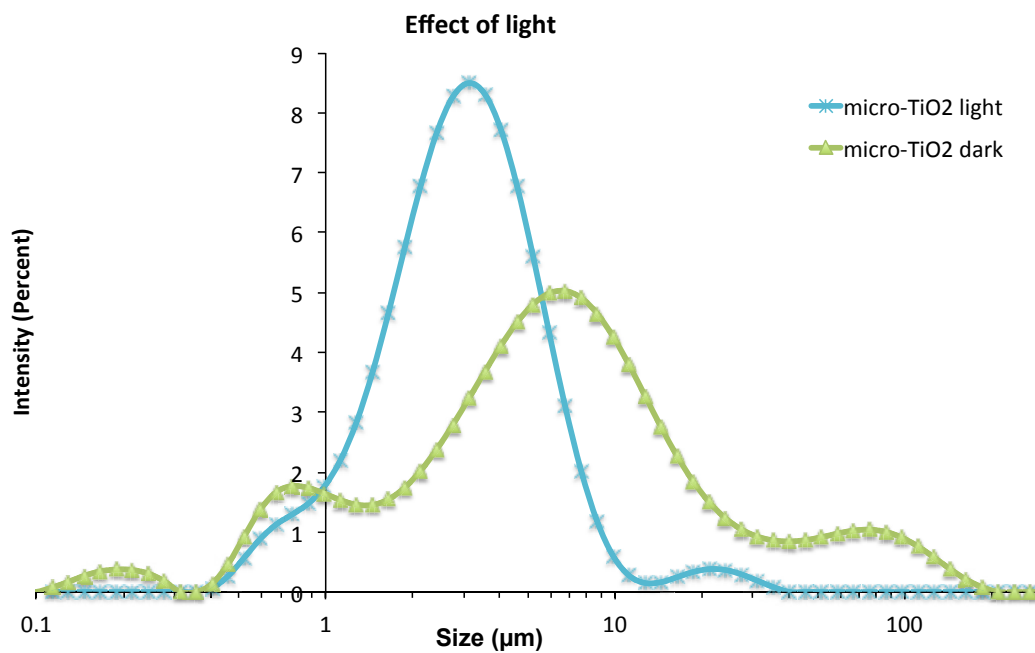


Fig. 3.12. Size distribution of Micro-TiO₂ under light and dark conditions in pure water. Agitation time 24 h.

So, a clear tendency to the size increase under dark conditions in comparison to light in pure water for both nano- and micro-TiO₂ was observed.

3.4.4. Effect of seawater

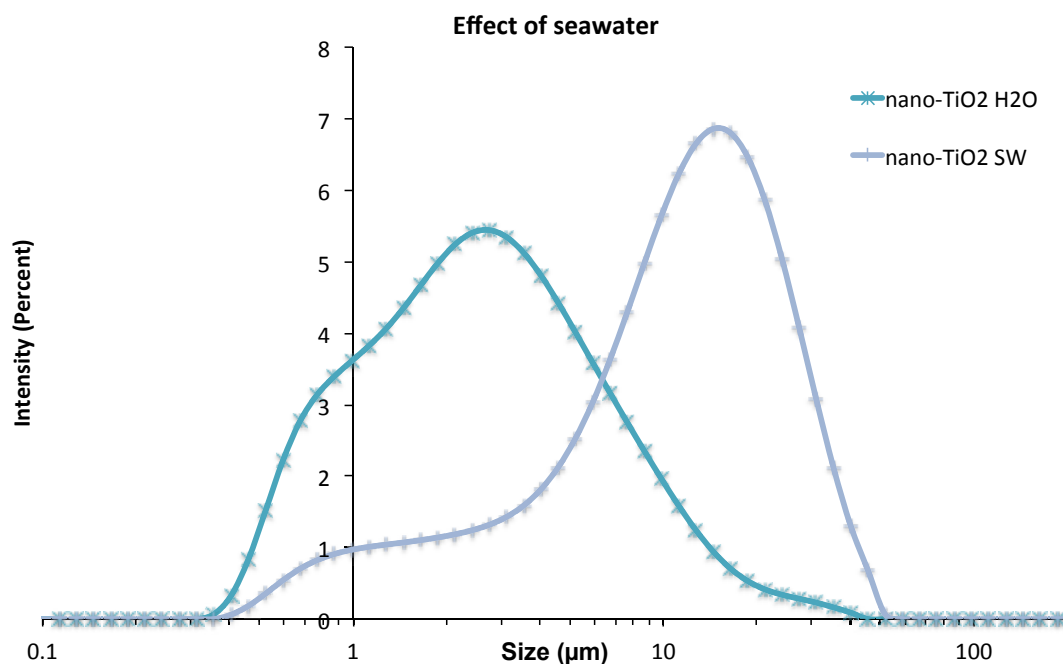


Fig. 3.13. Size distribution of Nano-TiO₂ under light conditions in pure and seawater. Agitation time 24 h.

To determine the effect of seawater TiO₂ particles aged under light conditions in pure and seawater were compared in term of size distribution. For nano-TiO₂ supernatant aged in seawater under light conditions (Fig. 3.13) a tendency is observed to the aggregation. The mean size is shifted from 5 to 9 microns. A lower concentration in nano-TiO₂ is measured in seawater due to the sedimentation and as a consequence lower obscuration values are measured (Table 3.1).

This is due to the increase in ionic strength in the system due to the presence of salt that facilitates NPs aggregation.

Table 3.1. Obscuration values (obtained by laser diffraction)

Obscuration (average)	Nano- TiO ₂ light	Nano- TiO ₂ dark	Nano- TiO ₂ light SW	Nano- TiO ₂ dark SW
30 min	38,31	17,33	11,16	10,21

24 h	25,36	30,17	9,32	7,53
48 h	24,69	33,79	1,13	0

Table 3.2. Obscuration values (obtained by laser diffraction)

Obscuration (average)	Micro-TiO ₂ light	Micro-TiO ₂ dark
30 min	21,98	19,37
24 h	19,32	20,59
48 h	37,93	54,66

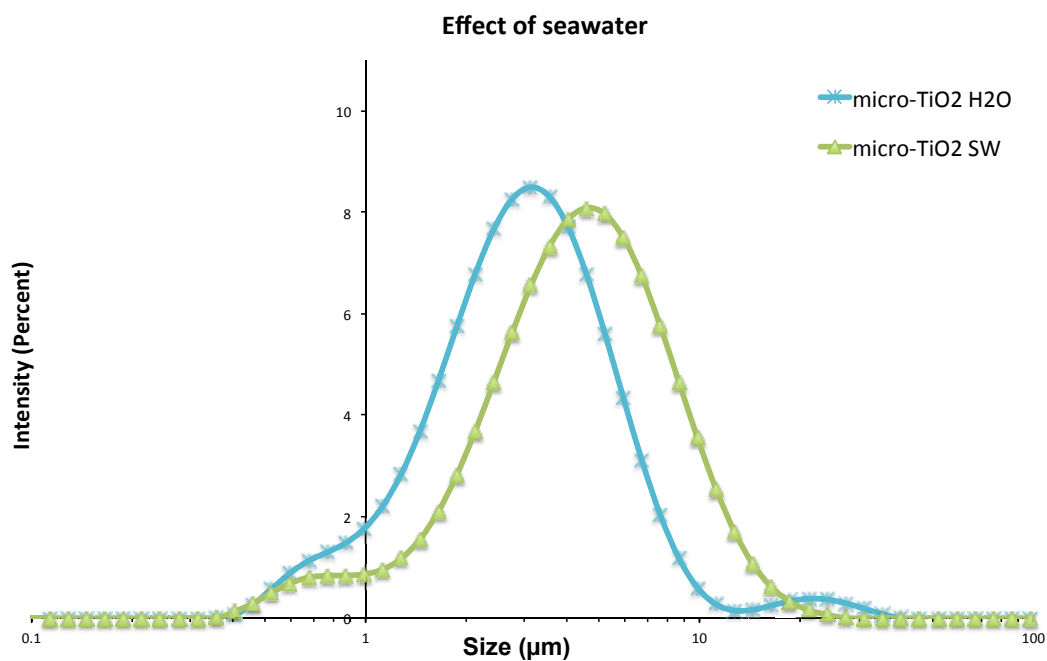


Fig. 3.14. Size distribution of Micro-TiO₂ under light conditions in pure and seawater. Agitation time 24 h.

As it is observed from Fig. 3.14 for micro-TiO₂ aged under light conditions there is a tendency to aggregation in seawater.

In seawater peaks corresponding to larger size are obtained for both nano- and micro-TiO₂ supernatant.

3.4.5. Effect of light in seawater

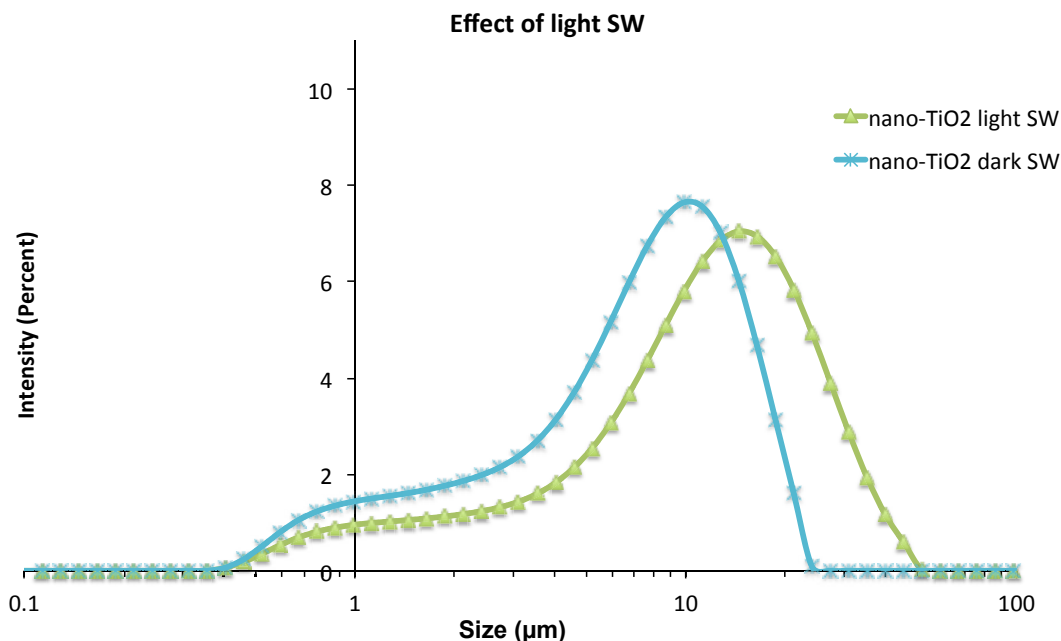


Fig. 3.15. Size distribution of Nano-TiO₂ under light and dark conditions in seawater. Agitation time 24 h.

To determine the effect of light in seawater TiO₂ particles aged under light conditions were compared in term of size distribution. Fig. 3.15 depicts size distribution of nano-TiO₂ in seawater. Contrary to what was observed in pure water, the size distribution in seawater is more shifted to larger values in light conditions than in dark condition. Approximately twofold aggregate sizes are obtained here in light condition, reaching 40 μm. On the other hand, the corresponding particle concentrations in the supernatant plotted in Figures 3.2 – 3.4 indicate similar values for both light and dark conditions, suggesting comparable fates.

Table 3.3. Obscuration values (obtained by granulometer)

Obscuration (average)	Micro- TiO ₂ light SW	Micro- TiO ₂ dark SW
30 min	7,46	1,15
24 h	10,60	0,46
48 h	9,462	0

For micro-TiO₂ in seawater a large difference is measured in the obscuration values between light and dark conditions, with 10.60 vs. 0.46 % at 24 h for light and dark respectively (Table 3.3). This suggests a much faster sedimentation in the dark condition, probably related to a faster aggregation. This is consistent with the low obscuration, derived count rate and TiO₂ concentration measured in its supernatant (Figs. 3.2, 3.3, 3.4). It is likely that peaks obtained at 35 μm correspond to the oil droplets as it is measured by ICP-AES no micro-TiO₂ particles in the suspension under dark conditions. However, due to the low concentration in micro-TiO₂ in the supernatant after aging under dark conditions, the size distribution measurement was not reproducible.

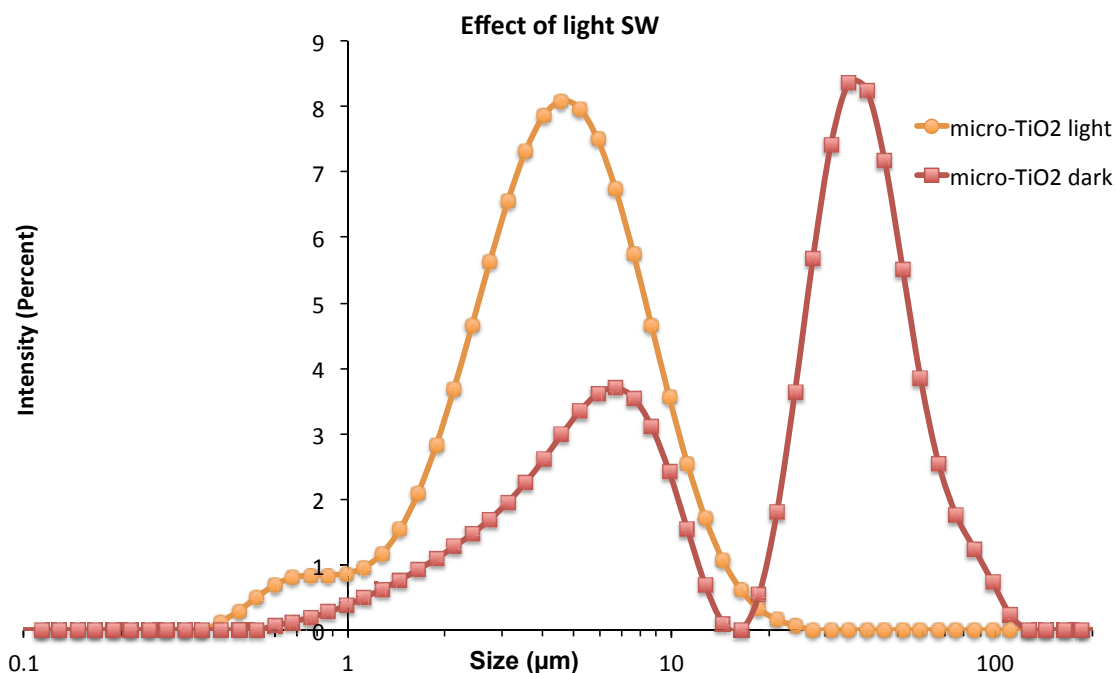


Fig. 3.16. Size distribution of Micro-TiO₂ under light and dark conditions in seawater. Agitation time 24 h.

3.5. Conclusion

To summarize the impact of different conditions and their combination on the NPs release in the water environment, a release of the colloidal residues containing TiO₂ nanoparticles from commercialized sunscreens into aquatic environment after an artificial aging was evidenced. It is revealed that TiO₂ releases into the aquatic environment as well as some organic part of the cream. The residues partly remain in the water suspension but a major part underwent the sedimentation especially in seawater. So, TiO₂ particles can be present both in suspension in pure water and in the sediments in seawater (where they are mainly settle down). Consequently, they will be exposed to both the planktonic and benthic organisms.

In pure water, larger aggregation and sedimentation takes place in light condition than in dark condition, probably due to the photodegradation of the organic components.

The nano- vs. micro-sized TiO₂ studied here did not show very contrasted behaviors under the aging conditions tested.

In seawater a tendency to larger aggregation and sedimentation than in pure water was observed due to a high ionic strength. No clear effect of light vs. dark conditions of aging could be shown due to the major sedimentation.

Under all conditions it is observed that residues from the sunscreen, TiO₂ particles with organic matrix remain in suspension. In order to study the influence for the real aquatic environment more complex systems have to be studied next to better mimic the environment.

Chapter 4. Interaction between TiO_2 nanoparticles and polysaccharide in suspension

4.1. Introduction

In the previous chapter, the aging and fate of two sunscreens with specified sizes of TiO_2 particles used as UV filter, were studied under different environmental conditions. This showed how the TiO_2 particles are transferred from the sunscreen to the water column or the sediment layer during aging and in dependence on their size and solution conditions. But this approach leaves open the question on how NPs interact with environmental components, and how this interaction may alter the NPs fate and impact on the biota. More particularly, this work focuses on the bacterial organic matter typically found in solution and at the surface of environmental collectors, and the effect that these natural components can have on the NPs fate.

As the sunscreen composition is too complex to study the precise interaction of NPs with environmental surfaces, we used in our approach some pure engineered TiO_2 NPs, as analogues of those typically released from sunscreen. Moreover, we used the pure polysaccharide YAS34 as analogue of bacterial gels. In this chapter, the interactions between TiO_2 NPs and EPS are studied under suspension conditions in aqueous medium. This should favor the probability of contact, and thus the interaction between NPs and EPS. In addition, when this interaction is attractive and some NPs-EPS associations take place, it is our hypothesis that such phenomenon should be detected by size measurement in suspension. In this context varying the solution conditions will help to clarify the mechanism driving the interaction between NPs and EPS.

4.2. Methodological approach

The size measurements of TiO_2 and EPS taken separately, or mixed together were performed using DLS. These studies were performed under conditions favorable or unfavorable for the interaction. For this purpose pure TiO_2 NPs and polyacrylate-coated TiO_2 (PAA- TiO_2) were used.

TiO_2 NPs are positively charged at pH lower than the IEP=4.9 and negatively charged at pH higher than IEP. So, favorable conditions were fixed at pH 4.3, giving positively charged TiO_2 NPs and negatively charged polysaccharide. This was

expected to favor attractive electrostatic interaction. PAA-TiO₂ NPs are negatively charged at pH > 3. They were used to create the unfavorable conditions at pH 8, where electrostatic repulsions were expected with the negatively charged polysaccharide. Of note, using the pure TiO₂ NPs with the EPS at pH = 8 would have also lead to repulsive interaction, i.e. unfavorable condition. However, this case study could not be used because the TiO₂ NPs did not remain individually dispersed at pH = 8 despite their negative surface charge.

Different NPs/EPS ratios were tested. TiO₂ suspension was prepared by diluting an aliquot of the 7.46 g/l TiO₂ stock suspension in 10⁻³ M NaCl, so that the final TiO₂ concentration in the mixture was 10 mg/l. The EPS solution was prepared by diluting 0.5 g/l EPS stock solution in 10⁻³ M NaCl. The EPS concentration in the mixture was varied at 1, 10 or 100 mg/l. The mixture solution was prepared by adding 5 ml of EPS and TiO₂ with the respective concentration. After the solution preparation it was placed in the DLS measurement cell for the initial measurements and 1 h later.

In a first part, the interaction between TiO₂ NPs and EPS was studied under Brownian motion, i.e., without any mechanical agitation. The mixture was prepared directly in the measuring cell for DLS and the size measurements were launched without any delay after mixing. A second size measurement is presented in the result section, corresponding to the time 1h after mixing.

In a second part, in order to distinguish and separate the EPS free in suspension from that bounded to NPs, a fractionation by ultracentrifugation was processed. The EPS bounded to NPs was supposed to sediment within the centrifugation and the free EPS to remain in the supernatant. The mixture of the components was prepared by adding 5 ml of the NPs to 5 ml of the EPS solution with the desired respective concentration. Just after mixing and manual handshaking for homogenization, the mixture was ultracentrifuged. An Optima L-100 XP Ultracentrifuge (Beckman Coulter) was used. Centrifugation speed was maximum possible 80000 rpm, corresponding to G-force (RCF) 548352 g. 1 h or 2 h ultracentrifuge duration was tested in the aim of removing all the TiO₂ NPs form the supernatant. The removal efficiency was controlled using DLS count rate and size measurement on 2 ml of supernatant.

4.3. Favorable conditions

4.3.1. Interaction between TiO_2 and EPS under Brownian motion

The DLS results below are presented in term of intensity weighted size distribution and of the corresponding correlograms. Due to the sample polydispersity, the typical DLS algorithms, Cumulant and NNLS, were sometime unable to correctly fit and interpret the correlograms measured on our samples. Nevertheless those correlograms may carry useful information such as the number of distinct size populations present in the supernatant and their relative diffusion times. Only the correlograms are presented below when the size distribution returned were not returned by a correct fit.

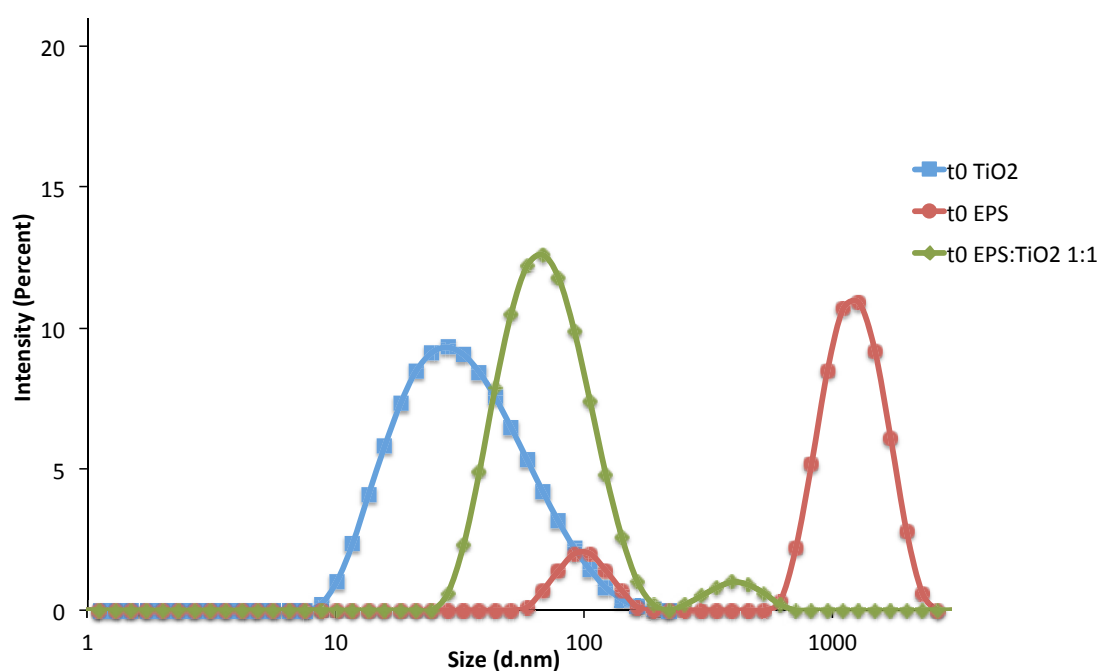


Fig. 4.1 a. Size distribution of the EPS and TiO_2 and their mixture $\text{EPS}:\text{TiO}_2=1:1$ at the start time (t_0) of interaction, 10^{-3} M NaCl, pH 4.3.

Fig. 4.1a shows size distribution of the TiO_2 and EPS taken separately and of their mixture at the initial time of interaction. The dispersed nanoparticle size distribution is clearly identified when taken separately, ranging from 10 to 100 nm. The EPS alone displays a size larger and wider in distribution, from 100 nm to few

micrometers. In the mixture, the two components are difficult to distinguish. The correlograms corresponding to the same measurements under the same conditions are presented in Fig. 4.1b.

The image was shown cut after 3000 nm (5000 nm is a limit detection for DLS) as a distribution fit was not good after this size.

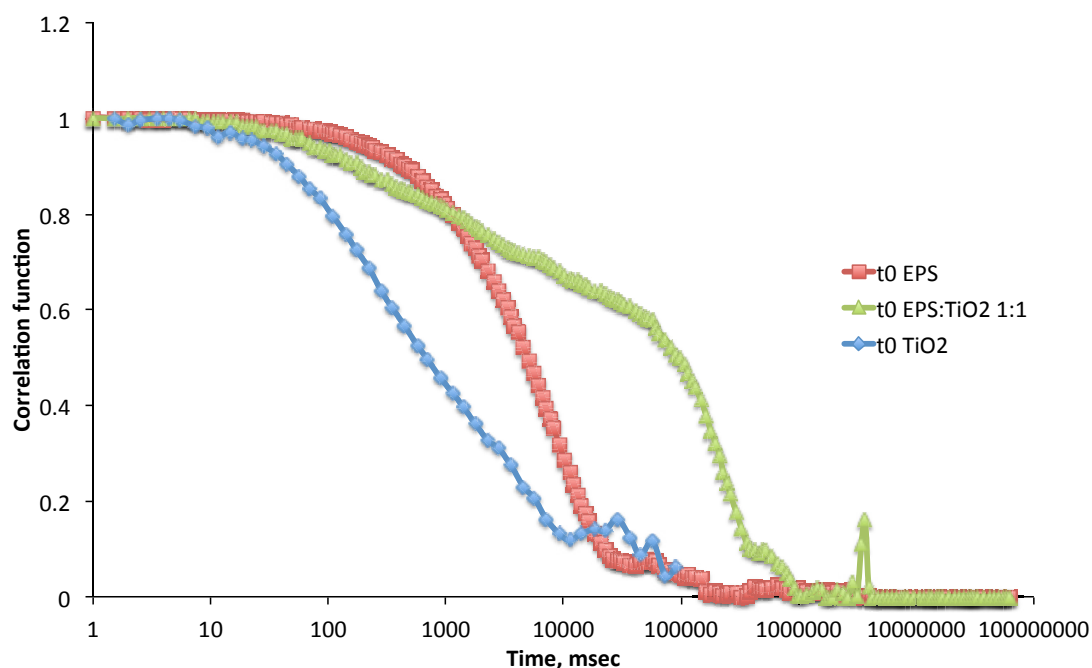


Fig. 4.1 b. Correlograms of the EPS and TiO_2 and their mixture $\text{EPS}:\text{TiO}_2=1:1$ at the start time (t_0) of interaction, 10^{-3} M NaCl, pH 4.3.

The correlogram of the mixture at time 0 depicts two shoulders that correspond to two distinct populations diffusing differently in the mixture. According to the respective signals shown separately in Fig. 4.1b, the range of 10-100 msec in diffusion time corresponds to the dispersed NPs. The signal from the polysaccharide alone is expected below 10^4 msec, but is not anymore distinguished in the correlograms of mixture. The population observed here in the 10^5 msec diffusion time corresponds to some diffusing entities larger than the EPS, likely NPs/EPS aggregates of few μm in size. These large aggregates were formed instantaneously after NPs and EPS mixing, as their signal is most measured at time 0.

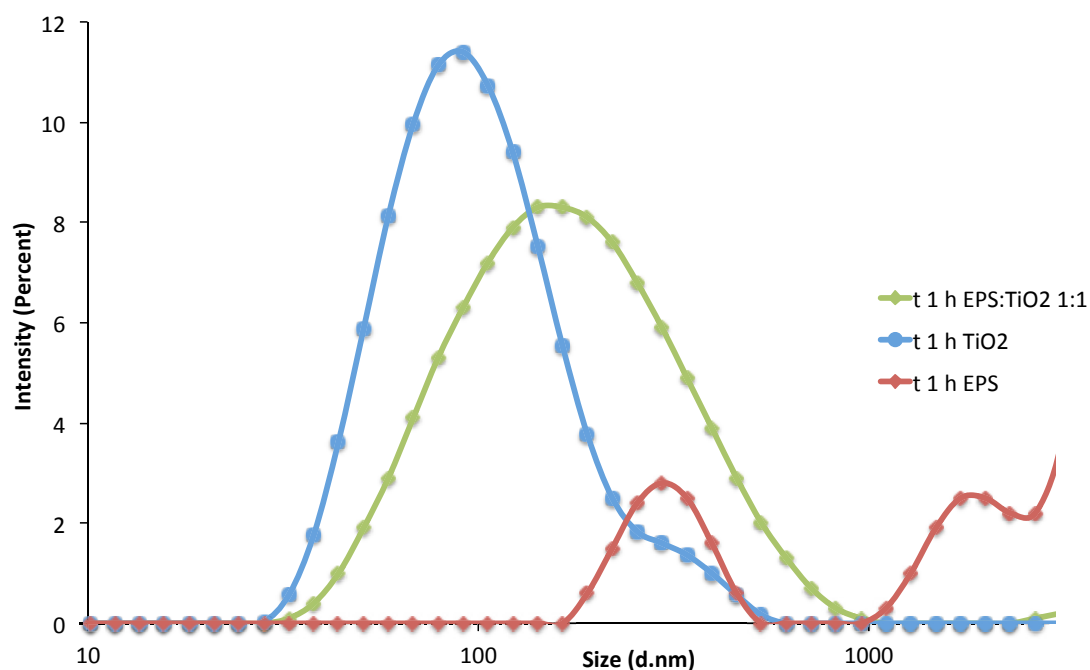


Fig. 4.2 a. Size distribution of the EPS and TiO₂ and their mixture EPS:TiO₂=1:1 at the end time (t_{1h}) of interaction, 10^{-3} M NaCl, pH 4.3.

DLS measurements after 1 h depict a shift of the size distribution corresponding to the TiO₂ alone from 28 nm at t_0 to more than 90 nm with the presence of larger size around 300 nm (Fig. 4.1a, 4.2a). This increase in NPs size may be due to aggregation within this time due to close IEP which is at pH 4.9 and working pH is 4.3.

In the mixture the size distribution is shifted to higher values after 1h, characterized by a peak at 165 nm. This is likely due to both the NP homoaggregation and the NPs heteroaggregating with the EPS.

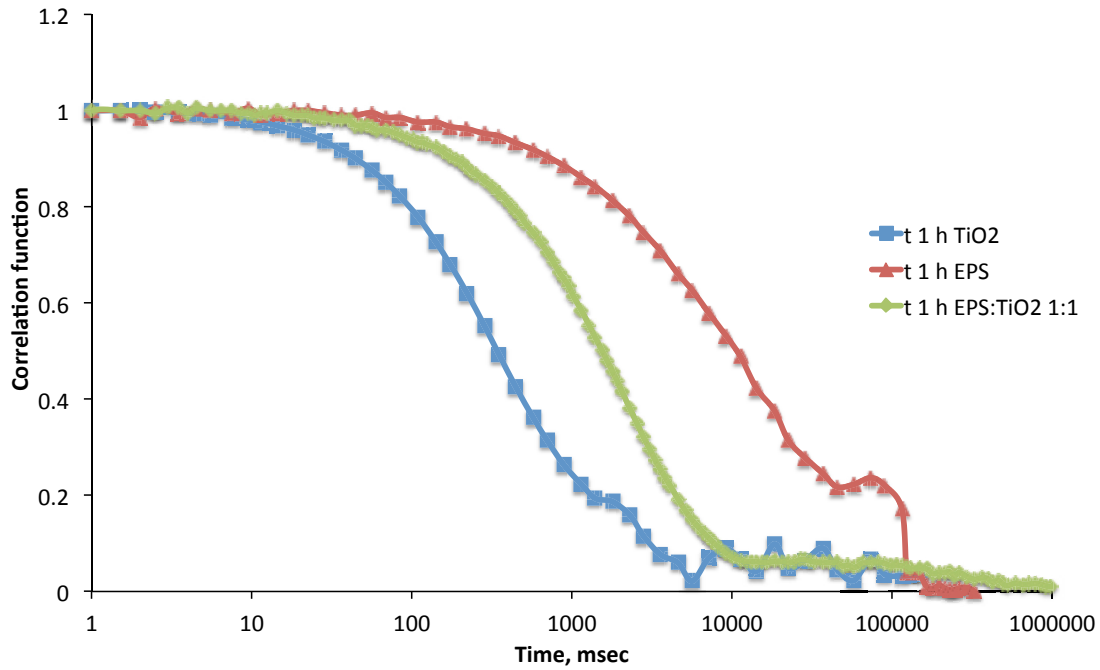


Fig. 4.2 b. Correlation function of the EPS and TiO_2 and their mixture $\text{EPS}:\text{TiO}_2=1:1$ at the end time (t_{1h}) of interaction, 10^{-3} M NaCl, pH 4.3.

The correlogram of the mixture at 1 h does not show large aggregates anymore. This suggests that, along with the interaction time, the largest aggregates settled down (Fig. 4.2b), and only the smallest units remain in suspension. Indeed, the absence of agitation in the present condition favored the settling of larger and denser entities displaying characteristics out of the colloidal limits. Moreover, such sedimentation process is confirmed by the decrease in the count rate measured between initial and final time from 1300 to 800.

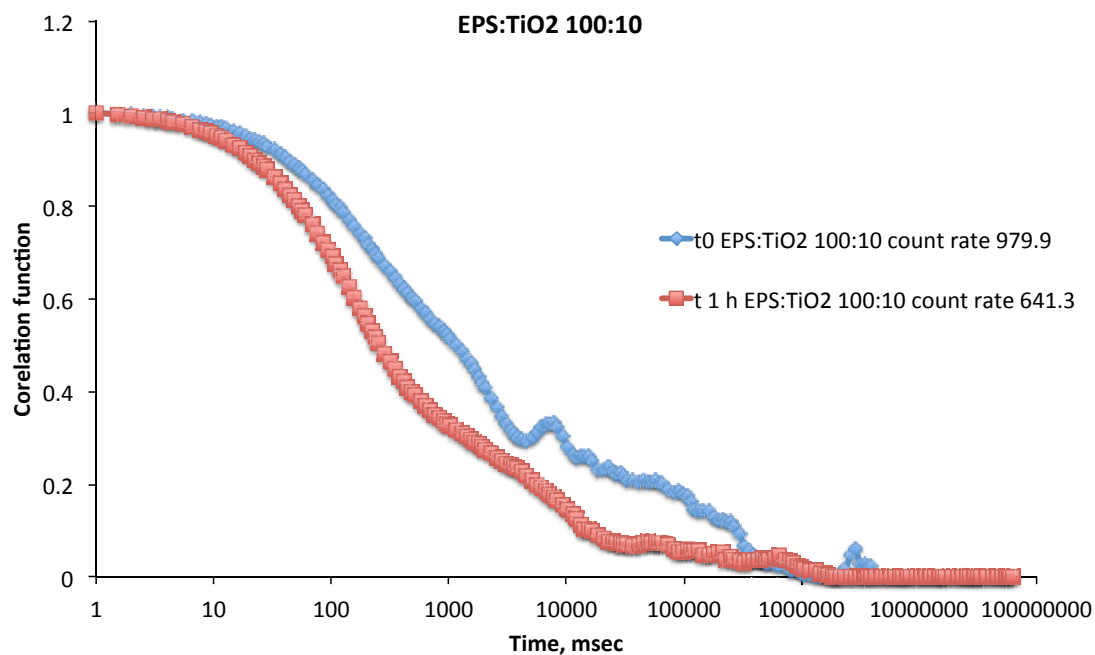


Fig. 4.3. Correlation function of the EPS:TiO₂=100:10 mixture at the start and end time of interaction, 10^{-3} M NaCl, pH 4.3.

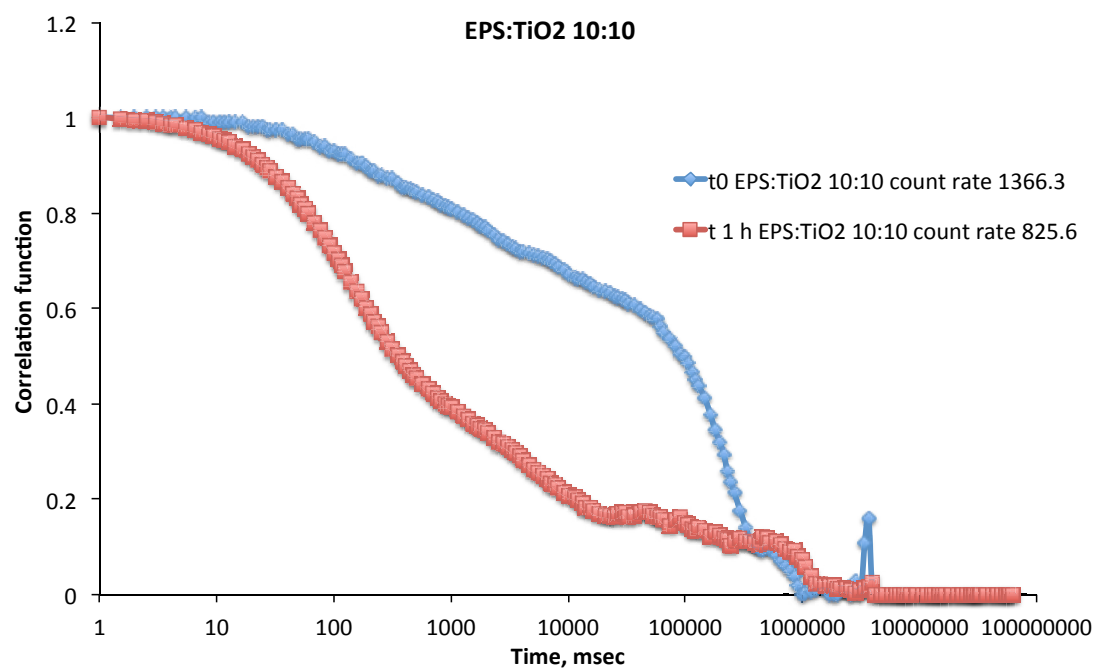


Fig. 4.4. Correlation function of the EPS:TiO₂=10:10 mixture at the start and end time of interaction, 10^{-3} M NaCl, pH 4.3.

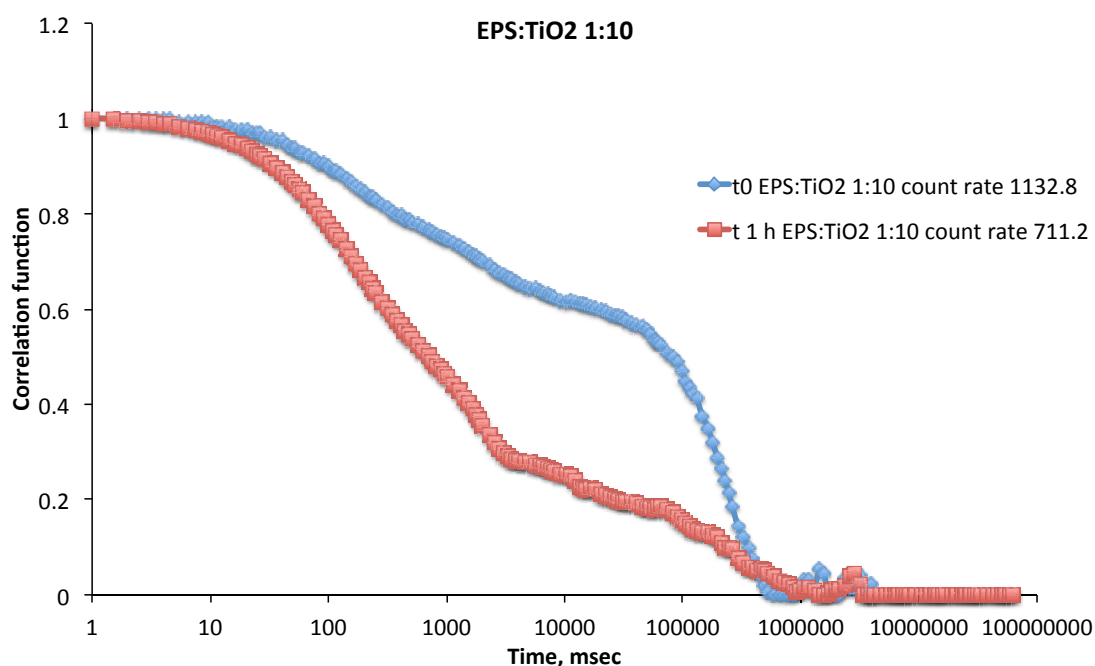


Fig. 4.5. Correlation function of the EPS:TiO₂=1:10 mixture at the start and end time of interaction, 10⁻³ M NaCl, pH 4.3.

In Figures 4.3, 4.4, 4.5 the correlograms measured on the mixtures at times 0 and 1h are plotted for different EPS:TiO₂ ratio, respectively 100:10, 10:10 and 1:10. In the three cases, the correlograms show the disappearance at time 1h of the shoulder corresponding to the slow diffusing units, i.e. the largest aggregates. Moreover, the decrease in count rate in the same time confirms that these large aggregates have settled down along with the interaction time. Nevertheless, despite rapid aggregation and sedimentation, after one hour of interaction, the correlograms still display significant contributions of both individual NPs and large aggregates remaining in suspension. This suggests that only a fraction of the nanoparticles have been involved in the aggregation process.

Looking at the different ratios tested it appears that they have a very limited effect on fate of the NPs. In all cases a tendency to significant aggregation was observed, well pronounced at 10:10 and 1:10, but more limited at 100:10. This is likely due to the saturation of the NPs surface by the adsorbed EPS.

4.4. Unfavorable conditions

4.4.1. Interaction between PAA-TiO₂ and EPS under Brownian motion

To create unfavorable condition for interaction between NPs and EPS, pure TiO₂ NPs at pH 8 were tested first, as under these conditions they are negatively charged as well as EPS. However, changing the pH of the TiO₂ suspension from acidic to basic pH implied passing the IEP of the NPs, which inevitably induced irreversible homoaggregation. This increased average size of NPs to around 300 nm. As the EPS hydrodynamic size is in the same range, it would have been hard to distinguish by DLS between the NPs homoaggregates and the EPS. For this reason, PAA-TiO₂ NPs were used in this section. Indeed, their polyacrylate coating stabilises the NPs dispersion with a negative surface charge in a large pH range.

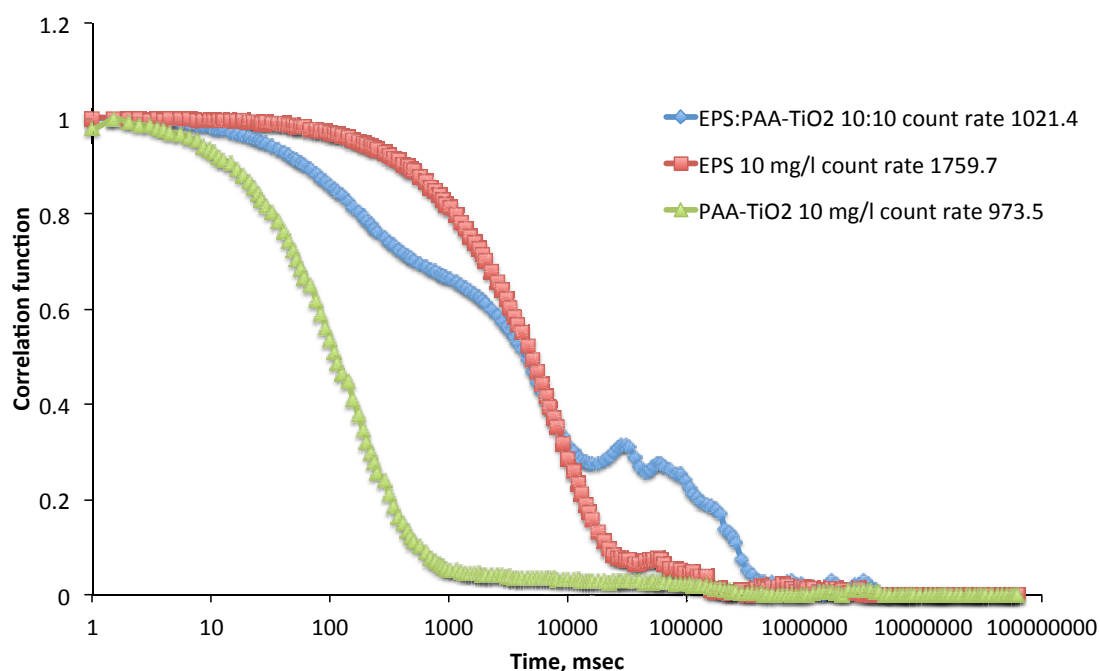


Fig. 4.6. Correlation function of the EPS:PAA-TiO₂=10:10 mixture, PAA-TiO₂ and EPS at the start of interaction, 10⁻³ M NaCl, pH 8.

The correlograms measured at time 0 for the TiO₂ and EPS taken separately and for their mixture at the ratio 10:10 are displayed in Fig. 4.6. The correlogram for the mixture clearly depicts three distinct entities among the diffusing objects. The

smallest size population, diffusing in the range of 100 msec, likely corresponds to the individual dispersed nanoparticles, as also observed on the separate TiO_2 measurement. The intermediate size population ranges within the diffusion time 1000-10000 msec, which corresponds to the polysaccharide free in solution, as also observed on the EPS measured alone. The largest size population displays diffusion time around 10^6 msec, which suggests the presence of aggregates, in the μm size range, behaving in suspension. In the present conditions, some large aggregates are still formed despite the expected repulsive interaction between EPS and NPs. The reason for this partial attractive interaction remains unclear to us now. Some inhomogeneities in the polysaccharide structure may cause such dual fate. It is worthwhile noting that some free polysaccharide remains detectable in solution at time zero of the interaction. This was not observed under favorable conditions with pure TiO_2 where rapid attractive interactions took place. Here, some attractive but weaker interactions prevail.

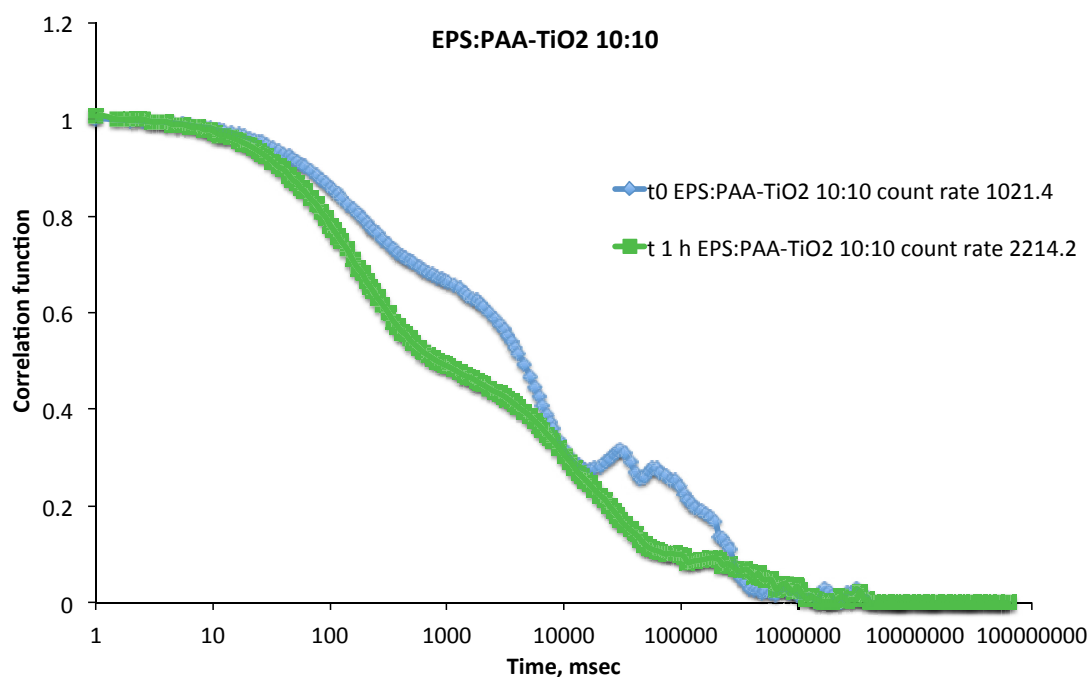


Fig. 4.7. Correlation function of the EPS:PAA- TiO_2 =10:10 mixture at the start and end time of interaction, 10^{-3} M NaCl, pH 8.

After one hour of interaction, as already observed under favorable condition, the large aggregates have settled out and did not contribute to the measurement anymore.

However, the signals corresponding respectively to the free polysaccharide and the nanoparticles remain clearly distinguishable, indicating their distinct behaviors in suspension.

At the EPS:PAA-TiO₂ 1:10, a trend similar as that at 10:10 is observed (Fig. 4.7), where some large μm aggregates are rapidly formed at the initial time of interaction, and then removed from the suspension at 1h time. These large aggregates are likely to be removed from the suspension due to their sedimentation. In this case, the free polysaccharide is more difficult to distinguish as it is tenfold diluted as compared to the 10:10 ratio.

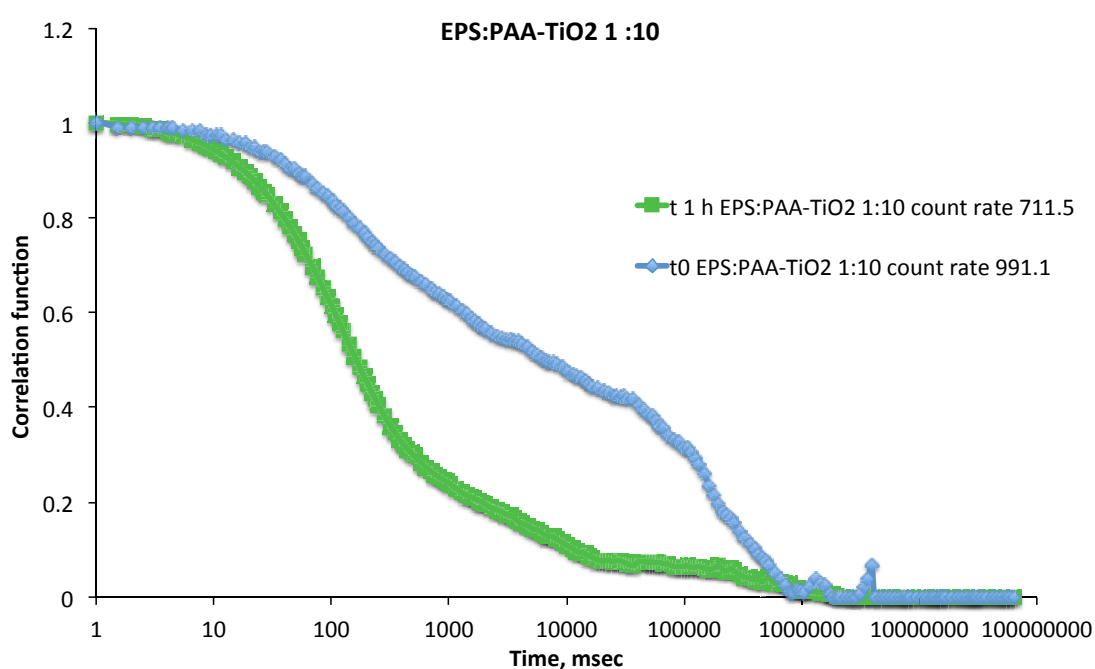


Fig. 4.8. Correlation function of the EPS:PAA-TiO₂=1:10 mixture at the start and end time of interaction, 10^{-3} M NaCl, pH 8.

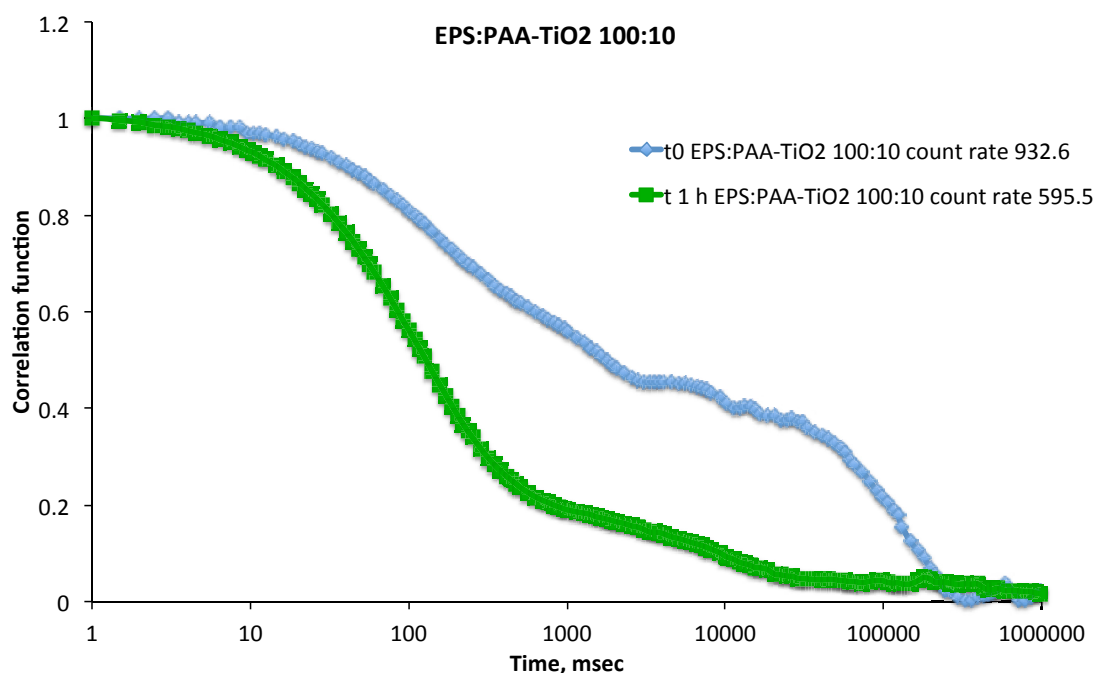


Fig. 4.9. Correlation function of the EPS:PAA-TiO₂=100:10 mixture at the start and end time of interaction, 10⁻³ M NaCl, pH 8.

To the contrary, at the 100:10 ratio, a more intense signal is logically detected in the large size domain of the correlogram, corresponding to the polysaccharide. However, this does not alter the overall time evolution of the mixture, showing distinct behaviors of the two components at time 1h.

As depicted by the correlation functions for the different EPS:NPs ratios studied under favorable or unfavorable conditions, some similar processes are registered by DLS, which proceeded during the interaction time. The EPS is likely to interact with a certain fraction of the nanoparticles in suspension, leading to the formation of large aggregates which tend to sediment. In the same time, especially under unfavorable conditions, some polysaccharide remains also free in solution.

Such tendency to the aggregation and sedimentation was studied here under Brownian motion. In the section 4.4.3 below, mechanical agitation was used in order to maintain the interacting materials in suspension during the 1h time frame, and to study then their ultimate fate.

Moreover, since DLS analysis gives a size measurement based on the light intensity, it always favors the detection of larger objects that are stronger scatterers. As a

consequence, it is sometime difficult to distinguish the different populations cohabitating in a polydisperse sample, particularly the smallest sizes may be hidden by the larger ones. For this reason, in the next section, ultracentrifugation was used to remove the NPs either free or bound to the EPS, before the DLS analysis, in order to verify the presence of any free polysaccharide in the supernatant.

4.4.2. Interaction between PAA-TiO₂ and EPS with centrifugation approach

Ultracentrifugation of the mixtures was performed at 80000 rpm during 1 or 2 h, started at time zero of interaction.

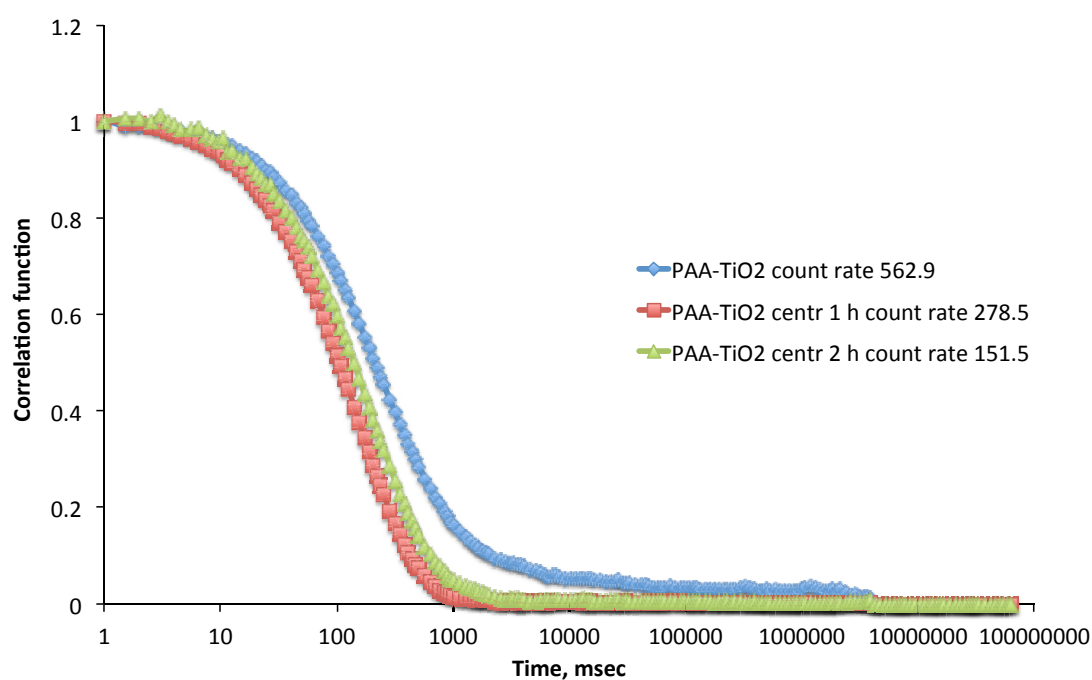


Fig. 4.10 a. Correlation functions of the PAA-TiO₂ before and after 1 and 2 h centrifugation, 10⁻³ M NaCl, pH 8.

First, in order to verify that the suspended NPs are well removed by the ultracentrifugation step, the suspension of NPs alone was centrifuged during 1h or 2h and the resulting supernatant was analyzed in term of the DLS count rate. Fig. 4.10a and 4.10b shows the corresponding correlograms and count rate values. The derived

count rate decreases from 562 to 278 and 151 kilo-counts after 1h and 2h of ultracentrifugation respectively. This suggests, that the NPs are not totally removed by the ultra-centrifugation but a minor fraction, constituted of the finest units, remains in suspension. The polydispersity observed in the initial suspension is indeed removed after centrifugation. Despite this limited NPs removal, this approach was followed on the EPS:NPs mixtures in order to verify the occurrence or not of free EPS after interaction with the NPs under these conditions.

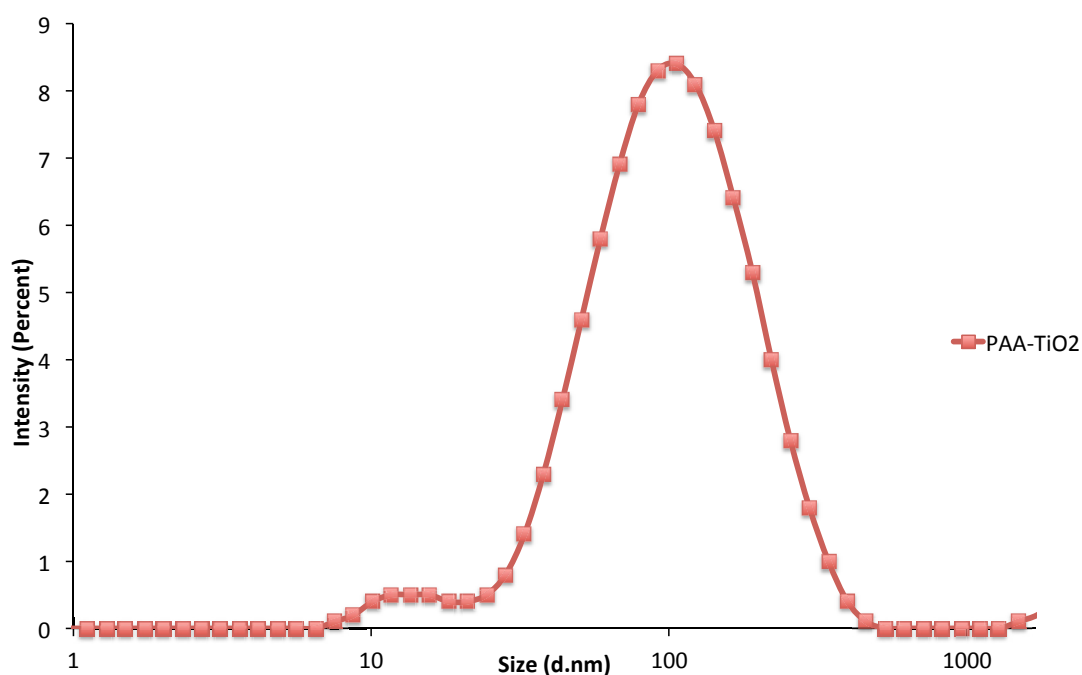


Fig. 4.10 b. Size distribution of the PAA-TiO₂, 10⁻³ M NaCl, pH 8.

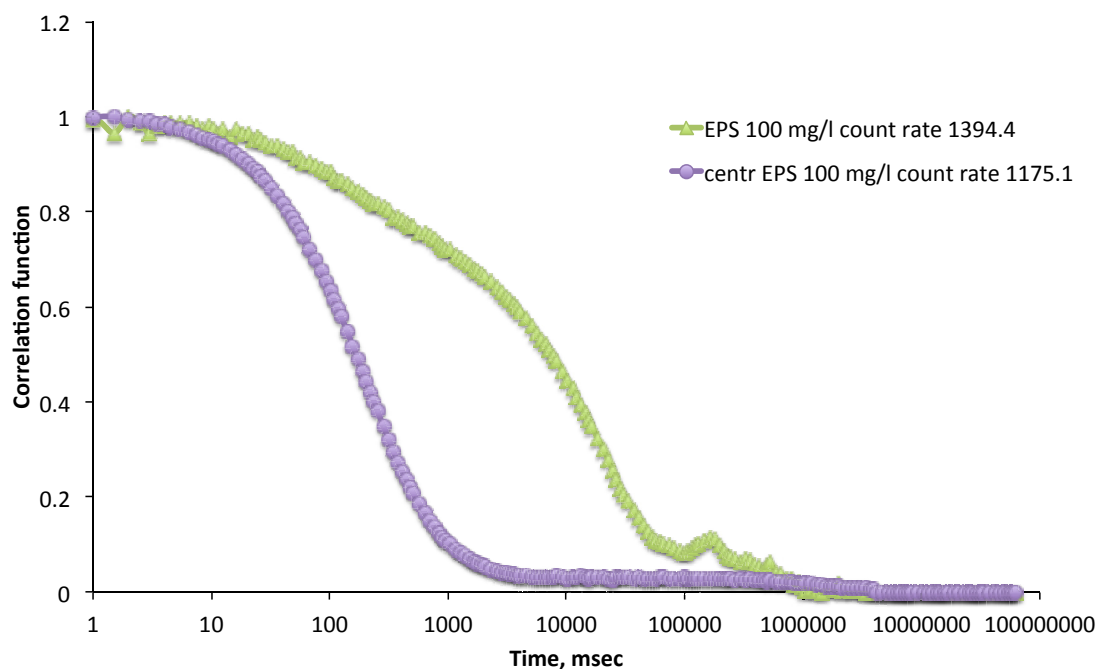


Fig. 4.11. Correlation function of the EPS before and after 1 h centrifugation, 10^{-3} M NaCl, pH 8.

Regarding, the effect of the ultracentrifugation on the EPS solution alone, the correlograms clearly show the presence of large EPS aggregates in the initial solution, that are removed after centrifugation. The corresponding count rate decrease remains minor, from 1394 to 1175 kilo-counts. This indicates that the EPS in solution does not sediment but remains suspended through the ultracentrifugation step.

Fig. 4.12 to 4.17 illustrate the size distribution and correlograms of EPS:PAA-TiO₂ mixtures before and after 2h of ultracentrifugation, for the ratio, 10:10, 100:10 and 1:10. In the three cases studied, the ultracentrifugation step, i.e. the major removal of NPs, lead to a general size increase. This is due to the larger size of the EPS remaining in suspension than that of the NPs removed. Such sample fractionation is also comforted by the decrease in count rate after centrifugation which corresponds to the decrease of NPs number in the suspension.

This effect is clearly observed with the ratio 10:10, in which the correlogram before ultracentrifugation displays a significant signal for dispersed NPs around 10 msec, in addition to larger units ranging in diffusion times from 100 to more than 10^6 msec. After ultracentrifugation, the NPs signal has largely decreased and the EPS pattern

has become overexpressed. This evidences the distinct fates of the NPs and the EPS in the mixture, at least for a certain fraction of these materials fractionated here. This is also evidenced by the size distribution depicted in Figure 4.13, with a shift of the mean peak roughly 80 nm to 200 nm after centrifugation.

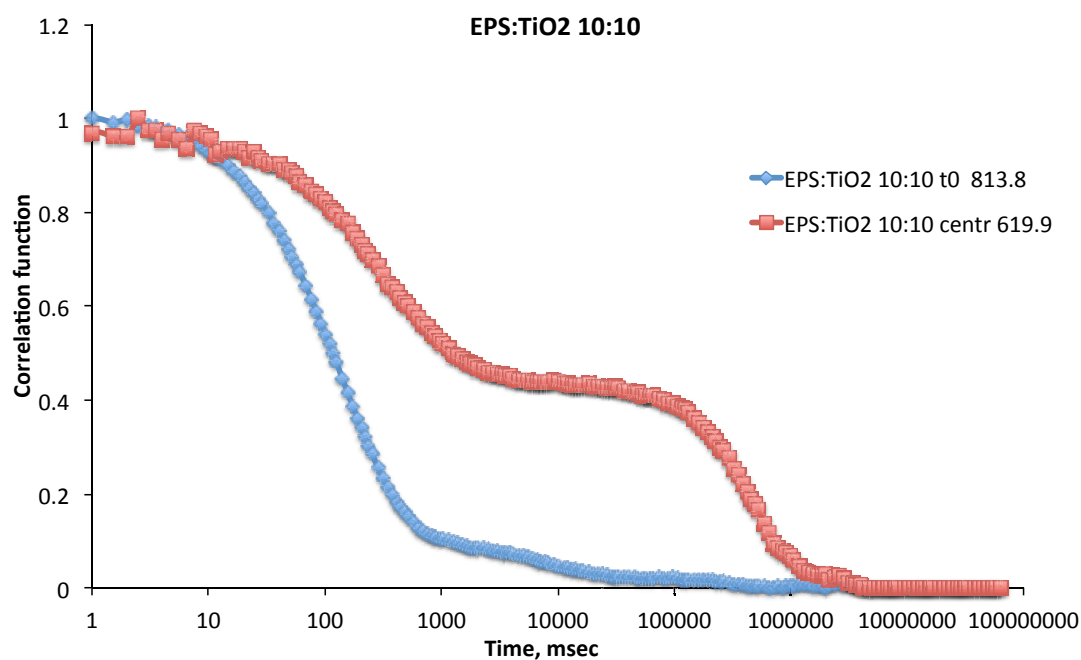


Fig. 4.12. Correlation function of the EPS:PAA-TiO₂=10:10 mixture before and after 2 h centrifugation, 10⁻³ M NaCl, pH 8.

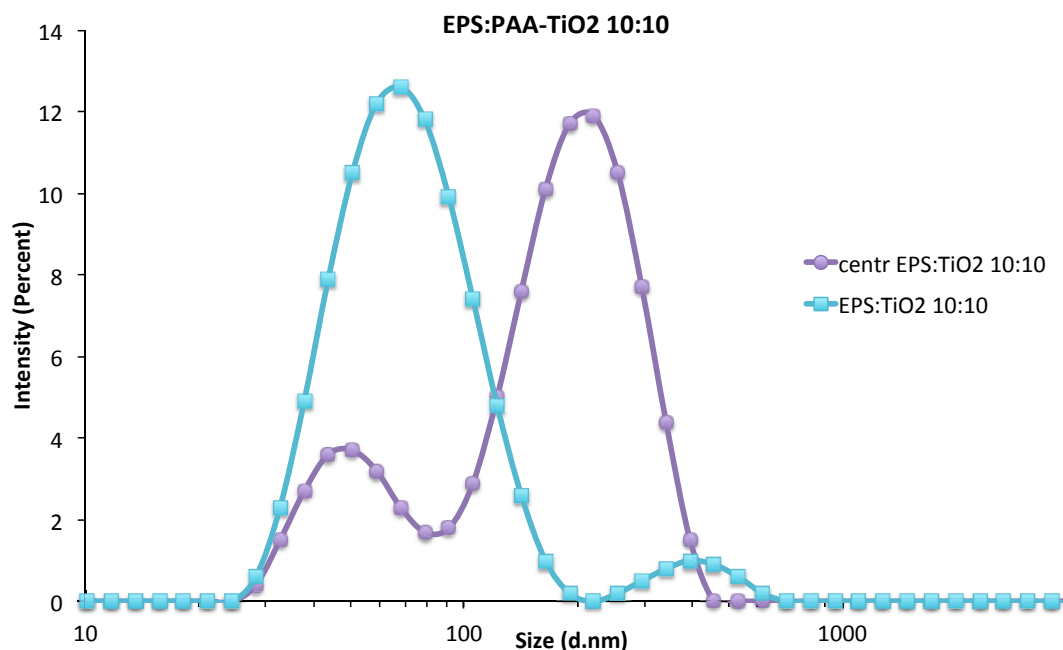


Fig. 4.13. Size distribution of the EPS:PAA-TiO₂ 10:10 mixture before and after 2 h centrifugation, 10⁻³ M NaCl, pH 8.

At the EPS:PAA-TiO₂ ratio = 100:10, the fractionation effect by ultracentrifugation is not as evident as with the 10:10 ratio due to the minor signal attributed to the NPs in the initial sample. The tenfold concentration in EPS indeed hides the NPs signal. Moreover, some large aggregates, identified with to diffusion time around 10⁵ msec are mostly removed. These aggregates are likely some EPS homoaggregates. However they may also involved a fraction of NPs associated with the EPS, as discussed in the previous section.

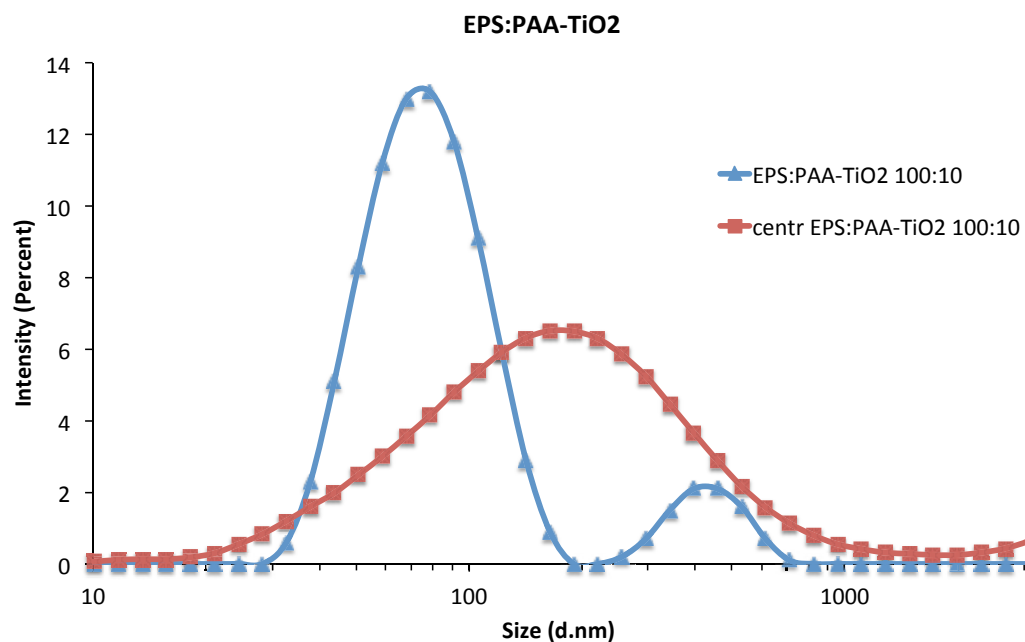


Fig. 4.14. Size distribution of the EPS:PAA-TiO₂ 100:10 mixture before and after 2 h centrifugation, 10^{-3} M NaCl, pH 8.

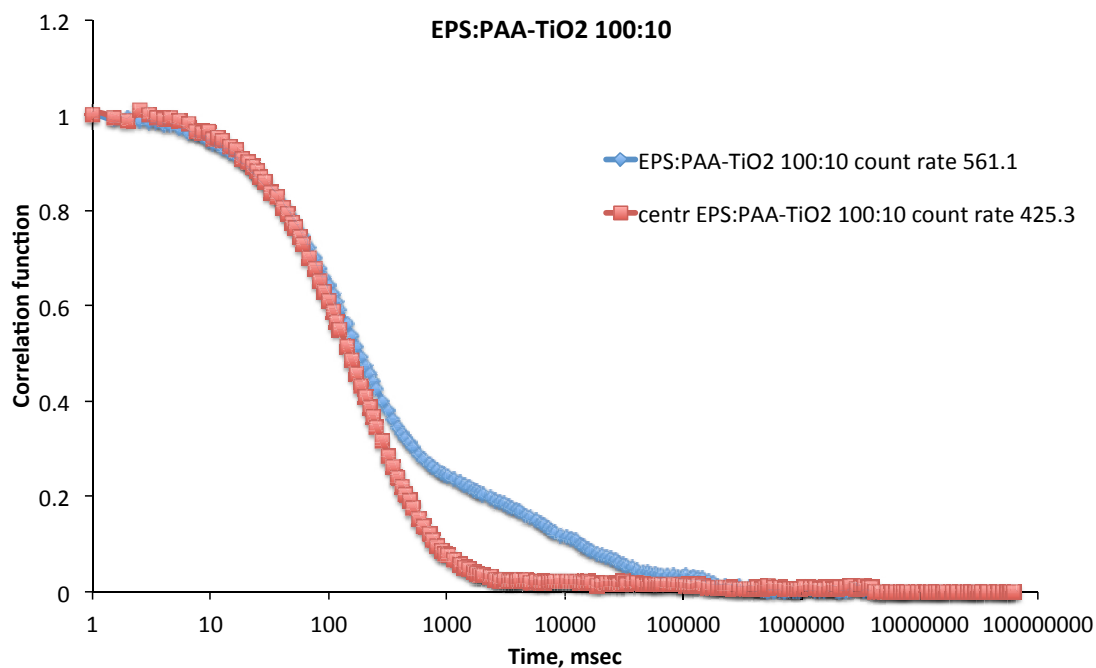


Fig. 4.15. Correlation function of the EPS:PAA-TiO₂=100:10 mixture before and after 2 h centrifugation, 10^{-3} M NaCl, pH 8.

At the EPS:PAA-TiO₂ ratio 1:10, the EPS signal is now under expressed and the signal attributed to the NPs can be clearly identified. The shift of both the size distribution and the correlograms toward larger sizes through ultracentrifugation likely suggests two contrasted initial and final states. Before centrifugation, the dispersed NPs constitute the majority of the suspended units. After centrifugation, mostly only EPS remain in the supernatant and is measured.

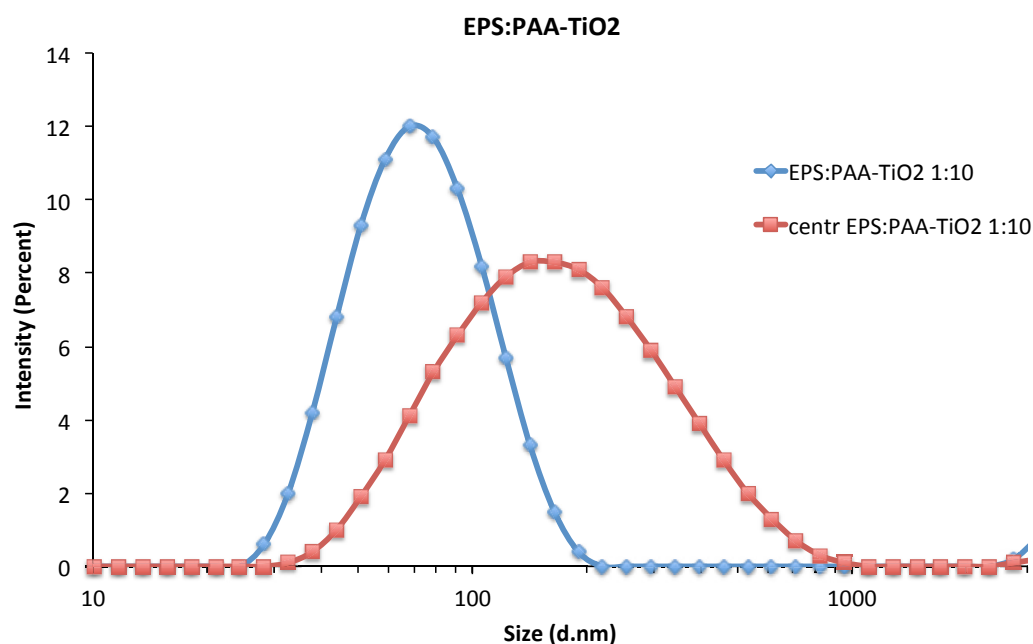


Fig. 4.16. Size distribution of the EPS:PAA-TiO₂ 1:10 mixture before and after 2 h centrifugation, 10⁻³ M NaCl, pH 8.

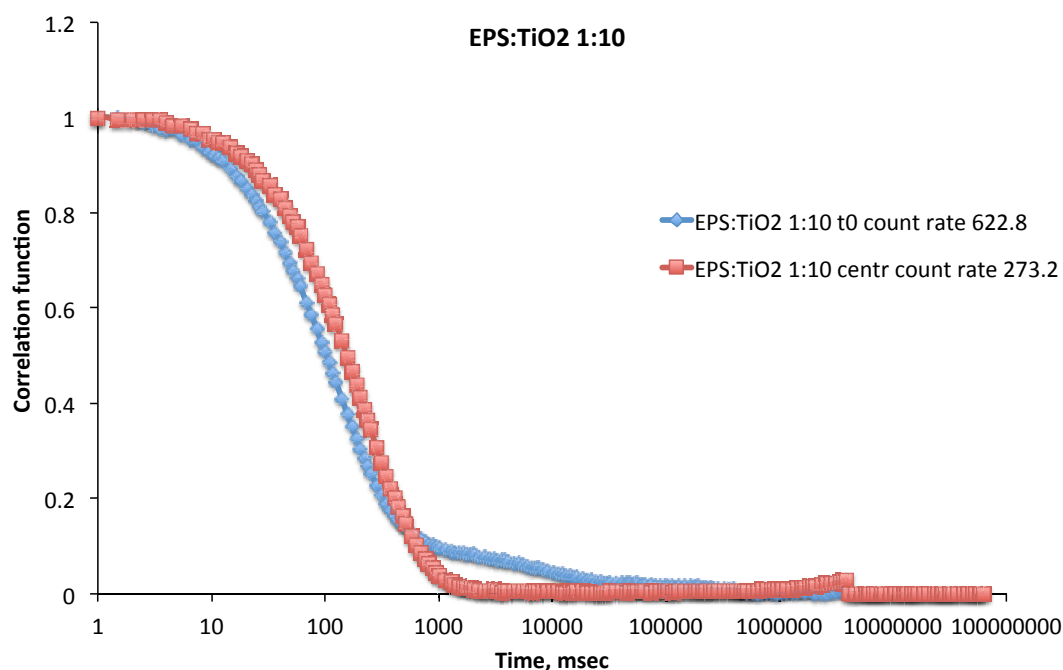


Fig. 4.17. Correlation function of the EPS:PAA-TiO₂=1:10 mixture before and after 2 h centrifugation, 10⁻³ M NaCl, pH 8.

To summarize the effectiveness of the centrifugation approach to separate NPs and free EPS, we can conclude that this procedure enabled us to remove the majority of NPs from the suspension and to measure only the remaining supernatant. The EPS underwent a minor fractionation through this process, with larger homoaggregates being removed and well dispersed macromolecules remaining stable in suspension/solution.

Analyzing the EPS:NPs mixture through this approach revealed distinct fates of the two materials through the centrifugation. Even if a fraction of NPs was shown in the previous section to heteroaggregate with the EPS despite the non favorable condition, the present approach evidences that a large fraction of the nanoparticles remains not associated with the EPS.

4.4.3. Interaction study of PAA-TiO₂ and EPS with agitation and centrifugation approach

Agitation step was performed in order to increase interaction time of the NPs and EPS. The procedure was as followed: the components were mixed at different ratios. They were covered with Aluminum foil and put in a dark place in order to prevent the alteration of the EPS by TiO₂ due to its UV activity and agitated upside down during 1 h.

Three DLS measurements were performed. One at time 0 of interaction, one after 1 h of agitation and one after 1 h of agitation followed by ultracentrifugation. Figures 4.18 to 4.20 show the correlograms measured for three EPS:PAA-TiO₂ ratios studied.

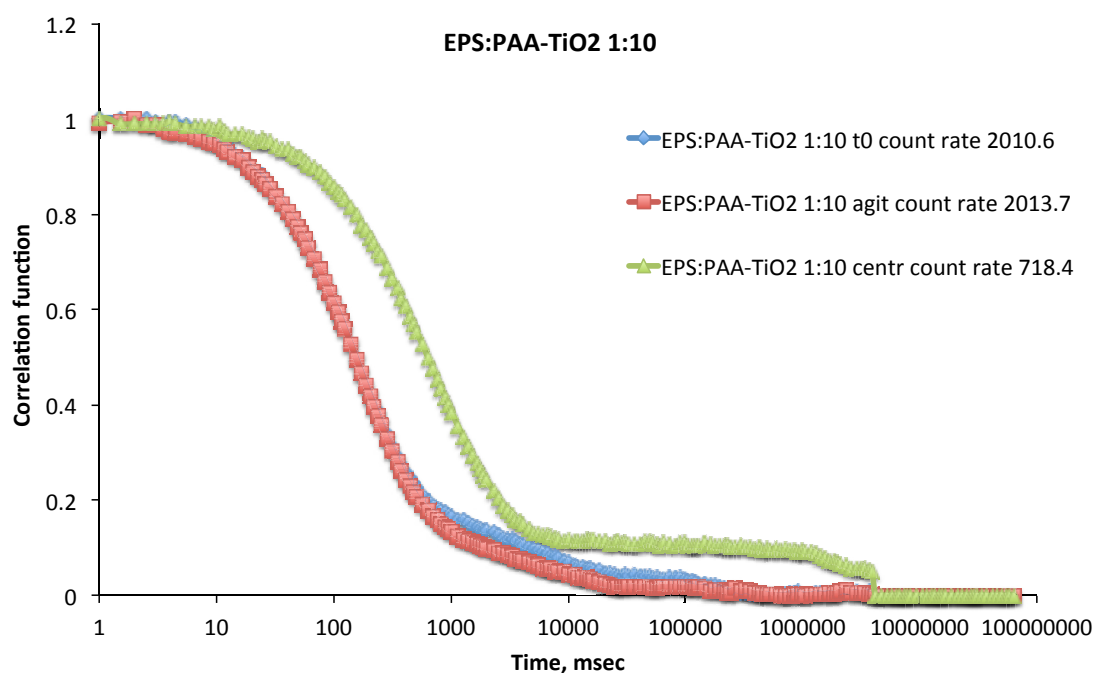


Fig. 4.18. Correlation function of the EPS:PAA-TiO₂=1:10 mixture before and after 1 h agitation and after 2 h centrifugation, 10⁻³ M NaCl, pH 8.

At the ration 1:10, as depicted in Fig. 4.18, after centrifugation, the NPs signal is removed and the signal of EPS appears around 1000 msec in diffusion time. This centrifugation effect is very similar to that observed in the previous section with the

same ratio but in absence of agitation. Here the 1h agitation time did not alter the DLS measurement as compared to the initial state.

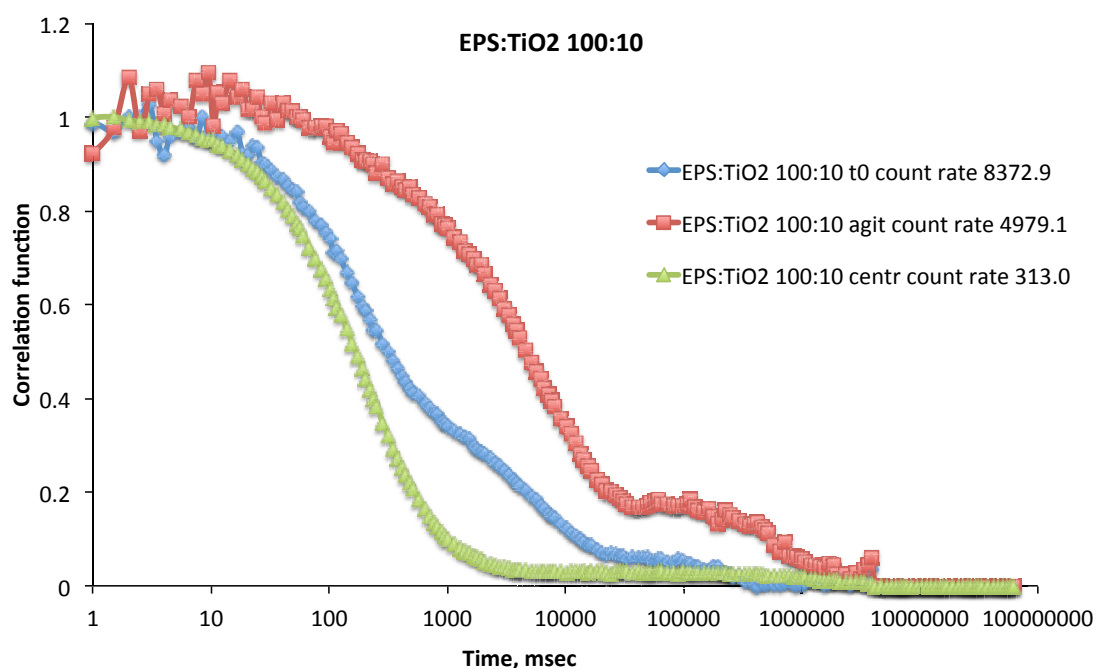


Fig. 4.19. Correlation function of the EPS:PAA-TiO₂=100:10 mixture before and after 1 h agitation and after 2 h centrifugation, 10⁻³ M NaCl, pH 8.

At the EPS:PAA-TiO₂ ratio 100:10, the correlograms shown in Fig. 4.19 indicate a progressive aggregation of the mixture along with the 1 h of agitation. This is evidenced by the increase diffusion time. In the same time, the measured count rate decreased from 8372 to 4979 despite the agitation supposed to maintain the material mass constant. This may be due to the inhomogeneity of the aggregated sample. After centrifugation, most of the aggregated material was removed from the suspension as suggested by the large decrease in count rate from 4979 to 313 k-counts. This was certainly favored by the aggregated state of the mixture just before centrifugation started. In the end, it is difficult to comment whether the material remaining suspended in the supernatant contains only well dissolved/dispersed EPS macromolecules that did not interact with the NPs, or some dispersed nanoparticles as well. The diffusion times measured are indeed very close to the typical small size or NPs. More probably, the majority of the NPs was involved in the aggregation process

evidenced with the EPS, and only the finest fraction of polysaccharide remained free and suspended.

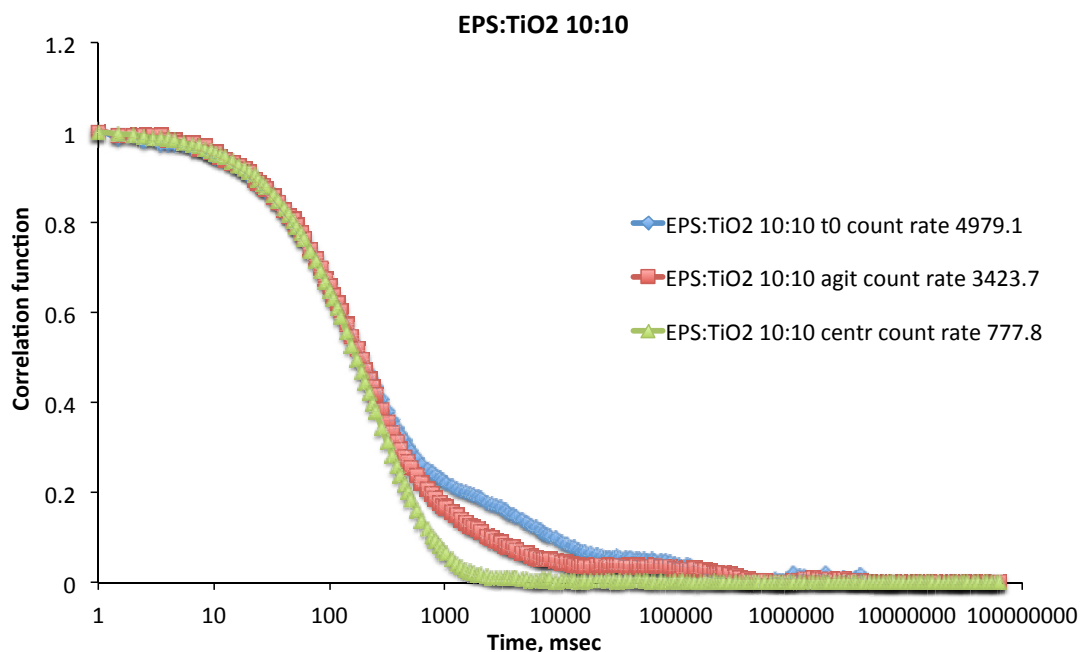


Fig. 4.20. Correlation function of the EPS:PAA-TiO₂=1:10 mixture before and after 1 h agitation and after 2 h centrifugation, 10⁻³ M NaCl, pH 8.

At the EPS:PAA-TiO₂ ratio 10:10, no clear fractionating effect was detected by DLS through either agitation time or centrifugation. The main population remains unchanged in a relatively low diffusion time range of 10-100 msec. Only the larger aggregates, already present in the initial mixture, are removed by the centrifugation process, as expected. These large aggregates may likely be constituted of the least dispersed fraction of the EPS and of the rapidly formed EPS-NPs heteroaggregates.

For all the ratios tested, the count rates measured after agitation decrease slightly in comparison with the initial mixture, while they decrease more than 3 times after centrifugation (Table 4.1).

Table 4.1. Derived count rates obtained for the mixtures

Conditions	Ratios EPS:TiO₂	100:10	10:10	1:10
after mixing		8372.9	4979.1	2020.6
after agitation		4979.1	3423.7	2013.7
after centrifugation		313.0	777.8	718.4

This indicates that agitation step during 1 hour influences the further process of centrifugation. Here, the NPs and EPS were maintained in suspension during 1h. This favored prolonged contact, and thus probably more and more material involved in the weak interaction occurring. This resulted after centrifugation in a larger removal of these materials from the supernatant in comparison to the partial removal obtained without agitation step.

To summarize the outcomes of the centrifugation process in our approach, the first limitation is that not the totality of the dispersed NPs is removed from the solution, even after 2 hours of ultracentrifugation. Approximately one quarter of the dispersed NPs remains in suspension. On the other hand, every material involved in any aggregate formation is removed from the suspension by the centrifugation step. This resulted also in a certain fractionation of the EPS as a function of its dispersion state. Large EPS aggregates, resulting from the partial dissolution in the EPS stock solution were removed, while the well dispersed macromolecules remained in the supernatant. Finally, even if ultracentrifugation enabled us to fractionate the materials in the EPS:NPs mixtures as a function of their dispersion state, coupling with DLS analysis for size measurement in the supernatant was not an approach really adapted to our system studied.

DLS does not distinguish clearly between NPs and EPS, as similar signal may be attributed either to aggregated NPs or dispersed EPS or the combination of both. This analytical approach gave us only a binary and preliminary view of the types of interaction likely occurring in our system.

For the EPS with UV measurements there were not obtained measured values with which it would be possible to quantify its amount in the solution. This is likely to that

detection limit is not compatible with measured values and is quite low 10 % of used concentration. Also EPS may not be left in the supernatant after the centrifugation in the enough quantity to measure. The possible concentration to determine with this method is between 10 and 70 mg/l which may not be the case of the used concentration also due to the detection limit.

4.5. Conclusion

Summarizing this study, under favorable conditions in all the mixture ratios studied, a tendency to significant aggregation of EPS with TiO_2 , and subsequent sedimentation of the aggregates formed, was observed. These aggregates were very rapidly formed, as already measured at the time 0 of interaction. Nevertheless, after 1 hour of such interaction under Brownian motion, a fraction of the nanoparticles still remained in suspension, and this was not involved in the aggregation process.

The same tendency were observed at 10:10 and 1:10, but a more limited aggregation occurred at 100:10 probably due to the saturation of NPs by the adsorbed EPS.

Under unfavorable conditions an unexpected aggregation was also observed between EPS and PAA- TiO_2 . Some aggregates formed rapidly, already detected at the initial time of the interaction. After one hour of interaction, they had settled out, but the some free polysaccharide and free nanoparticles remained clearly distinguishable, indicating their distinct behaviors in suspension. No clear effect of the EPS:PAA- TiO_2 ratio could be evidenced under theses conditions.

The nature of the attractive interaction between EPS and PAA- TiO_2 NPs under unfavourable conditions may be due to some heterogeneities in the structure of the EPS macromolecule. There may be some hydrophobic moieties in the EPS which favor interactions with the PAA- TiO_2 . The EPS may also compete with the PAA molecule for adsorption at the TiO_2 NP surface. The large molecular weight of the EPS is indeed expected to favor such competition at the expense of the PAA. In addition, the large molecular weight of the EPS may also favor physical interactions NPs, such as irreversible entrapment.

Chapter 5. Deposition of nanoparticles on a polysaccharide substrate

5.1. Introduction

In the natural environment EPS is mainly found as a film coating the surfaces in humid conditions (Flemming et al. 2007). That is why a next step of our investigation on the affinity of NPs to EPS is to study their deposition on an EPS gel surface. It was studied NPs deposition on a surface coated with bacterial polysaccharide compared to that on a bare SiO₂ mineral surface to estimate the effect of the collector colonization by bacteria on the NPs fate and to better understand the underlying mechanisms. The NPs surface functionalization, pH and salt concentration were varied to induce attractive or repulsive interactions of electrostatic or steric nature between the NPs and the substrate, and thus to examine how effective the NPs deposition was in various cases.

As in the previous chapter, TiO₂ NPs were selected in this work since they are widely used in different applications such as sunscreens and paints. Au NPs were studied here as well due to their stability in the suspension and in order to avoid the NPs aggregation to which TiO₂ NPs have a high tendency.

5.2. Attachment of TiO₂ NPs to a polysaccharide film immersed in a NPs suspension

The first part of this approach was to study the affinity of NPs for the gel by immersing a gel film in the NPs suspension and measuring the evolution in NPs concentration in the suspension with time. The aim of this approach was to evidence the NPs catchment by the gel film along with their random Brownian motion in suspension. Any decrease in the NPs concentration in suspension will indicate their removal from the suspension, due either to random catchment by the gel or to sedimentation following aggregation.

Both favorable and unfavorable conditions for the attachment were prepared. TiO₂ NPs were used at pH 4 and pH 8 and PAA-TiO₂ were used at pH 8. TiO₂ NPs are positively charged at pH 4 and negatively charged at pH 8. PAA-TiO₂ is negatively charged at pH 8.

Gel film was prepared with EPS solution at 15 g/l. A 10 g/l gel concentration was first tested, but resulted in an unstable film which tended to dissolve with time. 15 g/l was finally used in order to have stronger and more stable gel film. The gel film was prepared by pouring 0.15 ml of hot solution at 15 g/l on the bottom of a quartz UV cuvette with 1 cm optical path width. This lead to exactly 1 cm² of gel deposit, constituting the surface area possibly interacting with the NPs in the next step (Figure 5.1). When the gel was cooled down, 2.3 ml of NPs suspension (100 mg/l) was poured in the cuvette. The quantification of NPs behaving in suspension in the cuvette was achieved at different times after pouring, using UV absorbance spectroscopy at 270 nm wavelength.

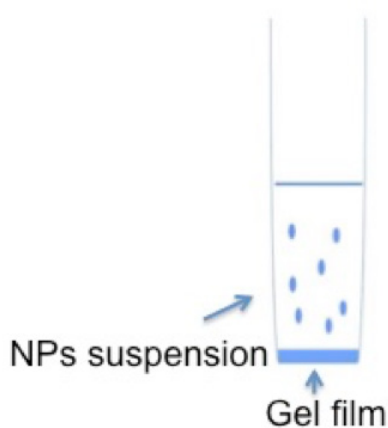


Fig. 5.1. Gel film location in the NPs suspension: made of 15 g/l EPS solution

As observed in Fig. 5.2, there is no change in concentration of PAA-TiO₂ at pH 8 with time. It remains stable in suspension in presence or absence of the gel film. This suggests that there is no attachment of the PAA-TiO₂ NPs to the gel film under these conditions. Indeed, electrostatic repulsive interactions were expected between the negatively charged EPS and the negatively charged NPs here. The effect of the pH on the extent of negative charge in the EPS and at the NPs surface, and thus on their relative affinity, could not be distinguished here between pH 5 and 8. Nevertheless, a slightly higher affinity can be detected at pH 5 using QCM-D (see next section).

Using TiO₂ NPs at pH 8, a decrease in concentration with time is measured, till almost complete NPs removal from the suspension. Nevertheless, these conditions are also expected as unfavorable since both EPS and TiO₂ NPs are negatively charged.

Here, the decrease in NPs concentration in suspension is actually due to their homoaggregation and sedimentation regardless to the presence or absence of the gel film. Indeed, adjusting the NPs suspension pH to 8 implied passing through the TiO_2 isoelectric point ($\text{IEP}=4.9$), which is accompanied by an irreversible aggregation of the NPs. Despite the net negative charge acquired at the NPs surface at the final pH 8, the NPs did not redisperse but remained aggregated and unstable in the suspension.

At pH 4, favorable conditions for TiO_2 NPs attachment to the gel are expected, due to their respective opposite surface charges. The NPs remained stable in suspension, as confirmed by the concentration time evolution in absence of gel. In the presence of the gel film, we did measure a decrease in the NPs concentration with time. Approximately half of the NPs remain in suspension after 1500 min of contact with the gel. This reveals that NPs diffusing in the medium attach to the gel, certainly due to electrostatic attractions. The NPs removal rate calculated from the highest slope measured in Fig. 5.2 was $2 \times 10^{-2} \text{ mg/cm}^2/\text{h}$. The total mass of the NPs deposited per gel mass was 56.22 mg/g.

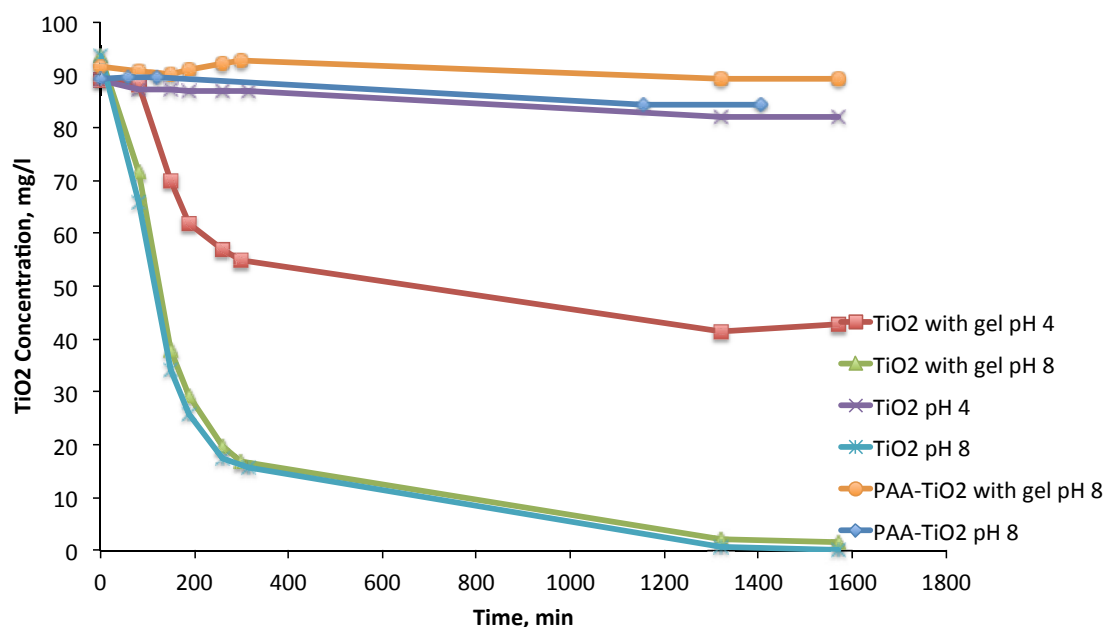


Fig. 5.2. Concentration of TiO_2 NPs dependence on time under favorable and unfavorable conditions

Finally, with this approach, it was not possible to accurately investigate the affinity and interaction of TiO_2 NPs and EPS under all favorable and unfavorable conditions due to the NPs sedimentation in the cuvette. This is why in the next experimental approach we measured the deposition of NPs directly in-situ on the polysaccharide layer.

5.3. Deposition of TiO_2 NPs on the polysaccharide and SiO_2 substrate

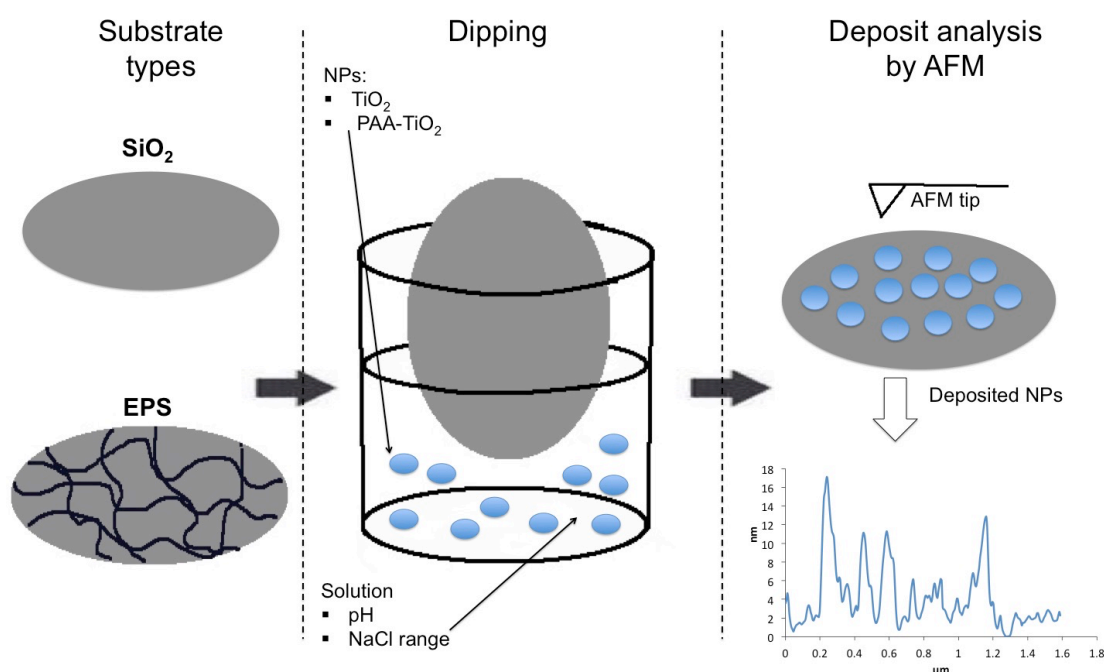


Fig. 5.3. Scheme of the experimental approach

NPs deposition was studied both by AFM after dipping the substrate in NPs suspension and by QCM-D in flow mode (Fig. 5.3). Favourable and unfavourable conditions for NPs deposition on the substrate were obtained by controlling the pH and salt concentration of the NPs dispersing medium.

For pure TiO_2 NPs with an IEP of 4.9, favourable conditions for deposition were obtained by adjusting the pH to 3. Although this condition is not environmentally relevant, our aim here was to obtain contrasting conditions that enable us to better

assess the deposition mechanism. At this pH, TiO₂ NPs are positively charged and stable in suspension, which favours a high affinity for the negatively charged substrate due to electrostatic attractions. However, the TiO₂ NPs repel each other when they are highly charged (Labille et al. 2015), which may limit the deposition density on the substrate. This effect was studied by varying the ionic strength of the solution and altering the type of electrolyte used; either NaCl or CaCl₂ electrolyte was used.

The PAA-coated TiO₂ has an IEP of 2.7. At pH 3, the PAA coating, as well as the polysaccharide and silica substrate surfaces, is weakly charged due to the protonation of the acidic moieties. This may favour weak hydrophobic interactions between the –OH groups of the organic molecules via hydrogen bonding (Anirudhan & Ramachandran 2006). On the other hand, at the natural pH = 5.6, unfavourable conditions for deposition are expected due to the negative charges, which result in electrostatic and steric repulsions between NPs and the substrate.

5.3.1. Deposition of NPs under attractive electrostatic interactions with the substrate

The deposition of NPs under attractive electrostatic interactions with the substrate was studied using positively charged pure TiO₂ NPs at pH = 3. Figure 5.4 shows AFM images of the polysaccharide-coated substrate surface after deposition of pure TiO₂ in NaCl 10⁻³ M or in NaCl-free solution. Both the topography and phase AFM images show less deposition of TiO₂ NPs in NaCl-free solution than in 10⁻³ M NaCl (θ_{AFM} = 12 vs. 40 %, Table 5.1). Figure 5.5 shows AFM images of the mineral substrate after deposition of TiO₂ NPs under the same conditions. As was previously observed with the polysaccharide substrate, the NPs deposition is more pronounced at the higher ionic strength (θ_{AFM} = 39.5 vs. 100 %, Table 5.1). Moreover, the density of the NPs deposit appears higher on the mineral substrate than on the polysaccharide substrate in both salt conditions. Finally, 10⁻³ M NaCl and mineral substrate appear to be the optimal conditions that lead to the highest NPs deposition density for a complete layer of NPs. The height profile indicates an average thickness of the NPs deposit of 8 nm in all conditions (Fig. 5.4 e, f and Fig. 5.5 e, f), which corresponds to the average NPs size at this pH. This suggests that NPs were not accumulated in patches but remained

distinct after deposition, and that a maximum of only one monolayer of NPs could be obtained in the optimal conditions.

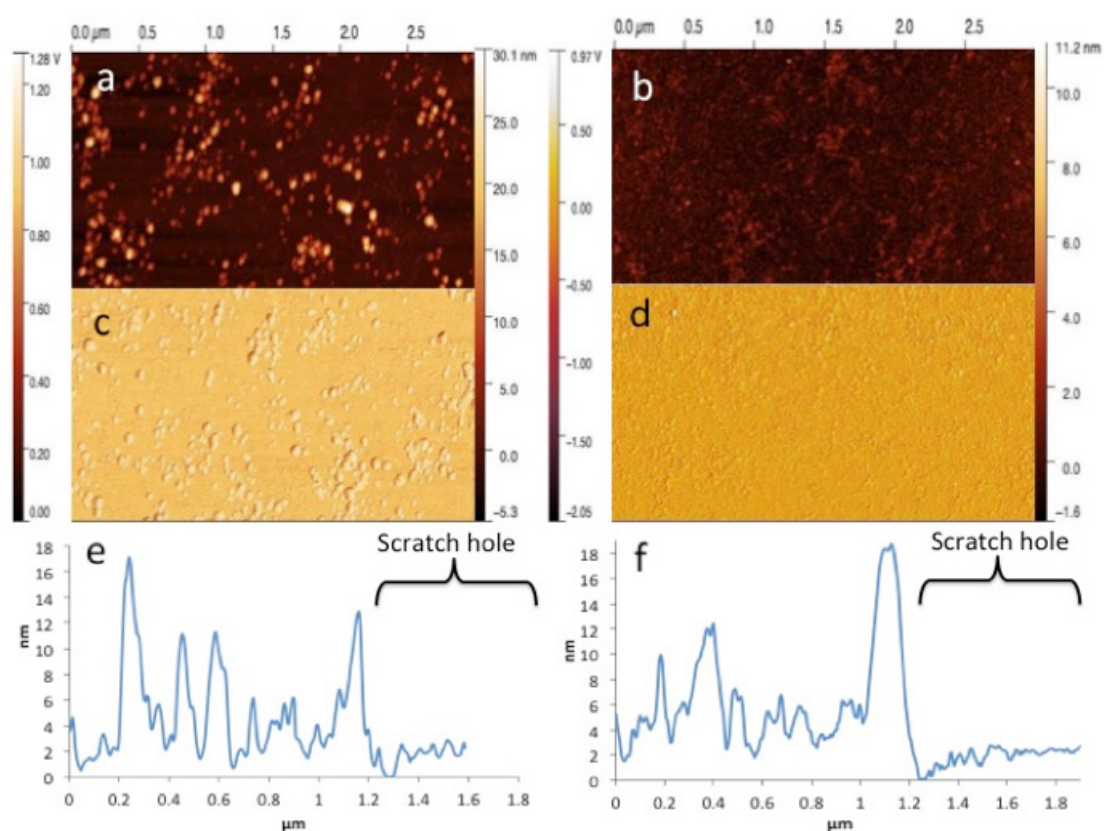


Fig. 5.4. AFM images of TiO₂ NPs deposition on the polysaccharide substrate in NaCl-free solution (a, c) or in 10⁻³ M NaCl (b, d) at pH = 3. Images display topographic mode (a, b), phase mode (c, d) and scratch height profile (e, f)

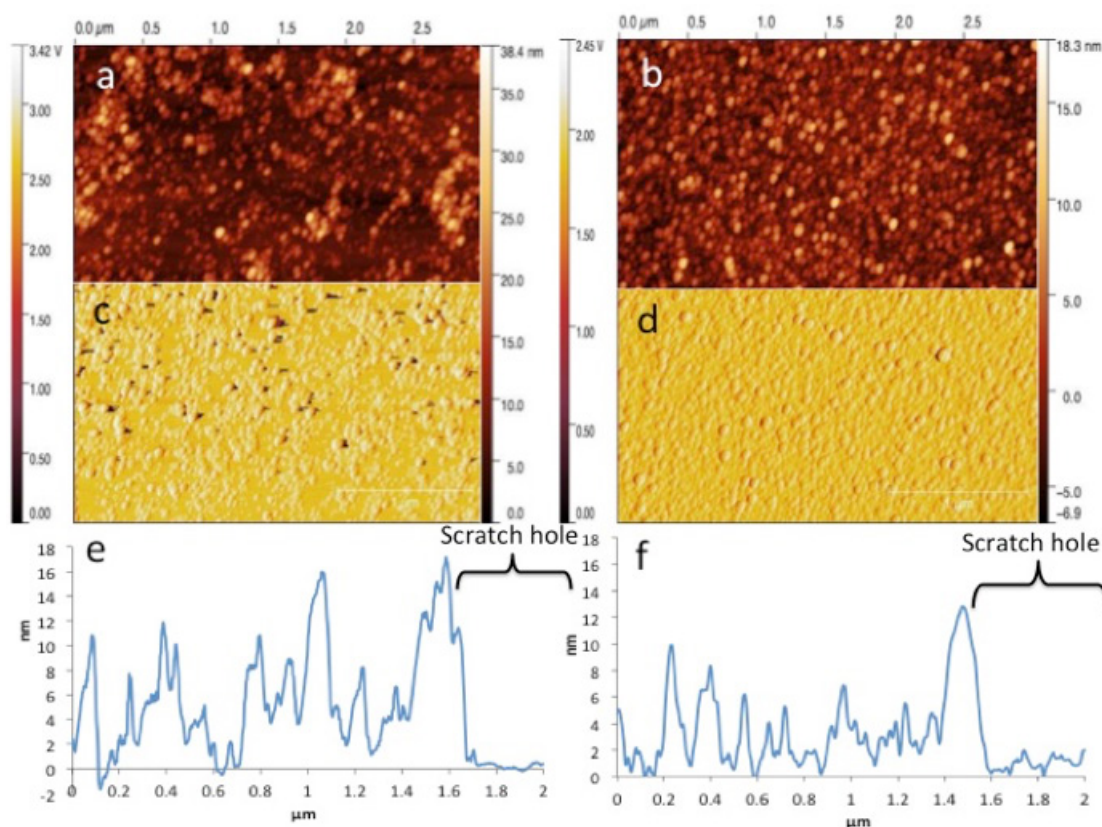


Fig. 5.5. AFM images of TiO₂ NPs deposition on the mineral surface in NaCl-free solution (a, c) or in 10⁻³M NaCl (b, d) at pH = 3. Images display topographic mode (a, b), phase mode (c, d) and scratch height profile (e, f)

Figure 5.6 illustrates the raw QCM-D data of the frequency variation with time, which corresponds to the deposited mass of NPs on the substrate, and the dissipation values, which correspond to the viscoelastic properties of the deposited layer. The zero-time corresponds to time at which the NPs are injected into the feed solution. The frequency recorded at the plateau corresponds to the total deposited mass of NPs on the substrate (eq. 2.2). Contrasting deposits are demonstrated as a function of the substrate type or the presence of salt. In agreement with AFM measurements, the NPs deposition is less pronounced on the polysaccharide substrate than on the silica substrate and in NaCl-free solution than in 10⁻³ M NaCl solution. Moreover, the low dissipation values measured in all cases indicate that the deposited layers are stiff during the entire experiment.

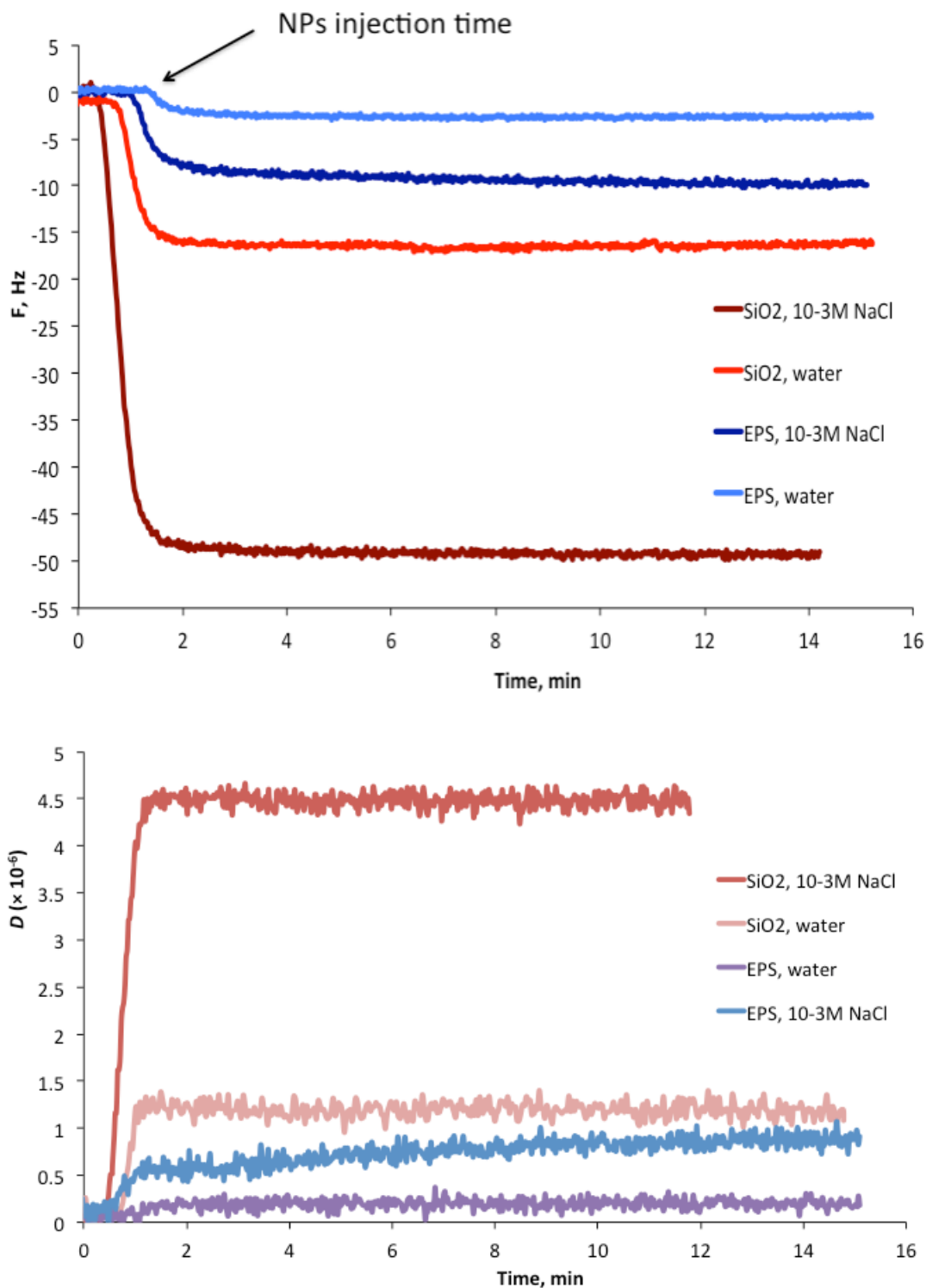


Fig. 5.6. Frequency change dependence a) and dissipation b) measured on QCM-D crystal when TiO₂ NPs deposit as a function of the electrolyte, water or 10⁻³ M NaCl and of the sensor surface chemistry, SiO₂ or polysaccharide (EPS). Time 0 corresponds to the NPs injection in the condition SiO₂, 10⁻³ M. Plots are shifted of 30 s from each other along the x axis for better distinction. pH = 3

The rinsing step and the second NPs injection, each performed after the frequency plateau for at least five minutes, did not lead to any measurable frequency change (Fig. 5.7). This suggests that the deposited NPs undergo strong interactions with both polysaccharide and silica substrates that cannot be eliminated as long as the same electrolyte condition is maintained, and it demonstrates that the number of NPs deposited at the plateau corresponds to a maximum under these conditions.

The underlying physicochemical mechanism that drives the NPs deposition will be discussed with regards to different characteristics of the system.

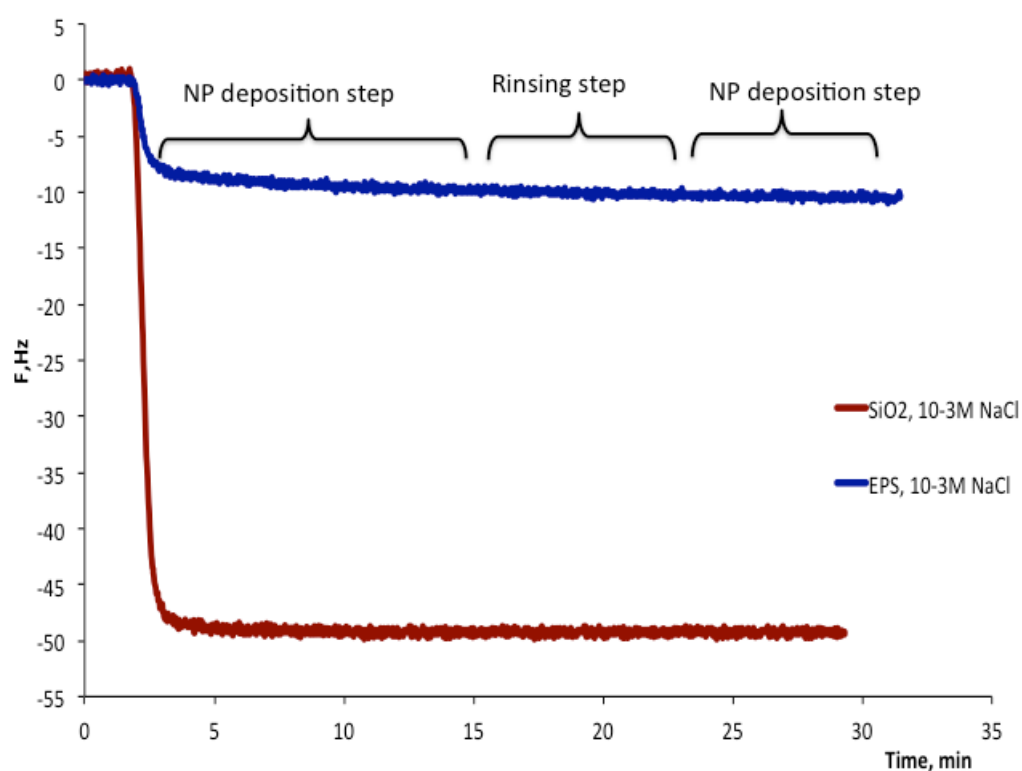


Fig. 5.7. Frequency change dependence measured on QCM-D crystal representing rinsing and second TiO₂ NPs deposition step as a function of the electrolyte 10⁻³ M NaCl and of the sensor surface chemistry, SiO₂ or EPS. pH = 3

Table. 5.1. Maximum surface coverage of TiO₂ NPs on the polysaccharide (polysac.) or silica substrate, as estimated from AFM and QCM-D measurements

Sample Characteristics	Thickness ⁱ , <i>nm</i>	Coverage $\theta_{\text{AFM}}^{\text{ii}}$, %	NP deposit mass QCM-D, <i>mg/m²</i>	Coverage $\theta_{\text{QCM-D}}^{\text{iii}}$, %
polysac., H ₂ O	7.7	12.0	0.51	4.3
polysac., 10 ⁻³ M NaCl	8	40.1	2.19	18.1
silica, H ₂ O	6	39.5	3.19	21.1
silica, 10 ⁻³ M NaCl	8	100	8.67	61.7

range of experimental error = ⁱ: +/- 6%; ⁱⁱ: +/- 5%; ⁱⁱⁱ: +/- 7%

Effect of the Ionic strength – The final surface coverage measured by both AFM (θ_{AFM}) and QCM-D ($\theta_{\text{QCM-D}}$) is plotted in Figure 5.8 as a function of the salt concentration. θ_{AFM} increases from 40 to 100 % on the silica substrate and from 12 to 40 % on the polysaccharide substrate as the NaCl concentration increases from 0 to 10⁻³ M.

The observed effect of the ionic strength can not be related to the attractive interaction between the NPs and the substrate, since according to the DLVO theory, under such condition of attractive electrostatic contribution, the deposition should increase when the ionic strength decreases. Actually, the interfacial forces driving the NPs deposition are not only between the NPs and the substrate, but also between the neighbour NPs on the substrate. The effect of ionic strength is likely due to the electrostatic repulsions between the neighbouring NPs, which are higher at low salt concentrations. However, the ζ potential that is measured separately on the nanoparticles suspended in the same electrolytes and in absence of polysaccharide or collector reveals that there is no significant evolution ($\zeta = +35 \pm 5$ mV) of the NPs surface charge in the moderate salt concentration range tested here 10⁻⁵-10⁻³ M (Table 5.2).

Table 5.2. Zeta Potential values of TiO₂ NPs at = pH 3 and different NaCl

NaCl Concentration, M	Zeta Potential ζ , mV
0	40
10^{-5}	40
10^{-4}	30
10^{-3}	35

Indeed, the NPs remain stable and dispersed under these conditions, i.e., with an attachment efficiency for homoaggregation (α_{NP-NP}) of zero (Labille et al. 2015). Nevertheless, the NPs deposition density increases as the ionic strength increases. This suggests that the electrostatic repulsions between neighbouring NPs are lower when they are deposited on an oppositely charged substrate than when they are in the suspension. This is due to a local effect of the negatively charged substrate, which partially balances the positive charge of the NPs. This has been evidenced on similar systems that the particle-particle interaction potential is not the same in the presence of a charged wall as in the bulk, especially in conditions of low $K.a$ values as here (where K is the inverse Debye length and a is the particle radius, Table 5.3) (Semmler et al. 1998), (Sadowska et al. 2016). The inverse Debye length is used to depict the effect of ionic strength on the collision efficiency. The Debye length (K^{-1}) is a characteristic length for the diffuse double layer thickness. This is due to the particle - wall interactions (Oberholzer et al. 1997). As a consequence, the salt effect, which was insignificant in the suspended NPs, becomes significant on the substrate where it can tune the deposition density. When the ionic strength increases, the electrostatic repulsions between neighbouring NPs reduce, which results in a denser NPs deposit.

Table 5.3. Calculation of the inverse Debye length K as a function of the ionic strength I of the system, a is the particle radius $6 \cdot 10^{-9}$ m.

I (M)	K (m^{-1})	$K.a$	$1/K$ (m)
10^{-5}	$1.03 \cdot 10^7$	$6.18 \cdot 10^{-2}$	$9.71 \cdot 10^{-8}$
10^{-4}	$3.25 \cdot 10^7$	$1.95 \cdot 10^{-1}$	$3.08 \cdot 10^{-8}$
10^{-3}	$1.03 \cdot 10^8$	$6.18 \cdot 10^{-1}$	$9.71 \cdot 10^{-9}$

Moreover, this effect is also observed and more pronounced in the presence of CaCl_2 electrolyte for the polysaccharide surface, where similar ranges for the θ_{AFM} (5 – 25%) and ζ potential are obtained when the CaCl_2 concentration increases from 10^{-5} to 10^{-4} M (Table 5.4). It should be noted that due to the positive surface charge of the NPs, the acting counterion is Cl^- . So changing the cation valence from 1 to 2 in a given salt concentration only affects the charge screening mechanism by doubling the counterion concentration.

At low ionic strengths, the value of the Debye length is high and repulsive double layer interactions extend to large distances. Since electrical double layer interactions arise from the overlap of diffuse double layers during the deposition process, the parameter K has a significant effect on the collision efficiency and deposition.

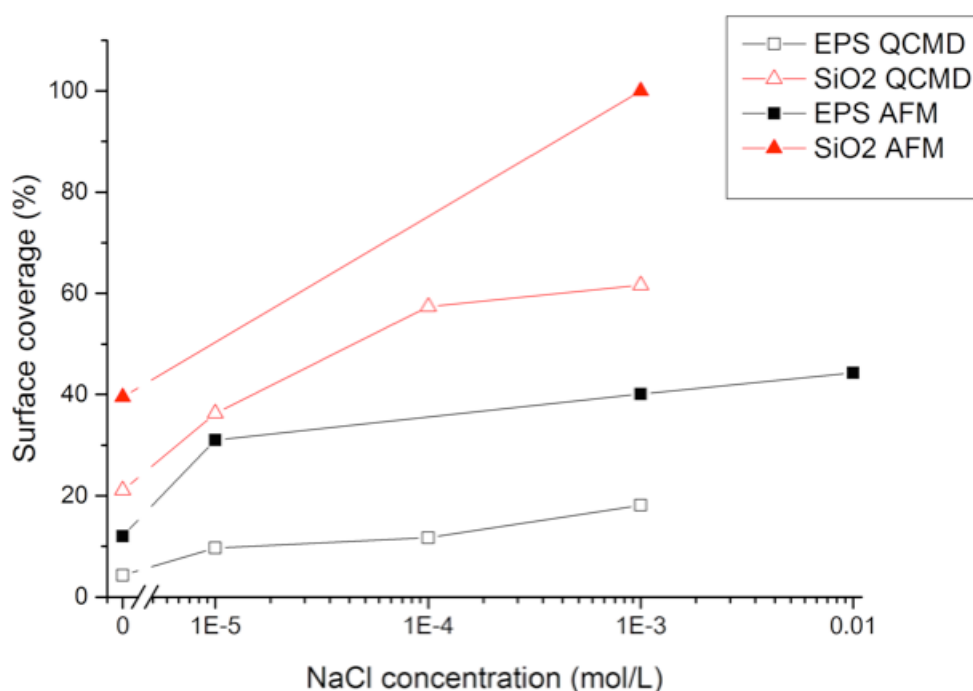


Fig. 5.8. Surface Coverage θ of the TiO_2 NPs deposited on the polysaccharide (EPS) or SiO_2 substrate, measured by QCM-D or AFM, as a function of NaCl. pH = 3

Effect of the substrate charge density - The polysaccharide substrate has approximately 2-3 times less surface coverage of NPs than the silica substrate (Table 5.1). This contradicts with the possible roughness effect of the polysaccharide substrate that, we could expect, would favour the NPs deposition. Here, the NPs – substrate interactions are electrostatically attractive, and thus they are likely limited by the respective surface charge densities of polysaccharide and silica substrates. At the silica surface, the Si-O⁻ group density was estimated 0.49 per nm² at pH = 3 (Duval et al. 2002). Normalized to the surface occupied by one NP, this results in 14 anionic groups potentially available per NP. With the polysaccharide substrate, the lower surface coverage measured suggests that the affinity for NPs deposition is lower, i.e. that the surface density of available anionic groups is lower than with silica. This surface density of reactive groups can be roughly estimated for the polysaccharide substrate. The proton titration of the polysaccharide indicates a charge density of 0.025 mmol/g (Fig. 2.2), and the QCM-D frequency change corresponding to the polysaccharide mass initially deposited on the crystal indicates 100 Hz, i.e. 17.7 mg/m² (data not shown). In turn, this gives approximately 0.27 anionic group per nm² on the polysaccharide substrate. Moreover, a fraction of these moieties may not be accessible to bind the depositing NPs. The polysaccharide substrate charge density is thus at least three times lower than that of silica, which agrees well with the lower surface coverage measured with the former.

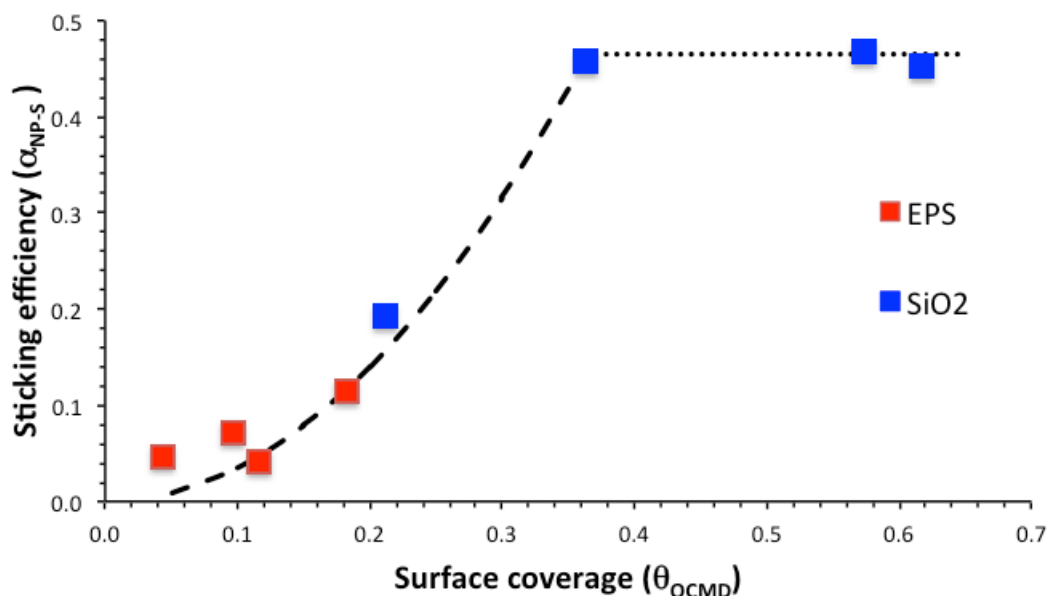


Fig. 5.9. Sticking efficiency of TiO_2 NPs on polysaccharide (EPS) and SiO_2 substrate as a function of the final surface coverage θ_{QCMD} . The dashed line corresponds to the reaction-limited regime, described by the equation $\alpha_{\text{NP-S}} = 3.5 \times \theta^2$. The dotted line corresponds to the plateau of the transport-limited regime

NPs deposition density – The surface coverage measured by AFM is higher than that calculated with the QCM-D method. This is because AFM and QCM-D data cannot be compared directly. The AFM probes the sample topography with a horizontal detection limit given by the 8 nm radius tip, so that some voids smaller than 8 nm between neighbouring NPs may remain inaccessible and appear occupied. On the other hand, QCM-D measures the exact mass of the NPs and the associated water that are deposited on the substrate. Of note, the maximum value θ_{QCMD} obtained in this work with 10^{-3} M NaCl and the SiO_2 substrate, 61.7 %, falls relatively close to the theoretical maximum value 54.7 % expected from the Random sequential adsorption model in two dimensions, i.e. the jamming limit (Hinrichsen et al. 1986). This suggests a very short distance between the neighbouring NPs. It was estimated here by considering a simple square arrangement for NPs deposited on the substrate, giving 3.1 nm for a 61.7 % surface coverage. Such a short distance is indeed not distinctly measured with the 8 nm radius AFM tip. Confirming the wall effect

previously described, these distances are shorter than the Debye lengths, $1/K = 9.7$ to 97 nm, calculated respectively in the range $10^{-3} - 10^{-5}$ M NaCl (Table 5.3).

Attachment efficiency - The deposition rates r_d of TiO_2 NPs on polysaccharide and SiO_2 substrates were estimated from the slope of the decrease in frequency for the raw QCM-D data (Table 5.4). A theoretical particle deposition rate, only limited by NPs transport, r_d^{SL} , was calculated using the Smoluchowski-Levich equation (eq. 2.3). Normalizing the experimental r_d to r_d^{SL} results in the actual sticking efficiency of NPs to the substrate, $\alpha_{\text{NP-S}}$. In particular, $\alpha_{\text{NP-S}}$ theoretically ranges from 0 when no deposition takes place to 1 when every colliding NP attaches to the collector. The obtained values are reported in Table 5.4 and in Figure 5.9 as a function of θ_{QCMD} . They range from 0.05 to 0.11 on the polysaccharide substrate and from 0.14 to 0.47 on the SiO_2 substrate.

In the presence of salt, r_d reaches a plateau at 170 g/min.m^2 on the SiO_2 substrate, which corresponds to the maximum sticking efficiency measured in all our studied conditions, $\alpha_{\text{NP-S}} = 0.47$ (Fig. 5.9). The fact that this experimental value is two times lower than the maximum theoretical value r_d^{SL} can reasonably be attributed to the NPs polydispersity between 5 and 20 nm. Note that by changing the NPs radius from 6 nm, used here in agreement with the AFM measurement, to 9 nm, eq. 2.3 would result in a r_d^{SL} value that is two times lower (186 g/min.m^2), which fits perfectly with the maximum r_d measured here. This reflects the high sensitivity of r_d^{SL} to the NPs size. Therefore, we can reasonably assume that the r_d plateau observed in Figure 5.9 corresponds to the regime of NPs deposition that is limited by transport. Within this regime, increasing θ , i.e. increasing the capacity of the substrate for NPs deposition, has no effect on the sticking efficiency, since the latter is limited by the NPs input (the concentration, flow rate, and diffusion coefficient).

Table 5.4. QCM-D data obtained from frequency change, in terms of the slope of the raw data, deposition rate and sticking efficiency, as a function of the substrate type, polysaccharide (polysac.) or silica

Salt used	Slope, Hz/min		Deposition rate, $r_d, \text{g/min.m}^2$		Sticking efficiency, α_{NP-S}	
	polysac.	silica	polysac.	silica	polysac.	silica
water	7.6	31.2	17.13	70.34	0.05	0.19
10^{-5}M NaCl	11.82	74.3	26.64	167.52	0.07	0.46
10^{-4}M NaCl	6.9	76.1	15.57	171.58	0.04	0.47
10^{-3}M NaCl	18.61	73.36	41.96	165.4	0.11	0.45
10^{-5}M CaCl_2	8.78	49.46	19.8	111.52	0.05	0.3
10^{-4}M CaCl_2	14.22	22.15	32.06	49.94	0.09	0.14

In Figure 5.9, α_{NP-S} values lower than 0.47 indicate the regime limited by the sticking reaction. In this domain, a correlation is observed between the sticking efficiency α_{NP-S} and the maximum surface coverage of the substrate θ_{QCM-D} . This is because for a given NPs input flow, a higher substrate capacity enables a higher deposition rate. In turn, this indicates that the interparticle repulsion forces drive not only the NPs arrangement density on the substrate but also their deposition rate. A power-2 relation describes the evolution of α_{NP-S} with θ_{QCM-D} , where $\alpha_{NP-S} = 3.5 \times \theta^2$. It is worthwhile to note that it likely reflects the dimensionality of the surface area in θ , while the factor of 3.5 can be attributed to the steric volume occupied by the NPs on the substrate.

At the beginning the first preliminary AFM studies for TiO_2 NPs in 10^{-3} M NaCl of 1 g/l concentration were performed. Favorable and unfavorable conditions were proceed at pH 4 and pH 8 respectively. Immersion time for each layer was 5 min.

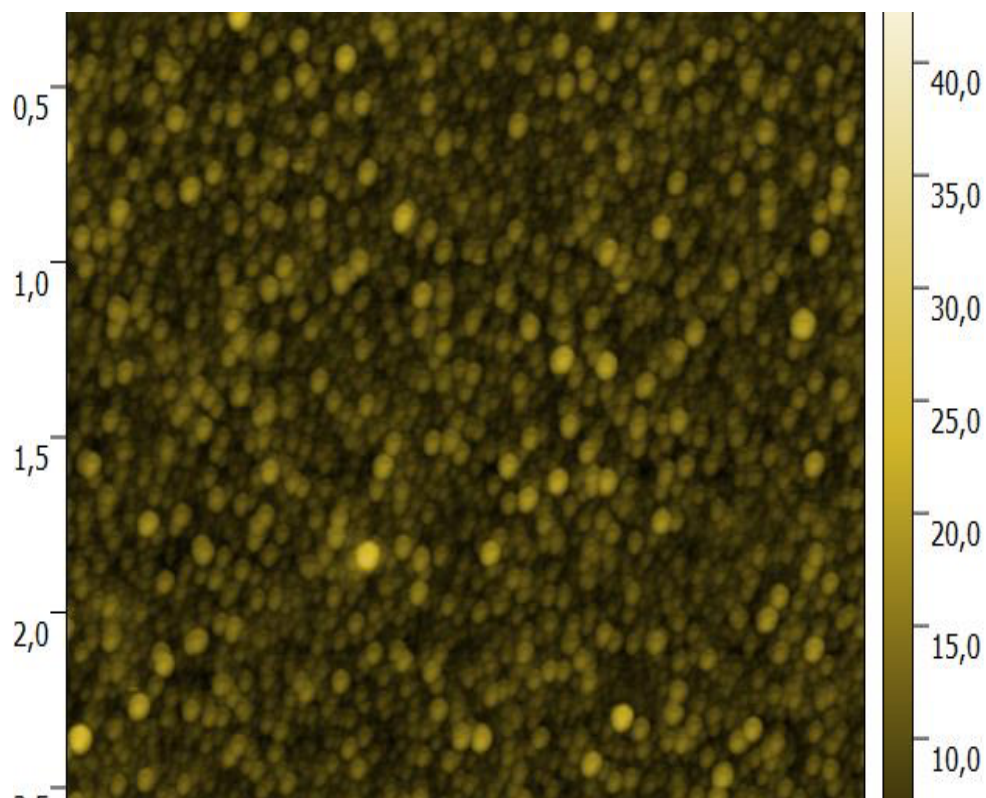


Fig. 5.10. AFM images (topographic mode) of TiO₂ NPs deposition on the polysaccharide substrate at pH = 4.

As it is revealed by AFM image (Fig. 5.10) there were observed high deposition on the polysaccharide layer under these favourable conditions in 10^{-3} M NaCl. AFM images of NPs deposition on PAH layer reveal no observed particles on the surface.

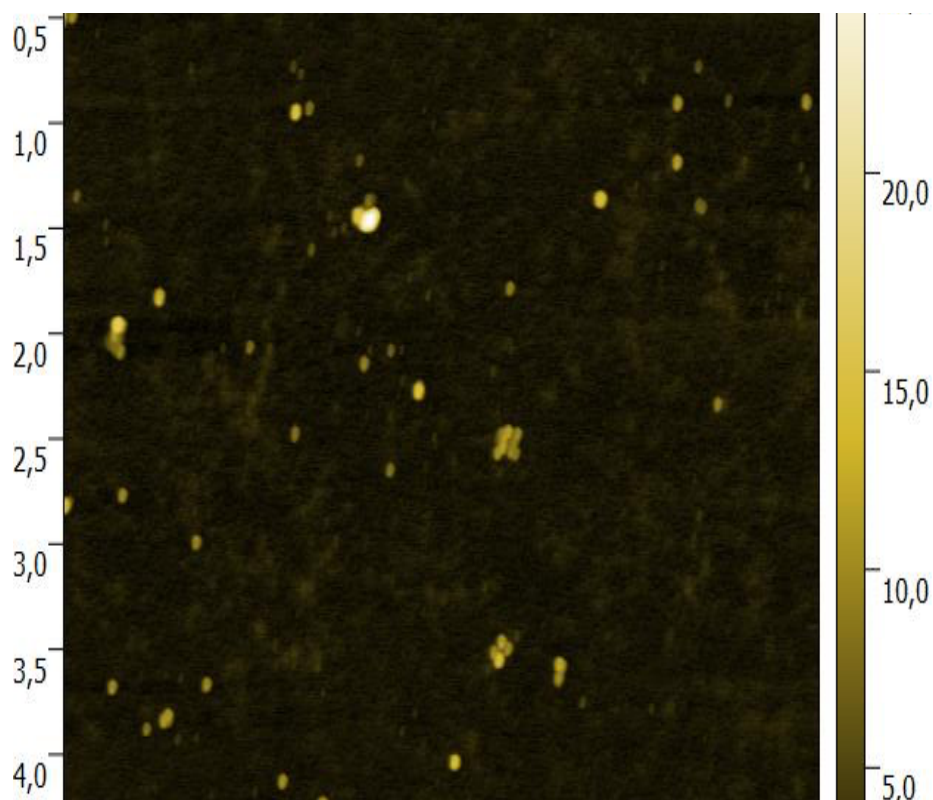


Fig. 5.11. AFM images (topographic mode) of TiO₂ NPs deposition on the polysaccharide substrate at pH = 8.

As it is observed under unfavourable conditions (Fig. 5.11) there is a slight deposition of TiO₂ NPs on the polysaccharide layer. This reveals that there is no interaction with a polysaccharide and TiO₂ NPs under these conditions while they adsorb on PAH layer that is positively charged (Fig. 5.12).

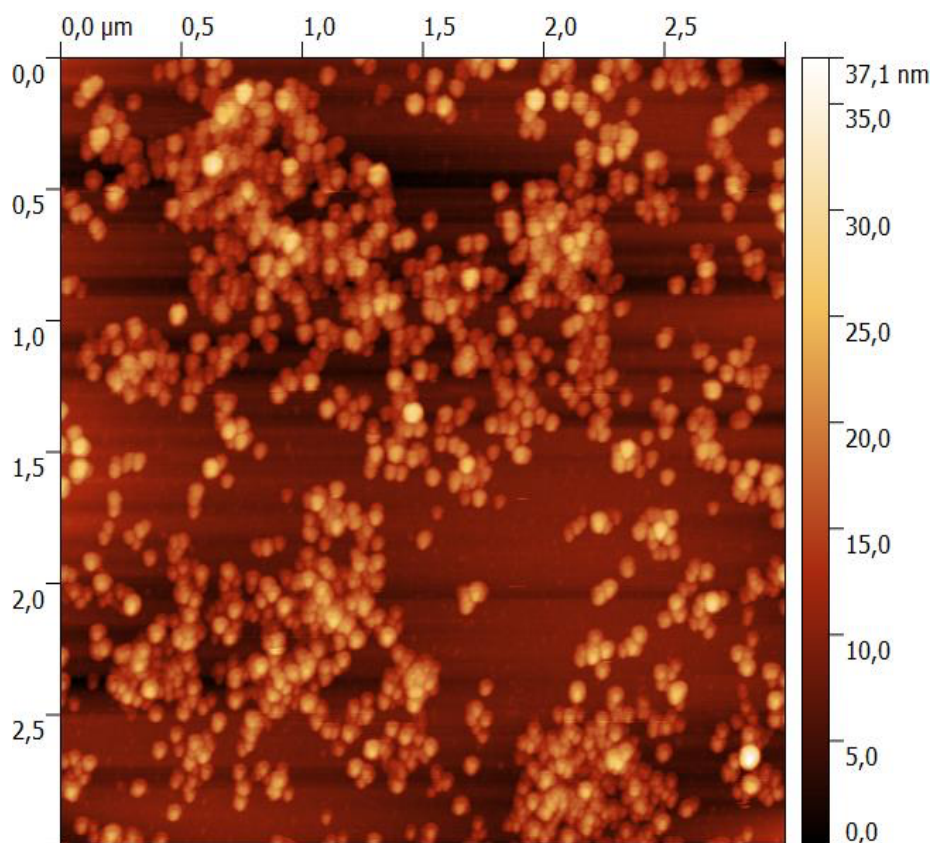


Fig. 5.12. AFM images (topographic mode) of TiO_2 NPs deposition on the PAH substrate at $\text{pH} = 8$.

Further study conditions were optimized to make them closer to the environmental concentrations.

5.3.2. NPs deposition under repulsive electrostatic interactions with the substrate

The deposition of NPs under electrostatic repulsive conditions was measured for PAA-coated TiO_2 NPs at $\text{pH} = 3$ and 5.6. The polyacrylate coating gives the NPs a pH_{IEP} of 2.7, i.e., a surface charge close to zero at $\text{pH} = 3$ and a more negative charge at $\text{pH} = 5.6$ (Fig. 2.4). This is expected to impact the deposition on the negatively charged substrate and result in less repulsive interactions at $\text{pH} = 3$ than at the natural pH . However, such a trend is not shown in Figure 5.13 where the raw QCM-D data for dissipation and frequency changes with time are plotted. A higher frequency change is measured for the NPs injection at $\text{pH} = 5.6$ than at $\text{pH} = 3$, which indicates a

higher deposition of the PAA-coated TiO_2 at higher pH. Moreover, the higher dissipation values measured at pH = 5.6 reveal a more viscoelastic behaviour. This is likely caused by a larger conformational extension of the PAA coating at the NPs surface, due to the more negative structural charge at pH = 5.6 (Laguecir et al. 2006). Moreover, the NPs also tend to aggregate at pH = 3 due to the lower surface charge, which decreases the viscoelastic behaviour.

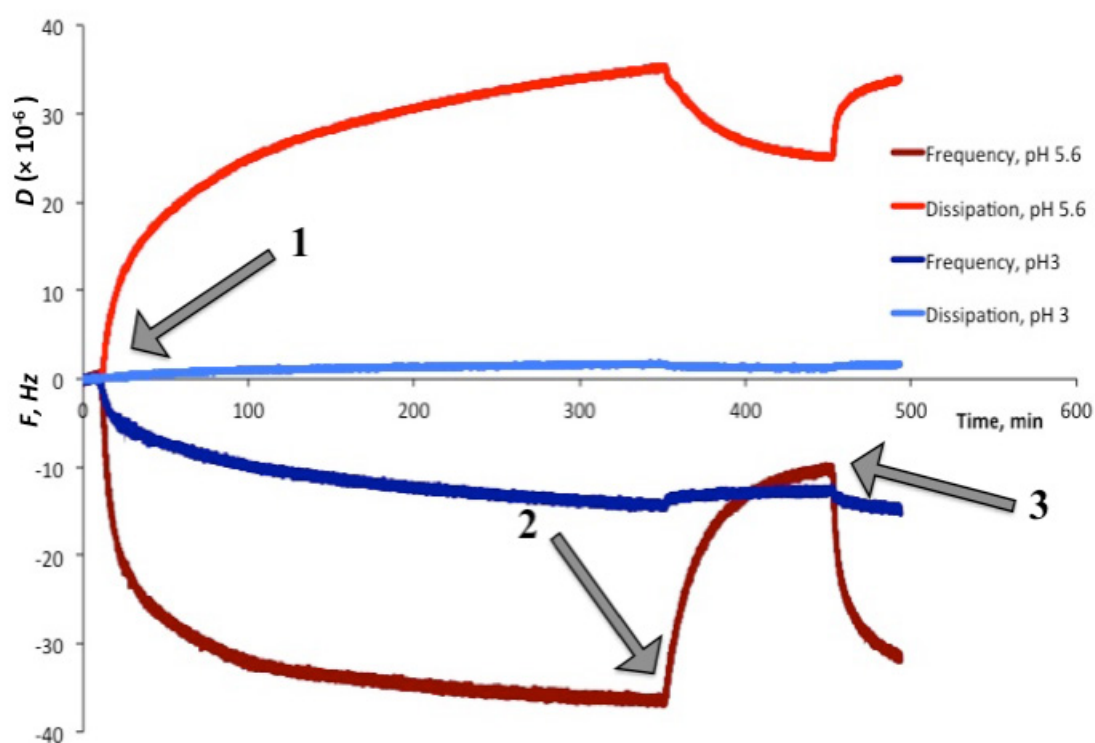


Fig. 5.13. Frequency and dissipation change dependence measured on QCM-D crystal when PAA-coated TiO_2 NPs deposit onto the polysaccharide substrate as a function of pH. Step 1 = NPs injection; step 2 = NPs-free electrolyte injection; step 3 = second NPs injection

The rinsing step and the second deposition step revealed partial reversibility of the frequency and dissipation values. The number of nanoparticles removed by rinsing is higher at pH = 5.6 (78 %) than at pH = 3 (21 %) (Table 5.5) which results in the same fraction of NPs remaining irreversibly attached at both pH values.

This suggests that two different processes likely drive the NPs deposition on the polysaccharide substrate. On one hand, some irreversible interactions contribute to NPs attachment to the same extent at both pH values. On the other hand, the reversible contribution, favoured at pH 5.6, likely corresponds to interactions between the PAA and the polysaccharide molecules, weakly bridging the NPs to the substrate. This results in an apparent steady state, which is rapidly cancelled when the NPs input is stopped.

Overall, the surface coverage values obtained for the PAA-coated TiO₂ under such unfavourable conditions ($\theta_{\text{QCMD, EPS, PAA-TiO}_2} = 2.9 - 3.9 \%$) (Table 5.5) always remain lower than the values obtained under favourable conditions with pure TiO₂ ($\theta_{\text{QCMD, EPS, TiO}_2} = 6 - 25 \%$) where electrostatic attraction drives the deposition.

The AFM analysis of PAA-coated TiO₂ NPs on the polysaccharide substrate at pH = 3 and at pH = 5.6 confirms that some NPs are deposited at both pH values (Fig. 5.14). The apparent surface coverage, θ_{AFM} is 12.4 and 10.9%, respectively, for pH = 3 and 5.6 (Table 5.5), which results in a divergence between θ_{AFM} and θ_{QCMD} on the same order as that detected for pure TiO₂ deposition on the polysaccharide substrate. Moreover, the presence of large aggregates is noticeable at pH = 3 (Fig. 5.14), which is in agreement with the size distribution measured by DLS (Fig. 2.3).

Table 5.5. Deposited mass of PAA-coated TiO₂ on the polysaccharide substrate before and after the rinsing step

pH	NP deposit mass θ_{QCMD} , mg/m^2		Coverage θ_{QCMD} , %		Coverage θ_{AFM} , %
	initial	rinsed	initial	rinsed	n.a.
3	2.39	1.89	5.0	3.9	12.4
5.6	6.35	1.40	13.2	2.9	10.9

In particular, the AFM analysis of the silica substrate after it was dipped in the PAA-coated TiO₂ NPs did not reveal any NPs deposition at pH 3 and 5.6. This is very likely due to the electrostatic repulsions that exist between the NPs and the flat substrate, which evidences the favourable polysaccharide effect on the NPs deposition measured here.

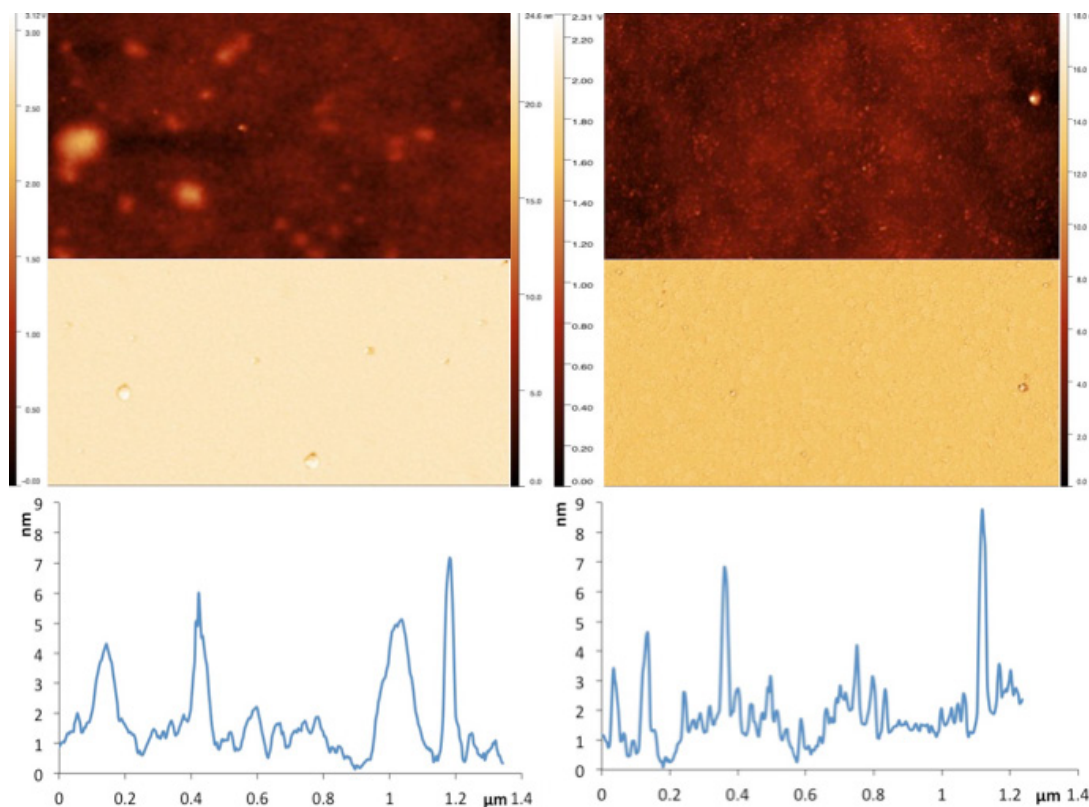


Fig. 5.14. AFM images of the PAA-coated TiO_2 NPs deposition on the polysaccharide substrate in pH = 3 (left) and in pH = 5.6 (right) in 10^{-3} M NaCl. Images display topographic mode, phase mode and scratch height profile from top to bottom.

5.4. Au NPs deposition on polysaccharide layer and SiO_2 substrate

Au NPs (coated with citrate, bPEI and silica) deposition was first studied by utilizing QCM-D. 0.05 mg/ml purchased solutions were diluted to 1 mg/l NPs by 10^{-3} M NaCl at pH 5.6. Deposition experiments were performed on silica surface and on a substrate coated with polysaccharide, following exactly the same procedure as described for TiO_2 NPs in the previous section.

In order to verify the possible reversibility in the NPs attachment after deposition on the substrate, a rinsing step was processed after a plateau was reached, by injecting

the same electrolyte free of NPs. The mass of NPs remaining deposited on the substrate after the rinsing step was recorded as well.

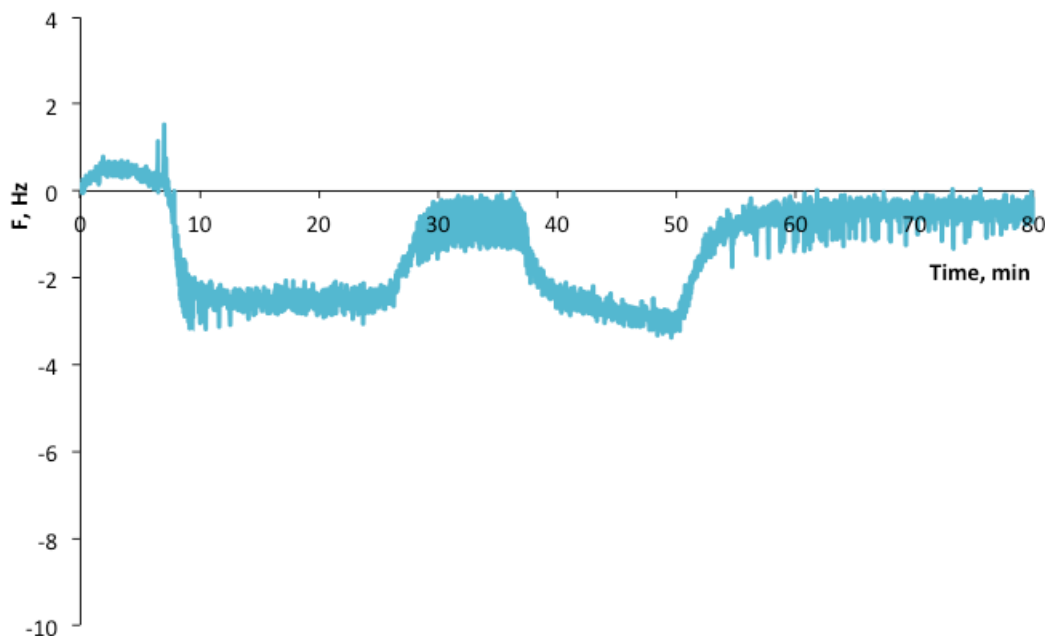


Fig 5.15. Deposition of cit-Au on the polysaccharide layer (raw QCM-D data). Decrease in F corresponds to the NPs deposition and increase to the rinsing step with electrolyte, pH 5.6

The raw QCM-D data for cit-Au NPs depositing on the EPS layer are presented in Figure 5.15. In this experiment, two consecutive cycles of NPs deposition followed by a rinsing step were processed. A reversible deposition was clearly shown under these conditions with the cit-Au NPs. Despite the negative surface charge of both the NPs and the substrate, some weak attractive interaction takes place between the EPS layer and the cit-Au NPs, similar to what was measured with the PAA-TiO₂ NPs. However, while the PAA-TiO₂ NPs deposition on the EPS was partially reversible through the rinsing step, here, the cit-Au NPs deposition is totally reversible.

The maximum mass deposited before and after rinsing is plotted in Figure 5.16 as a function of the substrate type and NPs coating. Surprisingly, the Si-Au NPs, which display a negative surface charge similar in zeta potential as that of the cit-Au NPs, did not deposit neither on the EPS substrate nor on the SiO₂ substrate. This is

certainly the result of the electrostatic repulsions between the negatively charged EPS and SiO_2 and the NPs. However, such electrostatic repulsions were overcome in the case of cit-Au NPs or PAA- TiO_2 NPs for which weak attractions took place. This suggests that these weak interactions between the EPS and the negatively charged NPs exist only when the NPs are coated with an organic layer, i.e. polyacrylic acid for TiO_2 or citrate for Au. This comforts the hypothesis that the weak attraction could result from the competition between the polysaccharide and the NPs organic coating, at the expense of the latter which is preferentially substituted by the EPS. Such substitution in the organic corona of the NPs is not possible in the case of the Si-Au NPs, for which the mineral coating is much more stable than any adsorbed organic molecule.

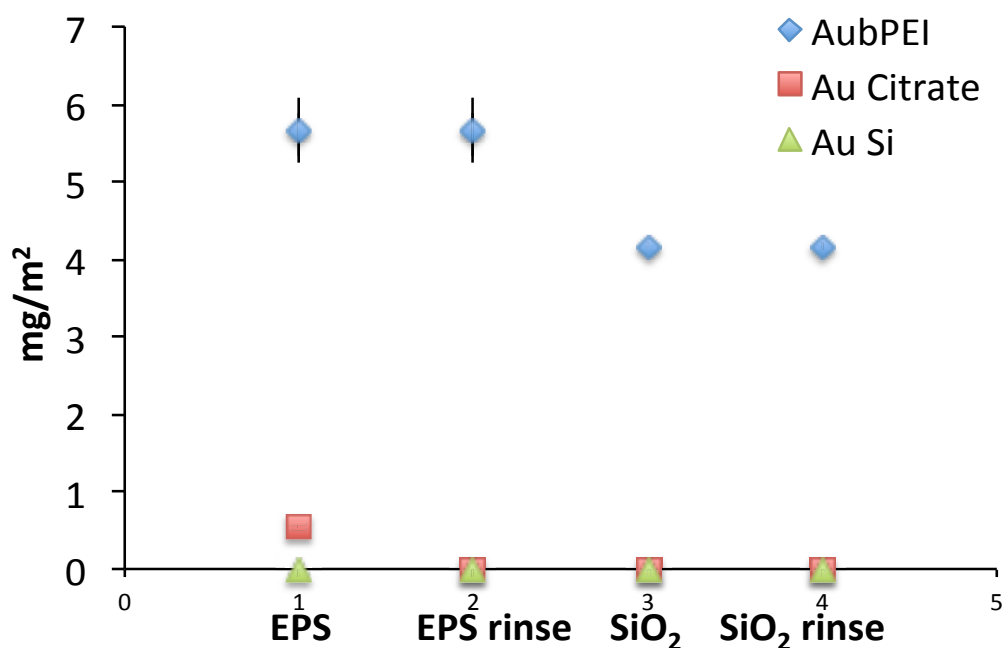


Fig. 5.16. Au NPs deposition on polysaccharide layer and SiO_2

As expected, the positively charged NPs, i.e. bPEI-Au NPs, undergo the maximum deposition density on the negatively charged substrates. However, the substrate type effect is opposite here to that observed under favorable conditions with TiO_2 NPs at $\text{pH} = 4$. Here, the deposited mass is higher on the EPS substrate than on the SiO_2 surface. This may be due to the deprotonation of the polysaccharide acidic moieties

here, which increases the EPS substrate charge density and favors a higher deposition density of the NPs by electrostatic attraction.

5.5. Stability of the EPS coating on the collector substrate

Prior to study the deposition of TiO₂ NPs on the EPS layer, the EPS stability at the collector surface was studied as a function of the ionic strength and pH. Its deposition was measured on gold crystal in QCM-D system from a 100 mg/l feed solution within a pH range from 3 to 10 and an ionic strength ranging from 10⁻³ M to 1 M of NaCl and CaCl₂. The stability was tested by changing the solution pH from 3 to 10 step by step. First, the EPS solution was deposited at pH 3 and rinsing step with a buffer was performed in order to measure the potential EPS release from the surface. Afterwards, the same was performed with a deposition prepared at pH 4. Further the buffer with increased pH to 10 was flowing in the system. pH tested were 3; 4; 6.5; 10. At pH 3 and pH 4 it was followed by the EPS deposition, the others pH by electrolyte rinsing. Each step was performed till obtaining a plateau with an average time for 15 min.

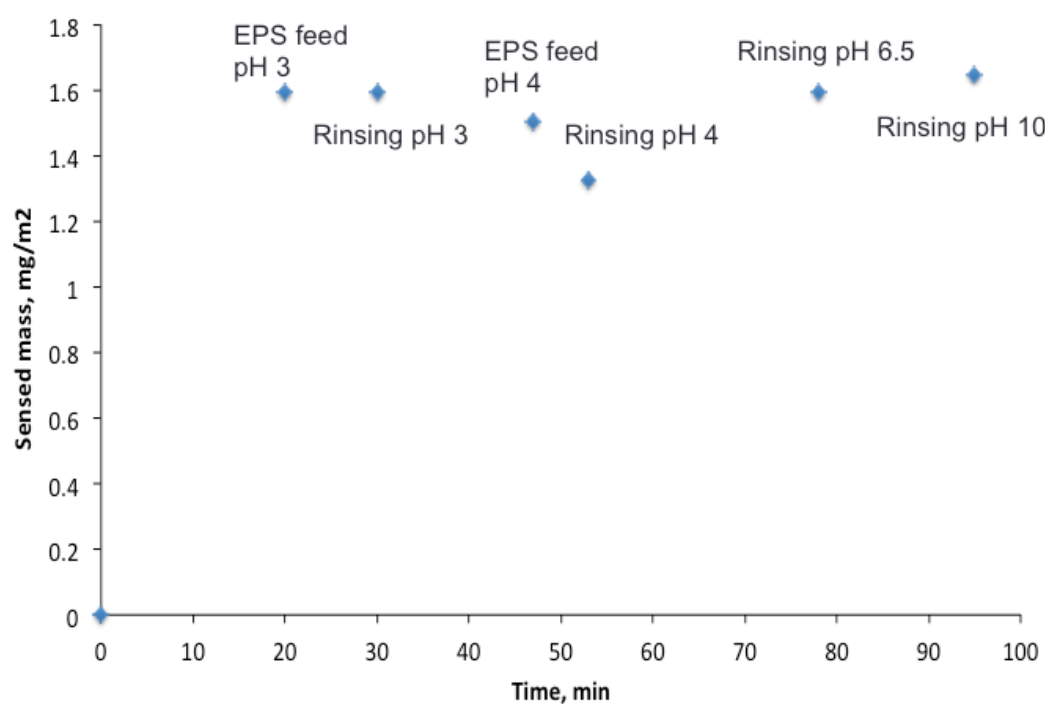


Fig. 5.17. Polysaccharide stability in 10^{-2} M NaCl solution within pH range 3 to 10

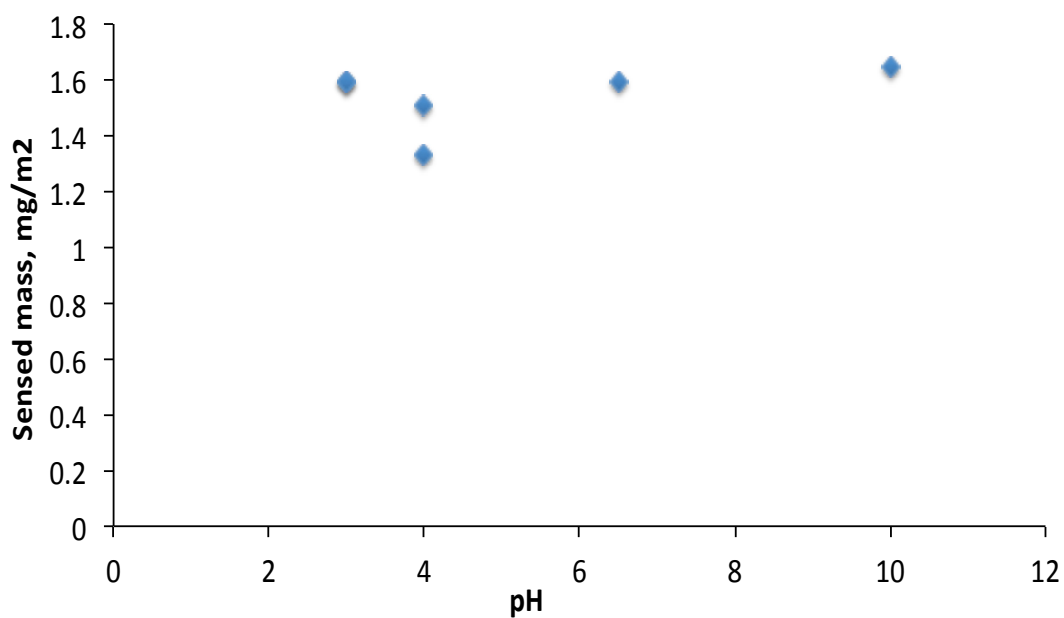


Fig. 5.18. Polysaccharide stability in 10^{-2} M NaCl solution within pH range 3 to 10

The mass of polysaccharide remaining deposited on the crystal after rinsing at increasing pH indicates that the prior deposition at pH 3 remains stable at higher pH. Indeed, no evident mass loss was detected after increasing the pH from 3 to 10. This suggests that the EPS bonds with the underlying PAH anchor on the crystal are irreversible under these conditions.

The effect of the ionic concentration and of the cation valency were also studied, using NaCl and CaCl₂ solutions at 10⁻³ or 10⁻² mol/l. (Fig. 5.19). The divalent cation strongly favors the polysaccharide adsorption at low salt concentration (10⁻³ mol/l), with 0,5 mg/m² deposited with NaCl vs. 3 mg/m² deposited with CaCl₂. This is probably the effect of the cationic bridges formed by the divalent cation between the PAH and the EPS.

When the salt concentration is increased tenfold, this valency effect is not that contrasted anymore. This is because increasing the NaCl concentration lead to increase six-fold the deposited mass on the crystal. This effect can be compared to that observed with the deposition of positively charged TiO₂ NPs on the negatively charged substrate in the previous section, where increasing the ionic strength resulted in a denser deposited layer due to the screened electrostatic repulsions between neighbor units.

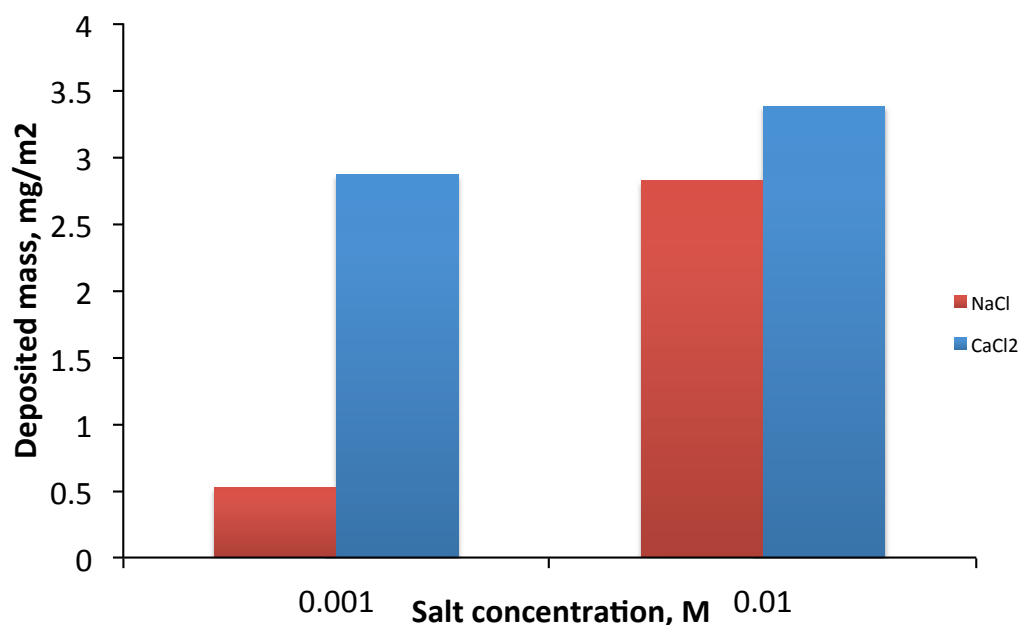


Fig. 5.19. Polysaccharide deposition vs ionic strength of NaCl and CaCl₂ solution

5.6. Conclusion

These results show that the physicochemical conditions strongly influence the mode of NPs deposition. Under attractive electrostatic interactions at pH 4, higher deposition for TiO₂ NPs was favoured on the mineral collector compared with the polysaccharide substrate for the same conditions because of the higher surface charge density of the former. Moreover, the nanoparticle deposition density for both substrate types increased as the ionic strength increased moderately. This was due to the partial screening of electrostatic repulsions between neighbouring nanoparticles by the oppositely charged substrate. The deposition rate was also shown to be dependent on the substrate capacity. The rate displayed regimes were either limited by the sticking reaction or by transport, in relation to the ionic strength and the substrate type.

For the positively charged bPEI-Au NPs the maximum deposition density was observed at pH 5.6 under favorable conditions as with TiO₂ NPs. In this case, the deposited mass was higher on the EPS substrate than on the SiO₂ surface that may be due to the deprotonation of the polysaccharide acidic moieties. This increases the EPS substrate charge density and favors a higher deposition density of the NPs by electrostatic attraction.

Under repulsive electrostatic interactions between the PAA-TiO₂ NPs and the substrate, an unexpected partially reversible deposition was observed only on the polysaccharide, suggesting the presence of bonds between the PAA coating molecule and the polysaccharide. The deposition was thus not only driven by electrostatic interactions with the collector but was also affected by the heterogeneity of the collector surface caused by the polysaccharide and by the interactions between the neighbouring NPs. With the cit-Au NPs, a reversible deposition was clearly shown. While the PAA-TiO₂ NPs deposition on the EPS was partially reversible through the rinsing step the cit-Au NPs deposition was totally reversible. However, for the Si-Au NPs the deposition was not observed neither on the EPS substrate nor on the SiO₂ substrate. Weak attractions took place only for the NPs coated with an organic layer, i.e. polyacrylic acid for TiO₂ or citrate for Au. They may likely result from the competition between the polysaccharide and the NPs organic coating, while for the mineral SiO₂ coating it is not possible.

The EPS layer on the substrate has been found to be stable in the solutions with different ionic strength ranging from 10^{-3} M to 1 M of NaCl and CaCl_2 and within a pH range from 3 to 10. The mass of polysaccharide remains deposited on the crystal the increasing pH and remains stable at higher pH. No evident mass loss was detected after increasing the pH from 3 to 10. This suggests that the EPS bonds with the underlying PAH anchor on the crystal are irreversible under these conditions.

Cation valency also had some influence at low ionic strength. The divalent cation strongly favors the polysaccharide adsorption at low salt concentration (10^{-3} mol/l), with $0,5 \text{ mg/m}^2$ deposited with NaCl vs. 3 mg/m^2 deposited with CaCl_2 . This is probably the effect of the cationic bridges formed by the divalent cation between the PAH and the EPS. When increasing tenfold the salt concentration this valency effect was not that contrasted anymore. This is because increasing the NaCl concentration lead to increase six-fold the deposited mass on the crystal. This effect can be compared to that observed with the deposition of positively charged TiO_2 NPs on the negatively charged substrate, where increasing the ionic strength resulted in a denser deposited layer due to the screened electrostatic repulsions between neighbor units.

In an environmental context, our results suggest that the bacterial polysaccharide coating on the collector surface tends to decrease the potential for NPs deposition when attractive electrostatic interactions exist between the NPs and the surface. This is clearly depending on the NPs types and probably their stability in the suspension as for the more stable Au NPs in comparison with TiO_2 this trend was not observed. Since a major part of environmental collectors is made of silica or displays a biofilm surface, i.e., a major part is negatively charged, this scenario will mostly occur for positively charged NPs. However, natural collectors, such as iron or aluminium oxides, may expose a positively charged surface that likely interacts with negatively charge NPs. On the contrary, under conditions of electrostatic repulsion, the NPs deposition is weaker and depends mainly on the respective surface heterogeneities and conformational changes of the organic coatings as a function of the physicochemical conditions. The NPs deposition on the bacterial polysaccharide surface also depends on the nature of their coating, organic coatings favours their deposition which is reversible while for the mineral coating no deposition is observed. As the deposition in the environment preferably happens in the suspension and some free polysaccharide must be present there also some processes of the interaction between NPs and EPS in the suspension have to contribute to the general interaction

of the NPs and EPS. As it is previously shown under favourable conditions a tendency to significant and rapid aggregation of EPS with TiO_2 , and subsequent sedimentation of the aggregates formed, was observed. Nevertheless, not all NPs react with the EPS but a fraction of the nanoparticles still remains in the suspension and is not involved in the aggregation and interaction process. This may be due to the saturation of NPs by the adsorbed EPS.

Under unfavorable conditions an unexpected aggregation was also observed between EPS and PAA- TiO_2 but some free polysaccharide and free nanoparticles remained clearly distinguishable, indicating their distinct behaviors in suspension in spite of the settling out the aggregates during the interaction.

Chapter 6. Transfer of nanoparticles through the polysaccharide gel

6.1. Introduction

In order to investigate the possible transport of NPs through the polysaccharide gel, a filtration experiment was designed. In this approach, due to steric hindrance favored in the gel, we selected Au NPs as surrogates having well-controlled and varying size and surface property. They were also selected in order to avoid the NPs homoaggregation as this would alter the studied permeability of the gel.

The experimental approach consisted in a percolation set up, where Au NPs were filtrated through a gel layer, using a syringe equipped with a membrane container, and connected in flow mode to UV spectroscopy for the NPs breakthrough quantification. The UV absorbance of the NPs left in filtrate was measured and converted to the NPs concentration. Based on this concentration, the amount of NPs retained by gel was calculated.

The different parameters studied and combined together are presented in Table 1. The resulting NPs concentrations measured in the filtrate are given as well.

6.2. Effect of the solution and NPs parameters on the breakthrough and gel retention capacity

Table 6.1. Effects of the studied parameters on the breakthrough of Au NPs. When the parameters are not indicated, the defaults conditions are gel concentration 10 g/l, cit-coated Au NPs 5 nm in water 1 mg/l, flow rate 1 ml/h

Parameters		
NPs Concentration (mg/l) in filtrate	Effect of gel concentration	
	10 g/l (cit-coated Au NPs 5 nm)	15 g/l (cit-coated Au NPs 5 nm)
	0,41	0,48
	Effect of salt concentration	
	cit-coated Au NPs 5 nm in	cit-coated Au NPs in 1M

	water after NaCl	NaCl
	0,3	0,36
	Effect of flow rate	
	1 ml/h (bPEI-Au 12 nm)	0,5 ml/h (bPEI-Au 12 nm)
	0,33	0,3
	Effect of NPs size	
	cit-coated Au NPs 5 nm	cit-coated Au NPs 12 nm
	0,41	0,31
	Effect of NPs charge	
	cit-coated Au NPs 5 nm	bPEI-Au 5 nm
	0,41	0,41

As observed in Table 6.1 the gel concentration 10 or 15 g/l has no evident effect on the relative retention of cit-coated Au NPs, which breakthrough at the concentration 0,41-0,48 mg/l. The salt NaCl 1 M vs. pure water, or the flow rate, has no evident effect neither. Even the NPs surface charge, negative citrate vs. positive bPEI, did not show any evident effect on the NPs retention.

The NPs size did have an impact on the retention by the gel. NPs with smaller size of 5 nm have a higher breakthrough, 0,41 mg/l, in comparison with 12 nm NPs at 0,31 mg/l.

Knowing the mass of NPs passed and the mass of polysaccharide in the gel layer, the mass and fraction of NPs retained by the gel could be estimated. The data obtained for all the conditions tested are summarized in Table 6.2. The lowest retention was measured for 5 nm citrate coated NPs in water. The retention was slightly higher in 1M NaCl than in water, and with 12 nm than with 5 nm size.

In order to estimate the possible reversibility in the NPs retention obtained in the presence of salt, after filtration with NaCl 1M, NPs water solution was passed through the gel layer in a second step, and the filtrate was analyzed again. Retained capacity of the gel under these conditions was in the same range as for the only water solution passed through the gel.

Table 6.2. NPs retention by the gel as a function of the parameters varied (default conditions are 1 mg/l NPs 5 nm, 10 g/l gel concentration, 1 ml/h flow rate)

NPs type and conditions	NPs retention by the Gel (mg/g)	Mass % of NPs retained
cit-coated Au NPs 5 nm in water	0,0588	59
cit-coated Au NPs 12 nm in water	0,0685	69
cit-coated Au NPs 5 nm in 1M NaCl	0,0636	64
cit-coated Au NPs 5 nm in water after 1M NaCl	0,07	70
bPEI-Au 5 nm in water	0,059	59
bPEI-Au 1ml/h	0,067	67
bPEI-Au 0,51ml/h	0,07	70

The similar retentions measured here with both positively and negatively charged NPs were unexpected. The negative charge of the polysaccharide gel was supposed to favor electrostatic attractions with the positively charged NPs, i.e. higher retention of the bPEI-coated Au NPs. This was actually not observed. Our hypothesis is that the gel capacity for NPs retention may be largely lower than the total amount of NPs injected in our working conditions. This could result in a fraction of bPEI-Au NPs retained not detected, because very low in comparison to that in the filtrate.

In order to verify this hypothesis, in the next part of this chapter, we measured the NPs breakthrough in the filtrate with time resolution. A syringe filtration system was used in-line instead of the filtration unit under vacuum, in order to measure the NPs breakthrough as a function of time.

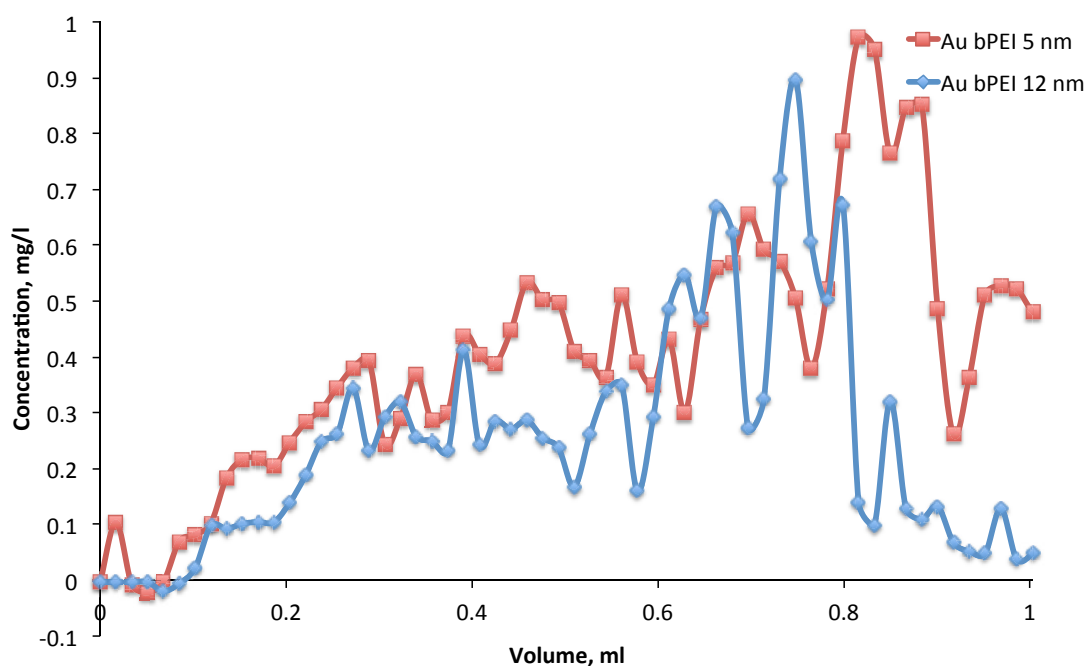


Fig. 6.1. bPEI-Au NPs 5 and 12 nm filtration, concentration in filtrate vs volume passed

Between 0 and 0.6 ml passed, the 5 nm NPs concentration in the filtrate increased from 0 to 0.5 mg/l. This indicates that along with the NPs injection, the fraction retained decreases progressively, probably because the gel saturates progressively. Between 0.6 and 1 ml, the NPs concentration in the filtrate is very irregular with pulses suggesting punctual NPs expulsions. This may be the consequence of the previous gel saturation.

Estimating the amount of NPs passed through the gel in 1 ml, the gel retention capacity for bPEI-Au NPs could be estimated basing on the concentration detection in the filtrate. The gel amount was calculated for the used volume of 10 g/l solution used for its preparation. Capacities of 0,038 mg/g for 5 nm and 0,050 mg/g for 12 nm were obtained. Thus the gel retention capacity is higher for the larger NPs.

We are far from gel porosity but gel fibers are saturated with NPs (solid fraction of gel). As the 1.5 ml gel volume used for the calculation is highly concentrated 10 g/l solution and 1 ml NPs volume passed is very diluted 1 mg/l suspension.

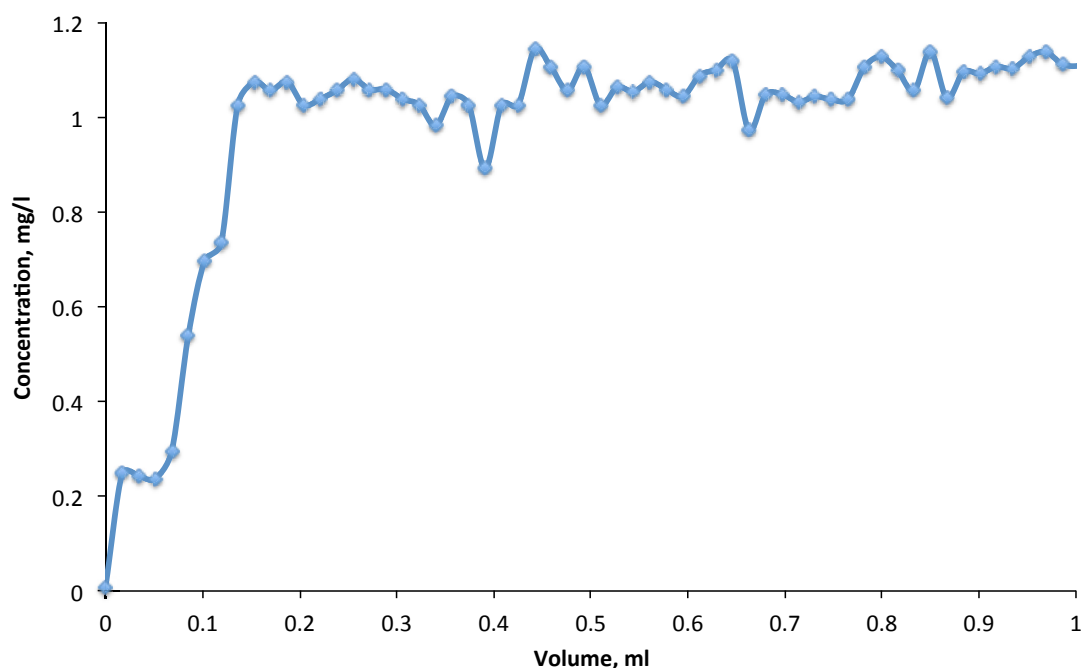


Fig. 6.2. Cit-coated Au NPs 5 nm filtration, concentration in filtrate vs volume passed

The breakthrough of cit-coated Au NPs is almost 100 %. The NPs concentration in the filtrate reaches that in the feed solution, 1 mg/l, after passing 0.15 ml of NPs suspension. The reason why the maximum NPs concentration in the filtrate is not instantaneously reached may be the gel tortuosity, which favors the NPs retention in dead-end pores. The gel retention capacity here was calculated at 0,012 mg/g.

Because in-line measurement of the UV-Vis absorbance using flow cell lead to a high noise and bad reproducibility in the obtained data, in the next part of this work, we decided to conduct the time resolved measurement of the absorbance in normal UV cuvette. The absorbance was measured periodically on approximately 0.7 ml samples collected along with the solution elution from the gel.

6.3. Effect of size and pH on the NPs transfer through the gel

Cit-coated Au NPs with 12 and 50 nm size were used in order to study the effect of size on their breakthrough in the gel layer. 50 mg/l stock suspension were diluted to 5

mg/l in 10^{-3} M NaCl at pH 5.6 and 4 ml of this NPs suspension were passed through the gel. Cit-coated Au NPs of 50 nm size suspension prepared at pH 3 and 5.6 were used to study the effect of pH on the NPs breakthrough. Syringe filtration was performed here. For NPs suspension passing through the gel and rinsing after that with free from the NPs electrolyte. After passing the NPs through the gel, the possible release of NPs from the gel was measured by passing through gel an electrolyte of same composition but free of NPs. The NPs then released in the filtrate were quantified by UV-Vis spectroscopy.

For Au 50 nm, a higher breakthrough is measured at pH 3 than at pH 5.6. The plateau is reached faster due to a higher slope in the breakthrough curve. This shows that cit-Au NPs 50 nm are more mobile in the gel at pH 3. At this pH, the polysaccharide charge is close to zero. This should decrease the electrostatic repulsion between the negatively charged citrate coated Au NPs and the polysaccharide chains, and thus increase the NPs retention. However, the opposite trend is observed. This could be due to the gel structure altered at pH 3 due to the charge neutralization. This may indeed favour the gel reticulation, which results in the gel fiber aggregation and leads to larger and more connected porosity.

At pH 3 the average amount of NPs passed is 0,015 mg, which corresponds to 75 % of totally injected amount in the NPs suspension volume. 0,005 mg of Au 50 nm NPs were retained at pH 3 corresponding to 25 % of the total passed NPs amount.

Almost the same amount of cit-coated Au NPs is released at pH 3 as at pH 5.6 which is equal 14,3 % of the retained NPs.

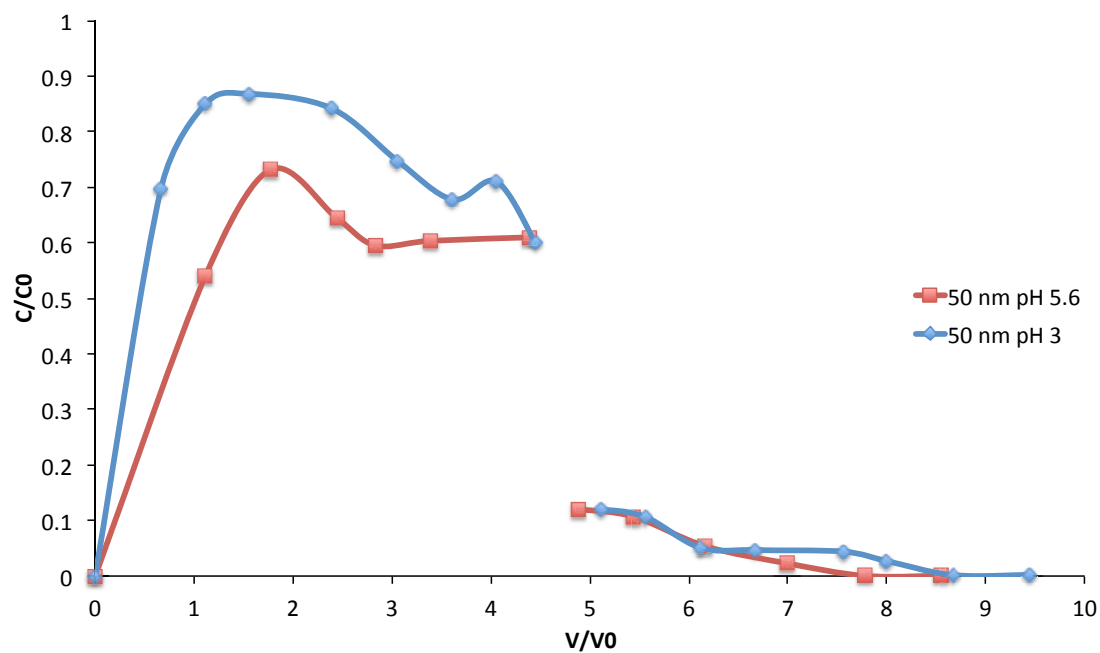


Fig. 6.3. Mobility and release profiles of cit-coated 50 nm Au NPs at pH 3 and 5.6 through the gel layer

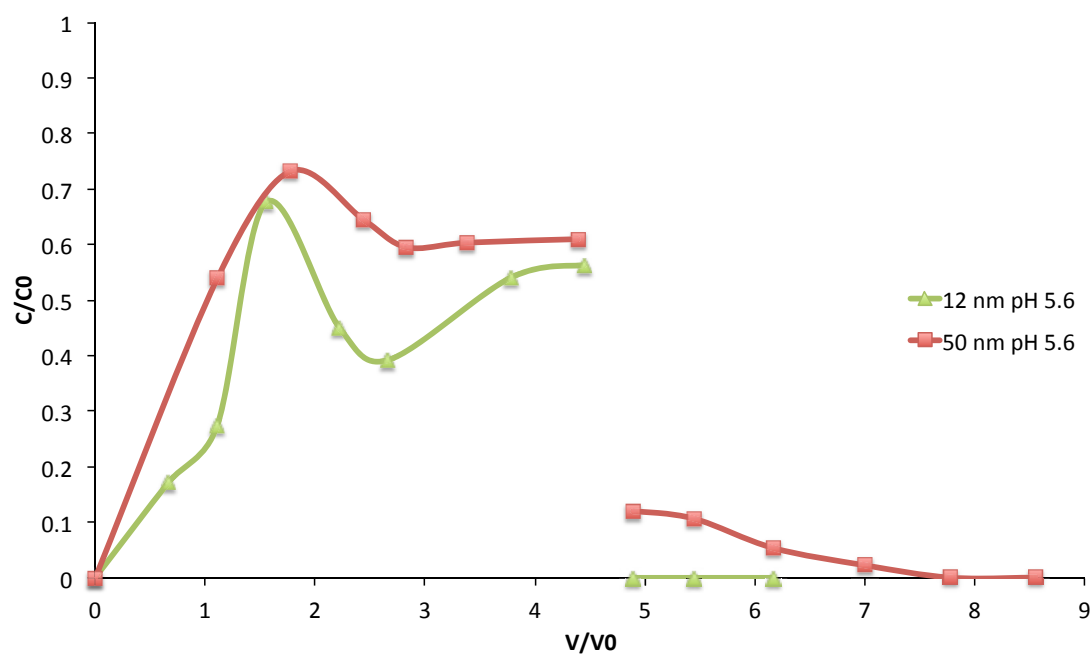


Fig. 6.4. Mobility and release profiles of cit-coated Au NPs of 12 and 50 nm at pH 5.6 through the gel layer

0,02 mg of cit-coated Au NPs were injected in total in the gel. An average amount of 0,012 mg passed through the gel at pH 5.6 for the 50 nm NPs, which corresponds to 61 % of the NPs injected. Thus 39 % were retained by the gel, corresponding to 0,008 mg of NPs.

The NPs release measured in a second step revealed that 0,0005 mg of previously retained NPs were released here with passing NaCl solution.

Amount of cit-coated Au NPs 12 nm were passed through the gel 0,009 mg of the total 0,02 mg and 0,011 mg respectively retained. This amount is in the same scale as for the 50 nm Au NPs. The 12 nm cit-coated Au NPs did not release. This could be due to the fact that the smaller NPs size favors deeper penetration into the smaller space of the gel porosity. This can also explain that they are previously retained in slightly higher amount than 50 nm Au NPs.

6.4. Conclusion

Under condition where NPs breakthrough was measured in one point after filtration, the results showed that the gel concentration has no evident effect on the relative retention of cit-coated Au NPs. The salt NaCl 1 M vs. pure water, or the flow rate, had no evident effect on the NPs transfer through the gel. The NPs surface charge, negative citrate vs. positive bPEI, unexpectedly, did not show any evident effect on the NPs retention neither. Our hypothesis was that the gel capacity for NPs retention is lower than the total amount of NPs injected in our working conditions, so that the fraction of bPEI-Au NPs retained was negligible. The NPs size had an impact on the retention by the gel, with smaller size having a slightly higher breakthrough.

A syringe filtration system used in-line instead of the filtration unit under vacuum enabled us to measure the NPs breakthrough as a function of time. Under favourable retention condition, with bPEI-Au NPs, the fraction retained decreased progressively along with the injection, probably because the gel was saturating. After the gel saturation, some irregular pulses of NPs expulsions were detected. The amount of NPs retained at the saturation suggests that the retention capacity by the gel is not limited by the pore volume but by the adsorption of NPs onto the gel fibers.

Under these conditions, it was confirmed that the NPs size has an influence on the NPs retention. For both types of coating bPEI or cit-coated the observed impact is the same, the gel retention is lower for the smallest NPs.

The breakthrough of cit-coated Au NPs was almost 100 %. The reason why the maximum NPs concentration in the filtrate is not instantaneously reached may be the gel tortuosity, which favors the NPs retention in dead-end pores.

Effect of size and pH on the NPs transfer through the gel was measured for cit-coated Au NPs. 50 nm cit-Au NPs are more mobile in the gel at pH 3 than at pH 5.6, probably because the polysaccharide charge is close to zero at pH 3, which collapses the gel structure, and leads to preferential pathways for the NPs.

The reversibility tests revealed that the same amount of the 50 nm cit-coated Au NPs is released at pH 3 and at pH 5.6 which is equal 14,3 % of the initially retained NPs. No reversibility was detected for 12 nm NPs, which could be due to the deeper penetration into the gel porosity of these smaller NPs. This can also explain that they are previously retained in slightly higher amount than 50 nm Au NPs.

Chapter 7. Conclusion and perspectives

The mobility and fate of NPs in the environment drive the exposure behaviour. This dissertation has investigated the interaction of variably charged and sized nanoparticles with bacterial polysaccharide. The interaction of the nanoparticles with the EPS in suspension and NPs deposition on its surface are key processes which influence their fate. The study was designed to determine the interaction between nanoparticles and bacterial polysaccharide in diluted conditions in suspension, deposition on the gel surface, and transfer through the gel, under favorable and unfavorable conditions. The release of nanoparticles from sunscreens under aqueous aging was also studied.

To summarize the impact of different conditions and their combination on the NPs release in the water environment the following conclusions can be drawn.

- Release of TiO₂ NPs from commercialized sunscreens into aquatic environment

Aging sunscreens, we evidenced the release of colloidal residues containing TiO₂ nanoparticles as well as organic part from commercialized sunscreens into aquatic environment after an artificial aging in water and in a saline water mimicking seawater under exposure to simulated sunlight or in dark conditions. Under all conditions the residues TiO₂ particles with organic matrix partly remained in the water suspension but a major part underwent aggregation and sedimentation especially in seawater.

This appears to be also dependent on TiO₂ particle size in the cream and aging conditions (light or dark). In pure water, larger aggregation and sedimentation took place in light condition than in dark condition, probably due to the photodegradation of the organic components. The hypothesis is that the nanoparticle coating underwent alteration and desorption in the aqueous media. This enables the nanoparticles to reorganize in the sunscreen residues and evolve toward a more hydrophilic character.

- Favorable interaction between TiO₂ NPs and an exopolysaccharide

Summarizing the study of the interaction of the nanoparticles and EPS in suspension, under favorable interaction conditions, it was revealed a tendency to significant aggregation of EPS with TiO₂, and subsequent sedimentation of the aggregates formed, in all the mixture ratios studied. These aggregates were very rapidly formed, as soon as the interaction was allowed, and the largest ones were removed from the

suspension after one hour of settling time. Nevertheless, despite this rapid evolution a significant part of both individual NPs and large aggregates remained in suspension after one hour of interaction. This suggests that only a fraction of the nanoparticles was involved in the aggregation process.

The different ratios tested appear to have a very limited effect on fate of the NPs. In all cases a tendency to significant aggregation was observed, well pronounced at 10:10 and 1:10, but more limited at 100:10. This is likely due to the saturation of the NPs surface by the adsorbed EPS.

The EPS is likely to interact with a certain fraction of the nanoparticles in suspension, leading to the formation of large aggregates which tend to sediment. In the same time, especially under unfavorable conditions, some polysaccharide remain also free in solution.

Under unfavorable conditions an unexpected aggregation was also observed between EPS and PAA-TiO₂. Some aggregates formed rapidly, already detected at the initial time of the interaction. After one hour of interaction, they had settled out, but some free polysaccharide and free nanoparticles remained clearly distinguishable, indicating their distinct behaviors in suspension. No clear effect of the EPS:PAA-TiO₂ ratio could be evidenced under these conditions.

The nature of the attractive interaction between EPS and PAA-TiO₂ NPs under unfavourable conditions is not yet clear. It may be due to some heterogeneities in the structure of the EPS macromolecule or to some competition between the EPS and the PAA coating to adsorb at the TiO₂ surface. These weak interactions were confirmed again in the other experimental approach followed in this work, based on NPs deposition to the EPS layer.

- Deposition of NPs on mineral collectors

The results of the deposition studies have shown that the physicochemical conditions strongly influence the mode of NPs deposition. Under attractive electrostatic interactions, higher deposition for TiO₂ NPs was favoured on the mineral collector compared with the polysaccharide substrate for the same conditions because of the higher surface charge density of the former. Moreover, the nanoparticle deposition density for both substrate types increased as the ionic strength increased moderately. This was due to the partial screening of the electrostatic repulsions between neighbouring nanoparticles by the oppositely charged substrate. The deposition rate

was also shown to be dependent on the substrate capacity. The rate displayed regimes were either limited by the sticking reaction or by transport, in relation to the ionic strength and the substrate type.

For the positively charged Au NPs a maximum deposition density was measured under favorable conditions as with TiO₂ NPs. In this case, the deposited mass was higher on the EPS substrate than on the SiO₂ surface. This can be attributed due to the deprotonation of the polysaccharide acidic moieties at the working pH 5.6, as compared to the favorable conditions used with TiO₂ at pH 4. This increases the EPS substrate charge density and favors a higher deposition density of the NPs by electrostatic attraction.

Under repulsive electrostatic interactions between the PAA-TiO₂ NPs and the substrate, a partially reversible deposition was observed only on the polysaccharide, indicating again the presence of bonds between these NPs and the polysaccharide.

A similar effect was observed with the cit-Au NPs, which underwent reversible deposition on the EPS substrate under unfavorable conditions. However, for the Si-Au NPs the deposition was not observed neither on the EPS substrate nor on the SiO₂ substrate. These weak attractions took place only for the NPs coated with an organic layer, i.e. polyacrylic acid for TiO₂ or citrate for Au. For this reason, our hypothesis is that they result from the competition between the polysaccharide and the NPs organic coating, where the latter is substituted by the former at the surface of the NPs. Such phenomenon could not take place with the Si-Au NPs which have a mineral coating more stable under these conditions.

In this chapter, our results thus evidenced that the deposition of NPs is not only driven by electrostatic interactions with the collector but is also affected by the heterogeneity of the collector surface caused by the polysaccharide and by the interactions between the neighbouring NPs.

A major part of environmental collectors is made of silica or is coated with a biofilm surface, i.e., a major part is negatively charged. Thus favourable conditions for NPs deposition will be encountered mostly with positively charged NPs. On an other hand, natural collectors, such as iron or aluminium oxides, may expose a positively charged surface that will likely interact with negatively charged NPs. On the contrary, under conditions of electrostatic repulsion, the NPs deposition is weaker and depends mainly on the stability of the NPs coatings. The NPs deposition on the polysaccharide

substrate is likely favoured with organic and releasable coatings, while it is unfavoured with mineral coating.

The transfer and breakthrough of the NPs through the gel was studied using filtration unit. It showed that under repulsive electrostatic interaction, i.e. favourable conditions for breakthrough, the NPs size had an impact on the retention by the gel. Cit-coated Au NPs with smaller size of 5 nm have a higher breakthrough in comparison with 12 nm NPs. The retention was also slightly higher in 1M NaCl than in water due to the screening of the electrostatic repulsions between the NPs and the gel fibers.

Using time-resolved breakthrough measurements, for bPEI-Au NPs 5 and 12 nm, i.e. under conditions favorable for electrostatic retention, the fraction of NPs retained decreased progressively with injection time, likely due to the gel progressive saturation. After saturation, the NPs concentration in the filtrate was very irregular suggesting punctual NPs expulsions. The amount of NPs retained at the saturation suggests that the gel capacity was actually limited by gel fibers surface area, and not by the gel pore volume.

Effect of size and pH on the NPs transfer through the gel was also measured for cit-coated Au NPs of 12 and 50 nm. For Au 50 nm, a higher breakthrough was measured at pH 3 than at pH 5.6. This trend could be due to the gel structure altered at pH 3 due to the charge neutralization, which favours the gel collapse, resulting in a higher permeability. For the smallest 12 nm NPs about 50 % was retained by the gel.

The reversibility test, processed by injecting in the gel an electrolyte free of NPs, revealed that almost the same amount of cit-coated 50 nm Au NPs is released at pH 3 as at pH 5.6, while no release was detected for 12 nm NPs. This could be due to the fact that the smaller NPs size favors deeper penetration into the smaller space of the gel porosity. This can also explain that they are previously retained in slightly higher amount than 50 nm Au NPs.

Some diffusion of the NPs in the diffusion cell set was only observed with a membrane support through it. It was very slow, concentration in the donor compartment was decreasing during 25 days and in the acceptor compartment increased slightly at the beginning and remained stable from the 3d day.

For gel diffusion measurements it was not possible to observe any tendency as there were not changes in UV absorbance within some measurements. Also there were encountered some problems with diffusion set experiment such as evaporation of the

solution in the compartments, leaking in the connection part of the compartments, bubbles in flow cell mode (saturation of UV spectrum due to this), agitation etc.

Also when there is an equilibration (the same concentration in donor and acceptor compartments), due to a pressure a liquid can penetrate and circulate which influences the diffusion.

For future studies of the NPs influence on the environment the aquatic systems studied may be complicated in order to consider more factors which may play a role in the release, mobility, and deposition processes. Concerning the release study of the different products in the environment, the residues of the sunscreens will be exposed consequently to both the planktonic and benthic organisms. In order to study the influence for the real aquatic environment more complex systems have to be studied next to better mimic the environment.

The distribution and mobility study of the NPs in the environmental conditions may be studied in the diffusion cell without pressure which may be considered to be closer to the environmental uninfluenced conditions. The methods of the quantification of the components (NPs and EPS) in the aquatic system may be used to determine the scale of the NPs distribution in the system.

Moreover, in addition to typical polysaccharide as studied in this work, biofilm matrix is also composed of e.g. various polymers, low molecular weight compounds and proteins, which contribute to local physicochemical conditions, and thus which may alter the affinity for NPs. The roles of these compounds could be considered in future works, considering both natural ecosystems and wastewater systems, where biofilm development on collectors is ubiquitous.

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Annex